Characterization of bioactive components in plants with respect to their efficacy in the treatment of selected water contaminants

Thesis submitted to the
University of Calicut in partial fulfillment of the
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DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL SCIENCE

By

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CERTIFICATE

This is to certify that the thesis entitled "**Characterization of bioactive components in plants with respect to their efficacy in the treatment of selected water contaminants", s**ubmitted to the University of Calicut by Mrs. Snisha S., in partial fulfillment of the award of the degree of Doctor of Philosophy in Environmental Science is a *bona fide* record of research work carried out by her under my supervision and guidance. No part of the present work has formed the basis for the award of any other degree or diploma previously.

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This is to certify that both the adjudicators have not cited any correction/modification in the thesis entitled "**Characterization of bioactive components in plants with respect to their efficacy in the treatment of selected water contaminants",, submitted by Mrs. Snisha, S., Research Scholar in Environmental Sciences, Department of Botany, University of Calicut.** Hence the thesis is submitted as such to the University of Calicut with reference to the letter No.1444837/RESEARCH-C-ASST-1/2017/Admn dated 9/2/2018.

Calicut University
28th February 2018

Dr. C. C. Harilal
(Supervising Teacher)

DECLARATION

The thesis entitled "**Characterization of bioactive components in plants with respect to their efficacy in the treatment of selected water contaminants",** submitted by me in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Environmental Science of the University of Calicut is an original research work carried out by me under the guidance and supervision of Dr. C.C. Harilal, Assistant Professor, Division of Environmental Science of the Department of Botany, University of Calicut. No part of the work has formed the basis for the award of any other degree or diploma of any University.

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Snisha S.

CONTENTS

LIST OF TABLES

Dedicated to

MY Family

ABBREVIATIONS

g	:	grams
mg	:	milligram
HAT	:	Hours after treatment
BCM	:	Billion cubic meter
NTU	:	Nephelometric Turbidity Unit
DOC	:	Dissolved organic carbon
COD	:	Chemical oxygen Demand
DO	:	Dissolved oxygen
BOD	:	Biological oxygen Demand
SPSS	:	Statistical Package for Social Sciences
FT-IR	:	Fourier Transform Infrared spectroscopy
HPTLC	:	High performance Thin Layer Chromatography
LC/Q-TOF/MS	:	Liquid Chromatography/Quadruple – Time of Flight/Mass Spectrometry
PPAE	:	Plantain Peelings Ash Extract

GENERAL INTRODUCTION

Water is an inevitable entity for sustaining life on Earth. It can be evident from history that early civilization blossomed and thrived beside a surplus water resource (Mishra and Clark, 2013). Of all natural resources accessible, water plays a significant role in ensuring the well-being of mankind (Gibbons, 1986). It is inextricably linked to the development of social, culture, economic and environmental aspects of life. Though water is a renewable resource, it is finite (WWAP, 2009).

Even though three fourth of the Earth is covered with water, it is unevenly distributed over time and space. Oceans hold major share of all water on Earth (97.5%), but it is saline and cannot be utilized in routine life. Freshwater accessible to mankind thus accounts for 2.5% of total water on Earth. Considering global fresh water, a large proportion is available away from the populace or in places where life is practically not possible (Glaciers - 68.7%). Rest of the fresh water exists as ground water (29.9%) and only 0.26% forms the lakes and river systems (Shiklomanov, 2016). Thus, the amount of fresh water accessible to humans is less than 1% of all freshwater and 0.01% of all water on Earth (UNEP, 2002). Also the distribution of fresh water is highly randomized on Earth. The largest share is confining to the continents of America (45%), followed by Asia (28%), Europe (15.5%) and Africa (9%) (FAO, 2003).

India lies in south central position of Asia. The country supports about 17% of total world population, but possess only 4% of all water resource (UNICEF, FAO and SaciWATERs, 2013). Unevenness in the distribution of water resource can also be observed within the country due to various geographical and geological reasons. For instance, rainfall is greater in states

lying in eastern region of the country namely Assam, Arunachal Pradesh, Manipur, Mizoram, Tripura, Meghalaya, Nagaland and Sikkim (Mall et al., 2006). However these places face seasonal droughts due to lesser water retention capacity owing to terrain characteristics. Snow melt of Himalayan glaciers from water replenishes the water needs of northern region of the country, which keeps almost all rivers in the northern region perennial. Southern states like Karnataka, Tamil Nadu and Kerala fulfill their water needs from monsoons and monsoon fed rivers (India Water Portal, 2017).

Much of extremities in weather pattern can be observed within the country and can be the cause of differences in water resources. For instance there are states which receives too little rainfall of 100mm per year to world's highest rainfall of 10,000mm annually (Ministry of Water Resources, 2014). Rain and snow received by the nation ranges around 4000 billion km^3 (BCM) annually (CPCB, 2010; Kumar et al., 2005; Ministry of Water Resources, 2011). Even with this, 20% of India's total land area is drought prone and 68% of the entire country is facing drought in varying degrees (Kumar et al., 2013). About 99 districts spread over 14 states have been identified as drought prone areas by Central Water Commission (CWC) (Mall et al., 2006). Most of the drought prone areas are concentrated in the states of Rajasthan, Karnataka, Andhra Pradesh, Maharashtra and Gujarat (Mall et al., 2006).

As per CPHEEO (2005), in India, about 70-80% of total water supplied for domestic purpose is turning to wastewater. Approximately 38,354 MLD (Million Liters per Day) sewage is generated from major Indian cities (Kaur et al., 2012). The total capacity of installed sewage treatment plants are below 12,000MLD leaving behind a gap of 26,468MLD (Kaur et al., 2012). Installing proper sewerage systems are capital intensive. It has been anticipated that for establishing treatment systems for the entire domestic wastewater in India costs around Rs. 7,560 crore, which is 10 times higher

than the Indian government planning to spend on such matters (Kaur et al., 2012).

About 21% of communicable diseases prevailing in India are mainly related to the deterioration in quality of water. Majority of inland rivers, which are the sources of drinking water in urban and rural areas are contaminated (Srikanth, 2009). It has been estimated that about 50 million cubic meters of untreated sewage is discharged into them each year (Rao and Mamatha, 2004). Apart from the microbial contamination, several states are facing increased levels of fluoride (fluorosis) and arsenic (arsenicosis) (Rao and Mamatha, 2004). Seventeen Indian states have been identified with the problem of excess fluoride in groundwater resources till 1999. The problem of fluorosis is severe in India as almost 80% of the rural population depends on untreated groundwater for potable water supplies. Several states like Northern Gujarat, Southern Rajasthan, Saurashtra, Coimbatore and Madurai districts of Tamil Nadu, Kolar district of Karnataka, the whole Royalseema region of Andhra Pradesh and parts of Punjab, Haryana and Uttar Pradesh have been detected with declination in terms of quantity of water in the range of 1-2m/year (Singh and Singh, 2002).

Though Kerala is regarded as one among the wettest places in India, the situation in the state is not much different from any other arid state in the country (Infochange, 2004). Kerala is adorned with 44 rivers and a flourishing monsoon, spreading over a period of six months. But still the state is heading for water crisis since 1980 (Infochange, 2004). The state receives annual rainfall ranging between 2000-3000mm per year, which is 2.5 times higher than the national average (NIDM, 2014). Despite all, recent study conducted by the Center of excellence in Environmental Economics (CEEE) of Kerala Agricultural University (KAU) has predicted that the state is going to face a severe water scarcity by 2021 (Santhosh, 2014). Due to high extent of

deforestation, sand mining from rivers etc. the time required for the water to get imbibed is reduced and water gets drained easily to sea within hours after downpour. The undulating topography and steep terrain of the state also accelerates the situation.

Earth possesses livable quantity of water. But unwarranted and uncontrolled uses lead to the present resource shortage. Apart from the naturally occurring disparity and unevenness in water resources, use and abuse of water still continues to quench the thirst of such an outsized world population. Factors like population surge, industrialization, urbanization, advancements in agriculture and food consumptions, etc. are also responsible for the present situation. Interestingly all these factors are interrelated and unless properly addressed will lead to an irreversible impact on life on Earth. Unconstrained water withdrawals had also put many regions of the world under severe water stress. According to WWAP (2014) global water withdrawals will increase by 55% by 2050 and 40% of the world population will be thriving in severe water stress areas by 2050. Water demand in the country are also expected to grow by 20% and 40%, in industrial and domestic sectors, respectively (Utamsingh et al., 2010). It is anticipated that strayed demand and supply of fresh water will create a large gap of 754BCM (Billion cubic meter) in the country by the year of 2021. Kerala is also going to face a gap of 1,268 billion liters of water between the demand and supply (UNICEF, FAO and SaciWATERs, 2013). Thus the challenge for the coming years will be to manage the balance between the needs of both people and ecosystems (Vorosmarty et al., 2005). Unless the condition is not restored, the world will face an increasingly severe global water deficit (WWAP, 2015).

Apart from issues related to quantity, water gets polluted from anthropogenic interferences like landfill and garbage dumps, improper sanitation, wastewater disposal, fertilizers and pesticides used in agriculture,

waste water expelled from mining, textile industries, tanneries etc. aggravate the situation further. Many cities in developing nations lack necessary infrastructure to treat wastewater. About 80% of water used is converted to waste water, creating an imbalance in hydrological cycle. It is estimated that 90% of all wastewater in developing nations are discharged directly into rivers, lakes or to the oceans, causing major environmental and health risks (UN-HABITAT, 2010). Volumes of water thus are getting contaminated in one way or other. It has been projected that about 2 million tons of sewage, industrial and agricultural wastes are discharged into the world's water every day. Similarly about 1500km^3 of wastewater is expelled annually, which is six times more than that exists in all the rivers of the world (UNWWAP, 2003). The spent or used water may contain harmful dissolved and suspended particulates that may hinder the recycling process.

Dirt and particulate matter can get into water during its flow. In such cases, surface water is more susceptible to contamination than ground water. Hence it became necessary for water to undergo appropriate treatment process before and after reaching a population. Usually water treatment process involves a series of steps like sedimentation, coagulation, flocculation, filtration and finally disinfection (Baghvand et al., 2010). Nowadays, apart from the conventional water treatment processes, multitude of other methods are available which include, precipitation, extraction, evaporation, adsorption on activated carbon, ion exchange, advanced oxidation, incineration, electro-floatation, electrochemical treatment, biodegradation and membrane filtration (Dąbrowski, 2001; Lefebvre et al., 2006; Matsumoto et al., 1996).

Coagulation / flocculation is one among the critical process involved in most surface water treatment process (Davis et al., 2014). It is employed to destabilize the colloids in water. Coagulation using chemical coagulants facilitate the aggregation of insoluble particles and / or dissolved organic

matter into large aggregates (Renault et al., 2009). Colloids impart murkiness to water and can sometimes harbor harmful microorganisms or chemical species (Davis et al., 2014). Colloids which get into water are difficult to settle because of their small size and surface charges. Generally colloids are negatively charged. Since they have similar charges, they repel each other and prevent themselves from settling. Thus for their removal, they have to undergo destabilization. Destabilizations of colloids are facilitated by the coagulants. Coagulants widely used are based on aluminium and iron, which can destabilize their surface charges and facilitate settling. They get hydrolyzed when added in water, generating numerous positively charged species which can bring about their settling. Recently pre-hydrolyzed salts like polyaluminium chloride (PACl) have gained attention because of their advantages over conventional hydrolyzing aluminium and iron salts (Wei et al., 2015). Though they have gained much popularity, their mode of action is not completely understood, especially with regard to their charge neutralization mechanism.

Apart from efficiency, the health threats heaved from the use of chemical coagulants has resulted in the resurgence of natural coagulants. It has been opined that residual aluminium in drinking water due to alum can trigger severe neuro-disorders like Alzheimer's disease, dementia etc. (Crapper et al., 1973; Perl, 1985; Rondeau et al., 2000). Further, cost of importing coagulants for conventional water treatment in developing nations might be high and at times prohibitive (Sanghi et al., 2006). Moreover the rural sector is completely deprived of such treatment facilities. Adverse impacts of such chemical coagulants on environment also compel to go for natural, affordable and environment friendly alternatives.

Natural coagulants were once the choice to remove impurities from water. But with the advent of chemical coagulants, their popularity

diminished and got confined to rural villages in developing nations. The use of seeds and earth as natural coagulants in villages of Sudan has been reported for the first time by German scientist Samia Al Azharia Jahn (1977). Sudanese women utilized these natural coagulants as a point of use of drinking water treatment. Jahn (1977) had mentioned that this mode of water purification methods where prevalent in ancient India and China in remote past.

As per WHO (2013) and UNICEF (2013) 0.78 billion people in entire World lack safe water to sustain their life. The urban (30%) and rural (90%) population in India depend on both surface and ground water for meeting their water requirements (Kumar, et al., 2005). Of this, 67% of the rural population does not have access to treated water, which is leading to various water related diseases. The reason underlying are shortages in water resources and the lack of reach of modern water treatment techniques owing to financial constraints (Wankhade, et al., 2014).

The fragile links between water, health and financial sectors could be improved by such in home water purification practices (Lantagne et al., 2006). In India, point use of disinfectants like chlorine and bleaching powder are promoted as a mode of disinfection of household and community water sources, including wells / tanks and other surface water bodies. These chemicals are being dumped indiscriminately with no guidelines for regulating dosage and contact time of chlorination, leading to excess residual chlorine (Srikanth, 2009). Apart from chlorination, no other practice is followed for the removal of other contaminants. The use of plant coagulants in water treatment in house hold systems among the rural population in developing nations, especially India, is reported in several literatures. The emergence of such innovative techniques can provide clean and safe water for the rural population in developing nations without much effort and cost. Such

efforts on the validation of plant based coagulants will be a scientific validation of those practices which were followed in the rural sector of our country in previous decades. The present study is an attempt in this direction. It is attempted with a set of objectives in the following manner.

Chapter I - Screening of plants / plant parts with potential stabilization / removal properties against selected water quality parameters /contaminants like pH, turbidity, iron and fluoride.

Chapter II - Characterization of Phyto-constituents in plant materials responsible for treatment efficiency.

CHAPTER I

SCREENING OF PLANTS / PLANT PARTS WITH POTENTIAL STABILIZATION / REMOVAL PROPERTIES AGAINST SELECTED WATER QUALITY PARAMETERS.

Introduction

Use of modern coagulants for water treatment is only more than a century old (Jiang, 2015). Before the advent of any forms of water treatment, inspecting the clarity of water alone was the criteria to judge its quality. Health concerns on drinking water evolved over time.

The inseparable link between water quality and health established only in 19th century with the report of Snow (1855) which demonstrated cholera epidemic as a result of water contamination. From then the water treatment industry has dedicated on preventing any water borne disease. However, from 1970, the objective of water treatment had become complex from rather preventing the disease outbreaks to abate chronic impact of emerging anthropogenic contaminants (Crittenden et al., 2012). Since then water treatment process like coagulation, flocculation, sedimentation and filtration were followed to remove contaminants from water resources (Teh et al., 2014). Subsequently, disinfection method was also included to check the harmful organisms in water. Coagulation is still one among the important step in wastewater and surface water treatment. It became inevitable as it facilitates the removal of particulates, microorganisms, natural and synthetic organic matter, precursors of disinfection by-products, inorganic and metal ions from water and wastewater (Choy et al., 2015; Jiang, 2015; Saritha et al., 2015).

The matter of risk in the use of chemical coagulants led to the search for environmental friendly natural coagulant alternatives. This paved way for the resurgence of plant derived coagulants, which were once popular among the rural population and became obsolete with the explicit use of chemical coagulants (Miller et al., 2008; Yin, 2010).

Most popular plant derived coagulants in use are from *Moringa oleifera* (Jahn and Dirar, 1979; Nkurunziza et al., 2009)*, Strychnos potatorum* (Mohamed et al., 2014; Packialaksmi et al., 2014)*, Opuntia* spp. (Miller et al., 2008; Mukhtar et al., 2015; Nougbode et al., 2013) etc. They have been worked out for their coagulation efficiency in removing several water contaminants like turbidity (Sciban et al., 2009), microbial population (Eilert et al., 1981), heavy metal (Mane et al., 2011), COD (Zhang et al., 2006) etc.

Though several works have endeavored to screen out potent plant coagulants, studies are mainly restricted to certain facets like limited number of plant candidates and recurring experiments on limited number of water contaminants. Here, in the present study, two dozen plants belonging to 17 families were screened for assessing their efficiencies in treating selected water quality parameters / contaminants like pH, turbidity, iron and fluoride. Plants with higher percentage removal efficiency were further subjected to standardization studies to optimize the conditions at which treatment efficiency is higher.

Review of Literature

The aim of water treatment is to remove physico chemical impurities and biological contaminants in order to meet the quality guidelines for drinking water (WHO, 2004). Treatment of water can be either physical, chemical, mechanical or even biological (Arnoldsson and Bergan, 2008). Mechanical means targets the removal of physical components from water.

Later, chemical coagulants gained wide acceptance as they are competent enough to remove suspended, colloidal and dissolved solids from water including hardness, colour, turbidity and even undesirable microorganisms (Greville, 1997). The present and conventional water treatment system comprises of methods like coagulation, flocculation, sedimentation, filtration, disinfection (Cañizares et al., 2009; Gidde and Bhalerao, 2010; Thakur and Choubey, 2014) etc. which are falling in the category of both physico-chemical and biological means.

Coagulation and flocculation have been the choice of removing colour, turbidity and natural organic matter (NOMs) from water (Aboulhassan et al., 2006; Chang et al., 2009). Generally the suspended and dissolved particulates are charged particles which repel and prevent each other from settling. Coagulants aid in neutralizing their charges and facilitate their settling. Most widely accepted and used coagulants are based on aluminium and iron (Abidin et al., 2013; Duan and Gregory, 2003).

Regardless of the superiority of chemical coagulants in treating polluted water, most of them violate the mandate of green chemistry. Constant use of such chemicals is found to be detrimental to nature as well as humans. Though the traditional chemical coagulants used in water treatment are considered to be cheap at price, there are even countries who can't afford it (Rahman et al., 2015). As the complexity of chemicals reaching water is high, additional treatment methods like reverse osmosis, proteolytic cleavage, nano-filtration, ultra-filtration, electro-dialysis etc. is suggested which make the treatment of water more expensive (Bodlund, 2013; Choy et al., 2014; Marobhe, 2008; Schutte, 2006). Also in the case of synthetic coagulants like PAC (Polyaluminum chloride), the unit cost is approximately 4-6 times higher than that of alum, hence they are not widely used (Elangovan, 2014). As per Simate et al., (2012) during 1960's the adverse effects of chemical

coagulants on human health were reported. Presence of residual aluminium in finished water are linked with the neuro disorders like Dementia, Alzheimer's disease etc. (Flaten, 2001; Gauthier et al., 2000; Nkhata, 2001; Rondeau et al., 2000).

Chandrasekaran et al., (2010) had reported higher concentrations of iron compounds in water can cause cancer, vascular diseases and neurological disorders. Health concerns over the use of alternative synthetic coagulants like polyacrylamide and poly diallyl-dimethyl ammonium chloride restricts their use (Hu et al., 2013). Various water treatments for removing the water contaminants are available in almost all developed nations. But the case is different in developing nations, where unaffordable cost and availability of chemicals for meeting the needs of such a huge population are acting as hindering factors (Oria-Usifo et al., 2014; Pritchard et al., 2009).

All these concerns like exorbitant price and adverse effects of chemical coagulants to human health and environment have diverted the attention of the scientific community to a cost effective, environment friendly water treatment devise. These deliberations resulted in the resurgence of natural coagulants. Utilizing plant materials as coagulant is not a new practice. It has been mentioned in several ancient texts that people have been depending on various plant parts, parts of Earth, metal, sunlight etc. to remove the dirt and suspended particulates from water (Schulz and Okun, 1983). With the advent of chemical coagulants, natural coagulants became infamous, but still popular among villages in developing nations where they lack modern water treatment amenities.

Several religious texts and early civilization had ascribed various methods of water treatment followed during ancient times. Baker (1948) has opined that earliest recorded knowledge on water treatment can be seen in the Sanskrit medical lore and Egyptian inscriptions. "Susruta Samhita", collection

of Indian medical lore probably dated back to 2000 B.C. had described techniques like boiling water over fire, expose to sunlight or dipping several times a piece of hot copper etc. to make foul smelling or turbid water clear and aesthetically pleasing (Baker, 1948). There are evidences that crushed kernels of almonds, apricots and peaches were once used in Egypt, Sudan, Tunisia, Lesotho, South Africa and in Bolivia for water clarification (Jahn, 1988). Various bean cultivars like peas, peanuts, lupines were also used for treating water in Egypt and North Sudan. Sap of certain cactus were also used for clarifying water in Peru, Chile and in Haiti (Gassenschmidt et al., 1995a). African nomads used the paste of the seeds as a natural coagulant for water purification in Sudan and parts of Africa in the past (Olayemi and Alabi, 1994). Rao (2005) have also reported that natural coagulants have been in use since ancient times. Powdered, roasted grains of *Zea mays* were used by soldiers of Peru for settling impurities in the 16^{th} and 17^{th} century.

Natural coagulants were popular among the village and tribal population of African and Asian countries. For instance, Jahn in 1977 had reported various traditional practices prevailed in riverine Sudan. They used traditional coagulants in the form of suspension or extract to achieve flocs. Along with the natural coagulants Sudanese also used muddy earth known as "Rauwaq" which was later identified as bentonite clay. For attaining successful coagulation "Rauwaq" or plant material were crushed and added to water in a small bowl, stirred for 10min and then poured into a jar (Olsen, 1987). Jahn had also reported the use of *Moringa* seeds as coagulant in African and Asian countries, where people can't afford the conventional water treatment techniques (Jahn, 1988). In India, seeds of *Strychnos potatorum*, commonly known as Nirmali tree were used to clarify water. People rub the seed inside the pot several times, releasing the active components attaining clarification of turbid water (Cohen et al., 1958; Subbaramiah and Rao, 1936).

Studies related to plant based natural coagulants were mainly focused on *Moringa oleifera*, Nirmali seeds, and *Opuntia ficus indicus* (Yin, 2010). Various aspects of coagulation using *Moringa oleifera* like optimum conditions, nature of the finished water, characterization of bioactive components etc. have been studied in detail by several authors. *Moringa* seeds were studied for its coagulation efficiency in surface water (Lea, 2014), wastewater (Vieira et al., 2010) and effluents from coffee plant (Padmapriya et al., 2015). Various contaminants extensively studied include turbidity (Abaliwano et al., 2008; Gidde and Bhalerao, 2010; Lea, 2014; Prasad and Rao, 2013), heavy metal (Ravikumar and Sheeja, 2013a), hardness (Muyibi and Evison, 1996), color removal (Prasad and Rao, 2013), microorganisms (Abaliwano et al., 2008; Lea, 2014), NOM (Natural Organic Matter) removal (Abaliwano et al., 2008) etc. In all the cases, parameters investigated were found to get reduced considerably with the addition of *Moringa* seeds. The seeds were also able to remove heavy metals like copper, lead, cadmium and chromium (Ravikumar and Sheeja, 2013a). The seeds were also found to have microbial disinfection property. It was able to reduce microbial content from surface water (80-99.5%) and accompanied by bacterial reduction (90% to 99. 99%) was reported (Lea, 2014). *Moringa oleifera* seeds have been reported for its iron removal capacity by Ravikumar and Sheeja, (2013b). Optimum dosage of 2.5g/L *Moringa* seeds achieved a 92% removal of 10mg/L of fluoride. Fresh leaves of *Azadirachta indica*, *Acacia catechu* and *Ficus religiosa* were studied for fluoride uptake. Jamode et al., (2004) investigated various pH (2, 4, 6 and 8) with a series of aqueous solutions with varying fluoride level. Fluoride gradually decreased to 0 mg/l within 180 min at 29 ± 0.5°C when the dose of adsorbent is 10 g/l in a sample of 50 ml volume.

Scientific study on the seeds of *Strychnos potatorum* was carried out in 1936, when Subbaramiah and Rao (1936) demonstrated that muddy water can

be clarified using the paste prepared from the seeds of *Strychnos potatorum*. High affinity of Nirmali (*Strychnos potatorum*) seeds for hydrophobic colloids such as turbidity was illustrated by Tripathi et al., (1976). Treatment studies at laboratory scale with the seeds exhibited substantial improvement in turbidity and microbial count. Babu and Chaudhuri (2005) opined that the seeds of *Strychnos potatorum* can be used as a point of use coagulant. They also suggested that these methods can give low risk water. The seeds were able to reduce turbidity at low dosages 0.25 to 3.5 mg/L. It acts as a good coagulant and a coagulant aid at higher turbidity 1000-3000 NTU (Deshmukh et al., 2013). The effectiveness of *Strychnos potatorum* in the removal of turbidity, total hardness, pH and Total Dissolved Solids (TDS) has been investigated by Packialaksmi et al., (2014). Cadmium (II) adsorption capacity of *Strychnos potatorum* seeds were studied and found that removal was pH dependent and the maximum removal was at pH 5.0 in a time span of 360 min (Saif et al., 2012). *Strychnos potatorum* seeds were also investigated for iron removal from steel plant effluent. Plant material was effective in removing pH, turbidity, TDS, BOD and coliforms (Maruthi et al., 2013).

The slimy mucilage obtained from *Abelmoscus esculentus* (Okra) seed pod was used as an effective natural coagulant in treating synthetic and industrial effluents (Agarwal et al., 2003, 2001; Al-Samawi and Shokralla, 1996; Anastasakis et al., 2009; Okolo et al., 2014). According to Al-Samawi and Shokralla (1996), okra extract is a powerful polyelectrolyte. They studied coagulation activity of okra extract derived from seed pod tips, sap, plant stalk and roots. A wide range of turbidities up to 3000 NTU has been studied. With the usage of alum in conjunction with okra mucilage minimized the alum consumption by 50% (Al-Samawi and Shokralla, 1996). According to Agarwal et al., (2003) gum of the plant material was found to be more effective. When tannery effluent was added with mucilage, about 95% suspended solid and 69% dissolved solid were removed from the effluent.

Similarly okra mucilage was found to reduce turbidity in fiber-cement industry effluent (Ani et al., 2012).

Zhang et al., (2006) demonstrated coagulant proficiency of cactus in removing turbidity. Yang et al., (2007) and Miller et al., (2008) also studied the turbidity removal capacity of *Opuntia*. While Mane et al., (2011) studied the heavy metal removal capacity of cactus mucilage, Sellami et al., (2014) investigated the bioflocculant property of cactus juice in treating industrial wastewater from different industries. Zhang et al., (2006) studied on the effects of factors like pH, temperature, alkalinity etc. on cactus. Removal efficiency was dependent on polyelectrolyte concentration and agitation speed while removing heavy metals from water (Mane et al., 2011). Efficiency of cactus was also dependent on the nature of the test water (Sellami et al., 2014). The optimum dosage was 10% (v/v) polyelectrolyte for removing both the Cr and Ni ions. The maximum removal capacity of polyelectrolyte was 68% and 88.4% for Cr and Ni ions respectively (Mane et al., 2011). Cactus juice was able to remove suspended solids and COD in the range 83.3% - 88.7% and 59.1% to 69.1% respectively (Sellami et al., 2014).

Another natural polysaccharide from fenugreek mucilage was reported for its coagulation potential by Mishra et al., (2003). It was able to remove almost 97% of suspended solids and 20% of TDS. Ghebremichael (2004) investigated coagulant efficiency of Pumice and *Moringa oleifera* seeds. Pumice seeds were found to be an alternative material for dual media filtration in both lab and pilot scale experiments. Sharma et al., (2006) had modified gum from *Cassia tora* and *Cyamopsis tetragonoloba*. This modified coagulant have been tested against kaolin suspension and compared with polyacrylamide based synthetic coagulant. Their work concluded that the modified seed gums have potential to replace synthetic coagulants.

Gunaratna et al., (2007) compared the coagulation activity of red bean, sugar maize and red maize with a known coagulant *Moringa oleifera*. They found that these coagulants are promising alternatives to *Moringa oleifera*. Marobhe et al., (2007) purified proteins of *Vigna unguiculata* and *Parkinsonia aculeata* seeds, which are indigenous water coagulants in rural areas of Tanzania. They were thermo-resistant with higher pH ranges of 8.5. Okonko and Shittu, (2007) showed the potential of latex of *Calotropis procera* in treating both domestic and industrial waste water. The results obtained were comparable with coagulants like *Moringa oleifera*, ferric chloride and alum.

Ash extract of plantain peeling was evaluated as a coagulant aid. Synthetic water with varying turbidity and pH was investigated. The effectiveness of plantain peelings was compared with alum. The addition of plantain peeling ash was found to be effective in reducing turbidity. pH was reduced with addition of alum, but an increase was observed with addition of the coagulant. High correlation coefficient values obtained when pH of the treated water was correlated with alum and PPAE dosage (Oladoja and Aliu, 2008).

Charcoal produced from coconut shell was found to be a good adsorbent in removing iron from drinking water. Optimum dosage was found to be 500ppm. Removal of iron dependent on particle size of coconut shell charcoal, removal efficiency was found to be directly proportional with the particle size. Optimum residence time was found to be 4hrs. Incorporation of Mn^{2+}enhanced the removal efficiency of coconut shell charcoal (Beenakumari, 2009).

Seeds of *Moringa oleifera*, *Arachis hypogea, Vigna unguiculata, Vigna mungo* and *Zea mays* were experimented in removing turbidity, hardness and heavy metals. *Moringa oleifera* seeds reduced turbidity to one fifth the

original turbidity levels within 2hrs of treatment. In the case of hardness *Moringa oleifera* seeds were able to remove 34% of hardness followed by peanut (25%), corn (19%), beans (22%) and urad (24%). In the case of heavy metal, *Moringa oleifera* seeds were found to be better than all other seeds in removing heavy metal. *Moringa* seeds were able to remove copper (90%), lead (80%) cadmium (60%), zinc (50%) and chromium (50%) from water (Nand et al., 2012; Sotheeswaran et al., 2011). Mango seed kernel was investigated in removing parameters such as coagulant dose, pH, turbidity of synthetic water. It was reported that 98% of turbidity removal was observed at 0.5ml dose and pH 13. The removal efficiency was improved with increase in pH. Crude extract removed turbidity of water up to 98% at an optimized dose of 0.5ml/L (Qureshi et al., 2011).

Phosphoric acid activated *Vetiveria zizanioides* root showed good fluoride adsorption capacity. Batch sorptive defluoridation was carried out at various conditions like pH, agitation time, dosage and particle size. Maximum defluoridation was achieved at pH 6. The percentage of fluoride removal increased with adsorbent dose and time at a given initial solute concentration (Harikumar, 2012).

Efficiency of *Jatropha curcas* solution, made from seeds and press cake in reducing turbidity of waste water through coagulation was investigated by Abidin et al., (2011). *Jatropha curcas* seed was found to be effective with more than 96% of turbidity removal at pH 1-3 and pH 11-12. Highest turbidity was observed at pH 3 using a dosage of 120mg/L. Flocs formed were bigger and settled faster. Comparable results were obtained with presscake of *J. curcas* seeds. They investigated parameters like pH, dosage, initial turbidity and blending time. Best performance was observed at 120mg/L at pH 3. They have opined that *Jatropha curcas* seeds are good coagulant for industrial waste water and water treatment.

Mucilage of *Coccinia indica* was reported as flocculent for the treatment of turbid water samples containing kaolin. Plant material was experimented at a dosage of 0.4mg/L at different pH range. Higher efficiency was observed with very high initial turbidity 100NTU (Patale and Pandya, 2012).

Vara (2012) investigated the effects of alum as coagulant in conjunction with bean, sago and chitin as coagulants on the removal of colour, turbidity, hardness and *E. coli* from water. The study was conducted at three different pH conditions 6, 7 and 8. The dosages chosen were 0.5, 1, 1.5 and 2mg/L. Reduction in turbidity and *E. coli* were observed with coagulants tested. Hardness reduction (93%) was also observed at pH 7 with 1mg/L concentration of alum. While chitin was stable at all pH ranges. It showed the highest removal at 1 and 1.5mg/L with pH 7. The study revealed considerable savings in chemicals and sludge handling cost when using natural coagulants.

Seeds of three plants *Moringa oleifera, Cicer aretinum,* and *Dolichos lablab* were investigated for their coagulation activity. An amount of 10g/L of the smaller fraction was suspended in distilled water. Coagulation experiment was carried out using synthetic turbid water, Turbidity was retained at 90-120NTU (high), 40-50NTU (medium) and 25-35NTU (low) levels. Of various coagulant experimented *Cicer arietinum* was found to be most effective in removing 90% of the turbidity (Choubey et al., 2012).ranged between high (90-120), medium (40-50) and lower (25-35) NTU of synthetic turbid water. It was also noticed that the plant materials were capable of reducing 89-96% of total coliforms.

Coagulant efficiency of seeds of *Moringa oleifera,* Okra gum and mucilage isolated from the dry flowers of *Calotropis procera* was investigated at low (15, 30 and 50NTU), medium (100NTU) and high (250NTU and 500NTU) turbidity levels. Varying dosages of plant material

was experimented (0, 2.5, 5, 7.5, 10, 12.5, 15.0, mg/L). The results were compared with alum and found that turbidity obtained after treatment was nearly equal to potable range (Renuka, 2013).

Cicer arientinum, *Moringa oleifera* and cactus were used as natural coagulant to treat untreated tannery effluents. The optimum dosage of *Cicer arietinum, Moringa oleifera* and cactus were found to be 0.1, 0.3 and 0.2g in 500ml respectively. The percentage removal of these three plants was found to be 81.20%, 82.02% and 78.54% for *Cicer arietinum, Moringa oleifera* and cactus respectively. *Moringa oleifera* was able to reduce turbidity and COD to 82.02% and 90% respectively than rest of the three plants (Kazi et al., 2013).

Kopytko et al. (2014) investigated the coagulation property of *Aloe vera* in conjunction with alum. It proved to be a primary coagulant, but when used as coagulant aid, removal turbidity at varying degrees. For the removal of 45.5NTU turbidity 5mg/L of *Aloe vera* was needed along with 56mg/L of alum, while water with a high-level turbidity (101 NTU) required 24mg/L of alum with 14mg/L of *Aloe vera* blend as a coagulation aid, in order to achieve more than 90% turbidity removal.

Mohamed et al. (2014) evaluated the efficiency of different types of chemical coagulant (alum and ferrous sulphate) and natural coagulants *Moringa oleifera and Strychnos potatorum* for treating car wash wastewater. Parameters like pH, Chemical Oxygen Demand (COD), phosphorous, Total Suspended Solids (TSS), and turbidity were studied. The removal efficiency of both the natural coagulants were found to be highly effective at lower dosages (60-80mg/L).

Phyllanthus emblica was investigated for its removal efficiency of physico-chemical parameters like colour, odour, taste, pH, acidity, alkalinity, total hardness, calcium, magnesium, chloride, nitrate, PO_4, SO_4, bacteria and

fungi. Certain contaminants like hardness were completely removed with *P. emblica* (Padmapriya et al., 2014).

Cicer arientinum showed 95.5% turbidity reduction efficiency. Jar test experiments were carried out for high level (100NTU), medium level (40NTU) and low level (20NTU) turbidity for setting time 30min, 60min and 120min. Natural coagulant was better in removing medium and low turbidity water. Statistical analysis of experiment showed major factor contribution as significant. Coagulation efficiency was maximum at neutral pH (Rahane and Navale, 2015).

Beyene et al. (2016) compared the effectiveness of cactus powder, alum and a mixture of both. Turbidity was found to be decreased with increase in dosage. The results also revealed that cactus powder was more effective in maintaining the pH, TDS and salinity removal from water than alum. But when used in combination, cactus powder and alum, parameters like turbidity, salinity, conductivity, TDS was found to get reduced, but marginal effect was observed on DO value. Thus it has been concluded that synergistic effect was countable as coagulant than used individually (Beyene et al., 2016).

Ghanmode and Chavan (2015) have evaluated the efficiency of few natural coagulants in removing turbidity from water. Plant parts from *Abelmoschus esculentus* (seeds), *Trigonella foenum-graecum* (seeds), *Terminalia bellirica* (seeds), *Cyamopsis tetragonoloba* (Pods) and *Moringa oleifera* (Seeds) were compared with Alum. Of these seeds experimented *Abelmoschus esculentus*, *Terminalia bellirica* and *Moringa oleifera* seeds were found to be more effective with percentage removal ranging between 84% to 93% followed by guar gum seeds 70-80% at a dose of 20mg/L.

Several factors influence the coagulation process like pH, dosage, temperature, coagulant type etc. (Ma et al., 2001). Hence all these factors should together be considered for accomplishing effectual and cost effective coagulation step.

Optimization is very much important as unoptimized conditions may lead to either wastage of coagulants or will get a reverse effect when used in excess. According to Bratby (1981), in the case of dosage chemical coagulants directly influence the settling characteristics and the coagulant cost. Almost all natural coagulants so far identified have been optimized for its favourable conditions like *Cicer arietinum* (Rahane and Navale, 2015), unmodified rice starch (Teh et al., 2014) and sago and chitin (Saritha et al., 2015). Most important parameters considered for optimization include pH (Baghvand et al., 2010; Rahane and Navale, 2015; Teh et al., 2014), dosage (Saritha et al., 2015; Teh et al., 2014), settling time (Teh et al., 2014) and effective mixing speed (Saritha et al., 2015).

Ndabigengesere and Narasiah (1996) opined that the coagulation process is highly influenced by factors like temperature, mixing, pH, cation–anion concentration etc. Even initial dosage and initial concentration of contaminant has a strong correlation with the coagulation activity (Muyibi and Evison, 1996).

Ndabigengesere and Narasiah (1998) compared the quality of water treated with *Moringa oleifera* seeds and alum, Results of pH, conductivity, alkalinity, cation and anion concentration showed that *Moringa* seeds have not altered the quality of water treated. Similar result was mentioned by Yang et al. (2007), when they used dry *Opuntia* powder as coagulant. In addition they also compared coagulation activity of *Opuntia* powder to the prevailing chemical coagulants. The amount of cactus powder added was well correlated with the turbidity removal. The plant material was able to remove 70-80% of

turbidity from water. Another work done by Miller et al., (2008) in evaluating the turbidity removal capacity of cactus mucilage recommended that the plant material is comparable to the well known coagulant *Moringa oleifera*. Mucilage was able to remove turbidity up to 98%. Equilibrium time required was 18h for the removal of heavy metals (Mane et al., 2011).

Optimum dosage of okra mucilage was found to be 0.04mg/L and maximum solid removal efficiency from tannery effluent was observed at initial hours of contact time (Agarwal et al., 2003). Whereas for brewery effluent, optimum efficiency of 92.6% was recorded at 30th minute. The optimal dosage was found to be 200mg/L and pH 2 for brewery effluent treatment (Okolo et al., 2014).

Zhang et al. (2006) studied the effects of cactus mucilage on factors like pH, temperature, alkalinity etc. and found that removal efficiency was dependent on polyelectrolyte concentration and agitation speed while removing heavy metals from water (Mane et al., 2011). Its efficiency was also dependent on the nature of the test water (Sellami et al., 2014). The optimum dosage was 10% (v/v) polyelectrolyte and 150rpm at 30^0 C for removal of both the Cr and Ni ions. The maximum removal capacity of polyelectrolyte was 68% and 88.4% for Cr and Ni ions respectively (Mane et al., 2011). Cactus juice was able to remove suspended solids and COD in the range 83.3% -88.7% and 59.1% to 69.1% respectively (Sellami et al., 2014).

Fenugreek mucilage was able to remove 97% of suspended solids and 20% of TDS at a dosage of 0.16mg/L and maximum removal was observed at 1-3h of contact time (Mishra et al., 2003). Best performance of *Jatropha curcas* seeds was observed at 120mg/L at pH 3. It was found to be a good coagulant for industrial waste water and water treatment (Abidin et al., 2011). Crude extract of mango pit removed turbidity of water up to 98% at an optimized dosage of 0.5ml/L (Qureshi et al., 2011).

In contrast to chemical coagulants, plant derived coagulants are safe and eco-friendly (Asrafuzzaman et al., 2011; Bratby, 2006). Natural coagulants are found to produce less sludge even five times lower than the chemical coagulants and are degradable (Ndabigengesere et al., 1995). Hence raw plants which are available under local conditions can be employed as a low cost alternative to chemical coagulants (Choy et al., 2014). The list of plants which are effective in this direction, as listed by Choy et al. (2015, 2014) is given in table 1.

Table 1. List of plants which were attempted to find out their effectiveness in the treatment of water contaminants Choy et al. (2015, 2014)

Sl. No.	Name of the plant	Parts used	Parameters experimented	Reference
1	*Coccinia indica*	Fruit	Turbidity	(Patale and Parikh, 2010) (Patale and Pandya, 2012)
2	*Abelmoschus esculentus*	Seed pod, tips, sap, plant stalk and roots	Turbidity	(Al—Samawi and Shokralla, 1996) (Agarwal et al., 2001)
3	*Luffa cylindrica*	Seeds	DOC (Dissolved Organic Carbon), TDS	(Sowmeyan et al., 2011)
4	*Arachis hypogea*	Seeds	Turbidity, Hardness, lead, chromium, zinc,	(Mbogo, 2008; Sotheeswaran et al., 2011)
5	*Cicer arietinum*	Seeds	Total coliforms	(Asrafuzzaman et al., 2011)
6	*Dolichos biflorus*	Seeds	Turbidity	(Bhole, 1985)
7	*Glycine max*	Seeds	Turbidity	(Bhole, 1985)
8	*Guar gum*		Feacal coliforms	(Pritchard et al., 2009)

9	*Lablab pupureus*	Seeds, peels	Turbidity, total coliform count,	(Asrafuzzaman et al., 2011; Bhole, 1985; Unnisa et al., 2010)
10	*Phaseolus angularis*	Seeds	Turbidity	(Gunaratna et al., 2007)
11	*Phaseolus mungo*	Seeds	Turbidity, lead, copper, chromium	(Mbogo, 2008)
12	*Phaseolus vulgaris*	Seeds	Turbidity, COD	(Šćiban et al., 2005)(Antov et al., 2010; Prodanović et al., 2011)
13	*Pisum sativum*	Seeds	Turbidity	(Mbogo, 2008)
14	*Vigna unguiculata*	Seeds	Turbidity, Chromium, lead, Zinc	(NJ Marobhe et al., 2007; Mbogo, 2008)
15	*Carica papaya*	Seeds	Turbidity	(Yongabi et al., 2011)
16	*Citrus sinensis*	Peel/skin	Alkalinty, turbidity	(Sowmeyan et al., 2011)
17	*Feronia limona*	Seeds	Turbidity	(Bhole, 1985)
18	*Hylocereus undatus*	foliage	Turbidity, COD, BOD, SS	(Som et al., 2007)
19	*Mangifera indica*	Seeds, seed kernels	Total coliform, turbidity	(Ali et al., 2008)
20	*Phoenix dactylifera*	Seeds, pollen sheath	Turbidity	(Yongabi et al., 2011)
21	*Prunus armeniaca*	Seeds	Turbidity, Total coliforms, fecal coliforms	(Ali et al., 2008)
22	*Tamarindus indica*	Seeds	Turbidity, TSS, DO.	(Bhole, 1985)

Several natural coagulants reported so far for have coagulation activity (Choy et al., 2015, 2014). Apart from the listed natural coagulants, little is known about other plants with noticeable water clarification properties. Identifying such potential and promising plants for tackling water contaminants like iron, fluoride etc... are scarce (Rao, 2005). Sciban et al.,

(2006) has also suggested that availability of plant species investigated may differ with countries and it will be advantageous to search for new candidates which are abundantly available in specific countries. The present attempt is to bring more members to this category. Also it is attempted to assess the potentialities of generally reported plants in their effectiveness to manage specific water contaminants like iron, fluoride etc.

Materials and Methods

Plant selection and processing

Two dozen (24) plant species belonging to 17 families were selected for assessing their efficiency in stabilizing / removing selected water contaminants like pH, turbidity, hardness, iron and fluoride. Details of plant material selected for the study are depicted in table 2.

Table 2: Details of plants used for the study.

SI. No.	Scientific name	Family	Common name
1	*Abelmoschus esculentus* L. Moench	Malvaceae	Okra, ladies finger, Vendakka (Mal), bhindee (Hindi)
2	*Aloe barbadensis* Mill.	Liliaceae	Kattarvazha (Mal), Aloe vera
3	*Azadirachta indica* A. Juss.	Meliaceae	Neem, Aryavepu (Mal),
4	*Bacopa monnieri* (L.) Wettst.	Scrophulariaceae	Water hyssop, Brahmi (Mal),
5	*Cyamopsis tetragonoloba* (L.) Taub.	Fabaceae	Cluster beans, Kothamara (Mal), Guar (Hindi)
6	*Euphorbia antiquorum* L.	Euphorbiaceae	Triangular spurge, chathurakalli (Mal), Tridhara (Hindi)
7	*Hemidesmus indicus* (L.) R.Br.	Asclepiadaceae	Indian sarsaparilla, Nannaari (Mal), Kshirini (Hindi)
8	*Lagenandra toxicaria* Dalzell	Araceae	Andavazha (Mal),

9	*Mangifera indica* L.	Anacardiaceae	Mango, Maanga (Mal), Aam (Hindi)
10	*Mentha arvensis* L.	Lamiaceae	Mint, Pudina (Hindi)
11	*Momordica charantia* L.	Cucurbitaceae	Bitter gourd, Kayappakka (Mal), Karela (Hindi)
12	*Musa paradisiaca* L.	Musaceae	Plantain, Vaazha (Mal), kela (Hindi)
13	*Opuntia dillenii* (Ker Gawl.) Haw.	Cactaceae	Prickly pear , Kallichedi (Mal), naghhana (Hindi)
14	*Phyllanthus emblica* L.	Euphorbiaceae	Indian Goose berry, Nellikka (Mal)
15	*Plectranthus amboinicus* (Lour.) Spreng.	Lamiaceae	Indian borage, Pannikkurkka (Mal), Patta ajavayin (hindi)
16	*Ricinus communis* L.	Euphorbiaceae	Castor bean, Avanakku (Mal), Arandi (Hindi)
17	*Strychnos potatorum* L.f.	Loganiaceae	Clearing nut tree, Nirmali, Thettamprel (Mal)
18	*Tamarindus indica* L.	Fabaceae	Tamarind, Puli (Mal), Imili (hindi)
19	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Bellirica myrobalan, Thanni (Mal), Baheda (Hindi)
20	*Terminalia chebula* Retz.	Combretaceae	Chebulic myrobalan, Kadukka (Mal)
21	*Theobroma cacao* L.	Malvaceae	Cocoa tree, Kokkoo (Mal), Haritaki (Hindi)
22	*Trigonella foenum-graecum* L.	Fabaceae	Fenugreek, Uluva (Mal), Methi (Hindi)
23	*Vetiveria zizanioides* (L.) Nash	Poaceae	Khaskhas grass, Ramacham (Mal), Khus (Hindi)
24	*Zea mays* L.	Poaceae	Corn, cholam (Mal)

Plants / plant parts experimented include leaves, stem, twigs, roots, rhizome, fruits, seeds, seed kernel and other modifications like cladode. As plant materials varied, the methods of processing were also devised accordingly. Details of processing of plant parts are given in the table 3.

Table 3: Various methods employed for the processing of plant materials.

Sl. No..	Scientific name	Parts used	Method adopted for processing
1	*Abelmoschus esculentus*	Fruit	Fruits were chopped and weighed. The weighed pieces were crushed using a mortar and pestle. This has been transferred to respective jars.
2	*Aloe vera*	Leaves	Thick mucilaginous portions were taken and weighed. The chopped and weighed pieces were crushed using a mortar and pestle. This has been transferred to respective jars.
3	*Azadirachta indica*	Leaves	Leaves were chopped into small pieces and weighed. It was then crushed using a mortar and pestle. This has been washed off and transferred to respective jars
4	*Bacopa monnieri*	Twigs	Plant materials were chopped and weighed. They were then immediately crushed using a mortar and pestle. This was then washed and transferred to respective jars.
5	*Cyamopsis tetragonoloba*	Fruits	Fruits were chopped and weighed. Weighed fruits were then crushed using a mortar and pestle. This has been transferred to respective jars.
6	*Euphorbia antiquorum*	Stem	Central thick mucilaginous portions were chopped out and desired quantities were weighed. These pieces were then crushed using a mortar and pestle, washed off and transferred to respective jars.
7	*Hemidesmus indicus*	Root	Material was chopped into small pieces. Desired quantities were weighed and crushed using a mortar and pestle. This has been washed and transferred to respective jars.
8	*Lagenandra toxicaria*	Rhizome	Rhizome was washed, chopped into small pieces and weighed. This was then crushed using a mortar and pestle and has been transferred to respective jars.

9	*Mangifera indica*	Seed kernel	Seeds were cut open to obtain the kernels. The kernels were chopped, weighed and macerated. This was then transferred to respective jars.
10	*Mentha arvensis*	Leaves	Leaves were cleaned and chopped. Desired quantities were weighed and pound gently. Washed off and transferred to respective jars.
11	*Momordica charantia*	Fruit	Fruits were chopped into small pieces, weighed and crushed using a mortar and pestle. This was then transferred to respective jars.
12	*Musa paradisiaca*	Pseudostem	Scape (peduncle) was chopped into small pieces. Chopped pieces were weighed and crushed using mortar and pestle. Washed off to respective jars.
13	*Opuntia dillenii*	Cladode	Central mucilaginous portion was chopped into small pieces and weighed. These pieces were then crushed using mortar and pestle. This was then transferred to respective jars.
14	*Phyllanthus emblica*	Dried fruits	Dried fruits were obtained and weighed. Weighed pieces were crushed using a mortar and pestle. These were then transferred to respective jars.
15	*Plectranthus amboinicus*	Leaves	Leaves were chopped into small pieces and were weighed. They were then macerated and transferred to respective jars.
16	*Ricinus communis*	Seeds	The hard seed coats were removed and the seeds were separated. The weighed seeds were crushed using a mortar and pestle. This has been transferred to respective jars.
17	*Strychnos potatorum*	Seeds	Seeds were broken into pieces using a kitchen blender (stone). Uniform pieces were weighed and transferred to respective jars.
18	*Tamarindus indica*	Seeds	The seed coats were removed and the seeds are separated. They were then weighed and crushed using a mortar and pestle. The crushed and pulverized seeds were transferred to respective

			jars.
19	*Terminalia bellirica*	Dried fruits	Dried fruits were weighed and weighed pieces were crushed using a mortar and pestle. This was then transferred to respective jars.
20	*Terminalia chebula*	Dried fruits	Dried fruits were weighed and crushed using a mortar and pestle. They were then transferred to respective jars.
21	*Theobroma cacao*	Seeds	Seeds were separated from the flesh and seed coats were removed. The kernels were then weighed and crushed using a mortar and pestle. They were then transferred to respective jars.
22	*Trigonella foenum-graecum*	Seeds	Seeds were separated, weighed and crushed using a mortar and pestle. They were then transferred to respective jars.
23	*Vetiveria zizanioides*	Roots	Roots were cleaned and chopped into small pieces. They were then weighed and the representative samples were crushed using a mortar and pestle. These were then transferred to respective jars.
24	*Zea mays*	Seeds	Seeds were cleaned, weighed and crushed using a mortar and pestle. They were then transferred to respective jars.

Raw water from a natural pond located in the Botanical garden of the University of Calicut was selected for the study. Approximately 30L of water sample from the pond was collected and brought to the laboratory, prior to experimentation on each parameter. The general physico-chemical quality of water confining to the pond is given in Table 4.

Table 4: Physico-chemical quality of water confining to the pond

Parameters	Range
pH	6.5-8.5
Turbidity	20-200NTU
Iron	< 0.01mg/L
Fluoride	< 0.01mg/L

The concentration of water quality parameters under investigation (pH, turbidity, iron and fluoride) was monitored in the pond water prior to each experiment. Contaminants were artificially imparted in the pond water, if their relative concentration was below the detection / experimentation level. For imparting acidity and alkalinity, 1N HCl and 1N NaOH were used. Similarly, iron and fluoride were imparted using ferrous ammonium sulphate and sodium fluoride respectively. With ferrous ammonium sulphate, a final iron concentration of 0.3mg/L and with sodium fluoride a final fluoride concentration of 1.5mg/l was maintained. As the turbidity of raw water obtained from the pond was higher and within detectable levels, it has been subjected to experimentation directly. The methods by which the parameters are assessed are given below:

pH

pH is a measure of acidity or basicity of water. It can be defined as the negative logarithm of the hydrogen ion activity (APHA, 2012). pH was measured using digital pH meter (EI make). Stipulated pH range of potable water is pH 6.5-9.5 (WHO, 2007).

Turbidity

Clarity of water is important for consumption as well as for several designated uses. Turbidity measures the cloudiness in water. It is an optical property of water and represents the amount of light scattered by the suspended matter in the water (APHA, 2012). Turbidity in water is usually attributed by silt, sand, mud, bacteria and several chemical substances. Higher the intensity, higher will be the turbidity. Turbidity of water in the present study was measured using a turbidity meter (EI make) and was expressed in Nephelometric Turbidity Units (NTU). Permissible levels of turbidity in potable water is <5NTU (APHA, 2012).

Iron

Iron was estimated using modified thiocyanate method (Goswami and Kalita, 1988). To 5ml of the sample 0.5 ml of ceric ammonium sulphate (0.25%) and 40% potassium thiocyanate were added and shaken well. Exactly 5 minutes after addition of thiocyanate solution, the absorbance of ferric thiocyanate complex is measured at 480 nm using a spectrophotometer (Scanning spectrophotometer, ELICO SL1157). The results were represented in mg/L. Iron content of the unknown sample was evaluated using a standard graph. Levels of 0.3-3mg/L iron in water are acceptable.

Fluoride

Fluoride was estimated using SPADNS colorimetric method (APHA, 2012). To 50ml of sample, 10ml of SPADNS reagent was added and shaken well. The absorbance of resultant solution was read spectrophotometrically at 570nm using a scanning spectrophotometer (ELICO SL1157), immediately after the addition of SPADNS reagent. The concentration of fluoride in the sample was elucidated from a standard graph. The results were represented in mg/L. Fluoride levels up to 0.7 mg/L are beneficial but harmful once it exceeds the limit of 1.5mg/L (APHA, 1999).

Experimental layout

Each plant material was analyzed for its stabilization / coagulation property against all the 4 selected water contaminants. Both preliminary and secondary screening studies were carried out for the selection of plants showing better stabilization / treatment efficiency.

(i) Preliminary screening

Batch treatment was carried out for each plant material at varying dosages (0.5, 1, 2 and 4g) and retention time (1.5, 3, 6, 12 and 24 Hours after

treatment (HAT). The treatment facility is shown in Plate 2. For the analysis of each water quality parameter, 25 glass jars were arranged in such a way that each column represents a specific retention time and each row a specific dosage. 1.0L each of of raw water, whose water quality parameter under study has been adjusted to the detectable limit, was dispensed into each jar of 2.0 L capacity. The quality of initial water sample is recorded. Respective dosage of plant material was added to the raw water and stirred manually for 5 minutes. A control was maintained for each set of experiment. After specific retention time, representative water samples were collected without turbulence from a distance of 5cm below the water level, using a siphon. Samples thus collected were analyzed for specific parameters, immediately.

The results of the preliminary screening studies were used for the optimization of batch treatment conditions.

(ii) Secondary screening

Batch treatment was carried out with those plant materials with highest stabilizing/ removal percentage. Each of the plant materials were studied at varying dosages (0.5g, 1g, 2g and 4g) and retention time (1.5, 3, 6, 12, and 24HAT). After stipulated time interval, representative samples from each of the treatment sets were analyzed for each of the selected water contaminants (pH, turbidity, iron and fluoride).

Statistical analysis

Statistical analysis was performed using SPSS V. 16.0. Analysis of variance (ANOVA) using General Linear Model (GLM) was performed to assess whether there exist significant variations among the treatment sets conducted to evaluate the effectiveness of plant material. Post hoc analysis using LSD were run to confirm where the exact differences occurred between groups concerning to dosage and retention time. The term “response of the

plant material" used in the statistical analysis collectively represents the changes brought about by the plant material on selected parameters like pH (acidic and alkalinity), turbidity, iron and fluoride at specific dosages and retention time, with respect to their control.

Results and Discussion

The results of the present study are represented in two sections: primary screening and secondary screening. In preliminary studies, 24 plants / plant parts were screened for their efficacy in removing / adjusting the selected water quality parameters like pH, turbidity, iron and fluoride. Plants having highest removal efficiencies were selected from preliminary screening and subjected to secondary screening. The results concerning secondary screening were statistically analyzed and those plants / plant parts which are significant in removing each of the selected contaminants were selected for characterization studies.

Preliminary screening studies

In preliminary screening, each of the plant materials were evaluated for their efficiency in stabilizing / removing all the four selected water contaminants such as pH (acidic and alkaline pH), turbidity, iron and fluoride. All of the two dozen plants exhibited varying results in the percentage removal / stabilization of water quality parameters with respect to control. Results thus obtained are tabulated in table 5.

Table5: Percentage change brought about by plant materials at specific retention time and dosages on selected water quality parameters like pH (acidity and alkalinity), turbidity, iron and fluoride.

Dosage (g)	Retention time (hours)					
	1.5	3	6	12	24	Mean
Abelmoschus esculentus						
Acidity						
0.5	-0.31898	-0.15576	0.607903	4.035874	1.492537	1.132314
1	-1.75439	-0.93458	1.215805	4.035874	1.940299	0.900603
2	-0.79745	-1.55763	0.911854	4.633782	6.567164	1.951544
4	-1.27592	-0.31153	4.863222	4.783259	1.343284	1.880464
Alkalinity						
0.5	0	1.651842	-0.26316	2.013423	4.261364	1.532694
1	0.123916	2.541296	3.815789	2.684564	7.954545	3.424022
2	1.982652	6.480305	11.57895	8.053691	10.22727	7.664574
4	6.071871	7.115629	11.44737	13.15436	6.534091	8.864664
Turbidity						
0.5	3.726708	-7.95455	-4.0404	1.219512	33.62069	5.314392
1	-9.93789	-14.7727	-7.07071	-7.31707	31.03448	-1.61278
2	-9.31677	-23.8636	-1.0101	-8.53659	11.2069	-6.30404
4	-0.62112	-120.455	-11.1111	-12.1951	2.586207	-28.3591
Iron						
0.5	-4.52261	-12.7168	-8.64198	-80.5556	-351.613	-91.61
1	-2.01005	-1.7341	-20.3704	-125	-351.613	-100.145
2	-2.01005	-17.341	-23.1481	-156.944	-364.516	-112.792
4	0	-15.3179	-17.284	-156.944	-422.581	-122.425
Fluoride						
0.5	9.859155	-13.6986	-57.1429	-41.3793	20.54795	-16.3627
1	-26.7606	-8.21918	-28.5714	-12.069	5.479452	-14.0281
2	-2.8169	-20.5479	7.142857	15.51724	31.50685	6.16042
4	18.30986	28.76712	-88.0952	-36.2069	36.9863	-8.04777
Aloe barbadensis						
Acidity						
0.5	3.660566	3.054662	1.100629	1.410658	2.143951	2.274093
1	6.156406	1.446945	1.72956	1.567398	2.756508	2.731364
2	1.497504	1.92926	2.672956	2.194357	2.45023	2.148862
4	21.13145	12.54019	10.37736	9.404389	8.575804	12.40584

Alkalinity						
0.5	2.564103	1.761518	5.479452	3.617945	1.490313	2.982666
1	4.183536	3.523035	8.90411	6.367583	1.639344	4.923522
2	8.77193	7.452575	8.356164	6.078148	3.278689	6.787501
4	13.09042	12.87263	13.42466	9.696093	6.259314	11.06862
Turbidity						
0.5	4.545455	7.936508	1.666667	-8.69565	18.91892	4.874379
1	10.60606	6.349206	3.333333	-6.52174	16.21622	5.996615
2	7.575758	6.349206	0	-6.52174	10.81081	3.642807
4	7.575758	4.761905	-1.66667	-19.5652	5.405405	-0.69776
Iron						
0.5	-2.5	-5.71429	-14.7059	-54.5455	-163.636	-48.2204
1	-2.5	-14.2857	-17.6471	-77.2727	-200	-62.3411
2	-10	-14.2857	-17.6471	-81.8182	-209.091	-66.5684
4	-2.5	-14.2857	-19.1176	-36.3636	-218.182	-58.0898
Fluoride						
0.5	16.66667	8.695652	62.16216	-57.5	-16.2162	2.761653
1	-43.3333	-86.9565	5.405405	-50	24.32432	-30.112
2	73.33333	-65.2174	45.94595	25	18.91892	19.59616
4	33.33333	47.82609	-5.40541	22.5	-89.1892	1.812965
Azadirachta indica						
Acidity						
0.5	0	0.331675	0.162338	2.733119	5.864198	1.818266
1	-0.1634	-0.99502	0.162338	4.501608	6.018519	1.904808
2	0.980392	-0.33167	1.785714	4.340836	8.487654	3.052584
4	2.614379	1.824212	2.435065	4.501608	9.722222	4.219497
Alkalinity						
0.5	4.860267	5.679012	9.587217	9.165527	10.21277	7.900958
1	8.262454	12.71605	11.05193	14.50068	13.47518	12.00126
2	13.24423	13.82716	15.84554	17.23666	13.61702	14.75412
4	17.98299	20.12346	17.97603	21.34063	18.01418	19.08746
Turbidity						
0.5	4.123711	-4.90798	-3.26797	-9.45946	11.96581	-0.30918
1	5.670103	-0.6135	0.653595	-2.02703	19.65812	4.668259
2	-0.51546	-6.74847	-2.61438	-3.37838	-11.1111	-4.87356
4	2.061856	0	-0.65359	0.675676	-10.2564	-1.63449
Iron						
0.5	6.060606	3.448276	0	-8.33333	0	0.23511
1	3.030303	10.34483	7.142857	-12.5	-15.7895	-1.5543
2	3.030303	10.34483	5.357143	-12.5	-28.9474	-4.54302

4	6.060606	0	14.28571	-8.33333	-21.0526	-1.80793
Fluoride						
0.5	13.58025	40.74074	26.41509	-15.5556	-20	9.036105
1	-11.1111	6.17284	5.660377	-44.4444	5	-7.74447
2	9.876543	25.92593	7.54717	-37.7778	-75.8333	-14.0523
4	18.51852	37.03704	7.54717	4.444444	20	17.50943
Bacopa monnieri						
Acidity						
0.5	-1.58172	-1.22592	-1.53061	0.330033	3.271028	-0.14744
1	-2.28471	-7.18039	-0.68027	0.990099	4.361371	-0.95878
2	-2.46046	-2.45184	-1.36054	1.815182	6.074766	0.323422
4	-2.46046	-2.45184	-1.02041	1.980198	6.23053	0.455605
Alkalinity						
0.5	-4.5392	-2.7933	-3.32871	4.557292	-0.58394	-1.33757
1	-3.43879	-2.09497	-1.52566	5.208333	1.167883	-0.13664
2	-0.55021	-2.7933	-0.97087	9.895833	0.437956	1.203883
4	0.550206	0.27933	3.32871	11.84896	3.50365	3.902171
Turbidity						
0.5	-1.92308	-7.35294	2.307692	2.564103	7.446809	0.608517
1	-2.5641	-9.55882	1.538462	-3.4188	8.510638	-1.09853
2	-1.92308	-7.35294	-2.30769	-5.12821	11.70213	-1.00196
4	-55.7692	-11.7647	-2.30769	-6.83761	1.06383	-15.1231
Iron						
0.5	-2.7027	-2.94118	-25.9259	-23.0769	-3.7037	-11.6701
1	0	-5.88235	-7.40741	-19.2308	-11.1111	-8.72633
2	2.702703	-2.94118	-22.2222	-30.7692	-14.8148	-13.6089
4	8.108108	0	-14.8148	-30.7692	-7.40741	-8.97667
Fluoride						
0.5	26.66667	0	-6.66667	-14.2857	61.22449	13.38776
1	26.66667	9.52381	-50	16.66667	38.77551	8.326531
2	6.666667	30.95238	-20	16.66667	38.77551	14.61224
4	20	30.95238	36.66667	9.52381	67.34694	32.89796
Cyamopsis tetragonoloba						
Acidity						
0.5	0.661157	2.070064	0.648298	8.695652	8.21485	4.058004
1	-0.33058	2.547771	1.134522	12.23833	10.42654	5.203316
2	-0.33058	3.503185	2.917342	11.75523	14.21801	6.412638
4	-1.32231	3.343949	9.076175	18.84058	15.95577	9.178831
Alkalinity						
0.5	0.768246	-0.92838	2.834008	2.456647	4.864092	1.998922

1	-1.79257	-0.79576	3.508772	1.011561	4.72103	1.330607
2	-0.6402	0.132626	3.238866	4.190751	7.58226	2.90086
4	-2.1767	1.724138	11.06613	9.682081	7.296137	5.518357
Turbidity						
0.5	-0.30817	9.966216	-1.21704	7.594937	16.93811	6.594812
1	-2.15716	2.871622	-3.24544	3.037975	20.52117	4.205634
2	-0.46225	-11.4865	-8.31643	12.65823	9.120521	0.302717
4	4.006163	-2.53378	-0.20284	-1.01266	2.931596	0.637696
Iron						
0.5	22.07792	0	3.703704	-5.26316	-8.49057	2.40558
1	12.33766	0	0	-5.26316	-8.49057	-0.28321
2	15.58442	7.142857	0	-9.64912	-8.49057	0.917517
4	15.58442	0	-6.66667	-5.26316	-13.2075	-1.91059
Fluoride						
0.5	28.43137	19.76744	13.82979	4.166667	31.42857	19.52477
1	26.47059	15.11628	0	44.44444	8.571429	18.92055
2	36.27451	25.5814	-17.0213	16.66667	28.57143	18.01454
4	26.47059	9.302326	19.14894	-4.86111	0	10.01215
Euphorbia antiquorum						
Acidity						
0.5	0.330579	0.813008	2.083333	1.292407	4.96124	1.896113
1	0.165289	1.626016	25.32051	2.907916	6.976744	7.399296
2	1.157025	2.601626	31.89103	4.361874	8.372093	9.676729
4	2.31405	2.113821	31.08974	5.654281	11.62791	10.55996
Alkalinity						
0.5	-0.72551	-8.98734	4.591837	8.827404	6.320225	2.005322
1	-5.68319	0	-0.89286	10.54018	8.707865	2.5344
2	-4.11125	1.772152	1.658163	13.83399	10.11236	4.653084
4	-0.96735	-1.39241	14.15816	14.88801	14.04494	8.146272
Turbidity						
0.5	-7.55208	-0.63694	-5.15464	-25.1101	11.32075	-5.42661
1	3.645833	-3.82166	-1.71821	-16.2996	12.73585	-1.09155
2	-4.94792	-10.1911	-5.15464	4.405286	19.33962	0.690254
4	-14.3229	-8.59873	2.749141	17.18062	6.603774	0.722378
Iron						
0.5	0	0	-8.33333	-19.1489	-125	-30.4965
1	0	7.5	-2.77778	-40.4255	-160	-39.1407
2	-2.5641	10	0	-40.4255	-170	-40.5979
4	-2.5641	0	-5.55556	-57.4468	-210	-55.1133

Fluoride						
0.5	-40	34.61538	38.57143	65.71429	78.57143	35.49451
1	-50	28.84615	60	25.71429	44.28571	21.76923
2	16.66667	-17.3077	61.42857	58.57143	65.71429	37.01465
4	-33.3333	42.30769	42.85714	40	68.57143	32.08059
Hemidesmus indicus						
Acidity						
0.5	-1.37457	2.177554	0.500835	3.414634	10.37736	3.019162
1	-1.89003	1.172529	-1.00167	3.577236	11.79245	2.730103
2	-1.89003	1.172529	1.502504	2.276423	13.52201	3.316687
4	-2.23368	2.177554	0	6.829268	17.45283	4.845195
Alkalinity						
0.5	0.947867	6.588235	11.44945	6.380028	8.34512	6.74214
1	0.592417	2	11.32765	9.015257	10.74965	6.736994
2	4.146919	4.705882	16.80877	10.67961	17.11457	10.69115
4	14.81043	12	17.29598	12.06657	16.5488	14.54436
Turbidity						
0.5	26.08696	9.803922	-5.88235	-10	-30.4348	-2.08525
1	34.78261	9.803922	2.941176	-10	-73.913	-7.27707
2	18.84058	11.76471	-5.88235	-16.6667	-86.9565	-15.7801
4	18.84058	1.960784	-8.82353	-33.3333	-200	-44.2711
Iron						
0.5	11.11111	-12.4324	-8.64198	-51.2	-13.0435	-14.8414
1	11.11111	-12.4324	-18.5185	-47.2	-50.4348	-23.4949
2	11.11111	-12.4324	-44.4444	-66.4	-103.478	-43.1288
4	11.11111	-12.4324	-28.3951	-53.6	-64.3478	-29.5328
Fluoride						
0.5	23.33333	47.82609	23.07692	23.25581	-14.2857	20.64129
1	40	17.3913	36.53846	16.27907	-14.2857	19.18462
2	45	8.695652	7.692308	16.27907	28.57143	21.24769
4	46.66667	32.6087	46.15385	30.23256	64.28571	43.9895
Lagenandra toxicaria						
Acidity						
0.5	-0.48544	-0.96154	-1.58479	0	0.297177	-0.54692
1	-1.45631	-0.64103	-1.58479	1.226994	-0.89153	-0.66933
2	-2.26537	-1.92308	-0.63391	-1.07362	0.148588	-1.14948
4	-2.589	-2.88462	-1.58479	-0.76687	0.445765	-1.4759
Alkalinity						
0.5	1.524778	-1.77596	-1.36799	-0.41322	-0.14245	-0.43497
1	2.287166	0.409836	-1.91518	-0.68871	-1.85185	-0.35175

2	2.160102	-2.73224	0.273598	3.030303	-0.8547	0.375412
4	4.193139	2.04918	1.915185	0.688705	-2.42165	1.284911
Turbidity						
0.5	-10.7143	12.12121	-8	31.03448	20.58824	9.005929
1	7.142857	16.66667	6	-117.241	-23.5294	-22.1923
2	-5.35714	19.69697	-10	-110.345	-38.2353	-28.8481
4	0	48.48485	66	-110.345	-5.88235	-0.34847
Iron						
0.5	0	4.166667	-8.69565	-4	-4.34783	-2.57536
1	-3.84615	4.166667	-17.3913	-8	7.826087	-3.44894
2	0	-8.33333	-21.7391	-12	-13.0435	-11.0232
4	0	-8.33333	-25.2174	-12	-8.69565	-10.8493
Fluoride						
0.5	31.25	25	26.66667	26.66667	38.57143	29.63095
1	29.16667	-22.5	6.666667	11.11111	34.28571	11.74603
2	-25	30	-10	28.88889	24.28571	9.634921
4	-25	20	-26.6667	15.55556	68.57143	10.49206
Mangifera indica						
Acidity						
0.5	2.702703	4.64	1.72144	7.934132	5.405405	4.480736
1	3.81558	6.56	4.538341	11.67665	7.957958	6.909705
2	18.60095	11.68	3.12989	16.91617	21.92192	14.44979
4	25.59618	25.44	28.95149	35.47904	33.93393	29.88013
Alkalinity						
0.5	0.240674	4.086538	6.359102	5.149051	7.972028	4.761479
1	-0.36101	9.375	10.22444	6.097561	8.951049	6.857408
2	10.58965	5.769231	13.96509	9.891599	11.60839	10.36479
4	10.95066	13.46154	13.21696	14.63415	11.88811	12.83028
Turbidity						
0.5	-16	-5.61224	-9.67742	-22.2222	-20	-14.7024
1	-15.5	-10.7143	-9.03226	-10.3175	-17.7778	-12.6684
2	-20	-11.7347	-27.0968	-25.3968	-23.3333	-21.5123
4	-36	-9.18367	-16.129	-12.6984	-50	-24.8022
Iron						
0.5	-25.9259	-19.5122	-36.1111	-121.053	-240	-88.5204
1	-51.8519	-14.6341	-52.7778	-194.737	-580	-178.8
2	0	-19.5122	-61.1111	-215.789	-1100	-279.283
4	-33.3333	-21.9512	-61.1111	-236.842	-840	-238.648

Fluoride						
0.5	14.47368	-6.66667	-20.9677	4.545455	16.27907	1.53276
1	17.10526	-10	-17.7419	-16.6667	11.62791	-3.13509
2	14.47368	-30	-16.129	13.63636	15.11628	-0.58054
4	10.52632	-25	-8.06452	-37.8788	8.139535	-10.4555
Mentha arvensis						
Acidity						
0.5	-0.17889	-0.72333	-1.98556	-0.70547	1.647446	-0.38916
1	-1.78891	-1.98915	-1.98556	-2.1164	0.164745	-1.54306
2	-0.71556	-2.71248	-3.2491	-1.23457	0.823723	-1.4176
4	-1.9678	-3.25497	-4.33213	-1.5873	8.237232	-0.58099
Alkalinity						
0.5	1.711491	3.030303	3.703704	11.05193	4.234973	4.74648
1	3.178484	5.934343	8.068783	11.58455	6.967213	7.146676
2	5.012225	10.4798	10.58201	12.38349	9.289617	9.549428
4	13.20293	14.77273	14.28571	13.18242	13.79781	13.84832
Turbidity						
0.5	-3.125	-1	-12.1951	-17.0732	30.30303	-0.61805
1	-2.08333	-5	-43.9024	-30.4878	-59.0909	-28.1129
2	-8.33333	-4	-53.6585	-53.6585	-66.6667	-37.2634
4	-9.375	-16	-78.0488	-91.4634	-225.758	-84.129
Iron						
0.5	0	-3.6036	-3.6036	-5.68182	-10.2273	-4.62326
1	0	-3.6036	-3.6036	-5.68182	9.090909	-0.75962
2	-4	-3.6036	-12.6126	-36.3636	15.90909	-8.13415
4	8	-3.6036	-8.10811	37.5	20.45455	10.84857
Fluoride						
0.5	-16.6667	30.35714	-23.6842	2.678571	-28.9474	-7.25251
1	4.761905	12.5	-18.4211	12.5	-10.5263	0.162907
2	-54.7619	25	-23.6842	16.07143	-63.1579	-20.1065
4	-2.38095	39.28571	-2.63158	66.07143	65.78947	33.22682
Momordica charantia						
Acidity						
0.5	-1.36286	1.821192	3.908795	5.750799	5.939005	3.211386
1	-1.36286	0	4.560261	6.86901	6.581059	3.329494
2	-2.38501	0.331126	6.677524	10.70288	8.507223	4.766748
4	-0.51107	2.980132	6.188925	13.57827	7.865169	6.020286
Alkalinity						
0.5	0	5.341615	13.71795	14.12894	10.13025	8.663751
1	1.835985	6.086957	13.58974	17.96982	10.41968	9.980438

2	3.059976	6.956522	21.79487	18.38134	12.59045	12.55663
4	9.424725	18.63354	20.12821	21.94787	14.6165	16.95017
Turbidity						
0.5	13.51351	16.19835	0.612245	15.49296	2.166065	9.596626
1	20	9.421488	0.816327	-2.53521	7.942238	7.128968
2	-6.66667	6.280992	-1.83673	5.915493	8.66426	2.471469
4	9.009009	5.950413	-5.71429	-12.3944	-41.5162	-8.9331
Iron						
0.5	-5	-2.89855	-14.0625	-52.381	-180	-50.8684
1	-5	-5.7971	-14.0625	-69.0476	-226.667	-64.1148
2	-5	-18.8406	-17.1875	-69.0476	-273.333	-76.6818
4	0	-8.69565	-14.0625	-73.8095	-120	-43.3135
Fluoride						
0.5	-50	-41.8182	-1.40845	-72.4138	-43.3962	-41.8073
1	-50	18.18182	25.35211	-5.17241	-7.54717	-3.83713
2	-57.1429	-56.3636	-21.1268	27.58621	0	-21.4094
4	-19.0476	12.72727	32.39437	31.03448	16.98113	14.81793
Musa paradisiaca						
Acidity						
0.5	-0.33167	0.489396	1.923077	2.978056	1.088647	1.2295
1	-0.66335	0.652529	0.801282	3.134796	2.488336	1.282719
2	-0.16584	1.305057	4.00641	6.583072	5.443235	3.434387
4	0.165837	3.425775	6.891026	9.717868	8.864697	5.813041
Alkalinity						
0.5	1.201923	3.431373	4.252577	6.69344	4.64135	4.044133
1	3.125	6.372549	9.407216	9.906292	5.907173	6.943646
2	6.850962	12.62255	13.14433	13.92236	9.845288	11.2771
4	9.735577	16.42157	17.78351	16.06426	12.93952	14.58889
Turbidity						
0.5	13.54167	1.369863	-16.6667	-6.66667	-10.3448	-3.75333
1	10.41667	-6.84932	-16.6667	-17.7778	-10.3448	-8.24438
2	7.291667	-2.73973	-15	-26.6667	-20.6897	-11.5609
4	14.58333	-6.84932	-23.3333	-37.7778	-31.0345	-16.8823
Iron						
0.5	11.95652	-18.4524	-15.0289	-24.6154	-84.3373	-26.0955
1	-0.54348	-23.8095	-17.341	-46.1538	-78.3133	-33.2322
2	11.95652	-13.0952	-15.0289	-53.0769	-118.072	-37.4634
4	0	-20.8333	-15.0289	-46.1538	-128.916	-42.1863

Fluoride						
0.5	13.72549	2.564103	-13.3333	-15.7895	-7.31707	-4.03006
1	19.60784	15.38462	-63.3333	5.263158	-46.3415	-13.8838
2	25.4902	12.82051	-43.3333	-15.7895	-70.7317	-18.3088
4	19.60784	10.25641	-50	-34.2105	-26.8293	-16.2351
Opuntia dillenii						
Acidity						
0.5	0.328947	0.487805	-0.48154	-0.46948	-0.3268	-0.09221
1	0.164474	-0.4878	1.444623	0.625978	-0.1634	0.316774
2	2.302632	1.463415	3.691814	0.938967	0.980392	1.875444
4	2.631579	4.552846	4.333868	3.442879	4.901961	3.972627
Alkalinity						
0.5	4.046997	2.7894	4.801097	4.329609	3.612717	3.915964
1	5.613577	1.67364	6.721536	5.167598	5.346821	4.904634
2	5.874674	2.37099	8.504801	9.357542	5.202312	6.262064
4	10.18277	7.949791	9.465021	11.45251	8.381503	9.486319
Turbidity						
0.5	-1.33333	8.333333	6.896552	-23.8095	17.64706	1.546817
1	0	6.944444	10.34483	-9.52381	11.76471	3.906034
2	2.666667	8.333333	5.172414	-7.14286	8.823529	3.570617
4	1.333333	5.555556	13.7931	-28.5714	-120.588	-25.6955
Iron						
0.5	0	0	-2.77778	-12.8571	-10.7692	-5.28083
1	0	6.329114	-9.72222	-12.8571	-10.7692	-5.4039
2	-2.53165	3.164557	-9.72222	-12.8571	-17.6923	-7.92775
4	3.164557	0	-2.77778	-12.8571	-21.5385	-6.80177
Fluoride						
0.5	-21.875	41.17647	-40.625	46.03175	0	4.941643
1	-21.875	17.64706	9.375	38.09524	2.325581	9.113576
2	-40.625	11.76471	12.5	-58.7302	20.93023	-10.832
4	43.75	-23.5294	46.875	-19.0476	-74.4186	-5.27413
Phyllanthus emblica						
Acidity						
0.5	21.15385	29.66252	39.69336	39.65812	45.18272	35.07011
1	23.95105	33.39254	35.26405	36.75214	45.84718	35.04139
2	33.74126	35.7016	39.35264	42.5641	45.34884	39.34169
4	37.23776	40.31972	42.93015	43.58974	45.84718	41.98491
Alkalinity						
0.5	18.3922	23.16176	24.96879	34.00538	26.37037	25.3797
1	24.96955	32.35294	29.08864	31.45161	39.55556	31.48366

2	32.52132	38.35784	53.05868	47.98387	45.03704	43.39175
4	53.10597	53.30882	55.55556	54.03226	50.07407	53.21534
Turbidity						
0.5	-33.3333	-9.25926	-12.2449	-43.5897	-30.303	-25.7461
1	-17.4603	-16.6667	-20.4082	-76.9231	-69.697	-40.231
2	-66.6667	-44.4444	-42.8571	-100	-203.03	-91.3997
4	-168.254	-101.852	-85.7143	-248.718	-166.667	-154.241
Iron						
0.5	-1.44231	-0.97087	0	-23.1481	-31.5385	-11.42
1	24.03846	14.56311	1.621622	-23.1481	-42.3077	-5.04653
2	32.69231	23.30097	9.189189	-5.55556	-10.7692	9.771536
4	35.57692	21.35922	4.864865	0	-35.3846	5.283279
Fluoride						
0.5	-52.6316	-24.5283	-68.4211	24.39024	-1.17647	-24.4734
1	-26.3158	-50.9434	-38.5965	17.07317	-2.35294	-20.2271
2	-21.0526	-13.2075	-31.5789	0	-15.2941	-16.2266
4	-10.5263	-35.8491	-15.7895	14.63415	-4.70588	-10.4473
Plectranthus amboinicus						
Acidity						
0.5	-2.16606	0.534759	-2.10896	-5.12367	1.779935	-1.4168
1	-2.34657	-1.42602	-2.10896	-4.59364	-0.48544	-2.19213
2	-3.97112	-1.78253	-2.9877	-7.77385	-1.61812	-3.62666
4	-2.52708	-4.45633	-6.67838	-12.8975	-2.75081	-5.86202
Alkalinity						
0.5	-1.02433	-1.83727	-2.20994	2.678571	1.438849	-0.19082
1	-1.02433	0.787402	-1.38122	4.209184	2.877698	1.093748
2	-1.66453	0.393701	0.138122	4.591837	2.302158	1.152257
4	0.256082	3.28084	2.071823	8.290816	4.604317	3.700776
Turbidity						
0.5	-4.34783	1.273885	5.46875	18.46154	6.097561	5.390782
1	8.695652	-5.73248	-3.125	13.07692	8.536585	4.290335
2	3.26087	-18.4713	-10.1563	0	10.97561	-2.87822
4	-1.63043	-10.1911	-19.5313	-1.53846	7.317073	-5.11483
Iron						
0.5	9.375	-2.85714	-6.06061	42.85714	-287.5	-48.8371
1	-6.25	-5.71429	-12.1212	0	-281.25	-61.0671
2	0	-11.4286	-21.2121	-11.4286	-337.5	-76.3139
4	-12.5	-14.2857	-18.1818	-14.2857	-325	-76.8506

Fluoride						
0.5	-200	-4.47761	25.37313	36.36364	-25.4902	-33.6462
1.0	-66.6667	-5.97015	7.462687	9.090909	11.76471	-8.8637
2.0	-191.667	23.8806	17.91045	20.45455	-11.7647	-28.2372
4	-266.667	11.9403	26.86567	36.36364	0	-38.2994
Ricinus communis						
Acidity						
0.5	-2.33813	-1.7762	-3.29289	-5.55556	-0.48544	-2.68964
1	-5.03597	-1.95382	-3.98614	-5.55556	0.323625	-3.24157
2	-3.23741	-3.37478	-3.29289	-6.90236	-0.16181	-3.39385
4	-5.7554	-4.35169	-5.28596	-9.68013	0	-5.01464
Alkalinity						
0.5	-1.71053	0.682128	1.923077	7.480315	7.922078	3.259414
1	-1.84211	1.909959	3.983516	9.973753	7.532468	4.311518
2	-2.5	0.272851	3.296703	8.136483	7.922078	3.425623
4	-1.71053	0.545703	6.181319	7.742782	11.16883	4.785622
Turbidity						
0.5	-25	-40.5405	-19.3182	-31.25	-114.634	-46.1486
1	-30.3571	-41.4414	-31.8182	-60	-151.22	-62.9673
2	-23.2143	-27.9279	-32.9545	-78.75	-200	-72.5694
4	-54.4643	-32.4324	-44.3182	-103.75	-531.707	-153.334
Iron						
0.5	-1.53846	-2.85714	-18.1818	-94.5455	-153.125	-54.0496
1	-10.7692	-2.85714	-22.7273	-94.5455	-153.125	-56.8048
2	-7.69231	5.714286	-22.7273	-85.4545	-181.25	-58.282
4	-10.7692	-2.85714	-22.7273	-127.273	-245.313	-81.7878
Fluoride						
0.5	12.19512	-14.6341	-15	-7.14286	27.63158	0.609939
1	-12.1951	-12.1951	-55	-19.6429	28.94737	-14.0171
2	7.317073	-7.31707	-12.5	-12.5	21.05263	-0.78947
4	7.317073	-19.5122	-2.5	10.71429	19.73684	3.151201
Strychnos potatorum						
Acidity						
0.5	3.107861	0	1.245552	-0.35398	4.785479	1.756982
1	2.010969	0.914077	1.245552	-0.17699	3.30033	1.458787
2	2.193784	0.365631	0	-1.76991	5.115512	1.181003
4	2.010969	-2.01097	-1.9573	-2.65487	2.970297	-0.32837
Alkalinity						
0.5	-0.25974	-3.77095	5.562579	5.531915	9.439528	3.300666
1	0.649351	1.675978	11.75727	10.78014	10.0295	6.978448

2	5.064935	3.212291	12.26296	11.20567	10.17699	8.38457
4	5.714286	-1.39665	14.28571	15.74468	20.94395	11.0584
Turbidity						
0.5	12.04188	11.72638	-14.8315	19.01639	10.30043	7.650726
1	8.726003	22.8013	-5.16854	9.180328	1.716738	7.451167
2	-8.02792	17.91531	-20	-0.32787	-7.72532	-3.63316
4	4.363002	8.794788	6.741573	-17.0492	-9.87124	-1.40421
Iron						
0.5	-7.69231	-12	-28.5714	-150	-720	-183.653
1	-7.69231	-4	-31.4286	-158.333	-300	-100.291
2	-7.69231	-4	-31.4286	-150	-740	-186.624
4	-3.84615	-8	-31.4286	-158.333	-760	-192.322
Fluoride						
0.5	-9.45148	-75.2617	-103.019	-43.155	-43.5629	-54.8899
1	-5.06329	-103.35	-66.2152	-12.9364	8.183633	-35.8762
2	-0.77637	-63.1193	-130.96	8.967596	-1.37226	-37.452
4	1.637131	-108.339	-123.142	-40.3165	-58.9072	-65.8136
Tamarindus indicus						
Acidity						
0.5	2.512563	-5.50622	-1.15702	2.28013	0	-0.37411
1	1.842546	-7.81528	-0.82645	1.791531	-0.57748	-1.11702
2	2.177554	-6.03908	-0.82645	2.442997	0.224575	-0.40408
4	1.005025	-6.39432	-0.99174	2.28013	0.064164	-0.80735
Alkalinity						
0.5	1.911589	1.73482	4.987212	4.987212	9.039548	4.532076
1	3.106332	1.239157	6.905371	6.649616	9.887006	5.557496
2	6.332139	3.469641	9.71867	8.056266	12.14689	7.944722
4	3.942652	6.071871	8.567775	9.335038	12.57062	8.097592
Turbidity						
0.5	3.726708	-7.95455	-4.0404	1.219512	33.62069	5.314392
1	-9.93789	-14.7727	-7.07071	-7.31707	31.03448	-1.61278
2	-9.31677	-23.8636	-1.0101	-8.53659	11.2069	-6.30404
4	-0.62112	-120.455	-11.1111	-12.1951	2.586207	-28.3591
Iron						
0.5	1.408451	-35.8491	-46.9136	36.92308	-161.111	-41.1084
1	1.408451	-32.0755	-29.6296	22.30769	-37.037	-15.0052
2	1.408451	-35.8491	-72.8395	-115.385	-418.519	-128.237
4	1.408451	-32.0755	-72.8395	-115.385	-433.333	-130.445
Fluoride						
0.5	8.108108	0	41.66667	23.68421	27.02703	20.0972

1	-5.40541	10	22.91667	7.894737	-94.5946	-11.8377
2	8.108108	-103.333	20.83333	-65.7895	-35.1351	-35.0633
4	-21.6216	-50	29.16667	10.52632	-24.3243	-11.2506
Terminalia bellirica						
Acidity						
0.5	0.862069	8.006536	8.103728	8.471761	13.7987	7.848559
1	1.724138	13.39869	12.64182	9.302326	25	12.41339
2	7.586207	27.94118	28.52512	25.24917	33.27922	24.51618
4	26.03448	32.35294	34.84603	33.55482	34.9026	32.33817
Alkalinity						
0.5	10.8508	11.64295	18.21705	16.18037	15.77465	14.53316
1	23.92109	20.56921	19.37984	17.10875	28.87324	21.97043
2	13.5635	19.40492	34.49612	22.9443	27.04225	23.49022
4	36.86806	30.9185	30.74935	46.15385	36.47887	36.23373
Turbidity						
0.5	-5.55556	-7.04225	-12.069	-12.7273	-78.0488	-23.0886
1	-6.94444	-12.6761	-22.4138	-50.9091	-160.976	-50.7838
2	-41.6667	-14.0845	-22.4138	-20	-346.341	-88.9013
4	-70.8333	-25.3521	-46.5517	-49.0909	-502.439	-138.853
Iron						
0.5	-7.69231	-27.7108	1.960784	-10.8434	-179.412	-44.7395
1	-10.7692	-68.6747	-27.451	-26.506	-226.471	-71.9743
2	-7.69231	-62.6506	9.803922	-22.8916	-235.294	-63.7449
4	-3.84615	-68.6747	-17.6471	-89.1566	-361.765	-108.218
Fluoride						
0.5	-47.8261	9.259259	-27.907	-13.4615	15.87302	-12.8125
1	-34.7826	-27.7778	-32.5581	11.53846	22.22222	-12.2716
2	26.08696	68.51852	6.976744	11.53846	65.07937	35.64001
4	39.13043	42.59259	39.53488	-3.84615	68.25397	37.13315
Terminalia chebula						
Acidity						
0.5	12.4792	6.393443	10	29.69005	33.8141	18.47536
1	18.96839	18.85246	14.59016	17.12887	41.34615	22.17721
2	23.12812	29.18033	29.83607	30.01631	41.50641	30.73345
4	33.61065	37.21311	35.2459	39.31485	42.62821	37.60254
Alkalinity						
0.5	25.09554	25.03161	19.32432	30.29872	30.04418	25.95887
1	29.29936	23.00885	21.08108	23.32859	32.98969	25.94152
2	24.20382	29.20354	28.78378	35.56188	42.41532	32.03367
4	34.3949	43.23641	40.81081	44.09673	38.88071	40.28391

Turbidity						
0.5	-17.7419	-2.67857	-7.92079	-3.125	-30.1587	-12.325
1	-3.22581	-3.57143	-13.8614	-20.8333	-52.381	-18.7746
2	-12.0968	-7.14286	-15.8416	-27.0833	-95.2381	-31.4805
4	-5.64516	-34.8214	-51.4851	-39.5833	-74.6032	-41.2276
Iron						
0.5	7.142857	0	-17.3913	-187.273	-71.6981	-53.8439
1	3.571429	-8	-21.7391	-194.545	-213.208	-86.7841
2	7.142857	-8	-13.0435	-205.455	-74.5283	-58.7767
4	7.142857	-8	-17.3913	-230.909	-44.3396	-58.6994
Fluoride						
0.5	-18.4615	-10.1449	20.25316	25	23.07692	7.944724
1	-6.15385	8.695652	5.063291	17.1875	33.84615	11.72775
2	-3.07692	4.347826	13.92405	14.0625	40	13.85149
4	4.615385	52.17391	8.860759	25	52.30769	28.59155
Theobromo cocoa						
Acidity						
0.5	5.472637	-1.60428	0.701754	-1.54374	-0.67797	0.469682
1	4.809287	-2.6738	-0.17544	-2.22985	0.508475	0.047736
2	5.638474	-2.31729	-0.52632	-0.34305	0.338983	0.55816
4	5.970149	1.069519	6.140351	5.317324	-0.67797	3.563875
Alkalinity						
0.5	2.143758	-1.60858	4.095112	5.866667	4.72879	3.04515
1	3.026482	0.268097	2.377807	7.6	9.040334	4.462544
2	3.278689	-0.13405	5.151915	9.333333	11.12656	5.751291
4	3.278689	-0.13405	6.472919	11.46667	16.13352	7.443549
Turbidity						
0.5	0.625	6.545455	2.30608	15.10297	25.90529	10.09696
1	0.625	6.545455	-2.09644	4.118993	16.43454	5.12551
2	4.53125	-3.45455	-9.85325	3.89016	20.89136	3.200996
4	6.71875	-0.18182	-9.64361	-8.46682	8.635097	-0.58768
Iron						
0.5	-5.40541	-8.33333	-9.52381	-30.6818	-17.3913	-14.2671
1	-9.72973	-12.5	-19.0476	-42.0455	-29.3478	-22.5341
2	-7.83784	-4.16667	-28.5714	-47.7273	-35.8696	-24.8346
4	-12.4324	-12.5	-19.0476	-47.7273	-35.8696	-25.5154
Fluoride						
0.5	27.8481	-4.6875	-4.7619	-19.6078	0	-0.24183
1	20.25316	0	-44.4444	-17.6471	1.388889	-8.08989
2	16.4557	-4.6875	-9.52381	-3.92157	5.555556	0.775675

4	5.063291	9.375	0	-19.6078	-5.55556	-2.14502
Trigonella foenum-graecum						
Acidity						
0.5	-1.5025	-1.31363	-0.65359	1.577287	8.035714	1.228655
1	-0.50083	-2.79146	-2.28758	2.050473	10.11905	1.317929
2	-1.66945	-2.29885	-0.98039	3.470032	11.45833	1.995935
4	-1.33556	-1.80624	-0.4902	4.258675	11.45833	2.417003
Alkalinity						
0.5	-1.2926	1.525822	-0.1233	-3.81356	6.76259	0.61179
1	-1.52761	0.938967	1.233046	0.141243	8.057554	1.768639
2	-1.52761	1.29108	1.35635	0.988701	9.928058	2.407315
4	-1.52761	1.173709	4.192355	4.378531	9.064748	3.456346
Turbidity						
0.5	-1.61031	24.53152	-7.87037	18.4492	8.088235	8.317655
1	-4.34783	6.132879	-10.8796	12.83422	2.941176	1.336165
2	-2.73752	16.01363	-10.4167	9.893048	0.735294	2.697557
4	-1.28824	25.3833	-11.8056	-0.53476	9.926471	4.336243
Iron						
0.5	-6	-4.9505	-40.9091	-309.302	-1128.57	-297.947
1	-6	-4.9505	-51.5152	-318.605	-1214.29	-319.071
2	-10.5	-4.9505	-53.0303	-309.302	-1285.71	-332.699
4	0	-9.40594	-53.0303	-318.605	-1342.86	-344.78
Fluoride						
0.5	15.09434	-155	20.33898	-31.3725	3.773585	-29.4331
1	22.64151	-150	-10.1695	13.72549	-15.0943	-27.7794
2	5.660377	-175	6.779661	-5.88235	11.32075	-31.4243
4	11.32075	-135	18.64407	-35.2941	-7.54717	-29.5753
Vetiveria zizanioides						
Acidity						
0.5	1.647446	0.665557	-2.16306	0.493421	1.134522	0.355577
1	0.329489	-0.83195	-2.99501	0.328947	2.106969	-0.21231
2	0.988468	-0.83195	-1.99667	3.125	5.672609	1.391492
4	-1.4827	-1.66389	-1.4975	1.973684	4.213938	0.308705
Alkalinity						
0.5	9.135802	6.052963	3.018373	4.037685	7.671602	5.983285
1	11.23457	8.575032	9.186352	7.671602	8.07537	8.948585
2	10.12346	7.818411	9.317585	11.17093	13.18977	10.32403
4	11.7284	9.962169	9.84252	13.18977	14.67026	11.87862
Turbidity						
0.5	-7.25806	0.833333	-4.80769	-3.8835	-1.0989	-3.24296

1	-14.5161	-1.66667	-5.76923	-5.82524	21.97802	-1.15985
2	-29.0323	-4.16667	-9.61538	-11.6505	9.89011	-8.91494
4	-19.3548	-16.6667	-24.0385	-25.2427	4.395604	-16.1814
Iron						
0.5	-100	-4.47761	46.07843	-2.77778	12.63158	-9.70908
1	-91.3043	-4.47761	41.17647	-21.2963	-31.5789	-21.4961
2	-80.8696	-0.74627	-27.451	-20.3704	-21.0526	-30.098
4	-64.3478	2.985075	-1.96078	3.703704	7.368421	-10.4503
Fluoride						
0.5	48.07692	-263.636	0	-58.6207	-27.2727	-60.2906
1	48.07692	-109.091	51.11111	-144.828	-9.09091	-32.7643
2	28.84615	27.27273	15.55556	20.68966	-81.8182	2.109182
4	32.69231	-18.1818	37.77778	48.27586	69.69697	34.05222
Zea Mays						
Acidity						
0.5	1.132686	-0.79618	-2.71132	0.778816	4.769001	0.6346
1	0.647249	0.636943	-2.39234	2.336449	7.749627	1.795585
2	0.485437	0	0.956938	10.5919	9.388972	4.284649
4	-0.97087	1.751592	2.870813	6.697819	11.17735	4.30534
Alkalinity						
0.5	3.640777	3.522013	3.608247	6	-1.58046	3.038115
1	5.218447	2.264151	3.994845	7.2	2.729885	4.281466
2	3.15534	5.031447	10.30928	11.06667	8.045977	7.521742
4	5.582524	8.679245	14.17526	12.66667	8.333333	9.887405
Turbidity						
0.5	-6.14887	4.054054	-0.70671	-11.4286	6.626506	-1.52072
1	-17.7994	-12.5	-13.4276	12.38095	31.92771	0.11635
2	-12.945	-9.7973	-8.12721	2.857143	-9.03614	-7.4097
4	-26.8608	-17.2297	-20.1413	-50.4762	-6.62651	-24.2669
Iron						
0.5	-3.84615	0	0	0	-12.9032	-3.34988
1	0	0	-3.84615	-4.34783	-23.6559	-6.36998
2	0	0	-3.84615	-4.34783	-18.8172	-5.40224
4	0	0	-3.84615	-13.0435	-23.6559	-8.10911
Fluoride						
0.5	52.17391	18.86792	3.846154	53.42466	-30.9524	19.47205
1	-100	24.5283	34.61538	12.32877	38.09524	1.913538
2	-73.913	35.84906	34.61538	38.35616	-21.4286	2.695798
4	-30.4348	24.5283	63.46154	50.68493	38.09524	29.26705

Positive values indicate % decrease and negative values indicate % increase with respect to control.

In the case of pH, plants / plant parts were investigated for their pH stabilizing capacity, both in the acidic and alkaline range.

When the efficiency of plant materials were experimented at acidic range of pH, they exhibited varying levels of stabilization capacity. Out of 24 plants experimented, 21 plants exhibited appreciable effectiveness at higher retention time (6 - 24HAT) and higher dosages (2-4g). Of the two dozen plants investigated, a total of 6 plants exhibited acidic pH stabilizing capacity and is given in table 6. Highest stabilization efficiency was observed with dry fruits of *Phyllanthus emblica* (45.84%, 1g, 24HAT), seed kernels of *Mangifera indica* (35.47%, 4g, 12HAT) and dry fruits of *Terminalia chebula* (42.62%, 4g, 24HAT). Other plants which showed comparatively better results were dry fruits of *Terminalia bellirica* (34.90%, 4g, 6HAT), fruits of *Abelmoschus esculentus* (25.32%, 2g, 24HAT), stem of *Euphorbia antiquorum* (25.32%, 1g, 6HAT), leaves of *Aloe barbadensis* (21.3%, 4g, 1.5HAT), fruits of *Cyamopsis tetragonoloba* (18.84%), roots of *Hemidesmus indicus* (17.45%, 4g,24HAT), fruits of *Momordica charantia* (13.57%, 4g, 12HAT) and seeds of *Trigonella foenum-graecum* (11.4%, 2g, 24HAT). Comparatively low levels of acidic pH stabilizing capacity was observed with seeds of *Strychnos potatorum* (5.11%, 2g, 24HAT), roots of *Vetiveria zizanioides* (5.67%, 2g, 24HAT), peduncle of *Musa paradisiaca* (9.7%, 4g, 12HAT), cladode of *Opuntia dillenii* (4.90%, 4g, 24HAT), seeds of *Theobroma cacao* (6.14%,4g, 6HAT), leaves of *Azadirachta indica* (9.72%, 4g, 24HAT), twigs of *Bacopa monnieri* (6.23%, 4g, 24HAT), leaves of *Mentha arvensis* (8.23%, 4g, 24HAT), seeds of *Tamarindus indica* (2.51%,0.5g,3HAT) and rhizome of *Lagenandra toxicaria* (1.22%, 1g, 12HAT).

Table 6. Dosages and retention time of plants/ plant parts at which highest acidic pH stabilizing capacity was observed.

Sl. No.	Plant	Percentage change with respect to control (%)	Quantity (grams)	Retention time (In hours)
1	*Phyllanthus emblica*	45.84	1	24 HAT
2	*Terminalia chebula*	42.62	4	24 HAT
3	*Mangifera indica*	35.47	4	12 HAT
4	*Terminalia bellirica*	34.90	4	24 HAT
5	*Euphorbia antiquorum*	31.89	2	6 HAT
6	*Aloe barbadensis*	21.13	0.5	1.5 HAT

Plants / plant parts with higher stabilizing capacity in the alkaline range are given in table 7. Comparatively higher percentage stabilizing capacity was observed with dry fruits of *Phyllanthus emblica* (55.55%, 4g, 6HAT), *Terminalia bellirica* (46.15%, 4g, 12HAT) and *Terminalia chebula* (44.09%, 4g, 12HAT). Moderate levels of stabilizing efficiency was observed with fruits of *Momordica charantia* (21.79%), leaves of *Azadirachta indica* (21.34%) and seeds of *Strychnos potatorum* (20.94%). Other plant materials with better results were leaves of *Mentha arvensis* (14.77%), stem of *Euphorbia antiquorum* (14.88%), seed kernels of *Mangifera indica* (14.63%), leaves of *Aloe barbadensis* (13.42%), fruits of *Cyamopsis tetragonoloba* (11.06%), roots of *Hemidesmus indicus* (17.29%), peduncle of *Musa paradisiaca* (17.18%), seeds of *Zea mays* (14.17%), fruits of *Abelmoschus esculentus* (13.15%), twigs of *Bacopa monnieri* (11.84%) and cladode of *Opuntia dillenii* (11.45%). Comparatively lower levels of percentage removal was observed with rhizome of *Lagenandra toxicaria* (4.19%), leaves of *Plectranthus amboinicus* (8.29%); seeds of *Ricinus communis* (11.16%);

Tamarindus indicus (12.57%), *Theobroma cacao* (16.13%) and roots of *Vetiveria zizanioides* (14.67%).

Table 7:- Dosages and retention time of plants/ plant parts at which highest alkaline pH stabilizing capacity was observed.

Sl. No.	Plant	Percentage change with respect to control (%)	Quantity (in grams)	Retention time (In hours)
1	*Phyllanthus emblica*	55.55	4	6
2	*Terminalia bellirica*	46.15	4	12
3	*Terminalia chebula*	44.09	4	12
4	*Momordica charantia*	21.94	4	12
5	*Azadirachta indica*	21.34	4	12
6	*Strychnos potatorum*	20.94	4	24

In the case of turbidity, maximum removal efficiency was observed both at lower (1.5 and 3HAT) and higher (12 and 24 HAT) retention time (Table 8). In general, maximum removal efficiency was observed at a retention time of 24HAT and at dosages of 0.5, 1 and 2g. Plant materials like rhizome of *Lagenandra toxicaria* (66%, 4g, 6HAT), roots of *Hemidesmus indicus* (34.78%, 1g, 1.5HAT) and fruits of *Abelmoschus esculentus* (33.62%, 0.5g, 24HAT) were found to be effective. Rest of the materials like seeds of *Theobroma cacao* (25.90%, 0.5g, 24HAT) and *Trigonella foenum-graecum* (25.38%, 4g, 3HAT); fruits of *Cyamopsis tetragonoloba* (20.52%, 1g, 24HAT), roots of *Vetiveria zizanioides* (21.97%, 1g, 24HAT), seeds of *Strychnos potatorum* (19.06%, 0.5g, 12HAT), leaves of *Aloe barbadensis* (18.91%, 0.5g, 24HAT), cladodes of *Opuntia dillenii* (17.64%, 0.5g, 24HAT), fruits of *Momordica charantia* (16.19%, 0.5g, 3HAT), peduncle of *Musa paradisiaca* (14.58%, 4g, 1.5HAT), twigs of *Bacopa monnieri* (11.70%, 2g,

24HAT) and stem of *Euphorbia antiquorum* (19.33%, 2g, 24HAT) showed varying results.

Table 8:- Dosages and retention time of plants/ plant parts at which highest percentage removal of turbidity was observed.

Si. No	Plant	Percentage change with respect to control (%)	Quantity (in grams)	Retention time (In hours)
1	*Lagenandra toxicaria*	66	4	6
2	*Hemidesmus indicus*	34.78	1	1.5
3	*Abelmoschus esculentus*	33.62	0.5	24
4	*Tamarindus indica*	33.62	0.5	24
5	*Zea mays*	31.92	1	24

Iron removal from water was observed at the early hours of treatment. Only 12 plants out of 24 plants selected exhibited efficiencies in removing iron from water. The plants which exhibited maximum removal efficiency are detailed in table 9. Highest percentage removal was observed with the roots of *Vetiveria zizanioides* (46.07%), leaves of *Mentha arvensis* (37.50%) and seeds of *Tamarindus indica* (36.92%). Other plant materials with better removal efficiencies were fruits of *Cyamopsis tetragonoloba* (22.07%); dry fruits of *Phyllanthus emblica* (24.03%) and *Terminalia chebula* (17.14%); leaves of *Azadirachta indica* (14.28%), roots of *Hemidesmus indicus* (11.11%), peduncle of *Musa paradisiaca* (11.95%), twigs of *Bacopa monnieri* (8.10%), cladode of *Opuntia dillenii* (6.32%), dry fruits of *Terminalia bellirica* (9.80%) and rhizome of *Lagenandra toxicaria* (7.82%).

Table 9:- Dosages and retention time of plants/ plant parts at which highest percentage removal of iron content was observed.

Sl. No.	Plant	Percentage change with respect to control (%)	Quantity (in grams)	Retention time (In hours)
1	*Vetiveria zizanioides*	46.07	0.5	6
2	*Mentha arvensis*	37.5	4	12
3	*Tamarindus indica*	36.92	0.5	12
4	*Phyllanthus emblica*	35.57	4	1.5
5	*Cyamopsis tetragonoloba*	22.07	0.5	1.5

Of 24 plants screened, 16 plants exhibited efficiencies n the removal of fluoride at lower dosages and four plants with highest efficiencies are depicted in Table 10. Highest removal percentage was observed with the plant material derived from the stem of *Euphorbia antiquorum* (78.57%, 0.5g, 24HAT), roots of *Vetiveria zizanioides* (69.69%, 4g, 24HAT) and leaves of *Aloe barbadensis* (73.33%, 2g , 1.5HAT). Plant materials like dry fruits of *Terminalia bellirica* (68.51%,2g,3HAT) and *Terminalia chebula* (52.30%, 4g, 24HAT); leaves of *Mentha arvensis* (66.07%, 4g, 12HAT), twigs of *Bacopa monnieri* (67.346%, 4g, 24HAT), rhizome of *Lagenandra toxicaria* (68.571%, 4g, 24HAT), seeds of *Zea mays* (63.46%, 4g, 6HAT), *roots of Hemidesmus indicus* (64.28%,4g, 24HAT), leaves of *Azadirachta indica* (40.74%, 2g, 24HAT), seeds of *Tamarindus indica* (41.67%, 0.5g, 6HAT), cladode of *Opuntia dillenii* (46.87%, 4g, 6HAT), fruits of *Abelmoschus esculentus* (36.98%, 4g, 24HAT); fruits of *Cyamopsis tetragonoloba* (44.44%, 2g, 6HAT) and *Momordica charantia* (32.39%, 4g, 6HAT); seeds of *Theobromo cacao* (27.84%, 0.5g, 1.5HAT) and *Ricinus communis* (27.63%,

1g, 12HAT); dry fruits of *Phyllanthus emblica* (24.39%, 0.5g, 12HAT), leaves of *Plectranthus amboinicus* (36.36%, 0.5g, 12HAT), peduncle of *Musa paradisiaca* (25.49%, 2g, 1.5HAT), seeds of *Trigonella foenum-graecum* (22.64%, 1g, 1.5HAT) seed kernels of *Mangifera indica* (17.10%, 1g, 1.5HAT) and seeds of *Strychnos potatorum* (8.96%, 2g, 12HAT) exhibited appreciable levels of fluoride removal.

Table 10:- Dosages and retention time of plants/parts at which highest percentage change was observed in removing fluoride content.

Sl. No.	Plant	Percentage change with respect to control (%)	Quantity (in grams)	Retention time (In hours)
1	*Euphorbia antiquorum*	78.57	0.5	24
2	*Vetiveria zizanioides*	69.69	4	24
3	*Aloe barbadensis*	73.33	2	1.5
4	*Terminalia bellirica*	68.51	2	3

Secondary screening was carried out with the plant material which exhibited highest removal / stabilization efficiency in preliminary screening. Three plants, which are highly effective against each of the five water contaminant / quality parameter (acidity, alkalinity, turbidity, iron and fluoride) were selected. Batch treatment was carried out with each of the plant material. List of plant materials with higher removal efficiency obtained from preliminary screening are given in the table 11. Statistical analysis was carried out on the data thus obtained. Data on statistical analysis are given in tables 12-76.

Table 11: List of plants/plant materials selected from secondary screening

Sl. No	Plant	Percentage change with respect to control (%)	Quantity (in grams)	Retention time (In hours)
Acidity				
1	*Phyllanthus emblica*	45.84	1	24
2	*Terminalia chebula*	42.62	4	24
3	*Mangifera indica*	35.47	4	12
Alkalinity				
1	*Phyllanthus emblica*	55.55	4	6
2	*Terminalia bellirica*	46.15	4	12
3	*Terminalia chebula*	44.09	4	12
Turbidity				
1	*Lagenandra toxicaria*	66	4	6
2	*Hemidesmus indicus*	34.78	1	1.5
3	*Abelmoschus esculentus*	33.62	0.5	24
Iron				
1	*Vetiveria zizanioides*	46.07	0.5	6
2	*Mentha arvensis*	37.5	4	12
3	*Tamarindus indica*	36.92	0.5	12
Fluoride				
1	*Euphorbia antiquorum*	78.57	0.5	24
2	*Vetiveria zizanioides*	69.69	4	24
3	*Aloe barbadensis*	73.33	2	1.5

All the three plant materials with higher stabilizing percentage were selected. They are *Phyllanthus emblica*, *Terminalia chebula* and *Mangifera indica*. Batch experiments were conducted in triplicates. Samples were

collected at respective intervals. The results obtained were then statistically analyzed and tabulated.

In studies on acidic pH with dry fruits of *Phyllanthus emblica,* an increase in retention time and dosages resulted in subsequent stabilization in acidic pH (Table 12). With an increase in retention time in the treatment set, a gradual reduction in pH was noticed. A gradual increase in dosage was observed with a steep reduction in pH after 3HAT. From the ANOVA table (Table 13) dosage and time were found to have a significant effect on the response of the plant material (Dosage: - F value = 19.081; p value <0.000; Time: - F value = 4.464, p= 0.004). The interactive effect of both dosage and retention time was found to be insignificant because p value was >0.05 (Table 13). In the case of dosage and retention time, higher dosages (2 and 4g) and retention time (24HAT) were found to be significant in stabilizing acidic pH of the water (Table 14 and 15).

Table 12: Response of dry fruits of *Phyllanthus emblica* at varying dosages and retention time.

Time	Control	Dosage			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	6.44±0.04	5.76±0.99	5.60±1.07	5.37±1.14	4.78±0.77
3HAT	6.45±0.14	6.20±0.23	6.12±0.11	5.80±0.23	5.21±0.55
6HAT	6.45±0.18	6.30±0.17	5.97±0.36	5.60±0.18	4.46±0.71
12HAT	6.46±0.10	5.92±0.40	5.48±0.74	5.29±1.15	3.97±0.25
24HAT	6.51±0.41	5.25±0.77	4.93±0.71	4.32±1.09	4.19±0.70

Table 13: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III sum of Squares	df	Mean Square	F	Sig
Corrected Model	42.659	24	1.777	4.350	<0.001
Intercept	2311.964	1	2311.964	5.658E3	<0.001
Dosage	31.189	4	7.797	19.081	<0.001
Time	7.296	4	1.824	4.464	0.004
Dosage*Time	4.173	16	0.261	0.638	0.837
Error	20.432	50	0.409		
Total	2375.055	75			
Corrected total	63.091	74			

a. R Squared = .676 (Adjusted R Squared = .521)

Table 14:- Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.5747*	0.017
	1.0g	0.8407*	<0.001
	2.0g	1.1853*	<0.001
	4.0g	1.9387*	<0.001
0.5g	1.0g	0.2660	0.260
	2.0g	0.6107*	0.012
	4.0g	1.3640*	<0.001
1.0g	2.0g	0.3447	0.146
	4.0g	1.0980*	<0.001
2.0g	4.0g	0.7533*	0.002

* Mean difference is significant at the 0.05 level

Table 15:- Significant mean differences at varying retention time of plant material

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	-0.3687	0.121
	6HAT	-0.1647	0.484
	12HAT	0.1660	0.480
	24HAT	0.5500*	0.022
3HAT	6HAT	0.2040	0.386
	12HAT	0.5347*	0.026
	24HAT	0.9187*	<0.001
6HAT	12HAT	0.3307	0.163
	24HAT	0.7147*	0.004
12HAT	24HAT	0.3840	0.106

* Mean difference is significant at the 0.05 level

With studies on acidic pH with dry fruits of *Terminalia chebula,* a gradual reduction in response of the plant material was observed with an increase in dosage and retention time (Table 16). Lower dosages were found to have a significant effect on the response of the plant material, while time beyond 3HAT showed an insignificant effect on the response (Table 14). In the case of retention time, significant reduction was observed up to 6HAT followed by a steep reduction. From ANOVA table (table 17) dosage and retention time was found to have a significant effect on the response of the plant material. Dosage of the plant material was significant because p value was <0.001 (F value = 116.835). In the case of retention time also, p value was significant (F value =23.409; p value <0.001) (Table 17). Interactive effect of dosage and retention time was also found to be significant (F value = 3.900; p value<0.001). Almost all dosages were found to be significant (Table 18), while in the case of retention time lower retention time was found to be significant (Table 19).

Table 16:- Response of dry fruits of *Terminalia chebula* at varying dosages and retention time.

Time (hrs)	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	6.26±0.02	5.96±0.09	5.92±0.13	5.50±0.32	4.81±0.17
3HAT	6.31±0.01	5.75±0.32	4.73±0.46	4.50±0.10	4.84±0.67
6HAT	6.36±0.08	5.34±0.15	4.36±0.43	4.01±0.14	3.94±0.24
12HAT	6.47±0.11	5.36±0.32	4.37±0.23	4.28±0.36	3.84±0.08
24HAT	6.47±0.18	4.91±0.54	4.47±0.20	4.37±0.57	4.18±0.18

Table 17. Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III sum of Squares	df	Mean Square	F	Sig
Corrected Model	55.698	24	2.321	25.974	<0.001
Intercept	1945.246	1	1945.246	2.177E4	<0.001
Dosage	41.757	4	10.439	116.835	<0.001
Time	8.366	4	2.092	23.409	<0.001
Dosage*Time	5.575	16	0.348	3.900	<0.001
Error	4.468	50	0.089		
Total	2005.412	75			
Corrected total	60.166	74			

a. R Squared = .926 (Adjusted R Squared = .890)

Table 18: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.9053*	<0.001
	1.0g	1.6007*	<0.001
	2.0g	1.8380*	<0.001
	4.0g	2.0487*	<0.001
0.5g	1.0g	0.6953*	<0.001
	2.0g	0.9327*	<0.001
	4.0g	1.1433*	<0.001
1.0g	2.0g	0.2373*	0.034
	4.0g	0.4480*	<0.001
2.0g	4.0g	0.2107	0.059

* Mean difference is significant at the 0.05 level

Table 19: Significant mean differences at varying dosages of plant material

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	0.4653*	<0.001
	6HAT	0.8887*	<0.001
	12HAT	0.8260*	<0.001
	24HAT	0.8127*	<0.001
3HAT	6HAT	0.4233*	<0.001
	12HAT	0.3607*	0.002
	24HAT	0.3473*	0.003
6HAT	12HAT	-0.0627	0.568
	24HAT	-0.0760	0.489
12HAT	24HAT	-0.0133	0.903

* Mean difference is significant at the 0.05 level

Studies on acidic pH with seed kernels of *Mangifera indica* showed reductions at higher retention time and higher dosage in treatment sets (Table 20). From ANOVA table (Table 21), dosage of plant material was found to

have significant effect on the response of the plant material (F value= 42.985; p value < 0.001). Retention time of the treatment sets were also found to be of significant effect on the response of the plant material (F value=10.137; p value <0.001). Interactive effect of both dosage and retention time was found to be significant (F value =4.441; p value <0.001) (Table 21). Almost all dosages and retention time were found to be significant (Table 22 and 23).

Table 20: Response of seed kernels of *Mangifera indica* at varying dosages and retention time.

Time (hrs)	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	6.29±0.09	6.26±0.01	6.29±0.04	6.26±0.06	6.00±0.22
3HAT	6.22±0.02	6.26±0.05	6.22±0.04	6.17±0.03	6.04±0.07
6HAT	6.19±0.03	6.22±0.09	6.14±0.05	5.99±0.05	5.86±0.05
12HAT	6.42±0.17	6.22±0.02	6.09±0.06	5.91±0.09	5.71±0.21
24HAT	6.52±0.15	6.11±0.07	5.98±0.10	5.82±0.06	5.50±0.36

Table 21: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	3.683	24	0.153	11.814	<0.001
Intercept	2797.953	1	2797.953	2.154E5	<0.001
Dosage	2.233	4	0.558	42.985	<0.001
Time	0.527	4	0.132	10.137	<0.001
Dosage*Time	0.923	16	0.058	4.441	<0.001
Error	.649	50	.013		
Total	2802.285	75			
Corrected Total	4.332	74			

a. R Squared = .850 (Adjusted R Squared = .778)

Table 22: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.1147*	0.008
	1.0g	0.1840*	<0.001
	2.0g	0.2993*	<0.001
	4.0g	0.5060*	<0.001
0.5g	1.0g	0.0693	0.102
	2.0g	0.1847*	<0.001
	4.0g	0.3913*	<0.001
1.0g	2.0g	0.1153*	<0.001
	4.0g	0.3220*	<0.001
2.0g	4.0g	0.2067*	<0.001

* Mean difference is significant at the 0.05 level

Table 23: Significant mean responses of the plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	0.0393	0.349
	6HAT	0.1393*	0.002
	12HAT	0.1507*	0.001
	24HAT	0.2347*	<0.001
3HAT	6HAT	0.1000*	0.020
	12HAT	0.1113*	0.010
	24HAT	0.1953*	<0.001
6HAT	12HAT	0.0113	0.786
	24HAT	0.0953*	0.026
12HAT	24HAT	0.0840*	0.049

* Mean difference is significant at the 0.05 level

Table 24: Multiple comparison of plants after LSD post hoc analysis

Response LSD		Mean difference (I-J)	SE	Sig	95% confidence level	
(I) Plant	(J) Plant				Lower bound	Upper bound
M. indica	*P. emblica*	0.5557*	0.06740	0.000	0.4226	0.6889
	T. chebula	1.0151*	0.06740	0.000	0.8819	1.1482
P. emblica	*M. indica*	-0.5557*	0.06740	0.000	-0.6889	-0.4226
	T. chebula	0.4593*	0.06740	0.000	0.3262	0.5925
T. chebula	*M. indica*	-1.0151*	0.06740	0.000	-1.1482	-0.8819
	P. emblica	-0.4593*	0.06740	0.000	-0.5925	-0.3262

*. The mean difference is significant at the .05 level.

Upon generalization, out of the three plants selected, *Terminalia chebula* was found to be highly significant with highest mean difference (Table 24). Hence for subsequent studies, dry fruits of *Terminalia chebula* were considered.

For studies on alkaline pH with the plant materials selected from preliminary screening, three plants / plant materials were selected. They are dry fruits of *Phyllanthus emblica*, *Terminalia bellirica* and *Terminalia chebula*.

In studies on alkaline pH with dry fruits of *Phyllanthus emblica*, a gradual reduction in alkaline pH was observed with an increase in dosage and retention time (Table 25). From the mean response of the plant material, it was evident that plant material retained the pH within the potable limit (table 25). From ANOVA table, dosage of the plant material was found to have a significant effect on the response of the plant material because p value was <0.001 (F value = 22.353; p value <0.001). Similarly influence of time on response of the plant material was also found to be significant with a p value

<0.05 (F value = 3.989; p value= 0.007). Interactive effect of both dosage and time was insignificant with p value >0.05 (F value = 0.696; p value = 0.784) (Table 26). Almost all dosages were found to be significant. Lower dosages of the plant material was found to be significant than higher dosages (table 27). Whereas in the case of retention time, lower retention time was found to be significant (table 28).

Table 25: Response of dry fruits of *Phyllanthus emblica* on alkaline pH at varying dosages and retention time.

Time (hrs)	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	7.32±0.23	6.11±0.33	5.73±0.67	5.90±0.34	3.88±2.86
3HAT	7.25±0.26	6.39±0.09	5.54±0.42	4.68±0.95	5.16±1.01
6HAT	7.28±0.16	6.38±0.29	5.29±0.83	4.87±1.28	5.20±0.91
12HAT	7.34±0.28	5.82±0.28	4.81±0.71	4.44±0.55	4.42±0.64
24HAT	6.55±0.91	4.77±0.46	4.42±0.67	4.29±0.68	3.95±0.85

Table 26: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	85.625[a]	24	3.568	4.854	<0.001
Intercept	2278.661	1	2278.661	3.100E3	<0.001
Dosage	65.715	4	16.429	22.353	<0.001
Time	11.727	4	2.932	3.989	0.007
Dosage*Time	8.184	16	0.511	0.696	0.784
Error	36.749	50	0.735		
Total	2401.035	75			
Corrected Total	122.374	74			

a. R squared = 0.700 (Adjusted R squared = 0.556)

Table 27: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	1.2533*	<0.001
	1.0g	1.9920*	<0.001
	2.0g	2.3107*	<0.001
	4.0g	2.6240*	<0.001
0.5g	1.0g	0.7387*	0.022
	2.0g	1.0573*	<0.001
	4.0g	1.3707*	<0.001
1.0g	2.0g	0.3187	0.314
	4.0g	0.6320*	0.049
2.0g	4.0g	0.3133	0.322

Table 28: Significant mean responses of the plant material at varying retention time

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	-0.0140	0.965
	6HAT	-0.0140	0.965
	12HAT	0.4240	0.182
	24HAT	0.9940*	0.003
3HAT	6HAT	0.0000	1.000
	12HAT	0.4380	0.168
	24HAT	1.0080*	0.002
6HAT	12HAT	0.4380	0.168
	24HAT	1.0080*	0.002
12HAT	24HAT	0.5700	0.075

In studies on alkaline pH with dry fruits of *Terminalia bellirica,* significant reduction of alkaline pH was observed at higher dosages and retention time, when compared with the control (Table 29). From the results of ANOVA, dosage and retention time were found to have a significant effect on the response of the plant material with F value = 54.476; p value <0.001 and F value = 30.749; p value <0.001 respectively. But interactive effect of both dosage and time on response of the plant material was insignificant because p value was <0.05 (F value= 0.498; p value = 0.936) (Table 30). Almost all dosages and retention time were found to be significant (Table 31 and 32).

Table 29: Response of dry fruits of *Terminalia bellirica* on alkaline pH at varying dosages and retention time.

Time	Control	Dosage (g)			
		0.5g	**1.0g**	**2.0g**	**4.0g**
1.5HAT	7.75±0.28	7.24±0.16	6.76±0.56	6.22±0.46	5.80±0.17
3HAT	7.60±0.60	6.76±0.39	6.18±0.42	5.83±0.64	5.03±1.25
6HAT	6.95±0.24	6.58±0.42	6.15±0.51	5.04±0.89	4.46±0.55
12HAT	6.43±0.28	5.99±0.29	4.79±0.34	4.30±0.13	4.06±0.15
24HAT	6.31±0.42	5.74±0.46	5.21±0.18	4.62±0.58	4.09±0.09

Table 30: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	83.655[a]	24	3.486	14.536	<0.001
Intercept	2553.834	1	2553.834	1.065E4	<0.001
Dosage	52.251	4	13.063	54.476	<0.001
Time	29.492	4	7.373	30.749	<0.001
Dosage*Time	1.912	16	0.119	0.498	0.936
Error	11.989	50	0.240		
Total	2649.478	75			
Corrected Total	95.644	74			

Table 31: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.546*	0.004
	1.0g	1.189*	<0.001
	2.0g	1.807*	<0.001
	4.0g	2.319*	<0.001
0.5g	1.0g	0.643*	0.001
	2.0g	1.261*	<0.001
	4.0g	1.773*	<0.001
1.0g	2.0g	0.618*	0.001
	4.0g	1.130*	<0.001
2.0g	4.0g	0.512*	0.006

Table 32: Significant mean responses of the plant material at varying retention time

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	3HAT	0.473*	0.011
	6HAT	0.917*	<0.001
	12HAT	1.636*	<0.001
	24HAT	1.558*	<0.001
3HAT	6HAT	0.444*	0.016
	12HAT	1.163*	<0.001
	24HAT	1.085*	<0.001
6HAT	12HAT	0.719*	<0.001
	24HAT	0.641*	0.001
12HAT	24HAT	-0.078	0.665

In studies on alkaline pH using dry fruits of *Terminalia chebula,* a gradual reduction in alkaline pH was noticed with an increase in retention time and dosage (Table 33). Steady reduction in alkaline pH was observed at lower dosages (Table 33). From ANOVA (Table 32), dosage (F value 14.331; p value <0.001) and retention time (F value = 4.621; p value = 0.003) of the treatment sets were found to have a significant effect on the response of the plant material. Interactive effect of both dosage and retention time were insignificant with p value >0.05 (F value= 0.399 and p value = 0.977) (Table 34). Almost all dosages of the plant material was found to have significant response (Table 35 and 36).

Table 33: Response of dry fruits of *Terminalia chebula* at varying dosages and retention time.

Time (hrs)	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	7.43±0.20	6.82±0.17	6.66±0.66	6.70±0.91	5.38±1.54
3HAT	7.15±0.62	6.64±0.60	6.78±0.77	6.24±0.82	4.71±1.12
6HAT	7.29±0.28	6.29±0.23	6.60±0.12	6.05±0.72	5.62±1.05
12HAT	7.28±0.31	6.09±0.92	5.91±1.06	5.83±1.57	5.11±1.20
24HAT	6.97±0.27	5.64±0.51	5.14±0.85	5.12±0.50	4.30±0.46

Table 34: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	53.149[a]	24	2.215	3.425	<0.05
Intercept	2836.687	1	2836.687	4.387E3	<0.05
Dosage	37.067	4	9.627	14.331	<0.05
Time	11.951	4	2.989	4.621	<0.05
Dosage*Time	4.131	16	0.258	0.399	0.977
Error	32.331	50	0.647		
Total	2922.168	75			
Corrected Total	85.481	74			

a. R squared =0.622 (adjusted R squared 0.440)

Table 35: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.9260*	0.003
	1.0g	1.0060*	<0.001
	2.0g	1.2360*	<0.001
	4.0g	2.1987*	<0.001
0.5g	1.0g	0.0800	0.786
	2.0g	0.3100	0.296
	4.0g	1.2727*	<0.001
1.0g	2.0g	0.2300	0.437
	4.0g	1.1927*	<0.001
2.0g	4.0g	0.9627*	0.002

Table 36: Significant mean responses of the plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
Control	3HAT	0.2927	0.324
	6HAT	0.2267	0.444
	12HAT	0.5540	0.065
	24HAT	1.1633*	<0.001
3HAT	6HAT	-0.0660	0.823
	12HAT	0.2613	0.378
	24HAT	0.8707*	0.005
6HAT	12HAT	0.3273	0.270
	24HAT	0.9367*	0.002
12HAT	24HAT	0.6093*	0.043

On an overall assessment (Table 37) *Terminalia chebula* was found to be significant with highest mean difference compared to other two plants in stabilizing alkaline pH. Hence dry fruits of *Terminalia chebula* were selected for further studies.

Table 37: Multiple comparisons between the response of the plant material after LSD post hoc analysis

Response LSD		Mean difference (I-J)	SE	Sig	95% confidence level	
(I) Plant	**(J) Plant**				**Lower bound**	**Upper bound**
P. emblica	*T. bellirica*	-0.3233*	0.12005	0.008	-0.5605	-0.0861
	T. chebula	-0.6380*	0.12005	0.008	-0.8752	-0.4008
T. bellirica	*P. emblica*	0.3233*	0.12005	0.008	-0.0861	-0.5605
	T. chebula	-0.3147*	0.12005	0.008	-0.5519	-0.0775
T. chebula	*P. emblica*	0.6380*	0.12005	0.008	-0.4008	0.8752
	T. bellirica	0.3147*	0.12005	0.008	-0.0775	0.5519

*the mean difference significant at 0.05 level

From preliminary screening studies, three plants with highest removal percentage were selected for removing turbidity from water. They were fruits of *Abelmoschus esculentus,* roots of *Hemidesmus indicus* and rhizome of *Lagenandra toxicaria.*

In the case of *Abelmoschus esculentus*, reduction in turbidity was observed with an increase in dosage and retention time (Table 38). Reduction became almost stable after a dosage of 2g at all retention time (Table38). From ANOVA table (Table 39) the dosage of plant material has an insignificant effect on the response of the plant material, because p value was >0.05 (F value = 1.190: p value=0.327). While retention time got a significant effect on the response of the plant material with p value <0.05 (F value=29.729; p value <0.001). Interactive effect of both dosage and retention

time were also found to have a significant effect on the response of the plant material (F value = 2.659; p value <0.001). Significant effect was not observed with mean differences of dosages (Table 40), while statistical significance was observed with retention time (Table 41).

Table 38: Response of fruits of *Abelmoschus esculentus* at varying dosages and retention time.

Time	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	13.10±2.40	9.60±1.51	8.43±2.48	10.17±2.78	8.77±3.32
3HAT	7.37±0.72	6.57±0.31	6.63±0.25	6.17±0.29	7.03±0.15
6HAT	7.30±0.20	6.90±0.44	6.40±0.75	6.47±0.50	7.13±0.42
12HAT	4.87±0.55	5.87±0.12	5.70±0.36	6.20±0.72	6.57±0.40
24HAT	4.10±0.96	4.97±0.29	6.00±0.26	6.00±0.72	7.67±0.60

Table 39: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	253.178[a]	24	10.549	6.926	<0.001
Intercept	3715.712	1	3715.712	2.439E3	<0.001
Dosage	7.250	4	1.812	1.190	0.327
Time	181.134	4	45.283	29.729	<0.001
Dosage*Time	64.794	16	4.050	2.659	0.004
Error	76.160	50	1.523		
Total	4045.050	75			
Corrected Total	329.338	74			

Table 40: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.5667	0.214
	1.0g	0.7133	0.120
	2.0g	0.3467	0.445
	4.0g	-0.0867	0.848
0.5g	1.0g	0.1467	0.746
	2.0g	-0.2200	0.628
	4.0g	-0.6533	0.153
1.0g	2.0g	-0.3667	0.420
	4.0g	-0.8000	0.082
2.0g	4.0g	-0.4333	0.341

* The mean difference is significant at the 0.05 level

Table 41: Significant mean responses of the plant material at varying retention time

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	3.2600*	<0.001
	6HAT	3.1733*	<0.001
	12HAT	4.1733*	<0.001
	24HAT	4.2667*	<0.001
3HAT	6HAT	-0.0867	0.848
	12HAT	1.0000*	0.048
	24HAT	1.0933*	0.030
6HAT	12HAT	-1.0000*	0.031
	24HAT	1.0933*	0.019
12HAT	24HAT	0.0933	0.837

* The mean difference is significant at the 0.05 level

In the case of *Hemidesmus indicus*, reduction in turbidity was observed with an increase in dosage and retention time (Table 42). However, after a dosage of 1g, prominent changes in turbidity were not observed (Table 42). Steep increase followed by a gradual reduction in turbidity was observed at higher retention time (Table 42). From the ANOVA (Table 43), dosage and retention time were found to have a significant effect on the response of the plant material (Dosage: F value = 7.648; p value <0.001; Time: F value = 40.418; p value <0.001). Interactive effect of both dosage and time on the response of the plant material was also found to be significant at 0.05 level (p value =0.04).Almost all mean differences of both dosages and retention time were found to be significant at 0.05 level (Table 44 and 45).

Table 42:- Response of roots of *Hemidesmus indicus* at varying dosages and retention time.

Time	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	5.63±0.78	5.43±0.25	5.60±0.26	5.90±0.70	5.83±0.55
3HAT	5.03±0.06	4.77±0.12	5.00±0.17	4.87±0.40	4.83±0.15
6HAT	5.63±0.32	5.73±0.15	5.27±0.70	5.93±0.42	6.13±0.06
12HAT	4.97±0.32	5.07±0.21	4.90±0.56	5.90±0.26	6.57±0.32
24HAT	3.50±0.10	3.83±0.15	4.17±0.64	4.43±0.68	4.60±0.53

Table 43: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	38.894[a]	24	1.621	9.271	<0.001
Intercept	2013.466	1	2013.466	1.152E4	<0.001
Dosage	5.347	4	1.337	7.648	<0.001
Time	28.261	4	7.065	40.418	<0.001
Dosage*Time	5.286	16	0.330	1.890	0.044
Error	8.740	50	0.175		
Total	2061.100	75			
Corrected Total	47.634	74			

Table 44: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	-0.0133	0.931
	1.0g	-0.333	0.828
	2.0g	-0.4533*	0.005
	4.0g	-0.6400*	<0.001
0.5g	1.0g	-0.0200	0.896
	2.0g	-0.4400*	0.006
	4.0g	-0.6267*	<0.001
1.0g	2.0g	-0.4200*	0.008
	4.0g	-0.6067*	<0.001
2.0g	4.0g	-0.1867	0.227

* The mean difference is significant at the 0.05 level

Table 45: Significant mean responses of the plant material at varying retention time

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	0.7800*	<0.001
	6HAT	-0.0600*	0.696
	12HAT	0.2000	0.196
	24HAT	1.5733*	<0.001
3HAT	6HAT	-0.8400*	<0.001
	12HAT	-0.5800*	<0.001
	24HAT	0.7933*	<0.001
6HAT	12HAT	0.2600	0.095
	24HAT	1.6333*	<0.001
12HAT	24HAT	1.3733*	<0.001

* The mean difference is significant at the 0.05 level

In studies on turbidity removal efficiency with the rhizome of *Lagenandra toxicaria,* though gradual reduction in turbidity was observed with a dosage of 1g, significant changes were not observed at higher dosages (Table 46). At 3, 6 and 12 HAT, gradual reduction was compared with control (table 46). From ANOVA (Table 47), dosage of the plant material was found to have no significant effect on the response of the plant material, as p value >0.05 (F value = 1.838: p value=0.136). Retention time of the treatment sets were found to have a significant effect on the response of the plant material with p value <0.001 and F value =49.268. Almost all mean differences of retention time were found to be significant, rather than the dosages of plant material (Table 48 and 49).

Table 46:- Response of rhizome of *Lagenandra toxicaria* on turbidity at varying dosages and retention time.

		Dosage (g)			
Time	**Control**	**0.5g**	**1.0g**	**2.0g**	**4.0g**
1.5HAT	5.13±0.23	5.30±0.40	5.07±0.40	5.47±0.35	5.20±0.46
3HAT	5.60±0.20	5.43±0.12	5.37±0.21	5.33±0.25	5.63±0.15
6HAT	6.43±0.67	6.43±0.12	6.27±0.23	6.20±0.00	6.40±0.17
12HAT	5.30±0.10	5.43±0.12	5.17±0.06	5.10±0.17	5.60±0.10
24HAT	5.03±0.45	4.83±0.15	4.90±0.36	5.00±0.20	5.27±0.35

Table 47: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected model	17.397[a]	24	0.725	8.971	<0.001
Intercept	2248.993	1	2248.993	2.783E4	<0.001
Dosage	0.594	4	0.149	1.838	0.136
Time	15.923	4	3.981	49.268	<0.001
Dosage*Time	0.879	16	0.055	0.680	0.799
Error	4.040	50	0.081		
Total	2270.430	75			
Corrected Total	21.437	74			

a. R squared = 0.812 (adjusted R squared = 0.721)

Table 48: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	0.0133	0.898
	1.0g	0.1467	0.164
	2.0g	0.8000	0.444
	4.0g	-0.1200	0.253
0.5g	1.0g	0.1333	0.205
	2.0g	0.0667	0.524
	4.0g	-0.1333	0.205
1.0g	2.0g	-0.0667	0.524
	4.0g	-0.2667*	0.013
2.0g	4.0g	-0.2000	0.060

Table 49: Significant mean differences of plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	-0.2400*	0.025
	6HAT	-1.1133*	<0.001
	12HAT	-0.0867	0.408
	24HAT	0.2267	0.034
3HAT	6HAT	-0.8733*	<0.001
	12HAT	0.1533	0.146
	24HAT	0.4667*	<0.001
6HAT	12HAT	1.0267*	<0.001
	24HAT	1.3400*	<0.001
12HAT	24HAT	0.3133*	0.004

Table 50: Multiple comparison of mean difference of all plants after LSD post hoc analysis.

Response LSD		Mean difference (I-J)	SE	Sig	95% confidence level	
(I) Plant	**(J) Plant**				**Lower bound**	**Upper bound**
A. esculentus	*H. indicus*	1.8573*	0.12574	0.000	1.6089	2.1058
	L. toxicaria	1.5627*	0.12574	0.000	1.3142	1.8111
H. indicus	*A. esculentus*	-1.8573*	0.12574	0.000	-2.1058	-1.6089
	L. toxicaria	-0.2947*	0.12574	0.000	-0.5431	-0.0462
L. toxicaria	*A. esculentus*	-1.5627*	0.12574	0.000	-1.8111	-1.3142
	H. indicus	0.2947*	0.12574	0.000	0.0462	0.5431

*The mean difference is significant at the .05 level.

On an overall assessment, out of the three plants selected, *Hemidesmus indicus* was found to be statistically significant in the removal of turbidity at 0.05 levels (table 50). Hence the plant was considered for further studies.

From preliminary screening studies, three plants with highest removal percentage were selected for removing iron from water. They were leaves of *Mentha arvensis*, seeds of *Tamarindus indica* and roots of *Vetiveria zizanioides.*

In studies on iron removal efficiency with the leaves of *Mentha arvensis,* reduction in iron concentration was observed at all dosages except at a dosage of 0.5g. Better removal was observed with an increase in retention time, instead of increase in dosages (Table 51). From the two way ANOVA analysis, effect of dosage on the response of the plant material was insignificant because p value was >0.05 (F value = 0.768; p value = 0.551). Retention time of the treatment sets showed significant p value <0.001 (F value =10.073; p value <0.001). The interactive effect between dosage and

retention time on response of the plant material was found to be insignificant with p value >0.05 (F value= 0.471; p value =0.950) (Table 52). Mean differences of varying retention time was found to be statistically significant rather than dosage of the plant material (Table 53 and 54).

Table 51: Response of leaves of *Mentha arvensis* in removing iron at varying dosages and retention time.

Time	Control	Dosage (g)			
		0.5g	1.0g	2.0g	4.0g
1.5HAT	0.48±0.10	0.57±0.13	0.45±0.05	0.44±0.06	0.34±0.14
3HAT	0.40±0.05	0.50±0.12	0.48±0.21	0.44±0.10	0.43±0.19
6HAT	0.29±0.05	0.35±0.12	0.31±0.03	0.29±0.06	0.25±0.06
12HAT	0.34±0.13	0.45±0.22	0.40±0.22	0.40±0.12	0.39±0.15
24HAT	0.24±0.09	0.17±0.06	0.24±0.13	0.27±0.06	0.25±0.01

Table 52: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	0.732[a]	24	0.030	2.121	0.13
Intercept	10.091	1	10.091	702.041	<0.001
Dosage	0.044	4	0.011	0.768	0.551
Time	0.579	4	0.145	10.073	<0.001
Dosage*Time	0.108	16	0.007	0.471	0.950
Error	0.719	50	0.014		
Total	11.541	75			
Corrected Total	1.450	74			

Table 53: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	-0.0567	0.0201
	1.0g	-0.0280	0.525
	2.0g	-0.0167	0.705
	4.0g	0.0140	0.750
0.5g	1.0g	0.0287	0.516
	2.0g	0.0400	0.365
	4.0g	0.0707	0.113
1.0g	2.0g	0.0113	0.797
	4.0g	0.0420	0.342
2.0g	4.0g	0.0307	0.487

* The mean difference is significant at the 0.05 level

Table 54: Significant mean responses of the plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	0.0073	0.868
	6HAT	0.1600*	<0.001
	12HAT	0.0613	0.167
	24HAT	0.2240*	<0.001
3HAT	6HAT	0.1527*	<0.001
	12HAT	0.0540	0.223
	24HAT	0.2167*	<0.001
6HAT	12HAT	-0.0987*	0.029
	24HAT	0.0640	0.150
12HAT	24HAT	0.1627*	<0.001

* The mean difference is significant at the 0.05 level

In studies on iron removal efficiency with the seeds of *Tamarindus indica,* significant reduction in iron was not observed (Table 55). Varying dosages of plant material doesn't show any significant effect on mean responses. But almost all dosages brought down the iron content after 6HAT and failed to keep it stable (Table 55). Gradual increase in iron concentration was observed with the treatment sets. This might be due to restabilization of iron particles due to ineffectiveness of plant material. From the ANOVA results (table 56), dosage of the plant material was found to be insignificant because p value was >0.05 (F value = 2.242; p value = 0.078). While in the case of retention time, a significant effect on the response of plant material with p value <0.05 (F value= 14.378; p value <0.001) was noticed. Interactive effect of both dosage and retention on the response of the plant material was found to be insignificant with p value > 0.05 (F value = 0.321; p value = 0.992). Significant mean differences were observed in retention times rather than dosages of the plant material (Table 57 and 58).

Table 55:- Response of seeds of *Tamarindus indica* in removing iron at varying dosages and retention time.

Time	Control	0.5g	1.0g	2.0g	4.0g
1.5HAT	0.02±0.06	0.09±0.07	0.07±0.10	0.22±0.08	0.15±0.12
3HAT	0.06±0.08	0.27±0.04	0.39±0.11	0.45±0.14	0.28±0.12
6HAT	0.83±0.53	0.80±0.19	0.97±0.18	0.89±0.26	0.71±0.22
12HAT	0.39±0.44	0.43±0.14	0.94±0.69	0.89±0.63	0.70±0.53
24HAT	0.58±0.50	0.57±0.26	0.77±0.19	0.82±0.23	0.67±0.31

Table 56: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	6.943[a]	24	0.289	2.984	<0.001
Intercept	20.124	1	207.561	207.561	<0.001
Dosage	0.869	4	2.242	2.242	0.078
Time	5.576	4	14.378	14.378	<0.001
Dosage*Time	0.498	16	0.321	0.321	0.992
Error	4.848	50			
Total	31.915	75			
Corrected Total	11.791	74			

a R squared = 0.589 (Adjusted R squared = 0.392)

Table 57: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	-0.0573	0.616
	1.0g	-0.2507*	0.032
	2.0g	-0.2780*	0.018
	4.0g	0.1240	0.281
0.5g	1.0g	-0.1933	0.095
	2.0g	-0.2207	0.058
	4.0g	-0.0667	0.560
1.0g	2.0g	-0.0273	0.811
	4.0g	0.1267	0.271
2.0g	4.0g	0.1540	0.182

Table 58: Significant mean responses of the plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	-0.1813	0.117
	6HAT	-0.7307*	<0.001
	12HAT	-0.5600*	<0.001
	24HAT	-0.5713*	<0.001
3HAT	6HAT	-0.5493*	<0.001
	12HAT	-0.3787*	0.002
	24HAT	-0.3900*	<0.001
6HAT	12HAT	0.1707	0.140
	24HAT	0.1593	0.167
12HAT	24HAT	-0.113	0.921

In studies on iron removal efficiency with the roots of *Vetiveria zizanioides,* an increase in iron content was observed in early hours of the treatment, but later a reduction was observed with increase in retention time. Gradual decrease in the iron content of the treatment set was observed with increase in retention time (Table 59). From the results of ANOVA, dosage and time were found to have a significant effect on the response of the plant material in removing iron content from the treatment set. Dosage and time were observed with a p value < 0.05 (Dosage (F value= 3.124; p value= 0.023) Time (F value=66.345; p value <0.001)). The interactive effect of dosage and time on the response of the plant material was also found to have a significant effect on the response of the plant material (F value =2.941; p value <0.005) (Table 60). Retention time was found to be significant than dosage of the plant material (Table 61 and 62).

Table 59:- Response of roots of *Vetiveria zizanioides* in removing iron at varying dosages and retention time.

Time	Control	0.5g	1.0g	2.0g	4.0g
1.5HAT	0.50±0.08	0.62±0.08	0.61±0.12	0.56±0.06	0.50±0.13
3HAT	0.34±0.05	0.25±0.12	0.23±0.08	0.25±0.14	0.14±0.03
6HAT	0.13±0.03	0.17±0.05	0.25±0.08	0.24±0.09	0.13±0.02
12HAT	0.24±0.08	0.36±0.04	0.25±0.08	0.40±0.04	0.35±0.03
24HAT	0.09±0.01	0.25±0.01	0.25±0.02	0.26±0.02	0.35±0.03

Table 60: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	1.613[a]	24	0.067	13.539	<0.001
Intercept	7.146	1	7.146	1.440E3	<0.001
Dosage	0.062	4	0.016	3.124	0.023
Time	1.317	4	0.329	66.345	<0.001
Dosage*Time	0.234	16	0.015	2.941	<0.005
Error	0.248	50	0.005		
Total	9.006	75			
Corrected Total	1.861	74			

Table 61: Significant mean differences at varying dosages of plant material

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	-0.0700*	<0.05
	1.0g	-0.0573*	<0.05
	2.0g	-0.0800*	<0.05
	4.0g	-0.0327	0.210
0.5g	1.0g	0.0127	0.625
	2.0g	-0.0100	0.699
	4.0g	0.0373	0.153
1.0g	2.0g	-0.0227	0.382
	4.0g	0.0247	0.342
2.0g	4.0g	0.0473	0.072

Table 62: Significant mean differences of plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	0.3180*	<0.001
	6HAT	0.3760*	<0.001
	12HAT	0.2407*	<0.001
	24HAT	0.3187*	<0.001
3HAT	6HAT	0.0580*	<0.05
	12HAT	-0.0773*	<0.05
	24HAT	0.0007	0.979
6HAT	12HAT	-0.135.*	<0.001
	24HAT	-0.0573*	<0.05
12HAT	24HAT	0.0780*	<0.05

Table 63: Multiple comparison of mean difference of all plants after LSD post hoc analysis.

Response LSD		Mean difference (I-J)	SE	Sig	95% confidence level	
(I) Plant	**(J) Plant**				**Lower bound**	**Upper bound**
M. arvensis	*T. indica*	-0.1512*	0.03215	0.000	-0.2147	-0.0877
	V. zizanioides	0.0581	0.03215	0.073	-0.0054	0.1217
T. indica	*M. arvensis*	0.1512	0.03215	0.000	0.0877	0.2147
	V. zizanioides	0.2093*	0.03215	0.000	0.1458	0.2729
V. zizanioides	*M. arvensis*	-0.0581	0.03215	0.073	-0.1217	0.0054
	T. indica	-0.2093*	0.03215	0.000	-0.2729	-0.1458

As an overall assessment, roots of *V. zizanioides* was found to be statistically significant than the other two plant materials in removing iron from water (Table 63). Hence plant material of *V. zizanioides* was selected for subsequent studies.

From preliminary screening studies, three plants with highest removal percentage was selected for removing fluoride from water. They were leaves of *Aloe barbadensis,* stem of *Euphorbia antiquorum and* roots of *Vetiveria zizanioides*. In the case of fluoride, plant material was effective till six hours of treatment hence data obtained up to 6HAT was subjected to statistical analysis.

In the studies on fluoride removal efficiency of leaves of *Aloe barbadensis, w*hen compared with control, a stable reduction was observed at 3HAT. Significant results were not observed at rest of the dosages and retention time (Table 64). Results obtained from ANOVA also demonstrated that the dosage of plant material has insignificant effect on the response of the

plant material in reducing fluoride from water treatment sets because p value >0.05 (F value = 0.138; p value = 0.967). But retention time of the treatment sets was highly significant with a p value <0.001 (F value=79.61905; p value<0.001). Interactive effect of both dosage and retention time on response of plant material in reducing fluoride was found to be insignificant because of p value > 0.05 (F value= 0.873; p value= 0.550) (Table 65). Dosage of plant material on fluoride removal was not significant, whereas retention time was found to be significant (Table 66 and 67).

Table 64: Response of leaves of *Aloe barbadensis* in removing fluoride at varying dosages and retention time.

Time	Control	0.5g	1.0g	2.0g	4.0g
1.5HAT	35.93±5.14	34.01±8.47	39.97±17.51	36.22±9.28	30.60±1.38
3HAT	82.84±14.39	75.93±5.02	70.55±16.19	75.57±11.27	71.53±3.07
6HAT	32.53±13.60	31.61±4.59	37.66±1.20	37.24±7.55	44.32±8.16

Table 65: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	16168.22[a]	14	1154.873	11.91219	<0.001
Intercept	108489	1	108489	1119.034	<0.001
Dosage	53.53041	4	13.3826	0.138038	0.967
Time	15437.95	2	7718.973	79.61905	<0.001
Dosage*Time	676.7453	8	84.59316	0.872555	0.550
Error	2908.464	30	96.94882		
Total	127565.7	45			
Corrected Total	19076.69	44			

[a] R squared = 0.845 (Adjusted R squared = 0.771)

Table 66: Significant mean responses of plant material at varying dosages.

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	3.249	0.489
	1.0g	1.037	0.825
	2.0g	0.757	0.872
	4.0g	1.617	0.730
0.5g	1.0g	-2.212	7.267
	2.0g	-2.492	6.987
	4.0g	-1.632	7.847
1.0g	2.0g	-0.280	9.200
	4.0g	0.580	10.060
2.0g	4.0g	0.860	10.339

Table 67: Significant mean responses of plant material at varying retention time.

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	-39.9380*	<0.001
	6HAT	-1.3273	0.715
3HAT	6HAT	38.6107*	<0.001

*The mean difference significant at 0.001 level

In studies on fluoride removal efficiency of stem of *Euphorbia antiquorum, a* gradual reduction was observed at higher dosages and retention time (Table 68), when compared with the control. With an increase in retention time, reduction in fluoride was found to be significant. From the ANOVA analysis (Table 69) dosage of the plant material was found to have insignificant effect on the response of the plant material because p value >0.05 (F value=0.213; p value=0.950). But retention time was found to be

highly significant having a p value >0.05 (F value=23.865; p value<0.001). Combined effect of both was insignificant with p value >0.05 (F value=1.253; p value=0.264).Though dosages of the plant material were statistically insignificant (Table 70) dosages were found effective in removing fluoride up to 6HAT (Fig 71).

Table 68: Response of stem of *Euphorbia antiquorum* in removing fluoride at varying dosages and retention time.

Time	Control	0.5g	1.0g	2.0g	4.0g
1.5HAT	95.25±5.03	96.84±15.34	106.84±14.27	93.17±15.00	63.17±49.12
3HAT	61.93±22.10	43.89±15.66	47.45±5.99	49.58±14.40	64.80±41.88
6HAT	40.65±4.12	37.23±12.81	45.65±4.89	48.71±6.18	45.96±8.32

Table 69: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	23300.32	14	1664.309	4.062479	<0.05
Intercept	177138.4	1	177138.4	432.3843	<0.001
Dosage	549.5357	4	137.3839	0.335346	0.852
Time	18767.93	2	9383.966	22.90571	<0.001
Dosage*Time	3982.851	8	497.8564	1.215238	0.324
Error	12290.34	30	409.6781		
Total	212729	45			
Corrected Total	35590.66	44			

a. R squared = 0.699 (Adjusted R squared = 0.555)

Table 70: Significant mean responses of plant material at varying dosages

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	6.623	0.493
	1.0g	-0.703	0.942
	2.0g	2.125	0.825
	4.0g	7.966	0.410
0.5g	1.0g	-7.326	0.449
	2.0g	-4.498	0.641
	4.0g	1.343	0.889
1.0g	2.0g	2.828	0.769
	4.0g	8.669	0.371
2.0g	4.0g	5.841	0.545

*The mean difference is significant at the 0.05 level

Table 71: Significant mean responses of plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	37.5224*	<0.001
	6HAT	47.4116*	<0.001
3HAT	6HAT	9.8892	0.191

*The mean difference is significant at the 0.05 level

In studies on fluoride removal efficiency of roots of *Vetiveria zizanioides, s*ignificant reduction in fluoride was not observed with increase in dosage, whereas gradual reduction is observed at higher retention time (Table 72). From the results of ANOVA, dosage of the plant material was found to have an insignificant effect on the response of the plant material because p value > 0.05 (F value = 0.563; p value = 0.691), while retention time has a significant effect on the response of the plant material with p value <0.001 (F value =289.729) which is <0.05 (Table 73). Significant mean

responses were observed with retention time rather than dosages (table 74 and 75).

Table 72: Response of roots of *Vetiveria zizanioides* in removing fluoride at varying dosages and retention time.

Time	Control	0.5g	1.0g	2.0g	4.0g
1.5HAT	69.29±20.35	74.59±11.93	65.95±11.56	75.33±7.09	62.05±1.17
3HAT	78.81±12.51	69.01±18.52	92.86±15.25	84.85 ±17.47	84.36±12.54
6HAT	44.81±16.39	46.91±6.10	33.49±4.48	48.20±12.77	37.97±3.77

Table 73: Results of Two way ANOVA Univariate analysis (GLM) (Dependent variable - Response)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	13921.43	14	994.3879	5.985293	<0.001
Intercept	184508.5	1	184508.5	1110.57	<0.001
Dosage	314.341	4	78.58525	0.47301	0.755
Time	11672.31	2	5836.154	35.12823	<0.001
Dosage*Time	1478.447	8	184.8059	1.11236	0.383
Error	4984.157	30	166.1386		
Total	210079.8	45			
Corrected Total	18905.59	44			

Table 74: Significant mean responses of plant material at varying dosages

Dosage (I)	Dosage (J)	Mean Difference (I-J)	Sig
Control	0.5g	-2.28	0.71
	1.0g	0.20	0.97
	2.0g	-5.16	0.40
	4.0g	2.84	0.64
0.5g	1.0g	2.48	0.69
	2.0g	-2.88	0.64
	4.0g	5.12	0.41
1.0g	2.0g	-5.36	0.38
	4.0g	2.64	0.67
2.0g	4.0g	8.00	0.20

Table 75: Significant mean responses of plant material at varying retention time

Time (I)	Time (J)	Mean Difference (I-J)	Sig
1.5HAT	3HAT	-12.2143*	<0.05
	6HAT	27.8195*	<0.001
3HAT	6HAT	40.0338*	<0.001

Table 76: Multiple comparison of mean difference of all plants after LSD post hoc analysis.

Response LSD		Mean difference (I-J)	SE	Sig	95% confidence level	
(I) Plant	(J) Plant				Lower bound	Upper bound
Aloe barbadensis	*E. antiquorum*	-13.6402*	3.15704	0.000	-19.9122	-7.3682
	V. zizanioides	-16.0785*	3.15704	0.000	-22.3505	-9.8065
E. antiquorum	*Aloe barbadensis*	13.6402*	3.15704	0.000	7.3682	19.9122
	V. zizanioides	-2.4383	3.15704	0.442	-8.7103	3.8337
V. zizanioides	*Aloe barbadensis*	16.0785*	3.15704	0.000	9.8065	22.3505
	E. antiquorum	2.4383	3.15704	0.442	-3.8337	8.7103

When compared to other two plant materials, stem of *E. antiquorum* was found to be significant at a level of 0.05 in removing fluoride from treatment sets (Table 76). Hence *E. antiquorum* was selected for subsequent studies.

The present study is an attempt to screen out plants / plant parts having efficiency for stabilization / removal of selected water quality parameters / contaminants like pH (acidic and alkaline), turbidity, iron and fluoride from water. From the preliminary screening conducted with two dozen plants / plant parts, those plants with highest removal / stabilization percentages were selected. Accordingly, dry fruits of *Phyllanthus emblica* (45.84%, 1g, 24HAT) and *Terminalia chebula* (42.62%, 4g, 24HAT); seed kernels of *Mangifera indica* (35.47%, 4g, 12HAT) were selected for stabilizing acidic pH. Likewise dry fruits of *Phyllanthus emblica* (55.55%, 4g, 6HAT),

Terminalia bellirica (46.15%, 4g, 12HAT) and *Terminalia chebula* (44.09%, 4g, 12HAT) were selected for stabilizing alkaline pH. Similarly, *Lagenandra toxicaria* (66%, 4g, 6HAT), *Hemidesmus indicus* (34.78%, 1g, 1.5HAT) and *Abelmoschus esculentus* (33.62%, 0.5g, 24HAT) were selected for removing turbidity from water. In the case of iron removal from water *Vetiveria zizanioides* (46.07%, 0.5g, 6HAT), *Mentha arvensis* (37.5%, 4g, 12HAT) and *Tamarindus indica* (36.92%, 0.5, 12HAT) were selected. Similarly plant parts like stem of *Euphorbia antiquorum* (78.57%, 0.5g, 24HAT) roots of *Vetiveria zizanioides* (69.69%, 4g, 24HAT) and *Aloe barbadensis* (73.33%, 2g, 1.5HAT) were found to be significant in removing fluoride from water.

Though pH is not a water contaminant, it can influence the hydrolysis of chemical coagulants (WHO, 2007; Zhao et al., 2009), charged groups of polysaccharide chains of natural coagulants (Patel and Vashi, 2011) and surface charge of the colloidal particles in water / waste water (Rasool et al., 2016). Several studies are available stating the role of pH in coagulation process (Kazi et al., 2013; Vara, 2012). Also, unlike chemical coagulants it has been regarded that natural coagulants do not alter the pH of the water (Gunaratna et al., 2007; Madhukar and Yogesh, 2013). Limited works are available on natural coagulants influencing the pH of water. The present study emphasizes that natural coagulants are capable of stabilizing pH of the water to specific ranges. A total of three plants each were selected from preliminary screening studies for stabilizing acidic and alkaline pH. Dry fruits of *Phyllanthus emblica* (45.84%, 1g, 24HAT) and *Terminalia chebula* (42.62%, 4g, 24HAT) and seed kernels of *Mangifera indica* (35.47%, 4g, 12HAT) were selected for stabilizing acidic pH. Likewise dry fruits of *Phyllanthus emblica* (55.55%, 4g, 6HAT), *Terminalia bellirica* (46.15%, 4g, 12HAT) and *Terminalia chebula* (44.09%, 4g, 12HAT) were selected for stabilizing alkaline pH. Based on the statistical analysis *Terminalia chebula* and *Terminalia bellirica* was found to be significant for stabilizing acidic and

alkaline pH respectively. With an increase in dosage and retention time gradual reduction in pH was observed. Similar case has been reported with *Acacia catechu* (Thakur and Choubey, 2014). The nature of plant materials in influencing pH in the present study can be related with the presence of various phyto-constituents. Various phyto-constituents from plants would have seeped into the water resulting in the stabilization of pH (Choy et al., 2014).

Out of 24 plants screened, reduction in turbidity was observed with various plant materials screened. From preliminary screening studies, *Lagenandra toxicaria* (66%, 4g, 6HAT), *Hemidesmus indicus* (34.78%, 1g, 1.5HAT) and *Abelmoschus esculentus* (33.62%, 0.5g, 24HAT) were selected for removing turbidity from water. Maximum removal percentage of turbidity in preliminary screening was observed with rhizome of *Lagenandra toxicaria* (66%) which was comparable with the turbidity removal efficiency of chitosan and *Lab lab purpureus* seeds, which exhibited removal percentage between 60-70% (Bina et al., 2009; Unnisa et al., 2010). Plant materials in the present study showed better efficiency than *Phaseolus vulgaris* and *Acacia catechu* in removing turbidity from water (Antov et al., 2010; Thakur and Choubey, 2014). Varying retention time ranging between half to 1 hour has been experimented in several related studies (Asrafuzzaman et al., 2011; Khodapanah et al., 2013). When compared with *Vigna unguiculata*, plant material in the present study was able to remove 66% turbidity within six hours of treatment but the latter attained it only after 24hours of treatment. Upon statistical analysis, out of the three plants selected from preliminary screening, roots of *Hemidesmus indicus* was found to be significant in removing turbidity, based on the varying dosages and retention time. *Hemidesmus indicus* was able to remove turbidity in the initial hours of the treatment. Hence the roots of *Hemidesmus indicus* can be regarded as a new promising candidate to remove turbidity from water. Beyond an optimum

range of time and dosage, increase in turbidity was observed in treatment sets with all plant materials and this can be attributed to particle restabilization (Choy et al., 2015).

Several works are available stating the adsorption of iron by a wide range of materials (Beenakumari, 2009; Thakuria and Buddharatna, 2016). Similarly, numerous adsorption studies on fluoride using activated or modified, natural as well as synthetic materials are also available (Alagumuthu et al., 2010; Miretzky and Cirelli, 2011; Mohapatra et al., 2009; Satyanaryana and Sudheera, 2015; Thakuria and Buddharatna, 2016; Vázquez-Guerrero et al., 2016). Studies mentioning the removal of iron and fluoride by the bioactive components from plants are scarce. Out of the three plants selected from preliminary screening *Vetiveria zizanioides* (46.07%, 0.5g, 6HAT), *Mentha arvensis* (37.5%, 4g, 12HAT) and *Tamarindus indica* (36.92%, 0.5, 12HAT) were selected. From statistical analysis *Vetiveria zizanioides* was found to be significant based on varying dosages and retention time in removing iron from water. In the present study gradual reduction of iron content in water was observed at higher retention time. Gradual reduction was observed up to a retention time of 6 hours, later a gradual increment in iron content was observed. Similar increment in iron content was observed at the initial stages of the treatment sets too.

In the case of fluoride, three plant materials *Euphorbia antiquorum* (78.57%, 0.5g, 24HAT), *Vetiveria zizanioides* (69.69%, 4g, 24HAT) and *Aloe barbadensis* (73.33%, 2g, 1.5HAT) were subjected to secondary screening. Stem of *Euphorbia antiquorum* was found to be a significant plant material among the three plants studied. Gradual reduction in fluoride content was observed up to a retention time of 6 hours. An increment in fluoride thereafter was observed as in iron removal. *Vetiveria zizanioides* and *Euphorbia antiquorum* are new candidates with iron and fluoride removal capacity. It is

supposed that the removal of iron and fluoride is due to the bioactive components released from the plant materials. Bioactive components can be either low molecular weight proteins or carbohydrates (Choy et al., 2015). Apart from proteins and carbohydrates, certain other plant derived compounds like polyphenols (Chang et al., 2009), tannins (Ozacar and Sengil, 2003) etc. are also capable of bring down the level of pollutants in water. It has also been reported that the synergistic effects of sugars or sugar acids promote coagulation (Choy et al., 2014; Adinolfi et al., 1994) in addition to various functional groups (Yin, 2010). Iron and fluoride are charged molecules. The synergistic effects of either of the aforementioned charged functional groups of plant constituents like polyphenols, tannins etc. might have been influencing the removal of the pollutants from water.

Summary and Conclusion

The aim of the present study was to screen out promising plant coagulants which are capable of stabilizing / removing water quality parameters like pH, turbidity, iron and fluoride. A total of 24 plants belonging to 17 families were screened for this purpose. Batch treatment has been followed with the addition of specific dosages of processed plant materials in water samples having specific configuration. The efficiency of the plant materials were assessed for 24hrs within a stipulated time interval.

Based on the removal percentage in preliminary screening three plants each were selected for each of the selected for assessing their efficiencies in secondary treatment studies. Accordingly dry fruits of *Phyllanthus emblica* and *Terminalia chebula*; and seed kernel of *Mangifera indica* was selected for acidic pH stabilizing capacity. Similarly in the case of stabilizing alkaline pH dry fruits of *Phyllanthus emblica, Terminalia bellirica* and *Terminalia chebula* were selected for secondary screening studies. Rhizome of *Lagenandra toxicaria,* roots of *Hemidesmus indicus* and fruits of

Abelmoschus esculentus were selected for studies on turbidity removal. Plant materials like roots of *Vetiveria zizanioides,* leaves of *Mentha arvensis* and seeds of *Tamarindus indicus* were selected for iron removal studies. In the case of fluoride removal, stem of *Euphorbia antiquorum,* leaves of *Aloe barbadensis* and roots of *Vetiveria zizanioides* were selected for further studies. Upon statistical analysis, plant materials like, dry fruits of *Terminalia chebula* and *Terminalia bellirica* was found to be significant in stabilizing acidic and alkaline pH respectively. Likewise, roots of *Hemidesmus indicus* and *Vetiveria zizanioides*; and stem of *Euphorbia antiquorum* was found to be significant in removing turbidity, iron and fluoride respectively. Thus selected statistically significant plant materials obtained for each of the selected water contaminant were characterized for their phyto-constituents.

CHAPTER II

CHARACTERIZATION OF PHYTO-CONSTITUENTS IN PLANT MATERIALS RESPONSIBLE FOR TREATMENT EFFICIENCY.

Introduction

Chemical coagulants have been serving us in the area of water purification for more than a century. The most effective coagulants are derived from either iron or aluminum salts. When used in excess or for a longer period of time, they have ill effects on both environment and humans. Recently these chemicals, especially alum, have been linked with the Alzheimer's disease (Crapper et al., 1973; Flaten, 2001). As urban areas of both developed and developing nations are equipped with treatment facilities, the rural areas are still deprived off such facilities. Apart from these, higher cost of treatment facilities and procurement costs of these coagulants are hindering their wide spread use in under developed nations. These reasons resulted in the resurgence of indigenous technologies of water treatment, especially in rural areas, in which the use of plant based natural coagulants is one such option.

Most widely accepted and studied natural coagulants are from *Moringa oleifera*. Other plant materials that have been studied are *Strychnos potatorum* (Babu and Chaudhuri, 2005; Saif et al., 2012), *Opuntia dillenii* (Nougbode et al., 2013), *Vigna unguiculata* (Marobhe, 2008), *Cassia alata* (Aweng, et al., 2012) etc. Knowing the exact nature of the active biomolecules, which are acting as coagulant is of essential importance (Gassenschmidt et al., 1995a) in upbringing such rural technologies. Several promising plants have been characterized for their bioactive components. Gassenschmidt et al., (1995a) had isolated the bioactive component from *Moringa oleifera.* Likewise

Fatombi et al., (2013) had isolated natural coagulant from *Cocos nucifera.* Most of the work highlighted the significance of protein as the coagulant (Gassenschmidt et al., 1995a; Ndabigengesere et al., 1995). But Okuda et al., (2001) have regarded that the active component is a polyelectrolyte. Apart from these, plant components like starch (Teh et al., 2014), poyphenols (Chang et al., 2009), tannins (Beltrán-Heredia et al., 2010; Ozacar and Sengil, 2003; Thakur and Choubey, 2014), polysaccharide (Kim et al., 1999; Pal et al., 2009) etc. are reported to have coagulant properties.

In light of this, characterization of the bioactive components associated with five plants which are effective in stabilizing / removing water quality parameters / contaminants have been attempted using FT-IR, HPTLC and LC/Q-TOF/MS studies.

Review of literature

Though several investigations are carried out to find out new plant material with coagulation activity, characterization of the bioactive components responsible for coagulation are not well attempted (Choy et al., 2014). It is being stated that direct extraction of these compounds could enhance the effectiveness of the overall coagulation process. Also searches for coagulant dosage required to achieve optimum coagulation can also be significantly reduced (Nancy Marobhe et al., 2007). Thus studies emphasizing the characterization and quantification of bioactive components are significant.

Though most of the plant materials which are having aromatic / medicinal properties have been subjected to the estimation of biochemical constituents, not much efforts have been carried out on the screening of biochemical constituents of plants with respective to their efficiencies in water treatment. Ndabigengesere et al., (1995) elucidated the active agents

responsible for coagulation in *Moringa oleifera*. Study stated that the active agent is a dimeric cationic protein having a molecular weight of 13 KDa and isoelectric points between 10 and 11. Gassenschmidt et al., (1995b) isolated a flocculating protein from *Moringa oleifera* by extracting with phosphate buffer followed by cation exchange chromatography. The molecular mass of the protein determined by SDS-PAGE was about 6.5 KDa , and the isoelectric point was above pH 10.

Okuda et al., (2001) reported that the active component responsible for coagulation from *Moringa oleifera* is not a protein, but a polysaccharide or a lipid. They opined that it s a polyelectrolyte with a molecular weight of 3KDa . In 2005, Ghebremichael et al., isolated a cationic protein from extracts of *Moringa oleifera* with a molecular mass less than 6.5 KDa. Marobhe et al., (2007) reported a 6KDa coagulant protein from *Vigna unguiculata* and *Parkinsonia aculeata* seeds. They mentioned that this isolated coagulant protein was similar to the coagulant protein isolated from *Moringa oleifera*. Santos et al., (2009) reported a coagulant protein lectin from *Moringa oleifera* seeds capable using in water treatment. A natural coagulant protein was extracted from endosperm of *Cocos nucifera*. Molecular weight determined by size exclusion chromatography was about 5.6 KDa (Fatombi et al., 2013).

The above literature reveals that the exact coagulation mechanism behind most of the natural coagulants has yet to be understood. At the same time, determination of the exact chemical constituents responsible for inducing specific property is extremely difficult. This is due to the complexity of the process and probable synergistic effects among the components present (Choy et al., 2014). Since characterization is an important step for in-depth studies of bioactive components responsible for coagulation, the present study is attempted.

Materials and Methods

From two dozen plants screened out,five plant materials which were statistically significant in adjusting / removing water quality parameters / contaminant were selected for characterization of bioactive components. The plant materials selected include dry fruits of *Terminalia chebula* and *Terminalia bellirica*; roots of *Hemidesmus indicus and Vetiveria zizanioides* and stem of *Euphorbia antiquorum*, which were found to be effective in stabilizing / removing acidity, alkalinity, turbidity, iron and fluoride, respectively.

Characterization was carried out with the help of FTIR (Fourier Transform Infrared Spectroscopy), HPTLC (High performance thin layer chromatography) and LC/QTOF/MS (Liquid chromatography quadrupole-Time of Flight- Mass spectrometer). Prior to characterization, these plants were subjected to serial soxhlet extraction. Descriptions of these five plants are depicted below.

(i) *Terminalia chebula* Retz.

Kingdom : Plantae

Order : Myrtales

Class : Magnoliopsida

Family : Combretaceae

It is a medium to large sized, up to 25 m tall, deciduous tree of variable appearance, with a usually short cylindric bole of 5-10 m length, 60-80 cm in diameter at breast height; crown rounded, with spreading branches; bark dark brown, usually longitudinally cracked with woody scales; branchlets rusty-villous or glabrescent(Plate 3a). Leaves alternate or opposite, thin-coriaceous,

ovate or elliptic-obovate, 7-12 cm x 4-6.5 cm, rounded at base, obtuse to subacute at apex, entire, pubescent beneath; petiole up to 2 cm long, provided with 2 glands at the base of the leaf blade. Flowers in axillary 5-7 cm long spikes, simple or sometime branched, about 4 mm across, yellowish-white and unpleasantly scented; calyx 5-lobed, corolla absent; stamens 10, exserted; ovary inferior, 1-celled. Fruit an obovoid or oblong-ellipsoid drupe, 2.5-5 cm long, faintly 5-angular, yellow to orange-brown when ripe, glabrous. It is distributed throughout India and Southeast Asia, especially in deciduous forests (Rathinamoorthy and Thilagavathi, 2014; Srivastava et al., 2012).

(ii) *Terminalia bellirica* Roxb.

Kingdom : Plantae

Order : Myrtales

Class : Magnoliopsida

Family : Combretaceae

Terminalia bellirica is a large deciduous tree to 50 m tall and a diameter of 3 m with a rounded crown (Plate 3b.). The frequently buttressed bole at the base is branchless up to 20 m. The bark is bluish or ashy-grey covered with numerous fine longitudinal cracks, the inner bark yellowish. Leaves large, glabrous, alternate, broadly elliptic to obovate-elliptical, 4-24 cm x 2-11 cm, base rounded to cuneate, rufous-sericeous but soon glabrescent, with 6-9 pairs of secondary veins. Secondary and tertiary venation prominent on both surfaces, clustered towards the ends of branchlets. Petiole 2.5-9 cm long. Young leaves copper-red, soon becoming parrot green, then dark green. Flowers solitary, small, 3-15 cm long, greenish white, simple, axillary spikes; calyx tube densely sericeous or tomentulose; flowers appear along with new leaves and have a strong honey-like smell. Fruit sub-

globular to broadly ellipsoid, 2-4 x 1.8-2.2 cm, densely velutinous or sericeous, light-yellow, obscurely 5-angled and minutely brown tomentosa (Deb et al., 2016).

(iii) *Hemidesmus indicus* (L.) R. Br.

Kingdom : Plantae

Order : Gentianales Juss.ex Bercht. & J.Presl

Class : Equisetopsida

Family : Appocyanaceae

The stems and branches which twine anticlockwise are profusely lactiferous, elongate, narrow, terete and wiry of a deep purple or purlplishbrown colour with the surface slightly ridged in the nodes (Plate 3c). Leaves: simple, petioled, exstipulate, opposite, entire, apiculate acute or obtuse, dark green above but paler and sometimes pubescent below. Leaves of the basal parts of the shoots are linear to lanceolate. Flowers: Greenish yellow to greenish purple outside, dull yellow to light purplish inside, calyx deeply five lobed, corolla gamopetalous, about twice the calyx. Stamens five, inserted near base of corolla with a thick coronal scale. Stamens five, insereted near base of corolla with distinct filaments and small connate oblong anthers endng in inflexed appendages. Pistil bicarpellary, ovaries free, many ovuled with distinct styles. Fruit two straight slender narrowly cylindrical widely divergent follicles. Seeds many, flat, oblong, with a long tuff of white silky hairs (George et al., 2008).

(iv) *Vetiveria zizanioides* (Linn.) Nash

Kingdom : Plantae

Order : Poales small

Class : Equisetopsida C. Agardh

Family : Poaceae

Vetiveria zizanioides is a densely tufted grass with the culms arising from an aromatic rhizome up to 2 m tall; the roots are stout, dense and aromatic; leaves are narrow, erect, keeled with scabrid margins; inflorescence is a panicle, up to 15-45 cm long of numerous slender racemes in whorls on a central axis; 440 spikelets are grey to purplish, 4-6 mm long, in pairs, one sessile the other pedicelled; 2-flowered; the lower floret is reduced to a lemma, upper bisexual in sessile, male in the pedicelled spikelet; glumes are armed with stout, tubercle-based spines, lemmas awnless, palea minute (Plate 3d). It grows wild in almost all plain states in India up to an elevation of 1200 m (Rao and Suseela, 2000).

(v) *Euphorbia antiquorum* L.

Kingdom : Plantae

Order : Malpighiales

Class : Equisetopsida C. Agardh

Family : Euphorbiaceae

It is one of the largest armed trees with an average height of 5-7m; stems are 5-7cm thick, green, glabrous, having branching from upper parts; upward curving and segmented (Plate 3e). The odour of its latex is highly pungent and lingering. Ribs are prominent generally three sometimes four to

five (4-5), wing like, up to 1-3cm wide, 3-5mm thick, prominently triangular shaped. Leaves are few, borne on the ridges, succulent, alternate, apically clustered, petiole very short, leaf blade obovate, to oblanceolate to spathulate in shape 2-5(- 10)×1-2 cm, base attenuate, margin entire, quite insignificant and fall off quickly Apex is rounded or obtuse with pointed projection, base gradually narrowing downward. Leaves are long in the young seedling, margins deeply sinuate. Flowers are cyathia yellowish green to pinkish in colour, subterminal, axillary and single or in triads or 3-4 individual cyathia together; peduncles are reddish brown; primary peduncle 1-1.5 cm long, cyathia peduncle 2-3mm; all cyathia bisexual; anthers pinkish. Male flower with one stamen, filamentous; female flower lies at the center of the cyathium, protruding beyond the involucre, styles generally three, not joined to each other, each style forking towards the tip. Blooming season of flowers and fruit is throughout the year. They are full of honey that attracted bees. Fruit is capsules, glabrous, obscurely lobed, smooth about 8-10mm in diameter and become deep red on maturity year (Kumar and Saikia, 2016).

Collection of plants

All the five plants / plant parts were collected from varying locations. Plants collected were authenticated with the help of standard keys (Gamble, 1967) and experts. Herbaria of all the five plants were prepared.

Plant processing and solvent extraction

All the five plants/ plant parts were shade dried and were pulverized separately to get coarse powder. Samples of this powder were subjected to soxhlet extraction using solvents on the basis of polarity. Accordingly petroleum ether, chloroform and ethanol were used as solvents.

Petroleum ether extraction: 250ml of the solvent was used for a single extraction. 5.0g of the plant material was weighed and transferred to a pre weighed filter paper pouch so that any fine particles getting into the thimble can be avoided. Temperature was adjusted near to the boiling point of petroleum ether (60-80^{0}C).

Chloroform extraction: 250ml of the solvent was added to the marc left out after petroleum ether extraction. The marc was allowed to dry before the next extraction. Here boiling point was maintained at 55-61.5^0 C.

Ethanol extraction: Marc from the previous two extractions were dried and added with 250ml of ethanol and kept for 3-6 hrs for hot extraction. Boiling point was adjusted near to 78.37^{0}C.

The extracts thus obtained were concentrated using a rotary evaporator (Scilogex RE-100). These extracts were kept under refrigeration until required for phyto-constituent analysis using HPTLC and LC/Q-TOF/MS.

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

Infrared analysis of all the five plant materials was carried out using the facility available at the Department of Nanotechnology, University of Calicut. The instrument used was "PerkinElmer Spectrum Two" model equipped with DTGS (Deuterated Tri Glycine Sulfate) detector. About 1.0 mg, dried powder from fruits of *Terminalia chebula* and *Terminalia bellirica*; roots of *Hemidesmus indicus* and *Vetiveria zizanioides*; stem of *Euphorbia antiquorum* was individually mixed thoroughly with KBr to form a pellet and infrared spectra for each of the plant material were recorded at room temperature in the mid infrared region of 4000-500cm^{-1}. Spectrum thus obtained was depicted in fig 1-5.

High Performance Thin Layer chromatography (HPTLC)

All the five plants were subjected to solvent extraction using petroleum ether, chloroform and Ethanol. HPTLC analysis was carried out at Center for Medicinal Plants Research (CMPR) at Kottakkal Arya Vaidya sala, Malappuram, Kerala. The instrument used was CAMAG HPTLC system (Switzerland). Samples were applied on aluminum backed pre-coated silica gel plates Merck 60 F 254 (0.2 mm thickness) using CAMAG ATS 4. Samples were applied to the plates as bands at 10mm from the bottom of the plate. The plate was developed up to 80mm in ascending mode in solvent system of Toluene and Ethyl acetate in a ratio of 8:2 (v/v) at room temperature (28 ± 2°C) in a Twin Trough Chamber (Camag, Switzerland) which was previously saturated with mobile phase. After development the air dried plate was scanned at 254 nm, 366 nm and 550nm, in CAMAG TLC SCANNER 3 using Deuterium lamp with winCATS software.

LC/Q-TOF/MS Analysis

Ethanolic extracts of all the five plants selected were subjected for LC/Q-TOF/MS analysis. LC/Q-TOF/MS analysis was carried out at the Inter-University Instrumentation Center at Mahatma Gandhi University, Kottayam, Kerala. The Instrument used was Acquity H class (Waters) Ultra Performance Liquid Chromatography and Xevo G2 (Waters) Quadrapole-Time-of-Flight (Q-TOF). BEH C18 column with a configuration of 50 mm × 2.1 mm × 1.7 µm was used at a flow rate of 0.3 ml/min. The total run time was 8 min. The source type was Electro- Spray Ionization (ESI) with the capillary temperature of 135°C. Capillary voltage of positive mode of ESI was 3.50 KV and for negative mode, it was 2.50 KV. The mobilization gas flow was nitrogen at 0.3 ml/min, approximately(Maya and Benjamin, 2016).

Results and discussion

All the five plants / plant parts which were statistically significant in stabilizing / removing each of the selected water quality parameter / contaminants were subjected to Soxhlet extraction using solvents like petroleum ether, chloroform and ethanol, based on polarity. The resultant extracts were concentrated in a rotary evaporator and subjected to HPTLC analysis. FTIR analysis was carried out with dried plant parts. LC/Q-TOF/MS was done with the ethanolic extracts of all five plants selected.

Characterization using FT-IR

Infra red analysis of all the five dried plant materials gave numerous characteristics peaks for various functional groups. The FT-IR profiles of each of the plants are depicted in figures 1to 5.

Fig1: Characteristic peaks obtained for *Terminalia chebula* after FT-IR analysis

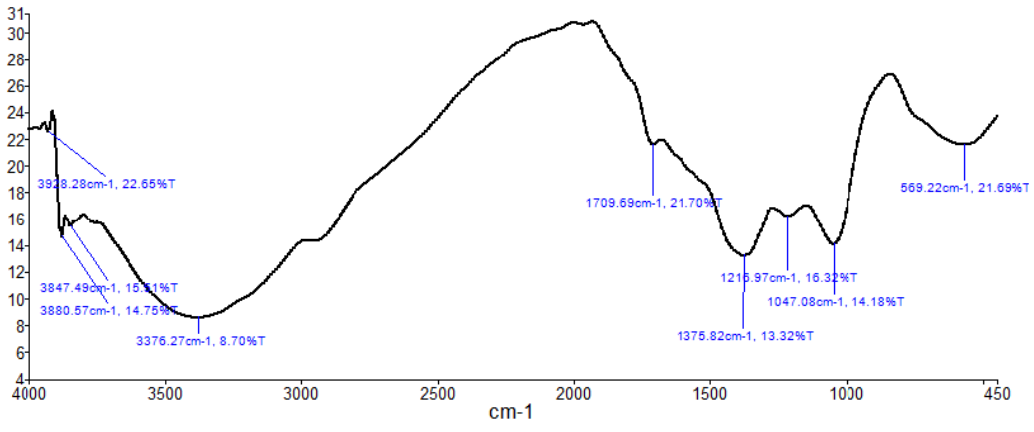


Fig2: Characteristic peaks obtained for *Terminalia bellirica* after FT-IR analysis

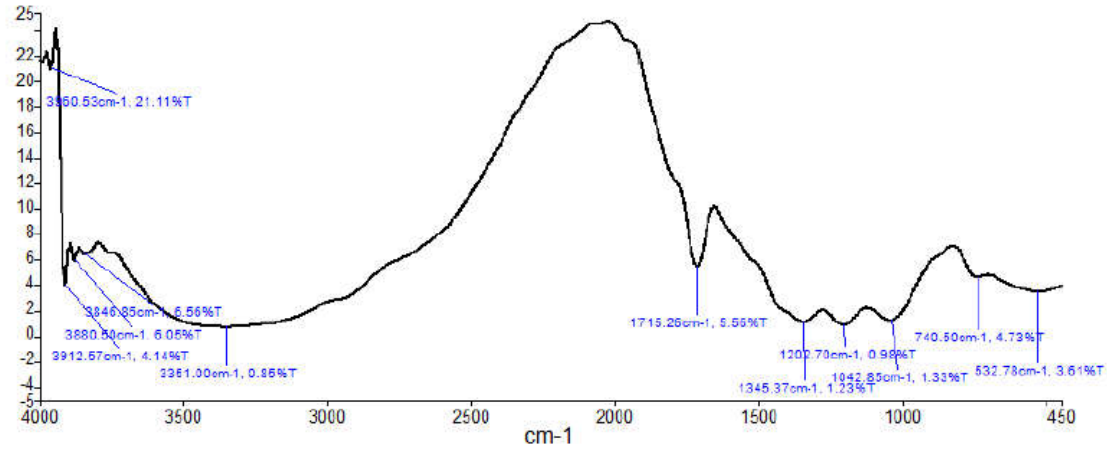


Fig 3: Characteristic peaks obtained for *Hemidesmus indicus* after FT-IR analysis

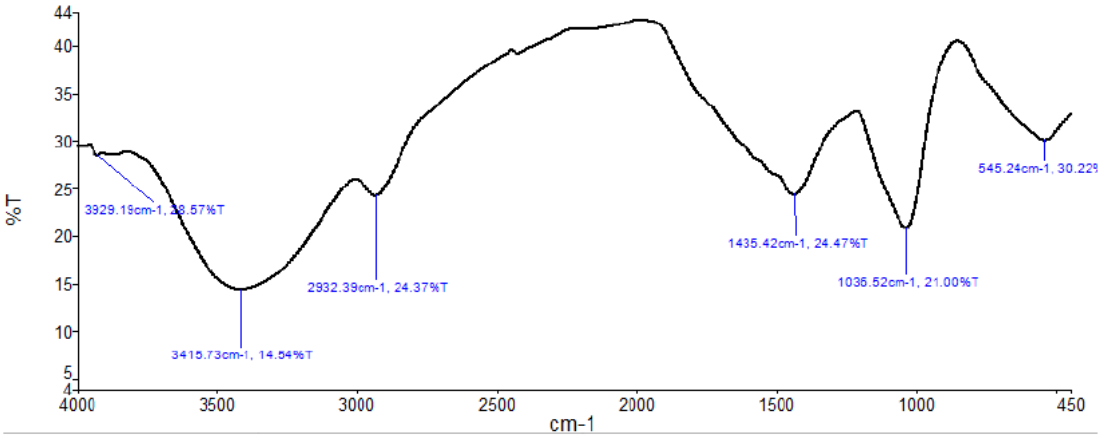


Fig4 : Characteristic peaks obtained for *Vetiveria zizanioides* after FT-IR analysis

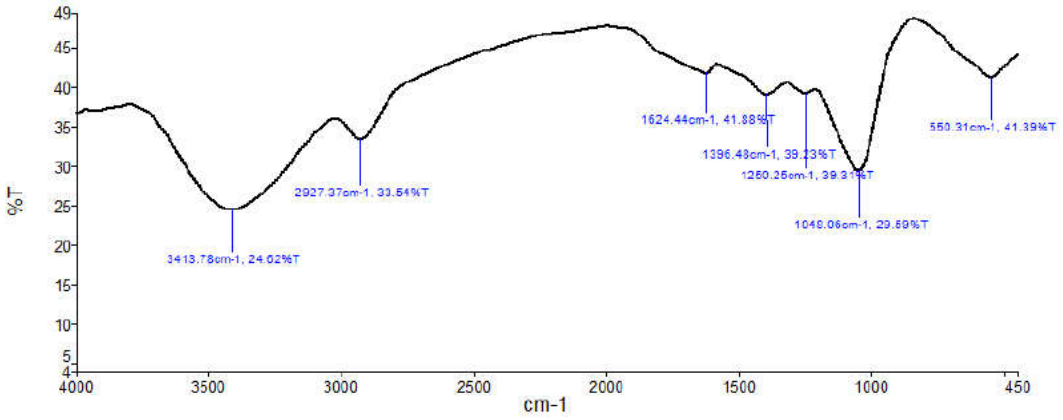
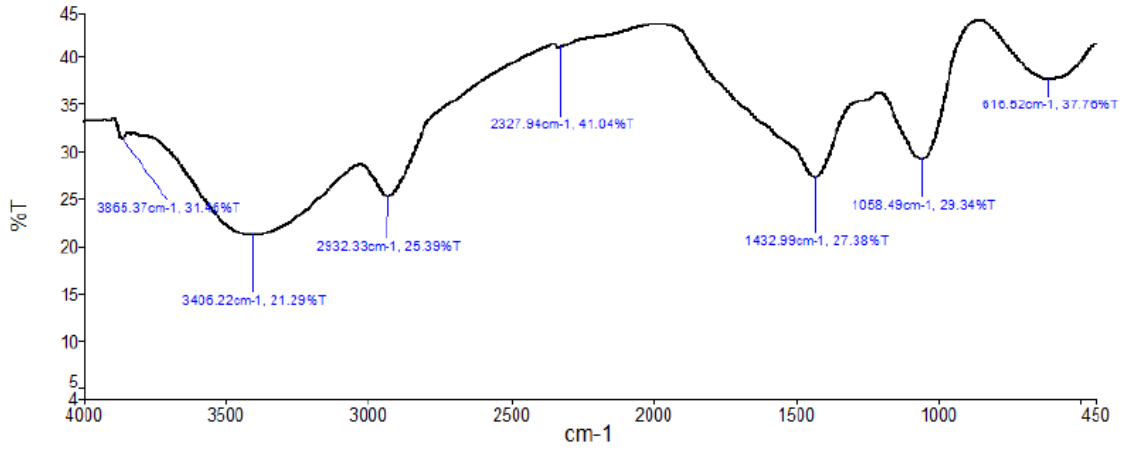


Fig 5 : Characteristic peaks obtained for *Euphorbia antiquorum* after FT-IR analysis



Upon FT-IR analysis, each of the plant material gave characteristic peaks and details of the peak obtained are given below.

For dry fruits of *Terminalia chebula,* 9 characteristic peaks were obtained at 3928.28 $cm^{-1,}$ 3847.49cm^{-1},3880.57cm^{-1}, 3376.27 cm^{-1}, 1709.69

cm^{-1}, 1375.82 cm^{-1}, 1216.97 cm^{-1}, 1047.08 cm^{-1} and 569.22 cm^{-1}. Similarly dry fruits of *Terminalia bellirica* gave eleven characteristic peaks at 3960.53 cm^{-1}, 3880.50 cm^{-1}, 3912.57 cm^{-1}, 3846.85 cm^{-1}, 3351.00 cm^{-1}, 1715.26 cm^{-1}, 1345.37 cm^{-1}, 1202.70 cm^{-1}, 1042.85 cm^{-1}, 740.50 cm^{-1} and 532.78 cm^{-1}. Roots of *Hemidesmus indicus* gave six characteristic peaks. They were 3929.19 cm^{-1}, 3415.73 cm^{-1}, 2932.39 cm^{-1}, 1435.42 cm^{-1}, 1036.52 cm^{-1} and 545.24 cm^{-1}. Similarly with the roots of *Vetiveria zizanioides,* seven characteristic peaks were obtained. They were 3413.78 cm^{-1}, 2927.37 cm^{-1}, 1624.44 cm^{-1}, 1396.48 cm^{-1}, 1250.25 cm^{-1}, 1048.06 cm^{-1} and 550.31 cm^{-1}. When stem of *Euphorbia antiquorum* were subjected to infrared analysis, seven characteristic peaks were obtained, which include 3865.37 cm^{-1}, 3406.22 cm^{-1}, 2932.33 cm^{-1}, 2327.94 cm^{-1}, 1432.99 cm^{-1}, 1058.49 cm^{-1} and 616.93 cm^{-1}.

Upon FTIR analysis, several characteristic peaks were obtained from all the five plant materials. Broad peaks at 3000 cm^{-1} represents the presence of –OH groups (Coates, 2000). Absorption peak above 3000 cm^{-1} are most likely to be due to the presence of unsaturated or aromatic functional groups (Coates, 2000). All the five plants exhibited peaks with absorption maximum above 3000 cm^{-1}. Bands found in 3500-3100 cm^{-1} are characteristic to the presence of polyphenolic compounds (Grasel et al., 2015). All the five plants exhibited these peaks. Hence it can be inferred that all the five plant material possess aromatic secondary metabolites like polyphenolic compounds. Band lying in 2960-2925 cm^{-1} occur due to stretching vibrations of CH, CH_2 or CH_3, which corresponds to the presence of polysaccharides. These bands were observed in all three, except in *Terminalia chebula* and *Terminalia bellirica* (Coates, 2000; Geethu et al., 2014; Grasel et al., 2015). Peaks at 1736-1706 cm^{-1} are due to C=O stretching (Amala and Jeyaraj, 2014). Specifically these stretching are due to the C=O bonds in the esters of hydrolysable tannins like Gallic acid (Grasel et al., 2015). *Terminalia chebula* and *Terminalia bellirica*

exhibited these two peaks in their IR spectrum. IR spectrum lying in 1618-1449 cm^{-1} represents the C=C-C aromatic bond which is another stretching vibrations characteristic to tannins (Coates, 2000; Grasel et al., 2015). These bands were prominent in *Hemidesmus indicus, Vetiveria zizanioides and Euphorbia antiquorum.* Nitrogen compounds with motile bonds have absorbance at 2300-1990 cm^{-1}. Single band was observed in *Euphorbia antiquorum*. IR spectrum of *Terminalia chebula* and *Terminalia bellirica* were observed with C-O bond in 1368-1158 cm^{-1}, which is characteristic to hydrolysable tannins (Fernandez and Agosin, 2007).

Peaks ~1050 cm^{-1} represents the presence of C-O stretch of primary alcohol (Amala and Jeyaraj, 2014). All the plant materials exhibited this peak (Coates, 2000). Most probably IR peak in ~1200 cm^{-1} corresponds to C-O stretch of phenol. This peak was observed in all three plant materials except *H. indicus* and *E. antiquorum*. Usually thiol groups are found in the near range between 500-430 cm^{-1} (Coates, 2000). This band was found in all plants except *Euphorbia antiquorum*.

Phyto-constituent profiling of each of the plant material was carried out using HPTLC. All the three extracts of each of the plant material was scanned at 254, 366 and 550nm.

HPTLC profile of petroleum ether extracts of *Terminalia chebula* gave 7 spots, chloroform extracts gave 5 spots and ethanolic extract gave 4 spots when scanned at 254nm (Fig 6-8). While scanned at 366nm petroleum ether extract gave 6 spots, chloroform extract gave 5 spots and 1 spot in ethanolic extract (Fig 11-13). Likewise when scanned at 550nm petroleum ether extract gave 14 spots, chloroform extract gave 9 spots and ethanolic extract gave 7 spots (Fig 16-18). Three dimensional densitogram representing all the three extracts at varying absorption maxima are depicted in the fig 9, fig 14 and fig 19. Similarly chromatogram with characteristic bands scanned at 254nm,

366nm and 550nm are shown in fig10, fig 15 and fig 20 respectively. Compounds corresponding to specific Rf value are given in the table 77.

Fig 6: HPTLC profile of petroleum ether extract of *Terminalia chebula*. Details of Rf values of compounds and their area percentage obtained when scanned at 254nm

Track 1, ID: PE T

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	0.4 AU	-0.03 Rf	13.0 AU	3.24 %	-0.02 Rf	0.1 AU	115.6 AU	1.81 %	unknown *
2	-0.02 Rf	0.2 AU	-0.00 Rf	138.0 AU	34.45 %	0.02 Rf	24.2 AU	1246.1 AU	19.49 %	unknown *
3	0.09 Rf	11.8 AU	0.10 Rf	26.4 AU	6.59 %	0.14 Rf	2.8 AU	359.0 AU	5.62 %	unknown *
4	0.15 Rf	3.0 AU	0.19 Rf	131.1 AU	32.72 %	0.22 Rf	1.1 AU	2525.4 AU	39.50 %	unknown *
5	0.22 Rf	1.1 AU	0.24 Rf	13.3 AU	3.32 %	0.27 Rf	3.2 AU	235.6 AU	3.68 %	unknown *
6	0.39 Rf	4.5 AU	0.42 Rf	11.6 AU	2.90 %	0.47 Rf	3.0 AU	394.4 AU	6.17 %	unknown *
7	0.72 Rf	3.8 AU	0.76 Rf	67.3 AU	16.78 %	0.79 Rf	2.3 AU	1516.6 AU	23.72 %	unknown *

Fig 7: HPTLC profile of chloroform extract of *Terminalia chebula,* Rf values of compounds and their area percentage obtained when scanned at 254nm

Track 2, ID: CL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	4.9 AU	-0.00 Rf	718.5 AU	73.42 %	0.11 Rf	17.3 AU	12616.2 AU	68.13 %	unknown *
2	0.15 Rf	11.1 AU	0.19 Rf	183.1 AU	18.71 %	0.23 Rf	3.9 AU	4035.8 AU	21.80 %	unknown *
3	0.31 Rf	2.5 AU	0.34 Rf	13.5 AU	1.38 %	0.37 Rf	2.0 AU	295.2 AU	1.59 %	unknown *
4	0.37 Rf	2.3 AU	0.39 Rf	12.4 AU	1.26 %	0.42 Rf	4.5 AU	260.7 AU	1.41 %	unknown *
5	0.78 Rf	6.7 AU	0.81 Rf	51.2 AU	5.23 %	0.86 Rf	0.5 AU	1308.8 AU	7.07 %	unknown *

Fig 8: HPTLC profile of ethanolic extract of *Terminalia chebula* and Rf values of compounds their area percentage when scanned at 254nm

Track 3, ID: ET

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	4.3 AU	-0.01 Rf	782.1 AU	41.24 %	0.00 Rf	39.5 AU	17627.9 AU	44.86 %	unknown *
2	0.01 Rf	724.0 AU	0.01 Rf	822.7 AU	43.39 %	0.06 Rf	39.9 AU	15877.5 AU	40.41 %	unknown *
3	0.06 Rf	201.3 AU	0.07 Rf	206.9 AU	10.91 %	0.12 Rf	21.9 AU	3682.8 AU	9.37 %	unknown *
4	0.14 Rf	20.6 AU	0.17 Rf	84.6 AU	4.46 %	0.21 Rf	5.0 AU	2105.9 AU	5.36 %	unknown *

Fig 9: HPTLC three dimensional densitogram of all three solvent extracts of *Terminalia chebula* at 254nm

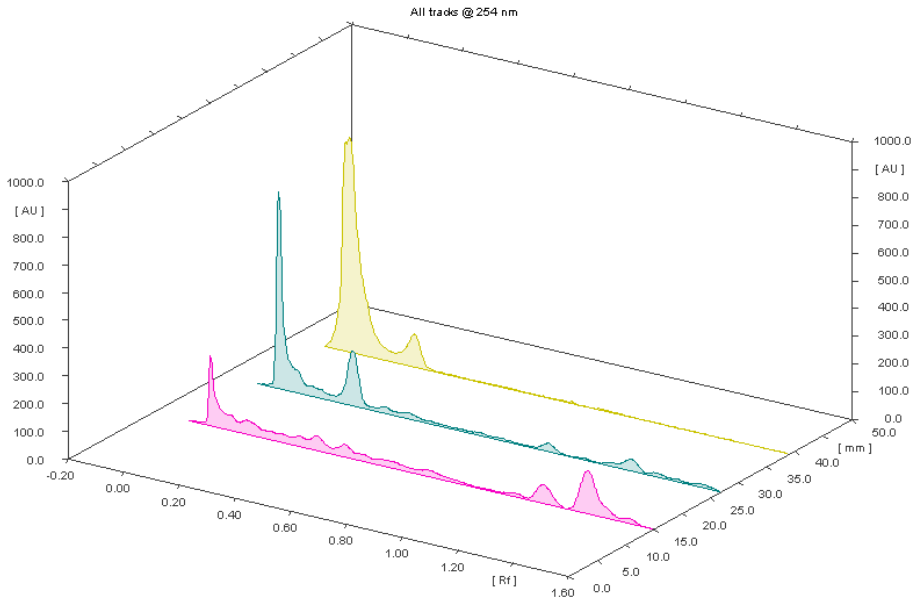


Fig 10: HPTLC image after derivitization observed at 254nm

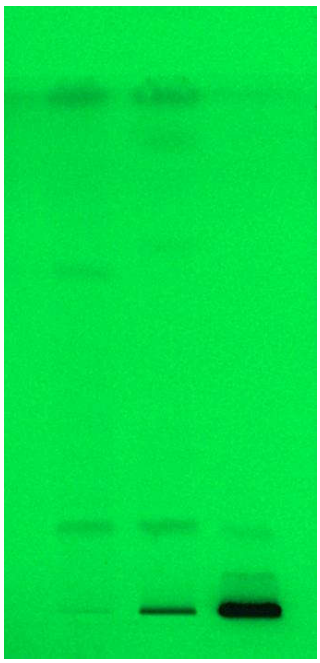


Fig 11: HPTLC profile of petroleum ether extract of *Terminalia chebula* and Rf values of compounds obtained when scanned at 366nm.

Track 1, ID: PE T

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	0.3 AU	-0.00 Rf	51.9 AU	25.53 %	0.02 Rf	7.9 AU	566.7 AU	13.05 %	unknown *
2	0.32 Rf	1.0 AU	0.35 Rf	15.0 AU	7.36 %	0.36 Rf	10.7 AU	298.6 AU	6.88 %	unknown *
3	0.41 Rf	8.6 AU	0.44 Rf	16.7 AU	8.21 %	0.48 Rf	4.2 AU	561.0 AU	12.92 %	unknown *
4	0.66 Rf	3.6 AU	0.69 Rf	14.2 AU	7.00 %	0.71 Rf	6.8 AU	331.5 AU	7.63 %	unknown *
5	0.71 Rf	7.1 AU	0.75 Rf	58.8 AU	28.94 %	0.80 Rf	2.6 AU	1483.2 AU	34.16 %	unknown *
6	0.90 Rf	0.5 AU	0.94 Rf	46.6 AU	22.95 %	0.99 Rf	1.7 AU	1101.4 AU	25.37 %	unknown *

Fig 12: HPTLC profile of chloroform extract of *Terminalia chebula* and Rf values of compounds obtained when scanned at 366nm.

Track 2, ID: CL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	4.8 AU	-0.00 Rf	527.9 AU	88.45 %	0.06 Rf	18.9 AU	7838.1 AU	85.70 %	unknown *
2	0.06 Rf	19.0 AU	0.06 Rf	20.7 AU	3.47 %	0.09 Rf	9.1 AU	327.9 AU	3.59 %	unknown *
3	0.17 Rf	6.8 AU	0.19 Rf	11.4 AU	1.91 %	0.22 Rf	0.5 AU	180.9 AU	1.98 %	unknown *
4	0.32 Rf	2.3 AU	0.35 Rf	14.3 AU	2.40 %	0.37 Rf	3.7 AU	276.3 AU	3.02 %	unknown *
5	0.91 Rf	5.9 AU	0.94 Rf	22.5 AU	3.77 %	0.97 Rf	6.4 AU	522.9 AU	5.72 %	unknown *

Fig 13: HPTLC profile of ethanolic extract of *Terminalia chebula* and Rf values of compounds obtained when scanned at 366nm

Track 3, ID: ET

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.9 AU	0.01 Rf	828.9 AU	100.00 %	0.06 Rf	18.4 AU	25037.9 AU	100.00 %	unknown *

Fig14 : HPTLC three dimensional densitogram of all the three solvent extracts of *Terminalia chebula* at 366nm

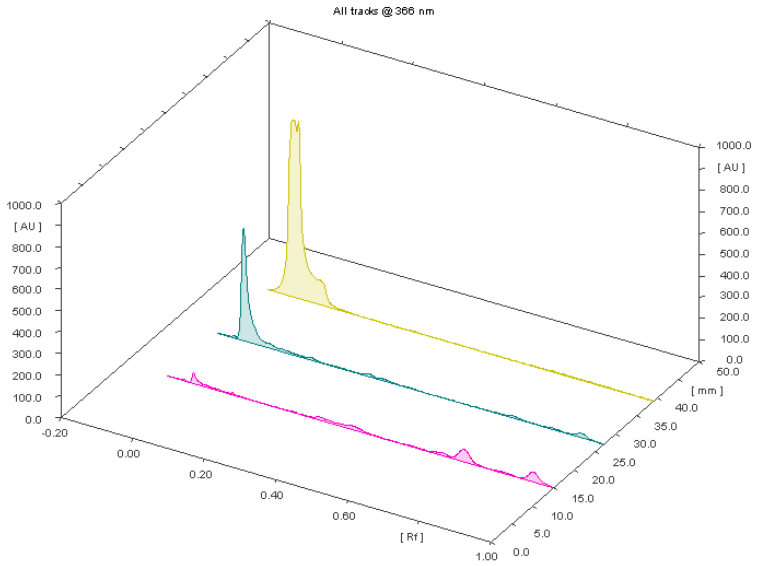


Fig 15: HPTLC image after derivitization observed at 366nm

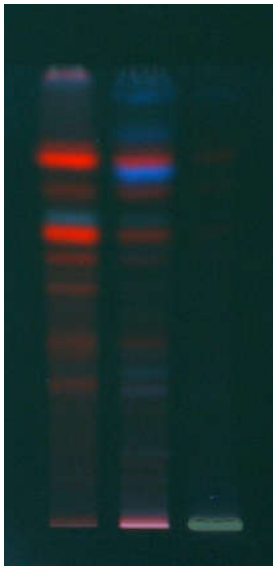
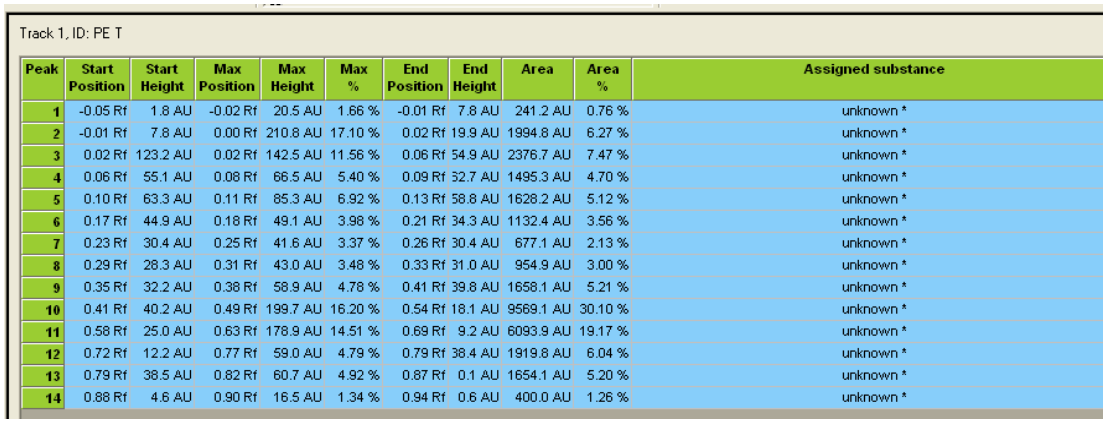


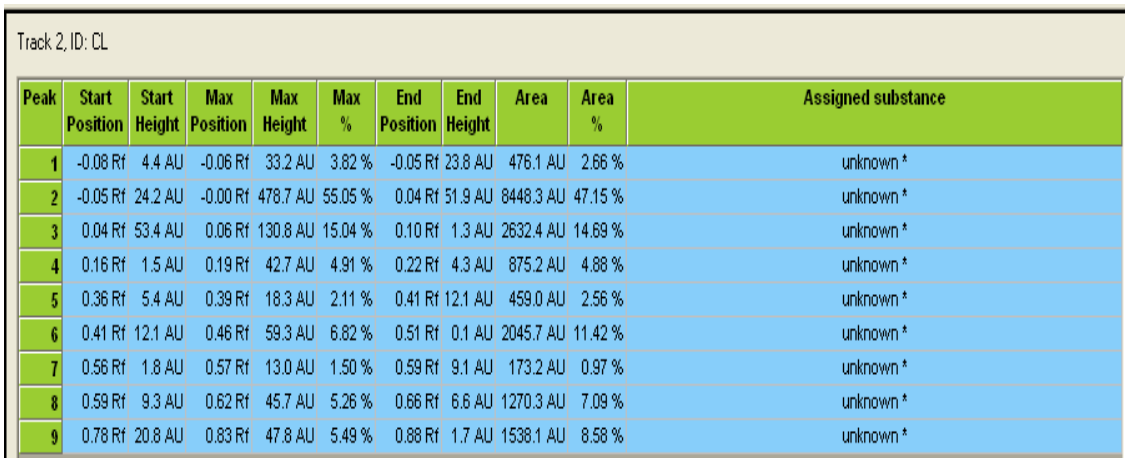
Fig 16. HPTLC profile of petroleum ether extract of *Terminalia chebula* and Rf values of compounds obtained when scanned at 550nm



Track 1, ID: PE T

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.05 Rf	1.8 AU	-0.02 Rf	20.5 AU	1.66 %	-0.01 Rf	7.8 AU	241.2 AU	0.76 %	unknown *
2	-0.01 Rf	7.8 AU	0.00 Rf	210.8 AU	17.10 %	0.02 Rf	19.9 AU	1994.8 AU	6.27 %	unknown *
3	0.02 Rf	123.2 AU	0.02 Rf	142.5 AU	11.56 %	0.06 Rf	54.9 AU	2376.7 AU	7.47 %	unknown *
4	0.06 Rf	55.1 AU	0.08 Rf	66.5 AU	5.40 %	0.09 Rf	32.7 AU	1495.3 AU	4.70 %	unknown *
5	0.10 Rf	63.3 AU	0.11 Rf	85.3 AU	6.92 %	0.13 Rf	58.8 AU	1628.2 AU	5.12 %	unknown *
6	0.17 Rf	44.9 AU	0.18 Rf	49.1 AU	3.98 %	0.21 Rf	34.3 AU	1132.4 AU	3.56 %	unknown *
7	0.23 Rf	30.4 AU	0.25 Rf	41.6 AU	3.37 %	0.26 Rf	30.4 AU	677.1 AU	2.13 %	unknown *
8	0.29 Rf	28.3 AU	0.31 Rf	43.0 AU	3.48 %	0.33 Rf	31.0 AU	954.9 AU	3.00 %	unknown *
9	0.35 Rf	32.2 AU	0.38 Rf	58.9 AU	4.78 %	0.41 Rf	39.8 AU	1658.1 AU	5.21 %	unknown *
10	0.41 Rf	40.2 AU	0.49 Rf	199.7 AU	16.20 %	0.54 Rf	18.1 AU	9569.1 AU	30.10 %	unknown *
11	0.58 Rf	25.0 AU	0.63 Rf	178.9 AU	14.51 %	0.69 Rf	9.2 AU	6093.9 AU	19.17 %	unknown *
12	0.72 Rf	12.2 AU	0.77 Rf	59.0 AU	4.79 %	0.79 Rf	38.4 AU	1919.8 AU	6.04 %	unknown *
13	0.79 Rf	38.5 AU	0.82 Rf	60.7 AU	4.92 %	0.87 Rf	0.1 AU	1654.1 AU	5.20 %	unknown *
14	0.88 Rf	4.6 AU	0.90 Rf	16.5 AU	1.34 %	0.94 Rf	0.6 AU	400.0 AU	1.26 %	unknown *

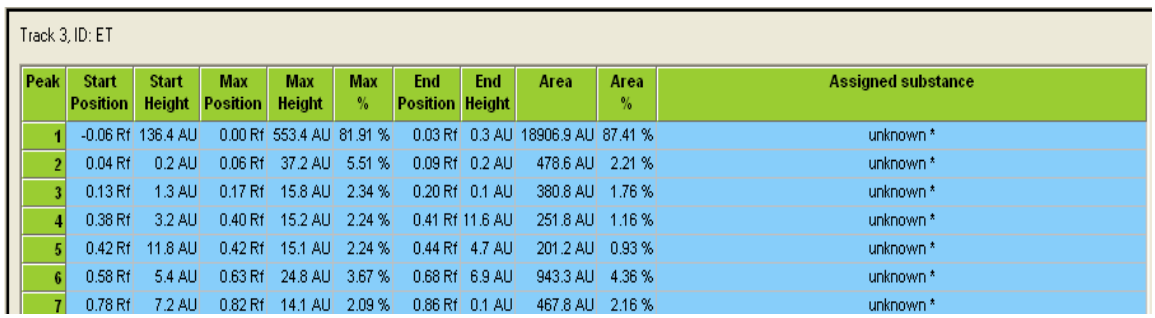
Fig 17. HPTLC profile of chloroform extract of *Terminalia chebula* and Rf values of compounds obtained when scanned at 550nm



Track 2, ID: CL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.08 Rf	4.4 AU	-0.06 Rf	33.2 AU	3.82 %	-0.05 Rf	23.8 AU	476.1 AU	2.66 %	unknown *
2	-0.05 Rf	24.2 AU	-0.00 Rf	478.7 AU	55.05 %	0.04 Rf	51.9 AU	8448.3 AU	47.15 %	unknown *
3	0.04 Rf	53.4 AU	0.06 Rf	130.8 AU	15.04 %	0.10 Rf	1.3 AU	2632.4 AU	14.69 %	unknown *
4	0.16 Rf	1.5 AU	0.19 Rf	42.7 AU	4.91 %	0.22 Rf	4.3 AU	875.2 AU	4.88 %	unknown *
5	0.36 Rf	5.4 AU	0.39 Rf	18.3 AU	2.11 %	0.41 Rf	12.1 AU	459.0 AU	2.56 %	unknown *
6	0.41 Rf	12.1 AU	0.46 Rf	59.3 AU	6.82 %	0.51 Rf	0.1 AU	2045.7 AU	11.42 %	unknown *
7	0.56 Rf	1.8 AU	0.57 Rf	13.0 AU	1.50 %	0.59 Rf	9.1 AU	173.2 AU	0.97 %	unknown *
8	0.59 Rf	9.3 AU	0.62 Rf	45.7 AU	5.26 %	0.66 Rf	6.6 AU	1270.3 AU	7.09 %	unknown *
9	0.78 Rf	20.8 AU	0.83 Rf	47.8 AU	5.49 %	0.88 Rf	1.7 AU	1538.1 AU	8.58 %	unknown *

Fig 18: HPTLC profile of ethanolic extract of *Terminalia chebula* and Rf values of compounds obtained when scanned at 550nm



Track 3, ID: ET

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	136.4 AU	0.00 Rf	553.4 AU	81.91 %	0.03 Rf	0.3 AU	18906.9 AU	87.41 %	unknown *
2	0.04 Rf	0.2 AU	0.06 Rf	37.2 AU	5.51 %	0.09 Rf	0.2 AU	478.6 AU	2.21 %	unknown *
3	0.13 Rf	1.3 AU	0.17 Rf	15.8 AU	2.34 %	0.20 Rf	0.1 AU	380.8 AU	1.76 %	unknown *
4	0.38 Rf	3.2 AU	0.40 Rf	15.2 AU	2.24 %	0.41 Rf	11.6 AU	251.8 AU	1.16 %	unknown *
5	0.42 Rf	11.8 AU	0.42 Rf	15.1 AU	2.24 %	0.44 Rf	4.7 AU	201.2 AU	0.93 %	unknown *
6	0.58 Rf	5.4 AU	0.63 Rf	24.8 AU	3.67 %	0.68 Rf	6.9 AU	943.3 AU	4.36 %	unknown *
7	0.78 Rf	7.2 AU	0.82 Rf	14.1 AU	2.09 %	0.86 Rf	0.1 AU	467.8 AU	2.16 %	unknown *

Fig 19: HPTLC three dimensional densitogram of different solvent extract of *Terminalia chebula* scanned at 550nm

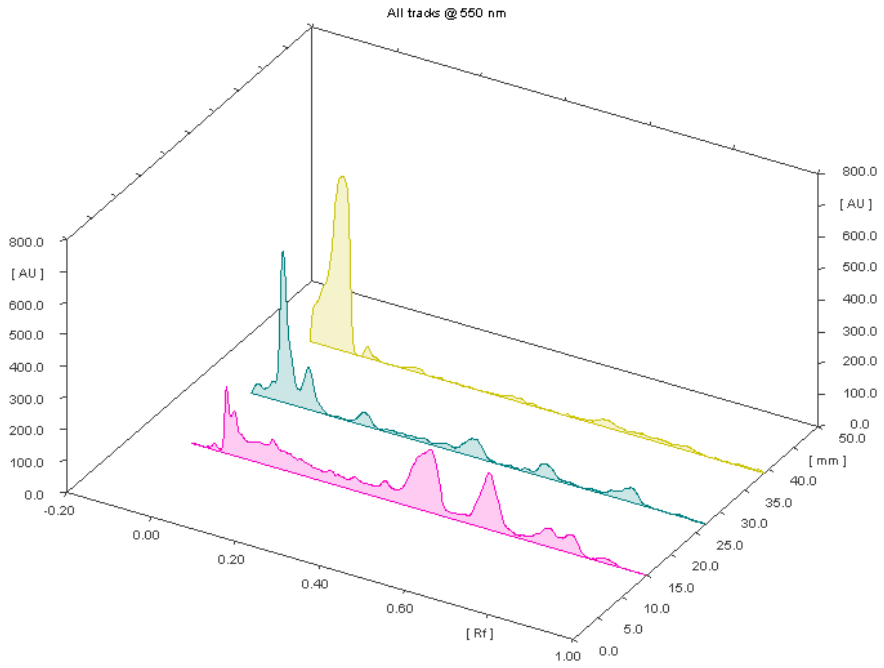


Fig 20: HPTLC image after derivitization observed at 550nm

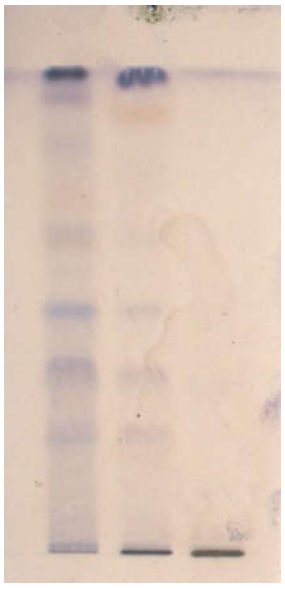


Table 77: HPTLC profile of all the three extracts of *Terminalia chebula* scanned at 254nm, 366nm and 550nm and tentative compounds according to Rf value obtained

Terminalia chebula								
254nm								
Rf value	**Petroleum ether extract**	**Reference**	**Rf value**	**Chloroform extract**	**Reference**	**Rf value**	**Ethanolic extract**	**Reference**
-0.02	Flavonoid	(Karthika et al., 2014)	0.11	Alkaloid	(Kalaiselvi et al., 2012)	0.00	Ellagic acid	(Ferry and Larson, 1991)
0.02	Flavonoid	(Karthika et al., 2014)	0.23	Alkaloid	(Kalaiselvi et al., 2012)	0.06	Terpenoid	(Yamunadevi, et al., 2011)
0.14	Phenol	(Banu and Nagarajan, 2014)	0.37	Terpenoids	(Biradar, 2013)	0.12	Flavanoid	(Jyothi, 2016)
0.22	Saponins	(Biradar, 2013)	0.42	Flavanoid	(Gomathi et al., 2012a)	0.21	Alkaloid	(Banu and Nagarajan, 2014)
0.27	Phenol	(Banu and Nagarajan, 2014)	0.86	Terpenoid	(Gomathi et al., 2012b)			
0.47	Steroids	(Gomathi et al., 2012b)						
0.79	Phenol	(Banu and Nagarajan, 2014)						

366nm								
Rf value	**Petroleum ether extract**	**Reference**	**Rf value**	**Chloroform extract**	**Reference**	**Rf value**	**Ethanolic extract**	**Reference**
0.02	Flavonoid	(Karthika et al., 2014)	0.06	Steroids	(Gomathi et al., 2012b)	0.06	Steroids	(Gomathi et al., 2012b)
0.36	Terpenoids	(Biradar, 2013)	0.09	Gallic acid (Flavanoid)	(Asha et al., 2015)			
0.48	Flavanoid	(Gomathi et al., 2012a)	0.22	Saponins	(Biradar, 2013)			
0.71	Terpenoid	(Yamunadevi, et al., 2011)	0.37	Terpenoids	(Biradar, 2013)			
0.80	Alkaloid	(Kalaiselvi et al., 2012)	0.97	Phenol (3,4-Dimethylphenol)	(Ferry and Larson, 1991)			
0.99	Steroids	(Gomathi et al., 2012b)						
550nm								
Rf value	**Petroleum ether extract**	**Reference**	**Rf value**	**Chloroform extract**	**Reference**	**Rf value**	**Reference**	**Ethanolic extract**
-0.01	Phenol	(Banu and Nagarajan, 2014)	-0.05	Alkaloid	(Kalaiselvi et al., 2012)	0.03	Alkaloid	(Banu and Nagarajan, 2014)
0.02	Flavonoid	(Karthika et al., 2014)	0.04	Tannic acid	(Ferry and Larson,	0.09	Gallic acid (Flavanoid)	(Asha et al., 2015)

					1991)			
0.06	Terpenoid	(Yamunadevi, et al., 2011)	0.10	Terpenoid	(Gomathi et al., 2012b)	0.20	Flavanoid	(Biradar, 2013)
0.09	Gallic acid (Flavanoid)	(Asha et al., 2015)	0.22	Saponins	(Biradar, 2013)	0.41	Phenolics	(Kalaiselvi et al., 2012)
0.13	Alkaloid	(Asante IK et al., 2016)	0.41	Phenolics)	(Kalaiselvi et al., 2012	0.44	Terpene	(Asante IK et al., 2016)
0.21	Alkaloid	(Banu and Nagarajan, 2014)	0.51	Alkaloid	(Asante IK et al., 2016)	0.68	Phenol Dihydroxy benzaldehyde	(Ferry and Larson, 1991)
0.26	Phenolics	(Kalaiselvi et al., 2012)	0.59	Alkaloid	(Kalaiselvi et al., 2012)	0.86	Terpenoid	(Gomathi et al., 2012b)
0.33	Flavanoid	(Biradar, 2013)	0.66	Terpenoid	(Gomathi et al., 2012b)			
0.41	Phenolics	(Kalaiselvi et al., 2012)	0.88	Terpenoid	(Gomathi et al., 2012b)			
0.54	Saponin	(Biradar, 2013)						
0.69	Flavonoid	(Biradar, 2013)						
0.79	Phenol	(Banu and Nagarajan, 2014)						
0.87	Terpenoid	(Biradar, 2013)						
0.94	Flavonoid	(Asante IK et al., 2016)						

Each of the three solvent extracts of dry fruits of *Terminalia chebula* was scanned at 254nm, 366nm and 550nm respectively. Major components obtained from all the three extracts with highest area percentage from dry fruits of *Terminalia chebula* are given in the table78.

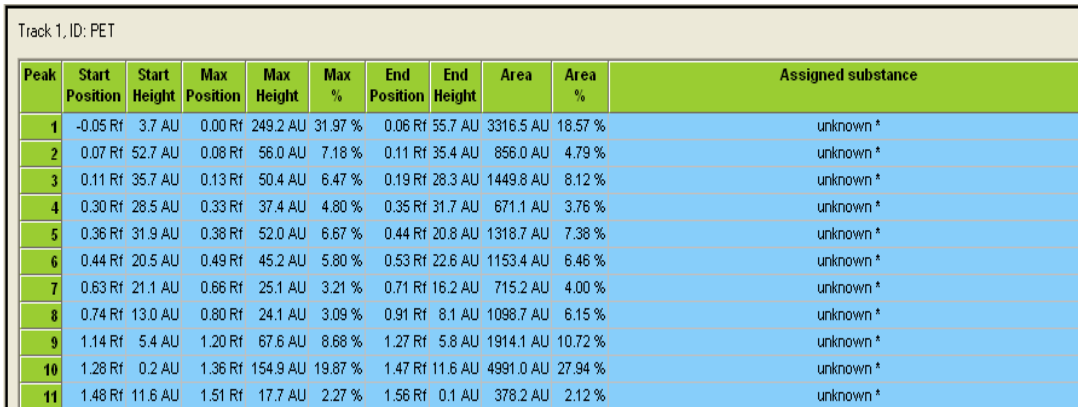
Table 78:- Major components obtained from *Terminalia chebula* from HPTLC analysis

Terminalia chebula				
	Scanned Absorbances	Petroleum ether extract	Chloroform extract	Ethanolic extract
	254nm	Saponins (39.50%) Phenolics (23.27%)	Alkaloids (68.13%) Terpenoids (7.07%)	Ellagic acid (44.86%) Terpenoid (40.41%)
	366nm	Alkaloids (34.16%) Steroids (25.37%)	Terpenoids (85.70%) Phenols (5.72%)	Terpenoids (100%)
	550nm	Phenolic compound (30.10%) Terpenoid (7.47%)	Tannic acid (47.15%)	Alkaloid (87.41%) Phenolic compounds (4.36%)

All the three extracts of plant material when analyzed with HPTLC, each extract gave several spots corresponding to particular compounds. Petroleum ether extracts of *Terminalia bellirica* gave 11 spots, chloroform extracts gave 8 spots and ethanolic extract gave 2 spots when scanned at 254nm (Fig 21-23). When they were scanned at 366nm petroleum ether gave 10 spots, chloroform gave 8 spots and ethanolic extract gave 2 spots (Fig 26-28). While scanned at 550nm petroleum ether extract was observed with 12 spots, chloroform with 10 spots and ethanolic extracts with 9 spots (Fig 31-33). Three dimensional densitogram representing all the three extracts at

254nm, 366nm and 550nm are depicted in fig24, fig29 and fig34 respectively. HPTLC image obtained for all the three extracts after derivitization are depicted in fig 25, fig 30 and fig35. Based on the Rf value obtained, the compounds tentatively identified are given in the table 17.

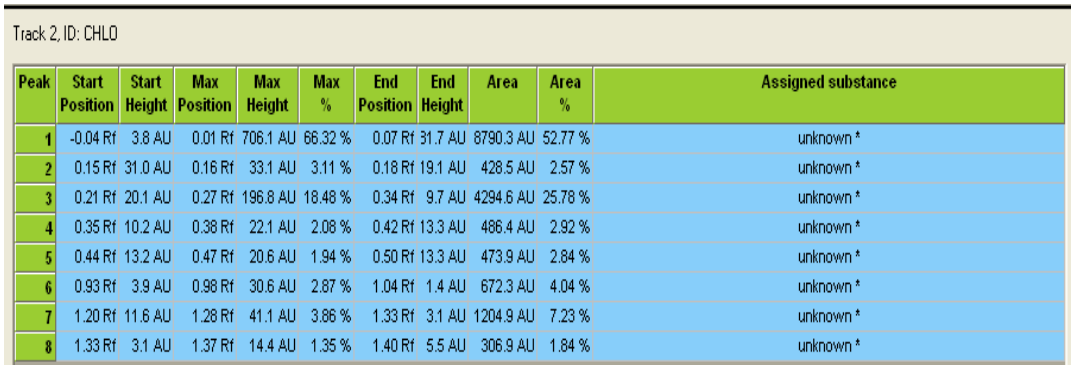
Fig21. HPTLC profile of petroleum ether extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 254nm



Track 1, ID: PET

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.05 Rf	3.7 AU	0.00 Rf	249.2 AU	31.97 %	0.06 Rf	55.7 AU	3316.5 AU	18.57 %	unknown *
2	0.07 Rf	52.7 AU	0.08 Rf	56.0 AU	7.18 %	0.11 Rf	35.4 AU	856.0 AU	4.79 %	unknown *
3	0.11 Rf	35.7 AU	0.13 Rf	50.4 AU	6.47 %	0.19 Rf	28.3 AU	1449.8 AU	8.12 %	unknown *
4	0.30 Rf	28.5 AU	0.33 Rf	37.4 AU	4.80 %	0.35 Rf	31.7 AU	671.1 AU	3.76 %	unknown *
5	0.36 Rf	31.9 AU	0.38 Rf	52.0 AU	6.67 %	0.44 Rf	20.8 AU	1318.7 AU	7.38 %	unknown *
6	0.44 Rf	20.5 AU	0.49 Rf	45.2 AU	5.80 %	0.53 Rf	22.6 AU	1153.4 AU	6.46 %	unknown *
7	0.63 Rf	21.1 AU	0.66 Rf	25.1 AU	3.21 %	0.71 Rf	16.2 AU	715.2 AU	4.00 %	unknown *
8	0.74 Rf	13.0 AU	0.80 Rf	24.1 AU	3.09 %	0.91 Rf	8.1 AU	1098.7 AU	6.15 %	unknown *
9	1.14 Rf	5.4 AU	1.20 Rf	67.6 AU	8.68 %	1.27 Rf	5.8 AU	1914.1 AU	10.72 %	unknown *
10	1.28 Rf	0.2 AU	1.36 Rf	154.9 AU	19.87 %	1.47 Rf	11.6 AU	4991.0 AU	27.94 %	unknown *
11	1.48 Rf	11.6 AU	1.51 Rf	17.7 AU	2.27 %	1.56 Rf	0.1 AU	378.2 AU	2.12 %	unknown *

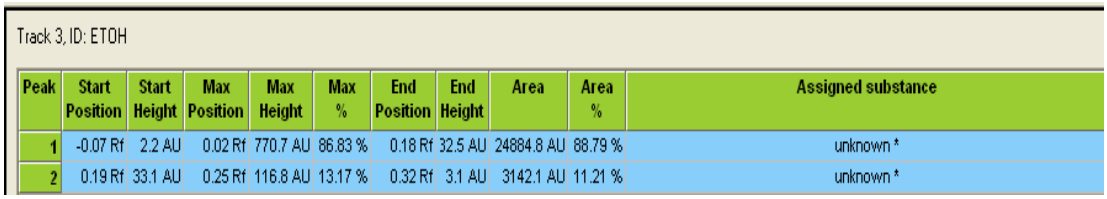
Fig 22. HPTLC profile of chloroform extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 254nm



Track 2, ID: CHLO

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	3.8 AU	0.01 Rf	706.1 AU	66.32 %	0.07 Rf	31.7 AU	8790.3 AU	52.77 %	unknown *
2	0.15 Rf	31.0 AU	0.16 Rf	33.1 AU	3.11 %	0.18 Rf	19.1 AU	428.5 AU	2.57 %	unknown *
3	0.21 Rf	20.1 AU	0.27 Rf	196.8 AU	18.48 %	0.34 Rf	9.7 AU	4294.6 AU	25.78 %	unknown *
4	0.35 Rf	10.2 AU	0.38 Rf	22.1 AU	2.08 %	0.42 Rf	13.3 AU	486.4 AU	2.92 %	unknown *
5	0.44 Rf	13.2 AU	0.47 Rf	20.6 AU	1.94 %	0.50 Rf	13.3 AU	473.9 AU	2.84 %	unknown *
6	0.93 Rf	3.9 AU	0.98 Rf	30.6 AU	2.87 %	1.04 Rf	1.4 AU	672.3 AU	4.04 %	unknown *
7	1.20 Rf	11.6 AU	1.28 Rf	41.1 AU	3.86 %	1.33 Rf	3.1 AU	1204.9 AU	7.23 %	unknown *
8	1.33 Rf	3.1 AU	1.37 Rf	14.4 AU	1.35 %	1.40 Rf	5.5 AU	306.9 AU	1.84 %	unknown *

Fig 23. HPTLC profile of ethanolic extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 254nm



Track 3, ID: ETOH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	2.2 AU	0.02 Rf	770.7 AU	86.83 %	0.18 Rf	32.5 AU	24884.8 AU	88.79 %	unknown *
2	0.19 Rf	33.1 AU	0.25 Rf	116.8 AU	13.17 %	0.32 Rf	3.1 AU	3142.1 AU	11.21 %	unknown *

Fig 24: HPTLC three dimensional densitogram of all the three solvent extract of *Terminalia bellirica* scanned at 254nm

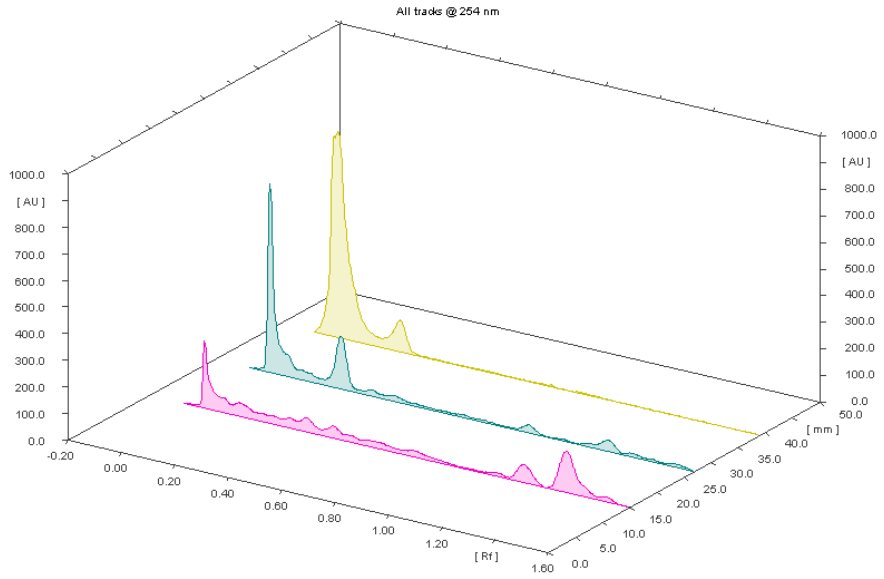


Fig 25:HPTLC image after derivitization observed at 254nm

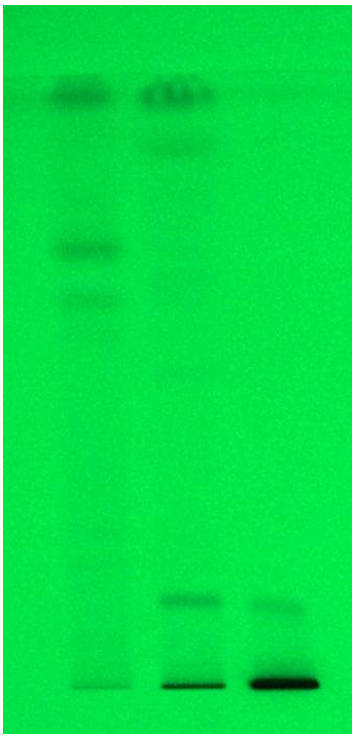


Fig 26. HPTLC profile of petroleum ether extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 366nm

Track 1, ID: PET

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	0.2 AU	0.00 Rf	136.4 AU	20.09 %	0.05 Rf	48.5 AU	1921.5 AU	11.14 %	unknown *
2	0.05 Rf	48.8 AU	0.07 Rf	58.9 AU	8.67 %	0.10 Rf	27.5 AU	1133.8 AU	6.57 %	unknown *
3	0.11 Rf	27.7 AU	0.14 Rf	51.7 AU	7.61 %	0.21 Rf	14.1 AU	1502.2 AU	8.71 %	unknown *
4	0.38 Rf	13.4 AU	0.42 Rf	24.5 AU	3.61 %	0.47 Rf	9.7 AU	624.9 AU	3.62 %	unknown *
5	0.65 Rf	11.5 AU	0.70 Rf	29.6 AU	4.36 %	0.74 Rf	15.3 AU	846.3 AU	4.90 %	unknown *
6	0.81 Rf	14.9 AU	0.82 Rf	17.7 AU	2.61 %	0.88 Rf	6.3 AU	377.5 AU	2.19 %	unknown *
7	1.05 Rf	3.7 AU	1.11 Rf	59.9 AU	8.83 %	1.15 Rf	15.0 AU	1508.1 AU	8.74 %	unknown *
8	1.15 Rf	15.6 AU	1.22 Rf	192.7 AU	28.40 %	1.32 Rf	3.5 AU	6657.8 AU	38.58 %	unknown *
9	1.33 Rf	0.2 AU	1.39 Rf	10.3 AU	1.52 %	1.43 Rf	0.8 AU	238.5 AU	1.38 %	unknown *
10	1.45 Rf	0.3 AU	1.52 Rf	97.0 AU	14.29 %	1.59 Rf	2.1 AU	2445.7 AU	14.17 %	unknown *

Fig 27. HPTLC profile of chloroform extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 366nm

Track 2, ID: CHLO

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	2.7 AU	0.01 Rf	668.1 AU	62.01 %	0.14 Rf	23.7 AU	9933.9 AU	50.18 %	unknown *
2	0.14 Rf	24.1 AU	0.16 Rf	30.9 AU	2.87 %	0.19 Rf	19.8 AU	548.6 AU	2.77 %	unknown *
3	0.20 Rf	17.2 AU	0.27 Rf	177.0 AU	16.43 %	0.33 Rf	9.4 AU	4006.8 AU	20.24 %	unknown *
4	0.93 Rf	5.7 AU	0.98 Rf	32.6 AU	3.02 %	1.03 Rf	1.6 AU	731.7 AU	3.70 %	unknown *
5	1.05 Rf	1.8 AU	1.11 Rf	24.8 AU	2.30 %	1.15 Rf	3.1 AU	550.4 AU	2.78 %	unknown *
6	1.16 Rf	3.8 AU	1.22 Rf	60.3 AU	5.60 %	1.30 Rf	4.0 AU	1831.5 AU	9.25 %	unknown *
7	1.33 Rf	0.4 AU	1.39 Rf	19.7 AU	1.83 %	1.42 Rf	10.2 AU	487.1 AU	2.46 %	unknown *
8	1.45 Rf	8.7 AU	1.52 Rf	63.9 AU	5.93 %	1.59 Rf	3.6 AU	1707.1 AU	8.62 %	unknown *

Fig28 . HPTLC profile of ethanolic extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 366nm

Track 3, ID: ETOH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	2.4 AU	0.01 Rf	834.3 AU	89.30 %	0.14 Rf	28.3 AU	18633.5 AU	85.90 %	unknown *
2	0.16 Rf	29.0 AU	0.25 Rf	100.0 AU	10.70 %	0.32 Rf	2.2 AU	3057.7 AU	14.10 %	unknown *

Fig 29: HPTLC three dimensional densitogram of different solvent extracts of *Terminalia bellirica* scanned at 366nm

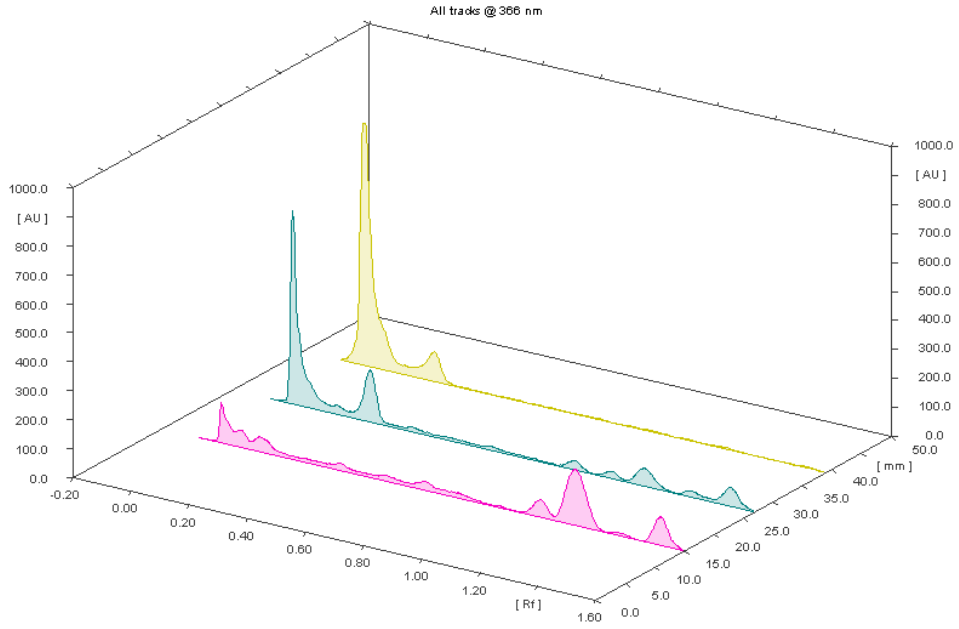


Fig30 : HPTLC image after derivitization observed at 366nm

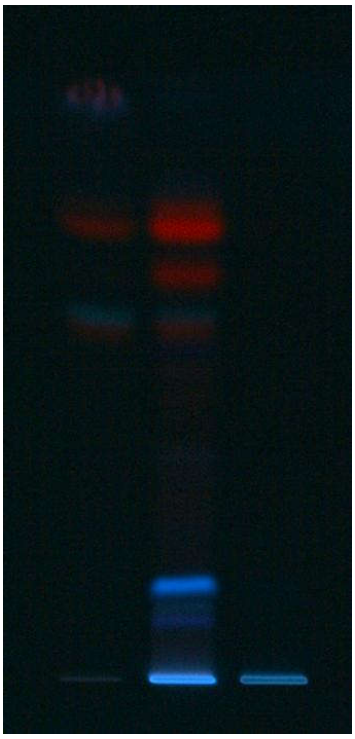
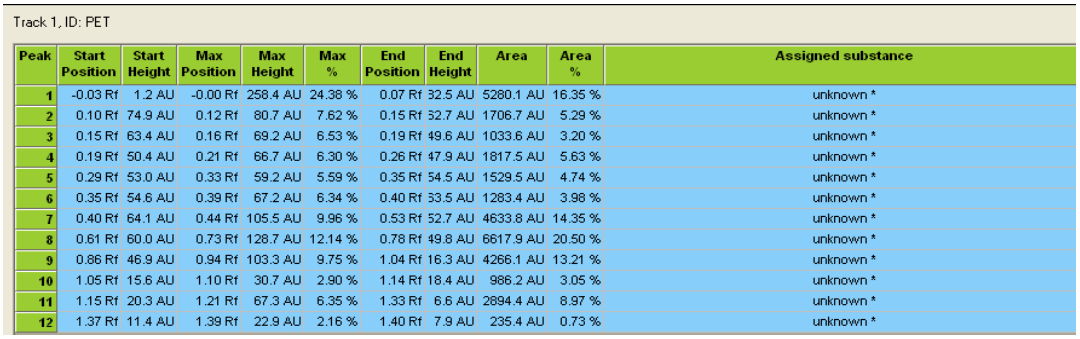


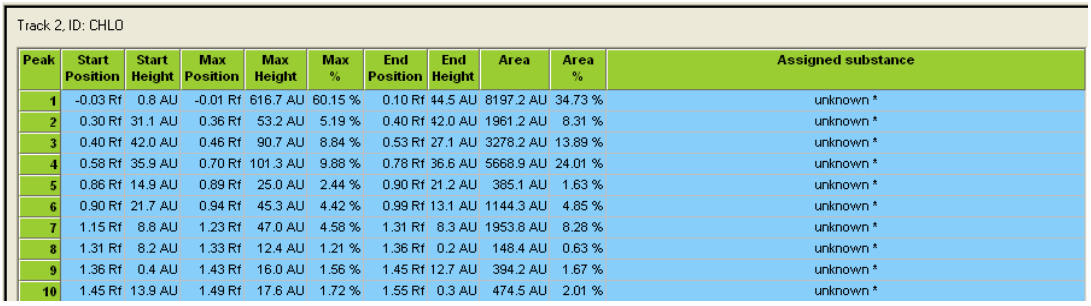
Fig 31. HPTLC profile of petroleum ether extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 550nm



Track 1, ID: PET

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	1.2 AU	-0.00 Rf	258.4 AU	24.38 %	0.07 Rf	32.5 AU	5280.1 AU	16.35 %	unknown *
2	0.10 Rf	74.9 AU	0.12 Rf	80.7 AU	7.62 %	0.15 Rf	52.7 AU	1706.7 AU	5.29 %	unknown *
3	0.15 Rf	63.4 AU	0.16 Rf	69.2 AU	6.53 %	0.19 Rf	49.6 AU	1033.6 AU	3.20 %	unknown *
4	0.19 Rf	50.4 AU	0.21 Rf	66.7 AU	6.30 %	0.26 Rf	47.9 AU	1817.5 AU	5.63 %	unknown *
5	0.29 Rf	53.0 AU	0.33 Rf	59.2 AU	5.59 %	0.35 Rf	54.5 AU	1529.5 AU	4.74 %	unknown *
6	0.35 Rf	54.6 AU	0.39 Rf	67.2 AU	6.34 %	0.40 Rf	33.5 AU	1283.4 AU	3.98 %	unknown *
7	0.40 Rf	64.1 AU	0.44 Rf	105.5 AU	9.96 %	0.53 Rf	52.7 AU	4633.8 AU	14.35 %	unknown *
8	0.61 Rf	60.0 AU	0.73 Rf	128.7 AU	12.14 %	0.78 Rf	49.8 AU	6617.9 AU	20.50 %	unknown *
9	0.86 Rf	46.9 AU	0.94 Rf	103.3 AU	9.75 %	1.04 Rf	16.3 AU	4266.1 AU	13.21 %	unknown *
10	1.05 Rf	15.6 AU	1.10 Rf	30.7 AU	2.90 %	1.14 Rf	18.4 AU	986.2 AU	3.05 %	unknown *
11	1.15 Rf	20.3 AU	1.21 Rf	67.3 AU	6.35 %	1.33 Rf	6.6 AU	2894.4 AU	8.97 %	unknown *
12	1.37 Rf	11.4 AU	1.39 Rf	22.9 AU	2.16 %	1.40 Rf	7.9 AU	235.4 AU	0.73 %	unknown *

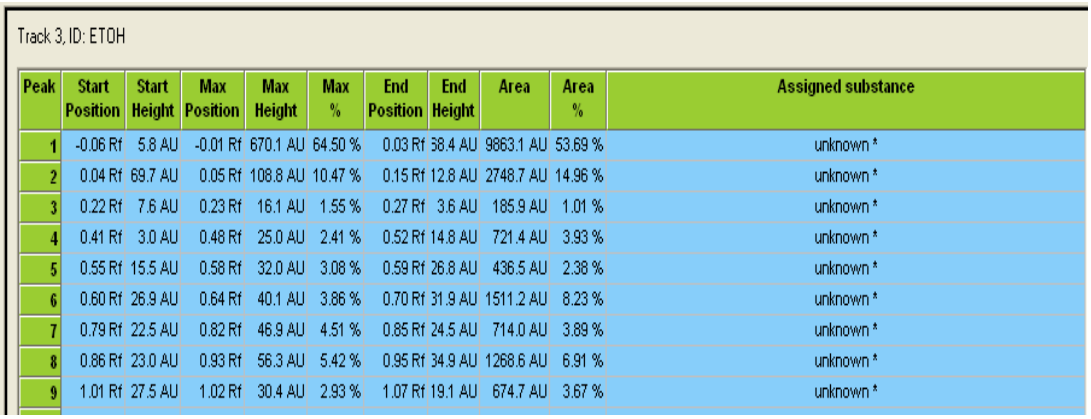
Fig 32. HPTLC profile of Chloroform extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 550nm



Track 2, ID: CHLO

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	0.8 AU	-0.01 Rf	616.7 AU	60.15 %	0.10 Rf	44.5 AU	8197.2 AU	34.73 %	unknown *
2	0.30 Rf	31.1 AU	0.36 Rf	53.2 AU	5.19 %	0.40 Rf	42.0 AU	1961.2 AU	8.31 %	unknown *
3	0.40 Rf	42.0 AU	0.46 Rf	90.7 AU	8.84 %	0.53 Rf	27.1 AU	3278.2 AU	13.89 %	unknown *
4	0.58 Rf	35.9 AU	0.70 Rf	101.3 AU	9.88 %	0.78 Rf	36.6 AU	5668.9 AU	24.01 %	unknown *
5	0.86 Rf	14.9 AU	0.89 Rf	25.0 AU	2.44 %	0.90 Rf	21.2 AU	385.1 AU	1.63 %	unknown *
6	0.90 Rf	21.7 AU	0.94 Rf	45.3 AU	4.42 %	0.99 Rf	13.1 AU	1144.3 AU	4.85 %	unknown *
7	1.15 Rf	8.8 AU	1.23 Rf	47.0 AU	4.58 %	1.31 Rf	8.3 AU	1953.8 AU	8.28 %	unknown *
8	1.31 Rf	8.2 AU	1.33 Rf	12.4 AU	1.21 %	1.36 Rf	0.2 AU	148.4 AU	0.63 %	unknown *
9	1.36 Rf	0.4 AU	1.43 Rf	16.0 AU	1.56 %	1.45 Rf	12.7 AU	394.2 AU	1.67 %	unknown *
10	1.45 Rf	13.9 AU	1.49 Rf	17.6 AU	1.72 %	1.55 Rf	0.3 AU	474.5 AU	2.01 %	unknown *

Fig 33. HPTLC profile of ethanolic extract of *Terminalia bellirica* and Rf values of compounds obtained when scanned at 550nm



Track 3, ID: ETOH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	5.8 AU	-0.01 Rf	670.1 AU	64.50 %	0.03 Rf	38.4 AU	9863.1 AU	53.69 %	unknown *
2	0.04 Rf	69.7 AU	0.05 Rf	108.8 AU	10.47 %	0.15 Rf	12.8 AU	2748.7 AU	14.96 %	unknown *
3	0.22 Rf	7.6 AU	0.23 Rf	16.1 AU	1.55 %	0.27 Rf	3.6 AU	185.9 AU	1.01 %	unknown *
4	0.41 Rf	3.0 AU	0.48 Rf	25.0 AU	2.41 %	0.52 Rf	14.8 AU	721.4 AU	3.93 %	unknown *
5	0.55 Rf	15.5 AU	0.58 Rf	32.0 AU	3.08 %	0.59 Rf	26.8 AU	436.5 AU	2.38 %	unknown *
6	0.60 Rf	26.9 AU	0.64 Rf	40.1 AU	3.86 %	0.70 Rf	31.9 AU	1511.2 AU	8.23 %	unknown *
7	0.79 Rf	22.5 AU	0.82 Rf	46.9 AU	4.51 %	0.85 Rf	24.5 AU	714.0 AU	3.89 %	unknown *
8	0.86 Rf	23.0 AU	0.93 Rf	56.3 AU	5.42 %	0.95 Rf	34.9 AU	1268.6 AU	6.91 %	unknown *
9	1.01 Rf	27.5 AU	1.02 Rf	30.4 AU	2.93 %	1.07 Rf	19.1 AU	674.7 AU	3.67 %	unknown *

Fig34 :HPTLC three dimensional densitogram of all the three solvent extract of *Terminalia bellirica* scanned at 550nm

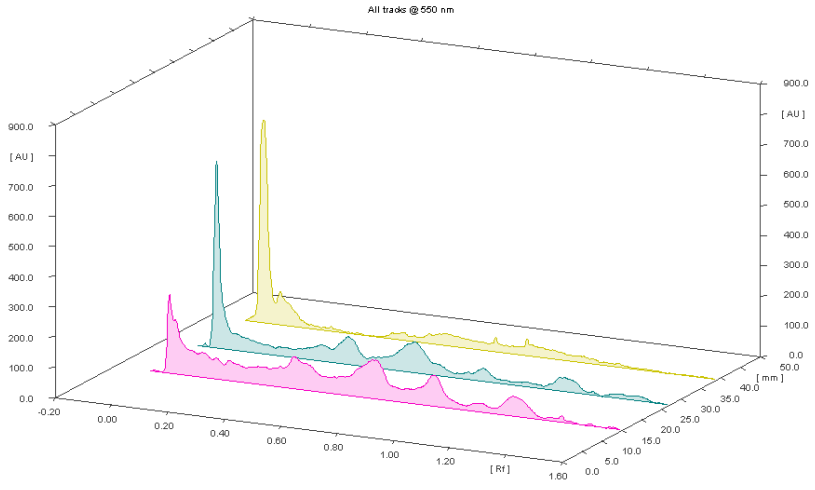


Fig 35 :HPTLC image after derivitization observed at 550nm

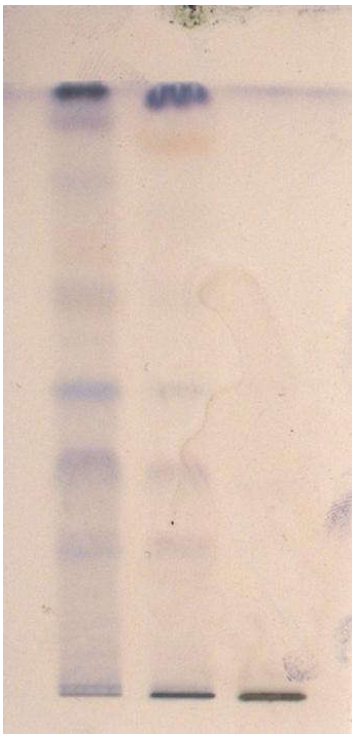


Table 79:- Rf values and tentatively identified compounds obtained from petroleum ether, chloroform and ethanolic extracts of *Terminalia bellirica*.

Terminalia bellirica								
254nm								
Rf value	**Petroleum ether extract**	**Reference**	**Rf value**	**Chloroform extract**	**Reference**	**Rf value**	**Ethanolic extract**	**Reference**
0.06	Steroids	(Gomathi et al., 2012b)	0.07	Flavonoid	(Gomathi et al., 2012a)	0.18	Flavonoid	(Asante IK et al., 2016)
0.11	Alkaloid	(Kalaiselvi et al., 2012)	0.18	Flavonoid	(Asante IK et al., 2016)	0.32	Flavonoid	(Asante IK et al., 2016)
0.19	Tannin	(Banu and Nagarajan, 2014)	0.34	Steroids	(Gomathi et al., 2012b)			
0.35	Terpenoid	(Yamunadevi, et al., 2011)	0.42	Flavanoid	(Gomathi et al., 2012a)			
0.44	Terpene	(Asante IK et al., 2016)	0.50	Alkaloid	(Biradar, 2013)			
0.53	Phenolics	(Kalaiselvi et al., 2012)	1.04	unknown				
0.71	Terpenoid	(Yamunadevi, et al., 2011)	1.33	unknown				
0.91	Flavonones Naringenin	(Ferry and Larson, 1991)	1.40	unknown				
1.27	unknown							
1.47	unknown							
1.56	unknown							
366nm								

Rf value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
0.05	Alkaloid	(Kalaiselvi et al., 2012)	0.14	Alkaloid	(Banu and Nagarajan, 2014)	0.14	Alkaloid	(Banu and Nagarajan, 2014)
0.10	Saponin	(Biradar, 2013)	0.19	Tannin	(Banu and Nagarajan, 2014)	0.32	Flavonoid	(Asante IK et al., 2016)
0.21	Alkaloid	(Banu and Nagarajan, 2014)	0.33	Flavonoid	(Biradar, 2013)			
0.47	Steroids	(Gomathi et al., 2012b)	1.03	unknown				
0.74	Gallic acid	(Savitha and Arivukkarasu, 2014)	1.15	unknown				
0.88	Terpenoid	(Gomathi et al., 2012b)	1.30	unknown				
1.15	unknown		1.42	unknown				
1.32	unknown		1.59	unknown				
1.43	unknown							
1.59	unknown							
550nm								
Rf value	**Petroleum ether extract**	**Reference**	**Rf value**	**Chloroform extract**	**Reference**	**Rf value**	**Ethanolic extract**	**Reference**
0.07	Flavonoid	(Karthika et al., 2014)	0.10	Saponin	(Biradar, 2013)	0.03	Alkaloid	(Banu and Nagarajan, 2014)
0.15	Gallic acid)	(Ferry and Larson, 1991	0.40	Steroids	(Gomathi et al., 2012b)	0.15	Gallic acid	(Ferry and Larson, 1991)

0.19	Tannin	(Banu and Nagarajan, 2014)	0.53	Phenolics	(Kalaiselvi et al., 2012)	0.27	Phenol	(Banu and Nagarajan, 2014)
0.26	Phenolics	(Kalaiselvi et al., 2012)	0.78	Tannic acid	(Savitha and Arivukkarasu, 2014)	0.52	Polyphenol	(Sasikumar et al., 2009)
0.35	Alkaloid	(Gomathi et al., 2012a)	0.90	Terpenoid	(Gomathi et al., 2012b)	0.59	Alkaloid	(Kalaiselvi et al., 2012)
0.40	Steroids	(Gomathi et al., 2012b)	0.99	Steroids	(Gomathi et al., 2012b)	0.70	Lupeol (Triterpenoid)	(Leela and Saraswathy, 2013)
0.53	Phenolics	(Kalaiselvi et al., 2012)	1.31	unknown		0.85	Flavanoid	(Jyothi, 2016)
0.78	Tannic acid	(Savitha and Arivukkarasu, 2014)	1.36	unknown		0.95	Flavanoid	(Jyothi, 2016)
1.04	unknown					1.07	unknown	
1.14	unknown							
1.33	unknown							
1.40	unknown							

All the three extracts (petroleum ether, chloroform and ethanol) of *Terminalia bellirica* where scanned at 254nm, 366nm and 550nm. Details of major compounds obtained from all these extracts are given in the table 80.

Table 80:- Major components obtained from *Terminalia bellirica* from HPTLC analysis

Terminalia bellirica				
	Scanned Absorbances	Petroleum ether extract	Chloroform extract	Ethanolic extract
	254nm	steroids (18.57%) alkaloid (8.12%	Flavonoid (52.77%) Steroid (25.78%)	Flavanoid (88.79%)
	366nm	Alkaloid (11.14%)	Alkaloid (50.18%) Flavonoid (20.74%)	Phenolic compounds (85.90%) Flavonoid (14.10%)
	550nm	Tannic acid (24.01%) Phenolics (14.35%)	Terpenoid (34.73%) Tannic acid (24.01%)	Alkaloid (53.89%) Gallic acid (14.96%)

When three extracts of *Hemidesmus indicus* was analyzed for phyto constituents, each extract gave several spots on chromatogram. Petroleum ether extracts gave 5 spots, chloroform extracts gave 11spots and ethanol extract gave 5 spots when examined under 254nm (Fig 36-38). When scanned at 366nm, petroleum ether extract gave 6spots, chloroform extract gave 9 spots and ethanolic extract gave 1 spot (Fig 41-43). Petroleum ether gave 11 spots, chloroform gave 13 spots and ethanol gave 10 spots when scanned at 550nm (Fig46-48). Three dimensional densitogram depicting all the three extracts are given in the figure 39, 44 and 49. Chromatogram obtained when scanned at 254nm, 366nm and 550nm are given in figures 40, 45 and 50.

Tentative compounds were identified with the help of Rf value obtained and compounds obtained are listed in table 81.

Fig 36: HPTLC profile of petroleum ether extract of *Hemidesmus indicus*. Details of Rf values of compounds and area percentage obtained when scanned at 254nm

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.05 Rf	8.8 AU	-0.04 Rf	21.8 AU	9.84 %	-0.02 Rf	0.0 AU	245.7 AU	10.11 %	unknown *
2	-0.01 Rf	0.5 AU	0.00 Rf	159.0 AU	71.84 %	0.02 Rf	9.2 AU	1244.8 AU	51.22 %	unknown *
3	0.40 Rf	4.7 AU	0.44 Rf	17.5 AU	7.93 %	0.47 Rf	4.9 AU	477.9 AU	19.67 %	unknown *
4	0.68 Rf	2.1 AU	0.70 Rf	11.6 AU	5.26 %	0.72 Rf	1.6 AU	212.6 AU	8.75 %	unknown *
5	0.78 Rf	8.3 AU	0.80 Rf	11.4 AU	5.14 %	0.83 Rf	0.6 AU	249.3 AU	10.26 %	unknown *

Fig 37: HPTLC profile of chloroform extract of *Hemidesmus indicus*. Details of Rf values of compounds and area percentage obtained when scanned at 254nm

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	3.1 AU	-0.04 Rf	21.0 AU	1.58 %	-0.03 Rf	8.5 AU	198.3 AU	0.78 %	unknown *
2	-0.03 Rf	8.6 AU	-0.00 Rf	631.1 AU	47.44 %	0.02 Rf	59.7 AU	9945.7 AU	39.10 %	unknown *
3	0.02 Rf	261.7 AU	0.03 Rf	268.9 AU	20.21 %	0.06 Rf	77.3 AU	4885.8 AU	19.21 %	unknown *
4	0.06 Rf	77.9 AU	0.08 Rf	111.6 AU	8.39 %	0.10 Rf	23.0 AU	2343.3 AU	9.21 %	unknown *
5	0.10 Rf	23.4 AU	0.11 Rf	27.6 AU	2.08 %	0.13 Rf	7.0 AU	346.7 AU	1.36 %	unknown *
6	0.13 Rf	7.1 AU	0.17 Rf	47.9 AU	3.60 %	0.23 Rf	10.2 AU	1464.5 AU	5.76 %	unknown *
7	0.28 Rf	1.5 AU	0.31 Rf	11.1 AU	0.84 %	0.34 Rf	0.0 AU	223.7 AU	0.88 %	unknown *
8	0.36 Rf	1.0 AU	0.44 Rf	153.1 AU	11.51 %	0.48 Rf	15.9 AU	4538.3 AU	17.84 %	unknown *
9	0.48 Rf	15.2 AU	0.51 Rf	24.5 AU	1.84 %	0.55 Rf	1.9 AU	663.5 AU	2.61 %	unknown *
10	0.75 Rf	4.9 AU	0.80 Rf	17.1 AU	1.28 %	0.84 Rf	0.2 AU	545.8 AU	2.15 %	unknown *
11	0.87 Rf	2.6 AU	0.90 Rf	16.4 AU	1.23 %	0.92 Rf	1.2 AU	280.6 AU	1.10 %	unknown *

Fig 38: HPTLC profile of ethanolic extract of *Hemidesmus indicus*, corresponding Rf values of compounds and area percentage obtained when scanned at 254nm

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	2.9 AU	-0.04 Rf	21.0 AU	3.34 %	-0.03 Rf	5.2 AU	225.7 AU	2.49 %	unknown *
2	-0.02 Rf	5.7 AU	-0.00 Rf	550.4 AU	87.82 %	0.03 Rf	16.5 AU	7683.4 AU	84.62 %	unknown *
3	0.04 Rf	16.9 AU	0.04 Rf	20.5 AU	3.27 %	0.09 Rf	2.1 AU	479.7 AU	5.28 %	unknown *
4	0.28 Rf	19.6 AU	0.29 Rf	20.0 AU	3.20 %	0.33 Rf	3.0 AU	394.8 AU	4.35 %	unknown *
5	0.94 Rf	4.4 AU	0.96 Rf	14.9 AU	2.37 %	0.99 Rf	3.5 AU	295.8 AU	3.26 %	unknown *

Fig 39: HPTLC three dimensional densitogram of different solvent extract of *Hemidesmus indicus* at 254nm

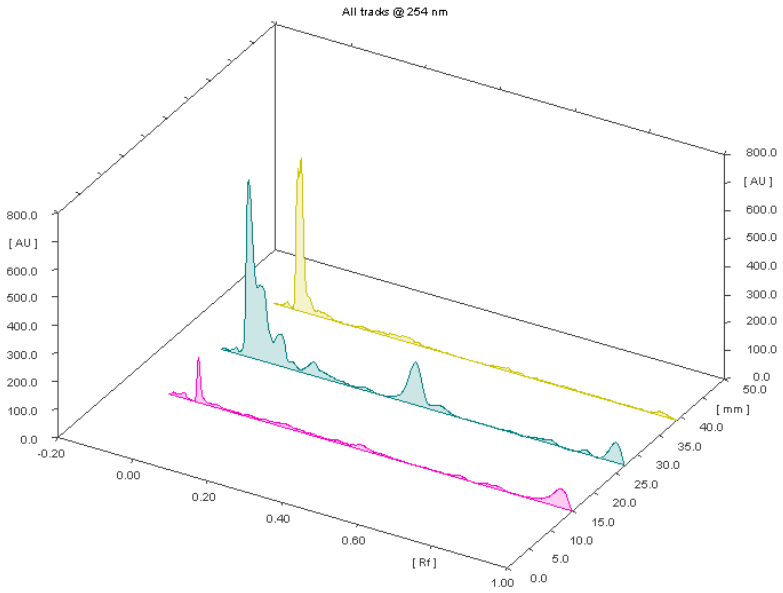


Fig 40: HPTLC image of *Hemidesmus indicus* after derivitization observed at 254nm

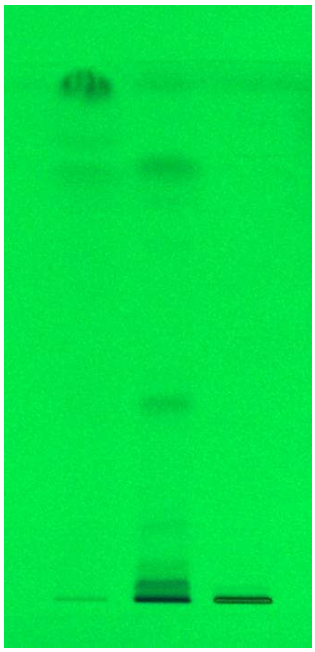


Fig41 . HPTLC profile of petroleum ether extract of *Hemidesmus indicus*, Rf values of compounds and area percentage obtained when scanned at 366nm

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	0.3 AU	-0.04 Rf	11.5 AU	8.27 %	-0.02 Rf	0.7 AU	114.0 AU	4.86 %	unknown *
2	-0.01 Rf	0.1 AU	0.00 Rf	60.2 AU	43.37 %	0.03 Rf	4.1 AU	490.1 AU	20.91 %	unknown *
3	0.20 Rf	2.6 AU	0.23 Rf	11.9 AU	8.59 %	0.26 Rf	1.7 AU	248.0 AU	10.58 %	unknown *
4	0.66 Rf	0.7 AU	0.70 Rf	10.4 AU	7.52 %	0.75 Rf	0.4 AU	336.5 AU	14.36 %	unknown *
5	0.87 Rf	1.9 AU	0.91 Rf	22.1 AU	15.95 %	0.93 Rf	13.5 AU	523.7 AU	22.35 %	unknown *
6	0.94 Rf	14.4 AU	0.96 Rf	22.6 AU	16.30 %	1.00 Rf	3.8 AU	631.4 AU	26.94 %	unknown *

Fig 42. HPTLC profile of chloroform extract of *Hemidesmus indicus*, Rf values of compounds and area percentage obtained when scanned at 366nm

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	1.7 AU	-0.00 Rf	595.2 AU	64.92 %	0.08 Rf	7.7 AU	11530.3 AU	60.48 %	unknown *
2	0.16 Rf	6.7 AU	0.19 Rf	28.1 AU	3.06 %	0.21 Rf	5.5 AU	568.2 AU	2.98 %	unknown *
3	0.27 Rf	0.7 AU	0.31 Rf	15.8 AU	1.73 %	0.35 Rf	0.3 AU	395.7 AU	2.08 %	unknown *
4	0.35 Rf	1.6 AU	0.41 Rf	48.8 AU	5.32 %	0.43 Rf	46.8 AU	1380.5 AU	7.24 %	unknown *
5	0.43 Rf	48.9 AU	0.44 Rf	52.6 AU	5.74 %	0.48 Rf	7.2 AU	1051.2 AU	5.51 %	unknown *
6	0.49 Rf	7.9 AU	0.50 Rf	12.1 AU	1.32 %	0.54 Rf	3.4 AU	271.3 AU	1.42 %	unknown *
7	0.67 Rf	0.9 AU	0.70 Rf	13.0 AU	1.41 %	0.73 Rf	1.7 AU	291.4 AU	1.53 %	unknown *
8	0.76 Rf	0.9 AU	0.80 Rf	41.5 AU	4.53 %	0.85 Rf	0.3 AU	1036.3 AU	5.44 %	unknown *
9	0.86 Rf	0.3 AU	0.90 Rf	109.7 AU	11.96 %	0.93 Rf	20.0 AU	2541.1 AU	13.33 %	unknown *

Fig 43. HPTLC profile of ethanolic extract of *Hemidesmus indicus*, Rf values of corresponding compounds and area percentage obtained when scanned at 366nm

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	4.3 AU	-0.00 Rf	483.2 AU	100.00 %	0.03 Rf	5.9 AU	6036.7 AU	100.00 %	unknown *

Fig 44: HPTLC three dimensional densitogram of different solvent extract of *Hemidesmus indicus* scanned at 366nm

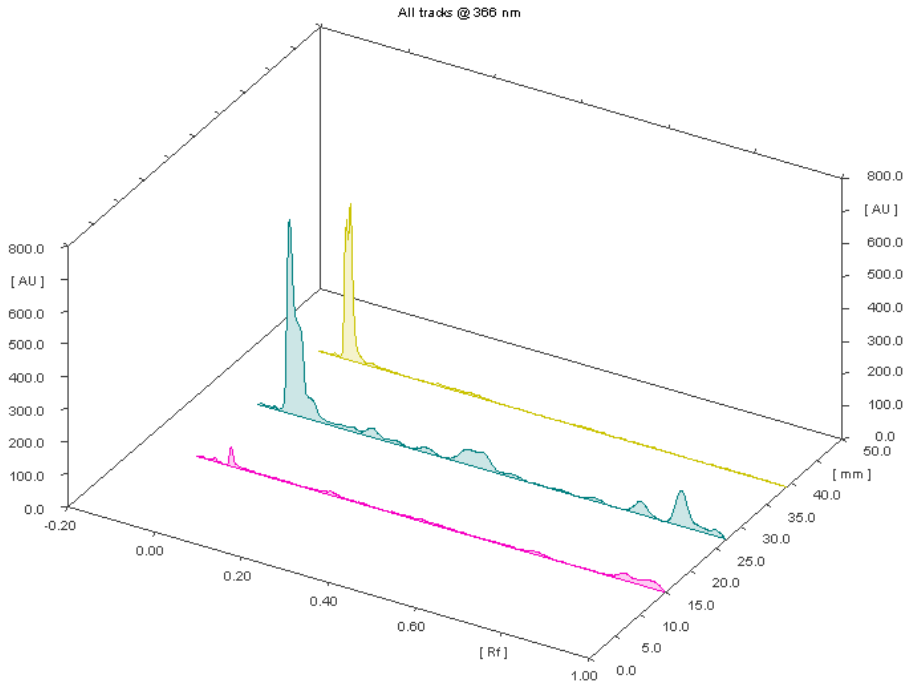


Fig 45:HPTLC image obtained after derivitization observed at 366nm

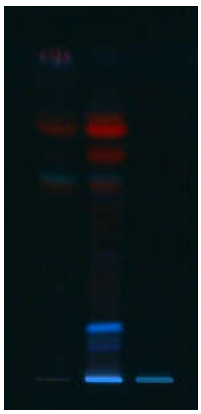


Fig 46. HPTLC profile of petroleum ether extract of *Hemidesmus indicus*, Rf values of compounds and area percentage occupied by the corresponding compound when scanned at 550nm

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	0.0 AU	-0.03 Rf	19.0 AU	1.23 %	-0.01 Rf	0.3 AU	158.8 AU	0.39 %	unknown *
2	-0.00 Rf	0.6 AU	0.01 Rf	518.7 AU	33.42 %	0.05 Rf	71.0 AU	6439.0 AU	15.74 %	unknown *
3	0.08 Rf	67.7 AU	0.09 Rf	70.1 AU	4.51 %	0.13 Rf	54.9 AU	2181.9 AU	5.33 %	unknown *
4	0.28 Rf	48.1 AU	0.34 Rf	126.2 AU	8.13 %	0.38 Rf	39.1 AU	5560.7 AU	13.60 %	unknown *
5	0.39 Rf	38.6 AU	0.40 Rf	40.8 AU	2.63 %	0.42 Rf	26.5 AU	525.6 AU	1.28 %	unknown *
6	0.42 Rf	26.8 AU	0.42 Rf	28.6 AU	1.84 %	0.45 Rf	18.4 AU	544.3 AU	1.33 %	unknown *
7	0.52 Rf	24.8 AU	0.57 Rf	155.2 AU	10.00 %	0.62 Rf	21.4 AU	4678.1 AU	11.44 %	unknown *
8	0.64 Rf	20.9 AU	0.70 Rf	95.3 AU	6.14 %	0.72 Rf	34.7 AU	3257.9 AU	7.97 %	unknown *
9	0.72 Rf	84.7 AU	0.77 Rf	459.9 AU	29.63 %	0.83 Rf	2.0 AU	16657.4 AU	40.73 %	unknown *
10	0.86 Rf	3.5 AU	0.90 Rf	22.4 AU	1.44 %	0.94 Rf	0.3 AU	613.4 AU	1.50 %	unknown *
11	0.96 Rf	4.1 AU	0.98 Rf	16.2 AU	1.05 %	0.99 Rf	4.7 AU	284.7 AU	0.70 %	unknown *

Fig 47. HPTLC profile of Chloroform extract of *Hemidesmus indicus*, Rf values of compounds and area percentage occupied by the corresponding compound obtained when scanned at 550nm

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.6 AU	-0.02 Rf	56.5 AU	4.09 %	-0.01 Rf	40.9 AU	1170.3 AU	3.18 %	unknown *
2	-0.01 Rf	42.6 AU	0.01 Rf	745.6 AU	53.94 %	0.08 Rf	42.1 AU	21792.5 AU	59.26 %	unknown *
3	0.08 Rf	142.8 AU	0.10 Rf	223.9 AU	16.20 %	0.16 Rf	20.3 AU	5500.3 AU	14.96 %	unknown *
4	0.16 Rf	20.7 AU	0.18 Rf	56.5 AU	4.08 %	0.22 Rf	9.1 AU	1285.2 AU	3.49 %	unknown *
5	0.25 Rf	8.8 AU	0.26 Rf	13.1 AU	0.95 %	0.29 Rf	1.2 AU	285.0 AU	0.77 %	unknown *
6	0.29 Rf	1.3 AU	0.36 Rf	78.3 AU	5.66 %	0.39 Rf	7.8 AU	2277.5 AU	6.19 %	unknown *
7	0.49 Rf	3.3 AU	0.50 Rf	13.8 AU	1.00 %	0.51 Rf	4.7 AU	157.6 AU	0.43 %	unknown *
8	0.52 Rf	4.8 AU	0.57 Rf	76.2 AU	5.52 %	0.60 Rf	19.9 AU	2097.8 AU	5.70 %	unknown *
9	0.61 Rf	22.3 AU	0.61 Rf	41.4 AU	3.00 %	0.64 Rf	3.1 AU	356.7 AU	0.97 %	unknown *
10	0.66 Rf	6.0 AU	0.70 Rf	15.6 AU	1.13 %	0.73 Rf	7.2 AU	460.8 AU	1.25 %	unknown *
11	0.74 Rf	4.6 AU	0.77 Rf	20.0 AU	1.45 %	0.80 Rf	6.1 AU	537.1 AU	1.46 %	unknown *
12	0.80 Rf	6.5 AU	0.83 Rf	20.0 AU	1.44 %	0.86 Rf	3.4 AU	403.5 AU	1.10 %	unknown *
13	0.89 Rf	5.1 AU	0.91 Rf	21.3 AU	1.54 %	0.94 Rf	2.3 AU	450.8 AU	1.23 %	unknown *

Fig 48. HPTLC profile of ethanolic extract of *Hemidesmus indicus*, Rf values of compounds and area percentage occupied by corresponding compounds obtained when scanned at 550nm

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	0.2 AU	-0.02 Rf	44.5 AU	5.05 %	-0.01 Rf	36.5 AU	811.8 AU	4.39 %	unknown *
2	-0.01 Rf	37.1 AU	0.03 Rf	671.9 AU	76.18 %	0.08 Rf	13.4 AU	14924.6 AU	80.75 %	unknown *
3	0.31 Rf	8.5 AU	0.32 Rf	14.5 AU	1.65 %	0.34 Rf	4.2 AU	178.9 AU	0.97 %	unknown *
4	0.51 Rf	1.6 AU	0.54 Rf	11.5 AU	1.30 %	0.55 Rf	9.1 AU	185.3 AU	1.00 %	unknown *
5	0.55 Rf	9.6 AU	0.57 Rf	19.4 AU	2.20 %	0.58 Rf	16.0 AU	256.2 AU	1.39 %	unknown *
6	0.63 Rf	11.6 AU	0.64 Rf	25.0 AU	2.83 %	0.66 Rf	15.4 AU	389.7 AU	2.11 %	unknown *
7	0.66 Rf	16.2 AU	0.68 Rf	34.0 AU	3.85 %	0.71 Rf	21.2 AU	822.5 AU	4.45 %	unknown *
8	0.71 Rf	21.5 AU	0.72 Rf	28.7 AU	3.26 %	0.73 Rf	17.4 AU	385.9 AU	2.09 %	unknown *
9	0.73 Rf	17.4 AU	0.75 Rf	20.6 AU	2.33 %	0.76 Rf	4.8 AU	309.0 AU	1.67 %	unknown *
10	0.86 Rf	4.2 AU	0.88 Rf	11.8 AU	1.34 %	0.91 Rf	3.3 AU	218.1 AU	1.18 %	unknown *

Fig49 :HPTLC three dimensional densitogram of different solvents of *Hemidesmus indicus* scanned at 550nm

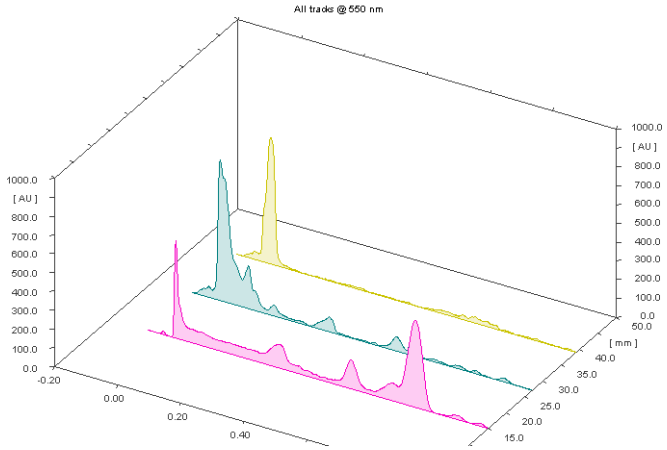


Fig 50: HPTLC image obtained after derivitization observed at 550nm

Table 81: HPTLC profile of all the three extracts of *Hemidesmus indicus* at 254nm, 366nm and 550nm and tentative compounds according to Rf value obtained

Hemidesmus indicus								
254nm								
Petroleum ether			Chloroform			Ethanol		
Rf Value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
-0.02	Flavonoid	(Karthika et al., 2014)	-0.03	Alkaloid	(Banu and Nagarajan, 2014)	-0.03	Alkaloid	(Banu and Nagarajan, 2014)
0.02	Flavonoid	(Karthika et al., 2014)	0.02	Flavonoid	(Karthika et al., 2014)	0.03	Alkaloid	(Banu and Nagarajan, 2014)
0.47	Steroids	(Gomathi et al., 2012b)	0.06	Terpenoid	(Yamunadevi, et al., 2011)	0.09	Gallic acid (Flavanoid)	(Asha et al., 2015)
0.72	Saponin	(Asante IK et al., 2016)	0.10	Terpenoid	Gomathi et al., 2012b)	0.33	Flavanoid	(Biradar, 2013)
0.83	Flavonoid	(Asha et al., 2015)	0.13	Alkaloid	(Asante IK et al., 2016)	0.99	Steroids	(Gomathi et al., 2012b)
			0.23	Alkaloid	(Kalaiselvi et al., 2012)			
			0.34	Steroids	(Gomathi et al.,			

					2012b)			
			0.48	Flavanoid	(Gomathi et al., 2012a)			
			0.55	Alkaloid	(Biradar, 2013)			
			0.84	Rutin (Flavanoid)	(Asha et al., 2015)			
			0.92	Phenol	(Ferry and Larson, 1991)			
366nm								
Rf Value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
-0.02	Flavonoid	(Karthika et al., 2014)	0.08	Saponin	(Biradar, 2013)	0.03	Alkaloid	(Banu and Nagarajan, 2014)
0.03	Alkaloid	(Banu and Nagarajan, 2014)	0.21	Alkaloid	(Banu and Nagarajan, 2014)			
0.26	Phenolics	(Kalaiselvi et al., 2012)	0.35	Alkaloid	(Gomathi et al., 2012a)			
0.75	Flavanoid	(Gomathi et al., 2012a)	0.43	Phenol	(Banu and Nagarajan, 2014)			
0.93	Terpenoid	(Gomathi et al., 2012b)	0.48	Flavanoid	(Biradar, 2013)			
1.00	Unknown		0.54	Alkaloid	(Biradar, 2013)			

			0.73	Phenolics	(Kalaiselvi et al., 2012)			
			0.85	Flavanoid	(Jyothi, 2016)			
			0.93	Terpenoid	(Gomathi et al., 2012b)			
550nm								
Rf Value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
-0.01	Phenol	(Banu and Nagarajan, 2014)	-0.01	Phenol	(Banu and Nagarajan, 2014)	-0.01	Phenol	(Banu and Nagarajan, 2014)
0.05	Alkaloid	(Kalaiselvi et al., 2012)	0.08	Saponin	(Biradar, 2013)	0.08	Saponin	(Biradar, 2013)
0.13	Alkaloid	(Asante IK et al., 2016)	0.16	Flavanoid	(Biradar, 2013)	0.34	Steroids	(Gomathi et al., 2012b)
0.38	Phenol	(Banu and Nagarajan, 2014)	0.22	Saponins	(Biradar, 2013)	0.55	Alkaloid	(Biradar, 2013)
0.42	Flavanoid	(Gomathi et al., 2012a)	0.29	Phenolics	(Kalaiselvi et al., 2012)	0.58	Polyphenol	(Sasikumar et al., 2009)
0.45	Flavanoid	(Jyothi, 2016)	0.39	Terpenoid	(Biradar, 2013)	0.66	Terpenoid	(Gomathi et al., 2012b)

0.62	Alkaloid	(Kalaiselvi et al., 2012)	0.51	Flavonoid	(Biradar, 2013)	0.71	Terpenoid	(Yamunadevi, et al., 2011)
0.72	Saponin	(Asante IK et al., 2016)	0.60	Flavanoid	(Jyothi, 2016)	0.73	Phenolics	(Kalaiselvi et al., 2012)
0.83	Flavanoid	(Jyothi, 2016)	0.64	Phenolics	(Kalaiselvi et al., 2012)	0.76	Terpenoid	(Gomathi et al., 2012b)
0.94	Terpenoid	(Gomathi et al., 2012b)	0.73	Phenolics	(Kalaiselvi et al., 2012)	0.91	Flavonones Naringenin	(Ferry and Larson, 1991)
0.99	Steroids	(Gomathi et al., 2012b)	0.80	Tannic acid	(Savitha and Arivukkarasu, 2014)			
			0.86	Terpenoid	(Gomathi et al., 2012b)			
			0.94	Steroids	(Gomathi et al., 2012b)			

Each of the three extracts of *Hemidesmus indicus* were scanned at 254nm, 336nm and 550nm. Major components obtained in HPTLC profiling are given in the table 82.

Table 82: Major components obtained from HPTLC analysis of *Hemidesmus indicus*.

Hemidesmus indicus				
	Scanned Absorbances	Petroleum ether extract	Chloroform extract	Ethanolic extract
	254nm	Flavonoid (51.22%) Steroids (19.67%)	flavonoid (39.10%)	Alkaloid (84.62%) Gallic acid (5.26%)
	366nm	terpenoid (22.35%) Alkaloid (20.91%)	Saponin (60.48%) terpenoid (13.33%)	alkaloid (100%)
	550nm	flavonoid (40.73%) alkaloid (15.74%)	saponin (59.26%) flavonoid (14.96%)	Saponin (80.75%) terpenoid (4.45%)

HPTLC analysis of all three extracts of *Vetiveria zizanioides* gave 16 spots in petroleum ether extracts, 10 spots in chloroform extracts and 7 spots in ethanol extracts, when scanned at 254nm (Figure 51-53). When scanned at 366nm and 550nm, petroleum ether extracts gave 7 and 3 spots (Figure 56-58); chloroform extracts gave 8 and 11 spots and alcohol extracts gave 4 and 7 spots respectively (Figure 61-63). Three dimensional graphs of all three extracts are depicted in fig 54, fig 59 and fig 64. Chromatogram obtained for each of the three extracts scanned under 254nm, 366nm and 550nm are given in fig 55, 56, fig 60 and fig 65. Based on the Rf value obtained, tentative compounds were identifies and are depicted in table 83.

Fig 51. HPTLC profile of Petroleum ether extract and Rf values of compounds obtained when scanned at 254nm

Track 1, ID: petroluem ether

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.1 AU	-0.05 Rf	13.4 AU	0.34 %	-0.03 Rf	0.7 AU	147.0 AU	0.11 %	unknown *
2	-0.02 Rf	0.1 AU	-0.00 Rf	353.0 AU	8.87 %	0.01 Rf	57.4 AU	4805.8 AU	3.67 %	unknown *
3	0.01 Rf	258.4 AU	0.03 Rf	298.4 AU	7.50 %	0.09 Rf	35.5 AU	12349.8 AU	9.42 %	unknown *
4	0.09 Rf	196.6 AU	0.13 Rf	411.4 AU	10.34 %	0.17 Rf	35.4 AU	14554.3 AU	11.11 %	unknown *
5	0.17 Rf	195.4 AU	0.18 Rf	210.0 AU	5.28 %	0.20 Rf	77.8 AU	3939.2 AU	3.01 %	unknown *
6	0.20 Rf	178.5 AU	0.22 Rf	230.3 AU	5.79 %	0.27 Rf	37.0 AU	9634.0 AU	7.35 %	unknown *
7	0.27 Rf	207.3 AU	0.31 Rf	261.0 AU	6.56 %	0.34 Rf	23.2 AU	10726.4 AU	8.19 %	unknown *
8	0.34 Rf	223.3 AU	0.36 Rf	298.4 AU	7.50 %	0.40 Rf	39.1 AU	10420.9 AU	7.95 %	unknown *
9	0.41 Rf	207.8 AU	0.44 Rf	223.9 AU	5.63 %	0.46 Rf	15.1 AU	6522.5 AU	4.98 %	unknown *
10	0.46 Rf	218.0 AU	0.49 Rf	233.4 AU	5.87 %	0.53 Rf	15.1 AU	8693.4 AU	6.63 %	unknown *
11	0.53 Rf	115.4 AU	0.55 Rf	129.4 AU	3.25 %	0.58 Rf	11.2 AU	3966.3 AU	3.03 %	unknown *
12	0.60 Rf	114.9 AU	0.67 Rf	364.3 AU	9.16 %	0.69 Rf	31.9 AU	13477.8 AU	10.29 %	unknown *
13	0.69 Rf	302.4 AU	0.70 Rf	316.2 AU	7.95 %	0.73 Rf	48.3 AU	7482.7 AU	5.71 %	unknown *
14	0.73 Rf	148.4 AU	0.79 Rf	467.7 AU	11.75 %	0.84 Rf	45.1 AU	19886.3 AU	15.18 %	unknown *
15	0.84 Rf	45.4 AU	0.88 Rf	147.2 AU	3.70 %	0.92 Rf	0.1 AU	4010.1 AU	3.06 %	unknown *
16	0.93 Rf	0.3 AU	0.95 Rf	21.3 AU	0.53 %	0.99 Rf	0.0 AU	417.2 AU	0.32 %	unknown *

Fig 52. HPTLC profile of Chloroform extract and Rf values of compounds obtained when scanned at 254nm

Track 2, ID: chloroform

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.1 AU	-0.00 Rf	764.4 AU	27.30 %	0.03 Rf	32.4 AU	23387.2 AU	23.01 %	unknown *
2	0.03 Rf	493.2 AU	0.04 Rf	502.7 AU	17.95 %	0.08 Rf	32.9 AU	13415.6 AU	13.20 %	unknown *
3	0.08 Rf	293.1 AU	0.12 Rf	413.0 AU	14.75 %	0.18 Rf	53.3 AU	17263.4 AU	16.99 %	unknown *
4	0.18 Rf	163.9 AU	0.21 Rf	185.2 AU	6.61 %	0.25 Rf	52.2 AU	8036.6 AU	7.91 %	unknown *
5	0.26 Rf	152.5 AU	0.28 Rf	169.6 AU	6.06 %	0.31 Rf	31.1 AU	5882.1 AU	5.79 %	unknown *
6	0.31 Rf	159.9 AU	0.34 Rf	174.5 AU	6.23 %	0.40 Rf	41.9 AU	9077.3 AU	8.93 %	unknown *
7	0.44 Rf	77.6 AU	0.53 Rf	143.8 AU	5.14 %	0.55 Rf	19.7 AU	8848.4 AU	8.71 %	unknown *
8	0.55 Rf	120.0 AU	0.58 Rf	160.1 AU	5.72 %	0.63 Rf	21.7 AU	4471.9 AU	4.40 %	unknown *
9	0.66 Rf	21.0 AU	0.74 Rf	251.9 AU	8.99 %	0.79 Rf	25.0 AU	9966.8 AU	9.81 %	unknown *
10	0.79 Rf	21.7 AU	0.84 Rf	34.8 AU	1.24 %	0.88 Rf	0.4 AU	1279.9 AU	1.26 %	unknown *

Fig 53. HPTLC profile of ethanolic extract and Rf values of compounds obtained when scanned at 254nm

Track 3, ID: ethanol

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	2.0 AU	-0.05 Rf	15.9 AU	1.78 %	-0.04 Rf	3.3 AU	171.6 AU	1.42 %	unknown *
2	-0.02 Rf	8.6 AU	-0.01 Rf	738.4 AU	82.78 %	0.05 Rf	22.8 AU	9530.6 AU	78.98 %	unknown *
3	0.08 Rf	22.4 AU	0.08 Rf	23.3 AU	2.61 %	0.11 Rf	8.5 AU	392.7 AU	3.25 %	unknown *
4	0.15 Rf	8.0 AU	0.17 Rf	23.3 AU	2.61 %	0.20 Rf	8.6 AU	521.5 AU	4.32 %	unknown *
5	0.35 Rf	14.7 AU	0.35 Rf	22.5 AU	2.52 %	0.38 Rf	10.7 AU	279.1 AU	2.31 %	unknown *
6	0.49 Rf	11.7 AU	0.51 Rf	31.1 AU	3.49 %	0.52 Rf	20.7 AU	467.7 AU	3.88 %	unknown *
7	0.54 Rf	22.5 AU	0.56 Rf	37.5 AU	4.20 %	0.58 Rf	11.6 AU	704.5 AU	5.84 %	unknown *

Fig 54: HPTLC three dimensional densitogram of all the three solvent extracts of *Vetiveria zizanioides* at 254nm

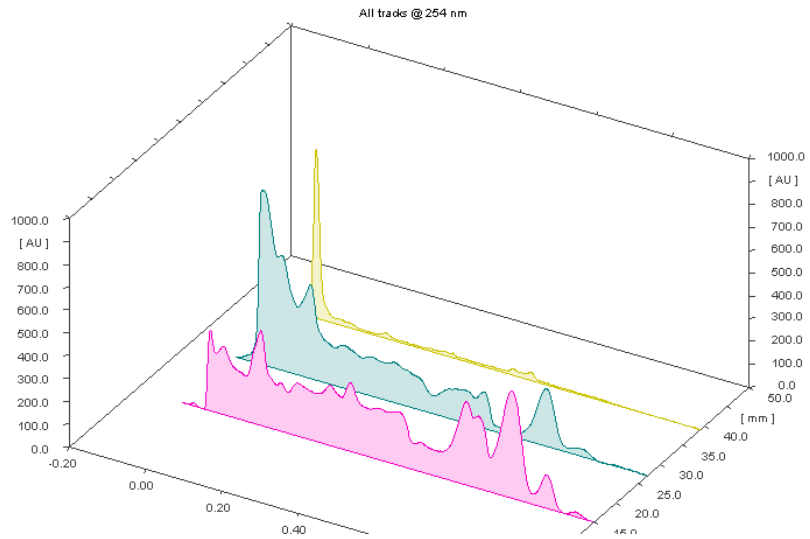


Fig 55:HPTLC image after derivitization observed at 254nm

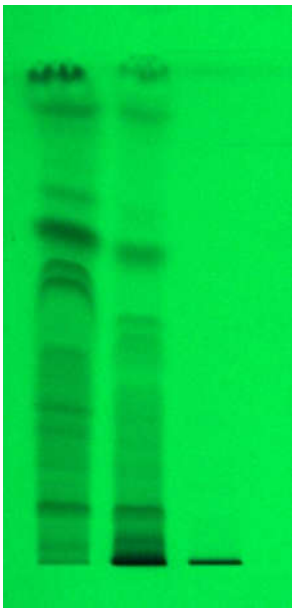
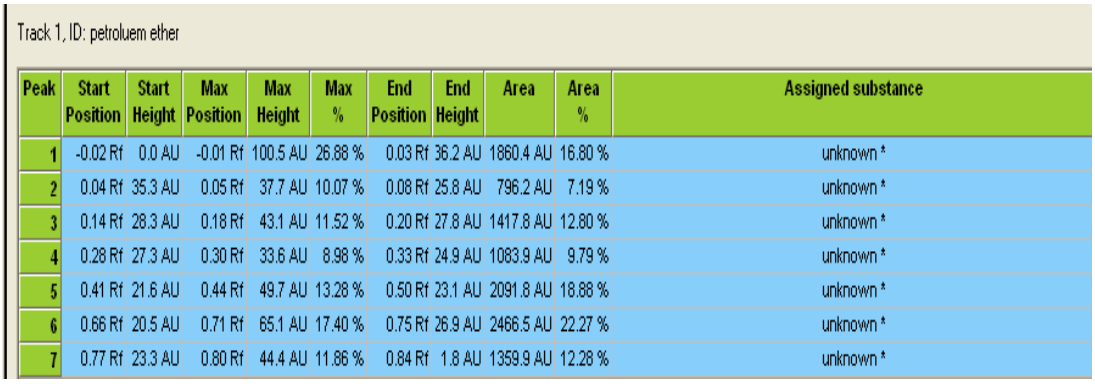


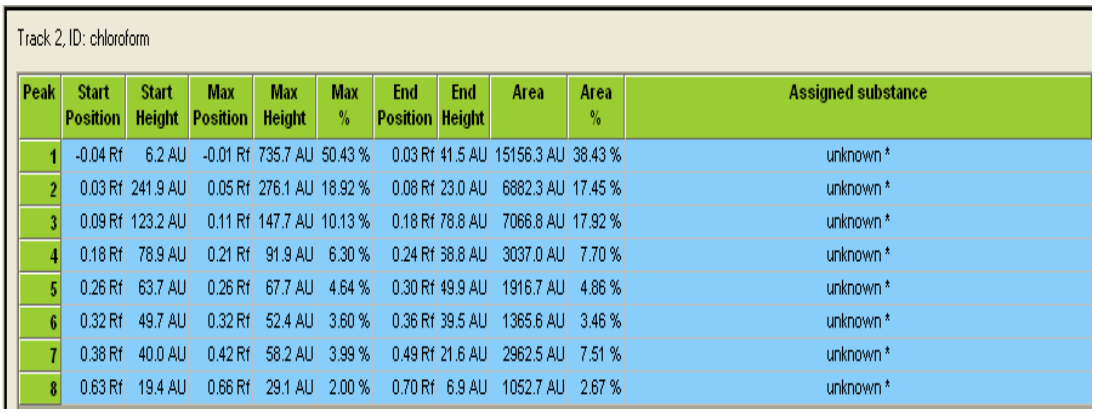
Fig56 . HPTLC profile of petroleum ether extract of *Vetiveria zizanioides* and Rf values of compounds obtained when scanned at 366nm



Track 1, ID: petroluem ether

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	0.0 AU	-0.01 Rf	100.5 AU	26.88 %	0.03 Rf	36.2 AU	1860.4 AU	16.80 %	unknown *
2	0.04 Rf	35.3 AU	0.05 Rf	37.7 AU	10.07 %	0.08 Rf	25.8 AU	796.2 AU	7.19 %	unknown *
3	0.14 Rf	28.3 AU	0.18 Rf	43.1 AU	11.52 %	0.20 Rf	27.8 AU	1417.8 AU	12.80 %	unknown *
4	0.28 Rf	27.3 AU	0.30 Rf	33.6 AU	8.98 %	0.33 Rf	24.9 AU	1083.9 AU	9.79 %	unknown *
5	0.41 Rf	21.6 AU	0.44 Rf	49.7 AU	13.28 %	0.50 Rf	23.1 AU	2091.8 AU	18.88 %	unknown *
6	0.66 Rf	20.5 AU	0.71 Rf	65.1 AU	17.40 %	0.75 Rf	26.9 AU	2466.5 AU	22.27 %	unknown *
7	0.77 Rf	23.3 AU	0.80 Rf	44.4 AU	11.86 %	0.84 Rf	1.8 AU	1359.9 AU	12.28 %	unknown *

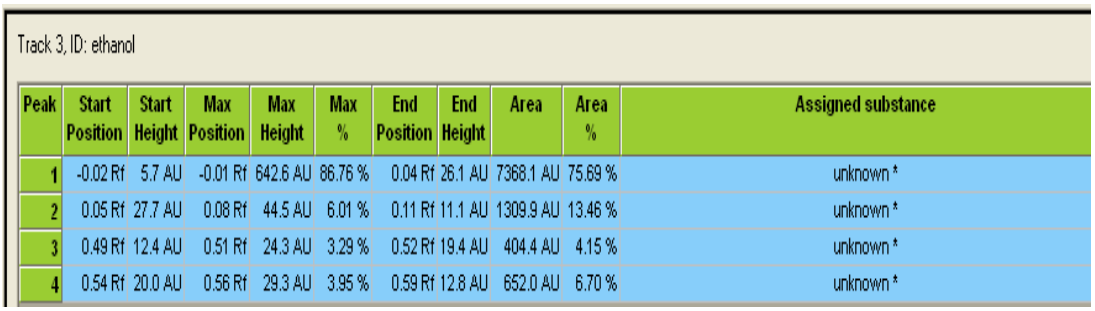
Fig 57. HPTLC profile of chloroform extract of *Vetiveria zizanioides* and Rf values of compounds obtained when scanned at 366nm



Track 2, ID: chloroform

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	6.2 AU	-0.01 Rf	735.7 AU	50.43 %	0.03 Rf	41.5 AU	15156.3 AU	38.43 %	unknown *
2	0.03 Rf	241.9 AU	0.05 Rf	276.1 AU	18.92 %	0.08 Rf	23.0 AU	6882.3 AU	17.45 %	unknown *
3	0.09 Rf	123.2 AU	0.11 Rf	147.7 AU	10.13 %	0.18 Rf	78.8 AU	7066.8 AU	17.92 %	unknown *
4	0.18 Rf	78.9 AU	0.21 Rf	91.9 AU	6.30 %	0.24 Rf	38.8 AU	3037.0 AU	7.70 %	unknown *
5	0.26 Rf	63.7 AU	0.26 Rf	67.7 AU	4.64 %	0.30 Rf	49.9 AU	1916.7 AU	4.86 %	unknown *
6	0.32 Rf	49.7 AU	0.32 Rf	52.4 AU	3.60 %	0.36 Rf	39.5 AU	1365.6 AU	3.46 %	unknown *
7	0.38 Rf	40.0 AU	0.42 Rf	58.2 AU	3.99 %	0.49 Rf	21.6 AU	2962.5 AU	7.51 %	unknown *
8	0.63 Rf	19.4 AU	0.66 Rf	29.1 AU	2.00 %	0.70 Rf	6.9 AU	1052.7 AU	2.67 %	unknown *

Fig 58. HPTLC profile of ethanolic extract of *Vetiveria zizanioides* and Rf values of compounds obtained when scanned at 366nm



Track 3, ID: ethanol

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	5.7 AU	-0.01 Rf	642.6 AU	86.76 %	0.04 Rf	26.1 AU	7368.1 AU	75.69 %	unknown *
2	0.05 Rf	27.7 AU	0.08 Rf	44.5 AU	6.01 %	0.11 Rf	11.1 AU	1309.9 AU	13.46 %	unknown *
3	0.49 Rf	12.4 AU	0.51 Rf	24.3 AU	3.29 %	0.52 Rf	19.4 AU	404.4 AU	4.15 %	unknown *
4	0.54 Rf	20.0 AU	0.56 Rf	29.3 AU	3.95 %	0.59 Rf	12.8 AU	652.0 AU	6.70 %	unknown *

Fig 59: HPTLC three dimensional densitogram graph of all the three extracts at 366nm of *Vetiveria zizanioides*

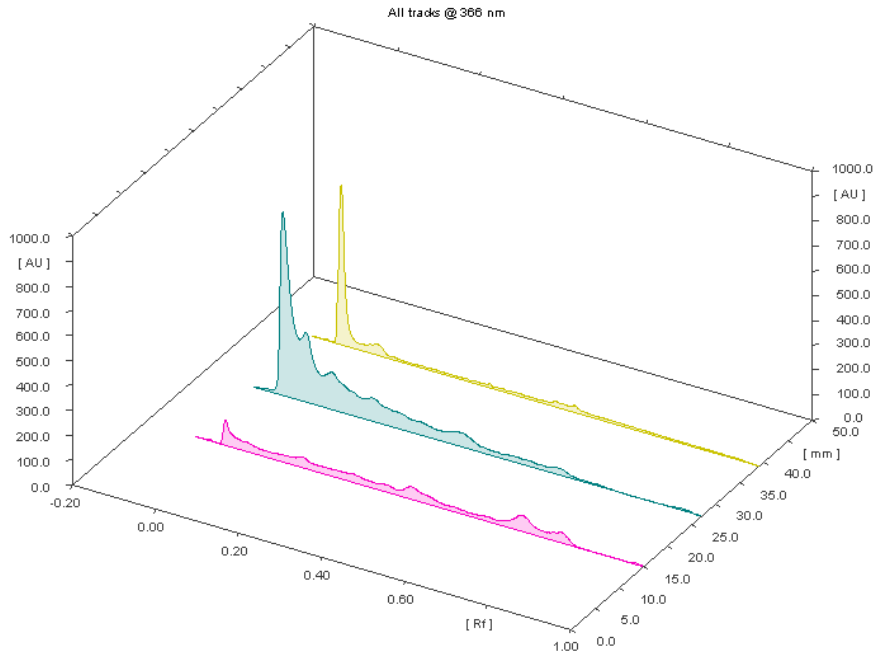


Fig 60 :HPTLC image after derivitization observed at 366nm

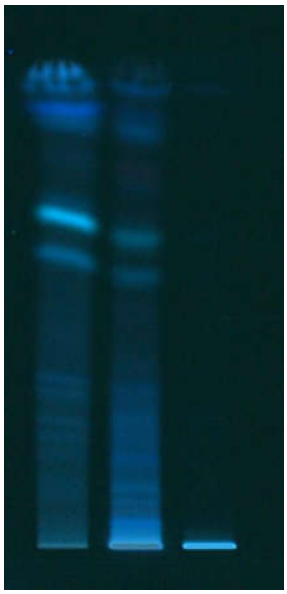
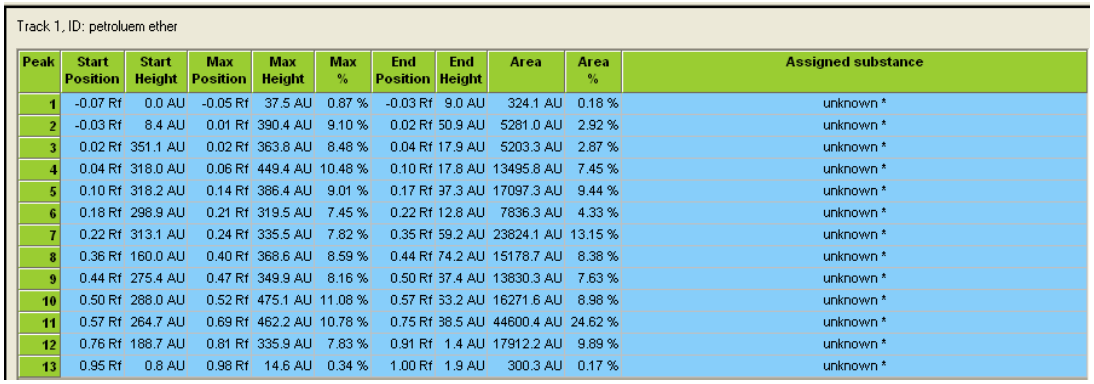


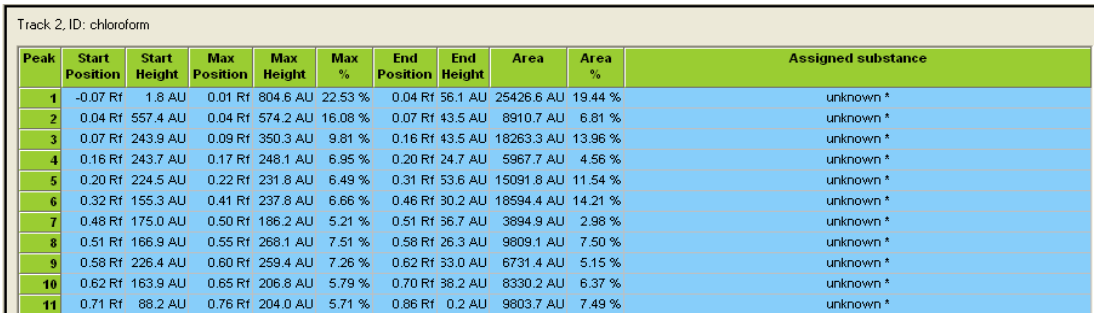
Fig 61. HPTLC profile of Petroleum ether extract and Rf values of compounds obtained when scanned at 550nm



Track 1, ID: petroluem ether

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.0 AU	-0.05 Rf	37.5 AU	0.87 %	-0.03 Rf	9.0 AU	324.1 AU	0.18 %	unknown *
2	-0.03 Rf	8.4 AU	0.01 Rf	390.4 AU	9.10 %	0.02 Rf	50.9 AU	5281.0 AU	2.92 %	unknown *
3	0.02 Rf	351.1 AU	0.02 Rf	363.8 AU	8.48 %	0.04 Rf	17.9 AU	5203.3 AU	2.87 %	unknown *
4	0.04 Rf	318.0 AU	0.06 Rf	449.4 AU	10.48 %	0.10 Rf	17.8 AU	13495.8 AU	7.45 %	unknown *
5	0.10 Rf	318.2 AU	0.14 Rf	386.4 AU	9.01 %	0.17 Rf	37.3 AU	17097.3 AU	9.44 %	unknown *
6	0.18 Rf	298.9 AU	0.21 Rf	319.5 AU	7.45 %	0.22 Rf	12.8 AU	7836.3 AU	4.33 %	unknown *
7	0.22 Rf	313.1 AU	0.24 Rf	335.5 AU	7.82 %	0.35 Rf	59.2 AU	23824.1 AU	13.15 %	unknown *
8	0.36 Rf	160.0 AU	0.40 Rf	368.6 AU	8.59 %	0.44 Rf	74.2 AU	15178.7 AU	8.38 %	unknown *
9	0.44 Rf	275.4 AU	0.47 Rf	349.9 AU	8.16 %	0.50 Rf	37.4 AU	13830.3 AU	7.63 %	unknown *
10	0.50 Rf	288.0 AU	0.52 Rf	475.1 AU	11.08 %	0.57 Rf	33.2 AU	16271.6 AU	8.98 %	unknown *
11	0.57 Rf	264.7 AU	0.69 Rf	462.2 AU	10.78 %	0.75 Rf	38.5 AU	44600.4 AU	24.62 %	unknown *
12	0.76 Rf	188.7 AU	0.81 Rf	335.9 AU	7.83 %	0.91 Rf	1.4 AU	17912.2 AU	9.89 %	unknown *
13	0.95 Rf	0.8 AU	0.98 Rf	14.6 AU	0.34 %	1.00 Rf	1.9 AU	300.3 AU	0.17 %	unknown *

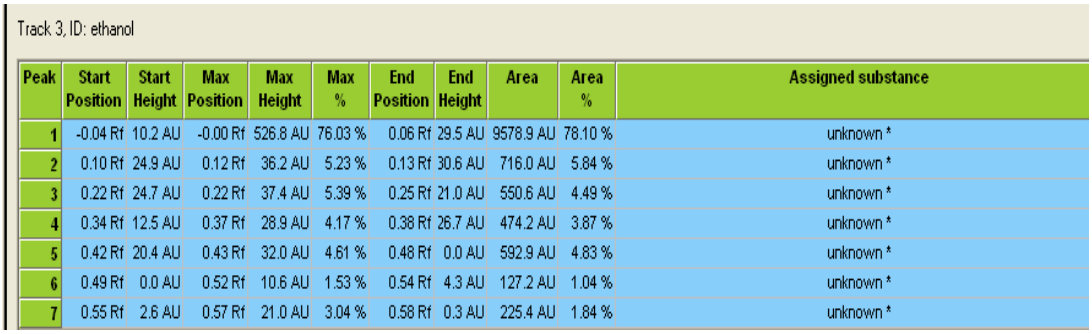
Fig 62. HPTLC profile of chloroform extract and Rf values of compounds obtained when scanned at 550nm



Track 2, ID: chloroform

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	1.8 AU	0.01 Rf	804.6 AU	22.53 %	0.04 Rf	56.1 AU	25426.6 AU	19.44 %	unknown *
2	0.04 Rf	557.4 AU	0.04 Rf	574.2 AU	16.08 %	0.07 Rf	43.5 AU	8910.7 AU	6.81 %	unknown *
3	0.07 Rf	243.9 AU	0.09 Rf	350.3 AU	9.81 %	0.16 Rf	43.5 AU	18263.3 AU	13.96 %	unknown *
4	0.16 Rf	243.7 AU	0.17 Rf	248.1 AU	6.95 %	0.20 Rf	24.7 AU	5967.7 AU	4.56 %	unknown *
5	0.20 Rf	224.5 AU	0.22 Rf	231.8 AU	6.49 %	0.31 Rf	53.6 AU	15091.8 AU	11.54 %	unknown *
6	0.32 Rf	155.3 AU	0.41 Rf	237.8 AU	6.66 %	0.46 Rf	30.2 AU	18594.4 AU	14.21 %	unknown *
7	0.48 Rf	175.0 AU	0.50 Rf	186.2 AU	5.21 %	0.51 Rf	36.7 AU	3894.9 AU	2.98 %	unknown *
8	0.51 Rf	166.9 AU	0.55 Rf	268.1 AU	7.51 %	0.58 Rf	26.3 AU	9809.1 AU	7.50 %	unknown *
9	0.58 Rf	226.4 AU	0.60 Rf	259.4 AU	7.26 %	0.62 Rf	33.0 AU	6731.4 AU	5.15 %	unknown *
10	0.62 Rf	163.9 AU	0.65 Rf	206.8 AU	5.79 %	0.70 Rf	38.2 AU	8330.2 AU	6.37 %	unknown *
11	0.71 Rf	88.2 AU	0.76 Rf	204.0 AU	5.71 %	0.86 Rf	0.2 AU	9803.7 AU	7.49 %	unknown *

Fig 63. HPTLC profile of ethanolic extract of *Vetiveria zizanioides*and Rf values of compounds obtained when scanned at 550nm



Track 3, ID: ethanol

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	10.2 AU	-0.00 Rf	526.8 AU	76.03 %	0.06 Rf	29.5 AU	9578.9 AU	78.10 %	unknown *
2	0.10 Rf	24.9 AU	0.12 Rf	36.2 AU	5.23 %	0.13 Rf	30.6 AU	716.0 AU	5.84 %	unknown *
3	0.22 Rf	24.7 AU	0.22 Rf	37.4 AU	5.39 %	0.25 Rf	21.0 AU	550.6 AU	4.49 %	unknown *
4	0.34 Rf	12.5 AU	0.37 Rf	28.9 AU	4.17 %	0.38 Rf	26.7 AU	474.2 AU	3.87 %	unknown *
5	0.42 Rf	20.4 AU	0.43 Rf	32.0 AU	4.61 %	0.48 Rf	0.0 AU	592.9 AU	4.83 %	unknown *
6	0.49 Rf	0.0 AU	0.52 Rf	10.6 AU	1.53 %	0.54 Rf	4.3 AU	127.2 AU	1.04 %	unknown *
7	0.55 Rf	2.6 AU	0.57 Rf	21.0 AU	3.04 %	0.58 Rf	0.3 AU	225.4 AU	1.84 %	unknown *

Fig64 : HPTLC three dimensional densitogram of all the three solvent extracts of *Vetiveria zizanioides* observed at 550nm

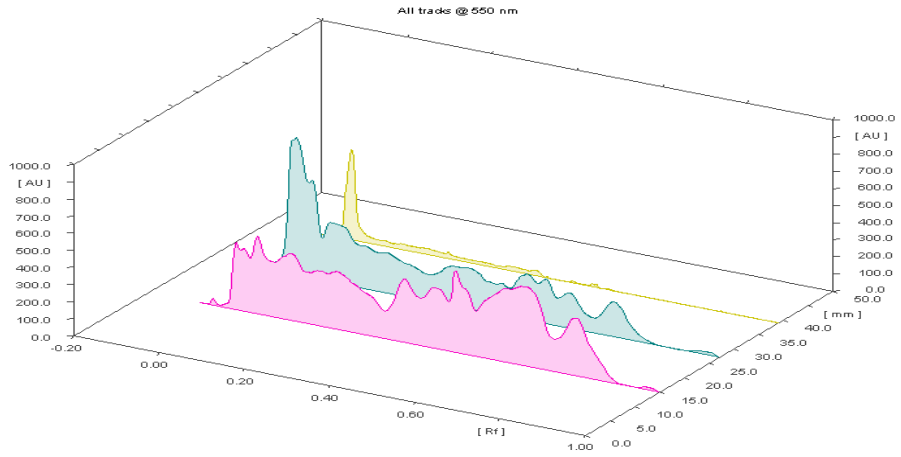


Fig 65:HPTLC image after derivitization observed at 550nm

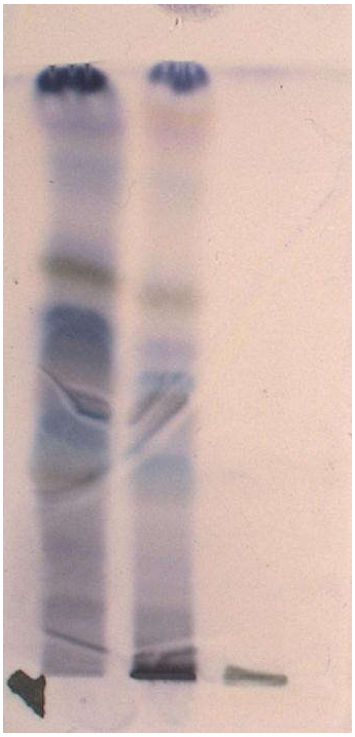


Table 83: - HPTLC profile of all the three extracts of *Vetiveria zizanioides* at 254nm, 366nm and 550nm and tentative compounds according to Rf value obtained.

Vetiveria zizanioides								
254nm								
Rf Value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
-0.03	Alkaloid	(Banu and Nagarajan, 2014)	0.03	Alkaloid	(Banu and Nagarajan, 2014)	-0.04	Tannic acid	(Ferry and Larson, 1991)
0.01	Phenol	(Banu and Nagarajan, 2014)	0.08	Saponin	(Biradar, 2013)	0.05	Alkaloid	(Kalaiselvi et al., 2012)
0.09	Gallic acid	(Flavanoid) (Asha et al., 2015)	0.18	Flavonoid	(Asante IK et al., 2016)	0.11	Alkaloid	(Kalaiselvi et al., 2012)
0.17	Phenolics	(Kalaiselvi et al., 2012)	0.25	Ferulic acid (Flavanoid)	(Asha et al., 2015)	0.20	Flavanoid	(Biradar, 2013)
0.20	Flavanoid	(Biradar, 2013)	0.31	Terpenoid	(Gomathi et al., 2012b)	0.38	Phenol	(Banu and Nagarajan, 2014)
0.27	Phenol	(Banu and Nagarajan, 2014)	0.40	Steroids	(Gomathi et al., 2012b)	0.52	Polyphenol	(Sasikumar et al., 2009)
0.34	Steroids	(Gomathi et al., 2012b)	0.55	Alkaloid	(Biradar, 2013)	0.58	Polyphenol	(Sasikumar et al., 2009)
0.40	Steroids	(Gomathi et al., 2012b)	0.63	Chlorogenic acid (Flavanoid)	(Asha et al., 2015)			
0.46	Terpenoid	(Yamunadevi, et al., 2011)	0.79	Phenol	(Banu and Nagarajan, 2014)			

0.53	Phenolics	(Kalaiselvi et al., 2012)	0.88	Terpenoid	(Gomathi et al., 2012b)			
0.58	Polyphenol	(Sasikumar et al., 2009)						
0.69	Triterpenoid	(Leela and Saraswathy, 2013)						
0.73	Phenolics	(Kalaiselvi et al., 2012)						
0.84	Rutin	(Flavanoid) (Asha et al., 2015)						
0.92	Phenol	(Ferry and Larson, 1991)						
0.99	Steroids	(Gomathi et al., 2012b)						
336nm								
Petroleum ether			Chloroform			Ethanol		
Rf Value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
0.03	Alkaloid	(Banu and Nagarajan, 2014)	0.03	Alkaloid	(Banu and Nagarajan, 2014)	0.04	Tannic acid	(Ferry and Larson, 1991)
0.08	Saponin	(Biradar, 2013)	0.08	Saponin	(Biradar, 2013)	0.11	Alkaloid	(Kalaiselvi et al., 2012)
0.20	Flavanoid	(Biradar, 2013)	0.18	Flavonoid	(Asante IK et al., 2016)	0.52	Polyphenol	(Sasikumar et al., 2009)

0.33	Flavanoid	(Biradar, 2013)	0.24	Flavanoid	(Gomathi et al., 2012a)	0.59	Alkaloid	(Kalaiselvi et al., 2012)
0.50	Polyphenol	(Sasikumar et al., 2009)	0.30	Alkaloid	(Kalaiselvi et al., 2012)			
0.75	Flavanoid	(Gomathi et al., 2012a)	0.36	Terpenoids	(Biradar, 2013)			
0.84	Rutin	(Flavanoid) (Asha et al., 2015)	0.49	Rutin	(Kalaiselvi et al., 2012)			
			0.70	Lupeol (Triterpenoid)	(Leela and Saraswathy, 2013)			
550nm								
Rf Value	Petroleum ether extract	Reference	Rf value	Chloroform extract	Reference	Rf value	Ethanolic extract	Reference
-0.03	Alkaloid	(Banu and Nagarajan, 2014)	0.04	Tannic acid	(Ferry and Larson, 1991)	0.06	Terpenoid	(Yamunadevi, et al., 2011)
0.02	Flavonoid	(Karthika et al., 2014)	0.07	Flavanoid	(Biradar, 2013)	0.13	Alkaloid	(Asante IK et al., 2016)
0.04	Tannic acid)	(Ferry and Larson, 1991	0.16	Flavanoid	(Biradar, 2013)	0.25	Ferulic acid (Flavanoid)	(Asha et al., 2015)
0.10	Terpenoid	(Gomathi et al., 2012b)	0.20	Flavanoid	(Biradar, 2013)	0.38	Phenol	(Banu and Nagarajan, 2014)
0.17	Phenolics	(Kalaiselvi et al., 2012)	0.31	Terpenoid	(Gomathi et al., 2012b)	0.48	Flavanoid	(Biradar, 2013)
0.22	Saponins	(Biradar, 2013)	0.46	Terpenoid	(Yamunadevi, et al., 2011)	0.54	Polyphenol	(Sasikumar et al., 2009)

0.35	Alkaloid	(Gomathi et al., 2012a)	0.51	Flavonoid	(Biradar, 2013)	0.58	Polyphenol	(Sasikumar et al., 2009)
0.44	Terpene	(Asante IK et al., 2016)	0.58	Polyphenol	(Sasikumar et al., 2009)			
0.50	Flavonoid	(Biradar, 2013)	0.62	Alkaloid	(Kalaiselvi et al., 2012)			
0.57	Flavanoid	(Biradar, 2013)	0.70	Lupeol (Triterpenoid)	(Leela and Saraswathy, 2013)			
0.75	Flavanoid	(Gomathi et al., 2012a)	0.86	Terpenoid	(Gomathi et al., 2012b)			
0.91	Flavonones Naringenin	(Ferry and Larson, 1991)						

Varying amount of phyto constituents were observed with *Vetiveria zizanioides*. Plant components observed at highest area percentage was observed at different absorption maxima (254nm, 366nm and 550nm) and is tabulated.

Table 84: Major components obtained from all the three extracts with highest area percentage from *Vetiveria zizanioides*

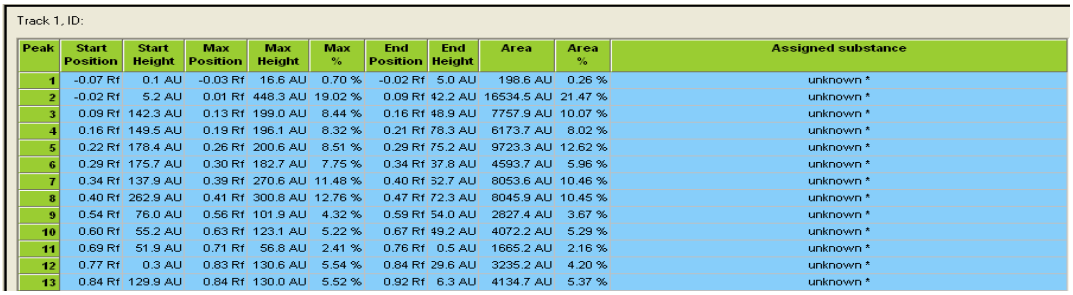
Vetiveria zizanioides				
	Scanned wavelength	Petroleum ether extract	Chloroform extract	Ethanolic extract
	254nm	Flavonoid (15.18%) Phenolics (11.11%)	Alkaloid (23.01%) Flavonoid (16.99%)	Alkaloid (78.98%) Polyphenols (5.84%)
	366nm	Flavonoid (22.27%) Polyphenols (18.88%)	Alkaloid (38.43%) Flavonoid (17.92%)	Tannic acid (75.69%) Alkaloid (13.46%)
	550nm	Flavonoid (24.62%) Alkaloid (13.15%)	Tannic acid (19.44%) Terpenoid (14.21%)	Terpenoids (78.10%) Alkaloid (5.84%)

All the three extracts of *Euphorbia antiquorum* was subjected to HPTLC analysis. Petroleum ether extracts when scanned at 254nm gave 12spots, chloroform extracts gave 10 spots and ethanolic extracts gave 4 spots (Fig 66-68). Likewise when scanned at 366nm Petroleum ether extracts gave 12 spots, chloroform extracts gave 11 spots and ethanolic extracts gave 6 spots (Fig 71-73). Petroleum ether extracts gave 13 spots, chloroform extracts gave 9 spots and ethanoilc extracts gave 5 spots when scanned at 550nm (Fig 76-78). Three dimensional densitogram representing all the three extracts scanned at 254nm, 366nm and 550nm are depicted in fig 69, 74 and 79. Chromatograms obtained for each of the solvent extracts are given in the fig

70,fig 75 and fig 80. From the Rf value obtained for various compounds were identified tentatively and are tabulated (Table 85).

Each of the extracts was scanned at 254nm, 366nm, and 550nm. Compounds having highest area percentage from the HPTLC analysis are given in the table 86.

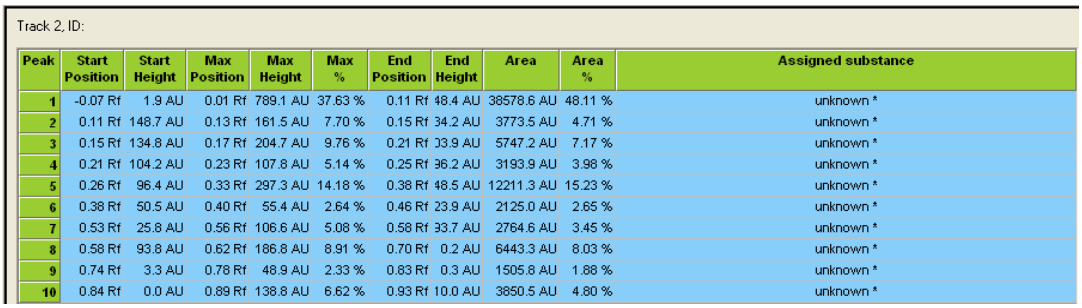
Fig 66. HPTLC profile of petroleum ether extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 254nm



Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.1 AU	-0.03 Rf	16.6 AU	0.70 %	-0.02 Rf	5.0 AU	198.6 AU	0.26 %	unknown *
2	-0.02 Rf	5.2 AU	0.01 Rf	448.3 AU	19.02 %	0.09 Rf	42.2 AU	16534.5 AU	21.47 %	unknown *
3	0.09 Rf	142.3 AU	0.13 Rf	199.0 AU	8.44 %	0.16 Rf	48.9 AU	7757.9 AU	10.07 %	unknown *
4	0.16 Rf	149.5 AU	0.19 Rf	196.1 AU	8.32 %	0.21 Rf	78.3 AU	6173.7 AU	8.02 %	unknown *
5	0.22 Rf	178.4 AU	0.26 Rf	200.6 AU	8.51 %	0.29 Rf	75.2 AU	9723.3 AU	12.62 %	unknown *
6	0.29 Rf	175.7 AU	0.30 Rf	182.7 AU	7.75 %	0.34 Rf	37.8 AU	4593.7 AU	5.96 %	unknown *
7	0.34 Rf	137.9 AU	0.39 Rf	270.6 AU	11.48 %	0.40 Rf	32.7 AU	8053.6 AU	10.46 %	unknown *
8	0.40 Rf	262.9 AU	0.41 Rf	300.8 AU	12.76 %	0.47 Rf	72.3 AU	8045.9 AU	10.45 %	unknown *
9	0.54 Rf	76.0 AU	0.56 Rf	101.9 AU	4.32 %	0.59 Rf	54.0 AU	2827.4 AU	3.67 %	unknown *
10	0.60 Rf	55.2 AU	0.63 Rf	123.1 AU	5.22 %	0.67 Rf	49.2 AU	4072.2 AU	5.29 %	unknown *
11	0.69 Rf	51.9 AU	0.71 Rf	56.8 AU	2.41 %	0.76 Rf	0.5 AU	1665.2 AU	2.16 %	unknown *
12	0.77 Rf	0.3 AU	0.83 Rf	130.6 AU	5.54 %	0.84 Rf	29.6 AU	3235.2 AU	4.20 %	unknown *
13	0.84 Rf	129.9 AU	0.84 Rf	130.0 AU	5.52 %	0.92 Rf	6.3 AU	4134.7 AU	5.37 %	unknown *

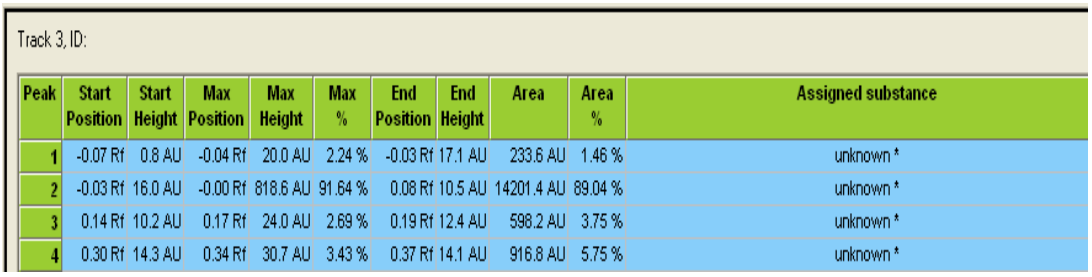
Fig 67. HPTLC profile of chloroform extract of *Euphorbia antiquorum* and Rf values of compounds obtained at 254nm



Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	1.9 AU	0.01 Rf	789.1 AU	37.63 %	0.11 Rf	48.4 AU	38578.6 AU	48.11 %	unknown *
2	0.11 Rf	148.7 AU	0.13 Rf	161.5 AU	7.70 %	0.15 Rf	34.2 AU	3773.5 AU	4.71 %	unknown *
3	0.15 Rf	134.8 AU	0.17 Rf	204.7 AU	9.76 %	0.21 Rf	33.9 AU	5747.2 AU	7.17 %	unknown *
4	0.21 Rf	104.2 AU	0.23 Rf	107.8 AU	5.14 %	0.25 Rf	36.2 AU	3193.9 AU	3.98 %	unknown *
5	0.26 Rf	96.4 AU	0.33 Rf	297.3 AU	14.18 %	0.38 Rf	48.5 AU	12211.3 AU	15.23 %	unknown *
6	0.38 Rf	50.5 AU	0.40 Rf	55.4 AU	2.64 %	0.46 Rf	23.9 AU	2125.0 AU	2.65 %	unknown *
7	0.53 Rf	25.8 AU	0.56 Rf	106.6 AU	5.08 %	0.58 Rf	33.7 AU	2764.6 AU	3.45 %	unknown *
8	0.58 Rf	93.8 AU	0.62 Rf	186.8 AU	8.91 %	0.70 Rf	0.2 AU	6443.3 AU	8.03 %	unknown *
9	0.74 Rf	3.3 AU	0.78 Rf	48.9 AU	2.33 %	0.83 Rf	0.3 AU	1505.8 AU	1.88 %	unknown *
10	0.84 Rf	0.0 AU	0.89 Rf	138.8 AU	6.62 %	0.93 Rf	10.0 AU	3850.5 AU	4.80 %	unknown *

Fig 68. HPTLC profile of ethanolic extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 254nm



Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.8 AU	-0.04 Rf	20.0 AU	2.24 %	-0.03 Rf	17.1 AU	233.6 AU	1.46 %	unknown *
2	-0.03 Rf	16.0 AU	-0.00 Rf	818.6 AU	91.64 %	0.08 Rf	10.5 AU	14201.4 AU	89.04 %	unknown *
3	0.14 Rf	10.2 AU	0.17 Rf	24.0 AU	2.69 %	0.19 Rf	12.4 AU	598.2 AU	3.75 %	unknown *
4	0.30 Rf	14.3 AU	0.34 Rf	30.7 AU	3.43 %	0.37 Rf	14.1 AU	916.8 AU	5.75 %	unknown *

Fig 69: HPTLC three dimensional densitogram of all the three extracts of *Euphorbia antiquorum* at 254nm

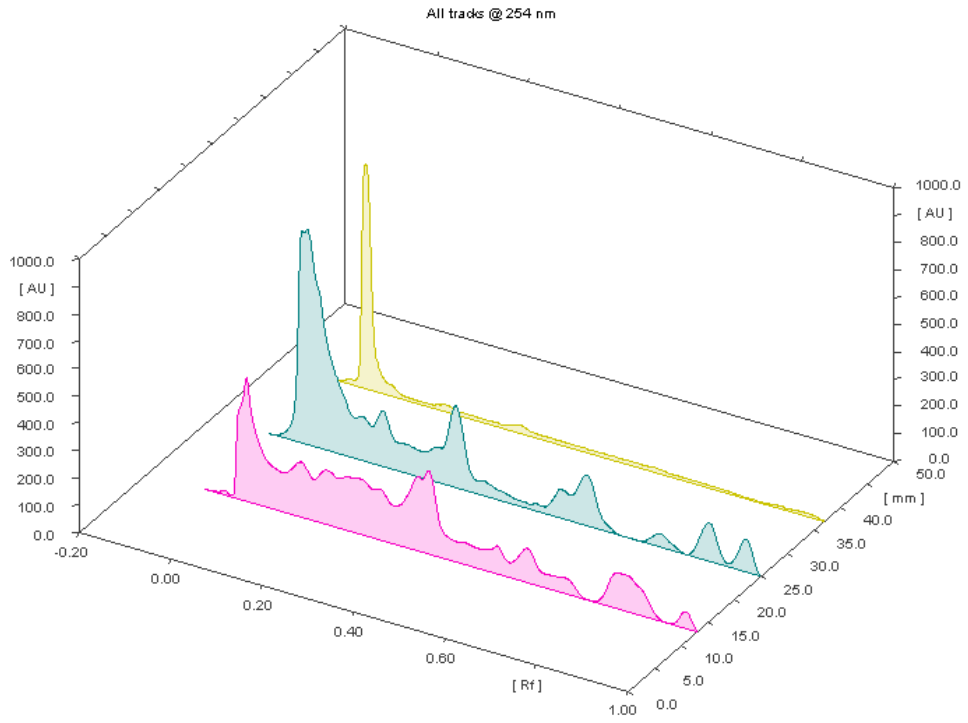


Fig 70:HPTLC image after derivitization observed at 254nm

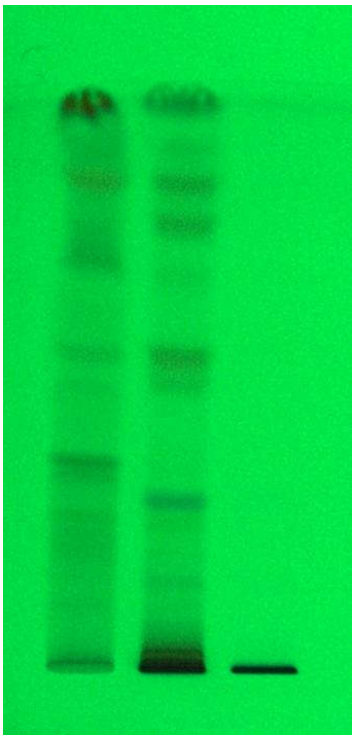


Fig 71: HPTLC profile of petroleum ether extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 366nm

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	4.1 AU	0.01 Rf	294.0 AU	21.52 %	0.07 Rf	74.1 AU	8374.3 AU	19.64 %	unknown *
2	0.07 Rf	74.1 AU	0.09 Rf	83.7 AU	6.13 %	0.14 Rf	32.5 AU	3626.9 AU	8.51 %	unknown *
3	0.18 Rf	67.3 AU	0.24 Rf	123.3 AU	9.02 %	0.27 Rf	35.7 AU	5173.8 AU	12.14 %	unknown *
4	0.27 Rf	86.2 AU	0.30 Rf	216.3 AU	15.83 %	0.34 Rf	30.6 AU	6536.3 AU	15.33 %	unknown *
5	0.34 Rf	91.1 AU	0.35 Rf	98.3 AU	7.20 %	0.38 Rf	53.7 AU	2278.4 AU	5.34 %	unknown *
6	0.38 Rf	54.0 AU	0.41 Rf	67.6 AU	4.95 %	0.44 Rf	57.2 AU	2429.7 AU	5.70 %	unknown *
7	0.44 Rf	56.6 AU	0.45 Rf	59.5 AU	4.36 %	0.48 Rf	47.8 AU	1187.2 AU	2.78 %	unknown *
8	0.48 Rf	48.0 AU	0.50 Rf	53.6 AU	3.92 %	0.53 Rf	27.2 AU	1610.9 AU	3.78 %	unknown *
9	0.54 Rf	25.2 AU	0.56 Rf	44.9 AU	3.29 %	0.58 Rf	27.2 AU	971.1 AU	2.28 %	unknown *
10	0.58 Rf	27.1 AU	0.63 Rf	179.3 AU	13.13 %	0.70 Rf	6.9 AU	6449.9 AU	15.13 %	unknown *
11	0.76 Rf	0.4 AU	0.83 Rf	130.4 AU	9.55 %	0.88 Rf	0.6 AU	3784.6 AU	8.88 %	unknown *
12	0.88 Rf	0.1 AU	0.90 Rf	15.1 AU	1.11 %	0.92 Rf	0.4 AU	211.1 AU	0.50 %	unknown *

Fig 72. HPTLC profile of chloroform extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 366nm

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	2.0 AU	0.00 Rf	780.1 AU	23.94 %	0.02 Rf	39.9 AU	17744.3 AU	17.44 %	unknown *
2	0.03 Rf	612.4 AU	0.03 Rf	681.8 AU	20.92 %	0.10 Rf	29.9 AU	16696.2 AU	16.41 %	unknown *
3	0.14 Rf	98.5 AU	0.17 Rf	145.3 AU	4.46 %	0.19 Rf	25.7 AU	4437.0 AU	4.36 %	unknown *
4	0.19 Rf	125.8 AU	0.22 Rf	165.9 AU	5.09 %	0.25 Rf	16.7 AU	5648.7 AU	5.55 %	unknown *
5	0.25 Rf	117.3 AU	0.29 Rf	220.4 AU	6.76 %	0.31 Rf	31.8 AU	6809.5 AU	6.69 %	unknown *
6	0.31 Rf	163.6 AU	0.33 Rf	234.9 AU	7.21 %	0.38 Rf	42.2 AU	6392.1 AU	6.28 %	unknown *
7	0.40 Rf	48.1 AU	0.44 Rf	85.9 AU	2.64 %	0.47 Rf	53.1 AU	2957.8 AU	2.91 %	unknown *
8	0.47 Rf	53.3 AU	0.48 Rf	55.1 AU	1.69 %	0.50 Rf	37.9 AU	1085.1 AU	1.07 %	unknown *
9	0.52 Rf	31.9 AU	0.62 Rf	518.1 AU	15.90 %	0.70 Rf	14.1 AU	28128.7 AU	27.65 %	unknown *
10	0.74 Rf	11.0 AU	0.79 Rf	92.3 AU	2.83 %	0.83 Rf	0.0 AU	2630.5 AU	2.59 %	unknown *
11	0.83 Rf	0.8 AU	0.89 Rf	278.6 AU	8.55 %	0.92 Rf	31.2 AU	9216.3 AU	9.06 %	unknown *

Fig 73. HPTLC profile of ethanolic extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 366nm

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	3.6 AU	-0.00 Rf	857.6 AU	85.36 %	0.04 Rf	27.2 AU	11672.3 AU	75.79 %	unknown *
2	0.04 Rf	27.3 AU	0.05 Rf	32.8 AU	3.27 %	0.08 Rf	9.1 AU	540.7 AU	3.51 %	unknown *
3	0.14 Rf	11.1 AU	0.18 Rf	19.9 AU	1.99 %	0.19 Rf	13.0 AU	547.4 AU	3.55 %	unknown *
4	0.30 Rf	16.5 AU	0.34 Rf	27.8 AU	2.77 %	0.36 Rf	15.2 AU	866.8 AU	5.63 %	unknown *
5	0.61 Rf	18.4 AU	0.63 Rf	49.1 AU	4.89 %	0.68 Rf	16.6 AU	1397.8 AU	9.08 %	unknown *
6	0.94 Rf	4.9 AU	0.97 Rf	17.4 AU	1.73 %	1.00 Rf	0.3 AU	375.2 AU	2.44 %	unknown *

Fig 74: HPTLC three dimensional densitogram of all the three extracts of *Euphorbia antiquorum* at 366nm

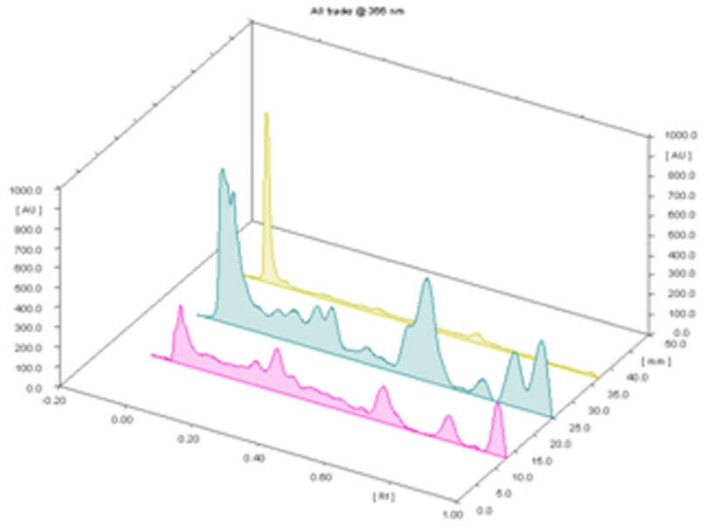


Fig 75: HPTLC image after derivitization viewed at 366nm

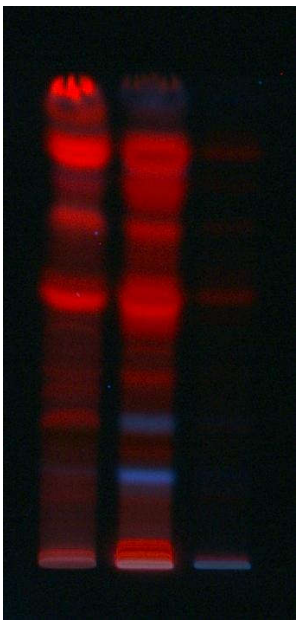


Fig 76. HPTLC profile of petroleum ether extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 550nm

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.2 AU	-0.05 Rf	12.4 AU	0.34 %	-0.03 Rf	0.8 AU	177.2 AU	0.10 %	unknown *
2	-0.03 Rf	1.1 AU	0.02 Rf	574.4 AU	15.73 %	0.11 Rf	75.0 AU	28650.6 AU	16.39 %	unknown *
3	0.12 Rf	275.4 AU	0.15 Rf	285.7 AU	7.82 %	0.19 Rf	58.7 AU	13881.1 AU	7.94 %	unknown *
4	0.19 Rf	258.8 AU	0.23 Rf	313.9 AU	8.59 %	0.27 Rf	36.6 AU	14390.7 AU	8.23 %	unknown *
5	0.28 Rf	208.3 AU	0.32 Rf	268.1 AU	7.34 %	0.35 Rf	39.7 AU	11283.6 AU	6.45 %	unknown *
6	0.35 Rf	240.8 AU	0.39 Rf	446.9 AU	12.23 %	0.46 Rf	79.6 AU	20554.9 AU	11.76 %	unknown *
7	0.46 Rf	179.9 AU	0.50 Rf	273.1 AU	7.48 %	0.52 Rf	29.9 AU	9779.4 AU	5.59 %	unknown *
8	0.52 Rf	230.3 AU	0.53 Rf	231.8 AU	6.35 %	0.58 Rf	78.3 AU	8313.3 AU	4.75 %	unknown *
9	0.58 Rf	178.7 AU	0.62 Rf	314.3 AU	8.61 %	0.68 Rf	52.0 AU	14682.2 AU	8.40 %	unknown *
10	0.68 Rf	152.2 AU	0.73 Rf	255.6 AU	7.00 %	0.75 Rf	45.7 AU	10450.5 AU	5.98 %	unknown *
11	0.75 Rf	246.7 AU	0.81 Rf	427.8 AU	11.71 %	0.90 Rf	31.1 AU	34003.2 AU	19.45 %	unknown *
12	0.90 Rf	92.5 AU	0.94 Rf	248.8 AU	6.81 %	1.00 Rf	1.4 AU	8674.8 AU	4.96 %	unknown *

Fig 77. HPTLC profile of chloroform extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 550nm

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.05 Rf	2.9 AU	0.00 Rf	788.3 AU	27.50 %	0.01 Rf	36.2 AU	11922.1 AU	10.50 %	unknown *
2	0.01 Rf	769.3 AU	0.02 Rf	801.1 AU	27.94 %	0.17 Rf	57.0 AU	35598.6 AU	31.36 %	unknown *
3	0.20 Rf	154.1 AU	0.21 Rf	159.6 AU	5.57 %	0.27 Rf	31.9 AU	5677.1 AU	5.00 %	unknown *
4	0.27 Rf	92.3 AU	0.30 Rf	141.7 AU	4.94 %	0.33 Rf	55.7 AU	4661.7 AU	4.11 %	unknown *
5	0.34 Rf	55.8 AU	0.37 Rf	103.7 AU	3.62 %	0.41 Rf	40.4 AU	3656.5 AU	3.22 %	unknown *
6	0.42 Rf	39.9 AU	0.49 Rf	168.1 AU	5.86 %	0.54 Rf	0.3 AU	7922.0 AU	6.98 %	unknown *
7	0.54 Rf	0.1 AU	0.61 Rf	132.2 AU	4.61 %	0.67 Rf	37.5 AU	6589.1 AU	5.80 %	unknown *
8	0.67 Rf	37.7 AU	0.82 Rf	503.1 AU	17.55 %	0.88 Rf	17.3 AU	35629.4 AU	31.38 %	unknown *
9	0.88 Rf	17.5 AU	0.90 Rf	68.9 AU	2.40 %	0.96 Rf	6.1 AU	1874.4 AU	1.65 %	unknown *

Fig 78. HPTLC profile of ethanolic extract of *Euphorbia antiquorum* and Rf values of compounds obtained when scanned at 550nm

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	6.9 AU	0.00 Rf	580.1 AU	69.92 %	0.07 Rf	25.5 AU	9254.4 AU	41.04 %	unknown *
2	0.12 Rf	17.8 AU	0.26 Rf	88.3 AU	10.64 %	0.35 Rf	49.1 AU	9201.1 AU	40.81 %	unknown *
3	0.41 Rf	40.6 AU	0.42 Rf	45.7 AU	5.50 %	0.46 Rf	27.8 AU	1214.2 AU	5.38 %	unknown *
4	0.53 Rf	30.0 AU	0.53 Rf	33.3 AU	4.02 %	0.58 Rf	0.3 AU	717.4 AU	3.18 %	unknown *
5	0.77 Rf	7.6 AU	0.83 Rf	65.7 AU	7.92 %	0.86 Rf	0.4 AU	2008.2 AU	8.91 %	unknown *
6	0.96 Rf	2.7 AU	0.97 Rf	16.6 AU	2.00 %	0.98 Rf	0.3 AU	152.1 AU	0.67 %	unknown *

Fig 79: HPTLC three dimensional densitogram of all the three extracts of *Euphorbia antiquorum* at 550nm

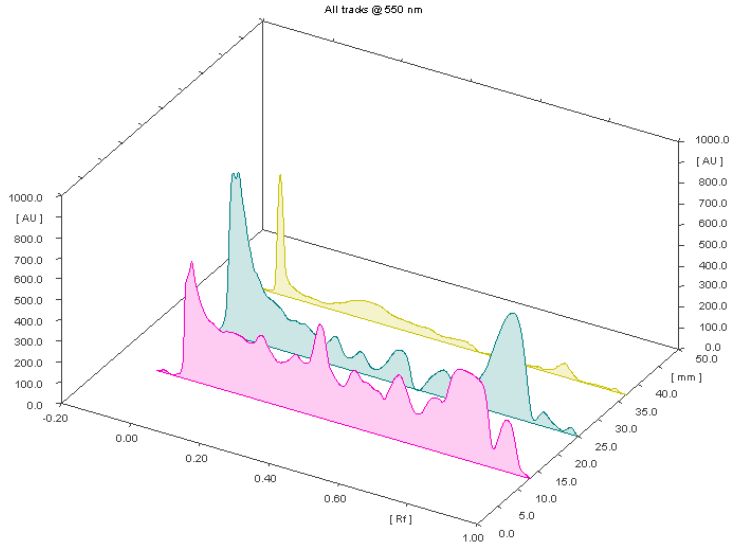


Fig 80: HPTLC image after derivitization observed at 550nm

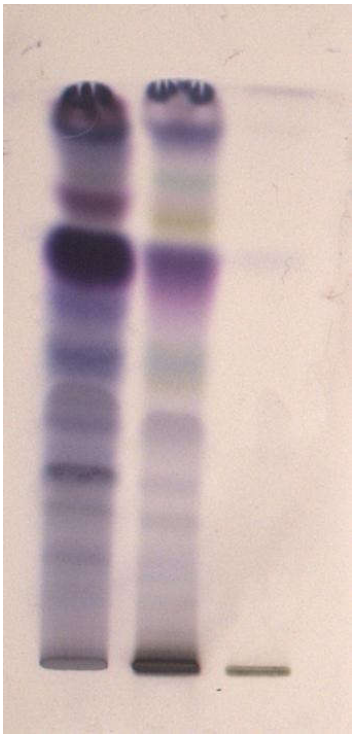


Table 85- HPTLC profile of all the three extracts of *Euphorbia antiquorum* at 254nm, 366nm and 550nm and tentative compounds according to Rf value obtained

Euphorbia antiquorum								
254nm								
Petroleum ether extract			**Chloroform extract**			**Ethanolic extract**		
Rf Value	**Tentative compound**	**Reference**	**Rf value**	**Tentative compound**	**Reference**	**Rf value**	**Tentative compound**	**Reference**
-0.02	Flavonoid	(Karthika et al., 2014)	0.11	Alkaloid	(Kalaiselvi et al., 2012)	-0.03	Alkaloid	(Banu and Nagarajan, 2014)
0.09	Gallic acid	(Asha et al., 2015)	0.15	Gallic acid	(Ferry and Larson, 1991)	0.08	Saponin	(Biradar, 2013)
0.16	Flavanoid	(Biradar, 2013)	0.21	Alkaloid	(Banu and Nagarajan, 2014)	0.19	Tannin	(Banu and Nagarajan, 2014)
0.21	Alkaloid	(Banu and Nagarajan, 2014)	0.25	Ferulic acid (Flavanoid)	(Asha et al., 2015)	0.37	Terpenoids	(Biradar, 2013)
0.34	Steroids	(Gomathi et al., 2012b)	0.38	Phenol	(Banu and Nagarajan, 2014)			
0.40	Steroids	(Gomathi et al., 2012b)	0.46	Terpenoid	(Yamunadevi, et al., 2011)			
0.47	Steroids)	(Gomathi et al., 2012b	0.58	Polyphenol	(Sasikumar et al., 2009)			
0.59	Alkaloid	(Kalaiselvi et al., 2012)	0.70	Lupeol (Triterpenoid)	(Leela and Saraswathy,			

					2013)			
0.67	Alkaloid	(Asante IK et al., 2016)	0.83	Flavanoid	(Asha et al., 2015)			
0.76	Terpenoid	(Gomathi et al., 2012b)	0.93	Terpenoid	(Gomathi et al., 2012b)			
0.84	Rutin (Flavanoid)	(Asha et al., 2015)						
0.92	Phenol	(Ferry and Larson, 1991)						
366nm								
Petroleum ether extract			**Chloroform extract**			**Ethanolic extract**		
Rf value	**Tentative compound**	**Reference**	**Rf value**	**Tentative compound**	**Reference**	**Rf value**	**Tentative compound**	**Reference**
0.07	Flavanoid	(Asha et al., 2015)	0.02	Flavonoid	(Karthika et al., 2014)	0.04	Tannic acid	(Ferry and Larson, 1991)
0.14	Phenol	(Banu and Nagarajan, 2014)	0.10	Saponin	(Biradar, 2013)	0.08	Saponin	(Biradar, 2013)
0.27	Phenol	(Banu and Nagarajan, 2014)	0.19	Tannin	(Banu and Nagarajan, 2014)	0.19	Tannin	(Banu and Nagarajan, 2014)
0.34	Steroids	(Gomathi et al., 2012b)	0.25	Ferulic acid (Flavanoid)	(Asha et al., 2015)	0.36	Terpenoids	(Biradar, 2013)
0.38	Phenol	(Banu and Nagarajan, 2014)	0.31	Terpenoid	(Gomathi et al., 2012b)	0.68	Phenol Dihydroxy benzaldehyde	(Ferry and Larson, 1991)
0.44	Terpene	(Asante IK et al., 2016)	0.38	Phenol	(Banu and Nagarajan,	1.00	unknown	

					2014)			
0.48	Flavanoid	(Biradar, 2013)	0.47	Terpenoid	(Yamunadevi, et al., 2011)			
0.53	Phenolics	(Kalaiselvi et al., 2012)	0.50	Flavonoid	(Biradar, 2013)			
0.58	Polyphenol	(Sasikumar et al., 2009)	0.70	Lupeol (Triterpenoid)	(Leela and Saraswathy, 2013)			
0.70	Lupeol (Triterpenoid)	(Leela and Saraswathy, 2013)	0.83	(Flavanoid)	(Asha et al., 2015)			
0.88	Terpenoid	(Gomathi et al., 2012b)	0.92	Phenol	(Ferry and Larson, 1991)			
0.92	Phenol	(Ferry and Larson, 1991)						
550nm								
Petroleum ether extract			Chloroform extract			Ethanolic extract		
Rf value	**Tentative compound**	**Reference**	**Rf value**	**Tentative compound**	**Reference**	**Rf value**	**Tentative compound**	**Reference**
-0.03	Alkaloid	(Banu and Nagarajan, 2014)	0.01	Phenol	(Banu and Nagarajan, 2014)	0.07	Steroids	(Gomathi et al., 2012b)
0.11	Alkaloid	(Kalaiselvi et al., 2012)	0.17	Phenolics	(Kalaiselvi et al., 2012)	0.35	Alkaloid	(Gomathi et al., 2012a)
0.19	Tannin	(Banu and Nagarajan, 2014)	0.27	Phenol	(Banu and Nagarajan, 2014)	0.46	Terpenoid	(Yamunadevi, et al., 2011)
0.27	Phenol	(Banu and Nagarajan,	0.33	Flavanoid	(Biradar, 2013)	0.58	Polyphenol	(Sasikumar et al., 2009)

		2014)						
0.35	Alkaloid	(Gomathi et al., 2012a)	0.41	Phenolics	(Kalaiselvi et al., 2012)	0.86	Terpenoid	(Gomathi et al., 2012b)
0.46	Terpenoid	(Yamunadevi, et al., 2011)	0.54	Phenolics	(Kalaiselvi et al., 2012)	0.98	Phenol	(Ferry and Larson, 1991)
0.52	Polyphenol	(Sasikumar et al., 2009)	0.67	Alkaloid	(Asante IK et al., 2016)			
0.58	Polyphenol	(Sasikumar et al., 2009)	0.88	Terpenoid	(Gomathi et al., 2012b)			
0.68	Phenol Dihydroxy benzaldehyde	(Ferry and Larson, 1991)	0.96	Phenol	(Ferry and Larson, 1991)			
0.75	Flavonoid	(Gomathi et al., 2012a)						
0.90	Phenol	(Banu and Nagarajan, 2014)						

Table 86: Major components obtained from all the three extracts with highest area percentage from *Euphorbia antiquorum*

Euphorbia antiquorum				
	Scanned Absorbances	**Petroleum ether extract**	**Chloroform extract**	**Ethanolic extract**
	254nm	Gallic acid (21.47%) Steroids (10.46%)	Alkaloid (48.11%) Phenol (15.23%)	Saponin (89.04%) Terpenoids (5.75%)
	366nm	Flavonoid (19.64%) Steroid (15.33%)	Flavonoids (17.44%) Terpenoid (16.41%)	Tannic acid (75.79%) Phenols (9.08%)
	550nm	Phenol (19.45%) Alkaloid (16.39%)	Phenolics (31.36%) Terpenoids (31.38%)	Alkaloid (41.04%) Terpenoids (40.81%)

Liquid Chromatography Quadruple –Time of Flight Mass spectrometry (LC/Q-TOF/MS)

LC/Q-TOF/MS analysis of ethanolic extracts of all the five plants gave varying number of compounds. Each of the extracts was scanned at positive and negative mode. A total of 22 plant related compounds were obtained from the ethanolic extracts of dry fruits of *Terminalia chebula*. While 44 plants related compounds were tentatively identified from both positive and negative mode from *Terminalia bellirica*. In the case of ethanolic extracts of *Hemidesmus indicus,* 21 plant related compounds were obtained from both positive and negative mode and were tentatively identified (Table 12). When ethanolic extracts of *Vetiveria zizanioides* were analysed, a total of 13 compounds from both positive and negative mode was obtained. Likewise, 32 plant related compounds were tentatively identified from the ethanolic extracts of *Euphorbia antiquorum*. List of the tentative plant related compounds obtained from ethanolic extracts of all the five plant materials, namely *Terminalia chebula, Terminalia bellirica, Hemidesmus indicus, Vetiveria zizanioides* and *Euphorbia antiquorum* are given in the table 87-91.

Table 87: Plant related compounds tentatively identified from LC/Q-TOF/MS analysis of ethanolic extracts of *Terminalia chebula*

LC/Q-TOF/MS analysis of Ethanolic extracts of *Terminalia chebula*				
Sl. No.	**Peaks obtained**	**Compound**	**Class**	**Reference**
Plant related compounds obtained at Negative mode				
1	111.0092	Quercetin 3D xyloside	Flavonoid	MASS BANK PR100447
2.	178.9998	Rutin	Glycoside	MassBank Record: MPR02803
3.	124.0176	Quercetin	Polyphenol	MassBank Record: PR040051
4	275.0218	Galloyl-HHDP-glucose	Phenolic component	(Hanhineva et al., 2008b)
5	365.0547	7,8,-dimethoxy-3',4'-methylene dioxyflavone	Non phenolic flavone	(Koteswara Rao et al., 2002)
6	113.0253	$C_{20}H_{20}O_4$-O-gluA		(Jliang Geng et al., 2014a)
7	189.0567	Phloretin	Natural Phenol	MassBank record BML00595
8	261.0059	Dihydrocaffeic acid sulfate	Phenolic acid	(Zhao et al., 2015)
9	161.0616	6'-O-sinapoylgeniposide	Glucoside	(Liu et al., 2016)
10	461.1143	Rhamnetin-3-O-rhamnoside	Flavanol derivative	(H. H. Song et al., 2016)
11	147.0459	Dihydroquercetin	Flavonoid	MassBank Record: BML01279
Plant related compounds obtained at Positive mode				
12	493.1342	Involcranoside A	Phenolic compound	(Wu et al., 2008)
13	208.1332	1,1-Dimethyl-6-methoxy-7-hydroxyl-1,2,3,4-tetrahydroisoquinoline	Alkaloid	(Zheng et al., 2013)

14	260.1649	Crassostreaxanthin A	Carotenoid	MassBank record 000034
15	299.0757	Hydroxybenzoic glucoside	Phenolic acid	(Ledesma-Escobar et al., 2015)
16	303.0492	Taxifolin	Flavanoid	(Yinjun Yang et al., 2016)
17	249.1483	Coriolopsin B	Sequeterpene	(Chen et al., 2017)
18	689.3870	Quadronoside III	Triterpene	(Mencherini et al., 2007)
19	923.5004	Soyasaponin Ɣg	Saponin	(Lee et al., 2017)
20	451.3204	Tomentodione	Terpenoid	(Zhang et al., 2017)
21	273.2203	Sigmasterol	Sterol	(Hussain et al., 2008)
22	315.1572	Volvalerenal C	Sesqueterpenoid	(Wang et al., 2010)

Table 88: Plant related compounds tentatively identified from LC/Q-TOF/MS analysis of ethanolic extracts of *Terminalia bellirica*

LC/Q-TOF/MS analysis of Ethanolic extracts of *Terminalia bellirica*				
Sl. No.	**Peaks obtained**	**Compound**	**Class**	**Reference**
Plant related compounds obtained at Negative mode				
1	169.0141	Gallic acid	Phenolic acid	(Wang et al., 2017)
2.	111.0088	Furoic acid	Carboxylic acid	(Fernanda et al., 2016)
3.	124.0162	Methyl Gallate	Phenolic compound	(Kumar et al., 2015)
4	197.0456	Rosmarinic acid	Ester of caffeic acid	(H. Song et al., 2016)
5	275.0201	HHDP-glucose (hexahydroxydiphenyl)	Phenolic acid derivative	(Hanhineva et al., 2008a)
6	785.0853	Digalloyl-HHDP-hexose	Gallotannin	(Pukalskiene et al., 2015)
7	137.0237	Malabaricone B	Acyl Phenol	(Pandey et al., 2016)
8	193.0495	Isocopoletin	Coumarin	(Pubchem, 2017)
9	243.1236	Iso-butyl α-L-rhamnopyranoside	glycoside	(Hricovíniová and Hricovíni, 2014)
10	112.9845	Loganin	Iridoid glycoside	MassBank Record: MPR02388
11	337.0936	4-O-P-Coumarylquinic acid	Quinic acid derivative	(Jaiswal et al., 2012)
12	343.0467	Quercetin-6-C-glucoside	Polyphenol derivative	(Ouyang et al., 2016)
13	649.3986	Triterpenoid hexose	Terpenoid derivative	(Hanhineva et al., 2008b)
14	351.0735	Noscapine	benzylisoquinoline alkaloid	MassBank Record: EQ302404

15	965.5450	Rotundioside	Glycoside	(Atsunaga et al., 2006)
16	116.9280	Sakosaponin	Saponin derivative	MassBank Record: MPR01197
17	487.3456	Yarumic acid	triterpene	(Guillermo et al., 2013)
18	265.1490	Phaseic acid	Terpenoid catabolite	(Arbona et al., 2015)
19	471.3498	Glycyrrhetinic acid	Triperpenoid derivative	(Woldegiorgis et al., 2015)
Plant related compounds obtained at Positve mode				
20	157.0496	Astragaloside	Xylose residue	(Liu et al., 2009)
21	208.1332	1,1-Dimethyl-6-methoxy-7- hydroxyl-1,2,3,4-tetrahydroisoquinoline	Alkaloid	(Zheng et al., 2013)
22	181.0131	Isocarthamidin -7-O –β-d-glucuronide	Flavones	(He et al., 2016)
23	221.0453	Cimifugin	Anthraquinones	MassBank Record: MPR00101
24	341.0875	Caffeoyl-hexose	Phenolic compound	(Abu-reidah et al., 2015)
25	331.0445	Laricitrin-glucuronide	Flavanol	(Ancillotti et al., 2016)
26	513.2019	Nimilin	Limonoid	(Pan et al., 2014)
28	390.1546	Usenamine B	Usnic acid dervative	(Yu et al., 2016)
29	488.1508	Caffeic acid rutinoside	Phenolic acid	(Bar-Akiva et al., 2010)
30	203.1795	*N*–Arachidonyl- ϒAminobutyric Acid	Arachidonic acid derivative	(Huang et al., 2001)
31	249.1486	Dihydrogranuloinden	Sesquiterpene	(Nord et al., 2015)
32	469.3320	Oxoglycyrrhetinic acid	Terpenoid	(Abu-Reidah et al., 2015)
33	443.3524	Crellasterone A	Sterol derivative	(Ragini et al., 2017)
34	318.3008	(2*S*,3*S*,4*R*)-2-Aminooctadecane-1,3,4-triol (1, D-*ribo*-phytosphingosine)	Phospholipid	(Yoon et al., 2007)
35	217.1587	Glycyrrhetinic Acid	Terpenoid	MassBank Record:

				EQ326302
36	439.3203	3b-[(O-b-D-glucopyranosyl-(1/3)-O-[a-L-rhamnopyranosyl-(1/2)]-a-L-arabinopyranosyl)oxy]-30-norolean-12-en-28-oic acid,	Steroidal saponin	(Ling, 2016)
37	287.1265	4β,10α-Dihydroxy-5αH-guai-1(2),11(13)-dien-12,8α-olide (9):	Sesquiterpene derivative	(Qin et al., 2011)
38	393.0781	Fraxin	Glucoside	(Patil et al., 2016)
39	451.3220	(23R,24R)-7-en-25-anhydrocimigenol-3-O-β-D-xylopyranoside	Sugar dervative	(Ma et al., 2011)
40	497.3232	25-Hydroxyporicoic acid H	Triterpene	(Wang et al., 2014)
41	149.0238	Siphulellic acid	Derivatives of ester	(Castro et al., 2017)
42	279.1597	4,5-Dimethoxyillicinone G	Phenylpropanoids	(Balkwill et al., 2009)
43	337.1437	Tephroapollin C	Flavonoid	(Khalafallah et al., 2009)
44	496.3406	Lysophospatidyl choline	Phosphatidylcholines derivative.	(Sun et al., 2016)

Table 89: Plant related compounds tentatively identified from LC/Q-TOF/MS analysis of ethanolic extracts of *Hemidesmus indicus*

LC/Q-TOF/MS analysis of Ethanolic extracts of *Hemidesmus indicus*				
Plant related compounds obtained at Negative mode				
Sl. No.	**Peaks obtained**	**Compound**	**Class**	**Reference**
1	447.1205	Kaempferol-3-O-(6"- malonyl-)-β-D-glucopyranoside	flavonoid	(Mekky et al., 2015)
2.	191.0580	Quinic acid	Cyclic polyol	(García-Salas et al., 2015)
3.	887.2861	Marruboside	phenylethanoid glycoside	(Amessis-Ouchemoukh et al., 2014)
4	463.0952	Quercetin 3-glucoside	Flavonoid	(Zhang and Zhu, 2015)
5	149.0619	Hydrastine;	Alkaloid	MassBank Record: BML00621
6	161.0258	Cistanoside D	Phenylethanoid (phenolic compound)	(Cui et al., 2017)
7	301.0026	Isorhamnetin3Oglucoside;	Flavonoid	MassBank Record: PR040095
8	515.1277	dicaffeoylquinic acid	Polyphenol	(Taşkın et al., 2017)
9	123.0461	Catechin	Flavonol	(Taşkın et al., 2017)
10	311.2275	13-Hydroxy-9-oxo-11-octadecenoic acidc	Fattyacid	(Zhang et al., 2014)
11	551.3140	Hypersampsone G	benzoylphloroglucinol	(Zeng et al., 2012)
Plant related compounds obtained at Positive mode				
12	209.0444	7,8-dihydroxy-6- methoxycoumarin (fraxetin)	O-methylated coumarin	(Sisó-Terraza et al., 2016)
13	274.2738	Soyasaponin III	Saponin derivative	(Lee et al., 2017)
14	739.1732	malyl-[α-Rha(1→6)-β-Glc]-	Flavonoid	(Fang et al., 2013)

15	175.0381	Isoerulic acid	Chlorogenic acid	(J. liang Geng et al., 2014)
16	207.0647	Geniposide	iridoid glycoside	(Liu et al., 2016)
17	461.2328	*4-Hydroxyartolakoochol*	flavonoid	(Sritularak et al., 2010)
18	535.3060	Ochrocarpinone	Coumarin	(Chaturvedula et al., 2002)
19	495.3054	Sarcinaxanthin;	Carotenoid	MassBank Record: CA000128
20	274.2738	Soyasaponin	Saponin derivative	(Lee et al., 2017)
21	149.0230	Gyrophobic acid	Polyphenolic compound	(Cornejo et al., 2016)

Table 90: Plant related compounds tentatively identified from LC/Q-TOF/MS analysis of ethanolic extracts of *Vetiveria zizanioides*

LC/Q-TOF/MS analysis of Ethanolic extracts of *Vetiveria zizanioides*				
Plant related compounds obtained at Negative mode				
Sl. No.	**Peaks obtained**	**Compound**	**Class**	**Reference**
1	111.2768	3'-O-didehydrolutein	carotenoid	MassBank Record: CA000074
2.	739.1930	3-*O*glucoside-rhamnoside-7-*O*-rhamnoside	Sugar derivative	(Oszmiański et al., 2015)
3.	191.0364	Asperuposide	Asperuloside	(Oszmiański et al., 2015)
4	395.1975	Akkummiline	Alkaloid	(Benayad et al., 2016)
5	125.0982	9,12,13-Trihydroxy-octadecadienoic acidc	Lipid derivative	(Zhang et al., 2014)
6	139.1124	13-Hydroxy-9-oxo-11-octadecenoic acidc	Fatty acid derivative	(Zhang et al., 2014)
7	269.0484	Apigenin 7-O-b-glucuronide	glycoside derivatives	(Li et al., 2014)
8	299.0595	Asteroidenone	Carotenoid	Asteroidenone
9	183.1408	9, 12-Dihydroxy- 10,13-epoxystaric acid	fatty acid	(Geng et al., 2014)
Plant related compounds obtained at Positive mode				
10	109.1008	Chaetoconvosin	Alkaloids	(Xu et al., 2015)
11	142.1222	pelletierine,	Alkaloids	(Suzuki et al., 2015)
12	301.0702	3_,4_,8-Trihydroxy-7-methoxyisoflavone	Flavonoid	(Goto et al., 2009)
13	279.1586	(*S*)-1′-Methylhexyl caffeate	Phenolic compunds	(Cabanillas et al., 2010)

Table 91: Plant related compounds tentatively identified from LC/Q-TOF/MS analysis of ethanolic extracts of *Euphorbia antiquorum*

LC/Q-TOF/MS analysis of Ethanolic extracts of *Euphorbia antiquorum*				
Plant related compounds obtained at Negative mode				
Sl. No.	**Peaks obtained**	**Tentative Compound**	**Class**	**Reference**
1	180.0611	N-acetyl glucosamine	Glucose derivative	(Blaas and Humpf, 2013)
2.	419.1311	(2S)-5,7,40-Trihydroxyflavan-5-Ob-D-glucopyranoside	Phenolic derivative	(Xin et al., 2017)
3.	163.0388	p-coumaroyl-o-galloyly-glucose	Phenolic compounds	(Fu et al., 1988)
4	193.0517	Ferulic acid	Phenolic compound	(Mekky et al., 2015)
5	371.0976	Dihydroferulic acid glucouronide	Phenolic compound	(Lang et al., 2013)
6	593.1492	Tectorigenin-7-O-xylosylglucoside	Flavonoid	(Lu et al., 2013)
7	319.0448	Myricetin	Polyphenolic compound	Pub Chem database CID 5281672
8	491.0822	Isorhamnetin-glucouronide	Polyphenolic compound	(Caseys et al., 2012)
9	197.0453	Syringic acid	Phenolic derivative	(Chen et al., 2015)
10	301.0349	quercetin	Polyphenolic derivative	(P. Yang et al., 2016)
11	447.0923	Kaempferol malonyl dihexoside	Flavonols	(Mekky et al., 2015)
12	187.0959	7-Hydroxy-10-deoxyeucommiol	iridiods	(Goudaa et al., 2003)

13	437.1080	Bellidifolin-8-O-β-D-glucopyranoside	Glycoside compound	(Jia et al., 2017)
14	269.1392	Rutin	Glycoside derivative	MassBank Record: MPR00105
15	343.0447	Quercetin-3-7-O-digluoside	Flavonod derivative	(Wang et al., 2015)
17	235.1690	Guignarderemophilane	Sesquiterpene	(Liu et al., 2015)
18	279.2322	Soyasaponin αg III	Saponin	(Mekky et al., 2015)
19	439.1768	Ugonin	flavonoid	(Huang et al., 2009)
20	471.3473	19α-Hydroxyursolic acid	Triterpene	(Hui Li et al., 2017)
Plant related compounds obtained at Positive mode				
22	161.1329	β-Cedrene	sesquiterpene	(Lee et al., 2014)
23	209.0705	Preussochromone F	Coumarin derivative	(Gonzalez-Menendez et al., 2017)
24	151.0747	benzoylpaeoniflorin	monoterpene glycosides	(Liu et al., 2014)
25	287.0552	Luteolin	Flavone	(Iqbal et al., 2017)
26	129.0545	Kaemfrritin	Flavanoid	MassBank Record: PR101009
27	303.0506	rutin	Glycoside	(Xu et al., 2016)
28	449.1076	Luteolin-8-C-glucoside	Polyphenol	(Ying Yang et al., 2016)
29	675.1529	8-Formylisorhamentin 3-O-robinobioside	Flavaonol	(Xin et al., 2017)
30	274.2745	protocatechuic acid	Polyphenol	(Zheng et al., 2017)
31	279.1590	Hyrtiosenolide	Sesqueterpene	(Youssef et al., 2004)

Present study was an attempt to characterize phyto-constituents, which are acting as coagulants / bioactive molecules in the stabilization / removal of selected quality parameters / contaminants from water. Statistically significant plant materials were subjected to various characterization studies like FT-IR, HPTLC and LC/Q-TOF/MS for determining the range of bioactive molecules responsible for treatment efficiency. Presence of various bioactive compounds is evident in all the three characterization studies.

FTIR analysis of all the five plants inferred the presence of functional groups characteristic to aromatic, polyphenolic compounds, tannins and gallic acid (Coates, 2000; Fernandez and Agosin, 2007; Geethu et al., 2014; Grasel et al., 2015).

Phyto-constituents belonging to the class of alkaloids and phenolic compounds were detected in *Terminalia chebula* (Hedina et al., 2016). Rathinamoorthy and Thilagavathi, (2014b) had reported the presence of tannin and ellagic acids in the plant material, which was concurrent with the HPTLC results obtained in the present study. Presence of steroid and saponins in *Terminalia chebula* has been reported by (Ram et al., 2015) and this was also parallel with the HPTLC results obtained in the present study. Confirmation of the presence of all secondary plant compounds, namely terpenoids, alkaloids, saponins, steroids, phenolic compounds obtained from HPTLC was confirmed with the LC/Q-TOF/MS analysis.

Whereas flavonoids (88.79%), phenolic compounds (85.90%), alkaloids (53.89%), steroids (25.78%), and tannic acid (24.01%) where found to be at higher percentage in dry fruits of *Terminalia bellirica* at various solvents and absorption maxima. Similar compounds like flavonoids, phenolics, alkaloids, steroids, and tannins in *Terminalia bellirica* have been reported (Abraham et al., 2014; Chandel et al., 2016; Devi et al., 2014; Tanaka et al., 2016). Compounds obtained from HPTLC include alkaloids,

derivatives of phenolic compounds, glycosides, polyphenolic compounds, terpenes and saponin derivatives. Presence of these compounds and their derivatives has been tentatively confirmed using LC/Q-TOF/MS analysis.

Roots of *Hemidesmus indicus* were detected with saponins (80.75%), alkaloids (100%), flavonoids (51.22%) and terpenoids (22.35%) at higher concentration in various solvents and absorption maxima. Presence of these compounds in the roots of *Hemidesmus indicus* have been mentioned in the reports of Manjulatha et al. (2014) and Seniya and Khan, (2012). Alkaloids, flavonoids, polyphenolic compounds, carotenoids, coumarins, saponins and their derivatives have also been tentatively identified in the LC/Q-TOF/MS analysis.

In the case of roots of *Vetiveria zizanioides*, alkaloids (78.98%), terpenoids (78.10%), tannic acidic (75.69%) and flavonoids (24.62%) were detected with higher area percentage at various solvents and absorption maxima in HPTLC analysis. Sonkusale, and Tale (2013) and Prajna et al., (2013) reported the presence of similar compounds in the roots of *Vetiveria zizanioides*. Alkaloids, flavonoids, phenolic compounds, carotenoids and their derivatives were tentatively identified using LC/Q-TOF/MS analysis.

From the stem of *Euphorbia antiquorum*, major plant components with higher peak area obtained at various solvents and absorption maxima were saponin (89.04%), tannic acid (75.79%), alkaloid (48.11%), phenolics (31.36%) and gallic acids (21.47%). Related compounds were identified in the stem of *Euphorbia antiquorum* (Anjaneyulu and Ravi, 1989; Sake et al., 2013). From LC/Q-TOF/MS analysis, polyphenoilc compounds, glyocoside derivatives, saponins, flavanols, coumarins, terpenes and their derivatives have been tentatively identified.

Most of the coagulation or related mechanisms occur by the electrostatic interaction of functional groups and contaminants / quality parameters in accordance with the charge they possess (Chang et al., 2009). From all the three phytochemical characterization studies, presence of secondary metabolites was evident. Major components detected belonged to alkaloids, flavonoids, polyphenols, tannins, terpenes, saponins, sugar derivatives, etc. Usually protein components were regarded to have the coagulation ability because of the charges they possess on their functional groups. Presence of functional groups, like -COOH and free –OH surface groups present on lipids, carbohydrates and alkaloids have reported to enhance the coagulation capability (Yin, 2010; Rao and Sastry, 1973). Further Adinolfi et al. (1994) have reported that a mixture of polysaccharide from galactomannan and galactan are capable of reducing 80% of turbidity from kaolin treated water. Clarification of muddy water by paste prepared from *Strychnos potatorum* seeds is due to the combined action of colloids and alkaloids present in the seeds. The albumin and other colloids sensitize the suspension and the coagulation is then caused by the alkaloid ions (Subbaramiah and Rao, 1936). It has also been reported that synergistic effects of sugars like arabinose, galactose, and rhamnose with galactouronic acid (sugar acid) also promotes coagulation (Choy et al., 2014). Polyphenolic compound with charged hydroxyphenyl groups can also promote coagulation because of the charge they possess. Similar synergistic effect might be the reason behind the coagulation efficiency of phyto-constituents from each of the plant material.

Prior to the utilization of above plant materials in the treatment of water quality parameters / contaminants, intensive studies on their phyto toxicity need to be carried out.

SUMMARY AND CONCLUSION

All the five plants, which are statistically significant in removing / adjusting selected water quality parameters / contaminants like pH, turbidity, iron and fluoride were characterized with the help of FT-IR, HPTLC and LC/Q-TOF/MS analysis. Compounds obtained were tentatively identified with the help of authentic samples, published data and data from computer library.

FT-IR analysis was carried out to preliminarily assess the various functional groups in the plant extracts. Characteristic peaks obtained were identified to be belonging to various classes of compounds like alkaloids, flavonoids, terpenes, tannic acid and polyphenols. Presence of these compounds was also well evident in HPTLC analysis. From LC/Q-TOF/MS analysis 22, 44, 21, 13 and 31 plant related compounds were identified from *Terminalia chebula, Terminalia bellirica, Hemidesmus indicus, Vetiveria zizanioides* and *Euphorbia antiquorum*. They were belonging to the class of alkaloids, flavonoids, terpenes, saponins and sterols. Hence these compounds are reported to have the coagulation proficiency, the property of all the five plant materials in stabilizing / removing the contaminants from water is supposed to be due to the synergistic effect of these phyto constituents characterized.

Prior to the utilization of above plant materials in the treatment of water quality parameters / contaminants, intensive studies on their phyto toxicity need to be carried out.

GENERAL SUMMARY AND CONCLUSION

Clean and safe water is a prerequisite for all living beings on Earth. It plays an important role in maintaining the socio economic stature of a nation. But with an increase in industries and population, water sector is facing severe crisis both in quantity and quality. Several water treatment techniques have been developed in the past centuries in this direction. For more than hundred years, chemical coagulants like alum, ferric chloride etc. is under use for water treatment and disinfection. But their continuous use is reported to cause several ill effects in humans. Excessive aluminum resulting from alum has been linked with Alzheimer's disease and other neurological disorders. Sludge produced from these chemicals is also a menace to environment. Moreover, most of these water treatment techniques are not affordable to undeveloped nations due to high capital cost.

It is the need of every nation to develop natural, cost effective and environment friendly water treatment practices to cater to the requirements of the people, especially living in the rural sector. Coagulation with the help of organic substances of plant and animal origin has been popular before the advent of chemical coagulants. Several plants have been under use in previous decades in water treatment owing to their coagulation efficiency. A lot more are still unexplored. Under these circumstances the present study has been proposed to screen out promising plants/parts which are effective in the coagulation of water contaminants.

In light of this, present study "Characterization of bioactive components in plants with respect to their efficacy in the treatment of selected water contaminants" suggests that plants / plant parts can be utilized as an

alternative mode of in home water purification by developing nations lacking the modern amenities. The study has been consolidated in two chapters.

- **Chapter I**

  Screening of plant/ plant products or derivatives with potential removal / stabilization properties against selected water contaminants / quality parameters like pH, turbidity, iron and fluoride.

- **Chapter II**

  Characterization of bioactive components from plant materials responsible for the removal of water contaminants.

- **Chapter I**

  Screening of plant/ plant products or derivatives with potential removal / stabilization properties against selected water contaminants / quality parameters like pH, turbidity, iron and fluoride.

Based on literature and traditional knowledge, two dozen plants were selected. They were collected from pollution free environments and identified with the help of standard keys and experts. Herbaria of identified specimens were maintained. Specimens from seeds, stem, roots, rhizome, whole plant etc. were taken for treatment studies.

Batch treatments were carried out with each of the plants/parts at varying dosage (0.5g, 1g, 2g and 4g) and varying retention time (1.5, 3, 6, 12, and 24HAT (Hours After Treatment))to find out their efficacy in removing /stabilizing selected water contaminants like pH (acidity and alkalinity), turbidity, iron and fluoride. Removal efficiency (in percentage) was calculated

against control and plants material with highest percentage removal was selected for further studies.

Out of the two dozen plants, dry fruits of *Phyllanthus emblica* (45.84%, 1g, 24HAT), dry seeds of *Terminalia chebula* (42.62%, 4g, 24HAT), seed kernel of *Mangifera indica* (35.47%, 4g, 12HAT), dry seeds of *Terminalia bellirica* (34.90%, 4g, 24HAT), cladode of *Euphorbia antiquorum* (31.89%, 2g, 6HAT) and leaves *Aloe barbadensis* (21.13%, 0.5g, 1.5HAT) were found to have highest capacity on stabilizing acidic pH. Similarly, in the case of alkaline pH, dry seeds *Phyllanthus emblica* (55.55%, 4g, 6HAT), dry seeds of *Terminalia bellirica* (46.15%, 4g, 12HAT), dry seeds of *Terminalia chebula* (44.09%, 4g, 12HAT), fruits of *Momordica charantia* (21.94%, 4g, 12HAT), leaves of *Azadirachta indica* (21.34%, 4g, 12HAT) and seeds of *Strychnos potatorum* (20.94%,4g, 24HAT) had highest alkaline pH stabilizing capacity.

In the case of turbidity, out of 24 plant screened, rhizome of *Lagenandra toxicaria* (66%, 4g, 6HAT), roots of *Hemidesmus indicus* (34.78%, 1g, 1.5HAT), fruits of *Abelmoschus esculentus* (33.62%, 0.5g, 24HAT), seeds of *Tamarindus indica* (33.62%, 0.5g, 24HAT) and seeds *Zea mays* (31.92%, 1g, 24HAT) were found to have maximum turbidity removal percentage.

Plant materials like roots of *Vetiveria zizanioides* (46.07%, 0.5g, 6HAT), leaves of *Mentha arvensis* (37.5%, 4g, 12HAT), seeds of *Tamarindus indicus* (36.92%, 0.5, 12HAT), seeds of *Phyllanthus emblica* (35.57%, 4g, 1.5HAT) and fruits of *Cyamopsis tetragonoloba* (22.07%, 0.5g, 1.5HAT) were found to be efficient in removing iron.

In the case of fluoride removal, cladode of *Euphorbia antiquorum* (78.57%, 0.5g, 24HAT), roots of *Vetiveria zizanioides* (69.69%, 4g, 24HAT),

leaves of *Aloe barbadensis* (73.33%, 2g, 1.5 HAT) and dry seeds of *Terminalia bellirica* (68.51%, 2g, 3HAT) was found to have effective removal percentages.

Three plant materials with highest removal percentages were selected for further studies. Accordingly *Phyllanthus emblica, Terminalia chebula,* and *Mangifera indica* were selected for stabilizing acidic pH. *Phyllanthus emblica, Terminalia bellirica,* and *Terminalia chebula* were selected for stabilizing alkaline pH. In the case of turbidity, *Lagenandra toxicaria, Hemidesmus indicus* and *Abelmoschus esculentus* were chosen. Similarly *Vetiveria zizanioides*, *Mentha arvensis* and *Tamarindus indicus* were selected for removing iron from water. Fluoride was found to be removed by the plants *Euphorbia antiquorum, Vetiveria zizanioides* and *Aloe barbadensis*.

Upon statistical analysis in the case of pH, *Terminalia chebula* was found to be significant for acidic pH at varying dosages (F value 116.835; p value <0.001) and retention time (F value-23.409; p value<0.001), likewise *Terminalia bellirica* was found to be significant at alkaline pH at varying dosages (F value-54.476; p value<0.001) and retention time (F value-30.749; p value<0.001). Turbidity removal of *Hemidesmus indicus* was found to be significant at varying dosages (F value-7.648; p value <0.001) and retention time (F value – 40.418; p value<0.001). In the case of iron removal, *Vetiveria zizanioides* was found to be significant at specific dosages (F value – 3.124; p value <0.05) and retention time (F value – 66.345; p value <0.001). *Euphorbia antiquorum* was found to be significant in removing fluoride at varying dosages (F value- 0.335, p value>0.05) and retention time (F value -22.90; p value<0.001).

Out of 24 plants screened for their efficiency in removing selective water contaminants / water quality parameter, three plants each with higher removal / stabilization efficiency against each of the selected water

contaminants namely, pH, turbidity, iron and fluoride were selected. Statistically significant plants / plant parts, namely *Terminalia chebula, Terminalia bellirica, Hemidesmus indicus, Vetiveria zizanioides* and *Euphorbia antiquorum* were characterized obtain phyto-constituents.

❖ **Chapter II**

Characterization of bioactive components from plant materials responsible for the removal of water contaminants.

Soxhlet extraction of the plant / parts with highest treatment efficacy with respect to each parameters were carried out with polar, moderately polar and non polar solvents, namely petroleum ether, chloroform and ethanol.

For characterizing the phyto constistuents from plant materials, FT-IR, HPTLC and LC/Q-TOF/MS were performed. Infrared analysis of crude extracts revealed several characteristic peaks for carbonyl, aldehyde, hydroxyl groups etc. which corresponds to various phyto-constituents. HPTLC revealed the presence and subsequent quantification of the phyto constituents like flavonoid, alkaloid, saponins, terpenoids, steroids etc. in the plant extracts. Tentative compounds were identified by comparing the Rf value with the previously published data. Likewise LC/Q-TOF/MS of ethanolic extracts of five plants gave 22, 44, 21, 13 and 31 compounds for *Terminalia chebula, Terminalia bellirica, Hemidesmus indicus, Vetiveria zizanioides* and *Euphorbia antiquorum* respectively. From the result of characterization, it is evident that similar compounds have been detected in both HPTLC and LC/Q-TOF/MS results. Major components quantified from HPTLC include Tannin (44.86%), Flavonoid (88.79%), Alkaloid (84.62 and 78.98%) and Saponin (89.04%) from *Terminalia chebula, Terminalia bellirica, Hemidesmus indicus, Vetiveria zizanioides* and *Euphorbia antiquorum* respectively. Hence it is assumed that individual or synergistic effects of these

secondary metabolites might have contributed to the treatment efficacy of contaminants of water.

GENERAL CONCLUSION

Two dozen plants have been screened for their activity in removing /adjusting selected water quality parameters like pH (Acidic and alkaline), turbidity, iron and fluoride. Three plants which showed better efficiencies in the removal of selected water contaminants / water quality parameter were further subjected to secondary screening. Statistically significant plant materials were characterized for their bioactive components. Characterization of the five (*Terminalia chebula, Terminalia bellirica, Hemidesmus indicus, Vetiveria zizanioides* and *Euphorbia antiquorum*) plants which are effective in the removal of specific contaminants revealed the presence of alkaloid, flavonoids, terpenoids, tannic acid, polyphenolic compounds and saponins in major quantities. It can be assumed that the treatment efficacy associated with these plants with respect to various water contaminants can be due to the presence of the secondary metabolites.

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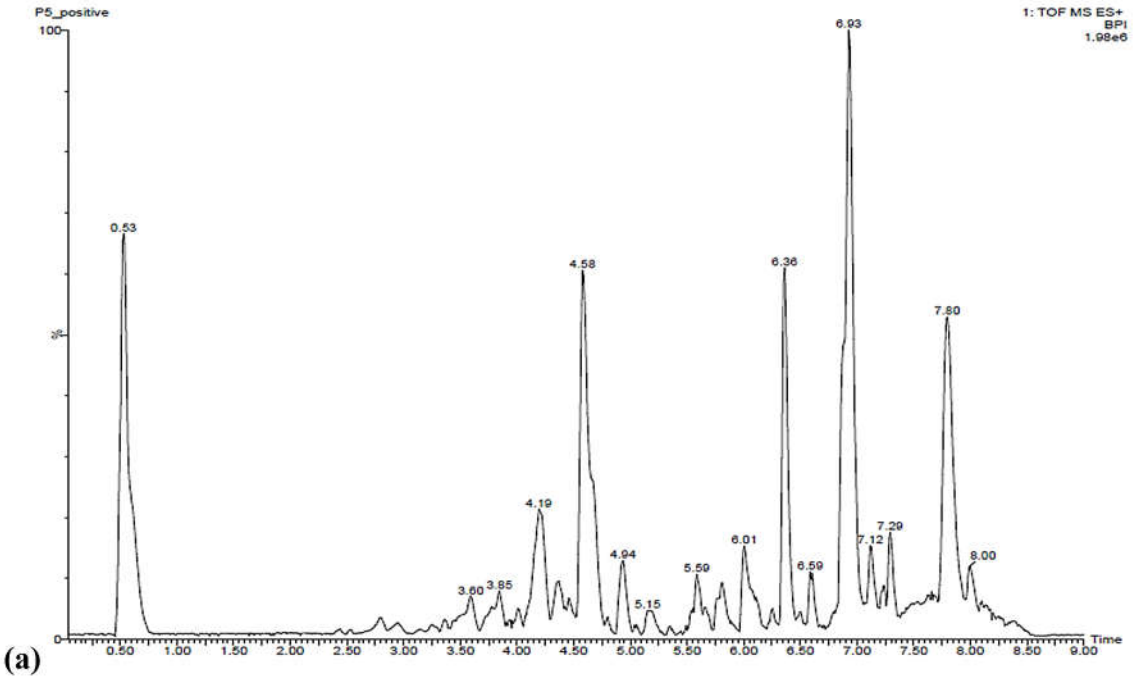

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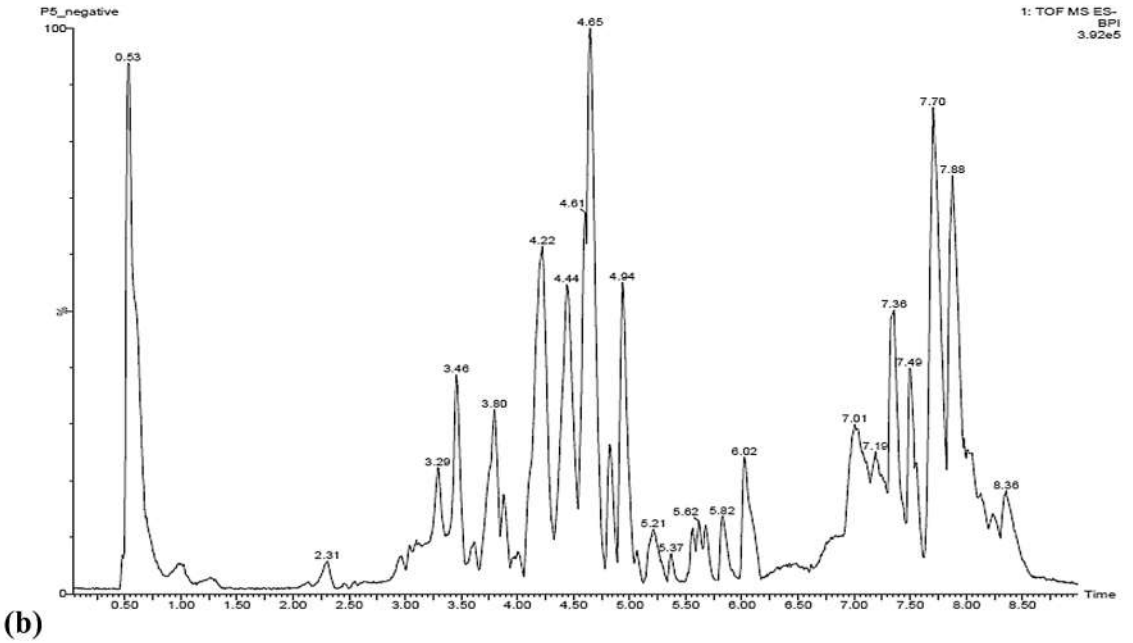


(b)

Plate 5e: Total chromatogram of of ethanolic extracts of stem *Euphorbia antiquorum* at (a) positive and (b) negative mode after LC/Q-TOF/MS analysis.

Plate 1a: Plant / plant parts selected for stabilizing/coagulation studies
(a) *Abelmoschus esculentus* (Malvaceae); (b) *Aloe barbadensis* (Liliaceae); (c) *Azadirachta indica* (Meliaceae); (d) *Bacopa monnieri* (Scrophulariaceae); (e) *Cyamopsis tetragonoloba* (Leguminosae); (f) *Euphorbia antiquorum* (Euphorbiaceae); (g) *Hemidesmus indicus* (Asclepiadaceae); (h) *Lagenandra toxicaria* (Araceae); (i) *Mangifera indica* (Anacardiaceae); (j) *Mentha arvensis* (Lamiaceae); (k) *Momordica charantia* (Cucurbitaceae); (l) *Musa* X *paradisiaca* (Musaceae).

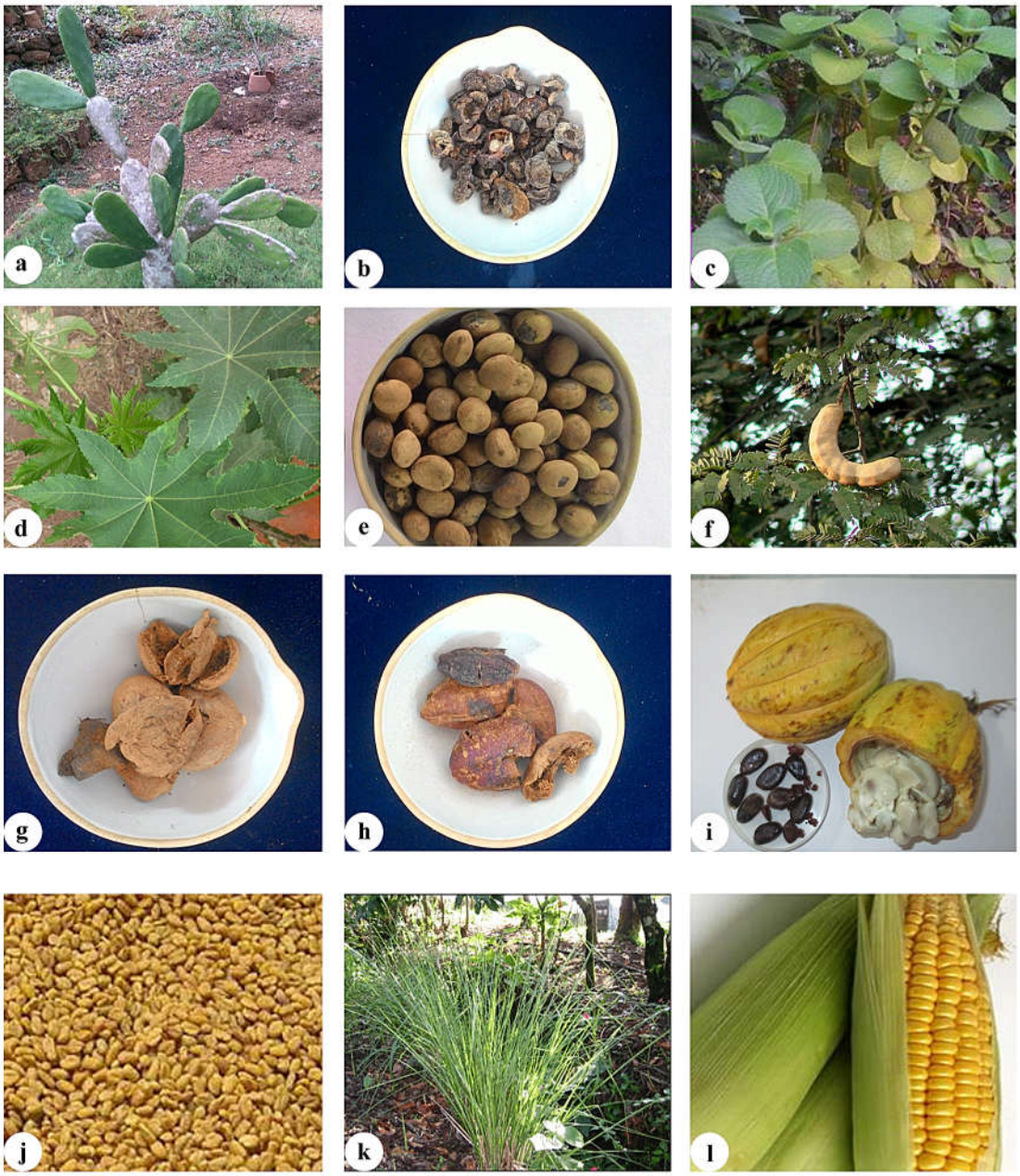


Plate 1 (b): Details ofdetails of plants used for the study
(a) ***Opuntia dillenii*** **(Cactaceae); (b)** ***Phyllanthus emblica*** **(Euphorbiaceae); (c)** ***Plectranthus amboinicus*** **(Lamiaceae); (d)*Ricinus communis*** **(Euphorbiaceae); (e)** ***Strychnos potatorum*** **(Loganiaceae); (f)*Tamarindus indica*** **(Leguminosae);(g)*Terminalia bellirica*** **(Combretaceae); (h)*Terminalia chebula*** **(Combretaceae); (i)*Theobromo cacoa*** **(Malvaceae); (j)** ***Trigonella foenum-graecum*** **(Leguminosae); (i)*Vetiveria zizanioides*** **(Poaceae); (j)** ***Zea mays*** **(Poaceae)**

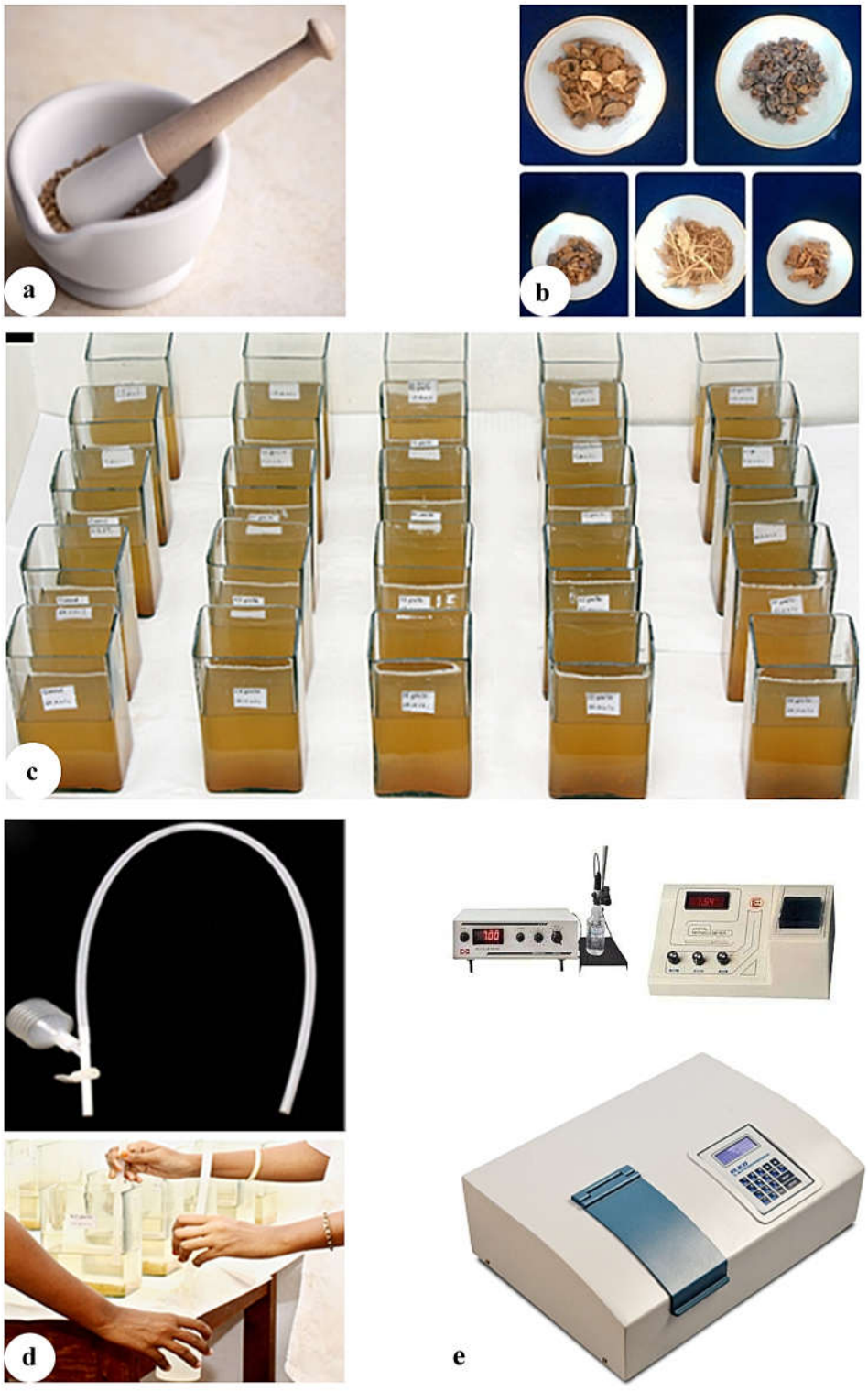


Plate 2: Experimental lay out (a) Mortar and pestle; (b) Processing of plant materials; (c) Experimental set up; (d) Collection of water sample; (e) Analysis of water sample.

(a) *Terminalia chebula* Retz.
Kingdom: Plantae
Order: Myrtales
Class: Magnoliopsida
Family: Combretaceae

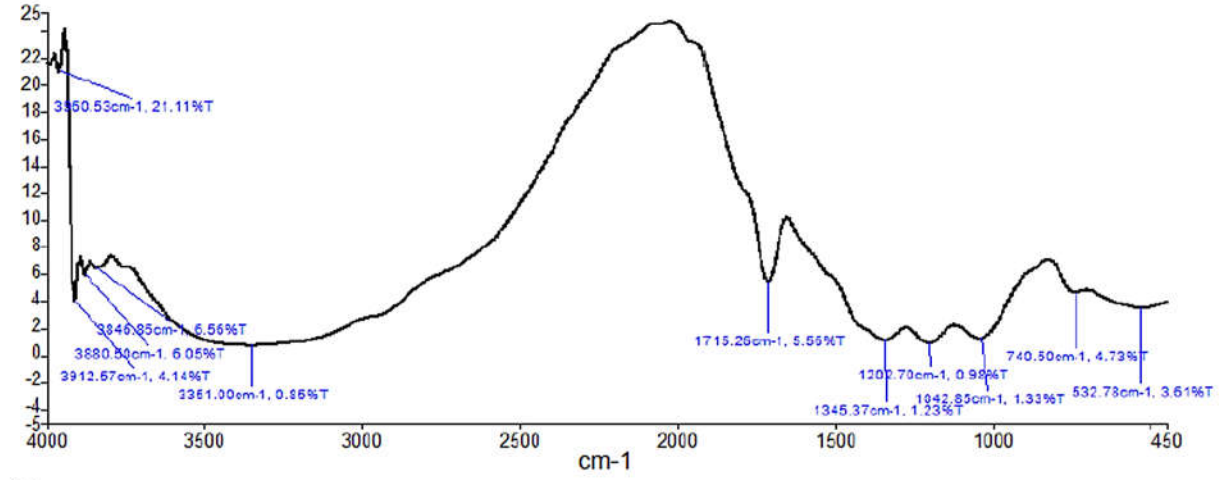
(b) *Terminalia bellirica* Roxb.
Kingdom: Plantae
Order: Myrtales
Class: Magnoliopsida

(c) *Hemidesmus indicus (L.) R. Br.*
Kingdom: Plantae
Order: Gentianales Juss.ex Bercht. & J.Presl
Class: Equisetopsida

(d) *Vetiveria zizanioides* (Linn.) Nash
Kingdom: Plantae
Order: Poales small
Class: Equisetopsida C. Agardh
Family: Poaceae

(e) *Euphorbia antiquorum L.*
Kingdom: Plantae
Order: Malpighiales
Class: Equisetopsida C. Agardh
Family: Euphorbiaceae

Plate 3: Systematic position of plant parts used for characterization



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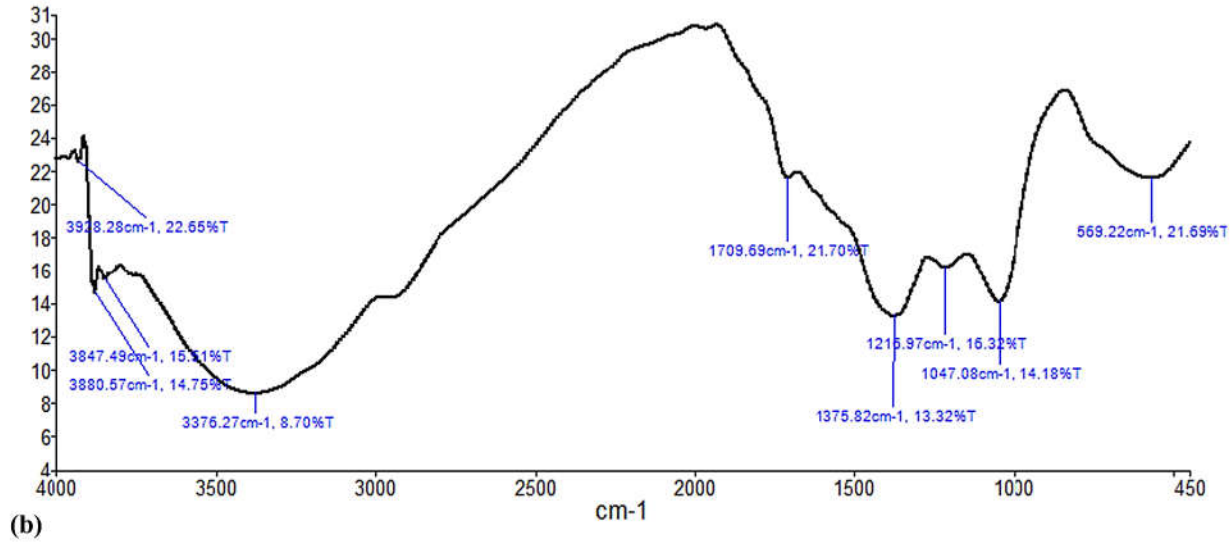

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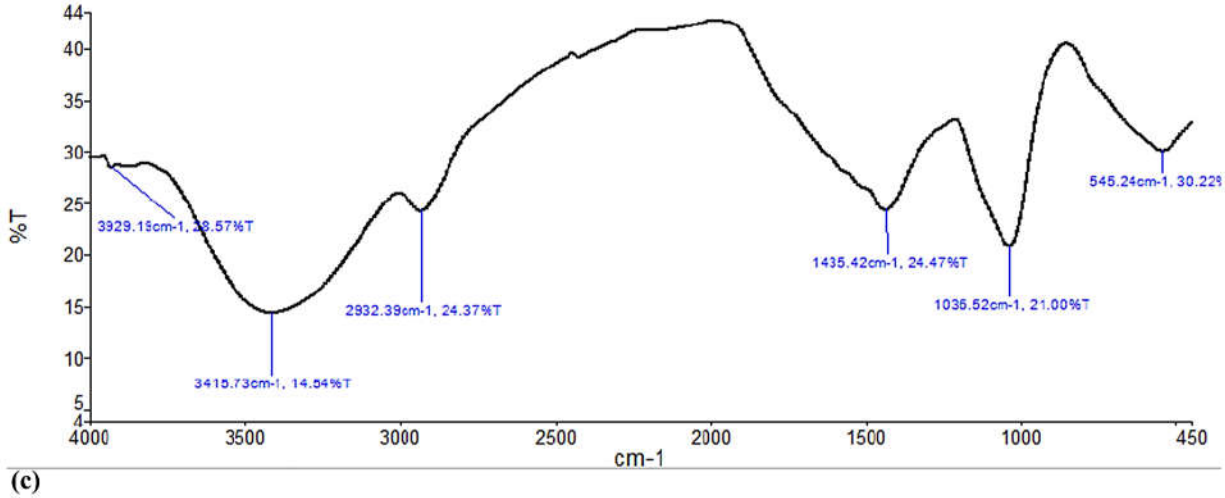
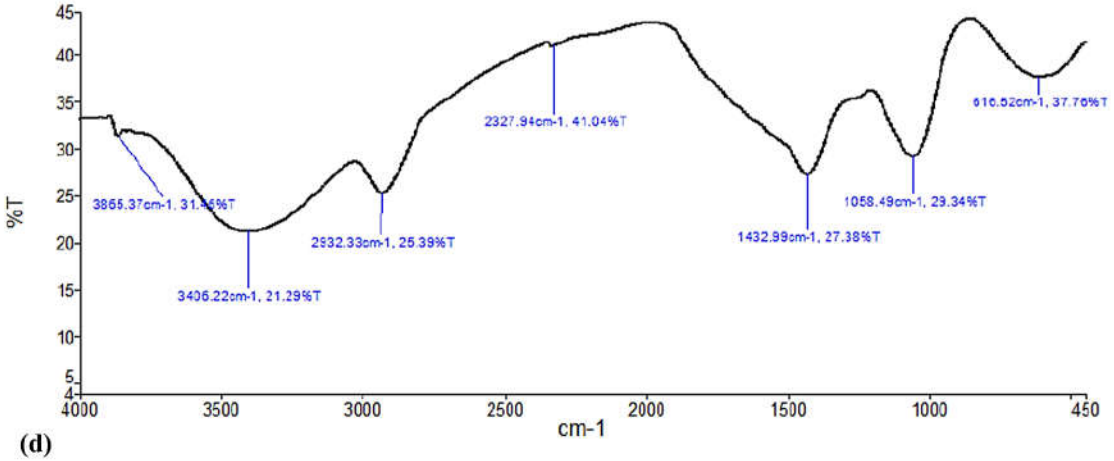
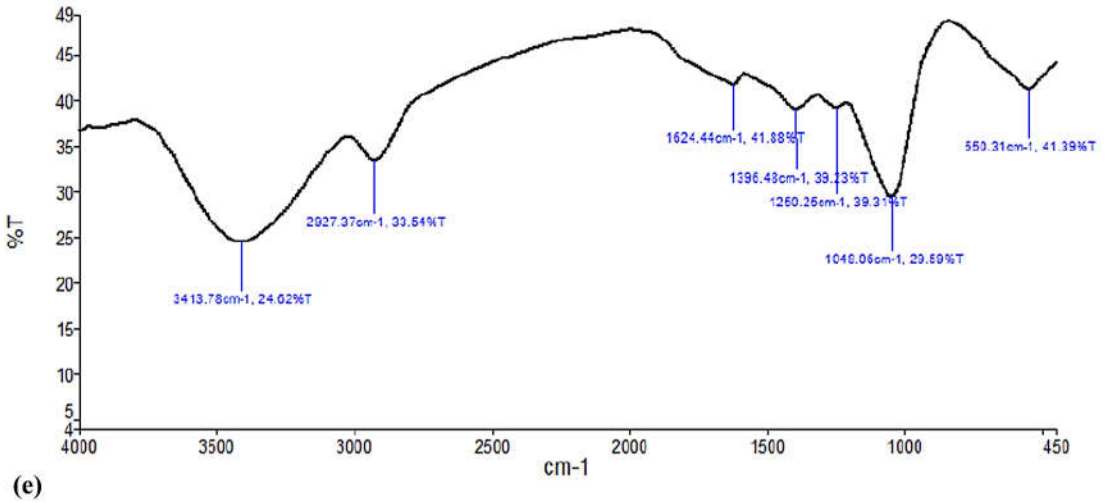


(c)

Plate 4a: Characteristic peaks obtained for dry fruits of (a) *Terminalia chebula* and (b) *Terminalia bellirica;* and roots of (c) *Hemidesmus indicus* after FT-IR analysis.

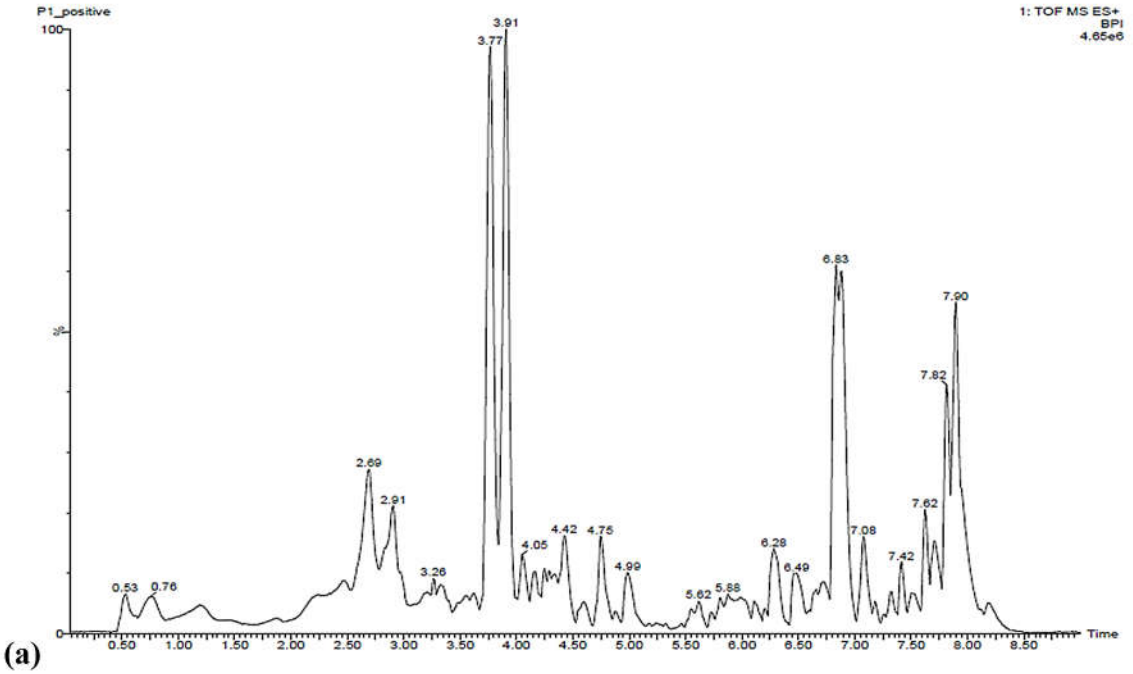


(d)

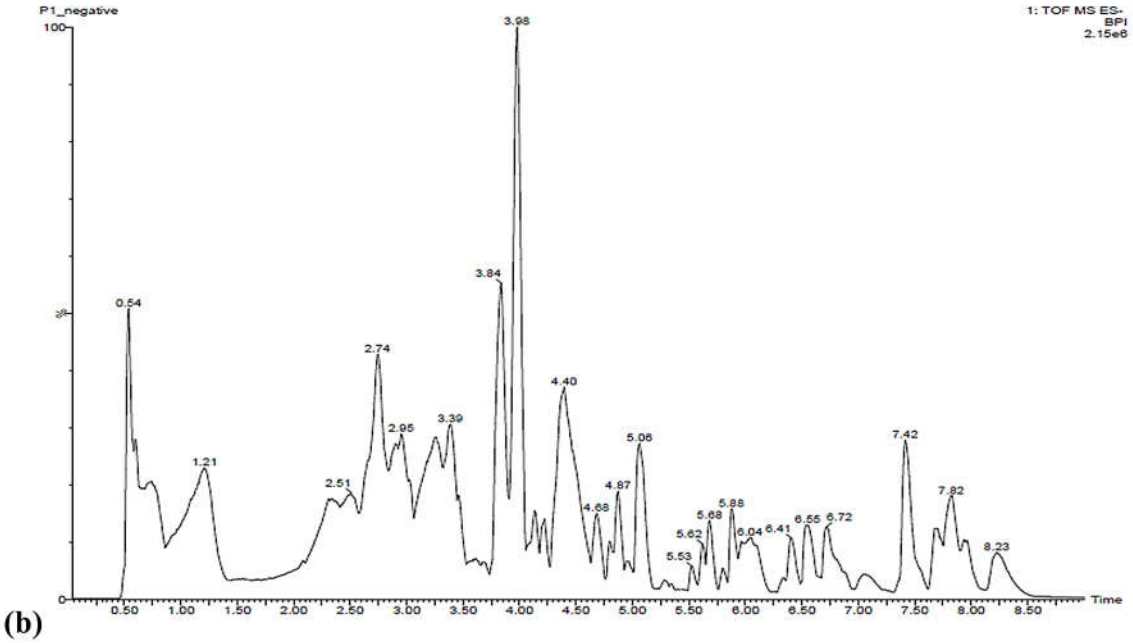


(e)

Plate 4b: - Characteristic peaks obtained from roots of (d) *Vetiveria zizanioides* and stem of (e) *Euphorbia antquorum* after FT-IR analysis.



(a)



(b)

Plate 5a: Total chromatogram of ethanolic extracts of dry fruits of *Terminalia chebula* at (a) positive and (b) negative mode after LC/Q-TOF/MS analysis.

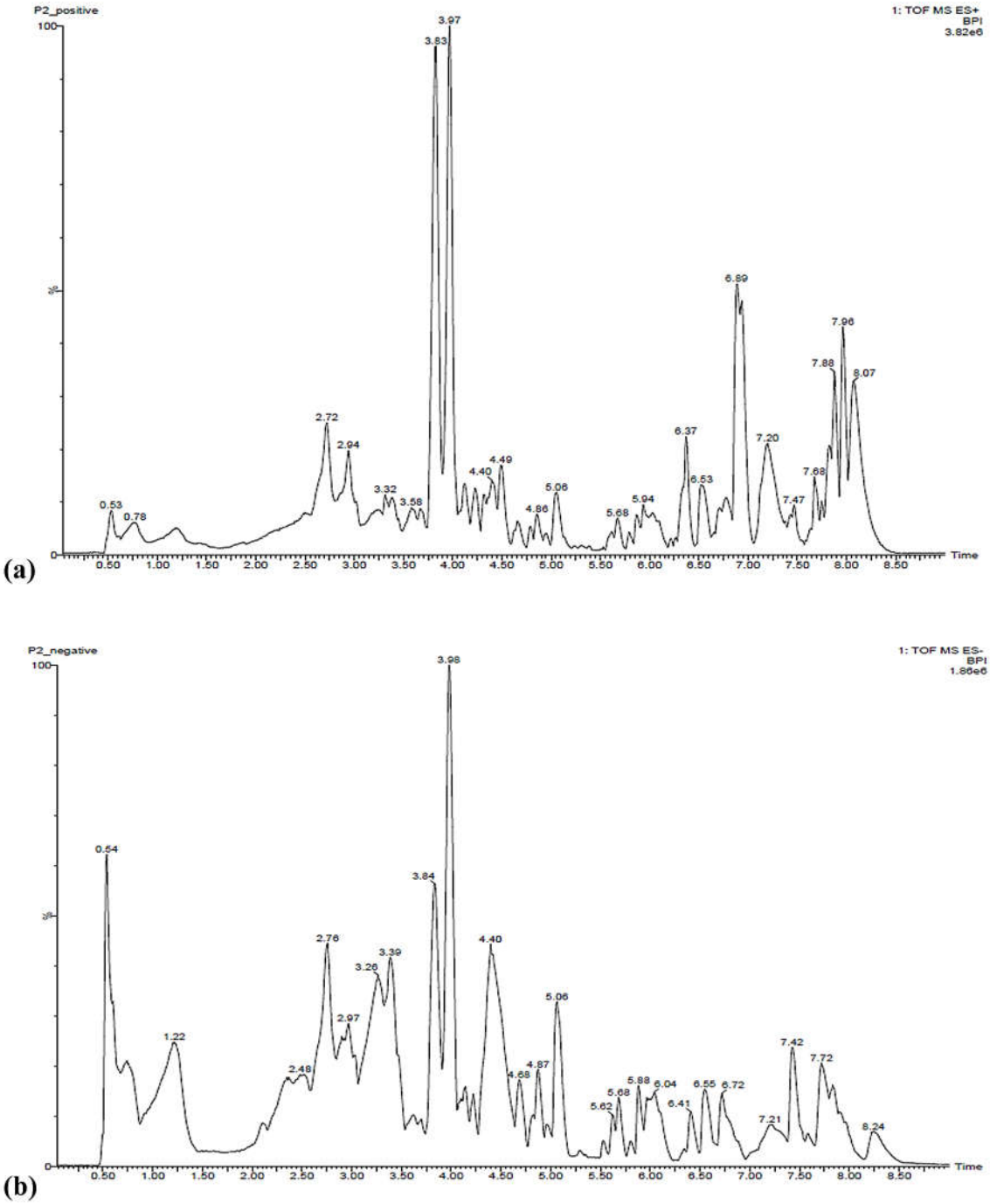
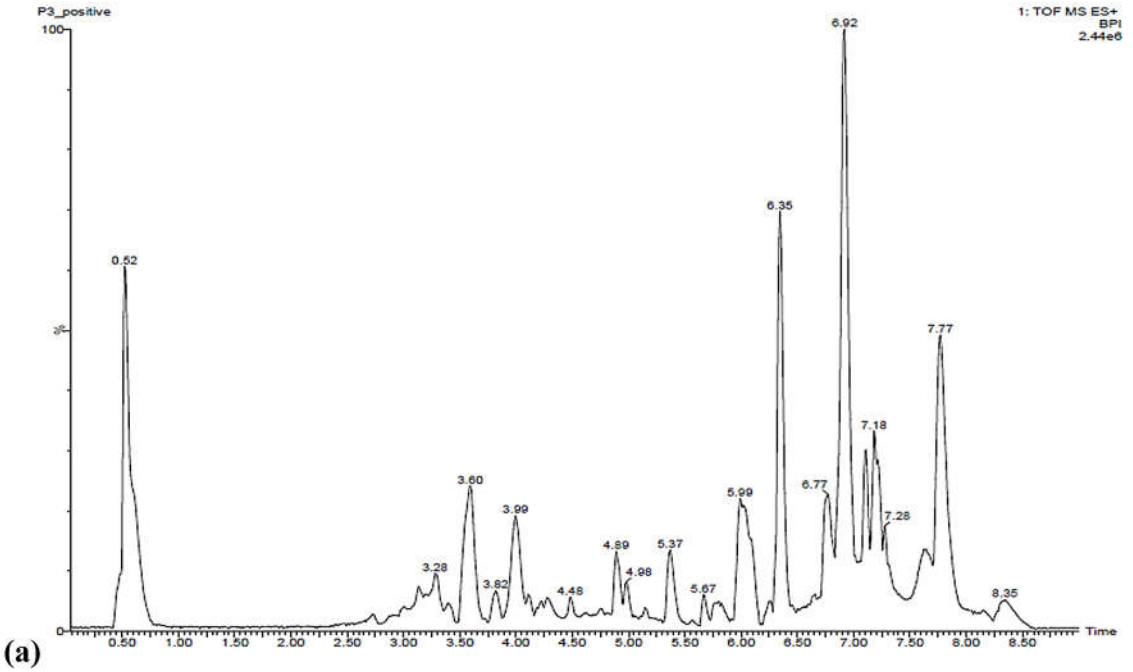
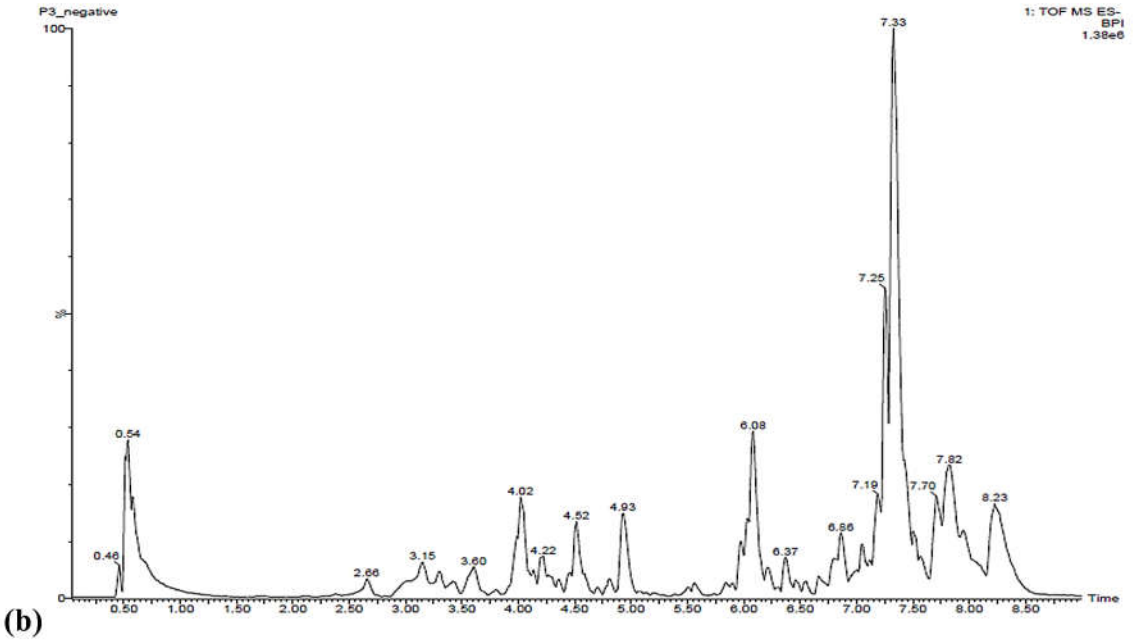


Plate 5b: Total chromatogram of ethanolic extract of dry fruits of *Terminalia bellirica* at (a) positive and (b) negative mode of ethanolic extracts after LC/Q-TOF/MS analysis.



(a)



(b)

Plate 5c: Total chromatogram of ethanolic extracts of roots of *Hemidesmus indicus*at (a) positive and (b) negative mode after LC/Q-TOF/MS analysis.

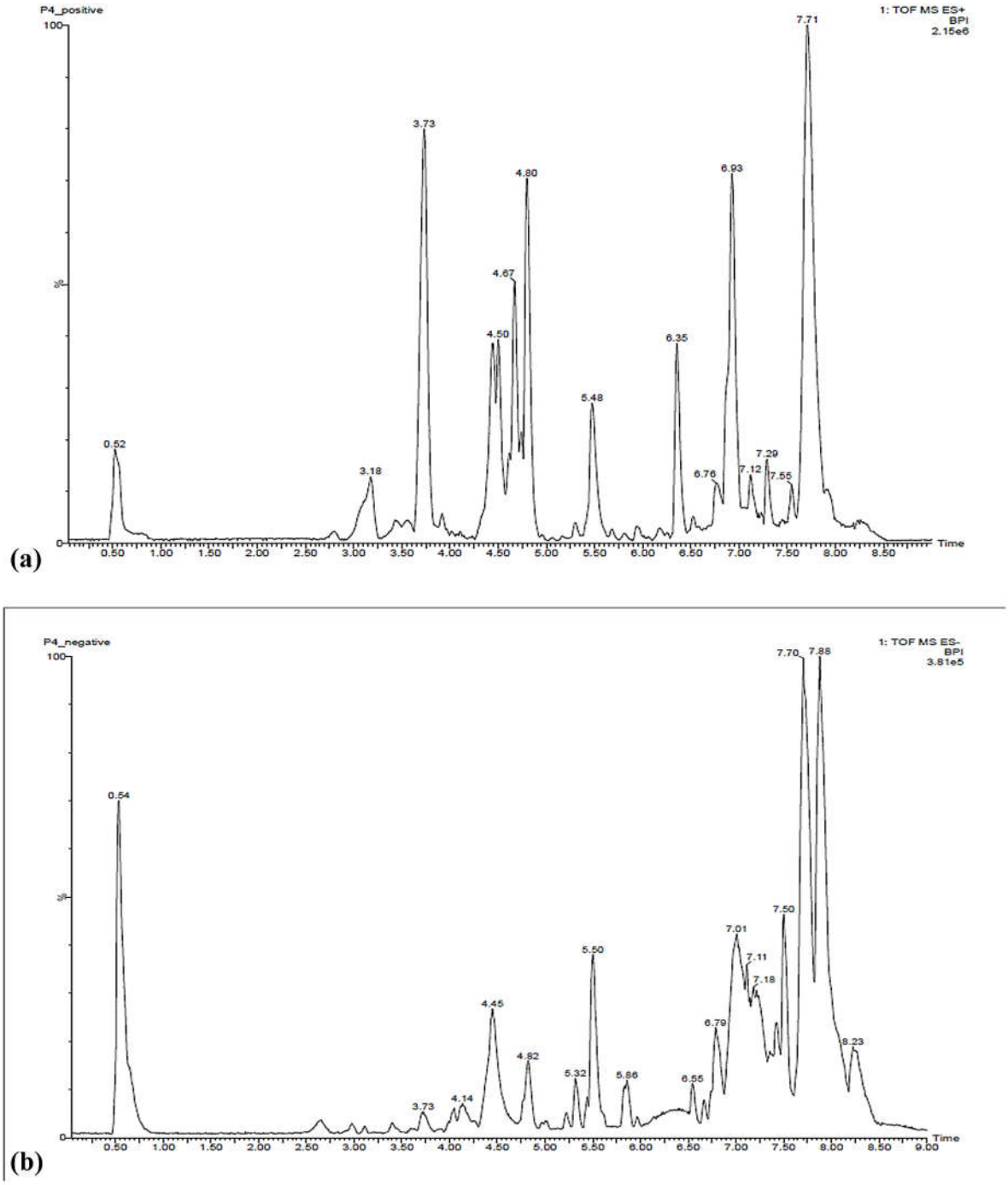


Plate 5d: Total chromatogram of ethanolic extracts of roots of *Vetiveria zizanioides* at (a) positive and (b) negative mode after LC/Q-TOF/MS analysis.