THEORETICAL INVESTIGATION ON THE RADICAL SCAVENGING ACTIVITY AND SOME OTHER APPLICATIONS OF NATURAL POLYPHENOLS

Thesis Submitted to
the University of Calicut for the award
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DOCTOR OF PHILOSOPHY IN CHEMISTRY

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2018

DECLARATION

I hereby declare that the thesis entitled **"THEORETICAL INVESTIGATION ON THE RADICAL SCAVENGING ACTIVITY AND SOME OTHER APPLICATIONS OF NATURAL POLYPHENOLS"** is the bonafide report of the original work carried out by me under the supervision of Dr. K. MuraleedharanDepartment of ,Professor , ,University of Calicut ,Chemistry for the award of the degree of ,sDoctor of Philosophy in Chemistry Under the Faculty of Science The content of this thesis have not .Kerala ,University of Calicut been submitted to any other institute or University for the award of .any degree or diploma

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CERTIFICATE

This is to certify that the thesis entitled "**Theoretical investigation on the radical scavenging activity and some other applications of natural polyphenols**", submitted to the University of Calicut, in partial fulfillment of the requirements for the award of **Degree of Doctor of Philosophy in Chemistry** is a bonafied record of research work done by **Ms. Vijisha K. Rajan**, during the period 2014-2018 in the Department of Chemistry at University of Calicut, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or other similar title to any candidate of any University.

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Vijisha K. Rajan

Dedicated to

All My Teachers and My Beloved Ones

PREFACE

Free radicals are short living-highly reactive fragments, have unpaired electrons in their outermost shell. They react rapidly with the cellular membrane and cause degenerative diseases which may ultimately lead to death. The generation of free radicals in biological system leads to oxidative stress (OS) and the free radicals are either Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS). In order to fight against these ROS/RNS, our body have some inbuilt defence mechanism by a set of compounds called antioxidants. But due to our modern life activities, the inbuilt antioxidants like Vitamin C & E are insufficient to fight against the reactive free radicals. In this scenario, development of new potent antioxidants is particularly important. But synthetic drugs have lots of side effects and due to their toxic nature, their use has been restricted so that researchers drives to discover new antioxidants with natural origin and this become a wide and noble area in research. Antioxidants fight against the ROS/RNS to protect the living cells from oxidative damage. The OS may lead the regular/normal life activities to an abnormal level and leads to ageing, arthritis, asthma, cancer, cataract, diabetes, neurodegenerative disorders, Alzheimer's, Parkinson's disease, etc.

Flavonoids are low molecular weight polyphenolic compounds having potential radical scavenging activities. The presence of C2-C3 double bond, presence of 5 or 3 –OH groups, presence of carbonyl group in ring [C], presence of electron withdrawing or releasing groups are the important structural factors which are taken into account when

dealing with flavonoids. In most of the flavonoids, the ring [B] is found to be the most reactive. The present work mainly focuses on the radical scavenging capacities of different classes of flavonoids. Further the UV filtering capacity, and capacity to act as pH indicators have also been evaluated. The electron donating/accepting capacities of each class of flavonids are well explained by evaluating global reactive descriptors. The radical scavenging capacities are analysed by means of suitable mechanisms and the most suitable one has been selected. The structural effects on the radical scavenging activities are also evaluated. Along with these, the pharmacokinetic parameters and bioactivities have been discussed for all the studied category of flavonoids. The molecular docking has been employed to evaluate the interaction of the studied flavonoids with the protein mono amine oxidase-B (MAO-B). Toxicological analysis of all the studied flavonoids has been carried out through the OSIRIS property explorer.

The present work employed a computational approach to evaluate the radical scavenging capacities of different classes of flavonoids including flavanones, flavanols, flavonols, flavones and anthocyanidins. Potential energy scanning has been carried out to elucidate the most stable conformers and is employed for further studies. All the computational works are carried out through Gaussian 09 (G09) software. Some online software like molinspiration, OSIRIS, etc., have also been used to analyse the toxicological and pharmacokinetic parameters of the studied flavonoids. The major objectives of the work are:

- To evaluate the global reactive descriptors of each class of flavonoids and thereby making an idea about their donor-acceptor interactions.
- Analyzing the UV-Visible spectrum obtained by the TDDFT tool in G09 to screen potential UV filters.
- To evaluate the structural changes of anthocyanidins with pH and understand about their capacity to act as pH indicator.
- To study the interaction of studied flavonoids with MAO-B *via* molecular docking and their capacity to prevent the oxidative stress caused by the action of MAO-B.
- To evaluate the toxicological properties of flavonoids by means of OSIRIS property explorer.
- To evaluate the pharmacokinetic properties and bioactivities against some specified targets *via* Molinspiration online software.
- To correlate the quantum chemical parameters or the computed BDE values with available experimental TEAC values of flavonoids.

All the computational works are carried out through G09 software by DFT-B3LYP functional and 6-31+G (d, p) as basis set. The solvent phase calculations are done through IEF-PCM model. Other softwares used are Chemcraft, OSIRIS property explorer, Molinspiration, Autodock Vina and Open Babel. At first a potential

energy scanning has been employed to get the lowest energy conformer of each category of flavonoids and the lowest energy conformer is then modified to get different flavonoids. These are then optimized by DFT-B3LYP functional and 6-31+G (d, p) as basis set. The solvent phase calculations have also been made. All the quantum chemical or global reactive descriptors are computed by suitable equations. The molecular orbitals (HOMO and LUMO) are analysed to get the band gap and the electrophilic and nucleophilic centres in each flavonoids are shown by plotting the ESP maps. The radical scavenging capacities have been evaluated by different mechanisms which include both hydrogen atom transfer (HAT) and electron transfer (SET). The competition between HAT and SET are well explained by means of DAM-FEDAM plots. By this way, the flavonoids are classified into antioxidants and antireductants. The inhibition activity of MAO-B by flavonoids has been analyzed by molecular docking studies. The toxicological properties are computed through OSIRIS and the pharmacokinetic properties are computed through Molinspiration.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

- *Introduce flavonoids, their applications such as antioxidant, UV filtering, pH indicating capacities, etc.*
- *Review on the recent experimental and theoretical works on the specific applications of flavonoids.*

1.1 Introduction

About 32 years ago; i.e., in 1985, the concept of Oxidative Stress (OS) has been introduced in the Redox biology and medicine as the introductory chapter 1 in the book 'Oxidative Stress' [1,2]. Later in a review article on 'Biochemistry of oxidative stress' [3], explain the world of antioxidants and their influence in biological system and then onwards 'Redox biology' becomes an interesting area in research among various disciplines like Chemistry, Medicinal Chemistry, Pharmaceutics, Biology, Radiation Biology, etc [4].

Free radicals are short living-highly reactive fragments, have unpaired electrons in their outermost shell. They react rapidly with the cellular membrane and cause degenerative diseases which may ultimately lead to death. The generation of free radicals in the biological system leads to OS and the free radicals are either Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS). In order to fight against these ROS/RNS, our body has inbuilt defense mechanism by a set of compounds called antioxidants. However, due to our modern life activities, the inbuilt antioxidants like Vitamin C & E are insufficient to fight against the reactive free radicals. In this scenario, the development of new potent antioxidants is particularly important. But synthetic drugs have lots of side effects and due to their toxic nature, their use has been restricted so that researchers drive to discover new antioxidants with the natural origin and this becomes a wide and noble area in research. Antioxidants fight against the ROS/RNS to protect the living cells from oxidative damage. The OS may lead the regular/normal life activities to an abnormal level and

leads to aging, arthritis, asthma, cancer, cataract, diabetes, neurodegenerative disorders, Alzheimer's, Parkinson's disease, etc [5–12]. Antioxidants fall into 3 general classes:

1) 1st line defense antioxidants: Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione reductase (GR) and minerals like Se, Cu, Zn.

2) 2nd line defense antioxidants: Glutathione (GSH), Vitamin C & E, albumin, carotenoids and flavonoids.

3) 3rd line defense antioxidants: a complex group of enzymes for repair of damaged DNA, damaged protein and oxidized lipids/peroxides (like lipases, proteases, methionine, etc.) [13,14].

The normal antioxidants present in our body are insufficient to cope up with the OS and its consequences. For example, Vitamin E can protect the outer fatty layers of cells but insufficient to protect the genetic material. To reduce/prevent these kinds of situations, medical experts prescribe antioxidants. These kinds of antioxidants now a day are widely synthesized from natural and synthetic sources. Antioxidants have lots of applications in the maintenance of normal life activities. In our body, the brain is the part of high metabolic rate, lipid peroxidation, elevated levels of poly unsaturated lipids, etc., so that brain is highly vulnerable to OS. So scientists are eagerly involved in research to screen out the most potent radical scavenger to treat brain injury.

As the lifestyle of human being goes on changing day by day, their food habits are also changed greatly. In olden days people cultivate rice and vegetables for their need and cook themselves. However, lifestyle has so changed that peoples were interested in ready to eat food items/junk food. This opens the way to lots of deadly diseases and health problems. So in this scenario, the intake of some protective food products called antioxidants is essential. Antioxidants prevent food spoilage. Some herbs in our diet act as antioxidants directly or indirectly, because the compound/s present in them have radical scavenging capacity which protects the fatty acids in food from oxidative damage. The natural antioxidants act as reducing agents, terminate the free radical chain reaction by neutralizing or inactivating them thereby preventing the off-odors and bad taste of food material [15–18].

Natural polyphenols have picked up a considerable measure of recognition in the current years given their potential as prophylactic and therapeutic agents and accordingly, scientists were enormously centered on their antiradical capacities. The epidemiological data shows the widespread acceptance of the use of herbal medicines including polyphenols in the field of OS, cardiovascular and neurodegenerative disorders, diabetes, tumor, and so forth [19]. Scientists in various places are effectively associated in the examination of the mode of action of polyphenols in protecting the living system from these sorts of maladies and thereby revealing the impact of polyphenols in controlling human health [20].

The distribution of phenolics in plants is not uniform; the dissolvable phenolics are found in vacuoles while the insoluble phenolics are found in the cell membrane and cell films. The dissolvable phenolics are fundamental to the plant's physiology as they are associated with various functions such as structure, pigmentation, pollination, pathogen and herbivore resistance, as well as growth and development. The insoluble phenolics supply mechanical strength and are associated with the development and growth of the plant. Plants synthesize a huge number of phenolic mixes which are considered as the secondary metabolites with diversified structural features and mostly contain hydroxyl benzene moieties [20]. The natural products from plants have been discovered to synthesize and design new drugs with potential activities. Even though their presence is not crucial for the developments and activities of plants but is essential as they are actively involved in the defense mechanism against any harmful environment and the interspecies protection. Hence they are synthesized by plants as secondary metabolites [19,21]

Up to now, more than 9000 polyphenolics have been discovered, in which, half of the contribution is from flavonoids. Flavonoids are further divided into several groups with varying structural configuration, which includes flavonols, flavanols, flavanones, flavanonols, flavones, anthocyanidins, and isoflavones (Fig.1.1.).

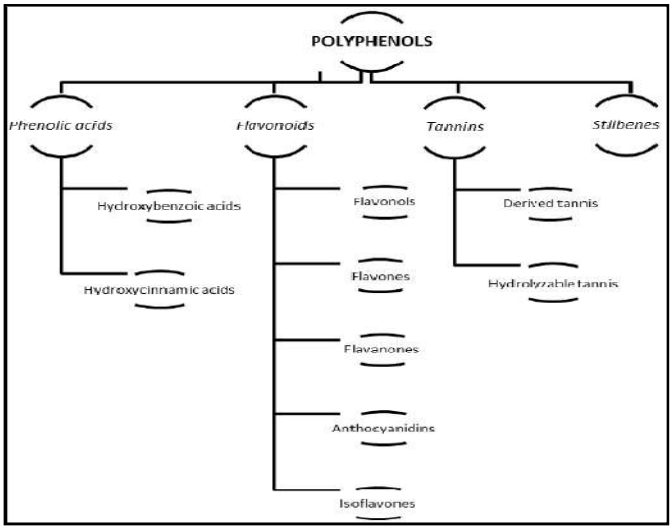


Fig.1.1 Classification of polyphenols

Flavonoids are low molecular weight polyphenolic compounds having potential radical scavenging activities. They also have antitumor, antimicrobial, anti-inflammatory, etc., effects [15,22,23]. It has been observed that antioxidants are highly useful for liver associated diseases also [24]. The wide range of health benefits of flavonoids opens a new world in research which includes various applications of flavonoids. The studies may be experimental or theoretical; theoretical studies have been used since for a few years. All these were mainly focused on the antioxidant, anticancer, antimicrobial, etc., effects of flavonoids. Flavonoids are polyphenols and the hydroxyl functionalities make it bioactive. The core structure of flavonoids consists of 15 carbon atoms in C6-C3-C6 fashion. There are two aromatic rings (ring [A] & ring [B]) with a heterocyclic ring [C] (Fig.1.2). They may or may not completely conjugate. The variation in substitution in the ring [C] forms the basis of classification

of flavonoids. Again, the different substituents in the ring [A] and [B] give different compounds in each flavonoids family.

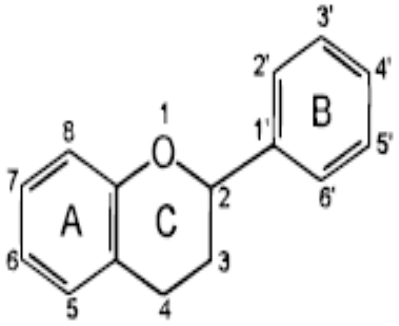
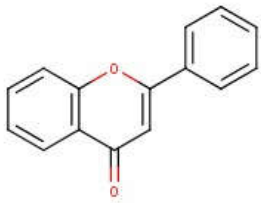


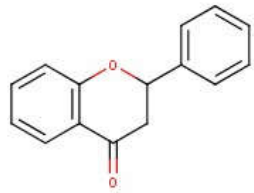
Fig.1.2 Basic structure of flavonoid

All categories of flavonoids are equally good for exhibiting various applications. The most important application among these is their radical scavenging activities and they do so either by acting as an antioxidant or as an antireductant. Different classes of flavonoids are shown in Fig.1.3. The presence of a C2-C3 double bond, the presence of 5 or 3 –OH groups, the presence of carbonyl group in the ring [C], the presence of electron withdrawing or releasing groups are the important structural factors which are taken into account when dealing with flavonoids. In most of the flavonoids, the ring [B] is found to be the most reactive [25].

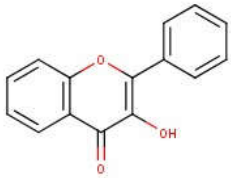
Flavone



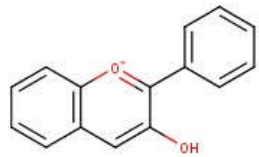
Flavanone



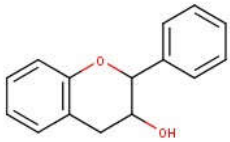
Flavonol



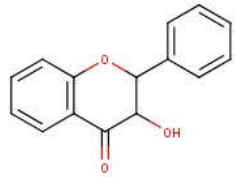
Anthocyanidin



Flavanol



Flavanonol



Isoflavone

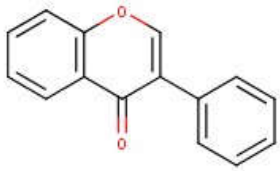


Fig.1.3 Basic structures of different classes of flavonoids

1.2 Applications of antioxidants

1.2.1 Antioxidants *vs*. Diabetes

An increased OS has been observed both in insulin dependent (Type-1) and non-insulin dependent diabetic (Type-2) patients. The free radical generation increases and antioxidant potential decreases in such conditions so that the body is unable to perform cellular activities to normal level [26]. The auto-oxidation of glucose is the most important factor responsible for the increased production of free radicals in diabetic patients. Besides this, production of Ferritin and Homocysteine are also increased.

Studies have shown that people with a large number of serum tocoferols (antioxidants) show a lower risk of type-2 diabetes. The primary defense against OS in a cell includes reduced glutathione and glutathione dismutase [27]. Plants are rich sources of antioxidants which plays a significant role in screening drugs for Diabetes Mellitus [23,28]. Scientists are actively involved in screening the most efficient antioxidants from plants to make drugs for the treatment of diabetes [29].

1.2.2 Antioxidants *vs.* Premature Infants

Enzymatic and non-enzymatic antioxidants decrease injuries from excess production of ROS, particularly in disorders like Bronkopulmanary Displasia, retinopathy of prematurity, periventricular leukomalaria and necrotiziny enterocolitis [30]. Enzymatic antioxidant level decreases in premature infants. Over

expression of antioxidants in such conditions reduces the effect of ROS. Either increased dose of Mn-SOD or Cu-Zn-SOD reverses the growth of inhibitory effect of hyperpoxia in any epithelial cells [31]. Non-enzymatic antioxidants also get depleted in ROS mediated stress conditions. It is a precursor of antioxidants GSH.

1.2.3 Antioxidants *vs.* Cancer

Most of the natural antioxidants like flavonoids, Se, Lanthanoids, Vitamin C & E, GSH, Lycopene, etc., have multiple functions like anticancer, antibacterial, antimicrobial, anti-inflammatory, etc., effects. Uses of natural drugs are highly promoted as the synthetic ones have lots of toxic side effects. Many of the cancer drugs are metal complexes, particularly that of Lanthanoids as they have pharmacological effects for the use in radio-immuno and photodynamic therapy. They have better pharmacological properties and serves as boarder range of antitumor activity [32–37].

1.2.4 Natural *vs.* Synthetic antioxidants

Discovery of natural antioxidants replaces the use of synthetic antioxidants because of some facts [38–45]. The natural antioxidants are of low cost, compatibility with dietary intake and no harmful effects in the biological system [13].

1.3 Other applications of flavonoids

The primary application of flavonoids is their radical scavenging activity. By this way, they find use in the treatment of various diseases which are already discussed above. Besides, some

classes of flavonoids like flavanones and flavones are efficient UV filters so that they find use in the production of sunscreen lotions and other related cosmetics. Further, anthocyanidins are powerful food colourants and efficient pH indicators. Flavaonoids are efficient corrosion inhibitors as they could bind with metal/metal ions.

1.3.1. Flavonoids as UV filters

UV light reaching on earth comprises mainly of UV-A (315-400 nm) and a little UV-B (280-315 nm) as the UV-C (100-280 nm) is almost entirely absorbed by the ozone layer. The UV-B is responsible for the delayed tanning and burning and it cannot penetrate deep into the skin while UV-A has a long wavelength, can penetrate the skin and is responsible for the immediate tanning, wrinkling, and aging of the skin. To protect cells from the dangerous UV radiations, plants have some inbuilt chemicals with them which acts as UV filters. These are mainly polyphenols like flavonones, flavones, etc. Using natural plant extracts containing these kinds of compounds people make sunscreen lotions and other cosmetics to protect their skin from the damages caused by UV-A and UV-B radiations [46]. The conjugated pi-electron system in these kinds of polyphenols makes them able to excite an electron from the ground state to the excited state by absorbing radiations in the UV region of the solar spectrum [47,48].

1.3.2. Flavonoids as food colourants and pH indicators

Unlike chlorophyll and carotene, the pigment anthocyanidins, a major class of flavonoids, are not membrane bound pigments but dissolved in the cell sap. In plants, in the presence of light, the

anthocyanidins are produced as a result of the reaction between protein and sugar in the cell sap. As the concentration of anthocyanidins increases, plants become brightly coloured. For example, the apple appears red on the side where light falls and green on the other side. The vital feature of anthocyanidins is the presence of chromenylium/flavylium ion. Due to this, effective π-conjugation significantly enlarges in comparison to flan-3-ols. This π-conjugation is responsible for its strong absorption around 500 nm [49] and thus they are brightly coloured; this, in turn, enables them to be used as a natural food colourant as the use of artificial colourants have been expanded to a splendid extent and become a extremely good risk to human fitness due to the fact of their toxicity. So, the development of some herbal meals colourant is especially relevant in the area of the food industry. From literature, it has been seen that anthocyanidins could act as pH indicators [50,51]. It has been observed that anthocyanidins are highly sensitive to pH values of the medium in which they are present and they undergo some structural reorganization with pH values. As a result, they exhibit different colours at different pH values so that they could act as pH indicators. At low pH values (in acidic media) the flavylium cations exist and are red in colour. As the pH values increase this cationic form undergo several structural deformations to form quinoidal bases and then to chalcones [52].

1.3.3. Flavonoids as corrosion inhibitors

Flavonoids have fascinating phytochemical properties (Cody, Middleton, and Harborne, 1986) and play multiple roles in the ecology

of plants. The presence of electronegative oxygen enables them to undergo metal chelation. This enables the accumulation of metals in the peripheral tissues of plants. These metal accumulations in plant tissues prevent the migration of these toxic metal ions to the ecosystem resulting in the suppression of metal toxicity. This is useful to both ecosystems as well as the plant since the deposited toxic metal ions prevent the attack of pathogens and plant eaters. This is the principle that is used in colourimetric titrations in the detection of metal traces [54]. There are lots of synthetic inhibitors available for metal corrosion. However, due to their side effects, toxicities and cost, researchers are in a hunt of some natural corrosion inhibitors. Reports have shown that polyphenols have the capacity to chelate metals and thereby they can provide 3 types of uses viz., 1) removal of toxic metals, 2) retardation of redox reactions of metal in acid and 3) prevention of metal catalyzed lipid oxidation and release of free radicals [55–57].

1.3.4. Flavonoids in optical communications

Materials possessing nonlinear optical (NLO) properties change the propagation characteristics (phase, frequency, amplitude, polarization, etc.) of the incident light and have a wide variety of applications. Recently the focus on new materials with NLO properties has been diverted from traditional inorganic solids to organic molecules, due to their chemical flexibility and variety of synthetic strategies. Organic materials are expected to possess high NLO properties as they have delocalized electronic systems. Advancements in the area of optical communications forces researchers to find out

more optical materials. In addition to inorganic materials, certain organic materials are also discovered with potential NLO properties. Because of the presence of a considerable number of electrons and its delocalized nature makes organic materials to possess high NLO properties and which in turn enhance its responsiveness in optical communication [58].

1.4. Recent studies on flavonoids

James and his coworkers had considered a theoretical calculation of the effect of substituents on the –OH bond dissociation energies (BDE) of phenolic antioxidants in comparison with the inbuilt antioxidant Vitamin E. They start with phenol and followed by substitution with methoxy, methyl and amino functionalities. The calculations were carried out through Gaussian 94. Their computed results through DFT-B3LYP/6-31G (d, p) were in good agreement with the experimental results. It has been found that as the structure under investigation are large and have several rings with them, then smaller basis sets like 6-31G should provide fairly good –OH BDE values [59].

One of the important factors to which the –OH BDE values depends is the planarity of –OH group with respect to the phenyl ring. This is also affected by the nearby substituents in the ring. For example, when both the *ortho*- positions are substituted, the –OH group become out of the plane. Moreover, if these substituents are oxygen or nitrogen containing groups, there is a chance of hydrogen bonding too. If the phenoxyl radical can form hydrogen bonding with

neighboring groups, corresponding –OH BDE value decreases. However, if the neutral phenolic compound is forming hydrogen bonding, then that particular –OH group has higher BDE value. Another factor is the orientation of –OH group. James and coworkers found that, when the –OH group and the o-methyl group are in toward-configuration, the BDE value decreases and consequently, the phenoxyl radicals are stable. The amino group has strong –OH bond weakening effect than methyl or methoxy groups. So, the amino substituted phenolic compounds may act as potential antioxidants through hydrogen atom transfer (HAT) [59].

Katarzyna and coworkers conducted a computational study on the influence of pH on antioxidant properties of some hydroxyl flavones. They proposed a Qualitative Structure Activity Relationship (QSAR) model to compute the pKa values of hydroxyl flavones and compared it with the pH dependent antioxidant profile through the Trolox Equivalent Antioxidant Capacity (TEAC) assay. They found that deprotonation leads to an increase in TEAC value. They found that HAT mechanism is the most favorable mechanism for explaining the radical scavenging capacity of hydroxyl flavones. The results from the DFT-B3LYP/6-31+ G (d, p) based evaluation of the antioxidant properties of hydroxyl flavones from our laboratory also consistent with these results. The authors also reported that the mode of action of radical scavenging activity of hydroxyflavones changes on deprotonation [25].

Katarzyna and coworkers employ DFT-B3LYP/6-31G (d) based study on hydroxyflavones by using Gaussian 98 programs. On

the evaluation of the pKa values, it has been found that the deprotonation depends on the position of the –OH group and the easiness follows 5 < 3 < 3' < 7 < 4'. Also, the hydrogen bond between 5 or 3 –OH groups with the carbonyl oxygen in position 4 of the ring [C] restricts the deprotonation. Generally, additional –OH groups have no significant effect on pKa values. For hydroxyflavones, the pKa values of 3 and 5 –OH groups are found to be greater than the physiological pH value and these sites may be responsible for the low BDE at the physiological pH value. So the study of pH dependent antioxidant studies is important and the pKa values at position 4' and 7 have to be considered. Often, the common antioxidant Trolox, whose antioxidant capacities are independent at the physiological pH values, taken as reference for the TEAC assay [60,61]. For 3/5 mono-hydroxyflavones, the deprotonation gets restricted due to the presence of hydrogen bonding with carbonyl oxygen even though their TEAC values increases with the pH value. This is further attributed to their higher solubility and electron transfer is favorable for them rather than the HAT. We have attempted to evaluate the selectivity of a particular mechanism by a compound regarding its global descriptive parameters [19]. For poly hydroxyflavones, the antioxidant activity at the physiological pH value is due to a combination of activity of both neutral and deprotonated forms with definite molar ratios. We have also evaluated the dependence of pH and pKa values of flavonoids to explain the deprotonation [62].

Stanislaw and coworkers studied the antiradical properties of 42 flavonoids which include flavonols, flavones, flavanones,

dihydroxyflavones, biflavanones, isoflavones and coumestrols. They experimentally determined the antioxidant and antiradical activities by heat induced oxidation in a β-carotene and linoleic acid medium and by the DPPH decolourisation test. The antiradical properties vary from category to category and are also depends on the number of free –OH groups, the presence of electron withdrawing or releasing substituents, etc. Thus, the radical scavenging capacity of flavonoids can be tailored by suitable substitution. Moreover, the inhibitory activity to β-carotene oxidation can be reduced by substitution at position 3. This effect is not seen on substituting at other positions of flavonols [63].

The presence of C2-C3 double bond and C3 –OH group reduces the antioxidant capacity as they are sensitive to oxidation which causes their rapid oxidation and structural modifications as that seen in anthocyanidins. They get partially decomposed during the course of measurement of antioxidant capacity. This is observed for other flavonoids also [63].

Henryk and coworkers evaluated the BDE of phenolic antioxidants through a computational approach. They examined the effect of substitution on the BDE values and have found that the BDE values decreases with electron releasing substituents and vice versa [64].

The electron withdrawing substituents increase the BDE values and increase the reactivity of resulting phenoxyl radical which reduces the antioxidant activity. The experimental work by Brigati in 2002 is unable to focus on the substituent effect on BDE values while the

quantum chemical calculations are efficient in these cases. Also, the computed results seem to be more accurate when thermal correction to enthalpy values have been implemented in calculations rather than the electronic energy [65]. This further enhances the utility of theoretical methods.

For polyphenols like flavonoids, relatively less expensive theoretical methods based on B3LYP functional are enough to produce reliable results with negligible errors in comparison with experimental values. This is an advantage of theoretical methods. Theoretical calculations provide quantitative predictions of the behavior of organic compounds [66].

Among flavonoids, anthocyanidins occupy an important space as they are widely used as natural food colourants since historical times. In 2005, a DFT based investigation on anthocyanidins had performed. Even though they exist as their glycoside derivatives called anthocyanins; the anthocyanidins are equally important because of their number of applications [67].

In theoretical works, the most important reactivity parameters are the energies of Highest Occupied and Lowest Unoccupied Molecular Orbitals (HOMO & LUMO). From these, the band gap in a molecule can be computed. Another importance of HOMO-LUMO energies are the calculation of global reactive descriptors by Koopmans's theorem [68,69]. This gives lots of information regarding the global hardness, softness, electronegativity, electrophilicity, etc., of the molecule. Moreover, it also helps to plot the Donor Acceptor Maps

(DAM) & Full Electron Donor Acceptor Maps (FEDAM) [70–78] and thereby enable the classification of radical scavengers into antioxidants and antireductants. This further helps in determining the most suitable mechanism for explaining the mode of radical scavenging action.

In 2007, Sebastian et al. conducted a DFT based study on quercetin and its several structural forms called activated forms, which are involved in the radical scavenging and prooxidant activities of quercetin [67]. Quercetin is the most widely studied flavonoid for its antioxidant activity. Depending upon the affinity towards environmental factors like pH, metal concentration, light, temperature, etc., some flavonoids, especially antocyanidins, undergo several changes in their structure [79,80]. Similarly, quercetin also undergoes structural deformations and they are quinine, semiquinone and deprotonated forms. So it is interesting to know, which among these are responsible for the antioxidant and prooxidant activity of quercetin. Theoretical calculations provide a quantitative prediction of the behavior of organic compounds [67].

Recently, it has been observed that these polyphenolic antioxidants, reacts with ROS/RNS and itself converted to phenoxide radicals called pro-oxidants. This, in turn, causes cell death and damage to DNA. Also, they mobilize chromatin bound copper ions and which in turn catalyse lipid peroxidation resulting in the generation of hydroxyl free radicals. The antioxidant in our body like vitamin C and E are also exhibited pro-oxidant activity. It has been observed that the pro-oxidant activities of polyphenols are significantly observed in the presence of Cu ions. Cu is an important redox active site in our body

and is often found associated with DNA bases especially Guanine. The mobilization of Cu ions by polyphenols leads to the generation of free radicals and in turn leads to DNA damage. At first, this is considered to be a severe issue in prescribing polyphenols as medicine, but now studies have shown that this pro-oxidant activity has some advantage and is not a side effect. Hadi et al. reported that this pro-oxidant activity makes polyphenols to become potential anticancer drugs. The level of chromatin bound Cu increases during cancer and the redox cycle between polyphenols and Cu facilitates the free radical production resulting in the cell death and consequently curing cancer. Also the cell death is observed only for cancer cells and not for normal cells [81–83]. Flavonoids have anticancer activity and it may also attribute to their pro-oxidant activity and it need not be a side effect. Moreover, the pro-oxidant activity is observed in high concentrations of polyphenols and so, in adequate concentrations, only their radical scavenging activity has been observed.

Sebastian et al. also investigated the influence of copper ion on the electronic properties on quercetin. The work gives a hint to select a particular reaction which is likely to occur most probably from a wide range of reactions. Moreover, the quantum chemical descriptors ensure quantitative predictions of their chemical reactivity and physicochemical properties. Thus, for each family of flavonoids, electronic and energetic analysis at the molecular level can be easily achieved through computational tools. For example, the reactive sites in quercetin are independent of each other. The structural modifications by proton, hydrogen or electron are so weak that the

localized spin densities explain the high reactivity of radical and charged forms of quercetin. The feasibility of each possible reaction can be predicted through the thermo-chemistry part of computed results. This can then be employed to study various antioxidants mechanisms associated with a particular molecule [67].

In 2007, a theoretical study on the antioxidant capacities of carotenoids had been performed by Annia Galano [84]. The donor-acceptor interactions and its necessary parameters [75–78] are utilized to explain the antioxidant properties of carotenoids.

Laura and Ricardo evaluated the structure and antioxidant properties of a well known anthocyanidin, 'Delphinidin,' through the DFT-B3LYP level of theory and 6-31++ G (d, p) basis set. The calculations are carried out in both gaseous and aqueous phases by considering the neutral, anionic and cationic forms of delphinidin. The site for most stable radical formation has found at position 4'. However, position 3 is also seen to be reactive in some other anthocyanidins [85]. Anthocyanidins are well known for their use as colour pigments as well as pH indicators [52]. Besides this, they are one among the most important natural antioxidant [86].

Ana Martinez et al. have conducted theoretical studies to evaluate the donor-acceptor behavior of some antioxidants like carotenoids, melatonin and vitamins. They do so by the implementation of DAM-FEDAM plots. They evaluated the electron donating and accepting capacities of the studied compounds with respect to Na (as good electron donor) and F (as a good electron

acceptor) respectively. By this way, the antiradical can be classifiedinto antioxidants and antireductants[70].

In 2009, Ana Martinez evaluated the antiradical behavior of anthocyanidins and pistacofulvins by means of DAM plots. Both the classes of compounds are regarded as natural colour pigments. In some cases, the donor-acceptor interactions do not alter much in solvent phases. However, for anthocyanidins, they behave as effective electron acceptors in aqueous media rather than electron donors. Substances with high electron affinity (EA) behave as good antireductants while those with low ionization energy (IP) are good antioxidants. However, IP and EA are considered only when there is complete electron transfer. In biological systems, often there are fractional electron transfer taking place and in such situations, we could adopt DAM plots [87].

Besides the antioxidant property, anthocyanidins are also important for their other properties like pH indicator, metal complexation, copigmentation, heavy metal removers, etc [49].

In 2009, Rosa et al. evaluated the antioxidant properties of anthocyanidins using B3LYP/6-31 G (d, p) method. It focuses on the HAT mechanism only and has computed the BDE values for all possible sites. The number of –OH groups and nature of substituents, their position, orientations, all together determine the overall antioxidant behavior of various anthocyanidins. In most of the article, the 6 anthocyanidins are commonly studied, which are Pelargonidin, Peonidin, Petunidin, Cyanidin, Malvidin and Delphinidin. According

to Rosa et al, the antioxidant behavior of anthocyanidins follows, Cyanidin > Malvidin > Auratinidin > Delphinidin > Peonidin > Pelrgonidin [88].

Fifenet al., in 2009, examined HAT reaction of some metal associated phenolic acids with hydroxyl radicals. They have carried out a DFT based computational study with 6-31 + G (d) basis set and NBO tool in Gaussian 03 program. The metal ions they studied are Mg^{2+}, $Ca^{2+}$and Cu^{2+}. The metal chelated phenolic acid complexes are evaluated for their charge transfer, BDE values and affinity towards hydroxyl radicals. In polyphenols, the presence of –OH groups enable them to chelate metal/metal ions, thereby forming stable metal complexes, which in turn prevent the metal catalyzed autoxidation and lipid peroxidations. The reactions involved are explained by the Fenton mechanism and the feasibility of this reaction for a given molecule can be studied through the thermodynamic parameters associated with the reaction. In polyphenol-metal complexes, there occurs some charge transfer between polyphenol and the metal ion. The direction of charge transfer can be easily understood by the analysis of charge by Mulliken method and Natural Population analysis (NPA). The possibility of charge transfer to metal ion increases with the electronegativity of the metal ion as well as with the decreasing ionization energies of polyphenols [89].

The electron donation capacity of a molecule is characterized by its HOMO values. Higher the HOMO Eigen values, higher will be donating capacity and higher will be the antioxidant behavior. Similarly, lower the LUMO level, higher will be the electron accepting

antireductant capacity. The chelation with metal ions does not alter the antiradical capacity to a significant extent. Moreover, the presence of metal ion increases the overall antioxidant behavior of phenolic acid towards the hydroxyl radical [89].

Dragan and Bono in 2010 develop a QSAR model to predict TEAC and expansion (VCEAC) values of structurally similar flavonoids. The computational method that they have used is semi empirical (PM6 and RM1). They correlate the experimental TEAC and VCEAC values with descriptors like computed BDE values, number of –OH groups, etc., and developed a QSAR model. BDE is selected, as the HAT mechanism is found to be suitable for almost all flavonoids. Even though there are lots of antioxidant assays available, none of them can be used as a standard; in this scenario; the successful usage of QSAR/QSPR procedures is undoubtful. But Dragan and Bono showed that the descriptors like a number of –OH groups and the computed BDE values in association with experimental expansion (ABTS) assay produce reliable QSAR models [90].

Lucas et al. also carried out a QSAR study on the antioxidant activity of phenolic compounds. Here also, one of the descriptors is BDE value. The others are IP, logP and logD values. The experimental pIC_{50} values are predicted with an R^2 value of 0.885. Both BDE and IP are computed through DFT method [91].

The global interest in antioxidants increases day by day. The discovery of some potential antioxidant with natural origin has become

a noble area in research. The natural antioxidants are all present in our food products. So they have less or negligible toxic effects.

The quantum chemical descriptors and the related studies on phenolic antioxidants have remarkable importance in the study of antioxidant behavior. Among these descriptors, the reactivity indices like electro-accepting/donation capacities (ω^+ and ω^-) and the –OH BDE values are very important. The discovery of ω^+ and ω^- and the related Ra and Rd have produced a significant effect in the analysis of antiradicals, particularly where electron transfer mechanisms like SET, SPLETand SETPT are important. Through this one could easily categorize the antiradicals to antioxidant and antireductant as well as into good, worst and best antiradicals [92].

Molecular modeling on the antioxidant activity of Kaempferol in the gas phase has been performed by Mohammad et al. They have carried out a DFT-B3LYP calculation to compute the –OH BDE values. Also, they have analyzed the influence of certain groups like –CN, -Cl, -OH, NH_2, $-CH_3$, etc., on the antioxidant behavior of Kaempferol and also check their orientation and position effect [93].

Jorge and coworkers employed FEDAM plots of antiradicals with IP and EA as descriptors. They have also calculated the rate constants for SET reaction for a number of flavonoids. The falvonoids are soluble in water and thus they have analyzed the reactions in the aqueous phase. The quantitative analysis of polyphenols like flavonoids through experiments is unreliable as they are unstable. The newly designed free radicals by Jorge and coworkers enable to

distinguish different antiradical compounds. Their computed results are in good agreement with the available experimental results. Moreover, they could suggest some experimental conditions with respect to the pH values to design proper antioxidant assays [94].

A QSAR study based on the fukui descriptors has performed by Houria and coworkers to predict the antioxidant behavior of flavonoids. This includes some flavones, flavanols, flavanones and flavonols. They carried out a DFT-B3LYP approach to compute charges and other structural parameters. They compute charge by both Mulliken and NPA approach. According to them, the uses of quantum chemical calculations are highly relevant and the resulting QSAR models are sufficient enough to screen good antiradicals. So, computational studies are of significant importance in drug designing prior to synthesis [95].

All these studies show the importance of flavonoids in the relief of OS in a biological system. Studies include both experimental and theoretical. The radical scavenging capacity of flavaonids makes them able to play multiple roles in a biological system as explained above. Each category of flavonoids is potent antiradicals by one or other way. Computational analysis enables the detailed structural analysis and gives a clear cut idea about the mechanisms by which each class of flavonoids becomes antiradical.

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CHAPTER 2

COMPUTATIONAL METHODOLOGY

- *Theories of computational chemistry.*
- *Details of various software, computational tools and computer power used for this work.*
- *An idea about the methodology adopted for doing this research work*

2.1 Introduction

Computational chemistry is the study of chemical problems using quantum mechanics with the help of suitable computer programs on a computer. The words exact/perfect cannot be used here, but almost all parameters can be predicted qualitatively or approximate quantitatively. Just like all spectra are not completely resolvable, yet they are highly useful for predicting adequate results, computational chemistry also has particular relevance in the field of research in various disciplines [1].

Quantum mechanics gives the mathematical description of the behavior of electrons which are never wrong but cannot be exactly solved for systems other than Hydrogen atom. Most of the quantum mechanical calculations are complex, but some are accurate than any other experimental results that are discovered so far. Most of the calculations are derived from a set of approximations. At first, a computational chemist must have knowledge of approximation methods, secondly; requires powerful computers and suitable software and finally, the implementation of mathematical description on to these PCs [1].

Computational calculations and approximations are doing for getting accurate results within the error limit less than 1 kcal/mol. This is sufficient to describe the weakest interaction in chemistry called the van der Waals interactions.

Computational tools can be used in a number of ways with respect to our intention; for example, molecular modeling prior to

synthesis the compound/s in the laboratory. However, computational models are not 100% accurate, but it is useful to rule out about 90% of unsuitable models or molecule for the particular solution from a wide range of models or compounds. This minimizes or removes tedious laboratory works, toxic waste production and time loss. The unwanted and excess use of raw materials has also been eliminated.

The second main use of computational chemistry is that we can understand a problem more completely. Most of the experimental results can be computed with a considerable degree of accuracy, more easily within a short period of time. The results, regarding molecular bonding, have not obtained from any experimental methods; can be retrieved from the computational calculations. So, most of the researchers in experimental fields of various disciplines, are forced to adopt some computational calculations, to get a clear molecular picture of the compound that they have synthesized. We cannot say that the computational calculations are simple, because, only an expert can understand and explain all the computed results in a satisfactory manner [1,2].

Energy is the most basics in science. All the computational experts are doing their calculations to get the most stable (lower energy) conformer. Energy can be kinetic and potential. For studying energetics, we must have a reference system with zero energy. This may be different for different approximations, for example; *Ab initio* and Density Functional Theory (DFT), which model all the electrons in a system, zero energy corresponds to all nuclei and electrons at an infinite distance from one another. However, the Semi Empirical (SE)

methods use valance energy that corresponds by removing valence electrons and the resulting ions at an infinite distance. In Molecular Mechanics (MM), chemical standard states or stainless molecule is taken as zero energy. But often all theses total energy values relative to the corresponding zero energy are inaccurate. This is due to the occurrence of a systematic error. However, the energy difference can be accurate so that energy difference between conformers, bond dissociation energies (BDE), etc., are extremely accurate [1]. The basic tools in computational chemistry are:

2.2 Molecular Mechanics (MM)

MM is fast and undemanding in its hardware requirements. It considers atoms as balls and bonds as sticks/springs so that it is also called the ball and stick model. If we know the spring lengths and angle connecting them, we could calculate the energy of molecules like a steroid in a very small amount of time. As it ignores electrons, electronic properties cannot be computed. Because of its speed and availability of parameters for almost all elements, regardless of its low accuracy, the optimized structures from MM can be used as inputs for *Ab initio*, SE, or DFT calculations to reduce the time of calculation and is one of the major advantages of MM.

If we optimize a compound with MM, the IR spectral calculation of the compound by same MM can be accurate. In MM atoms are considered to be in a force field and mathematical treatment of these force fields gives the energy of the atom in terms of geometrical parameters. So this is also called force field method. By

such force field parameterization, MM calculate the electronic properties by analogy and may or may not be accurate. MM is silent about electrons, shapes of orbitals and the related properties. The force fields for one class of compounds may be inaccurate for another set of compounds. Transfer of parameters from one force field to another is invalid.

The optimized structures in MM may or may not be a global minimum. The force fields and parameters are sometimes inaccurate. The solvation and neighboring ions are neglected so that electronic correlations or polar compounds cannot be studied through MM. In MM, potential energy is expressed as a sum of terms involving bond stretching, angle bending, dihedral angles and non-bonded interactions. Giving these terms explicit mathematical forms constitutes devising a force field and giving actual numbers to the constants in the field constitutes parameterization of the field. Calculations of bio-molecules are the major applications of MM.

2.3 *Ab initio* Methods

These are based on the fundamental physical equation, the Schrödinger equation, without empirical adjustments. They use some quantum chemical approximations and if these approximations are not too severe for the problem at hand, they could give accurate results. This is slower than MM calculations but is accurate than the later. The levels of theory for a particular application with considerable accuracy can be achieved through trial and error methods or experience. A consequence of the absence of the empirical parameters is that the *Ab*

initio calculations can be applied to any kind of system including transition states, non-stationary points, rather than only species for which empirical parameters are available and is a great advantage. The reliability and generality are the strengths of *Ab initio* calculations and is slower than the other computational tools.

Unlike MM calculations, *Ab initio* calculations demand hardware requirements. The computer resource requirements increase with the level of calculations, but these are overcome by the implementation of super computers. The simplest *Ab initio* method is Hartree-Fock (HF); in which the wave function is approximated as a Slater determinant composed of occupied spin orbitals.

Writing the molecular energy as expectation values and solving the related Schrödinger equation, then differentiating the energy with respect to spin orbitals that composes the wave function, we could get the HF equations. To use these in practical applications, these are approximated as a linear combination of basis functions, which can be any mathematical functions that could give a reasonable wave function.

The main defect of the HF method is that it does not consider electron correlations. Each electron in HF is considered to be in an average potential of the rest of ions. However, in actual practice, one electron considers the other electron as a moving particle and they adjust themselves to minimize the interactive repulsion energy. However, electron correlation is studied in Post-HF calculations like Moller-Plessel (MP), Configuration interaction (CI) and Coupled

Cluster (CC) methods. These methods lower the electron correlation energy by allowing electrons to occupy not just in conveniently occupied molecular orbitals, but also in formally unoccupied molecular orbitals called virtual molecular orbital.

Energy, IR-UV-NMR spectra, electron affinity, ionization enthalpy, dipole moments, etc., can be computed through *Ab initio* method. Since most of the enzyme-substrate interaction depends on the shape, charge distribution, etc., and depends in turn on electronic properties and energy differences, *Ab initio* calculations are important.

2.4 Semi Empirical Methods (SE)

These are faster than *Ab initio* and density functional theory method but slower than the MM. Due to the speed of calculation, the accuracy in results is lower than that obtained through *Ab initio* and density functional theory methods. However, for comparative studies, SE results are quite enough. The most commonly used SE methods like Austin Method (AM1) and Parametric Method (PM3) could produce reliable results. All the SE methods use some experimental results so that the name semi empirical, and some of the integrals in the quantum mechanical calculation procedure can be omitted with the help of these available experimental results. This is called parameterization method. But when the molecule under study is outside these parameterization set, the results will be unreliable. In SE methods, errors of 10 kJ/mol may occur in the calculation of enthalpy of formations of compounds. AM1 and PM3 sometimes underestimate steric repulsion and nucleophillicity, but it overestimates basicity.

Sometimes it also gives unreasonable charges and structures. AM1 give better energies while more reliable structures are obtained from PM3. PM3 shows some improvement in tracing hydrogen bonds. However, neither AM1 nor PM3 is suitable for studying hydrogen bond energies. Errors obtained from SE methods are less systematic than *ab initio* and are thus harder to correct.

SE methods are also based on the Schrödinger equation. Here repeated diagonalization of Fock matrix refines the wave function and the molecular energy. The simple and extended Hückel methods need only one matrix diagonalization because their Fock matrix elements are not calculated by using a wave function guess. The speed is high because some integrals are reduced and some are approximated with the help of experimental results. The Pariser–Parr–Pople (PPP) method, Complete Neglect of Differential Overlap (CNDO) method, Intermediate Neglect of Differential Overlap (INDO) method, Neglect of Diatomic Differential Overlap (NDDO) method, etc., are some Self Consistent Field (SCF) SE tools. PPP is limited to pi-electrons while others are extended to valence electrons. All these are the Zero Differential Overlap (ZDO) where the differential overlap integral is zero so that the number of integrals is greatly reduced.

2.5 Density Functional Theory (DFT)

Despite the fact that the DFT isn't solely the space of quantum science, it has been broadly utilized even sometime before the Hohenberg-Kohn and Kohn-Sham papers. DFT has been broadly utilized in all the field of science like material sciences, chemical

sciences, biological sciences, and so forth. The likenesses among the different uses of DFT are because of the presence of variational principles. The energy which isn't related with an external potential is a universal functional (it is the same for all the external potentials) in all the fields where DFT is utilized. Be that as it may, the functional which are utilized here are from various approximations and the correct functional is obscure and are unpredictable in nature. The best possible decision of a functional is constantly connected with some insight, scientific capacity and a mix of numerical, test and investigative fittings. The electronic properties estimated by DFT are chiefly centered on the Hohenberg-Kohn hypothesis and Kohn-Sham strategies. The most present day utilization of DFT is the arrangement of Schrödinger conditions for electronic frameworks and is the most exact first principle method of calculation of the energy of chemical interest [3].

There are two noteworthy ways to deal with the Schrödinger wave equation; the wave function based techniques and density based strategies. Both the techniques portray the many-body framework as the single molecule equations. DFT focuses on the Slater technique, i.e., it depends on the densities of electronic frameworks rather inferring the wave function straight forwardly. The energy of a framework is communicated as a function of electronic density which is thus a function of 3 space directions and accordingly called a functional. In 1964, Hohenberg and Kohn demonstrated that whatever be a ground state property, it can be characterized by the electron density. The nature of exchange-correlation functional is a challenge in

Kohn- Sham (KS) strategy. It must be approximated in various approaches to get the best fitting functional bringing about the local, gradient-corrected, or hybrid functional. The most well-known exchange-correlation functional depends on the Local Density Approximations (LDA) where the functional depends locally on the electron density.

2.5.1 Electron density

Electron density is the focal quantity in DFT. It can be computed as:

$$\rho(\vec{r}) = N\iint |\psi(\vec{x}_1, \vec{x}_2, ..., \vec{x}_N)|^2 d\vec{x}_1 d\vec{x}_2 ... d\vec{x}_N \qquad (2.1)$$

Where $\rho(\vec{r})$ is the probability of finding an electron within the volume element dr. 'ρ(r)' is a positive function of only 3 spatial variables and vanishes at infinity. It can be measured experimentally. At any position of an atom, the gradient of $\rho(\vec{r})$ has a discontinuity and a cusp [1,2,4,5].

2.5.2 Thomas-Fermi model: The first DFT

This model, regarding as the first DFT, is proposed in 1927. According to them, the kinetic energy (KE) is given by;

$$T_{TF}\left[\rho(\vec{r})\right] = (3/10)(3\pi^2)^{2/3}\int \rho^{5/3}(\vec{r})d\vec{r} \qquad (2.2)$$

The total energy is given by;

$$E_{TF}\left[\rho(\vec{r})\right] = (3/10)(3\pi^2)^{2/3}\int \rho^{5/3}(\vec{r})d\vec{r} - Z\int \frac{\rho(\vec{r})}{r}d\vec{r} + \frac{1}{2}\iint \frac{\rho(\vec{r}_1)\rho(\vec{r}_2)}{r_{12}}d\vec{r}_1 d\vec{r}_2 \quad (2.3)$$

Where, the last two terms are derived from the concept of nuclear-electron and electron-electron potential respectively. So the energy of the system can be completely expressed in terms of density [1,2,4,5].

2.5.3 The first Hohenberg-Kohn theorem

They express the Hamiltonian operator in terms of density and determine all the properties of a system. They state that the external potential $V_{ext}(\vec{r})$ is a function of $\rho(\vec{r})$ and $V_{ext}(\vec{r})$ fixes and thus the full many particle ground state is now a unique function of $\rho(\vec{r})$. The ground state energy can be expressed as;

$$E[\rho] = E_{Ne}[\rho] + \mathrm{T}[\rho] + \mathrm{E}_{ee}[\rho] = \int \rho(\vec{r})V_{Ne}(\vec{r})d\vec{r} + F_{HK}[\rho] \quad (2.4)$$

$$F_{HK}[\rho] = T[\rho] + E_{ee} \quad (2.5)$$

If the functional $F_{HK}[\rho]$ is known exactly, one could solve the Schrödinger equation completely. As the functional is independent of the system, it can be equally applied to hydrogen and all the complex molecules like protein, DNA, etc. However, the clear-cut form of $T[\rho]$ and the non-classical contribution of energy by electron-electron interaction is a major challenge in DFT [1,2,4,5].

2.5.4 The second Hohenberg-Kohn theorem

The search for getting the true or exact or the most probable density functional for a system under consideration opens the way to the second Hohenberg-Kohn theorem. According to this, the lowest energy delivers only when the input density is the true ground state density.

$$E_0 \leq E[\tilde{\rho}] = T[\tilde{\rho}] + E_{Ne}[\tilde{\rho}] + E_{ee}[\tilde{\rho}] \tag{2.6}$$

$$\left\langle \tilde{\psi} \middle| \hat{H} \middle| \tilde{\psi} \right\rangle = T[\tilde{\rho}] + E_{ee}[\tilde{\rho}] + \int \tilde{\rho}(\vec{r}) V_{ext} d\vec{r} = E[\tilde{\rho}] \geq E_0[\rho] = \left\langle \tilde{\psi}_0 \middle| \hat{H} \middle| \tilde{\psi}_0 \right\rangle \tag{2.7}$$

However, the variational principle that we have used so far is applicable to ground state only and thus we cannot extend this to situations in which the system under consideration is an excited one [1,2,4,5].

2.5.5 The Kohn-Sham equations

In Thomas-Fermi model, the approximation for kinetic energy is not so good that it is not widely accepted and in order to get a good approximation to kinetic energy, Kohn and Sham proposed an equation in 1965. According to them, the kinetic energy is given by;

$$T_s = \frac{1}{2} \sum_{i}^{N} \left\langle \psi_i \middle| \nabla^2 \middle| \psi_i \right\rangle \tag{2.8}$$

where, ψ_i are the orbitals of the non-interacting system. However, T_s is not the exact or true kinetic energy of the system. Kohn and Sham introduce another functional $F[\rho]$ as:

$$F[\rho] = Ts[\rho] + J[\rho] + E_{xc}[\rho] \tag{2.9}$$

where $E_{xc}[\rho]$ is the exchange-correlation energy, the calculation of it is really difficult as the terms in its equation are all unknown. After a long series of mathematical salvation procedures and the implementation of the variational principle, the well known Kohn-Sham (KS) equations are developed.

$$\left(-\frac{1}{2}\nabla^2 + \left[\int \frac{\rho(\vec{r}_2)}{r_{12}} + V_{XC}(\vec{r}_1) - \sum_A^M \frac{Z_A}{r_{1A}}\right]\right)\psi_i = \left(-\frac{1}{2}\nabla^2 + V_S(\vec{r}_1)\right)\psi_i = \varepsilon_i\psi_i \tag{2.10}$$

$$V_S(\vec{r}_1) = \int \frac{\rho(\vec{r}_2)}{r_{12}} d\vec{r}_2 + V_{XC}(\vec{r}_1) - \sum_A^M \frac{Z_A}{r_{1A}} \tag{2.11}$$

Once the terms in Eqs. (2.10) and (2.11) are known, the ground state density and energy can be determined [1,2,4,5].

2.6 DFT in Bioactivity Study

DFT has got honorable consideration in the field of pharmaceutical and therapeutic science to screen potential compounds with different biological applications. DFT advances step by step as the approximations utilized as a part of it are sufficiently high to get the best exchange-correlation functional. So the utilization of DFT has been upgraded in electronic structure particularly in solid state material sciences and all fields of biology and chemistry. The headway of supercomputers and different programming additionally shares in the advancements of DFT and computational science. In the present investigation, the bioactivity that we have examined is the antiradical capacity. Heaps of studies have been accounted both experimentally

and computationally for antiradical activity. However, the trial examines are a monotonous errand as it requests an extensive number of chemicals, both lethal and costly, and furthermore require various test methodology. Moreover, still, at the end of the day, we get just the net BDE esteem or a solitary BDE esteem for a given antiradical. So we cannot think about the reactivities at all the conceivable sites in an antiradical. Here we could look at the antiradical capacity of various particles as it were. Yet, in the computational examination, we could get the reactivities at all the possible sites in the antiradical compound and in this way we can look at the reactivities at different sites in the same molecule as well as between different molecules. Likewise, computational investigations did not require any poisonous or costly chemicals. We get the outcomes in a brief time frame and once the software has installed, there is no more cost or prerequisites for examination. We can screen the particles, as well as can be expected to be doled out or to be chosen. On the off chance that we give the most possibly dynamic medication like atom/set of particles to an experimentalist, he/she need to blend just that specific atom/set of particles in a commercial scale. This evacuates the undesirable advances, chemicals, time misfortune and furthermore the unsafe well being perils caused by these chemicals. Besides the antiradical capacity, UV filtering capacity, pharmacokinetic properties, toxicological analysis, etc., of flavonoids is also evaluated in this thesis.

2.7 Basis Set

A set of mathematical functions from which the wave function has been derived is called basis set. In HF, each molecular orbital can be expressed as the linear combination of a set of basis functions and their coefficients can be iteratively solved by HF-SCF formulation. This is then arranged to form a Slater determinant with the occupied molecular orbitals. A limit called HF limit has been achieved by using an infinite basis set and it addresses the optimal description of electron probability density. However, it is impractical to use an infinite basis set. So one always try to get an optimal basis set which can produce close proximity of HF limit as efficient as possible [2,4,6].

By Linear Combination of Atomic Orbitals (LCAO) approach, suitable basis functions, representing atomic orbitals, are combined to give useful representations of molecular orbitals. The physical significance of basis function relies on the electronic distribution around an atom and their combination gives the corresponding electronic distribution in molecules.

In Simple Hückel and Extended Hückel Methods (SHM and EHM), we have used the simplest form of basis functions. Here simple Hückel method consists of only p-atomic orbitals, while EHM consists of only the valence orbitals. In SHM, we need not worry about the mathematical description of basis functions and are represented in the Fock matrix. However, in EHM they are represented *via* Slater functions [2,4,6].

2.7.1 Gaussian functions

One could adopt several ways to address the electron distribution around an atom, which may be based on the solution of the Schrödinger equation, polynomial based function, Slater functions, and Gaussian functions. Among these the last two are mathematically simpler than the others and they are currently in use. In EHM, and other semi empirical methods, Slater functions have been used while in modern *Ab initio* methods Gaussian type functions have been used. This does not mean that we cannot use Slater type functions for *Ab Initio* calculations. The problem is that several 2-e integrals need much excessive computational time and cost, even though they are good approximations [2,4,6].

There are four functions in 2-e integrals which may involve 4 centers also. Hence this kind of integrals with three or four functions and three or four nuclei are extremely difficult to solve and are highly expensive and time consuming. This can be overcome by the use of Gaussian type orbitals (GTO). Here the easiness comes from the fact that the product of two GTOs on 2 centers is a GTO on a third center.

Consider real s-type GTO on nuclei A and B:

$$g_A = a_A e^{-\alpha_A |r - r_A|2} \tag{2.12}$$

$$g_B = a_B e^{-\alpha_B |r - r_B|2} \tag{2.13}$$

$$g_A g_B = a_C e^{-\alpha_C |r - r_C|2} = g_C \tag{2.14}$$

i.e., the product of g_A and g_B are again a GTO g_C centered at r_C.

The electron repulsion integral is given by:

$$\langle r_s | t_u \rangle = \iint \frac{\phi_s^*(1)\phi_s(1)\phi_t^*(2)\phi_t(2)}{r_{12}} dv_1 dv_2 \tag{2.15}$$

Considering φ as a single real GTO we will get:

$$\langle v | w \rangle = \iint \frac{\phi_v(1)\phi_w(2)}{r_{12}} dv_1 dv_2 \tag{2.16}$$

i.e., the four center 2-e integrals with 4 basis functions now simplified to 2-c integral with two functions and can be solved easily.

However, the use of single GTO often leads to poor results so that we always use a combination of several GTOs as per our necessities [2].

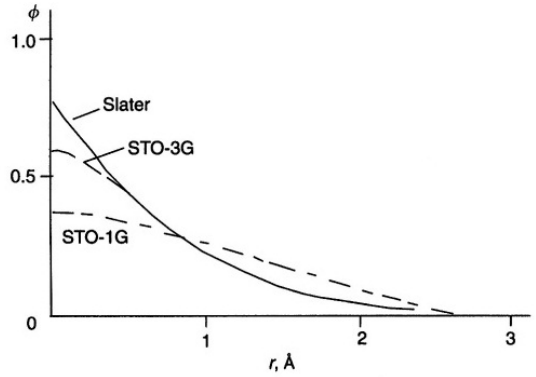

Fig.2.1 STO versus GTO

From Fig.2.1, it is clear that GTOs decays faster than STOs as 'r' increases. This difficulty can be overcome by using several GTOs for a single STO (e.g., STO3G, STO1G, etc).

STO-nG means Slater type orbital with n-GTOs. These are contracted Gaussian with n-primitive Gaussians. The use of contracted Gaussian instead of Slater functions, speeds up the calculations to a great extend. Usually, a linear combination of Gaussian functions is used. The commonly used basis sets are those described by Pople and co-workers [7]. On the basis of the number and type of GTOs used, the basis sets are named as 3-21G, 6-31G*, 6-31+G**, 6-311G* etc. Here the '*' sign denotes addition of polarization functions while the '+' sign denoted addition of diffuse functions. Polarization function is implemented to include orbitals of higher angular momentum for increasing the flexibility of basis sets, i.e., to consider asymmetry of molecular orbitals. Diffuse functions use more extensive versions of s- and p-type functions. These functions improve the accuracy of calculations [1]. In the present study, the basis set employed is 6-31+G (d, p) as it is a common basis set used for flavonoids to get reliable results [8].

2.8 Software

2.8.1 Computational software

- **Gaussian 09 & Gaussview-5.0**

Gaussian 09 software package is developed by John Pople and his research group at Carnegie Mellon University in the earliest of 1970s with the name Gaussian 70 [9]. The software has been updated continuously since then and we had used the Gaussian 09 version for the present work. The input structures are drawn by using the Gaussview-5.0 graphical user interface [10] and are given to Gaussian

09 software package [11] to get the output. Different computational tools like simple molecular mechanics (such as Amber force field), semi-empirical methods (such as CNDO), Hartree-Fock (restricted and unrestricted), MPn (Mollar-Plesset perturbation theory of order n = 2, 3, 4), CI (Configuration-Interaction), CC (Coupled-Cluster), Multi-configurational SCF (such as CAS-SCF) and various DFT methods are incorporated in the software.

Gaussian software helps in the electronic structure and quantum chemical calculations. Spectral data like NMR chemical shifts, Infra Red spectra, Raman spectra, ultraviolet spectra can be easily reproduced by this software. A wide range of molecular properties that can be computed includes NLO, thermochemistry, PES, IRC plots, etc. Gaussian is one of the very popularly used computational software among scientists of various disciplines like physics, chemistry and biology.

- **Autodock Vina**

Molecular Graphics Lab at The Scripps Research Institute develops an open source platform called Autodock-Vina software with multi-core capability, high performance, and enhanced accuracy for drug discovery, molecular docking and virtual screening [9]. The results from Autodock Vina are easy to interpret. Other commercial softwares like Glide, Schrodinger, etc., are also available to do the molecular docking, but, Autodock Vina is a free software, we have used it for our studies.

Autodock Vina uses an iterated Local search global optimizer rather providing the user with a choice of the search algorithm. Parameters like Partial charges, solvation parameters, etc., are not required for Autodock Vina. AutoDock distributes an interactive graphics software by which we can prepare the receptor and ligands for molecular docking.

- **Open Babel**

Structures downloaded from PubChem [12] database are in sdf format. We have to convert it into Gaussian input files (GJF) in order to execute them in Gaussian 09. This is done by the open software called Open Babel. Open Babel is a chemical toolbox which allows searching, analyzing or converting data for research [13].

2.8.2 Visualization softwares

- **Gauss View 5.0**

Gauss View 5.0 or simply the gauss view is the graphical user interface basically designed to prepare the input structures for submission to the Gaussian software. Gauss View allows sketching even huge molecules in a very short period of time. It also allows to rotate, translate and zoom in on these molecules. Gauss View also helps to examine the results of Gaussian calculations using a variety of graphical techniques. The current work has a heavy dependence on Gauss View for creating inputs for analyzing output structures [10].

- **Chemcraft**

Chemcraft is a graphical program for working with quantum chemistry calculations [14]. It acts as a convenient tool for visualizing computed results and preparing new jobs for a calculation. It helps to render 3-dimensional pictures of molecules, visualization of molecular orbitals in the form of iso-surfaces or coloured planes and many more. In the current work, Chemcraft has been used mainly to create some publication-ready images of molecules in customizable display modes and to construct NLMOs and NLMO clusters which are discussed in successive chapters.

2.8.3 Online softwares

- **Molinspiration**

Molinspiration is an online cheminformatics tool supporting molecule manipulation and processing. It helps in the calculation of various molecular properties like logP, bioactivities, etc., needed for doing QSAR, molecular modeling and drug design [15].

- **Osiris Property Explorer**

The OSIRIS Property Explorer, now available as DataWarrior helps to calculate various drug-relevant properties like lipophilicity, solubility, toxicity, etc., on a valid input structure. Different properties significant for the drug action are computed and are summed up to give the drug-likeness score and drug score. Besides this, the toxicity risk of the given molecule can be easily interpreted [16,17].

2.9 Computer Power

All the calculations included in this thesis are performed using:

- Lenovo Thinkstation with processor Intel®Xeon®CPU E5-2660 v3 @2.60 GHz and 32.0 GB RAM.

2.10 Computational Methodology

The following methods have been employed for interpreting the results obtained from various resources:

2.10.1 Optimization of Structures

Molecular orbital energies; Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) are the important quantum chemical descriptors. These are the most popular descriptors which play a crucial role in governing the chemical reactions, electronic band gaps in solids [18–20] and global descriptive parameters (by Koopmans's theorem) [21]. The Frontier Molecular orbital theory of chemical reactivity tells that the transition states are formed due to the interaction between HOMO and LUMO orbitals [19,22]. So these orbitals are separately studied than the other orbitals in governing the chemical reactions [22]. The HOMO energy level is characterized by the susceptibility of a molecule towards the attack of an electrophile whereas the LUMO energy level characterizes the susceptibility towards a nucleophile and thus the HOMO and LUMO energy levels respectively give the Ionization Energy (IE) and Electron Affinity (EA) of molecules. These are important in reactions involving radicals also [23,24]. The hard and soft nucleophile and electrophile

concept are directly derived from the HOMO-LUMO energy levels. A hard nucleophile and a hard electrophile are characterized by low energy HOMO and high energy LUMO respectively while soft nucleophile and soft electrophile is characterized by high energy HOMO and low energy LUMO respectively [25].

The difference between the HOMO and LUMO energy levels, the energy gap, is significant in studying the electronic and charge transfer reactions. A high energy gap corresponds to the low chemical reactivity and high stability of a molecule and vice versa [18]. The concept of activation energy in molecular reactions is also defined from the HOMO-LUMO energy gap [18,26]. The activation hardness distinguishes the reaction rates at different sites in a compound and is crucial for studying the orientation effects [18]. Since the lower energy gap makes the polarization easier, the qualitative hardness is closely related to the polarizability [26].

The HOMO-LUMO energy levels of all the studied flavonoids have been analyzed and the energy gap values are computed. The level of theory adopted is B3LYP, which consists of Becke's exchange functional [27] in conjunction with Lee-Yang-Parr correlational functional [28]. The studies in solvent media are done through IEF-PCM model which is an Integral Equation Formalism for solving relevant Self-Consistent Reaction Field (SCRF) equations which facilitates the computation of gradients and molecular response properties an extension to permit application to infinite periodic systems in one and two dimensions, and an extension to liquid/liquid and liquid/vapour interfaces [5,6]

2.10.2 Electrostatic Potential Map (ESP)

The total energy along a particular pathway is the sum of energies of particles on interaction with the electric components along that pathway. It is different in different positions on the surfaces of components. So it will take large time to get the full information about the ESP. But it is easy when we plot the graph showing ESP.

ESP maps, also known as electrostatic potential energy maps, or molecular electrical potential (MEP) surfaces, are analyzed which illustrate the charge distributions of a molecule three dimensionally. These maps allow us to visualize variably charged regions of a molecule. Knowledge of the charge distributions can be used to determine how molecules interact with each other. They enable us to visualize the charge distributions of molecules and charge related properties of molecules. In organic chemistry, ESP maps are invaluable in predicting the behavior of complex molecules. Electrostatic potential energy is fundamentally a measure of the strength of the nearby charges, nuclei and electrons, at a particular position [29].

Here the red region denotes the areas of lower potential and hence higher electron density while the blue region is the area with high electrostatic potential and with a low concentration of electrons. ESP map is also useful for determining the nature of chemical bonds between two atoms based on the electronegativity differences. The colours other than red and blue represents the electronegativity difference. These are seen in between red and blue coloured region.

When this region with colours green-yellow increases, the charge is very polarized and there is a significant difference in the electronegativity values. In such cases complete red/blue region is seen on the molecular surfaces.

2.10.3 Global Descriptive Parameters

The utility of global descriptive parameters of a molecule opens a way to get the relation between the chemical reactivity of a molecule and its sensitiveness to structural perturbations and responses to the changes in external conditions. The global descriptive parameters include the chemical potential, electronegativity, hardness, softness, electrophilicity index, etc. These quantities are corresponding to the linear responses of the electron density with respect to the changes in the external potential and number of electrons [30]. Thus global descriptors are of particular relevance in comparing the properties of different molecules. The global hardness reflects the overall stability of the system [31]. The chemical hardness fundamentally signifies the reluctance towards the deformation or polarization of the electron cloud of the atoms, ions or molecules under small perturbation encountered during chemical processes. Chemical Softness is the measure of the capacity of a molecule to receive electrons, more precisely it is related to the groups or atoms present in that molecule and inversely proportional to chemical hardness [32]. The chemical potential in DFT, measures escaping tendency of an electron from equilibrium, is accounted by the first derivative of energy with respect to the number of electrons [1] and is also the negative of electronegativity, which is a measure of the tendency to attract

electrons in a chemical bond [32]. The electrophilic index tells us about the strength of electrophilicity of the species [21]. Global reactive descriptors can be calculated by two different methods, one is based on the difference in total electronic energy of neutral molecule and its corresponding anion and cation, obtained from the geometry of the neutral molecule in order to keep the external potential constant, and usually call it as 'energy vertical' [33]. The global properties are computed by using the equations[29]:

$$\text{Vertical Ionization Potenial (VIP)} = E_{cation} - E_{neutral} \quad (2.17)$$

$$\text{Vertical Electron Affinity (VEA)} = E_{neutral} - E_{anion} \quad (2.18)$$

where E_{HOMO} is the energy of HOMO and E_{LUMO} is the energy of LUMO

$$\text{Hardness } (\eta) \approx \frac{IP-EA}{2} \quad (2.19)$$

$$\text{Electronegativity } (\chi) \approx \frac{IP+EA}{2} \quad (2.20)$$

$$\text{Softness(S)} \approx \frac{1}{2\eta} \quad (2.21)$$

$$\text{Chemical potential } (\mu) \approx -\chi \quad (2.22)$$

$$\text{Electrophilicity index } (\omega) \approx \frac{\mu^2}{2\eta} \quad (2.23)$$

2.10.4 Full Electron Donor Acceptor Map (FEDAM)

IE and EA values are the two important parameters to explain the one electron transfer mechanism of antiradicals. The Full Electron Donor Acceptor Map (FEDAM) helps to study the electron donating/accepting tendency simultaneously [34]. For that, we have

computed two parameters called Relative IE (RIE) and Relative EA (REA) as:

$$RIE = IE_L / IE_{Na} \tag{2.24}$$

$$REA = EA_L / EA_F \tag{2.25}$$

where L is the antiradical and F and Na are selected as a powerful electron acceptor and donor respectively. The IE and EA values of F and Na are $IE_F = 17.54$; $IE_{Na} = 5.14$; $EA_F = 3.40$; and $EA_{Na} = 0.54$.The reference FEDAM is shown in Fig.2.2 [35].

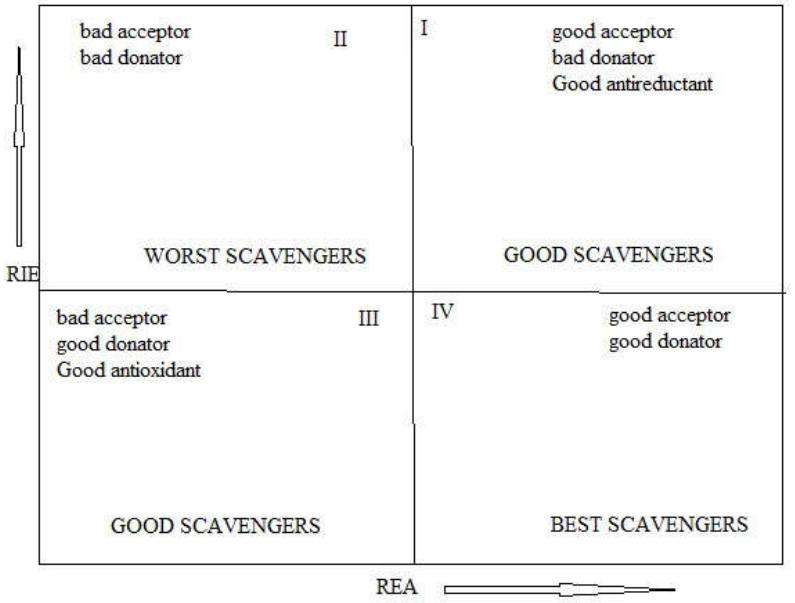

Fig.2.2. Reference FEDAM

However, in some situations, there is only fractional charge transfer takes place from antiradical to free radical or vice versa. In such cases, we cannot adopt FEDAM and then we define another set of

parameters called Electroaccepting power (ω+) and Electrodonating power (ω-) and these parameters can be computed as:

$$\omega^{-} = (3IE + EA)^2 / 16(IE - EA) \tag{2.26}$$

$$\omega^{+} = (IE + 3EA)^2 / 16(IE - EA) \tag{2.27}$$

The tendency of a compound to accept or donate charge is expressed by using ω+ and ω-. As ω- decreases the compound will have a strong tendency to donate electrons, while a large value of ω+ implies a strong tendency to accept electrons. Even though IE and EA are also related to the electron donating and accepting the capacity of a compound, ω+ and ω- are more suitable for explaining the charge transfer mechanisms of antiradicals. It is because of that sometimes there is only a fractional charge transfer taking place. The former parameters are significant only when there is a complete transfer of electrons.

2.10.5 Donor Acceptor Map (DAM)

DAM is a plot which simultaneously shows the electron acceptance and electron donating power of studied molecules [35–37]. Electron accepting index with respect to F (Ra) and Electron donating index with respect to Na (Rd) is the respective parameters for DAM and are calculated as:

$$R_a = \omega_L^{+} / \omega_F^{+} \tag{2.28}$$

$$R_d = \omega_L^{-} / \omega_{Na}^{-} \tag{2.29}$$

where;

$\omega_L^+ \cong \omega_F^+ \Rightarrow$ L and F are equally efficient electron acceptors.

$\omega_L^+ >> \omega_F^+ \Rightarrow R_a > 1 \Rightarrow$ L is more efficient than F.

$\omega_L^- \cong \omega_{Na}^- \Rightarrow$ L and Na are equally efficient electron donors.

$\omega_L^- << \omega_{Na}^- \Rightarrow R_d < 1 \Rightarrow$ L is more efficient than Na.

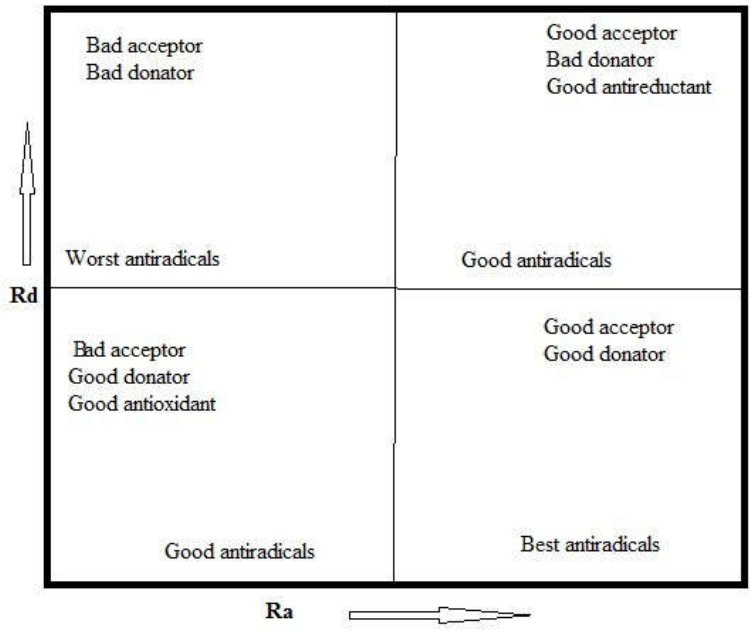

Fig.2.3. Reference DAM

2.10.6 Mechanisms of antioxidant capacity

A number of molecular descriptors are available to screen the best antioxidant molecule. These are HOMO-LUMO energies,

Adiabatic Ionization Potential (AIP), Electron Transfer Enthalpy (ETE), Bond Dissociation Energies (BDE), etc. There are three antioxidant mechanisms which describe antioxidant reactions [38–46]:

1) HAT (*Hydrogen atom transfer*) mechanism

$$ArOH + X^{\bullet} \rightarrow ArO^{\bullet} + XH \tag{2.30}$$

According to this mechanism, phenolic antioxidant reacts directly with a free radical, which has neutralized, and a radical form of phenolic antioxidant appears. A numerical parameter associated with this mechanism is BDE. The lower BDE parameter characterizes the better antioxidant property.

2a) SET (*Single electron transfer*) mechanism

$$ArOH + X^{\bullet} \rightarrow ArOH^{\bullet+} + X^{-} \tag{2.31}$$

The numerical parameter related to the SET mechanism is AIP.

2b) SET-PT (*Single-electron transfer followed by proton transfer*)

$$ArOH^{\bullet+} \rightarrow ArO^{\bullet} + H^{+} \tag{2.32}$$

This mechanism is a two-step reaction. In the first step, a phenolic antioxidant molecule reacts with the free radical, and a cationic radical form of the phenolic antioxidant and an anionic form of the radical appear. This reaction is a thermodynamically significant step of this two-step mechanism. In the second step, the cationic radical form of the phenolic antioxidant decomposes into a phenolic radical and proton. A numerical parameter related to the SET-PT

mechanism is AIP for the first step and PDE (*Proton Dissociation Enthalpy*) for the second step.

3) SPLET (*Sequential Proton Loss Electron Transfer*)

$$1.\ ArOH \rightarrow ArO^- + H^+ \tag{2.33}$$

$$2.\ ArO^- + X^{\bullet} + H^+ \rightarrow ArO^{\bullet} + XH \tag{2.34}$$

This mechanism also consists of a two-step reaction. In the first step, the phenolic antioxidant dissociates into an anionic form and proton, and then ions created in the first reaction react with the free radical. In this reaction, a radical form of the phenolic antioxidant and a neutral molecule appear. A numerical parameter related to this mechanism is for the first reaction step: PA (*Proton affinity*) and for the second step: ETE.

The parameters can be computed as:

$$BDE = H_{ArO^{\bullet}} + H_{H^{\bullet}} - H_{ArOH} \tag{2.35}$$

$$AIP = H_{ArOH^{\bullet+}} - H_{ArOH} \tag{2.36}$$

$$PDE = H_{ArO^{\bullet}} + H_{H^+} - H_{ArOH^{\bullet+}} \tag{2.37}$$

$$PA = H_{ArO^-} + H_{H^+} - H_{ArOH} \tag{2.38}$$

$$ETE = H_{ArO^{\bullet}} - H_{ArO^-} \tag{2.39}$$

2.10.7 Time-Dependent Density Functional Theory (TDDFT)

Electronic excitations and their transitions are highly important in studying various spectral characteristics, especially the UV-Visible spectra of compounds. An atom, molecule or compound, if placed in a fluctuating linear electric field experiences an electric field of:

$$E = r\cos\omega t \tag{2.40}$$

where 'r' is the position vector,'t' is the time and 'ω' is the fluctuating frequency. The frequency dependent polarizability can thus compute as:

$$\langle\alpha\rangle = \sum_{i\neq 0}^{states} \frac{\left|\langle\psi_0|r|\psi_i\rangle\right|^2}{\omega-(E_i-E_0)} \tag{2.41}$$

If 'ω' becomes exactly equal to the difference between the energies of excited and ground states, the denominator vanishes.

Using the propagator methodology, one could easily compute the frequency-dependent polarizability without computing all the excited states. In the Random Phase Approximations (RPA), the integrals that are necessary to compute the excited states are essentially the same as those required to fill the CI matrix, as CI matrix contains all the single and double excitations and transition dipole moment between the ground state and all the single excitation configurations.

RPA methods are more accurate than CIS as RPA method includes double excitations. However, CIS gives a formal wave function while RPA does not. RPA method can apply to HF or

MCSCF wave functions. The error will be high in CIS in comparison with the RPA method.

For studying the excited states in DFT, there is a method exists, analogous to RPA, which is called Time-Dependent Density Functional Theory (TDDFT). In the DFT based computation of the UV-Visible spectra of compounds, a time-dependent approach has been employed. UV-Visible spectra are results from the electronic excitations between the ground state orbitals and the higher energy orbitals called the excited states. As DFT has essentially confined to ground state properties, one may think that it is not suitable for studying the excited states. However, it is not so. An alternative DFT called the TDDFT method has implemented to solve this problem. Here the studied molecule is placed in a fluctuating electric field and analyze the properties with respect to time. In this work we have used the time-dependent version of KS equations, which in turn derives from the time-dependent Schrödinger equation.

$$i\frac{\partial}{\partial t}\phi_i(r,t)=\left(-\frac{1}{2}\nabla_i^2+v(r,t)+\int d^3r\frac{\rho(r',t)}{|r-r'|}+\frac{\partial A_{XC}[\rho]}{\partial\rho(r,t)}\right)\phi_i(r,t)=\vec{F}^{KS}\phi_i(r,t) \tag{2.42}$$

with

$$\rho(r,t)=\rho_S(r,t)=\sum_i^N\left|\phi_i(r,t)\right|^2 \tag{2.43}$$

In the TDDFT matrix, elements of the Hamiltonian have replaced by the KS orbital energies and various exchange integrals. As KS orbital energies for orbitals that are high up in the virtual manifold

are very poor so that TDDFT calculations are reliable only for low energy excitations. If the excitation energies are smaller than the molecular ionization potential and promotions into the orbitals with positive KS Eigenvalues are restricted, TDDFT could produce the most reliable results. A promising improvement in TDDFT has been made to study high energy excitations and the charge transfer Circular Dichroism (CD) of compounds in inorganic chemistry.

Two different methodologies can be employed to obtain the excitation energies and the oscillator strengths by employing the TD-KS: one is to propagate the TD-KS wave function in time called the real-time TDDFT and the second is the analysis of the linear response of the TD-KS equation. TDDFT calculations give the wave functions of MOs that oscillate between the ground and excited states. TDDFT has also called time-dependent density functional response theory (TDDFRT). TDDFT has recently implemented in many of the quantum mechanical software packages and is an advantage of TDDFT. It is implemented in Gaussian by Stratman et al. [11].

In the TDDFT approach, only properties of the ground state and their corresponding orbital energies are involved and thus the excitation energies have expressed in terms of the ground state. To do the calculations of electronic excitations in Gaussian 09, the keyword TD must be given in the route section so that the program will automatically select the TD-HF or TDDFT based on the level of theory selected. The outfile contains the excitation energy, wavelength values, oscillator strength values and the MOs involved in the electronic transitions. Analyzing the outfile, one could easily interpret the nature

of electronic transitions in the molecule. Transitions with maximum oscillator strength value are considered as λmax.

In spectroscopy, the oscillator strength is a dimensionless quantity that expresses the probability of absorption or emission of electromagnetic radiations, in transitions between the energy levels in atoms or molecules. It can also be taken as the ratio between the quantum mechanical transition rate and the classical absorption/emission rate of a single electron oscillator with the same frequency as the transition. The transition rate is proportional to the dipole matrix element so that for the discussion of intensities, it is convenient to use a dimensionless quantity called oscillator strength.

$$f_{ba} = \frac{2m\nu_{ba}}{3\hbar}\left|\langle \psi_b | r | \psi_a \rangle\right|^2 \tag{2.44}$$

where, the quantity ' f_{ba} ' is the oscillator strength which characterizes the intensity of transition between energy levels 'a' and 'b'. A knowledge of ' f_{ba} ' for an atomic or molecular system shows us to determine the transition rates for absorption, spontaneous and stimulated emission and lifetimes of electronic levels as well as static polarizabilities. For the characterization of optical properties of atoms or molecules the ' f_{ba} ' values therefore plays an important role. It is related to the experimental intensity of transition as:

$$f = 1.44 * 10^{-19} \int \varepsilon(\nu) d\nu \tag{2.45}$$

where ν is the frequency, and $\varepsilon(\nu)$ is the molar absorptivity at each frequency in units of L mol^{-1} cm^{-1}. Thus, the oscillator strength can be determined from the area under the quantitative absorption spectrum of a given transition. The oscillator strength is a measure of the probability of a transition. Oscillator strength ~1 implies that a transition is completely allowed.

2.10.8 UV Filters

Green plants prepare food by photosynthesis in the presence of sunlight. During this, they have exposed to UV radiation in sunlight. UV radiation, constituting the non-ionizing part of the electromagnetic spectrum, partakes about 8-9 % of the total solar spectrum. Generally, the entire UV radiation has divided into three regions depending upon the wavelengths. They are UV-C (200-280 nm), UV-B (280-320 nm) and UV-A (320-400 nm). Among these, the UV-C radiation is particularly harmful to living systems. The UV-B and UV-A radiations are relevant to study through the former partake of about 1.5 % of the total spectrum and the latter constitutes about 6.3 % of the total solar spectrum. This is attributed to their toxic effects and it follows the order UV-C > UV-B > UV-A.

All the UV-C radiation and most of the UV-B radiations have been absorbed by the ozone layer. However, as the wavelength of radiation increases, the absorption coefficient of the ozone layer decreases and approaches zero as the wavelength becomes 330 nm. Thus, the UV-A radiation completely reaches on earth. It was reported that, for every unit percent decrease in the ozone layer concentration,

about 1.3 to 1.8 % UV-B reaches on the earth. As the UV radiation has harmful effects on the living system, it must be filtered from sunlight to protect the living system [47–53].

Now a day, increased growth in skincare is observed because of the modern lifestyle and habits, most of the skin problems are due to the generation of free radicals and their consequent reaction on skin cells and tissues. The free radicals are generated because of the UV irradiation on the skin. Due to human activities, the ozone layer depletion increases day by day and which in turn causes the harmful UV-B and UV-A radiation to reach on the earth. This increases the generation of free radicals in the skin and the oxidative stress in the body increases. This leads to serious damage to skin cells and tissues and the development of aging, tanning, burning, vision loss, skin cancer, eczema, etc. The UV-B radiation does not penetrate deep into the cells as it affects mostly on the surfaces of skin and causes conditions like UV-B induced erythema. However, unlike UV-B, the UV-A radiation could penetrate deep into the dermal matrix of our skin tissues and leads to chronic skin effects like skin cancer. Therefore, the primary need to save our skin is to use some UV filters on the skin. This is what our sunscreen lotions and the related cosmetics do when we have applied them on our skin. The UV filters in these sunscreens protect our skin from the harmful effects of UV radiation [47–53]. It has been reported that the compounds in the categories of flavones, anthocyanidins, and flavanones are capable of protecting the living system from the harmful UV-A and UV-B radiations so that they can be used as potential UV filters [48,49,54–59].

Two types of UV filters are available; inorganic and organic. Inorganic filters used in sunscreens reflect and scatter the UV radiation. An example of this is metal oxides (Zn and Ti oxides) which protect the body from the UV radiation by reflecting it. As these are chemically inert, they did not cause any allergic problem to the skin and thus they are highly recommended. However, they could protect the skin only to a small extent. Organic UV filters protect the skin by absorbing UV radiation. Currently, there are lots of organic UV filters or sunscreens available in the market with both natural and synthetic origin. But, the synthetic sunscreens cause allergic sensations and cannot recommend. However, the sunscreen with natural origin are mostly polyphenols with potential antioxidant capacity. They not only absorb 95% of UV radiation of 290-320 nm wavelengths, protecting against erythema and skin wrinkling but also from the free radical generation and associated OS. Polyphenols having electron releasing groups have considerable UV filtering capacity. The introduction of flavonoids like rutin, quercetin, etc., could increase the sun protection factor (SPF) of sunscreens to a great extent [60,61].

The basic structure of flavonoids consists of two benzene rings [A] & [B] in conjugation with a ring [C]. The rings [A] & [B] and their conjugation to ring [C] attribute the absorption characteristics of flavonoid compounds. The difference in substitution and presence of glucose linkages give tentative information in their characteristic UV-Visible spectrum and this helps to differentiate between each category of flavonoids. All flavonoids consist of an absorption band at 240-290 nm due to π-π^* transition (Band II) and this band has a great affinity towards conjugation in the ring [A] and which in turn altered on

conjugation in the ring [A] and its substituents. If the rings [B] and [C] have conjugated with a double bond, then there is another absorption band near to 300-550 nm due to n-π^* transition (Band I). This band has observed at 460-560 nm for anthocyanidins while it is at 310-370 nm for flavones and flavonols. The band, I absorption for flavonols, are found to be greater than that of flavones and is the factor to differentiate those two categories of flavonoids [62–64].

The positions at which bands I and II have seen depend on the number of –OH groups in rings [A] and [B] and the glycoside linkages. Generally, the glycoside substitution of –OH groups decreases the absorption band I. Even though the actual glucose group does not affect the UV spectrum of flavonoids, but the acyl groups in the glucose moiety, if any, can cause some altering in the absorption bands [62–64].

2.10.9 Natural Bond Orbital (NBO) analysis

The NBO analysis, a unique peculiarity of computational studies which is otherwise not possible by experiments, enables chemists to see an intuitive picture of both electron orbitals and population analysis. NBO analysis is based on an approach which optimally transforms a given wave function of molecules into a localized form that corresponds to single-center [lone pair(LP)] and two centered [natural bond and antibonding orbitals (BD and BD* respectively)] elements in a Lewis structural representation [29]. It gives a deep insight into the intra and intermolecular orbital interaction in the molecules between filled donor and empty acceptor NBOs, which enable us to give a quantitative evaluation and thereby results in a qualitative conclusion of donor-acceptor properties of substituents

[65]. Each donor(i) and acceptor(j) interactions are quantitatively expressed by means of second-order perturbation interaction energy $E^{(2)}$ associated with delocalization i → j and is estimated as

$$E^{(2)} = q_i \frac{F^2(i.j)}{\varepsilon_j - \varepsilon_i} \quad (2.46)$$

where q_i is the electronic occupancy in donor orbital, F(i, j) is the off diagonal NBO Fock matrix element and ε_i and ε_j are diagonal elements in orbital energies. By analyzing the interactions between occupied Lewis NBO (bond pair or lone pair) as a donor and an unoccupiednon-Lewis NBO (anti-bonding or Rydberg) as acceptor with their second order perturbation energy will give clear information regarding the origin of stabilization of that molecule. If the stabilization energy $E^{(2)}$ associated with interaction is large, more will be the extent of stabilization. The NBO analysis provides an efficient method for studying inter and intra molecular bonding interactions and also extenta convenient basis for investigating charge transfer or conjugative interactions in the molecular system [66,67].

The bonding efficiency in a molecule is often explained by bond order values. This can also be obtained from the NBO output file. The strength of each bond is obtained from the result and the weakest bond which is broken first can be predicted. Antioxidants act *via* a free radical scavenging mechanism and in this scenario, the stability of radicals is relevant, which in turn signifies the study of bond orders in a molecule [29].

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CHAPTER 3

EVALUATION ON THE ANTIOXIDANT AND UV FILTERING PROPERTIES OF FLAVANONES

- *Evaluation on the antioxidant and UV filtering properties of 7 flavanones.*
- *The major applications discussed are their radical scavenging and UV filtering capacity.*
- *The donor-acceptor, toxicological, pharmacokinetic properties and the Lipinski's rule of 5.*
- *Molecular docking studies.*

3.1 Introduction

The taste is one of the important factors to be taken care of while synthesizing a drug. In pharmaceutical industries, flavors like lemon, orange, natural taste masking agents like flavonoids, etc., are employed to compensate for the unpleasant taste and flavor of the drug [1]. Flavanones (Fig. 3.1) are the major contents in the citrus fruits which are responsible for the pleasant taste and which in turn make them able to use as natural taste masking agents. For example, Rosehip is a fruit used in beverages and pharmaceutical industries to mask the bitter taste. The primary content in the rosehip is a flavanone called eriodictyol which is responsible for the bitter masking ability. Chapter 3 attempts to evaluate the UV filtering, and antioxidant capacity of four potentially active flavanones namely; eriodictyol, hesperidin, hesperetin, and naringenin. Besides this, three monohydroxy flavanones have also been studied to get a clear cut idea about the effect of structural features on the bioactivities that they have shown.

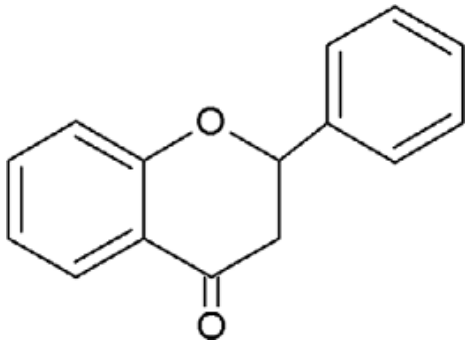


Fig.3.1 Basic structure of flavanone

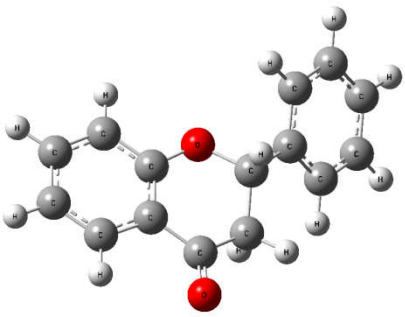
Eriodictyol is 5, 7, 3', 4'-tetrahydroxyfiavanone. Eriodictyol is the principal flavonoid of lemons after Hesperidin and is the principal content in the rosehip, which is widely used in making wine and is also present in certain plant materials, particularly the lemon, occurring therein in its glycoside form. Rosehip extract is added to soft drinks to compensate for their bitter taste. It is well known for its antioxidant [1] anti-inflammatory and antimicrobial activity. Hesperetin is a 4'-methoxy derivative of eriodictyol. Its 7-O-glycoside derivative is called Hesperidin and is the flavonoid found in citrus fruits especially the lemons and sweet oranges. The name is derived from 'Hesperidium,' a plant giving citrus fruits and hesperidin is the major content in it. Hesperidin has a major role in the defense mechanism of plants. It was first isolated from the inner layers of citrus peels. Hesperidin is also found in peppermint with considerable amounts. Naringenin is a bitter flavanone and is the predominant flavanone in grapefruit.

Plants have some inbuilt chemicals with them which act as UV filters to protect cells from the dangerous UV radiations. These are mainly polyphenols like flavanones, flavones, etc. Using natural plant extracts containing these kinds of compounds people make sunscreen lotions and other cosmetics to protect their skin from the damages caused by UV-A and UV-B radiations [2]. The conjugated pi-electron system in these kinds of polyphenols makes them able to excite an electron from the ground state to the excited state by absorbing radiations in the UV region of the solar spectrum [3,4].

Besides this, the radical scavenging activity makes flavonoids to be the right candidates for photo-protection [4]. The flavonoids are potent for their antiradical activity. Oxidative stress, produced by reactive oxygen/nitrogen species (ROS/RNS), damage the proteins, lipids, DNA, etc., in a living system and leads to some diseases like cancer, diabetes, Alzheimer's, etc. In order to fight against these reactive free radicals, we include lots of fruits and vegetables in our diet. They provide enough antioxidants for us. Polyphenols including flavonoids have both UV filtering and radical scavenging capacity, and there are lots of reports available for that also [3,5–7].

3.2 Structural features of flavanones

The basic structure of flavanone has been drawn by GaussView5 and has been subjected to Gaussian 09 for a PES calculation (Fig.3.2). The lowest energy conformer has been identified from the scan result and has been employed for future studies. This stable conformer has been modified to get different flavanones studied and optimized with the DFT-B3LYP level of theory and 6-31+G (d, p) as a basis set. The 13 conformers obtained on scanning the dihedral connecting the rings [B] and [C] have shown in Fig. 3.2, and among them, conformer four is having the lowest energy, and the dihedral angle is -89.677˚.



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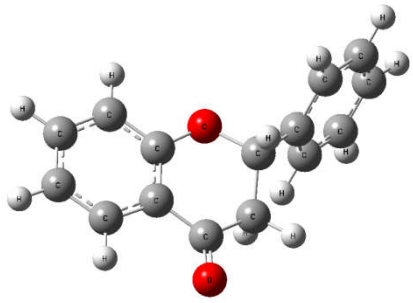

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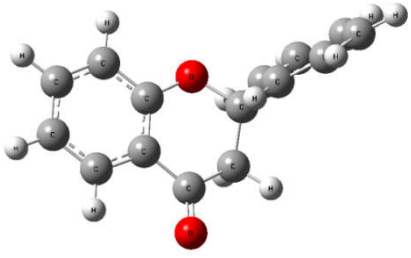

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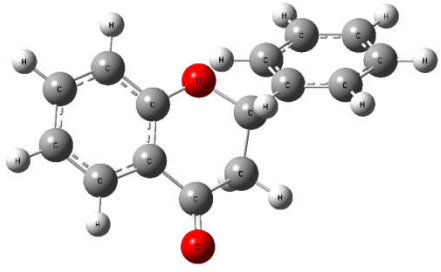

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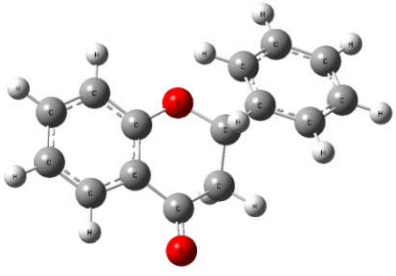

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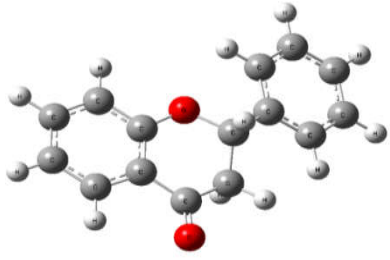

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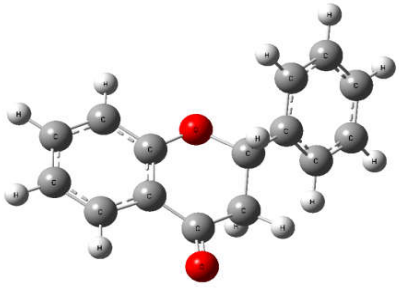

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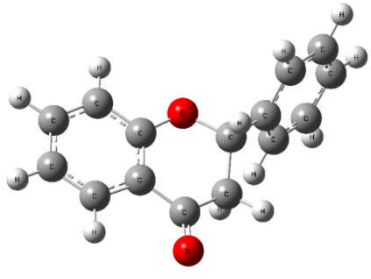

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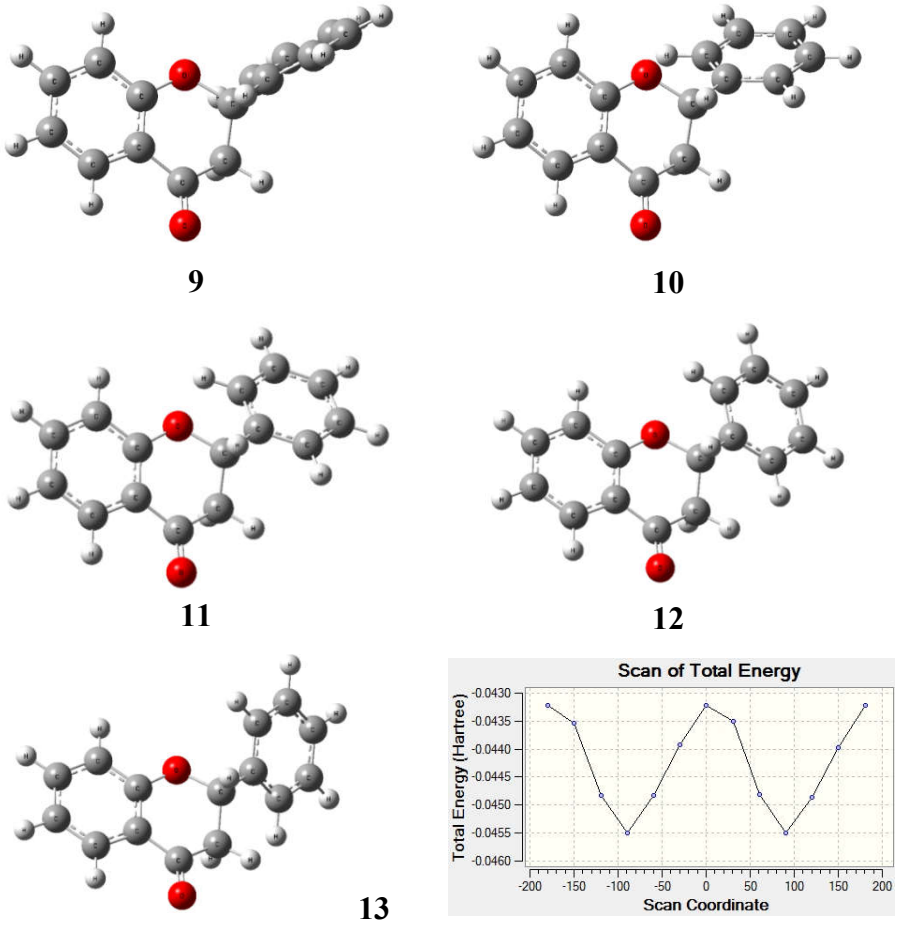
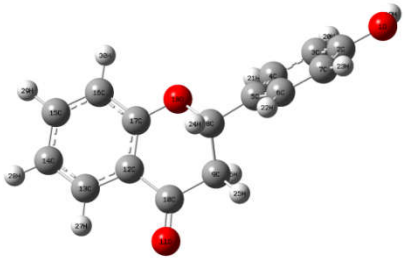


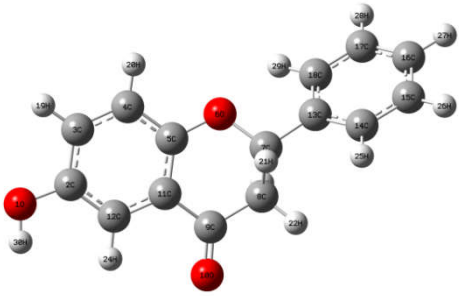
Fig.3.2 PES diagram and different conformers of flavanone

Individual flavanones, obtained by modifying the lowest energy conformer, are optimized, and the structures are shownin Fig. 3.3. Among them, eriodictyol, hesperetin, hesperidin and naringenin have hydrogen bonding between the carbonyl oxygen and the –OH group at position 5 (Fig.3.4).

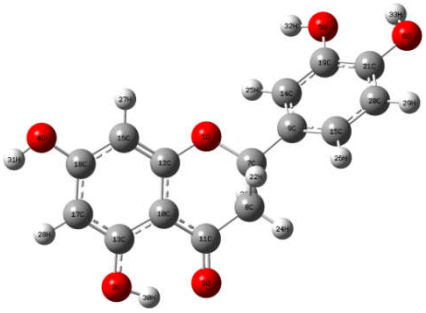
2'-Hydroxyflavanone



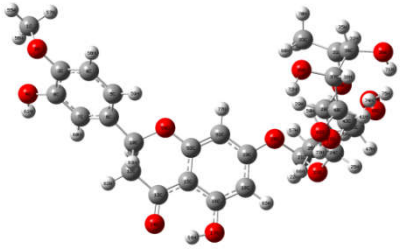
4'-Hydroxyflavanone



6-Hydroxyflavanone

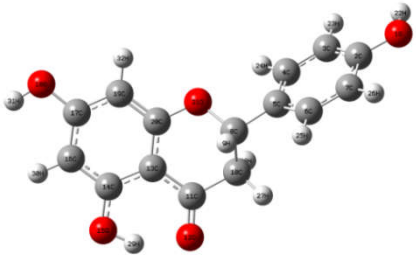


Eriodictyol



Hesperidin

Hesperetin



Naringenin

Fig.3.3 Optimized structures of Flavanones

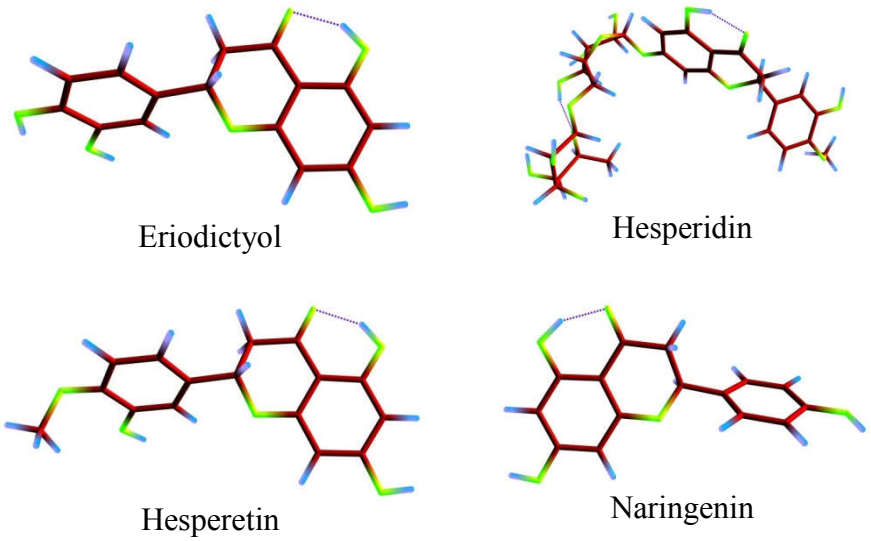
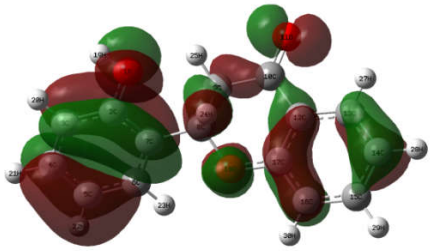
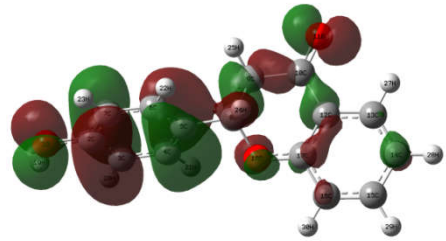


Fig.3.4 Hydrogen bonding in the studied flavanones

For all the seven flavanones, the LUMOs have delocalized over ring [A] and ring [C]. However, the HOMOs are not so. For 2'-Hydroxyflavanone, the HOMO is completely delocalized. The HOMO of 4'-Hydroxyflavanone is delocalized all over the molecule except half of the ring [A]. For both 6-Hydroxyflavanone and Naringenin, LUMOs and HOMOs are delocalized over the rings [A] and [C]. The HOMO of eriodictyol has been delocalized completely over the ring [B] and partially on ring [C]. This is the same for hesperetin also. For hesperidin, the HOMO is delocalized over the entire three rings except over the glucose group. The HOMOs and LUMOs of studied flavanones are shown in Figs. 3.5 & 3.6 respectively.



2'-Hydroxyflavanone



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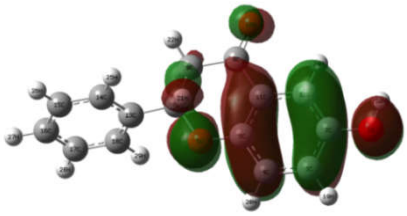
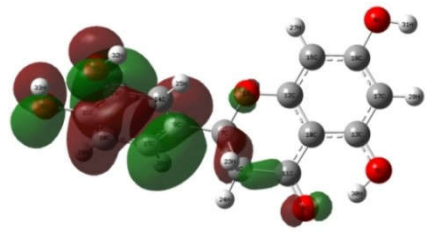
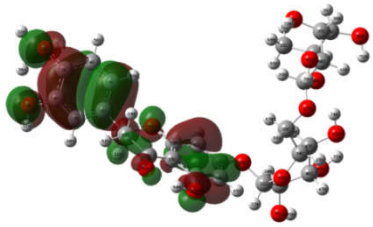


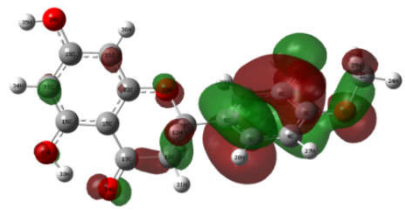
6-Hydroxyflavanone



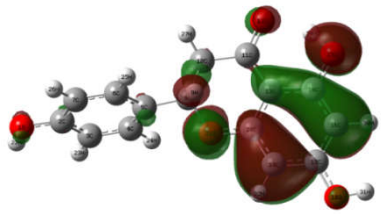
Eriodictyol



Hesperidin

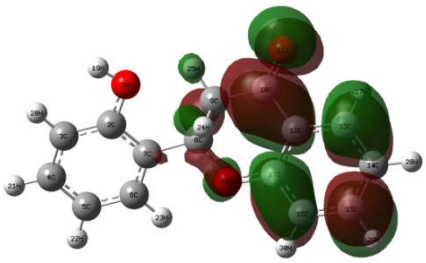


Hesperetin

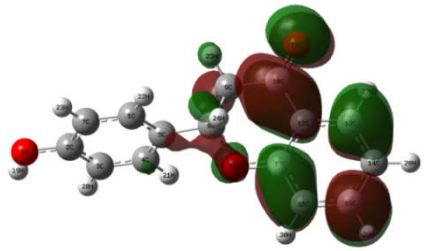


Naringenin

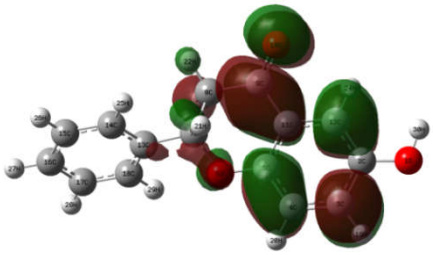
Fig.3.5 HOMO of Flavanones



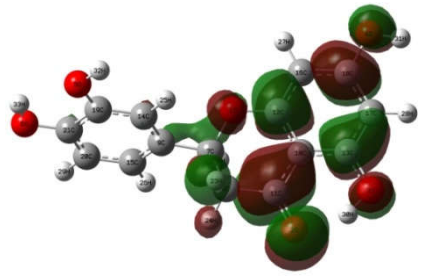
2'-Hydroxyflavanone



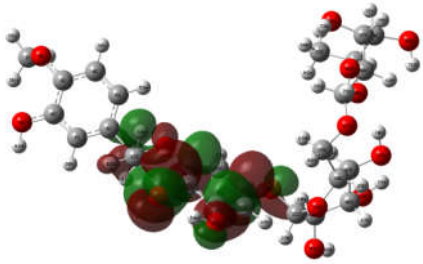
4'-Hydroxyflavanone



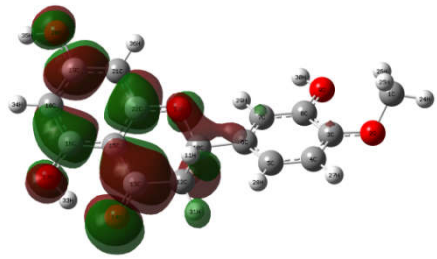
6-Hydroxyflavanone



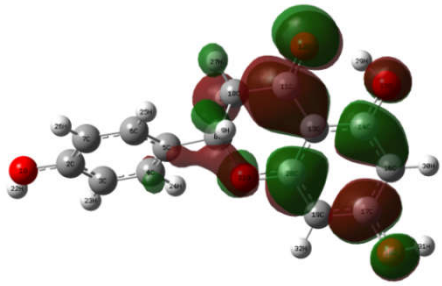
Eriodictyol



Hesperidin



Hesperetin



Naringenin

Fig.3.6 LUMO of Flavanones

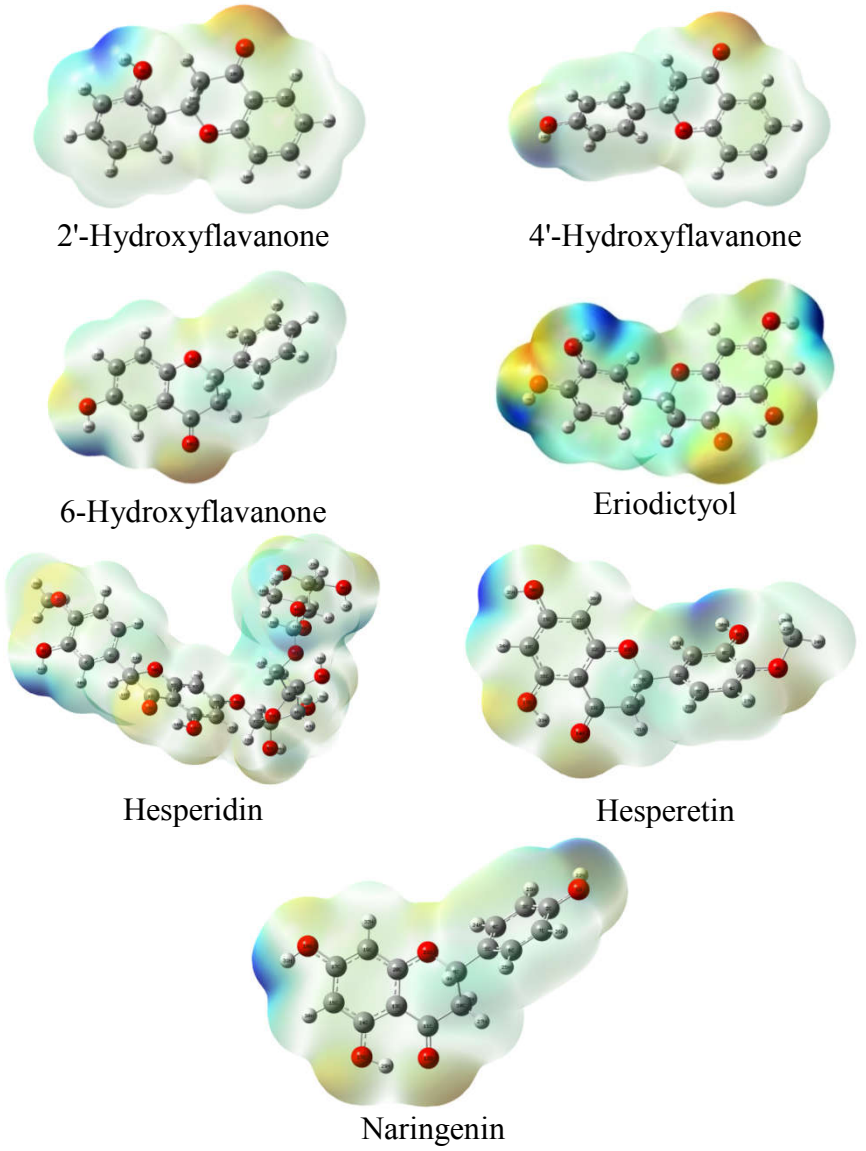


Fig.3.7 ESP of Flavanones

The ESP diagrams are easy to interpret and are essential to get the idea of electrophilic and nucleophilic centers in a compound. The electrostatic potentials are shown in Fig.3.7. The red regions are

electron rich while blue is electron deficient. So, the red colour locates on oxygen atoms while blue locates on hydrogen atoms.

3.3 Global reactive descriptors of flavanones

The reactivities of a set of compounds can be easily demonstrated using global reactive descriptors. Table 3.1 displays the global reactive descriptors of the studied flavanones.

Table 3.1

Global reactive descriptors (eV) of flavanones

No	Compound	ΔE	IE	EA	η	S	χ	μ	ω	Q_{max}	ω-	ω+
1	Eriodictyol	4.39	5.86	1.47	2.19	0.23	3.67	-3.67	3.06	1.67	5.17	1.50
2	2'-Hydroxyflavanone	4.73	6.15	1.42	2.37	0.21	3.79	-3.79	3.03	1.60	5.22	1.43
3	4'-Hydroxyflavanone	4.58	6.10	1.51	2.29	0.22	3.80	-3.80	3.16	1.66	5.35	1.54
4	Hesperetin	4.54	6.03	1.49	2.27	0.22	3.76	-3.76	3.12	1.66	5.28	1.52
5	Hesperidin	4.50	6.08	1.58	2.25	0.22	3.83	-3.83	3.26	1.70	5.45	1.62
6	Naringenin	4.60	6.03	1.43	2.30	0.22	3.73	-3.73	3.03	1.62	5.19	1.45
7	6-Hydroxyflavanone	4.22	5.83	1.60	2.11	0.24	3.71	-3.71	3.27	1.76	5.39	1.67

2'-hydroxyflavanone has the highest and 6- hydroxyflavanone has the lowest band gap values. All the studied flavanones have relatively high IE and low EA values, so that are less efficient electron donors than Na and less efficient electron acceptors than F. However, we could compare the donor-acceptor capacities among themselves by plotting the DAM. The 'R_a' denotes the electron accepting while 'R_d' indicates the electron donating power. It is the fractional charge transfer that is considered here to plot the DAM (Fig.3.8).

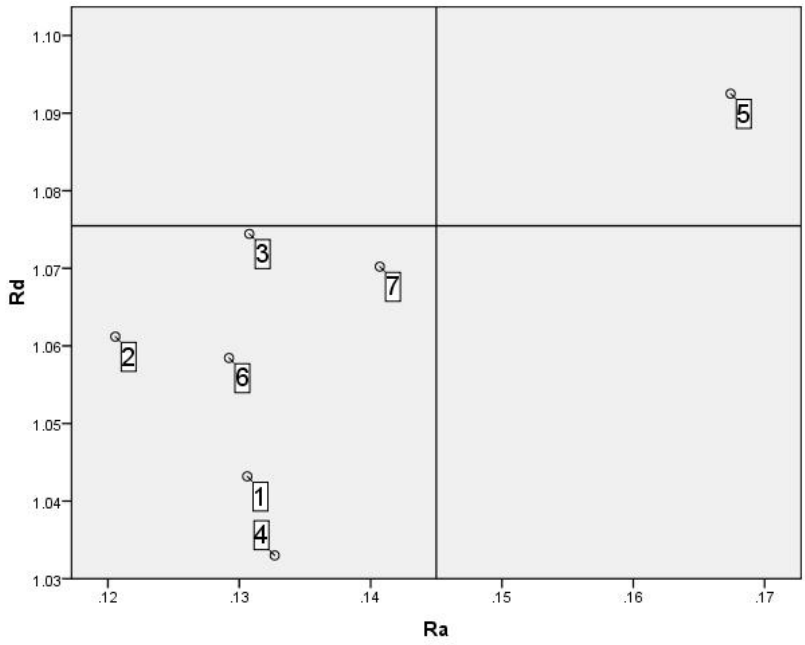


Fig.3.8 DAM of flavanones

The DAM of flavanones shows that the 7 flavanones studied here lie in two quadrants of DAM. The flavanone 5, i.e., hesperidin lies in the quadrant of good antireductants and is attributed to the highest EA and Qmax values of hesperidin which favors the charge transfer from free radical to hesperidin. The remaining flavanones lie in the quadrant of good antioxidants as they have low IE values.

3.4 UV-Visible spectral characteristics of flavanones

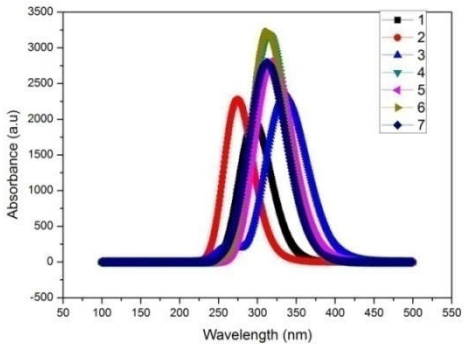
As the conjugations in flavanones are low in comparison with other classes of flavonoids, the absorbance values are lower than that of others. The conjugation gets lost in the ring [C] due to the presence of sp3 carbon atom at position 3. So the band gap values are high in comparison with other flavonoids. However, the loss in conjugation

makes them able to filter the dangerous UV-A and UV-B radiations. This enhances the use of flavanones in sunscreen lotions and other cosmetic products. The UV-Visible spectra of all the studied flavanones are computed *via* the TDDFT tool in Gaussian 09 software package and the spectra in and methanol media are shown in Fig.3.9.

Table 3.2

UV-Visible spectral characteristics of flavanones in the gas phase

Flavanone	Energy (eV)	λ_{max} (nm)	Oscillator strength	MO involved	% Contribution
2'-Hydroxyflavanone	3.67	338	0.03	HOMO-2-LUMO	64.38
	4.17	**298**	**0.04**	**HOMO-1-LUMO**	**46.41**
	4.32	287	0.01	HOMO-1-LUMO	46.47
4'-Hydroxyflavanone	3.64	341	0.01	HOMO-2-LUMO	56.86
	4.13	**300**	**0.03**	**HOMO-1-LUMO**	**55.55**
	4.26	291	0.03	HOMO-1-LUMO	38.14
6-Hydroxyflavanone	3.64	341	0.02	HOMO-LUMO	44.70
	3.73	**332**	**0.04**	**HOMO-LUMO**	**51.57**
	4.74	262	0.01	HOMO-3-LUMO	73.32
Eriodictyol	3.84	323	0.02	HOMO→LUMO	83.06
	3.93	**315**	**0.08**	**HOMO-1→LUMO**	**87.92**
	4.24	293	0.01	HOMO-3→LUMO	77.26
Hesperidin	**3.85**	**322**	**0.05**	**HOMO-1-LUMO**	**55.58**
	3.94	314	0.02	HOMO-LUMO	41.08
	4.23	293	0.01	HOMO-5-LUMO	57.16
Hesperetin	3.91	317	0.03	HOMO-1-LUMO	60.90
	3.99	**311**	**0.05**	**HOMO-LUMO**	**47.67**
	4.25	292	0.03	HOMO-4-LUMO	59.22
Naringenin	**3.91**	**317**	**0.05**	**HOMO-LUMO**	**90.25**
	4.03	307	0.02	HOMO-1-LUMO	58.56
	4.40	282	0.01	HOMO-1-LUMO	39.66



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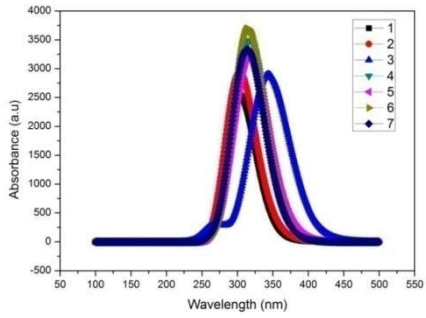

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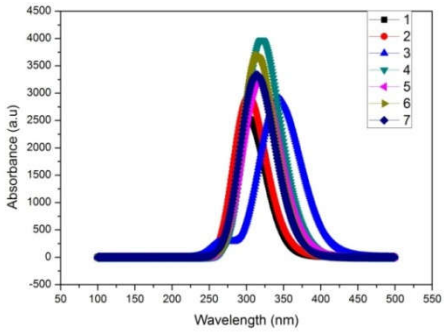


c

Fig.3.9 UV-Visible spectrum of flavanones in (a) gas, (b) water and (c) methanol

Table 3.3

UV-Visible spectral characteristics of flavanones in the aqueous phase

Flavanone	Energy (eV)	λ_{max} (nm)	Oscillator strength	MO involved	% Contribution
2'-Hydroxyflavanone	3.74	331	0.01	HOMO-LUMO	36.86
	4.04	307	0.02	HOMO-LUMO	47.62
	4.12	**301**	**0.04**	**HOMO-1-LUMO**	**77.46**
4'-Hydroxyflavanone	3.69	336	0.02	HOMO-LUMO	56.36
	4.05	**306**	**0.04**	**HOMO-1-LUMO**	**53.01**
	4.14	299	0.03	HOMO-1-LUMO	41.72
6-Hydroxyflavanone	**3.59**	**345**	**0.07**	**HOMO-LUMO**	**92.90**
	3.78	328	0.01	HOMO-1-LUMO	52.25
	4.62	268	0.01	HOMO-3-LUMO	53.72
Eriodictyol	3.88	320	0.02	HOMO-1→LUMO	66.12
	3.91	**317**	**0.07**	**HOMO→LUMO**	**62.51**
	4.22	293	0.02	HOMO-3-LUMO	72.32
Hesperidin	3.84	323	0.03	HOMO-1-LUMO	53.52
	3.89	**319**	**0.04**	**HOMO-LUMO**	**51.66**
	4.23	293	0.01	HOMO-5-LUMO	67.13
Hesperetin	3.89	319	0.02	HOMO-1-LUMO	59.18
	3.96	**313**	**0.07**	**HOMO-LUMO**	**55.99**
	4.27	290	0.01	HOMO-4-LUMO	66.67
Naringenin	3.89	319	0.03	HOMO-LUMO	49.32
	3.98	**311**	**0.05**	**HOMO-1-LUMO**	**50.09**
	4.37	284	0.03	HOMO-4-LUMO	81.15

For 2'-Hydroxyflavanone, 4'-Hydroxyflavanone and Hersperitin, the MOs involved in the transition giving λ_{max} are same in all the three phases. However, for all others, the MOs corresponding to λ_{max} is different in different media. This may be attributed to the difference in the interaction of these flavanones with solvent media. The significant transitions involved are HOMO-LUMO and HOMO-1-LUMO.

All of them have absorption in the range of 260-345 nm which lies in the UV-A and UV-B region and consequently they can be

employed as potential UV filters. In order to understand the absorption characteristics in more detail, NBO-NLMO study has been performed on Eriodictyol as a representative example.

Table 3.4

UV-Visible spectral characteristics of flavanones in methanol

Flavanone	Energy (eV)	λ_{max} (nm)	Oscillator strength	MO involved	% Contribution
2'-Hydroxyflavanone	3.74	331	0.01	HOMO-2-LUMO	35.86
	4.04	307	0.02	HOMO-LUMO	47.75
	4.12	**301**	**0.04**	**HOMO-1-LUMO**	**76.50**
4'-Hydroxyflavanone	3.69	336	0.01	HOMO-LUMO	55.75
	4.05	**306**	**0.04**	**HOMO-1-LUMO**	**52.46**
	4.14	299	0.03	HOMO-1-LUMO	42.28
6-Hydroxyflavanone	3.78	328	0.01	HOMO-LUMO	92.69
	3.59	**345**	**0.06**	**HOMO-1-LUMO**	**52.43**
	4.62	268	0.01	HOMO-3-LUMO	54.1
Eriodictyol	3.79	327	0.02	HOMO→LUMO	93.17
	3.88	**319**	**0.08**	**HOMO-1→LUMO**	**93.39**
	4.25	292	0.01	HOMO-3→LUMO	77.11
Hesperidin	**3.84**	**323**	**0.03**	**HOMO-1-LUMO**	**53.41**
	4.23	293	0.01	HOMO-LUMO	51.5
	3.89	319	0.04	HOMO-5-LUMO	67.26
Hesperetin	3.89	319	0.02	HOMO-1-LUMO	58.69
	3.96	**313**	**0.07**	**HOMO-LUMO**	**55.49**
	4.27	291	0.01	HOMO-4-LUMO	66.72
Naringenin	3.89	319	0.04	HOMO-LUMO	49.94
	3.98	**311**	**0.05**	**HOMO-1-LUMO**	**50.54**
	4.37	284	0.01	HOMO-4-LUMO	80.84

The NBO analysis has been performed on Eriodictyol, and corresponding NLMOs has also been analyzed. The spatially and energetically related NLMOs are coupled into molecular orbital clusters and are shown in Fig.3.11. The main transitions seen in UV-Visible spectra are those between HOMO, HOMO-1, HOMO-3 levels and LUMO level and are analyzed with NBO tool in G09. The HOMO is formed from a cluster of 4 spatially and energetically closer molecular orbitals of ring B in the compound. Similarly, HOMO-1 and

HOMO-2 are formed from 4 molecular orbitals of atoms in ring A and C respectively. The HOMO-3 is formed from 3 molecular orbitals of atoms in ring B.The HOMO and HOMO-3 are delocalized on ring B, in which HOMO is more delocalized than HOMO-3. The LUMO cluster is formed from two antibonding molecular orbitals, which are entirely delocalized over ring C and partially on ring A.

Thus the HOMO-LUMO transitions in Eriodictyol involve electronic transitions between ring B and C while those of HOMO-1 and HOMO-3 to LUMO involves transitions between ring A to C and ring B to C respectively. The HOMO-LUMO transitions are due to the transfer of lone pairs of electrons of oxygen atoms in ring B to the π* orbitals of oxygen atom of ring C.The HOMO-1 to LUMO transitions are between the lone pairs of atoms of ring A to π* orbitals of oxygen atom of ring C. The three carbon atoms (C13, C14, C15) on ring B interacts with π* orbitals of oxygen atom of ring C resulting in the HOMO-3 to LUMO transition in the compound.

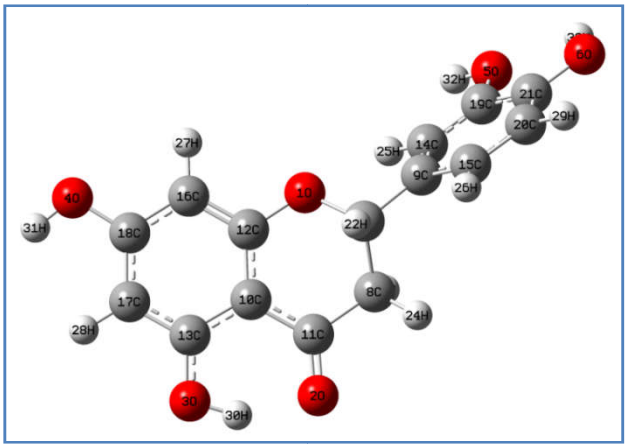


Fig.3.10 Numbering in the stable conformer of Eriodictyol

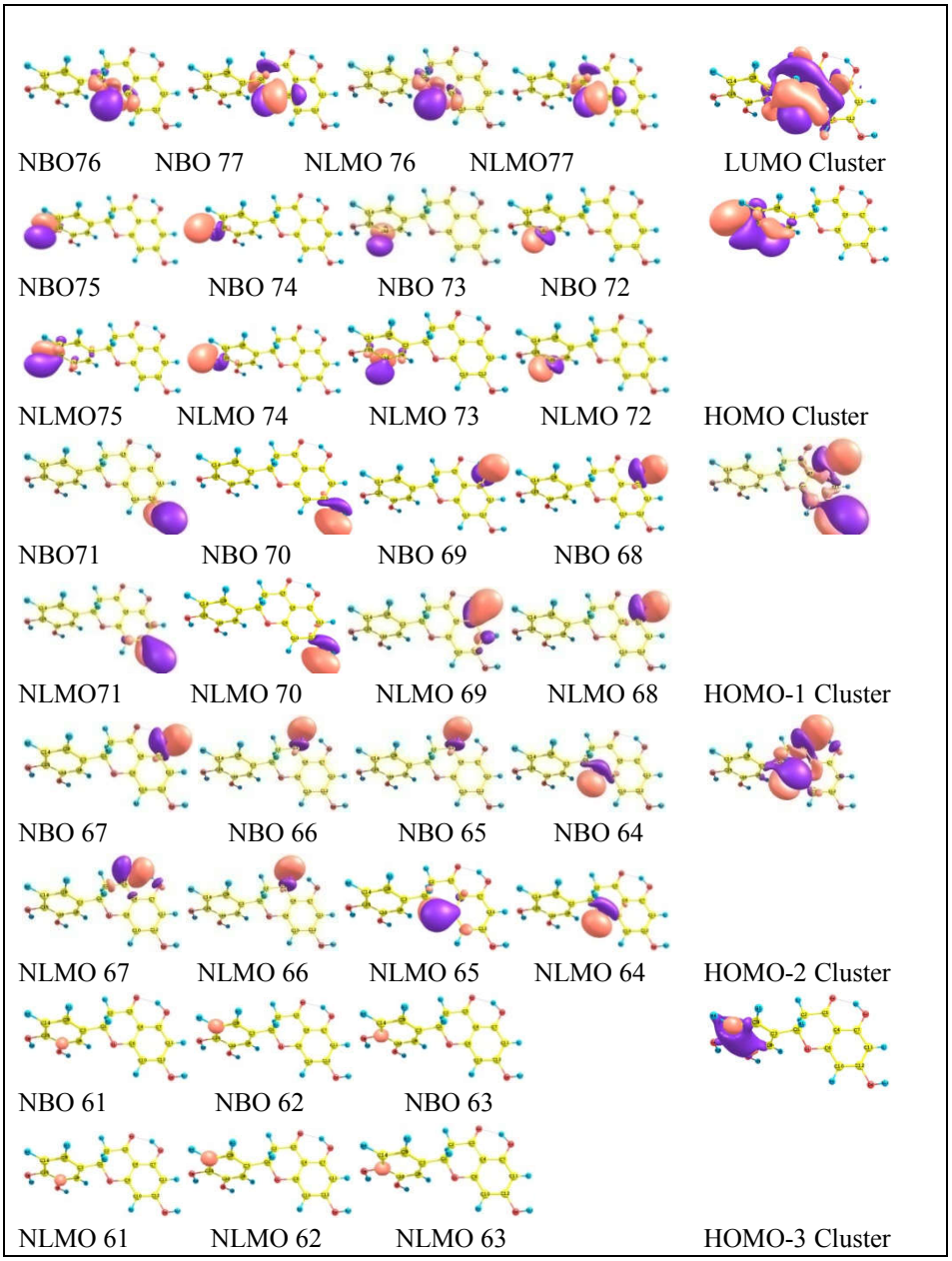


Fig.3.11. NBOs and NLMOs of Eriodictyol

3.5 Antioxidant properties of flavanones

Three antioxidant mechanisms are discussed here, which involve either transfer of hydrogen, proton, or electron or both. When the parameters associated with a particular mechanism are low, then that mechanism is favorable for that compound. Again a comparison of BDE and AIP values of these polyphenols with phenol gives an idea about the selectivity of the compound towards a particular mechanism.

Table 3.5

Parameters (in kcal/mol) related to antiradical mechanisms

Flavanones	BDE	AIP	PDE	PA	ETE	ΔBDE	ΔAIP
Eriodictyol	74.1	167.3	220.5	-1.0	388.9	-8.8	-24.7
4'-Hydroxyflavanone	82.2	173.8	222.9	0.4	396.3	-0.7	-18.2
Hesperetin	73.3	164.5	225.6	-1.9	391.9	-9.6	-27.5
Hesperidin	72.5	163.7	225.6	-7.2	396.5	-10.4	-28.3
Naringenin	77.4	170.8	226.1	-0.3	397.1	-5.5	-21.2
6-Hydroxyflavanone	84.1	169.5	225.3	-2.0	396.7	1.2	-22.5
2'-Hydroxyflavanone	82.9	175.0	220.0	2.4	392.5	0.0	-17.1

Table 3.5 shows the parameters related to the three mechanisms. The lowest BDE value has been observed for hesperidin and is attributed to the presence of $–OCH_3$ and glucose groups in it. The electron releasing inductive effect of $–OCH_3$ group decreases the BDE values of hesperetin than the eriodictyol, even though eriodictyol has the highest number of –OH groups. Next lowest BDE value has been observed for naringenin as it has 3 –OH groups. The remaining flavanones have only one –OH group,and hence they have relatively higher BDE values. Among them, the BDE value of 6-

hydroxyflavanone is the highest and other two have lower BDE value than the former. This indicates the higher reactivity of ring [B] in flavanones and the –OH groups present in the ring [B] has lower BDE values. So the number and position of –OH groups and presence of electron releasing/withdrawing groups together determine the order of BDE values in polyphenols. Hence by HAT mechanism, hesperidin is the most potent antioxidant among the studied flavanones.

Table 3.5 clearly shows that the ring [B] is more reactive for flavanones and the reactivity follows as 3' > 4' > 2' > 6 > 7 > 5. For naringenin, the ring [B] has only one –OH group and which lies at position 4' and is,in turn, having the lowest BDE value. Flavanones which contain a 3'-OH group, have their lowest BDE value at this position followed by position 4'. The higher BDE value at position 5 is attributed to the presence of hydrogen bonding and is explained by the bond order and interaction energy values obtained through the NBO analysis.

Table 3.6 shows the bond order values of –OH groups in the flavanones with the respective BDE values. Generally, as bond order decreases, the BDE value also decreases. However, here some exceptions are seen for position 5. For all other sites, the order is the same as we expected. This is due to the hydrogen bonding at position 5, which increases the bond length of –OH group but restricts the breakage of bond to form phenoxide radical at this position. The carbonyl oxygen act as the donor NBO and the hydrogen atom the –OH at position 5 acts as the acceptor NBO and interact with each other

resulting in the high interaction energies at this position and restricts the bond from breakage.

Table 3.6

BDE values and bond orders at different positions in flavanones

Flavanones	**Position**	**Bond order**	**BDE**
Eriodictyol	4'	0.7166	82.73
	5	0.6158	99.11
	7	0.7408	88.90
	3'	0.7308	74.09
4'-Hydroxyflavanone	4'	0.7453	82.24
Hesperetin	7	0.7381	89.15
	3'	0.7285	73.34
	5	0.6170	92.80
Hesperidin	5	0.6198	78.73
	3'	0.7393	72.51
Naringenin	4'	0.7348	77.67
	5	0.6170	89.67
	7	0.7484	88.89
6-Hydroxyflavanone	6	0.7482	84.09
2'-Hydroxyflavanone	2'	0.7477	82.94

For SET and 1st step of SET-PT, the parameter associated is AIP value. Lower AIP values are favourable for good antioxidants with good electron donating capacity. Here, among the studied flavanones, hesperidin, hesperetin,anderiodictyol have lower AIP values than the remaining so that they are susceptible to electron transfer reactions. For SET mechanism also, the hesperidin is the most potent antioxidant among the studied flavanones. However, the SET-

PT mechanism is not feasible as the 2nd step of SET-PT is highly energy demanding.

Similarly, even though the PA values of all the studied flavanones are low, the SPLET mechanism is not suitable as its 2nd step requires a large amount of energy. So HAT and SET are the two possible mechanisms. Among these, the selectivity for a particular mechanism has been explained as follows.

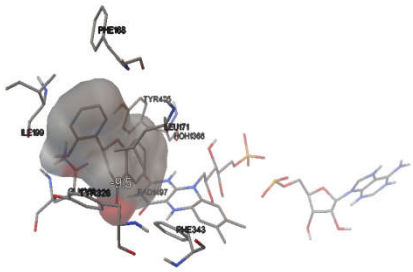
The selection of two mechanisms HAT or SET can be identified by evaluating ΔBDE and ΔAIP values, where ΔBDE and ΔAIP values are the difference in the respective parameters from that of phenol. BDE and AIP of phenol are respectively 82.9 and 192.05 kcal/mol in the gas phase. When the ΔBDE ~ -42 kJ/mol (-10.03 kcal/mol) and ΔAIP < -151 kJ/mol (-36.08 kcal/mol) the mechanism is dominated by hydrogen transfer (BDE)[8–10]. Among the studied flavanones, all except 2'-hydroxyflavanone and 6- hydroxyflavanone have ΔBDE values less than -10.03 kcal/mol and ΔAIP < -36.08 kcal/mol,so that HAT mechanism is favorable than SET. For hesperidin, the ΔAIP value is less than -36.08 kcal/mol, but its ΔBDE values are -10.4 kcal/mol. Taking into the consideration of lowest BDE value, HAT mechanism is favorable for it also. For the two flavanones showing an exception to this,may be due to their high BDE value. However, for them also, the ΔAIP values are less than -36.08 kcal/mol.

3.6 Molecular docking studies

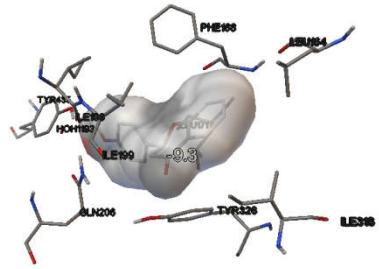
For a better understanding of the antioxidant behavior of flavanones, molecular docking studies have been employed. The molecular docking studies have been performed through AutoDock Vina software and the binding energy of flavanones with the active site of the protein target MAO-B has been computed. The active site of MAO-B is surrounded by specific amino acids Phe168, Ile199, Tyr326, Phe343, Leu171, Tyr435, Phe166, Leu164, Ile316, Gln206, Ile198, Arg100, Thr478, Glu483, Pro102, Phe103, Thr202 and some water molecules, wherein they contribute to ligand binding. The binding energies of bounded ligand, SAG and 7 flavanones are given in Table 3.7 and are shown in Fig.3.12. All the interacting residues lie in the range of 1 Å. All the studied compounds have affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and the related free radical generation and oxidative stress can be minimized. Among the studied flavanones, hesperetin has the highest binding capacity. Hesperidin forms a hydrogen bond with Arg100.

Table 3.7
Binding energies (kcal/mol) of flavanones with MAO-B

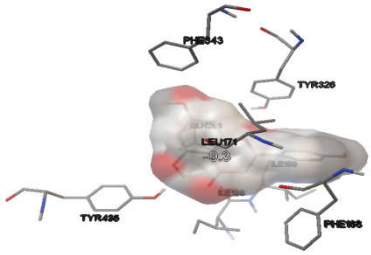
Flavanone	**Binding energy**
2'-Hydroxyflavanone	-9.5
4'-Hydroxyflavanone	-9.3
Eriodictyol	-9.9
Hesperetin	-10.2
Hesperidin	-10.1
Naringenin	-9.3
6-Hydroxyflavanone	-9.3
SAG	-9.4



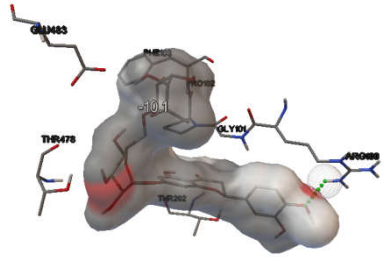
2'-Hydroxyflavanone



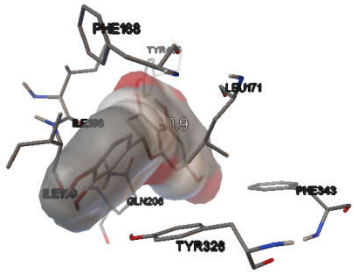
4'-Hydroxyflavanone



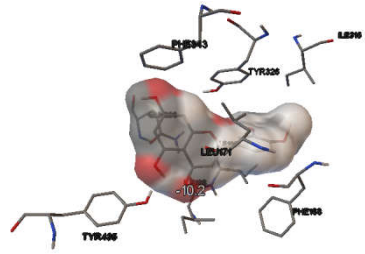
Naringenin



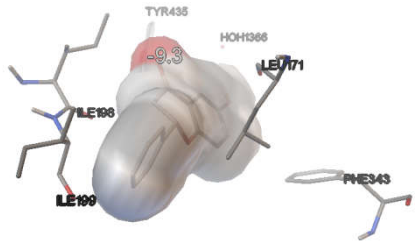
Hesperidin



Eriodictyol



Hesperetin



6-Hydroxyflavanone

Fig.3.12 Flavanones in the active site of MAO-B

3.7 Pharmacokinetic properties of flavanones

The pharmacokinetic properties of studied flavanones have been carried out through Molinspiration online software, and the Lipinski rule of 5 (RO5) has been validated. According to the rule, an orally admissible drug-like molecule must have:

1. HBD < 5
2. HBA < 10
3. MW < 500 Dalton
4. LogP < 5
5. ROTB < 10 (added by Veber)

Even though this rule does not predict whether a compound is pharmacologically active, it would help to get a deep knowledge about the pharmacokinetics including Absorption, Distribution, Metabolism, and Excretion (ADME) of the molecule under investigation. This makes researchers or pharmaceutics to screen out the best orally admissible drug like candidates with good ADME characteristics. For medicinal chemistry students or those who are involved in drug designing or the related studies, this RO5 become an excellent tool to screen out the investigated molecules [11]. All the studied flavanones except hesperidin have a molecular weight less than 500 Dalton, and all have LogP values less than 5. The pharmacokinetic properties offlavanones are given in Table 3.8. Here the numbers 1-7 are respectively 2'-Hydroxyflavanone, 4'-Hydroxyflavanone, 6-Hydroxyflavanone, Eriodictyol, Naringenin, Hesperetin, and Hesperidin.

Table 3.8

Pharmacokinetic properties of flavanones

No.	1	2	3	4	5	6	7
miLogP	3.12	2.70	2.68	1.63	2.12	1.94	-0.55
TPSA	46.53	46.53	46.53	107.22	86.99	96.22	234.3
nAtoms	18	18	18	21	20	22	43
MW	240	240	240	288	272	302	610
nHBA	3	3	3	6	5	6	15
nHBD	1	1	1	4	3	3	8
nViolations	0	0	0	0	0	0	3
nROTB	1	1	1	1	1	2	7
Volume	214.23	214.23	214.23	238.28	230.26	255.81	511.79

Table 3.8 clearly shows that all the studied flavanones except hesperidin obey RO5. The violation of hesperidin is in the nHBA, nHBD,and MW, which is due to the glucose group in it.

The insight into the drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability,and blood-brain barrier penetration are retrieved from the values of polar surface area (TPSA). For a druggable molecule, the TPSA must be less than 140 A^2 [12]. Here the TPSA values are lower than this value for all the flavanones except hesperidin. The number of rotatable bonds (nROTB) decides the conformational flexibility of a molecule. This is quite important for analyzing the conformational changes that can be undergone by a molecule and ultimately it is essential in the binding of molecules to receptors or channels. Oral viability criteria have set the number of rotatable bonds to be less than or equal to 10 [13–15]. All the studied flavanones have nROTB less than 10.

All flavanones studied, were analyzed under four criteria of known successful drug activity; ion channel modulation, GPCR ligand, kinase inhibition, and nuclear receptor ligand activity. Also, their activity as protease and enzyme inhibitors is studied. A positive value of bioactivity score indicates considerable biological activity whereas a score between −0.5 and 0.00 indicates moderate activity and less than −0.5 is considered inactive. Table 3.9 clearly shows that all the flavanones have considerable activity as an enzyme inhibitor (Here also the numbers 1-7 denotes the same flavanones as in Table 3.8).

Table 3.9

Bioactivity scores of the molecules against drug targets

No.	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-0.05	-0.35	-0.52	0.17	-0.28	0.15
2	-0.01	-0.32	-0.47	0.24	-0.26	0.15
3	-0.02	-0.33	-0.49	0.25	-0.28	0.14
4	0.07	-0.20	-0.22	0.46	-0.09	0.21
5	0.03	-0.20	-0.26	0.42	-0.12	0.21
6	0.04	-0.26	-0.20	0.38	-0.13	0.16
7	-0.01	-0.59	-0.36	-0.20	0.09	0.06

Eriodictyol, Naringenin, and Hesperetin have considerable activity towards GPCR ligand while all others have moderate activity. In all the cases except as an enzyme inhibitor, the monohydroxy flavanones have moderate activity. Only hesperidin shows considerable activity as a protease inhibitor while all others have moderate activity. All the flavanones have moderate activity towards the targets Ion channel modulator and Kinase inhibitor. Similarly all

but Hesperidin has considerable activity against nuclear receptor ligand.

3.8 Toxicological studies of flavanones

For an orally admissible drug, the drug-likeness qualitatively assesses the probability of a molecule to become an oral drug concerning the bioavailability. Drug-likeness has been implemented from the physicochemical inspections of development compounds advanced enough to consider as oral drug candidates. This has been employed to omit molecules with the incompatible pharmacokinetic profile [11]. The toxicological studies like mutagenicity, tumorigenicity, etc., are carried out through Datawarrior (new version of OSIRIS) property explorer. The results are given in Table 3.10 (numbering is same as that of Table 3.8).

Table 3.10

Parameters from Datawarrior

Flavanone	1	2	3	4	5	6	7
cLogP	2.85	2.85	2.85	1.81	2.16	2.09	-0.8
cLogS	-3.23	-3.23	-3.23	-2.34	-2.64	-2.66	-2.75
DL	-0.22	-0.22	-0.22	-0.22	-0.22	-0.08	2.04
Mutagenic							
Tumorigenic							
Irritant							
DS	0.62	0.62	0.62	0.66	0.65	0.66	0.54

Table 3.10 clearly shows that all the studied flavanones are nonmutagenic, nontumorigenic and nonirritant (green colour indicates zero risks for toxicity). Solubility is vital in the evaluation of drug

absorption and distribution characteristics. Low solubility implies low absorption. For most of the commercially available drugs, the solubility is found to be higher than -4.00 [15]. All the flavanones have solubilities in the range -2.3 to -3.3, indicates good solubility. The positive values for drug score indicate that all of them can act as a potential drug. Thus flavanones can be used as potential antioxidants and UV filters without any side effects to the biological system.

Conclusion

A computational investigation of UV filtering and radical scavenging properties of 7 flavanones has been performed. Among the studied flavanones, 2'-hydroxyflavanone has the highest and 6-hydroxyflavanone has the lowest band gap values. All the studied flavanones have relatively high IE and low EA values, so that are less efficient electron donors than Na and less efficient electron acceptors than F. Hesperidin acts as an antireductantand is attributed to the highest EA and Qmax values which favor the charge transfer from free radical to hesperidin. The remaining flavanones act as good antioxidants as they have low IE values. The major transitions involved are HOMO-LUMO and HOMO-1-LUMO. All of them have absorption in the range of 260-345 nm which lies in the UV-A and UV-B region and consequently they can be employed as effective UV filters. By HAT mechanism, hesperidin is the most powerful antioxidant among the studied flavanones. Flavanones which contain a 3'-OH group, have their lowest BDE value at this position followed by position 4'. The higher BDE value at position five is attributed to the presence of hydrogen bonding. The selection of two mechanisms HAT or SET can be identified by evaluating ΔBDE and ΔAIP values.

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CHAPTER 4

EVALUATION ON THE ANTIOXIDANT, UV FILTERING AND TOXICOLOGICAL PROPERTIES OF FLAVANOLS

- *The evaluation of the antioxidant, UV filtering and toxicological properties of 7 flavanols in green tea.*
- *The radical scavenging properties are evaluated through various mechanisms and molecular docking studies.*
- *The UV filtering capacity, donor-acceptor, toxicological and pharmacokinetic properties.*

4.1 Introduction

Modern life habits drive people to depend on ready to eat food items, resulting in obesity. The increased fat content in the body leads the cellular activities to an abnormal level and causes lots of disorders. Consequently, people have to do daily workouts more seriously. Besides this, proper diet should also be maintained. Scientifically, Green tea is one of the most welcome nutritional drinks which must be included in the diet of peoples who wish to burn out their excess fat. As a result, the general consumption of green tea by general population especially patients population increases to a great extends. In olden days, the Chinese and Japanese peoples used to drink green tea as a beverage [1–5].

Tea is a non-alcoholic drink that is consumed by people in worldwide. Different varieties of tea available like green tea, lime tea, Tulsi tea, etc., of which the benefits of green tea are surprisingly high, and it comprises about 20% of total tea production. The catechins present in the green tea partakes this complement and are potential bioactive molecules with particular relevance in the pharmaceutical industry. Green tea is made from the leaves and buds of the plant *Camellia sinensis*. Green tea consumption reduces cholesterol and obesity and protects the cardiovascular system. Catechins are potentantioxidants, and this makes them pharmaceutically crucial from the historic times [6–8].

Catechins are flavanols with multiple bioactivities like antioxidant [7], anticancer [9], antifungal [10], antibacterial [10], etc,.

Catechins have very high antioxidant activity against peroxy radicals. Because of the enormous benefits of catechins, their uses in clinical trials are being developed. Catechins have been clinically tested for the control of plaque in the oral cavity and found that catechin gel is superior to the solution [8]. Various studies are reported for the antioxidant activities of catechins which include both experimental and computational.

Chemically catechins are flavanoids with the 3-hydroxy group and sp^3 carbon at position-3 in the flavanoid backbone (Fig. 4.1). Like flavanones, here also the presence of sp^3 carbon atom at position-3 in the ring [C] reduces the conjugation resulting in the decrease in the absorption wavelength in comparison to flavonols.

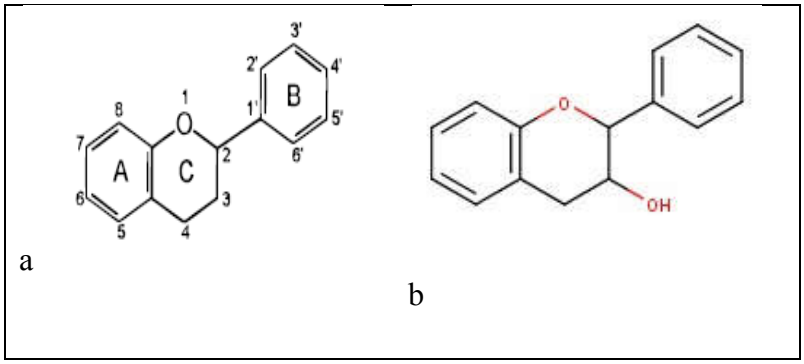

Fig.4.1. Basic structure of (a) flavonoids and (b) flavanol

The work presented here explain the antioxidant activities of 7 catechins including Catechin (C), Catechin gallate (CG), Epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin (EGC), Epigallocatechin gallate (EGCG) and Gallocatechin (GC), computationally with the suitable antioxidant mechanism. Along with

this, the UV filtering capacity and molecular docking studies have also been performed. Most of the reports on food-related polyphenols like catechins are about their bioactivities. However, only very few are reported for their toxicity studies. In this scenario, we have conducted a toxicological investigation on the seven catechins using some online software like OSIRIS and Molinspiration. The results are well explained in the following sections.

4.2 Optimization of structures of catechins

The structures of 7 Catechins have optimized with DFT-B3LYP level of theory and 6-31+G (d, p) as basis set and are shown in Fig.4.2. All the optimized structures are those which correspond to the global minimum and are confirmed by the absence of imaginary frequency. The energy gaps of catechins are given in Table 4.1, and the values show that all the studied catechins are stable.

Table 4.1

Energy gap in catechins

No	Compound	ΔE (eV)	Dipole moment (Debye)
1	(-) Epicatechin (EC)	5.64	3.50
2	Catechin (C)	5.46	4.03
3	Catechin Gallate (CG)	4.90	4.02
4	Epigallocatechin (EGC)	5.56	4.42
5	Epigallocatechin Gallate (EGCG)	4.54	4.68
6	Epicatechin Gallate(ECG)	4.88	3.92
7	Gallocatechin (GC)	5.79	4.01

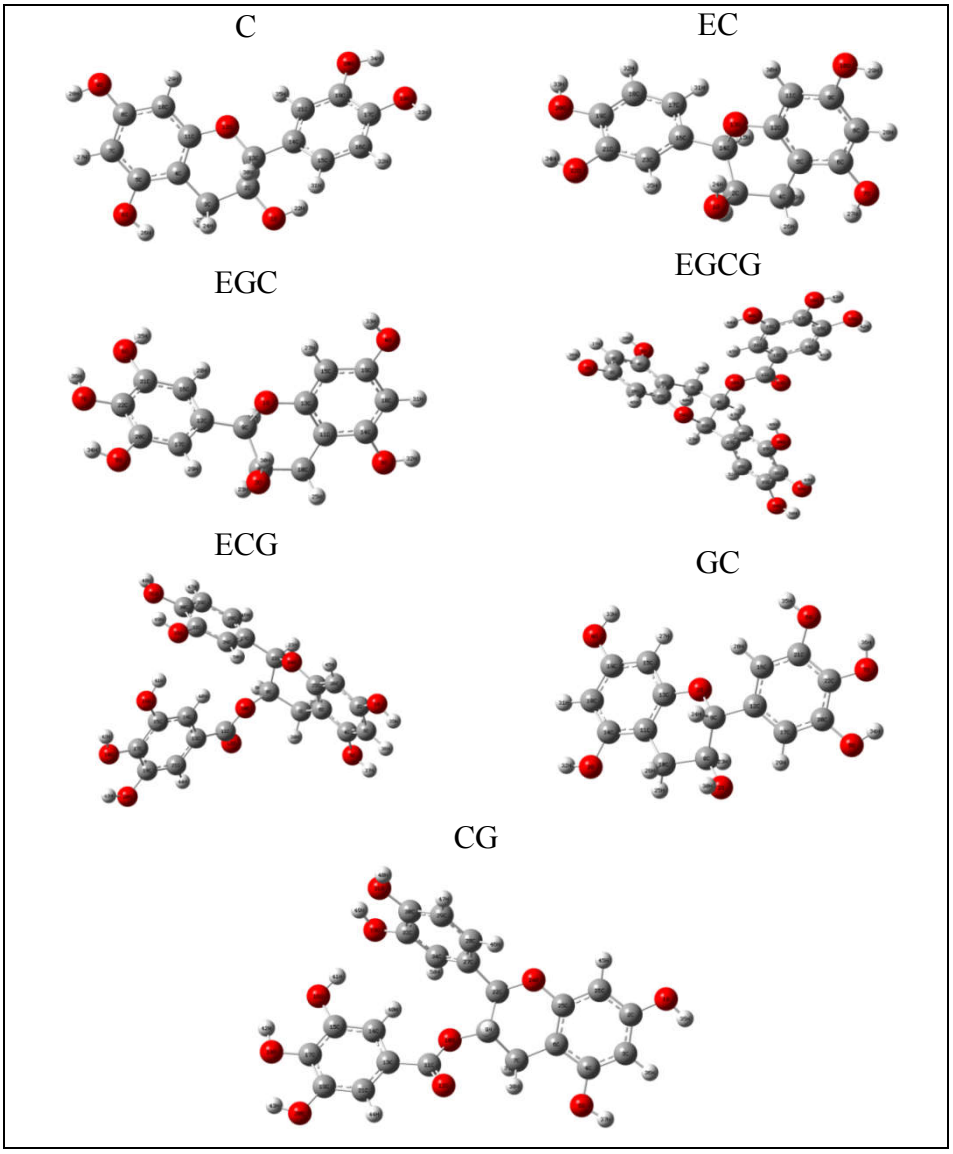

Fig.4.2 Optimized structures of catechins

For C, the HOMO has delocalized over the ring [A] and partially over ring [C] in the flavanol moiety (Figs.4.3&4.4). In EC, the HOMO is wholly delocalized. In EGC, the delocalization is complete over the ring [B] and partially over ring [C]. However, the

HOMO of EGCG is delocalized over the gallate unit. In ECG, the delocalization is over half of the flavanol moiety and on the gallate unit. The HOMO of GC is delocalized over the gallate unit and half of the ring [C]. In CG, the HOMO is delocalized over half of flavanol and gallate moiety. The energy gap (given in Table 4.1) is highest for GC and lowest for EGCG. So, one can expect the same order in their reactivity too.

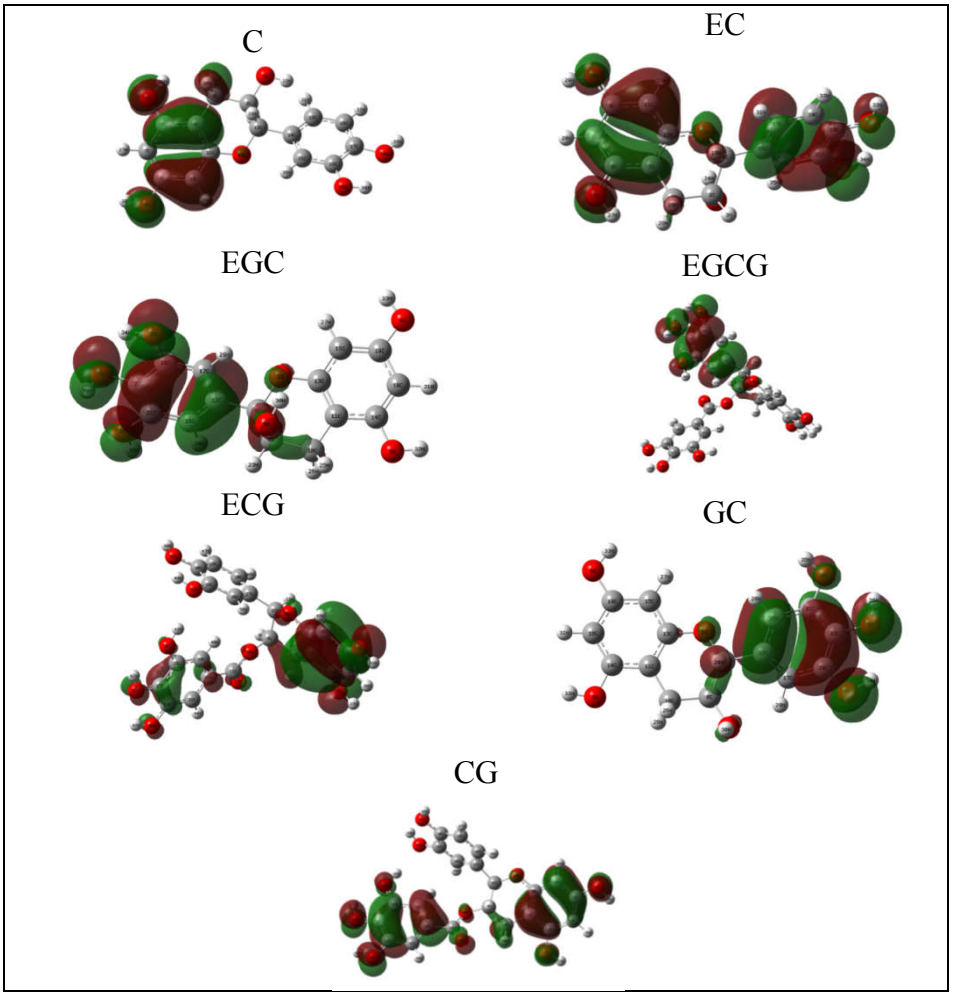

Fig.4.3 HOMO of catechins

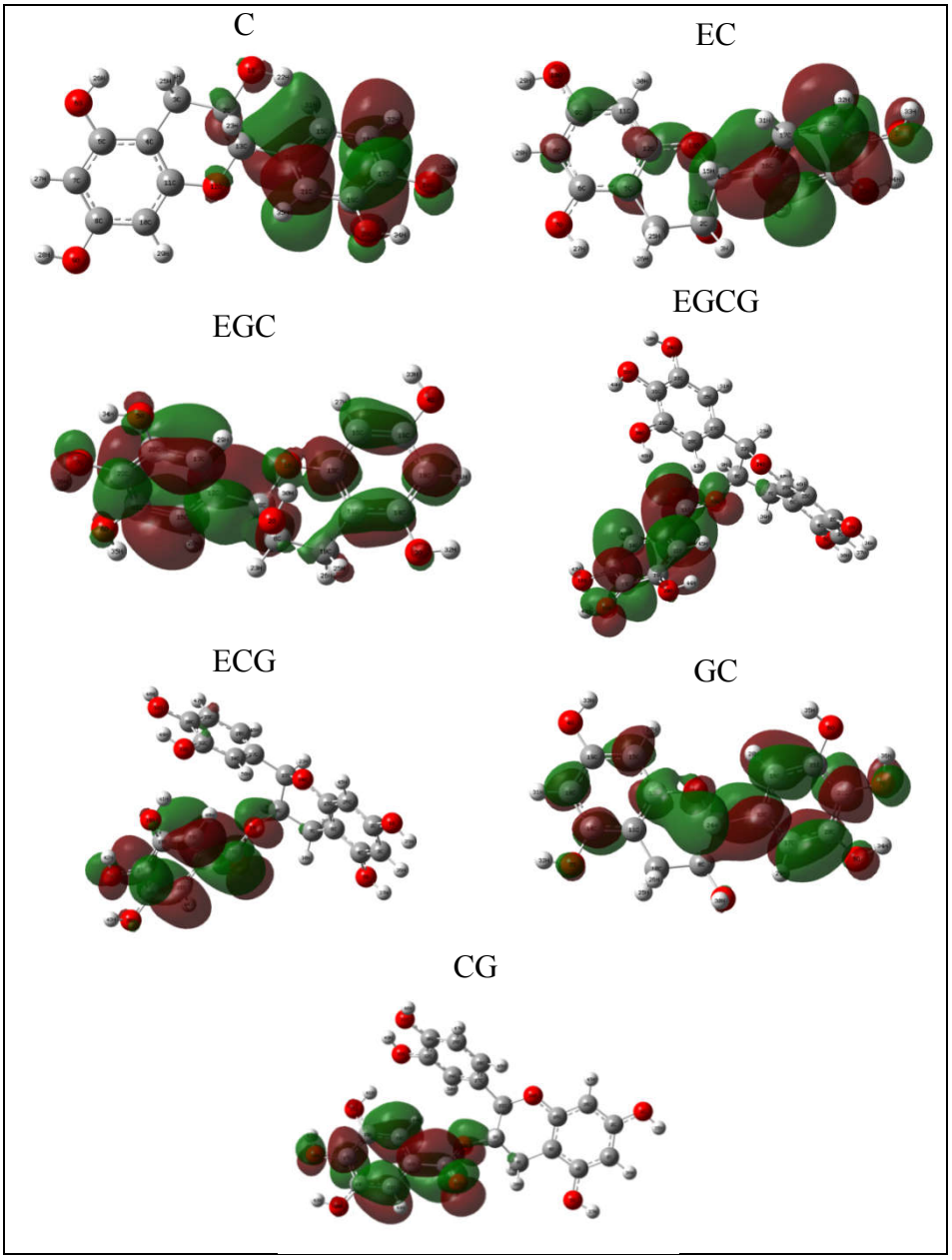

Fig.4.4 LUMO of catechins

The knowledge of charge distribution gives information on how a molecule interacts with one another and is very important in organic chemistry. The sum of energies of molecular particles on interaction with the electric components along a particular pathway gives the total electrostatic potential (ESP) energy along that particular pathway. It varies with positions on the surfaces of components. However, one has to be patient enough to get the full information about the ESP.

Nevertheless, if we plot the graph showing ESP, the analysis will be more accessible. The variably charged regions of a molecule have been visualized from these maps and the knowledge of the charge distributions can in turn used to determine how the molecules interact with each other. They are also enabled to visualize the charge distributions of molecules and charge related properties of molecules. Electrostatic potential energy is fundamentally a measure of the strength of the nearby charges, nuclei, and electrons, at a particular position [11]. The ESP maps of studied flavanols are shown in Fig.4.5.

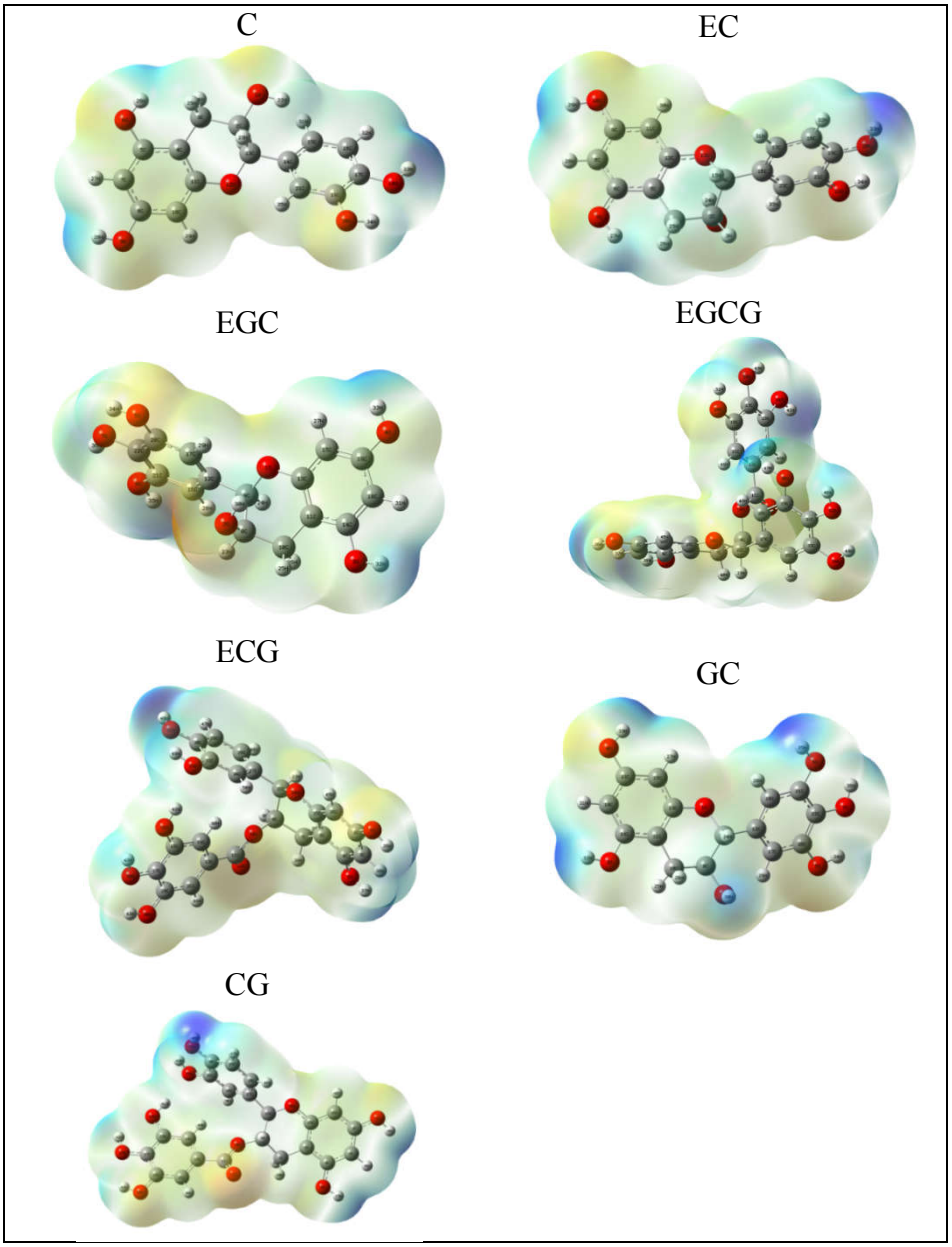

Fig.4.5 ESP map of catechins

The red region denotes the areas of lower potential and hence higher electron density while the blue region is the area with high

electrostatic potential and with a low concentration of electrons. The atoms present in our compounds are C, H, and O. The red region locates on the most electronegative oxygen atom. The ESP map is also useful for determining the nature of chemical bonds between two atoms based on their electronegativity differences. The colors other than red and blue represents the electronegativity difference. These have seen between red and blue colored region. When this region with colors green-yellow is increased, the charge is very polarized, and there is a significant difference in the electronegativity values. In such cases, a complete red/blue region has seen.

4.3 Global reactive descriptors of catechins

The global reactive descriptors enable one to understand the mode of charge transfer in a molecule and are often employed to compare the reactivities of a set of molecules. The global reactive descriptors of the seven studied catechins are given in Table.4.2

Table 4.2

Global reactive descriptors (eV) of Flavanols

No	Compound	IE	EA	η	S	χ	μ	ω	Q_{max}	ω-	ω+
1	EC	7.04	0.25	3.39	0.15	3.65	-3.65	1.96	1.07	4.20	0.56
2	C	7.03	-0.45	3.74	0.13	3.29	-3.29	1.45	0.88	3.56	0.27
3	CG	6.86	-0.44	3.65	0.14	3.21	-3.21	1.41	0.88	3.47	0.26
4	EGC	7.00	-0.26	3.63	0.14	3.37	-3.37	1.56	0.93	3.71	0.33
5	EGCG	6.77	-0.15	3.46	0.14	3.31	-3.31	1.58	0.96	3.67	0.36
6	ECG	6.82	-0.47	3.64	0.14	3.17	-3.17	1.38	0.87	3.42	0.25
7	GC	6.85	0.06	3.39	0.15	3.46	-3.46	1.76	1.02	3.91	0.46

EGCG have the lowest and EC have the highest IE values. So the efficient electron donor among the studied flavanols is EGCG. Similarly, EC, having the highest EA values, will be the efficient electron acceptor. All of them have moderate hardness values and are thus relatively stable and are less reactive. This is further supported by the low softness values. The charge holding capacity indicated by the Qmax values is found to be high for EC and GC indicates that they are dominant electron acceptors and they may accept electrons rather than donating them in charge transfer reactions with metal ions or free radicals. This is clear from the IE *vs.* EA plot shown in Fig.4.6.

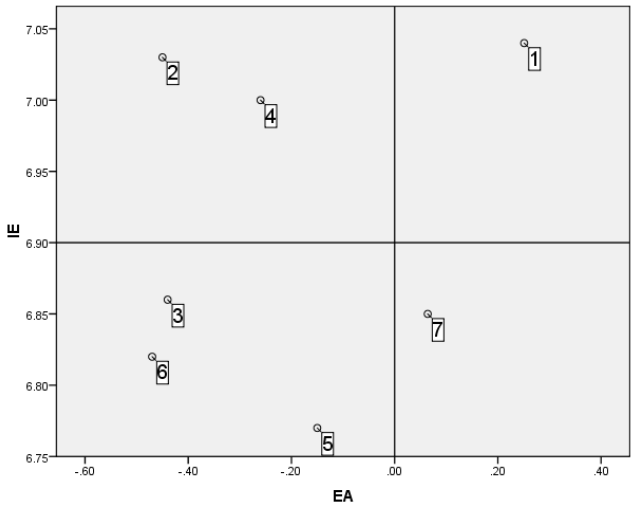

Fig.4.6 IE *vs.* EA plot of catechins

From Fig. 4.6 it is clear that the flavanols 2 (C) and 4 (EGC) show high IE and low EA values which indicates that they are not suitable to donate or accept electrons in comparison with others. The flavanols, 3 (CG), 5 (EGCG), and 6 (ECG) have low IE and EA values that show that they are capable of donating electrons. The falvanol1can

accept electrons only as it has high EA and IE values but flavanol 7 (GC) is capable of both accepting and donating electrons in comparison to others.

In some cases, there will be only a fractional charge transfer taking place, and in such conditions, we cannot evaluate the donor-acceptor properties by their IE and EA values. In such situations, we adopt the DAM (Fig.4.7) [12–14].

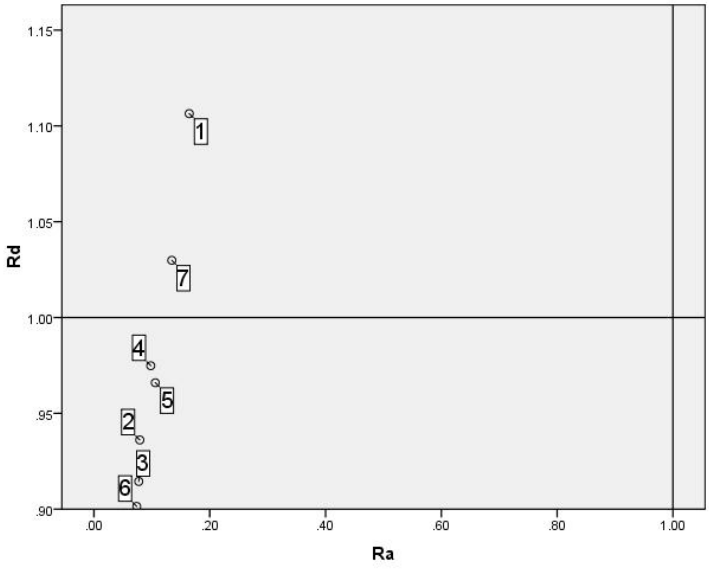

Fig.4.7 DAM of catechins

Fig.4.7 shows the donor-acceptor map (DAM) of studied catechins. Here the catechins 1 (EC) and 7 (GC) falls in the worst antiradical quadrant while all others fall in the good antioxidants region as they have low Rd values. Thus in comparison to Na, the catechins, 2 (C), 3 (CG), 4 (EGC), 5 (EGCG), and 6 (ECG) are more

efficient electron donors while catechins 1 (EC) and 7 (GC) are less efficient.

4.4 UV filtering capacity of catechins

The UV filtering ability of flavanols is attributed to their absorbance in the range of UV-A (315-400 nm) and UV-B (280-315 nm) radiations. All the studied flavanols can filter UV-B radiations completely as their absorbance lies in the range of 230-320 nm. For C, the λ_{max} is due to the transition between HOMO-1 and LUMO levels in both gaseous and aqueous phases. For all others, the transitions bringing λ_{max} are different in both the media. This is due to the difference in interactions of catechins with aqueous media. The necessary parameters regarding the UV spectra are given in Table4.3a & 4.3b. For most of the catechins, the important transitions are between HOMO, HOMO-1 and LUMO levels. Since all of them have an absorbance in the UV-B region, they can be used as effective UV filters in sunscreen lotions. The UV spectra of all the catechins are shown in Fig.4.8. As medium changes from gaseous to aqueous, the introduction of gallate groups increases the λ_{max} values for CG, ECG and EGCG, while for others like C, EC and EGC, the changes in λ_{max} are low and if so, they are decreased in aqueous media. In same media, also the gallate group increases the λ_{max} values, as C has a λ_{max} value of 256 nm while it is at 272 nm for CG. This is same for EC-ECG and EGC-EGCG pairs also.

Table 4.3a

Parameters of UV-Visible spectroscopy in the gas phase

Flavanols	E (eV)	λ_{max} (nm)	OS	MO involved
C	4.8	258	0.02	HOMO-2-LUMO+1
	4.84	**256**	**0.03**	**HOMO-1-LUMO**
	4.85	255	0.01	HOMO-LUMO
CG	4.34	286	0.02	HOMO-3-LUMO
	4.37	284	0.02	HOMO-LUMO
	4.55	**272**	**0.06**	**HOMO-2-LUMO**
EC	4.79	259	0.01	HOMO-1-LUMO+1
	4.84	**256**	**0.09**	**HOMO-1-LUMO**
	4.91	252	0.02	HOMO-LUMO
ECG	4.29	289	0.01	HOMO-LUMO
	4.34	**285**	**0.02**	**HOMO-3-LUMO**
	4.46	278	0.01	HOMO-2-LUMO
EGC	4.67	266	0.01	HOMO-LUMO
	4.86	255	0.01	HOMO-3-LUMO
	4.91	**252**	**0.03**	**HOMO-3-LUMO+1**
EGCG	**4.03**	**308**	**0.02**	**HOMO-LUMO**
	4.17	298	0.01	HOMO-1-LUMO
	4.26	291	0.01	HOMO-2-LUMO
GC	5.03	246	0.01	HOMO-LUMO+3
	5.07	244	0.03	HOMO-1-LUMO
	5.42	**229**	**0.04**	**HOMO-LUMO**

Table 4.3b

Parameters of UV-Visible spectroscopy in the aqueous phase

Flavanols	E (eV)	λ_{max} (nm)	OS	MO involved
C	**4.87**	**254**	**0.08**	**HOMO-1-LUMO**
	4.95	250	0.02	HOMO-LUMO+1
	5.12	242	0.01	HOMO-LUMO
CG	4.15	299	0.01	HOMO-LUMO
	4.18	297	0.01	HOMO-1-LUMO
	4.29	**289**	**0.03**	**HOMO-4-LUMO**
EC	**4.85**	**256**	**0.12**	**HOMO-LUMO**
	4.94	251	0.01	HOMO-1-LUMO
	5.1	243	0.02	HOMO-1-LUMO
ECG	4.07	304	0.01	HOMO-LUMO
	4.15	299	0.01	HOMO-1-LUMO
	4.25	**292**	**0.03**	**HOMO-2-LUMO**
EGC	4.92	252	0.01	HOMO-3-LUMO
	4.96	**250**	**0.01**	**HOMO-LUMO+2**
	4.96	250	0.01	HOMO-1-LUMO+1
EGCG	3.9	318	0.01	HOMO-LUMO
	3.97	313	0.01	HOMO-1-LUMO
	4.12	**301**	**0.03**	**HOMO-3-LUMO**
GC	5.03	246	0.02	HOMO-3-LUMO
	5.08	244	0.01	HOMO-1-LUMO+1
	5.36	**231**	**0.05**	**HOMO-1-LUMO**

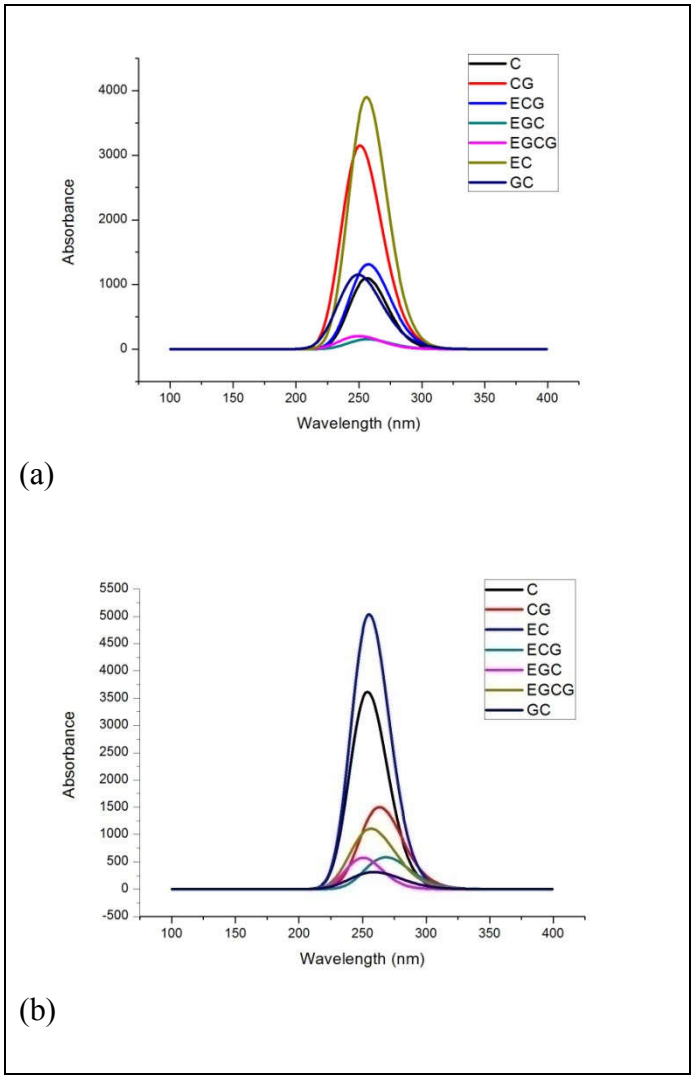

Fig.4.8 UV-Visible spectrum of flavanols in (a) gaseous and (b) aqueous phase

4.5 The antiradical capacity of catechins

To understand the mode of action of the studied antiradicals towards free radicals, three antiradical mechanisms are evaluated. The

necessary parameters for the three mechanisms are given in Table 4.4. A mechanism is feasible when parameters associated with it are low.

Table 4.4 reveals that the PA values are the lowest for all the catechins. However, the SPLET mechanism is not suitable for these catechins as the ETE values for the second step are very high. The SETPT mechanism is also unfavorable as the PDE values are high. So the possible mechanisms are the SET and HAT. According to the energy concern, HAT is preferable than the SET mechanism. For HAT mechanism, the BDE values decrease with an increase in antioxidant capacity. So the introduction of gallate units increases the BDE values. This can be seen for CG, ECG, and EGCG as compared to those without gallate (C, EC and EGC). However, the literature reports (experimental) show that the catechins with gallate groups are more effective antiradicals even though the BDE values are high.

Table 4.4

Parameters of antioxidant mechanisms in kcal/mol

No.	Flavanols	BDE	AIP	PDE	PA	ETE
1	EC	73.1	162.0	224.9	-8.1	395.0
2	C	73.3	161.6	225.4	8.5	378.5
3	CG	73.9	157.7	230.0	8.5	379.2
4	EGC	73.3	160.9	226.1	-8.5	395.6
5	EGCG	73.5	155.5	231.8	2.4	384.8
6	ECG	73.8	156.7	230.9	9.1	378.5
7	GC	72.2	157.4	228.5	-3.0	389.0
8	G	77.0	188.0	204.0	-4.0	396.0

The reason can be attributed to the competition between SET and HAT mechanisms. Considering the SET mechanism, the catechins with gallate groups are having lower AIP values than the corresponding catechins without gallate group. So SET and HAT are equally possible for explaining the radical scavenging activity of catechins. For comparison, the Gallic acid (G) is also considered. Looking the values of BDE of C, G, CG, and GC, it has been found that, the introduction of catechin group to G reduces the BDE while the introduction of gallate group to catechins increases the BDE value.

The lowest BDE value has been observed for GC, which is attributed to the presence of the highest number of –OH groups in ring [B]. In all the catechins the number of –OH groups in ring [A] are same but differ in ring [B] resulting in the variation of BDE values. In CG, ECG, and EGCG, the 3-OH group has been replaced by gallate units, and it increases the BDE values. The exceptional lower BDE value of GC even though it contains the gallate unit is due to the presence of the 3-OH group. So the presence of the 3-OH group is an essential structural feature in determining the antioxidant behavior of catechins through HAT mechanism. Moreover the BDE values of G (77 kcal/mol) [15] decreases on complexation with flavanols.

Table 4.5

BDE values (kcal/mol) at different sites

Flavanols	Position	BDE	Flavanols	Position	BDE
EC	3'	80.14	EGCG	3'	75.21
	4'	**73.10**		**4'**	**73.50**
	5	82.82		5	82.42
	7	83.35		5'	79.87
	3	99.92		7	81.13
C	5	83.14		m	81.48
	3'	79.81		p	75.23
	7	83.61		m	76.54
	4'	**73.30**	GC	**4'**	**72.20**
	3	100.8		3'	73.49
CG	**4'**	**73.90**		3	97.36
	3'	80.29		5	82.60
	7	81.30		5'	80.38
	5	80.49		7	81.93
	m	78.55	ECG	**4'**	**73.80**
	m	83.55		5	80.92
	p	74.79		3'	80.21
EGC	5	82.28		7	81.17
	3'	74.13		m	78.15
	4'	**73.30**		p	74.74
	5'	81.91		m	83.38
	3	99.65			
	7	83.19			

Table 4.5 describes the BDE values at different sites in all the seven catechins. The values shown in red color are those in the *meta*- and *para*- positions of gallate unites in position-3 of flavanol skeleton and the values given in bold are the lowest BDE values. It has been observed that the lowest BDE value is at position 4'. Generally, the BDE values at different positions in the ring [B] of flavanol are lower than the others indicating high reactivity of ring [B]. The highest BDE values have been observed at position-3 and are due to the lower

acidity of sp^3-carbon. For the gallate units, the lowest BDE values have been seen at the *para-* position.

The competition between SET and HAT can be explained as follows. The AIP *vs.*BDE plot shows that GC has low AIP and BDE values. So both HAT and SET (from catechin to free radicals). For CG, ECG, and EGCG the AIP values are low, but BDE values are high, so only SET (from catechin to free radicals) mechanism is favorable for them.For EC, C and EGC both HAT and SET (from catechin to free radicals) mechanisms are unfavorable (Fig.4.9).

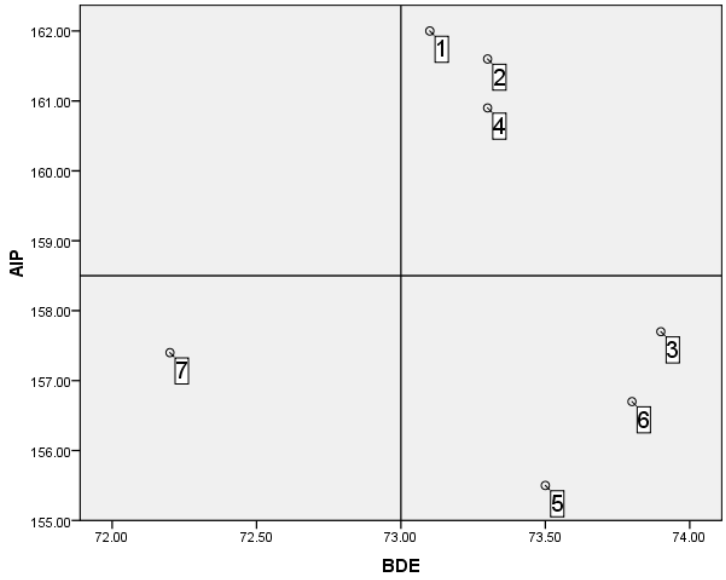

Fig.4.9 BDE *vs.* AIP plot

Competition between SET (from free radicals to catechin) and HAT is explained with the help of Fig.4.9. Catechin 7 undergoes both HAT and SET reactions as it has high EA and low BDE values in

comparison to other catechins. Catechin 1 can undergo only SET as it has high EA and BDE value (Fig.4.10).

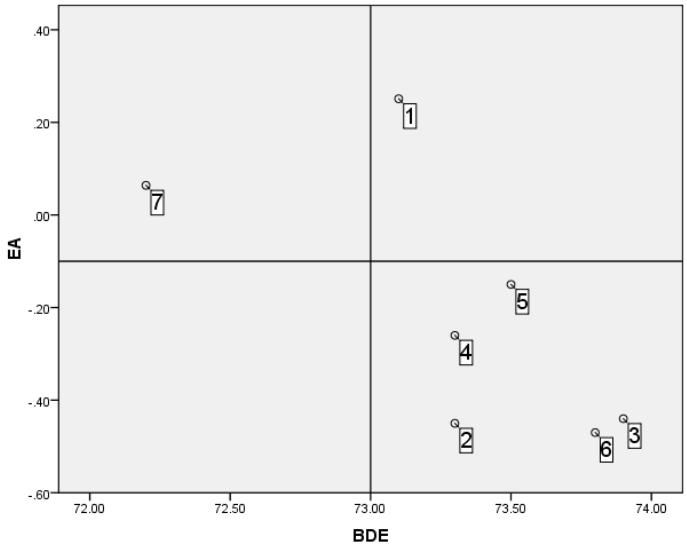

Fig.4.10 BDE *vs.* EA plot

4.6 Molecular docking analysis of catechins

For a better understanding of the antioxidant behavior of catechins, molecular docking studies have been implemented. The molecular docking studies have been performed through AutoDock Vina software, and the binding energy of all possible conformers of catechins with the active site of the protein target MAO-B has been computed. Specific amino acids surround the active site of MAO-B are Asp123, Arg127, Ile477, Thr479, Trp119, His115, and Phe103, wherein they contribute to ligand binding. Amino acids like Thr202, Glu84, Thr478, Ser200, Arg100, and Pro476 also appear in the docking site when doing molecular docking. The binding energies of

bounded ligand SAG and different conformers of 7 catechins are given in Table 4.6, and the conformers with highest binding energy values are shown in Fig.4.11. In Table 4.6 the numbers 1-9 denotes different conformers of catechins. All the interacting residues lie in the range of 1 Å. All the studied compounds have an affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and the related free radical generation, and oxidative stress can be minimized. Among the studied flavanols, C has the highest binding capacity while EC has the lowest. In the case of CG, the binding energy is decreased than that of C. However, for ECG and EGCG, the binding capacity increases than that of EC and EGC respectively.

Table 4.6

Binding energies (kcal/mol) of catechins and SAG

Conformer	**Binding energy**						
	C	**CG**	**EC**	**ECG**	**EGC**	**EGCG**	**GC**
1	**-10.0**	**-8.3**	**-7.7**	**-9.2**	**-7.9**	**-8.9**	**-8.0**
2	-10.0	-8.1	-7.5	-9.1	-7.9	-8.5	-7.9
3	-7.3	-8.1	-7.5	-9.0	-7.4	-8.4	-7.8
4	-7.3	-8.0	-7.5	-8.4	-7.0	-8.1	-7.6
5	-7.2	-7.9	-6.9	-8.3	-7.0	-8.0	-7.5
6	-7.1	-7.9	-6.8	-7.7	-7.0	-8.0	-7.5
7		-7.7	-6.7	-7.6	-6.8	-7.8	-7.2
8		-7.3	-6.7	-7.1	-6.7	-7.1	-6.9
9		-6.8	-6.6	-6.9	-6.6	-7.0	-6.9
SAG	**-9.4**						

Here all the catechins except C form 1 or 2 hydrogen bond with the amino acid residues of MAO-B. CG forms a hydrogen bond with Thr202 while EC forms three hydrogen bonds with Glu84, Thr202, and Thr478. ECG forms hydrogen bonds with Thr478 and Glu84. EGC

forms hydrogen bonds with Glu84 and Ser200 while EGCG forms a hydrogen bond with Pro476. GC forms a hydrogen bond with Glu84 and Arg100.

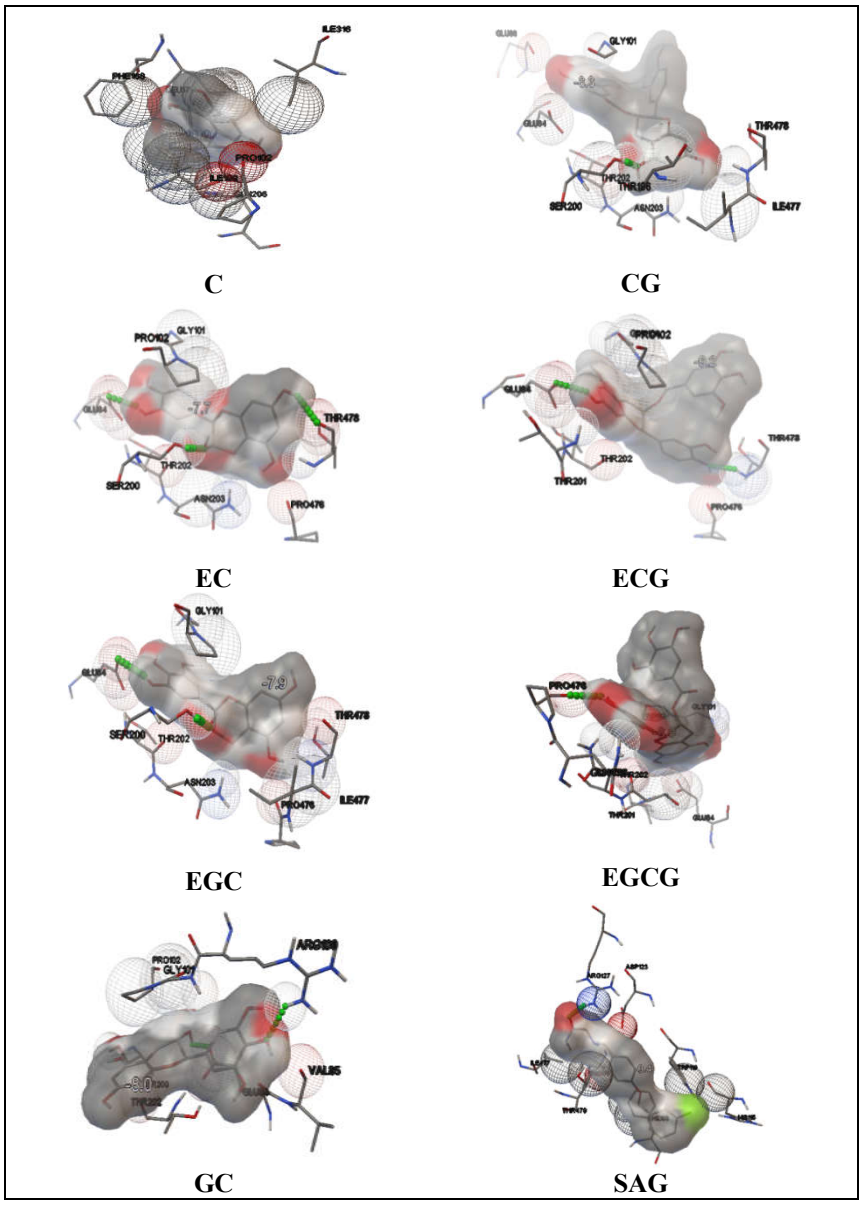

Fig.4.11 Catechins and SAG in the active site of MAO-B

4.7 Pharmacokinetic properties of flavanols

The pharmacokinetic properties of studied flavanols have been carried out through Molinspiration online software, and the Lipinski rule of 5 (RO5) has been validated. The rule states that an orally admissible drug-like molecule must have:

1. HBD < 5
2. HBA < 10
3. MW < 500 Dalton
4. LogP < 5
5. ROTB < 10 (added by Veber)

Even though this rule does not predict whether a compound is pharmacologically active, it would help to get a deep knowledge about the pharmacokinetics including Absorption, Distribution, Metabolism, and Excretion (ADME) of the molecule under investigation. This makes researchers or pharmaceutics to screen out the best orally admissible drug like candidates with good ADME characteristics. For medicinal chemistry students or those who are involved in drug designing or the related studies, this RO5 become an excellent tool to screen out the investigated molecules [16]. All the studied catechins have a molecular weight less than 500 Dalton and LogP values less than 5. The pharmacokinetic properties of flavanols are given in Table 4.7.

Table 4.7

Pharmacokinetic properties of flavanols

Property	GC	C	CG	EC	ECG	EGC	EGCG
miLogP	1.08	1.37	2.54	1.37	2.54	1.08	2.25
TPSA	130.6	110.3	177.1	110.3	177.1	130.6	197.3
nAtoms	22	21	32	21	32	22	33
MW	306	290	442	290	442	306	458
nHBA	7	6	10	6	10	7	11
nHBD	6	5	7	5	7	6	8
nROTB	1	1	4	1	4	1	4
nViolations	1	0	1	0	1	1	2
Volume	252.2	244.1	359.5	244.1	359.5	252.2	367.5

Flavanols C and EC do not show any violation to Lipinski rule of 5. GC, CG, ECG, and EGC show one violation to the rule and are in the number of hydrogen bond donor (nHBD). However, this will get reduced as they form phenoxide radicals on reaction with free radicals. The compound EGCG shows violation both in HBD and hydrogen bond acceptors (nHBA). This is due to the presence of the gallate group in it. However, the violations are not so significant that all these can be taken orally, especially C and EC.

The polar surface area of flavanols is also given in Table 4.7. It gives an insight into the drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood-brain barrier penetration. For a druggable molecule, the TPSA must be less than 140 A^2 [17]. Here the TPSA values are higher than this value for CG, ECG, and EGCG due to the presence of gallate units. However, the TPSA values of C, EC, and GC are less than 140 A^2. The number

of rotatable bonds (nROTB) decides the conformational flexibility of a molecule. This is quite important for analyzing the conformational changes that can be undergone by a molecule and ultimately it is essential in the binding of molecules to receptors or channels. Oral viability criteria have set the number of rotatable bonds to be less than or equal to 10 [18–20]. All the studied flavanols have nROTB less than 10.

Table 4.8

Bioactivity scores of the molecules against drug targets.

Target	GC	C	CG	EC	ECG	EGC	EGCG
GPCR ligand	0.40	0.41	0.17	0.41	0.17	0.4	0.16
Ion channel modulator	0.14	0.14	0.02	0.14	0.02	0.14	0.02
Kinase inhibitor	0.14	0.09	0.05	0.09	0.05	0.14	0.06
Nuclear receptor ligand	0.57	0.60	0.34	0.60	0.34	0.57	0.33
Protease inhibitor	0.29	0.26	0.13	0.26	0.13	0.29	0.13
Enzyme inhibitor	0.49	0.47	0.25	0.47	0.25	0.49	0.25

All compounds, studied, were analyzed under four criteria of known successful drug activity; ion channel modulation, GPCR ligand, kinase inhibition, and nuclear receptor ligand activity. Also, their activity as protease and enzyme inhibitors is studied. A positive value of bioactivity score indicates considerable biological activity whereas a score between −0.5 and 0.0 indicates moderate activity and less than −0.5 is considered inactive. Table 4.8 clearly shows that all the flavanols have a positive drug score for all the specified targets. Here also the score decreases as we move from C, EC, and EGC to CG,

ECG, and EGCG respectively. However, all have significant bioactivity.

4.8 Toxicological analysis of catechins

For an orally admissible drug, the drug-likeness qualitatively assesses the probability of a molecule to become an oral drug concerning the bioavailability. Drug-likeness has been implemented from the physicochemical inspections of development compounds advanced enough to consider as oral drug candidates. This has been employed to omit molecules with the incompatible pharmacokinetic profile [16].

The octanol-water partition coefficient, logP has been used as the standard descriptor for lipophilicity. It has been included as a dedicated descriptor in explaining the pharmacokinetics of drug-like molecules because of its importance in the pharmacokinetics of drug discovery. The primary requirement of a drug is the ease of handling and formulation. This was achieved through the solubility of the drug molecule. Moreover, for an orally admissible drug, the solubility plays a crucial role in its absorption. As well, a drug meant for parenteral usage has to be highly soluble in water to deliver a sufficient quantity of its active ingredient in the small volume of such pharmaceutical dosage [16].

Catechins are the critical ingredients of the widely accepted health drink green tea and are expected to have very low or zero toxicity. To confirm this, free online software called OSIRIS Property

Explorer (now available as DataWarrior) has been implemented. The results are given in Table 4.9.

Table 4.9

Parameters from Datawarrior

Property	C	CG	EC	ECG	EGC	EGCG	GC
Drug score	0.73	0.55	0.73	0.55	0.74	0.55	0.74
clogP	1.51	2.40	1.51	2.40	1.16	2.05	1.16
Mutagenic							
Tumorigenic							
Irritant							
Druglikeness	0.32	-0.33	0.32	-0.33	0.32	-0.33	0.32
Solubility	-1.76	-2.46	-1.76	-2.46	-1.47	-2.16	-1.47

Table4.9 clearly shows that all the studied catechins are nonmutagenic, nontumorigenic and nonirritant (green color indicates zero risks for toxicity). Solubility is vital in the evaluation of drug absorption and distribution characteristics. Low solubility implies low absorption. For most of the commercially available drugs, the solubility is found to be higher than -4.00 [20]. All the catechins have solubilities in the range -1.4 to -2.5, indicates good solubility. The positive values for drug score indicate that all of them can act as a potential drug. Thus catechins can be used as potential antioxidants and UV filters without any side effects to the biological system. This may be the reason behind the health benefits of green tea.

Conclusion

A computational evaluation of the antioxidant, UV filtering and toxicological analysis of green tea catechins has been performed. The energy gap is highest for GC and lowest for EGCG. The efficient electron donor among the studied flavanols is EGCG. All of them have

high hardness values and are thus relatively stable and are less reactive. This is further supported by the low softness values. High Qmax values for EC and GC, indicates that they are dominant electron acceptors. The falvanol 1(EC) can accept electrons only as it has high EA and IE values but flavanol 7 is capable of both accepting and donating electrons in comparison to others. All the studied flavanols can filter UV-B radiations completely as their absorbance lies in the range of 230-320 nm. For most of the catechins, the important transitions are between HOMO, HOMO-1 and LUMO levels. The lowest BDE value is seen at position 4' and ring [B] is more reactive in flavanols. The introduction of gallate units increases the BDE values. However, the catechins with gallate groups have lower AIP values than the corresponding catechins without gallate group. The competition between HAT and SET mechanism are clearly explained. All the studied compounds have an affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and can reduce the related free radical generation and oxidative stress. Flavanols C and EC do not show any violation to Lipinski rule of 5. GC, CG, ECG, and EGC show one violation to the rule and are in the number of hydrogen bond donor. All the flavanols have a positive drug score for all the specified targets. Here also the score decreases as we move from C, EC, and EGC to Cg, ECG and EGCG respectively. All the studied catechins are nonmutagenic, nontumorigenic and nonirritant. All the catechins have solubilities in the range -1.4 to -2.5, indicates good solubility. The TPSA and nROTB values of all the catechins are under the limit of druggable molecules. Thus catechins can be used as potential antioxidants and UV filters without any toxicity to the biological system. This may be the reason behind the health benefits of green tea.

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CHAPTER 5

EVALUATION ON THE STRUCTURE, RADICAL SCAVENGING, pH INDICATOR AND TOXICOLOGICAL PROPERTIES OF ANTHOCYANIDINS

- *Evaluation on the structure, radical scavenging, ph indicator and toxicological properties of 12 anthocyanidins.*
- *The DAM-FEDAM plots: Antireductuant properties.*
- *The toxicological parameters and the pharmacokinetic properties.*

5.1 Introduction

Today the use of synthetic colourants have been increased to a great extent and become a significant threat to human health because of their toxicity. In this scenario the development of some natural food, colourants are particularly relevant in the area of the food industry. Carotenoids and anthocyanidins are the widely used natural food colourants. Anthocyanidins are water soluble, less stable than carotenoids and are widely seen in grapes, berries, red cabbage, apples, radishes, tulips, roses, and orchids. Anthocyanidins are responsible for the bright colour of the fruits, flowers, and leaves of plants listed above [1].

Anthocyanidins are the major constituents of vascular plants and are harmless water-soluble natural pigments. This makes their wide acceptance as an excellent natural food colourant. The anthocyanidins are not only useful as food colourant but also used as antioxidant, anticancer agents, etc., therapeutics. They also found to have the capacity to chelate metal ions in biological systems. Due to this wide range of uses, there are some research articles available in the literature about the anthocyanidins. These are widely distributed class of flavonoids and are unique with their attractive colour. The acylglycosides of these are called anthocyanins. Pelargonidin (PEL), Cyanidin (CYA), Delphinidin (DEL), Peonidin (PEO) and Malvidin (MAL) are the most common anthocyanidins. Studies have shown that they have antioxidant [2–5], anticancer, and antidiabetic capacities [6–10]. However, the relationship between the structures of

anthocyanidins and the properties that they have shown is not entirely understood.

The basic structure of anthocyanidins consists of an aromatic ring [A] bonded to a heterocyclic ring [C] that contains oxygen which is also bonded by a C-C bond to a third aromatic ring [B] (Fig.5.1.) [11].

The critical feature of anthocyanidins is the presence of chromenylium ion. Due to this, the effective π-conjugation significantly enlarges in comparison to flan-3-ols. So HOMO-LUMO gap decreases than flavan-3-ols. This π-conjugation is responsible for its strong absorption around 500 nm. They are known to have the capacity to protect the DNA from the damage caused by UV-A and UV-B light. Partially they may also have the capacity to alter the interaction of plants with insects because of their optical properties. The variation in the chemical structure of anthocyanidins, alter the colour of plants/plant parts accordingly. Depending upon the –OH or -OCH_3 or glycosidic groups that they contain, they modulate UV-Visible absorption. This also depends on the pH, metal adsorption, long-range intermolecular interactions (co-pigmentation), etc. In optoelectronic devices, the optical absorption or emission must be well controlled, and since the theoretical predictions of UV-Visible spectra of anthocyanidins show good agreement with experimental results, they can be used as dyes in optoelectronic devices [12,13].

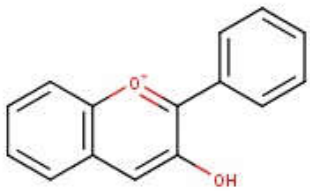


Fig. 5.1 Structure of anthocyanidin

There are a large variety of anthocyanidins differing in the number of –OH groups and glycosidic linkages. The isolated anthocyanidins are highly unstable because of their affinity towards pH, metal ions, temperature, concentration, light, etc., makes them unstable in the free state. Besides this, they are potential pH indicators as they change their structure with the pH of the medium [14–16].

The objective of the present chapter is to investigate computationally the structure and some potential applications of 12 important anthocyanidins including, CYA, DEL, PEO, PEL, MAL, Petunidin (PET), Apigenidin (API), Diosmetinidin (DOS), Fisetinidin (FIS), Luteolinidin (LUT), Kuromanin (KUR) and Quercetagetinidin (QUE).

5.2 Structural features of anthocyanidins

The basic structure of anthocyanidin has been drawn by Gaussview5 [17] and is subjected to Gaussian 09 [18] for a PES calculation. The lowest energy conformer has been identified from the scan result and is employed for future studies. This stable conformer has been modified to get different anthocyanidins studied and are optimized with DFT-B3LYP level of theory and 6-31+G (d, p) as a basis set. The 13 conformers (Fig 5.2) obtained on scanning the

dihedral connecting the rings [B] and [C] are shown below, and among them, conformer 1 has the lowest energy with dihedral angle 180˚.

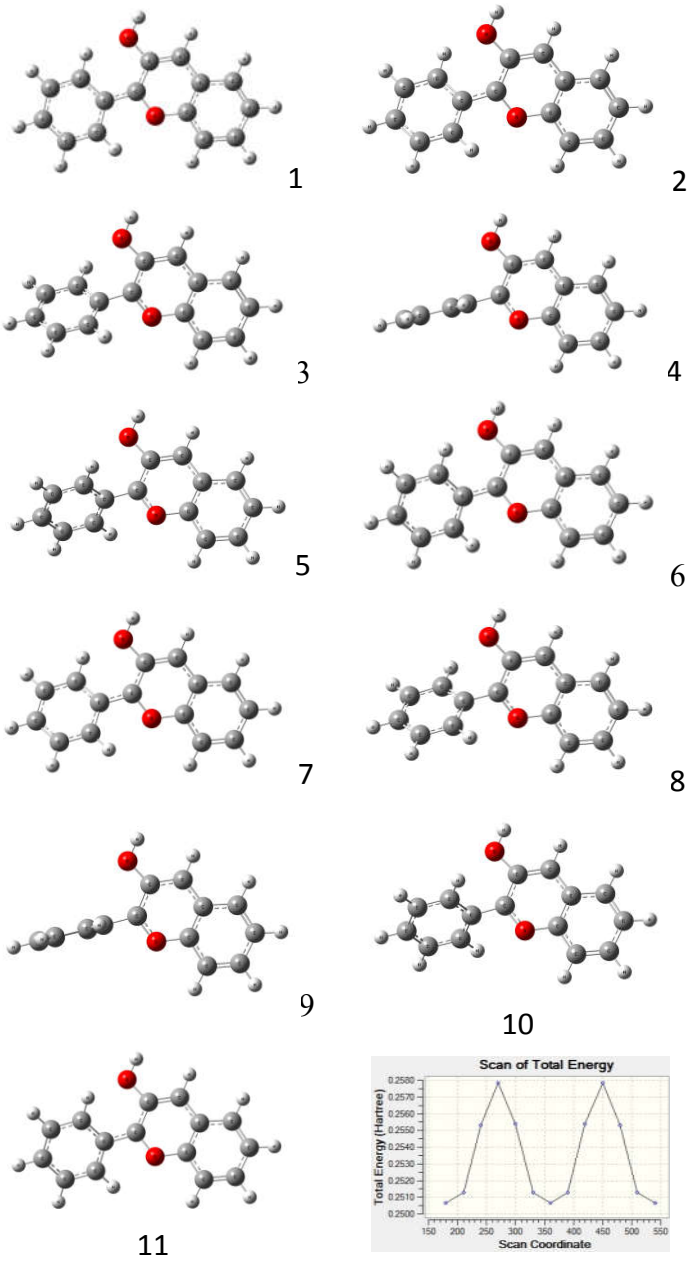
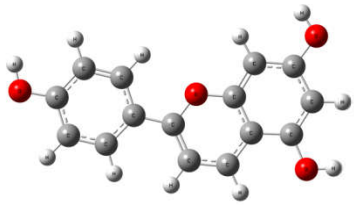
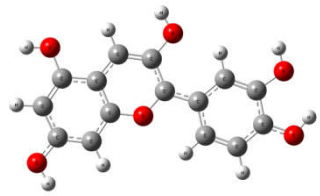


Fig.5.2 Different conformers and PES diagram of anthocyanidin

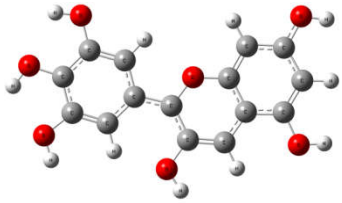
Individual anthocyanidins obtained by modifying the lowest energy conformer and are optimized and the structures are shown in Fig. 5.3. PEO has the highest and MAL has the lowest energy gap. Among the studied anthocyanidins, KUR has one glucose linkage at position 3. The –OH group at position 3 is absent in LUT, API, and DOS. The HOMOs and LUMOs of all the anthocyanidins are delocalized over the entire molecule almost in a similar manner (Figs 5.4 & 5.5).



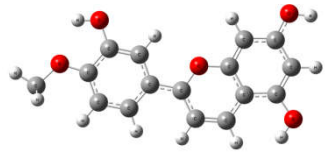
Apigenidin



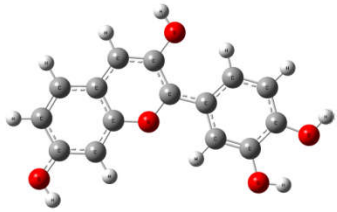
Cyanidin



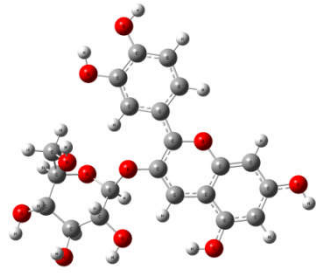
Delphinidin



Diosmetinidin



Fisetinidin



Kuromanin

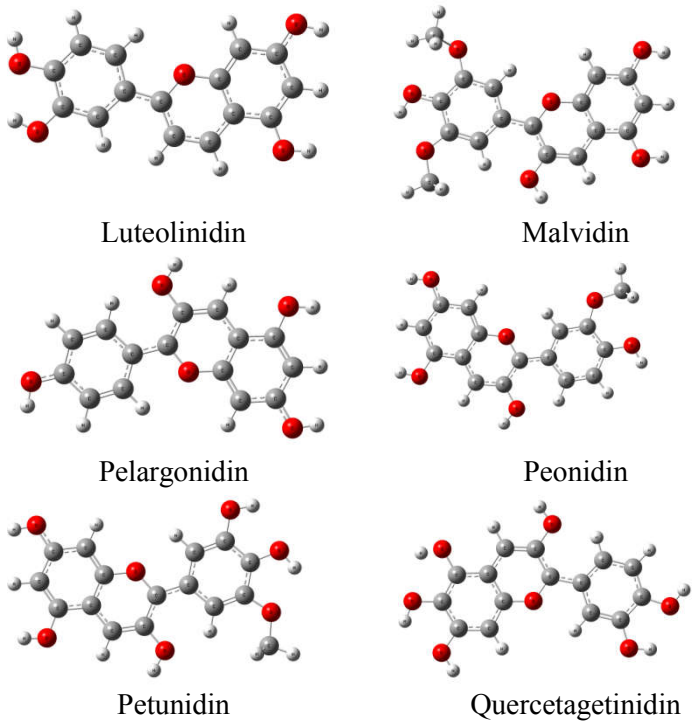


Fig.5.3 Optimized structures of anthocyanidins

Table 5.1

Band gap (eV) of anthocyanidins

No	Compound	ΔE	No	Compound	ΔE
1	API	3.09	7	LUT	2.85
2	CYA	2.67	8	MAL	2.55
3	DEL	2.60	9	PEL	2.82
4	DOS	2.72	10	PEO	5.97
5	FIS	2.65	11	PET	2.57
6	KUR	2.75	12	QUE	2.68

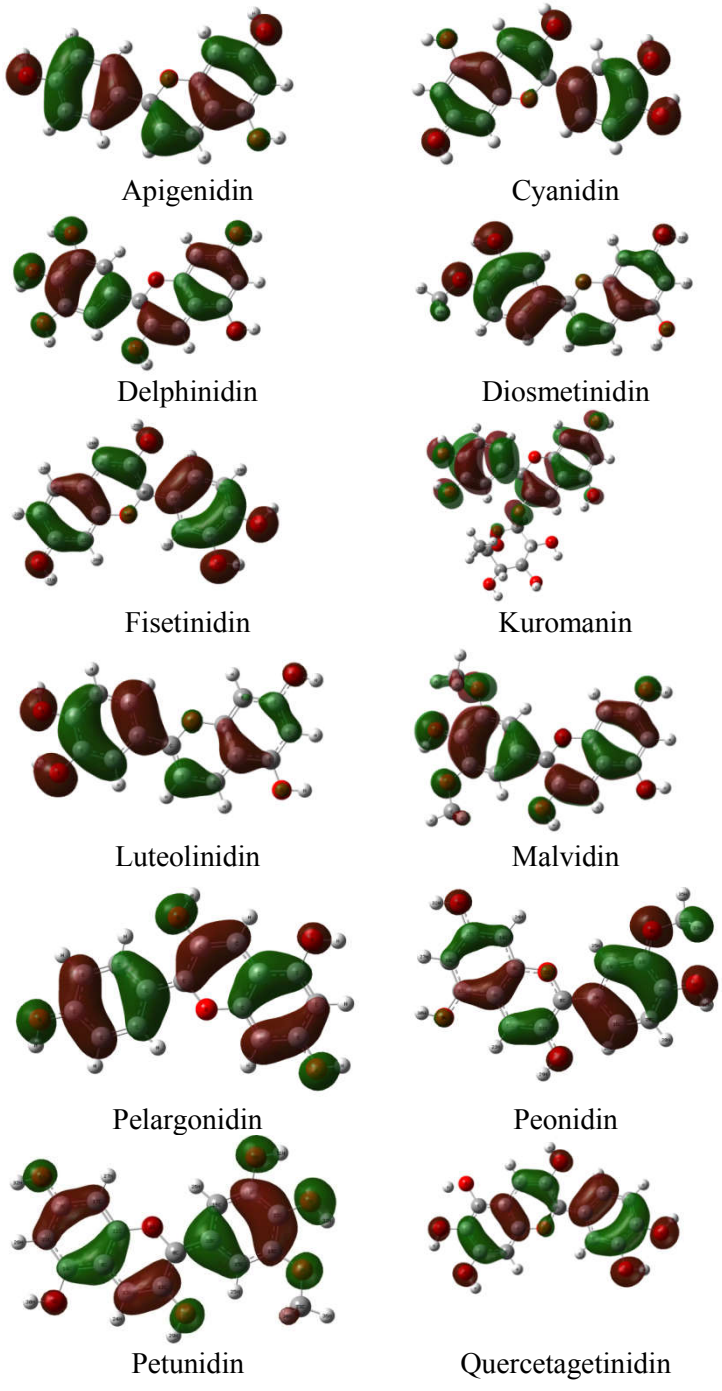


Fig.5.4 HOMO of anthocyanidins

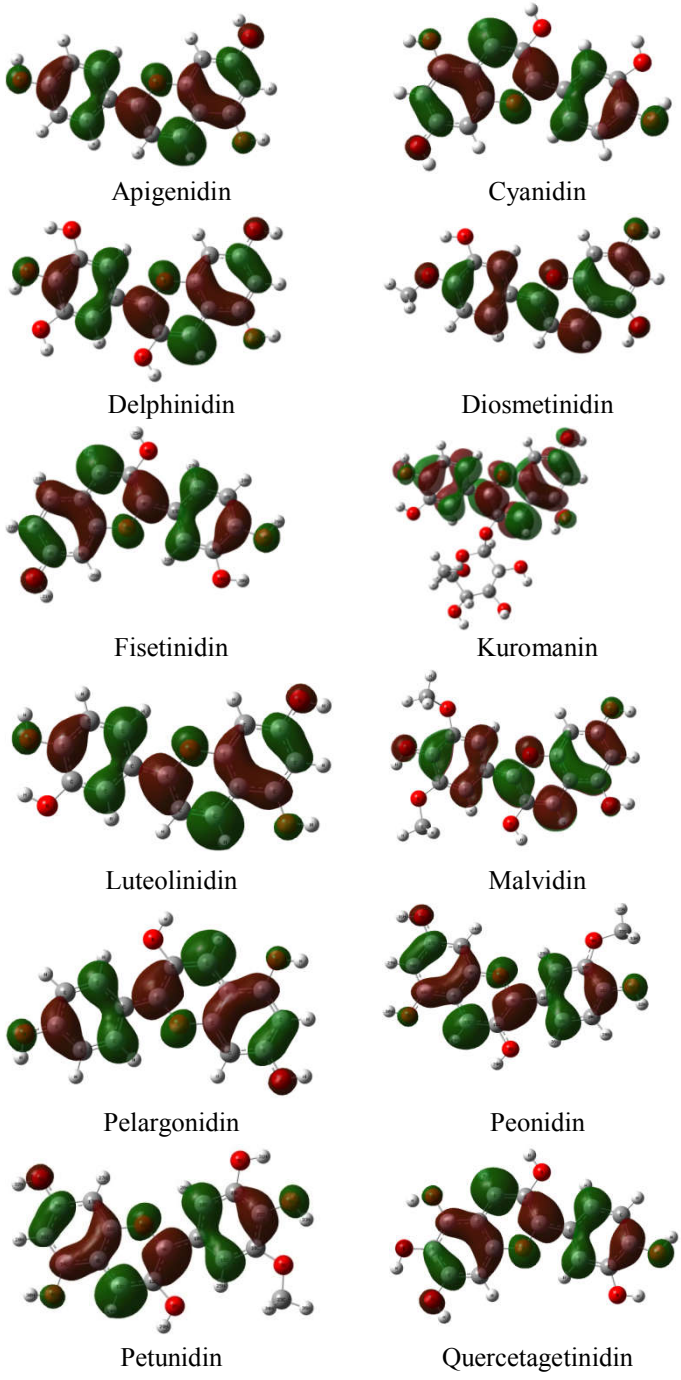


Fig.5.5 LUMO of anthocyanidins

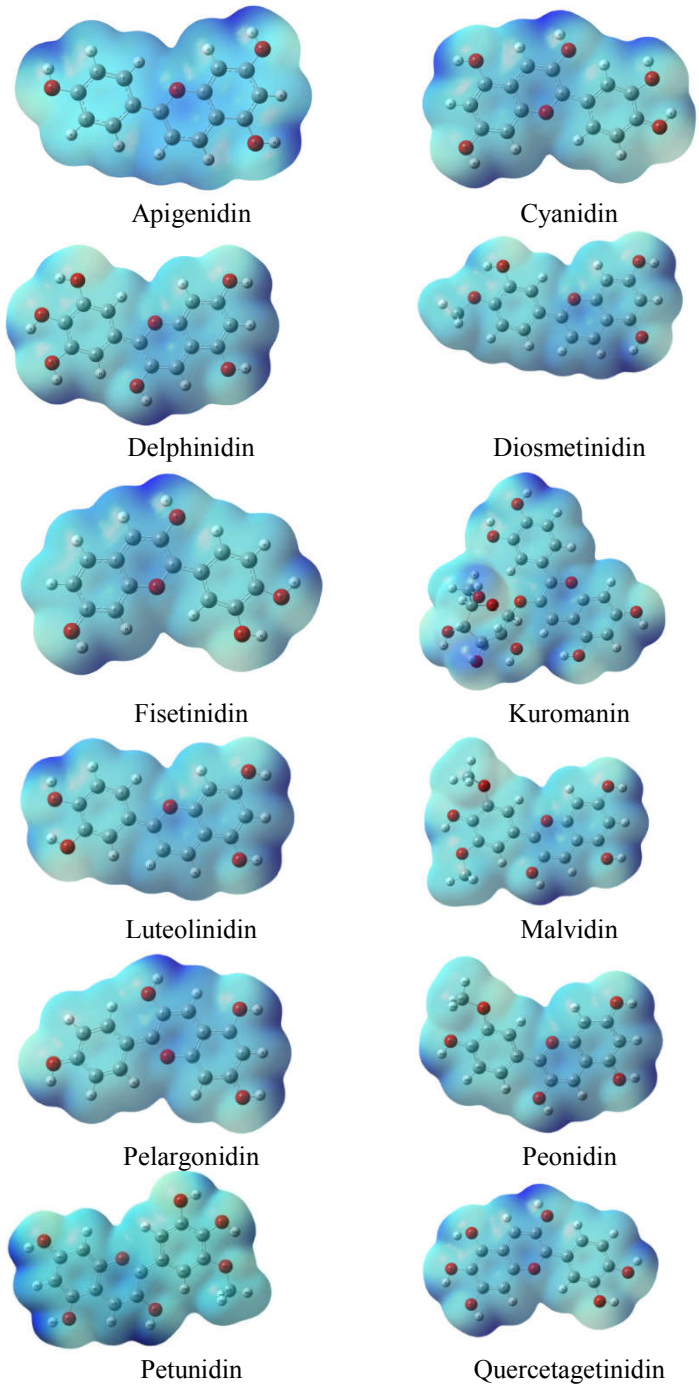


Fig.5.6 ESP map of anthocyanidins

The ESP diagrams (Fig 5.6) are easy to interpret and are essential to get the idea of electrophilic and nucleophilic centers in a compound. The electrostatic potentials are plotted here. The red regions are electron rich while blue is electron deficient. So, the red colour locates on oxygen atoms while blue locates on hydrogen atoms. However, here the red colour is not so pronounced as in the case of the other flavonoids. The blue colour predominates and is spread over the entire molecule. When this happens, such molecules are said to be highly polar. This gives an indication about the charge accepting tendency of anthocyanidins and their pronounced antireductant character in comparison with other flavonoids. This is confirmed in the following sections.

5.3 Global reactive descriptors of anthocyanidins

The reactivities of a set of compounds can be easily demonstrated using global reactive descriptors. Table 5.2 displays the global reactive descriptors of the studied anthocyanidins. Compared to the other classes of flavonoids, anthocyanidins have very high EA and Qmax values. This confirms the results from ESP maps and these compounds are characterized by their charge accepting capacity (Qmax). The reactivities are high as indicated from the high value for softness except for PEO which is due to its high energy gap.

Table 5.2

Global reactive descriptors (eV) of anthocyanidins

No	Compound	IE	EA	η	S	χ	μ	ω	Q_{max}	ω-	ω+
1	API	9.49	6.40	1.54	0.32	7.95	-7.95	20.46	5.15	24.63	16.68
2	CYA	8.94	6.26	1.34	0.37	7.60	-7.60	21.62	5.69	25.58	17.98
3	DEL	8.83	6.23	1.30	0.38	7.53	-7.53	21.82	5.79	25.75	18.21
4	DOS	9.01	6.29	1.36	0.37	7.65	-7.65	21.46	5.61	25.46	17.81
5	FIS	9.04	6.39	1.33	0.38	7.71	-7.71	22.44	5.82	26.46	18.74
6	KUR	8.72	5.97	1.38	0.36	7.35	-7.35	19.62	5.34	23.46	16.11
7	LUT	9.09	6.24	1.42	0.35	7.67	-7.67	20.63	5.38	24.64	16.98
8	MAL	8.65	6.10	1.28	0.39	7.38	-7.38	21.31	5.78	25.16	17.78
9	PEL	9.09	6.27	1.41	0.35	7.68	-7.68	20.91	5.44	24.92	17.24
10	PEO	11.60	5.60	2.99	0.17	8.59	-8.59	12.34	2.87	17.01	8.419
11	PET	8.73	6.16	1.29	0.39	7.44	-7.44	21.54	5.79	25.42	17.98
12	QUE	8.89	6.20	1.34	0.37	7.55	-7.55	21.22	5.62	25.16	17.61

The DAM (Fig. 5.7) shows that all the studied anthocyanidins are effective electron acceptors than the most electronegative element F and are worst electron donor as compared to Na. Among the studied anthocyanidins, molecule 5, i.e., FIS has the highest electron accepting tendency. All the anthocyanidins discussed here fall in the quadrant of good antireductants as they are good electron acceptors.

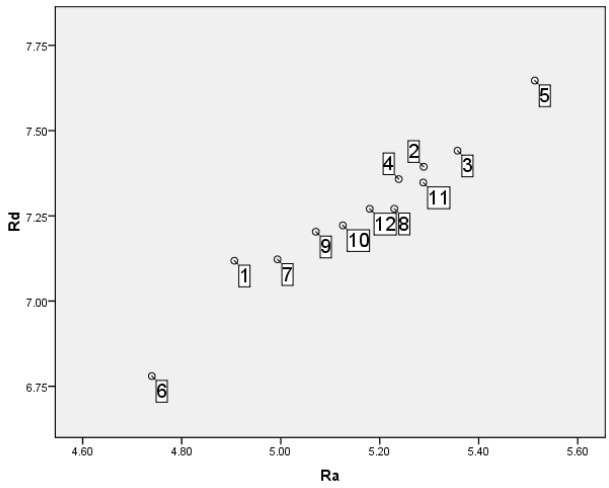
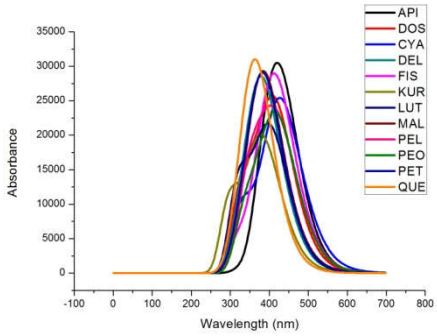


Fig. 5.7 DAM of anthocyanidins

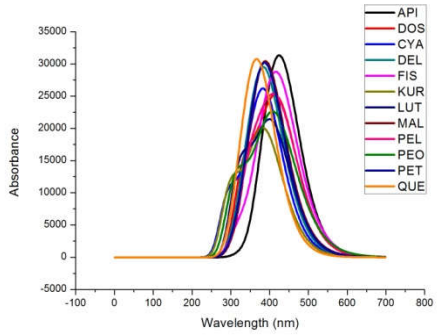
5.4 UV-Visible spectroscopy of anthocyanidins

From literature, it has been seen that anthocyanidins could act as pH indicators [19,20]. It has been observed that anthocyanidins are highly sensitive to pH values of the medium in which they are present, and they undergo some structural reorganization with pH values. As a result, they exhibit different colours at different pH values so that they could act as pH indicators. For example, Peonidin exhibits different colours concerning the pH values of the environment where the plants are lived. At low pH values (in acidic media) the flavylium cations exist and are red. As the pH values increase this cationic form undergo several structural deformations to form quinoidal bases and then to chalcones.

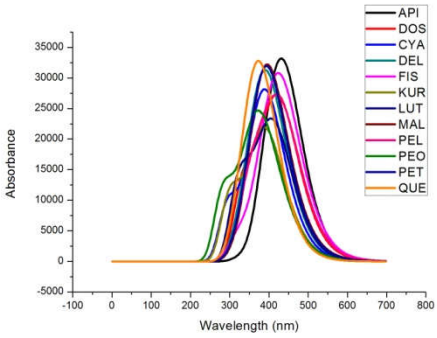
At first, the UV-Visible spectra of all the anthocyanidins are computed in water, acetic acid and aniline by TDDFT tool in Gaussian 09 software package. The spectra are shown in Fig.5.8, and the respective parameters are given in Tables 5.3-5.5.



(a)



(b)



(c)

Fig.5.8 UV-Visible spectra of anthocyanidins in (a) water, (b) acetic acid and (c) aniline

The possible transitions in all the anthocyanidins are found to be those between HOMO, HOMO-1, and HOMO-2 with LUMO energy level.

Table 5.3

UV-Visible spectral characteristics in water

Compound	Energy (eV)	λ_{max} (nm)	Oscillator strength	MO involved	% contribution
CYA	2.57	483	0.49	HOMO-LUMO	92.38
	2.82	440	0.01	HOMO-1-LUMO	96.39
	3.22	386	0.26	HOMO-2-LUMO	90.49
DEL	2.42	512	0.03	HOMO-1-LUMO	87.78
	2.56	484	0.54	HOMO-LUMO	76.68
	2.90	428	0.11	HOMO-2-LUMO	82.75
DOS	2.50	497	0.32	HOMO-LUMO	90.53
	2.85	435	0.05	HOMO-1-LUMO	92.44
	3.16	392	0.42	HOMO-2-LUMO	85.35
FIS	2.48	500	0.25	HOMO-LUMO	78.56
	2.87	432	0.41	HOMO-1-LUMO	79.55
	3.37	368	0.09	HOMO-2-LUMO	90.31
KUR	2.49	498	0.27	HOMO-LUMO	90.51
	2.67	464	0.07	HOMO-1-LUMO	93.37
	3.18	390	0.17	HOMO-2-LUMO	57.86
LUT	2.53	489	0.18	HOMO-LUMO	90.96
	2.92	425	0.21	HOMO-1-LUMO	86.35
	3.34	371	0.31	HOMO-2-LUMO	85.21
MAL	2.39	518	0.01	HOMO-2-LUMO	95.25
	2.52	491	0.62	HOMO-LUMO	89.40
	2.86	433	0.03	HOMO-2-LUMO	89.09
PEL	2.64	470	0.42	HOMO-LUMO	82.87
	3.11	399	0.32	HOMO-1-LUMO	78.62
	3.47	357	0.03	HOMO-2-LUMO	97.76
PEO	2.55	486	0.41	HOMO-LUMO	86.99
	2.73	453	0.04	HOMO-1-LUMO	96.48
	3.15	394	0.32	HOMO-2-LUMO	85.85
PET	2.42	512	0.03	HOMO-2-LUMO	99.01
	2.54	488	0.61	HOMO-LUMO	89.94
	2.88	431	0.07	HOMO-2-LUMO	85.67
QUE	2.44	508	0.01	HOMO-1-LUMO	98.26
	2.52	492	0.27	HOMO-LUMO	79.58
	2.92	425	0.45	HOMO-2-LUMO	78.25
API	2.91	426	0.46	HOMO-LUMO	69.82
	3.17	391	0.27	HOMO-1-LUMO	68.08
	3.65	339	0.01	HOMO-2-LUMO	89.47

Table 5.4

UV-Visible spectral characteristics in Acetic acid

Anthocyanidin	Energy (eV)	λ_{max} (nm)	Oscillator strength	MO involved	% Contribution
API	2.83	437	0.58	HOMO-LUMO	80.51
	3.14	394	0.29	HOMO-1-LUMO	78.84
	3.75	331	0.01	HOMO-2-LUMO	53.38
DEL	2.48	499	0.67	HOMO-LUMO	91.34
	2.61	474	0.02	HOMO-1-LUMO	99.19
	2.90	428	0.14	HOMO-2-LUMO	86.51
DOS	2.58	481	0.58	HOMO-LUMO	94.62
	2.90	427	0.01	HOMO-1-LUMO	96.25
	3.21	386	0.35	HOMO-2-LUMO	94.90
FIS	2.56	485	0.61	HOMO-LUMO	95.49
	2.90	428	0.17	HOMO-1-LUMO	94.31
	3.38	367	0.10	HOMO-2-LUMO	92.61
KUR	2.49	497	0.46	HOMO-LUMO	96.06
	2.72	455	0.01	HOMO-1-LUMO	97.42
	3.19	389	0.28	HOMO-2-LUMO	94.00
LUT	2.64	470	0.43	HOMO-LUMO	96.21
	2.89	429	0.09	HOMO-1-LUMO	96.20
	3.34	372	0.33	HOMO-2-LUMO	93.04
MAL	2.46	505	0.71	HOMO-LUMO	92.93
	2.62	474	0.01	HOMO-1-LUMO	98.56
	2.87	432	0.09	HOMO-2-LUMO	88.60
PEL	2.56	484	0.54	HOMO-LUMO	90.90
	3.07	403	0.34	HOMO-1-LUMO	87.81
	3.61	344	0.02	HOMO-2-LUMO	95.89
PET	2.47	503	0.69	HOMO-LUMO	92.09
	2.61	474	0.01	HOMO-1-LUMO	98.70
	2.88	430	0.11	HOMO-2-LUMO	87.68
QUE	2.45	505	0.02	HOMO-1-LUMO	98.30
	2.58	480	0.64	HOMO-LUMO	95.47
	2.95	420	0.19	HOMO-2-LUMO	92.39
CYA	2.51	495	0.63	HOMO-LUMO	94.92
	2.84	437	0.01	HOMO-1-LUMO	94.70
	3.25	382	0.24	HOMO-2-LUMO	95.60
PEO	2.33	533	0.50	HOMO-LUMO	95.02
	2.58	481	0.05	HOMO-1-LUMO	97.70
	3.05	406	0.30	HOMO-2-LUMO	93.54

Table 5.5

UV-Visible spectral characteristics in Aniline

Anthocyanidin	Energy (eV)	λ_{max} (nm)	Oscillator strength	MO involved	% Contribution
API	2.80	442	0.66	HOMO-LUMO	84.84
	3.13	396	0.26	HOMO-1-LUMO	83.17
	3.75	331	0.01	HOMO-2-LUMO	51.93
DEL	2.45	507	0.72	HOMO-LUMO	93.17
	2.62	474	0.01	HOMO-1-LUMO	99.16
	2.89	430	0.13	HOMO-2-LUMO	88.37
DOS	2.55	486	0.64	HOMO-LUMO	95.55
	2.90	427	0.01	HOMO-1-LUMO	96.26
	3.20	388	0.34	HOMO-2-LUMO	96.07
FIS	2.52	491	0.68	HOMO-LUMO	97.30
	2.89	429	0.14	HOMO-1-LUMO	95.59
	3.37	368	0.09	HOMO-2-LUMO	93.04
KUR	2.47	502	0.51	HOMO-LUMO	96.80
	2.72	456	0.01	HOMO-1-LUMO	97.44
	3.18	390	0.28	HOMO-2-LUMO	95.12
LUT	2.62	474	0.49	HOMO-LUMO	96.97
	2.89	430	0.08	HOMO-1-LUMO	96.73
	3.32	373	0.33	HOMO-2-LUMO	94.41
MAL	2.42	512	0.75	HOMO-LUMO	94.42
	2.62	473	0.01	HOMO-1-LUMO	98.90
	2.86	433	0.08	HOMO-2-LUMO	89.97
PEL	2.53	490	0.60	HOMO-LUMO	93.05
	3.06	406	0.32	HOMO-1-LUMO	90.11
	3.61	343	0.02	HOMO-2-LUMO	95.62
PET	2.43	511	0.74	HOMO-LUMO	93.81
	2.62	474	0.01	HOMO-1-LUMO	99.04
	2.87	432	0.10	HOMO-2-LUMO	89.29
QUE	2.45	506	0.03	HOMO-1-LUMO	97.41
	2.55	486	0.70	HOMO-LUMO	96.22
	2.94	421	0.17	HOMO-2-LUMO	93.93
CYA	2.47	502	0.68	HOMO-LUMO	95.99
	2.83	437	0.01	HOMO-1-LUMO	95.12
	3.24	383	0.24	HOMO-2-LUMO	96.45
PEO	2.30	539	0.56	HOMO-LUMO	96.31
	2.58	481	0.04	HOMO-1-LUMO	98.01
	3.04	408	0.29	HOMO-2-LUMO	94.77

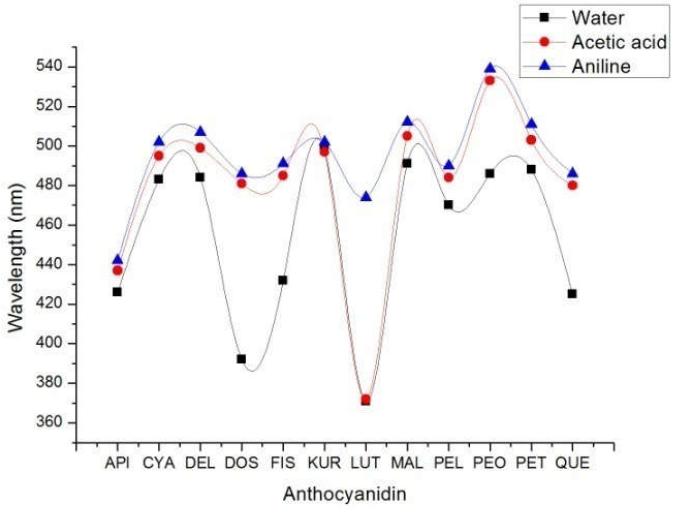


Fig.5.9 Variation of λ_{max} value with media

The variation of λ_{max} values with different media is shown in Fig.5.9. The λ_{max} values are high in aniline for all the anthocyanidins. Peonidin, the second most abundant anthocyanidin, is an O-methylated anthocyanidin and is considered as a primary plant pigment and is thus selected here as a reference sample for studying the pH indicator properties of anthocyanidins. Peonidin gives purple-red hue to the flowers of Peony while it gives a blue colour to the flowers of Morning glory (Fig. 5.10). It is interesting that the same pigment gives two different colours to the flowers of two plants. Morning glory is found to grow in alkaline soil whereas Peony preferably grows in acidic soil. So there is a difference in pH values and which may be the reason for their colour change [14].

Fig.5.10. (a) Morning glory, (b) Peony names

The basic structure of Peonidin is the anthocyanidin ring with extended conjugation. Generally, anthocyanidins are stable at lower pH and thus have bright colours. However, Peonidin and Petunidin are found to be stable up to a pH value of 8. This enables them to be used as a natural food colourant in addition to their antiradical capacity. The structural deformations according to the pH values results in the change in colour, whereby they can act as pH indicators. The different structural isomers of Peonidin at different pH value is shown in Fig.5.11; the structural forms are obtained from the work of Birse, Raymond Brouillard and Jacques-Emile Dubois [16] and Chantal Houbiers and et al. [21].

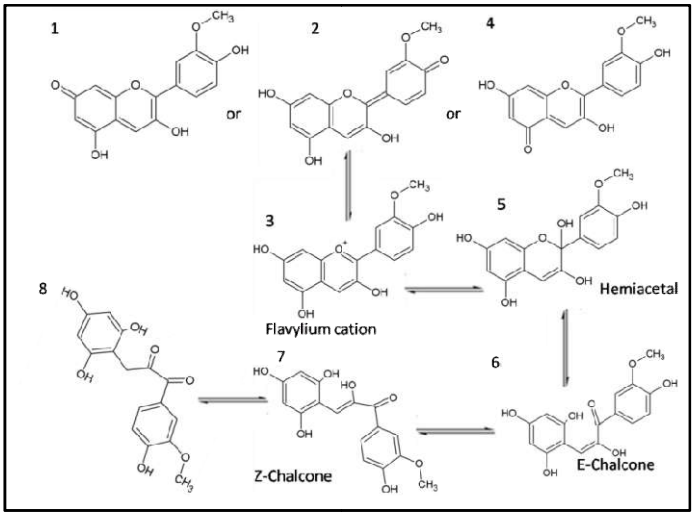


Fig.5.11. Structural isomers of Peonidin at different pH values

Here the compound 3 is in the form of flavylium cation, compounds 1, 2 and 4 are in quinoidal base form, and rests are in the form of chalcones. At lower pH values, the anthocyanidins are often in their flavylium cationic form, and consequently, anthocyanidins are intensely coloured in acidic media. As the pH value increases, there occur some structural variations, and they change their colour from purple-red to navy blue. Most of the anthocyanidins undergo degradation when the pH values approach to 8 and beyond, due to the instability of flavylium cation. Peonidin and Petunidin are stable up to the pH 8 and are in blue at this pH value. The existences of quinoidal bases and chalcones are highly favored in the alkaline media. As the pH value increases, proton losses from the flavylium cation rapidly, and form quinoidal bases, which are also coloured. So in acidic media anthocyanidins prefer to exist in their flavylium cationic form whereas in higher pH values they prefer to exist in the quinoidal bases and chalcone form [20,22]. Like the colour of flavylium cation, quinoidal bases and chalcones are different; they show a colour change by the

pH of the medium and this property makes anthocyanidins to use as pH indicators.

5.4.1 Colour of Peonidin

Peonidin is seen in two plants namely, Peony and morning glory. Peonidin imparts pink-purple colour to peony flower while it gives a bluecolour to the flower of morning glory. The reason is that peonidin exists in two different structural forms in the two plants. Peony grows only in acidic media so that Peonidin exist in it as flavylium cation (compound 3). This is the reason for the pink colour of peony flower as flavylium cation of peonidin is pink in colour. On the other hand, peonidin exist as its quinoidal base (compound 4) form in morning glory, which prefers to grow in alkaline soil, so that it appears blue/violet-blue [14].

All the structural forms in Fig.5.11are optimized at DFT-B3LYP/6-31+G (d, p) level of theory, and UV-Visible spectra are computed for all of them. The results are given in Table 5.6.

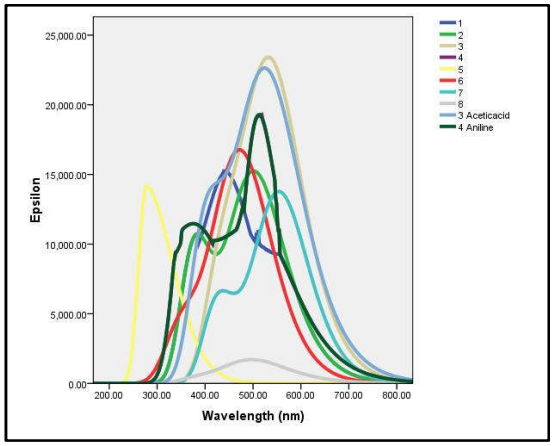


Fig.5.12UV-Visible spectrum of different structural forms of Peonidin

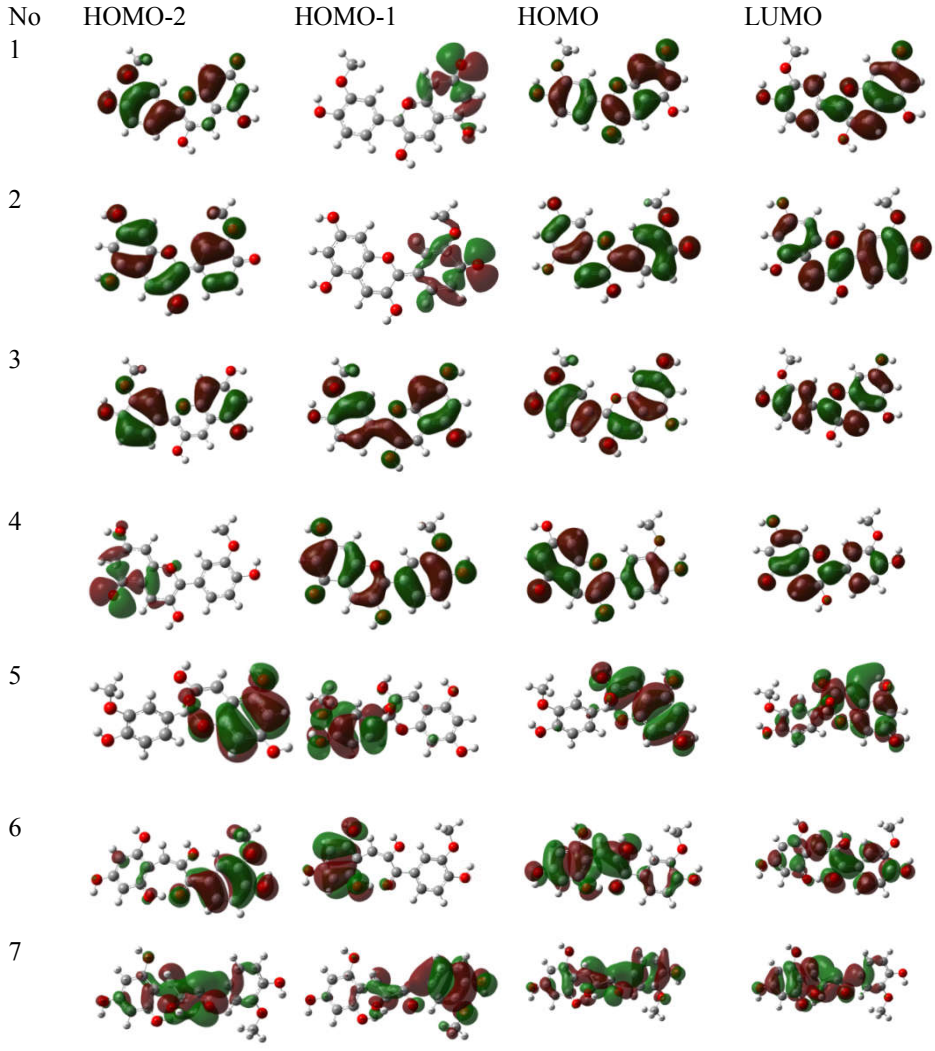
Table 5.6

UV-Visible spectral details of different isomers of Peonidin

Isomers	λ_{max} (nm)	Oscillator strength	Contribution of MO (%)	MO involved
1	439	0.74	94.18	HOMO-LUMO
	422	0.01	95.90	HOMO-1-LUMO
	370	0.08	84.51	HOMO-2-LUMO
2	430	0.37	94.01	HOMO-LUMO
	425	0.01	97.21	HOMO-1-LUMO
	381	0.25	84.32	HOMO-2-LUMO
3	480	0.54	89.12	HOMO-LUMO
	434	0.01	90.69	HOMO-2-LUMO
	391	0.24	87.08	HOMO-1-LUMO
4	575	0.45	95.68	HOMO-LUMO
	470	0.01	98.01	HOMO-2-LUMO
	400	0.15	94.28	HOMO-1-LUMO
5	278	0.33	88.79	HOMO-LUMO
	272	0.01	45.71	HOMO-1-LUMO
	262	0.02	45.24	HOMO-2-LUMO
6	416	0.40	97.76	HOMO-LUMO
	387	0.01	97.23	HOMO-1-LUMO
	337	0.11	87.55	HOMO-2-LUMO
7	432	0.33	98.02	HOMO-LUMO
	361	0.04	87.44	HOMO-1-LUMO
	330	0.13	78.99	HOMO-2-LUMO
8	512	0.04	88.37	HOMO-LUMO
	448	0.01	47.28	HOMO-2-LUMO
	409	0.01	50.81	HOMO-1-LUMO
9 (3 in Acetic acid)	533	0.50	95.02	HOMO-LUMO
	480	0.05	97.69	HOMO-2-LUMO
	406	0.30	93.54	HOMO-1-LUMO
10 (4 in Aniline)	570	0.47	97.10	HOMO-LUMO
	427	0.01	98.07	HOMO-2-LUMO
	416	0.27	96.01	HOMO-1-LUMO

Table 5.6 clearly shows that the flavylium cation (isomer 3) has an absorbance at 480 nm and is in the blue-green region of electromagnetic spectrum so that it appears as red. The compound 4, a quinoidal base, has an absorbance at 575 nm, which is in the yellow-orange region of the electromagnetic spectrum and thus it appears as blue [14,23–25]. These results are in agreement with the observed

colour of flowers of peony and morning glory. In order to confirm this, the absorption characteristics of compound 3 in acetic acid is carried out and has been observed that the λ_{max} is at 533 nm and this is responsible for the purple-pink colour of peony flower. The absorption of compound 4 in aniline changes to 570 nm and is responsible for the violet-blue colour of morning glory [14].



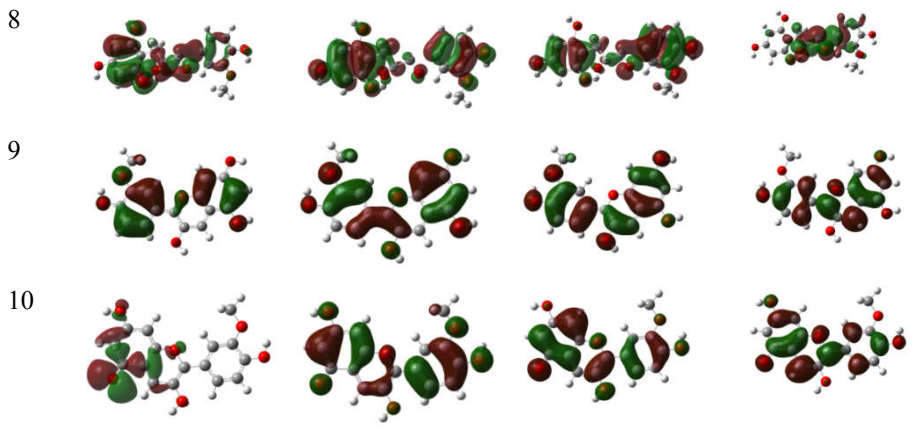


Fig.5.13 MOs of compounds involved in the transitions

The Molecular Orbital (MO) picture in Fig.5.13 clearly explains the possibility of different transitions involved in the studied compounds. The value of oscillator strength reveals the intensity of the corresponding transition. In all compounds, λ_{max} is found to be due to the transitions between HOMO and LUMO energy levels. The other transitions possible for the studied compounds are HOMO-1 to LUMO and HOMO-2 to LUMO levels. When the MOs are delocalized in similar manner, the transition becomes more feasiblea . Here HOMO and LUMO of all the compounds except five are delocalized almost over the entire molecule. In 5, LUMO is delocalized almost on the entire molecule, but HOMO, HOMO-1, and HOMO-2 are localized on some parts of the molecule only. Its HOMO is localized over the entire [A] and [C] ring, while HOMO-1 localized over the [B] ring and HOMO-2 localized over the entire [A] ring and partially on rings [C]. Due to this, the chances of transition from HOMO to LUMO will be high while that between HOMO-1 and LUMO is low. In compounds 1 and 2, the HOMO-1 is localized on [B] ring and [A] ring respectively, while the other three MOs of them are delocalized over the entire

molecule, and consequently the intensity of transition between HOMO-1 and LUMO is very low. Similarly, the transition between HOMO-2 and LUMO are less intense for compounds 3 and 4. This is the same for these compounds in acetic acid and also in aniline [14]. The contribution of MO in percentage has been given in Table 2 and is obtained by multiplying the square of the coefficient of MO by 200 [26].

5.5 Antioxidant properties of anthocyanidins

The radical scavenging capacities of anthocyanidins are carried out using three mechanisms namely, HAT, SET/SETPT, and SPLET. The necessary parameters are given in Table 5.7.

Table 5.7

Parameters (kcal/mol) of different antioxidant mechanism

No.	Anthocyanidin	BDE	AIP	PDE	PA	ETE
1	API	90.30	248.35	155.71	-114.06	518.12
2	CYA	80.73	242.31	152.18	-122.83	517.32
3	DEL	80.05	239.88	154.53	-122.54	516.94
4	DOS	81.16	238.01	159.84	-115.89	513.75
5	FIS	80.76	239.16	155.35	-116.71	511.22
6	KUR	53.90	225.01	166.46	-114.14	505.61
7	LUT	84.11	241.44	153.47	-113.41	508.33
8	MAL	54.90	225.54	143.11	-112.24	480.90
9	PEL	82.02	245.47	150.31	-122.21	517.98
10	PEO	81.50	235.37	159.89	-120.49	515.75
11	PET	80.02	236.4	157.41	-120.93	514.74
12	QUE	80.66	234.72	159.25	-113.03	507.01

Generally, for anthocyanidins, position3 is found to be most reactive to form stable radicals. However, here there are some exceptions for DOS, LUT, API, and KUR. In DOS, API, and LUT, the 3-OH group is absent while it is replaced by glucose moiety in KUR.

For all other anthocyanidins, the 3-OH group is present and is the site for most stable radical formation. Next, to position 3, position 4' is also reactive in anthocyanidins. However, in DOS, as this site is methylated, the reactive site is found to be position 3'.

Among the studied anthocyanidins, KUR is having lowest BDE value, so that it is the most potent antioxidant according to HAT mechanism. Thisis attributed to the glucose group. Generally, BDE values decrease with the number of –OH groups, and again the BDE value decreases with electron-releasing substituents in an adjacent position to the –OH groups. Among the studied anthocyanidins, DEL and QUE have the highest number of –OH groups (6). But the lowest BDE value is not observed for these molecules. This can be explained as follows. In KUR, there are 4 –OH groups in the anthocyanidin moiety and one glucose group in the ring [C]. This is responsible for its lowest BDE value. The total number of –OH groups are same in DEL and QUE, but the number of –OH groups in the ring [B] is higher in DEL than QUE resulting in the lower BDE values of DEL than QUE. In MAL, there are 4 –OH groups and 2 $–OCH_3$ groups resulting in the lower BDE value of MAL after KUR. In PET, there are 5 –OH groups and one $–OCH_3$ group. Consequently, its BDE value is less than that of DEL & QUE. The CYA has lower BDE values due to the presence of 5 –OH groups.

In order to confirm the fact that the presence of a glucose group decreases the BDE, glucose group has been incorporated in one of the anthocyanidins, say PET, and has observed that its BDE value decreases. Moreover, the replacement of glucose group in KUR by –OH, it resembles CYA. The BDE value of CYA is higher than that of KUR.

In order to study the structural features on BDE values, the BDE values were analyzed at all possible sites in the anthocyanidins. Table 5.8 clearly shows that, for all the anthocyanidins except those without 3-OH groups, the stable radical is formed at position 3. The second reactive site is 4'. Thisis confirmed from the bond order values obtained from the NBO analysis. In some case, the ring [B] is reactive after position 3, while in some cases, the ring [A] is reactive after position 3 in radical formation. Anthocyanidins without 3 –OH group, the position 4' is having least bond order and thus having low BDE values.

Table 5.8

BDE values (kcal/mol) at different sites

Compound	Position	BDE	Bond order	Compound	Position	BDE	Bond order
API	5	92.41	0.7691	DOS	**3'**	**84.11**	0.6978
	4'	**90.3**	**0.7468**		5	90.1	0.7132
	7	93.44	0.7834		7	94.18	0.7765
CYA	3'	90.52	0.7451	PET	4'	81.76	0.6945
	4'	83.35	0.6989		3'	91.46	0.7564
	7	90.42	0.7352		7	89.54	0.7432
	3	**80.73**	**0.6542**		5	85.91	0.7123
	5	88.59	0.7231		**3**	**80.05**	**0.6745**
DEL	5	86.23	0.7430	QUE	**3**	**80.22**	0.6734
	7	90.04	0.7245		7	89.81	0.7765
	3'	91.73	0.7512		3'	81.61	0.7345
	4'	82.81	0.6895		6	83.95	0.7612
	5'	83.75	0.7312		4'	81.44	0.7012
	3	**80.66**	0.6567		5	81.83	0.7132
FIS	**3**	**80.76**	0.6576	PEL	5	92.17	0.7865
	7	90.23	0.7246		**3**	**82.02**	0.6985
	4'	81.69	0.6887		4'	86.49	0.7456
	3'	90.18	0.7123		7	90.24	0.7613
KUR	**4'**	**53.9**	0.6123	PEO	7	89.57	0.7467
	3'	55.32	0.6549		5	85.91	0.7314
	5	59.08	0.6984		**3**	**81.5**	0.6873
	7	62.36	0.7123		4'	81.69	0.6998
LUT	**4'**	**81.16**	0.6753	MAL	**3**	**54.9**	0.6204
	3'	88.47	0.7232		4'	57.69	0.6654
	7	93.1	0.7653		7	64.27	0.7002
	5	90.22	0.7412		5	60.86	0.6897

The possibilities of undergoing electron transfer reaction by anthocyanidins are also considerable in comparison to other classes of flavonoids as anthocyanidins have very high EA and Qmax values. This promotes them to undergo charge transfer reactions with free radicals and if so they tend to accept electrons from free radicals. The competition between the two mechanisms HAT and SET can be achieved by comparing the BDE and AIP values of studied compounds with that of phenol. The results are given in Table 5.9. BDE and AIP of phenol are 82.9 and 192.05 kcal/mol respectively. When the ΔBDE ~ -42 kJ/mol (-10.03 kcal/mol) and ΔAIP < -151 kJ/mol (-36.08 kcal/mol) the mechanism is dominated by hydrogen transfer (BDE).

Table 5.9

Competition between HAT and SET

Anthocyanidin	**ΔBDE**	**ΔAIP**
PET	-2.88	44.35
PEO	-1.40	43.32
MAL	-28.00	33.49
DEL	-2.85	47.83
CYA	-2.17	50.26
PEL	-0.88	53.42
API	7.40	56.30
QUE	-2.24	42.67
LUT	1.21	49.39
KUR	-29.00	32.96
FIS	-2.14	47.11
DOS	-1.74	45.96

Here the ΔBDE values obey the condition for HAT mechanism but ΔAIP values does not. So, depending upon the conditions of the surroundings, anthocyanidins prefer either HAT or SET to scavenge free radicals. Fig 5.14 shows that the EA values of most of the

anthocyanidins except PEO are high so that they prefer SET (from free radical to anthocyanidin) mechanism. However, KUR and MAL prefer both the mechanism as they have low BDE and high EA values.

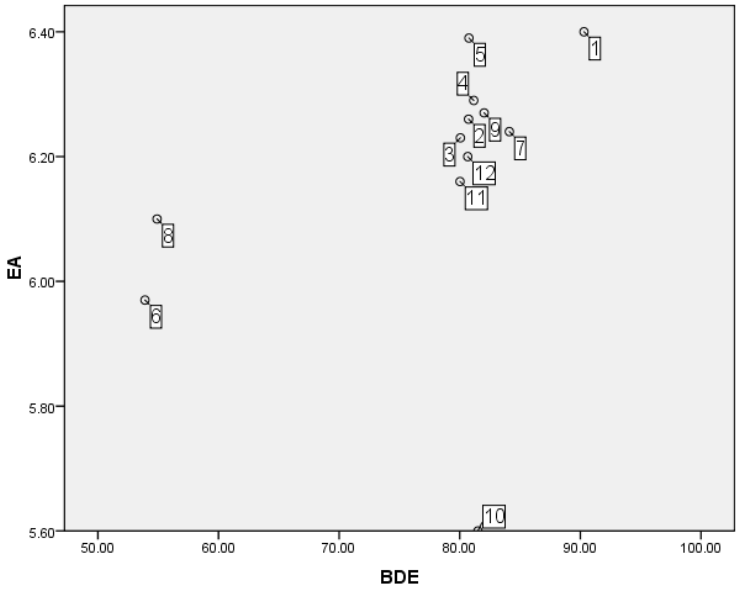


Fig. 5.14 SET *vs.* HAT mechanism

5.6 Molecular docking studies

The efficiency of anthocyanidins to prevent oxidative stress in a biological system has been validated through molecular docking studies. The molecular docking studies have been performed through AutoDock Vina software, and the binding energy of all the studied anthocyanidins with the active site of the protein target MAO-B has been computed. The active site of MAO-B is surrounded by specific amino acids Asp123, Arg127, Ile477, Thr478, Trp119, His115, and Phe103, wherein they contribute to ligand binding. Amino acids like Thr202, Glu84, Tyr435, Ser200, Arg100, Gln206, Tyr326, Phe99,

Ile199, Ile316, Cys172, Pro102, etc., are also appeared in the docking site when doing molecular docking. The binding energies of bounded ligand, SAG and 12 anthocyanidins are given in Table 5.10, and the conformers with the highest binding energy values are shown in Fig.5.15.All the interacting residues lie in the range of 1 Å. All the studied compounds have an affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and the related free radical generation, and oxidative stress can be minimized.

Table 5.10

Binding energy values (kcal/mol) against MAO-B

No	Compound	2V5Z	No	Compound	2V5Z
1	CYA	-9.0	7	MAL	-7.4
2	DEL	-8.4	8	PEL	-9.0
3	DOS	-8.9	9	PEO	-8.2
4	FIS	-7.6	10	PET	-7.8
5	KUR	-8.5	11	QUE	-8.5
6	LUT	-8.7	12	API	-9.3

The CYA forms 2 hydrogen bonds with Gln206 and Tyr326 while PEO forms a hydrogen bond with Arg100. All other anthocyanidins did not form a hydrogen bond with the amino acid residues of MAO-B.

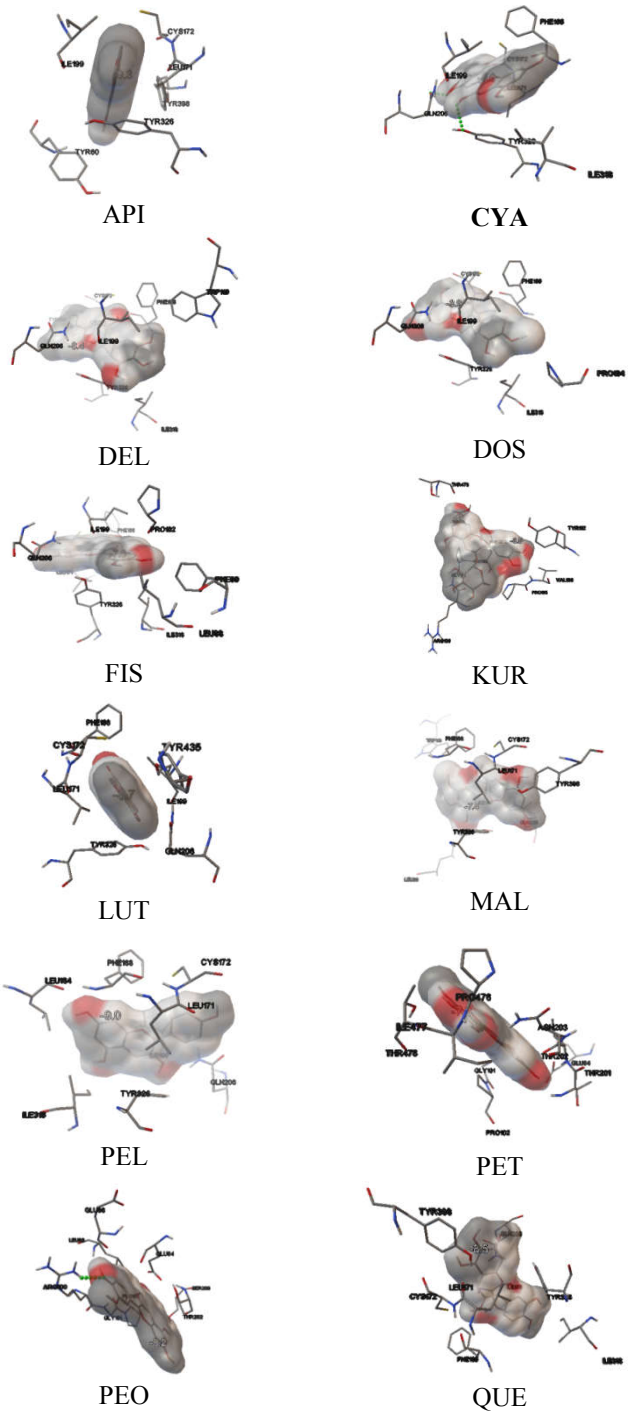


Fig.5.15 Anthocyanidins in the active site of MAO-B

5.7 Pharmacokinetic properties of anthocyanidins

The pharmacokinetic properties of studied anthocyanidins have been carried out through Molinspiration online software. Through this, the Lipinski rule of 5 (RO5) has been validated for all the 12 anthocyanidins. According to the rule, an orally admissible drug-like molecule must have:

1. HBD < 5
2. HBA < 10
3. MW < 500 Dalton
4. LogP < 5
5. ROTB < 10 (added by Veber)

Even though this rule does not predict whether a compound is pharmacologically active, it would help to get a deep knowledge about the pharmacokinetics including Absorption, Distribution, Metabolism, and Excretion (ADME) of the molecule under investigation. This makes researchers or pharmaceutics to screen out the best orally admissible drug like candidates with good ADME characteristics. For medicinal chemistry students or those who are involved in drug designing or the related studies, this RO5 become an excellent tool to screen out the investigated molecules [27]. All the studied anthocyanidins have a molecular weight less than 500 Dalton and LogP values less than 5. The pharmacokinetic properties of anthocyanidins are given in Table 5.11.

Table 5.11

Pharmacokinetic properties of anthocyanidins

Property	miLogP	TPSA	nAtoms	MW	nHBA	nHBD	nViolations	nROTB	Volume
DEL	1.37	130.6	22	303	7	6	1	1	242.83
CYA	1.66	110.4	21	287	6	5	0	1	234.81
API	0.03	71.85	19	255	4	3	0	1	218.77
QUE	1.19	130.6	22	303	7	6	1	1	242.83
PEO	1.97	99.38	22	301	6	4	0	2	252.34
PEL	2.15	90.15	20	271	5	4	0	1	226.79
PET	-0.73	121.5	23	317	7	5	0	2	260.36
DOS	2.30	79.15	21	285	5	3	0	2	244.32
KUR	-0.04	189.5	32	449	11	8	2	4	366.93
LUT	2.00	90.15	20	271	5	4	0	1	226.79
FIS	1.75	90.15	20	271	5	4	0	1	226.79
MAL	1.99	108.6	24	331	7	4	0	3	277.88

Only three anthocyanidins show violation to Lipinski's rule of 5. DEL and QUE show violation because of the presence of one excess number of HBD site, and KUR shows violation in nHBA and nHBD. All the others obey the rule of 5 and are orally admissible.

The polar surface area of anthocyanidins is also given in Table 5.11. It gives an insight into the drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood-brain barrier penetration. For a druggable molecule, the TPSA must be less than 140 A^2 [28]. Here the TPSA values are lower than this value except for KUR due to the presence of a glucose unit. The number of rotatable bonds (nROTB) decides the conformational flexibility of a molecule. This is quite important for analyzing the conformational changes that can be undergone by a molecule and ultimately it is essential in the binding of molecules to receptors or channels. Oral viability criteria have set the number of rotatable bonds to be less than

or equal to 10 [29–31]. All the studied anthocyanidins have nROTB less than 10 which again support the oral absorption of the studied anthocyanidins.

Table 5.12

Bioactivity scores against different drug targets

Anthocyanidins	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
QUE	-0.14	-0.14	-0.35	-0.06	-0.25	0.07
API	-0.29	-0.17	-0.21	-0.09	-0.44	-0.11
DEL	-0.12	-0.14	-0.31	0.04	-0.21	0.1
MAL	-0.15	-0.21	-0.31	-0.03	-0.22	0.04
CYA	-0.14	-0.16	-0.39	0.04	-0.27	0.06
PEO	-0.17	-0.23	-0.36	-0.02	-0.3	0.01
PEL	-0.2	-0.17	-0.45	-0.02	-0.3	0.05
PET	-0.15	-0.17	0.03	0.01	-0.29	-0.01
LUT	-0.06	-0.12	-0.32	0.06	-0.18	0.16
KUR	0.17	0.01	-0.12	0.05	0.04	0.36
FIS	-0.26	-0.22	-0.47	-0.1	-0.41	-0.04
DOS	-0.08	-0.19	-0.29	0.01	-0.22	0.11

All compounds, studied, were analyzed under four criteria of known successful drug activity; GPCR ligand activity, ion channel modulation, kinase inhibition activity, and nuclear receptor ligand activity. Also, their activity as protease inhibitors and enzyme inhibitors are studied. A positive value of bioactivity score indicates considerable biological activity whereas a score between −0.5and 0.00 indicates moderate activity and less than −0.5 is considered inactive. Table 5.12 clearly shows that all the anthocyanidins have activity against all the specified targets. KUR shows significant bioactivity against GPCR while all the others show moderate activity against

GPCR. Similarly, all the anthocyanidins show moderate activity as an ion channel modulator as well as a kinase inhibitor. DEL, CYA, PET, KUR, LUT, and DOS show considerable activity towards the nuclear receptor ligand while all the others show moderate activity. Only KUR shows considerable activity as a protease inhibitor whereas all others have moderate inhibition activity. All the anthocyanidins, except API, PET and FIS, show considerable enzyme inhibition activity. So, all the studied anthocyanidins can be used as potential drugs against these specified targets.

5.8 Toxicological studies on anthocyanidins

For an orally admissible drug, the drug-likeness qualitatively assesses the probability of a molecule to become an oral drug concerning the bioavailability. Drug-likeness has been implemented from the physicochemical inspections of development compounds advanced enough to consider as oral drug candidates. This has been employed to omit molecules with the incompatible pharmacokinetic profile [27].

The octanol-water partition coefficient, logP has been used as the standard descriptor for lipophilicity. It has been included as a dedicated descriptor in explaining the pharmacokinetics of drug-like molecules because of its importance in the pharmacokinetics of drug discovery. The primary requirement of a drug is the ease of handling and formulation. Thiswas achieved through the solubility of the drug molecule. Moreover, for an orally admissible drug, the solubility plays a crucial role in its absorption. As well, a drug meant for parenteral

usage has to be highly soluble in water to deliver a sufficient quantity of its active ingredient in the small volume of such pharmaceutical dosage [27].

Anthocyanidins are the key ingredients of widely accepted natural food colourants and are thus expected to have very low or zero toxicity. To confirm this, free online software called OSIRIS Property Explorer (now available as DataWarrior) has been implemented. The results are given in Table 5.13.

Table 5.13

Parameters from DataWarrior

Name	cLogS	Drug-likeness	Mutagenic	Tumorigenic	Irritant	Drug Score
API	-2.67	-1.02				0.55
CYA	-2.31	-1.15				0.56
DEL	-2.01	-1.15				0.56
DOS	-2.69	-0.84				0.56
FIS	-2.60	-1.15				0.55
KUR	-2.01	-4.26				0.40
LUT	-2.37	-1.02				0.56
MAL	-2.64	-0.97				0.55
PEL	-2.60	-1.15				0.55
PEO	-2.62	-0.97				0.56
PET	-2.32	-0.97				0.57
QUE	-2.01	-1.15				0.56

Table 5.13 clearly shows that all the studied anthocyanidins are nonmutagenic, nontumorigenic and nonirritant (green colour indicates zero risks for toxicity). Solubility is vital in the evaluation of drug

absorption and distribution characteristics. Low solubility implies low absorption. For most of the commercially available drugs, the solubility is found to be higher than -4.00 [31]. All the anthocyanidins have solubilities in the range -2.0 to -2.7, indicates good solubility. The positive values for drug score indicate that all of them can act as a potential drug.

Conclusion

Anthocyanidins are the major constituents of vascular plants and are harmless water-soluble natural pigments. The critical feature of anthocyanidins is the presence of chromenylium ion. PEO has the highest and MAL has the lowest energy gap. Among the studied anthocyanidins, KUR has one glucose linkage at position 3. The –OH group at position 3 is absent in LUT, API, and DOS. Compared to the other classes of flavonoids, anthocyanidins have very high EA and Qmax values. All the anthocyanidins discussed here fall in the quadrant of good antireductants as they are good electron acceptors. At low pH values (in acidic media) the flavylium cations exist and are red. As the pH values increase this cationic form undergo several structural deformations to form quinoidal bases and then to chalcones. The possible transitions in all the anthocyanidins are found to be those between HOMO, HOMO-1, and HOMO-2 with LUMO energy level. The λ_{max} values are high in aniline for all the anthocyanidins. Among the studied anthocyanidins, KUR is having lowest BDE value, so that it is the most potent antioxidant according to HAT mechanism. For all the anthocyanidins except those without 3-OH groups, the stable radical is formed at position 3. The second reactive site is 4'.The EA

values of most of the anthocyanidins except PEO are high so that they prefer SET (from free radical to anthocyanidin) mechanism. However, KUR and MAL prefer both the mechanism as they have low BDE and high EA values. All the studied compounds have an affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and the related free radical generation, and oxidative stress can be minimized. Only three anthocyanidins show violation to Lipinski's rule of 5. DEL and QUE show violation because of the presence of one excess number of HBD site, and KUR shows violation in nHBA and nHBD. All the others obey the rule of 5 and are orally admissible. All the studied anthocyanidins can be used as potential drugs against the specified targets like, Kinase. All the studied anthocyanidins are nonmutagenic, nontumorigenic and nonirritant. All the anthocyanidins have solubilities in the range -2.0 to -2.7, indicates good solubility. The positive values for drug score indicate that all of them can act as a potential drug.

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CHAPTER 6

EVALUATION OF STRUCTURE, RADICAL SCAVENGING, UV FILTER, TOXICOLOGICAL AND PHARMACOKINETIC PROPERTIES OF FLAVONES

- *The evaluation of structure, radical scavenging, UV filter, toxicological and pharmacokinetic properties of 11 flavones.*
- *The effect of structural features on the radical scavenging properties.*
- *Toxicological parameters and the pharmacokinetic properties.*

6.1 Introduction

A class of flavonoids with a backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) is called flavones [1,2] (Fig 6.1). They are mainly found in spices, red-purple fruits and vegetables. Flavones are potential phytochemicals, which are classes of compounds with significant health promoting benefits with lots of bioactivities. This is attributed to their radical scavenging and metal chelating abilities [3]. In addition to this, they also protect the living system from the harmful UV radiations [4]. Flavones have an affinity towards CYP (P450) activity, which is the enzyme that metabolizes most drugs in our body [5,6]. In this work, 11 flavones are studied computationally for their bioactivity analysis. They are 3, 7-Dihydroxyflavone, 5-Hydroxyflavone, 6-Hydroxyflavone, 7-Hydroxyflavone, 7, 8-Dihydroxyflavone, Baicalein, Chrysin, Luteolin-4'-glucoside, Isovitexin, Luteolin and Luteolin-3', 7-diglucoside. These flavones include both hydroxyl and glycoside derivatives.

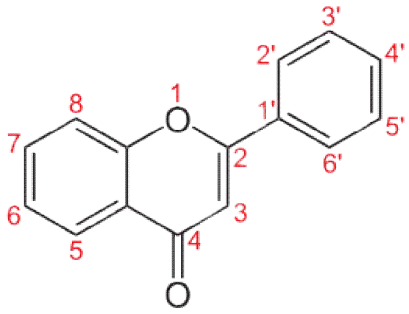


Fig. 6.1 Basic structure of flavones

6-Hydroxyflavone is a noncompetitive inhibitor of P450 C29 and is also used as a potential drug for the treatment of anxiety-related

disorders. It has been found in the leaves of *Barleria prionitis* found in India [7]. 7, 8-Dihydroxyflavone, also called 7, 8-DHF, is found in *Godmania aesculifolia, Tridax procumbens*, and also in the Primula tree leaves [8–10]. 7, 8-DHF is an orally bioavailable natural drug and have also been reported to serve as receptors for tropomyosin receptor kinase B (TrkB) and neurotrophin brain-derived neurotrophic factor (BDNF) [11–13] and it can penetrate through the blood-brain barrier [14,15]. Because of its importance and potential activity, a pro-drug of 7, 8-DHF has been in development for the treatment of Alzheimer's disease [16]. It is also useful for the disorders of the central nervous system [13]. Besides aromatase, it also found to inhibit aldehyde dehydrogenase and estrogen sulfotransferase [11].

Baicalein is chemically 5, 6, 7-trihydroxyflavone and has been isolated from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora* and is also found in *Oroxylum indicum* or Indian trumpet flower. Its glycone derivative is termed as Baicalin. Sho-Saiko-To, a Chinese herbal supplement used for enhancing liver health has believed to contain Baicalein as one of the active ingredients. Baicalein is a positive allosteric modulator. In mice, Baicalein shows anxiolytic effects without causing sedation or myorelaxation [17–20]. It acts as an antagonist for estrogen receptor and shows inhibitory action towards lipoxygenases. Moreover, it also has anti-inflammatory and anti-proliferative effects. In animals, it also imparts some anti-depressant action.

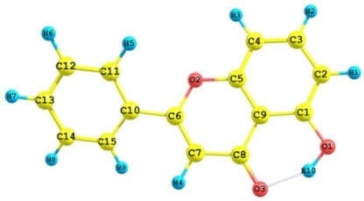
Chrysin is also an important flavone, found in propolis, honeycomb, *Passiflora caerulea* and *Oroxylum indicum* [21]. It is an

active ingredient in the dietary supplements. It is a dihydroxy flavone present in carrots and some flowers. Its concentration in propolis has found to be greater than that in honey. It has low oral bioavailability but has rapid excretion. Studies have shown that a daily oral intake of about 0.5 to 3.0 g Chrysin is safe [22]. Its potential in the treatment of Parkinson's disease is under investigation. Isovitexin is apigenin-6-glucoside found in passionflowers, cannabis, and Acai palm. It is also an active ingredient in fenugreek seeds. Luteolin is an important flavone seen in the leaves and barks of plants. The sources of Luteolin are celery, green pepper, parsley, etc.

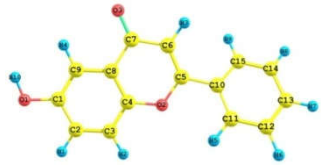
In the following sections, the structural features, radical scavenging capacity, the possible mechanisms for radical scavenging, the global reactive descriptors, toxicity and pharmacokinetics analysis of the 11 flavones are evaluated through a DFT based computational approach.

6.2 Structural characterization of flavones

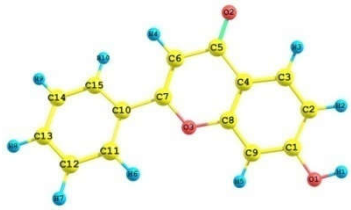
All the flavones have optimized to get the global minimum structure. Initially, a potential energy scanning (PES) is carried out on the basic flavone structure and the most stable structural form has been deduced and has been used for further studies. All the flavones were modified from this basic structural moiety and optimized to get the global minimum structures with the DFT/-B3LYP level of theory and 6-31+G (d, p) as the basis set [23,24]. All the computational calculations were done by using Gaussian 09 software package [25]. The optimized structures of studied flavones have shown in Fig 6.2.



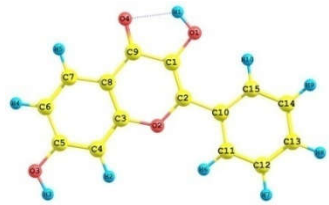
5-Hydroxyflavone



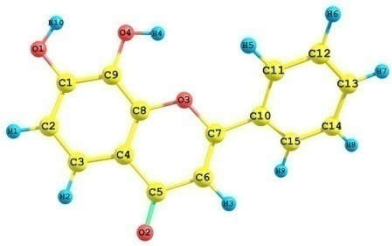
6- Hydroxyflavone



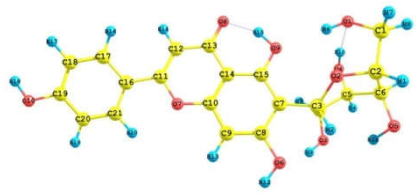
7- Hydroxyflavone



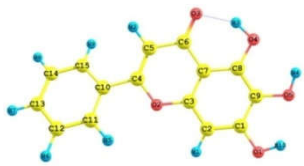
3, 7-Dihydroxyflavone



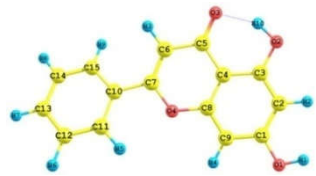
7, 8-Dihydroxyflavone



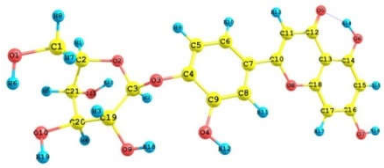
Isovitexin



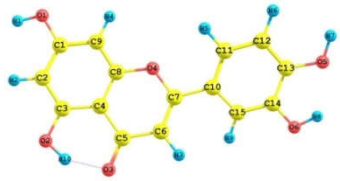
Baicalein



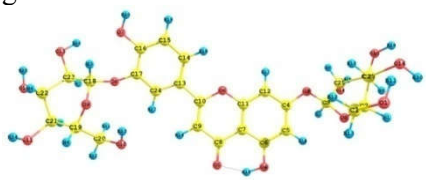
Chrysin



Luteolin-4'-glucoside



Luteolin



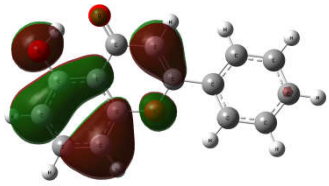
Luteolin-3', 7-diglucoside

Fig.6.2 Optimized structures of studied flavones

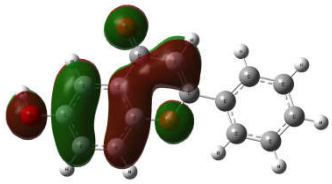
The molecular structures of flavones are highly delocalized. The conjugation passes from ring [A] to ring [B] through the C2-C3 double bond of the ring [C]. The flavones have said to possess a planar structure. The effect of hydrogen bonds can also see in its properties, which have been discussed later in this chapter.

6.3 Frontier Molecular Orbital (FMO) analysis

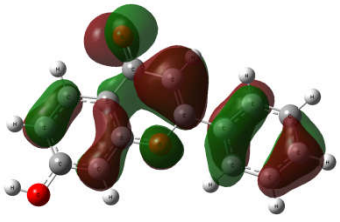
Here the energies of different molecular orbitals have discussed. The energies of HOMO and LUMO of a molecule are important quantum chemical descriptors as their difference serves as band gap in solids. These are of particular relevance in the study of charge-transfer complex formation reactions. The HOMO and LUMO orbital pictures of studied flavones have shown in Figs. 6.3 and 6.4.



5-Hydroxyflavone



6- Hydroxyflavone



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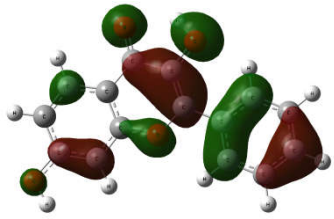
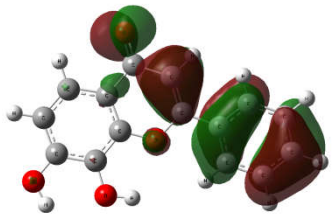
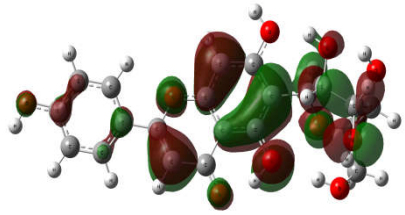


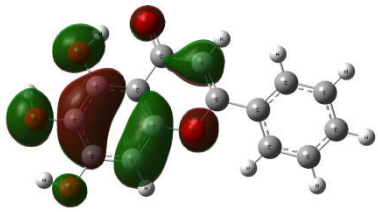
3, 7-Dihydroxyflavone



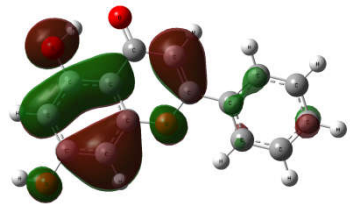
7, 8-Dihydroxyflavone



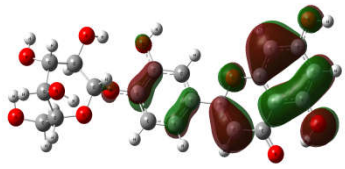
Isovitexin



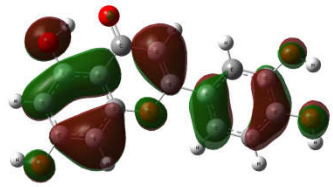
Baicalein



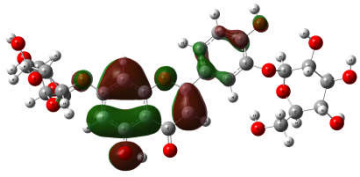
Chrysin



Luteolin-4'-glucoside



Luteolin

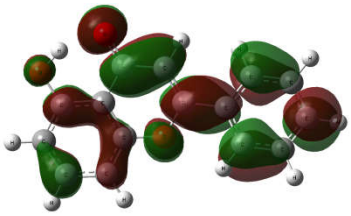


Luteolin-3', 7-diglucoside

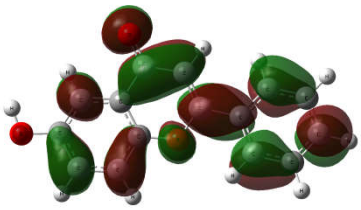
Fig.6.3 HOMO of studied flavones

The HOMO of 5-Hydroxyflavone and 6-Hydroxyflavone have delocalized over rings [A] and [C] while that of 7-Hydroxyflavone has been delocalized over the entire molecule. In the case of 3, 7-Dihydroxyflavone, the HOMO is delocalized completely over the rings [B] and [C] and partially over the ring [A]. In 7, 8-Dihydroxyflavone, the HOMO is delocalized over the ring [B] and 3/4th of the ring [C]. In

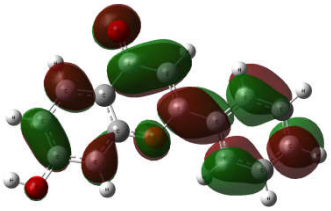
Isovitexin the HOMO is delocalized over rings [A] and [C] along with the glucose moiety. For Baicalein and Chrysin, the HOMO has found to delocalize over the rings [A] and [C]. In the case of Luteolin-4'-glucoside, the delocalization of HOMO has been found over the ring [B] and its substituents. For Luteolin, the HOMO of completely delocalized over the molecule while the delocalization has been found to decrease on Luteolin-3', 7-diglucoside where the HOMO is delocalized over the rings [A] and [C] and some parts of the ring [B]. However, the delocalization has not seen over its glucose moieties.



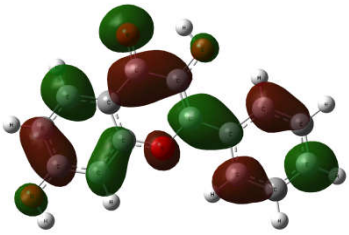
5-Hydroxyflavone



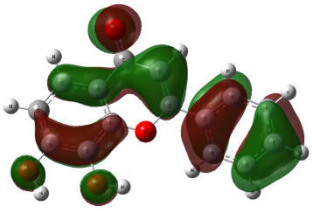
6- Hydroxyflavone



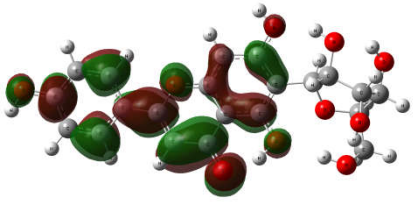
7- Hydroxyflavone



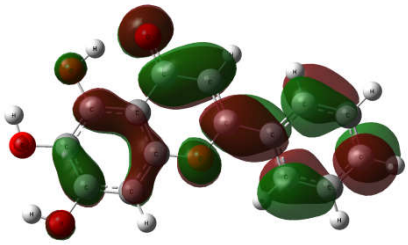
3, 7-Dihydroxyflavone



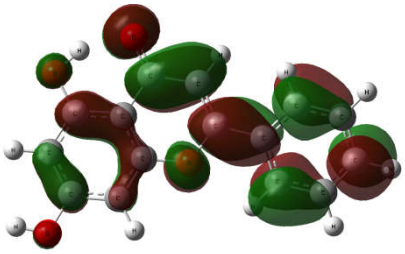
7, 8-Dihydroxyflavone



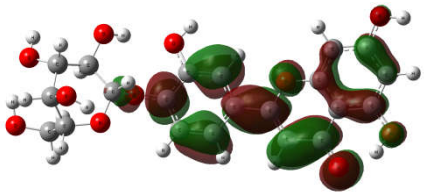
Isovitexin



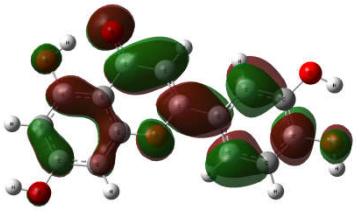
Baicalein



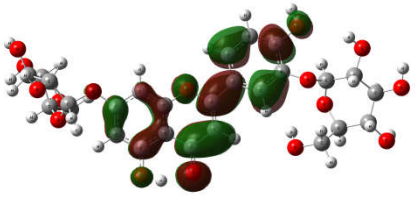
Chrysin



Luteolin-4'-glucoside



Luteolin



Luteolin-3', 7-diglucoside

Fig.6.4 LUMO of studied flavones

Unlike the HOMO, the LUMO of 5-Hydroxyflavone and 6-Hydroxyflavone have delocalized over the entire molecule. The same has been found for 7-Hydroxyflavone, 3, 7-Dihydroxyflavone and 7, 8-Dihydroxyflavone. This makes the transition between HOMO and LUMO levels of 7-Hydroxyflavone and 3, 7-dihydroxyflavone more feasible and is confirmed through the TDDFT tool in Gaussian 09 software package. The interaction of HOMO and LUMO orbitals of these two compounds are highly feasible so that the corresponding transition has highest oscillator strength in the UV-Visible spectrum of

these compounds. For Isovitexin, LUMO delocalized over the entire molecule, except on the glucose moiety. In the case of Baicalein and Chrysin, unlike the HOMO, LUMO is completely delocalized. Similarly, for Luteolin-4'-glucoside also, the LUMO is delocalized over the entire molecule, but not over the glucose moiety. For Luteolin and Luteolin-3', 7-diglucoside, the delocalization is similar to that of HOMO. This has also confirmed through the TDDFT studies. The most intense transition in the UV-Visible spectrum of Luteolin and Luteolin-3', 7-diglucoside are thus those between HOMO and LUMO levels.

The HOMO-LUMO energies, band gaps and dipole moments of studied flavones have given in Table 6.1

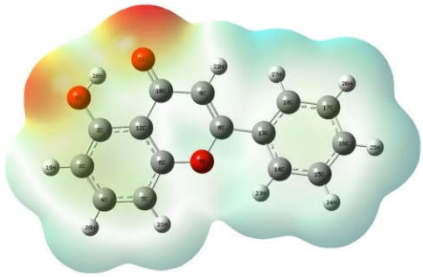
Table 6.1

Band gaps and dipole moments of studied flavones

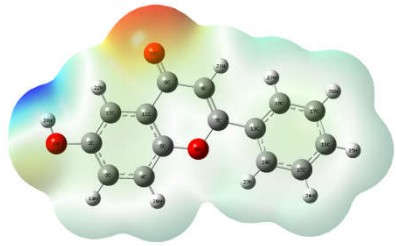
No.	Compound	Energy gap (eV)	Dipole moment (Debye)
1	3, 7-Dihydroxyflavone	3.9016	4.0486
2	5-Hydroxyflavone	4.0134	5.0067
3	6-Hydroxyflavone	4.2539	3.1019
4	7-Hydroxyflavone	4.5963	3.2463
5	7, 8-Dihydroxyflavone	4.2434	5.6249
6	Baicalein	3.8379	2.9069
7	Chrysin	4.1103	3.6893
8	Luteolin-4'-glucoside	4.0629	4.9487
9	Isovitexin	4.1353	11.2212
10	Luteolin	4.1459	6.4460
11	Luteolin-3', 7-diglucoside	4.0528	10.0249

Table 6.1 clearly shows that the Baicalein has the lowest and 7-Hydroxyflavone has the highest energy or band gap among the studied flavones. Isovitexin has the highest dipole moment while Baicalein has the lowest. The polar nature of a molecule increases with its dipole moment. Flavonoids without the –OH group at position 3 (flavones) are found to be more polar than those with an –OH group at position 3 (flavonols). So Isovitexin is the most and Baicalein is the least polar flavone among the studied flavones. Knowing the dipole moment of a molecule, one could predict the interaction of the molecule with biological systems, i.e., with the molecular targets like enzymes or proteins. Even a small change in orientations of functional groups in a molecule will produce a remarkable change in its dipole moment.

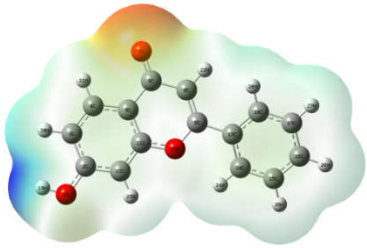
Electrostatic potential energy is fundamentally a measure of the strength of the nearby charges, nuclei, and electrons, at a particular position [26]. The ESP of all the compounds has shown in Fig.6.5.

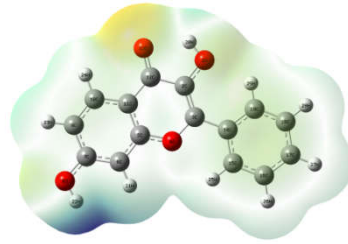


5-Hydroxyflavone



6- Hydroxyflavone





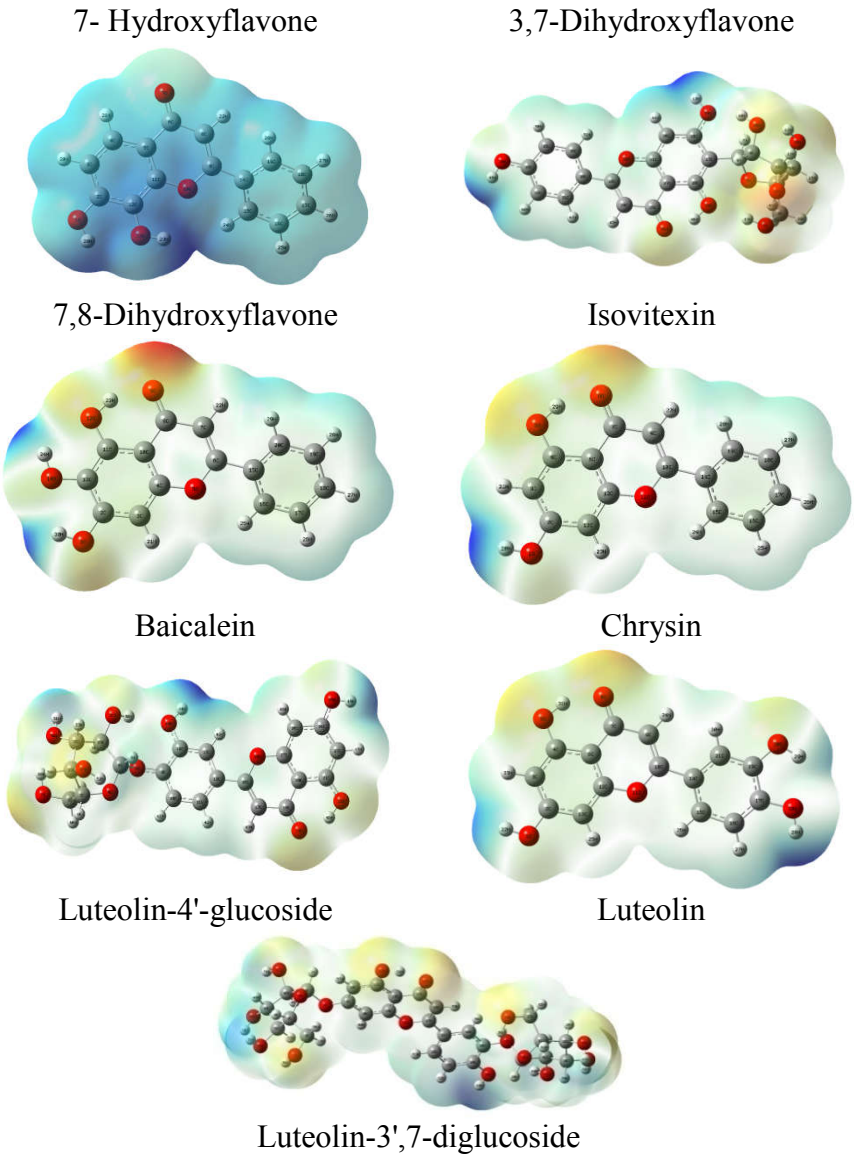


Fig.6.5 ESP of studied flavones

Fig 6.5 clearly represents the electrostatic distribution of studied compounds. The knowledge of charge distribution gives information on how a molecule interacts with one another and is very important in organic chemistry. Here the red region denotes the areas

of lower potential and hence higher electron density while the blue region is the area with high electrostatic potential and with a low concentration of electrons. Here the atoms present in our compounds are C, H, and O; the red region locates on the most electronegative oxygen atom. The ESP map is also useful for determining the nature of chemical bonds between two atoms based on their electronegativity differences. The colours other than red and blue represents the electronegativity difference. These have seen in between red and blue coloured region. When this region with colours green-yellow is increased, the charge is very polarized and there is a significant difference in the electronegativity values. In such cases, a complete red/blue region has seen. This has been observed for 7, 8-Dihydroxyflavone. For all other flavones, the red region locates on an oxygen atom while the blue region locates over the hydrogen atom.

6.4 UV-Visible spectra of flavones

The most interesting area in the electromagnetic spectrum is the visible region as it gives colour to our day-to-day life. This was obtained from the absorption spectrum of molecules. The UV-Visible spectral characteristics of flavones are given in Tables 6.2 and 6.3 and are shown in Fig. 6.6.

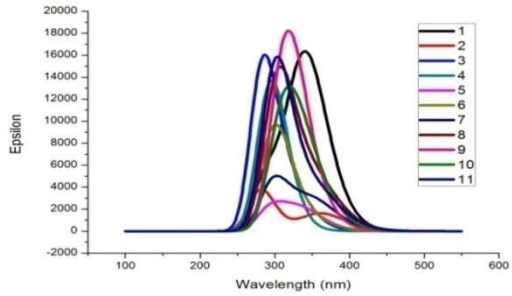
Table 6.2

UV-Visible spectral properties of studied flavones in the gas phase

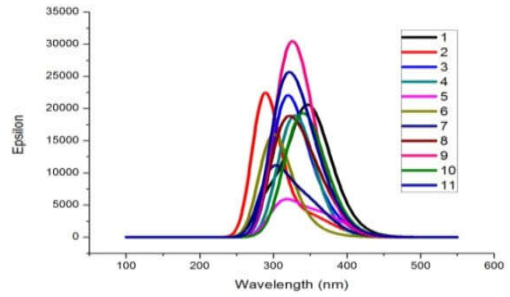
Compound	Energy (eV)	λ_{max} (nm)	Oscillator strength	MOs involved	% contribution
3,7-dihydroxyflavone	3.63	342	0.39	HOMO-LUMO	95.64
	4.11	302	0.01	HOMO-4-LUMO	96.57
	4.28	290	0.13	HOMO-2-LUMO	80.58
5-hydroxyflavone	3.43	362	0.04	HOMO-LUMO	97.57
	3.87	321	0.02	HOMO-4-LUMO	81.24
	4.40	282	0.09	HOMO-3-LUMO	38.76
6-hydroxyflavone	3.51	353	0.02	HOMO-2-LUMO	73.13
	3.83	324	0.08	HOMO-LUMO	93.83
	4.35	285	0.38	HOMO-3-LUMO	65.04
7-hydroxyflavone	3.51	353	0.01	HOMO-3-LUMO	85.97
	3.68	337	0.04	HOMO-2-LUMO	97.70
	4.19	296	0.05	HOMO-LUMO	73.94
7,8-dihydroxyflavone	3.51	354	0.04	HOMO-1-LUMO	52.61
	4.16	298	0.26	HOMO-3-LUMO	63.83
	4.27	290	0.09	HOMO-LUMO	66.81
Baicalein	3.30	376	0.02	HOMO-LUMO	94.61
	3.84	323	0.24	HOMO-2-LUMO	94.47
	3.98	311	0.02	HOMO-4-LUMO	92.01
Chrysin	3.51	353	0.07	HOMO-LUMO	97.74
	3.93	316	0.02	HOMO-4-LUMO	90.90
	4.14	300	0.12	HOMO-2-LUMO	89.86
Luteolin-4'-glucoside	3.47	357	0.10	HOMO-LUMO	96.84
	3.89	318	0.01	HOMO-5-LUMO	90.23
	4.06	306	0.35	HOMO-2-LUMO	76.75
Isovitexin	3.59	346	0.04	HOMO-LUMO	92.90
	3.90	318	0.38	HOMO-2-LUMO	79.60
	3.98	312	0.05	HOMO-7-LUMO	51.65
Luteolin	3.57	347	0.11	HOMO-2-LUMO	88.94
	3.95	314	0.22	HOMO-LUMO	68.79
	3.97	313	0.05	HOMO-5-LUMO	68.24
Luteolin-3',7-diglucoside	3.47	357	0.08	HOMO-2-LUMO	96.67
	3.87	321	0.01	HOMO-4-LUMO	92.90
	4.10	303	0.38	HOMO-LUMO	70.60

Here in the studied flavones, there are three flavones with glucose linkages. Among them the Luteolin-3', 7-diglucoside is the

compound with glucose substitution on Luteolin at 3' and 7 positions. The glucose substitution decreases the λ_{max} of Luteolin from 314 nm to 302 nm in Luteolin-3', 7-diglucoside. The flavones with only one –OH groups are 5-hydroxyflavone, 6-hydroxyflavone and 7-hydroxyflavone. For the first two compounds, the λ_{max} is due to the HOMO to LUMO and HOMO-3 to LUMO transition respectively, while for the third; it is due to the transition between HOMO-2 and LUMO. The other two peaks are due to the transition between HOMO-LUMO and HOMO-4 – LUMO. The λ_{max} value follows the order 5-hydroxyflavone < 6-hydroxyflavone < 7-hydroxyflavone. This indicates the position of –OH group has some influence on the absorption characteristics of flavones. The absorption due to transition between HOMO-LUMO levels is high for 5-hydroxyflavone while is low for 6-hydroxyflavone. The flavones 7, 8-Dihydroxyflavone and 3, 7-Dihydroxyflavone, there are two –OH groups. In the former one, the –OH groups are adjacent to each other while they are far apart in the latter. The λ_{max} for 7, 8-Dihydroxyflavone is due to HOMO-3-LUMO while the λ_{max} of 3, 7-Dihydroxyflavone is due to HOMO-LUMO transition. However, the λ_{max} increases on introducing the second –OH group. In most cases, the λ_{max} is due to the transition between HOMO-2 and LUMO. For others, it is due to HOMO-LUMO or HOMO-3 to LUMO transitions.



a



b

Fig. 6.6 Computed UV-Visible spectrum of studied flavones in (a) gas and (b) water

In the aqueous phase, λ_{max} is due to HOMO-1 to LUMO transition for most of the studied flavones while HOMO to LUMO transition has also observed for some flavones for their λ_{max} values. Here also the λ_{max} value decreases on glucose linkage as can be seen for Luteolin-3', 7-diglucoside and Luteolin. For flavones containing only one –OH group, the order of λ_{max} is different from that in the gas phase. The λ_{max} value of 6-Hydroxyflavone id found to be greater than that of 7-Hydroxyflavone. However the as the number of –OH group increases, the λ_{max} value also increased. This is clear from the λ_{max} value of 3, 7-dihydroxyflavone and 7, 8-dihydroxyflavone. The trend is the same as that in the gas phase.

Table 6.3

UV-Visible spectral properties of studied flavones in the aqueous phase

Compound	E (eV)	λ_{max} (nm)	oscillator strength	% contribution	MOs involved
3,7-dihydroxyflavone	3.56	348	0.50	96.12	HOMO-LUMO
	4.20	295	0.161	83.56	HOMO-1-LUMO
	4.24	293	0.001	96.59	HOMO-4-LUMO
5-hydroxyflavone	3.52	352	0.076	97.29	HOMO-LUMO
	4.05	306	0.01	85.38	HOMO-4-LUMO
	4.29	289	0.55	85.85	HOMO-1-LUMO
6-hydroxyflavone	3.73	332	0.005	90.88	HOMO-2-LUMO
	3.78	328	0.108	94.37	HOMO-LUMO
	4.23	293	0.51	87.76	HOMO-1-LUMO
7-hydroxyflavone	3.67	338	0.08	97.81	HOMO-LUMO
	3.78	328	0.04	80.15	HOMO-3-LUMO
	4.26	291	0.14	51.26	HOMO-1-LUMO
7,8-dihydroxyflavone	3.75	331	0.03	91.34	HOMO-2-LUMO
	4.05	306	0.40	91.03	HOMO-LUMO
	4.28	290	0.09	80.45	HOMO-1-LUMO
Baicalein	3.35	370	0.03	94.94	HOMO-LUMO
	3.85	322	0.39	95.23	HOMO-1-LUMO
	4.14	299	0.01	89.94	HOMO-4-LUMO
Chrysin	3.57	347	0.12	97.62	HOMO-LUMO
	4.11	302	0.06	71.30	HOMO-4-LUMO
	4.14	299	0.20	77.34	HOMO-1-LUMO
Luteolin-4'-glucoside	3.53	352	0.20	95.22	HOMO-LUMO
	3.94	315	0.37	91.55	HOMO-1-LUMO
	4.10	302	0.02	91.10	HOMO-5-LUMO
Isovitexin	3.65	340	0.31	82.35	HOMO-LUMO
	3.89	319	0.51	84.24	HOMO-1-LUMO
	4.18	296	0.01	36.43	HOMO-7-LUMO
Luteolin	3.59	345	0.36	76.73	HOMO-LUMO
	3.78	328	0.14	77.50	HOMO-1-LUMO
	4.16	298	0.01	89.64	HOMO-4-LUMO
Luteolin-3',7-diglucoside	3.56	348	0.25	91.48	HOMO-LUMO
	3.94	315	0.51	89.45	HOMO-1-LUMO
	4.11	302	0.02	65.37	HOMO-5-LUMO

All the studied flavones have absorption in the range 285-375 nm, which is in the UV-A and UV-B region. This confirms the use of flavones as UV filters in sunscreen lotions and other related cosmetic

products. For most of the studied flavones, the λ_{max} values have observed in the range of UV-B radiation. Some of the flavones have λ_{max} values in the UV-A region also. In all the media studied here (gaseous and water), the flavones act as UV filters.

6.5 Analysis of global descriptive parameters

There are two types of parameters namely global and local descriptive parameters to predict the chemical reactivity of a compound of which global parameters have discussed here. The global descriptive parameters including chemical hardness, chemical softness, chemical potential, electronegativity, electrophilicity, vertical ionization energy and vertical electron affinity have computed for the 11 flavones and have evaluated and are given in Table 6.4. Here the numbers from 1-11 are respectively 3, 7-Dihydroxyflavone, 5-Hydroxyflavone, 6-Hydroxyflavone, 7-Hydroxyflavone, 7-8-Dihydroxyflavone, Baicalein, Chrysin, Luteolin-4'-glucoside, Isovitexin, Luteolin, and Luteolin-3', 7-diglucoside.

Table 6.4

Global reactive descriptors (eV) of flavones

No	IE	EA	η	S	χ	μ	ω	Q_{max}	ω-	ω+
1	7.16	0.47	3.34	0.15	3.82	-3.8	2.18	1.14	4.5	0.69
2	7.28	0.69	3.29	0.15	3.98	-4	2.41	1.21	4.81	0.83
3	7.61	0.46	3.57	0.14	4.03	-4	2.28	1.13	4.74	0.71
4	7.74	0.41	3.66	0.14	4.08	-4.1	2.27	1.11	4.77	0.69
5	7.6	0.53	3.53	0.14	4.07	-4.1	2.34	1.15	4.82	0.75
6	6.8	0.68	3.06	0.16	3.74	-3.7	2.28	1.22	4.54	0.8
7	7.51	0.64	3.44	0.15	4.08	-4.1	2.42	1.19	4.89	0.81
8	7.08	0.98	3.05	0.16	4.03	-4	2.66	1.32	5.05	1.03
9	7.25	1.09	3.08	0.16	4.17	-4.2	2.82	1.35	5.29	1.12
10	7.24	-6	6.61	0.08	0.63	-0.6	0.03	0.09	1.17	0.54
11	6.98	1	2.99	0.17	3.99	-4	2.66	1.33	5.02	1.04

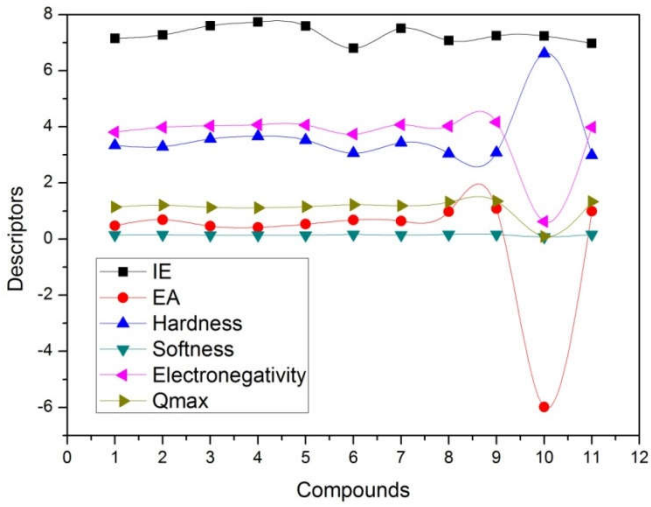


Fig.6.7 Global descriptors of flavones

The global reactive descriptors show that the compound 10 (Luteolin) has maximum value for hardness, which indicates that it is the least reactive molecule among the studied flavones. The electron affinity, charge-holding capacity (Q_{max}) and the electronegativity are low for molecule 10. So for molecule 10, it is better to donate electron than to accept. This is a clear indication of a potent antioxidant capacity of the molecule. All others have almost comparable reactivities.

6.5.1 Full Electron Donor Acceptor Map (FEDAM)

To explain the one-electron transfer mechanism of antiradicals, IE and EA are the two suitable parameters. The Full Electron Donor-Acceptor Map (FEDAM) has been employed to study the electron donating/accepting tendency simultaneously [27]. The FEDAM of studied compounds have shown in Fig.6.8.

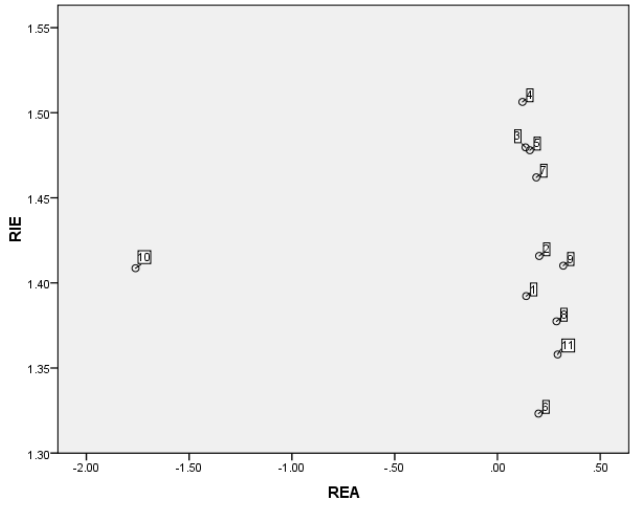


Fig.6.8. FEDAM of studied compounds.

If the RIE value is less than one then the flavones will have a greater capacity to donate electron than Na. Similarly, if the REA value is greater than one then the flavones will be more effective electron acceptors than F. However, here, none of the studied flavones satisfies these two conditions implying that the studied flavones are less efficient electron donors and acceptors than Na and F respectively. However, we could have a comparison between the studied flavones by themselves and could reach a generalization by using FEDAM. The flavone 10 has the lowest EA value so that it does not accept an electron during the radical scavenging reaction. This confirms that flavone 10 is exclusively an antioxidant. All others have high EA in comparison with flavones 10. However, the flavones 3, 4, 5, and 7 have high IE and EA values indicate that they can accept electron than to donate. Therefore, they are antireductant. The flavones 1, 2, 6, 8, 9, and 11 have low IE and high EA value so that they can act as both antioxidant and antireductant.

Nevertheless, in most of the situations, we are not sure that there occurs complete electron transfer. Sometimes, there is only a fractional charge transfer takes place from antiradical to free radical or vice versa. In such circumstances, we cannot adopt FEDAM and then we define another set of parameters called Electroaccepting power (ω^{+}). The tendency of a compound to accept or donate charge has expressed by using ω+ and ω-. As ω- decreases, the compound will have a strong tendency to donate electrons, while a large value of ω+ implies a strong tendency to accept electrons. Even though IE and EA have also related to, the electron donating and accepting a capacity of a compound, ω+ and ω- are more suitable for explaining the charge transfer mechanisms of antiradicals. It is because of that sometimes there is only a fractional charge transfer takes place. The former parameters are significant only when there is a complete transfer of electrons [28].

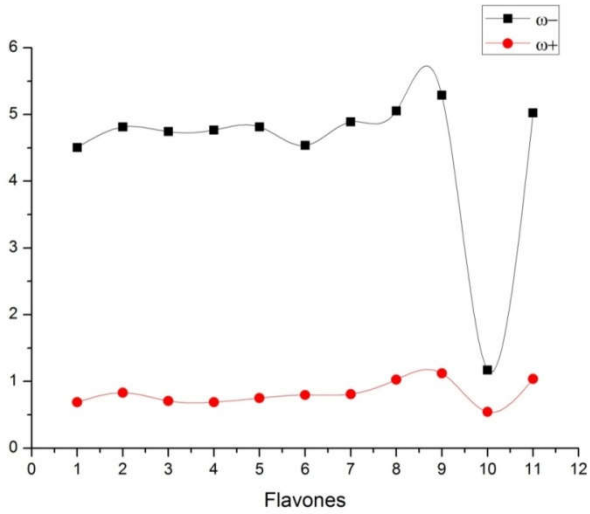


Fig.6.9. ω^{+} and ω^{-} values of studied flavones

6.5.2 Donor Acceptor Map (DAM)

DAM is a plot which simultaneously shows the electron acceptance and electron donating power of studied molecules [29–32]. Electron-accepting index with respect to F (R_a) and Electron donating index with respect to Na (R_d) is the respective parameters for DAM.

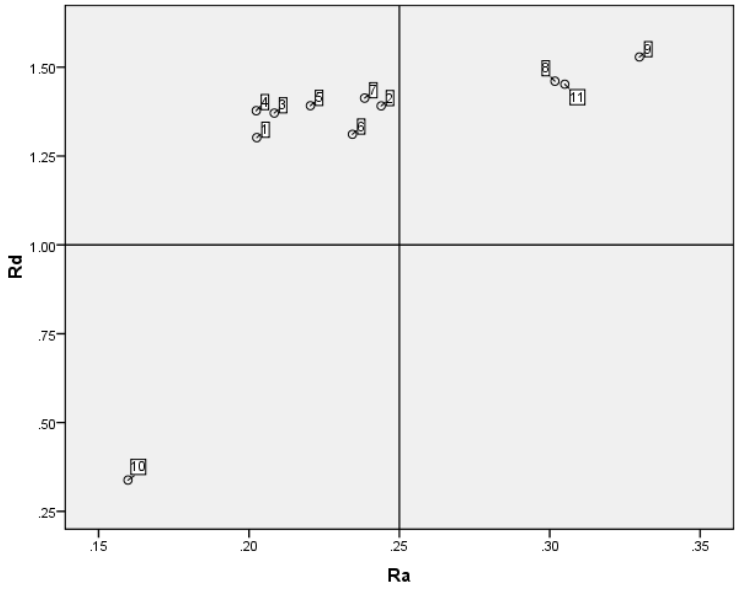


Fig.6.10. DAM of studied compounds

DAM gives a more detailed idea of charge transfer and it has observed that the flavone 10 falls in a quadrant of good antioxidants. It has a high tendency to donate a fraction of electrons to the free radicals than sodium. All others have less capacity to donate or accept electrons than Na/F as obtained from FEDAM. However, here also we can arrive at a conclusion. For fraction electron transfer, flavones 8, 11, and 9 have electron-accepting tendency and are good antireductants.

However, the remaining flavones show neither electron accepting nor donating tendency.

6.6 Analysis of antiradical capacity of flavones

There are three antiradical mechanisms discussed here. The HAT mechanism is purely hydrogen atom transfer while others involve electron transfer too. There will be competition between the hydrogen atom transfer and electron transfer mechanism. Before that, we must have an idea about the ease of hydrogen atom and electron transfer. Table 6.5 displays all the necessary parameters for the three mechanisms. The BDE values related to HAT mechanism falls in the range of 70-100 kcal/mol. As the BDE, value decreases, the antioxidant capacity will also increase. There is an inverse relationship between the BDE value and the radical scavenging capacity. Here the studied flavones include both hydroxyl and glycoside derivatives so that the effect of structural features on the BDE values can easily demonstrate.

Table 6.5

Parameters (kcal/mol) of different antiradical mechanisms

Compound	BDE	AIP	PDE	PA	ETE
3,7-Dihydroxyflavone	83.34	164.97	232.13	-11.95	409.05
5-Hydroxyflavone	99.54	167.49	245.80	-17.29	430.59
6-Hydroxyflavone	85.52	174.62	222.69	-11.68	408.99
7-Hydroxyflavone	83.79	177.56	201.99	-10.46	390.01
Luteolin-4'-glucoside	72.75	157.20	230.65	-17.52	405.36
Chrysin	77.96	171.88	229.83	-16.24	417.95
Luteolin-3',7-diglucoside	71.44	166.34	220.85	-13.33	400.53
Luteolin	75.95	164.72	231.99	-26.26	422.97
Baicalein	76.04	162.56	227.24	-23.51	413.31
Isovitexin	74.90	159.71	229.94	-24.16	413.82
7,8-Dihydroxyflavone	83.08	174.68	211.83	-13.18	399.69

The order of BDE values follows as: 5-Hydroxyflavone > 6-Hydroxyflavone > 7-Hydroxyflavone > 3, 7-Dihydroxyflavone > 7, 8-Dihydroxyflavone > Chrysin > Baicalein > Luteolin > Isovitexin > Luteolin-4'-glucoside > Luteolin-3', 7-diglucoside. Therefore, in accordance with the HAT mechanism, the order of radical scavenging capacity will be the reverse of the above-mentioned order. In Luteolin, there are four –OH groups; two each in the ring A and B of Luteolin. Among the studied flavones without glycoside moiety, Luteolin is having a maximum number of –OH groups in B ring. The radical scavenging capacity increases with the number of –OH groups as it decreases the BDE value. Moreover, this effect will more pronounce if the –OH groups are present in the ring B. This makes Luteolin have the lowest BDE value among the studied flavones. Also adjacent –OH groups decreases the BDE value more than that when they are far apart. This is seen in 3, 7-Dihydroxyflavone and 7, 8-Dihydroxyflavone. Here the-OH groups are far apart in 3, 7-Dihydroxyflavone while they are adjacent to each other in 7, 8-Dihydroxyflavone. Moreover, the –OH group in position 3 of 3, 7-Dihydroxyflavone makes a hydrogen bond with the oxygen atom of the carbonyl group in it. However, there is no such hydrogen bonding in 7, 8-Dihydroxyflavone. Consequently, their BDE value follows the order 3, 7-Dihydroxyflavone > 7, 8-Dihydroxyflavone. Generally, electron-releasing groups decrease BDE while electron-withdrawing groups increase the BDE value. Hence, one can tailor the radical scavenging capacity of the compounds by making a suitable substitution. This has seen in flavones with glycoside units in them. In Isovitexin and Luteolin-4'-glucoside there are three –OH groups; two in ring A and one in ring B and one glycoside group. The glycoside group is present in ring A for Isovitexin and that in ring B for Luteolin-

4'-glucoside. The groups in ring B have more effect as ring B is found to be reactive for flavones than the ring A. consequently the glycoside moiety in ring B decreases the BDE value more than that when it is in ring A. this results in the order of BDE values as Isovitexin > Luteolin-4'-glucoside. In Luteolin-3',7-diglucoside, there are two glycoside moieties; one each in ring A and ring B. this decreases the BDE value of parent Luteolin more profoundly and hence is having lowest BDE value among the studied flavones. The remaining flavones have only one –OH groups with them and in all of them, it is present in ring A. The reactivities at different positions and the possibility of hydrogen bonding determine the order of BDE value in such compounds. The reactivity at different site follows the order 7 > 6 > 5. This can explain as follows. There is a chance of hydrogen bonding between the hydrogen of –OH group at position 5 and carbonyl oxygen in ring C. this increase the bond length of –OH at position 5 but restricts the hydrogen atom transfer. This explains the order of BDE values as 5-Hydroxyflavone > 6-Hydroxyflavone > 7-Hydroxyflavone. The effect of hydrogen bonding has discussed through NBO analysis later in this chapter.

Up to this moment, we have discussed only the HAT mechanism. Before going to the rest, we have to find out which mechanism is more suitable for a given compound. For that, we have to get the BDE and AIP values of phenol. In the case of polyphenols including flavonoids, the selection of two mechanisms HAT or SET/SET-PT can be identified by evaluating ΔBDE and ΔAIP values, where ΔBDE and ΔAIP values are the difference in the respective parameters from that of phenol [26]. The BDE and AIP values of phenol are 82.9 and 192.05 kcal/mol in the gas phase and 97.12 and

145.14 kcal/mol in water respectively. When ΔBDE ~ -42 kJ/mol (-10.03 kcal/mol) and ΔAIP < -151 kJ/mol (-36.08 kcal/mol) the mechanism is dominated by hydrogen transfer (HAT). Here two compounds5-Hydroxyflavone and 6-Hydroxyflavone follow electron transfer mechanism while others prefer HAT mechanism (Table 6.6). Among them, Luteolin-3',7-diglucoside and Luteolin-4'-glucoside prefer both the mechanism according to the surrounding [33–35]. For the third mechanism to be suitable, both the parameters should be low. However, here for all the compounds ETE values are high enough to rule the mechanism. However, the first step of SPLET involves a proton transfer and for all the studied compounds, the value of PA has found to be low. So they can undergo a proton transfer easily. However, for the second step energy requirement is quite high so that they can go for it only if they get such a high amount of energy. Nevertheless, within this time they can undergo either of the other two mechanisms.

Table 6.6

Selection of antiradical mechanism with respect to phenol

Compound	ΔBDE	ΔAIP	Mechanism
3,7-Dihydroxyflavone	-1.1935	-27.08	HAT
5-Hydroxyflavone	14.6890	-24.56	SET
6-Hydroxyflavone	0.9438	-17.43	SET
7-Hydroxyflavone	-0.7523	-14.49	HAT
Luteolin-4'-glucoside	-11.5759	-34.85	HAT/SET
Chrysin	-6.4680	-20.17	HAT
Luteolin-3',7-diglucoside	-12.8602	-25.71	HAT/SET
Luteolin	-8.4386	-27.33	HAT
Baicalein	-8.3504	-29.49	HAT
Isovitexin	-9.4680	-32.34	HAT
7,8-Dihydroxyflavone	-1.4484	-17.37	HAT

6.6.1 Natural Bond Orbital (NBO) analysis

The NBO analysis, a unique peculiarity of computational studies that are otherwise not possible by experiments, enables chemists to see an intuitive picture of both electron orbitals and population analysis. The bonding efficiency of a molecule has often explained by bond order values. This can also obtain from the NBO output file. The strength of each bond has obtained from the result and the weakest bond, which is broken, first can predict. The optimization studies show that the radical formed from third oxygen is most stable than the other radicals. This result has further supported by bond order values. Table 6.7 shows that O3-H15 bond is having the least bond order and is the weakest one. Therefore, this bond breaks easily that the radical thus formed is more stable. Antioxidants act via a free radical scavenging mechanism and in this scenario, the stability of radicals is relevant, which in turn signifies the study of bond orders in a molecule [26].

Table 6.7

Bond orders of –OH groups in the studied Flavones

Flavone	Position	Bond order	BDE
3, 7-Dihydroxyflavone	3	0.53	85.35
	4	0.55	83.34
5-Hydroxyflavone	5	0.61	99.54
6-Hydroxyflavone	6	0.74	85.52
7, 8-Dihydroxyflavone	7	0.71	83.78
	8	0.71	83.08
7-Hydroxyflavone	7	0.74	83.79
Baicalein	5	0.59	85.53
	6	0.70	76.04
	7	0.71	89.69
Chrysin	5	0.61	100.65
	7	0.73	77.96
Luteolin-4'-glucoside	7	0.74	100.86
	5	0.61	88.16
	4'	0.73	72.75
Isovitexin	7	0.72	74.90
	5	0.57	83.89
	4'	0.73	103.99
Luteolin	5	0.60	84.28
	7	0.73	105.92
	3'	0.71	75.95
	4'	0.72	87.55
Luteolin-3', 7-Diglucoside	5	0.61	100.72
	4'	0.71	71.44

Table 6.7 clearly shows that the –OH groups with the lowest bond order have the lowest BDE value. This is not true for all the compounds. The compounds showing an exception to this was attributed to the presence of hydrogen bonding in them. In these compounds, the lowest bond order has seen on the position, where the hydrogen atom involved in the hydrogen bonding with the adjacent

carbonyl oxygen. The hydrogen bonding makes the bond weak but restricts the bond to break and limits the formation of radical at the same site. The presence of hydrogen bonding in these compounds can verify from the donor-acceptor interaction energy values obtained from the NBO analysis.

Table 6.8

Donor-acceptor interactions in hydrogen-bonded flavones

Flavones	**Donor NBO**	**Acceptor NBO (j)**	**E(2) kcal/ mol**	**E(j)-E(i) a.u.**	**F(i, j) a.u.**
Baicalein	LP (2) O at position 5	σ*O 12 - H 23	25.36	0.69	0.120
Chrysin	LP (2) O at position 5	σ* O 5 - H 29	26.33	0.70	0.123
Luteolin-4'-glucoside	LP (2) O at position 5	σ*O 22 - H 46	25.65	0.70	0.122
Isovitexin	LP (2) O at position 5	σ*O 29 - H 46	55.27	0.46	0.156
Luteolin	LP (2) O 8 at position 5	σ*O 5 - H 31	26.74	0.70	0.124
3, 7-Dihydroxyflavone	LP (2) O at position 3	σ*O 1- H 20	6.82	0.65	0.061

Table 6.8 shows the donor-acceptor interactions and their corresponding energy values of flavones in which Hydrogen bonds have seen in position 5. The high interaction energy values make the Hydrogen bonds between the lone pairs (LP) of carbonyl oxygen and the respective –OH groups strong and this, in turn, restrict the same –OH bond to undergo breaking and radical formation. Therefore, even though their bond orders are low, the high interaction energy due to the Hydrogen bond at position 5 makes the formation of radical at this

position difficult. This is same for 3, 7-Dihydroxyflavone also and the difference is that here the hydrogen bond is between the carbonyl oxygen and the hydrogen atom –OH group at position 3.

6.7 Druggability and toxicity study of flavones

The toxicological properties of studied flavones are given in Table 6.9. Here the numbers from 1-11 are respectively 3, 7-Dihydroxyflavone, 5-Hydroxyflavone, 6-Hydroxyflavone, 7-Hydroxyflavone, 7-8-Dihydroxyflavone, Baicalein, Chrysin, Luteolin-4'-glucoside, Isovitexin, Luteolin, and Luteolin-3', 7-diglucoside. A positive drug score indicates the druggability of a compound. All the studied flavones have positive drug score implies that all of them can use as drugs. The toxicological results show that all the studied flavones except 3, 7-dihydroxyflavone, 7, 8-dihydroxyflavone and Luteolin-4'-glucoside is non-irritant, non-mutagenic and non-Tumorigenic. So the probability of using 3', 7-dihydroxyflavone, 7, 8-dihydroxyflavone and Luteolin-4'-glucoside as drugs may be fatal. Low solubility implies low absorption. For most of the commercially available drugs, the solubility is found to be greater than -4.00 [36].

Table 6.9

Drug-like and toxicological parameters of studied flavones

No	MW	clogP	clogS	DL	M	T	I	R	DS
1	254	2.53	-3.38	-0.08					0.38
2	238	3.03	-3.45	0.28					0.66
3	238	3.03	-3.45	0.28					0.66
4	238	3.03	-3.45	0.28					0.66
5	254	2.68	-3.15	-0.28					0.41
6	270	2.34	-2.86	0.28					0.70
7	254	2.68	-3.15	0.27					0.68
8	448	-0.15	-2.45	-3.25					0.25
9	432	-0.08	-2.27	-1.99					0.46
10	286	1.99	-2.56	0.28					0.71
11	610	-1.99	-2.33	-3.25					0.31

All the studied flavones have solubility greater than -4.00 which indicate good solubility and thus good absorption in a biological system. Among the studied flavones, Luteolin has maximum drug score while Luteolin-4'-glucoside has minimum drug score. The drug score of mutagenic flavones is founded to be low. Among them the drug score of Luteolin-4'-glucoside is the least and may attribute to the presence of a glucose moiety in it. The drug score of Isovitexin and Luteolin-3', 7-diglucoside are also low. This is also due to the presence of a glucose moiety. However, in Luteolin-3', 7-diglucoside there are two glucose moieties, even then it has a drug score which is higher than Luteolin-4'-glucoside which has only one glucose moiety. Therefore, there may some other structural factors, which determine the drug score of compounds.

6.8 Pharmacokinetic properties of flavones

The pharmacokinetic properties of studied flavones have been carried out through Molinspiration online software. Through this the Lipinski rule of 5 (RO5) has been validated for all the 11 flavones. All the studied flavones except Luteolin-3', 7-diglucoside have molecular weight less than 500 Dalton and all have LogP values less than 5. The pharmacokinetic properties of flavones are given in Table 6.10 (numbering is same as that of Table 6.9).

Table 6.10

Pharmacokinetic properties of flavones

No.	miLogP	TPSA	nAtoms	MW	nHBA	nHBD	nViolations	nROTB	volume
1	2.94	70.67	19	254.24	4	2	0	1	216.03
2	3.47	50.44	18	238.24	3	1	0	1	208.01
3	3.23	50.44	18	238.24	3	1	0	1	208.01
4	3.23	50.44	18	238.24	3	1	0	1	208.01
5	2.97	70.67	19	254.24	4	2	0	1	216.03
6	2.68	90.89	20	270.24	5	3	0	1	224.05
7	2.94	70.67	19	254.24	4	2	0	1	216.03
8	-0.04	190.28	32	448.38	11	7	2	4	364.19
9	0.52	181.04	31	432.38	10	7	1	3	355.2
10	1.97	111.12	21	286.24	6	4	0	1	232.07
11	-1.83	269.43	43	610.52	16	10	3	7	496.31

Luteolin-3', 7-diglucoside shows violation in nHBD, nHBA and MW. Luteolin-4'-glucoside show violation in nHBD and nHBA while isovitexin shows violation in nHBD. All the other flavones obey Lipinski's rule of 5. The polar surface area of flavones is also given in Table 5.11. For a druggable molecule, the TPSA must be less than 140 A^2 [37]. Here the TPSA values of Luteolin-3', 7-diglucoside, Luteolin-4'-glucoside and isovitexin are higher than this value due to the presence of glucose unit. The number of rotatable bonds (nROTB) decides the conformational flexibility of a molecule. Oral viability criteria has set the number of rotatable bonds to be less than or equal to 10 [36,38,39]. All the studied flavones have nROTB less than 10 which again support the oral absorption of the studied flavones.

All compounds, studied, were analyzed under four criteria of known successful drug activity; GPCR ligand activity, ion channel modulation, kinase inhibition activity and nuclear receptor ligand activity. Also their activity as protease inhibitors and enzyme inhibitors are studied. A positive value of bioactivity score indicates considerable biological activity whereas a score between −0.5 and 0.00 indicates moderate activity and less than −0.5 is considered inactive. The bioactivity score towards specific targets are given in Table 6.11 (numbering is same as that of Table 6.9).

Table 6.11

Bioactivity scores against different drug targets

No.	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-0.22	-0.31	0.06	0.1	-0.46	0.17
2	-0.17	-0.06	0.06	0.15	-0.34	0.21
3	-0.16	-0.13	0.04	0.1	-0.43	0.15
4	-0.2	-0.17	0.1	0.1	-0.45	0.14
5	-0.18	-0.15	0.11	0.08	-0.41	0.22
6	0.1	-0.01	0.18	0.28	-0.02	0.43
7	-0.11	-0.08	0.15	0.3	-0.3	0.26
8	-0.12	-0.18	0.19	0.17	-0.35	0.26
9	0.12	0.02	0.15	0.23	0.04	0.47
10	-0.02	-0.07	0.26	0.39	-0.22	0.28
11	-0.03	-0.5	-0.11	-0.06	-0.04	0.07

Here all the flavones could act as enzyme inhibitor with considerable activity. Similarly, all flavones except Luteolin-3', 7-diglucoside have considerable activity against kinase inhibitor and Nuclear receptor ligand. All flavones except isovitexin have moderate activity as iron channel modulator. Luteolin and Baicalein have considerable activity against GPCR while all others have moderate activity.

6.9 Molecular docking studies

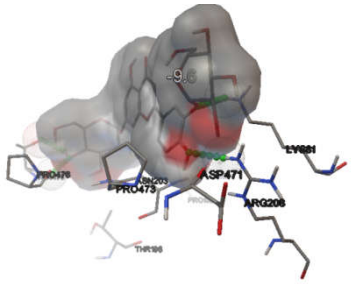
The interaction of the studied flavones with the protein MAO-B has been validated by means of molecular docking studies via Autodock Vina. Flavones binds with several amino acid residues including Cys172, Tyr398, Leu164, Trp119, Phe343, Ile316, Ile199, Leu171, Gln206, Tyr326, Phe168, Tyr435, Lys81, Arg206, Asn203, Pro473, Thr478, etc., and one water molecule HOH1366. Luteolin-3',

7-diglucoside forms two hydrogen bonds with Thr478 and one with each Lys81 and Arg206. The binding energies are given in Table 6.12 and the interactions are shown in Fig. 6.11.

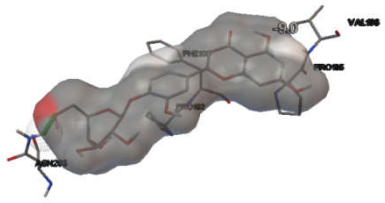
Table 6.12

Binding energies (kcal/mol) of flavones with MAO-B

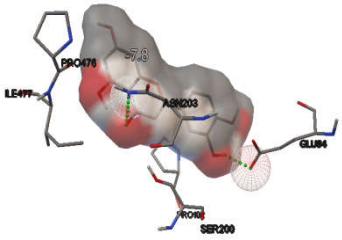
Flavones	Binding Energy	Flavones	Binding Energy
Luteolin-3', 7-diglucoside	--9.6	7, 8-Dihydroxyflavone	-7.4
Luteolin	-7.8	7-Hydroxyflavone	-7.6
Isovitexin	-8.7	6-Hydroxyflavone	-10.1
Luteolin-4'-glucoside	-9.0	5-Hydroxyflavone	-10.6
Chrysin	-7.2	3, 7-Dihydroxyflavone	-8.8
Baicalein	-7.9	SAG	-9.4



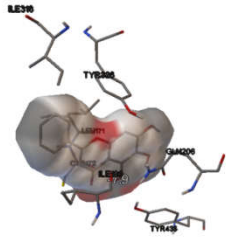
Luteolin-3', 7-diglucoside



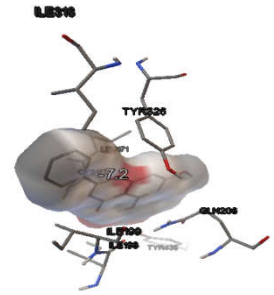
Luteolin-4'-glucoside



Luteolin



Baicalein



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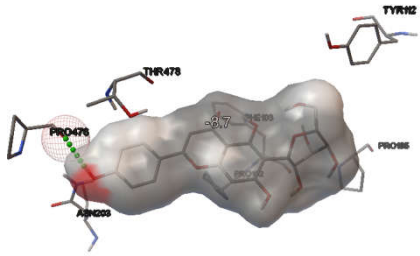
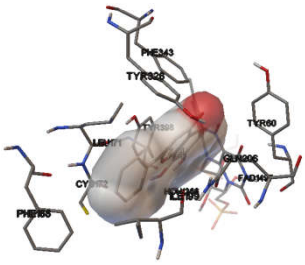
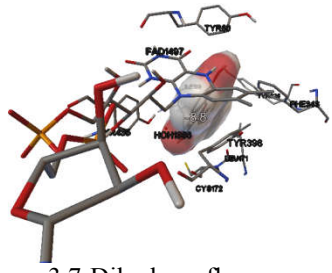


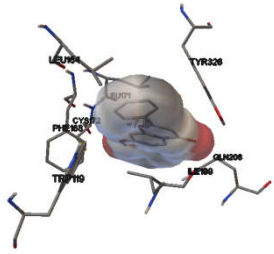
Isovitexin



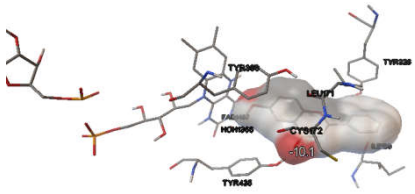
7, 8-Dihydroxyflavone



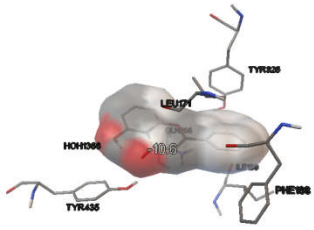
3,7-Dihydroxyflavone



7-Hydroxyflavone



6-Hydroxyflavone



5-Hydroxyflavone

Fig.6.11 Flavones in the active site of MAO-B

Conclusion

Flavones are potential phytochemicals with lots of bioactivities. The flavones have said to possess a planar structure. Baicalein has the lowest and 7-Hydroxyflavone has the highest energy or band gap among the studied flavones. Isovitexin has the highest dipole moment while Baicalein has the lowest. Here in the studied flavones, there are three flavones with glucose linkages. The glucose substitution decreases the $\lambda_{max.}$ The position of –OH group has some influence on the absorption characteristics of flavones. In most cases, the λ_{max} is due to the transition between HOMO-2 and LUMO. For others, it is due to HOMO-LUMO or HOMO-3 to LUMO transitions. All the studied flavones have absorption in the range 285-375 nm, which is in the UV-A and UV-B region. This confirms the use of flavones as UV filters in sunscreen lotions and other related cosmetic products. The global reactive descriptors show that the compound 10 (Luteolin) has maximum value for hardness, which indicates that it is the least reactive molecule among the studied flavones. For fraction electron transfer, flavones 8, 11, and 9 have electron-accepting tendency and are good antireductants. However, the remaining flavones show neither electron accepting nor donating tendency. The BDE values related to HAT mechanism falls in the range of 70-100 kcal/mol. The order of BDE values follows as: 5-Hydroxyflavone > 6-Hydroxyflavone > 7-Hydroxyflavone > 3, 7-Dihydroxyflavone > 7, 8-Dihydroxyflavone > Chrysin > Baicalein > Luteolin > Isovitexin > Luteolin-4'-glucoside > Luteolin-3', 7-diglucoside. All the studied flavones effectively bind in the active site of MAO-B and can inhibit the free radical generation

from MAO-B. The toxicological results show that all the studied flavones except 3, 7-dihydroxyflavone, 7, 8-dihydroxyflavone and Luteolin-4'-glucoside is non-irritant, non-mutagenic and non-tumorigenic. All the studied flavones except Luteolin-3', 7-diglucoside have molecular weight less than 500 Dalton and all have LogP values less than 5. The pharmacokinetic and toxicological properties of flavones are well explained.

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CHAPTER 7

EVALUATION ON THE STRUCTURE, GLOBAL PARAMETERS, ANTIOXIDANT, TOXICOLOGICAL AND PHARMACOKINETIC PROPERTIES OF FLAVONOLS

- *The evaluation of structure, radical scavenging, toxicological and pharmacokinetic properties of 23 flavonols.*
- *The effect of structural features on the radical scavenging properties.*
- *The toxicological parameters and the pharmacokinetic properties.*

7.1 Introduction

Plants in nature are gifted with lots of highly beneficial phytochemicals called flavonoids. Among the different category of flavonoids, flavonols are the most wide spread class which are distributed in various food items [1–9]. Quercetin is the most commonly studied flavonol for its antiradical property [1,6,10–15]. The proportion of flavonols in fruits and vegetables vary with varieties, seasonal changes, etc. The chemical structures of flavonols make them potent antioxidants and this favors the activity of SODs, catalase, etc., so that the oxidative defense mechanism in our body become highly active and consequently the amount of oxidation, peroxidation, etc., are greatly reduced [1,2,16,17]. Quercetin is commonly used for the protection of blood vessels.

The basic skeleton of flavonols (3-hydroxy-2-phenylchromen-4-one) (Fig.7.1) consists of 3-hydroxyflavone backbone. The C2-C3 double bond makes them distinct from flavanols (catechins).

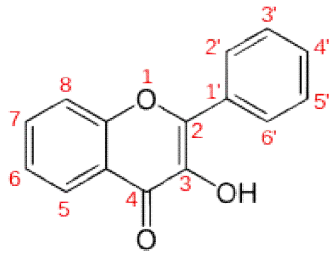


Fig.7.1 Basic structure of flavonol

As, flavonols are abundant in food items, we have been often consume about 20-50 mg per day through our diet [18]. The property

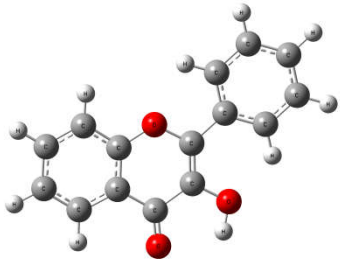
of dual fluorescence and tautomerism in flavonols and their glycosides enable them to involve in the UV protection and colour of flowers in plants [19]. Reports have shown that flavonols could act as an inhibitor for CYP2C9 [20] and CYP3A4 [18], which are enzymes that metabolize most drugs in the body.

Along with the basic flavonol moiety, 22 other flavonols including Fisetin, Azaleatin, Galangin, Gossypetin, Gossypin, Hibifolin, Isoquercetin, Isorhamnetin, Kaempferide, Kaempferol, Morin, Rutin, Rhamnetin, Rhamnazin, Quercetrin, Quercetin, Quercetagetin, Patuletin, Pachypodol, 3, 6-Dihyrdoxyflavonol, Myricitrin and Myricetin have been studied here for their antioxidant properties [21]. At first, the structures of flavonols have been analyzed and the respective quantum mechanical properties are computed. All the computational works are carried out through Gaussian 09 software package and the theory adopted is DFT-B3LYP/6-31+G (d, p). Along with the antioxidant properties, the toxicological and pharmacokinetic properties have also been evaluated. The molecular docking studies are also implemented to analyze the interaction of flavonols with MAO-B.

7.2 Structural features of flavonols

The basic structure of flavonol has been drawn by Gaussview5 and is subjected to Gaussian 09 for a PES calculation. The lowest energy conformer has been identified from the scan result and is employed for future studies. This stable conformer has been modified to get different flavonols studied and are optimized with DFT-B3LYP level of theory and 6-31+G (d, p) as basis set. The 13 conformers

obtained on scanning the dihedral connecting the rings [B] and [C] are shown below and among them conformer 4 is having the lowest energy and the dihedral angle is -179.677˚.



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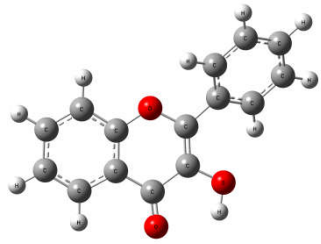

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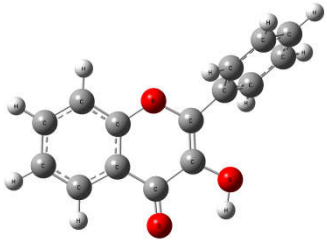

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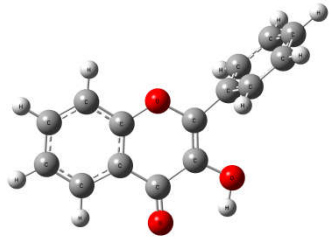

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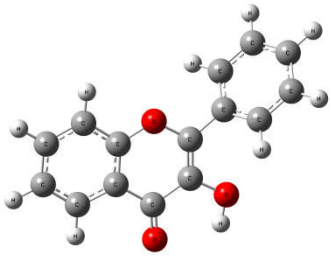

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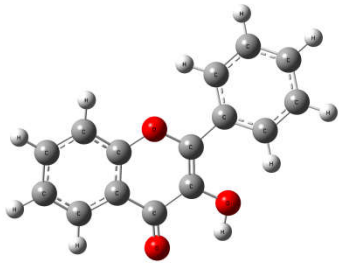

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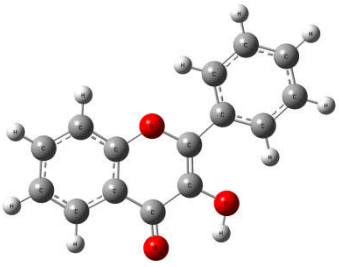

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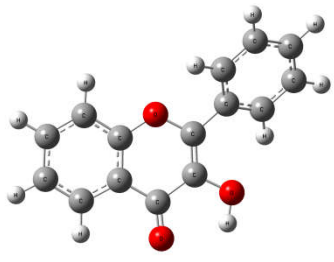

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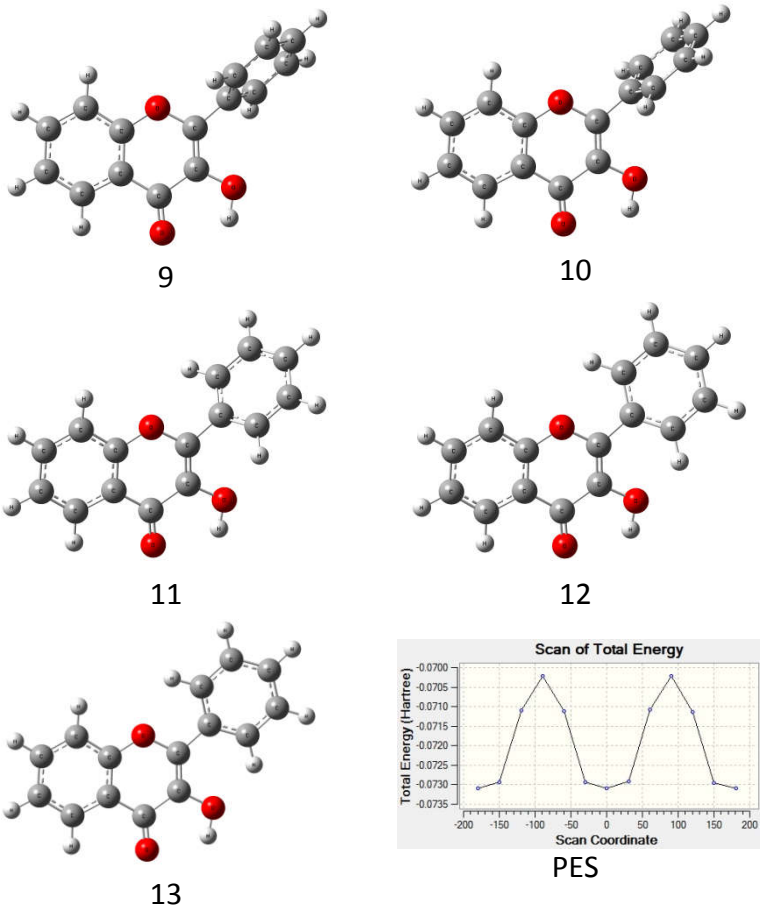
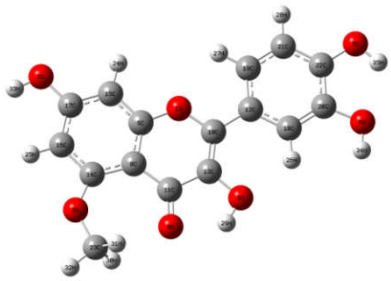
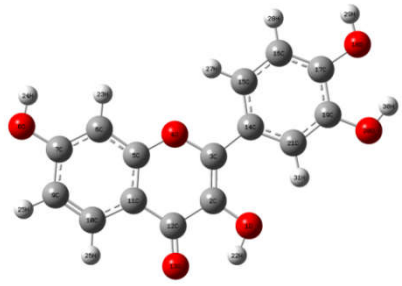


Fig.7.2. Conformers and PES diagram of flavonols

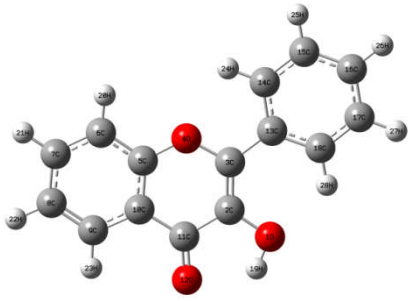
The optimized structures of flavonols are shown below. In some flavonols, glucose groups are present while in some cases hydrogen bonds are also present. This will influence the properties of flavonols; discussed later in this chapter.



Azaleatin



Fisiten



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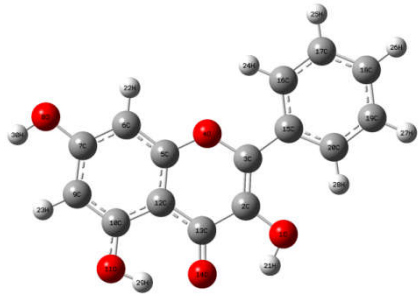

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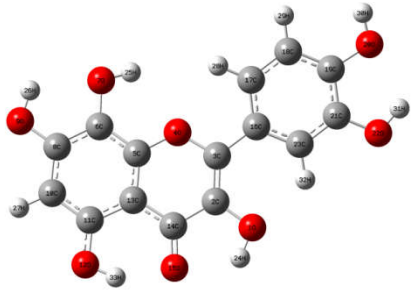

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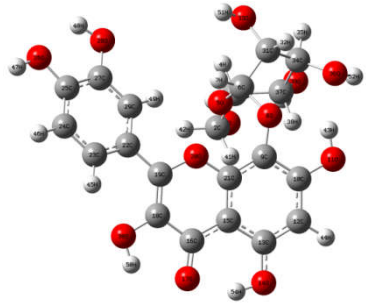

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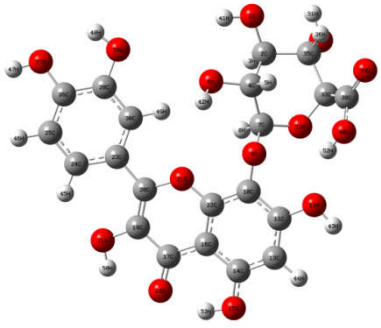
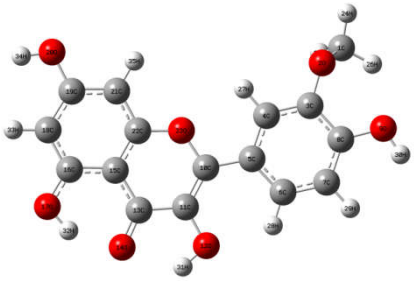


Hibifolin

Isoquercetin



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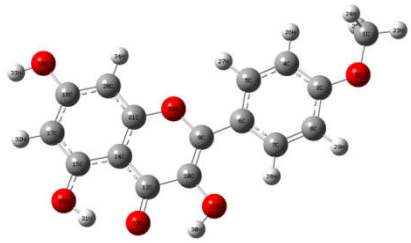

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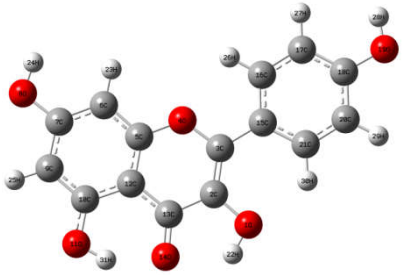

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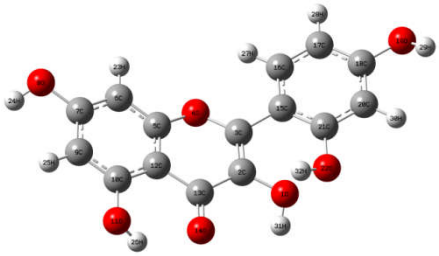
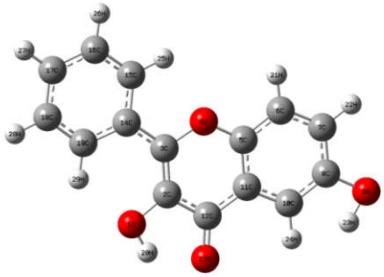


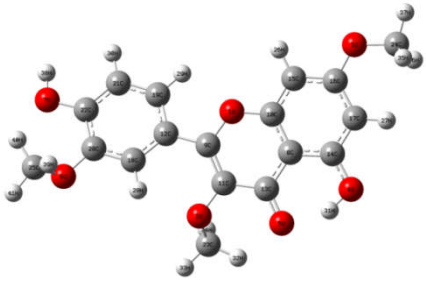
Morin

Myricetin

Myricetrin



3,6-Dihyrdoxyflavonol



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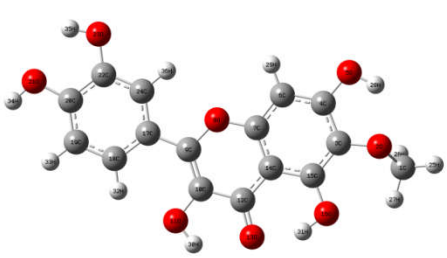

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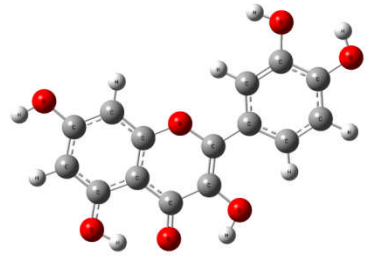

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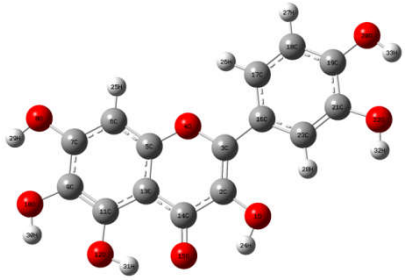

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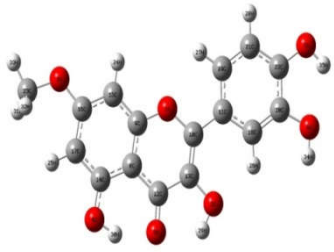
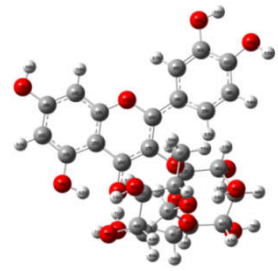


Rhamnetin



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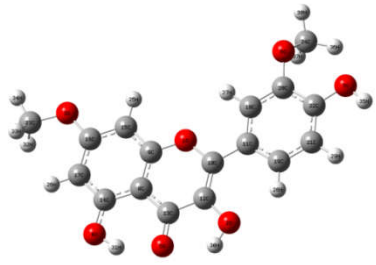
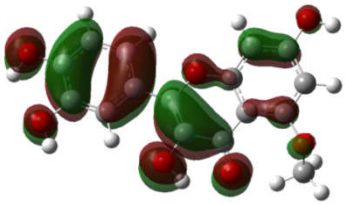


Rhamnazin

Fig.7.3. Optimized structures of flavonols

The HOMOs and LUMOs are important as their difference gives the band gap and besides this the analysis of MOs helps in the study of charge transfer reactions. The HOMO-LUMO of all the studied flavonols are shown in Figs.7.4 & 7.5. For all the flavonols the HOMOs and LUMOs are delocalized almost over the entire molecule except over the glucose groups if any.



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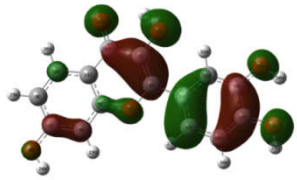

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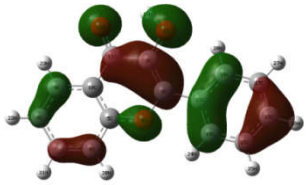

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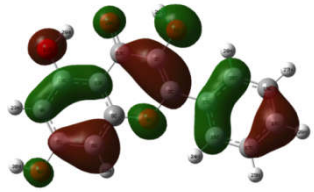

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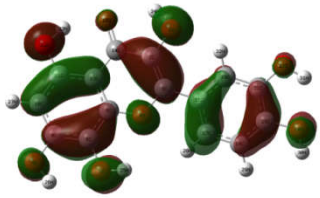

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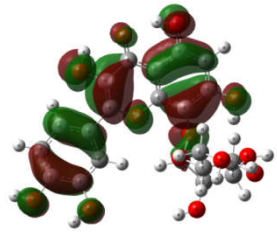

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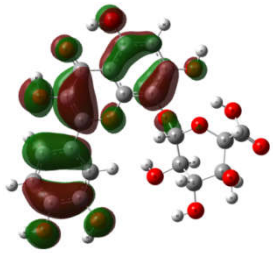
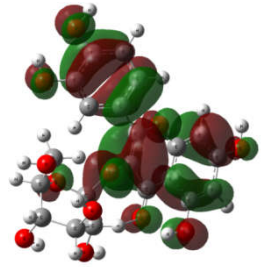


Hibifolin



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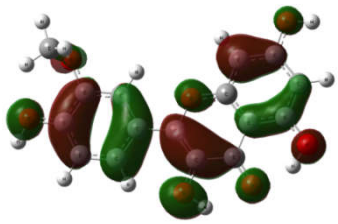

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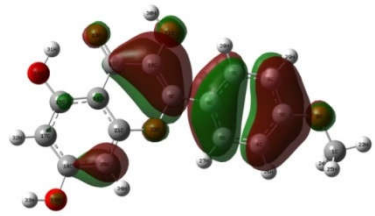

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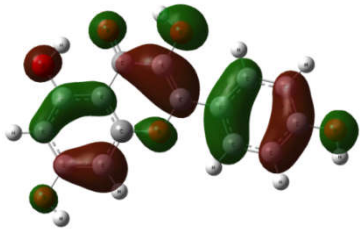

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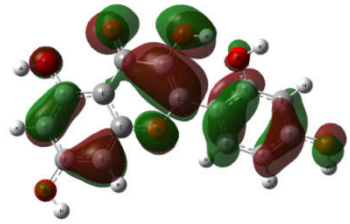
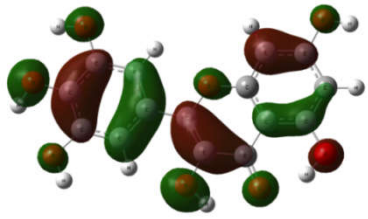
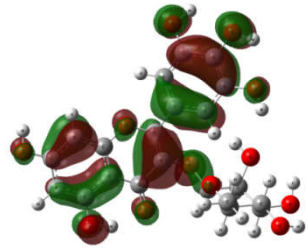


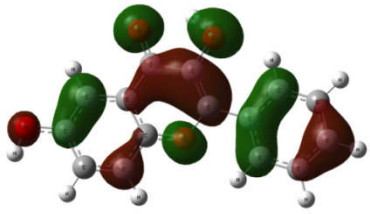
Morin



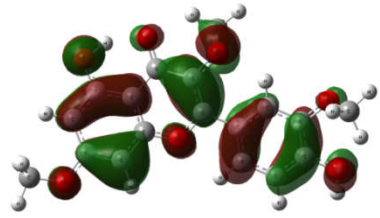
Myricetin



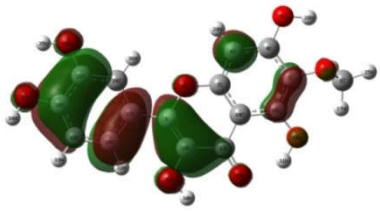
Myricetrin



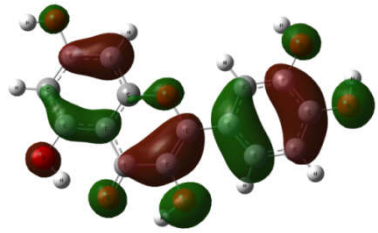
3,6-Dihyrdoxyflavonol



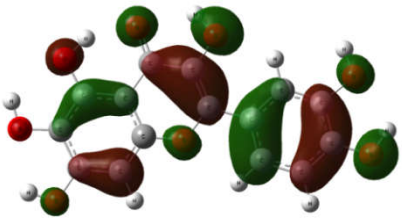
Pachypodol



Patuletin



Quercetin



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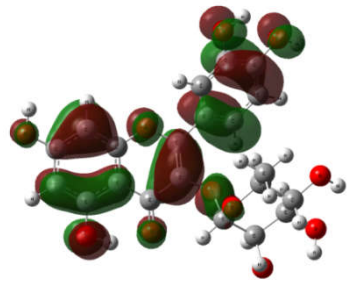

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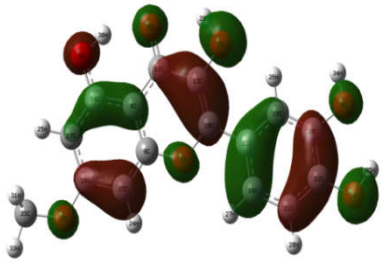
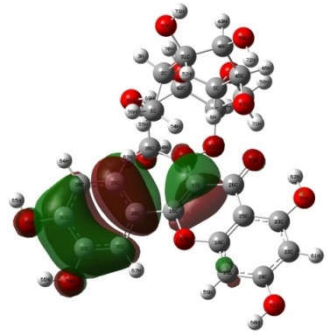
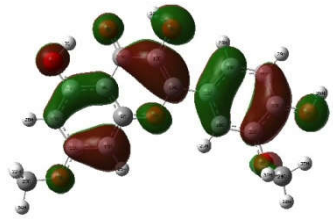


Rhamnetin

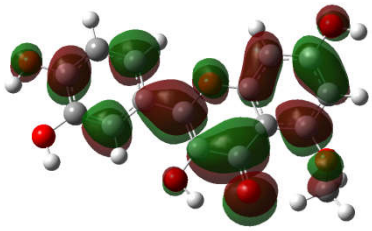


Rutin



Rhamnazin

Fig.7.4. HOMO of flavonols



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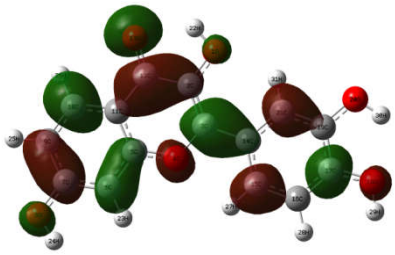

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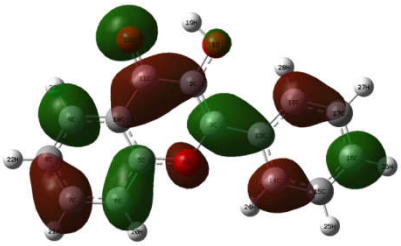
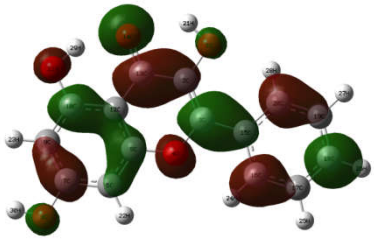
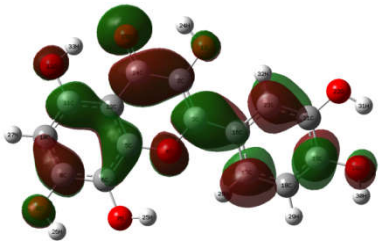


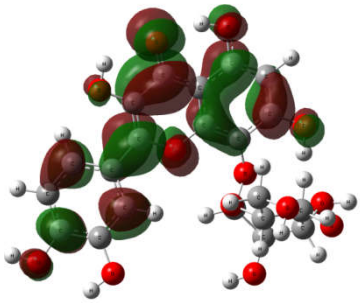
Flavonol



Galangin



Gossypetin



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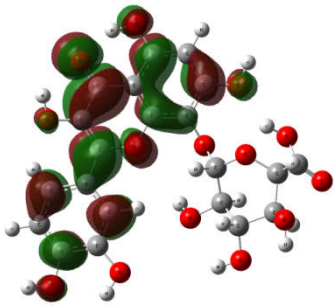

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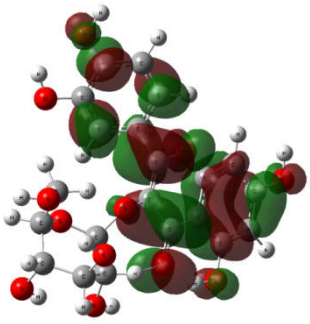

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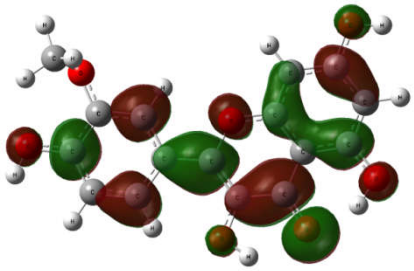

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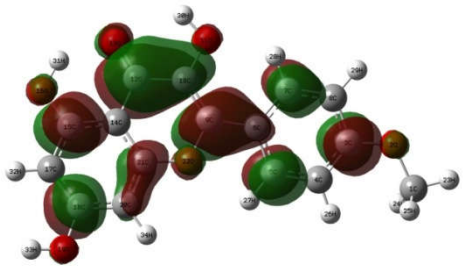

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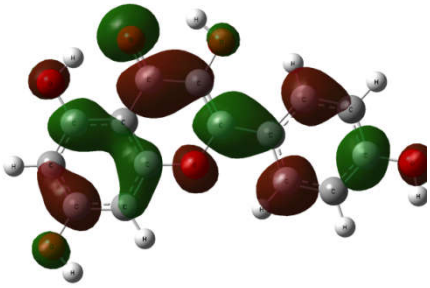

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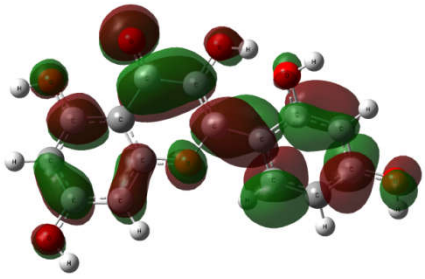
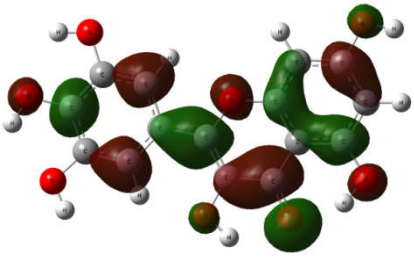
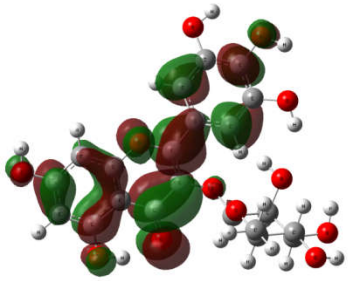


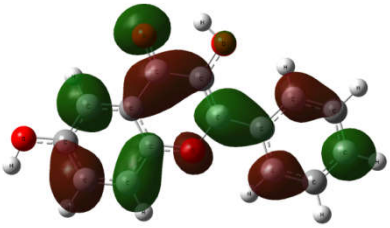
Morin



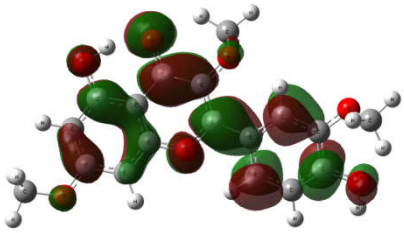
Myricetin



Myricetrin



3,6-Dihyrdoxyflavonol



Pachypodol

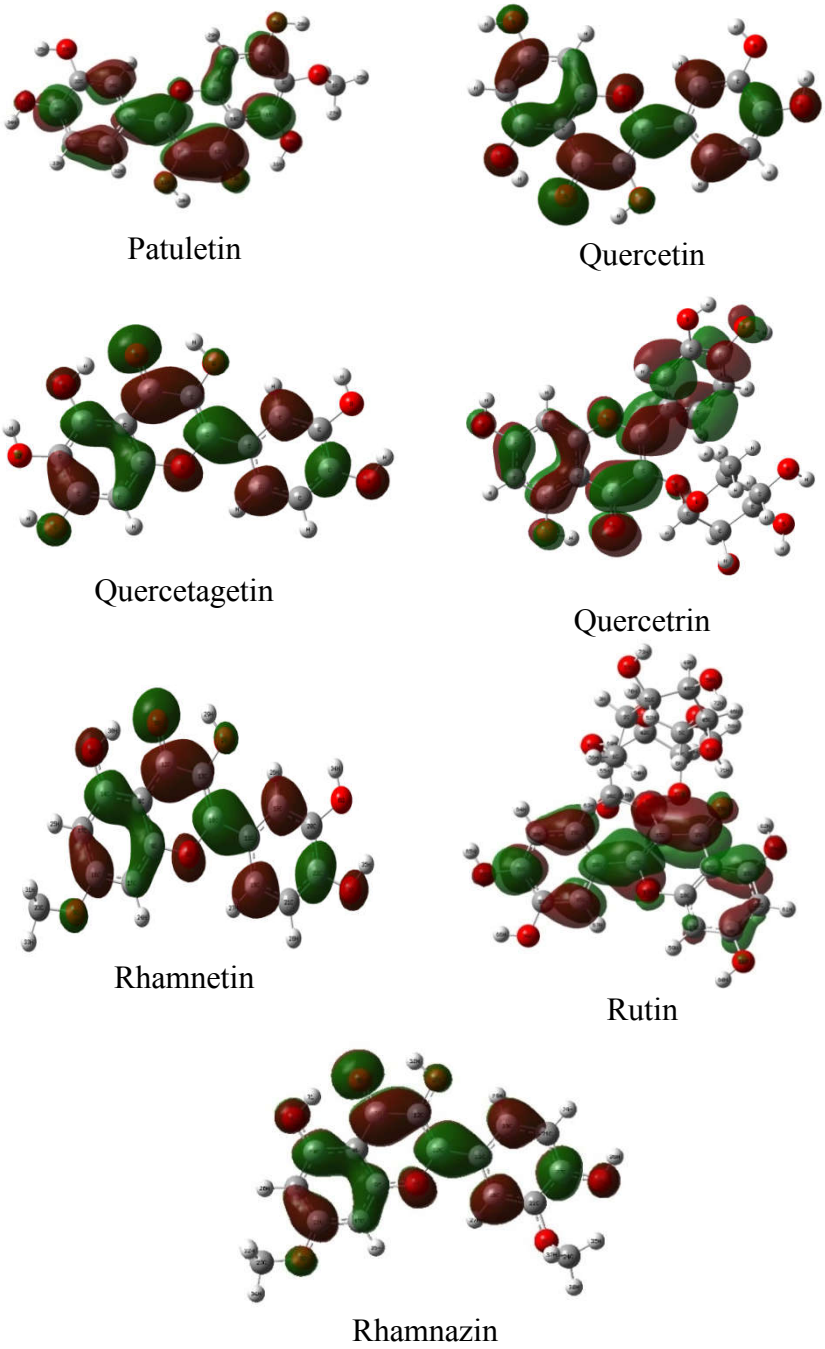


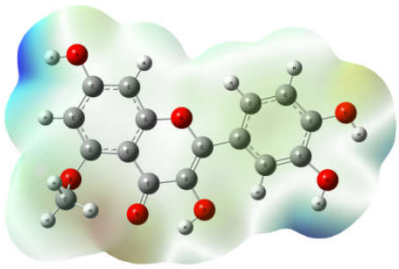
Fig.7.5. LUMO of flavonols

Table 7.1
Band gap of flavonols

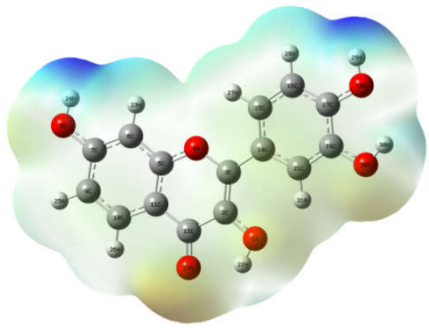
Compound	Dipole moment (Debye)	ΔE (eV)	Compound	Dipole moment (Debye)	ΔE (eV)
Azaleatin	1.83	3.64	Myricetin	1.53	3.63
Fisetin	6.65	3.74	Myricitrin	4.37	3.98
Flavonol	3.06	3.83	3,6-Dihyrdoxyflavonol	4.51	3.76
Galangin	2.09	3.79	Pachypodol	4.93	3.94
Gossypetin	7.01	3.58	Patuletin	2.71	3.68
Gossypin	4.84	3.81	Quercetagetin	1.31	3.64
Hibifolin	6.62	3.61	Quercetin	4.09	3.61
Isoquercetin	9.23	3.89	Quercetrin	5.89	4.03
Isorhamnetin	2.27	3.69	Rhamnazin	2.23	3.67
Kaempferide	3.81	3.69	Rhamnetin	0.57	3.62
Kaempferol	5.64	3.75	Rutin	5.94	3.83
Morin	7.22	4.21			

The band gap is highest for morin and lowest for gossypetin. Among the studied flavonols, Isoquercetin and rhamnetin has the highest and lowest dipole moment respectively.

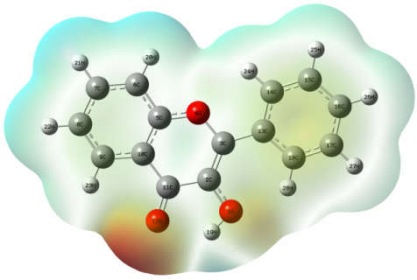
The ESP diagram (Fig.7.6) makes the analysis of electrophilic and nucleophilic centers in a molecule easier and the knowledge of ESP is important in identifying the electron rich centers in a molecule. The red regions are electron rich while blue are electron deficient. ESP also tells about the polar nature of a molecule. When the ESP diagram of a molecule is completely blue or red or orange coloured, such molecules are said to be highly polar. Here, Kaempferide, Patuletin and Rutin fall in this category and are thus highly polar in nature.



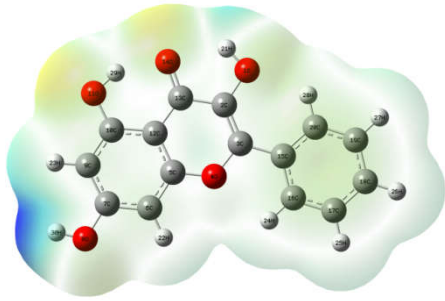
Azaleatin



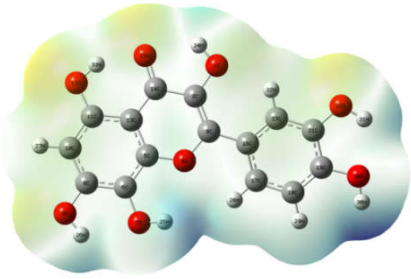
Fisiten



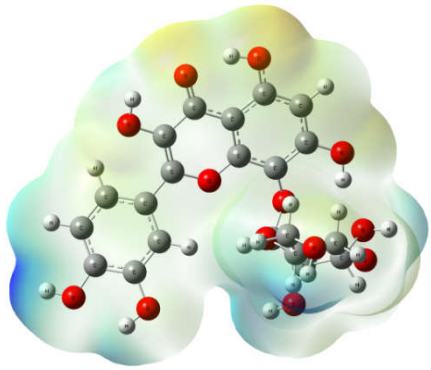
Flavonol



Galangin



Gossypetin



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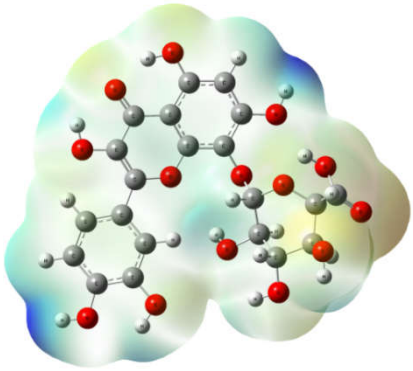
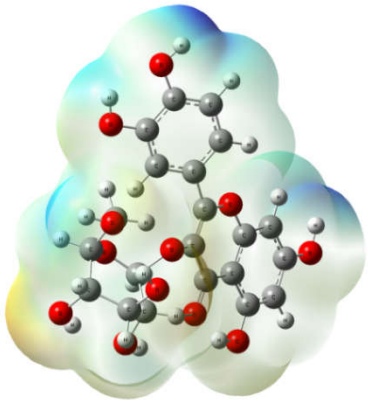
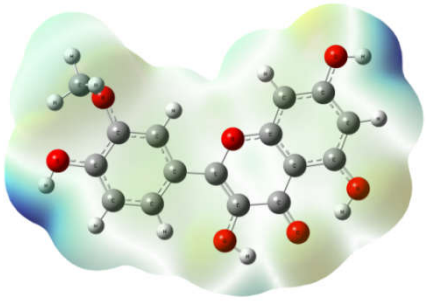


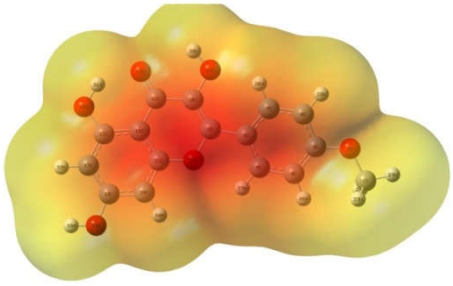
Hibifolin



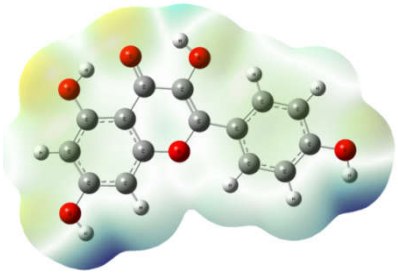
Isoquercetin



Isorhamnetin



Kaempferide



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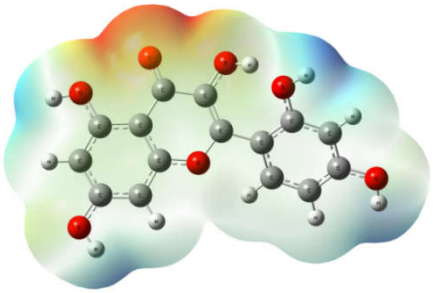
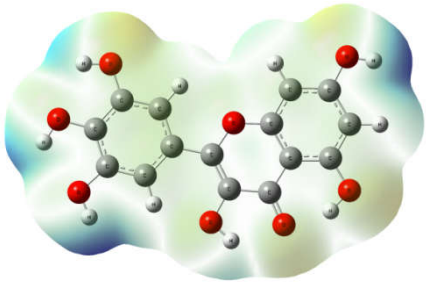
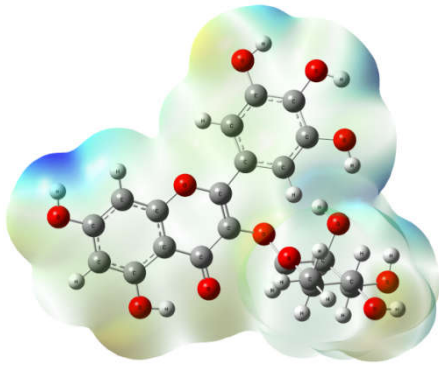


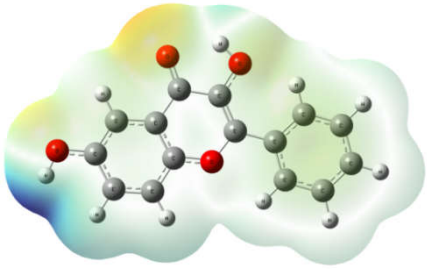
Morin



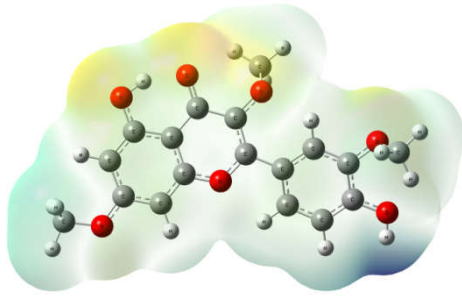
Myricetin



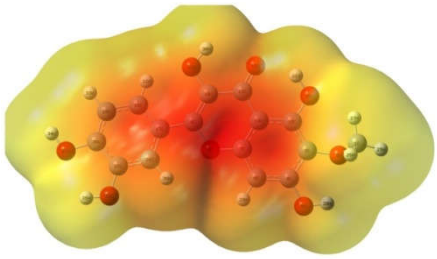
Myricetrin



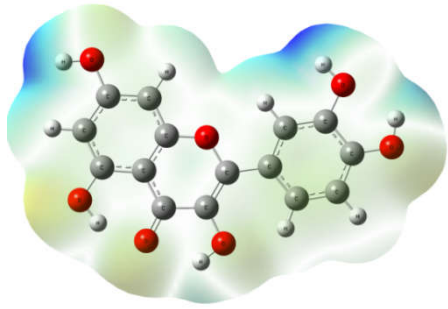
3,6-Dihyrdoxyflavonol



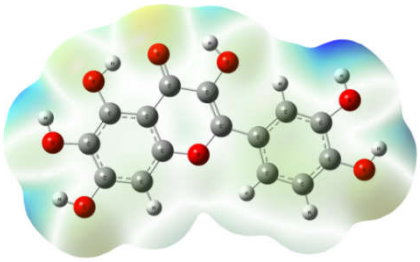
Pachypodol



Patuletin



Quercetin



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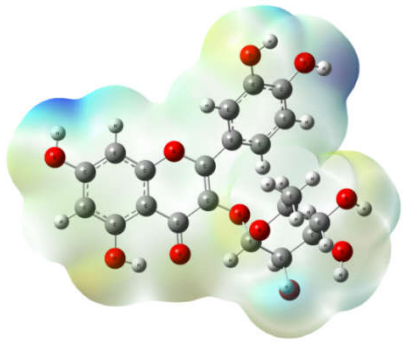

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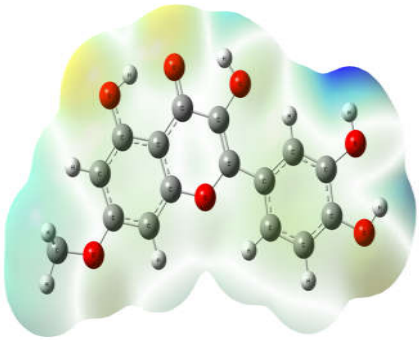
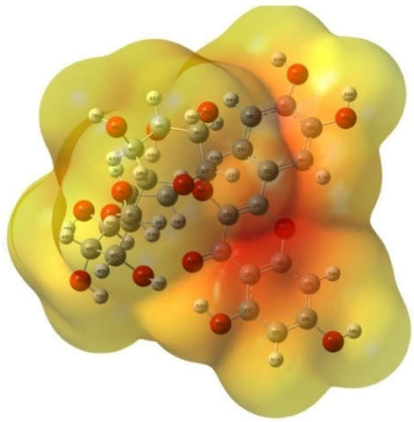
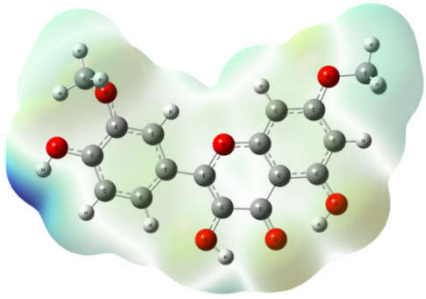


Rhamnetin



Rutin



Rhamnazin

Fig.7.6 ESP map of flavonols

7.3 Global reactive descriptors of flavonols

Analysis of global reactive descriptors helps in comparing the reactivities of studied flavonols. It also account for the nature and direction of charge transfer reactions which is followed by a particular compound. The global reactive descriptors of flavonols are given in Table 7.2.

Rutin has the highest and gossypin has the lowest IE value among the studied flavonols. Similarly Rutin posses maximum EA while Morin has the minimum EA value. All the flavonols have similar reactivities as they have almost same value for softness. The charge holding capacity Qmax is also high for Rutin so that it can accept electrons during reactions.

As Quercetin is the most widely studied flavonol, for the bioactivity studies like antioxidant, it is taken as a reference sample and the IE and EA values of others are compared with that of Quercetin. The results are ploted in Figs.7.7 & 7.8. Flavonol, Galangin, Gossypetin, Hibifolin, Isoquercetin, 3, 6-Dihydroxyflavonol and Rutin have EA values higher than that of Quercetin, indicating that they are powerful electron acceptors than Quercetin. Similarly gossypin, Azaleatin, Fisetin, Gossypetin, Morin, Rhamnetin and Rhamnazin have IE values lower than that of Quercetin so that they could act as powerful electron donor than Quercetin.

Table 7.2
Global reactive descriptors (eV)of flavonols

No	Compound	IE	EA	η	S	χ	μ	ω	Q_{max}	ω-	ω+
1	Azaleatin	5.36	1.71	1.82	0.27	3.53	-3.53	3.43	1.94	5.42	1.89
2	Fisetin	5.43	1.69	1.87	0.27	3.56	-3.56	3.39	1.91	5.41	1.85
3	Flavonol	5.80	1.96	1.92	0.26	3.88	-3.88	3.92	2.02	6.10	2.23
4	Galangin	5.74	1.95	1.90	0.26	3.85	-3.85	3.90	2.03	6.06	2.21
5	Gossypetin	5.45	1.87	1.79	0.28	3.66	-3.66	3.75	2.05	5.81	2.14
6	Gossypin	5.23	1.42	1.90	0.26	3.32	-3.32	2.90	1.75	4.80	1.48
7	Hibifolin	5.74	2.13	1.81	0.28	3.94	-3.94	4.29	2.18	6.48	2.54
8	Isoquercetin	5.81	1.91	1.95	0.26	3.86	-3.86	3.82	1.98	6.00	2.14
9	Isorhamnetin	5.51	1.81	1.85	0.27	3.66	-3.66	3.62	1.98	5.68	2.02
10	Kaempferide	5.48	1.79	1.85	0.27	3.64	-3.64	3.58	1.97	5.62	1.99
11	Kaempferol	5.58	1.83	1.87	0.27	3.70	-3.70	3.66	1.98	5.74	2.04
12	Morin	5.34	1.12	2.11	0.24	3.23	-3.23	2.48	1.53	4.36	1.12
13	Myricetin	5.46	1.83	1.82	0.28	3.65	-3.65	3.67	2.01	5.72	2.07
14	Myricitrin	5.79	1.81	1.99	0.25	3.80	-3.80	3.63	1.91	5.78	1.98
15	3, 6-Diydroxyflavonol	5.71	1.95	1.88	0.27	3.83	-3.83	3.91	2.04	6.06	2.23
16	Pachypodol	5.61	1.68	1.97	0.25	3.64	-3.64	3.37	1.85	5.44	1.80
17	Patuletin	5.47	1.79	1.84	0.27	3.63	-3.63	3.59	1.98	5.64	2.00
18	Quercetagetin	5.49	1.85	1.82	0.27	3.67	-3.67	3.70	2.02	5.77	2.10
19	Quercetin	5.46	1.85	1.81	0.28	3.65	-3.65	3.69	2.02	5.74	2.09
20	Quercetrin	5.85	1.82	2.02	0.25	3.84	-3.84	3.65	1.90	5.82	1.98
21	Rhamnazin	5.44	1.77	1.84	0.27	3.61	-3.61	3.55	1.97	5.58	1.97
22	Rhamnetin	5.42	1.80	1.81	0.28	3.61	-3.61	3.60	2.00	5.64	2.02
23	Rutin	5.99	2.16	1.92	0.26	4.08	-4.08	4.34	2.13	6.62	2.54

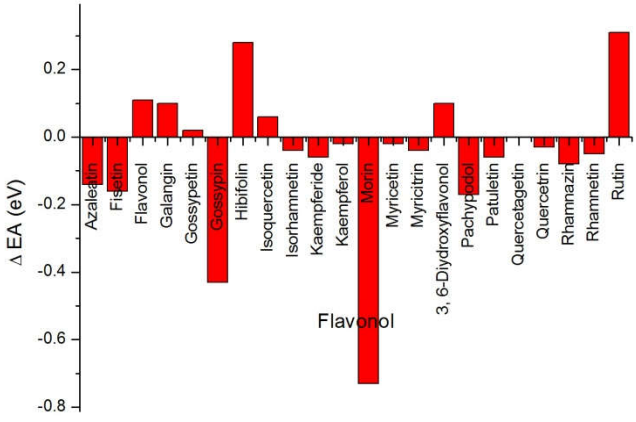


Fig.7.7 EA with respect to Quercetin

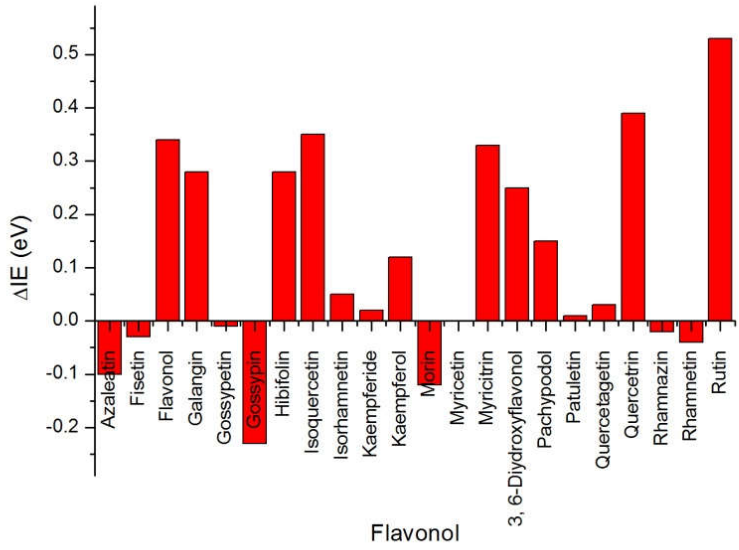


Fig.7.8 IE with respect to Quercetin

Again, the basic 'flavonol' is also taken as a reference samples as it is the simplest molecule among the studied flavonols. Here only Hibifolin and Rutin could behave as efficient electron acceptors than basic flavonol while all the flavonols except Isoquercetin, Myricetrin, Rutin and Quercetrin could act as powerful electron donor than the basic flavonol.

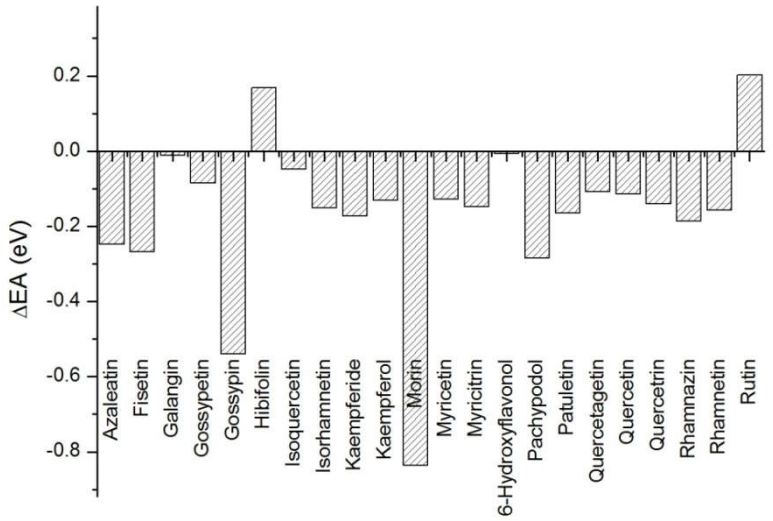


Fig.7.9 EA with respect to flavonol

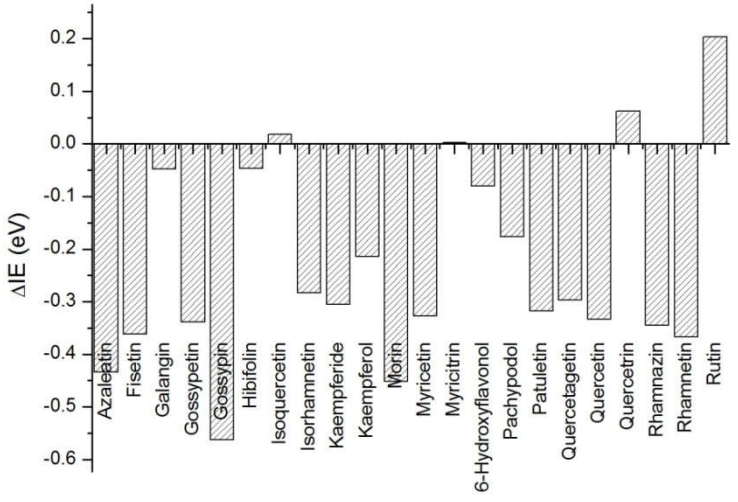


Fig.7.10 IE with respect to flavonol

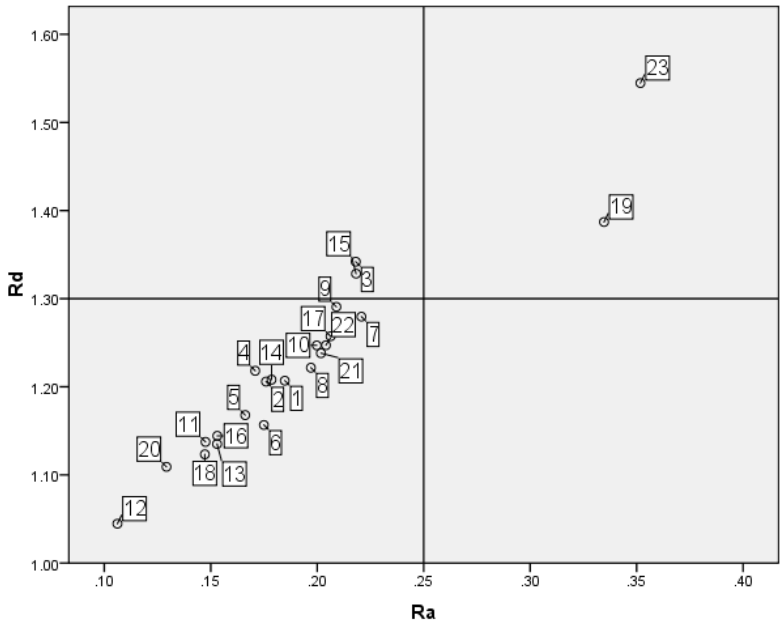


Fig.7.11 DAM of flavonols

The DAM in Fig.7.11 shows that the flavonols Quercetin (19) and Rutin (23) are good antireductants while all others except 3 and 15 are good antioxidants. The flavonols 3, 6-Dihydroxyflavonol (15) and basic flavonol (3) are in the quadrant of worst antiradicals and may due the absence of more number –OH and glucose groups to increase the chemical reactivities for undergoing charge transfer reaction. However, they may scavenge free radicals *via* some other pathway like HAT.

7.4 UV-Visible characteristics of flavonols

The aromatic conjugated systems in polyphenols like flavonoids make them able to absorb in the UV-Visible region. For flavonols the absorption maximum has been found at 310-370 nm and is usually higher than that of flavones. This makes the distinction

between flavonols and flavones become easier [22]. The UV-Visible spectral characteristics are analyzed *via* the TDDFT tool in Gaussian 09 software. The results are given in Table 7.3. The λ_{max} values of all the flavonols are found to be due to the transition between HOMO and LUMO levels. This is clear from the MO diagrams (Figs. 7.4 & 7.5) as both the MOs are delocalized in a similar manner so that they could interact to a maximum extend.

Table 7.3

UV Spectral characteristics of flavonols

No	Compound	E (eV)	λmax (nm)	Oscillator strength	MOs involved	% Contribution
1	Galangin	3.70	335	0.35	HOMO-LUMO	93.86
2	Morin	3.84	323	0.28	HOMO-LUMO	84.07
3	Rhamnetin	3.30	375	0.47	HOMO-LUMO	92.37
4	Gossypin	3.43	362	0.32	HOMO-LUMO	91.57
5	Quercetagetin	3.47	358	0.36	HOMO-LUMO	93.72
6	Gossypetin	3.37	368	0.22	HOMO-LUMO	90.45
7	Fisetin	3.46	358	0.41	HOMO-LUMO	96.14
8	Hibifolin	3.41	364	0.39	HOMO-LUMO	96.51
9	Patuletin	3.34	371	0.36	HOMO-LUMO	88.93
10	Myricetin	3.46	358	0.47	HOMO-LUMO	96.28
11	Isoquercetin	3.64	341	0.32	HOMO-LUMO	91.73
12	Quercetrin	3.82	325	0.31	HOMO-LUMO	91.03
13	Myricitrin	3.68	337	0.15	HOMO-LUMO	47.88
14	Rhamnazin	3.32	373	0.43	HOMO-LUMO	92.14
15	Quercetin	3.51	353	0.44	HOMO-LUMO	95.87
16	Isorhamnetin	3.37	368	0.44	HOMO-LUMO	92.30
17	Rutin	3.39	366	0.26	HOMO-LUMO	88.56
18	Pachypodol	3.45	359	0.25	HOMO-LUMO	89.69
19	Azaleatin	3.38	367	0.50	HOMO-LUMO	96.94
20	Kaempferol	3.62	343	0.45	HOMO-LUMO	95.20
22	Kaempferide	3.37	368	0.48	HOMO-LUMO	93.00
22	3, 6-Dihydroxyflavonol	3.49	355	0.25	HOMO-LUMO	95.26
23	Flavonol	3.38	346	0.36	HOMO-LUMO	96.36

Generally, in case of flavonids, the λ_{max} values increases with number of –OH groups. But here the trend is not strictly followed and which may be due to the difference in position of –OH groups and difference in substitution. Usually the glycosylation of –OH group decreases [22] the λ_{max} values and is clear from the pairs such as Quercetin-Quercetrin and Myricetin-Myricetrin.

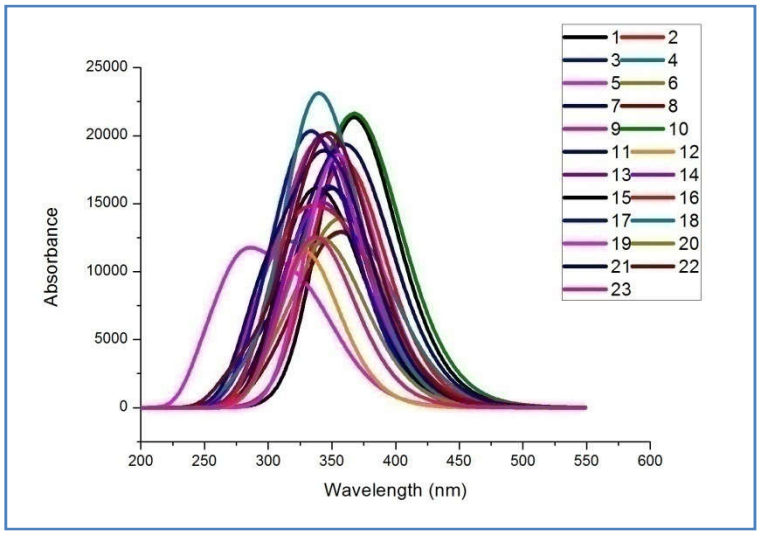


Fig.7.12. UV-Visible spectrum of flavonols

7.5 Antioxidant properties of flavonols

Three mechanisms namely, HAT, SET/SETPT and SPLET are discussed here for analyzing the radical scavenging capacity of 23 flavonols. The studied flavonols contains –OH, -OCH_3 and glucose moieties. For a mechanism to be suitable for explaining the antioxidant capacities of a compound, the associated parameters should be small. All the values are computed in aqueous phase.

Table 7.4

Parameters (kcal/mol) of different antioxidant mechanism

No	Flavonol	BDE	AIP	PDE	PA	ETE
1	Azaleatin	76.65	154.14	240.24	-10.72	405.10
2	Fisetin	78.62	156.83	227.53	-9.74	394.10
3	Flavonol	83.38	167.1	230.03	-13.82	410.95
4	Galangin	81.44	164.46	230.73	-15.47	410.66
5	Gossypetin	72.99	156.11	229.04	-15.31	400.45
6	Gossypin	71.99	159.77	225.2	-13.75	398.72
7	Hibifolin	71.34	159.74	226.16	-22.59	408.49
8	Isoquercetin	74.34	157.26	241.48	-20.10	418.84
9	Isorhamnetin	75.72	159.65	228.33	-13.34	401.31
10	Kaempferide	78.01	156.43	237.62	-12.57	406.62
11	Kaempferol	80.70	159.64	234.82	-13.00	407.46
12	Morin	77.41	153.17	224.54	1.6805	376.03
13	Myricetin	72.15	156.14	230.60	-13.53	400.27
14	Myricitrin	71.22	169.83	231.50	4.4189	396.91
15	3, 6-Dihydroxyflavonol	82.13	164.65	231.23	-14.04	409.92
16	Pachypodol	74.72	153.70	235.14	-6.775	395.61
17	Patuletin	71.39	156.01	229.08	-13.98	399.07
18	Quercetagetin	73.08	156.86	230.71	-14.26	401.84
19	Quercetin	76.99	156.50	237.41	-13.63	407.54
20	Quercetrin	74.21	169.54	217.79	4.4672	382.86
21	Rhamnazin	75.63	154.23	233.64	-13.01	400.89
22	Rhamnetin	76.75	155.08	233.59	-13.18	401.86
23	Rutin	73.58	156.49	231.6	-27.98	416.07

According to HAT mechanism, lower BDE values indicate higher antioxidant capacity. The BDE values are highly affected by the structural features. Generally, as the number of –OH groups increases the BDE values decreases. Again, the electron releasing substituents

decreases the BDE value while electron withdrawing substituents increases the BDE value. So the number of –OH groups, nature and position of substituents together determines the order of BDE values for a set of compounds with similar structure. Here we have discussed 23 flavonols. Here the maximum number of –OH groups seen in the flavonol skeleton is 6 (Gossypetin, Myricetin and Quercetagetin). Among Gossypetin, Myricetin and Quercetagetin, the number of –OH groups in ring [B] is higher for Myricetin and is responsible for its lower BDE value. In flavonols, Gossypin, Hibifolin, Myricetrin and Patuletin, glucose and -OCH_3 groups are present in addition to 5 –OH groups. The electron releasing capacity of these substituents decreases the BDE values than those with 6 –OH groups. Among them, Myricetrin with 3 –OH groups in ring [B] along with the glucose group has the lowest BDE value. The next set of flavonols having a total of 5 substituents (-OH, -OCH_3 and glucose groups together) includes, Rutin, Rhamnazin, Rhamnetin, Quercetin, Quercetrin, Pachypodol, Morin, Isoquercetin, Isorhamnetin and Azaleatin. Among these flavonols, Rutin, Quercetrin and Isoquercetin have 2 –OH groups each in ring [A] & [B]. In Rutin (Quercetin-3-Rutinoside), a disaccharide Rutinoside is present in position 3 while Quercetrin and Isoquercetin are respectively querctin-3-rhamnoside and Quercetin 3-glucoside. The presence of three -OCH_3 groups decreases the BDE value of Pachypodol than the others with less number of -OCH_3 groups. Quercetin and Morin have no such groups and thus have higher BDE values. The flavonols, Fisetin, Kaempferide and Kaempferol have respectively 4, 3 and 4 –OH groups. The presence of one -OCH_3 group decreases the BDE value of Kaempferide than the other 2 flavonols.

Again the presence of 2 –OH groups in ring [B] of Fisetin decreases its BDE value than that of Kaempferol. The remaining flavonols Galangin and 3, 6-Dihydroxyflavonol has respectively 3 and 2 –OH groups and hence the BDE values are higher than the above flavonols. Thus, according to HAT mechanism, Myricetrin has the lowest BDE value and hence having highest antioxidant capacity.

In order to evaluate the influence of structural features on the BDE values of flavonols, the BDE values at all possible sites in the studied compounds have been computed and are given in Table 7.5.

Table 7.5

BDE values (kcal/mol) at different sites

Flavonol	Position	BDE	Flavonol	Position	BDE
Azaleatin	4'	76.65	Morin	3	88.14
	3'	83.18		5	95.86
	3	87.87		7	84.60
	7	84.02		4'	77.41
Galangin	5	95.84		2'	78.39
	3	93.44	Gossypin	3'	80.69
	7	81.44		4'	71.22
Rhamnetin	5	95.00		5	92.15
	4'	76.75		7	88.76
	3'	81.92		3	89.58
	3	89.19	Gossypetin	3	90.78
Quercetagetin	6	79.96		8	80.78
	3'	74.23		5	93.57
	4'	73.08		7	83.63
	3	91.82		4'	72.99
	5	94.83		3'	74.03
	7	85.92	Patuletin	3'	80.40
Fisetin	7	84.65		7	88.16
	4'	78.62		5	99.01
	3'	82.46		3	90.28
	3	87.65		4'	71.39
Hibifolin	3'	73.79	Isoquercetin	5	98.28
	7	80.16		4'	72.15
	3	91.00		3'	76.07

	5	94.76		7	89.03
	4'	71.34	Myricitrin	5	106.10
Myricetin	5'	75.98		7	87.86
	7	86.97		3'	80.72
	4'	72.99		4'	74.21
	3'	73.94		5'	77.76
	5	95.50	Rhamnazin	3	88.00
	3	93.94		5	95.03
Quercetrin	5	99.51		4'	75.63
	7	87.51	Isorhamnetin	4'	75.72
	4'	73.58		3	90.23
	3'	84.00		7	86.90
Quercetin	3'	78.75		5	94.22
	4'	76.99	Kaempferol	7	86.42
	7	86.68		4'	80.74
	3	90.16		5	95.78
	5	95.86		3	90.70
Rutin	4'	73.58	Kaempferide	7	78.01
	5	97.65		3	90.30
	7	88.28		5	95.74
	3'	74.05	3,6-Dihydroxyflavonol	3	83.19
Pachypodol	5	105.70		6	82.13
	4'	74.72	Flavonol	3	83.38

Table 7.5 clearly shows that the BDE values are found to be low in ring [B] for all the studied flavonols. Again, the BDE values at position 3 and 5 are very high than that at other positions and is due to the presence of hydrogen bonding. The –OH groups in position 3 and 5 forms hydrogen bonds with the carbonyl oxygen in position 4. Hydrogen bond increases the bond length but restricts the bond breakage so that the BDE values increases.

Compared to anthocyanidins, the AIP values of flavonols are low so that they can donate electrons while undergoing electron transfer reactions like SET.

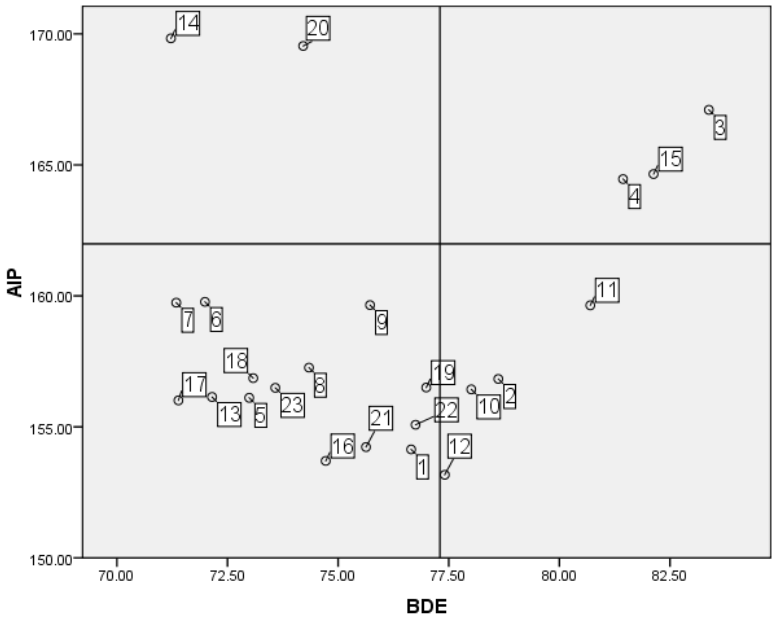


Fig.7.13. HAT *vs* SET

The graph shows the competition between HAT and SET mechanism. Here, the flavonols 3 (Flavonol), 4 (Galangin) and 15 (3, 6-Dihydroxyflavonol) lies in the 1st quadrant. As they have high BDE and AIP values, they neither undergo HAT nor SET. Flavonols 14 (Myricetrin) and 20 (Quercetrin) have low BDE value but high AIP value so that they prefer to undergo HAT than SET mechanism. Similarly, the flavonols 11 (Kaempferol), 2 (Fisetin), 10 (Kaempferide) and 12 (Morin) has low AIP value but high BDE values so that they undergo SET than HAT mechanism. The remaining flavonols have both AIP and BDE values are so low that they can undergo either HAT or SET reaction depending upon the circumstances.

The other two mechanisms SET-PT and SPLET are not suitable for explaining the antioxidant capacities of studied flavonols as one of the step in both the mechanisms are highly energy demanding.

7.6 Molecular docking study

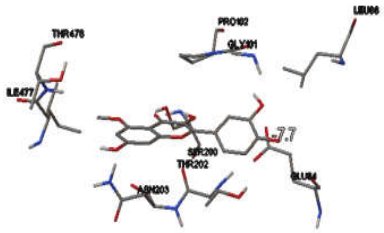
For the better understanding of the antioxidant behavior of flavonols, molecular docking studies have been employed. The molecular docking studies have been performed through Auto Dock Vina software and the binding energy of flavonols with the active site of the protein target MAO-B has been computed. The active site of MAO-B is surrounded by specific amino acids Phe168, Ile199, Tyr326, Phe343, Leu171, Tyr435, Phe166, Leu164, Ile316, Gln206, Ile198, Arg100, Thr478, Glu483, Pro102, Phe103, Thr202 and some water molecules, wherein they contribute to ligand binding. The binding energies of bounded ligand, SAG and 23 flavonols are given in Table 7.6 and the conformers with highest binding energy values are shown in Fig.7.14. All the interacting residues lie in the range of 1 Å.

Among the studied flavonols, Quercetrin and Rhamnetin form a hydrogen bond with the amino acid residue Glu207. Rutin forms one hydrogen bond each with Thr478 & Pro476, Quercetagetin forms hydrogen bond with Ser200 & Asn203, Quercetin with Arg100 & Val85, Isoquercetin with Val82 & Arg208, Gossypetin with Glu84 & Arg100 and Myricetin with Ser200. Myricetrin and Gossypin forms hydrogen bonds with Thr478 and Asn203 while Pachypodol forms two hydrogen bonds with Thr478. Rhamnazin forms three hydrogen bonds, one each with Ser200, Gly101 and Arg100. Highest binding energy

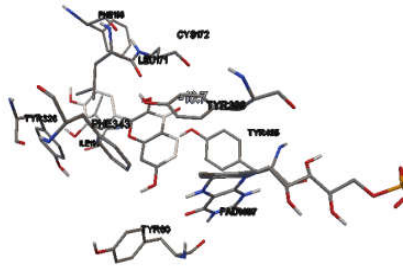
has been observed for Patuletin. However, all the studied compounds have affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and the related free radical generation and oxidative stress can be minimized.

Table 7.6
Binding energy values (kcal/mol) against MAO-B

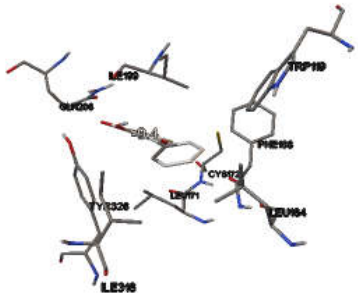
No	Compound	Binding energy	No	Compound	Binding energy
1	Azaleatin	-7.7	13	Myricetin	-8.2
2	Fisetin	-10.7	14	Myricitrin	-8.5
3	Flavonol	-9.4	15	3, 6-Dihydroxyflavonol	-9.9
4	Galangin	-10.1	16	Pachypodol	-7.5
5	Gossypetin	-10.3	17	Patuletin	-11.8
6	Gossypin	-8.4	18	Quercetagetin	-8.6
7	Hibifolin	-9.4	19	Quercetin	-8.0
8	Isoquercetin	-9.0	20	Quercetrin	-7.7
9	Isorhamnetin	-10.8	21	Rhamnazin	-7.5
10	Kaempferide	-7.9	22	Rhamnetin	-8.2
11	Kaempferol	-8.0	23	Rutin	-8.6
12	Morin	-7.9			



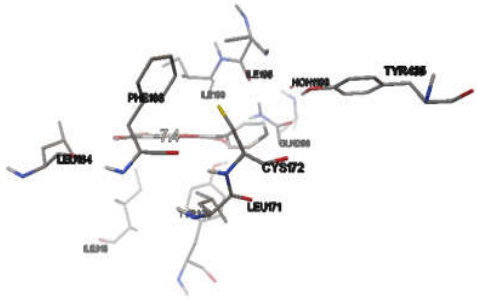
Azaleatin



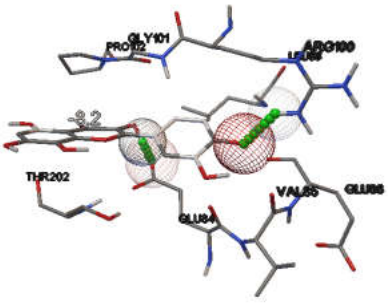
Fisiten



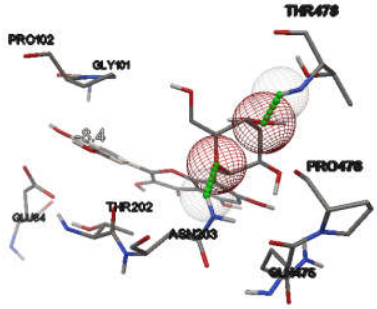
Flavonol



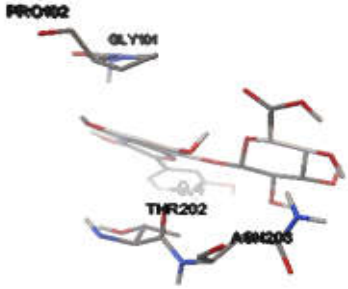
Galangin



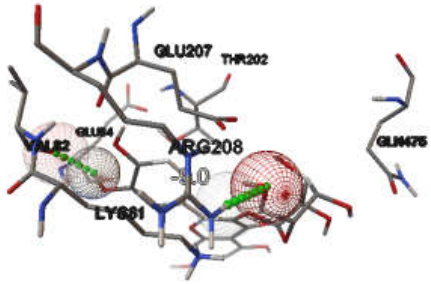
Gossypetin



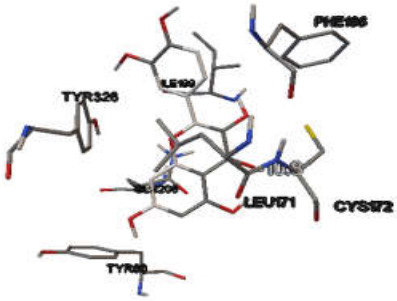
Gossypin



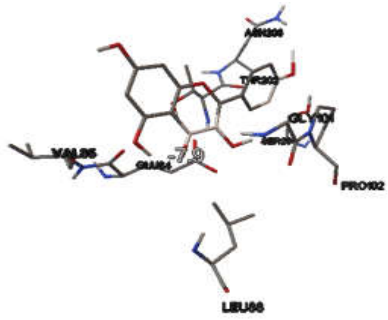
Hibifolin



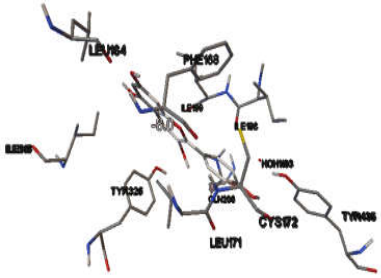
Isoquercetin



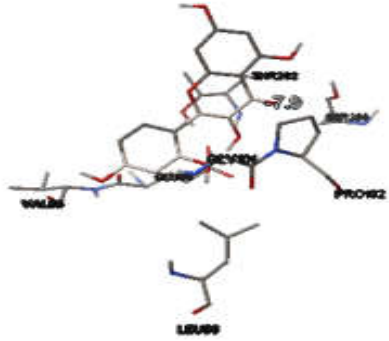
Isorhamnetin



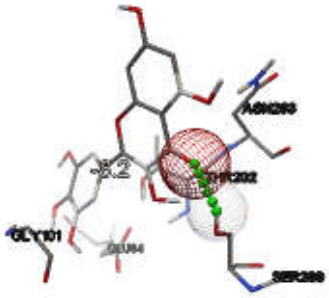
Kaempferide



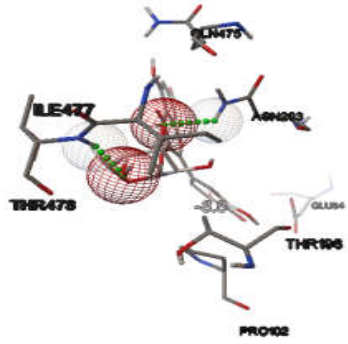
Kaempferol



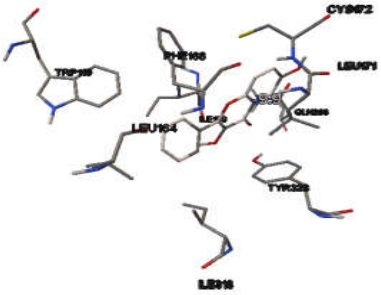
Morin



Myricetin



Myricetrin



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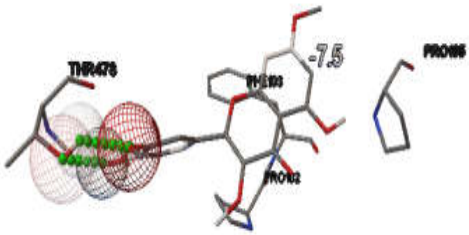
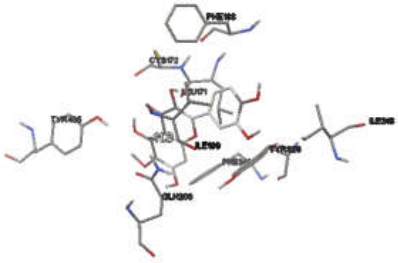
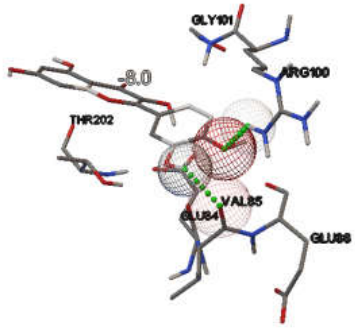


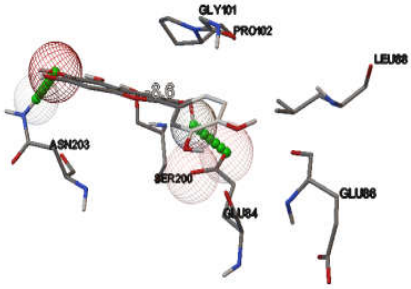
Pachypodol



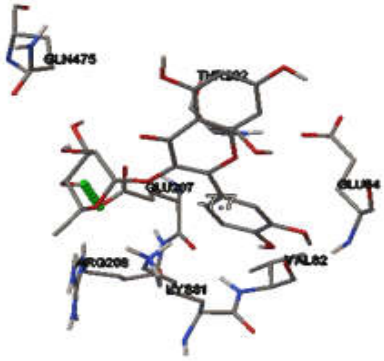
Patuletin



Quercetin



Quercetagetin



Quercetrin

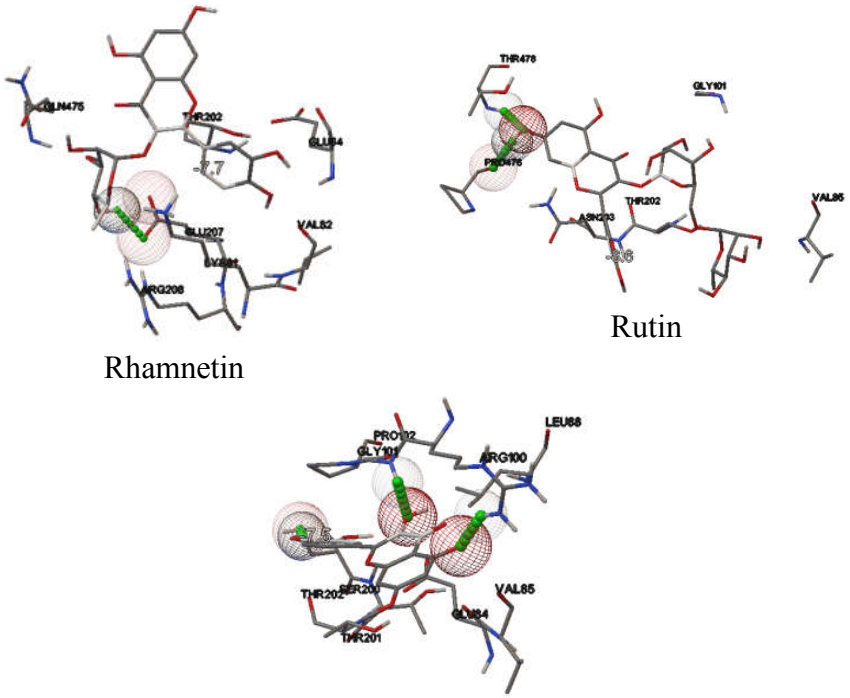


Fig.7.14. Flavonols in the active site of MAO-B

7.7 Toxicological analysis of flavonols

The value of drug-likeness (DL) qualitatively assesses the probability of a molecule to become an oral drug with respect to the bioavailability. DL has been implemented from the physicochemical inspections of development compounds advanced enough to consider as oral drug candidates. This has been employed to omit molecules with the incompatible pharmacokinetic profile [23].

The logP value (octanol-water partition coefficient), has been used as the classical descriptor for lipophilicity. Similarly, the ease of

handling and formulation of a drug was achieved through its solubility as it plays a crucial role in the absorption of a drug [23]. The toxicological properties of flavonols along with their lopP and solubility values are given in Table.7.7 (numbering is same as that if Table 7.6). These are the results obtained from Datawarrior.

Here the numbers 1-23 denotes respective flavonols in Table 7.6. All the studied flavonols are non-irritant and non-Tumorigenic in nature. But most of the flavonols are mutagenic in nature. Only 3 (Flavonol), 8 (Isoquercetin), 14 (Myricetrin), 20 (Quercetrin) and 23 (Rutin) are non-mutagenic in nature.

The logP values of all the flavonols are less than 5 indicates their bioavailability. Similarly the solubility values of the flavonols are found to be higher than -4.0 confirm their considerable absorption. All of them have positive drug score and the maximum DS is found for Myricetrin.

As per the OSIRIS property explorer, it is better to get molecules with positive DL value as most of the drug molecules used in their database has positive DL value. Here, the flavonols 1, 9, 10, 14, 17, 20, 21, 22 and 23 have positive while all others have negative DL value. The maximum DL has been observed for Rutin.

Table 7.7

Properties from Datawarrior

Flavonols	cLogP	cLogS	DL	Mutagenic	Tumorigenic	Irritant	DS
1	1.77	-2.81	0.06				0.40
2	1.84	-2.79	-0.08				0.40
3	2.87	-3.68	-0.08	None			0.61
4	2.18	-3.08	-0.08				0.39
5	1.14	-2.20	-0.08				0.41
6	-0.80	-2.08	-3.71				0.23
7	-1.20	-2.06	-0.11				0.33
8	-0.30	-2.19	-3.67	None			0.40
9	1.77	-2.81	0.06				0.40
10	2.11	-3.10	0.06				0.24
11	1.84	-2.79	-0.08				0.40
12	1.49	-2.49	-0.08				0.40
13	1.14	-2.20	-0.08				0.41
14	0.23	-2.40	1.93	None			0.72
15	-0.80	-2.08	-3.71				0.23
16	2.47	-3.25	-0.11				0.37
17	1.42	-2.51	0.06				0.41
18	1.14	-2.20	-0.08				0.41
19	1.49	-2.49	-0.08				0.24
20	0.58	-2.70	1.93	None			0.73
21	2.04	-3.12	0.06				0.39
22	1.77	-2.81	0.06				0.40
23	-1.30	-2.40	1.93	None			0.55

7.8 Pharmacokinetic parameters of flavonols

The pharmacokinetic properties of studied flavonols have been carried out through Molinspiration online software and are employed to validate the Lipinski rule of 5 (RO5). As per the rule, an orally admissible drug-like molecule must have:

1. nHBD < 5
2. nHBA < 10
3. MW < 500 Dalton
4. LogP < 5
5. nROTB < 10 (added by Veber)

Table 7.8

Pharmacokinetic parameters of flavonols

No.	miLogP	TPSA	nAtoms	MW	nHBA	nHBD	nViolations	nROTB	Volume
1	1.96	120.36	23	316	7	4	0	2	257.61
2	1.97	111.12	21	286	6	4	0	1	232.07
3	3.45	50.44	18	238	3	1	0	1	208.01
4	2.65	90.89	20	270	5	3	0	1	224.05
5	1.42	151.58	23	318	8	6	1	1	248.10
6	-0.62	230.73	34	480	13	9	2	4	380.22
7	-0.75	247.8	35	494	14	9	2	4	382.4
8	-0.36	210.50	33	464	12	8	2	4	372.21
9	1.99	120.36	23	316	7	4	0	2	257.61
10	2.71	100.13	22	300	6	3	0	2	249.59
11	2.17	111.12	21	286	6	4	0	1	232.07
12	1.88	131.35	22	302	7	5	0	1	240.08
13	1.39	151.58	23	318	8	6	1	1	248.10
14	0.35	210.50	33	464	12	8	2	3	371.96
15	2.94	70.67	19	254	4	2	0	1	216.03
16	2.80	98.37	25	344	7	2	0	4	292.67
17	1.70	140.59	24	332	8	5	0	2	265.63
18	1.42	151.58	23	318	8	6	1	1	248.10
19	1.68	131.35	22	302	7	5	0	1	240.08
20	0.64	190.28	32	448	11	7	2	3	363.95
21	2.53	109.36	24	330	7	3	0	3	275.14
22	2.22	120.36	23	316	7	4	0	2	257.61
23	-1.06	269.43	43	610	16	10	3	6	496.07

All these would help to get a deep knowledge about the pharmacokinetics including Absorption, Distribution, Metabolism, and

Excretion (ADME) of the molecule under investigation [23]. All the studied flavonols except Rutin (No. 23) have molecular weight less than 500 Dalton and LogP values of all are less than 5.

Flavonols 5, 13 and 18 have nHBA > 5 so that show one violation to Lipinski's rule of 5 while flavonols 6, 7, 8, 14 and 20 shows 2 violations from Lipinski's rule and are in nHBA and nHBD. In addition to the high values of nHBA and nHBD the flavonol 23 have high MW also and violated the rule. The presence of glucose groups increases the total polar surface area (TPSA) of flavonols. TPSA value gives an insight in to the drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. For a druggable molecule, the TPSA must be less than 140 A^2 [24]. Among the studied flavonols, 5, 6, 7, 8, 14, 18, 20 and 23 have TPSA values greater than 140 A^2. All the others obey the rule of 5 completely so that can be taken orally. Again, the number of rotatable bonds (nROTB) decides the conformational flexibility of a molecule. In our case all the studied flavonols have nROTB < 10. This is quite important for analyzing the conformational changes that can be undergone by a molecule and ultimately it is important in the binding of molecules to receptors or channels.

Molinspiration also allows us to compute the bioactivities of compounds against some specified targets as given in Table 7.9. All the flavonols except 5, 6 and 7 have moderate activity as GPCR ligand while 5, 6 and 7 have considerable activity. All have moderate activity as ion channel modulator while all have considerable activity as enzyme inhibitor. Similarly all flavonols show moderate activity as

protease inhibitor and all but 3 and 23 show considerable activity against nuclear receptor ligand. All the flavonols except 3, 7 and 23 shows considerable activity towards kinase inhibitor.

Table 7.9

Bioactivities of flavonols towards specified targets

No.	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-0.07	-0.24	0.24	0.29	-0.28	0.19
2	-0.11	-0.27	0.18	0.20	-0.36	0.20
3	-0.28	-0.30	-0.02	-0.05	-0.51	0.13
4	-0.13	-0.21	0.19	0.28	-0.32	0.28
5	-0.09	-0.18	0.30	0.30	-0.23	0.30
6	0.05	0.00	0.12	0.14	-0.09	0.42
7	0.02	-0.03	-0.02	0.27	-0.07	0.42
8	0.06	-0.04	0.13	0.20	-0.06	0.42
9	-0.10	-0.26	0.25	0.28	-0.30	0.22
10	-0.12	-0.28	0.19	0.30	-0.28	0.20
11	-0.10	-0.21	0.21	0.32	-0.27	0.26
12	-0.09	-0.22	0.22	0.34	-0.27	0.28
13	-0.06	-0.18	0.28	0.32	-0.20	0.30
14	-0.02	-0.08	0.08	0.14	-0.06	0.38
15	-0.18	-0.27	0.10	0.11	-0.44	0.18
16	-0.12	-0.22	0.14	0.14	-0.27	0.16
17	-0.14	-0.34	0.21	0.13	-0.35	0.17
18	-0.11	-0.28	0.26	0.22	-0.30	0.26
19	-0.06	-0.19	0.28	0.36	-0.25	0.28
20	-0.01	-0.08	0.08	0.17	-0.06	0.37
21	-0.12	-0.28	0.21	0.23	-0.27	0.18
22	-0.11	-0.27	0.21	0.27	-0.27	0.20
23	-0.05	-0.52	-0.14	-0.23	-0.07	0.12

Conclusion

Along with the basic flavonol moiety, 22 other flavonols have been studied for their antioxidant properties. All the computational works are carried out through Gaussian 09 software package and the theory adopted is DFT-B3LYP/6-31+G (d, p). Along with the antioxidant properties, the toxicological and pharmacokinetic properties have also been evaluated. The molecular docking studies are also implemented to analyze the interaction of flavonols with MAO-B. For all the flavonols the HOMOs and LUMOs are delocalized almost in the same manner so that the λ_{max} values of all the flavonols are found to be due to the transition between HOMO and LUMO levels. The band gap is highest for Morin and lowest for Gossypetin. Kaempferide, Patuletin and Rutin are highly polar in nature. All the flavonols have similar reactivities as they have almost same value for softness. The charge holding capacity Qmax is also high for Rutin so that it can accept electrons during reactions. Flavonol, Galangin, gossypetin, Hibifolin, Isoquercetin, 3, 6-dihydroxyflavonol and Rutin have EA values higher than that of Quercetin, indicating that they are powerful electron acceptors than Quercetin. Similarly gossypin, Azaleatin, Fisetin, Gossypetin, Morin, Rhamnetin and Rhamnazin have IE values lower than that of Quercetin so that they could act as powerful electron donor than Quercetin. The DAM shows that the flavonols Quercetin and Rutin are good antireductants while all others except flavonol and 3, 6-Dihydroxyflavonol are good antioxidants. According to HAT mechanism, Myricetrin has the lowest BDE value and hence having highest antioxidant capacity. The BDE values are

found to be low in ring [B] for all the studied flavonols. The BDE values at position 3 and 5 are very high than that at other positions and is due to the presence of hydrogen bonding. The Flavonol, Galangin and 3, 6-Dihydroxyflavonol have high BDE and AIP values, they neither undergo HAT nor SET. Myricetrin and Quercetrin have low BDE value but high AIP value so that they prefer to undergo HAT than SET mechanism. Similarly, Kaempferol, Fisetin, Kaempferide and Morin have low AIP value but high BDE values so that they undergo SET than HAT mechanism. The remaining flavonols have both AIP and BDE values are so low that they can undergo either HAT or SET reaction depending upon the circumstances. All the studied compounds have affinity towards the MAO-B and could bind in the active site of the protein so that all of them can inhibit the action of MAO-B and the related free radical generation and oxidative stress can be minimized. Some flavonols have positive while some others have negative DL value. The maximum DL has been observed for Rutin. Only 3 Flavonols among the studied flavonols are non-mutagenic in nature.

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SUMMARY

A computational study of 5 classes of flavonoids (flavanones, flavonols, flavones, anthocyanidins and flavanols) has been conducted. All the computational calculations were carried out through Gaussian 09 software package and the level of theory adopted was DFT-B3LYP with 6-31+ G (d, p) as basis set. Other online software like OSIRIS property explorer (Datawarrior), Molinspiration, etc., have also been employed for the toxicological and pharmacokinetic property analysis. The molecular docking studies have been implemented through Autodock Vina software. The major application studied here was the antiradical capacity of different classes of flavonoids. However, some other applications like, UV filtering and pH indicating properties have also been studied. The major outcomes of the research work are pointed out below.

Flavanones

- The presence of sp^3 carbon at position 3 makes the flavanones coplanar.
- All the studied flavanones have relatively high IE and low EA values,so that are less efficient electron donors than Na and less efficient electron acceptors than F.
- Hesperidin acts as an antireductant and is attributed to the highest EA and Qmax values which favor the charge transfer from free radical to hesperidin. The remaining flavanones act as good antioxidants as they have low IE values.

- All of them have absorption in the range of 260-345 nm which lies in the UV-A and UV-B region and consequently they can be employed as effective UV filters. The major transitions involved are HOMO-LUMO and HOMO-1-LUMO.

- By HAT mechanism, hesperidin is the most powerful antioxidant among the studied flavanones.

- Flavanones which contain a 3'-OH group, have their lowest BDE value at this position followed by position 4'. The higher BDE value at position 5 is attributed to the presence of hydrogen bonding.

Flavanols

- The planarity of flavanols loses due to the presence of sp^3 carbon at position 3.

- High Qmax values for EC and GC, indicates that they are powerful electron acceptors.

- All the studied flavanols are able to filter UV-B radiations completely as their absorbance lies in the range of 230-320 nm. For most of the catechins, the important transitions are between HOMO, HOMO-1 and LUMO levels.

- The lowest BDE value is seen at position 4' and ring [B] is more reactive in flavanols. The introduction of gallate units increases the BDE values.

- Flavanols C and EC do not show any violation to Lipinski rule of 5 and all have positive drug score towards the specified targets.

Anthocyanidins

- The important feature of anthocyanidins is the presence of chromenylium ion. PEO has the highest and MAL has the lowest energy gap.
- Compared to the other classes of flavonoids, anthocyanidins have very high EA and Qmax values and are thus good antireductants.
- At low pH values (in acidic media) the flavylium cations exist and are red in colour. As the pH values increase this cationic form undergo several structural deformations to form quinoidal bases and then to chalcones.
- Among the studied anthocyanidins, KUR is having lowest BDE value, so that it is the most powerful antioxidant according to HAT mechanism.
- For all the anthocyanidins except those without 3-OH groups, the stable radical is formed at position 3. The second reactive site is 4'.
- DEL and QUE show violation because of the presence of one excess number of HBD site and KUR shows violation in nHBA and nHBD.

- The positive values for drug score indicate that all of them can act as a potential drug.

Flavones

- The flavones have said to possess a planar structure.
- All the studied flavones have absorption in the range 285-375 nm, which is in the UV-A and UV-B region. The glucose substitution decreases the λ_{max} values. The position of –OH group has some influence on the absorption characteristics of flavones. In most cases, the λ_{max} is due to the transition between HOMO-2 and LUMO.
- The BDE values related to HAT mechanism falls in the range of 70-100 kcal/mol.

flavonols

- For all the flavonols the HOMOs and LUMOs are delocalized almost in the same manner so that the λ_{max} values of all the flavonols are found to be due to the transition between HOMO and LUMO levels.
- Kaempferide, Patuletin and Rutin are highly polar in nature.
- The charge holding capacity 'Qmax' is high for rutin so that it can accept electrons during reactions.
- All the studied flavonols except morin and gossypin have EA values higher than that of quercetin, indicating that they are

powerful electron acceptors than quercetin. Similarly only gossypin could act as powerful electron donor than quercetin.

- The DAM shows that the flavonols quercetin and rutin are good antireductants while all others except flavonol and 3, 6-dihydroxyflavonol are good antioxidants.

- According to HAT mechanism, Myricetrin has the lowest BDE value and hence have the highest antioxidant capacity.

- The BDE values are found to be low in the ring [B] for all the studied flavonols. The BDE values at position 3 and 5 are higher than that at other positions and are due to the presence of hydrogen bonding.

- The maximum DL has been observed for Rutin. Only 3 Flavonols among the studied flavonols are non-mutagenic in nature.

➢ All the studied flavonoids have considerable capacity to inhibit the action of MAO-B so that they could reduce the OS caused by MAO-B.

➢ Global reactive descriptors and ESP maps are effective tools to evaluate the donor-acceptor interactions in a molecule. Similarly, the NBO program is a powerful tool to compute the bond orders.

In order to evaluate the efficiency of the present study, the experimental TEAC values [1] of available flavonoids were compared

with their computed BDE values and the results shows high correlation and the model equation is:

$$TEAC_{value} = 28.67596(\pm 2.4015) - 0.32463(\pm 0.02944)BDE \qquad (1)$$

Table 1

Statistical Parameters

Parameter	Values
SEE	0.5055
R2	0.8294
Adj. R2	0.8226
PRESS	6.3882
F	121.5679
Q2	0.7792
MAE	0.2375
SD	0.1814
Quality	GOOD

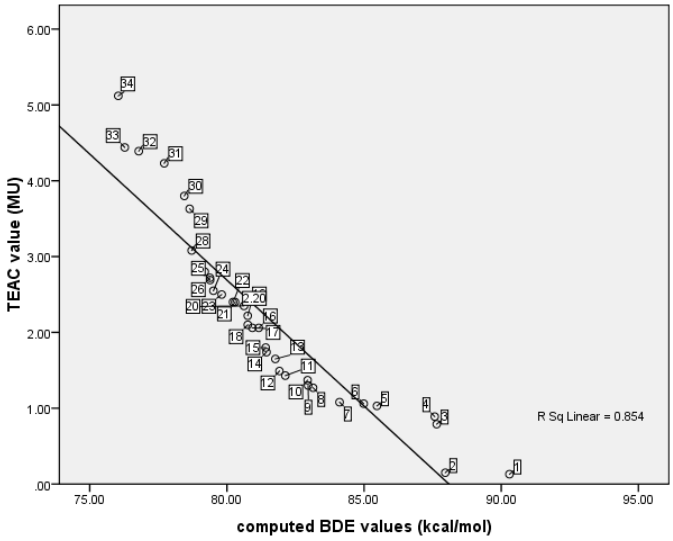

Fig.1. correlation between TEAC value and BDE value

Reference

[1] B. Luc, D. Amic, Reliability of bond dissociation enthalpy calculated by the PM6 method and experimental TEAC values in antiradical QSAR of flavonoids ˇ ic, Bioorg. Med. Chem. 18 (2010) 28–35. doi:10.1016/j.bmc.2009.11.015.

FUTURE PERSPECTIVES

- Computing the NLO properties of the flavonoids.
- Evaluating the metal chelating capacity of all the flavonids and the related metal catalysed peroxidations *via* Fenton mechanism.
- Analyzing the multi target drug activities of flavonoids.
- Developing best QSAR/QSPR models based on virtual screening, machine learning, etc.
- Evaluating the copigmentation properties of anthocyanidins.
- Studying the metal binding and the colour dependence of anthocyanidins-metal complexes with pH, temperature and concentration.
- Studying the corrosion inhibiting capacity of flavonoids towards several metals like Fe, Al, Cu, etc.

DETAILS OF PUBLICATIONS

Journal Publications:

Details of published papers				
a. In International journals				
No	Details of paper	Journal	Impact factor	ISSN/ ISBN
1	**Vijisha K. Rajan**; K.Muraleedharan. The pKa values of amine based solvents for CO2capture and itstemperature dependence—An analysis by density functional theory.	International Journal of Greenhouse Gas Control: 58, 2017, 62-70.	4.764	ISSN: 1750-5836
2	**Vijisha K. Rajan**; K.Muraleedharan. A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, Gallic acid.	Food chem.: 220, 2016, 93-99.	4.946	ISSN: 0308-8146
3	**Vijisha K. Rajan**; C. K. Hasna; K.Muraleedharan. The natural food colorant Peonidin from cranberries as a potential radical scavenger- A DFT based mechanistic analysis.	Food chem.: 262, 2018, 184-190.	4.964	ISSN: 0308-8146
4	**Vijisha K. Rajan**, Shameera Ahamed T.K, K. Muraleedharan: Studies on the UV filtering and radical scavenging capacity of the bitter masking flavanone Eriodictyol.	JPHOTOBIOL: 185, 2018, 254-261.	3.337	ISSN: 1011-1344
5	**Vijisha K. Rajan**, Shameera Ahamed T.K., Hasna C.K, K. Muraleedharan: A non toxic natural food colorant and antioxidant 'Peonidin' as a pH indicator: A TDDFT analysis.	Journal of computational biology and chemistry: 76, 2018,	1.437	ISSN: 1476-9271

		202-209.		
6	**Vijisha K. Rajan**; K. Muraleedharan; V.M.Abdul Mujeeb. Green synthesis of pure and doped semiconductor nanoparticles of ZnS and CdS:	Trans. Nonferrous Met. Soc. China: 25, 2014, 3265-3270.	1.891	ISSN: 1003-6326
7	**Vijisha K. Rajan**, Shameera Ahamed T.K, K. Muraleedharan: Studies on the UV filtering and radical scavenging capacity of the bitter masking flavanone Eriodictyol:	Data in Brief 20, 2018, 981-985	0.287	ISSN: 2352-3409
8	**Vijisha K. Rajan**; K. Muraleedharan. Calculation of p*K*a Values of Alkanolamines – A DFT-B3LYP Computational Analysis.	International Journal of Cheminformatics Research: 1(1), 2016, 1-8.	-	-
9	K.Sarada; **Vijisha K. Rajan**; K.Muraleedharan; Exploration of the thermal decomposition of oxalates of copper and silver by experimental and computational methods.	Journal of Analytical and Applied Pyrolysis: 120, 2016, 207-214.	4.324	ISSN: 0165-2370
10	Bassila Hassan; **Vijisha K. Rajan**; V.M.Abdul Mujeeb; K. Muraleedharan. A DFT baser analysis of adsorption of Hg^{2+} ion on chitosan monomer and its citralidene and salicylidene derivatives: Prior to the removal of Hg toxicity.	International Journal of Biological Macromolecules: 99(1), 2017, 549-554.	3.929	ISSN: 0141-8130
11	Shameera Ahamed T.K., **Vijisha K. Rajan**, Sabira. K, K. Muraleedharan QSAR classification-based virtual screening followed by molecular docking studies foe	Journal of computational biology and chemistry	1.437	ISSN: 1476-9271

	identification of potential inhibitors of 5-Lipoxygenase	77, 2018, 154-166		
12	Amitha G S, **Vijisha K. Rajan**, K Muraleedharan, Suni Vasudevan, Novel 4,4'-fluoresceinoxy bisphthalonitrile showing aggregation-induced enhanced emission and fluorescence turn off behavior to Fe3+ ions	Journal of Fluorescence doi: 10.1007/s10895-018-02338-0	1.665	ISSN: 1053-0509
13	Shameera Ahamed. T. K, **Vijisha K. Rajan**, Sabira. K, K. Muraleedharan, DFT and QTAIM based Investigation on the structure and antioxidant behavior of lichen substances Atranorin, Evernic acid and Diffractaic acid	Journal of computational biology and chemistry doi.org/10.1016/j.compbiolchem.2019.03.009	1.437	ISSN: 1476-9271
14	Vintu M, **Vijisha K. Rajan**, Muraleedharan K, Unnikrishnan Gopalakrishnapanicker, Suzuki coupling derived indolocarbazole based macromolecule as a solid phase/ solution phase sensor for Hg2+: Experimental and Theoretical explorations	European Polymer Journal	3.795	ISSN: 0014-3057
15	T.K. Shameera Ahamed, **Vijisha K. Rajan**, K. Muraleedharan: QSAR modeling of benzoquinone derivatives as 5-lipoxygenase inhibitors	Food Science and Human Wellness, 8, 53-62, 2019	-	ISSN:2213-4530
16	K.P Safna Hussan, M. Shahin Thayyil, **Vijisha K. Rajan**, K.Muraleedharan. DFT studies on global parameters, antioxidant mechanism and molecular docking of amlodipine besylate	Journal of computational biology and chemistry, 80, 46-53, 2019	1.437	ISSN: 1476-9271

17	K.P Safna Hussan, M. Shahin Thayyil, **Vijisha K. Rajan**, K.Muraleedharan. Experimental and theoretical studies on a double active pharmaceutical ingredient, benzalkonium ibuprofenate	Journal of computational biology and chemistry, 72, 2018, 113-121.	1.437	ISSN: 1476-9271
18	T. Noushad; P. Alikutty; H. Basila; **Vijisha K. Rajan**; K. Muraleedharan; V. M. Abdul Mujeeb. A comparative study on the druggability of Schiff bases and dithiocarbamate derivatives of chitosan.	Polym. Bull.: 73 (8). 2016, 2165-2177:	1.589	ISSN: 0170-0839
19	K.P.Safna Hussan, M. Shahin Thayyil, S.K.Deshpande, T.V.Jinitha, **Vijisha K. Rajan**, K.L.Ngai. Synthesis and molecular dynamics of double active pharmaceutical ingredient Benzalkonium Ibuprofenate.	Journal of Molecular liquids, 223, 2016, 1333-1339.	4.513	ISSN: 0167-7322
20	K.P. Safna Hussan, Mohamed Shahin Thayyil, M. Binesh, S.K. Deshpande, **Vijisha K. Rajan**. Molecular dynamics in amorphous pharmaceutically important protic ionic liquid–benzalkonium chloride.	Journal of Molecular Liquids, 251, 2018, 487-491.	4.513	ISSN: 0167-7322
21	**Vijisha K. Rajan**, Ragi. C. K, K. Muraleedharan: A computational exploration into the structure, antioxidant capacity, mechanism of radical scavenging and druglikeness of the natural food colorant Petunidin from Berries and grapes	Heliyon		ISSN: 0955-2863
22	**Vijisha K. Rajan**, Ragi. C, Hasna. C. K, K. Muraleedharan: Evaluation of donor, acceptor and radical scavenging capacity of	Journal of computational biology and	1.437	ISSN: 1476-9271

	flavonols and anthocyanidins: A DFT based comparative study towards hydroxyl free radicals	chemistry		
23	Amitha G. S., **Vijisha K. Rajan**, K Muraleedharan, Suni Vasudevan, Amritha B, Betti base and its modified phthalonitrile derivative for the turn on fluorimetric detection of Hg2+ and Cr3+ ions.	JPHOTOBIOL: 382, 2019, 111904	3.337	ISSN: 1011-1344
b. In National journals				
1	**Vijisha K. Rajan**; K. Muraleedharan. Calculation of p*K*a Values of Alkanolamines	Devagiri Journal of Science 2(1), 113-117	-	ISSN 2454-2091
Details of papers under revision				
1	**Vijisha K. Rajan**, K. Muraleedharan, K.P Safna Hussan. A theoretical investigation on the global descriptive parameters, antioxidant, UV filtering and NLO properties of some flavanones.	Food Science and Human Wellness	-	ISSN: 2213-4530
2	**Vijisha K. Rajan**, K. Muraleedharan, Theoretical evaluation of the antioxidant activities of flavonoids: A review.	Journal of pharmaceutical analysis	1.780	ISSN: 2095-1779
3	**Vijisha K. Rajan**, Shameera Ahamed. T. K, K. Muraleedharan, A computational evaluation on the antioxidant, UV filtering, pharmacokinetic and Toxicological properties of Green tea Catechins.	Food chemistry	4.946	ISSN: 0308-8146

Book

No	Title	Publication	ISBN
1	Antiradical properties of some ployphenols & Gallic acid- A computational study	LAMBERT Academic Publishing, Germany, 2017	978-3-330-07848-2
2	A computational investigation on the structure, radical scavenging capacity and toxicological analysis of some potent flavones	Cambridge Scholars Publishing, England (in press)	

Book chapters

No	Chapter	Book	Publication	ISSN/ISBN
1	**Vijisha K. Rajan**; K. Muraleedharan. Study of pKa values of alkylamines based on density functional theory.	Computational Chemistry Methodology in structural Biology and Material Sciences, (Part I, Chapter 1)	Taylor & Fransis.CRC Press	ISBN: 9781771885683
2	**Vijisha K. Rajan**; K.Muraleedharan; Computational study of complexes of delphinidin with Al^{3+} and Ni^{2+} - prior to reduce the metal toxicity.	Heavy metals and metalloids inbiosphere-Impacts and assessment; ENVBOOK series	DPH Pvt. Ltd. 2016.	ISBN: 978-93-5056-860-6.
3	**Vijisha K. Rajan**, K. Muraleedharan, K.P Safna Hussan. Theoretical structural evaluation and toxicological study of a bitter masking bioactive flavanone, 'Eriodictyol' from Rose hip.	Polyphenols: Prevention and Treatment of Human diseases. (Volume 2, Chapter 5)	Elsevier, 2018	ISBN: 978-0-12-813008-7

4	**Vijisha K. Rajan,** Sarada K, K. Muraleedharan. Electroless palladium plating on silver powder.	Advanced materials in Chemistry (chapter 4)	Department of Chemistry, University of Calicut	

Seminar presentations

International	
1	Vijisha. K. Rajan; K. Muraleedharan: Presented a poster on Preparation and characterization of Pure and Doped Semiconductors of ZnS and CdS: International Conference on advances in new materials conducted by Department of Inorganic Chemistry, University of Madras, Chennai-600025. (Published abstract in the proceedings) ISSN = 978-81-89843-57-1.
2	Vijisha. K. Rajan; K. Muraleedharan: Presented a paper on Determination of Gas phase Gibbs free energy of amines and alkanolamines- A Computational study: International Seminar "Saturnalia of Crystallograghy" organized by the Department of Chemistry, Little Flower College, Guruvayoor, Thrissur on 24th July 2014. (Published abstract in the proceedings)
3	Vijisha. K. Rajan; K. Muraleedharan: Presented a poster on Study of antiferromagnetic coupling interactions by Density Functional Theory: International conference on Nanomaterials for energy, environment, catalysis and sensors (ICNEECS-2015), at Department of Physical Chemistry, Madurai kamaraj Univeristy, Madurai on 11-12 December, 2015. (Published abstract in the proceedings)
4	K.P. Safna Hussan, M. Shahin Thayyil, S.K. Deshpande, T.V. Jinitha, Vijisha K. Rajan, K.L. Ngai: Molecular Dynamics of double active pharmaceutical ingredient- benzalkonium ibuprofenate studied by Broadband Dielectric Spectroscopy; 8th International Discussion Meeting on Relaxations in Complex Systems, Wista, Poland, 2017. ISBN 978-83-226-3251-2

National	
1	Vijisha. K. Rajan; K. Muraleedharan: presented a paper on DFT-B3LYP study of electronic properties of Gallic acid: UGC sponsored National Seminar on Recent Advances in Chemistry, organized by the Department of Chemistry, Kandaswami Kandar's College, Tamil Nadu, on 13-14 August 2015. (Published full paper in the proceedings) ISBN: 978-93-84443-51-1.
2	Vijisha. K. Rajan; K. Muraleedharan: Presented a paper on DFT based calculation of Gas phase basicity and proton affinity of alkylamines: National Seminar on Recent Advances in Chemistry (NSRAC-15), at Department of Chemistry, St.Mary's College, Thrissur on 19th August 2015. (Published full paper in the proceedings)
3	Vijisha. K. Rajan; K. Muraleedharan: presented a paper on DFT-B3LYP Study of electronic properties and uv-visible spectra of triamterene molecule: National Seminar on Recent Advances in Material Science (RAMS-2015), at Department of Physics, T. M. Govt. College, Tirur on 30th Sep- 1st Oct, 2015. (Published full paper in the proceedings)
4	Vijisha. K. Rajan; K. Muraleedharan: Presented a paper on Sterric hindrance and carbamate stability of amines – a DFT-B3LYP computational analysis: National Seminar on Chemistry for Sustainable Future, at Department of Chemistry, Little Flower College, Guruvayoor on 13-14 October, 2015. (Published abstract in the proceedings)
5	Sarada. K; Vijisha. K. Rajan; K. Muraleedharan: Presented a paper on Electronic structure calculation of anhydrous Copper and Silver oxalate to predict the thermal decomposition: National Seminar on Chemistry for Sustainable Future, at Department of Chemistry, Little Flower College, Guruvayoor on 13-14 October, 2015. (Published abstract in the proceedings)
6	Vijisha. K. Rajan; K. Muraleedharan: Presented a paper on Natural bond orbital analysis of Gallic acid by Density Functional Theory: National conference on Colloquium on exotic materials and its implication in societal life, at Department of Chemistry, Sri Vyasa N S S College, Wadakkanchery, on 17-18 December, 2015.
7	Vijisha. K. Rajan; K. Muraleedharan: Presented a paper on Electroless Palladium plating on Silver powder: UGC Sponsored Graduate Seminar on Advances in Materials Chemistry, University of Calicut at the Seminar hall of the Department on 5th December 2014.

Seminars attended:

1. Attended UGC sponsored one day seminar on Natural Radioactivity and its Significances organized by Department of Physics, Chemistry and Botany at SVNSS College, Wadakanchery.
2. Participated in the national conference on Advances in Organic and Physical Chemistry (AOPChem-2012), organized by Department of Chemistry, Calicut University.
3. Attended in the Science Academies' Lecture Workshop on Advances in molecular Spectroscopy organized by Department of Chemistry, Calicut University.
4. Participated in the national conference on New Materials in Chemistry (NMC-2015), organized by Department of Chemistry, Calicut University during 30-31 January 2015.
5. Participated in the national conference on Enchanting Developments in Advanced Materials (EDAM-2015), organized by Department of Chemistry, Calicut University on 27 July 2015.
6. Participated in the National seminar on Frontiers in Chemistry 2017 organized by Department of Chemistry, Calicut University on 28-30 March 2017.