Molecular Studies on Selected Members of the Family Zingiberaceae

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RAJESH KUMAR, T.

BIOTECHNOLOGY DIVISION
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
KERALA, INDIA
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DECLARATION

I hereby declare that this thesis entitled '**Molecular studies on selected members of the family Zingiberaceae'** submitted by me for the award of the degree of Doctor of philosophy in Botany of the University of Calicut, contains the results of bonafide research work done by me at Department of Botany, Calicut University, under the guidance of **Dr. A Yusuf** (Assistant Professor, Department of Botany, University of Calicut). This thesis or part of it has not been submitted to any other university for the award of any other degree or diploma. All sources of help received by me during the course of this work have been duly acknowledged.

C. U. Campus **Rajesh Kumar, T.**

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LIST OF ABBREVIATIONS USED

Abbreviation	Full form
AFLP	Amplified fragment length polymorphism
BM	British Museum (Natural History), London.
BLAT	Blatter Herbarium, St. Xavier's College, Bombay, India
BPH	Botanico-Periodicum-Huntianum
CAL	Central National Herbarium
CALI	Calicut University Herbarium, Kerala, India
CTAB	Cetyl Trimethyl Ammonium Bromide
dNTP's	Dinucleotide triphosphate
EDTA	Ethylene diamine tetra acetic acid
FAA	Formaldehyde: Acetic acid: Alcohol
GC-MS	Gas chromatography – mass spectrometry
g /l	Gram per litre
ICBN	International code of Botanical nomenclature
JSI	Jaccard's similarity index
K	Herbarium of the Royal Botanic Garden, England
Kb	Kilobase
LINN	Herbarium of the Linnean Society of London, Britain
M	Molar
MH	Madras Herbarium
Mg/l	Milligram per litre
mM	Millimole
min	Minutes
OD	Optical Density
PCR	Polymerase chain reaction
PDA	Herbarium of the Royal Botanic Garden, Peradeniya, Sri Lanka
Pg	picogram
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction fragment length polymorphism
SING	Herbarium of Singapore Botanic Gardens, Singapore
TAE	Tris Acetic acid EDTA
Taq	Thermophile aquaticus
TE	Tris EDTA

UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultra violet
V	Volume
W/v	Weight/ volume
µM	Micromole
µl	Microlitre
µg	Microgram

LIST OF PUBLICATIONS

1. Yusuf A, **Rajesh Kumar T**, Nikilesh T S and Rao P S (2011). Effects of antioxidents and gelling agents on regeneration, *in vitro* conservation and genetic stability of *Bacopa monnieri* (L.) Pennell. *International Journal of Ayurvedic and Herbal medicine*.1(3): 92-103.

2. Yusuf A, and **Rajesh Kumar T** (2013). Micropropagation and assessment of genetic stability of wild *Curcuma* species and *C. longa* morphotypes using Random Amplified Polymorphic DNA. *Biologia Plantarum* (Accepted).

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4. Sanoj E, Sabu M and **Rajesh Kumar T** (2010). *Hedychium forrestii* (Zingibereaceae) with a new synonymy and a new variety from India. *J. Bot. Res. Institute Texas* 4(2): 633-639.

5. Sanoj E, Thomas V P, Sabu M and **Rajesh Kumar T**. (2008). *Stahlianthus involucratus* (King *ex* Baker) R.M. Smith (Zingiberaceae): A new generic record for India. *Folia malaysiana* 9(1): 43-50.

INTRODUCTION

Flowering plants (Angioperms) comprises of about 90 percent of the Kingdom Plantae. The total number of species is estimated to be in the range of 250,000 to 400,000 and many tropical species are yet to be unnamed (Thorne, 2002, Scotland and Wortley, 2003).This includes around 12,000 species of mosses (Goffinet *et al*., 2004) and 11,000 species of pteridophytes (Raven *et al*., 2005).According to APG III (2009) the number of families in flowering plants are 415, showing that the flowering plants are more diverse. Angiosperms are well known for their incredible diversity in species number, range of habitat and morphology (Taylor and Hickley, 1997). They occupy every habitat on earth except extreme environments such as the highest mountaintops, the regions immediately surrounding the poles and the deepest oceans. India is renowned as one of the mega biodiversity countries of the world and nurtures huge plant diversity, the vascular plants form the most dominant and conspicuous vegetation cover comprising of over 17,500 species of angiosperms belonging to 4000 genera which represents more than 7% of the world's known flowering plant species (Mudgal and Hajra 1997, Karthikeyan, 2000). These species are occurring in different ecosystems from the humid tropics of Western Ghats to the Alpine zones of the Himalayas and from Mangrooves of tidal Sunderbans to the dry desert of Rajasthan. Biogeographically India represents two of the major realms (Palaearctic and Indo-Malayan) and three biomes. Considering the vastness of the country and climatic variation pattern in different areas, the country is divided into ten botanical regions with distinct bioclimatic conditions. These include: Coromandal coast, Malabar, Indus plain, Indian desert, Gangetic plain, Assam, Eastern Himalaya, Central Himalaya, Western Himalaya, Andaman and Nicobar Islands and Lakshdweep and Minicoy group of Islands.

Twenty two percent of angiosperms are monocotyledons with about 56,000 species (Simpson, 2006). The cladistic analysis of molecular and non-molecular data

have recognized a well-supported lineage of four orders of monocotyledonae known as commelinoid monocots (Chase *et.al*., 2000) which includes Commelinales, Poales, Arecales, Zingiberales and one with special distinction as a family Dasypogonaceae (Zona, 2001).

The order Zingiberales includes many conspicuous taxa, like 'bananas' (Musaceae), 'bird of paradise' (Sterilitziaceae), 'heliconias'(Heliconiaceae), 'gingers' (Zingiberaceae) etc. (Kress *et al*., 2002). Zingiberales are monophyletic clade of eight families and they are almost entirely restricted to tropical regions. The order is widely accepted by most taxonomists and phylogenists and included them in a distinctly circumscribed "natural" or monophyletic lineage. No morphological characters are in conflict with the acceptance of the Zingiberales as a monophyletic group.

The family Zingiberaceae is the largest of the eight families in the order, with 53 genera and over 1377 species (Kong *et al*., 2010). They are mainly distributed in tropics and subtropics with the centre of distribution in the Indo-Malayan region, but extending through tropical Africa to central and South America (Tomlinson 1969, Kress *et al*., 2002, Kong *et al*., 2010).Zingiberaceae are one among the largest monocotyledonous families in India, and are represented by 21 genera and about 180 species (Jain and Prakash, 1995).The earliest published record of Zingiberaceous taxa in India is *Hortus Indicus Malabaricus* by Hendrick Andriean Van Rheede (1692), in which 7 genera and 15 species of Zingiberaceae are recorded. There is a considerable difference of opinion regarding the number of constituent taxa within the family. Schumann (1904) recognized Zingiberaceae as having 38 genera and 800 species, whereas Bailey (1949) recognized 40 genera and only 400 species, 50 genera and about 1000 species by Dahlgren *et al*. (1985) and Willis (1948) about 45 genera and 800 species.Works by Larsen (1997), Williams *et al*. (2004), Skornickova and Sabu (2005) and Kress *et al*. (2010) reduced the total number of genera to 18. Recent additions of *Plagiostachys* Ridl.(Sabu *et al*., 2008), *Stahlianthus* Kuntze (Sanoj *et al*., 2008) and *Larsenianthus* W.J. Kress and Mood

(Kress *et al*., 2010) again raised the total Indian generic representation to 20, and are mainly found in North-Eastern, Peninsular and Andaman and Nicobar regions (Fig.1).

The knowledge about South Indian Zingiberaceae is fragmentary and scattered. About 40% of the genera are represented in the native flora of India. Of the 21 indigenous genera of Zingiberaceae present in India 10 are represented in South India, which form about 50% of total genera occurring in the whole of India. Among the four subfamilies, two *i.e.,* Zingiberoideae and Alpinioideae, are represented in these areas. The Zingiberoideae is well represented in South India with maximum number of 6 genera and 46 species including some exotic ornamentals. The Alpinioideae is the second largest tribe, with 3 genera and 19 species, of which 11 taxa are endemic to this region (Skornickova *et al*., 2004).

The family consists of rhizomatous perennial herbs with well-developed aerial shoots. Inflorescence is terminal on a leafy shoot or on a short, separate leafless shoot arising directly from the rhizome. Flower is zygomorphic, epigynous and bisexual. The family is characterized by the fusion of the lateral staminodes of the inner staminal whorl into labellum, presence of two epigynous glands and presence of cells containing essential or ethereal oils are unique features which distinguish this family from other families of Zingiberales.

The family is related to its sister families of the order Scitamineae (Zingiberales or Arillatae): Musaceae, Cannaceae and Marantaceae of Bentham and Hooker and form a natural group as they have common features such as rhizomatous herbaceous habit, imbricate bases of sheathing petioles, sheath being open or nearly close, calyx and corolla in separate whorls and inferior ovary. This order is a very advanced group and representing the climax of one line of development of the division in which the calyx and corolla remained in separate whorls (Hutchinson, 1934). He also considered this as a parallel group to

orchidales, a climax group of the petaloid Monocotyledons, with regard to a reduction to one stamen in both.

The families of the order show an interesting trend in the reduction in the number of stamens and the number of ovules. The Musaceae approach more nearly the common monocotyledonous arrangement in floral features, in Zingiberaceae and Costaceae, a single stamen is fertile, but in the Cannaceae and Marantaceae only one half-anther is functional, the rest of the stamens being petaloid. In Marantaceae the number of ovules is reduced to just one in each chamber or sometimes two of the three chambers abort, while other families have trilocular ovary.

The family Zingiberaceae, generally known as 'Spice family', form an important group with considerable economic potential, with genera such as *Aframomum*, K.Schum., *Alpinia* Roxb., *Amomum* Roxb., *Curcuma* L., *Elettaria* Maton., *Kaempferia* L. and *Zingiber* Boehm. Many members of this family have been used in Ayurvedic and other natural system of medicine from time immemorial and some are well known spices. They are also used as medicinal, traditional, food and ornamental plants. Nearly 250 species of gingers are used as ornamentals in different parts of the world.

The Zingiberaceae are classified into four subfamilies (Kress *et al*., 2002) based on the evidences from molecular data, viz. Siphonochiloideae, Tamijioideae, Alpinioideae and Zingiberoideae. The subfamily Alpinioideae is further divided in to two tribes, viz. Riedelieae and Alpinieae. The subfamily Zingiberoideae is also divided into two tribes, viz. Zingibereae and Globbeae.

Zingiberaceae have been a taxonomically neglected group mainly because of the inaccessible nature of the wet evergreen forests in which they grow. The short flowering period coincides with the monsoon season, makes their collection much laborious. Moreover, huge vegetative part and massive underground parts makes the preparation of herbarium specimens tedious. Consequently, most

herbarium specimens are fragmentary and the treatments in most of the floras, based on these dried specimens, are truncated accounts. Due to the delicate nature of flowers, loss of color and formation of a gummy mass soon after collection, the study of floral morphology based taxonomical problems, are much difficult to solve especially in the genera *Curcuma* and *Zingiber* and are the most difficult materials for plant hunters, herbarium technicians as well as taxonomists. For field characters and diagnostic features of rhizomes, tubers, nature of the peduncle, the colour of the bracts at different stages of the inflorescence, life cycle and colour of the labellum etc. one has to depend upon the cursory notes prepared by earlier collections, which would be wanting in many details. To make the matters worse, the flowers are evanescent and even flower specimens in the herbaria cannot be of much help to a critical taxonomist. The determination of correct identity is necessary for proper utilization and conservation of Zingiberaceous crops. So proper characterization of the taxa by using molecular and phytochemical methods are needed to solve the problem of delimitation of some taxa. The vulnerability of many of these species and imminent danger of their extinction makes it more urgent. However, strong molecular support is lacking to study the diversity of genera *Zingiber* and *Curcuma* in India.

Traditionally, plant taxonomy is mainly dependent on comparative external morphological characters. Taxonomic confusion is reported to be prevailing in the family Zingiberaceae which is often difficult to be discriminated based on conventional taxonomic tools. A few studies based on morphological, anatomical and biochemical characterization of *Curcuma* and *Zingiber* species have been attempted earlier (Jiang *et al.,* 2006, Zhou *et*. *al*., 2007, Policegoudra and Aradhya 2008, Paramasivam *et al*., 2009, Bua-in and Paisooksantivatana, 2010). Relying much on the morphological characters alone in species delimitation has its own limitations since they are not always completely representative of the genetic structure. Conventional taxonomic techniques in conjunction with molecular biology and biochemical tools may go a long way in providing accurate and

powerful methods for analyzing genetic relationship among the species in the family Zingiberaceae. However, concerted effects are not been taken on molecular characterization in *Curcuma* and *Zingiber* species. Molecular markers assume great significance, as these methods detect polymorphisms by assaying subsets of the total amount of DNA sequence variation in a genome (Das *et al*., 2011).

Molecular phylogenetics is the study of evolutionary relationship among organisms or genes and is done by a combination of molecular biology tools and statistical methods. It is commonly called as molecular systematics, if the relationships of organisms are under scrutiny. Molecular systematics is included under molecular evolution which comprises of three areas of study. First comprises of the evolution of macromolecules which includes the rate and patterns of change in genetic material and its encoded products during the evolutionary period and the mechanisms responsible for such changes,second includes the reconstruction of evolutionary history of genes and organisms, also known as molecular phylogenetics (Molecular systematics) and the third area of study deals with prebiotic evolution or the “Origin of life”.

The present investigation attempts to study the selected members of thegenera *Curcuma* and *Zingiber* in India by means of morphological characters, biochemical and molecular markers, and to develop more convenient identification methods and classify them based on molecular data.

IMPORTANCE OF THE STUDY

A comprehensive global taxonomic revision of *Curcuma* and *Zingiber* have not yet been attempted. The major problems encountered in the taxonomic studies of the genera are lack of type specimens and illustrations of old species, lack of protologues with finer details in the earlier literature, absence of important floral parts in the herbarium specimens, incomplete descriptions of the rhizome features in the herbarium sheets, fleshy and perishable aerial portions etc.

(Sasikumar, 2005). It is now believed that atleast some of the species may be synonyms and may not be true species as described earlier.

Conventional taxonomic techniques in conjunction with molecular biology tools may go a long way in resolving the taxonomic confusion prevailing in the genera. Though few studies on the morphological and anatomical characterization of *Curcuma* species and cultivars have been attempted, not much is done on molecular characterization (Syamkumar and Sasikumar, 2007).

Relying much on the morphological characters alone in species delimitation has its own limitations in *Curcuma* and *Zingiber* as described above; hence,molecular biology techniques like ISSR/RAPD markers assume significance.

The present work is the first attempt in molecular characterization of selected Indian *Curcuma* and *Zingiber* species and adds relevance in the present ongoing context of the taxonomic revision of the genera. Not many effortsare done to classify *Curcuma* and *Zingiber* species by using molecular and phytochemical markers. The development of highly reliable molecular marker system for assessing the genetic diversity within the genus could help in crop improvement programme through molecular breeding.

MOLECULAR MARKERS

Molecular markers are useful in comparing sequence level variation in the DNA from different samples for the characterization of plants. Visible morphological variation is known to occur at a much lower frequency than at the DNA level (Cloutier and Landry, 1994). DNA isolated from the leaves is mostly used in investigations because of the ease of acquisition and preparation (Jarret, 1986). As a result of the high specificity of DNA, molecular markers are able to identify a particular fragment of DNA sequence that is associated to a part of the genome and comparisons are usually made on the basis of the presence or absence of a DNA band. Besides, the use of isozymes which are relatively quicker and cheaper,

direct DNA sequencing, single nucleotide polymorphisms and microsatellites are now available for more informative marker systems. The work by Botstein *et al*. (1980) on the construction of genetic maps using restriction fragment length polymorphism (RFLP) was the first reported molecular marker technique in detecting DNA polymorphism. Presently, a number of molecular techniques are available to detect sequence variation between closely related organisms and also the differences between source plants and somaclones. RFLPs, isozyme, cytological methods and polymerase chain reaction based techniques such as Random amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP) and microsatellite markers are the various molecular detection methods used in the analysis of genetic diversity of plants (Bairu *et al*, 2011).

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

Random amplified polymorphic DNA involves the use of single short primers of arbitrary nucleotide sequences to reproducibly amplify segments of target genomic DNA. These short primers referred to as genetic markers are used to reveal polymorphisms among the amplification products which are seen as visible bands with the aid of ethidium bromide-stained agarose gel electrophoresis (Williams *et al*, 1990). Arbitrary primed PCR (AP-PCR), arbitrary amplified DNA (AAD) and DNA amplification finger printing (DAF) are other variants of Random amplified polymorphic DNA (RAPD). For example, in AP-PCR, a single primer (10-15 nucleotides long) is used and involves amplification for initial two cycles at low stringency. Subsequently, the remaining cycles are performed at higher stringency by increasing the annealing temperature (Welsh and McClelland, 1990). Although AP-PCR is not widely accepted because it involves the use of autoradiography, it has been simplified and fragments can now be fractionated with the use of agarose gel electrophoresis (Agarwal *et al*, 2008). With the DAF technique, shorter single arbitrary primers (less than 10 nucleotides long) are used for amplification and the fragments are analysed using polyacrylamide gel with silver staining (Caetano-Anolles and Bassam, 1993). Technically, RAPD has been described as the simplest

version of PCR with arbitrary primers used for detecting DNA variation and for convenience, all RAPD variants are commonly referred to as RAPD (Weising *et al*, 2005).

Besides providing an efficient technique for polymorphism that allows rapid identification and isolation of chromosome-specific DNA fragments, RAPD markers are also useful for genetic mapping, DNA finger printing, plant and animal breeding (Venkatachalam *et al*, 2008). The use of RAPD markers are especially beneficial to discriminate between materials that are genetically similar, to evaluate genetic variability within a collection and to choose the components of the core collection (Piola *et al*, 1999; Bernardo Royo and Itoiz, 2004).

RAPD and ISSR techniques have been successfully used to assess genetic relationship in many plants, for example, sugar cane (Devarumath *et al*, 2007), sorghum (Singh *et al*, 2006) and apple (Bernardo Royo and Itoiz, 2004). Furthermore many authors have found RAPD technique useful in examining tissue culture induced variation. For instance, it has been used to identify somaclonal variants in peach (Hashmi *et al*, 1997), sugarcane (Taylor *et al*, 1995), moth orchids (Chen *et al*, 1998) and bananas (Bairu *et al*, 2006). RAPD have attracted researchers when financial investment is limited because the input cost is cheaper than other molecular markers such as AFLP and microsatellites (Belaj *et al*, 2003; Weising *et al*, 2005).

GAS CHROMATOGRAPHY – MASS SPECTROMETRY (GC-MS)

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances/compounds within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. Additionally it can identify trace elements in materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely heralded as a gold standard for

forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred McLafferty. The development of affordable and miniaturized computers has helped in the simplification of this instrument, as well as allowed great improvements in the amount of time taken to analyse a sample. GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatography utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase proper (eg. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules are retained by the column and then eluted from the column at different times and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. These two components, used together allow a much finer degree of substance identification than either used singly. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone.

Gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that are vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture and the relative amount of such components can also be determined.

In some situations, GC may also help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture. Gas chromatography is also known as vapor-phase chromatography (VPC), or gas – liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently used in scientific literature.

Chromatography dates to 1903 in the work of a Russian Scientist, Mikhail Semenovich Tswett. German graduate student Fritz Prior developed solid state gas chromatography in 1947. Archer John Porter Martin, who was awarded the Nobel prize for his work in developing liquid – liquid (1941) and paper (1944) chromatography, laid the foundation for the development of gas chromatography and later produced liquid – gas chromatography (1950). A gas chromatograph is a chemical analysis instrument for separating chemicals from a complex sample.

PHYTOCHEMISTRY

Plants represent an unlimited source of phytochemicals such as the metabolites of primary and secondary metabolism. Secondary metabolites are compounds that are biosynthetically derived from the primary metabolites and their distribution in the plant kingdom is restricted. These compounds are generally detected in a lower volume compared to the primary metabolites and possess significant biological activities. Therefore they are also termed as the higher value – lower volume products or speciality chemicals (Roja and Rao, 1998).

Secondary metabolism in a plant plays a critical role for its survival by producing attractants for pollinators; chemical defense against predators and diseases and also an important component of our food, taste, colour and scent. Others such as alkaloids, anthocyanins, flavonoids, quinines, lignans, steroids and terpenoids have commercial applications in the pharmaceutical and biomedical fields and are part of drugs, dyes, flavours, fragrances and insecticides (Veerpoorte *et al*., 2002).

Volatile oil containing drugs and essential oils has been used for a long time both in folk medicines and in therapeutics, both traditional and alternative. Essential oils, the volatile secondary metabolites responsible for the odours of aromatic plants are used in perfumery, as aroma products, flavouring agents in food and beverages, in cosmetic products and as drugs. There is an increasing global trend in the consumption of self-prescribed herbal and natural products for treating numerous ailments such a cancer and even by healthy individuals as preventive measures (Teixeirada Silva, 2004).

Essential oils are frequently referred to as the "life force" of plants unlike fatty oils, they are volatile, highly concentrated substances extracted from flowers, leaves, stems, roots, seeds, bark, resin and fruit rinds. The amount of essential oil found in these plants can range from 0.01% to 10% of the total, necessitating huge quantities of plant material for obtaining meager quantities of oil. These oils have potent antimicrobial factors having 200-300 therapeutic constituents. Most of the essential oils cannot be substituted with alternative chemical synthesis. Only pure oils contain the full spectrum of compounds which cannot be imitated. Essential oils have unique properties that are prized worldwide for thousands of years, being used therapeutically in early Roman, Greek, Egyptian, Indian and Chinese civilizations.

Essential oils are effectively used in aromatherapy. Aromatherapy is the use of pure essential and absolute oils for psychological and physical well-being. Essential oils are believed to stimulate the olfactory nerves and exert influence on the brain centre that controls emotion (Mabey, 1988). They are used as natural rejuvenating and antiwrinkling agents in aromatherapy (Varshney, 1991). It is used in the treatment of pain, psychological disturbances, allergies, skin diseases, gastro intestinal disorders, cardio vascular problems, urinary disorders, gynecological disturbances, cancer etc. (Jamil, 1997).

The versatile use of several aromatic plants in food, cosmetic and pharmaceutical industries demand an extensive screening of essential oils and their components. Individual chemicals isolated from essential oils are more often used than the oils (Brud and Gora, 1989). Therefore, identification of trace components is very helpful to reveal the quality of the oil. Analysis of essential oils can be easily done using Gas chromatography and mass spectrometry (GC-MS). With the help of GC-MS technique, it has now been possible to analyze directly the fragrances of natural or artificial materials without the use of heat or solvents and directly by the use of head space analysis (Thappa *et al*., 1982). GC-MS differs from other types of spectral analysis in that the sample does not absorb radiation from the electromagnetic spectrum. It is highly sensitive and only a small quantity of the sample required.

When coupled with separation techniques like GC or HPLC (High performance Liquid Chromatography), it is a highly specific way to identify organic compounds (Smith and Busch, 1999). A GC-MS machine with computerized library search discs are regarded as the best tool for essential oil analysis (Jose and Rajalakshmi, 2005).

ESSENTIAL OILS

Essential or volatile oils are a complex mixture of organic compounds responsible for the aroma and involved in the defense mechanisms of many plants. Depending on the plant family, volatile oils can be produced by special secretory structures, such as secretory cells, secretory cavities, or secretory ducts (Zizovic *et al*., 2007). These compounds can be stored in several organs, such as flowers (orange, bergamot), leaves (lemon grass, *Eucalyptus* and menthe), bark (Cinnamon), wood (sandal wood, rose wood), rhizomes (*Curcuma*, ginger), fruits (star anise, fennel), or seeds (nutmeg) (Simoes *et al*., 1999). Although all parts of the plant contain essential oils, their composition and quantity may vary with location. Other factors such as the harvesting time, cultivation practice, soil

nutrients, climatic conditions, and genetics can also affect the quality of the volatile oil (Pereira *et al*., 2008; Simoes *et al*., 1999).

The main constituents of essential oils may be classified into two groups: hydrocarbons and oxygenated compounds derived from these hydrocarbons, including alcohols, aldehydes, esters, ketones, phenols, and oxides. The terpenoids contain isoprene (C_5) units: monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), etc. The terpenic compounds found most frequently in essential oils are monoterpenes (90% of essential oils) and sesquiterpenes.

Essential oils contain an important group of bioactive compounds. Several pharmacological properties have been attributed to essential oils, such as antiviral, anti-bacterial, anti-inflammatory, antioxidant, anti-spasmodic and others (Kim *et al*., 1995; Sivropoulou *et al*., 1997; Mimica-Dukic *et al*., 2004; Chao *et al*., 2005;Tuberoso *et al*., 2005; Jirovetz *et al*., 2006; Gornemann *et al*., 2008;). Due to these properties, essential oils have been used as ingredients of cosmetics, food, and pharmaceutical products.

USES OF THE MEMBERS OF ZINGIBERACEAE:

SPICE

Amomum subulatum* Roxb*.: Large cardamom is one of the major cash crops of India andthe ingredients for flavour and fragrance in food and perfumery industry.Dried seed of cardamom form one of the important ingredients used in the masticatory throughout India. In both Indian and European medicines it is a frequent adjunct to other stimulants, bitters and purgative, used in the form of tincture or powder.

Common Name: Large cardamom; Economic part: Dried fruits; Uses: Flavoring food, Ayurvedic medicine

***Elettaria cardamomum* (L.) Maton**: Generally known as 'Queen of Spices' is one of the most economically valuable crops of South India and it constitutes the second most important 'National Spice' of India.In India, Kerala state has the largest area under cardamom cultivation (about 62% of the total).

Common Name: Cardamom; Economic part: Dried fruits; Uses: Flavoring food, Ayurvedic medicine

***Zingiber officinale* Roscoe**: It is one of the five most important major spices of India and about 70% of the total ginger production is confined to Kerala. The name *Zingiber* itself has its origin in Malayalam/Tamil, the language of Kerala from 'inchiver' (*inchi* meaning ginger plant and *ver* meaning root or rhizome).

Common Name: Ginger; Economic part: Rhizome; Uses:Flavoring food, Ayurvedic medicine

***Curcuma longa* L**.: Turmeric is known as 'spice of life'; the orange-yellow rhizome was regarded as the 'herb of the sun' by the people of the vedic period. It is one of the very important spice of India and a traditional item of export from ancient times. 93.7 % of the total world production of turmeric is from India. It is used as an antiseptic for fresh wounds and ulcers, coloring material in pharmacy, social, cultural and religious functions and rituals in India. The powder of turmeric is used for food preparation, poojas and against poisoning. The yellow colour of turmeric is due to the presence of group of compounds called curcuminoides, of which the most important one is curcumin.

Common Name: Turmeric; Economic part: Rhizome; Uses:Spice, Ayurvedic medicine

MEDICINAL

***Alpinia calcarata* (Haw.) Rosc.:** The rhizome, with a sharp odour and a pleasant taste, is used in the form of an infusion for fever, rheumatism and catarrhal

affections. It is also supposed to improve voice. The rhizomes form a major ingredient of several Ayurvedic preparations such as *Rasnadi kashayam, Rasnadhi churnam, Rasnadi thailam* and *Aswagandharishtam.*

Common Name: Chittaratha; Economic part: Rhizome; Uses: Ayurvedic Medicine

***Alpinia galanga* (L.) Retz.:** The rhizomes of this species is aromatic, pungent and bitter. It improves appetite, taste and voice. It is also used to treat head-ache, lumbago, rheumatic pains, sore-throat, stuttering, chest pain, diabetis, burning sensation of the liver and disease of the kidney. The drug stimulates digestion and purifies blood.

Common Name: Aratha; Economic part: Rhizome; Uses: Ayurvedic Medicine

***Curcuma aeruginosa* Roxb.**: The rhizomes are widely used in South India for the extraction of East Indian arrowroot or Travancore starch. It is used as a medicine for stomach disorders and as an ingredient in various cuisines.

Common Name: Neela Kua; Economic part: Rhizome; Uses: Ayurvedic Medicine, Arrowroot extraction

***Curcuma aromatica* Salisb.:** The rhizomes are used medicinally, being regarded as tonic and carminative. It is used externally for scabies and the eruption of small pox. It is made into a paste with benzoin and is applied to the forehead for headache. When applied externally to the skin, it gives a peculiar lively tinge to the natuarally dark complexion and a delicious fragrance.

Common Name: Kasthuri manjal; Economic part: Rhizome; Uses: Ayurvedic Medicine

***Kaempferia galanga* L.:** It is a reputed remedy for respiratory ailments like cough, bronchitis and asthma. The powder extracted from the rhizome is mixed with honey and given for coughs and pectoral infections. The tuber is boiled in oil and applied externally to treat blocked nasal tract.

Common Name: Kachola kizhangu; Economic part: Rhizome; Uses: Ayurvedic Medicine

***Kaempferia rotunda* L.:** The powder extracted from this species is made into an ointment and is used for healing fresh wounds. It is taken internally to remove coagulated blood or purulent matter within the body. It is also used in many Ayurvedic preparations.

Common Name: Chengazhinir kizhangu; Economic part: Rhizome; Uses: Ayurvedic Medicine

ORNAMENTAL

***Alpinia purpurata* (Vieill) K. Schum**. (Red ginger): A very popular ornamental plant in India. It is widely used as cut flowers around the world. Plants with red, pink, white and even double coloured bracts are cultivated in garden.

***Alpinia vittata* W. Bull.** (Striped Narrow Leaf Ginger): Grows up to 5' (1.50m) high in medium sun. Narrow variegated leaves make this plant attractive.

***Etlingera elatior* (Jack) R.M.Sm**.(Torch ginger): A very attractive plant with beautiful large torch like inflorescence. Several varieties of this species are widely cultivated. Good cut flower and much used in cut flower industry.

***Curcuma roscoeana* Wall**.*:* Commonly called as Jewel of Burma or pride of Burma. In India it is widely distributed in the forests of Andaman Islands. The species can be easily recognized by its bright orange bracts and cream coloured flowers.

Curcuma thoreli: (Chiang mai snow): The pretty small to mid-sized species. This species is the most utilized species of *Curcuma*. The fertile bracts are relatively small and green embracing a flower with violet labellum, but the coma bracts are white in colour, much larger and spreading. It is widely used as a cut flower throughout the world.

***Curcuma petiolata* Roxb**.*:* A medium sized plant grown for its foliage and used in landscaping. The terminal inflorescence has white bracts tinged with pink at tips. This plant is also grown as a cut flower crop and as a potted plant.

***Curcuma alismatifolia* Gagnep**. : Peduncle long, slender and stiff. The coma bracts pink, large and erect. The trade name for this species is known as “Siam Tulip”. Its bulbs are exported to Japan.

***Curcuma aurantiaca* Zijp**. (Rainbow ginger). The inflorescence, which appears in different hues, is a promising material as cut flowers and ground cover.

***Curcuma inodora* Blatter** (Hidden purple ginger). The inflorescence with variously coloured and shaped comma bracts, and labellum adds more beauty to our garden. The labellum shows range of colours from dark purple, white, yellow and golden.

Curcuma sparganifolia (Pink pearls): A narrow leaved *Curcuma* growing wild in Cambodia and Thailand. The inflorescence is small and globose with a few dark tipped bright pink bracts. The plant can be grown as a cut flower crop. The inflorescence has high export value.

***Zingiber capitatum* Roxb**.: A beautiful *Zingiber* with terminal inflorescence. The yellow flowers and green bracts with narrow distichously arranged leaves add beauty to the plant. The rhizomes once planted will reproduce the aerial shoot and flowers for several years.

***Zingiber zerumbet* (L.) Rosc.** (Shampoo ginger): Widely distributed throughout India. Lateral Globose or oblong dark green inflorescence, on a leafless long peduncle turns red when mature,is a promising material as cut flowers.

***Globba schomburgkii* Hook. f.** (Dancing ladies):Inflorescence with lax bracts, orange pedicellate flowers make the plant attractive. Widely grown as an ornamental and naturalized in Asian countries.

***Globba winitii* Wright.** (White dragon): Commonly called as *Dancing ladies*, the rhizomes are seasonally dormant and blooms in summer. Flowers are yellow with violet or white reflexed bracts. Grown for cut flowers. Inflorescence with white flowers are also common.

***Hedychium coccineum* Buch.-Ham.** (Scarlet ginger): The flowers are small, dull orange to brick red. The inflorescence is large 25-30 cm long with 6-7 rows of lax bracts on the peduncle. The plant is also attractive in vegetative stage due to the purple shade on the lower side of the leaf. The plant can be grown as a hedge plant.

Boesenbergia siphonantha* (Baker) Sabu *et al. This is a very beautiful small plant found growing in the moist deciduous forests of Andaman Islands. The plant can be grown as an ornamental ground cover. The lip tinged with pink white flowers blooms out of the dark green leaves adds more aesthetic value to our garden.

OBJECTIVES

Major objectives of the study are:-

1. Taxonomic and molecular characterization of the selected members of the genera *Curcuma* and *Zingiber* (Zingiberaceae) in India.

2. Extensive survey of literature and critical study of herbarium specimens deposited at major herbaria in India.

3. To study the variation among different taxa at molecular level by using RAPD and ISSR and phytochemical studies by using GC-MS.

4. Conservation of all taxa under study in the Calicut university Botanic Garden.

5. Preparation of a detailed taxonomic and molecular level identification key, using NTSYS-pc software and UPGMA clustering.

REVIEW OF LITERATURE

The members of the family Zingiberaceae are perennial rhizomatous herbs growing in shady habitats and are characterized by the possession of a tuberous, often creeping rhizome with an aerial shoot, often covered by sheathing leaf bases. The word 'gingers' refers to the members of the family Zingiberaceae, whereas "spiral gingers" to the members of the family Costaceae (Sabu, 2006). The inflorescence is usually a spike or raceme. The flowers are very delicate and fleshy so that in most cases they wither and crumble forming a gummy mass soon after collection, making it difficult to study floral morphology unless fresh flowers are readily available. In majority of the constituent taxa, flowering period is very short and usually associated with rainy season. As most of the *Zingibers* grow in open areas or as undergrowth in dense forests, usually impenetrable during rainy season, collection of fresh materials is very difficult. The earliest published record of Zingiberaceae taxa in India is in *Hortus Malabaricus* by Hendrik Andriaan van Rheede (1678-1693). In the eleventh volume of this monumental work ten species of Zingiberaceae are described.

The family Zingiberaceae consists of about 53 genera and more than 1300 species, distributed mainly in tropics and subtropics with the centre of distribution in the Indo-Malayan region, but extending through tropical Africa to Central and South America (Kress *et al*., 2002). There is considerable difference of opinion regarding the number of constituent taxa within the family. Schumann (1904) recognized Zingiberaceae having 38 genera and 800 species, whereas Bailey (1949) recognized only 40 genera and 400 species, 50 genera and about 1000 species by Dahlgren *et al* (1985) and Willis (1948) about 45 genera and 800 species. The number of valid genera as accepted today is 53 with about 1377 species (Kong *etal*., 2010). It forms one of the most interesting and natural groups of monocotyledons and the species are remarkable for their wide range of morphological features.

Zingiberaceae forms an important group with considerable economic potential, with plants such as *Aframomum, Amomum, Curcuma, Elettaria, Kaempferia* and *Zingiber.* Many members of this group have been used in Ayurveda and other native systems of medicine from time immemorial and some are well known spices. *Elettaria cardamomum* is far more important economically than others and constitutes the second most important 'National Spice' of India which is known as the 'Queen of Spices' and forms one of the most valuable crops of South India. In India, Kerala state has the largest area under cardamom cultivation (about 62% of the total), followed by Karnataka (31%) and Tamil Nadu (7%). *Zingiber officinale* also constitutes one of the five most important major spices of India and about 70% of the total ginger production is confined to Kerala alone, which produces best quality ginger. *Curcuma longa* is another very important spice of India and a traditional item of export from ancient times (Pruthi, 1976).

The west- coast of South India including most of Kerala and the western parts of Karnataka and Maharashtra are the richest floristic regions in the country. On account of its Geographic location, Kerala and the western parts of Karnataka have a warm and humid climate with heavy rainfall, and support dense vegetation. However, intense human interference during the present century is slowly depleting the vegetation particularly along the ghats giving way to agricultural land and plantations. The eastern side gradually merges into the arid land with the attenuated flora of the elevated plateau of Deccan (Mudgal and Hajra, 1977).

The flora of the south western part of South India is strikingly similar to that in certain areas of south western Sri Lanka. Species belonging to monocot familiessuch as Araceae, Poaceae, Orchidaceae and Scitamineae are very common in both the regions (Chatterjee, 1956). Addition of *Curcuma oligantha* Trimen and *Alpinia fax* B.L. Burtt and R.M.Sm. (Prasanth Kumar *et al.*, 2002), *Amomum masticatorium* Thwaites (Bhat, 1988) to the ginger flora of India confirm this view. The flora of this area also shows affinity with the distant Assam, Thailand and Malesia. Apart from the already reported taxa common to both regions, the

discovery of *Curcuma aurantiaca*, *Hedychium spicatum* var. *acuminatum* and *Globba schomburgkii* from South India, hitherto reported only from North East India, Thailand and Malaysia, supports this view. The two areas of tropical evergreen forests of South Western- South India and Assam and Malaysia are separated by about 3000-4000 Km. apart. Yet, certain common species are found in both these areas and are absent in the intervening areas. Another feature of the area of the present study is that it comprises coastal areas on the west and east followed by Western and Eastern Ghats with the central plateau incorporated by numerous loosely connected and isolated hill masses, which aids in the study of variations within the same species occurring in varied environments.

So far the knowledge about South Indian Zingiberaceae is fragmentary and scattered. About 40% of the genera are represented in the native flora of India. Of the 21 indigenous genera of Zingiberaceae present in India, 10 are represented in South India, which form about 50% of total genera occurring in the whole of India. The tribe *Zingibereae* is well represented in South India with maximum number of genera (5) and species (42).

The *Alpinieae* is the second largest tribe, with 3 genera and 19 species, including 7 endemic and 2 exotic species.Tribe *Globbeae* is represented by a single genus and 4 species. *Curcuma* is the largest genus in this area with 20 species, of which 11 taxa, *C. bhatii* (R.M. Sm.) Skornickova and Sabu; *C. coriacea,* Sabu and Mangaly, *C. decipiens,* Dalzell; *C. haritha,* Mangaly and Sabu*, C. karnatakensis,* Amalraj *et al., C. kudagensis,* Velayudhan *et al., C. mutabilis,* Skornickova *et al., C. neilgherrensis,* Wight*, C. raktakanta,* Mangaly and Sabu*, C. vamana,* Sabu and Mangaly, and *C. oligantha* var. *lutea,* (Ansari *et al.*) K.G. Bhat) are endemic to this region (Mangaly and Sabu, 1993; Skornickova *et al.* 2004).

***Curcuma* Sp.**

The genus *Curcuma* L., comprises of around 120 species mainly distributed in the Indo-Malayan region includes a homogenous group of rhizomatous

perennials. The genus is easily recognized by its inflorescence, a spike with prominent spiral bracts, which laterally fuse or adnate to the peduncle and form pouches, each subtending a cincinnus of flowers and a cluster of often coloured, sterile, terminal bracts called coma (Skornickova and Sabu, 2002; Skornickova *et al*., 2004).

The species belonging to the genus *Curcuma* can be grown in diverse tropical conditions from sea level to a height of 1500m on the hilly slopes, in temperatures ranging from 20 to 30^0C. Rainfall of 150 cm or more or an equivalent amount of irrigation is essential for optimum growth and development of *Curcuma* species. Ideal soil requirements for growing *Curcuma* are loose, friable loamy or alluvial soil suitable for irrigation that should have efficient drainage capacity. The species are naturally found in mixed deciduous tropical forests and tropical broad-leaved evergreen forests of the tropical and subtropical regions. The geographical distribution of the genus spreads from India to Thailand, Indonesia, Malaysia and finally to northern Australia (Apavatjrut *et al*., 1999; Sirirugsa, 1999).

There is no available documented literature about the origin and distribution of African and South American *Curcuma* species. The members of the genus in these regions are important resources and have great potentials in terms of commercial value as source of spices, medicines and horticultural products (Purseglove 1974; Apavatjrut *etal*., 1999; Cao *etal*; 2001; Yusuf *etal*; 2001; Sasaki *etal*; 2002; Cao and Komatsu, 2003;Maciel and Criley, 2003; Joe *etal*; 2004; Sasaki *etal*; 2004).

The genus *Curcuma* was first put forward by Linneaus (1753). Roxburgh (1820) divided *Curcuma* into two sub genera, depending on the position of spikes (lateral and central). Horaninow (1862) distinguished three sections namely Exantha (spikes always lateral), Mesantha (Spikes invariably terminal) and Amphiantha (spikes both terminal and lateral) in the genus. Baker (1890) accepted section Exantha and Mesantha, rejected section Amphiantha and introduced a new

section Hitcheniopsis and described 27 species in the *Flora of British India*, the section Exantha consists of 14 species including turmeric and other economically important species such as *C. angustifolia* Roxb., *C. aromatica* Salis., and *C.zedoaria* Rosc. Schumann (1904) rejected the sectional classification based on spike position but recognized two sub genera, subgenus Eucurcuma and subgenus Hitcheniopsis based on the presence or absence of spur on anthers. Purseglove (1968) and Harlan (1975) suggested the origin of *Curcuma* in the Indo-Malayan region considering the great diversity of the genus represented by over 80 species in the region. Velayudhan *et al*. (1999) also suggested that over 40 species are indigenous to India is more supportive to its Indian origin. Scientists have different opinion regarding the number of the species. Larsen *et al*. (1998) suggested 80 species and as around 100 by Sirirugsa (1996). In India about 29 species have been distributed in almost all the states; many are cultivated and naturalized and the main centres of distribution are South West India, North East India and Andaman and Nicobar Islands (Karthikeyan *et al*., 1989; Sabu, 2006). Velayudhan *et al*. (1999) reported the occurrence of 20 species of *Curcuma* in Kerala. From South India 16 species of *Curcuma* were reported, out of which 9 are endemic to India (Sabu, 2006).

Properties and Uses

Turmeric (*C .longa* L.) is an important medicinal plant, which is known for its aromatic, stimulant, carminative and anthelmintic properties (Satyavati *et al*., 1976). It is used as a colouring material in pharmaceuticals, confectionary and food industries. The finest Indian arrow root derived from *C. angustifolia* Roxb. has been used as a source of starchy food for centuries (Das *et al*., 1999). A number of *Curcuma* species have beautiful inflorescence and luxurious foliages that have an immense commercial value in floriculture as a versatile ornamental crop used as cut flowers, pot and landscape plant (Maciel and Criley, 2003; Paisooksantivatana *et al;* 2001a and 2001b). Among them *C. alismatifolia* is recognized and popular in international trade as cut flower (Paisooksantivatana *et al;* 2001b). Some other species such as *C. aeruginosa*, *C. amada*, *C. angustifolia*, *C. ceasia*, *C. elata*, *C.*

petiolata, *C. rubescense*, *C. zanthorrhiza* and *C. zedoaria* have also received considerable attention as cut flowers and tropical glass house ornamentals. The rhizomes of *C.aeruginosa* are widely used in South India for the extraction of East Indian arrowroot or Travancore starch. It is used as a medicine for stomach disorders and as an ingredient in various cuisines. The tubers of *C.amada* are regarded as cooling and as useful in prurigo. Tubers, made into a paste with spirit and egg-white is applied for chronic rheumatism and bruises. Roots are expectorant and astringent, useful for treating diarrhea and gleet. Tubers are also used as condiment and vegetable. The rhizomes of *C.aromatica* are used medicinally, being regarded as a tonic and carminative. It is used externally for scabies and the eruption of small pox. It is made into a paste with benzoin and is applied on the forehead for headache. When applied externally to skin, it gives a peculiar lively tinge to the naturally dark complexion and a delicious fragrance (Watt, 1972).

The fresh root of *C. zanthorrhiza* checks leucorrhoeal and gonorrheal discharges and purifies blood. The juice of the leaf is given in dropsy (Rheede, 1692). Zedoary oil derived from the rhizomes of *C. zedoaria* is used as a spice, tonic and perfume. The terminal bracts from a sterile cluster called coma, often brightly coloured and in case of some species these coma bracts are rich in volatile oil that are also used to produce perfumes and cosmetics. Recently, leaf essential oils of *C. longa* and *C. aromatica* have been analyzed by Behura *et al*., (2002). They found several important essential oils such as a-phellandrene, 1, 8 cineole, c 8-aldehyde and Linlol. These essential oils are valuable material for pharmaceutical and cosmetic industries.

***Zingiber* Sp.**

The generic epithet *Zingiber* was derived from Malayalam/Tamil 'ingiver' meaning ginger rhizome. This term spread to ancient Greece and Rome through the Arab traders and from them to Western Europe (Ravindran and Babu, 2005).

Some authors believed that the name was derived from the Sanskrit word 'Singabera', which mean 'horn-root' (rhizome) and later from Arabic word 'Zanjabil' which gave rise to the classical German Zingiberi and finally to Zingiber in Latin. As the Sanskrit language was not popular in the region in those days, the second opinion is of no relevance. Some are of the opinion that the horn – shaped long arching anther hood and still others considers the two lateral staminodes, which are fused with the labellum pointing forward resemble horns (Valeton, 1918; Watt, 1972).

Zingiber is distinct from other genera of the family in the presence of a single anther with a beak or horn-like appendage, which embraces the upper part of the style. The inflorescence usually arises at the base of the leafy stem, on a long or subterranean peduncle. The bracts are overlapping and each subtends a non-tubular bracteole and a single flower. In many species the bracts are green when young, turning to red in the fruiting stage. The flowers are very delicate and fragile and last only for a few hours. The genus can be recognised in the vegetative stage by the presence of a pulvinus between the base of the petiole and ligule.

The genus is represented by 141 species, distributed mainly in tropical Asia (Theilade, 1999b; Theilade and Mood, 1999). The genus in India was first studied more than a century ago by Roxburgh (1810) and reported 11 species and placed all the species under two sections based on the nature of the spike, *i.e*, Sect. I. Spikes radical and Sect. II. Spikes terminal. Baker, (1892) described 24 species from British India. He placed them under 4 sections *viz*. Cryptanthium Horan; Lampuzium Horan; Pleuranthesis Benth. and Dymczewiczia (Horan.) Benth. This infra generic classification was followed by Schumann (1904) in the revision of Zingiberaceae in which he recognized 55 species. Fischer (1928) recorded 7 species from Western Ghats of South India. Jha and Varma (1995) revised the genus in Bihar, whereas Kumar (2001) recorded 7 species from Sikkim.

There is still, a lot of confusion in the delimitation of South Indian species. The descriptions in many of the earlier treatments are insufficient and data appeared to be necessary for a proper chracterization of the species, are often omitted. The genus *Zingiber* is one of the most difficult material of Zingiberaceae to collect and work out satisfactorily due to the colour of the bracts, which deteriorates and changes in due course of time, peduncle at the different stages of the inflorescence, only one or two flowers open at a time and it is difficult to detach them from the bract without damaging, life cycle and varying colour of the labellum and poorly preserved herbarium etc.

Karthikeyan *et al*. (1989) in *Flora indicae Enumeratio Monocotyledonae*, listed 21 species of *Zingiber*. Jain and Prakash (1995) studied the phytogeography and endemism of Zingiberaceae in India and listed 130 species under 22 genera in which 88 species were endemic to India. Sabu and Mangaly (1996) revised Zingiberaceae of South India and listed 11 genera and 55 species.

Mohanan and Sivadasan (2002) recorded 12 taxa under 7 genera of Zingiberaceae and 3 species of *Zingiber* in Flora of Agasthyamala. Nayar *et al*. (2006) enumerated 48 species under 9 genera of Zingiberaceae which included 6 species of *Zingiber*.

PROPERTIES AND USES

A hot infusion of ginger (*Z. officinale*) known as ginger tea is considered to have diaphoretic effect on colds (Datta and Mukerji, 1950). Prakash and Mehrotra (1996) discussed the importance of *Z. officinale* for cough, bronchitis, asthma, heart and abdominal troubles, piles, elephantiasis, scorpion sting, snake bite, appetizer, stomachic, aphrodisiac, carminative and its uses as spice, condiment and preservative. In traditional medicine, ginger is extensively used for its specific action in rheumatism and inflammation of liver (Aiyer and Kolammal, 1964; Kuruppu *et al*., 1979). Rhizome of *Z. montanum* is given in diarrhea, colic etc. and alsocan be used as a stimulant, carminative, flavoring agent and an antidote to

snake bite. Rhizome of *Z. roseum* is used in cold, cough and rheumatism. *Z. zerumbet* rhizome is given in cough, asthma, stomach ache, vermifuge, leprosy and other skin deseases and also used as a substitute for true gingers (Rajendran *et al*., 1997; Srivastava., 2003). It is also known as "shampoo ginger", since the mucilaginous substance present in the inflorescence is used as shampoo and the flowers are used in cut flower industry (Sabu, 2006).

MOLECULAR CHARACTERIZATION

During the last few decades, use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing part in plant systematics and their genetic studies. There are different types of markers *viz*. morphological, biochemical and DNA based molecular markers. These DNA based markers are differentiated in to two types, non PCR (RFLP) and PCR based markers (RAPD, AFLP, SSR, SNP etc.). Day by day development of new and specific types of markers makes their importance in understanding the genomic variability and the diversity between the same as well as different species of the plants (Kumar *et al.,* 2009). Due to the rapid developments in the field of molecular genetics, varieties of different techniques have emerged to analyze genetic variation during the last few decades. These genetic markers may differ with respect to important features, such as genome abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and financial investment. No marker is superior to all others for a wide range of applications. The most appropriate genetic marker depends on the specific application, the presumed level of polymorphism, the presence of sufficient technical facilities and know how, time constraints and financial limitations (Weising *et al*., 1995).

MOLECULAR MARKERS

Molecular markers are DNA sequences that are readily detected and whose inheritance can be easily monitored. The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis for designing

strategies to exploit specific purposes. Different types of molecular markers used in plant diversity analysis, their advantages, disadvantages etc. are represented in Table-1 and 2.A marker can be polymorphic; if it exists in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker. Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two discontinuous variants or genotypes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. The first such DNA markers to be utilized are fragments produced by restriction digestion-the restriction fragment length polymorphism (RFLP) based gene marker. Consequently, several markers system has been developed (Kumar *et al.,* 2009).

Table -1. Different types of markers used in plant diversity analysis.

Sl.No.	Name of the Technique		Discoverer
A.	**Biochemical markers**	Allozymes	Tanksley and Orton 1983; May, 1992
B.	**Molecular markers**		
	1.Non-PCR based techniques	Restriction Fragment Length Polymorphisms (RFLP)	Botstein *et al*., 1980; Neale and Williams, 1991
		Minisatellites or Variable Number of Tandem Repeats (VNTR)	Jeffreys *et al*., 1985
	2.PCR-based techniques		
	DNA sequencing	Multi-copy DNA, Internal Transcribed Spacer regions of nuclear ribosomal genes (ITS)	Takaiwa *et al*., 1985; Dillon *et al*., 2001
		Single-copy DNA, including both introns and exons	Sanger *et al*., 1977; Clegg 1993a

Sl.No.	Name of the Technique		Discoverer
	Sequence-Tagged Sites (STS)	Microsatellites, Simple Sequence Repeats (SSR), Short Tandem Repeats (STR), Sequence Tagged Microsatellite (STMS) or Simple Sequence Length Polymorphism (SSLP)	Litt and Luty 1989; Hearne *et al*., 1992; Jarne and Lagoda, 1996
		Amplified Sequence Length Polymorphism (ASLP)	Maughan *et al*., 1995
		Sequence Characterized Amplified Region (SCAR)	Michelmore *et al*., 1991; Martin *et al*., 1991; Paran and Michelmore, 1993
		Cleaved Amplified Polymorphic Sequence (CAPS)	Akopyanz *et al*., 1992; Konieczny and Ausubel, 1993
		Single-Strand Conformation Polymorphism (SSCP)	Hayashi, 1992
		Denaturing Gradient Gel Electrophoresis (DGGE)	Riedel *et al*., 1990
		Thermal Gradient Gel Electrophoresis (TGGE)	Riesner *et al*., 1989
		Heteroduplex Analysis (HAD)	Perez *et al*., 1999
		Denaturing High Performance Liquid Chromatography (DHPLC)	Hauser *et al*., 1998; Steinmetz *et al*., 2000; Kota *et al*., 2001
	Multiple Arbitrary Amplicon Profiling (MAAP)		Caetano-Anolles, 1996
		Random Amplified Polymorphic DNA (RAPD)	Williams *et al*., 1990; Hadrys *et al*., 1992
		DNA Amplification Fingerprinting (DAF)	Caetano-Anolles *et al*.,1991
		Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)	Welsh and McClelland, 1990; Williams *et al*., 1990

Sl.No.	Name of the Technique		Discoverer
		Inter-Simple Sequence Repeat (ISSR)	Williams *et al*., 1990, Zietkiewicz *et al*., 1994
		Single Primer Amplification Reaction (SPAR)	Staub *et al*., 1996
		Directed Amplification of Minisatellites DNA (DAMD)	Heath *et al*., 1993; Somers and Demmon, 2002
		Amplified Fragment Length Polymorphism (AFLP)	Vos *et al*., 1995
		Selectively Amplified Microsatellite Polymorphic Loci (SAMPL)	Witsenboer *et al*., 1997

Table-2. Advantages and disadvantages of some commonly used molecular markers

Type of markers	Advantages	Disadvantages
Restriction Fragment Length Polymorphism (RFLP)	-High genomic abundance -Co-dominant markers -Highly reproducible -reusability of membrane filters -Good genome coverage -Can be used across species -No sequence information -Can be used in plants reliably (well-tested) -Needed for map based cloning	-Need large amount of good quality DNA -Laborious (compared to RAPD) -Difficult to automate -Need radioactive labeling -Cloning and characterization of probe are required

Type of markers	Advantages	Disadvantages
Random Amplified Polymorphic DNA (RAPD)	-High genomic abundance -Good genome coverage -No sequence information -Ideal for automation -Less amount of DNA (poor DNA acceptable) -No radioactive labeling -Relatively faster	-No probe or primer information -Dominant markers -Not reproducible -Cannot be used across species -Not very well-tested
Single Sequence Repeat (SSR)	-High genomic abundance -Highly reproducible -Fairly good genome coverage -High polymorphism -No radioactive labeling -Easy to automate -Multiple alleles	-Cannot be used across species -Need sequence information -Not well-tested
Amplified Fragment Length Polymorphism (AFLP)	-High genomic abundance -High polymorphism -No need for sequence information -Can be used across species -Work with smaller RFLP fragments -Useful in preparing contig maps	-Very tricky due to changes in patterns with respect to materials used -Cannot get consistent map (not reproducible) -Need to have very good primers
Sequence-Tagged Site (STS)	-Useful in preparing contig maps -No radioactive labeling -Fairly good genome coverage	-Laborious -Cannot detect mutations out of the target sites -Need sequence information -Cloning and characterization of probe are required

Type of markers	Advantages	Disadvantages
	-Highly reproducible -Can use filters many times	
ISOZYMES	-Useful for evolutionary studies -Isolation lot easier than that of DNA -Can be used across species -No radioactive labeling -No need for sequence information	-Laborious -Limited in polymorphism -Expensive(each system is unique) -Have to know the location of the tissue -Not easily automated

Among the diverse DNA markers identified during the past decades RAPDs with the potentially unlimited number of markers allow finer distinction. The use of molecular markers is becoming widespread for the identification of genotypes and also to quantify the extent of genetic variation in a given population. The polymerase chain reaction (PCR) has been the basis of a growing range of newer techniques (Saiki *et al*., 1988). PCR allows specific amplification of DNA sequences making it ideal for the identification of plant genotypes. Amplification of a genotype – specific sequence can take advantage of some of the many features of PCR like speed, simplicity, specificity, sensitivity and cost (Henry, 1997). Molecular markers such as restriction fragment length polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) (Welsh and Mc Clelland, 1990; Williams *et al*., 1990) appears to be good, but when compared to RFLP, RAPD appears to provide a better basis for genetic characterization because of the simplicity of the necessary procedures (Baird *et al*., 1992).

Molecular markers are used for the identification of genotypes and to quantify the extent of genetic variation in any given population. While on one hand the approach of RAPD profiling has been useful in tissue culture methods for

detection and selection of somaclonal variants (Munthali *et al*., 1996). The molecular technique, with the same logic, is directly utilizable for assessing the population of micropropagated clones from any given explant for genetic uniformity. Using PCR with short primers of arbitrary sequences, RAPD markers are shown to be sensitive for detecting variations among individuals between and within species (Carlson *et al*., 1991; Roy *et al*., 1992). This is an alternative approach for finding new DNA based polymorphic markers among closely related genotypes (Welsh and Mc Clelland, 1990; Nymbom *et al*, 1990; Lindout *et al*., 1999). RAPD analysis using PCR with arbitrary oligonucleotide primers (Williams *et al*., 1990) has the advantage of being non-radioactive, rapid and is a convenient assay of polymorphism that requires only a small amount of crude DNA.

The main issues associated with the use of these techniques are the problem of ensuring reproducibility of amplification profiles. The nature of the amplification process with short primers is such that many sites in the genome are potential templates and the profile obtained may be influenced by any variation in the method used to prepare the DNA template and the exact reaction composition and the conditions used in the PCR (Muralidharan and Wakeland, 1993). Obtaining reliable results depends upon standardizing the conditions or identifying combination of conditions that give consistent results, even when variations in the key reaction conditions are encountered. A key requirement for reliable and reproducible RAPD results is dependent upon sample preparation and DNA isolation. Both the quality and quantity of the template DNA preparation have the potential to substantially influence the result.

Polymorphism results from either base change at the primer binding site (point mutation) or chromosomal changes in the amplified regions (insertions, deletions or inversions) which alter the size or prevent the successful amplification of a target DNA. Southern hybridizations are not required and polymorphisms can also be detected in fragments containing highly repeated sequences, which are

recalcitrant to RFLP analysis. The extent of polymorphism detected by RAPDs is therefore greater than that is observed by RFLPs (Williams *et al*., 1990).

Random Amplified Polymorphic DNA (RAPD) markers have been applied in woody species (Goto *et al*., 1998) to assess the reproduction of some segments of the genome, for rapid appraisal of tissue-culture-derived plants (Rani and Raina, 1998). They have been shown to enhance breeding efforts in annual and perennial crops (Rafalski *et al*., 1993). They are also effective for cultivar identification. The amplification of Random DNA segments is carried out with single primers (usually 10-mers) of arbitrary nucleotide sequence (Williams *et al*., 1990). RAPD reactions are not radioactive, requires only nanogram quantities of DNA and is applicable to a broad range of species. Allelic variation among individuals is detected as the presence or absence of the multiplication product visualized as a band after PCR and electrophoresis (Rafalski *et al*., 1993).

RAPD profiles generated by 11 operon primers as an index for estimating genetic fidelity of selected 'variants' among micropropagated and callus regenerated plants of ginger and indicated the generation of variants through tissue culture(Suja 2002; Nirmal Babu *et al.,* 2003). Earlier studies by Rout *et al.* (1998) indicated that RAPD did not indicate any polymorphism among the micropropagated plants.

An efficient protocol for the isolation of high molecular weight DNA from dry powdered samples of turmeric including market samples was described (Remya *et al*., 2004). This will help in PCR based detection of adulteration in marketed samples of turmeric. The method involves a modified CTAB (3% w/v) procedure with 2M NaCl, 0.3% (v/v) β-mercaptoethanol coupled with purification of DNA in 30% polyethylene glycol (8000). The yield of the DNA obtained from the samples varied from 2 to 4 µg/g tissue. The DNA obtained from the five different samples were consistently amplifiable with RAPD primers.

Molecular markers like RAPD, PCR-RFLP, and ISSR polymorphism are used to characterise 96 collections comprising important cultivars, varieties and related genera of cardamom to develop fingerprints and to study the inter-relationships (Nirmal Babu *et al*., 2005). The study indicated that there were no duplicates in the 100 lines characterized and the Kerala and Karnataka population were divergent in that they formed two separate clusters in the phylogram. Eleven species represented 5 major tribes viz., *Amomum subulatum*, *A. aromaticum, A. ghatium, A. microstephanum, Amomum involucratum, Alpinia galanga, A. purpurea, A. mutica, Aframomum melegueta, Hedychium coronarium* and *Elettaria cardamomum* were also profiled for polymorphism using RAPD and ISSR primers. The phylogram showed that *Elettaria cardamomum* is clustered with *Amomum subulatum* and *A. microstephanum* indicating that *Amomum* is closest to cultivated cardamom among the genera studied (Jayakumar *et al*., 2005).

Chen *et al.* (1999) used RAPD to differentiate within and among *Curcuma wenyujin*, *C. sichuanensis* and *C. aromatica* and also observed difficult to differentiate between *C. wenyujin*, *C. sichuanensis* at DNA level. The relationship between *C. wenyujin* and *C. aromatica* also analyzed and based on the morphological and phytochemical data, it was suggested that these two species should be combined into one and that classification based on peduncle central or peduncle lateral may not be correct.

Kress *et al*. (2002) studied the phylogeny of the gingers (Zingiberaceae) based on DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid mat K regions and suggested a new classification. Their studies suggested that at least some of the morphological traits based on which the gingers are classified are homoplasious and three of the tribes paraphyletic. The African genus *Siphonochilus* and Bornean genus *Tamijia* are basal clades. The former Alpinieae and Hedychieae for the most part are monophyletic taxa with the Globbeae and Zingiberae included within the later. They proposed a new classification of the Zingiberaceae that recognizes four subfamilies and four tribes: Siphonochiloideae

(Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingibereae, Globbeae).

Sasaki *et al*. (2002) used sequence analysis of Chinese and Japanese *Curcuma* drugs based on 18S rRNA gene and trnK gene and the application of amplification – refractory mutation system analysis for their authentication. The botanical origins of Chinese and Japanese *Curcuma* drugs were determined to be *Curcuma longa*, *C. phaeocaules*, the Japanese population of *C. zedoaria, C. kwangsiensis, C. wenyujin,* and *C. aromatica* based on a comparison of their 18s rRNA gene and trnK gene sequences with those of six *Curcuma* species reported previously. Moreover, to develop a more convenient identification method, amplification – refractory mutation system (ARMS) analysis of both gene regions was performed in plants. ARMS method for the 18S rRNA gene was established using two types of forward primers designed based on the nucleotide difference at position 234. When DNAs of four *Curcuma* species were used as templates, PCR amplification with either of the two primers only generated a fragment of 912 base pairs (bp). However, when DNA of the purple-cloud type of *C. kwangsiensis* and *C. wenyujin* were used, PCR amplifications with both primers generated the fragment, suggesting that these two were heterozygotes. The ARMS method for the trnK gene was also established using a mixture of four types of specific reverse primers designed on the basis of base substitutions and indels (insertion deletions) among six species, and common reverse and forward primers. *C. phaeoeaulis* or the chinese population of *C. zedoaria* or the purple cloud type of *C. kwrangsiensis*, the pubescent type of *C. kwrangsiensis* or *C. wenyujin,* and *C. aromatica* were found to show specific fragments of 730, 185, 527 or 528 and 641 or 642 bp, respectively All species including *C. longa* showed a common fragment of 897-904 bp. Using both ARMS methods, together with information on producing areas, the *Curcuma* plants were identified. This ARMS method for the trnK gene was also useful for authentication of *Curcuma* drugs.

Cao *et al*. (2003) used a molecular approach, trnK nucleotide sequencing, for identification of six medicinal *Curcuma – C. longa, C. phaeocaulis, C. sichuanensis, C. chuanyujin, C. chuanhuangjiang* and *C. chuanezhu* found in Sichuan, China. The matK gene (an intron embodied in trnK gene) sequence and the intron spacer region of the trnK gene have great diversity within these six medicinal *Curcuma* species. There were six single base substitutions between trnK coding region and matK region, the 9-bp deletion and 4-bp or 14-bp insertion repeat at some sites of matK region in each taxon. These relatively variable sequences were potentially informative in the identification for these six *Curcuma* species at the DNA level.

A phylogenetic analysis of the tribe Zingibereae (Zingiberaceae) was performed by Ngamriabsakul *et al.* (2003) using nuclear, ribosomal DNA (ITSI, 5.8S and ITS2) and chloroplast DNA (trnL (UAA) 5 [Prime Prime or minute) exon to trnF (GAA). The study indicated that tribe Zingibereae is monophyletic with two major clades, the *Curcuma* clade and the *Hedychium* clade. The genera *Boesenbergia* and *Curcuma* are apparently not monophyletic.

Sasaki *et al*. (2004) applied, single – nucleotide polymorphism analysis of the trnK gene for the identification of *Curcuma* plants. *Curcuma* plants and drugs derived from *Curcuma longa*, *C. phaeocaulis, C. zedoaria* and *C. aromatica* could be identified by the nucleotide differences at two sites and the existence of a 4-base indel on trnK gene. Thus single nucleotide polymorphism (SNP) analysis was developed to identify four *Curcuma* plants. Sasikumar *et al*. (2004) developed a PCR based method for detection of extraneous *Curcuma* species contamination in the powdered market samples of turmeric. The study revealed the presence of *Curcuma zedoaria* samples mixed with true turmeric (*C. longa*) samples.

Xia *et al.* (2005) undertook molecular genetics and chemical assessment of Rizoma *Curcumae*. Rhizoma curcumae (Ezhu) is a traditional Chinese medicine that has been used in removing blood stasis and alleviating pain for over a thousand

years. Three species of *Curcuma* rhizomes were used, which include *Curcuma wenyujin*, *C. phaeocaulis,* and *C. kwangsiensis*. Chemical finger prints were generated from different species of *Curcuma*, which could serve as identification markers. For molecular identification, the 5s-rRNA spacer domains of 5 *Curcuma* species, including the common adulterants of this herb, was amplified, and their nucleotide sequences were determined. Diversity in DNA sequences among various species was found in their 5s-rRNA spacer domains. Thus, the chemical fingerprint together with the genetic distinction could serve as markers for quality control of *Curcuma* species. Pinchai *et al*. (1999) reported association of a few isozymes markers in the identification of some of the early flowering *Curcuma* species.

Molecular markers provide an efficient way to screen the tissue culture induced variations as these markers are not affected by environmental factors (Peredo *et al*., 2009). Salvi *et al*. (2001) reported Random Amplified polymorphic DNA (RAPD) analysis of leaf base callus derived eight regenerated turmeric plants using 14 primers when separated on non-denaturing polyacrylamide gels showed 38 novel bands. About 51 bands present in the control were absent in the regenerants. The results indicated that variation at DNA level has occured during *in vitro* culture. RAPD and ISSR markers were chosen as reliable molecular markers because of simplicity and cost effectiveness and their efficiency in reliable monitoring of variability of DNA sequences among *in vitro* conserved plantlets (Zietkiewicz *et al*., 1994; Martins *et al*., 2004; Mohanty *et al*., 2008; Bhatia *et al*., 2009).

Komatsu *et al*. (2004 a, b) reported that the *Curcuma* sp. in Yakushima Islsand, Japan might be *Curcuma zedoaria* by 18S rDNA and trnK gene sequences analysis of several *Curcuma* plants in China and Japan. However, so far, a complete analysis of the ribosomal DNA sequence of *Curcuma aeruginosa* has not been reported and the aspect of the aerial part of the *Curcuma* sp. in Yakushima is very similar to those of *Curcuma aeruginosa* and *C. zedoaria*. Based on this background,

Kitamura *et al*. (2007) carried out a comparison of the *Curcuma* sp. in Yakushima Island, Japan, with *C. aeruginosa* and *C. zedoaria*, Java Island, Indonesia by the use of several gene analyses and gas chromatography mass spectrometry (GC-MS) techniques.

Nayak *et al.* (2011) used cytophotometric and random amplified polymorphic DNA (RAPD) as well as inter simple sequence repeats (ISSR) analysis to periodically monitor the genetic stability of micropropagated clones of *Curcuma longa* conserved *in vitro* up to 7 years at every 6 months interval. A total of eighteen RAPD and eight ISSR primers gave 45,537 distinct and reproducible bands, monomorphic across all 353 plants analyzed.

Singh *et al*. (2011) aimed to make comparative field evaluation of the drug yielding potential of tissue culture derived and conventionally grown turmeric by assessing genetic stability through molecular profiling. They confirmed by RAPD analysis that axillary multiplication is the safest mode of micropropagation to produce true-to-type plantlets in *C. longa* (cv. *Suroma*).

Banerjee *et al*. (2012) exploited the multifarious properties of *Luffa* sponge as a novel matrix for synthetic seed conservation of *Curcuma amada* and RAPD finger printing revealed 84.62% genetic similarity between randomly selected synthetic seed derived plantlets. This report strengthened the vital conservation approach of *C. amada* using inexpensive *Luffa* sponge as storage matrix and bavistin for eradication of contaminations.

Syamkumar and Sasikumar (2007) developed molecular genetic finger prints of 15 *Curcuma* species by using RAPD and ISSR markers to elucidate the genetic relatedness/diversity among the species. Cluster analysis of the data obtained using UPGMA algorithm placed the fifteen species into seven groups that were somewhat congruent with classification based on morphological characters by earlier workers.

Kuanar *et al*. (2012) subjected micropropagated turmeric plants to ISSR analysis. Eight ISSR primers utilized had successfully amplified turmeric DNA with reproducible banding pattern. Banding pattern of all the plants was similar showing monomorphic profile whereas three regenerants (P_4, P_{15}, and P_{24}) revealed polymorphism with different molecular profile. The callus derived regenerants have enough significance for producing improved varieties of turmeric.

ESSENTIAL OIL ANALYSIS

The analysis of secondary metabolites in plants is a challenging task because of their chemical diversity, usually low abundance and variability even within the same species. It is estimated that 100,000 – 200,000 metabolites occur in the plant kingdom (Oksman – Caldentey and Inze, 2004), and considering the fact that many traditional herbal preparations of Chinese or Indian origin contain not one but several medicinal plants, only highly selective and sensitive methods will be suitable for controlling their composition and quality. Gas chromatography (GC) and Mass spectrometry (MS) is a powerful tool for detecting the relative concentrations of the volatile components according to their elusion order on column (Steinmann and Ganzera, 2011).

Several epidemiological studies established a link between phytochemicals and the range of biological activities that impart health benefits to human beings. Scientific research supports, that biological activities of many of the phytochemicals persist only in their native forms. They were copiously used in Ayurveda and other traditional medicines (Moon *et al*. 2010), which dates back to Charaka Samhita (Gupta *et al*. 2010). Amongst the phytochemicals, several groups of polyphenols (anthocyanins, proanthocyanidins, flavanones, isoflavones, resveratrol and ellagic acid), non-nutrient chemical and dietary constituents are currently used in the pharmaceutical industry. The spices are considered to be the store house of large number of active phytochemicals. Various spices belonging to the genera *Curcuma* and *Zingiber* are well known for their multiple uses as

medicines, cosmetics, dyes, flavourings and neutraceuticals (Policegoudra *et al*. 2011).

CURCUMA

The aroma and flavour of turmeric are determined by the composition of its steam volatile oil. The yield of oil obtained on distillation and its physico-chemical properties can also vary between individual samples (Gildemeister and Hoffmann, 1956; Khalique and Das, 1968; Krishnamurthy *et al*., 1976). The differences can arise from a number of factors, which include the origin, and the method of curing the spice, its age, and the condition of distillation and the stage of maturity of the rhizome at harvest.

The essential oils obtained by hydrodistillation from the fresh rhizomes of two endemic species of *Curcuma viz*. *C. haritha* and *C. raktakanta* were studied by GLC analysis. Eleven components were identified from *C. haritha* of which camphor (21.24%) was the major component and ten components were identified from *C. raktakanta* of which ethyl p-methoxycinnamate (16.57%) was the major component. A- Pinene, camphor, terpinyl acetate, turmerone and ethyl P-methoxy-cinnamate were common to both the species (Mathew *et al*., 2002). Ibrahim *et al*. (1999) reported essential oil components of four *Curcuma* species, *Curcuma mangga*, *C. xanthorhiza, C. aeruginosa* and *C. longa* (collected from Malaysia), analyzed by GC- MS. Myrcene (81.4%) was the most abundant component in the essential oil of *C. mangga*. The essential oil of *C. zanthorrhiza* was made up mainly of sesquiterpenoids of which xanthorrhizol (44.5%) was the major constituent. 1, 8- Cineole (eucalyptol) (23.2%) and curzerenone (28.4%) were the predominant constituents of the essential oil of *C. aeruginosa.* The essential oil of *C. domestica* contained significant amounts of alpha tumerone (45.3%), linalool (14.9%) and beta-tumerone (13.5%).

Bordoloi *et al*. (1999) collected essential oils from steam distilled leaves and rhizomes of cultivated *Curcuma aromatica* (from India), and were investigated by

GC and GC-MS. About 50 compounds were identified, accounting for more than 85% of the essential oils. The major constituents of the leaf essential oil were camphor (28.5%) ar –turmerone (13.2%), curzerenone (6.2%), 1, 8 – cineole (eucalyptol) (6%) and alpha turmerone (2.5%). The rhizome essential oil consisted mainly of camphor (32.3%), curzerenone (11%), alpha-turmerone (6.7%), ar-turmerone (6.3%) and 1, 8 –cineole (5.5%).

Nguyen-Xuan-Dung *et al*. (1995) steam-distilled essential oil from leaves of *C. domestica* (*C. longa*) and analyzed by GC and GC-MS. More than 20 components were identified, of which the monoterpenes alpha-phellandrene (24.5%), 1, 8-cineole (eucalyptol) (15.9%), P-cymene (13.2%) and beta-pinene (8.9%) were the major ones.

The essential oil of turmeric besides having antimicrobial and antifungal activities is also used in aromatherapy and perfume industry (Sasikumar, 2005). Growing demand of this plant necessitated the development of new genotypes with improved yield and quality of essential oil. The composition of mango ginger (*Curcuma amada*) volatile oil was determined by various workers (Dutt and Tayal 1941; Golap and Bandyopadhyaya 1984; Rao *et al*. 1989; Chaudhary *et al*. 1996; Srivastava *et al*. 2001; Singh *et al*. 2003; Mustafa *et al*. 2005,Police Goudra *et al*. 2011). The mango flavour is mainly attributed to presence of Car-3-ene and cis-ocimene among the 68 volatile aroma components present in the essential oil of mango ginger rhizome.

Kitamura *et al*. (2007) compared essential oil components in rhizomes of three *Curcuma* Sp. by the GC-MS technique using nine authentic sesquiterpenes as standard samples. The total ion exchange chromatogram for essential oil fractions prepared from the rhizome of *Curcuma* Sp. in Yakushima was quite similar to that in *C. aeruginosa* in Java. On the other hand, peaks of Curcumenol, dehydrocurdione and (4s, 5s) – (+) – germacrone 4,5 – epoxide were not observed

in the chromatogram for *C. zedoaria* in Java, in contrast to that of *Curcuma* Sp. in Yakushima.

Nayak *et al*., (2011) micropropagated turmeric after being conserved for 7 years in vitro and transplanted to soil in field and drug yielding potential of tissue culture derived plants was evaluated in field through estimation of phyto constituents like Curcumin and essential oil content.

Kuanar *et al*., (2012) compared callus derived plants of turmeric with field grown clones on the basis of essential oil content. Among 24 plants of *Curcuma longa* screened, 3 somaclones (P_3, P_7, P_{11}) were selected on the basis of significantly high leaf oil content (0.73% - 0.93%) as compared to mean oil yield (0.48%) of source plants. Oil contents of other 21 plants were almost similar to that obtained is the source plants.

There were few reports on turmeric leaf oil composition from different origin. The proportion of ∝-phellandrene, 1-8-cineole, P-cymene and ∝-pinene in the leaf oil of *C. domestica* from Vietnem was 24.5, 15.9, 13.2 and 8.9 respectively (Dung *et al*., 1995, Raina *et al*., 2005). The calculation of proportion was based on peak area analyte/peak area total ion chromatogram. While that of a Nigerian chemo type for ∝-phellandrene and terpinolene was 47.7 and 28.9 respectively (Oguntimein *et al*., 1990). Similarly, the proportion of major constituents; ∝-phellandrene, 1,8-cineole, P-cymene and terpinolene in leaf oil of Bhutanese origin was 18.2, 14.6, 13.3 and 11.6 respectively. While the proportion of P-cymene, 1, 8-cineole, cis- sabinol and ∝-pinene in the leaf oil from North India was 25.4, 18.0, 7.4 and 6.3 respectively, as major constituents (Garg *et al*., 2002). Leela *et al*. (2002) obtained the proportion of ∝-phellandrene (32.6), terpinolene (26.0), 1, 8 – cineole (6.5) and P-cymene (5.9) in turmeric leaves cultivated in Calicut, India.

Zaibunnisa *et al*. (2009) studied the pressurized liquid extraction of turmeric leaves, optimised and compared with essential oil composition obtained by hydro-distillation and the extract from Soxhlet extraction using n-hexane. The PLE

method offers important advantages over conventional methods, namely shorter extraction time, less solvant volumes employed and lower cost.

Solid phase micro extraction – gas chromatographic mass spectrometric analysis of volatile compounds from *Curcuma wenyujin* was carried out (Cao *et al*., 2006) and a comparison between SPME-GC-MS and SD-GC-MS methods is made. In addition to the comparable results of the two methods, SPME-GC-MS method uses much less sample, shorter time and simpler procedure.

ZINGIBER

Essential oil, extracted from the rhizomes of ginger, was analyzed (He-Wenshan *et al*., 2001) by GC-MS. 35, 36 and 44 constituents were identified respectively from the methanol, ethyl acetate and hexane extracts. Principal constituents detected in 3 extracts were terpenes, but the composition and contents of the terpenes were different. Oil composition of fresh and dried ginger rhizomes of Nigeria was investigated by means of a combination of column chromatography, high resolution GC and GC – MS (Ekundayo *et al*., 1998). The essential oils contained mainly mono and sesquiterpinoids of which geranial, neral, 1, 8-cineole, zingiberene, β-sesquiphellandrene were the major components. Rhizomes of *Z. officinale* were sliced dried at 4 temperatures, viz 41^0C, 46^0C, 54^0C and 64.5^0C. The drying temperature had no effect on essential oil yield which ranged from 0.64 to 0.89% on dry weight basis (Maia *et al*., 1991). Chairgulprasert *et al*. (2005) reported the chemical constituents of essential oil and antibacterial activity of *Zingiber wrayi* var. *halabala*. Essential oils of 9 *Zingiber officinale* cultivars were evaluated using GC by Gopalam and Ratnambal (1989).

The recent research on secondary metabolites of ginger such as essential oils, oleoresin, gingerol, shogoal and its pharmacological effects described by Schubam and Franz (2000). Singh *et al*. (1999) studied on yield and quality characters in 15 local ginger cultivars.

Mathai (1975) studied seasonal accumulation of chemical constituents in seven *Z. officinale* varieties and trends in oleoresin accumulation using Ethyl alcohol and Acetone as solvents. Varieties exhibited a reduction in the amount of oleoresin which increased concomitant with maturity and alcohol was observed to be better oleoresin extractor than acetone.

Oliveros (1996) isolated the main constituent of the volatile oil of *Z. purpureum*. Terpinen 4-ol is most stable at pH 7.0 and temperature ranging from 23^0C – 60^0C. Zingisol and Zingiment have antibacterial and antifungal activities and it can be used as an alternative medication for skin infections caused by strains that have developed resistance to antibiotics. Volatile oil from rhizome contains terpinen -4-ol, which is used in folk medicine for the treatment of asthma, rheumatism, diarrhea, cough and skin diseases.

Sreekumar *et al*. (1999) developed a new commercially viable technology for recovery of ginger oil with fresh flavour from fresh rhizomes of *Z. officinale*.

Bordoloi *et al*. (1998) studied essential oils from the leaf and rhizome of *Zingiber officinale* by gas chromatography and found the major compounds such as zingiberene (16.05%) and geranial (12.05%) geranyl acetate (11.42%) geraniol (9.05%) in rhizome and 14-hydroxy-9 – epi – (E) caryophyllene (35.74%) geranyl acetate (10.60%), geranil (0.2%), geranial (8.78%) caryophyllene oxide (8.60%) in leaf while sesquiphellandrene (6.11%), bisabolene (5.50%), camphene (5.46%) and (E, E) – 9 farnesene (5.00%) are found in rhizome oil only.

Nishimura (2001) studied the fresh rhizomes of *Z. officinale* using multidimensional GC system in Japan and revealed that linalool, 4 – terpineol, isoborneol and borneol are present.

Fakim *et al*. (2002) studied the chemical composition of the essential oils obtained from the hydrodistillation of the rhizomes of *Z. officinale*, *H. coccineum* (Buch-Ham.) ex Smith, *H. flavescens* Carey ex Roscoe and *H. coronarium* Koeing by GC and GC/MS. Oil of *Z. officinale* was characterized by the presence of geranial

(16.3%), neral (10.3%), zingiberene (9.5%), β-sesquiphellandrene (6.3%), and ar-curcumene (5.1%).

Pino *et al*. (2004) studied the chemical composition of the essential oil obtained from the rhizomes of *Z. officinale* by combined GC and GC/MS. The oil was characterized by the presence of ar-curcumene, zingiberene, β-bisabolene and cadina 1, 4 diene.

Sabulal *et al*. (2006) isolated oil from the rhizomes of *Z. nimmonii* and conducted GC and GC-MS analysis. Major oil constituents were β- caryophyllene and ∝-humulene, ∝-caryophyllne as of isocaryophyllene. Oil contained 71.2% sesquiterpenes, 14.2% oxygenated sesquiterpenes, 8.9% monoterpenes, and 1.9% oxygenated monoterpenes and 1.3% non terpenoid constituents. The oil also showed inhibitory activity against certain fungi and bacteria.

Sabulal *et al*. (2007) worked out the oil constituents of *Z. neesanum* by GC and GC-MS. Major compounds are phenylbutanoids. (E) – 1- (3', 4' – dimethoxy phenyl) butadiene as anti-inflammatory compound, (E) – 1 - (3', 4' – dimethoxy phenyl) but -1-ene and (E) - β- ocimene, β- pinene and linalool are major terpenoid constituents of rhizome oil.

Prakash *et al*. (2006), studied phytochemical composition of essential oil from the seeds of *Z. roseum*. The major compounds being ∝ - pinene, β-pinene, limonene, P-cymene, ∝-terpinol and verticiole. The presence of mono-and sesquiterpene hydrocarbons which make about 82% of the oil, and the oil showed myorelaxant activity on isolated rat duodenal smooth muscle.

Nazrul *et al*. (2008) worked out the chemical components of *Z. cassumunar* by GC-MS and identified 64 compounds in the rhizome oil. The main component of leaf oil is sabinene (14.99%), β-pinene (14.32%) and caryophyllene (9.47%). The rhizome oil contained triquinacene 1, 4 – bis (methoxy) (26.47%), Z-ocimene (21.97%) and terpinene 4 – ol (18.45%).

CLASSIFICATION

Many Scientists have classified members of the family Zingiberaceae, based on both vegetative and floral characters, into 4 tribes (Peterson, 1889; Schumann, 1904; Holttum, 1950; Burtt and Smith, 1972; Larsen *et al*., 1998). Although a number of morphological features have been used to distinguish the tribes, the characters are often inconsistent and variable. Lestiboudois (1841) and Duchartre (1849) presented the Zingiberaceous taxa under six tribes *viz*., Kaempferiees, Hedychiees, Curcumees, Alpinees, Costoidees and Mantisiees.

Linnaeus in his *Species Plantarum* (1753) described 10 species under 5 genera of present day Zingiberaceae, some of which were based on the plates of Rheede's *Hortus Malabaricus*. The species are: *Amomum zingiber*, *A. zerumbet*, *A. cardamom*, *A. granparad*, *Costus arabicus*, *Alpinia racemosa*, *Curcuma rotunda*, *C. longa*, *Kaempferia galanga* and *K. rotunda*, all under his *Monandria Monogynia*. Subsequently, some of these species have been relegated to other genera, *Amomum zingiber* as *Zingiber officinale*, *A. zerumbet* as *Zingiber zerumbet*, *A. cardamom* as *Elettaria cardamomum* and *Curcuma rotunda* as *Boesenbergia rotunda*.

Bentham and Hooker (1862-1883) in their monumental work '*Genera plantarum*' proposed a natural classification and included four tribes under the order Scitamineae. They grouped 36 genera of the order Scitamineae under four tribes; Canneae (1), Maranteae (10), Musaceae (4) and Zingibereae (21). Later authors recognized these tribes as separate families and gave ordinal status to Scitaminae of Bentham and Hooker.

Petersen (in Engler and Prantl, 1889) raised Scitamineae to the ordinal status (Reihe) and the rank of the four tribes to families, Zingiberaceae, Marantaceae, Cannaceae and Musaceae and subdivided the Musaceae, which has a common character in the number of fertile anthers, into the tribes Museae

(containing *Musa*, *Ravenala* and *Strelitzea*) and Heliconieae (*Heliconia*). The solitary ovule per locule, septicidal fruit dehiscence, and inverted symmetry of the flowers distinguished *Heliconia* from the other members of Musaceae. *Orchidantha*, the sole genus in the Lowiaceae, was excluded from the Scitamineae of Bentham and Hooker, but recognized as a possible member of the group by Petersen. *Orchidantha* has always been considered as an unusual member of the Zingiberales due to the specialized leaf blade with mesophyll of irregularly arranged large and small cells, several pairs of longitudinal veins parallel to the distinct midrib and the elaboration of the adaxial petal into a large labellum. The Lowiaceae are among the most poorly known taxa in the order in terms of taxonomy, general morphology, embryology, chemistry and ecology. Peterson (1889), further divided Zingiberaceae into three tribes, Hedychieae, Zingibereae and Globbeae.

Earlier, Lestibodois (1841) circumscribed the present day Zingiberaceous taxa under six tribes (Kaempferiees, Hedychiees, Curcumees, Alpiniees, Costoidees and Mantisiees) and Duchartre (1849) in agreement with the above followed the same classification.

Schumann in *Das Pflanzenreich* (in Engler, 1900, 1902, 1904) further sub-divided the order Scitamineae into two subfamilies Zingiberoideae K. Schum. and Costoideae K. Schum., by segregating genera into subfamilies and retained three tribes circumscribed by Petersen (1889): Zingiberoideae contained three families Hedychieae, Zingibereae and Globbeae, and subfamilia Costoideae contained four genera *Costus* L., *Dimerocostus* Kuntze, *Monocostus* K. Schum and *Tapeinochilus* Miq.

Hutchinson (1934, 1959, 1973), used the ordinal name Zingiberales (after Nakai, 1941), accepted the divisions of Schumann, but raised it to the rank of family; the Strelitziaceae (including *Heliconia*) and Lowiaceae. He also further subdivided the Zingiberaceae into four tribes Zingibereae, Hedychieae, Globbeae and Costeae of equal status.

Nakai (1941) first suggested that the Costoideae and the Heliconieae be raised to the rank of family. The non-aromatic vegetative body, spirally arranged leaves, and anther appendages were cited by Nakai as characters separating the Costaceae from the Zingiberaceae. The uniovulate locules, exarillate seeds, and capitate stigmas of the Heliconiaceae distinguished this taxon from the other families of the order. Subsequently Tomlinson (1962, 1969), investigated the anatomy of the order, pointed out that the degree of morphological and anatomical differences among the eight entities is about the same, and therefore accepted the classification of Nakai. Stebbins (1974), Takhtajan (1980), Cronquist (1978, 1981), and Dahlgren and coworkers (1983, 1985) followed Nakai and Tomlinson in the recognition of eight families in the order.

Burtt and Smith (1972) recognized the family Zingiberaceae into two subfamilies, Costoideae and Zingiberoideae. Subfamily Zingiberoideae is further divided into four tribes; Hedychieae, Zingibereae, Alpinieae and Globbeae.

In Globbeae, the ovules are arranged on a parietal placenta and anther usually long, exerted on an arched ascending filament. Zingibereae is characterized by the distichy of leaves parallel to rhizome, style protruded beyond the anther, anther crest wrapped around the style and trilocular ovary with axile placentation. In Hedychieae, the prominent characters are: distichy of leaves parallel to the rhizome, style not exerted beyond the anther, anther crest not wrapped around the style, lateral staminodes petaloid and free from labellum, pseudostem short. In Alpinieae, pseudostem is well developed, distichy of leaves transverse to rhizome, style not exerted beyond the anther, lateral staminodes smalland teeth like or absent.

Vegetative and floral characters were considered in all the described classifications of Zingiberaceae. The classification of Bentham and Hooker stands unchanged even though the number and constitution of families changed with authors and the taxonomic status of scitamineae was raised to that of an order

(Zingiberales) or super order (Zingiberanae). It forms a very closely interrelated assemblage of monocotyledonous taxa, probably very distinct from other groups.

Larsen *et al.* (1998) divided Zingiberaceae in to 4 tribes viz. Hedychieae Petersen (19 genera), Globbeae Petersen (4 genera), Zingibereae Petersen (1 genus) and Alpinieae Meisn (21 genera).

The history of the classification of Zingiberales shows that as described, information on new characters becomes available, new hypotheses on relationship among the families and taxonomic rank are proposed. Kress (1990), re- evaluated the character state distributions and homologies coupled with the methods of phylogenetic systematics have provided a new classification based on cladistic hypotheses. However, many useful characters in the Zingiberales which can be evaluated phylogenetically remain to be studied carefully, appraised, and verified; he proposed a new phylogenetic classification based on the cladogram that recognizes eight families, two superfamilies and five suborders within the Zingiberales. (Figure-2)

Kress *et al.* (2002) used molecular sequence data of 104 species in 41 genera representing all four tribes of Zingiberaceae based on DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid matK regions to find out the phylogenetic relationship among the genera of Zingiberaceae in order to evaluate the past classification and proposed a new phylogenetic classification, which is followed in the present treatment. This classification represents natural groups and more convinced.

Key to the subfamilies and Tribes (Kress *et al*., 2002)

1. Plane of distichy of leaves perpendicular to rhizome..... 2

1. Plane of distichy of leaves parallel to the rhizome .....
.....Sub fam. 1. Zingiberoideae

2. Lateral staminode well developed and fused to the labellum 3

2. Lateral staminode reduced or absent .....Sub fam. 2. Alpinioideae

3. Plants ever green with fibrous rhizome; ovary unilocular with parietal placentation .....Sub fam. 3. Tamijioideae

3. Plants with seasonal dormancy period and fleshy rhizome; ovary trilocular with axile placentation .....Sub fam. 4. Siphonochiloideae

Sub fam. Zingiberoideae Hassk.

Zingiberoideae Hassk., Cat. Hort. Bot. Bogor 1844

Key to the tribes

1. Ovary trilocular with axile, basal or free columnar placentation; labellum usually not connate to the filament .....Zingibereae

1. Ovary unilocular with parietal placentation; labellum after connate to the filament in a slender tube ..... Globbeae

Tribe: Zingibereae Meisn.

Zingibereae Meisn., Pl. Vasc. Gen. Tab. Diagr. 388. Comm. 290. 1842.

Key to the genera

1. Stem well developed; primary bracts not adnate laterally; lateral staminodes adnate to the labellum; anther crest elongate, embracing the style .....*Zingiber*

1. Stem poorly developed or absent; primary bracts adnate to each other laterally forming a pouch; lateral staminodes free from the labellum; anther with or without a crest; not embracing the style .....*Curcuma*

SPECIES DESCRIPTION

***Curcuma* L.**

The genus name *Curcuma* was coined by Linnaeus in 1753 in his *Species Plantarum*.The name *Curcuma* originated from the Arabic word 'Kurkum', meaning yellow, which probably refers to the colour of the rhizome or the flowers. The genus *Curcuma* L., with around 120 species (Skornickova *et al*., 2004) distributed in tropical and subtropical Asia consists of a rather homogenous group of rhizomatous perennials. The genus is easily recognized by its inflorescence, a spike with prominent spiral bracts, which is laterally fused or adnate to the peduncle and form pouches, each subtending a cincinnus of flowers and a cluster of, often coloured, sterile, terminal bracts called 'coma'.

The genus *Curcuma* is mainly distributed in the Indo-Malayan region. Baker (1890) described 29 species in the *Flora of British India*. He subdivided the genus into three sections – Exantha, Mesantha and Hitcheniopsis. Hitcheniopsis differed from the rest of the genus in its spurless anthers. Schumann (1904) rejected the sectional classification based on spike position but recognised two subgenera [Subgen. Eucurcuma and subgen. Hitcheniopsis (Baker) K. Schum.] based on the presence or absence of spur on anthers.

In India, except for a few ubiquitous species, the genus is mainly concentrated in the South West and North East India, and has not been revised since Baker (1890) who reported 29 species (Karthikeyan *et al*., 1989; Jain and Prakash, 1995). From South West India, Fischer (1928) reported eight species. But this was proved as gross underestimate by the subsequent addition of twelve more species, bringing the total number of South West Indian species to 20 of which 12 taxa are endemic to this region (Sabu, 2006).

The species belonging to the genus *Curcuma* can be grown in diverse tropical conditions from sea level to a height of 1500 m on the hilly slopes, in the temperature range of 20 to 30^{o}C. A rainfall of 150 cm or more or an equivalent amount of irrigation is essential for optimum growth and development of *Curcuma* species. Ideal soil requirements for the growth of *Curcuma* are loose, friable loamy or alluvial soil suitable for irrigation that should have efficient drainage capacity. The species are naturally found in mixed deciduous tropical forests and tropical broad-leaved evergreen forests of the tropical and subtropical regions. There is no available documented literature about the origin and distribution of African and South American *Curcuma* species. The members of the genus in these regions are important resources and have great potential in terms of commercial values as source of spices, medicines and horticultural products. (Apavatjrut *et al*., 1999; Cao *et al*., 2001; Cao and Komatsu, 2003; Joe *et al*., 2004; Sasaki *et al*., 2004; Yusuf *et al*., 2001).

Curcuma aeruginosa Roxb., Asiat. Res. 11: 335. 1310, Fl. India 1: 27.1820.Roscoe. Monandr. Pl. t. 106. 1828; Horan., Monogr. 22. 1862; Baker in Hook. f., Fl. Brit. India 6: 212. 1890; K. Schum. In Engler, Pflanzenr. 4 (46): 112. 1904.

Iconotype: Roxburgh Ic. t. 1924 (CAL).

Type: Kerala, Thrissur, Pavaratty, *Velayudhan AV158* (MH).

Rhizome large, blue in the centre, verging towards grey, strongly aromatic; sessile tubers branched, condensed; root tubers many. Leafy shoot 70-100 cm tall; pseudostem 30-35 cm. Leaf lamina 30-40 x 10-12 cm, oblong-lanceolate, tip acute, base acuminate, glabrous, purple or reddish brown patch along the sides of the distal half of the midrib on upper side only, groove of the mid rib green. Inflorescence lateral; coma bracts large, pink to violet, fertile bract 18-20, each subtends a cincinnus of 8-10 flowers. Bracteoles large. Flower equal to or shorter than the bracts. Corolla tube longer than calyx, pink. Labellum emarginate at tip, yellow with a median deep yellow band. Lateral staminodes yellow. Anther

spurred at base, divergent. Stigma bilipped, slightly exerted above the anther lobes. Fruiting not common (Fig.3-A,B,C).

Distribution: Native of Myanmar. Also seen in Java and widely cultivated in Malaysia. It is wild in South India.

Curcuma amada *Roxb*., Asiat. Res. 11: 341. 1810. Baker in Hook. f., Fl. Brit. India 6: 213.1890; K.Schum. in Engler, Pflanzenr. 4 (46): 108. 1904; Cooke, Fl. Pres. Bombay 2: 731. 1907; Fischer in Gamble, Fl. Pres. Madras 8: 1483. 1928; V.S. Ramach. and V.J. Nair. Fl. Cannanore 467. 1988. Mangaly and M. Sabu, Rheedea 3(2): 143. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 77. 1993; Fl. Uduppi, 627. 2003; M. Sabu and Mangaly Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Iconotype: Roxburgh. Ic. t. 1760 (CAL).

Rhizome large, light yellow inside, white towards periphery, with the smell of green mango, sessile tubers thick, cylindric, branched; root tubers absent. Leafy shoot 65-75 cm high. Pseudostem 30-35 cm tall. Leaves 4-6; petiole 5-10 cm long; lamina 45-60 x 14-15 cm, oblong-lanceolate, without purple patch, lower surface puberulous, upper glabrous. Inflorescence lateral or central. Coma bracts spreading, light violet. Fertile bracts green, subtend 4 or 5 flowers. Flowers longer than bracts. Calyx 3-lobed at the tip. Corolla pale yellow; lobes, white. Labellum elliptic, 3 lobed; mid-lobe emarginate, recurved, pale yellow with median dark yellow band. Lateral staminodes without glandular hairs. Stamen white, spurred. Stigma closely appressed within the anther lobe (Fig.3. D., E., F.). Fruits not seen.

Distribution: Native of Bengal. It is now widely cultivated throughout India.

Curcuma aromatica Salisb., Parad. London t. 96. 1805. Wight, Icon. Pl. Ind. Or. 6: t. 2005. 1853; Baker in Hook. f., Fl. Brit, India 6: 210. 1890; Trimen. Handb. Fl. Ceylon 4: 241. 1898; K. Schum. in Engler, Pflanzenr. 4(46): 111. 1904; C.E.C. Fisch. Rec. Bot. Surv. India 9: 177. 1921, in Gamble. Fl. Pres. Madras 8: 1483. 1928; A.S. Rao and D.M. Verma. Bull. Bot. Surv. India 14: 122. 1972; B.L. Burtt and R.M. Sm. in Dassan..

Rev. Handb. Fl. Ceylon 4: 503. 1983; Manilal, Fl. Silent Valley 311. 1988; Mangaly and M. Sabu, Rheedea 3(2): 148. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 78. 1993; Fl. Uduppi, 627. 2003; M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Curcuma zedoaria auct. non (Christm.) Roscoe.; Roxb., Asiat. Res. 11: 333. 1810, Fl. Indica. 1: 23. 1820; Dalell. and A. Gibson, Bombay Fl. 274. 1861.

Iconotype: Roxburgh, Ic. t. 1010 (CAL).

Rhizome large, greyish yellow within, aromatic with many sessile tubers. Leafy shoot 1m or more. Leaves 5-7; lamina 50 x 10-14 cm, broadly lanceolate, acuminate, densely pubescent below. Inflorescence lateral. Coma bracts pink. Fertile bracts *ca*. 6 cm long, tip recurved. Calyx *ca*. 2 cm long, sparsely pubescent. Corolla longer than calyx, pinkish white. Labellum orbicular, obscurely 3-lobed, deep yellow. Lateral staminodes oblong, as long as the corolla lobes. Anther spurred. Stigma bilobed with a perforation in the centre (Fig.4. A., B.).

Distribution: Occurs in India, China and Sri Lanka. It is widely cultivated in South India for its rhizome.

Curcuma aurantiaca Zijp., Recueil Trv. Bot. Neerl. 12. 345. 1915.

Type: C. van *Zijp s.n.* (1911) (holo. BO)

Type: Kerala, Palakkad Dt, Kanjirapuzha, *Sivarajan and indu AVS 1497* (E; CALI).

Curcuma ecalcarata Sivar. and Indu, Notes Roy. Bot. Gard. Edinburgh 41: 321. 1983.

Rhizome small, conical, yellow inside; sessile tubers absent; root tubers fusiform. Leafy shoot 30-45 cm high. Leaves 6-8, petiolate; petiole 20-25 cm long, minutely pubescent; lamina 20-30 x 10-15 cm, broadly ovate, slightly unequally cordate at base; lower surface densely pubescent. Inflorescence central, with a distinct coma. Coma bracts bright rose or greenish white. Fertile bracts broadly rounded, tip recurved. Flowers longer than the bracts, yellow or orange yellow.

Corolla tube longer than calyx, light yellow-orange. Labellum 3-lobed; mid-lobe much exceeding the laterals. Lateral staminode, oblong – obtuse. Stamen yellow, anther ecalcarate; connective forms broad hood at the apex, glandular hairs on back (Fig.4. C., D., E.). Fruit obovoid.

Distribution: Common on the Western Ghats and midlands of Kerala.

Curcuma bhatii (R.M. Sm.) Sckornickova and Sabu, Gardens Bull. Singapore 57: 37-46. 2005.

Paracautleya bhatii R.M. Sm. Notes Roy. Bot. Gard. Edinburgh 35: 368. 1977.

Type: KARNATAKA, South Kanara Dt., Manipal, near Medical College, *K.G.Bhat 204* (CAL).

Rhizome very small, conical, erect, white inside; root tubers small, many. Leafy shoot up to 18 cm high. Leaves 2-7 in basal tuft; sessile, ligulate. Lamina 4-6 x 0.7-1.5 cm, linear lanceolate, tip acute, glabrous. Inflorescence central, bracts up to 25, free from each other. Flower longer than bracts, one in each bract, ebracteolate. Calyx companulate. Corolla tube slender, *ca*. 1 cm long. Labellum *ca*. 10 x 8 mm, obovate, deflexed, deeply emarginate. Lateral staminodes petaloid. Anther versatile, spurred; connective prolonged into a minute rounded crest. Ovary imperfectly trilocular, ovules attached to the base of the ovary (Fig.4. F., G.). Capsule *ca*. 1 cm long, subglobose with persistant calyx.

Distribution: Endemic to South India. Known only from type locality.

Curcuma coriacea Mangaly and Sabu, Notes Roy. Bot. Gard. Edinburgh 45: 429. 1989.Rheedea 3(2): 143. 1993; Sabu and Mangaly Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: Kerala, Idukki Dt., Painavu, 700 m, *Mangaly* and *Sabu 10337* (MH).

Rhizome small, cylindrical to conical, non-aromatic, white inside; sessile tubers absent; root tubers long, 10-18 cm. Leafy shoot 30-45 cm tall, pseudostem

10-15 cm long. Leaves straight; lamina 27-35 x 10-15 cm, elliptic, coriaceous, densely pubescent on both sides. Inflorescence lateral, later central; coma bracts deep pink to violet. Flowers as long as or slightly longer than bracts, yellow, 3-5 in each bract. Corolla bright yellow. Labellum *ca*. 2 x 2 cm, tip deeply split, deep yellow. Anther with small crest, spread at base. Stigma faintly 4-lobed, bilipped (Fig.5. A., B.). Seeds avoid or ellipsoid, smooth.

Distribution: Endemic to Kerala, known only from Idukki, Palakkad and Pathanamthitta Districts.

Curcuma decipiens Dalzell, Kew J. Bot. 2: 144. 1850.Dalzell and A. Gibson, Bombay Fl. 274. 1861; Baker in Hook. f., Fl. Brit. India 6: 215. 1890; K. Schum. in Engler. Pflanzenr. 4(46): 105. 1904; C.E.C.Fisch., Rec. Bot. Surv. India 9: 178. 1921, in Gamble, Fl. Pres. Madras 8: 1483. 1928; Mangaly and M. Sabu, Rheedea 3(2): 150. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 80. 1993; M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

TYPE: In original publication mentioned “Crescit in prov. Malwan”, but no type designated directly.

Rhizome small, ovoid, conical; root tubers ovoid. Leafy shoot 30-60 cm high. Leaves 2-4; lamina 10-30 x 5-15 cm, broadly ovate, base slightly cordate, tip deltoid, glabrous. Inflorescence both lateral and central. Coma bracts many, upper deep purple pink. Fertile bracts recurved, green with purple pink tips. Bracteoles two. Flower equal to the bracts, 1 or 2 in each bract. Calyx white with pink spots. Corolla tube longer than calyx, deep purple. Labellum *ca*. 1.5 x 15 cm, tip slightly 3-lobed; mid-lobe emarginate, purple towards base. Lateral staminoides oblong, yellow. Anther spurs with purple spots. Stigma exserted from the anther (Fig.5. C.) Fruits ovoid. Seeds brown with a white spot at the tip.

Distribution: Endemic to peninsular India, North Kerala and Karnataka.

Curcuma haritha Mangaly and Sabu, Rheedea 3(2): 152. 1993. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: India, Kerala, Calicut Dt., Kolathara. *Sabu 39113* (CALI).

Rhizome large, yellowish grey inside, non-aromatic; sessile tubers finger shaped, branched; root tubers present. Leafy shoot 70-100 cm high, pseudostem *ca*. 30 cm long, green with few pink dots. Leaves 4-6; lamina 30-50 x 10-14 cm, ovate elliptic, tip accuminate, base acute, leathery, densely puberulent on lower surface, sparsely hairy above, erect, semiplicate, without purple patch. Inflorescence lateral. Coma bright pink. Fertile bract, broadly ovate, flowers 3 or 4 in a bract. Corolla tube longer than calyx, white. Labellum *ca*. 2 x 2, shortly 3 lobed, middle lobes shortly emarginate, light yellow with a median dark yellow band. Lateral staminodes with a patch of glandular hairs at the centre. Anther spurred, white. Stigma bilipped, slightly exserted from anther (Fig.6. A., B .). Fruits not known.

Distribution: - Reported only from Kerala.

Curcuma inodora Blatter J. Proc. Asiat. Soc. Bengal 26: 357. 1930.Santapau, J. Bombay Nat. Hist. Soc. 51: 135. 1952; Phatak and Oza, J. Bombay Nat. Hist. Soc. 56: 368. 1959; Mangaly and M. Sabu, Rheedea 3(2): 154.1993; Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: Bombay Presidency, Moolgaum, Salsette, *Hallberg 12724* (BLAT).

Rhizome conical; root tubers ovoid. Leafy shoot 30-60 cm tall. Leaves 3-5; petiole 20-25 cm long; lamina 15-30 x 7-12 cm, elliptic, base oblique, tip acuminate, upper side minutely hairy along the prominent veins, lower surface glabrous. Inflorescence both lateral and central with violet coma. Fertile bracts pale green with purple patch at the tip, not recurved, each subtends 3 or 4 flowers. Bracteoles small, purple. Flowers equal to the bracts. Calyx white. Corolla tube longer than calyx, deep purple. Labellum obovate, obscurely 3-lobed, tip emarginate, purple

with a median bright yellow band. Lateral staminodes purple. Anther spurs bent inwards. Style pink, stigma bilobed (Fig.6. C., D., E., F.). Fruit globose to ovoid with persistent calyx. Seeds brown.

Distribution: Endemic to peninsular India, extending from Maharashtra upto North Karnataka.

Curcuma Karnatakensis Amalraj, Velayudhan and V.K. Murali, J. Econ. Tax. Bot. 15(2): 490.1991.

Type: Karnataka, Uttar Kannada Dt., 11.09. 90, Hirahalli, *Amalraj 807* (Holo. MH).

Rhizome small, ovoid, cream colour with slight mango ginger flavour; root tubers fusiform or conical. Leafy shoot *ca*. 40 cm high. Leaves upto *ca*. 26 x 7-14 cm, broadly ovate-elliptic, spreading, base unequal and slightly cordate; petiole *ca*. 6 cm long, petiole and sheath green or purple tinged. Inflorescence lateral or central, coma absent. Flowers longer than bracts, *ca*. 5.6 cm long. Calyx *ca*. 2 cm long, pale transparent green. Corolla tube longer than calyx. Labellum *ca*. 1.9 x 2 cm, white, three lobed, mid-lobe larger, deeply bifid, bright yellow band in the middle. Lateral staminodes multicoloured. Anther white, *ca*. 4.5 mm long, spurs rose coloured; filament pale yellow (Fig.7. A., B.).

Distribution: Endemic to Karnataka state, South India.

Curcuma longa L., Sp. Pl. 1:2. 1753.pro max. parte; Koenig in Retz., Obs. Bot. 3: 72. 1783; Roxb., Asiat. Res. 11: 340. 1810, Fl. Indica 1: 32. 1820; Baker in Hook. f., Fl. Brit. India 6: 214. 1890; K. Schum. in Engler, Pflanzenr. 4 (46): 108. 1904; C.E.C. Fisch. in Gamble, Fl. Pres. Madras 8: 1483. 1928; B.L. Burtt and R.M. Sm. Notes Roy. Bot. Gard. Edinburgh 31: 185. 1972, in Dassan., Rev. Handb. Fl. Ceylon 4: 500. 1983; B.L. Burtt, Notes Roy. Bot. Gard. Edinburgh 35: 209. 1977, in Manilal, Bot. Hist. Hort. Malab. 144. 1980; Nicolson. *et al*. Interpret. Hort. Malab. 317. 1988; Mangaly and Sabu, Rheedea 3(2): 155. 1993; K.G. Bhat, High Pl. Indian subcont. 4: 82. 1993;

Fl. Uduppi 627. 2003; Sabu and Mangaly Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Lectotype: Manjellakua Rheede, Hort. Malab. 11: 21. t. 11. 1692.

Rhizome medium sized, conical, deep orange-yellow inside, strongly aromatic; sessile tubers many, branched. Leafy shoot 80-120 cm high. Leaves 4-6; lamina 45-60 x 15-20 cm, oblong-lanceolate, tapering at both ends, glabrous, without purple patch. Inflorescence central, with distinct white coma. Fertile bracts many, tip recurved, subtends 1 or 2 flowers. Flowers equal to the bracts. Bracteoles 2. Calyx *ca*. 1 cm long, white. Corolla longer than calyx, white. Labellum *ca*. 2.2 x 2.5 cm, bilobed; midlobe emarginate, light yellow with a broad median dark yellow band. Lateral staminode without glandular hairs. Anther spurred. Stigma bilipped (Fig.7. C., D.). Fruits not seen.

Distribution: The plant is cultivated throughout the tropics.

Curcuma montana Roxb., Pl. Corom. 2: 28. t. 151. 1802.Asiat. Res. 11: 342. 1810, Fl. Indica 1: 35. 1820; Baker in Hook. f., Fl. Brit. India 6: 214. 1890 (in part); K. Schum. in Engler, Pflanzenr. 4 (46): 106. 1904; C.E.C. Fisch. in Gamble, Fl. Pres. Madras 8: 1483. 1928; B.L. Burtt and R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 31: 226. 1972; A.S. Rao and Verma, Bull. Bot. Surv. India 14: 122. 1972; Mangaly and Sabu, Rheedea 3(2): 158. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 82. 1993; Sabu and Mangaly Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Iconotype: Roxb. Pl. Corom. 2: t. 151. 1802.

Rhizome ovate- conical, uniform light orange-yellow within; sessile tubers branched; root tubers fusiform. Leafy shoot 80-100 cm tall. Leaves 15-17 cm long, oblanceolate, lower surface puberulent. Inflorescence central, with distinct coma. Coma white with pink in the distal half. Fertile bracts many, green with pink patch. Flowers as long as or smaller than bracts. Calyx *ca*. 8 mm long. Corolla much longer than calyx, white. Labellum 1.8-1.6 cm long; mid-lobe with an apical notch,

deep yellow. Lateral staminodes included within corolla lobe, without glandular hairs. Anther spurred; connective prolonged into a hood. Stigma bi-lipped included within the hood (Fig.7. E., F.). Fruits not seen.

Distribution: South India.

Curcuma mutabilis Sckornickova, Sabu and Prasanth Kumar, Gard. Bull. Singapore. 56: 43-54. 2004.

Type: India, Kerala, Malappuram District, Nilambur, *Skornickova* and *Prasanthkumar 84145* (holo. MH; iso. K, CALI, SING).

Rhizome, conical or cylindrical, light brown externally, faintly aromatic; root tubers ovoid. Leafy shoot 15-60 cm long. Leaves 7; petiole up to 20 cm long, green or with red tinge; lamina 14-35 x 7-11, ovate elliptical, hairy on prominent raised margin on upper surface of leaf, lower surface glabrous. Inflorescence both lateral and terminal. Bracts many, light green or with red tinge, tip rounded with a patch of deep violet colour; coma insignificant. Each bracts subtends a cincinnus of 2-4 flowers. Flowers exserted from bracts, 4.5-6 cm. Calyx *ca*. 1 cm long, white or tinged with pink or violet. Corolla longer than calyx, yellowish; lobes tinged with pink or violet. Labellum 1.4-1.6 x 1.5-1.9 cm, emarginate yellow with deep yellow in the centre. Lateral staminodes yellow with reddish base. Anther 3.5-4 mm long; spur pointing upwards. Stigma exerted (Fig.8. A., B., C., D.). Fruit dehiscent capsule, spherical. Seeds brown, shiny glabrous.

Distribution: Reported only from Nilambur, Malappuram Dt. Kerala.

Curcuma neilgherrensis Wight, Pl. Ind. Orient. t. 2006. 1853.Baker in Hook. f., Fl. Brit. India 6: 210. 1890; K. Schum. in Engler, Pflanzenr. 4(46): 109.1904; T. Cooke. Fl. Pres. Bombay 2: 728. 1907; Fyson, Fl. Nilgiri and Pulney Hilltops 1: 408. 1915, Fl. S. Indian Hill Stat. 2: 598. 1932; C.E.C. Fisch. in Gamble. Fl. Pres. Madras 8: 1482. 1928; Ramamoorthy in C.J. Saidanha and Nicolson, Fl. Hassan Dist. 766. 1976; Rao and Razi, Synopt. Fl. Tamil. Carnatic 2: 1615. 1983; Manilal, Fl. Silent Valley 312.

1988; V.S. Ramach. and V.J. Nair, Fl. Cannanore 468. 1988; Mangaly and M. Sabu, Rheedea 3(2): 160. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 83. 1993; Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: Not known.

Rhizome small, conical, white inside, sessile tubers absent; root tuber few. Leafy shoot 20-30 cm high. Leaves 6-9; petiole 10-15 cm long; lamina 12-18 x 6-9 cm, ovate-elliptic, base subequal, lower surface sparsely pubescent. Inflorescence both lateral and central, with a distinct coma. Coma bracts light to dark pink or violet. Fertile bracts green or with a pink or violet spot at the tip, slightly recurved. Bracteoles 2 or 3, triangular. Flowers longer than bracts, 3 or 4 in each bract, light yellow. Calyx 3-lobed at apex, violet spotted. Corolla tube light yellow. Labellum *ca*. 2 x 2 cm, with a median cleft, yellow with deep yellow median band. Anther hooded; spurs downwardly pointing. Stigma bilipped appressed within the anther thecae (Fig.8. E., F.). Fruit yellowish green, with persistent calyx. Seeds obovate.

Distribution: Endemic to South India.

Curcuma oligantha Trimen, J. Bot. 23. 245. 1885. Handb. Fl. Ceylon 4: 242 1898; Baker in Hook. f.. Fl. Brit. India 6: 215. 1890; K. Schum. in Engler, Pflanzenr. 4 (46): 109. 1904; B.L. Burtt and R.M. Sm. in Daasan., Rev. Handb. Fl. Ceylon 4: 504. 1983; K.G. Bhat, Indian J. For. 10: 66. 1987; V.S. Ramach. and V.J. Nair, Fl. Cannanore 468.1988; Mangaly and Sabu, Rheedea 3(2): 161. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 84. 1993; Fl. Uduppi, 629. 2003; Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: Uma Oya, *Trimens*. *n*. (PDA).

Rhizome small, 1-1.2 cm, conical, non-aromatic; sessile tubers absent; root tubers large, *ca*. 4 cm long. Ovate or fusiform. Leafy shoots 15-35 cm high. Leaves petiolate; petiole 7-10 cm long; lamina 10-20 x 7-14 cm, ovate elliptic, base oblique, glabrous or minutely pubescent. Inflorescence lateral or central, without distinct

coma. Fertile bracts 5-10, recurved at apex; green or with pinkish tinge. Flowers longer than bracts, 5.5-7 cm long. Corolla equal to or longer than bracts, light yellow. Labellum obovate, shortly 3 lobed, white or orange yellow. Anther spurred; connective prolonged into a small crest, crest orange yellow. Stigma bilipped, appressed within the thecae and crest (Fig.9. A., B., C.). Fruit subglobose, with persistent calyx. Seeds obovate with a basal depression.

Distribution: Sri Lanka and South India. In South India it occurs in North Kerala and Southern parts of Karnataka, along the west coast.

Curcuma pseudomantana J. Graham, Cat. Pl. Bombay. 210. 1839.C.E.C. Fisch., Rec. Bot. Surv. India 9: 177. 1921, in Gamble, Fl. Pres. Madras 8: 1483. 1928; Santapau, J. Bombay Nat. Hist. Soc. 45: 618. 1945, Fl. Khandala ed. 3: 273. 1967; Rao and Razi, Synopt. Fl. Mysore Dist. 581. 1931; Manilal and Sivar., Fl. Calicut 287. 1982; Mangaly and M. Sabu, J. Econ. Tax. Bot. 10: 159. 1987, Rheedea 3(2): 165. 1993; K.G. Bhat, High Pl. Indian Subcont. 4: 86. 1993; Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: Poona, *Prain* s.n. 467219 (CAL)

Rhizome small, conical, yellow in centre, white towards periphery, aromatic; sessile tubers absent; root tubers 2-10 cm long. Leafy shoot 80-125 cm. Leaves 6-7; petiole 60-70 cm long; lamina 40-50 x 6-9 cm, oblong-lanceolate, base tapering, margins and terminal half of upper surface pubescent. Inflorescence both lateral and central, with bright pink coma. Fertile bracts green with purple tinge or purple. Bracteoles pink. Flowers longer than bracts. Calyx membranous, white or pale yellow. Labellum *ca*. 1.5 – 1.8 x 1.7 cm, clearly 3-lobed, mid-lobe deeply cleft, bright yellow. Lateral staminodes bright yellow, without glandular hairs. Anther spurs divergent and pointed forward, connective prolonged into small crest. Stigma slightly exerted above anther lobes (Fig.9. D., E.) Fruits trigonous.

Distribution: Endemic to peninsular India.

Curuma raktakanta Mangaly and M. Sabu, J. Econ. Tax. Bot. 12: 475. 1988, Rheedea 3(2): 168. 1993;M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: Kerala, Ernakulam Dt., Neerickode. *Mangaly10346A* (MH).

Rhizome medium sized, conical, aromatic; sessile tubers finger shaped, branched; root tubers fusiform. Leafy shoot 65-75 cm high; pseudostem reddish purple. Leaves 4-6, spreading, lamina 35-45 x 10-12 cm, oblong-lanceolate, base and tip acuminate, glabrous. Inflorescence lateral with distinct coma, coma deep pink. Fertile bracts green with pink tip. Bracteoles many. Flowers as long as or slightly smaller than the bracts, 3-4 in each bract. Calyx white. Corolla light pink. Labellum *ca*. 2 x 2.2 cm, with median cleft, light yellow with a median dark yellow band. Lateral staminodes included within dorsal corolla lobe, with a patch of glandular hairs. Anther spurs divergent. Stigma exerted from the anther (Fig.10. A., B.). Fruits not seen.

Distribution: Endemic to Kerala, known to occur in Ernamkulam and Trissur districts only.

Curcuma vamana M. Sabu and Mangaly. J. Econ. Tax. Bot. 10. 307. 1988.Mangaly and Sabu, Rheedea 3(2): 167. 1993; M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 20. 1996.

Type: India, Kerala, Palghat Dt.: Mannarghat, Kanjirapuzha, Monsoon forest, *Sabu and Mangaly 37342* (MH).

Rhizome small, conical; orange within, stoloniferous; sessile tubers absent; root tubers few, spherical or ellipsoid. Leafy shoot upto 50 cm tall. Leaves 4 or 5; petiole 20-30 cm long; lamina 20-25 x 6-8 cm, oblong, base subequal. Inflorescence central, distinct coma absent. Bracts 4-8, loosely arranged, slightly recurved, subtends 2-4 flowers. Flower shorter than the bracts, 1.8-2 cm long. Calyx persistent. Corolla tube *ca*.8 mm long; lobes almost equal, yellowish white.

Labellum *ca*. 8 x 7 mm, tip notched, margin crisped, golden yellow. Anther thecae convergent at base to form a beak-like spur. Epigynous glands absent. Stigma exserted from the anther (Fig.10. C.,D.). Fruit obovoid with persistent calyx. Seeds brown when mature.

Distribution: Endemic to Kerala.

Curcuma zanthorrhiza Roxb., Fl. India 1: 25. 1820.K. Schum. in Engler Pflanzenr. 4(46): 112. 1904; Ridley, FI. Malay Penin. 4: 254. 1924; Holttum, Gard. Bull. Singapore 13: 72. 1950; Skornickova and M. Sabu, Garden's Bull. Singapore 57: 2005.

Lectotype: Icones Roxburghianae 2003 (K).

Rhizome large, 5-8 x 7-9 cm, broadly ovoid, with smell of camphor, yellow to deep yellow inside; sessile tubers branched; root tubers present. Leafy shoot 80-100 cm high. Leaves 4-6; lamina 40-60 x 15-20 cm, oblong-lanceolate, purple coloured patch on the upper side along the whole length of the midrib, glabrous. Inflorescence lateral, with dark pink coma. Fertile bracts 20-25, tip recurved, green with pink margin. Flowers almost equal to the bracts, 4 or 5 in each bract. Calyx greenish white. Corolla longer than calyx, white with pinkish tinge. Labellum 1.5-2 cm wide, shortly 3 lobed; mid-lobe emarginate, pale yellow with deep yellow band. Anther connective not produced into crest. Stigma bilipped (Fig.10. E., F.). Fruit ovoid, smooth.

Distribution: Cultivated and naturalised throughout India and S.E.Asia.

***ZINGIBER* Boehm.**

The name *Zingiber* Boehm.was originated from Malayalam/Tamil “ingiver” meaning ginger rhizome and the Arab traders spread it to Greece and Rome (Ravindran and Babu, 2005). It is also believed to be originated from Sanskrit word

singebera (horn-root) which gave rise to the classical Greek name Zingiberi and finally *Zingiber* in Latin.

The genus *Zingiber*, the type genus of the family Zingiberaceae, represented by 141 species (Theilade and Mood 1999) distributed mainly in tropical Asia. Baker (1892) reported 24 species from British India.

Zingiber species are perennial rhizomatous herbs with tuberous sympodial rhizomes. Aerial shoot is often covered by sheathing leaf bases. The inflorescence is usually a spike or raceme. *Zingiber* is distinct from other genera of the family by the presence of a single anther with a beak or horn-like appendage, which embraces the upper part of the style. The inflorescence usually arises at the base of the leafy stem on a short or long, aerial or subterranean peduncle. The bracts are overlapping; each subtends a non-tubular bracteole and a single flower. In many species the bracts are green when young, turning to red in the fruiting stage. The flowers are very delicate and fragile and last only for a few hours. The genus can be recognized in the vegetative stage by the presence of a pulvinous between the base of the petiole and ligule.

The common edible ginger, *Z. officinale* constitute one of the five most important species occurring in India and about 70% of the total ginger production is confined to Kerala alone. The dried rhizomes of ginger are used in Ayurvedic and other natural systems of medicine from time immemorial. In Ayurveda, ginger is used as a carminative, and digestive. It is pungent, hot, anodyne, antirheumatic, carminative, cooling, diuretic and aphrodisiac and also promotes digestive power. It is used in the treatment of anorexia, dyspepsia and for the suppression of inflammation. Dry ginger is used in the treatment of asthma, cough, diarrhoea, flatulence, nausea and vomiting (Datta and Mukerji, 1950). It is an important spice extensively used in the preparation of condiments, curries and syrups. The rhizome of *Z. montanum* is given in diarrhoea, also used as a stimulant, carminative, flavouring agent, and also as antidote to snake poison. Rhizome of *Z. roseum* is

used in cold, cough and rheumatism. *Z. zerumbet* rhizome is given in cough, asthma, stomach ache, vermifuge, leprosy and other skin diseases and also used as substitute for true gingers (Prakash and Mehrotra, 1996). The mucilage present in the inflorescence of *Z. zerumbet* is used as shampoo hence known as shampoo ginger. Many varieties of *Z. zerumbet* are now used as ornamental plants.

***Zingiber capitatum* Roxb. var *elatum* (Roxb.)** Baker in Hook. f., Fl. Brit. India 6: 249. 1892. Prain, Bengal Pl. 2: 785. 1963 (Rep. ed); Jha and Varma in A.K. Pandey (ed.), Taxonomy and Biodiversity 110. 1995; Kumar, Zingib. Sikkim 69. 2001; Sabu, Zingiberaceae and Costaceae of S. India 229. 2006.

Type: *Zingiber capitatum* Roxb. var *elatum* Roxb. Iconotype – Icones Roxburghianae t. 1509 (CAL).

Plant, a herb with perennial rhizome. Rhizome thick, yellow, sympodial, tuberous inside, aromatic, root tubers oblong. Plants 1-1.5 m high, internodes 2.2 cm long. Leaves many, lower smaller in size, upper 32-45 x 2-3.3 cm, narrow lanceolate, glabrous above, minutely hairy on lower side. Spike terminal, sessile, linear; deep green, oblong, many flowered, tip obtuse, 12-14 x 2-3.5 cm. Bracts green with red margins. Bracteoles 2-keeled. Flowers 4.8-5.2 cm long, pale yellow, 4-6 opens at a time, longer than bracts. Open after 3 p.m. Calyx white. Corolla tube deep yellow. Labellum yellow, tip bilobed. Dorsal and lateral lobes yellowish, lanceolate. Anther yellow; beak equal to the anther lobes. Stigma funnel-shaped with ciliate margin (Fig.11. A., B., C.). Capsule 3-sided, smooth, bright red. Seeds black, aril white.

Distribution: This taxon so far reported only from Bihar and Bengal (Jha and Varma, 1995) and Sikkim (Kumar, 2001). Sharma *et al*. (1984) reported *Z. capitatum* from Karnataka. This forms a new record for South India.

Zingiber cernuum Dalzell in Hook. Kew J. Bot. 4: 342, 1852. Dalzell and Gibson, Bombay Fl. 273. 1861. Baker in Hook. F., Fl. Brit. India 6.245.1892; K. Schum. In

Engler, Pflanzenr. 4(46). 182. 1904; T. Cooke, Fl. Pres. Bombay 2.734. 1907. Santapau, Fl. Khandala ed. 3. 274. 1967.

Type: To be typified. All efforts to locate the types at different herbaria ended as a futile exercise.

Plant a herb with perennial rhizome. Rhizome fleshy, subterranean, purplish-lilac inside, aromatic, roots many bearing ovoid tubers. Leafy shoot 65-90 cm tall, slightly bending, pseudostem 50-66 cm tall, ensheathed by green bracts. Leaves shortly petiolate, 11-15 in number, distance between leaves 6-6.8 cm long, pulvinate. Lamina 20-28 x 7-10 cm, oblong-lanceolate, glabrous above, densely pubescent beneath. Inflorescence emerges directly from the rhizome, peduncle short, green, ensheathed by reddish green sheaths, outer surface pubescent. Spike subglobose, 5-6.8 x 2-2.7 cm, reddish green, base submerged in soil. Bracts numerous, linear oblong, tip hooded, accuminate, greenish or with red streaks, outer densely pubescent. Bracteoles pale yellow, trilobed at the tip. Flowers 5.5-5.8 cm long, fragile, dark yellow with red spotted labellum, one or two opens at a time. Calyx tubular, pale yellow. Corolla tube 3-3.5 cm long, yellow. Dorsal and lateral lobes lanceolate, yellowish. Labellum shorter than corolla lobes, ovate, emarginate, dark yellow with purple red spots and streaks towards margin. Lateral staminodes yellow with red spots. Stamens yellow. Stigma slightly projecting from the anther crest. Ovary pubescent, trilocular (Fig.11. D., E.). Capsule ellipsoid, fleshy, green when young and turns to red at maturity. Seeds 6-8 x 2-4 mm, dark brown, striate, arillate.

Distribution: Endemic to India especially Kerala, Karnataka, Tamil Nadu and Maharashtra.

Zingiber montanum (K.D. Koenig) Link ex Dietr., Sp. Pl. 1: 52. 1831. B. L. Burtt and R. M. Sm., Notes Roy. Bot. Gard. Edinburgh 31: 194. 1972; Ramamamoorthy in C. J. Saldanha and Nicolson, Fl. Hassan Dist. 769. 1976; Theilade, Nord. J. Bot. 19(4): 396. 1999. M. Sabu, Zingiberaceae and Costaceae of S. India 231. 2006.

Type: Thailand, Phuket, Koenig s.n. (holo C).

Herbs with a perennial rhizome. Rhizome yellow inside. Leafy stem 1-1.5 m high, pseudostem *ca*. 9.5 m high. Leaves sub sessile; ligule membraneous, short, *ca*. 2 mm long. Lamina 23-41.5 x 3-3.5 cm, oblong-lanceolate, tip acute, base slightly rounded, upper surface glabrous, lower pubescent. Inflorescence separate, lateral spike from rhizome. Peduncle 10-25 cm long, sheathed by pubescent, brownish green sheaths. Spike 6-8 x 4 cm, ovate, purple, compact. Flowers pale yellow, 7.3 - 7.5 cm long, 1-2 open at a time. Bracts broadly ovate, purplish brown, pubescent, turn to red at maturity. Calyx truncate. Corolla tube pale yellow, lobes lanceolate; dorsal lobe cymbiform; lateral lobes linear, reflexed. Labellum sub-orbicular, 3 lobed, apex emarginated with crisped margin, yellowish white. Lateral staminodes oblong, yellow, fused with the labellum. Ovary pubescent (Fig.12. A., B., C.). Capsule *ca.* 1.5 cm long, ovoid. Seeds very small, purple.

Distribution

Native of India, seen throughout in India, Malay Peninsula and Java. Cultivated widely in tropical Asia.

Zingiber neesanum (J. Graham) Ramamoorthy in Saldanha and Nicolson, Fl. Hassan Dist., 769. 1976. Manilal, Fl. Silent Valley 314. 1988; K.G. Bhat, High. Pl. Indian subcont. 4: 70.1993, Fl. Udupi, 635. 2003; Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 21.1996. Sabu, Zingiberaceae and Costaceae of S. India 235. 2006.

Type: Not Known

Rhizome creeping just below the soil surface, yellow inside; root tubers fusiform. Leafy shoot 60-120 cm tall, enclosed within reddish – green vegetative bracts. Leaves shortly petiolate; ligule short, 2-3 mm long; lamina 15-25 x 2.3-3.5 cm, linear, oblong-lanceolate, base equal. Inflorescence separate from leafy shoot; peduncle 15-30 cm; Spike 8-20 x 1-1.5 cm, cylindrical, tapering to a narrow apex. Bracts reddish green. Calyx tubular, split on one side. Corolla tube slender.

Labellum equal to or slightly shorter than lateral corolla lobes, obovate, white with pink or purple spotted and striped. Filament short, connective has violet stripes on upper surface. Stigma with an apical circular aperture (Fig.12. D.E., F.). Fruit ellipsoid or sub-globose, 3-valved. Seed deep purple to black.

Distribution: Endemic to peninsular India. It is reported from Maharashtra, Karnataka and Kerala.

Zingiber nimmonii (J. Graham) Dalzell in Hook., Kew J. Bot. 4: 341. 1852, ('nimmoi'); Dalzell and A. Gibson, Bombay Fl. 273. 1861; Baker in Hook. f., Fl. Brit. India 6: 244. 1892; K. Schum. in Engler, Pflanzenr. 4(46): 184. 1904; T. Cooke, Fl. Pres. Bombay 2: 734. 1907; C.E.C. Fisch. in Gamble, Fl. Pres. Madras 8: 1489. 1928; Sabu, Folia Malaysiana 4(1): 36. 2003.

Type: Malabar, Concan, Law s.n. (K)

Rhizome small, purplish lilac inside, strongly aromatic; root tubers present. Leafy shoot 60-90 cm high, clothed by greenish or reddish bracts. Leaves almost sessile or shortly petiolate; ligule bilobed, coriaceous; lamina 20-25 x 8-10 cm, oblong-lanceolate, tip acuminate, base oblique, upper surface dark green, lower surface densely pubescent. Inflorescence produced directly from the rhizome; peduncle very short (0.5-3 cm) or absent; spike ovate or sub-globose. Bracteoles small, shortly trilobed. Flowers *ca*. 5.5 cm long. Calyx unilaterally split. Corolla tube slender. Labellum shorter than corolla lobes, light yellow with purple spots. Lateral staminodes half as long as midlobe, deep yellow with purple red spots. Anther sessile (Fig.13. A., B., C.). Fruit trigonous, exceeding bracts, whitish when young, turning deep red at maturity; seeds, 7-8 mm long, dark red, striate.

Distribution

Endemic to peninsular India; reported to occur in Karnataka, Kerala, Maharashtra and Tamil Nadu.

Zingiber officinale Roscoe, Trans. Linn. Soc. London 8: 348. 1807; Roxb., Asiat. Res. 11: 345. 1810, Fl. Indica 1: 46. 1820; Dalzell and A. Gibson, Bombay Fl. Suppl. 87. 1861; Baker in Hook. f., Fl. Brit. India 6: 246. 1892; K. Schum. in Engler, Pflanzenr. 4(46): 170. 1904; T. Cooke, Fl. Pres. Bombay 2: 736. 1907; C.E.C. Fisch., Rec. Bot. Surv. India 9: 178. 1921, in Gamble, Fl. Pres. Madras 8: 1489. 1928; Holttum, Gard. Bull. Singapore 13: 54. 1950; A.S. Rao and D.M. Verma, Bull. Bot. Surv. India 14: 137. 1972; B.L. Burtt and R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 31: 180. 1972, in Dassan. Rev. Handb. Fl. Ceylon 4: 498. 1983; B.L. Burtt in Manilal, Bot. His. Hort. Malab. 144. 1980; Nicolson et al., Interpret. Rheede Hort. Malab. 318. 1988; R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 45: 412. 1989; Kumar, Zingib. Sikkim 71. 2001; K.G. Bhat, High. Pl. Indian subcont. 4: 69. 1993, Fl. Udupi, 635. 2003; M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 21. 1996; Sabu, Folia Malaysiana 4(1): 39.2003.

Type: Herb. Hermann 4: 7. No.3 (BM) designated by Burtt (1993). No specimen at LINN or in Herb. Cliff. (BM)(Burtt, 1972; Theilade, 1999).

Rhizome thick, palmately lobed, greyish-yellow within, smell pungent. Leafy shoot *ca*. 1 m high. Leaves sessile; pulvinous prominent, ligulate; ligule 2-4 mm; lamina 25-30 x 1.5-2 cm, narrowly laneolate, accuminate, base attenuate, lower surface hairy, upper glabrous. Inflorescence lateral; peduncle 15-25 cm long; spike 4-8 x 2-3 cm long, ovoid. Bracts green with pale margin, lower ones mucronate, turning red at maturity. Flowers longer than the bracts. Corolla tube included within the bract, lobes equal. Labellum more or less round; dark purple spotted. Lateral staminodes smaller. Anther connective yellow, prolonged into a 5-8 mm long, dark purple crest. Stigma white (Fig.13. D., E., F.G.).

Distribution: Cultivated in tropical countries throughout the world. Some wild forms occur in evergreen forests of Kerala.

Zingiber roseum (Roxb.) Roscoe, Trans. Linn. Soc. London 8: 348. 1807; Roxb., Asiat.Res.11: 347. 1810, Fl. Indica 1: 49.1820; Baker in Hook. f., Fl. Brit. India 6:

244. 1892; K. Schum. in Engler, Plfanzenr. 4(46): 184. 1904; C.E.C. Fisch. in Gamble, Fl. Pres. Madras 8: 1489. 1928; Manilal, Fl. Silent Valley 314: 1988; K.G. Bhat, High. Pl. Indian Subcont. 4: 71. 1993; M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 21.1996. Sabu, Folia Malaysiana (1): 45. 2003.

Type: *Zingiber roseum* (Roxb.) Roscoe. Iconotype – Icones Roxburghianae. t. 502. (CAL).

Plant a herb with subterranean rhizome. Rhizome branched, white, tuberous, thick, stoloniferous, fibrous. Leafy shoot 90-110 cm long, pseudostem 60-70 cm high, dark green. Leaves 13-18 in number at a distance of 2.7-4.7 cm, sub-sessile; lamina 30-35 x 6.5-7.6 cm, lanceolate, upper glabrous, lower densely pubescent; petiole very small, pubescent beneath, green, pulvinate. Inflorescence very dense, arising from rhizomes, green with red streaks towards tip, base immersed in soil; spike globose, round at base, condensed, 4-6 x 25-30 cm. Bracts lanceolate, outer pubescent, base light green with red streaks towards tip, outer ones broadly ovate. Bracteole bilobed, white with red spotted towards tip. Flowers 6.2-6.8 cm long, 1-2 opens at a time. Calyx tubular, 3 toothed. Corolla tube, white, 3.5 cm, slender. Dorsal and lateral lobes linear, outer pubescent, red colour is prominant. Labellum orbicular, 3-lobed, white at the centre, yellow with red spots towards margin, lateral staminodes 0.2 cm long, yellow, orbicular, very small. Stamen arching over the labellum, yellowish. Anther thecae and crest yellow. Ovary pubescent, trilocular (Fig.14. A., B.). Fruit dehiscent capsule 3.5 x 2.6 cm, ovoid.

Distribution: Reported from Central India and Eastern Ghats.

Zingiber wightianum Thwaites, Enum. Pl. Zeyl. 315. 1861; Baker in Hook. f., Fl. Brit. India 6: 244. 1892; Trimen, Handb. Fl. Ceylon 4: 257. 1898; K. Schum. in Engler, Pflanzenr. 4(46): 186. 1904; C.E.C. Fisch., Rec. Bot. Surv. India 9: 178. 1921, in Gamble, Fl. Pres, Madras 8: 1489. 1928; B.L. Burtt and R.M. Sm. in Dassan., Rev. Handb. Fl. Ceylon 4: 496. 1983; K.G. Bhat, High. Pl. Indian Subcont. 4: 71. 1993, M.

Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 21. 1996; Sabu, Folia Malaysiana (1): 46. 2003.

Type: *Zingiber wightianum* Thwaites. Lectotype-C. P. 2286 (PDA).

Plants herbs with subterranean rhizome. Rhizome, 1.5-1.8 cm thick, fleshy and stolen like; leafy shoot 85-90 cm high. Pseudostem 60-70 cm. Leaves 15-17 in number at a distance of 5-6 cm, reddish colour ligule, oblong-lanceolate, pale green, pubescent below; lamina 28-33 x 6.2-7.8 cm, upper surface glabrous, lower pubescent, base obovate. Inflorescence ovate or oblong on separate leafless peduncle; peduncle 2.5-6 cm long, ensheathed by sterile bracts. Spike 8-8.5 cm long, oblong, greenish red, partly immersed in soil. Flower 6.1-6.4 cm long, 1-2 opens at a time, opens early in the morning. Bracts greenish red, pubescent. Calyx green with red markings. Corolla tube longer than calyx, yellowish green, pubescent. Corolla lobes equal, acuminate, yellow orange. Labellum *ca*. 3 cm long, cuneate, obovate, emarginate, yellow-orange, heavily marked deep purple red. Lateral staminodes *ca*.1 cm long. Stamen shorter than the lip, connective prolonged into dark purple – red beak. Ovary *ca*. 5 mm long, slightly pubescent (Fig.14. C., D.). Fruit *ca*. 2.5 cm long, oblong, red. Seeds black with white weil.

Distribution: Occurs in Southern peninsular India and Sri Lanka.

Zingiber zerumbet (L.)Smith, Exot. Bot. 2: 105, t. 112. 1806. Roxb., Asiat. Res. 11: 346. 1810, Fl. Indica 1: 47. 1820; Dalzell and A. Gibson, Bombay Fl. 272. 1861; Thwaites, Enum. Pl. Zeyl. 315. 1861; Baker in Hook. f., Fl. Brit. India 6: 267. 1892; Trimen, Handb. Fl. Ceylon 4: 259. 1898; K. Schum. in Engler, Pflanzenr. 4(46): 172. 1904; T. Cooke, Fl. Pres. Bombay 2: 734. 1907; C.E.C. Fisch., Rec. Bot. Surv. India 9: 178. 1921, in Gamble, Fl. Pres. Madras 8: 1490. 1928; Holttum, Gard. Bull, Singapore 13: 59. 1950; A.S. Rao and D.M. Verma, Bull. Bot. Surv. India 14: 137. 1972; B.L. Burtt and R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 31: 182. 1972, in Dassan., Rev. Handb. Fl. Ceylon 4: 495. 1983; Nicolson et al., Interpret. Rheede Hort. Malab. 319. 1988; R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 45: 418. 1989;

K.G. Bhat, High. Pl. Indian Subcont. 4: 72. 1993, Fl. Udupi, 635. 2003; M. Sabu and Mangaly, Proc. 2nd Symp. Fam. Zingiberaceae 21. 1996; Sabu, Folia Malaysiana 4(1): 47. 2003.

Type: This species remains to be typified. "Linnaeus" Amomum No.3 may be identified as *Z. zerumbet* but it has only been seen as microfiche" (Theilade, 1999).

Rhizome large, 10-15 x 4 cm, light yellow inside. Leafy shoot 1-2 m high. Leaves shortly petiolate; petiole 4-5 mm long; ligule 2.5-3.5 cm long, membranous, entire; lamina 10-35 x 5-10 cm, oblong-lanceolate, tip acuminate, upper surface glabrous, lower surface pubescent. Inflorescence lateral; peduncle 20-25 cm long; globose or oblong. Bracts *ca*. 3 x 5 cm, ovate-obovate, green. Flowers larger than bracts, *ca*. 5 cm long, yellow. Corolla as long as the bracts, white; dorsal lobe large. Lebellum pale yellow, dark yellow towards the centre, margin highly crumbled, unspotted; mid-lobe emarginate (Fig.15. A., B., C., D.) Fruit *ca*. 1.5 cm long, white with persistent calyx. seeds many, black, glabrous.

Distribution: Widely distributed throughout India, Malay Peninsula and Java. Grown extensively in Sri Lanka and other countries. This species is supposed to be native of India (Holtum, 1950).

MATERIALS AND METHODS

Taxonomy

Members of the family Zingiberaceae selected for the study were collected from different regions of India. Field observations such as habitat, frequency, association of vegetation, habit, colour and odour of plants were noted in the field book. Whole plants and their underground parts such as rhizome, roots and root tubers were collected. The rhizomes collected from different localities were planted in the Calicut University Botanical Garden (CUBG) for continued observations. Vegetative and flowering twigs were pickled in 50% Formaldehyde-Acetic acid-Alcohol solution (FAA) as soon as they were collected from the field and stored for further laboratory studies. Specimens were identified with the help of Floras, Revisions and Monographs and also referred to experts for accurate identification. The nomenclatural corrections were made according to the ICBN (Mc Neill *et al,* 2006) and for abbreviation of periodicals; the BPH (Lawrence, 1968) is followed. Brumitt and Powell (1992) is followed for the abbreviation of authors. The types and authentic materials available in Central National Herbarium (CAL), Kolkata and Madras Herbarium (MH) at Coimbatore were studied. Voucher specimens used for this investigation are deposited in the Herbarium of Calicut University (CALI).

Molecular Characterization

Isolation of Genomic DNA

Materials required

Mortar and Pestle, Meassuring cylinders (5-250ml.), Beakers, conical flasks, Variable volume pipettes (1-1000µl), electronic balance, Cooling centrifuges, Eppendorf tubes etc.

Genomic DNA from different species of *Curcuma, Zingiber* were isolated using the modified CTAB method of Ausubel *et al*., (1995). The procedure used for obtaining pure DNA is as follows.

1. Approximately 10 mg of fresh leaf tissues without midrib were ground to a fine powder in liquid nitrogen in a pre-chilled mortar and pestle.

2. The powder was immediately transferred to a centrifuge tube containing 2.0 ml pre-warmed (65^{o}C) extraction buffer containing 100mM Tris – HCl (pH 8.0), 20mM EDTA (pH 8.0), 1.4 M NaCl, 2% (w/v) Cetyl trimethyl ammonium bromide (CTAB) and 0.2% (v/v) β-mercaptoethanol. Added 1µl of 10 µg/µl proteinase K. (For preparation see Table-3&4)

3. The powder was emulsified gently and incubated at 65^{o}C for one hour with occasional mixing by gentle swirling.

4. The homogenate was extracted with equal volume of Chloroform: isomyl alcohol (24:1) and mixed by inversion to emulsify. The mixture was centrifuged at 10,000 rpm for 10 min., at 4^{o}C. The supernatant was collected.

5. Added 2/3 volume of Isopropanol and mixed by gentle inversions and kept at room temperature for 30 min. The precipitated DNA were scooped into eppendorf tubes containing 70% (v/v) ethanol. Spun at 5000 rpm for 2-5 minutes, discarded the supernatant and vacuum dried for 20 min. and dissolved in minimum TE buffer (10mM Tris, pH 8.0 and 1.0 mM EDTA). (If sufficient DNA strands are not formed, the DNA will be obtained by pelleting as described below).

6. Incubated at 4^{o}C for overnight after adding Isopropanol.

7. Centrifuged at 10,000 rpm for 15 minutes at 4^{o}C.

8. Discarded supernatant and added 70% (v/v) ethanol and washed the precipitate by gentle swirling for 3-4 minutes.

9. Spun at 10,000 rpm for 15 min. at 4^{o}C. Poured off supernatant, inverted the tubes for 15 min. to drain off excess alcohol and left the pellet to air dry overnight.

10. To the dried pellet added minimum of TE to dissolve the DNA and transfer the solution to 1 ml sterile microfuge tubes.

Purification of DNA

1. The DNA thus obtained was subjected for RNase treatment. Added 1 µl of 10 µg/µl of RNase that is DNase free and incubated at 37^{o}C for 1 hour.

2. To the solution added equal volume of Phenol: Chlroform: Isomyl alcohol (25:24:1) and mixed well.

3. Spun at 10,000 rpm for 10 min. at 4^{o}C and the upper layer was transferred to fresh microfuge tubes.

4. Extracted with equal volume of Chloroform: Isoamyl alcohol (24:1), spun at 10,000 rpm and transferred the aqueous phase to fresh tubes.

5. Added 1/10 volume of 3M sodium acetate (pH 5.2) and 0.8 V of isopropanol and mixed gently and incubated at 4^{o}C overnight.

6. Centrifuged at 5,000 rpm for 5 minutes.

7. Decanted the supernatant carefully. Washed the pellet with 70% (v/v) ethanol.

8. Vacuum dried the pellet and dissolved in TE buffer.

Table-3 Preparation of buffers used for genomic DNA isolation (Sambrook *et al*., 1989)

	Buffer	Method of preparation	Comments
1	CTAB Extraction Buffer: 1 litre 100mM Tris HCl (pH 8.0) 20mM EDTA (pH 8.0) 1.4 M NaCl 2% (w/v) CTAB (Himedia) 0.2% (v/v) β-Mercapto ethanol (Himedia)	Measure 100 ml Tris (1M), 280 ml of NaCl, 40 ml of EDTA (0.5M). Mix with about 400 ml of hot distilled water, added 20 g of CTAB to this. Adjusted final volume to 1 litre. Dispensed to reagent bottles and autoclaved. Just before use, added 0.2% β-mercaptoethanol	CTAB will take time to dissolve. Avoid foaming
2.	TE (0.1 mM) buffer 100 ml, 100 mM Tris HCl (pH 8.0), 0.1 mM EDTA (pH 8.0)	Take 1 ml of Tris HCl (1M), 20 ml of EDTA (0.5M). Mixed with 99 ml of sterile distilled water taken in a reagent bottle, mixed thoroughly and autoclaved.	TE (0.1mM) is written since there is TE with 1mM EDTA also.
3.	TAE buffer 10x: 1 litre	Weighed 48.4 gm of Tris base; added 20 ml of EDTA (0.5M); 11.42 ml of Glacial acetic acid and around 150 ml of sterile distilled water. Dissolved the salt and adjusted volume to 1 litre. Autoclaved.	It will dissolve much easily in 500 ml solution.
4.	Gel loading buffer (6x) 100 ml 0.25% (w/v) Bromophenol blue (Himedia) 30% (v/v) Glycerol (Himedia)	Dissolved 0.25 g of BPB in 99 ml of 30% (v/v) Glycerol. Kept on a magnetic stirrer for several hours to get the dye completely dissolved. Dispensed to reagent bottles and kept in $4^{o}C$.	Strong dye handle carefully.
5.	Proteinase K – Storage Buffer. Glycerol (50ml.), 1M Tris-HCl, pH 7.5 (1ml.), $CaCl_2$(0.29 g) dd H_2O to 100ml.	Put 10 ml. storage buffer in a screw cap tube, add100mg of proteinase K,mix well and aliquote to 1.5 ml eppendorf tubes.	Store at $-20^{o}C$
6.	RNase A	Prepare a 10 mg/mL stock solutionin 10 mM sodium acetate buffer, pH 5.2. Heat to 100 ^{0}Cfor 15 minutes, allow to cool to room temperature, and then adjust to pH 7.4 using 0.1 volume of 1 M Tris-HCl, pH 7.4.	Aliquot and store at –20 ^{0}C.

Table-4.Preparation of stock solutions (Sambrook *et al*., 1989)

	Solutions	Method of preparation	Comments
1	1M Tris (pH 8.0), 500 ml	Dissolved 60.55 gm Tris base (Himedia) in 300 ml distilled water. Adjusted pH to 8 by adding conc. HCl. Made the volume to 500 ml. Dispensed to reagent bottles and sterilized by autoclaving.	pH of Tris solution is temperature dependent. Tris will completely dissolve only when pH becomes 8. Initial pH will around 11.
2	0.5 M EDTA pH 8.0	Dissolved 93.05 g of EDTA – disodium salt (Himedia) in 300 ml of water. Adjusted pH to 8 by adding NaOH pellets.Made the volume to 500 ml. Dispensed into reagent bottles and autoclaved.	The salt will take time to dissolve.
3.	5M NaCl 500 ml	Weighed146.1 g NaCl (Himedia) added 200 ml of water and mixed well. When the salts get completely dissolved, adjust the final volume to 500 ml. Dispensed into reagent bottles and autoclaved.	The salt will take much time to dissolve.
4.	3M Sodium acetate (pH 5.2) 250 ml	Dissolved 61.523 g of anhydrous Sodium acetate (Merck) in 200 ml of water and mixed well. When dissolved completely adjusted the pH of the solution to 5.2 with glacial acetic acid (99-100%). Dispensed to reagent bottles and autoclaved.	The salt will take much time to dissolve.
5.	Ethidium Bromide 100 mg/ml, 100 ml	Added 1g. Ethidium Bromide to 100 ml of distilled water. Kept on magnetic stirrer to ensure that the dye has dissolved completely. Dispensed to amber colored reagent bottle and stored at $4^{o}C$	Ethidium Bromide is a powerful mutagen and is moderately toxic. So handle carefully.
6	70% (v/v) ethanol, 500 ml	Take 355 ml of ethanol: mix with 145 ml of distilled water. Dispensed to reagent bottle and stored at $4^{o}C$.	Stock ethanol is 99% (v/v)., hence 355 ml is taken instead of 350 ml.
7	Chlroform: Isomyl alcohol (24:1), 500 ml	Measured 450 ml of chloroform and 20 ml of isomyl alcohol. Mixed well and stored in reagent bottle in room temperature.	Chloroform will evaporate so close the cap tightly.
8	1 M $MgCl_2$, 100 ml	Weighed 20.33 g of $MgCl_2$ (Himedia), dissolved in double distilled water, make up to 100 ml, dispensed in to reagent bottles.	

Quantification of DNA

DNA quantification was carried out by using UV scanning Thermo scientific Nano Drop^{TM} 2000/2000 C Spectrophotometer. OD at 260/280 nm was recorded and the amount of DNA was calculated based on its OD at 260 nm. 0.8% (w/v) agarose gel was used for DNA visualization for its quality and stored at -20^{o}C. The DNA samples were diluted with sample buffer containing Bromophenol blue and 20 µl of the samples were loaded to each well. The gel was incorporated with 1µl of (10µg/µl) ethidium bromide. 1kb DNA ladder was used as molecular weight marker. The gel was run in a horizontal electrophoretic machine containing 1X TAE buffer until the tracking dye reaches the bottom edge of the gel.The OD of the samples were measured at 280 nm and the 260/280 value for the DNA samples will be calculated to assure their quality. The quantity of DNA was calculated based on the assumption that 1OD of DNA = 50 ng of double stranded DNA.

Polymerase Chain Reaction

A 96 well thermal cycler with gradient block was used for RAPD and ISSR reactions (Eppendorf mastercycler pro S, Germany). The desalted custom primers for RAPD and ISSR were obtained from Sigma Genosys, U.S.A., Taq DNA polymerase, 10 X reaction buffer and magnesium chloride were obtained from Genei, Banglore, India. dNTPs and 1 Kb ladder were obtained from Sigma, USA. For gel electrophoresis, agarose was obtained from Hi-Media, India.

Template DNA quality

One of the important features of PCR is that the quantity and quality of the DNA sample subjected to amplification do not need to be high. When DNA of known concentration is available, amounts of 50-100 ng were typically used for amplifications of single copy loci (Innis & Gelfand, 1990). The quantified DNA samples of *Zingiber* and *Curcuma* were diluted to a final concentration of 25µg/ml and stored at -20^{o}C. For standardizing the optimum quantity of DNA required for

RAPD and ISSR reactions purified DNA of 20ng, 25ng, 30 ng and 50 ng were used as templates.

Taq polymerase Assay Buffer (10 x)

The *Taq* polymerase assay buffer contains 500 mM KCl, 200 mM Tris HCl (pH 8.4 at 25^{o}C), 1.5 mM $MgCl_2$, 100 μl/ml of gelatin and 0.1% Triton X-100 that will be adequate for the majority of genomic DNA PCR (Innis & Gelfand; 1990). 10 x buffer was obtained from Genei, Banglore, India

Deoxy Nucleotide Triphosphates

The four dNTPs – dATP, dTTP, dCTP and dGTP tested in concentrations between 100-200 μM each that result in the optimal balance among yield, specificity and fidelity. All the four dNTPs were used at equivalent concentrations to minimize incorporation errors in a final concentration in the range of 100-200 μM (Innis & Gelfand, 1990).

***Taq* Polymerase Enzyme**

The optimal concentration of *Taq* DNA polymerase depends on the quantity and quality of template and length of the PCR product. Usually 0.5-1 unit of DyNAzyme II DNA polymerase per 50 μl reaction volume gives good results, but the optimal amount can range from 0.5 to 2.0 units per 50 μl reaction. As a rule, concentrations in excess of 4 units tend to result in the accumulation of non specific amplification products, whereas amounts less than 1 U usually reduce the yield of the desired product (Innis & Gelfand; 1990). For optimizing the reaction products during RAPD reactions and ISSR of *Zingiber* and *Curcuma* Taq DNA polymerase was used in the concentration of 0.5,0.75 and 1U/reaction.

Primers

Desalted oligos obtained from Sigma Genosys, U.S.A, were diluted to 1 μM concentrations and working solutions were prepared from these stock. The stock

solutions were stored in -20^{o}C deep-freezer. Primer concentrations between 10-15 nm are generally optimal. Higher primer concentrations may promote mispriming and accumulation of non-specific product and may increase the probability of generating a template, independent artifact, termed primer – dimer. Nonspecific products and primer dimer artifacts are themselves substrates for PCR and compete with the desired product for enzyme, dNTPs and primers, resulting in a low yield of the desired product (Innis & Gelfand; 1990). For standardizing the primer concentrations for optimal results primers in the concentration of 10, 12, 14 and 15 pm were used.

The 7 ISSR primers were developed from the University of British Columbia website and were also obtained from Sigma Genosys, USA, the solutions of 1µM and working solutions were prepared from the stock. The oligos used for RAPD and ISSR and their sequences are given in Table-5.

Magnesium ion (Mg^{2+}) concentration

The Mg^{2+} concentration affects the capacity of primer annealing, strand dissociation temperature of template and PCR product, product specificity and formation of primer dimer artifacts, enzyme activity and fidelity. Taq DNA polymerase requires free Mg^{2+}which is the cofactor for DNA polymerase enzyme, primers and dNTPs. Accordingly, PCRs should contain 0.5-2.5 mM Mg^{2+} over the total dNTP concentration. The presence of EDTA or other chelators in the primer stocks or template DNA may disturb the apparent Mg^{2+}. Therefore, though the buffer contains magnesium, an additional Mg^{2+} also added for the reaction. In the reaction for standardising Mg^{2+} concentrations for obtaining intense bands, $MgCl_2$ in the concentrations of 0.25, 0.50, 0.75 and 1.0 mM was added separately to the reaction in addition to the 1.5 mM $MgCl_2$ present in the assay buffer.

Table-5.Oligos used in RAPD and ISSR analysis and their base sequences

S.No.	Oligo Name	Sequence (5' ⟶ 3')
1.	OPA 5	AGGGGTCTTG
2.	OPA 7	GAAACGGGTG
3.	OPA 8	GTGACGTAGG
4.	OPA 10	GTGATCGCAG
5	OPA 11	CAATCGCCGT
6	OPA 12	TCGGCGATAG
7	OPA 14	TCTGTGCTGG
8	OPA 15	TTCCGAACCC
9	OPA 17	GACCGCTTGT
10	OPA 18	AGGTGACCGT
11	OPA 19	CAAACGTCGG
12	OPB09	TGGGGGACTC
13	OPB 12	CCTTGACGCA
14	OPB 16	TTTGCCCGGA
15	OPC 07	GTCCCGACGA
16	OPC 08	TGGACCGGTG
17	OPC 09	CTCACCGTCC
18	OPC 10	TGTCTGGGTG
19	OPC 11	AAAGCTGCGG
20	OPC 12	TGTCATCCCC
21	OPC 13	AAGCCTCGTC
22	OPC 14	TGCGTGCTTG
23	OPC 16	CACACTCCAG
24	OPC 18	TGAGTGGGTG
25	OPC 20	ACTTCGCCAC
26	OPD 01	ACCGCGAAGG
27	OPD 03	GTCGCCGTCA
28	OPD 04	TCTGGTGAGG
29	OPD 05	TGAGCGGACA
30	OPD 07	TTGGCACGGG
31	OPD 08	GTGTGCCCCA
32	OPD 13	GGGGTGACGA
33	OPD 14	CTTCCCCAAG
34	OPD 15	CATCCGTGCT
35	OPD 16	AGGGCGTAAG
36	OPJ 10	AAGCCCGAGG
37	OPJ 16	CTGCTTAGGG
38	OPJ 17	ACGCCAGTTC
39	OPJ 18	TGGTCGCAGA

S.No.	Oligo Name	Sequence (5' ⟶ 3')
40	OPM 01	GTTGGTGGCT
41	OPM 07	CCGTGACTCA
42	OPM 20	AGGTCTTGGG
1	ISSRI	$(CT)_8TG$
2	ISSR 2	$(CT)_8AC$
3	ISSR 3	$(TCC)_5AG$
4	ISSR 4	$(AGC)_4GT$
5	ISSR 5	$(CAC)_3GC$
6	ISSR 6	$(CTC)_3GC$
7	ISSR 7	$(GACA)_3$
8	ISSR 8	$(GACA)_3GC$

The Reaction mixture for PCR

Component		**Volume (IX)**
Sterile double distilled water	:	11.75 µl
10 x PCR buffer	:	2.50 µl
dNTPs	:	3.75 µl
Primer	:	2.00 µl
$MgCl_2$	:	1.50 µl
Taq polymerase	:	0.5 µl
Template DNA	:	3.00 µl
Total reaction volume	:	25.00 µl

Optimization of PCR Programming

Temperature profile tested for optimizing PCR as follow as:

Reaction cycles: 3

Ist cycle: Denaturation of 92^{0}C for 3, 4 and 5 min.

IInd cycle (35X): Denaturation- 92^{0}C for 1 min.

Annealing-37, 38 and 39^0Cfor 30 sec. and 1min.

Extension- 72^0 C for 1 min.

IIIrd cycle: Final extension- 72^0C 10 min.

For ISSR, the annealing temperature was fixed at 55^0C with all the other reaction conditions same as RAPD.

Data scoring and analysis

The electrophoretic bands were visualized by "WUV-L 50" Dual wave length UV Transilluminator and the gels were photographed using Cell-bio Science's chemiluminescence system and documented. Scoring was carried out as 1/0 for the presence or absence of bands in each gel. To avoid taxonomic ambiguities, the intensity of bands was not taken to considerations, only the presence of band was taken as indicative. The data was entered into excel, spread sheet and the matrix obtained was loaded to the NTSYS-pc programme (Rohlf, 2000) and Jaccard's similarity index (JSI) between the species under study were calculated. A UPGMA dendrogram was constructed based on the derived JSI.

Extraction of essential oil by hydrodistillation (AOAC, 1975)

Materials used:-

1. Flask (Short neck, round bottom type, 500 ml or 1 litre with S.T 24/29 ground joint)
2. Electric heating mantle
3. Volatile oil traps (Clevenger type) with S.T 24/29 ground joints
4. West type condenser (400 mm in length with water-cooled drip tip and S.T 24/29 ground joint).

Methods used:-

Shade dried aerial plant parts and rhizomes of wild *Curcuma* and *Zingiber* species were hydrodistilled separately in a Clevenger-type apparatus (Clevenger,

1928) at 100^{o}C for 4 hours as prolonged extraction normally increases the yield (Gildemeister and Hoffman, 1961). Volatile oil was collected over water. The sample was cooled to room temperature and allowed to stand until oil layers were clear and finally the extracted oil was collected. The oils thus obtained were dried over anhydrous sodium sulfate and kept in refrigerator at 4^{0} C prior to analysis.

GC/MS analysis

Instrument details:

GC INSTRUMENT : 6850 NETWORK GC SYSTEM, AGILENT TECHNOLOGIES

COLUMN : HP5MS Nominal length: 30.0 m Nominal diameter: 250.00 µm Nominal film, thickness: 0.25 µm.

MS : 5975C VLMSD WITH TRIPLE AXIS DETECTOR, AGILENT TECHNOLOGIES

MS ACQUISITION PARAMETERS:-

COLUMN

Capillary Column		
Max temperature	:	325^{0} C
Nominal length	:	30.0 m
Nominal diameter	:	250.00 um
Nominal film thickness	:	0.25 um
Mode	:	constant flow
Initial flow	:	1.0 mL/min
Nominal init pressure	:	8.22 psi
Average velocity	:	37 cm/sec
Source	:	Inlet
Outlet	:	(unspecified)
Outlet pressure	:	vacuum

INJECTOR

Sample volume (uL)	:	1.000
Syringe size (uL)	:	10.0
Draw Speed (uL/min)	:	300
Dispense Speed (uL/min)	:	6000
Inject Speed (uL/min)	:	6000
Solv Draw Speed (uL/min)	:	300
Solv Dispense Speed (mL/min)	:	6000
Tune File	:	atune.u
Acquistion Mode	:	Scan
Solvent Delay	:	3.00 min
EMV Mode	:	Relative
Relative Voltage	:	0
Resulting EM Voltage	:	1212
[Scan Parameters]		
Low Mass	:	50.0
High Mass	:	800.0
Threshold	:	0
Sample #	:	2 A/D Samples 4
Plot 2 low mass	:	50.0
Plot 2 high mass	:	550.0
MS Source	:	230 C maximum 250 C
MS Quad	:	150 C maximum 200 C
TUNE PARAMETERS for SN	:	US10011008
EMISSION	:	34.610
ENERGY	:	69.922

REPELLER	:	30.123
IONFOCUS	:	81.616
ENTRANCE_LE	:	19.000
EMVOLTS	:	1211.765
Actual EMV	:	1211.77
GAIN FACTOR	:	0.28
AMUGAIN	:	2407.000
AMUOFFSET	:	127.188
FILAMENT	:	1.000
DCPOLARITY	:	0.000
ENTLENSOFFS	:	19.075
MASSGAIN	:	-304.000
MASSOFFSET	:	-39.000

Temperature programming: 5 min at 60^0 C, then rising at 5^0 C/min to 110^0C, then 3^0C from 110^0-200^0C, then 5^0C/min to 220^0C and maintained at 220^0C for 5 minutes. Helium was used as the carrier gas and sample was injected in split mode.

Identification of Compounds: Compounds were identified by comparing the retention indices of the peaks on a RTX wax column with literature values, computer matching against the library spectra built up using pure substances and components of known essential oils and finally confirmed by comparison of mass spectra of peaks and retention indices with published data (Mc – Carron *et al*., 1995; Adams, 1989; Swigar and Silverstein, 1987; Ramaswamy *et al*., 1988). The relative proportion of each individual component of the oil was expressed as a percentage relative to the total peak area.

RESULTS

The important characters studied to develop a taxonomic key for *Zingiber* and *Curcuma* sp. are represented in Tables-6&7

Key to the genera

1. Stem well developed; primary bracts not adnate laterally; lateral staminodes adnate to the labellum; anther crest elongate, embracing the style ..... *Zingiber*

1. Stem poorly developed or absent; primary bracts adnate to each other laterally forming a pouch; lateral staminodes free from the labellum; anther with or without a crest; not embracing the style ..... *Curcuma*

Key to the species based on morphological characters

Key to the species

Zingiber Boehm**.**

1. Spike terminal, on the leafy stem ..... 1. *Z. capitatum* var. *elatum*

1. Spike lateral, from the base of the leafy stem 2

2. Peduncle immersed in soil, not erect, 2-9.5 cm long or absent 3

2. Peduncle erect, 10-50 cm long 6

3. Labellum white with spots, *ca*. 3.2 cm long, oblong, cuneate, margin recurved, rhizome white inside ..... 6. *Z. roseum*

3. Labellum yellow or purple with spots, 2.4-2.7 cm long, obovate or sub ovate, rhizome purple-lilac or yellow inside 4

4. Rhizome purple-lilac inside, sympodial, ligule 5 m – 1 cm long, bilobed; labellum yellow with purple spots or streaks 5

4. Rhizome yellow inside, stoloriferous, ligule 3-3.5 cm long, deeply notched, labellum light violet with white spots ..... 8. *Z. wightianum*

5. Leafy shoot 65-90 cm tall, peduncle 0.8 cm long, flower 5.8 cm long .... 2. *Z. cernuum*

5. Leafy shoot 1.2-1.5 m tall, peduncle 9.5 cm long, flower 7.7 cm long .... 5. *Z. nimmonii*

6. Labellum pale yellow, unspotted 7

6. Labellum dark purple or purple spotted 8

7. Bracts greenish brown, spike ovate with acute tip ..... 3. *Z. montanum*

7. Bracts green, turn to red at maturity, spike globose with round tip .... 9. *Z. zerumbet*

8. Leaves lanceolate, stem dark red, spike cylindrical, dark red, labellum white with purple spots ..... 4. *Z. neesanum*

8. Leaves linear, narrowly lanceolate, stem green, labellum dark purple with creamy yellow blotches ..... 7. *Z. officinale*

***Curcuma* L.**

1. Anther lobes ecalcarate ..... *C. aurantiaca*

1. Anther lobes spurred 2

2. Leafy shoot 15-60 cm high 3

2. Leafy shoot 65-125 cm high 11

3. Inflorescence with or without inconspicuous coma 4

3. Inflorescence with well-developed coma 8

4. Rhizome stoloniferous; flowers shorter than the bracts ..... 18. *C. vamana*

4. Rhizome ovoid; flowers longer than the bracts 5

5. Leaves 0.7-1.5 cm broad; inflorescence only central ..... 5 *C. bhatii*

5. Leaves 7-14 cm broad; inflorescence both lateral and central 6

6. Leafy shoot upto 35 cm tall; flowers 5-5.7 cm long ..... 15 *C. oligantha*

6. Leafy shoot upto 60 cm tall; flowers 4.5-6 cm long 7

7. Labellum white with a median bright yellow band; anther 4.5 mm long ..... 10. *C. karnatakensis*

7. Labellum yellow or white with yellow center; anther 3.5-4 mm long .... 13. *C. mutabilis*

8. Root tubers cylindrical, 10-18 cm long; leaves coriaceous, densely pubescent on both sides ..... 6. *C. coriacea*

8. Root tuber small, spherical, ovoid or oblong; leaves not coriaceous, glabrous or sparsely pubescent 9

9. Flowers equal to the bracts; lip purple 10

9. Flowers longer than bracts; lip yellow ..... 14. *C. neilgherrensis*

10. Leaves broadly ovate, sub cordate at base; fertile bracts recurved; lip purple towards base ..... 7. *C. decipiens*

10. Leaves elliptic, base oblique; fertile bracts not recurved; lip deep purple with a bright yellow band.....9. *C. inodora*

11. Leaves with purple patch along the midrib..... 12

11. Leaves without a purple patch along midrib..... 13

12. Rhizome blue within; leaves with a purple patch on the distal half on the upper side only..... 1. *C. aeruginosa*

12. Rhizome yellow to deep yellow within; leaves with purple patch on both sides along the whole length of the midrib ..... 19. *C. zarthorrhiza*

13. Lateral staminodes with a patch of glandular hairs at centre 14

13. Lateral staminoides without glandular hairs..... 15

14. Pseudostem reddish purple; leaves spreading, oblong-lanceolate, glabrous below ..... 17. *C. raktakanta*

14. Pseudostem green with a few light pink dots; leaves erect, semiplicate, ovate-elliptic, densely pubescent on the lower surface ..... 8 *C. haritha*

15. Rhizome with the smell of green mango .....2. *C. amada*

15. Rhizome without the smell of green mango..... 16

16. Rhizome with sessile tubers..... 17

16. Rhizome without sessile tubers .....16. *C. pseudomontana*

17. Rhizome deep orange-yellow within; lip light yellow with a median dark yellow band .....4. *C. longa*

17. Rhizome yellow or light orange yellow within; lip deep yellow 18

18. Rhizome light orange-yellow within; corolla white; calyx 8 mm long ..... .12 ..... *C. montana*

18. Rhizome greyish-yellow within; corolla pinkish white; calyx 2 cm long ..... ……3. *C. aromatica*

Table-6.Characters selected from different species of *Curcuma* for morphological characterisation

Species	Ploidy level	Floral characters			Rhizome characters			Aerial characters	
		Spike position	Colour of calyx	Color of corolla	Colour of rhizome	Aroma of rhizome	Taste of rhizome	Colour of leaf sheath	Leaf midrib colour
C.karnatakensis	__	Lateral/Central	Transparent green	Pale rose	Flesh cream	Mango	Gingery	Green,purple tinged	Purple tinged
C.mutabilis	__	Vernal/Central	White with pink tinge	Variable colours	Light brown	Faintly aromatic	Slight bitter	Green with red tinge	Deep green
C.amada	42	Lateral/Central	Light purple	White	Light yellow/white	Mango	Gingery	Green	Green
C.neilgherrensis	42	Lateral/Central	White with violet dotted	Light yellow	White	Slight camphoraceous	Bitter	Green	Green
C.oligantha	__	Lateral/Central	White/light green	Yellow/pink tinge	Yellow	Non- aromatic	Bitter	Green	Green
C.haritha	42	Lateral	White with purple tinge	White	Yellowish-grey	Non- aromatic	Slight bitter	Green/light pink dots	Green
C.aromatica	42, 63,86	Lateral	White with purple tinge	White	Greyish yellow	Camphoraceous	Bitter	Green	Green
C.aeruginosa	63	Lateral	Light purple	Light purple	Blue in the centre, grey-periphery	Camphoraceous	Bitter	Green	Purple
C.zanthorrhiza	63, 64	Lateral	Greenish-white	Pale yellow	Deep yellow	Camphoraceous	Slight bitter	Green	Purple patch
C.coriacea	__	Lateral/Central	Light purple	Bright yellow	White	Non-aromatic	Bitter	Green with violet tinge	Green

Species	Ploidy level	Floral characters			Rhizome characters			Aerial characters	
C.longa	62, 63, 64	Central	White	White	Deep orange-yellow	Turmeric aroma/ Camphoraceous	Slight bitter	Green	Green
C.montana	__	Central	Light purple	White	Light orange-yellow	Camphoraceous	Bitter	Purple	Green
C.pseudomontana	__	Both Lateral and Central	White/pale yellow	Yellowish-White	Yellow in the centre, white -periphery	Pleasently aromatic/ Camphoraceous	No taste	Green/purple to violetish	Green
C.raktakanta	63	Lateral	White/purple tinge	Light pink	Greyish-yellow inside, whitish-periphery	Camphoraceous	Slight bitter	Reddish-purple	Green
C.decipiens	32, 42, 62	Both Lateral and Central	White with pink spots	Deep purple	Pale yellow-white	Camphoraceous	No taste	Greenish purple	Green
C.inodora	__	Both Lateral and Central	White	Deep purple	Pale yellow in the centre, whitish-periphery	Non-aromatic	Slightly bitter	Greenish purple	Green
C.aurantiaca	__	Central	White	Light yellow-orange	Yellow-white	Slight camphoraceous	Slight bitter	Green	Green
C.vamana	__	Central	White	Golden yellow	Orange	Slight camphoraceous	Slight bitter	Green	Green
C.bhatii	__	Central	White	Light yellow	White	Non-aromatic	Bitter	Green	Green

Table-7.Characters selected from different species of *Zingiber* for morphological

Species	Ploidy level	Floral characters			Rhizome characters			Aerial characters	
		Spike position	Colour of calyx	Color of corolla	Colour of rhizome	Aroma of rhizome	Taste of rhizome	Colour of leaf sheath	Leaf midrib colour
Z. roseum	22	Lateral on a separate leaf less peduncle	White	Prominent red	White to pale yellow	Faintly aromatic	Slight bitter	Green with red streaks	Dark green
Z. wightianum	22	Lateral on a separate leaf less peduncle	Light yellow	Pale yellow with pink towards tip	Yellow inside	Faintly aromatic	Gingery	Reddish green	Green
Z. nimmonii	22	Lateral, arise from rhizome	White	Light yellow	Purple lilac	Camphoraceous	Gingery, slightly bitter	Reddish green	Green
Z. cernuum	22	Lateral, arise from rhizome	White	Yellow-orange	Purplish-lilac	Camphoraceous	Gingery, Bitter	Reddish green	Green
Z. zerumbet	22	Lateral spike	White	Pale yellow	Yellowish	Ginger aroma	Gingery	Light green	Green
Z. officinale	22	Lateral on a separate spike	White	Yellow	Greyish yellow	Strongly ginger aroma	Pungent, gingery	Green	Green
Z. neesanum	22	Lateral, from base of the leafy shoot	White	White	Yellow inside	Camphoraceous	Slightly bitter	Reddish Green	Green
Z. montanum	22	Lateral spike	White	Light Yellow	Bright yellow	Camphoraceous	Bitter	Green	Green
Z. capitatum	22	Terminal spike	White	Yellow	Yellow	Faintly aromatic	Slightly bitter	Green	Green

Molecular characterization

Isolation of genomic DNA

The protocol used for the extraction of DNA worked for all the species, however, interspecific variations were observed in the quality and quantity of DNA. Good quality and quantity of DNA was extracted from uppermost first and second leaves of both *Curcuma* and *Zingiber* species. The isolated DNA from different species of *Curcuma* and *Zingiber* were quantified and the quantity/mg of leaf sample for each species is shown in the Tables –8 &9. The 260/280 ratio of all the samples were calculated and the samples having high protein content with a lesser 260/280 value were purified and the 260/280 values were reassessed. The quality of the DNA isolated was also checked by running the samples with sample buffer containing bromophenol blue on a 0.8% (w/v) agarose gel containing 1µg/ml of Ethidium bromide. The samples with higher DNA content were diluted so as to have a final concentration of DNA up to 25µg/ml for RAPD and ISSR analysis.

Table-8.Quantity and quality of DNA obtained from different species of *Curcuma*.

Sl. No.	Species Name	DNA Quantity (ng/µl)	OD 260/280	OD 260/230
1	*C. karnatakensis*	433.0	2.20	1.19
2	*C. mutabilis*	546.2	1.61	1.21
3	*C. amada*	159.1	2.49	0.63
4	*C. neilgherrensis*	743.4	2.54	0.80
5	*C. oligantha*	921.0	2.28	0.97
6	*C. haritha*	403.1	2.19	1.11
7	*C. aromatica*	121.1	1.76	1.13
8	*C. aeruginosa*	294.7	1.56	0.86
9	*C. zanthorrhiza*	513.2	2.25	1.03
10	*C. coriacea*	870.1	2.30	1.04
11	*C. longa*	844.9	2.24	1.36
12	*C. montana*	258.5	2.06	0.96
13	*C. pseudmontana*	295.2	2.06	0.95
14	*C. raktakanta*	574.6	2.15	1.41
15	*C. decipiens*	266.2	1.86	1.67
16	*C. inodora*	271.2	2.18	0.68
17	*C. aurantiaca*	690.6	2.16	1.31
18	*C. vamana*	372.0	1.14	1.69
19	*C. bhatii*	405.4	2.33	0.93

Table-9.Quantity and quality of DNA obtained from different *Zingiber* species.

Sl. No.	Species Name	DNA Quantity (ng/µl)	OD 260/280	OD 260/230
1	*Z. roseum*	303.2	2.43	0.70
2	*Z. wightianum*	243.8	2.63	1.73
3	*Z. nimmonii*	329.9	2.02	0.68
4	*Z. cernuum*	210.3	2.68	1.86
5	*Z. zerumbet*	842.3	1.97	0.84
6	*Z. officinale*	389.2	1.56	1.12
7	*Z. neesanum*	750.7	2.05	1.45
8	*Z. montanum*	971.6	2.13	0.92
9	*Z. capitatum*	166.1	2.32	1.68

STANDARDISATION OF CONDITIONS FOR PCR

Out of the different DNA concentrations used for RAPD and ISSR reactions 30ng of genomic DNA produced better amplification with bright bands. When 20 ng of DNA used the bands produced were diffuse and at the end of the run and bands were not clear. Both 25 and 50 ng of DNA produced bands during the run, but the band intensity and clarity of the bands were lesser.

Taq DNA polymerase at the concentration of 0.5 U for 25 µl of total reaction volume produced better results, however, an increasing concentration of *Taq* DNA polymerase to 0.75 and 1 U/ reactions did not affect the quality of the reaction. As the concentration of the enzyme increased, it doesn't affect the band intensity and the reaction.

The RAPD and ISSR primers at a concentration of 10pm/reaction produced better results, however, on increasing the concentration of the primer to 12, 14 and 15 pm produced unreacted nucleotides at the bottam end of the gel and the reaction products were not clear and the intensity of the bands were lesser.

Addition of $MgCl_2$ (0.50mM) to the reaction mixture in addition to the 1.5 mM present in the 10X reaction buffer produced high intensity bands, $MgCl_2$ at

0.25 mM produced feeble bands and 0.75 and 1.0 mM of $MgCl_2$ produced one or two thick bands and the bands diffused at the end of the gel run.

For the PCR cycles denaturation at 92^{o}C for 3 min. provided best results with good amplifications. When the denaturation time increased to 4 min. the amplification products were broken into smaller bands and at 5 min. no reaction products were obtained.

Annealing temperature for the primers were screened with 37^{o}C for 30 sec. and 1 min. provided high intense bands whereas increasing the annealing temperature to 38 and 39^{o}C for 30 sec. and 1 min. did not produce the desired bands. For ISSR primers only the anealing temperature was changed to 55^{0} C, and all other reaction conditions were the same as RAPD.

RAPD AND ISSR ANALYSIS OF *CURCUMA*

A total of 42 random decamer primers obtained from Sigma Genosys, U.S.A., were selected for RAPD analysis of which 20 primers produced polymorphic bands in all the species of *Curcuma*. Of the 8 ISSR primers used for analysis, 7 primers produced polymorphic bands and were selected for the characterization of the 19 *Curcuma* species. The sequence of the RAPD/ISSR primers used for the molecular fingerprinting of the 19 *Curcuma* species and RAPD/ISSR banding patterns expressed by the primers and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table- 10.

The 20 random decamer RAPD primers produced a total of 2226 scorable bands in the 19 species of *Curcuma* studied, out of which 1025 were polymorphic. The percentage polymorphism ranged from a maximum of 56.7% to a minimum of 36.5% (Table-11). Whereas, the seven ISSR primers studied, produced 800 bands and out of which 424 were polymorphic, the percentage polymorphism ranged from 60.78% to 48.14%. The representative figures for the gels with selected primers are presented (Fig.16-24).

Table-10.Total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by RAPD and ISSR primer in *Curcuma* species

Primer	*C.karnatakensis*		*C.mutabilis*		*C.amada*		*C.neilgherrensis*		*C.oligantha*		*C.haritha*		*C.aromatica*		*C.aeruginosa*		*C.zanthorriza*		*C.coriacea*	
	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M
OPA 05	7	9	4	7	7	7	7	2	3	2	7	5	6	6	3	5	2	2	1	0
OPA 07	5	8	2	3	3	6	4	8	3	7	7	5	5	4	6	6	2	8	6	4
OPA 08	6	3	3	3	4	4	3	3	3	4	3	3	7	6	2	3	5	5	4	8
OPA 10	4	6	3	5	5	4	2	3	2	5	2	2	3	4	4	2	4	2	3	3
OPA 11	4	5	3	5	5	3	2	4	4	2	2	2	3	0	3	2	3	1	2	2
OPA 12	4	3	2	1	2	2	3	7	1	4	5	3	5	5	7	3	2	3	2	1
OPA 14	7	5	3	5	4	4	3	3	5	4	3	3	5	2	4	3	3	4	3	2
OPA 15	4	7	5	6	4	4	4	2	3	3	2	2	3	1	3	7	4	3	2	3
OPA 17	6	3	2	1	2	4	4	8	2	3	6	2	6	5	8	5	2	4	3	1
OPA 18	7	7	2	6	9	4	7	5	4	7	4	5	8	7	3	6	5	3	5	2
OPA 19	2	0	4	3	2	1	4	2	1	1	8	6	2	1	4	7	4	5	5	4
OPB09	7	5	3	4	5	3	4	2	5	4	4	4	8	6	4	5	5	2	8	6

Primer	*C.karnata kensis*		*C.mutabilis*		*C.amada*		*C.neilghe rrensis*		*C.oligantha*		*C.haritha*		*C.aromatica*		*C.aeruginosa*		*C.zanthorriza*		*C.coriacea*	
OPB 12	8	5	3	5	7	7	2	6	7	5	2	3	2	4	5	7	2	3	5	3
OPB 16	7	4	5	3	6	5	5	7	8	4	6	2	4	1	2	1	1	3	1	2
OPC 07	6	4	3	3	8	4	6	3	2	1	4	8	5	6	4	4	7	5	5	2
OPC 08	7	7	7	5	6	2	5	3	7	2	4	5	6	1	6	2	6	2	3	3
OPC 09	3	1	3	1	6	2	3	2	2	1	4	1	2	1	6	2	3	2	3	2
OPC 10	7	2	5	1	8	4	5	4	6	1	8	3	5	3	9	1	7	4	4	4
OPC 11	5	2	12	1	2	1	4	1	1	0	5	2	5	1	4	2	5	2	4	2
OPC 12	7	5	6	5	6	2	6	6	4	6	6	4	4	1	6	3	5	1	4	3
ISSR I	6	1	4	2	2	1	4	2	1	0	9	4	4	4	4	4	11	4	5	3
ISSR 2	5	3	9	5	2	1	4	1	0	1	4	2	6	3	4	4	4	3	4	3
ISSR 3	6	6	4	7	5	3	5	7	3	6	5	3	4	1	5	5	4	3	4	2
ISSR 4	5	2	4	1	2	1	4	0	2	0	8	5	7	1	5	3	11	2	5	2
ISSR 5	6	2	5	1	8	2	5	1	10	5	2	1	3	1	1	0	5	2	6	1
ISSR 6	8	1	5	2	9	2	5	3	4	1	4	2	3	1	2	0	7	1	5	7
ISSR 7	3	2	7	5	3	2	3	1	9	2	5	3	4	2	7	3	4	2	2	0

Primer	*C.longa*		*C.montana*		*C.pseudomontana*		*C.raktakanta*		*C.decipiens*		*C.inodora*		*C.aurantiaca*		*C.vamana*		*C.bhatii*	
	P	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**
OPA 05	2	1	7	1	5	2	5	7	3	3	3	5	3	3	4	3	4	2
OPA 07	3	4	4	4	2	1	5	3	1	1	4	2	2	1	5	4	4	7
OPA 08	4	4	2	6	2	4	5	6	3	5	3	3	5	9	7	6	2	4
OPA 10	8	4	1	1	4	1	0	1	3	2	3	3	3	2	3	3	8	4
OPA 11	2	1	5	6	2	6	4	5	5	6	2	4	5	5	3	1	4	4
OPA 12	4	4	4	4	8	1	2	3	6	5	5	5	3	1	5	3	3	6
OPA 14	9	6	1	1	3	1	1	1	3	5	5	2	4	4	5	3	9	6
OPA 15	2	1	8	5	4	6	4	5	7	5	3	3	7	3	2	2	6	2
OPA 17	4	4	3	7	8	5	4	2	6	2	4	5	3	1	4	4	6	4
OPA 18	4	4	7	5	3	5	4	4	6	5	5	6	5	2	1	5	1	1
OPA 19	2	1	1	2	3	2	0	1	5	5	4	3	6	6	5	3	2	5
OPB09	4	6	5	8	5	6	5	3	5	1	6	1	2	0	7	7	2	0
OPB 12	4	4	5	1	8	4	5	4	6	4	7	5	3	1	2	4	5	8

OPB 16	4	1	0	1	3	1	1	1	2	2	5	8	4	6	2	0	3	1
OPC 07	5	8	4	6	4	5	5	8	2	1	7	2	4	3	21	1	5	6
OPC 08	2	0	4	1	2	1	3	2	2	1	6	5	1	1	2	1	2	1
OPC 09	1	1	9	5	2	2	6	5	3	2	6	3	4	2	2	2	2	1
OPC 10	6	1	7	1	6	2	2	1	7	1	2	0	6	1	2	1	6	2
OPC 11	3	4	5	1	9	5	8	4	5	2	6	2	9	5	9	2	10	2
OPC 12	4	2	7	5	3	7	6	6	3	3	8	1	3	3	7	3	4	1
ISSR I	3	1	9	6	2	1	3	2	1	0	6	6	4	4	3	4	9	5
ISSR 2	4	3	5	1	10	4	7	5	5	2	4	3	8	4	5	5	6	4
ISSR 3	4	1	5	5	3	7	7	6	3	3	5	4	4	2	8	4	3	2
ISSR 4	4	3	11	5	2	1	4	1	1	0	8	3	5	2	4	3	9	4
ISSR 5	4	2	4	1	2	0	5	1	2	1	7	6	7	5	5	4	4	2
ISSR 6	6	2	4	6	6	1	7	3	4	4	6	1	7	4	4	4	4	5
ISSR 7	3	1	1	0	8	5	4	2	9	4	5	2	4	3	2	1	4	0

P- Polymorphic; M-monomorphic

DIVERSITY ANALYSIS

The scoring was done from the gels after running the PCR products on an agarose gel, where the bands were clearly visible and amplified products were reproducible over three repeated amplifications. Amplified fragments were manually scored for presence (1) and absence (0) and the binary matrices were subjected to statistical analyses using NTSYS–*pc* (Numerical Taxonomic Multivariate Analysis System version 2.1) by Exceter softare (Rohlf, 2000). To compute pair-wise genetic similarities, Jaccard's similarity coefficients (J_{ij}) were calculated by using the formula, J_{ij}= a/(n-d) where *a* is the number of RAPD bands present in both *i* and *j* accessions, d is the number of bands absent in both *I* and *j* accessions, and *n* is the total number of RAPD bands. The similarity matrices were computed and corresponding dendrograms of genetic relatedness were constructed by applying Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm (Fig-25and Table- 12).

In the case of all the *Curcuma* species the JSI ranged from 0.184 to 0.4. The species were grouped by subjecting the similarity values to UPGMA clustering. Based on the dendrogram developed, the 19 species taken for the study were grouped into two main clusters. Cluster I had only one node containing one species, *i.e*. *Curcuma bhatii* and has 0.23 JSI with the all the other species which were taken for the study, indicating the conversion of *Paracautleya bhati* to *C.bhati* is illegitimate and the plant deserves a separate genus status. The cluster II is further subdivided into cluster IIA and cluster IIB at overall 25% similarity according to Jaccard's similarity coefficients. Cluster IIA contained five species of which C*. karnatakensis* and *C. mutabilis* which were 37.5% similar. *C. neilagherrensis* and *C.oligantha* which have 33.7% similarity. *C. amada* grouped with a 28% jaccard's similarity with the *C. neilgherrensis*/ *C. oligantha* cluster and had a distinct node indicating its special morphological characters and biochemical features are valid in molecular studies also. Cluster IIB contain two small subgroups which showed 27% similarity among themself. *C. haritha* and *C. aromatica* were 32% similar. *C.*

aeruginosa and *C. zanthorrhiza* showed 34.7% Jaccard's similarity coefficient. C*. coriacea* and *C. longa* were grouped together with a similarity coefficient of 32%. The second subgroup contained 7 species in which *C.montana* and C*. pseudomontana* were grouped together with maximum similarity between them and can be considered as the same species. Morphological characters supporting separate species identity for these two cannot find support from the RAPD- ISSR data. *C. decipiens* and *C.inodora* showed 38% similarity and C*. aurantiaca* grouped along with *C. decipiens/C.inodora* cluster with 36.8% similarity. *C. vamana* showed 29% similarity with all the other species in the cluster, and form a separate node.

Table-11. Total number of bands generated, number of polymorphic bands and percentage polymorphism exhibited by RAPD and ISSR primers in different *Curcuma* Sp.

Sl.No.	Primer	Sequence (5' → 3')	Total no.of bands	No. of polymorphic bands	Polymorphism (%)
1.	OPA 05	AGGGGTCTTG	97	46	47.42
2.	OPA 07	GAAACGGGTG	116	43	37.06
3.	OPA 08	GTGACGTAGG	116	48	41.37
4.	OPA 10	GTGATCGCAG	109	58	53.21
5	OPA 11	CAATCGCCGT	97	47	48.45
6	OPA 12	TCGGCGATAG	111	60	54.05
7	OPA 14	TCTGTGCTGG	134	76	56.71
8	OPA 15	TTCCGAACCC	132	57	43.18
9	OPA 17	GACCGCTTGT	133	59	44.36
10	OPA 18	AGGTGACCGT	116	60	51.72
11	OPA 19	CAAACGTCGG	80	41	51.25
12	OPB09	TGGGGGACTC	120	59	49.16
13	OPB 12	CCTTGACGCA	137	50	36.50
14	OPB 16	TTTGCCCGGA	89	34	38.20
15	OPC 07	GTCCCGACGA	117	44	37.61
16	OPC 08	TGGACCGGTG	80	32	40
17	OPC 09	CTCACCGTCC	76	40	52.63
18	OPC 10	TGTCTGGGTG	133	61	45.86
19	OPC 11	AAAGCTGCGG	122	54	44.26
20	OPC 12	TGTCATCCCC	111	56	50.45
		Total	2226	1025	46.04
1	ISSR I	$(CT)_8TG$	104	55	52.88
2	ISSR 2	$(CT)_8AC$	108	52	48.14
3	ISSR 3	$(TCC)_5TG$	148	73	49.32
4	ISSR 4	$(AGC)_4GT$	107	58	54.20
5	ISSR 5	$(CAC)_3GC$	102	54	52.94
6	ISSR 6	$(CTC)_3GC$	129	70	54.26
7	ISSR 7	$(GACA)_3$	102	62	60.78
		Total	800	424	53

Table-12. Similarity indices generated by the binomials for RAPD and ISSR primers of different *Curcuma* species

Rows/Cols	*C.karnatakensis*	*C.mutabilis*	*C.amada*	*C.neilgherrensis*	*C.oligantha*	*C.haritha*	*C.aromatica*	*C.aeruginosa*	*C.zanthorriza*	*C.coriacea*
C.karnatakensis	1.0000000									
C.mutabilis	0.3694779	1.0000000								
C.amada	0.2652330	0.2578125	1.0000000							
C.neilgherrensis	0.2089552	0.2415254	0.2978723	1.0000000						
C.oligantha	0.2605364	0.2735043	0.2550607	0.3640777	1.0000000					
C.haritha	0.2508834	0.2423077	0.2737643	0.2857143	0.2489960	1.0000000				
C.aromatica	0.2266187	0.2109375	0.2432432	0.2850877	0.2735043	0.3292181	1.0000000			
C.aeruginosa	0.2392857	0.2539683	0.2193309	0.2254098	0.2510288	0.3055556	0.3277311	1.0000000		
C.zanthorriza	0.2304833	0.2145749	0.2046332	0.2145923	0.2467532	0.3096234	0.2658228	0.3304348	1.0000000	
C.coriacea	0.2126866	0.2049180	0.2047244	0.2096070	0.2051282	0.2429150	0.2098765	0.2500000	0.2566372	1.0000000
C.longa	0.2384615	0.2226891	0.2520661	0.2177778	0.2345133	0.2991453	0.2876106	0.2638298	0.2217391	0.2331839
C.montana	0.2525952	0.2259259	0.3041825	0.2921811	0.2559055	0.3030303	0.3293173	0.2862595	0.2788845	0.2401575
C.pseudomontana	0.2599278	0.3195021	0.2595420	0.2918455	0.2857143	0.2779923	0.2619048	0.3170732	0.2520325	0.2226721
C.raktakanta	0.2611940	0.2685950	0.2364341	0.2500000	0.2394958	0.2260536	0.2134387	0.2570281	0.2638298	0.2597403
C.decipiens	0.2720588	0.2909836	0.2386364	0.2468619	0.2469136	0.2377358	0.2600000	0.2995951	0.2448980	0.2304527
C.inodora	0.2184300	0.2490421	0.1859649	0.2459677	0.2265625	0.2696629	0.2442748	0.3019608	0.2248062	0.2015504
C.aurantiaca	0.2344828	0.2528736	0.2194245	0.2400000	0.1842105	0.2454212	0.2674419	0.2566038	0.2579365	0.2390438
C.vamana	0.2183099	0.2256809	0.1978022	0.2163265	0.2023810	0.2377358	0.3347458	0.2539063	0.2298387	0.2510460
C.bhatii	0.2691030	0.2625899	0.2517241	0.2462687	0.2372263	0.3140794	0.3146067	0.2614841	0.2490842	0.2226277

C.longa	*C.montana*	*C.pseudomontana*	*C.raktakanta*	*C.decipiens*	*C.inodora*	*C.aurantiaca*	*C.vamana*	*C.bhatii*
1.0000000								
0.3448276	1.0000000							
0.2563025	0.3139535	1.0000000						
0.2151899	0.3279352	0.4000000	1.0000000					
0.2813853	0.2873563	0.3292181	0.3276596	1.0000000				
0.2738589	0.2899628	0.2324723	0.2868526	0.2929688	1.0000000			
0.2419355	0.2654545	0.2835249	0.3170732	0.3174603	0.3398438	1.0000000		
0.2813853	0.2537313	0.2617188	0.2580645	0.2749004	0.2537879	0.3441296	1.0000000	
0.2769231	0.2827586	0.3198529	0.3082707	0.3639847	0.3060498	0.3834586	0.3798450	1.0000000

RAPD AND ISSR ANALYSIS OF *ZINGIBER*

From the 42 random primers used for RAPD analysis, 18 primers were selected for RAPD analysis based on the polymorphic bands produced and 6 ISSR primers were selected for the characterization of 9 *Zingiber* species. The sequence of the RAPD/ISSR primers used for the molecular genetic finger printing of the 9 *Zingiber* species and RAPD/ISSR banding patterns expressed by oligo primers and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table - 13.

The 18 random decamer primers produced a total of 997 scorable bands in the 9 species of *Zingiber* studied out of which 660 were polymorphic. The percentage of polymorphism ranged from a maximum of 77.78% to a minimum of 54.54% (Table-14)Whereas in case of the 6 ISSR primers studied, the percentage of polymorphism ranged a maximum of 77.27% and a minimum 56.94%. The representative figures for the gels with selected primers are presented (Fig.26-33).

DIVERSITY ANALYSIS OF *ZINGIBER*

The similarity indices obtainedby converting the presence or absence of bands in the RAPD and ISSR are shown in Table- 15. All the nine species of *Zingiber* used for the study formed two clusters, cluster I and II (Fig.34). Cluster I formed by three species of *Zingiber, Z. capitatum* showed a Jaccard's similarity index of 0.29. *Z. neesanum and Z. montanum* showed a similarity index of 0.341. The entire cluster of this group showed JSI of 0.27. Cluster II comprised of 6 species and *Z. zerumbet* and *Z.officinale* were grouped together with a JSI of 0.36. *Z. nimnonii* and *Z.cernuum* showed a JSI of 0.41 and were grouped together with the same node indicating they are synonyms. The node comprising of *Z. roseum* and Z*. wightianum* and had a JSI of 0.41.

Table-13.Total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by RAPD and ISSR primers in *Zingiber* species

Primer	*Z.roseum*		*Z.wightianum*		*Z.nimmonii*		*Z.cernuum*		*Z.zerumbet*		*Z.officinale*		*Z.neesanum*		*Z.montanum*		*Z.capitatum*	
	P	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**
OPA 05	9	4	5	3	5	7	4	4	7	4	5	3	6	5	2	1	8	4
OPA 07	6	2	4	1	4	2	2	6	6	4	5	4	4	2	3	1	4	4
OPA 08	7	2	5	2	6	2	4	3	5	4	2	1	3	4	2	4	3	3
OPA 10	4	5	4	1	6	1	8	5	4	2	2	2	4	5	4	3	4	3
OPA 11	7	2	4	3	5	8	3	3	8	5	5	4	7	4	2	3	6	4
OPA 12	5	2	3	3	4	4	6	6	4	7	4	4	6	2	3	2	5	1
OPA 14	7	5	3	7	6	5	5	3	6	7	4	3	5	4	3	1	4	2
OPA 15	6	4	6	1	5	3	3	2	6	3	3	2	8	2	4	6	7	2
OPA 17	9	4	5	3	6	4	7	1	5	4	5	4	8	4	5	2	2	1
OPA 18	8	5	5	6	3	2	1	3	6	5	3	7	5	4	7	6	6	5
OPA 19	5	1	2	2	4	1	4	3	3	3	3	3	5	2	4	0	3	1
OPB09	7	6	6	4	7	4	4	4	6	2	4	4	4	6	3	7	3	4
OPB	6	4	5	3	9	4	3	5	4	1	2	3	2	0	2	1	3	1

12																		
OPB 16	3	3	3	1	6	5	3	3	6	2	5	2	3	1	5	1	4	1
OPC 07	8	3	6	3	4	4	3	3	2	0	4	2	4	2	2	1	1	2
OPC 08	9	2	7	1	2	1	4	1	3	3	6	1	5	1	7	7	5	2
OPC 09	6	7	4	5	3	1	5	1	5	1	8	2	8	3	7	2	7	2
OPC 10	9	3	6	6	5	4	2	1	8	3	5	3	4	4	4	4	3	3
ISSR I	6	6	6	3	6	8	5	5	5	2	9	5	8	7	6	2	3	3
ISSR 2	2	1	3	1	5	4	6	1	5	6	6	2	4	3	3	1	3	2
ISSR 3	9	4	5	6	3	4	5	4	3	8	4	3	6	6	4	7	6	4
ISSR 4	8	3	3	8	7	3	2	2	7	1	2	0	5	2	2	2	7	3
ISSR 5	8	1	5	2	5	1	5	3	2	1	4	1	8	3	3	2	4	3
ISSR 6	5	3	5	3	4	3	5	1	8	3	6	3	9	4	5	2	4	2

P- Polymorphic; M-monomorphic

Table-14.Total number of bands generated, number of polymorphic bands and percentage polymorphism exhibited by RAPD and ISSR primers in different *Zingiber* species

Sl.No.	Primer Name	Sequence (5 → ’3’)	Total no.of bands	No. of polymorphic bands	Polymorphism (%)
1.	OPA 05	AGGGGTCTTG	77	42	54.54
2.	OPA 07	GAAACGGGTG	52	39	75
3.	OPA 08	GTGACGTAGG	51	37	72.55
4.	OPA 10	GTGATCGCAG	47	32	68.09
5	OPA 11	CAATCGCCGT	73	42	57.53
6	OPA 12	TCGGCGATAG	58	35	60.34
7	OPA 14	TCTGTGCTGG	57	42	73.68
8	OPA 15	TTCCGAACCC	61	47	77.05
9	OPA 17	GACCGCTTGT	59	43	72.88
10	OPA 18	AGGTGACCGT	69	42	60.87
11	OPA 19	CAAACGTCGG	37	24	64.86
12	OPB09	TGGGGGACTC	62	39	62.90
13	OPB 12	CCTTGACGCA	46	32	69.57
14	OPB 16	TTTGCCCGGA	46	34	73.91
15	OPC 07	GTCCCGACGA	36	28	77.78
16	OPC 08	TGGACCGGTG	44	26	59.09
17	OPC 09	CTCACCGTCC	61	37	60.66
18	OPC 10	TGTCTGGGTG	61	39	63.93
		Total	997	660	66.19
1	ISSR I	$(CT)_8TG$	66	41	62.12
2	ISSR 2	$(CT)_8AC$	46	32	69.57
3	ISSR 3	$(TCC)_5TG$	72	41	56.94
4	ISSR 4	$(AGC)_4GT$	60	39	65
5	ISSR 5	$(CAC)_3GC$	44	34	77.27
6	ISSR 6	$(CTC)_3GC$	66	45	68.18
		Total	354	232	65.53

Table-15. Similarity indices generated by the binomials of RAPD and ISSR primers of different *Zingiber* species

Rows/Cols	*Z.roseum*	*Z.wightianum*	*Z.nimmonii*	*Z.cernuum*	*Z.zerumbet*	*Z.officinale*	*Z.neesanum*	*Z.montanum*	*Z.capitatum*
Z. roseum	1.0000000								
Z.wightianum	0.3967611	1.0000000							
Z.nimmonii	0.3097015	0.3215859	1.0000000						
Z.cernuum	0.3192308	0.2807018	0.4056604	1.0000000					
Z.zerumbet	0.3284672	0.2880658	0.2811245	0.3012552	1.0000000				
Z.officinale	0.2480916	0.2432432	0.2260870	0.2924528	0.3594470	1.0000000			
Z.neesanum	0.3246269	0.2614108	0.2757202	0.3073593	0.3076923	0.3302326	1.0000000		
Z.montanum	0.2682927	0.2253521	0.1973094	0.2634146	0.2556054	0.2272727	0.3415842	1.0000000	
Z.capitatum	0.3000000	0.2424242	0.2794760	0.2666667	0.2697095	0.2453704	0.2857143	0.3092784	1.0000000

GC-MS-BASED METABOLIC PROFILING

GC-MS analysis is a powerful tool to study the chemical components in the volatile oil. The present investigation detected a number of compounds from different species could be used as marker compounds to distinguish between the species. Essential oil yield was too low in some species, thus its chemical characterization was not possible. Moreover, the distribution of the species is scanty to repeat the experiment. The extraction of essential oils from both rhizome and leaves were not possible for all the species. In some species both the rhizome and leaf yielded essential oils, but in some cases either leaf or rhizome provided oil or in some cases neither of them yield good quality and quantity oil. The relative content of volatile compounds identified by GC-MS from *Curcuma* rhizomes/leaves are given in Table- 16. The major compounds detected from the essential oil are given below:

***Curcuma aeruginosa*- Rhizome**

The GC-MS profile of essential oil isolated from the rhizome of *C. aeruginosa* are represented in Figs. 35-40. The major compounds detected were 2-Pyridinamine,4,6-dimethyl-(28.84%),Eucalyptol (10.27%) 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)- (6.24%), Camphor(6.15%), 2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol(4.55%), Thiazole, 5-methyl-4-phenyl-(3.95%), Cyclohexanone, 2-methyl-5-(1-methylethenyl)-(3.78%), beta Pinene (2.76%), Camphene(2.06%), Isoborneol (2.19%),Caryophyllene (2.15%),Ar-tumerone (1.99%) andCurlone (1.54%).

***Curcuma amada* –Rhizome**

GC-MS analysis of essential oil of *C. amada* detected the following compounds. Beta-Pinene (0.99%), beta-Myrcene (4.36%), alpha-Phellandrene (1.28%), Caryophyllene oxide (1.43%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (6.16%), Caryophyllene (1.33%) Tumerone (4.93%), Benzofuran,

Hydrazine, 1H-Pyrazole-1-carboxylic acid (11.03%), gamma.-Elemene (2.89%), Artumerone (7.25%), 4,5,6,6a-Tetrahydro-2(1H)-pentalen one (18.45%),4,5,6,6a-Tetrahydro-2(1H)-pentalen one (1.40%), Benzofuran (1.66%), 3,7-Cyclodecadien-1-one, 3,7-dimethyl-10- (9.80%), Curlone (3.03%), 4-Toluenesulfonylmethylisocyanide (1.15%), Benzene, Naphthalene (2.50%), Phenol, Propane-1,2,3-triol (1.33%), 1(2H)-Naphthalenone (5.15%) and Naphthalene (11.37%). The GC- MS profile obtained by subjecting the essential oil of *C.amada* rhizome are presented in Figs.41-47.

***Curcuma aromatica* –Rhizome**

The GC-MS profile of essential oils obtained from the rhizome of *C. aromatica* are presented in Figs. 48-52. Major compounds detected were, Neocurdione (28.43%), Camphor (20.97%), 6,10-Dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione (10.53%), Bicyclo[2.2.1]heptan-2-one,1,7,7- trimethyl-,(1R)-,Borneol (8.09%),2-Cyclohexen-1-one, 2-methyl-5-(1- methylethenyl)-, (S) - (5.95%), Eucalyptol, Trifluoroacetyl-.alpha.-terpineol (5.55%), 1, 6-Octadien-3-ol, 3, 7-dimethyl (2.99%), Ethanone (2.60%), Cyclopentadecane (1.98%), Camphene (1.90%) and Benzofuran (1.89%).

***Curcuma aurantiaca*-Leaves**

The analysis of essential oil from the leaves of *C. aurantiaca* detected the following compounds. Caryophyllene (31.07%), 7,11-dimethyl-3-methylene-(E)-(17.30%), Camphene(4.25%), Beta.-Pinene (2.18%), Phytol (11.32%), Benzofuran (9.06%)1,6,10-Dodecatrien-3-ol (6.65%), alpha-Caryophyllene(3.49%),n-Hexadecanoic acid (2.69%)1, 6, 10-Dodecatriene, Naphthalene (2.12%), Caryophyllene oxide (1.83%). The GC-MS profile of the *C.aurantiaca* essential oils are presented in Figs. 53-57.

***Curcuma aurantiaca*-Rhizome**

Analysis of *C. aurantiaca* rhizome detected the following compounds as depicted in the chromatogram (Fig.58-63). The compounds detected were alpha-

Pinene (2.13%), Cyclohexene, 1-methyl-4-(1-methylethylidene)- (20.87%), Caryophyllene (14.41%), Camphene (10.01%), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- (8.70%), Eucalyptol (6.54%), D-Limonene (6.70%), 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (4.08%), Benzene, 1-methyl-2-(1-methylethyl)- (3.81%), Naphthalene (2.65%), alpha-Phellandrene (2.33%), beta -Myrcene (2.20%) and , Caryophyllene oxide (1.74%) .

***Curcuma haritha*-Rhizome**

The GC-MS analysis of *C. haritha* rhizome yielded the following compounds. 2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol, Imidazol-4-one, Ethanone (34.51%), Benzofuran, 5-Benzofuranacetic acid, Benzene (12.48%), Camphor (8.25%), 3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)- (6.66%), Borneol, Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-,formate, endo- Isoborneol (6.65%), Camphene (6.39%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1.alpha.,2.beta.,4.beta.)]- (5.64%), Anthracene, Borinic acid, 1,4-Benzenediamine (2.55%), Trifluoroacetyl-.alpha.-terpineol (2.53%), 1,4-Naphthalenedione (2.43%), Eucalyptol , gamma.-Elemene (2.25%), alpha.-Pinene (2.17 %), Neocurdione (2.05%). The GC-MS profile of the essential oil is represented in Figs.64-68.

***Curcuma inodora*- Leaves**

The leaf essential oil analysis detected the presence of beta-Pinene (4.09%), Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl) (3.04%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (11.50%), Caryophyllene (31.33%), gamma.-Elemene (1.88%), alpha-Caryophyllene (5.97%), 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl) (4.37%), Benzofuran, 5-Benzofuranacetic acid, alpha.-methylene-, methyl ester (21.29%), gamma.-Elemene (5.18%) , alpha-Bisabolol (2.32%), beta-Elemenone (3.09%), 2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol , Ethanone (2.7%) and Phytol (3.19%). The GC-MS profile is shown in Figs. 69-72.

***Curcuma longa* –Rhizome**

Rhizome analysis of *C. longa* by GC-MS detected compounds such as, Curlone (16.83%), 2-Pyridinamine, 4-Pyridinamine, 4,6-dimethyl- (10.28%), Benzoic acid, Benzene, 1-methyl-2-(1-methylethyl)-(8.58%), alpha.-Phellandrene (6.90%), Eucalyptol (5.33%), Phenol (4.36%), beta-Elemenone (3.13%) m-Toluicacid (3.04%) and Benzofuran (2.19%). The major compounds and their chromatographic profile are represented (Figs.73-76).

Curcuma mutabilis – Leaves

Leaves of *C. mutabilis* showed the presence of 1H-1-Silaindene, 2,3-dihydro-1-methyl-1-propyl- (15.96%), 2,4,6-Cycloheptatrien-1-one 2-Pyridinamine, 4,6-dimethyl- (9.64%), Caryophyllene (8.12%) 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-(7.91%), Benzofuran, Phenol, 5-Benzofuranacetic acid (7.04%) alpha.-Caryophyllene (6.68%), Naphthalene (5.78%), alpha.-Farnesene (5.55%), beta.-Elemenone (5.40%), Phytol (4.91%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (3.41%), Ar-tumerone (3.29%), Tumerone (2.53%), 1(2H)-Naphthalenone (2.46%), beta.-Pinene (2.12%), Caryophyllene oxide, Geranylgeraniol (1.91%), gamma.-Elemene (1.88%), Nerolidol 2, 1,6,10-Dodecatrien-3-ol (1.81%) and Curlone, Benzene, 1-ethynyl-4-fluoro-, Benzoic acid (1.35%) in GC- MS analysis. The peaks and the obtained chromatogram are represented in Figs. 77-82.

***Curcuma raktakanta* – Leaves**

C. raktakanta essential oil from leaves subjected to GC-MS analysis detected Hydrazine, phenyl-Chloroacetic acid (28.55%), 2(Benzothiazol-2-ylamino)-3H-imidazol-4-ol (24.89%), Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1methylethenyl)-[1S-(1.alpha.,2.beta.,4.beta.)]- (12.23%), beta.-Elemenone(6.17%), Caryophyllene (4.66%), Benzofuran (4.04%), Eucalyptol (2.68%), Neoisolongifolene (2.26%), gamma.-Elemene (1.94%), Phytol (1.79%) and

Thiourea (1.67%) as major compounds. The chromatographic profile obtained is represented in Figs.-83-87.

***Curcuma raktakanta*-Rhizome**

Rhizome oil of *C.raktakanta* yielded, alpha-Pinene (1.20%), Camphene (3.27%), beta-Pinene (1.37%), alpha.-Phellandrene (1.23%), D-Limonene (1.75%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (5.91%), Caryophyllene (1.51%), gamma.-Elemene (1.94%), 1,6-Cyclodecadiene, 1-methyl-5-methylene-8- (2.32%), Naphthalene (0.91%), Benzofuran, Benzene, Hydrazine (15.32%), beta-Panasinsene (1.47%), beta-Elemenone (3.77%), Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl- (1.27%), Globulol (0.95%), 1,4-Benzenediamine, N-(4-methoxyphenyl)- (3.17%), 3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene (17.04%), 1(2H)Phenanthrenone (1.77%), 2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol, Ethanone (14.56%), Methyl alpha.-cyano-4-nitrocinnamate (1.90%), 7H-Furo[3,2-g][1]benzopyran-7-one , Ethanone (1.10%), 2,4-Dimethyl-6-methoxy-8-nitroquinoline (1.49%), Naphthalene, Phenol (2.58%), Phytol (1.37%) and (3-Methoxyphenyl)acetonitrile (1.66%) as major components from the essential oil as shown in the profile (Figs. 88-95).

Zingiber Sp.

The relative content of volatile compound indetified by GC-MS based metabolic profiling from the leaf and rhizomeof different *Zingiber* species are given in Table-17.

***Zingiber montanum*- Rhizome**

The major compounds detected in GC- MS analysis were alpha.-Pinene (4.80%), beta-Phellandrene (33.94%), beta-Pinene (3.91%), Bicyclo[3.1.0]hex-2ene,4methyl1(1methylethyl)- beta.Phellandrene (2.08%), 1,3Cyclohexadiene,1-methyl-4-(1-methylethyl)- (5.59%), Bicyclo[3.1.0]hex-2-ene,4-methyl- (10.18%), 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (10.23%), Cyclohexene,1-methyl-4-(1-

methylethylidene) (2.08%), Caryophyllene (2.05%), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- (2.25%), 1H-Cyclopropa[a]naphthalene (1.75%), 2-Isopropenyl-4a, 8-dimethyl-1,2,3 4,4a,5,6,7-octahydronaphthalene, Naphthalene (2.58%), Cyclohexene, 3-(1,5-dimethyl-4-hex enyl)-6-methylene-, [S-(R*,S*)]- (4.57%),1,2-Dimethyl-6-nitroindolizine4-Methyl-3-phenyl-1,2, (3.58%), Thiourea, 2-Propenoic acid, 5,6,7-Trimethoxy-1-indanone (2.59%), Benzoic acid and Triquinacene (7.80%). The chromatogram obtained from the analysis of the essential oil is represented in Figs. 96-100 .

***Zingiber nimmonii*- Rhizome**

The major compounds detected in GC-MS were: Bicyclo [3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, (1.44%), alpha-Pinene (2.31%), beta.-Phellandrene(5.10%), beta.-Myrcene (1.51%), alpha-Phellandrene (2.57%), 3-Carene (12.26%), 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (1.22%), Benzene, 1-methyl-2-(1-methylethyl)- (3.62%), D-Limonene (2.19%), Naphthalene (1.07%), 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (2.62%), 4-tert-Butylcyclohexyl acetate (1.06%), 4-tert-Butylcyclohexyl acetate (3.05%), Copaene, alpha-Cubebene (1.44%), 1H-3a,7-Methanoazulene, Benzenemethanol (1.47%), 2,3,4,7,8,8a-hexahydro-(2.82%), Caryophyllene (20.52%), 3-Buten-2-one (1.21%), Bicyclogermacrene (1.21%), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (9.33%), 1H-Cycloprop[e]azulen-7-ol (1.35%), Caryophyllene oxide (11.64%), Benzene, 1,2,4-tributyl-(1.52%) and Benzoic acid (3.37%). The GC-MS profile is presented in Figs.- 101-108.

***Zingiber officinale*- Rhizome**

Major compounds detected were Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (20.18%), Naphthalene (2.26%), 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-, Benzene (36.72%), Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) (11.50%), Nerolidol 2 (2.70%), Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (2.23%), Di-epi-.alpha.-cedrene-(I), 1H-3a,7-

Methanoazulene, 1H-Benzocycloheptene (2.01%), (.+/-.)-Lavandulol and pentafluoropropionate (2.97%). The chromatographic profile is represented in Figs.109-112.

Zingiber cernuum: **Rhizome**

The GC-MS analysis of the essential oil yielded, Beta caryophyllene (18.6%), terpinene- 4-ol (13%), caryophyllene oxide (5.6%), beta phyllandrene (5.4%) and alpha humulene (9.8%).

Zingiber neesanum: **Rhizome**

The chromatographic profile of the rhizome essential oil yielded, (E)-1-(3', 4'-dimethoxy phenyl) butadiene (31.1%), (E)-1-(3', dimethoxy phenyl) but-1-ene (23.1%), trans-ocimene (12.7%), beta-pinene (7.4%) and linalool (6.8%).

Zingiber roseum: **Rhizome**

The major compounds obtained from the GC-MS analysis were Caryophllene oxide (21.4%), humulene epoxide II (2.5%), alpha-humulene (7.1%) and beta-caryophyllene (4.5%).

Zingiber wightianum: **Rhizome**

GC-MS analysis of the rhizome essential oil yielded, Beta-eudesmol (14.5%), germacrene D (12.1%) and alpha-cardinol (7.4%).

Zingiber zerumbet: **Rhizome**

Rhizome eseential oil GC-MS analysis detected Zerumbone (62.1%), humulene epoxide I (7.4%), camphene (5.2%), alpha-humulene (3.3%) as major components.

Table-16.Relative content of volatile compounds identified by GC/MS based metabolic profiling of *Curcuma* species (C1- *C. aurantiaca* (Leaves), C2- *C.aeruginosa* (Rhizome), C3- *C.aurantiaca* (rhizome), C4- *C.longa* (Rhizome), C5- *C.aromatica* (Rhizome), C6- *C.raktakanta* (Leaves), C7- *C.haritha* (Rhizome), C8- *C.mutabilis* (Leaves), C9- *C.raktakanta* (Rhizome), C10- *C.amada* (Rhizome), C11-*C.inodora* (Leaves).

Components	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Camphene	2	2	3	0	2	0	3	0	2	0	0
Bicyclo[3.1.1]heptane, 6,6-dimethy l-2-methylene-, (1S)	2	2	0	0	0	0	0	0	0	0	0
Beta.-Pinene	2	0	0	0	0	0	0	2	2	1	2
Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-methylethenyl)	1	0	0	0	0	0	0	0	0	0	0
Caryophyllene	3	2	3	0	0	2	0	3	2	2	3
alpha.-Caryophyllene	2	0	2	0	0	0	0	3	0	0	3
1, 6, 10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	3	3	0	0	0	0	0	3	0	2	0
Benzofuran	3	2	0	2	2	0	3	3	3	3	3
5-Chloropentanoic acid	1	0	0	0	0	0	0	0	1	0	0
5-Benzofuranacetic acid	0	0	0	0	2	0	0	1	0	0	0
Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-	2	0	0	0	0	0	0	0	0	0	0
Naphthalene	2	0	2	0	0	0	0	3	1	3	0
1H-Cycloprop[e]azulene	2	0	0	0	0	0	0	0	0	0	0
1,6,10-Dodecatrien-3-ol	3	0	2	0	0	0	0	0	0	0	0
Nerolidol 2	1	0	1	0	0	0	0	1	0	0	0
Caryophyllene oxide	2	0	2	0	0	0	0	0	0	2	0
n-Hexadecanoic acid	2	0	0	0	0	0	0	0	0	0	0

Tetradecanoic acid	3	0	0	0	0	0	0	0	0	0	0
Phytol	0	0	0	0	0	2	2	2	2	2	0
Eucalyptol	0	3	3	3	3	2	2	0	0	0	0
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-	0	3	0	0	0	0	0	0	0	0	0
Camphor	0	0	0	0	0	0	3	0	0	0	0
Isoborneol	0	2	0	0	0	0	0	0	0	0	0
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	0	2	0	0	0	0	0	0	2	0	0
2-Pyridinamine, 4, 6-dimethyl-	0	3	0	0	0	0	0	0	0	0	0
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1, 7-trimethyl-4-methylene-, [1ar-	3.25(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha .)]-	0	0	1	0	0	1	0	0	0	0	0
Ar-tumerone	0	2	0	0	0	0	0	2	0	3	0
3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	0	2	0	0	0	0	0	0	0	0	0
Curlone	0	2	0	0	0	0	0	2	0	2	0
3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	0	2	0	3	0	0	0	0	0	0	0
Thiazole, 5-methyl-4-phenyl-	0	2	0	0	0	0	0	0	0	0	0
Cyclopentan-1-al, 4-isopropylidene-2-methyl-	0	2	0	0	0	0	0	0	0	0	0
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	0	2	0	0	0	0	0	0	0	0	0
2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol	0	2	0	0	0	0	0	0	0	0	0
Tricyclo[2.2.1.0(2,6)]heptane, 1,7 ,7-trimethyl-	0	0	2	0	0	0	0	0	0	0	0
1R-.alpha.-Pinene	0	0	0	0	0	0	2	0	0	0	0
1S-.alpha.-Pinene	0	0	2	0	0	0	0	0	2	0	0
beta.-Myrcene	0	0	2	0	0	0	0	0	0	2	0
alpha.-Phellandrene	0	0	2	0	0	0	0	0	2	2	0
Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)-	0	0	2	0	0	0	0	0	0	0	0
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0	0	2	0	0	0	0	0	0	0	0
Benzene, 1-methyl-2-(1-methylethyl) -	0	0	2	0	0	0	0	0	0	0	0
D-Limonene	0	0	3	0	0	0	0	0	2	0	0

Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0	0	3	0	0	0	0	0	0	0	0
Bicyclo[2.2.1]heptan-2-one, 1,7,7- trimethyl-, (1R)-	0	0	2	0	0	0	0	0	0	0	0
2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	0	0	2	0	0	0	0	0	0	0	0
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	0	0	2	0	0	0	0	0	0	0	0
alpha.-Phellandrene	0	0	0	3	0	0	0	0	0	0	0
Benzene, 1-methyl-2-(1-methylethyl)-	0	0	0	3	0	0	0	0	0	0	0
m-Toluic acid, tridec-2-ynyl ester	0	0	0	2	0	0	0	0	0	0	0
2-Pyridinamine, 4,6-dimethyl-	0	0	0	3	0	0	0	0	0	0	0
beta.-Elemenone	0	0	0	2	0	0	0	3	0	0	2
phenol	0	0	0	2	0	0	0	0	2	0	0
1,6-Octadien-3-ol, 3,7-dimethyl-	0	0	0	0	2	0	0	0	0	0	0
Bicyclo[2.2.1]heptan-2-one, 1,7,7- trimethyl-, (1R)-	0	0	0	0	3	0	0	0	0	0	0
Borneol	0	0	0	0	3	0	3	0	0	0	0
endo-Borneol	0	0	0	0	2	0	0	0	0	0	0
2-Cyclohexen-1-one, 2-methyl-5-(1- methylethenyl)-, (S)-	0	0	0	0	3	0	0	0	0	0	0
Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-	0	0	0	0	2	0	0	0	0	0	0
6-Tert.butyl-2,3-dicyanonaphthalen	0	0	0	0	2	0	0	0	0	0	0
Neocurdione	0	0	0	0	3	0	0	0	0	0	0
6,10-Dimethyl-3-(1-methylethyl)-6- cyclodecene-1,4-dione	0	0	0	0	3	0	0	0	0	0	0
2-(Benzothiazol-2-ylamino)-3H-imid azol-4-ol	0	0	0	0	2	0	0	0	0	0	0
Cyclopentadecane	0	0	0	0	2	0	0	0	0	0	0
Trifluoroacetyl-.alpha.-terpineol	0	0	0	0	0	2	0	0	0	0	0
Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)	0	0	0	0	0	3	0	0	0	0	0
gamma.-Elemene	0	0	0	0	0	2	2	2	3	2	3
1,6-Cyclodecadiene, 1-methyl-5-met hylene-8-(1-methylethyl)-,	0	0	0	0	0	2	0	0	0	0	0
Hydrazine, phenyl-Chloroacetic acid	0	0	0	0	0	3	0	0	0	0	0
Cyclohexane, 1-ethenyl-1-methyl-2- (1-methylethenyl)-4-	0	0	0	0	0	3	0	0	0	0	0

Cyclohexanone, 5-ethenyl-5-methyl- 4-(1-methylethenyl)-2-	0	0	0	0	0	2	0	0	0	0	0
Neoisolongifolene	0	0	0	0	0	2	0	0	0	0	0
3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene	0	0	0	0	0	2	0	0	0	0	0
1,4-Benzenediamine, N-(4-methoxyphenyl)-	0	0	0	0	0	2	0	0	0	0	0
2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol	0	0	0	0	0	3	0	0	0	0	0
Thiourea, 1-(2,4,6-trimethylphenyl)-3-(2-propynyl)-	0	0	0	0	0	2	0	0	0	0	0
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	0	0	0	0	0	0	3	0	0	0	0
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-,	0	0	0	0	0	0	2	0	0	0	0
3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	0	0	0	0	0	0	3	0	0	0	0
Neocurdione	0	0	0	0	0	0	2	0	0	0	0
Anthracene	0	0	0	0	0	0	2	0	0	0	0
2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol	0	0	0	0	0	0	0	0	0	1	0
Imidazol-4-one	0	0	0	0	0	0	3	0	0	0	0
2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one	0	0	0	0	0	0	2	0	0	0	0
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	0	0	0	0	0	0	0	2	0	0	0
alpha.-Farnesene	0	0	0	0	0	0	0	3	0	0	0
Nerolidol 2	0	0	0	0	0	0	0	2	0	0	0
Geranylgeraniol	0	0	0	0	0	0	0	2	0	0	0
2,4,6-Cycloheptatrien-1-one	0	0	0	0	0	0	0	3	0	0	0
Cyclohexanecarboxylic acid	0	0	0	0	0	0	0	2	0	0	0
Fumaric acid	0	0	0	0	0	0	0	1	0	0	0
1(2H)-Naphthalenone, 3,4,5,6,7,8-hexahydro	0	0	0	0	0	0	0	2	0	0	0
3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)-	0	0	0	0	0	0	0	2	0	0	0
2-Acetoxy-4-phenylhex-2-en-5-one(3-Methoxyphenyl)	0	0	0	0	0	0	0	0	0	0	0
acetonitrile	1	0	0	0	0	1	0	0	0	0	0
1H-1-Silaindene, 2,3-dihydro-1-methyl-1-propyl-	0	0	0	0	0	0	0	3	0	0	0
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0	0	0	0	0	0	0	0	1	0	0

Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-	0	0	0	0	0	0	0	0	2	0	0
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,	0	0	0	0	0	0	0	0	3	0	0
1,4-Methanoazulene	0	0	0	0	0	0	0	0	2	0	0
beta.-Panasinsene	0	0	0	0	0	0	0	0	0	2	0
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-	0	0	0	0	0	0	0	0	2	0	0
Cyclohexanone	0	0	0	0	0	0	0	0	2	0	0
Globulol	0	0	0	0	0	0	0	0	1	0	0
3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene	0	0	0	0	0	0	0	0	3	0	0
4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl-3,3'-diene	0	0	0	0	0	0	0	0	2	0	0
1,4-Benzenediamine, N-(4-methoxyphenyl)-	0	0	0	0	0	0	0	0	2	0	0
1(2H)Phenanthrenone	0	0	0	0	0	0	0	1	0	1	0
Benzene, 1-methoxy-4-(4-methylphenoxy)-	0	0	0	0	0	0	0	0	2	0	0
2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol	0	0	0	0	0	0	0	0	0	0	1
Ethanone	0	0	0	0	0	0	0	0	3	0	0
Methyl .alpha.-cyano-4-nitrocinnamate	0	0	0	0	0	0	0	0	2	0	0
7H-Furo[3,2-g][1]benzopyran-7-one	0	0	0	0	0	0	0	0	2	0	0
Anthracene	0	0	0	1	0	0	0	0	0	0	0
2,4-Dimethyl-6-methoxy-8-nitroquinoline	0	0	0	0	0	0	0	0	2	0	0
(3-Methoxyphenyl)acetonitrile	0	0	0	0	0	0	0	0	2	0	0
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	0	0	0	0	0	0	0	0	0	3	0
4,5,6,6a-Tetrahydro-2(1H)-pentalen one	0	0	0	0	0	0	0	0	0	3	0
4,5,6,6a-Tetrahydro-2(1H)-pentalen one	2	0	0	0	0	0	0	0	0	0	0
6-Hydroxynicotinic acid	0	0	0	0	0	0	0	0	0	2	0
Tumerone	0	0	0	0	0	1	2	0	0	0	0
Benzeneethanamine	0	0	0	0	0	0	0	0	0	2	0
Phenol, o-(o-methoxyphenoxy)-	0	0	0	0	0	0	0	0	0	2	0
3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-	0	0	0	0	0	0	0	0	0	2	0

4-Toluenesulfonylmethylisocyanide	0	0	0	0	0	0	0	0	0	2	0
1,4-Dimethyl-2-cyclopentylbenzene	0	0	0	0	0	0	0	0	0	2	0
1(2H)-Naphthalenone, 3,4,5,6,7,8-h	0	0	0	0	0	0	0	0	0	0	0
exahydro-	0	0	1	0	0	0	0	2	0	0	0
2(3H)-Naphthalenone	0	0	0	0	0	0	0	0	0	2	0
Propane-1,2,3-triol	0	0	0	0	0	0	0	0	0	2	0
1(2H)-Naphthalenone	0	0	0	0	0	0	0	0	0	3	0
Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)	0	0	0	0	0	0	0	0	0	0	2
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,	0	0	0	0	0	0	0	0	0	0	3
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	0	0	0	0	0	0	0	0	0	0	2
alpha.-Bisabolol	0	0	0	0	0	0	0	0	0	0	2
2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol	0	0	0	0	0	0	0	0	0	0	2

Note: 1 indicates <0.5%, 2 indicates 0.5%-5% and 3 indicates > 5% of total integrated peak area of total ion chromatogram (TIC) of a particular sample.

Table-17.Relative content of volatile compounds identified by GC/MS based metabolic profiling of *Zingiber* species (Z1- *Z. nimmonii* (Rhizome), Z2- *C.officinale* (Rhizome), Z3- *C. montanum* (rhizome), Z4- *Z.cernuum* (Rhizome), Z5- *Z.neesanum* (Rhizome), Z6- *Z.roseum* (Rhizome), Z7- *Z.wightianum* (Rhizome), Z8- *Z.zerumbet* (Rhizome).

	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-,	2	0	0	0	0	0	0	0
1R-.alpha.-Pinene	0	1	1	0	1	0	0	0
1S-.alpha.-Pinene	2	0	2	0	0	0	0	0
beta.-Phellandrene	3	0	3	3	0	0	0	0
beta.-Myrcene	2	0	0	0	0	0	0	0
alpha.-Phellandrene	2	1	0	1	0	0	0	0
Cyclopentene, 3-isopropenyl-5,5-dimethyl-	2	0	0	0	0	0	0	0
3-Carene	3	0	0	0	0	0	0	2
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	2	0	0	1	0	0	0	0
Benzene, 1-methyl-2-(1-methylethyl)-	2	0	0	0	0	0	0	0
D-Limonene	2	0	0	0	0	0	0	2
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	2	0	0	0	0	0	1	0
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	2	0	0	0	0	0	0	0
4-tert-Butylcyclohexyl acetate	1	0	0	0	0	0	0	0
p-tert-Butyl cyclohexyl-acetate ci	2	0	0	0	0	0	0	0
4-tert-Butylcyclohexyl acetate	0	0	0	0	0	0	0	0
p-tert-Butyl cyclohexyl-acetate trans	2	0	0	0	0	0	0	0
Copaene	0	1	0	0	0	0	0	0
alpha.-Cubebene	2	0	0	0	0	0	0	0
1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-	0	0	1	0	0	0	0	0

3,6,8,8-tetramethyl-,	2	0	0	0	0	0	0	0
beta Caryophyllene	3	0	2	3	0	2	0	0
3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-,	2	0	0	0	0	0	0	0
Bicyclogermacrene	2	0	2	1	0	0	0	0
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-	2	0	2	0	0	0	0	0
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-	2	0	0	0	0	1	0	0
Benzenemethanol, .alpha.-(trichloromethyl)-, acetate	2	0	0	0	0	0	0	0
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	2	0	0	0	0	0	0	0
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,	2	0	0	0	1	0	0	0
Caryophyllene oxide	2	0	0	3	0	3	0	0
Benzene, 1,2,4-tributyl-	2	0	0	0	0	1	0	0
Benzoic acid, 2-hydroxy-, phenylmethyl ester	2	0	0	0	0	0	0	0
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	0	3	0	0	0	0	0	0
Naphthalene, decahydro-4a-methyl-1-methylene-7-	0	2	0	0	0	0	0	0
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-	0	3	0	0	0	0	0	0
Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	0	3	0	0	0	0	0	0
Nerolidol 2	0	2	0	0	0	0	0	0
Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-,	0	2	0	0	0	0	0	0
Di-epi-.alpha.-cedrene-(I)	0	0	1	0	0	0	0	0
1H-3a,7-Methanoazulene	0	2	0	0	0	0	0	0
(.+/-.)-Lavandulol, pentafluoropropionate	0	2	0	0	1	0	0	0
beta.-Pinene	0	0	2	0	3	0	0	0
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	0	0	2	0	0	0	0	0
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0	0	3	0	0	0	1	0
Bicyclo[3.1.0]hex-2-ene, 4-methyl-	0	0	3	0	0	0	1	0
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0	0	3	0	0	0	1	0
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0	0	2	0	0	0	0	0

3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	0	0	2	0	0	0	0	0
Cyclohexene, 3-(1,5-dimethyl-4-hex	1	0	0	0	0	0	0	0
enyl)-6-methylene-, [S-(R*,S*)]-	0	0	2	0	0	0	0	0
1,2-Dimethyl-6-nitroindolizine4-Methyl-3-phenyl-1,2,	0	0	2	0	0	0	0	0
Thiourea	0	2	0	0	0	0	0	0
2-Propenoic acid	0	0	2	0	0	0	0	0
Benzoic acid	0	0	3	0	0	0	0	0
alpha-humulene	0	0	0	3	0	3	0	0
linalool	0	0	0	0	3	0	0	2
(E)-1-(3',4'-dimethoxyphenyl)butadene	0	0	0	0	3	0	0	0
(E)-1-(3',4'-dimethoxyphenyl)but-1-ene	0	0	0	0	3	0	0	0
trans ocimene	0	0	0	0	3	0	0	0
humulene epoxide 11	0	0	0	0	0	3	0	0
Beta-eudesmol	0	0	0	0	0	0	3	0
germacrene D	0	0	0	0	0	0	3	0
alpha-cardinol	0	0	0	0	0	0	3	0
Zerumbone	0	0	0	0	0	0	0	3
humulene epoxide I	0	0	0	0	0	0	0	3
camphene	0	0	0	0	0	0	0	2

Note: 1 indicates <0.5%, 2 indicates 0.5%-5% and 3 indicates > 5% of total integrated peak area of total ion chromatogram (TIC) of a particular sample.

Based on the presence/absence of essential oil components from rhizome and leaf of *Curcuma* and *Zingiber* a dichotomous key was developed for classifying the species.

Curcuma

1. Nerolidol, D-limonene present ..... *C. aurantiaca*
1. Nerolidol, D-limonene absent 2
2. Ethanone and Cyclopentadrene present..... *C. aromatica*
2. Ethanone and Cyclopentadrene absent ..... *C. haritha*
3. β- myrcene, Naphthalenone found commonly .....*C. amada*
3. β- myrcene, Naphthalenone not found commonly..... 4
4. Azulene, Globulol, Imidazole present..... *C. raktakanta*
4. Azulene, Globulol, Imidazole absent .....*C. aeruginosa*
5. α-phellandrene, m-Toluinic acid and Curlone present.....*C. longa*
5. α-phellandrene, m-Toluinic acid and Curlone absent 6
6. Ar-tumerone, Tumerone, α-farnesene and Fumaric acid detected .... *C. mutabilis*
6. Ar-tumerone, Tumerone, α-farnesene and Fumaric acid not detected .... .C. *inodora*

Zingiber

1. Zerumbone detected as major compound .....*Z. zerumbet*
1. Zerumbone not detected 2
2. Nerolidol-2, Di-epi-α-cedrene and Lavandulol present..... *Z. officinale*
2. Nerolidol-2, Di-epi-α-cedrene and Lavandulol absent 3
3. β-caryophyllene, Caryophyllene oxide, β-phyllandrene present 4
3. β-caryophyllene, Caryophyllene oxide, β-phyllandrene absent..... 5
4. D-limonene and β-myrcene present .....*Z.nimmonii*
4. D-limonene and β-myrcene absent.....*Z.cernuum*
5. Humulene epoxide, α-humulene and β-caryophyllene present .....*Z. roseum*
5. Humulene epoxide, α-humulene and β-caryophyllene absent… ..... *Z.wightianum*

DISCUSSION

The family Zingiberaceae is generally considered as spice family since they include many spices such as cardamom, large cardamom, turmeric, ginger etc. They are widely used as traditional food, ornamental and has cultural, ritual and medicinal properties. This family is the largest in the order Zingiberales, consists of approximately 52 genera and 1,200 species and readily differentiated from other families in the order by their aromatic property. Their distribution is pantropical but concentrated in the Old World, especially in Southeast Asia. India has rich diversity of gingers with about 200 species belonging to 21 genera. In India they are mostly confined to Northeast India, South India and Andaman and Nicobar Islands (Kress *et al*., 2002). Because of the ephemeral flowers, taxonomic study of the family is difficult and a classification is still incomplete. The family has been variously divided into a number of tribes. Burtt and Smith (1972) recognized four tribes: Hedychieae, Zingibereae, Alpineae, and Globbeae. The fusion of the lateral staminodes of the inner staminal whorl into a labellum, the presence of two epigynous nectariferous glands at the base of the style, and the occurrence of cells containing essential or ethereal oils are autapomorphies of the family. Other floral characters normally associated with the zingiberaceae, are the presence of a single fertile tetrasporangiate anther and the slender style, which lies between the two pollen sacs, are derived characters shared with the Costaceae (Kress, 1990).

***CURCUMA* L.**

The genus *Curcuma* L., with around 120 species distributed in tropical and subtropical Asia, consists of a rather homogenous group of rhizomatous perennials.In India about 29 species, distributed almost all states. The genus can be easily recognized by its inflorescence, a spike with prominent spiral bracts, which laterally fuse or adnate to the peduncle and form pouches, each subtending a cincinnus of flowers, and a cluster of, often coloured, sterile, terminal bracts called

'coma'. The genus however is a taxonomically difficult one. It is a nightmare for plant hunters, herbarium technicians as well as taxonomists. Their occurrence as undergrowths in remote inaccessible forest areas and the extremely short period of flowering, hinders collection of materials with adequate character details. With their large size, fleshiness of rhizomes, tubers etc., they are difficult material for herbarium preparation. Consequently, most herbarium specimens are fragmentary and the treatments in most of the Floras, based on these dried specimens, are truncated accounts (Skornickova *et al*., 2004).

During this work, the area of study was extensively explored and detailed field notes were carefully made. The collected specimens were dissected and studied. Rhizomes were collected as planting materials for raising plants for further observation. To ensure the correct identification specimens from various herbaria in India were consulted.

Earlier taxonomists have attempted subgeneric classification of this genus. Roxburgh (1820) divided the genus into two sections, depending on lateral or central spikes while Horaninow (1862) distinguished 3 sections namely, *Exantha* (spikes always lateral), *Mesantha* (spikes invariably terminal) and *Amphiantha* (spikes both terminal and lateral). Baker (1890) accepted sections *Exantha* and *Mesantha* while rejecting section *Amphiantha* and introduced a new section *Hitcheniopsis*, which differed from the rest of the genus in its spurless anthers. Schumann (1904) rejected the sectional classification based on spike position but recognized two subgenera [Subgen. *Eucurcuma* and Subgene. *Hitcheniopsis* (Baker) K. Schum.] based on the presence or absence of spur on anthers. *C. aurantiaca* Zijp. (*C. ecalcarata* Sivar. and Indu.) the only South Indian species of the subgen. *Hitcheniopsis* having spurless anthers, the specific epithet 'ecalcarata' refers to its lack of spur on anthers. Jayasree (2007) studied the anatomy of South Indian *Curcuma* and proved the subgeneric classification into *Eucurcuma* and *Hitcheniopsis* based on the presence or absence of anther spur does not coincide with the occurrence of any particular anatomical characters.

However, authors of most Floras have continued to rely upon questionable characters like position of spikes, presence or absence of root tubers and the colour of coma for the identification of species in the genus *Curcuma.* Roxburgh (1810) had already pointed out that the positional difference of spikes is a matter of flowering season, the early spikes being lateral and later ones terminal. Santapau (1945, 1952) added that in *C.pseudomontana* at the beginning of the rainy season the plant has a large spike coming out from the side of the leaves. Gradually by beginning of August, this lateral spike decays and the central one appears surrounded by leaves, resulting in both central and lateral spikes in the same plant.

C. aurantiaca shows a high range of variation in floral characters, which gives a highly appreciable position to this species. The plant is medium sized with small rhizomes and tubers. Inflorescence is central in position and the main attraction is the incredibly colourful nature of the inflorescence. The bract colour ranges from green, white, brown, purple to rose. Its specific epithet comes from the unusual striking colour of the flowers.

The main attraction of the plant *C.inodora* is its beautiful and attractive spike. The inflorescence with variously coloured and shaped comma bracts, and labellum shows range of colours from dark purple, white, yellow and golden. The plant produces two inflorescence in a year; one lateral and one central. It is dormant during summer; starts sprouting by the end of April and fresh leaves appear. Hence named as hidden purple ginger. The species is closely related to *C.decipiens* Dalzell, and can be distinguished from it in the flowers equaling the bracts, 3-4 flowered cincinni, purple corolla and staminodes and labellum with a dark yellow band at the centre.

Identification of the species to some extent is possible by qualitative observation of leaf and rhizome shape, colour, size etc. Leaf length and breadth highest in *C. zanthorriza* followed by *C. aromatica, C. aeruginosa, C. longa, C.amada, C.coriacea, C.haritha, C.raktakanta* and *C.pseudomontana*. In *C.*

zanthorriza, purple colour is present throughout the midrib of the leaf. In *C. aeruginosa* the purple colour is present only towards the upper half. *C.raktakanta* closely resembles *C.aeruginosa* but differs from it in the yellowish to grey colour of the rhizomes instead of blue colour in the centre, purple coloured pseudostem and peduncle and absence of purple patch on leaves. *C. amada* closely related to *C.longa* but the characteristic smell and pale yellow colour of the rhizome, pale yellow flowers and light violet coma displays some degree of differentiation. The characteristic camphor smell of rhizome and densely haired lower surface of the leaf are differentiating characters of *C.aromatica*. *C.coriacea* is endemic to Kerala, this having coriaceous dense pubescent leaves, lateral inflorescence and bright yellow corolla and *C. decipiens* can be distinguished from other species of *Curcuma* by the deep purple corolla and coma, 2 flowered cincinni. From its close ally*, C.inodora* Blatter, *C. decipiens* differs in the flowers being longer than the bracts, 2-3 flowered cincinni, labellum purple with deep yellow band.

C.vamana produces the smallest flowers in the genus *Curcuma* reported so far from India. This plant having subequal leaf base, much shorter, condensed spike and bracts, few flowered cincinni, absence of anther crest and presence of spurs on fertile anther. This plant closely resembles *C.burtii*, but differs from other Indian species.

C.karnatakensis closely resembles *C.oligantha* but differs from it in the large size of the plants and large white flowers with a median yellow band (Amalraj *et al*., 1999).

C.oligantha was described as a new species under the name *C.cannanorensis* by Ansari *et al.* (1982). This plant is closely allied to *C.albiflora* Thw., but differs from it in the smaller size of leaves with acuminate and apiculate apex; the petiole always shorter than lamina, shorter spike and larger size of flowers. It is also related to *C.neilgherrensis* but differs from it mainly in the absence of a coma and smaller bracteoles. Subsequently, Bhat (1987) studied this

species in detail and identified as *C.oligantha*. Velayudhan *et al*. (1991) retained the specific status of *C.cannanorensis* without seeing the type and protologue of *C.oligantha*. As this is baseless and without any substantial proof this proposal was rejected.

C.mutabilis is one of the most interesting species due to the variations in colour of flowers. Corolla can vary from whitish pink, pink-red, reddish orange, dark pink to dark violet, labellum and lateral staminodes can be found in pure white colour, white with yellow or reddish streaks in the throat of labellum or base of the lateral staminodes, different shades ranging from creamy, light yellow to deep yellow colour. Since there is no correlation between colour of corolla and staminodes, combination of these two characters makes wide range and infact almost every individual possess slightly different look. Velayudhan *et al.* (1999) had described this species as *C.nilamburensis* based on a collection from Nilambur, Kerala. Unfortunately, the same is not validly published according to the St. Louis code (Greuter *et al.,*2000) because the description lacked a Latin diagnosis and a type was not designated. In addition, the publication by Velayudhan *et al*. (1999) is of limited circulation. Hence, the species was validly published under *C.mutabilis* (Skornickova *et al.,* 2004).

C.haritha is closely related to *C.aromatica* Salisb., but differs from it in the yellowish-grey, non-aromatic rhizome, leathery, semiplicate, erect leaves, white corolla, and light yellow lip with a median dark yellow band. It also resembled *C.raktakanta* but differs from it in having green pseudostem with light pink spots, white corolla lobes and swollen placenta.

Out of the 19 species studied *C.bhatii* shows some variations from all other species under study. *C.bhatii* was originally described under another genus *Paracautleya*in 1977 by R. M. Smith. Skornickova and Sabu (2005) found the characters were not sufficient to keep it under another genus so they merged *Paracautleya bhatii* R. M. Smith into *Curcuma bhatii* based on the type specimen

collected by K.G. Bhat from Udupi, Karnataka, South India. The genus *Paracautleya* was established by Smith (1977) based on the same specimen. She noted the affinity of *Paracautleya* with *Curcuma*, but pointed out that *Curcuma* flowers are borne in cincinni within pouches formed by adnate bracts. She confirmed the close affinity of *Paracautleya* with *Cautleya* and *Roscoea* mainly based on the singly borne ebracteolate flowers and leafy stems. However, she assigned generic status to this taxon mainly because of stemless habit, elongated naked peduncle and ovules attached at the base of the ovary. Anatomical and molecular studies revealed that this species shows some differences from other South Indian species. Hence, this generic delimitation considered as imperfect.

***ZINGIBER* BOEHM.**

The genus is represented by 141 species, distributed mainly in tropical areas in dense forests, open grass lands at high altitudes and in plains at lower elevations in regions like, Malaysia, Queensland, Japan, East Indies, Java, New Guinea, Thailand, Kampuchea, Cambodia, Laos, Philippines, China, Srilanka, India etc.

Zingiber is distinct from other genera of the family in the presence of a single anther with a beak or horn-like appendage, which embraces the upper part of the style. The inflorescence usually arises at the base of the leafy stem, on a long or subterranean peduncle. Rarely terminal inflorescence is also present. The bracts are overlapping and each subtends a non-tubular bracteole and a single flower. In many species the bracts are green when young, turning to red in the fruiting stage. The genus can be recognized in the vegetative stage by the presence of a pulvinous between the base of the petiole and ligule. The genus *Zingiber* is one of the most difficult material of Zingiberaceae to collect satisfactorily. Only one or two flowers open at a time and it is difficult to detach them from the bract without damage. Field notes such as the nature of the peduncle, the colour of the bracts at different stages of the inflorescence, life cycle and colour of the labellum are very important as these characters are rarely preserved in the herbarium.

` The vegetative stage of *Amomum* and *Zingiber* are very similar, but can be distinguished. The former has hard and woody leafy shoots (pseudostem) while the latter has a fleshy leafy shoot. In *Zingiber*, a swollen area or pulvinous is present at the base of the petiole. Anatomically this is formed by the collenchymatous thickening of the cells of the vascular bundle sheaths (Tomlinson, 1956). Where as in other zingiberaceous genera the bundle sheaths are sclerenchymatous.

Sharma *et al.,* (1984) reported *Z.capitatum* Roxb. var. *elatum* from Karnataka. This species is so far known only from central and Eastern Himalaya. There are no authentic specimens of this species collected from Karnataka in any herbaria in India. The identity of some herbarium specimens labeled as *Z.capitatum* needs confirmation. We collected *Z.capitatum* from Karnataka for this study. Roxburgh (1820) treated this as an independent species, whereas Baker (1892) treated it only as a variety of *Z.capitatum*. Horaninow (1862) placed this under a new genus *Dymczewiczia*. Subsequently, Baker (1892) raised this genus to the status of a section *Dymczewiczia* of the genus *Zingiber*. Schumann (1904) did not even consider *Z.elatum* neither as a variety nor as a species. Verma et al., (1991) confirmed Baker's view, *i.e*. varietal status of *Z.elatum* based on flavanoid spot pattern characters.

Zingiber cernuum was first described by Dalzell in Hookers Kew Journal of Botany in 1852 along with *Z.nimmonii*. Subsequently, Dalzell and Gibson (1861), Baker (1892), Schumann (1904) and Cooke (1907) followed the same treatment. Santapau (1967) treated both of them as conspecific and placed it under *Z.cernuum*. While Ramamoorthy (1976) officially transferred *Z.nimmonii* to *Z.cernuum*. Sabu (2003, 2006) based on the rule of priority (article 11. 4-ICBN. Mc Neill *et al*. 2006) treated *Z.nimmonii* as valid name and the *Z.cernuum* as synonym. Vasantha (2009) studied the specimens from South India using morphological, phytochemical, anatomical and palynological characters and reported these two species shows some difference. During present study we have studied in detail a large number of specimens and found that both specimens have many overlapping

morphological characters and slight differences in the case of leaf pubescence, flower colour etc.

Z.montanum resembles *Z.zerumbet* in yellow flowers with crisped margins but can be distinguished from the latter by the narrow leaves and stout bifid ligule. It also resembles *Z.officinale* in the vegetative stage, but differs from it in the presence of yellow with purple spotted labellum.

Z.roseum closely resembles *Z.wightianum* but differ in long bifid ligule, small white labellum with yellow markings. Haines (1961) suggested that *Z.roseum* may be a form of *Z.rubens* with a more robust inflorescence but treated them as distinct species. Later Babu (1977) suggested that they were closely related and differ only in the colour of the bracts and purple streaked lip. Jha and Varma (1995) studied this species and concluded that these two are distinct species. In the present study it was revealed that *Z.roseum* has distinct characters such as oblong, cuneate, recurved, labellum white at the centre and yellow with red spots towards margin. Anatomical studies by Jayasree (2007) revealed that it is very distinct from *Z.wightianum* with thick and fibrous rhizome, which is white inside and abaxial side of midrib V-shaped.

Molecular markers are used as an efficient tool to screen the species and at genetic level by using specific techniques like RAPD, SSRs, Microsatellites etc. These markers are not affected by the environmental factors (Peredo *et al*., 2009). DNA based markers become the markers of choice for studying the crop genetic diversity and tremendously affected the understanding of molecular basis of evolution of large number of plant groups. To have more precise understanding, techniques were developed which will give a clear cut understanding about the plant species by using different methods. A single molecular approach was never a solution to solve the problems of phylogenic analysis of wide groups of plants, but recent approach was to complement each other by combining different methods.

Some techniques are more appropriate than others for some specific applications like crop diversity and taxonomic studies.

Due to the rapid developments in the field of molecular genetics, varieties of different techniques have emerged to analyze genetic variations during the last few decades. Most of the genetic markers differ from each other in important aspects like genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirement and financial investment. No marker is superior over the others for a wide range of applications. Most appropriate genetic marker depends upon a specific application, the presumed level of polymorphism the marker can exhibit, in conjunction with the presence of sufficient technical facilities and know how, time constraints and financial limitations.

The importance of genetic variations in facilitating plant breeding and/or conservation strategies has long been recognized (Sehgal and Raina, 2008). Majority of the molecular markers have been developed either from genomic DNA library (eg. RFLPs or SSRs) or from random PCR amplification of genomic DNA (RAPDs), AFLPs or both (Varshney *et al*., 2007). Availability of large number of molecular markers and their modifications led to comparative studies among them in many crops including soya bean, wheat and barley (Powell *et al*., 1996, Russell *et al*., 1997; Bohn *et al*., 1999).

Among the different markers used RAPDs and SSRs are used routinely to detect the genetic variability among different Zingiberaceous plants including *Curcuma* and *Zingiber* (Syamkumar and Sasikumar, 2007). SSRs detect a high level of polymorphism, co-dominant, where as RAPD is a dominant marker and have a low-moderate polymorphism. Microsatellites (Litt and Luty, 1989) are also known as Short tandem repeats (STRs, Edwards *et al*., 1991) Simple sequence repeats (SSRs, Jacob *et al*., 1991) or Simple sequence length polymorphism (SSLP, Tautz, 1989) are tandem repeat motifs of 1-6 bp long which have a frequent occurrence in all prokaryotic and eukaryotic genomes (Zane *et al* ., 2002). Plants are rich in AT

repeats, where as in animals AC repeats are most common, and this can be used as distinguishing marker for the identification of plants from animal genome.

SSRs are present in the coding and non coding regions and are distributed throughout the nuclear genome. They are also reported from the chloroplast and mitochondrial genomes (Chung *et al*., 2006; Rajendrakumar *et al*., 2007). SSRs are characterized by a low degree of repetition/locus (5-100) random dispersed distribution of about (10^4-10^5) per genome (Tautz, 1993), and high degree of length polymorphism (Zane *et al* ., 2002). Due to the presence of high degree length polymorphism they can be easily detected and reproduced by using polymerase chain reaction. SSRs are classified as mono-hexa nucleotides based on the arrangement of nucleotides in the repeats motifs (Weber, 1990; Wang *et al*., 2009a).

RAPD is the enzymatic amplification of the target or random DNA sequences with arbitrary primers. The essential and important components of the reaction are quality and quantity of DNA, which should be free from salts and other impurities and should be available in adequate quantities.

For the extraction of DNA from *Curcuma* species taken for the study a modified CTAB method of Ausubel *et al*., (1995) was used. The material was swabbed with 70% ethanol to remove contaminants present on the leaf surface. The midrib and prominent veins of the leaves are removed so as to get maximum leaf tissue to extract good quality DNA. Younger leaves from the upper surface of the plants were found to be ideal for the extraction of good quality of DNA. A similar observation on the extraction of DNA was observed in other plant species (Yusuf *et al*., 2012). Optimization in the salt concentration and the organic components of the extraction buffer was critical in obtaining pure, dissolvable and stable DNA from the leaf samples. The organic salt quality and quantity affected the quality and quantity of DNA extracted from the leaf samples.

For RAPD PCR the annealing temperature selected for the primers, Mg^{2+}concentration and concentration of DNA play a critical role in obtaining reproducible banding pattern for all the species taken for the study. Of the 42 RAPD primers used 20 produced polymorphic bands showing the wide genetic basis of the *Curcuma* species selected for the study. The level of polymorphism detected in a cultivated species is dependent on a wide range of factors like breeding system, habitat specialization, intensity and direction of selection and type of genetic material used. The high polymorphism detected in this study displayed the resolving power of the RAPD and ISSR markers selected for genetic diversity analysis. Aptness of RAPD markers for determining the genetic diversity of Zingiberaceous plants are reported earlier (Rout *et al*., 1998; Palai and Rout, 2007). RAPD analysis of *Curcuma* with the primers which produced polymorphic bands yielded a total of 1025 bands with high resolving power.

The bands present/absent during RAPD/ISSR in each species were computed and converted into presence and absence binomial data. These binomials were used for overall similarity-dissimilarity assessment of each species and Jaccard's similarity indices (JSI) were calculated for each species. The JSI between and among each species are measures of their similarity coefficients and the nearest neighbour's can be joined together. This distance measures are based on nucleotide substitution (Nei and Kumar, 2000).Similarity- dissimilarity indices will form the initial grouping among the species and will discriminate the nearest similar to the farthest neighbour joining (N.J.) can be used as data sets of several hundred taxa to find tree, branch lengths and support (Susuki *et al*., 2002). In the present study with all the species of *Curcuma* the highest JSI was observed in the comparison of *C.pseudomontana* with C. *raktakanta*. In support to this under the boot strap method these two species gave a boot strap value of 82% corresponding to true clades in experimental phylogeny (Hillis and Bull, 1993).

The highest JSI was observed for *C. karnatakensis* and *C. mutabilis* indicating that this two species have the maximum closeness in the RAPD and ISSR banding

pattern. The geographical existance of this two species *ie*. *C. karnatakensis* is reported from Uttar kannad districts of Karnataka and *C. mutabilis* was collected from Nilambur of Malappuram District. However, the genetic similarity between these two species suggests that the primers used for our studies showed a common banding pattern for this two species. This suggests that the genetic polymorphism exhibited by the two species may be due to the environmental impacts as suggested by Das *et al*. (2011). *C. amada* shared Jaccard's similarity coefficient of 28% with the *C. neilgherrensis*/*C. oligantha* cluster suggesting that *C. amada* has divergent character from the other members of this cluster. *C. amada* in its morphological and biochemical features stands as a separate species with specific aromatic 'mango' flavour and specific secondary metabolites forms a specific branch in cluster II A. Thus the five species which are geographically very near and taxonomically grouped at similar level showed similarities with the members of the same group. *C. amada* showed its distinctiveness from the others in the group as described before (Syamkumar and Sasikumar, 2007). Cluster II B formed with two sub groups, the first with 6 species and the second with 7 species. The two sub clusters showed 27% Jaccard's similarity among themselves. *C. aromatica* and *C. haritha* shows a common ecological niche in the mid land and base of Western Ghats of India. Eventhough *C. aromatica* is a seed setting species (George, 1981) *C. haritha* and *C. aromatica* have the same somatic chromosome number 2n = 42 (Joseph *et al*., 1999). The geographical niche of the two species may also affect the profile obtained in RAPD analysis. The group formed together by *C. aeruginosa* and *C. zanthorrizha* have 34.7% similarity, have the implication on the genetic constitution of the species. Both the species have 2n = 63, 64 chromosomes and this ploidy level may be the basis for existance of the two species in the same group. The pairing of *C. aeruginosa* with *C. zanthorrizha* is very interesting as they show a 34% similarity between them.

The most cultivated species of *Curcuma*, *C. longa* is grouped along with *C. coriacea* and both of them showed 33% similarity with each other. The ecological

niche of this two species are identical and this may be contributing to the identical banding pattern and similarity of the two species.

Cluster II B formed with 7 species which shared similarity up to 27%. The species *C. montana* and *C. pseudomontana* shared equal distance among themselves. They were treated as two different species by earlier taxonomic investigations (Sabu and Mangaly, 1996). However, they showed the maximum JSI (40%) among the species taken for the study and can be assumed as synonyms. The *montana*/*pseudomontana* complex have a similarity index of 32% with *C. raktakanta*.

C. decipiens and *C. inodora* showed 38% similarity between the two species and the taxonomic classification treated them as two separate species. *C. aurantiaca* showed a JSI of 34-38% between the species is indicative of its relatedness with the other species geographically located in the same ecological niche. Of all the species *C. bhatii* stands alone, without exhibiting much similarity with all the other species. In earlier reports on taxonomic classification this species was treated as a separate genera, *Paracautleya* (Smith, 1977), however, studies by Skornickova and Sabu (2005) shown that depending on the bracts which forms pouches as in *Curcuma* classified it as *Curcuma bhatii.* But RAPD and ISSR data showed that *C. bhatii* exists as a separate entity, showing very less similarity with all the other *Curcuma* species, indicating its genetic identity as a separate genus.

The levels of polymorphism detected in cultivated and wild species is dependent on a wide range of factors like breeding system, habitat specialization, intensity and direction of selection,type of the genetic material and the type of genetic markers used. The high polymorphism detected in this study displayed the resolving power of the RAPD and ISSR markers selected for genetic diversity analysis. RAPD markers are used for the diversity analysis of *Zingiber* by various workers (Rout *et al*., 1998, Nayak *et al*., 2005, Palai and Rout, 2007). The average polymorphic loci produced by the RAPD primers are 36.6% indicating the wide

genetic base of the species taken for the study. Highest level of polymorphism was detected in the case of OPC-07 which produced 77.78% polymorphism, preceded by OPA-15 with 77.05% polymorphism. ISSR markers produced a total of 232 polymorphic bands with overall percentage polymorphism of 65.53%. Out of 6 different ISSR primers used ISSR-5 $(CAC)_3GC$ showed 77.27% polymorphism preceded by ISSR- 6 $(CTC)_3$ GC with 68.18% polymorphism.

Combining the RAPD and ISSR polymorphic data and converting them into with binomials and generating the similarity matrix created a Jaccards similarity index in which the highest JSI was observed in the case of *Z. roseum* and *Z. wightianum* and the lowest JSI between *Z. montanum* and *Z. nimmonii*. The similarity matrix generated, produced a dendrogram with two main clusters. Cluster I comprised of three species in which *Z. neesanum* and *Z. montanum* showed a JSI 0.34%. According to the morphological characterization these two species were treated close together (Sabu, 2006). The other species included in the group is *Z. capitatum* with specific distinction from all the other species taken for the study. It shared a JSI 0.29 with *Z. neesanum*/*Z. montanum* group and clustered with the cluster II with a JSI of 0.27. The Cluster II comprised of 6 species with *Z. roseum* and *Z. wightianum* showed a common JSI of 0.39. Morphological treatment based classification also treated this two species as close relatives (Sabu, 2006) based on the keys developed. *Z. nimmonii* and *Z. cernuum* showed the maximal similarity index 0.41 of the species taken for the study, and both of them can be considered as a single species even though morphological characters are different for both the species.

Z. zerumbet and *Z. officinale* showed a common JSI of 0.35 even though the morphological classification showed them as distantly apart based on the characters and for their classification.

Both the marker systems provide an efficient mode of classification for the species taken for the study. The nine wild species taken for the study showed

certain character dissimilarity but have only a maximal similarity of 41%. Habitat heterogeneity plays a prominent role in preserving the diversity by diversifying the selection, especially plants which are clonally propagated; in contrast to habitat homogeneity results in gradual decrease in diversity through directional selection (Hangelbroek *et al*., 2002). All the nine species taken for the study are geographically apart with preference for difficult ecological niches.

Clustering of *Z. roseum* with *Z. nimmonii* and *Z. cernuum* in the same group refers to their habitat homogeneity and distance occupied by *Z. capitatum* shows the habitat and character heterogeneity showed by the species. High genetic/molecular variance within the population/group in comparison to among population/group has been reported in both sexually and asexually reproducing crops like Ginger (Haldimann *et al*., 2003, Sreekumar and Renuka, 2005; Jatoi *et al*., 2008). Genotypes that form separate OTU dissimilar to remaining accessions are potential germplasm that may be exploited to broaden the genetic basis.

RAPD and ISSR markers confirm the wide genetic basis of *Zingiber* germplasm, and the binomial data obtained clearly differ in the case of *Z. nimmonii* and *Z. cernuum* which can be treated as a single species and the difference obtained for *Z. officinale* and *Z. zerumbet* was much higher than the taxonomic data.

GC-MS is a powerful tool for analysis of volatile oil, volatile oil usually contains heat-sensitive components which may degrade and may lead to wrong conclusions during GC analysis. For example, numerous 1,4-dienes represent this property, as their skeleton rearranges thermally through a [3.3]-sigmatropic reaction (Cope rearrangement). However, chemical properties of the components in volatile oil were unknown in most cases and the pure compounds were difficult to be obtained for the related studies. Therefore, a method should be developed for optimization of GC-MS conditions. Extracted oil was subjected to the analysis and compounds identified by the library search program as being ≥ 80% probability

were viewed as likely hits. Spectra for each eluting compound were then compared to the standard spectrum for the best hit to determine if the molecular ion peaks and the fragmentation patterns match.

In the present investigation we detected a number of compounds that were identified from the different species could be used as marker compounds to distinguish between the different species. Essential oil yield was too low in some species, thus its chemical characterization was not possible. Moreover, the distribution of the species is scanty to repeat the experiment. The extraction of essential oils from both rhizome and leaves were not possible for all the species. In some species both the rhizome and leaf extraction yielded essential oils, but in some cases either leaf or rhizome provided oil or in some cases neither of them yields good quality and quantity oil. Many compounds present in small quantities were not included in this analysis because they could not be readily identified due to insufficient mass spectrum quality or because their relative concentration could not be adequately evaluated. The composition of the essential oils from a particular species can differ between harvesting seasons, extraction methods and geographical sources and that those from the different parts of the same plant (Burt, 2004).

Several epidemiological studies established a link between phytochemicals and the range of biological activities that impart health benefits in human beings. Scientific research supports the biological activity of many of the phytochemicals in their native forms. They were copiously used in Ayurveda and other traditional medicines which dates back to Charaka Samhita (Moon *et al*., 2010). Amongst the phytochemicals, several groups of polyphenols (anthocyanins, proanthocyanidins, flavanones, isoflavones, resveratrol and ellagic acid), non-nutrient chemical and dietary constituents are currently used in the pharmaceutical industry. The spices are considered to be the storehouse of active phytochemicals. The various spices belonging to the genera *Curcuma* and *Zingiber* are well known for their multiple uses as medicines, cosmetics, dyes, flavourings and neutraceuticals.

Many of the compounds identified in the GC-MS analysis of the samples of *Curcuma* and *Zingiber* could be used as marker compounds to distinguish between the different species. Nineteen constituents were characterized by GC-MS analysis of *Curcuma aurantiaca* leaves, the major constituents of the oil were Caryophyllene (31.07%), 1, 6, 10-Dodecatriene, 7,11-dimethyl-3-methylene (17.30%), Phytol (11.32%), Camphene (4.25%), beta pinene (2.18%), Benzofuran (9.06%), alpha caryophyllene (3.49%).

21 compounds were detected from the essential oil of *C. aurantiaca* rhizome, the major constituents of the oil were Camphene (10.01%), Cyclohexene, 1-methyl-4-(1-methylethylidene)- (20.87%), Caryophyllene (14.41), Eucalyptol (6.54%), D-Limonene (6.70%),1,6,10-Dodecatrien-3-ol (8.70%), alpha.-Phellandrene (2.33%), beta.-Myrcene (2.20%) and Benzene, 1-methyl-2-(1-methylethyl) –(3.81%). The chemical profile shows the rhizome and leaf oil of *C. aurantiaca* wre the same in its oil components. Phytochemical compositions of essential oils in *C. aurantiaca* are reported for the first time.

GC-MS analysis of essential oil of *C.aeruginosa* rhizome showed the presence of 20 major components. 2-Pyridinamine, 4, 6-dimethyl (28.84%) comprised maximum peak area. Other identified compounds were Eucalyptol (10.27%), camphor (6.15%), Isoborneol (2.19%), caryophyllene (2.15%), 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)- (6.24%), beta.-Pinene (2.76%), camphene (2.06%), benzofuran (3.42%), cyclohexane (6.63%), thiazole (3.95%) etc.

Raj *et al*., (2008) isolated volatile oil from the rhizomes of *Curcuma haritha* from the Western Ghats, southern India and subjected to a combination of GC-FID, GC-MS, co-GC, database and literature search, linear retension indices, prep. TLC, IR, NMR and MS. Refractive index, specific rotation and specific gravity were measured and identified fifty constituents. camphor (36.0%), 1, 8-cineole (13.9%), isoborneol (10.6%), camphene (5.7%), linalool (4.7%) and borneol (4.6%) were the major monoterpenes. Germacrane-type sesquiterpenes, viz. curdione (6.9%),

furanogermenon (3.3%), germacrone (2.8%) and neocurdione (1.5%) were the major sesquiterpenoids in *C.haritha* rhizome oil. In the present study we detected 30 major constituents by GC-MS analysis. The prominent compounds were camphor (8.25%), camphene (6.39%), alpha-pinene (2.17%),borneol (2.85%), isoborneol (6.65%), neocurdione(2.05%), ethanone (34.51%), gamma elemene (2.25%), anthracene (2.55%) were the major monoterpenes and sesquiterpinoids. The presence of 1,8-cineole, with its low aroma threshold, contributes the camphoraceous, minty and sweet aroma in turmeric leaves. Oxygenated compounds are more stable and have strong odour and flavour of the original oil (Kirchner and Miller, 1952). Monoterpene compounds are less valuable than oxygenated compounds as they contribute to the fragrance of the oil only in a minor way. The concentration of oxygenated monoterpene 1,8-cineole obtained from this study decreased after extraction temperature reached 170^0 C, the optimization temperature of 1,8-cineole is 147^0C. So in this study the yield of this oxygenated compound is decreased due to their degradation.

Chatterjee *et al*., (2000) isolated the volatile essential oils from commercial samples of dry turmeric and samples γ-irradiated at a dose of 10 kGy using simultaneous distillation extraction technique and analyzed by GLC and GC-MS. Some of the major compounds identified by GC/MS were α-phellandrene, p-cymene, 1,8 cineol, β-caryophyllene, ar-curcumene, zingiberene, β-sesquiphellandrene, nerolidol, turmerone, ar-turmerone, curlone and dehydrozingerone. In this study we detected 15 major compounds from the rhizome oil of *C.longa* are alpha-Phellandrene (6.90%), Benzene, 1-methyl-2-(1-methylethyl)-(8.58%), Eucalyptol (5.33%), 2-Pyridinamine, 4,6-dimethyl-(10.28%), beta.-Elemenone (3.13%), Curlone (16.83%), Phenol (4.36%) and m-Toluic acid (3.04%).

C. aromatica is the highest camphor yielding species in the genus *Curcuma* (20.97%). Camphor has various traditional applications, such as the control of arthritic, rheumatic and back pains, coughs, use as insect repellent etc. It is used in

religious ceremonies in India. Further, fullerenes and carbon nanotubes, with applications in nanotechnology and electronics, use camphor as the major natural precursor. The other major compounds present in the volatile oil of *C. aromatica* were neocurdione (28.43%), 6,10-Dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione (10.53%), borneol (8.09%), isoborneol (3.61%), camphene (1.90%) andeucalyptol (5.55%). Neocurdione and curdione are stereoisomers and has anticancer, antioxidant, bile secretion promoting and hepatoprotective activities. Historically, the rhizomes of *C.aromatica* are used as tonic, carminative and externally for skin eruptions and infections and to improve complexion. *C. aromatic*a has been reported to exert various medicinal activities such as promoting blood circulation to remove blood stasis and for the treatment of cancer.

The volatile oil isolated from the leaves of *C. raktakanta* yielded Ethanone (34.51%), Camphor (8.25%), Camphene (6.39%), Benzofuran (12.48%), Neocurdione (2.05%), Gamma-elemene (2.25%), Borneol (2.85%), Isoborneol (6.65%), Alpha-pinene (2.17%), Eucalyptol (2.53%) as the major compounds. In the case of *C. raktakanta* rhizome Benzofuran (15.32%), Ethanone (14.56%), Gamma-elemene (5.88%), Camphene (3.27%) were identified, apart from these alpha.-Phellandrene (1.23%), D-Limonene (1.75%), Naphthalene (1.47%), 3,7-Cyclodecadien-1-one (17.04%), Sulfaguanidine (1.20%), Anthracene (3.17%), Phytol (1.37%) and Globulol (0.95%) were detected.

C. mutabilis is one of the most variable species we have ever come across within the genus. Most remarkable is difference in colour of flowers. This species so far known only from its type locality, Nilambur. The chemical profile of *C. mutabilis* have never been reported. In present study we isolated the volatile oil from the leaves and subjected to GC/MS analysis, the major compounds detected were 1H-1-Silaindene (15.96%), alpha.-Farnesene (5.55%), 5- Benzofuran acetic acid (7.04%), alpha.-Caryophyllene (6.68%), 1,6,10-Dodecatriene (7.91%), Caryophyllene (8.12%), beta.-Pinene (2.12%), beta.-Elemenone (5.40), Curlone (1.35%), Ar-

tumerone (3.29%), Tumerone (2.53%), 2,4,6-Cycloheptatrien-1-one (9.64%), Geranylgeraniol (1.91%), Nerolidol-2 (1.81%) and gamma.-Elemene (1.81%).

C. amada, commonly known as 'Mango ginger'is the second most important rhizomatous species of the genus *Curcuma* after *C.longa*. *C. amada* possesses a very exclusive chemical characteristic of having raw mango-like flavor blended with that of ginger, which distinguishes this species from the rest 80 species of this genus. Mango-ginger is gaining world-wide attention as a potential source of new drug molecule (s) to combat diverse ailments as it is credited with compounds having antimicrobial, antioxidant, anticancer, anti-inflammatory, antidepressant, anti-tubercular and platelet-aggregation inhibitory activities (Policegoudra *et al*., 2011). The mango flavour is mainly attributed to presence of car –3 –ene, *cis*-and *trans* hydroocimene, ocimene and myrcene which indicates that the aroma of mango ginger is a mixture of characteristic compounds found in both raw mango and turmeric (Rao *et al*., 1989). The major bioactive compounds of *C. amada* consist of curcuminoids, curcumin, demethoxy curcumin, bis-demethoxy curcumin and diterpene lambda-8 (17), 12-diene-15, 16-dial. In the present investigation we detected 50 compounds, including Ar-tumerone (7.25%), Tumerone (4.93%), Curlone (3.03%), 1(2H)-Naphthalenone (5.15%), Naphthalene (11.37%), 3,7-Cyclodecadien-1-one (9.80%), 4,5,6,6a-Tetrahydro-2(1H)-pentalen one (18.45%), Benzofuran (11.03%),beta.-Myrcene (4.36%), Beta-pinene (0.99%), gamma.-Elemene (2.89%), alpha.-Phellandrene (1.28%), Caryophyllene (1.33%), Caryophyllene oxide (1.43%) etc.

C.inodora is an endemic species to peninsular India from Maharashtra extending up to North Karnataka. The specific epithet inodora (Latin) refers to the non-aromatic nature of the rhizome. Size and quantity of rhizomes are very low, hence isolated the volatile oil from the air dried leaves. This is the first report of the leaf oil composition of *C. inodora* from India. The major constituents of the above oils werebeta.-Pinene (4.09%), Caryophyllene (31.33%), alpha-caryophyllene (5.97%), Benzofuran (21.29%),gamma.-Elemene (5.18%),alpha.-Bisabolol (2.32%),

beta.-Elemenone (3.09%), Ethanone (2.76%), Phytol (3.19%), 1,6-Cyclodecadiene (4.37%) and Cyclohexane (11.50%).

The chemical profile of the oil is with respect to its major constituents is very specific to each species of *Zingiber.* Based on the compounds that were detected or identified from the different species, we found that many of the compounds could be used as marker compounds to distinguish between the different *Zingiber* species. In *Z. officinale* the gingerols and their derivatives 1,3-Cyclohexadiene (36.72%), Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) (11.50%), Nerolidol-2 (2.70%), Di-epi-alpha cedrene-(1) (2.01%) and other compounds like Lavandulol, pentafluoropropionate (2.97%), Naphthalene (2.26%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (20.18%) were detected.

Similarly, a number of other compounds were present in *Z. nimmonii* essential oil, Caryophyllene (20.52%) as its major component followed by 3-Carene (12.26%), Caryophyllene oxide (11.46%), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (9.33%), D-Limonene (2.19%), beta-phellandrene (5.10%), alpha.-Pinene (2.31%), beta.-Myrcene (1.51%), Copaene (1.44%) and Benzoic acid (3.37%). *Z. cernuum* showed caryophyllene as its major component of the essential oil followed by Caryophyllene oxide makes the similar chemical support of these two species.

A higher concentration of humulene epoxide II (22.5%) makes *Z. roseum* distinct from its counterparts in India. The presence of beta-caryophyllene, Caryophyllene oxide and alpha-humulene indicates the chemical relationship between *Z. nimmonii, Z. cernuum* , *Z. roseum* and *Z. wightianum* but beta-caryophyllene and caryophyllene oxide is lower in *Z. wightianum* compared to the other three species.

At least 25 compouds were identified from the volatile oil of *Z. montanum*, beta.-Phellandrene (33.94%) as its major component followed by 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (10.23%), Bicyclo[3.1.0]hex-2-ene, 4-

methyl- (10.18%), alpha.-Pinene (4.80%), beta.-Pinene (3.91%), Caryophyllene (2.05%), Thiourea (2.59%) and Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)- (2.08%).

The chemical compounds isolated from each species were distinct and can be used to develop and identification tool or as a marker for each species.

SUMMARY

The family Zingiberaceae is the largest of the eight families in the order Zingiberales, with 53 genera and over 1377 species (Kong *et al*., 2010). They are mainly distributed in tropics and subtropics with the centre of distribution in the Indo-Malayan region, but extending through tropical Africa to central and South America (Tomlinson 1969, Kress *et al*., 2002, Kong *et al*., 2010). In India it is represented by 21 genera and about 180 species (Jain and Prakash, 1995). Zingiberaceae have been a taxonomically neglected group mainly because of the inaccessible nature of the wet evergreen forest habitats in which they grow. The short flowering period coincides with the monsoon season, makes the collection more laborious. Moreover, huge vegetative and massive underground parts make the preparation of herbarium specimens tedious. Consequently, most of the herbarium specimens are fragmentary, prepared from dried specimens, hence the descriptions in most of the Floras are inadequate. Due to the delicate nature of flowers, loss of color and formation of a gummy mass soon after collection, the study of floral morphology proved much difficult to solve the taxonomical problems, especially in the genera *Curcuma* and *Zingiber.*

Conventional taxonomic techniques in conjunction with molecular biology and biochemical tools may go a long way in providing accurate and powerful ways of analyzing genetic relationship among the species in the family Zingiberaceae. However, concerted efforts are not made on molecular characterization in *Curcuma* and *Zingiber* species at molecular level by using molecular markers and phytochemical studies. Molecular markers assume great significance, as these methods detect polymorphisms by assaying subsets of the total amount of DNA sequence variation in a genome (Das *et al*., 2011).

All taxa under study were collected from different regions of India. The rhizomes and plants from different collection area were planted in the Calicut

University Botanical Garden for continued observation. Specimens were identified, the nomenclatural corrections were made, the types and authentic materials were studied and a detailed description of each species was made, Photographs and colour plates were prepared. A detailed classification history and taxonomic key were developed for *Curcuma* and *Zingiber* species studied.

The genetic variability of 19 *Curcuma* species was studied with 42 random primers and 8 ISSR primers. RAPD/ISSR banding patterns expressed by the primers and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer were calculated. 20 random decamer RAPD primers developed polymorphic banding pattern and produced a total of 2226 scorable bands in the 19 species of *Curcuma,* out of which 1025 were polymorphic. The percentage polymorphism ranged from a maximum of 56.7% to a minimum of 36.5% within the species. Whereas in case of the seven ISSR primers studied, produced 800 bands and out of which 424 were polymorphic, the percentage polymorphism ranged from 60.78% to 48.14%.

Based on the UPGMA dendrogram developed, the 19 species taken for the study were grouped into two main clusters. Cluster I had only one node containing one species, *i.e*. *Curcuma bhatii* and has 0.23 JSI with the all the other species clarifying the questions raised against the circumscription of *C.bhatii* from *Paracautleya batii* The second subgroup contained 7 species in which *C.montana* and C*. pseudomontana* were grouped together with maximum similarity and are synonyms.

In the case of *Zingiber*, 18 random decamer primers produced a total of 997 scorable bands in the 9 species studied out of which 660 were polymorphic. The percentage polymorphism ranged from a maximum of 77.78% to a minimum of 54.54%. Whereas in case of the 6 ISSR primers studied, the percentage of polymorphism ranged a maximum of 77.27% and a minimum 56.94%.The sequence of the RAPD/ISSR primers used for the molecular genetic finger printing of the 9

Zingiber species and RAPD/ISSR banding patterns expressed by the primers and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer were evaluated.

All the nine species of *Zingiber* used for the study formed two clusters, cluster I and II. *Z. nimnonii* and *Z.cernuum* showed a JSI of 0.41 and were grouped together with the same node indicating the species status given by taxonomists to the two samples are not correct and both are synonyms.

GC-MS-BASED METABOLIC PROFILING

GC-MS analysis is a powerful tool to study the chemical components of the volatile oil. In the present investigation we detected a number of compounds that were identified from the different species of *Curcuma* and *Zingiber* could be used as marker compounds to distinguish between the different species. Essential oil yield was too low in some species, thus its chemical characterization was not possible. Moreover, the distribution of the species is scanty to repeat the experiment. The extraction of essential oils from both rhizome and leaves were not possible for all the species. In some species both the rhizome and leaf extraction yielded essential oils, but in some cases either leaf or rhizome provided oil or in some cases neither of them yields good quality and quantity oil. The major compounds detected and the chromatograms from the essential oil were obtained and a dichotomous key was developed based on the presence or absence of specific compounds.

The major findings of the study are:

1. Based on morphological characters a taxonomic key was developed for the identification of 19 *Curcuma* species and 9 *Zingiber*sp.

2. RAPD and ISSR analysis and similarity matrix created by the profiles generated by each primer derived a specific UPGMA clustering for both the

genus suggestive of the need for relooking the separate status given to some species.

3. GC-MS analysis detected the major compounds and a dichotomous key created for the species which yielded essential oil on hydrodistillation.

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Fig. 2. The 'Family tree'or 'Rhizogram' of the ginger order.

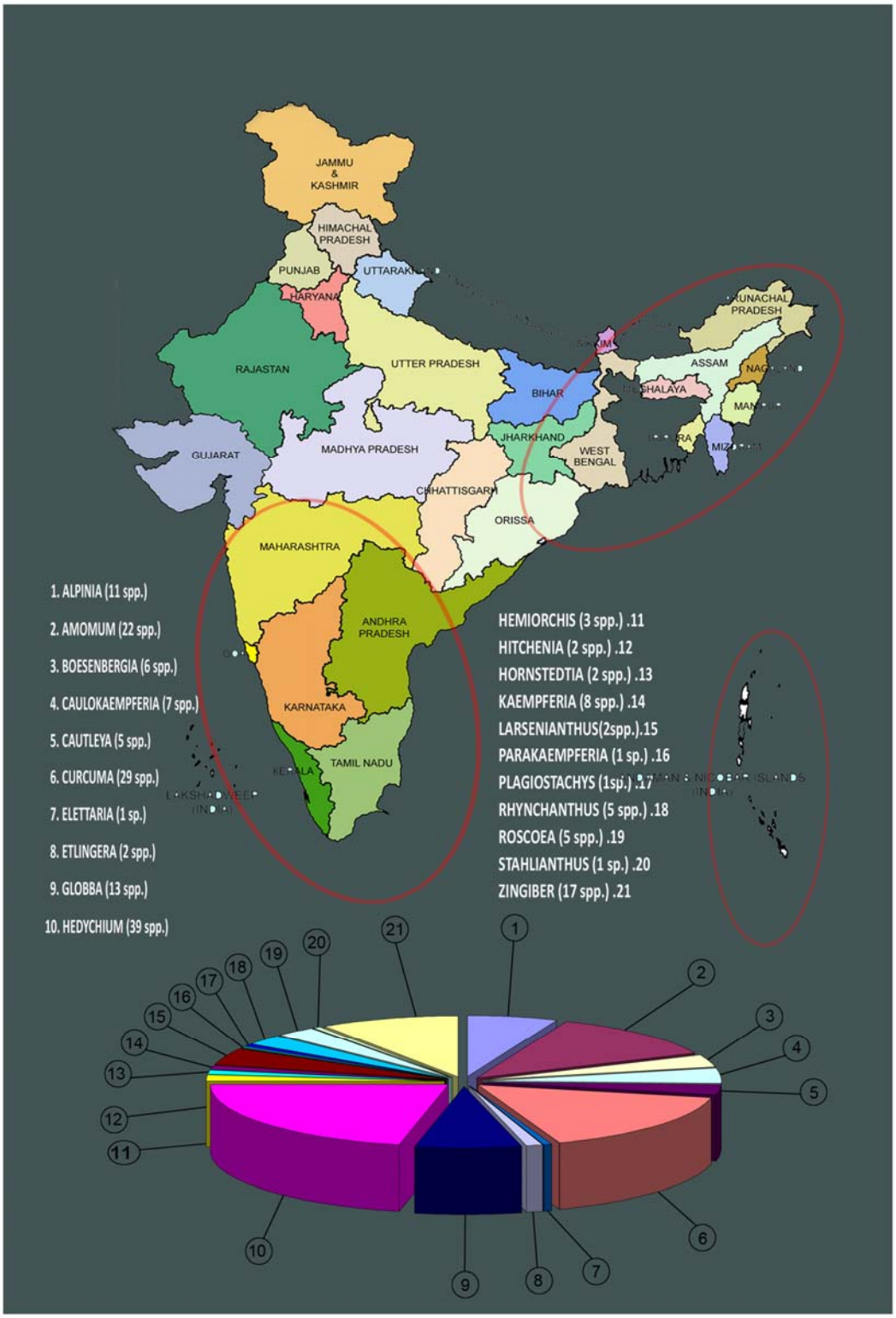


Fig. 1 Zingiberaceae - Indian scenario

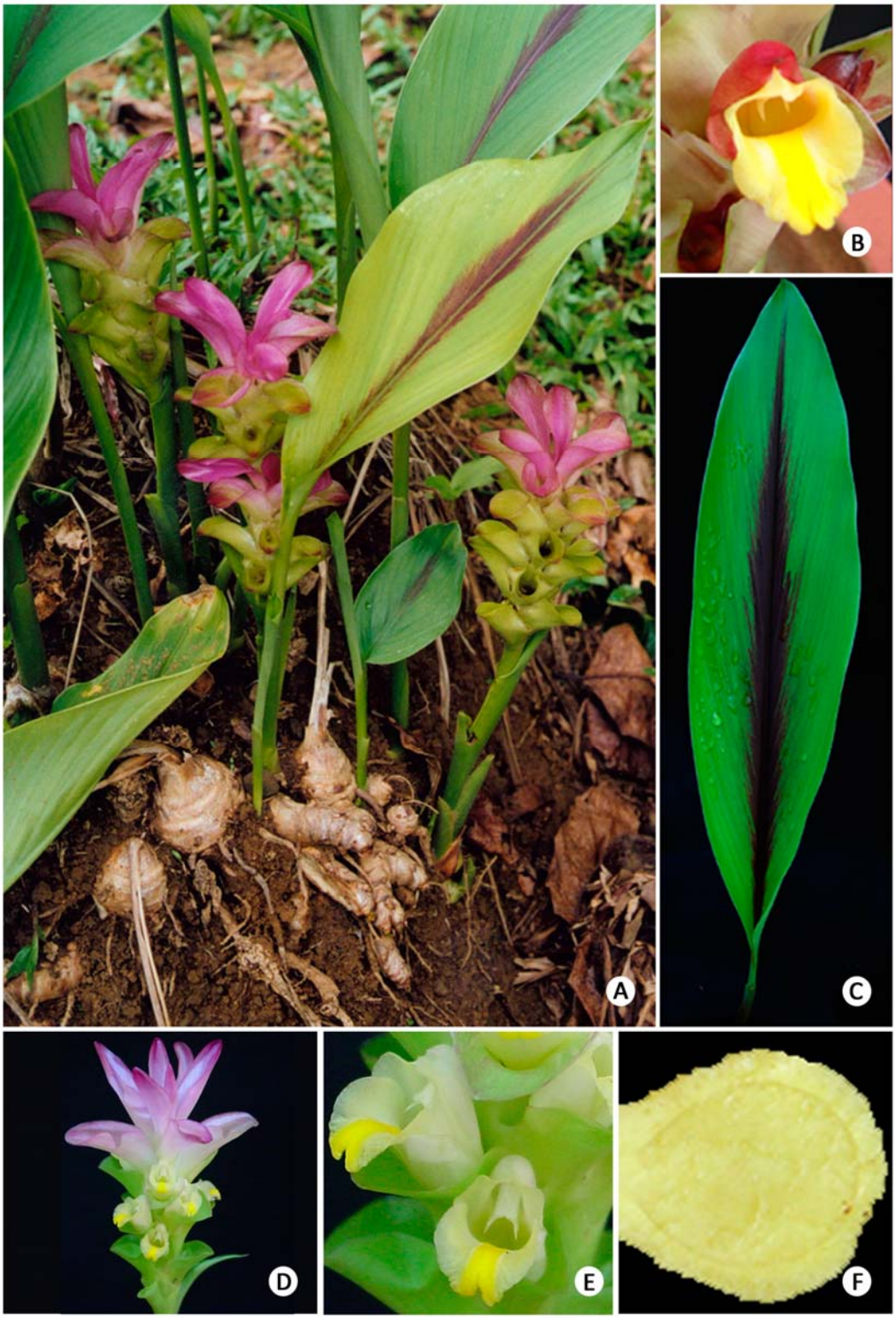


Fig.3. A-C *Curcuma aeruginosa*: A. Habit B. Single flower; C. Leaf; D-F *Curcuma amada*: D. Inflorescence; E. Flower; F. Rhizome T.S.

Fig.4. A&B. *Curcuma aromatica*: A. Habit; B. Inflorescence; C-D. *Curcuma aurantiaca*: Habit with Inflorescence; F&G. *Curcuma bhatii*: Habit with inflorescence.

Fig.4E. *Curcuma aurantiaca*: Inflorescence colour variations

Fig.5. A&B. *Curcuma coriacea*: A. Habit; B. Inflorescence; C. *Curcuma decipiens*: Habit.

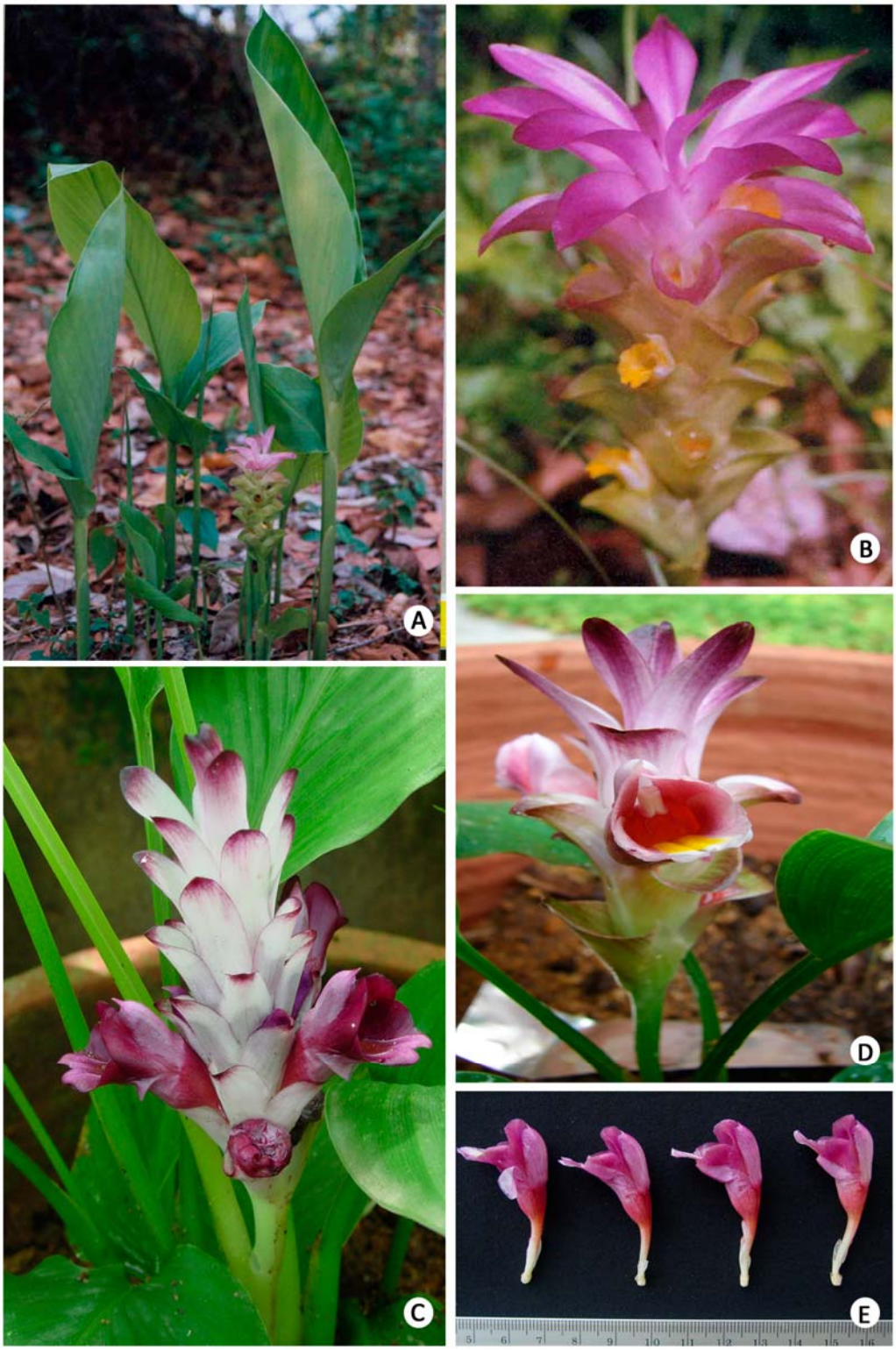


Fig.6.A&B. *Curcuma haritha*: A. Habit: B. Inflorescence; C-F. *Curcuma inodora*: C. Habit; D. Inflorescence; E. Flowers.

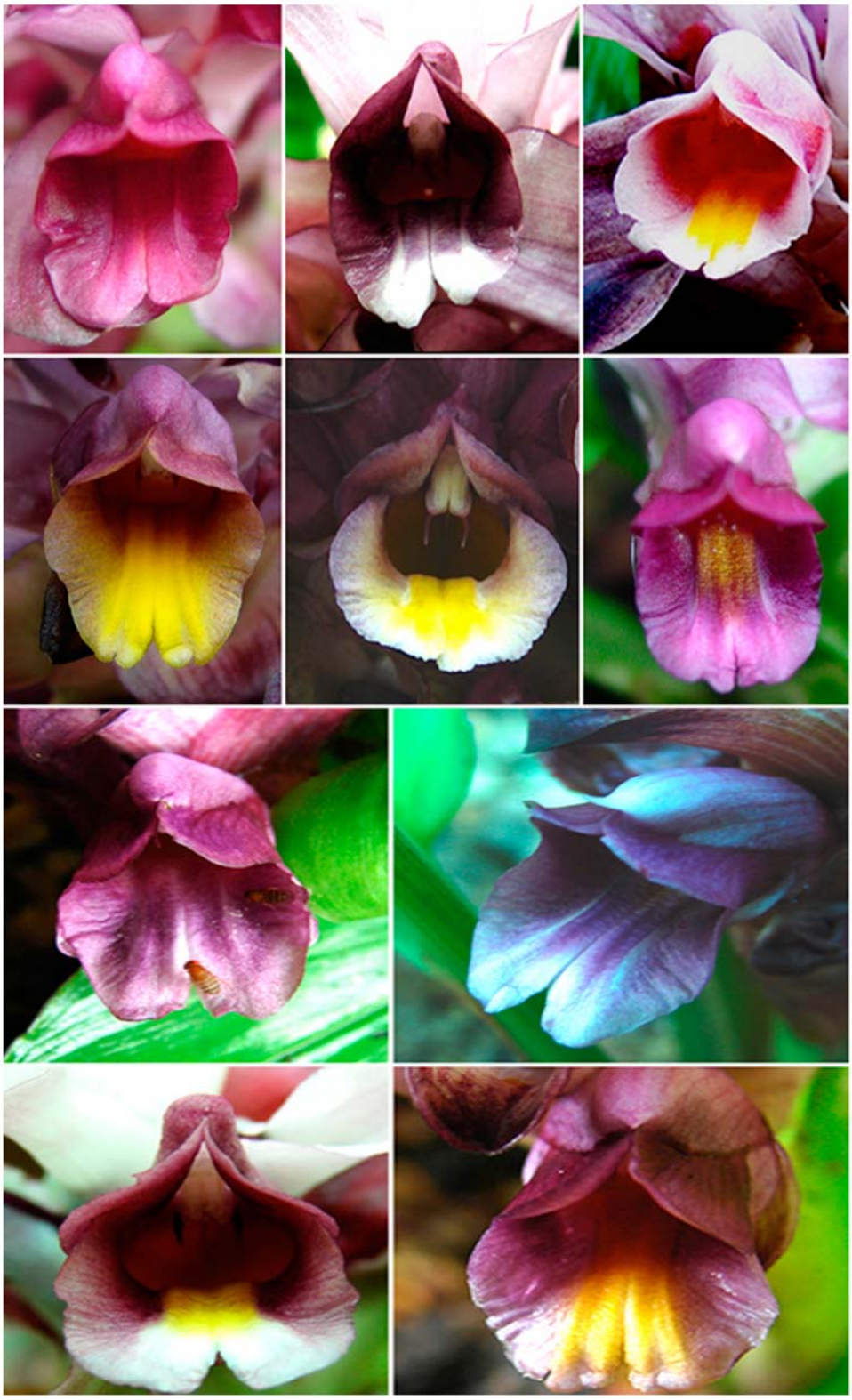


Fig.6F. *Curcuma inodora* Flower : Colour Variations

Fig.7. A&B. *Curcuma karnatakensis*: A. Inflorescence; B. Habit; C&D. *Curcuma longa*: C. Habit; D. Inflorescence; E&F. *Curcuma montana*; E. Habit; F. Inflorescence.

Fig.8. A-D. *Curcuma mutabilis*: A. Habit; B. C. & D. Inflorescence colour variation; E&F. *Curcuma neilgherrensis*: E. Inflorescence F. Habit.

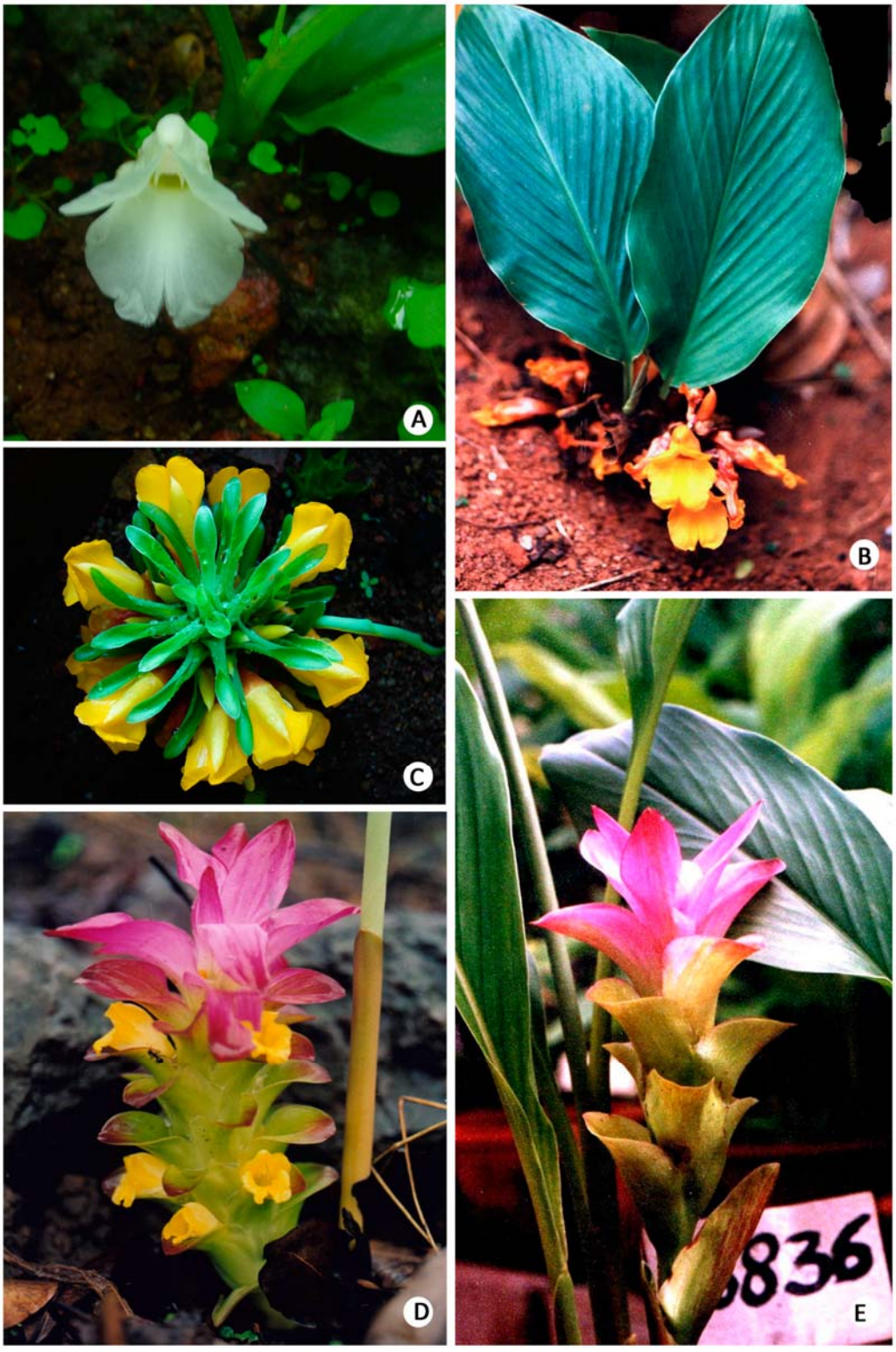


Fig.9. A. *Curcuma oligantha* var. *oligantha*: Habit; B. *C. oligantha* var. *lutea*; Habit; C. Inflorescence; D&E. *Curcuma pseudomontana*: D. Habit; E. Inflorescence.

Fig.10. A&B. *Curcuma raktakanta*: A. Habit; B. Inflorescence; C&D. *Curcuma vamana*: C. Habit; D. Inflorescence; E&F. *Curcuma zanthorrhiza*: E. Habit; F. Inflorescence.

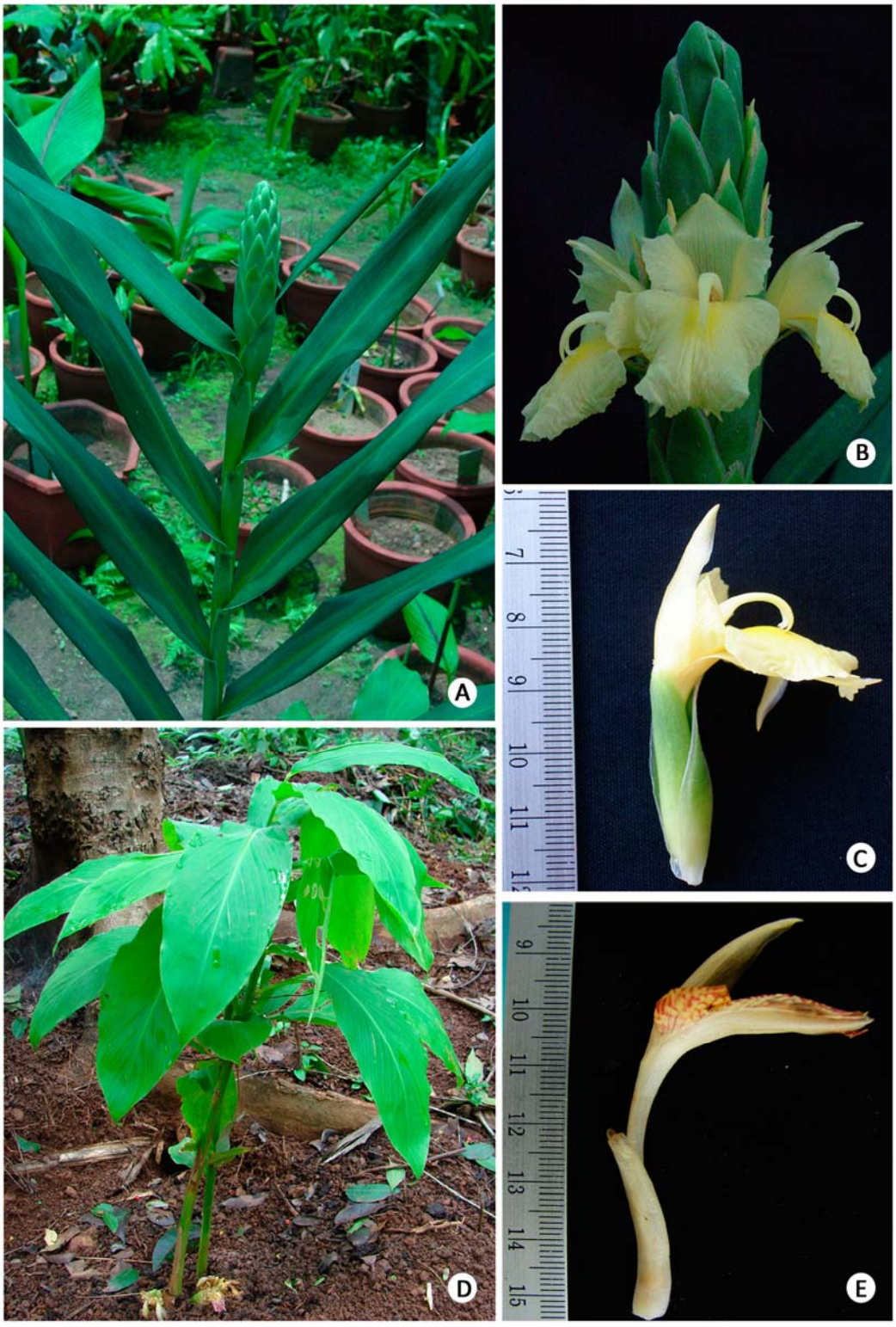


Fig.11. A-C. *Zingiber capitatum* var. elatum: A. Habit; B. Inflorescence; C. Flower; D&E. *Zingiber cernuum*; D. Habit; E. Flower.

Fig.12. A-C. *Zingiber montanum*; A. Habit; B. Inflorescence; C. Flower; D-F.*Zingiber neesanum*: D. Habit; E. Infrutescence; F. Flower.

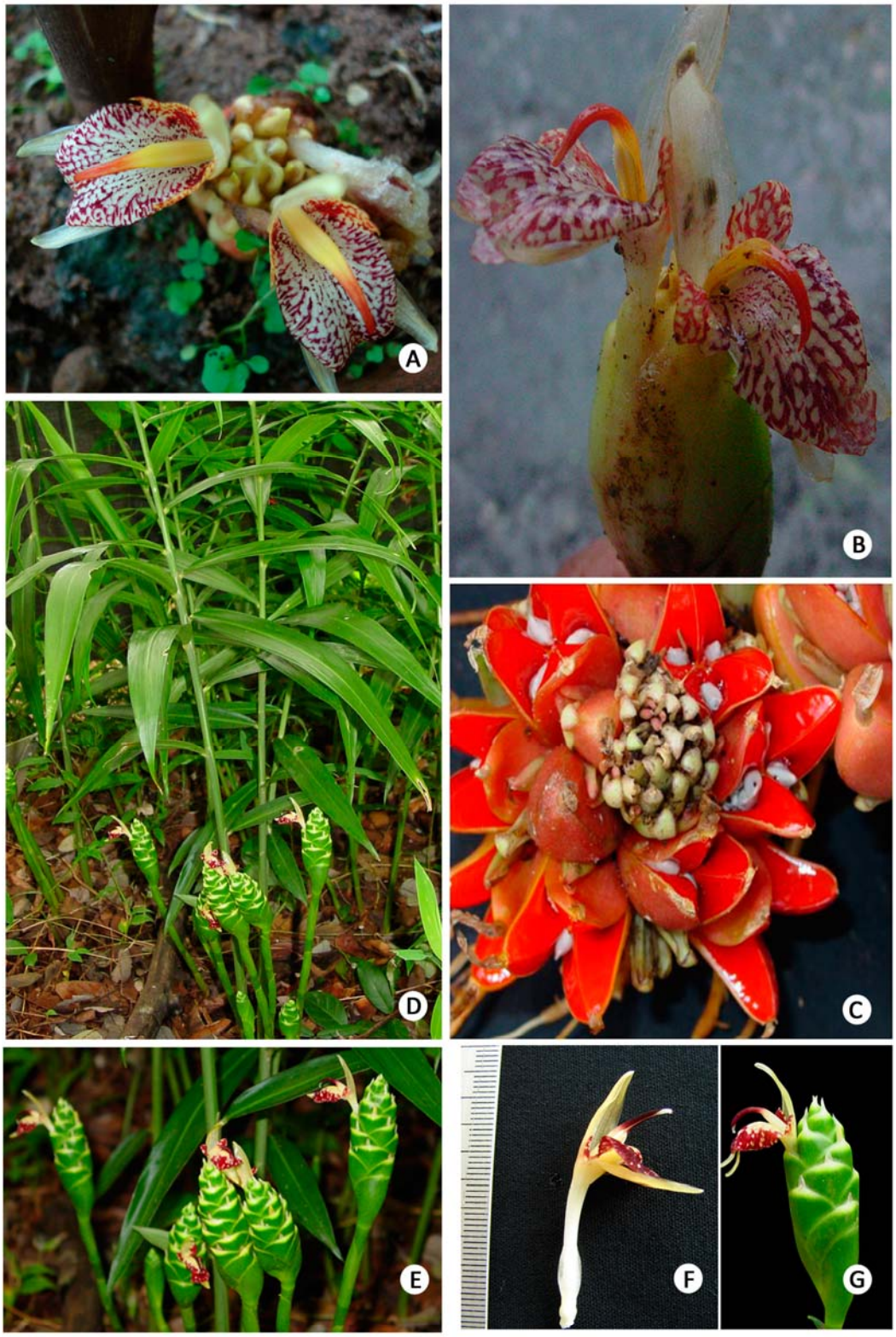


Fig.13. A-C. *Zingiber nimmonii*: A.Habit; B.Inflorescence; C.Infrutescence; D-G. *Zingiber officinale*: D. Habit; E & G. Inflorescence; F. Flower.

Fig.14. A&B. *Zingiber roseum*: A. Habit; B. Inflorescence; C&D. *Zingiber wightianum*: C. Habit; D. Inflorescence.

Fig.15. A-D. *Zingiber zerumbet*: A. Habit; B. Inflorescence; C. Infrutescence; D. Flower.

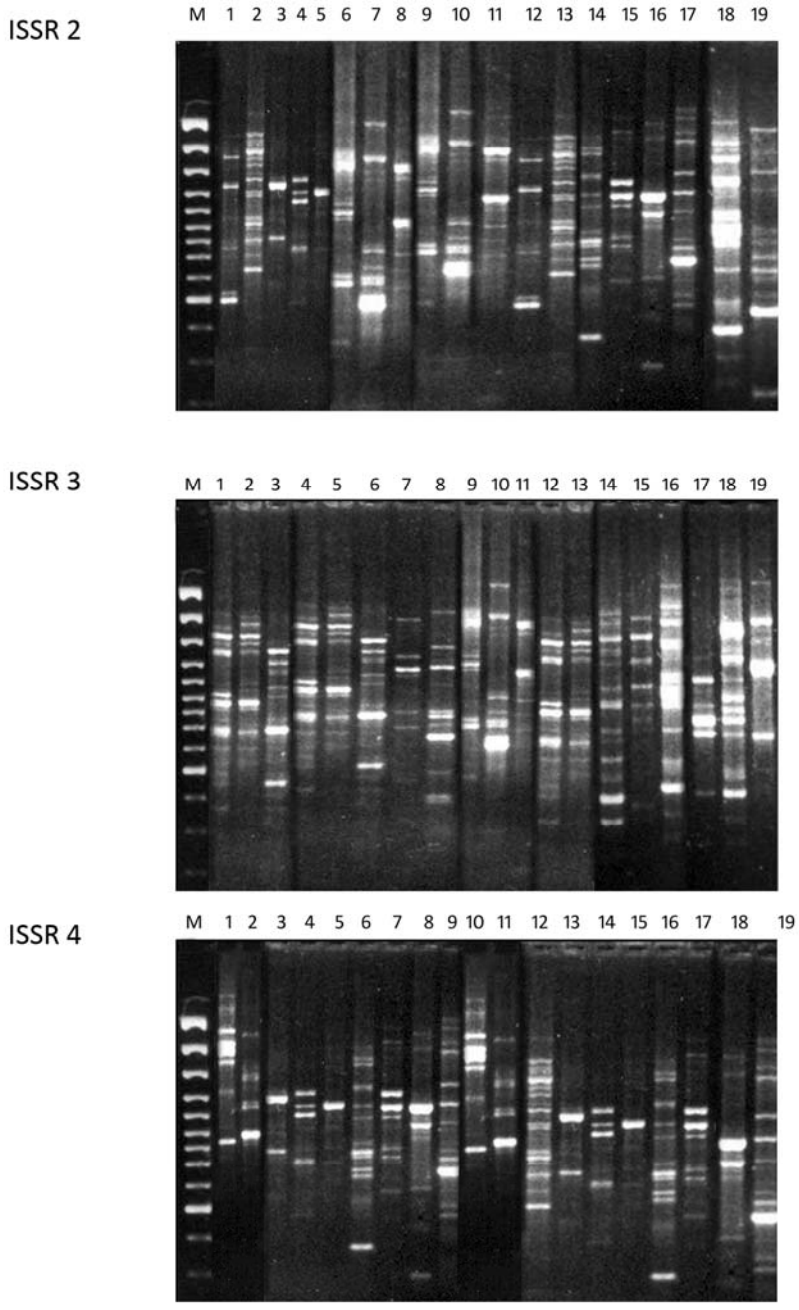


Fig.23.ISSR Profile generated in *Curcuma* **species by primers ISSR 2, ISSR 3 & ISSR 4. M-Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C. oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C. aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora***

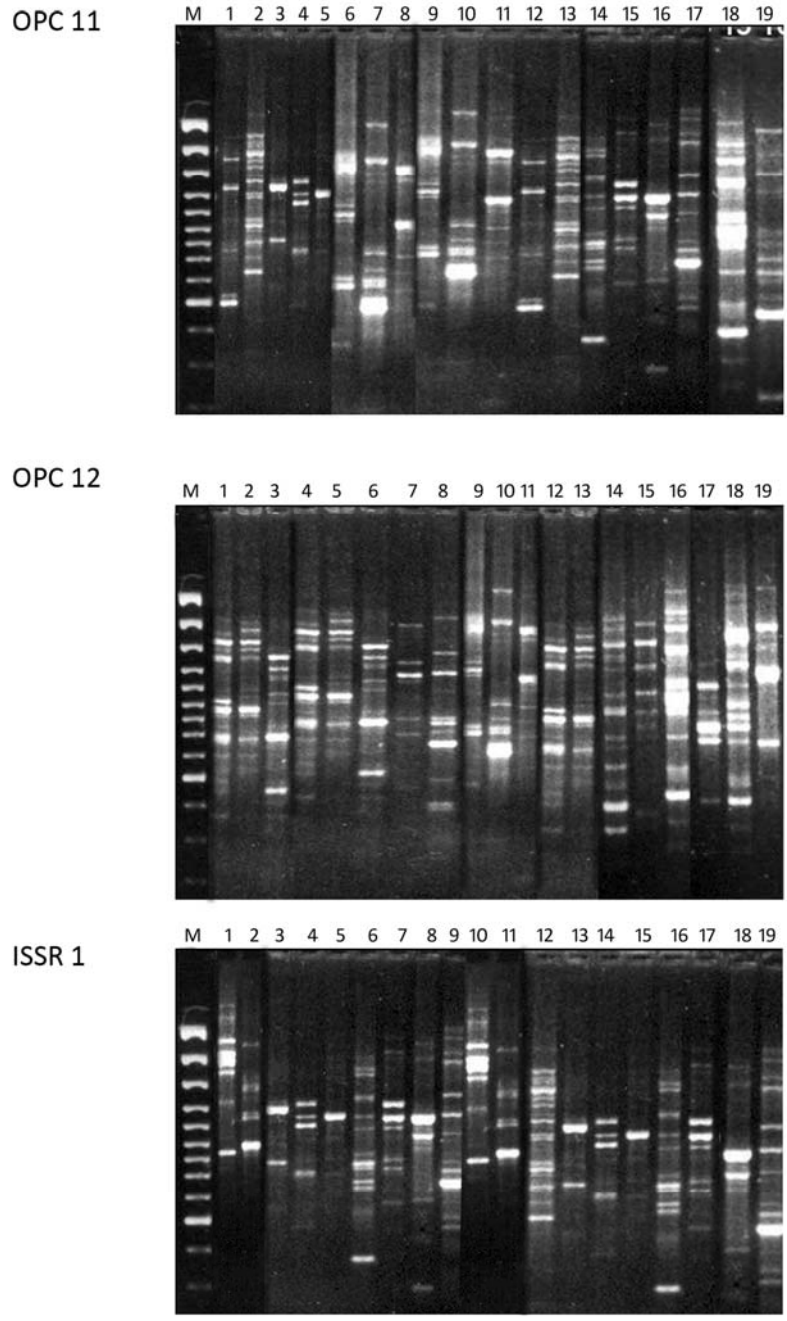


Fig.22.RAPD&ISSR profiles of *Curcuma* species obtained with primers OPC 11, OPC 12 & ISSR 1.M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C. neilgherrensis,* 5. *C.oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C.aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C.raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C. inodora*

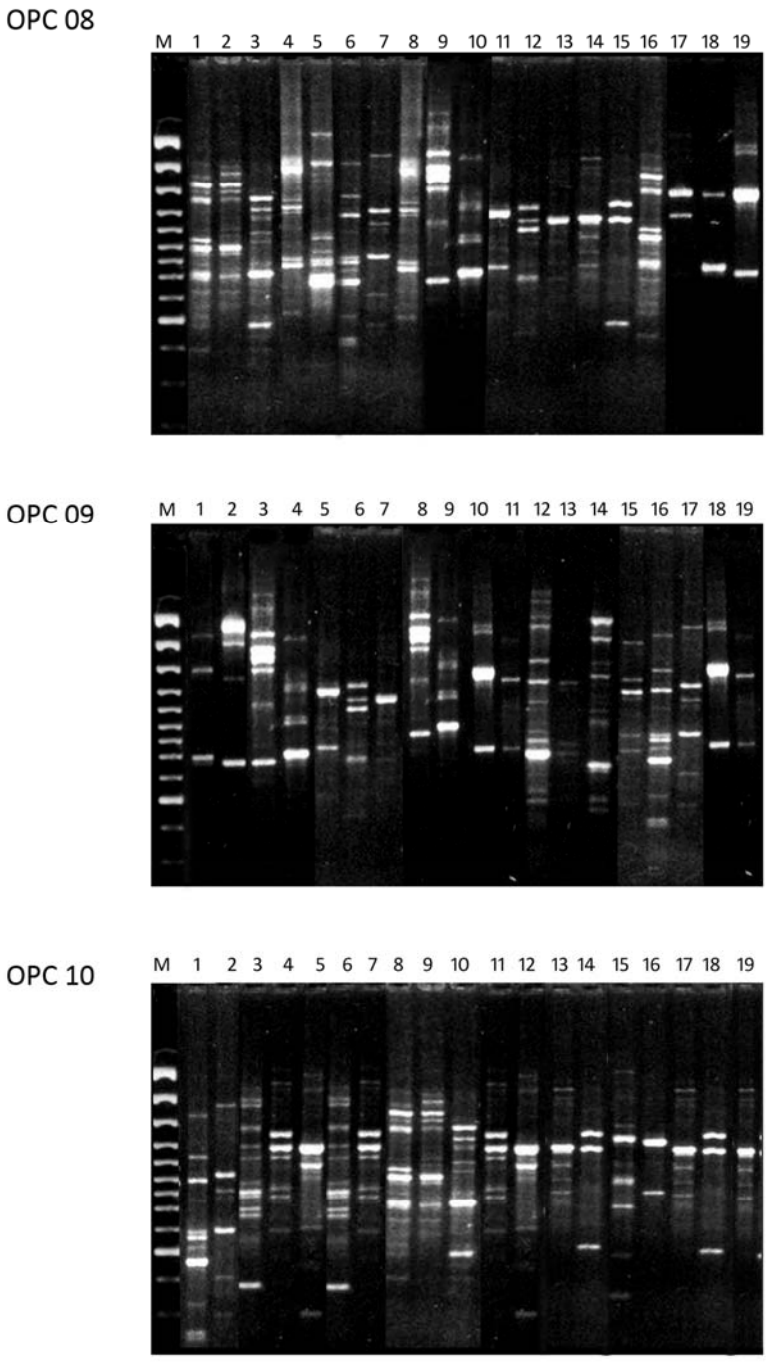
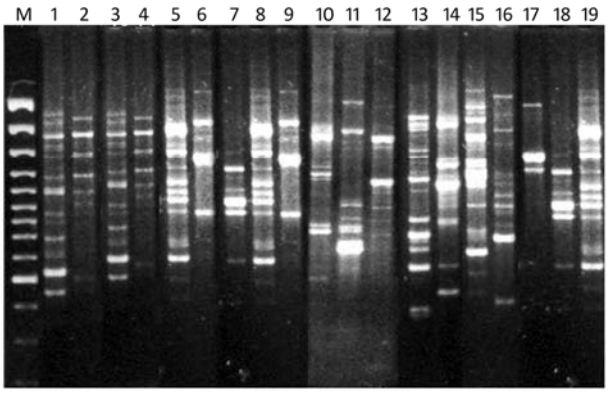


Fig.21.RAPD profile of *Curcuma* species obtained with primers OPC 08, OPC 09&OPC 10. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C.oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C.aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

OPB 12



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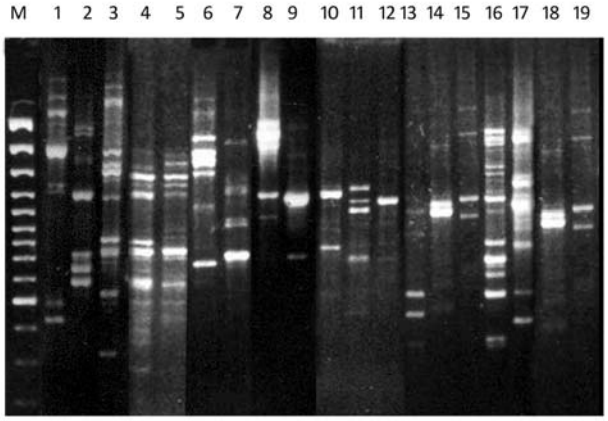


OPC 07

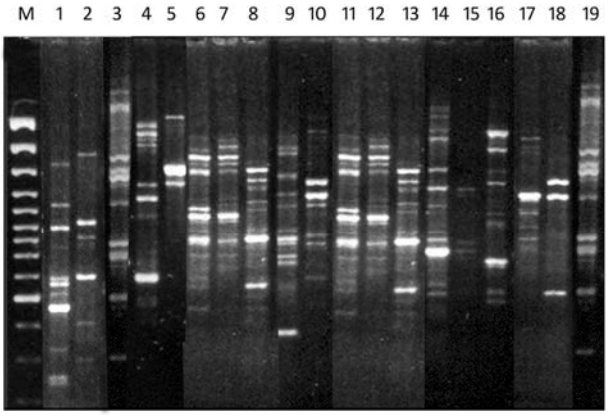
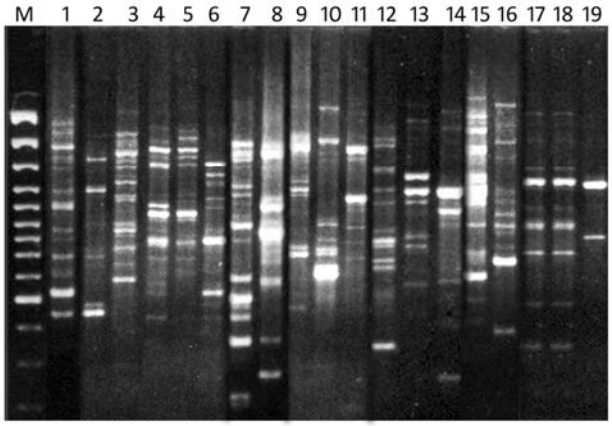


Fig.20.RAPD profile of *Curcuma* species obtained with primers OPB 12, OPB 16&OPC 07. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C.oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C.aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

OPA 18



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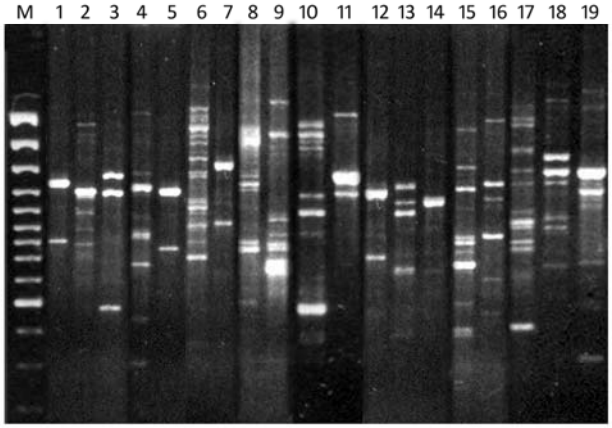

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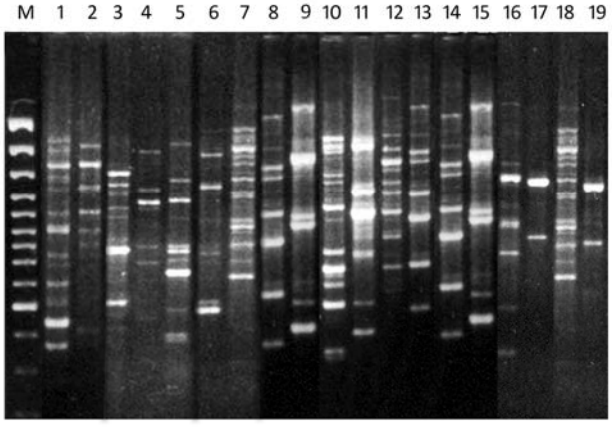


Fig.19.RAPD profile of *Curcuma* species obtained with primers OPA 18, OPA 19&OPB 09. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C. neilgherrensis,* 5. *C.oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C.aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C.raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

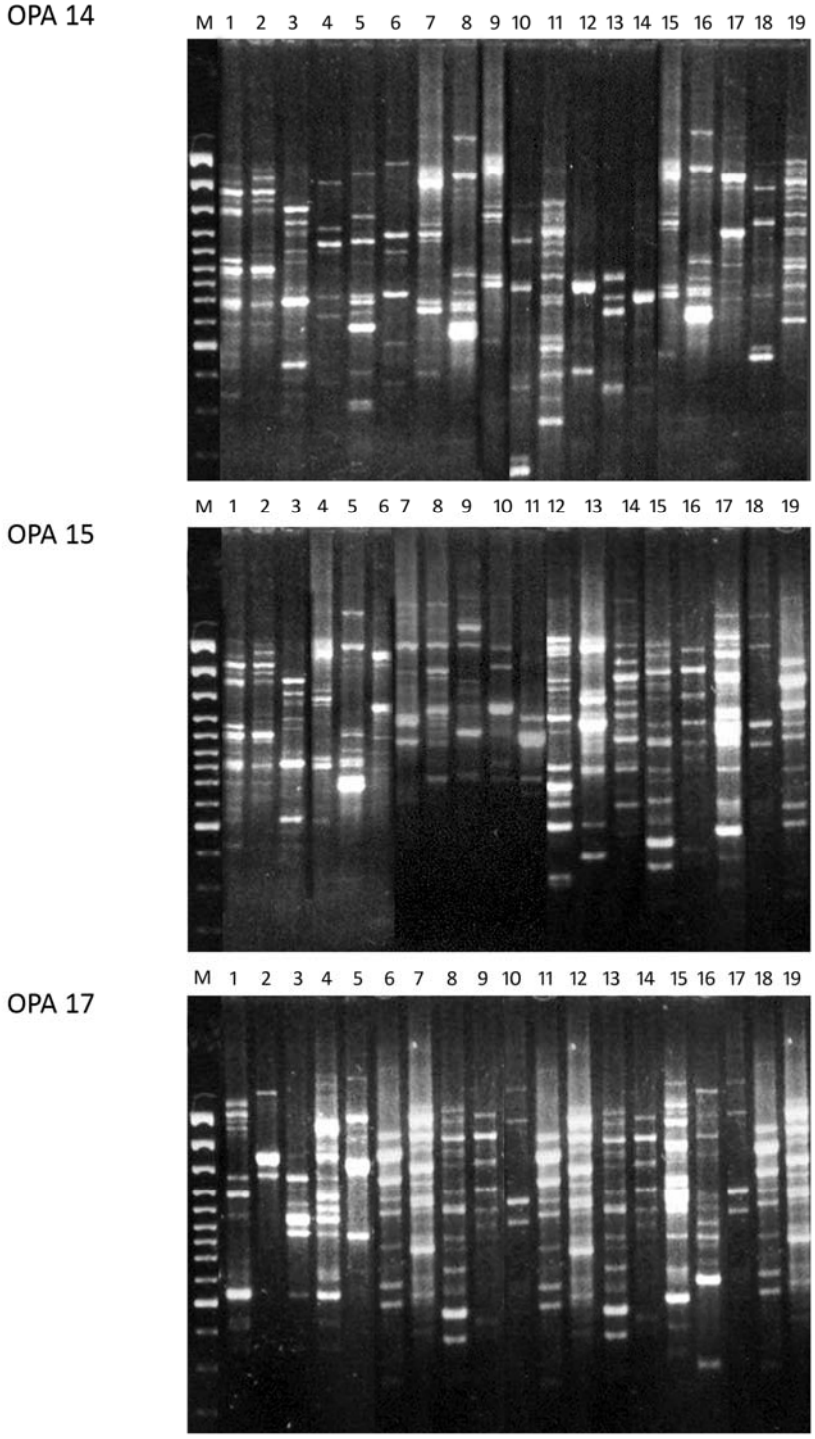
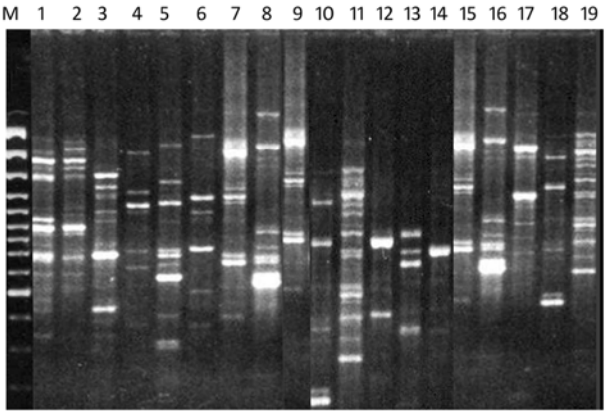


Fig.18.RAPD profile of *Curcuma* species obtained with primers OPA 14, OPA 15&OPA 17. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C. oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C. aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

OPA 10



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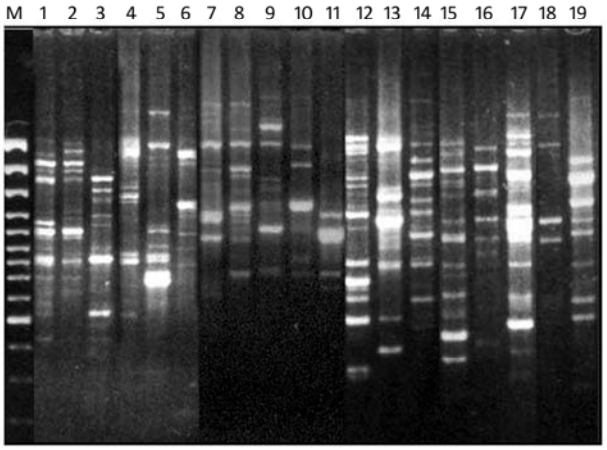

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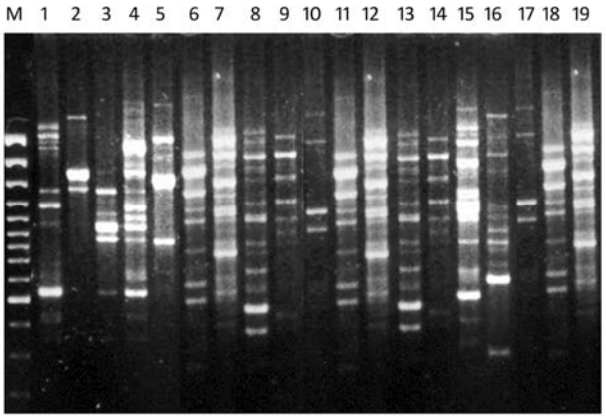
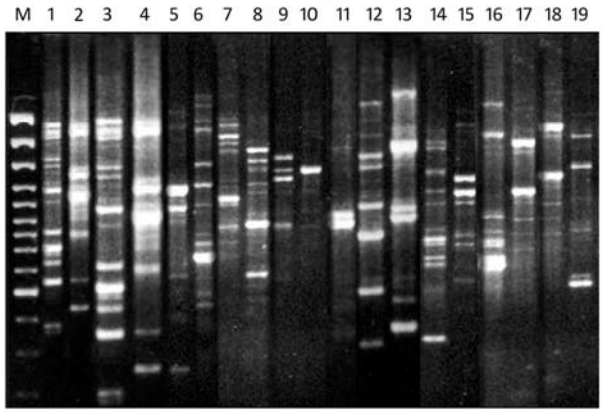


Fig.17.RAPD profile of *Curcuma* species obtained with primers OPA 10, OPA 11& OPA 12. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C.oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C.aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

OPA 05



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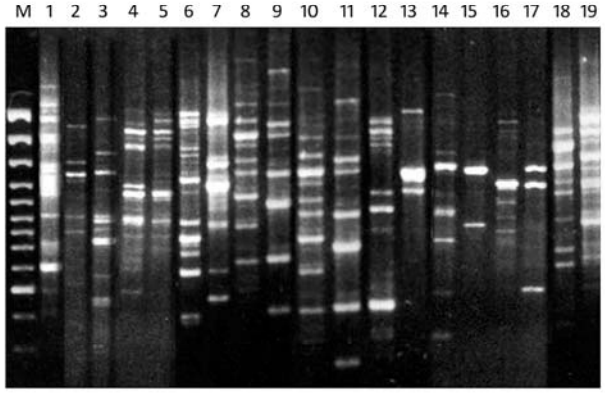

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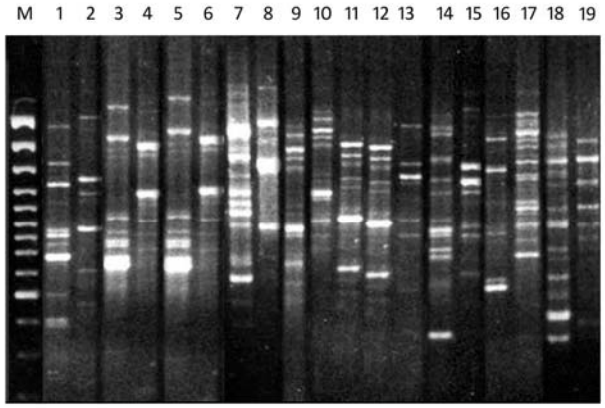

Fig.16.RAPD profile of *Curcuma* species obtained with primers OPA 05, OPA 07&OPA 08. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C.oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C.aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

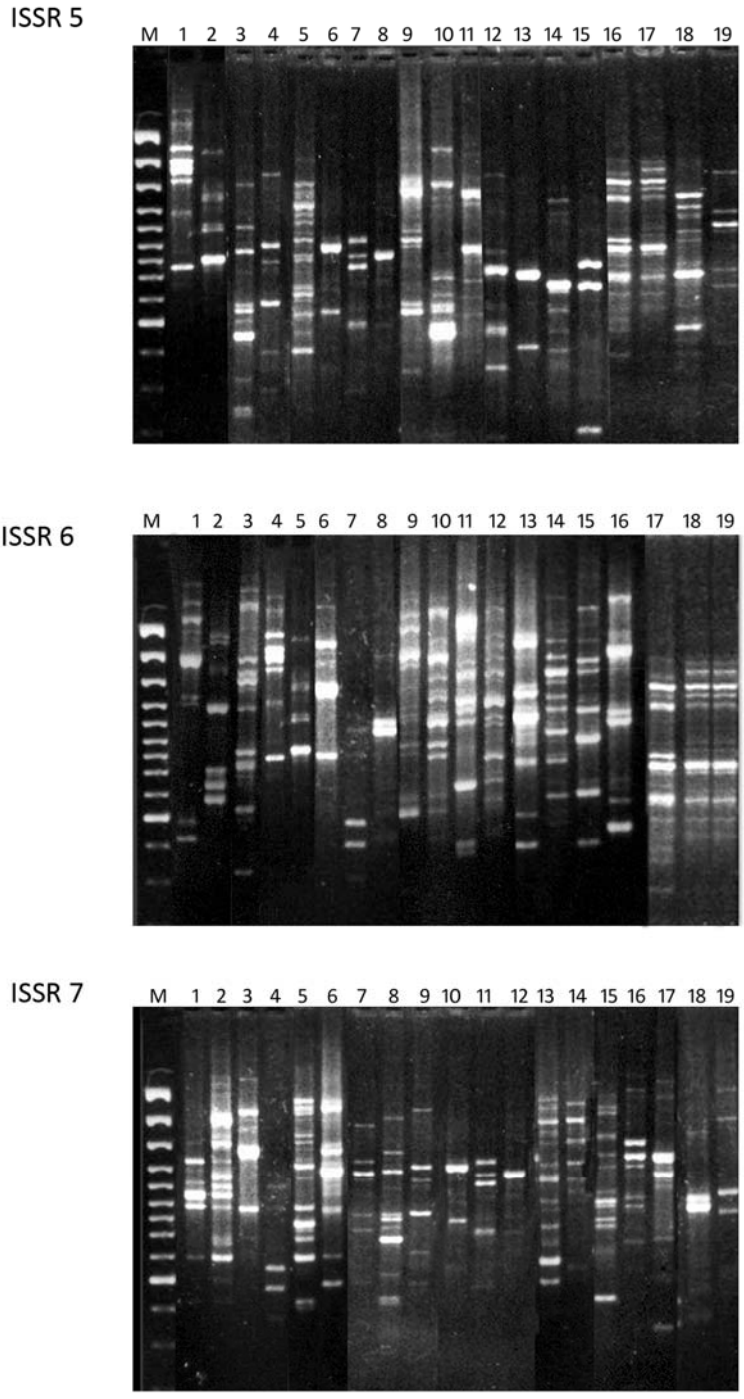

Fig.24.ISSR Profile generated in *Curcuma* species by primers ISSR 5, ISSR 6 & ISSR 7. M- Marker, 1. *C.karnatakensis*, 2. *C.mutabilis*, 3. *C.amada*, 4. *C.neilgherrensis,* 5. *C. oligantha,* 6. *C.haritha*, 7. *C.aromatica*, 8. *C. coriacea*, 9. *C. longa,* 10. *C.bhatii,* 11. *C. aeruginosa*, 12. *C.zanthorrizha,* 13. *C.montana*, 14. *C.pseudomontana*, 15. *C. raktakanta,* 16. *C.vamana,* 17. *C.decipiens*, 18. *C.aurantiaca*, 19. *C.inodora*

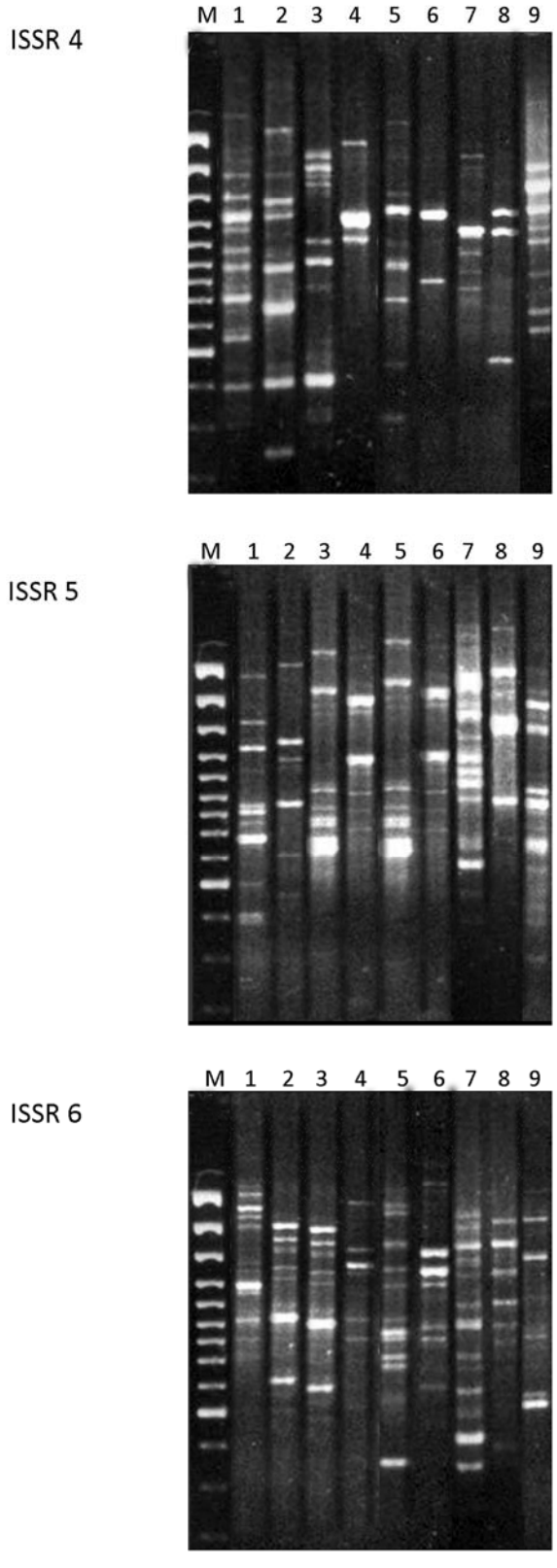

Fig.33.ISSR Profile generated in *Zingiber* species by primers ISSR 4, ISSR 5 & ISSR 6. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z.zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

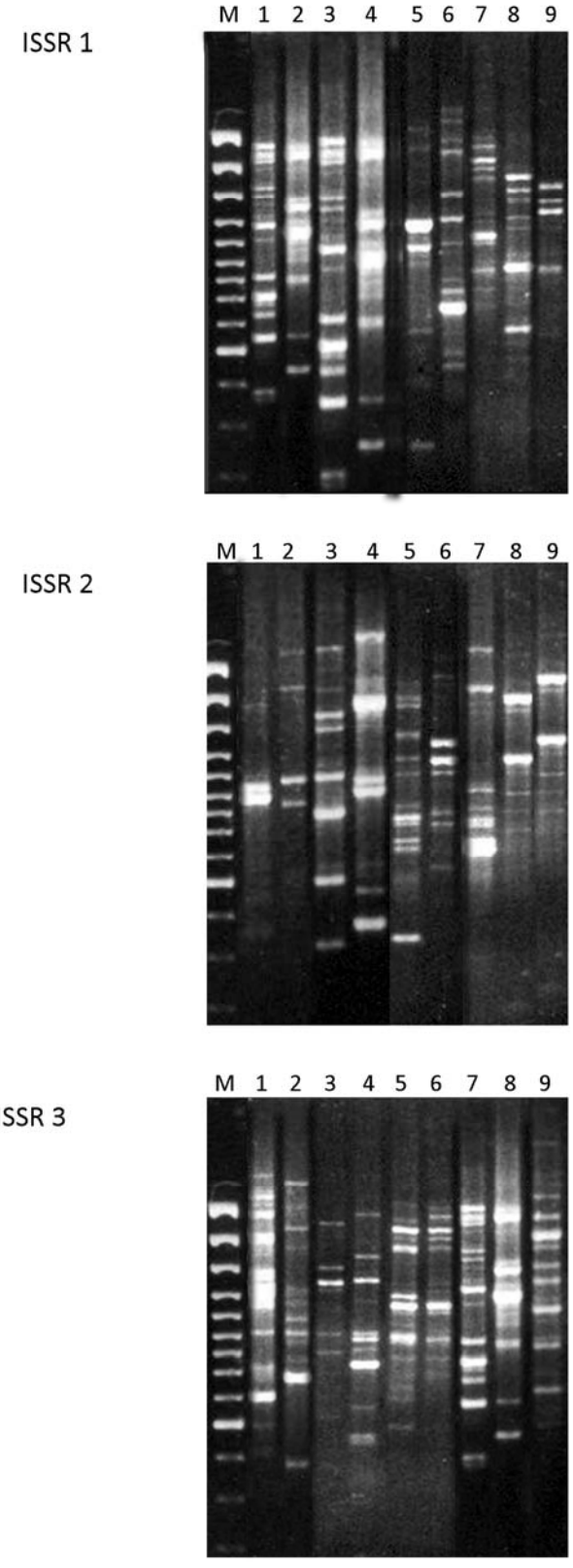

Fig.32.ISSR Profile generated in *Zingiber* species by primers ISSR 1, ISSR 2 & ISSR 3 M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z.zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

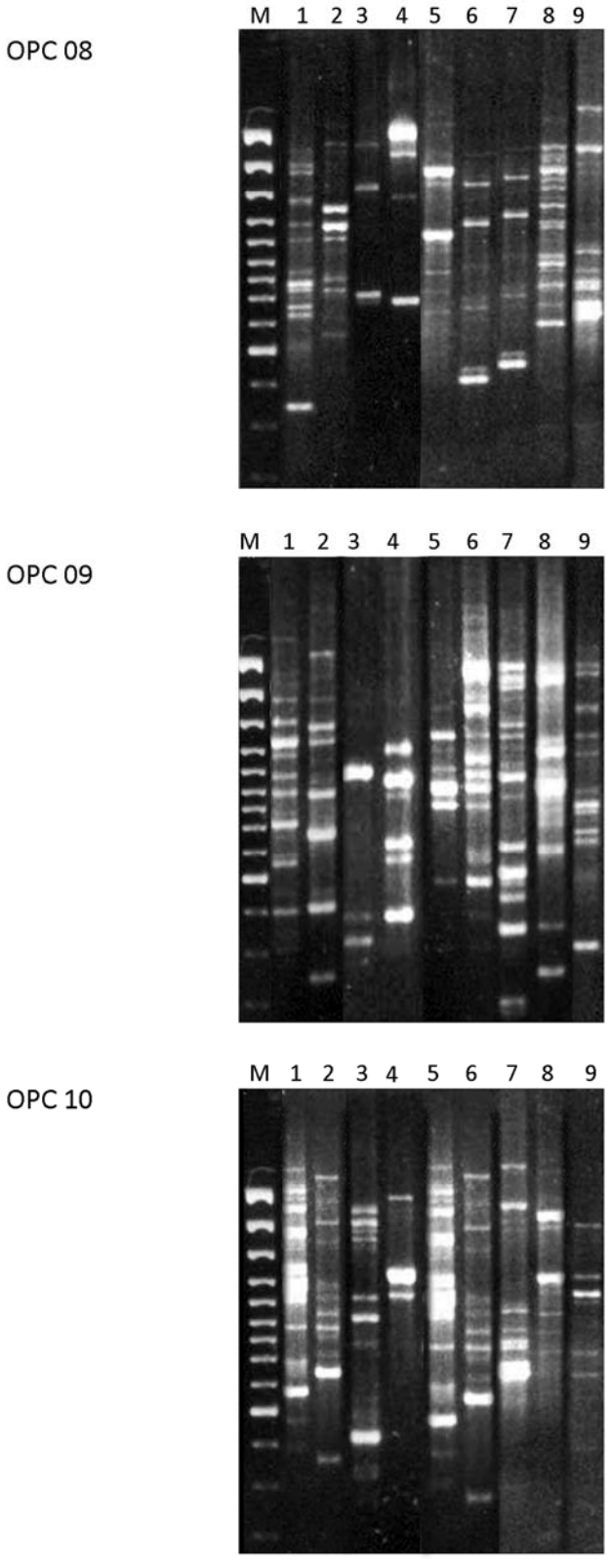

Fig.31.RAPD profile of *Zingiber* species obtained with primers OPC 08, OPC 09 &OPC 10. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z. zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

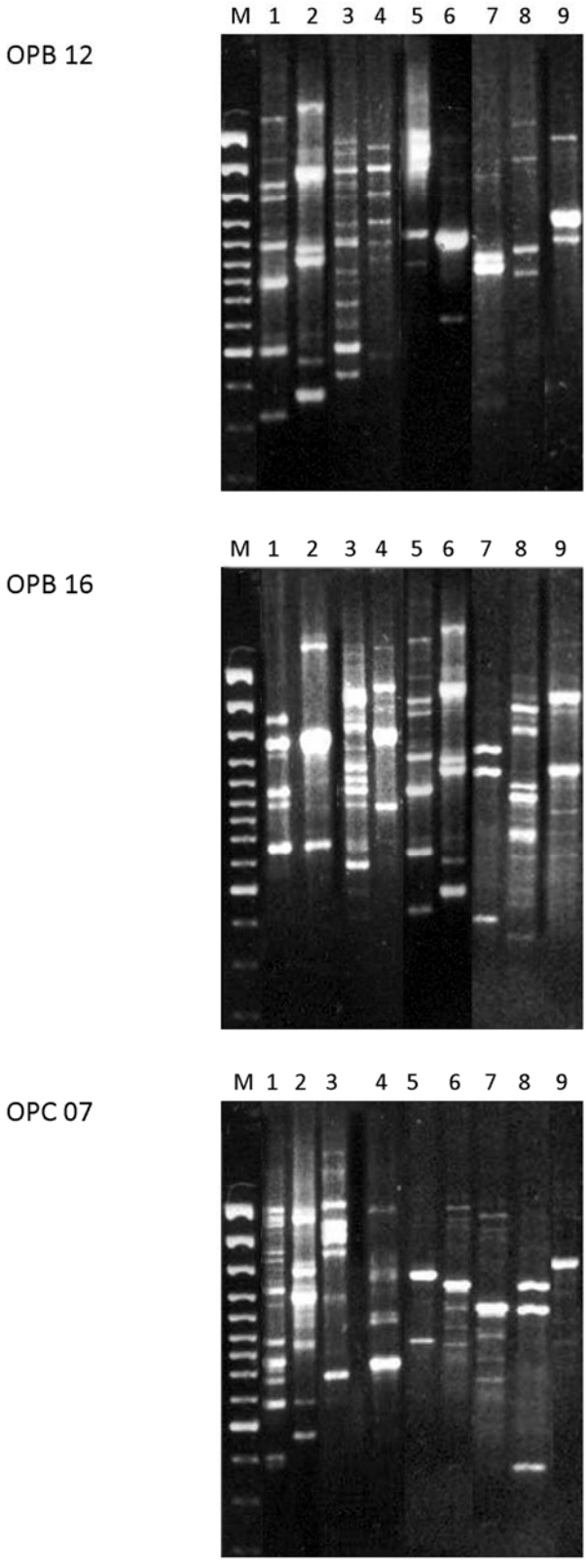

Fig.30.RAPD profile of *Zingiber* species obtained with primers OPB 12,OPB 16&OPC 07. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z. zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

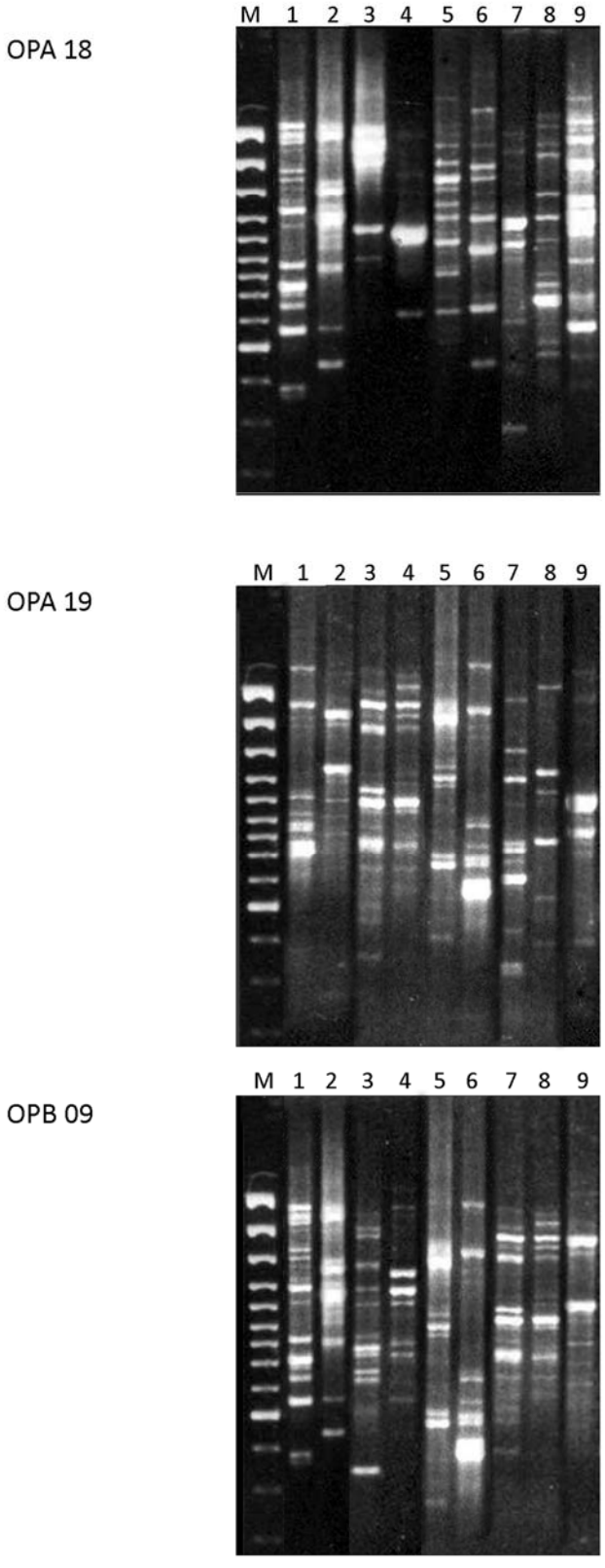

Fig.29.RAPD profile of *Zingiber* species obtained with primers OPA 18, OPA 19&OPB 09. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z. zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

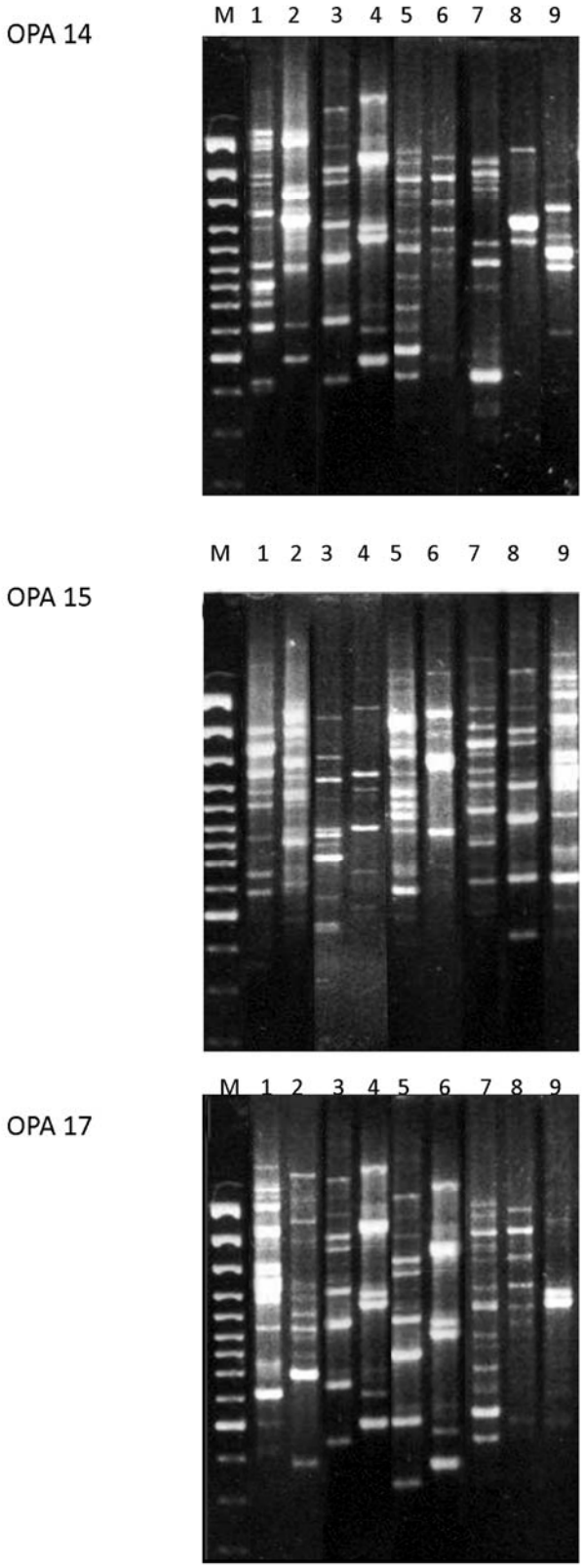

Fig.28.RAPD profile of *Zingiber* species obtained with primers OPA 14, OPA 15&OPA 17. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z. zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

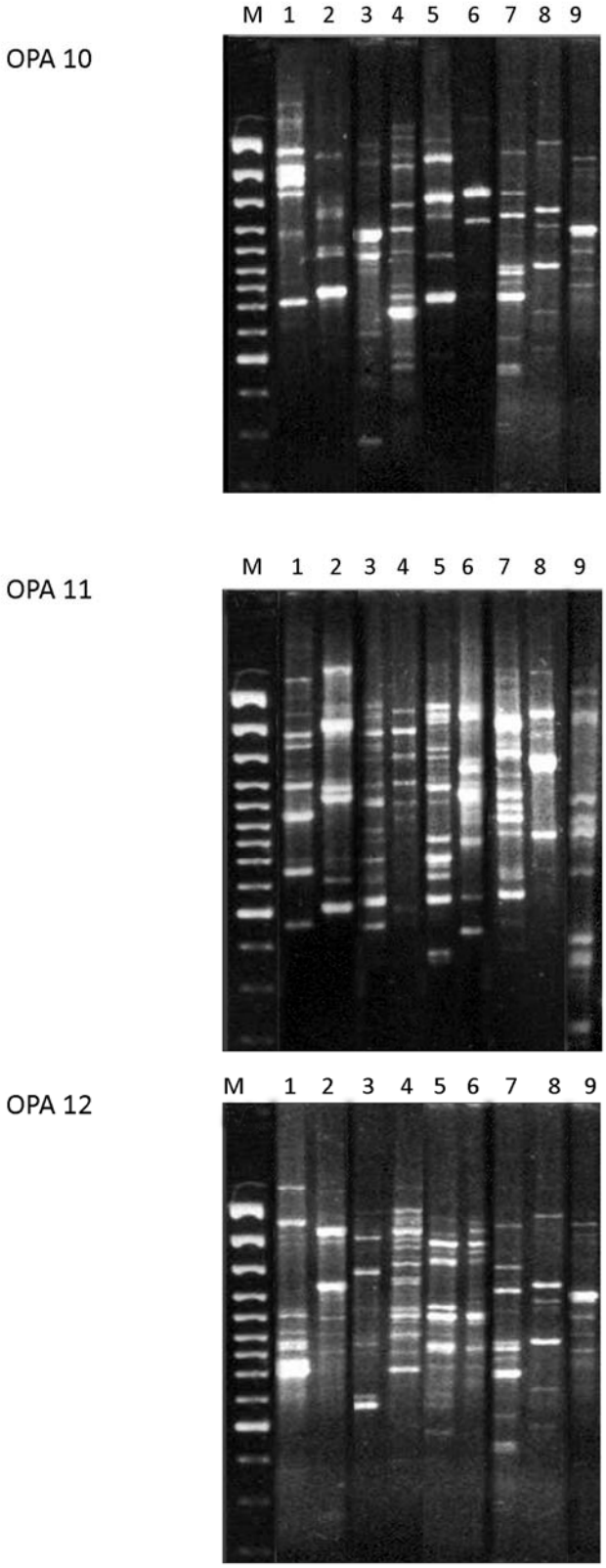

Fig.27.RAPD profile of *Zingiber* species obtained with primers OPA 10, OPA11&OPA 12. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z. zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

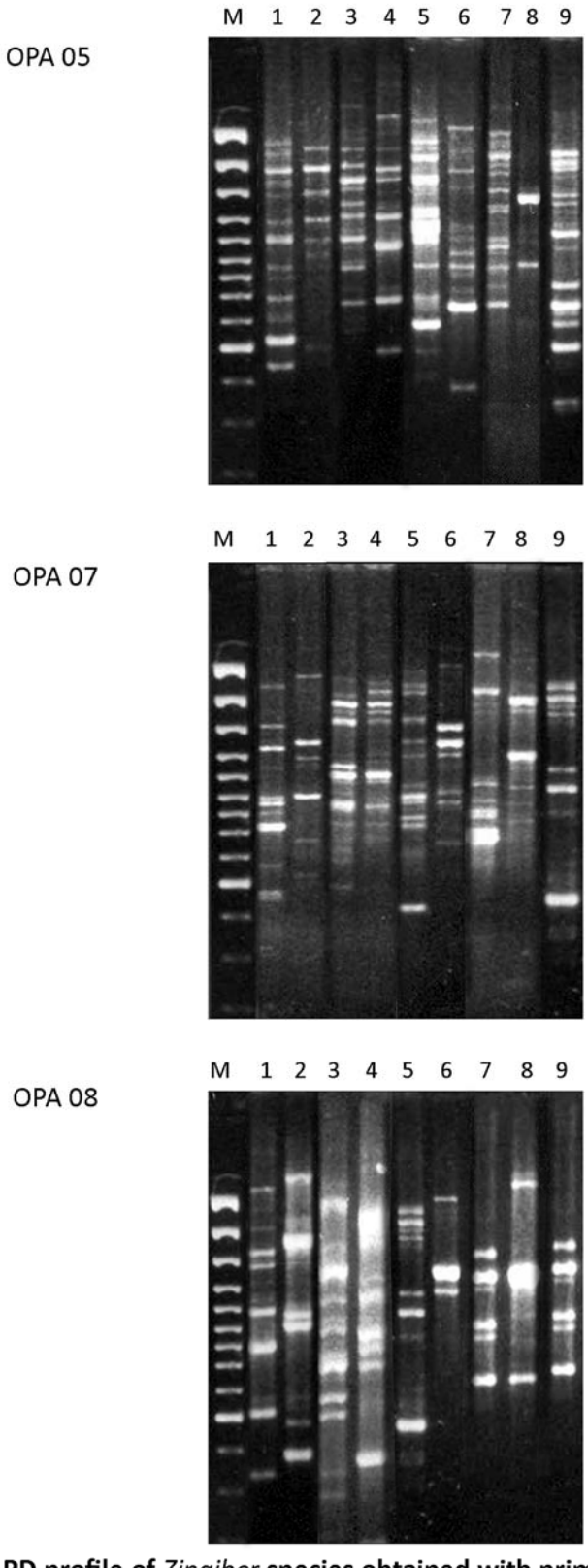

Fig.26.RAPD profile of *Zingiber* species obtained with primers OPA 05, OPA 07&OPA 08. M-Marker, 1 *Z.roseum*, 2. *Z.wightianum*, 3. *Z.nimmonii*, 4. *Z.cernuum* 5. *Z. zerumbet*, 6. *Z.officinale,* 7. *Z.neesanum*, 8. *Z.montanum,* 9. *Z.capitatum.*

Fig.15. A-D. *Zingiber zerumbet*: A. Habit; B. Inflorescence; C. Infrutescence; D. Flower.

Fig.14. A&B. *Zingiber roseum*: A. Habit; B. Inflorescence; C&D. *Zingiber wightianum*: C. Habit; D. Inflorescence.

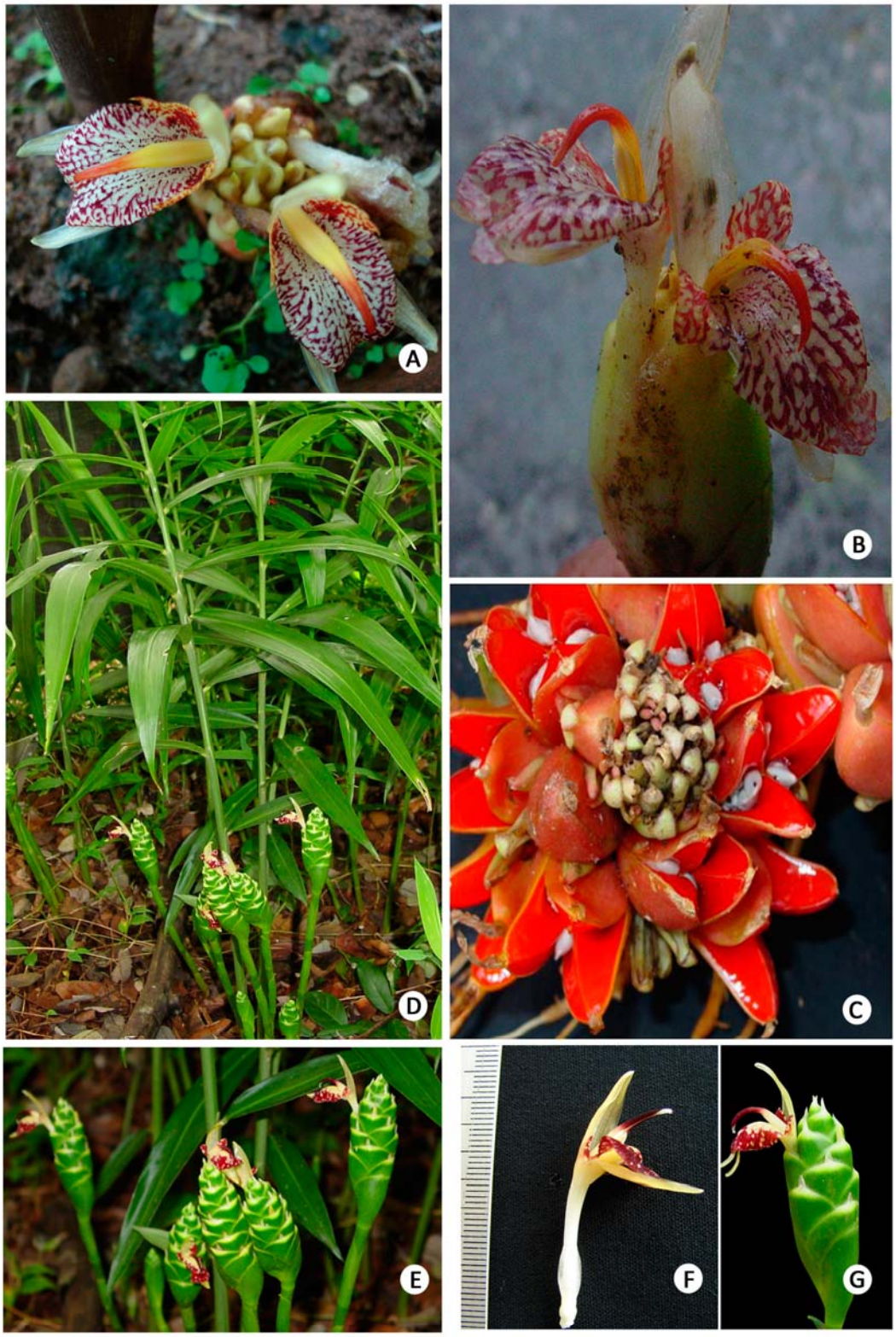

Fig.13. A-C. *Zingiber nimmonii*: A.Habit; B.Inflorescence; C.Infrutescence; D-G. *Zingiber officinale*: D. Habit; E & G. Inflorescence; F. Flower.

Fig.12. A-C. *Zingiber montanum*; A. Habit; B. Inflorescence; C. Flower; D-F.*Zingiber neesanum*: D. Habit; E. Infrutescence; F. Flower.

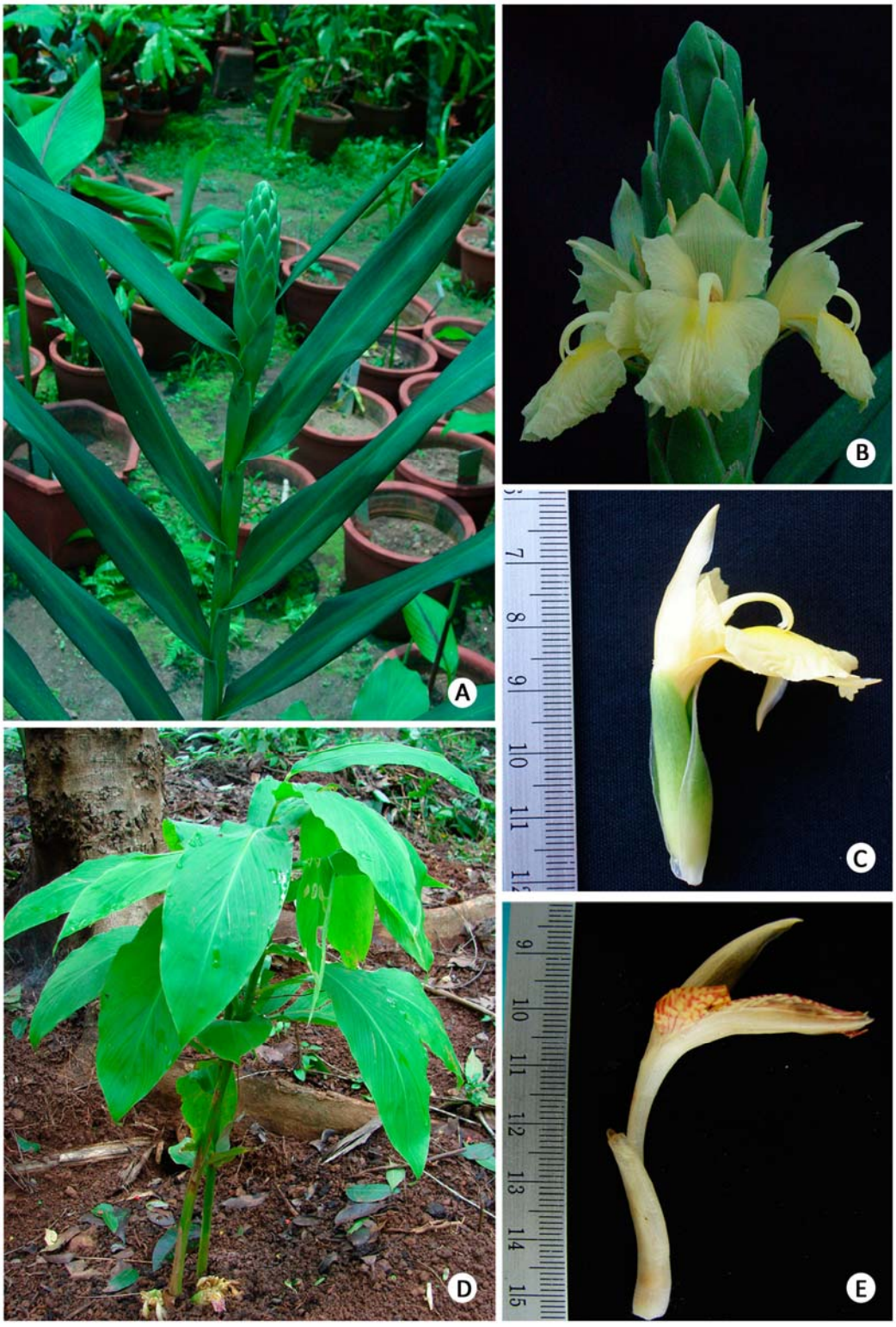

Fig.11. A-C. *Zingiber capitatum* var. elatum: A. Habit; B. Inflorescence; C. Flower; D&E. *Zingiber cernuum*; D. Habit; E. Flower.

Dr. A .YUSUF Ph.D.
Assistant Professor
Biotechnology Division

UNIVERSITY OF CALICUT
DEPARTMENT OF BOTANY
Calicut University P. O., 673 635,
Kerala, India.

CERTIFICATE

I hereby certify that the thesis entitled, **" Molecular Studies on selected members of the family Zingiberaceae "** submitted by **Mr. Rajesh Kumar, T.**, for the award of the degree of **Doctor of Philosophy** in Botany of the University of Calicut contains the results of bonafide research work carried out by him under my supervision and guidance. No part of the work has been submitted to any other university for the award of any other degree or diploma. All source of help received by him during the course of the investigation have been duly acknowledged. Certified that he has also passed the required qualifying examination.

C.U. Campus
14.05.2013

Dr. A. Yusuf

Mobile:09497192730 ; Email: akkara69@yahoo.co.in.