PHYTO-CHEMICAL STUDIES OF SELECTED PLANTS AS A SOURCE OF BIO-COAGULANT AGENT FOR WATER PURIFICATION

ty 'kf)dj.k gr to&LdUnd d L=kr d #i e p;fur iknik dk to&jklk;fud v/;;u

A THESIS

Submitted for the award of

DOCTOR OF PHILOSOPHY

In the faculty of Science

Of

UNIVERSITY OF KOTA, KOTA

by

Satish Kumar Sharma

Under Supervision

Of

Dr. Neerja Shrivastava
Associate Professor (Botany)
Department of Botany,
Government P.G. College, Kota (Raj.)

UNIVERSITY OF KOTA, KOTA
2018

Declaration

I, hereby, certify that the work, which is being presented in the thesis, entitled **"Phyto-Chemical Studies of Selected Plants as A Source of Bio-Coagulant Agent for Water Purification"** in partial fulfillment of the requirement for the award of the Degree of Doctor of Philosophy, carried under the supervision of **Dr. Neerja Shrivastava** and submitted to the University of Kota, Kota represents my ideas in my own words and where others ideas or words have been included. I have adequately cited and referenced the original sources. The work presented in this thesis has not been submitted elsewhere for the award of any other degree or diploma from any Institutions. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will cause for disciplinary action by the University and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Date : …….. (Satish Kumar Sharma)

This is to certify that the above statement made by **Satish Kumar Sharma** Enrolment No. UOK/2013/15598 is correct to the best of my knowledge.

Date : ……..

(Dr. Neerja Shrivastava)
Associate Professor,
Deptt. of Botany
Govt. P.G. College, Kota

CERTIFICATE

I feel great pleasure in certifying that the thesis entitled **"Phyto-Chemical Studies of Selected Plants as A Source of Bio-Coagulant Agent for Water Purification"** by **Satish Kumar Sharma** under my guidance. He has completed the following requirements as per Ph. D regulations of the University.

(a) Course work as per the university rules.

(b) Residential requirements of the university (200 days)

(c) Regularly submitted annual progress report.

(d) Presented his work in the departmental committee.

(e) Published/accepted minimum of one research paper in a referred research journal.

I recommend the submission of thesis.

Date : ……

(Dr. Neerja Shrivastava)
Associate Professor,
Deptt. of Botany
Govt. P.G. College, Kota

"A Words of Gratitude"

Though this thesis bears my name, the main spirit and dynamic force behind this work is the brilliant genius of my esteemed guide.

Dr. Neerja Shrivastava
Associate Professor,
Department of Botany,
Govt. P.G. College, Kota

I consider myself to be extremely lucky having worked under such a perfect and dynamic teacher. It is only because of her unfailing interest, constant inspiration, simplicity, wisdom, understanding, sympathy, logical approach and stimulating criticism that provided me impetus of this work.

I shell ever remain grateful for the encouragement which my able and affectionate guide gave me at every stage of my work. Words are poor medium for expression of thanks to my respected guide.

To her, I express much more than a mere sense of gratitude. I shall always gratefully cherish that sweet memory of the tender guardship for years to come. It is proud privilege to work under such a delightful and pleasant personality.

Best regards,

Satish Kumar Sharma

Acknowledgement

All the praise and respects to Almighty GOD who is very kind and merciful. I am obligated to pay my heart rendering thanks and gratitude to my respected supervisor **Dr. (Ms.) Neerja Srivastava**, Lecturer, Department of Botany, Govt. PG College Kota, Kota (Raj.), whose unremitting concentration, highly courteous behavior, scholastic guidance and valuable advices in planning and execution of the present research work and completion of the thesis. Without their sincere inspiration and keen interest, the completion of this work would have been difficult.

I have no words to express my deepest sense of gratitude and innumerous thanks to **Dr. (Mrs.) Rashmi Pant,** Head, Department of Botany. I am obligated to express my thanks to all the lecturers of Department of Botany, Govt. PG College Kota, Kota (Raj.) for their continuous encouragement and provision of all possible facilities to conduct this study.

I am very thankful to **Late Dr. L. K. Dadhich, Ex-Principal, JDB Govt. College, Kota (Raj.)** for their interest, technical guidance and support.

It will be unfair, if I don't mention the paramount cooperation and help of **Dr. (Mrs.) Pallavi Sharma, Mr. G. L. Tapariya, Shirin Akther, Anju Rani and Mr. Ravi Shringi** to fulfill this goal. At the same time, I am also thankful to all laboratory staff for assisting analytical works available at Department.

With humble regards, I also offer my deepest indebtedness to my honorable grandfather **Sh. Kalyanmal Sharma** and grandmother **Late Smt. Kamla Devi** for their prayers and continuous spiritual help during the course of the study.

I always feel freshness in life due to the love, entreaties and encouragement of my mother **Smt. Mohini Sharma** as well as inspiration from the reminiscences and elevated wishes of my father **Sh. R. N. Sharma** about my carrier. It really gave me strength to complete my research work with absolute confidence. I am grateful indebted to my other family members **Sh**. **K.C. Sharma** (Guruji), **Sangeeta Sharma, Sh. Arvind Sharma, Sarita Sharma, Sunil Sharma, Sunita Sharma, Gopal Sharma, Unnati Sharma.** I am also fortunate to have had the support of my life-mate **Mrs. Meenakshi Sharma**. Sweet thank is also for my son **Reyansh**.

At last but not least, I am grateful to all my friends and colleagues or their good wishes and ready help whenever needed.

Merely ending the acknowledgements here would be devoiding others of the credit who have remained behind the scene and always prayed for the successful completion of this. Even though the collective shoulders of all these kind persons are broader than time, I alone must take responsibilities of all errors and shortcomings.

Date :

Place : Kota

Satish Kumar Sharma

LIST OF ABBREVIATIONS

- **BOD** : Biochemical Oxygen Demand
- **CFU** : Colony-Forming Units
- **DO** : Dissolved Oxygen
- **GC-MS** : Gas Chromatography Mass Spectrometry
- **GUI** : Graphical User Interface
- **MPN** : Most Probable Number
- **NCBI** : National Center for Biotechnology Information
- **NTU** : Nephelometric Turbidity Unit
- **PDB** : Protein Data Bank
- **PPM** : Parts Per Million
- **WHO** : World Health Organization

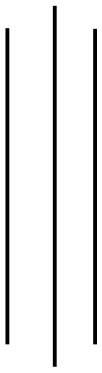
LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

CONTENTS

CHAPTER – 1



INTRODUCTION

INTRODUCTION

Water is one of the most important and abundant compounds of the ecosystem. All living organisms on the earth need water for their survival and growth. Water is a ubiquitously chemical substance vital to all known forms of life. In nature water exists in liquid, solid and gaseous states. Larger amount of water is present on the earth about three-quarters of the earth surface is covered with water occupying around 97% as seawater and 3% as fresh water. Around two-third of fresh water is icebergs and glaciers. Availability of fresh water for our daily life is only 0.8% of the total amount of water present on earth.

Water is a colorless, tasteless and odorless transparent liquid at ambient temperature. Water is a good solvent it is often called as the universal solvent. The polarity of water is an important factor in determining its solvent properties. Water dissolves most of inorganic substances and some organic substances having ionic bonds by dissociating and hydrating them. Uses of water comprise agricultural, industrial, household and environmental activities.

Drinking water is a vital resource for all aspects of human beings. Access to safe and clean drinking water is a major concern throughout the world. Ground water surface water and rainwater are often the major sources of water in a community. Ground Water is often the most appropriate source of water for drinking as long as it does not contain high mineral content. Ground water could be extracted through wells or bore holes. Surface Water requires treatment to make it safe for human consumption. Surface water is

almost always contaminated by people and animals who defecate in or near the water. Rain water is pure it can be collected in large storage basin or smaller containers. However rain water collected in dirty or unclean containers have to be treated to make it safe for drinking.

Natural waters occurring in the environment are not chemically pure waters. While circulating in the environment water contacts with atmosphere, rocks and soil. Due to physical, chemical and biological processes water passing through the ground undergoes purification. Physical processes include dilution, coagulation, precipitation and adsorption. Chemical processes include degradation, oxidation and hydrolysis while biological process includes biodegradation.

Natural water contains either inorganic or organic compounds. The quantity of inorganic compounds dissolved in natural waters is differentiated. Rainwater may contain as little as a few milligrams of dissolved matter is derived from different sources such as sulphate, sulphur dioxide etc. After falling onto the land the inorganic content of the water increased. Natural waters contain organic matter including decaying matter and industrial pollutents. The naturally dissolved organic matter is transformation biological products of proteins, amino acids, fats, sugar etc.

Due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity wateris highly polluted with different harmful contaminants. Therefore it is necessary that the quality of drinking water should be checked at regular time interval, because due to use of contaminated drinking

water, human population suffers from varied of water borne diseases. Contaminated water is a very turbid liquid with an offensive smell in most cases. Its composition varies from large floating or suspended solids to smaller suspended solids, very small solids in colloidal form due to microbes and chemical pollutants. The quantity of organic matter present in waste water determines the strength of waste water. The different components of waste water are of primary importance as it plays a crucial role in the design of the treatment plant. Waste water generally contains biological components including pathogenic organisms mostly of faecal origin and non-biological substances such as organic and inorganic compounds. Organic components include carbohydrates, protein and fats while inorganic components includes salts and metals.

A large numbers of pollutants can impart colour, tastes and odors of water thus making them unaesthetic and even unfit for domestic consumption. The changes in oxygen, temperature and pH affect the chemical property of waters often triggering chemicals reactions resulting in the formation of unwanted products. The addition of organic components results in depletion of oxygen. The direct addition of nutrient through various sources enhances the algal and other biological growth which further depletes the oxygen. The decomposition of excessive organic matter results in odorous unaesthetic condition due to accumulation of several obnoxious gasses like ammonia, hydrogen sulphide and methane. The algal photosynthesis consumes carbon dioxide and increases pH of the water due to formation of carbonates which gets precipitated as calcium carbonate often co-precipitating phosphorus with it.

However, pH can fall at the time of higher rate of organic matter decomposition and low phosphothetic activity which can bring back the precipitated calcium carbonate is solution from the sediments.

Producing potable water from surface water or ground water usually involves one or several treatment steps for removing unwanted substances. WHO estimates that about 85 percent of the rural population lack potable drinking water. About 80 percent of illnesses in developing countries are directly connected with contaminated drinking water. Reports also indicate that 90% of water in India is polluted. It has been estimated that 1.2 billion people do not have clean and safe drinking water. About 4 millions children die every year from water-borne diseases in India. According to a report of Indian Toxicology Research Center about 8,000 cases of cholera, 1 million cases of gastroenteritis and 7 million cases of dysentery were reported annually. Need of water treatment process is so important that we can avoid many possible water borne diseases like diarrhea, dysentery, amoebiassis, hepatitis, typhoid, Jaundice, cholera and so on. Water borne infections are responsible for more than 80% of the diseases in all over the world. Water quality is of concern to everyone. To control these diseases water needs to be purified in order to make it safe for human consumption.

Water is recognized as in important aspect in transmission of many diseases It is, therefore, of great importance to remove the pathogenic organisms from drinking or polluted water. Polluted waters especially those polluted by domestic sewage and discharge from hospitals and slaughterhouses etc., are effective source of infectious diseases. Water contains great number of bacteria, many of

which have important role in diseases causing. Pathogenic organisms from waste can be broadly classified as bacteria, fungi, viruses, protozoa and helminthes.

Water-borne bacterial disease (Table-1) cause a wide range of syndromes including: acute dehydrating diarrhea (cholera), prolonged febrile illness with abdominal symptoms (typhoid fever), acute bloody diarrhea (dysentery) chronic diarrhea (Brainerd diarrhea) food poisoning, botulism and anthrax.

Table : 1. Water Borne Bacterial Diseases

S. No	Disease	Pathogen	Rout of exposure	Mode of transmission
1.	Cholera	*Vibrio cholera*	Gastrointestinal	Often water borne
2.	Botulism	*Clostridium botulinum*	Gastrointestinal	Water borne
3.	Dysentery	*Shigella dysenteriae*	Gastrointestinal	Water borne
4.	Typhoid	*Salmonella typhi*	Gastrointestinal	Water borne
5.	Colon infection	*E. coli*	Gastrointestinal	Water borne

Water-borne bacterial infection accounts of diarrhea resulting in 1-2 million deaths per year. The death tends to be of infants and young children from dehydration, malnutrition and other complications of water borne bacterial infection. Contaminated river water sources and large poorly functioning municipal water

distribution systems contribute to transmission of water borne bacterial disease. Chlorination san safe water handling can eliminate the risk of water borne bacterial disease. But centralized water treatment and distribution systems are expensive and take years to complete.

The need for health care is very urgent due the excessive growth urban and rural population. It clear that some of the diseases can be greatly reduced at a relatively low cost through improved housing and living conditions. To reduce the death rate from Diarrhea disease improved availability of water and sanitation services and provisions for oral-rehydration solution are needed. These deaths can be prevented. This is true for both the urban and rural areas. Since in urban areas, there is higher income for the middle income groups, it appears that the situation is better. However, the conditions of the poor for people in the urban village areas are the same as those of the rural area.

Contaminated water may have off-tastes, odors or visible particles. However, some dangerous contaminants in water are not easy to detect. Accurate water testing is needed to determine safety and quality. Turbidity in water is caused by suspended matter such as clay, silt, finely divided organic and inorganic matter, planktons, and other microscopic organisms.

Water that contains diseases causing organisms so it is necessary for water to be purified so as to be made safe for drinking. To achieve this various methods have been employed but each method has its own setbacks in terms of efficiency, cost and

ecological suitability. There are several major water purification techniques some are more efficient in removing particular types of impurities. A combination of two or more technologies is better in a given situation. These Methods include; distillation, ion exchange, carbon adsorption, filtration, ultra filtration, reverse osmosis, electro deionization, ultraviolet (UV) radiation. Distillation is probably the oldest method employed in water treatment but it requires large amount of energy and water despite the fact that it removes a broad range of contaminants. It requires expert training and careful maintenance to ensure efficiency. Ion exchange is very efficient in removing organic contaminant from water. The Carbon adsorption is 99.99% efficient in removing suspended solids the pressure of a Millipore membrane filters of 0.22um track down all bacterial but cannot remove inorganic as well as colloidal particles. Ultrafiltration acts as molecular sieve, effectively removed all types of particles and microbes. Reversed osmosis can effectively remove all types of contaminants to some extent (particles, pyrogens, microorganisms, colloids and dissolves inorganic) through the flow rate is limited. Electrode ionization is a technology clone from electro dialysis and ion exchange, it's inexpensive to operate and absolutely efficient in removing inorganics but the set back is that the water requires pre-treatment for water. The adsorption of UV light by the DNA and proteins in the microbial cells results in cell inactivation but the method cannot remove particles, colliods or ions.

The target of drinking water treatment is to remove colloidal material and microorganisms demand to achieve the quality drinking water. Conventional technique is mostly used for surface water

treatment includes chemical coagulation followed by flocculation, sedimentation, filtration and disinfection. There are many steps in water treatment processes, one of them is water treatment processes which applying for removal of suspended particles and colloidal material in raw water.

Common artificial coagulants are aluminium sulphate, polyaluminium chlorides, ferric chloride, and synthetic polymers. All of these coagulants have in common the ability of producing charged ions when liquefied in water which can contribute to charge neutralization. Aluminium sulfate (alum) is a common coagulant generally utilized in water treatment. Alum increases concerns when introduced into the environment towards eco-toxicological impact regarding the application of artificial polymers and have many carcinogenic characteristic. Natural coagulants such as the seeds from many plants can also be used. Traditionally, surface water has been treated with the help of plants as natural coagulants for centuries in India. Coagulation is an important step in water treatment processes not only for adsorb particles but because it is also for removing the microorganisms that are often attached to the particles. The important process for removing turbidity is the coagulation. The aim today is how give other people access to uncontaminated drinking water by cost effective means, particularly the rural people who can not afford any water treatment chemicals without affecting the health. In view of the above quite a number of natural materials of plant origin have long been used by local communities in many developing countries in water treatment. Use of plants to reduce contaminants in environment known as phytoremediation. It is coast effective,

efficient, novel and ecofriendly technique. Phytoremediation includes the use of plants *in situ* or *ex situ* to partially or substantially remediate selected organic and inorganic contaminants in contaminated soil, sludge, sediment, ground water, surface water and waste water.

Phytoremediation has also been called green remediation, agroremediation and vegetative remediation. Phytoremediation is a variety of processes occurring to differing degrees for different conditions, media, contaminants and plants. Phytoremediation can be used to clean up heavy metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons and landfill leachates. Phytoremediation encompasses a number of different methods that can lead to contaminant degradation, removal through accumulation, dissipation and immobilization. Some of the effective coagulants (Table 2) used for water purification.

Table : 2. Natural coagulants can be used for water purification

S. No.	Name of Plant	Family	Used plant parts
1.	*Acrorus calamus*	Araceae	Roots
2.	*Anaphalis cunefolia*	Compositae	Entire plant
3.	*Eclipta aibba*	Compositae	Entire plant
4.	*Azadirachta Indica*	Meliaceae	Leaf
5.	*Moringa oleifera*	Moringaceae	Fruits, Roots, Stem
6.	*Stryctnos potatorum*	Loganiaceae	Seeds
7.	*Jatropha curcas*	Euphorbiaceae	Seeds
8.	*Cicer arietinum*	Fabaceae	Seeds

In recent years there has been considerable interest in the development of usage of natural coagulants which can be produced or extracted from plants. These plant based coagulants should be biodegradable and are presumed to be safe for human health. Natural coagulants produce readily biodegradable and less voluminous sludge. Natural coagulants have been used for domestic household for centuries in traditional water treatment in tropical rural areas. The usage of natural coagulants derived from plant based sources represents a vital development in sustainable environmental technology since it focuses on the improvement of quality of life for underdeveloped communities. Plant based coagulants act using adsorption and neutralization mechanism. Plant based coagulants contain phytochemical compounds such as lipids, proteins and secondary metabolites with carboxyl and hydroxyl groups which increase coagulation and flocculation for water purification. Phytochemical compounds can be separated. The separated compounds can be identified and quantified. To achieve this Gas Chromatography Mass Spectrometry (GC-MS) is used to separate volatile compounds in a mixture. GC can well separate complex mixtures and MS can detect these compounds. GC-MS has a relatively broad coverage of non-volatile compound classes mainly those involved in primary metabolism including organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates within the polar as well as lipophilic compounds such as fatty acids, fatty alcohols and sterols.

The separated compounds can be identified and quantified. To achieve the identification of different compounds various steps can be used during GC-MS.

Extraction of metabolites from the biological sample which should be complete as possible to avoiding degradation or modification of metabolites. Derivatisation of metabolites to make them volatile and amenable to gas chromatography. Injection depends on the sample gas, liquid or solid and compounds in a mixture need to be volatilized. Injection techniques may be split, split less, thermal desorption and solid phase micro extraction (SPME)

After injection of a mixture, separation is achieved in the capillary column. This column is coated with a fluid or a solid support the stationary phase. An inert gas also called the mobile phase is flowing through the column. Depending on the phase equilibrium between the stationary and mobile phase, compounds travel with different velocities through the column. The mixture becomes separated, and as a result, individual compounds reach the detector with a different retention time. By choosing a column, which separates on boiling point, polarity, size or stereochemistry, a wide range of compounds can be separated.

Ionization of compounds as they are eluted from the GC which is required for subsequent mass spectrometry. Electron impact (EI) ionization is most widely used it produces reproducible fragmentation patterns and molecular ions which simplifies the evaluation of resulting mass spectra.

Detection of molecular ions, which can be achieved with different mass-detection devices, including single quadrupole detectors (QUAD), ion trap technology (TRAP), and time-of-flight detectors (TOF).

Each component is identified by comparing its "retention time" that is the length of standard time that it remains in the column. The retention time of a vapor depends on the column temperature limits and ramp rate, the column length, type of stationary phase and carrier gas velocity. If these variables are kept constant, the retention time of a component may be tentatively identified by comparison to the retention time of a known standard run under identical operating conditions. If the response of the detector is linear, the area under a peak accurately represents the quantity of the component present. If it is not, calibration for detector response to the types of components expected to be in the analyte yields a set of response factors which convert the reported area percentages to quantitative weight percentages.

Evaluation of data begins by matching chromatographic retention times and mass-spectral fragmentation patterns to reference data in local and/or public databases. Software provided with GC-MS equipment facilitates this to a greater or lesser extent, depending on the platform used. The best software programmers support automated, comprehensive extraction of all mass spectra from a chromatogram, correction for co-eluting metabolites, calculation of GC retention time indices, and automated selection of suitable mass fragments for quantification.

After detection of compounds their orientation and conformation can be predicted by using molecular modeling. In the field of molecular modeling, docking is a method which predicts the favored orientation of one molecule to a second when bound to each other to form a stable complex. Docking means predicting the

bioactive conformation of a molecule in the binding site of a target structure. Knowledge of the chosen orientation in turn may be used to predict the strength of association or binding affinity between two molecules. In essence, this is equivalent to finding the global free energy minimum of the system consisting of the ligand and the target. Docking is used as a tool in structure-based drug design. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

The aim of molecular docking is to evaluate the practicable binding geometries of a putative ligand with a target whose 3D structure is known. The binding geometries include both the positioning of the ligand relative to the receptor (ligand configuration) and the conformational state of the ligand and the receptor. There are three basic tasks any docking procedure. Target Preparation for docking in structure based virtual screening identification of compounds for automated docking does a given receptor-binding pocket. The 3D structure of targets is typically obtained by X-ray crystallography, NMR and homology models can also be used. In some cases the target is not a protein but can be RNA or DNA. RASMOL is a public domain program that can be used to view molecular structures in 3-D perspective. It is an easy program to use. It does have research applications, and can be used to view the

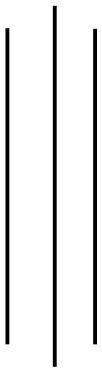
structure of macro-molecules like proteins. We shall be using this program to understand basic molecular structure principles.

Encapsulation use to enclose medicinal importance constituent in a relative stable shell known as capsule. Two main types of capsule are used one is hard shelled capsule and another is soft shelled capsule. Hard shelled capsule made up with gelatin and contain dry powdered ingredients. Lower part of capsule known as body and it is sealed with high diameter cap. Soft shelled capsule primarily used for oils and for active ingredients that are dissolved. To avoid contamination use aseptic manipulation techniques was followed.

Contaminated water contains diseases causing agents such as bacteria, viruses, protozoa. Water also possesses chemical parameters (Alkalinity, Dissolve oxygen, Biochemical oxygen demand) and physical parameters (Turbidity, pH, Colour etc.) these parameters are very important to decide the drinking water quality. Various studies indicate that water quality parameters affected by various water borne bacteria. As a small part of this study regarding anticoagulant activity of some plants for water purification were evaluated.

For the purification of surface water there is a need to develop cost effective, easier and environmental friendly process. In the light of above facts present study is selected to know "**Phyto-Chemical Studies of Selected Plants as A Source of Bio-Coagulant Agent For Water Purification.**" In recent years there has been considerable interest in the development of usage of plant natural coagulants. These coagulants are biodegradable and are supposed to be safe for human health.

CHAPTER – 2



REVIEW OF LITERATURE

Review of liteRatuRe

In India, most of the population is dependent on surface water as the only source of drinking water supply. The groundwater is believed to be comparatively much clean and free from pollution than surface water. But prolonged discharge of industrial effluents, domestic sewage and solid waste dump causes the groundwater to become polluted and created health problems (Raja *et al.*, 2002). The rapid growth of urban areas has further affected water quality due to overexploitation of resources and improper waste disposal practices. Hence, there is always a need for and concern over the protection and management of surface water and groundwater quality (Patil *et al.*, 2001).

Water pollution is the contamination of water bodies including lakes, rivers, oceans and ground water. Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds. Pollution is caused when a change in the physical, chemical or biological condition in the environment harmfully affect quality of human life including other animals life and plant (Lowel and Thompson, 1992 and Okoye 2002). Industrial, sewage, municipal wastes are been continuously added to water bodies hence affect the physiochemical quality of water making them unfit for use of livestock and other organisms (Dwivedi and Pandey, 2002).

Today water pollution is the biggest problem for human beings which deteriorate the water quality. Various human activities make

water unfit for drinking and domestic purposes. The main sources of water pollution are chemical fertilizers and pesticides getting in an untreated sewage and industrial effluents into rivers and streams, running close to the cities and low lands. Garg *et al.,* (2007) reported that many dangerous diseases are caused by using polluted water by reducing the incidence of many water borne communicable diseases. The diseases associated with contaminated water cause serious public health problems in India (Tambekar, 2007).

Water pollution can come from many different sources. If pollution comes from a single source it is called point source pollution. If pollution comes from a many source it is called non-point source pollution (Jain *et al.*, 1998). In most of the Indian cities, the major sources contributing to the pollution problems are land disposal of solids wastes, sewage disposal on land, agriculture activities, leakage and spills of effluent carrying culverts, deep well disposal of liquid waste, urban run-off and polluted surface water refuse is dumped in low lying area.

Alagmutuhu and Rajan, (2008) indicate that one of the greatest challenges of the coming time is to provide an ample supply of safe drinking water for house hold consumption to everyone. But the quality of water resources are unevenly distributed over the earth's surface and this is deteriorating due to anthropogenic activities, so in future even countries are going to suffer from scarcity of pure water. As we dump municipal wastes and industrial waste, heavy land and salt making properties of ground water change. Therefore, it is essential to analyze the ground to study the variations in connection with quality parameters. Based on the physico-chemical parameters

quality could be rated based on uses like drinking, agriculture and industrial etc.

According to Nevondo and Cloete, (1991) in areas where portable water supplies are provided, the supplies are unreliable and insufficient; forcing residence to reverse to traditional contaminated water sources. It is therefore imperative to monitor the physico-chemical and microbial quality of water supply in rural areas in other to highlight the quality of water supply and to provide the impetus for sustained government intervention.

According to a recent United Nations Children Federation (UNICEF) report, about 800 million people in Asia and Africa are living without access to safe drinking water. Consequently, this has caused many people to suffer from various diseases (Tanwir *et al.,* 2003). The quality of drinking water is of vital concern to mankind, since it is directly associated with human life. Fecal pollution of drinking water causes water-borne diseases, which wiped out entire population of cities (Farah *et al.,* 2002).

According to World Health Organization (WHO), (2003) drinking water supplies have a long history of being infected by a wide spectrum of microbes. Therefore, the primary goal of water quality management from health perspective is to ensure that consumers are not exposed to pathogens that cause disease. Protection of water source and treatment of water supplies have greatly reduced the incidence of these diseases in developed countries. Hasan, *et al.,* (2010) reported that the quality of the water consumed by our local

population is critical in controlling infectious diseases and other health problems.

According to WHO, (2003) reported that the biological contamination in drinking water is a major problem of public health in developing world. WHO estimates that about 1.1 billion people globally drink unsafe water and the vast majority of diarrheal diseases in the world (88%) is attributable to unsafe water, sanitation and hygiene.

Various researches indicate that waste water is spoiling the entire environmental conditions. This water may have pollutants of almost all kinds from simple nutrients and organic matter (Trivedi *et al.*, 2004). Waste water is responsible for polluting the ground water level and makes the water unsuitable for drinking purpose (Singh *et al.*, 2005). Singh *et a*l., (2005) concluded that the quality of various water sources in and around of Khagaul town showed the water pollution. The water pollution causes irreparable damage to the pollution, soil, plants and animals (Trivedi, 1990 and Chandrasekhar, 1997).

Heavy metal pollution in soil and aquatic bodies is a serious environmental problem threatening not only the aquatic ecosystems but also human health through contamination of drinking water. Unlike organic pollutants heavy metals are not degraded so requires removal for decontamination. In view of the above it is very important to remove the heavy metals from the contaminated sites. According to Chermisinoff *et al.*, (2002) safe drinking water should generally be free from heavy metals, turbidity, organic compounds

and pathogens. Turbidity may contain these compounds and also shields pathogens from chemical or thermal damage. It is also important to remove turbidity for the aesthetic values of the drinking water. Organic substances in water might originate from industrial and agricultural operations, which contribute with compounds such as chloroform, gasoline, pesticides and herbicides. Finally, protozoa, bacteria and viruses are all pathogens that can cause diseases.

According to WHO, (2008) among the coagulating agents used in water treatment, ferric sulphate or alum is most widely used salts. The salts acts as coagulants by neutralizing the charges of colloidal particles adsorb or trap them and facilitate the agglomeration of particles during slow mixing provided in flocculation.

Studies conducted by Stumm and Morgan, (1996) that conventional treatment of water often includes coagulation, flocculation, sedimentation, filtration and disinfection. Coagulation is the destabilization of particles, which means a changed state in the dispersion of colloidal particles. The stability of colloids is dependent of their surface charge. Polymers can affect the particle interaction by forming bridges between them or by sterically stabilizing them.

The cleanup technology involves a variety of techniques ranging from simple biological process to advanced engineering technologies. The use of clean up technologies without producing other harmful waste products is required as best option (Rajkumar *et al.*, 2008).

Phytoremediation that is using vegetation to remove, detoxify or stabilize persistent pollutant is an accepted tool for cleaning of

polluted soil and water. Phytoremediation includes group of technologies that use plants for remediating soils, sludge, sediments and water contaminated with organic and inorganic contaminants. Plants are unique equipped with remarkable metabolic and adsorption capabilities. Phytoremediation is an alternative technology that can be used in place of mechanical conventional technologies that require high capital inputs and labor. (Ali *et al.*, 2004, Anderson *et al.*, 1994).

Studies conducted by Lombi *et al.*, (2002), Cheng *et al.*, (2002), *Bhargava et al.*, (2008) conclude that attempts for developing cost-effective treatment approach always revolved around using the different types of treatment technologies. Techniques related to treatment cannot avoid chemical, mechanical and energy requirements except this they also generate a new category of pollutants. Using plants to purify soil and water has always fascinated researchers also trying for the same. Consequently many natural systems were developed such as oxidation ponds, lagoons, and constructed wetland etc. that use the ability of different plant species for the degrading or uptake of the pollutants. Use of the plants for such purpose is termed as phytoremediation.

Phytoremediation is divided into different areas such as phytoextraction, rhizofiltration, phytostabiilzation, phytodegradation, rhizodegradation and phytovolatilization. Phytoextraction also called by the name of phytoaccumulation refers to the uptake and translocation of metal contaminants in the soil by plant roots into the aerial parts of plants (Eaphan *et al.*, 2005). There are two basic strategies of phytoextraction as chelate assisted phytoextraction,

which may also be termed as induced phytoextraction and the other is long term known as continuous phytoextraction. Although chelate assisted phytoextraction has become more developed and also being implemented commercially.

The plant kingdom presents a wealth of chemical compounds of pharmaceutical nature. This has been resulted in the utilization of medicinal plants as flavours, odours, dyes, preservatives and a number of traditional and folklore medicines. Phytochemical derived from plants are not of recent origin, rather they have been provided by the Asian countries, India and China. In China, the medicinal record was as old as 5000-4000 B.C. In India the oldest record of the medicinal plants comes from 'Rigveda' (4500-1600 B.C.), the 'Atherveda' (4000-1600 B.C.) which provides a remarkable knowledge about Indian medicinal plants.

The importance of natural medicines can be indicated by the fact that one of the most "lifesaving drugs" (antibiotics) can only be obtained by plants. It was reported that use of phytodrugs has increased in the recent years, although synthetic compounds and microbial agents have a major contribution to the pharmaceutical industries, 25% of the prescribed drugs are of plant origin.

Most of the plants producing the valuable medicinal compounds are grown wildly in nature and some of them are cultivated. India's large quantity forest has provided a prominent position in export of medicinal plants and crude drugs. Such plants have often collected from the naturally occurring flora has created a gap in their balance over the earth.

From the time immemorial the medicinal importance of plant products is well known to human beings. Antimicrobials are defined as those secondary metabolites though not belonging to a specific class, which are capable of inhibiting the growth of other micro-organisms. Several plants have been screened for their various biological activities such as antibacterial, antifungal, antiviral, insecticidal properties. Skinner, (1995) has screened a number of vascular plants for their antimicrobial activity.

According to Yin, (2010) several plant-based coagulants have been studied scientifically some of them are listed as seeds of *Strychnos potatorum* has been used to clarify turbid surface waters for over 4000 years. *Moringa oleifera* has coagulant properties related to dimeric cationic proteins.

Clijsters *et al.*, (1985) reported that macrophytes based wastewater treatment systems have several potential advantages as compared to conventional treatment systems with the low operating costs. They are more flexible and less susceptible to shock, loading and have genetically homogeneous population which is small in size with abundance and rapid growth. Such treatment systems can often be established at the same site where the waste water is disposed.

According to Gray *et al.,* (2000) in case of developing countries like India, such macrophyte based technology may be extremely advantageous where a plenty of waste land requires the reclamation, so that it can be used for agriculture.

G. Vijayaraghavan *et al.,* (2011) reported that frequently studied plant-based coagulants include Nirmali seeds (*Strychnos*

potatorum), *Moringa oleifera*, Tannin and *Cactu*s represents important progress in sustainable environmental technology as they are renewable resources and their application is directly related to the improvement of quality of life for underdeveloped communities.

According to (Doppalapudi sandeep *et al.*, 2012) *Cicer arietinum* which is generally consumed as a seed food is a good source of protein. Phytochemical analysis indicates the presence of flavonoids, phenols and saponins in both the methanolic and ethanolic extracts. An extra presence of the tannins in methanolic extract may contribute somewhat major anti-edematous activity when compared to that of the ethanolic extract. There by the findings concluded that *Cicer arietinum* seeds exhibit an anti-inflammatory activity and further studies were suggested to isolate the active principles responsible for the activity.

Md. Asrafuzzaman *et al.*, (2011) reported that using some locally available natural coagulants for example *Moringa oleifera, Cicer arietinum, Dolichos lablab*, significant improvement in removing turbidity and total coliforms from synthetic raw water was found. Maximum turbidity reduction was found for highly turbid waters. After dosing, water-soluble extract of *Moringa oleifera*, *Cicer arietinum*, and *Dolichos lablab* reduced turbidity to 5.9, 3.9, and 11.1 NTU, respectively, from 100 NTU and 5, 3.3, and 9.5 NTU, respectively after dosing and filtration. It was also found that these natural coagulants reduced about 89–96% of total coliforms. Among the natural coagulants used in this study for turbidity reduction, *Cicer arietinum* was found most effective. It reduced up to 95.89% turbidity from the raw turbid water.

G. Muthuraman *et al.,* (2013) studied coagulation-flocculation followed by sedimentation and filtration is the most commonly used water treatment process in which turbidity or particles removal is strongly dependent on proper coagulant dosage, effect of pH ,effect of time, jar test and settling column tests. The maximum turbidity removal efficiency obtained for *Moringa oleifera* at 12hrs retention time in the settling column, for 100 NTU, 250 NTU, 500 NTU initial turbidity removal efficiency are as 95.93%, 95.10% and 99% respectively. Turbidity values were very similar although the dose of aluminum sulphate which was required to achieve this was greater than that of the *Moringa* oleifera and other coagulant.

According to Sankar Narayan Sinha (2012) in recent times the use of plants as a source of novel compounds to combat microbial infections has gained prominence. Study analyzed the phytochemical composition of activities of *Moringa* oleifera extracts using in vitro antibacterial screening techniques. The *Moringa* oleifera moreover studies were conducted to assess the antibacterial methanol extract of leaves, flowers, barks, seeds and fruits of this plant at a concentrations of 5 mg/ml exhibited antibacterial activities against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae and Shigella flexneri* .

Tasneembano Kazi *et al.*, (2013) reported that tanning industry is one of the oldest industries which is highly complex and is characterized by high BOD, COD, suspended solids, settleable solids, sulphide, chloride and chromium. C*icer arietinum, Moringa oleifera,* and *Cactus* were used as locally available natural coagulants in this study to reduce turbidity and COD of tannery wastewater. The

optimum dosage of *Cicer arietinum, Moringa* oleifera and *Cactus* were found as 0.1, 0.3 and 0.2gm/500ml respectively. The optimum pH value with *Cicer arietinum*, *Moringa oleifera*, and *Cactus* was found to be 5.5, 4.5 and 5.5 respectively. In case of *Cicer arietinum, Moringa oleifera*, and *Cactus maximum* reduction in turbidity were found to be 81.20%, 82.02% and 78.54%, and maximum reduction in COD were found to be 90%, 83.33% and 75%, respectively. Among the natural coagulants used in this study maximum turbidity reduction of 82.02% and COD reduction of 90% was found with *Moringa oleifera* and *Cicer aretinum* respectively.

Daniyan S. Y. *et al.*, (2011) studied the antimicrobial effects of the methanol, ethyl acetate and hexane extracts of *Jatropha curcas* seed at concentration ranging from 50-200mg/ml were tested against some pathogenic organisms using agar diffusion method. The extracts exhibited antimicrobial activities with the zones of inhibition ranging from 10-25, 8-23, 10-20 and 12-21(mg/ml) for *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Candida albican*, respectively. The Minimum Inhibitory Concentration (MIC) which ranges from 3.13-12.5mg/ml was determined using the broth dilution method. The minimum bactericidal concentration (MBC) ranges from 25-12.25mg/ml. The phytochemical analysis revealed the presence of alkaloid, glycosides,flavonoid and carbohydrate.

Studies conducted by Mangale S. M *et al.,* (2012) confirm the effectiveness of seed powder extracted from mature-dried *Moringa oleifera* seeds which are commonly available in most rural communities. Various doses of *Moringa* seed powder 50, 100 and 150 mg/l were taken and checked for the efficiency dose on raw water.

After treatment of seed powder with water samples were analyzed for different parameter like pH, Turbidity, TDS, TS, Hardness, Chlorides, Alkalinity, Acidity, MPN and SPC. All parameters were reduced with increasing dose of 50, 100 and 150 mg/l seed powder respectively (except alkalinity and pH). *Moringa oleifera* seeds acts as a natural coagulant, flocculent, absorbent for the treatment of drinking water. It reduces the total hardness, turbidity, acidity, alkalinity, chloride after the treatment. It also acts as a natural antimicrobial active against the micro-organisms which is present in the drinking water and decrease the number of bacteria. MPN test reading was reduced after treatment of higher dose at 150 mg/l of *Moringa* seed powder.

Sonal Choubey (2013) reported the performance of *Strychnos potatoram* seed extract as primary coagulant and compared with the performance of alum. *Strychnos potatoram* seed extract is effective as prime coagulant. Compared with alum (residual filtrate turbidity 2 NTU and residual color 3 TCU), it produces water with slightly higher residual filtrate turbidity (4 and 3 NTU) and residual color (15 and 13 TCU), but the residual turbidity and residual color are within the WHO drinking water guideline values for turbidity (5 NTU) and color (15 TCU). The effectiveness of *Strychnos potatoram* in the removal of turbidity, total hardness, pH, COD, BOD and total dissolved solids (TDS) has been investigated. *Strychnos potatoram* was found to be especially effective in reducing the parameters like turbidity, BOD, COD, hardness.

According to K. A. Yongabi (2010) the potentials of *Moringa oleifera, Jatropha curcas, Pleurotus tuberregium, Citruss aurontifolia, Strynos potatorium* coagulants with respect to turbidity

removal and disinfection of water borne diseases in comparison with chemical coagulants and disinfectants such as Alum and Chlorine have been presented. Studies conclusively demonstrates that biocoagulants especially *Moringa* oleifera seeds are as efficient as Alum is purifying water and wastewater at low cost. Heiras-Palazuelos MJ *et al.,* (2013) reported that *Cicer arietinum* is one of the most important grain-legume crops in the world used as nutraceutical. Chickpeas have high nutritional value due to high protein and dietary fibre. It has high antioxidant activity due to this it is a well known and potent nutraceutical.

N. Packialakshmi *et al.,* (2014) reported that the efficiencies of powdered seeds of *Strychnos potatorum* natural water treatment agents alternative to the use of synthetic chemicals. The optimum dosages and turbidities were observed to the alum and *Strychnos potatorum*. Seed extract is effective as a prime coagulant compared with alum. it produces water with slightly higher residual turbidity and residual color, but the residual turbidity and residual color are within the WHO drinking water guideline values for turbidity (5 NTU) and color (15 TCU). The effectiveness of *Strychnos potatoram* in the removal of turbidity, total hardness, pH, and total dissolved solids (TDS) has been investigated.

Various researchers Agarwal and Agarwal, (2007); Fairless, (2007); Akbar *et al.,(* 2009), indicate *Jatropha* L. (Euphorbiaceae) is a very diverse subtropical and tropical genus which includes succulents, caudiciform species, herbaceous perennials and woody trees. A number of *Jatropha* species especially *Jatropha curcas* yield oils of biofuel value. Kumar and Sharma, (2008); Openshaw, (2000);

Martinez-Herrera *et al*., (2006**)** reported that *Jatropha curcas* can be used to prevent and/or control erosion, to reclaim land, grown as a live fence especially to contain or exclude farm animals and be planted as a commercial crop. These characteristics along with its versatility make *Jatropha* of vital importance to developing countries.

According to Mamta Arora *et al.,* (2014) seeds of *Cicer arietinum* show antioxidant and antimicrobial activities. Antioxidant activity of various methanol extracts were assessed using three different methods (Reducing Power, Total antioxidant activity and Ferric reducing antioxidant power). Antimicrobial activity of methanolic and hydroalcoholic extracts were assessed by agar cup method with three different microbial strains *Staphylococcus aureus, E.coli* and *Pseudomonas alcaligenes.*

According to M Madhu *et al.,* (2016) plants play important roles in discovery associated with new beneficial therapeutic agents and have received significant focus because of their bio- active substances like antioxidants, hypoglycemic and hypolipidemic factors. Plant species were screened for their phytochemicals by using 4 different solvent extracted from their selected parts (leaves, stem, pericarp of the fruit and seeds cotyledons). All the plants which are selected for the study contains phytochemicals like alkaloids, flavonoids, steroids, phenols and saponins. The highest concentrations of alkaloids are observed in *L.officinale* leaf and *F.vulgare* stem extracts by using petroleum ether.

Waseem ahmad *et al.,* (2017) reported that phyotochemical analysis and antimicrobial study of any selected plant species is a

very significant way to establish that the selected plant species may be use as potent drugs. *Euphorbia hirta* is a potential source of useful bioactive compounds. Leaves and flowers extract of the plant contain large amount of alkaloids and flavanoids along with terpenoids, saponins, tannins and carbohydrate in small amount. Plant shows significant antibacterial activity against selected gram positive strains. Results clearly indicate that *Euphorbia hirta* as potent antibacterial drugs of natural origin.

According to Wilson Rwai Waweru *et al.,* (2017) the medicinal properties exhibited by various medicinal plants are driven by the phytochemicals present in the plants. Identification of the phytochemicals present in the selected plants such as Vernonia *mygdalina, Zehneria scabra, Leonotis nepetifolia, Tetradenia riparia, Aloe myrianantha.* Standard procedures for phytochemical screening were used to test for the presence of various phytochemicals. All the selected medicinal plants were found to contain tannins and flavanoids, saponnis and phenols. Alkaloids were also present in all the selected plants except *Aloe myriacantha* and *Eucalyptus camaldulensis.* The study concluded that medicinal plants used in Rwanda possessed various phytochemicals that aids in the medicinal properties of the studied plants.

According to Chapman and Kimstach, (1992) the pH is used to read the acid balance of a solution and it is defined as 'the negative of the logarithm to the base 10 of the hydrogen ion concentration'. The pH scale ranges from 0 to 14 (i.e., very acidic to very alkaline), and pH 7 indicates a neutral condition. The pH of natural water stays in between 6.0 and 8.5 but could be affected by chemicals entering the

waterways. This parameter can be used to evaluate the amount of effluent plume in the water body, while measuring the effects of an effluent discharge.

Various researches indicate that extremely high or low pH values of fresh water make it unsuitable for most aquatic organisms. Moreover, water with low pH values become corrosive on the other hand, water with high pH values reduces the availability of phosphate, sulphate, iron and manganese (Gambrell and Patrick 1988; Jackson *et al.*, 1993; Handreck and Black, 1994). Furthermore, at high pH levels most of the dissolved carbon dioxide is converted into bicarbonate (HCO_3-) or carbonate (CO_3^{2-}). This parameter has a direct effect on the treatability of the water. The pH value varying between 6.5 to 8.0 is required for a proper biological treatment of wastewater (Metcalf and Eddy, 1991).

According to World Health Organization (WHO) 1984 the desirable pH of drinking water is 7.0 to 8.5. The pH has no direct adverse effect on health, but at the same time alters the taste of water.

According to Chapman and Kimstach, (1992) the chemical oxygen demand (COD) is commonly used to measure the susceptible levels of oxidation of the organic and inorganic materials existent in water bodies as well as in the sewage and industrial effluents. It measures the O_2 equivalent of the organic matter present in a water sample that can be oxidized by a strong chemical oxidant, such as dichromate or permanganate. The concentration of COD observed in unpolluted surface water remain around 20 mg/l or less, while values are normally greater than 200 mg/l in effluents. Masters, (2004)

observed that COD measurements are usually higher than the BOD_5 measurements.

According to Chapman and Kimstach, (1992) and Liston and Maher, (1997) the Biochemical Oxygen Demand (BOD) is used to read the level of biochemically degradable organic matter or carbon loading in the water. It is usually defined by the amount of O_2 consumed by the aerobic micro-organisms present in the water sample for the purpose of oxidizing the organic matter and to convert it to a stable inorganic form. Hence, in water quality analysis this parameter is used to determine the biodegradable organic content of the waste in terms of O_2 which is required when the wastes are discharged into natural water where aerobic condition prevails.

According to Chapman and Kimstach, (1992) the BOD is usually determined through standardized laboratory procedures where the sample is incubated in the dark at a steady temperature of 20 ^{0}C for the duration of 5 days, thereby measuring the amount of O_2 consumed in this process. This explains the term BOD_5 (biochemical oxygen demand on five days). Unpolluted waters typically contain BOD_5 values of 2 mg/l or less, while raw sewage could have a BOD_5value of about 600 mg/l.

According to Odum, (1971) Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are the significant parameter of pollution. Various researches shows that due population explosion and rapid urbanization, people dependent on water sources of unconvinced quality in the absence of better alternatives, or due to economic and technological constraint to adequate treat the available

water before use (Anna and Adedipe, 1996; Calamari and Naeve, 1994).The scarcity of clean of water and pollution of fresh water has therefore led to a situation in which one - fifth of the urban dwellers in developing countries and three - quarter of their rural dwelling population do not have access to reasonably safe water supplies (Lloyd and Helmer, 1992).

Gamedze *et al.,* (2012) shows that colour, taste, smell and turbidity are the quality parameters mostly used by rural households to determine water suitability for domestic use. Most ground water sources were found to have saline water due to low ground water recharge in the area. Water quality remains a sustainable development challenge in the rural areas of Swaziland.

According to Abbasi *et al.,* (1996) and Rao *et al.,* (1985) an inverse relationship between Dissolved Oxygen (DO) and BOD and these values indicate the lowest biological activity in winter months. According to Rao, (1997) drinking water usually has a BOD of less than 1 mg/l and water is considered to be fairly pure with BOD of 3mg/l and of doubtful purity when the BOD values reach 5 mg/l.

According to Chapman and Kimstach, (1992) Dissolved Oxygen (DO) is used to measure the amount of gaseous oxygen dissolved in the water, which is crucial for all forms of aquatic life. DO in water mainly appear by diffusion from the atmosphere and also from the photosynthesis of aquatic plants.

Coliforms are the major microbial indicator of monitoring water quality (Brenner *et al.,* 1993 and Grant, 1997). The detection of *Escherichia coli (E. coli)* provides definite evidence of fecal

pollution; in practice, the detection of thermotolerant (faecal) *coliform* bacteria is an acceptable alternative (WHO, 1997).

According to the United Nations (UN) and WHO, (1996) data, more than five million people die annually from water borne diseases. Of these, about four million deaths (400 deaths/hr) are of children below age five (WHO, 1996). Most of the pollution in drinking water is caused by the uptake and distribution system, by insufficient upkeep of sewage system, by defects and breaks in the disinfection processes (Scoglio *et al.*, 1989), by human and animal fecal matter (Clark *et al.*, 1982). The fecal *coliform* and *E.coli* has been used an indicator for the potential presence of human enteric pathogen for many years, as it proliferates in the water distribution system (Moe *et al.*, 1991).

The parameters recommended by WHO, (2003) for the minimum monitoring of community supplies are those that ensure the hygienic state of water and reduce the risk of water-borne pathogens. The essential parameters of water quality are: (a) *Escherichia coli* and thermo-tolerant *coliforms* accepted as suitable substitutes, (b) chlorine residual (c) pH and (d) turbidity (WHO, 2003). The *coliform* groups of bacteria principally infect water used for domestic, industrial or other purposes (Zamaxaka *et al.*, 2004). High levels of *coliform* counts indicate a contaminated source and inadequate treatment deficiencies.

According to Watson and Cichra, (2006) organic matter also stimulates the growth of decomposers such as bacteria and fungi. Bacteria and fungi are very critical to the breakdown of the toxic

component of the effluent. It has been observed that dissolved oxygen in water is required during the decaying of the organic matter, which may lead to depletion of oxygen in the water body and cause harmful substance to accumulate.

Many researchers Tona *et al.*, (1998), Samy and Ignacimuthu, (2000), Palombo and Semple (2001**)**, Kumaraswamy *et al.*, (2002), Govindarajan *et al.*, (2006) indicated that plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Plants show antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties.

According to Indu *et al.*, (2006) that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains significant increase in the inhibitory zone was observed by increasing the concentration of the extract.

Purohit *et al.,* (1995) have screened out antimicrobial activity of various extracts of *Evolvulus alsinoides (*Convolvulaceae) and they found that there is no selective inhibition of any specific organism and all the extracts were showing less or moderate activity against all the bacteria and fungus.

Gislene, (2000) screened out the antibacterial activity of some plant extracts and phytochemicals on antibiotics resistant bacteria. The highest antibacterial potential was observed from the extracts of *Caryophyllus aromaticus* and *Syzygium jambolanum*.

Parvathamma and Shanthamma, (2001) was found that *A. sativum* possess maximum inhibitory action against *S. typhimurium* and *S. aureus*. It was concluded by him that *A. sativum* is a broad spectrum antimicrobial agent.

Rafi *et al.,* (2005) was tested antimicrobial activity of leaf extracts of *Moringa concanensis* against *Staphylococcus aureus, S. pneumonia, Streptococcus mutants, E. coli, S. typhi, K. pneumonia* and *P. aeruginosa*. It was revealed that that the ethanolic extract showed maximum inhibitory effect and *S. aureus* was most sensitive bacteria.

Parekh, *et al.,* (2006) was tested antibacterial activity of 12 medicinal plants against *Bacillus cereus, S. aureus, K. pneumonia, E. coli, Pseudomonas pseudolacaligenes.* The preliminary screening experiment revealed that methanol extract were more potent than aqueous extract and *Bauhinia variegata* exhibited.

According to Varghese *et al.*, (2009) Methanolic leaf extracts of *Typha angustifolia* and the isolated compounds were subjected to the antimicrobial activity against both bacteria like *E. coli* and *Staphylococcus aureus* by disc diffusion method. It was compared with standard drug. Methanolic extract and isolated compounds were found to possess potent antibacterial activity.

Phytochemical characterization using various solvent extracts was studied by Neha grover *et al.*, (2013) indicate that various extracts of the leaf and flower of *Woodfordia fruticosa* were screened for the presence of steroids, reducing sugars, alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, glycosides and

ascorbic acid by standard qualitative test procedures and further this study was extended by analyzing the potent bioactive compounds in the methanolic extract of *Woodfordia fruticosa* leaves using GC-MS analysis. It was found that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. The GC-MS analysis revealed the presence of twenty one compounds in the methanolic leaf extract of *Woodfordia fruticosa*. The major costituents were Di-N-Octyl Phthalate; Dibutyl phthalate; Hydrocinnamic acid; 3,5-bis(1,1-dimethylethyl)-4-hydroxy- ; 2-Propanol, 1-(2-butoxyethoxy)- and Caryophyllene Oxide, along with other minor constituents. Results confirmed the presence of therapeutically potent compounds in the leaf extract predominantly tannins and terpenoids.

Dalen M.B. *et al.,* (2009) reported that phytochemical analysis of *Moringa oleifera* seeds indicates the presence of saponnins, flavonoids and alkaloids. Insrumental analysis showed also the presence of sodium (15.21$\pm$0.10ppm), aluminum (12.21$\pm$0.012) potassium (14.21$\pm$0.013ppm) and sulphate (1.72$\pm$0.011 ppm). Jar test trials on raw water samples displayed favorably characteristics at 60% alum to 40% *Moringa oleifera* mg/l blend with total coliform count of $30ml^{-1}$ and turbidity of 3.2NTU below the WHO maximum permissible limit of 5NTU. The results indicate that *Moringa* oleifera has a double advantage compared to commercial alum because of the presence of phytochemicals which have been reported to possess antimicrobial properties with potentials for conjunctive use with alum for water purification in rural communities.

According to Lengauer and Rarey, (1996) in the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. In the context of molecular modeling, docking means predicting the bioactive conformation of a molecule in the binding site of a target structure.

Verkhivker *et al.*, (2000); Totrov and Abagyan, (1997) reported that docking is used as a tool in structure-based drug design as well as in SBVS. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Therefore docking is useful for predicting both the strength and type of signal produced. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs.

Kitchen *et al.*, (2004) given the biological and pharmaceutical significance of molecular docking considerable efforts have been directed towards improving the methods used to predict docking.

According to Rollinger *et al.*, (2008) virtual screening is a computational approach utilized inside medication discovery research. The in silico measurement of big ligand libraries associated with substance buildings in order to determine those structures almost

certainly to stimulation to drug target, protein receptor or enzyme. Virtual screening helps in the identification of small novel molecule that binds to target protein that useful for recent step in drug discovery.

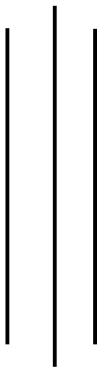
Bhavisha rabadiya and Paresh rabadiya (2013) reported that capsule is most preferable dosage form. Till now gelatin is widely used as a capsule shell material for the preparation of hard gelatin and soft gelatin capsule.

In developing countries people do not have access to adequate sanitation facilities and the most important problem is related to the disposal of non degraded waste and waste-water (Ayaz *et al.*, 2001). In case of developing countries high costs of infrastructure investment, continual replacement facilities, lack of efficient treatment technologies and financial problems become the main constraints in the path of pollutants management (Brix, 1994). Thus there is a critical need of cost-effective long-term treatment technologies to deliver public health and environmental protection in developing countries.

Hence an analysis and review of available literature reveals that biocoagulant activity of studied plants has been little explored. Antibacterial activity in general and studies about of the antibacterial activity the studied plants will be made considering and important aspect of medico Ethnobotany.

Review of literature indicate that there are lot of work done on effect of plant based coagulants on water quality but there was very little work regarding GCMS analysis and Molecular modeling.

CHAPTER – 3



MATERIAL & METHOD

Material and Methods

The materials and methods of present work on water purification are divided into four parts. The first part deals with seed collection of selected biocoagulant test plants *Cicer arietinum* L., *Jatropha curcas* L., *Moringa oleifera* L., *Strychnos potatorum* L. and their phytochemical and antimicrobial activity evaluation. The second part deals with water quality analysis including physiochemical parameters after treatment with biocoagulants.

Third parts deal with separation and identification of plants phytochemicals using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. Fourth parts deal with molecular docking of compounds identified by GC-MS that help to identify coagulants compounds. It also associated with formulation of seed powder capsule formulation.

PART-A

For the study of phytochemical analysis the extract of *Cicer arietinum* L., *Jatropha curcas* L., *Moringa oleifera* L., *Strychnos potatorum* L. plants is analyzed for the presence of Alkaloids, Phenolics, Saponins, Steroid and flavonoids, according to standard methods Odebiyi and sofowora, (1978); Sofowora, (1982); Williamson e (1996); Banso and Ngbede, (2006 and 2008).

Seed Collection: Riped fruits (pods) of *Cicer arietinum* L., *Jatropha curcas* L., *Moringa oleifera* L. and *Strychnos potatorum* L. collected from different certified seed suppliers.

Sample Treatment: For the study of phytochemical analysis, the ethanol extract of the plant seed powder prepared according to standard methods (Sofowora, 1982). The seeds are peeled to obtain the nuts and dried in an oven for 1hours and thereafter grind the dried seeds and sieved to mesh size of 150 pm. Transfer the powdered material in to solvent extractor and extract it with 95% ethanol for 72 hours. Dried extract kept in refrigerator at 4ºC for their future use in phytochemical analysis.

PHYTOCHEMICAL ANALYSIS:

In phytochemical evaluation solvent extracted seed powder of *Cicer arietinum* L., *Jatropha curcas* L., *Moringa oleifera* L., *Strychnos potatorum* L. are subjected to phytochemical screening of various plant constituents.

Plants synthesize an enormous range of organic compounds that are traditionally classified as primary and secondary metabolites. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, growth and development. These include phytosterols, lipids, nucleotides, amino acids and organic acids. Other phytochemicals which accumulate in surprisingly high concentrations in some species are referred to as secondary metabolites. These are structurally diverse and many are distributed among a very limited number of species within the plant kingdom such as alkaloids, glycosides, terpenoids, flavonoids and saponins.

Detection of alkaloids:

A small portion of the extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidal reagents such as Mayer's reagent, Dragondroff's reagent, Hager's reagent and Wagner's reagent. Appearance of cream coloured precipitate shows the presence of alkaloids.

Detection of phenolics:

Small quantity of various extracts were taken separately in water tested for the presence of phenolic compounds with dilute ferric chloride solution (5%) and lead acetate solution (10%). Appearance of deep blue-black colour and white precipitate indicate the presence of phenolics.

Detection of flavonoids:

A fraction of the extract treated with 1N aqueous NaOH solution and concentrated sulphuric acid. Appearance of yellowish orange colour shows the presence of flavonoids.

Detection of steroids and terpenoids:

A small amount of sample dissolved in 2ml of chloroform taken in a dry test tube. Add equal volume of concentrated sulphuric acid and shake gently. The presence of steroids and terpenoids indicated by the upper layer of chloroform turning red and lower layer showing yellow green fluorescence.

Detection of saponins:

In a test tube add about 5ml of extract and add a drop of sodium bicarbonate. Shake mixture vigorously and kept for 3minutes. The formation of a honey comb like froth showed the presence of saponins.

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT:

Successful prediction of phytochemical compounds from plant material is largely dependent on the types of solvent used the extraction procedure. Studies shows that methanol and ethanol extract of plants were certainly much better and powerful. This may be due to the better solubility of active compounds in organic solvent.

Preparation and dilution of plant sample:

Take 10g of the seed powder of each *Cicer arietinum* L., *Jatropha curcas* L., *Moringa oleifera* L., *Strychnos potatorum* L. plant samples and homogenized with 100ml of the respective solvents. The crude preparation left overnight in the shaker at room temperature and then centrifuged at 4000rpm for 20mins. Take supernatant the supernatant containing the plant extract. Transfer supernatant to a pre- weighed beaker and evaporate the solvent at 60°C. Weigh the crude extract and dissolved in a known volume of dimethyl sulphoxide to obtain a final concentration of sample.

Recovery of lyophilized cultures:

Disinfect the ampule by wiping with 70% ethanol. Heat the tip of the outer vial in a flame. Add a few drops of water on the hot tip to

crack the glass. Aseptically add 0.2–0.5 ml of sterile water. Using a sterile pipette, gently aspirate the contents several times to mix the suspension thoroughly. Let the suspension to rest for 15-30 minutes. Inoculate the suspension onto an appropriate medium and incubate.

Antibacterial assay:

Method used for the measurement of antibacterial activity of plants seed extracts against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* that was agar well diffusion assay.

Mueller-Hinton Agar media composition:

Beef infusion: 300.0 gm, Casamino acids: 17.5 gm, Starch: 1.5 gm, Agar: 17.0 gm

Distilled water: 1000 ml, pH:7.4

Procedure:

Prepare Muller Hinton Agar medium by dissolving all medium constituents.

1. Autoclaved the medium at 15 lbs pressure at 121°C for 15 minutes.
2. The autoclaved medium mixed well and add test organism and poured onto petriplates (25-30ml/plate) while still molten.
3. After solidification of medium cut wells and add 20 µl of the plant extracts to each well.
4. Incubate these plates at 37℃ for 24 hours.
5. Chloramphenicol disc used as a positive control.

6. Observed zone formed around the well to detect antimicrobial activity.

Measure the diameter of the inhibition zone formed around the well to detect antimicrobial activity and compared with control.

PART-B

For the present study, physic-chemical and bacteriological characters of water parameters of sample water were observed. For the analysis of various physico-chemical parameters were determined by following methods devised by APHA, (1976); Adoni, (1985); Trivedy and Goel, (1984) and Gupta, (2006).

Collection of Water Samples: -

Water samples were collected from Chambal river during rainy season. Precautions were taken to prevent any vertical disturbance during the collection. The water samples were collected in presterilized bottles. For collection of water following method is adopted. Samples were collected from at least 2 to 3 meter away from the boundaries of river. For this purpose a pre-sterilized bottles was tied at one end of a long bamboo pole and collect the sample after displaying surface water which might contain organic floating over it. After filling, the bottle the cap was placed tightly, the name, address, sample number, identification mark etc, was provided on the bottle. Various methods were used for physico-chemical analysis of the surface water.

WATER QUALITY ANALYSIS:

Water from selected sites of rivers was collected and analyzed for various physical parameters viz. clearity, odour, colour, pH, turbidity, alkalinity, dissolved oxygen and biochemical oxygen demand. Study to know about palatability of water the standard quality and was compared to the table approved by Bureau of Indian Standard for drinking water.

Jar Test

To perform jar test 1g each of plant powder dissolved in separate 100ml of distilled water as stock solutions. 200ml of raw sample water were measured and introduced into beakers labeled 1-7. With a calibrated pipette, each stock solution dosages of solutions were added onto the water samples in the beakers as rapidly as possible. Mix contents for 2 minute at a speed of 100rpm, followed by slow mixing for 8mins at 25 rpm. Observe the beakers and evaluated for specific dosages and flock quality. Turned off the jar test mixer and the flocks allowed to settle in the beakers for 30mins and observe flocks settling characteristics.

pH determination:

The term pH refers to the measure of hydrogen ion concentration in a solution and defined as the negative log of H^+ ions concentration in water and wastewater. The values of pH 0 to a little less than 7 are termed as acidic and the values of pH a little above 7 to 14 are termed as basic.

pH of water sample was measured by toshniwal manufactured CL 54 pH meter. Before using the pH meter it is necessary to eliminate the error. The meter was earlier calibrated against the known buffer solutions of 7.0 pH and 9.2 pH.

Turbidity of water:

Turbidity is the technical term referring to the cloudiness of a solution and it is a qualitative characteristic which is imparted by solid particles obstructing the transmittance of light through a water sample. Turbidity often indicates the presence of dispersed and suspended solids like clay, organic matter, silt, algae and other microorganisms.

Turbidity of water sample was measured by century made CTD 401 digital turbidity meter usind Nephelometry-US EPA method.

Method:

Take clean sample cell and add turbidity free distilled water up to the horizontal mark. Wipe gently with soft tissue. Place sample cell in the turbidity meter such that the vertical mark in the sample cell should coincide with the mark in the turbidity meter and cover the sample cell. Now adjust the reading to zero using the set zero knob. Prepare a standard 10 NTU, 100 NTU solutions by diluting the standard 4000 NTU solution. Add standard solutions to the sample cells up to the horizontal mark, wipe gently with soft tissue. Place it in the turbidity meter and cover the sample cell. Now check reading 10 NTU, 100 NTU. If the instrument is not showing 10 NTU, 100

NTU, using the calibration knob adjust the reading. Fill sample water in sample cell and check turbidity of sample.

Total alkinity determination:

Alkalinity was determined titrimetrically with (0.02 N) sulphuric acid, using the phenolphthalein and methyl orange as indictor. Carbonate and bicarbonate were calculated by the method as given by Adoni, (1985) and APHA, (1976).

Method:

Rinse the burette with 0.02N Sulphuric acid and discard the solution. Fill the burette with 0.02N sulphuric acid and adjust it to zero. Using a measuring cylinder exactly measure 100 ml of sample and pour it into a 250 ml of conical flask. Add few drops of phenolphthalein indicator to the contents of conical flask. The colour of the solution will turn to pink. This colour change is due to alkalinity of hydroxyl ions in the water sample. Titrate it against 0.02N sulphuric acid till the pink color disappears. This indicates that all the hydroxyl ions are removed from the water sample. Note down the titter value (V1). The value of titration is 0.5ml .This value is used in calculating the phenolphthalein alkalinity. To the same solution in the conical flask add few drops of mixed indicator. The colour of the solution turns to blue. This colour change is due to CO_3^{2-}& HCO_3^- ions in water sample. Continue the titration from the point where stopped for the phenolphthalein alkalinity. Titrate till the solution becomes red. The entire volume (V2) of sulphuric acid is noted down and it is accountable in calculating the total alkalinity.

Calculation:

$$\text{Total Alkalinity} = \frac{\text{Volume of } H_2SO_4 \text{ X Normality X 50X 1000}}{\text{Volume of sample taken}}$$

Normality of Sulphuric Acid = 0.02 N

Volume of Sample = 50 mL

Biological Oxygen Demand (BOD) determination:

The biochemical oxygen demand determination is a chemical procedure for determining the amount of dissolved oxygen needed by aerobic organisms in a water body to break the organic materials present in the given water sample at certain temperature over specific period of time. BOD of water or polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under standard condition at a standardized time and temperature.

All reagents needed for measuring of dissolved oxygen were also used in BOD estimation by APHA standerd method 5210B, (1976). Three oxygen bottles were filled with the water sample to be analyzed. To the first bottle, Winkler's reagents was added immediately just after the sample collection. The rest two bottles were sealed immediately without letting the air bubble go in and were kept in the incubator at 20 ^{o} C for five days (BOD_5). At the end of fifth days, the amount of dissolved oxygen was measured in each of the bottles. In the polluted samples, the dissolved oxygen was completely used up after five days incubation period, or 70 % of the initial oxygen is consumed, it was necessary to aerate and dilute the water sample.

Calculation:

Let D_O = DO in the sample bottle on 0^{th} day.

D_1 = DO in the sample bottle on 5^{th} day.

C_0 = DO in the blank bottle on 0^{th} day

C_1 = DO in the blank bottle on 5^{th} day.

$C_0 - C_1$ = DO depletion in the dilution water alone.

$D_0 - D_1$ = DO depletion in sample + dilution water.

$(D_0 - D_1) - (C_0 - C_1)$ = DO depletion due to microbes.

BOD mg/l = $(D_0 - D_1) - (C_0 - C_1)$ mg x Decimal fraction of sample used.

If the sample is seeded, find out BOD of seed in the above manner and apply correction, as per demonstration.

PART-C

The separation and screening of phytochemicals that have natural coagulant property present in seed powder of each *Cicer arietinum, Jatropha curcas, Moringa oleifera, Strychnos potatorum* plant samples was carried out by taking plant extract on Gas Chromatography Mass Spectroscopy.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis:

A sample of the analyte is introduced by syringe injection into the heated injector tube, where it is vaporized and mixed with carrier gas. As the sample vapor is carried through the column by the carrier gas, the analyte partitions between the gas and liquid phases according to the analyte components solubility in the liquid at the column operating temperature. Each component travels at a

characteristic rate, and if the column has sufficient length and resolving power, the sample will be completely separated by the time it reaches the detector.

The detector located at the column exit is the ITD mass spectrometer. It records the total number of ions entering the mass analyzer from the column. The chromatogram produced is called the total ion chromatogram. Each point in the chromatogram is a mass spectrum. Each component is identified by comparing its "retention time", the length of time that it remains in the column, to that of a standard. The retention time of a vapor depends on the column temperature limits and ramp rate, the column length, type of stationary phase, and carrier gas velocity.

The screening of phytochemicals was carried out by taking plant extract on Gas Chromatography Mass Spectroscopy (GC-MS - Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with Silica capillary column of 30 m length, 0.25mm diameter and 0.25µm film thickness. For GC detection an electron ionization system with ionization energy 50eV was used. Helium (99.99%) gas was used as carrier gas at a linear velocity 40.5 cm/sec. The total flow, column flow and purge flow were 16.3 ml/min, 1.21ml/sec and 3.0ml/min. respectively, at a pressure 81.9 KPa. The column oven temperature and injector temperature were set at 80.0^{o}C and 250.0^{o}C respectively. The identification of compounds was assigned by comparison of their retention indices and mass spectra fragmentation pattern obtained in GCMS chromatogram with those stored in computer libraries and also with published literature.

PART-D

Molecular docking of compounds identified by GC-MS:

Molecular docking is a method whish predicts the preferred orientation of one molecule to a second when bound to each other to form stable complex. Molecular docking predicts binding conformation of small molecule legend to appropriate target binding site. The obabel command line program converts chemical objects such as molecules or reactions from one file format to another. The Open Babel graphical user interface (GUI) is an alternative to using the command line and has the same capabilities. Since Open Babel is available cross-platform on Windows, Linux and MacOSX. Application includes interconversion of chemical file formats or representations, addition of hydrogens, generation of 3D molecular structures, calculation of partial charges and generation of molecular fingerprints.

Method:

1. Download .sdf file from NCBI and save in folder.
2. Select the type of the type of the input file from the dropdown list.
3. Click the “select” button and select the file. Its contents are displayed in the textbox below.
4. Choose the output format and file in a similar way. You can merely display the output without saving it by not selecting an output file or by checking “Output below only”.
5. Click the “Convert” button and save file.

Molecular visualization using RasMol:

RasMol is a public domain program that can be used to view molecular structures in 3-D perspective. It is an easy program to use. It does have research applications, and can be used to view the structure of macro-molecules like proteins, nucleic acid. We shall be using this program to understand basic molecular structure principles.

Procedure:

1. Get the software by using Internet browser.
2. Go to the course home page and click on software downloads click on RASMOL.
3. Notice or set where the file "RASMOL.EXE" gets saved. Then click ok. What you get is a "self exploding" compressed file.
4. Install the Software. On your own PC, use My Computer to locate RASMOL.EXE.
5. Double-left-click on the file name. Edit the "Extract To:" window to specify some NEW folder/directory, such as C:\Programs\Rasmol. Click "Extract" and then Click "Yes".
6. Double click the icon to open the program then click file, Open, select (click) any file (.pdb), click ok.
7. Click display ball & stick or spacefill. By using left-click drag across the top of the molecule to rotate it.

Capsule formulation of plant seed powder:

Encapsulation use to enclose medicinal importance constituent in a relative stable shell known as capsule. Empty hard gelatin used to pack plant seed powder. For preparation plant filtered seed powders weigh in appropriate amount and pour seed powder into the base. Place cover onto the base. To avoid contamination use aseptic manipulation techniques was followed.

To test the biocoagulant activity of medicinally important plants four plants were selected after screening.

***1. Cicer arietinum* L.:**

Belong to family Fabaceae is an annual herb is native of India. *Cicer arietinum* which is most commonly called as chick pea or Indian gram is an edible legume.

Medicinal Importance:

Cicer arietinum is found most effective natural coagulant of turbid water. *Cicer arietinum* is cheap, easily cultivable and available. On the other hand naturally occurring coagulants are biodegradable and presumed safe for human health. *Cicer arietinum* contains good amount of protein and is very much responsible for building body and it also promotes growth. It is also a good anti bacterial and anti fungal agent. It is very widely used in enhancing beauty. It is an excellent herbal supplement for hairs and also nourishes the scalp. It is also used in skin ailments. It is also a good liver stimulant and also keeps a check on the digestive system.

***2. Jatropha curcas* L.:**

Jatropha curcas, commonly known as physic nut and Ratanjot belongs to the Euphorbiaceae family. *Jatropha curcas* is a monoecious shrub or small tree of on average 3-5 and up to 8 m height. *Jatropha* grows in arid and semi-arid areas therefore known as drought tolerant, but for seed production sufficient water is needed.

Medicinal Importance:

The potential for *jatropha* to provide a renewable source of energy technically exists, and different forms of energy can be produced from different components of the plant. It also shows antimicrobial activity. *Jatropha* plant used for the treatment of different ailment. It also used for wound healing and inflammation.

***3. Moringa oleifera* L.:**

Moringa oleifera belongs to family Moringaceae is a nontoxic tropical multipurpose tree that is commonly known as the miracle tree found throughout India. Common name of *Moringa oleifera* is drumstick tree, horseradish tree and senjana.

Medicinal Importance:

Among many other properties, *M. oleifera* seeds contain a coagulant protein that can be used either in drinking water clarification or wastewater treatment. It is said to be one of the most effective natural. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of antioxidants, protein,

vitamins, beta-carotene, amino acids and various phenolics compounds. It also shows antibacterial and antifungal activity.

4. *Strychnos potatorum* L.:

Strychnos potatorum belons to family Loganiaceae. *Strychnos potatorum* is a moderate sized tree found in southern and central parts of India. Common name of Strychnos *potatorum* are clearing nut tree, nirmala and nirmali.

Medicinal Importance:

The plant has a variety of local medicinal uses and is also used locally to clarify water prior to drinking it. *Strychnos potatorum* reduce turbidity and used as a biocoagulant for water purification. Seeds of *Strychnos potatorum* used in the treatment of gonorrhea, leucorrhea, chronic diarrhea diabetes and eye disease. *Strychnos potatorum* show considerable antimicrobial activity against both bacteria and fungi.

PLATE - 1

1.1:- ***Cicer arietinum*** **L. Seeds and Plant**

1.2:- ***Jatropha curcas*** **L. Seeds and Plant**

Plant Seeds Selected for Analysis of Natural Coagulant Activity

PLATE - 2

2.1:- ***Moringa oleifera*** **L. Seeds and Plant**

2.2:- ***Strychnos potatorum*** **L. Seeds and Plant**

Plant Seeds Selected for Analysis of Natural Coagulant Activity

PLATE - 3

3.1:- Soxhlet Extractor

3.2:- Water Bath

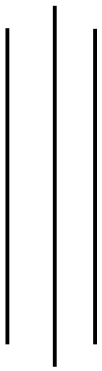
3.3:- Turbidity Meter

3.4:- pH Meter

3.5:- GC-MS Instrument

Showing Some Instruments Used During Phytochemical Analysis

CHAPTER – 4



OBSERVATION & RESULTS

observation and results

The observation and result of present work on water purification are divided into four parts. The first part deals with phytochemical and antimicrobial activity evaluation of biocoagulant test plants *Cicer arietinum, Jatropha curcas, Moringa oleifera* and *Strychnos potatorum*. The second part deals with water quality analysis including physiochemical parameters after treatment with biocoagulants. Third parts deal with separation and identification of plants phytochemicals using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. Fourth parts deal with molecular docking of compounds identified by GC-MS that help to identify coagulants compounds and formulation of seed powder capsule formulation.

PART-A

In present investigation various plant species has been selected as a biocoagulants belongs to different plant families and subjected to phytochemical screening of various plant constituents which are illustrated on figures.

Phytochemical analysis:

In phytochemical evaluation solvent extracted seed powder of *Cicer arietinum, Jatropha curcas, Moringa oleifera* and *Strychnos potatorum* (Table-3) are subjected to phytochemical screening of various plant constituents.

Table : 3. Plants Seed Selected for Biocoagulant Activity Analysis

S. No.	Name of plant
1.	*Cicer arietinum*
2.	*Jatropha curcas*
3.	*Moringa oleifera*
4.	*Strychnos potatorum*

The polarity of the solvent play an important role in the extraction of active plant extracts as shown by the higher yields obtained. Percent yield of secondary metabolite of each sample were estimated in ethanolic extract. For this purpose filtered plants seed powder was taken in the amount of 20 gm. Ethanolic extract of *Cicer arietinum* obtained was 1.12 gm and percent yield was 5.6. *Jatropha curcas* ethanolic extract obtained weight was 0.86 gm and percent yield of ethanolic extract was 4.3. *Moringa oleifera* weight of extract was 1.81gm and ethanolic extract gave percent yield 9.0. *Strychnos potatorum* obtained weight of extract was 2.12gm and percent yield was 10.6. Two types of texture were observed that is oily and gummy. Likewise colour of extract yellowish brown to brown colour was recorded (Table-4, Figure-3A).

Percent yield of secondary metabolite of each plant seed powder were also estimated in methanolic extract. For this purpose plant material of each sample was taken in the amount of 20 gm. Methanolic extract of *Cicer arietinum* obtained was 2.56 gm and percent yield was 12.8. *Jatropha curcas* mthanolic extract obtained weight was 0.72 gm and percent yield of methanolic extract was 3.6.

Moringa oleifera weight of extract was 2.24gm and methanolic extract gave percent yield 11.2. *Strychnos potatorum* obtained weight of extract was 2.48gm and percent yield was 12.4. Two types of texture were observed that is oily and gummy. Likewise colour of extract Yellowish brown to brown colour was recorded (Table 5, Plate- 4). This difference may be due to various reasons like differences in plant constituents in different plant species, time of sample collection or other geographical factors.

Table : 4. Percent Yield of Secondary Metabolites in Ethanolic Extract

Sample	Weight of plant material	Weight of extract	% Yield	Texture	Colour
Cicer arietinum	20	1.12	5.6 ± 0.29	Oily gum	Yellowish Brown
Jatropha curcas	20	0.86	4.3 ± 0.66	Oily gum	Dark Brown
Moringa oleifera	20	1.81	9.0 ± 0.76	Gummy	Brown
Strychnos potatorum	20	2.12	10.6±0.43	Gummy	Yellowish Brown

FIGURE-3

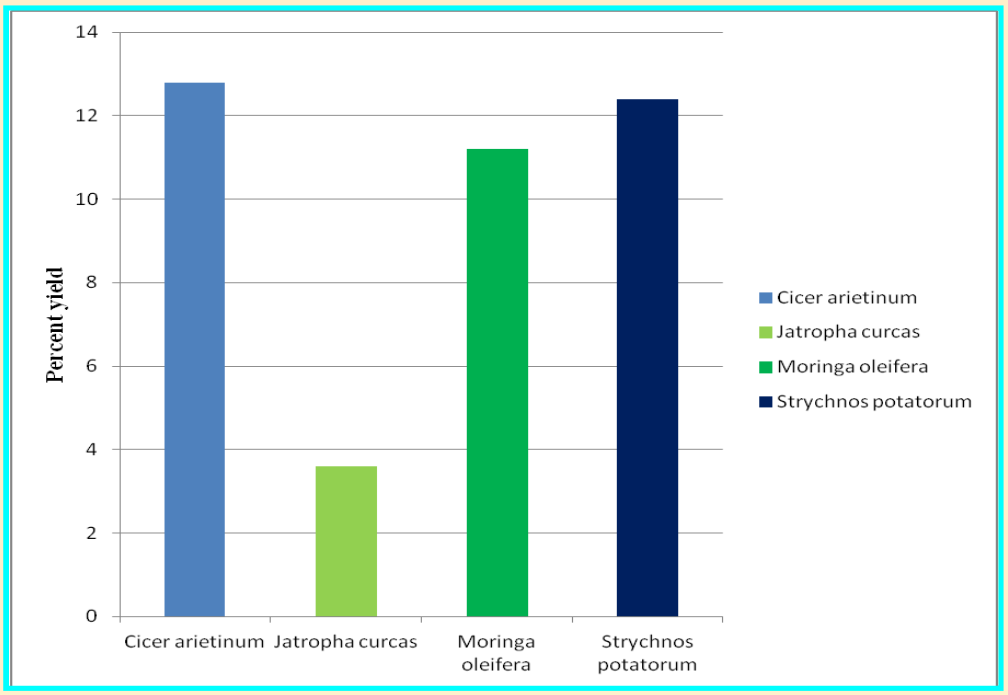


Fig-3A: Comparision of Percent Yield Obtain after Methanolic Solvent Extraction of Natural Plant Coagulants

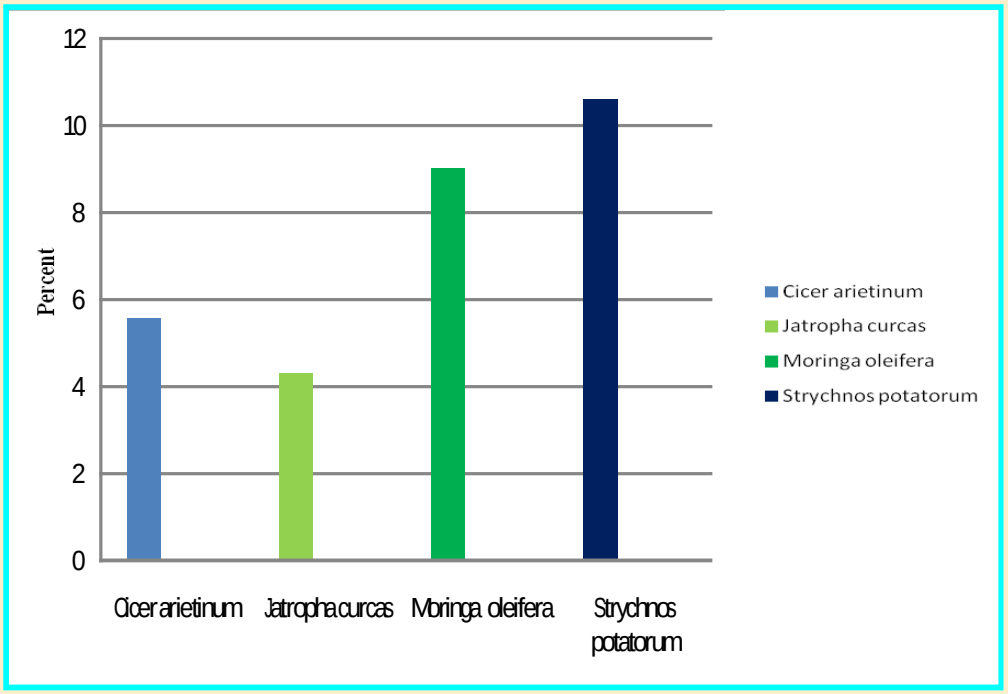


Fig-3B: Comparision of Percent Yield Obtain after Ethanolic Solvent Extraction of Natural Plant Coagulants

Table : 5. Percent Yield of Secondary Metabolites in Methanolic Extract

Sample	Weight of plant material	Weight of extract	% Yield	Texture	Colour
Cicer arietinum	20	2.56	12.8 ± 0.75	Oily gum	Yellowish Brown
Jatropha curcas	20	0.72	3.6 ± 0.63	Oily gum	Dark Brown
Moringa oleifera	20	2.24	11.2 ± 0.58	Gummy	Brown
Strychnos potatorum	20	2.48	12.4 ± 0.46	Gummy	Yellowish Brown

Various secondary metabolites or phytochemical constituents like Alkaloids, Flavanoids, Phenol, Saponin, and Steroids were also assessed for different plant seed solvent extract. In *Cicer arietinum* all component present except phenol in ethanolic extract. Alkaloid and steroids were absent in methanolic extract. In *Jatropha curcas* Saponin in ethanolic and methanolic extract was absent but Flavanoids and Phenol were present in higher amount in methanolic extract. In *Moringa oleifera* Saponin was absent in both ethanolic and methanolic extract. Whereas Phenol in ethanolic extract was present in higher amount. In *Strychnos potatorum* all components were shown their presence in both ethanolic and methanolic extract (Table 6, Plate 4 & 5).

Table : 6. Phytochemical Analysis of Components of Plant Seeds Solvent Extract

Phyto chemicals	***Cicer arietinum***		***Jatropha curcas***		***Moringa oleifera***		***Strychnos potatorum***	
	EtoH	MeoH	EtoH	MeoH	EtoH	MeoH	EtoH	MeoH
Alkaloids	+	-	+	+	+	+	+	+
Flavanoids	+	+	++	+	+	+	+	+
Phenol	-	+	++	+	++	+	+	+
Saponin	+	+	-	-	-	-	+	+
Steroids	+	-	+	+	+	+	+	+

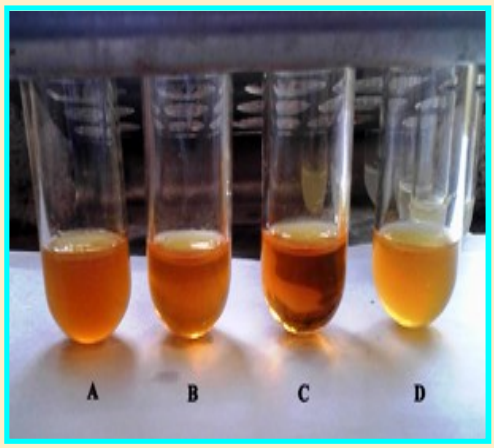
++: Highly present **+: Slightly present** **- : Absent**

Antibacterial activity of plant seed extract:

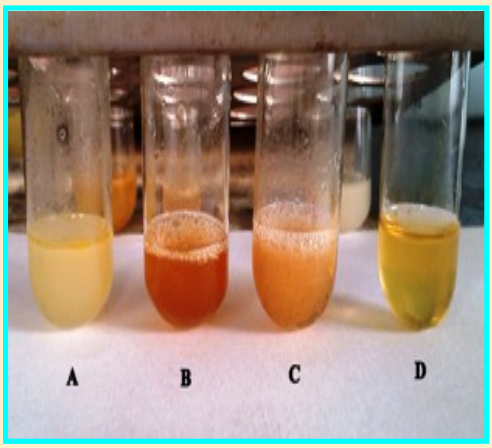
Antimicrobial properties of each experimented plants methanolic extract was also studied in present investigation. Preliminary screening of plant antibacterial activity was performed against *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Methanolic seed powder extract of *Cicer arietinum, Jatropha curcas, Moringa oleifera* and *Strychnos potatorum* in different concentration of 50, 100, 200 mg were tested for antibacterial activity against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* (Plate-7).

Following incubation the plates were examined for the presence of growth inhibition which was indicated by a clear zone surrounding of each well develops of zone called a zone of inhibition. For positive control erythromycin 25 mg/l was used.

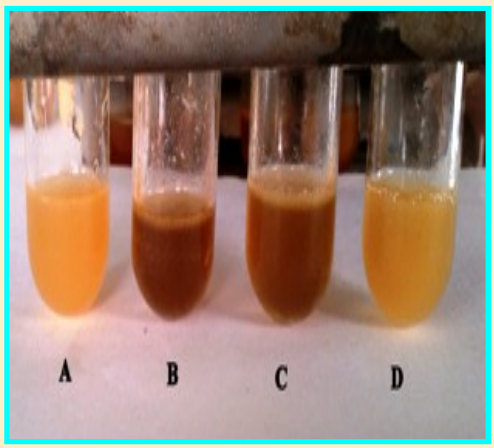
PLATE - 4



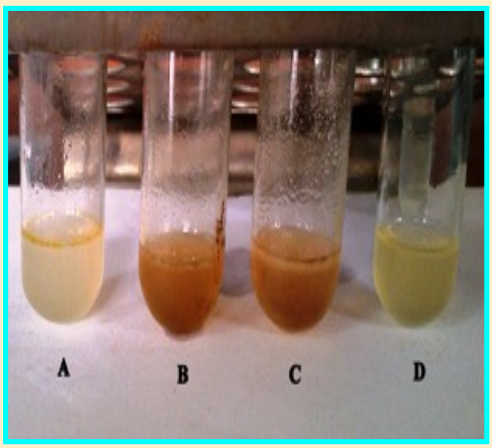
4.1:- Alkaloids Detection



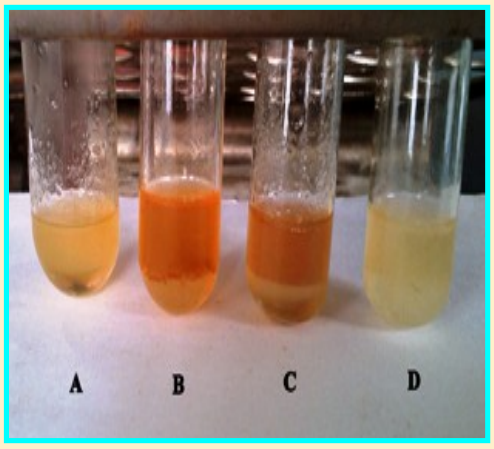
4.2:- Flavonoids Detection



4.3:- Phenol Detection



4.4:- Saponin Detection

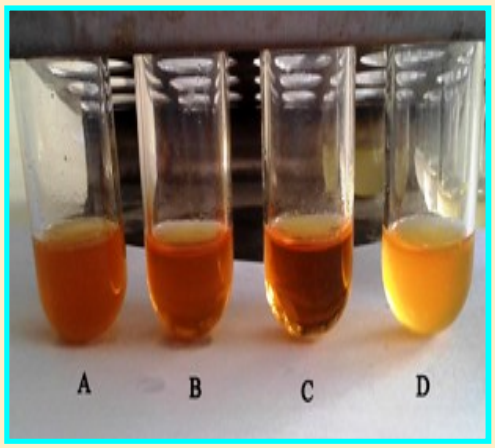


4.5:- Steroid Detection

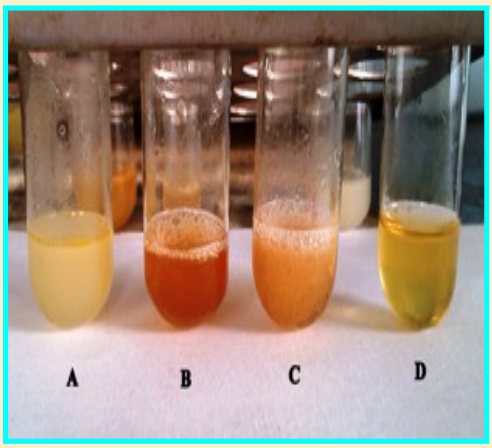
A: *Cicer arietinum* L. B: *Jatropha curcas* L.
C: *Moringa oleifera* L. D: *Strychnos potatorum* L.

Showing Phytochemical Analysis of Ethanolic Extract

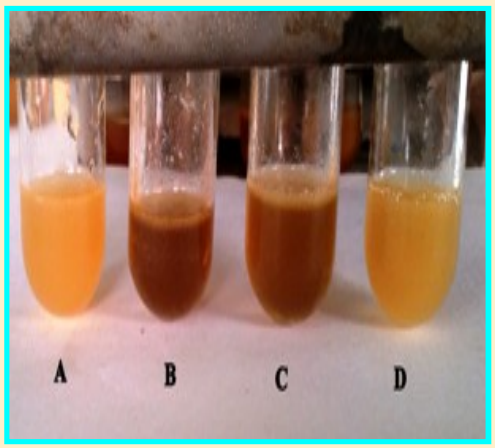
PLATE - 5



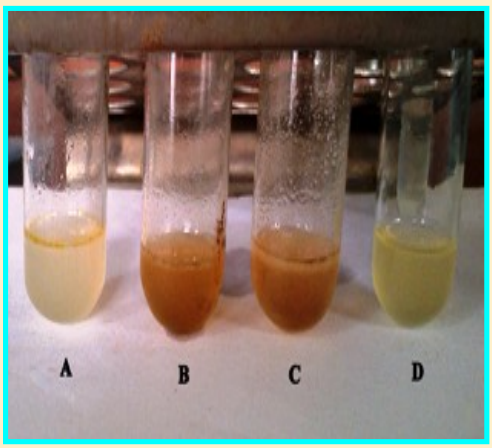
5.1:- Alkaloids Detection



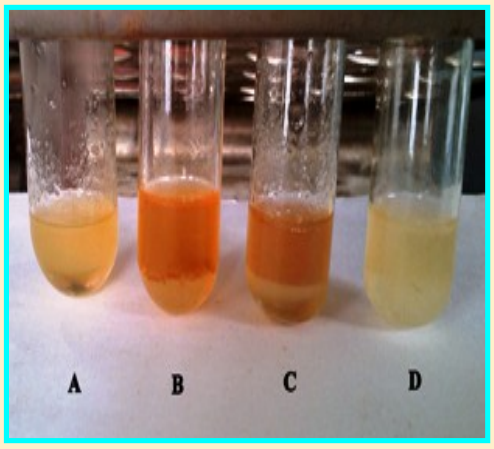
5.2:- Flavonoids Detection



5.3:- Phenol Detection



5.4:- Saponin Detection

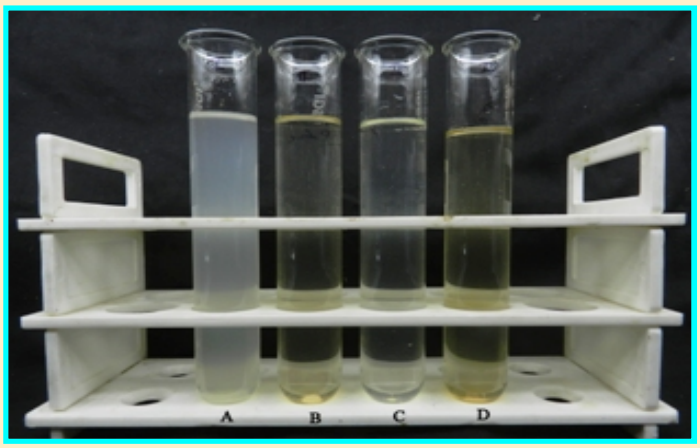


5.5:- Steroid Detection

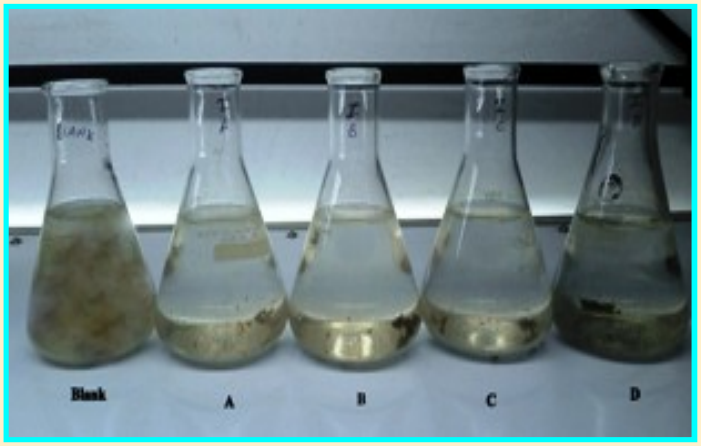
A: *Cicer arietinum* L. B: *Jatropha curcas* L.
C: *Moringa oleifera* L. D: *Strychnos potatorum* L.

Showing Phytochemical Analysis of Methanolic Extract

PLATE - 6



6.1:- Stock Solution of Plant Seed Powder



6.2:- Untreated and Treated Water Sample

6.3:- Alkalinity Determination Using Titration Method

A: *Cicer arietinum* L. B: *Jatropha curcas* L.
C: *Moringa oleifera* L. D: *Strychnos potatorum* L.

Showing Biochemical Parameters Testing of Treated and Untreated Water

Antimicrobial activity of *Cicer arietinum:*

Antimicrobial activity of *Cicer arietinum* maximum inhibitory zone size was reported in erythromycin against all bacterial strain and minimum was in 50 mg/ml concentration of extract (Table-7, Figure-1A).

Table : 7. Antimicrobial Properties of *Cicer arietinum* Seed Methanolic Extract

Con of extract Mg/ml	Zone of inhibition (mm)		
	BS	**EC**	**PA**
50	2	11	9
100	6	14	13
200	9	16	15
Eryt 25mg/ml	13	27	32

BS*: Bacillus subtilis*

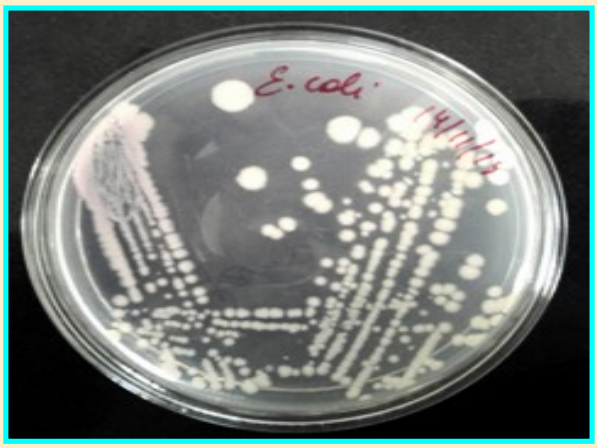
EC: *Escherichia coli*

PA: *Pseudomonas aeruginosa*

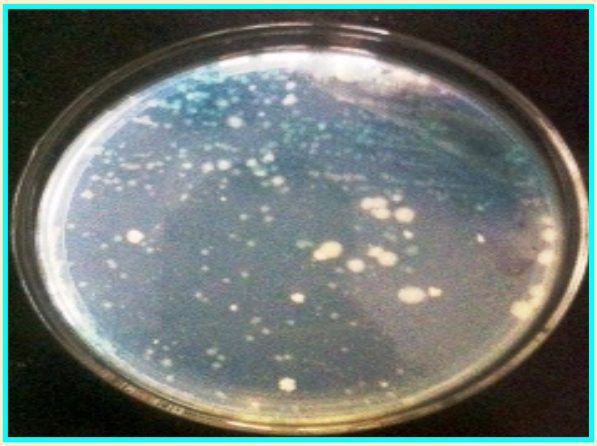
Antimicrobial activity of *Jatropha curcas:*

Antimicrobial activity of *Jatropha curcas* methanolic extract shown (0 mm) zone size of *Bacillus subtilis* and *Pseudomonas aeruginosa* were reported in 50 and 100 mg/ml concentration. Maximum inhibitory zone size (23 mm) was reported in 200 mg/ml against *Escherichia coli* bacterial strain (Table-8, Figure-1B).

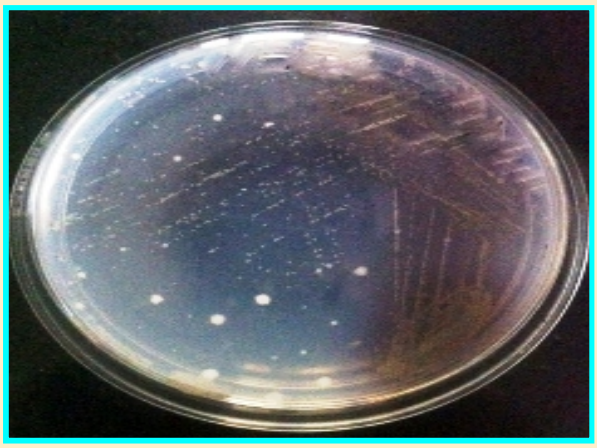
PLATE - 7



7.1:- ***Escherichia coli***



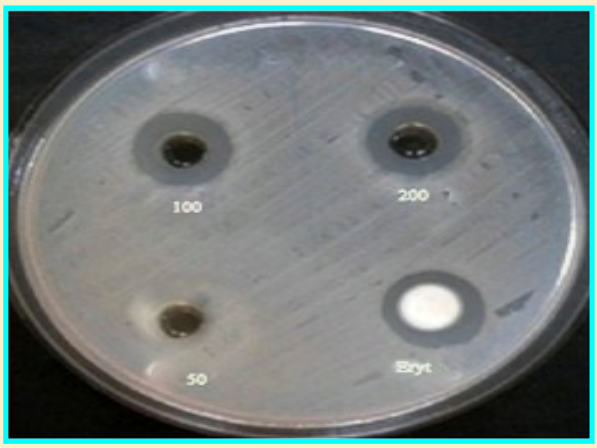
7.2:- ***Bacillus subtilis***



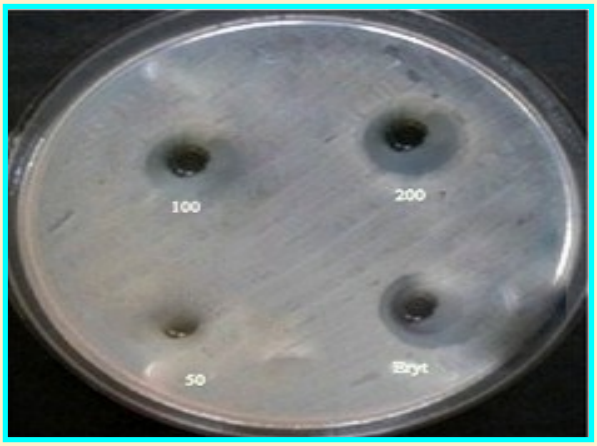
7.3:- ***Pseudomonas aeruginosa***

Showing Growth of Pure Culture of Test Organisms Used for Antibacterial Activity Analysis

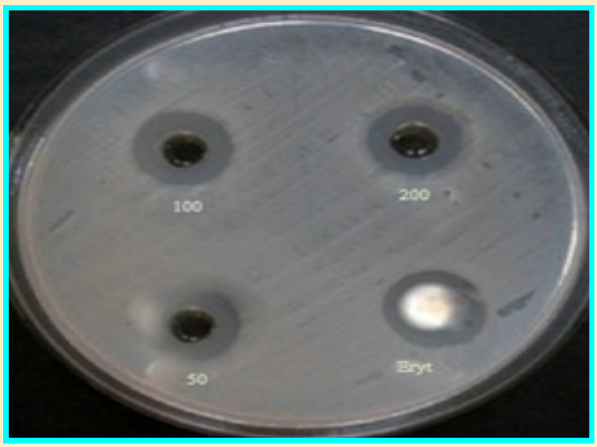
PLATE - 8



8.1:- ***Escherichia coli***



8.2:- ***Bacillus subtilis***



8.3:- ***Pseudomonas aeruginosa***

Showing Antibacterial Activity of *Cicer arietinum* L. Plant Seed Extract Against Different Bacteria

Table : 8. Antimicrobial Properties of *Jatropha curcas* Seed Methanolic Extract

Con of extract Mg/ml	Zone of inhibition (mm)		
	BS	**EC**	**PA**
50	0	8	0
100	0	12	0
200	15	23	10
Eryt 25mg/ml	15	20	10

BS*: Bacillus subtilis*

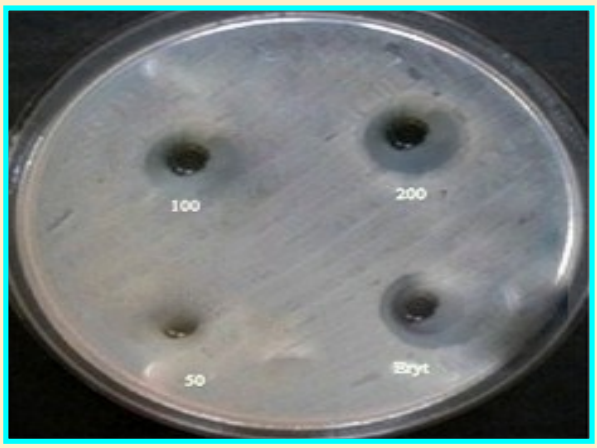
EC: *Escherichia coli*

PA: *Pseudomonas aeruginosa*

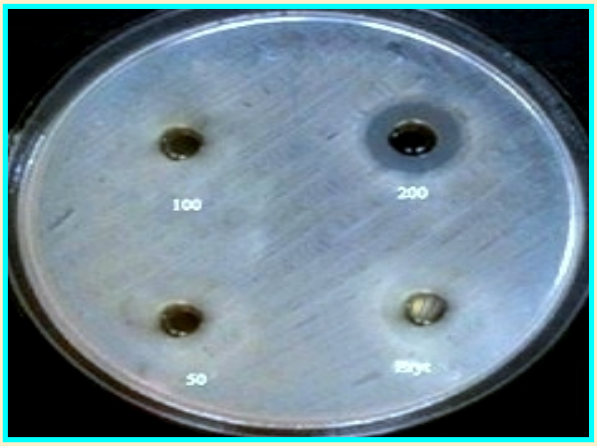
Antimicrobial activity of *Moringa oliferea:*

Antimicrobial properties of *Moringa oliferea* methanolic extract increasing pattern of zone of inhibition was observed for *Bacillus subtilis* and *Escherichia coli but Pseudomonas aeruginosa* maximum zone size (15 mm) was reported in 200 mg/l of extract. 50 mg/l concentration of extract ineffective against all test organisms (Table-9, Figure-2A).

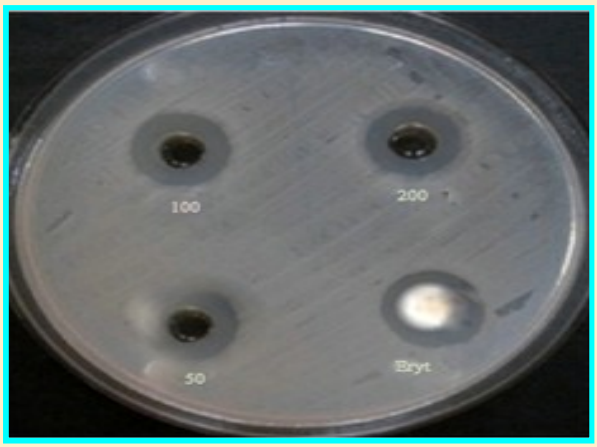
PLATE - 9



9.1:- ***Escherichia coli***



9.2:- ***Bacillus subtilis***



9.3:- ***Pseudomonas aeruginosa***

Showing Antibacterial Activity of *Jatropha curcas* L. Plant Seed Extract Against Different Bacteria

FIGURE-1

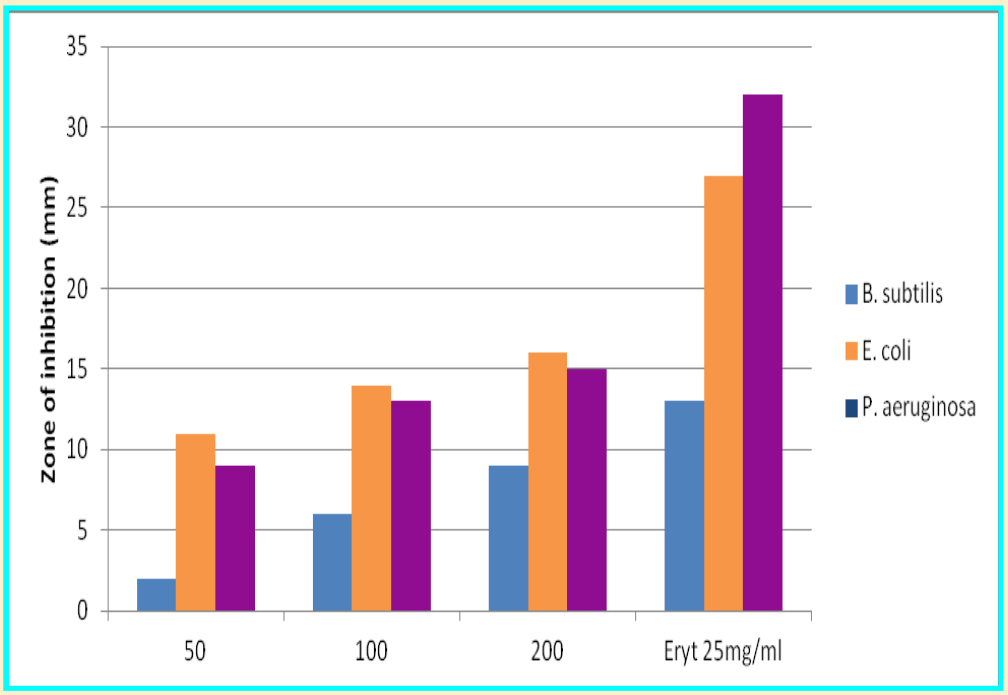


Fig-1A: Comparision of Antibacterial Activity of *Cicer arietinum* L. Methanolic Seed Extract Against Different Bacterial Strains

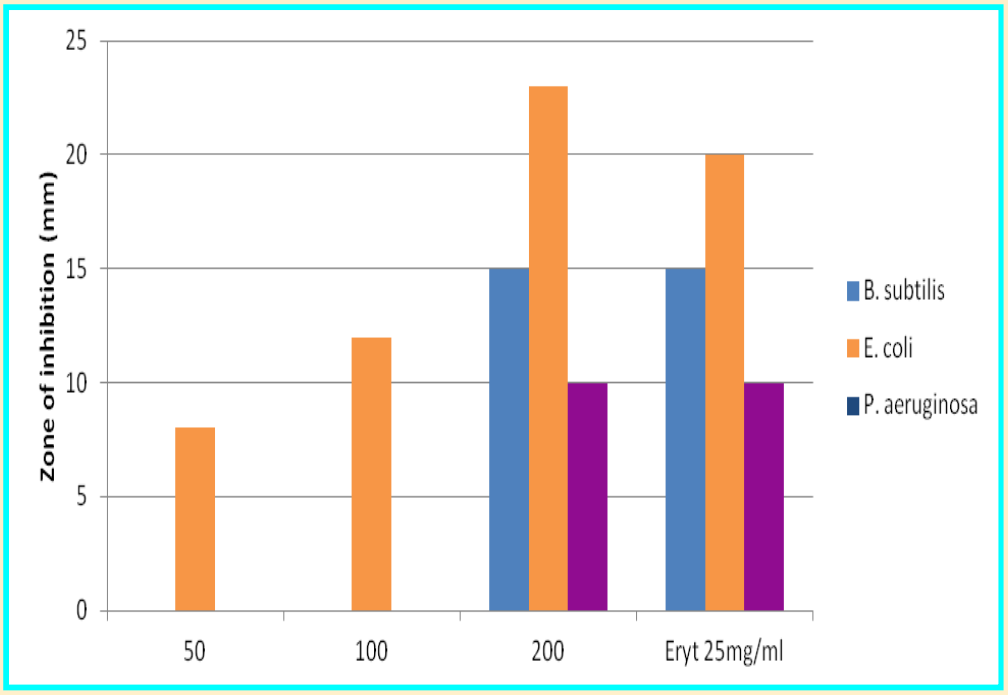


Fig-1B: Comparision of Antibacterial Activity of *Jatropha curcas* L. Methanolic Seed Extract Against Different Bacterial Strains

Table : 9. Antimicrobial Properties of Moringa *oliferea* Seed Methanolic Extract

Con of extract Mg/ml	Zone of inhibition (mm)		
	BS	**EC**	**PA**
50	0	0	0
100	14	12	11
200	16	14	15
Eryt 25mg/ml	18	16	13

BS*: Bacillus subtilis*

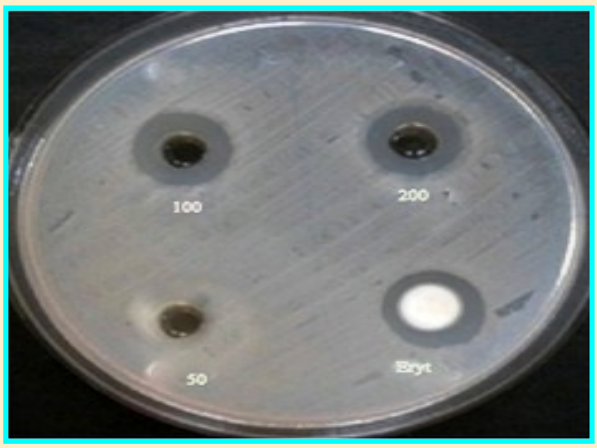
EC: *Escherichia coli*

PA: *Pseudomonas aeruginosa*

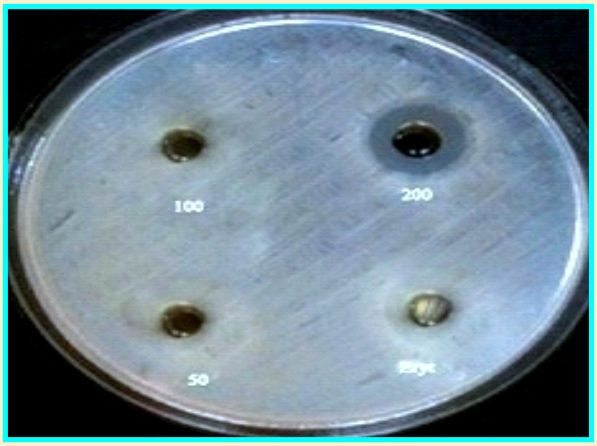
Antimicrobial activity of *Strychnos potatorum:*

Antimicrobial properties of *Strychnos potatorum* methanolic extract increasing pattern of zone of inhibition was observed for *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.* Maximum zone size (20 mm) was reported of erythromycin (Table-10, Figure-2B).

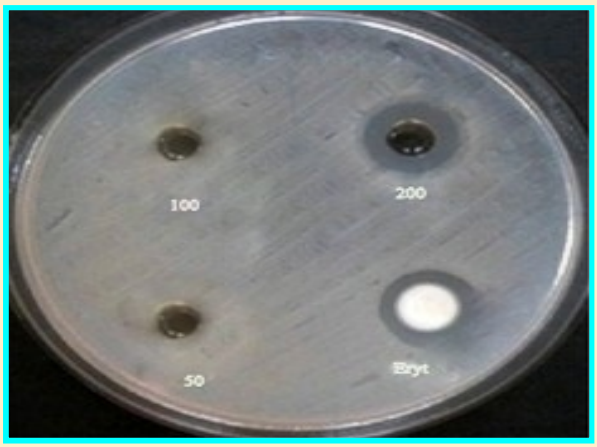
PLATE - 10



10.1:- ***Escherichia coli***



10.2:- ***Bacillus subtilis***



10.3:- ***Pseudomonas aeruginosa***

Showing Antibacterial Activity of *Moringa oleifera* L. Plant Seed Extract Against Different Bacteria

Table : 10. Antimicrobial Properties of *Strychnos potatorum* Seed Methanolic Extract

Con of extract Mg/ml	Zone of inhibition (mm)		
	BS	**EC**	**PA**
50	4	7	8
100	9	11	13
200	14	15	16
Eryt 25mg/ml	16	20	15

BS*: Bacillus subtilis*

EC: *Escherichia coli*

PA: *Pseudomonas aeruginosa*

PART-B

The water quality observations focused on indicator variables, which are illustrated on figures for each plant biocoagulant:

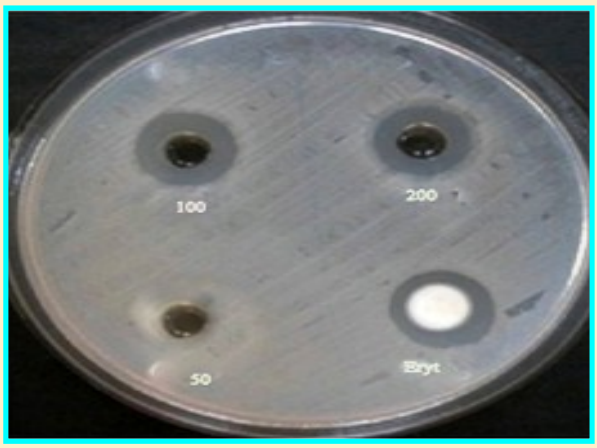
Water quality analysis:

Stock solutions of biocoagulants *Cicer arietinum, Jatropha curcas, Moringa oleifera* and *Strychnos potatorum* were prepared through seed powder for each sample was maintained in the amount of 100 mg/l.

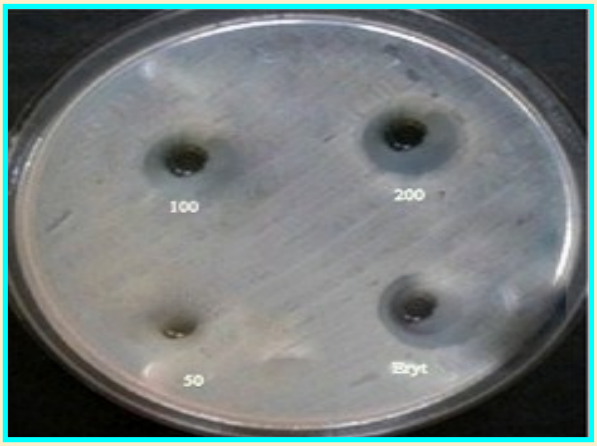
Physio-chemical parameters:

Water samples before and after treatment was estimated in the form of colour, odor and clearity. In all samples control was very

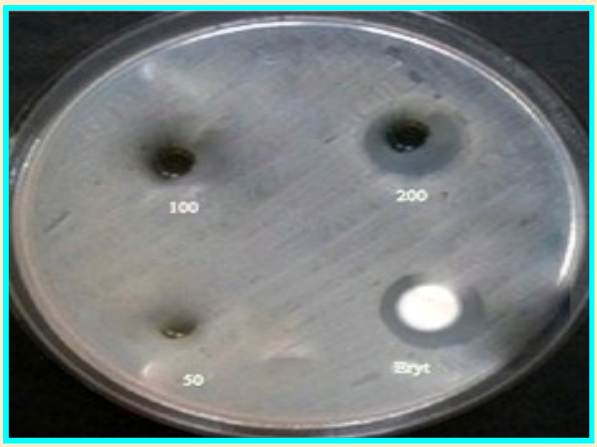
PLATE - 11



11.1:- ***Escherichia coli***



11.2:- ***Bacillus subtilis***



11.3:- ***Pseudomonas aeruginosa***

Showing Antibacterial Activity of *Strychnos potatorum* L. Plant Seed Extract Against Different Bacteria

FIGURE-2

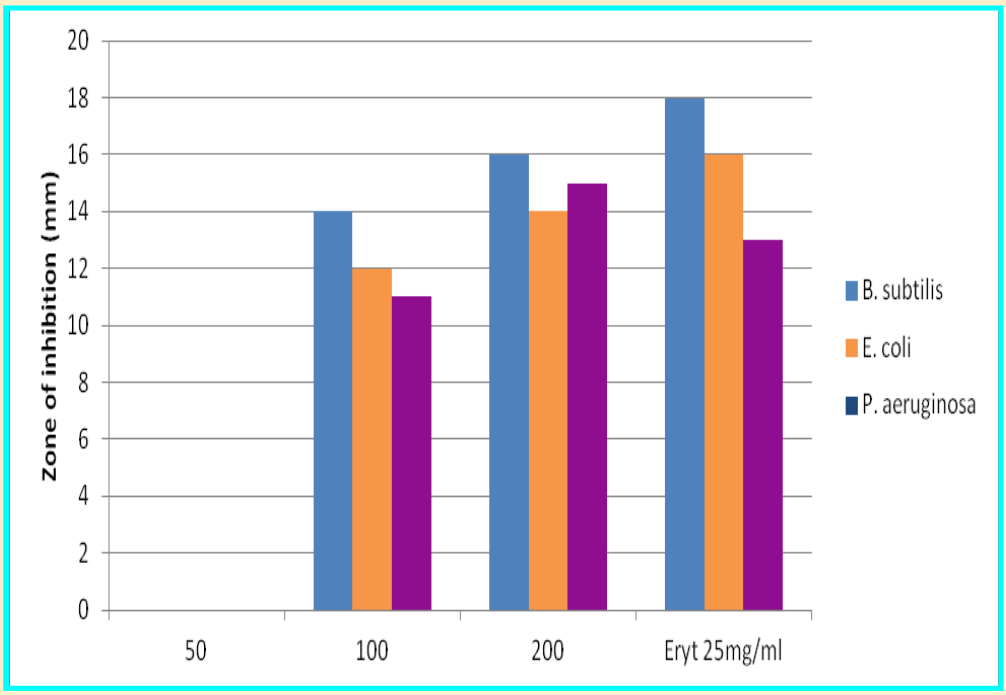


Fig-2A: Comparision of Antibacterial Activity of Moringa *oliferea* L. Methanolic Seed Extract Against Different Bacterial Strains

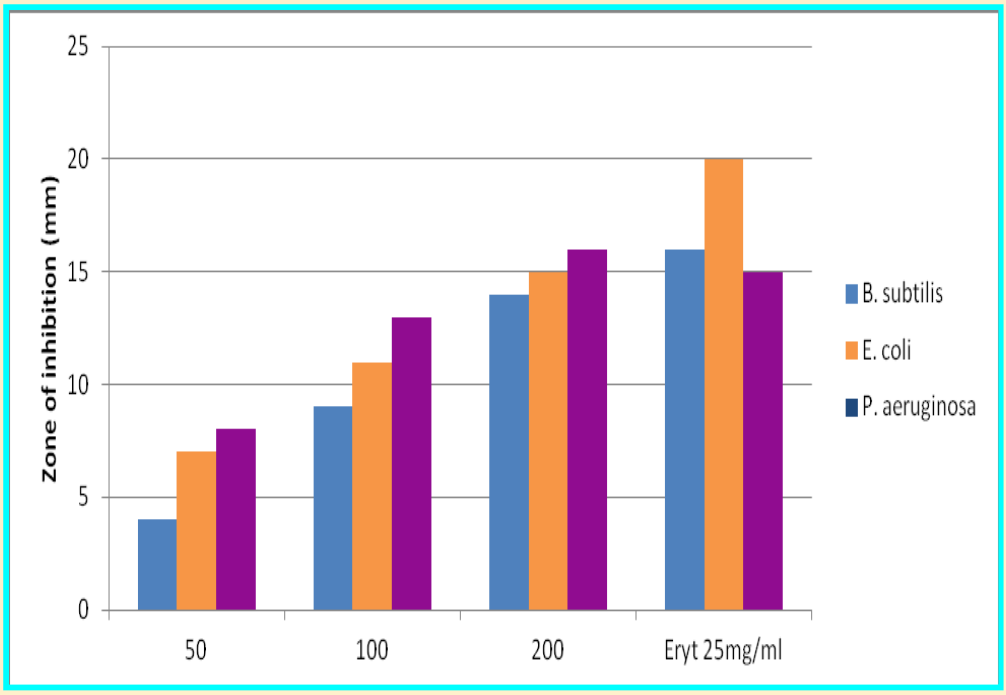


Fig-2B: Comparision of Antibacterial Activity of *Strychnos potatorum* L. Methanolic Seed Extract Against Different Bacterial Strains

cloudy in clearity offensive in odor and dirty brown in colour. Whereas *Moringa oleifera* and *Strychnos potatorum* was similar in clarity which was very clear, odorless in odor and totally colourless. *Jatropha curcas* was clear, slightly odorfull and light brown in colour. On the other hand sample *Cicer arietinum* was cloudy clear, slightly odorfull and light brown in colour (Table-11).

Table: 11. Physicochemical Parameters before and after Treatment of Water with Seed Powder

Sample	Clearity	Odour	Colour
Cicer arietinum	Cloudy	Slight	Light brown
Jatropha curcas	Clear	Slight	Colourless
Moringa oleifera	Very clear	Odourless	Colourless
Strychnos potatorum	Very clear	Odourless	Colourless
Control	Very cloudy	Offensive	Dirty brown

pH:

Hydrogen ion concentration plays an important role in the biological processes of almost all aquatic organisms. Low pH values indicate acidic water having corrosive properties. High pH values indicate alkaline properties. pH values between 6.5 to 8.5 are considered acceptable. Two different concentrations (50 mg/l and 100 mg/liter) were estimated on the behalf of pH before and after treatment of water sample. In all samples control shows maximum pH in both concentrations (8.22, 8.22). In concentration 50 mg/l of plant sample pH of *Strychnos potatorum* shown maximum pH (8.20)

followed by equally *Moringa oleifera* and *Cicer arietinum* (8.15) and the minimum concentration was 8.11 of *Jatropha curcas*.

On the other hand the concentration of 100 mg/l maximum pH was 7.95 of *Moringa oleifera* which was followed by *Strychnos potatorum* (7.70); *Jatropha curcas* (7.21) and *Cicer arietinum* (7.15) respectively (Table-12, Figure- 4B).

Table : 12. Comparative Analysis of pH before and after Treatment of Water with Seed Powder

Sample	pH	
	50 mg/l	**100 mg/l**
Cicer arietinum	8.15	7.15
Jatropha curcas	8.11	7.21
Moringa oleifera	8.15	7.95
Strychnos potatorum	8.20	7.70
Control	8.22	8.22

Turbidity:

Turbidity refers to the cloudiness of a solution and it is a qualitative characteristic which is imparted by solid particles obstructing the transmittance of light through a water sample. After treatment turbidity was maximum in *Jatropha curcas* (12.6 NTU) and minimum in *Moringa oleifera* (7.4 NTU). In *Cicer arietinum* and *Strychnos potatorum* turbidity was estimated (8.4 NTU) and (7.8 NTU) respectively. Maximum turbidity was examined in control that was (54.0 NTU) (Table-13, Figure- 6A).

Table : 13. Comparative Analysis of Turbidity Removal of Water before and after Treatment with Seed Powder Dosage range (100 mg/l)

Sample	Initial turbidity (Before Treatment)	Final turbidity (After Treatment)	% Removal
Cicer arietinum	54.0	8.4 ± 0.83	64.2
Jatropha curcas	54.0	12.6 ± 0.62	42.8
Moringa oleifera	54.0	7.4 ± 0.29	72.9
Strychnos potatorum	54.0	7.8 ± 0.29	69.2
Control	54.0	-	-

Alkalinity:

Comparative analysis of alkalinity of water before and after treatment with seed powder was observed in increasing order from *Cicer arietinum* to control. Maximum alkalinity was estimated (124 mg/l) for control and minimum was (98 mg/l) for sample *Cicer arietinum*. Whereas (100 mg/l, 116 mg/l, 120 mg/l) alkalinity were reported for *Jatropha curcas, Moringa oleifera* and *Strychnos potatorum* respectively (Table-14, Figure-6B).

Table : 14. Comparative Analysis of Alkalinity of Water before and after Treatment with Seed Powder Dosage range (100 mg/l)

Sample	Volume of Sample (ml)	Burette Reading (ml)		Volume of Sulphuric acid (ml)	Alkalinity (Mg/l)
		Initial	Final		
Cicer arietinum	50	0.0	4.9	4.9	98.0 ± 0.62
Jatropha curcas	50	4.9	9.9	5.0	100.0 ± 0.51
Moringa oleifera	50	10.0	15.8	5.8	116.0 ± 0.51
Strychnos potatorum	50	16.0	22.0	6.0	120.0 ± 0.43
Control	50	0.0	6.2	6.2	124.0 ± 0.72

Dissolved Oxygen (DO):

Dissolved oxygen in of considerable importance in water quality investigation and its concentration in water is an indicator of ability of a water body to support a well-balanced aquatic life. DO in water is replenished through photosynthesis, dissolution from the atmosphere and addition of oxygen rich water such as through run-off.

DO of all samples were examined before and after treatment with seed powder. DO of untreated water was (0.2 mg/l). Maximum DO was reported in *Cicer arietinum* (4.5 mg/l) which is followed by *Moringa oleifera* (3.0 mg/l); *Strychnos potatorum* (2.9 mg/l) respectively. Minimum DO was (2.6 mg/l) shown by *Jatropha curcas* (Table-15, Figure-5A).

Table : 15. Dissolve Oxygen of Water before and after Treatment with Seed Powder Dosage range (100 mg/l)

Sample	D_0	D_1	$DO = D_0 - D_1$ (Mg/l)
Cicer arietinum	8.1	3.6	4.5 ± 0.36
Jatropha curcas	8.6	6.0	2.6 ± 0.49
Moringa oleifera	7.2	4.2	3.0 ± 0.47
Strychnos potatorum	6.6	3.7	2.9 ± 0.43
Control (Without treatment)	4.2	4.0	0.2 ± 0.32
Blank	9.0 (C0)	1.6 (C1)	7.4

Biological Oxygen Demand (BOD):

BOD of water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under standard condition at a standardized time and temperature.

Maximum BOD was estimated in control (70 mg/l). In various treated *Cicer arietinum* show maximum BOD (41 mg/l) followed *Moringa oleifera* (26 mg/l); *Strychnos potatorum* (25 mg/l) respectively. Minimum BOD was represented by *Jatropha curcas* (22 mg/l) (Table-16, Figure-5B).

FIGURE-4

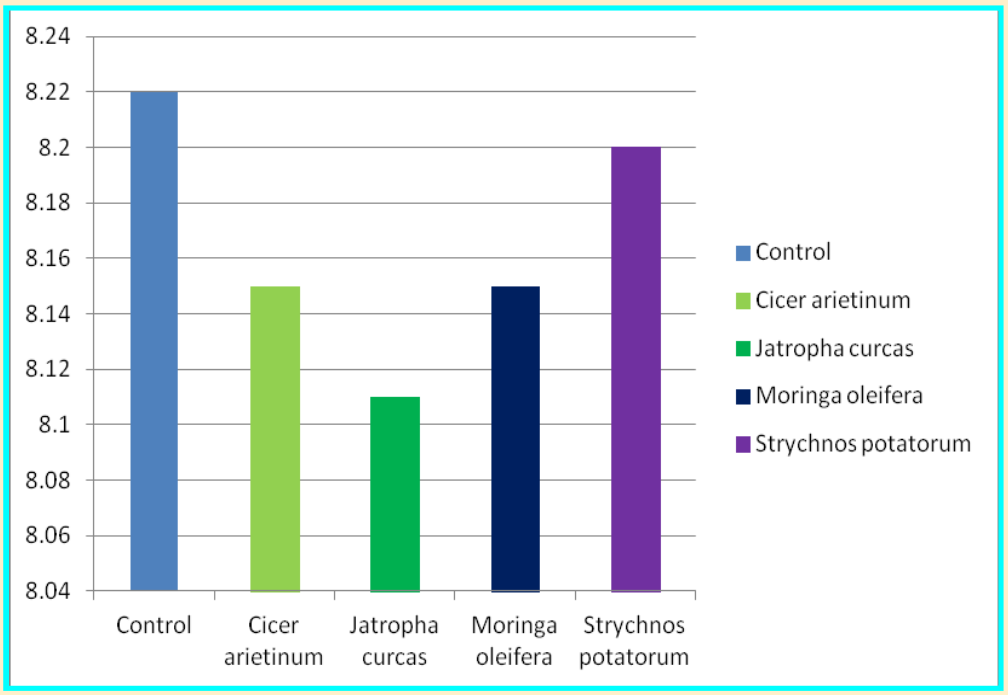


Fig-4A: Comparision of pH Reduction after using Plant Biocoagulants Concentration 50 mg/l

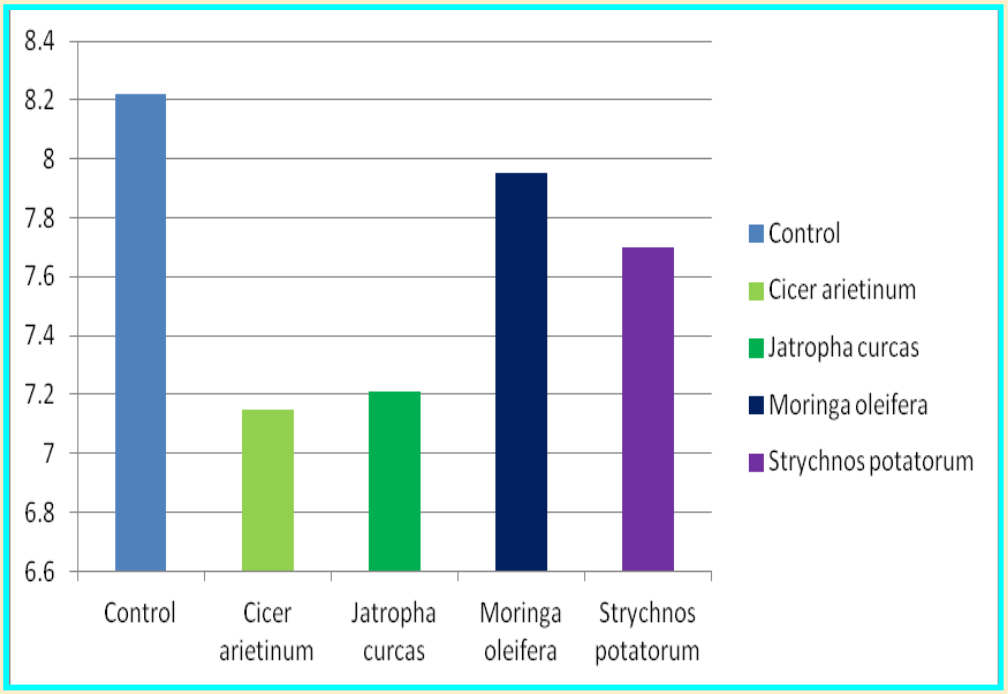


Fig-4B: Comparision of pH Reduction after using Plant Biocoagulants Concentration 100 mg/l

FIGURE-5

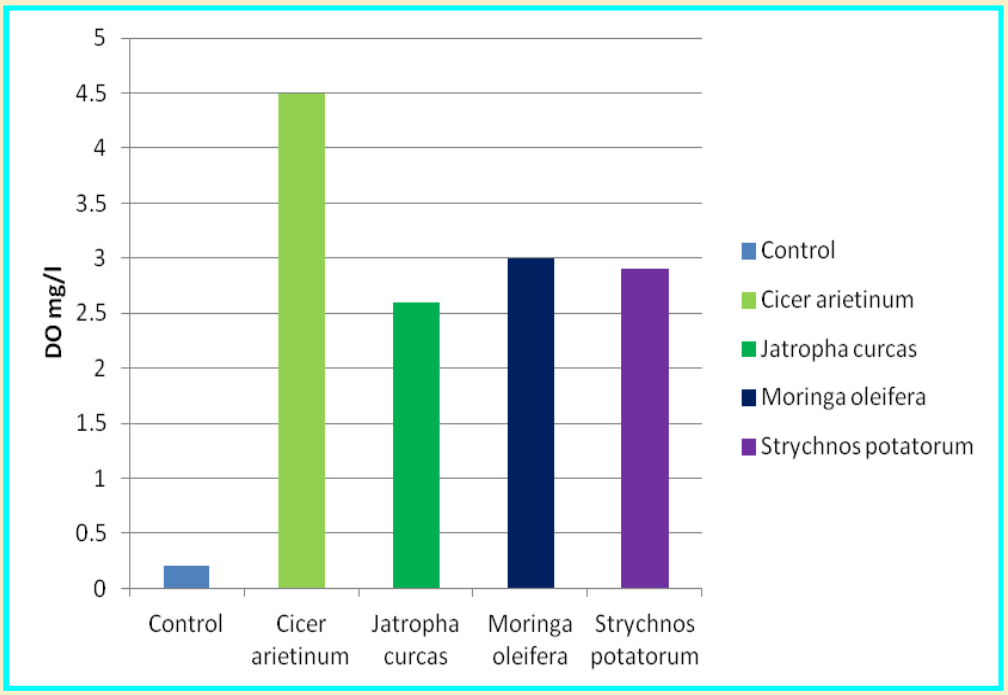


Fig-5A: Comparision of DO after using Plant Biocoagulants Concentration 100 mg/l

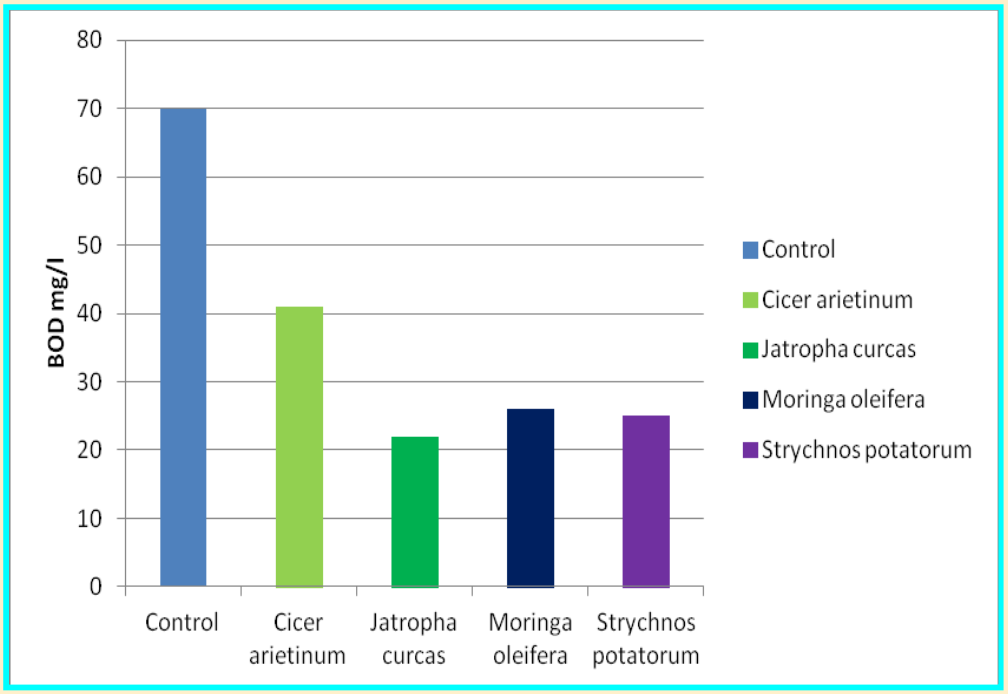


Fig-5B: Comparision of BOD after using Plant Biocoagulants Concentration 100 mg/l

FIGURE-6

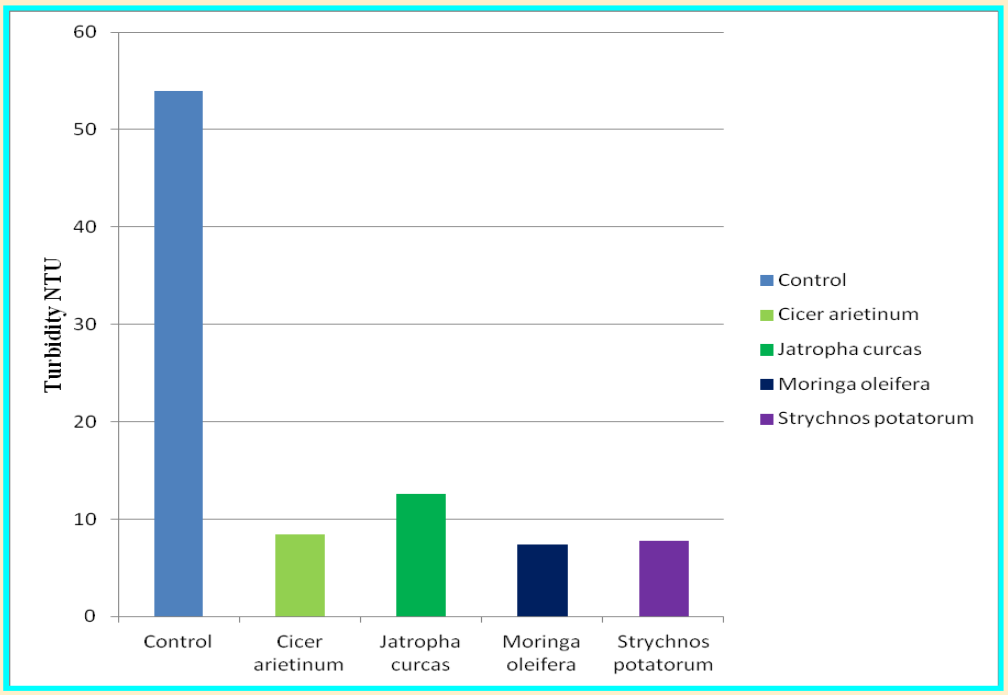


Fig-6A: Comparision of Turbidity Changes after using Plant Biocoagulant Concentration 100 mg/l

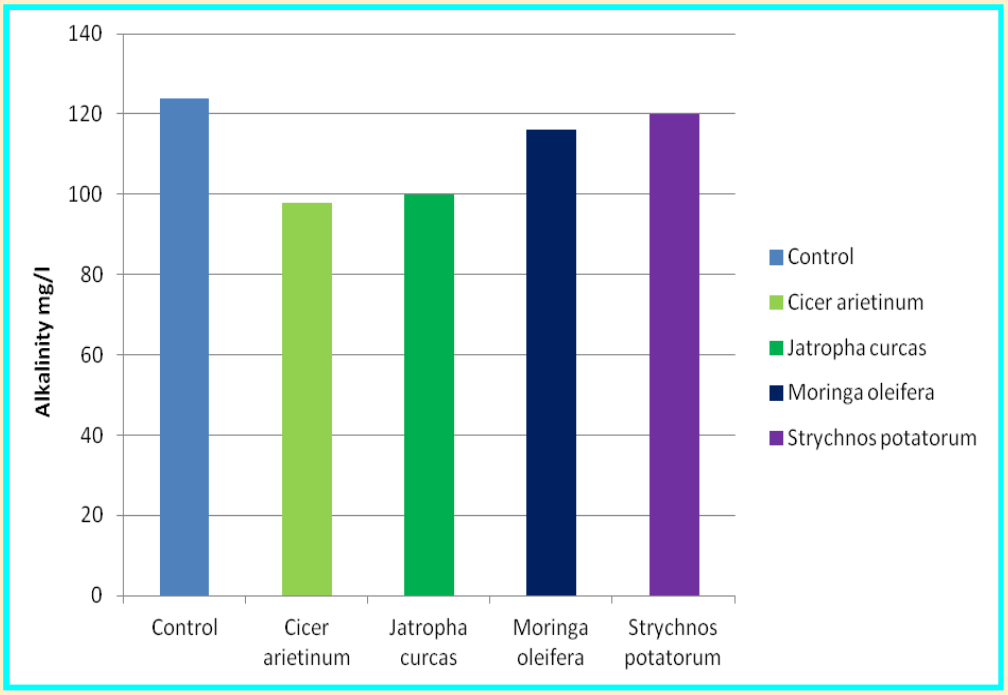


Fig-6B: Comparision of Alkalinity Changes after using Plant Biocoagulants Concentration 100 mg/l

Table : 16. BOD of Water before and after Treatment with Seed Powder Dosage range (100 mg/l)

Sample	DO = $D_0 - D_1$	($C_0 - C_1$)	Decimal fraction of sample	BOD (Mg/l)
Cicer arietinum	4.5	0.4	10	41 ± 1.3
Jatropha curcas	2.6	0.4	10	22 ± 0.42
Moringa oleifera	3.0	0.4	10	26 ± 0.53
Strychnos potatorum	2.9	0.4	10	25 ± 0.55
Control (Without treatment)	7.2	0.2	10	70 ± 0.46

PART-C

GC-MS analysis of plant seed methanol extract was performed. This investigation was carried out to determine the possible chemical compound from natural plant based biocoagulants. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with NIST, WILEY and PESTE library sources were used for matching the identified components from the plant material.

GC-MS analysis of *Cicer arietinum:*

The GC-MS analysis of *Cicer arietinum* seeds methanolic extract revealed the presence of various bioactive compounds such as 2-Propyl-tetrahydropyran-3-ol/ N-Acetylmannosamine (RT: 7.06); 4-O-Methylmannose (RT: 16.9); 5-Hydrxoymethylfurfural (RT: 9.03);

Linoleic acid (RT: 10.7); n-Hexadecanoic acid (RT: 17.4) Maltol (RT: 6.49); Piperidin-2-one-5-carboxylic acid (RT: 10.29) along with other minor constituents were also present (Table-17, Figure-7).

GC-MS analysis of *Jatropha curcas:*

The GC-MS analysis of *Jatropha curcas* seeds methanolic extract revealed the presence of many bioactive compounds such as Glycerin (RT: 10.8); 9,12-Octadecadienoic acid (RT: 19.0); Ethyl 2-acetylhexanoate (RT: 7.02); Methyl hexofuranoside (RT: 15.9); n-Hexadecanoic acid (RT: 17.3); 3-Hydroxy-4-methoxybenzoic acid (RT: 14.0) along with other minor constituents were also present (Table-18, Figure-8).

Table : 17. Shows GC-MS Analysis of Methanolic Seed Extract of *Cicer arietinu* L.

S. No.	Retention Time	Area %	Name of compound
1	6.217	0.16	Methyl 2-furoate
2	6.495	0.30	Maltol
3	7.064	2.65	2-Propyl-tetrahydropyran-3-ol
4	9.032	36.21	5-Hydrxoymethylfurfural
5	9.748	0.08	Trimethylsilyl ester of 3-methyl-furan-2-ca
6	10.294	0.81	Piperidin-2-one-5-carboxylic acid
7	11.737	0.11	Dimethyl phthalate
8	12.026	0.08	Methyl 2-methyltridecanoate
9	12.441	0.26	DI-T-butyl phenol
10	12.658	0.06	Benzoic acid, 4-ethoxy-, ethyl ester
11	13.086	1.36	Hexanoic acid
12	13.689	0.51	Benzoic acid
13	14.508	0.12	d,l-Xylitol, 1-O-undecanoyl-
14	14.821	0.42	Adipic acid, diallyl ester

15	15.108	0.13	1,3-Cyclohexanedicarbohydrazide
16	16.916	39.06	4-O-Methylmannose
17	17.404	3.49	n-Hexadecanoic acid
18	18.149	0.13	Cyclopentane, 1-ethenyl-3-ethyl-2-methyl-
19	18.552	1.12	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
20	19.063	10.72	Linoleic acid
21	20.293	0.19	Nonanoic acid
22	20.544	0.11	Hydroxyethylpalmitamide
23	20.664	0.05	8,11,14-Eicosatrienoic acid
24	21.657	0.05	7-tetradecenal
25	21.824	0.06	Glycine, N-butoxycarbonyl-, propyl ester
26	21.919	0.12	Palmitoyl chloride
27	22.050	0.03	Hexanoic acid, tridecyl ester
28	30.202	0.15	Cholesterol

Table : 18. Shows GC-MS Analysis of *Jatropha curcas* L. Methanolic Seeds Extract

S. No.	Retention Time	Area %	Name of compound
1	4.969	0.16	Phthalan
2	5.577	0.06	1,5-Anhydro-d-talitol
3	6.150	0.15	N-Nitrosooctamethyleneimine
4	6.525	0.18	1,2,3-Propanetriol, monoacetate
5	7.025	1.18	Ethyl 2-acetylhexanoate Ethyl 2-acetylhexanoate methyl-
6	10.838	94.30	Glycerin
7	11.898	0.06	Isosorbide
8	12.537	0.15	beta.-D-Glucopyranose
9	12.892	0.05	D-Allothreonine
10	13.506	0.16	beta.-D-Glucopyranos
11	14.016	0.24	3-Hydroxy-4-methoxybenzoic acid
12	15.093	0.13	Acetoxyacetic acid, 5-pentadecyl ester
13	15.913	1.01	Methyl hexofuranoside
14	17.207	0.04	cis-9-Hexadecenoic acid
15	17.379	0.44	n-Hexadecanoic acid
16	18.261	0.04	2,2-Dimethylpropanoic acid, nonyl ester
17	18.558	0.08	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
18	19.024	1.34	9,12-Octadecadienoic acid (Z,Z)-
19	20.357	0.11	Octanoic acid, 2-ethylcyclohexyl ester
20	21.924	0.01	Palmitoyl chloride
21	22.412	0.03	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester
22	23.306	0.04	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
23	23.860	0.06	1,3-Pentadiene, 2,4-di-t-butyl-

FIGURE-7

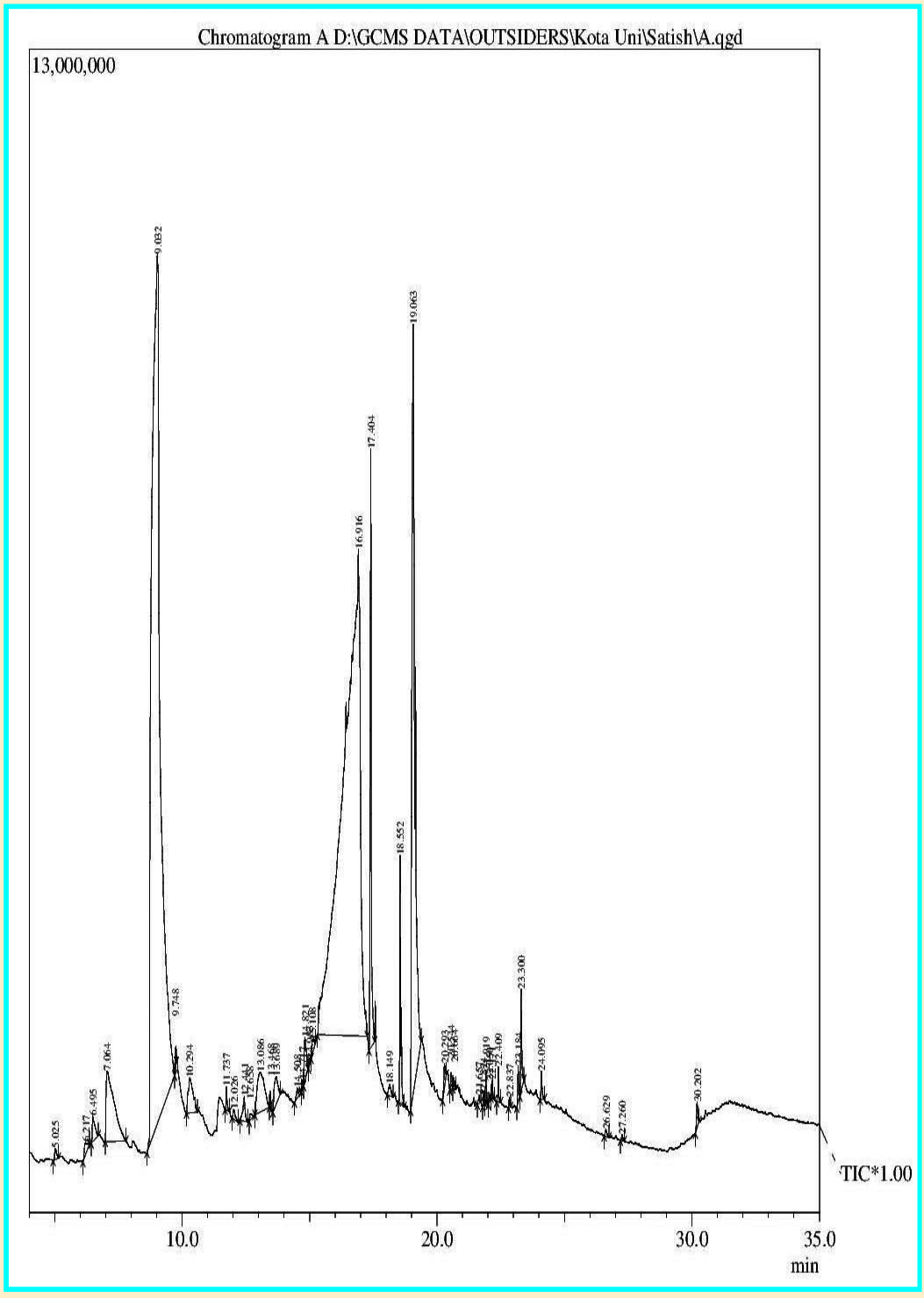


Fig 7: Shows GC-MS Chromatogram of Methanolic Seed Extract of *Cicer arietinum* L.

FIGURE-8

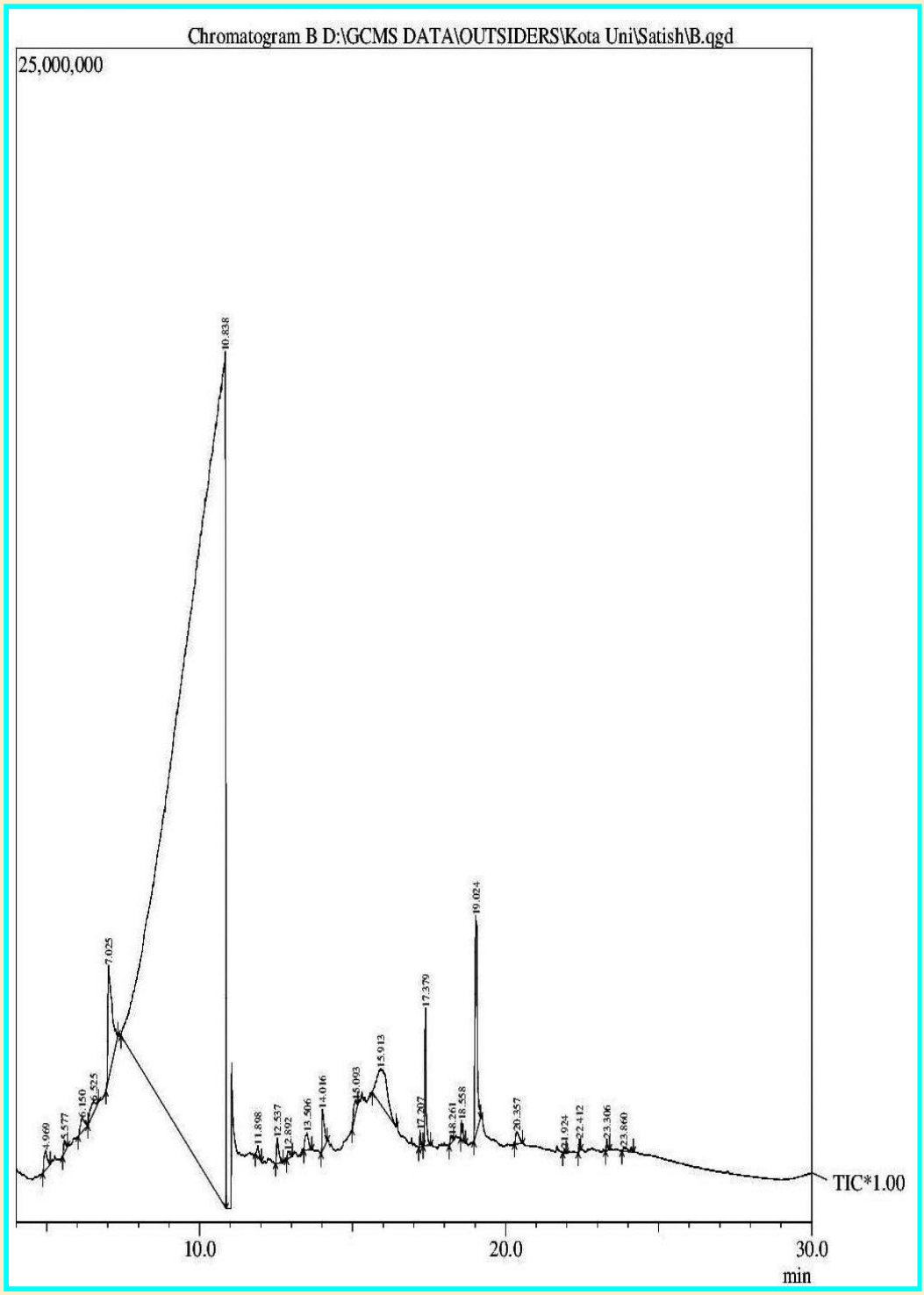


Fig 8: Shows GC-MS Chromatogram of Methanolic Seed Extract of *Jatropha curcas* L.

GC-MS analysis of *Moringa oleifera:*

The GC-MS analysis of methanolic seed extract of *Moringa oleifera* revealed the presence of many bioactive compounds such as 1,2,3-propanetriol/ glycerol (RT: 10.3); Benzeneacetonitrile (RT: 13.1); Oleic acid (RT: 17.2); n-Hexadecanoic acid (RT: 17.4); momeinositol (RT: 16.0) D-Sorbitol (RT: 11.5); 2-octanon (RT: 11.7) along with other minor constituents were also present. Results confirmed the presence of potent biocoagulant compounds in the seed extract (Table-19, Figure-9).

GC-MS analysis of *Strychnos potatorum:*

The GC-MS analysis of *Strychnos potatorum* seeds methanolic extract revealed the presence of many bioactive compounds such as Oleic acid (RT: 19.0); n-Hexadecanoic acid (RT: 16.9) ; Alpha amyrin, D-Allose (RT: 12.8); Nonanoic acid, Isosorbide (RT: 9.6); Tetradecanoic acid (RT: 15.3); Lupeol acetate (RT: 30.7) along with other minor constituents were also present (Table-20, Figure-10). The presence of phytocomponents reveals the importance of the plant as medicinally used. So it is recommended as a plant of phyto-pharmaceutical importance, however further studies will need to be undertaken to ascertain fully its pharmacological activity.

Table : 19. Shows Phytocompounds Identified by GC-MS in Methanolic Extract of *Moringa oleifera* L.

S. No.	Retention Time	Area %	Name of compound
1	4.987	0.74	2-octanon
2	5.892	0.02	2-Methoxyphenyl acetate
3	7.066	0.42	Ethyl 2-Ethyl 2-acetylhexanoate
4	10.348	62.44	1,2,3-Propanetriol
5	11.179	0.05	Ethyl butanoate
6	11.342	0.02	Nicotinic acid
7	11.562	0.58	D-Sorbitol
8	11.714	0.12	4-Octanol
9	12.082	0.15	2-Methyl-l-methylmannopyranoside
10	12.409	0.35	D-Allose
11	13.138	22.61	Benzeneacetonitrile
12	13.578	1.14	hexanoic acid
13	16.071	1.55	Momeinositol
14	17.218	0.76	Oleic Acid
15	17.419	1.87	n-Hexadecanoic acid
16	18.600	0.65	9-Octadecenoic acid, methyl ester, (E)-
17	19.175	5.72	Oleic acid
18	19.300	0.17	Steric acid
19	20.770	0.07	cis-13-Eicosenoic acid
20	21.781	0.07	13-Octadecenal
21	23.311	0.09	Oleoyl chloride

Table : 20. Shows Phytocompounds Identified by GC-MS in Methanolic Extract of *Strychnos potatorum* L.

Peak	Retention Time	Area %	Name of compound
1	4.655	4.89	Glycerin
2	5.766	0.45	Undacane
3	7.114	1.98	2-Propyl-tetrahydropyran-3-ol
4	9.397	1.78	Nonanoic acid
5	9.617	3.50	Isosorbide
6	12.478	0.57	Menthol
7	12.808	5.26	D-Allose
8	13.187	0.52	Dodecanoic acid
9	13.511	0.43	Diethyl Phthalate
10	13.746	3.12	Isocyanic acid, octadecyl ester
11	13.960	1.10	Oleyl alcohol, trifluoroacetate
12	15.277	0.94	Momeinositol
13	15.378	2.01	Tetradecanoic acid
14	16.441	0.78	Phthalic acid,
15	16.922	0.76	Hexadecanoic acid, methyl ester
16	17.204	0.34	cis-9-Hexadecenoic acid
17	17.398	19.78	n-Hexadecanoic acid
18	18.600	2.49	Elaidic acid, methyl ester
19	19.080	28.76	Oleic acid
20	19.243	1.72	Eicosanoic acid
21	22.750	0.83	Capsaicin
22	22.925	0.63	Dihydrocapsaicin
23	24.417	4.39	2,6,10,14,18,22-Tetracosahexaene
24	30.508	1.00	Methyl commate A
25	30.704	1.18	Lupeol acetate
26	30.929	8.09	Methyl commate E

FIGURE-9

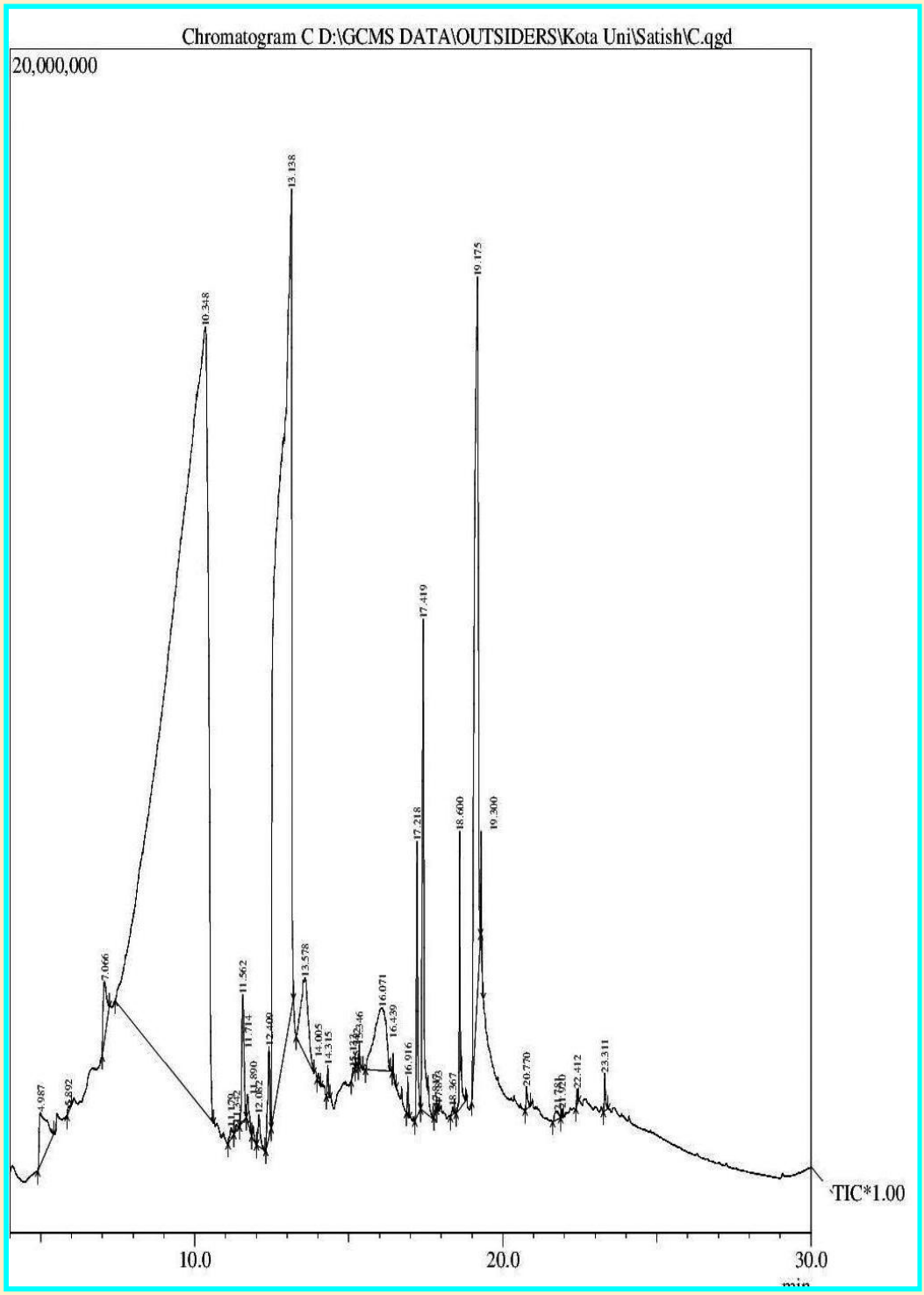


Fig 9: Shows GC-MS Chromatogram of Methanolic Seed Extract of *Moringa oleifera* L.

FIGURE-10

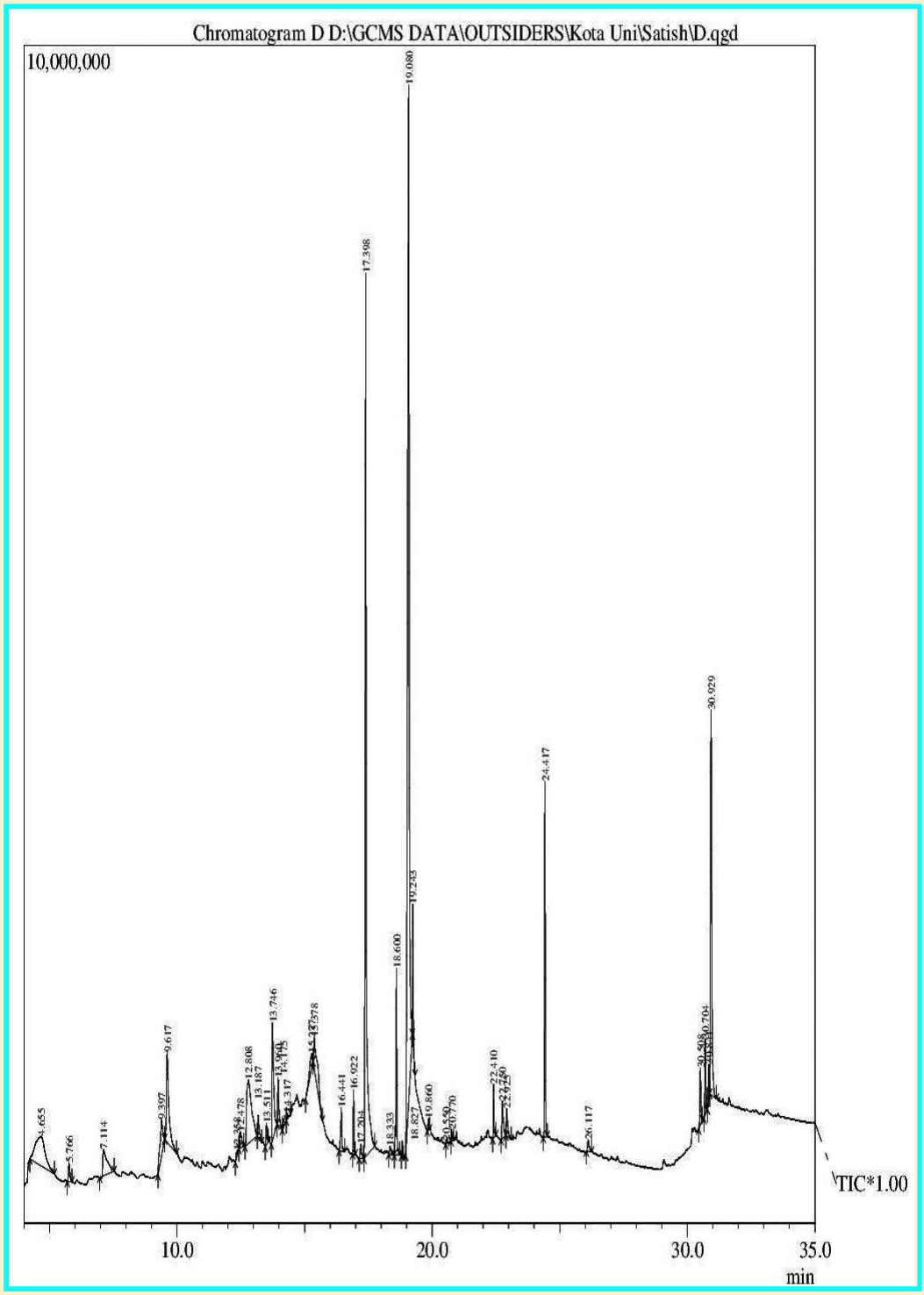


Fig 10: Shows GC-MS Chromatogram of Methanolic Seed Extract of *Strychnos potatorum* L.

PART-D

Properties of various bioactive compounds were obtained from RASMOL and Pubchem demonstrate structure, hydrogen bonds and other hydrophobic interactions that stabilize the ligands at the target site and help alter binding affinity.

linoleic acid obtained from RASMOL and Pubchem shown different chemical properties including number of hydrogen bond donor count (1); Hydrogen bond acceptor count (2) and number of covalently bounded unit was (1).

Properties of 9, 12-Octadecadienoic acid obtained from RASMOL and Pubchem shown by (Table 21) including number of hydrogen bond donor Count (1); Hydrogen bond acceptor count (2) and number of covalently bounded unit was (1). Rotatable bound counts were (14).

Table : 21. Properties of 9, 12-Octadecadienoic Acid Obtained from RASMOL and Pubchem

Molecular Weight	**280.44548 g/mol**
Molecular Formula	$C_{18}H_{32}O_2$
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	2
Rotatable Bond Count	14
Covalently-Bonded Unit Count	1

Computed Properties of Benzeneacetonitrile obtain from RASMOL and Pubchem shown absence of hydrogen bond donor but

hydrogen bond acceptor (2) and rotatable bound counts were (1) (Table 22).

Table : 22. Computed Properties of Benzeneacetonitrile obtain from RASMOL and Pubchem

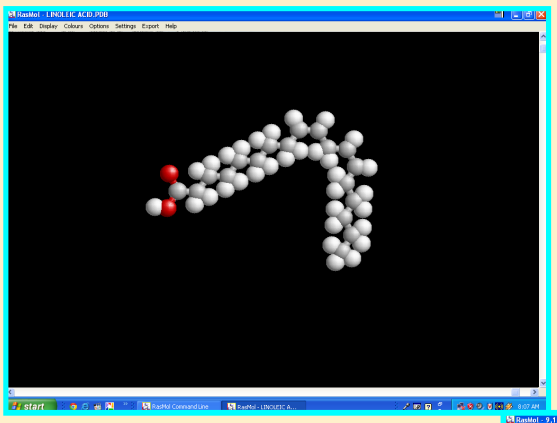
Molecular Weight	**117.14788 g/mol**
Molecular Formula	C_8H_7N
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	1
Rotatable Bond Count	1
Covalently-Bonded Unit Count	1

Table : 23. Properties of Linoleic acid Obtained obtain from RASMOL and Pubchem

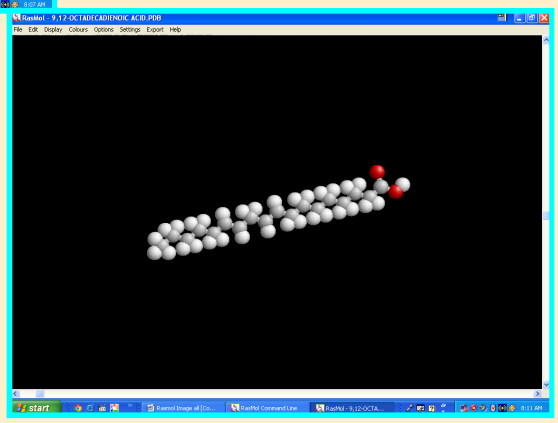
Molecular Weight	**280.44548 g/mol**
Molecular Formula	$C_{18}H_{32}O_2$
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	2
Rotatable Bond Count	14
Defined Bond Stereocenter Count	2
Covalently-Bonded Unit Count	1

Computed Properties of D- Allose obtain from RASMOL and Pubchem revealed that presence of hydrogen bond donor count was (5); Hydrogen bond acceptor count (6) and number of covalently bounded unit was (1) (Table 24).

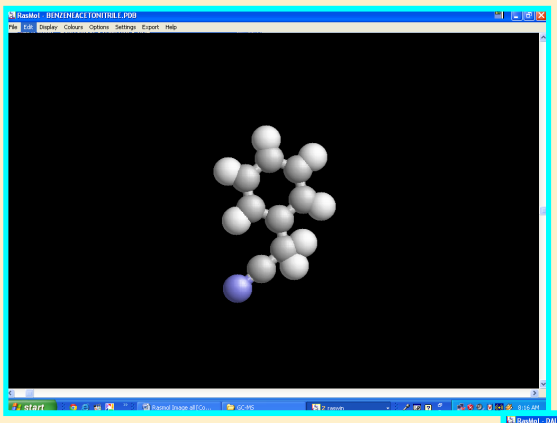
PLATE - 12



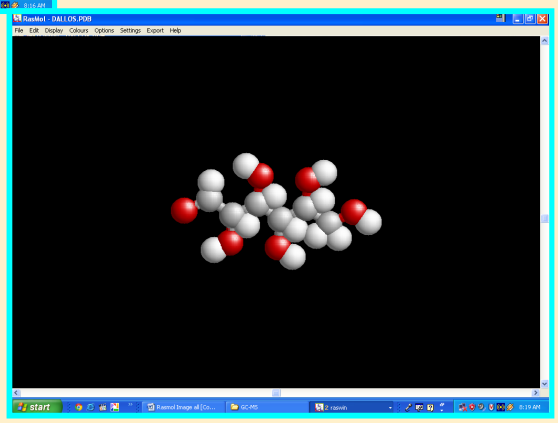
21.1:- Linoleic acid



12.2:- 9,12-Octadecadienoic acid



12.3:- Benzeneacetonitrile



12.4:- D- Allose

Showing Ball and Stick Model of Compounds Using RASMOL

Table : 24. Computed Properties of D- Allose obtain from RASMOL and Pubchem

Molecular Weight	**180.15588 g/mol**
Molecular Formula	$C_6H_{12}O_6$
Hydrogen Bond Donor Count	5
Hydrogen Bond Acceptor Count	6
Rotatable Bond Count	5
Covalently-Bonded Unit Count	1

(Table 25) shown computed properties of Ethyl 2-acetylhexanoate obtain from RASMOL and Pubchem absence of hydrogen bond donor but hydrogen bond acceptor (3) and rotatable bound counts were (7).

Table : 25. Computed Properties of Ethyl 2-acetylhexanoate obtain from RASMOL and Pubchem

Molecular Weight	**186.24812 g/mol**
Molecular Formula	$C_{10}H_{18}O_3$
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	3
Rotatable Bond Count	7
Covalently-Bonded Unit Count	1

(Table 26) reveled computed properties of Glycerin obtain from RASMOL and Pubchem number of hydrogen bond donor Count (3); Hydrogen bond acceptor count (3) and number of covalently bounded unit was (1).

Table : 26. Computed Properties of Glycerin obtain from RASMOL and Pubchem

Molecular Weight	**92.09382 g/mol**
Molecular Formula	$C_3H_8O_3$
Hydrogen Bond Donor Count	3
Hydrogen Bond Acceptor Count	3
Rotatable Bond Count	2
Isotope Atom Count	0
Covalently-Bonded Unit Count	1

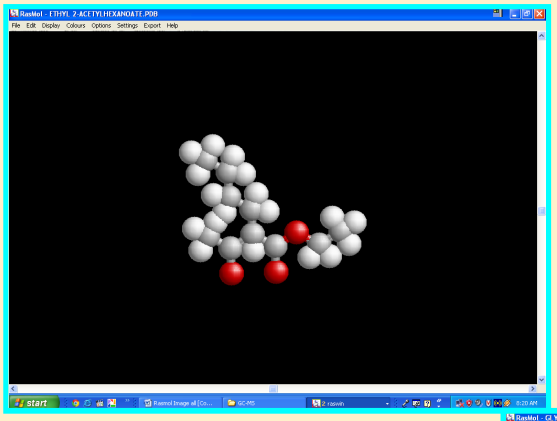
Computed Properties of Hexadecanoic acid obtain from RASMOL and Pubchem shown by (Table 27) including number of hydrogen bond donor Count (1); Hydrogen bond acceptor count (2) and number of covalently bounded unit was (1). Rotatable bound counts were (14).

Table : 27. Computed Properties of Hexadecanoic acid obtain from RASMOL and Pubchem

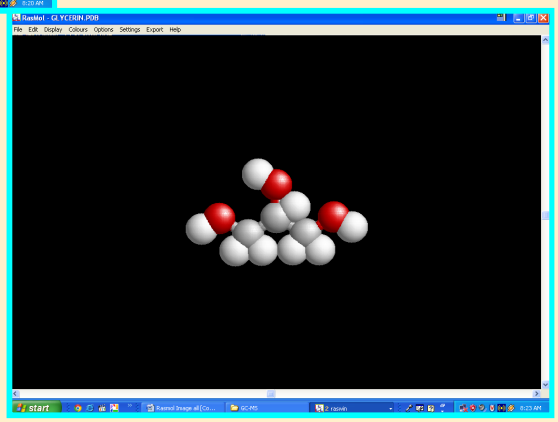
Molecular Weight	**256.42408 g/mol**
Molecular Formula	$C_{16}H_{32}O_2$
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	2
Rotatable Bond Count	14
Covalently-Bonded Unit Count	1

According to (Table28) Computed Properties of Linoleic Acid obtain from RASMOL and Pubchem shown presence of hydrogen bond donor was (1) and hydrogen bond acceptor (2) respectively.Whereas number of covalently bounded unit was (1). Rotatable bound counts were (14).

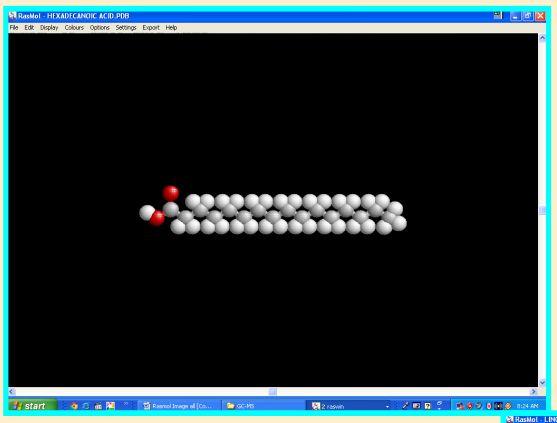
PLATE - 13



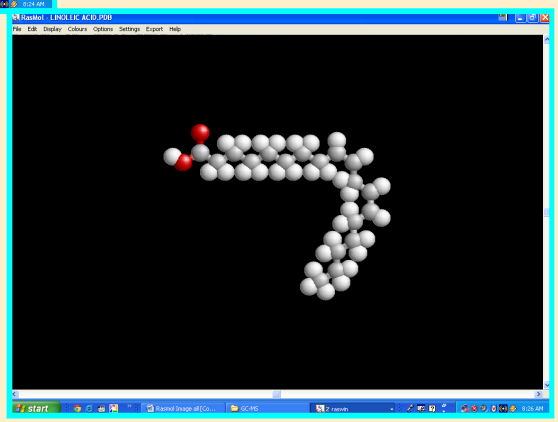
13.1:- Ethyl 2-acetylhexanoate



13.2:- Glycerin



13.3:- Hexadecanoic acid



13.4:- Linoleic Acid

Showing Ball and Stick Model of Compounds Using RASMOL

Table : 28. Computed Properties of Linoleic Acid obtain from RASMOL and Pubchem

Molecular Weight	**280.44548 g/mol**
Molecular Formula	$C_{18}H_{32}O_2$
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	2
Rotatable Bond Count	14
Covalently-Bonded Unit Count	1

Computed Properties of Oleic Acid (Table 29) obtain from RASMOL and Pubchem presence of hydrogen bond donor was (1) and hydrogen bond acceptor (2) respectively. Whereas number of covalently bounded unit was (1). Rotatable bound counts were (15).

Table : 29. Computed Properties of Oleic Acid obtain from RASMOL and Pubchem

Molecular Weight	**282.46136 g/mol**
Molecular Formula	$C_{18}H_{34}O_2$
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	2
Rotatable Bond Count	15
Covalently-Bonded Unit Count	1

(Table 30) shown Computed Properties of 2-Furaldehyde obtain from RASMOL and Pubchem absence of hydrogen bond donor but presence of hydrogen bond acceptor (2). Whereas number of covalently bounded unit was (1). Rotatable bound counts were (1).

Table : 30. Computed Properties of 2-Furaldehyde obtain from RASMOL and Pubchem

Molecular Weight	**96.08406 g/mol**
Molecular Formula	$C_5H_4O_2$
Hydrogen Bond Acceptor Count	2
Rotatable Bond Count	1
Covalently-Bonded Unit Count	1

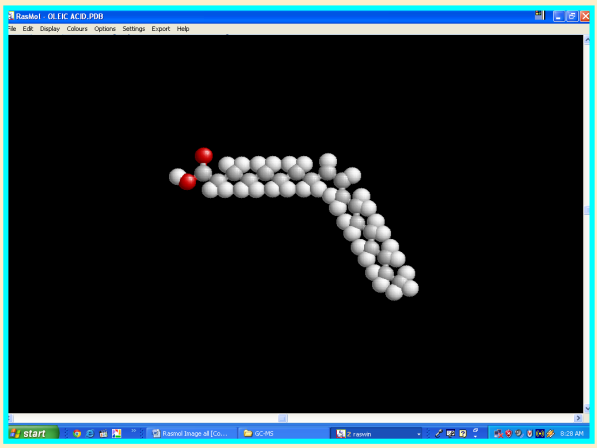
According to (Table 31) Computed Properties of 2-O-Methylhexos obtain from RASMOL and Pubchem shown presence of hydrogen bond donor was (4) and hydrogen bond acceptor (6) respectively. Whereas number of covalently bounded unit was (1). Rotatable bound counts were (6).

Table : 31. Computed Properties of 2-O-Methylhexos obtain from RASMOL and Pubchem

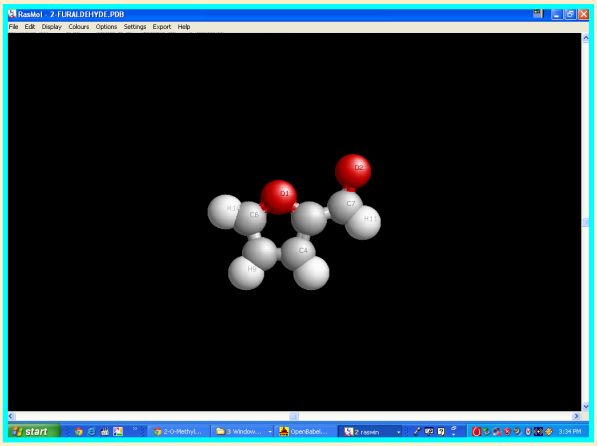
Molecular Weight	**194.18246 g/mol**
Molecular Formula	$C_7H_{14}O_6$
Hydrogen Bond Donor Count	4
Hydrogen Bond Acceptor Count	6
Covalently-Bonded Unit Count	1

Capsules are known to be the preferred delivery method of medications and supplements. They are quick dissolving, fairly inexpensive and easy to fill for both home and commercial purpose. Empty capsule are available in two distinct categories that is traditional gelatin capsule and vegetarian verities. Gelatin is most common material with less expensive option. Another factor to consider when choosing capsule was gelatin that gelatin capsule was only suitable for use with plant powder.

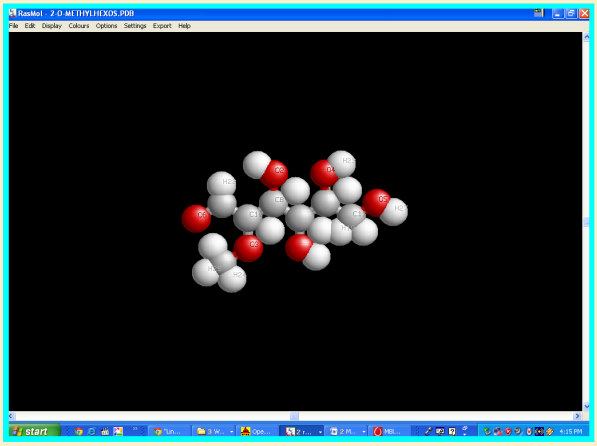
PLATE - 14



14.1:- Oleic Acid



14.2:- 2-Furaldehyde



14.3:- 2-O-Methylhexos

Showing Ball and Stick Model of Compounds Using RASMOL

PLATE - 15

C. arietinum **L. Seed Powder Capsule**

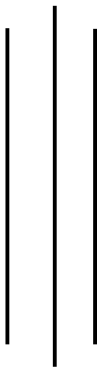
J.curcas **L. Seed Powder Capsule**

M. oleifera **L. Seed Powder Capsule**

S. Potatorum **L. Seed Powder Capsule**

Showing Encapsulated Dried Seed Powder Capsules

CHAPTER – 5



DISCUSSION & CONCLUSION

Discussion anD conclusion

The waste generated by modern society when discarded in nature can make the water unfit for human consumption. Thus to obtain drinking water is necessary to perform a physical-chemical treatment which allow the removal of the turbidity and organisms harmful to health. Various methods are used to make water safe to the consumer. The method employed depends on the character of the raw water. For the treatment of surface water some traditional chemicals are used during the treatment of surface water at its various steps. Commonly used chemicals for various treatment units are synthetic organic and inorganic substances. In most of the cases, these are expensive since they are required in higher dose and do not shows cost effectiveness. Many of the chemicals are also associated with human health and environmental problems. The use of clean up technologies without producing other harmful waste products is required as best option using vegetation to remove, detoxify or stabilize persistent pollutant is an accepted tool for cleaning of polluted soil and water. Natural coagulants have been used for domestic household for water treatment in rural areas. Now a day some reports describe natural coagulants from plants are used for natural water purification.

The use of plant seed materials is receiving attention for their effectiveness in wastewater treatment. The technologies involved are economical, traditional and easy to implement. These observations motivate me to analyze the biocoagulant property of some plant species *Cicer arietinum, Jatropha curcas, Moringa oleifera* and

Strychnos potatorum (Table: 3 & Plate: 1, 2) were selected for further study.

Operation and maintenance of plant-based technologies operating a plant-based water clarifier system is very simple with no major machinery or specialized labor required as observed by Kebreab, (2004) and Yongabi, (2006). Some previous studies have screened a number of plants as disinfectant for water treatment, *Acrorus calamus*, *Anaphalis cunefolia*, *Arnebial nobills*, *Eclipta aibba*, *Azadirachta indica*, *Moringa oleifera* (Jahn, 1981).

The seed powder of *Moringa oleifera* has been used in many african societies for water clarification for domestic use reported by Sutherland *et al.,* (1990); Lowell, (2001); Kebrea, (2004). The seed powder of physic nut (*Jatropha curcas*) is very useful in wastewater treatment. This plant belongs to the family Euphorbiaceae. Reports on the potentials of this plant in wastewater treatment existed by Yongabi K.A., (2004).

Rajendran *et al.,* (2013) reported that seeds of *Strychnos potatorum* and *Moringa* oleifera have shown promising result as the source of natural coagulant in the clarification of turbid water. Asrafuzzaman *et al.,* (2011) studied the reduction efficiencies of *Moringa oleifera*, *Dolichos lablab, Cicer* aretinum in treatment of synthetic water and reported that *Cicer* aretinum is most effective in reduction of turbidity.

During the period of present assessment percent yield of secondary metabolite of each sample were estimated after ethanolic and methanolic solvent extraction (Table: 4 & 5). Percent yield in

ethanolic extract observed maximum in *Strychnos potatorum* and minimum in *Jatropha curcas* where as percent yield in methanolic extract observed maximum in *Cicer arietinum* and minimum in *Jatropha curcas.* Extraction yields ranged from 4.3% to 10.6% for ethanolic extract and for methanolic extract extraction ranged from 3.6 % to 12.8%. The polarity of the solvent play an important role in the extraction of active plant extracts as shown by the higher yields obtained. It can also be found that the yield of the ethanolic extract is slightly less than that of methanolic extract.

The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters.

Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc. which have been found in vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswas *et.al.*,2002).

The present study was undertaken to compare the effect of using different extraction solvents to extract the active components like Alkaloids, Flavanoids, Phenol, Saponin and Steroids from the dried seed powder (Table: 6 & Plate: 4,5). During phytochemical analysis study of *Cicer arietinum* plant seed ethanolic solvent extract our result indicated that various secondary metabolites like alkaloids, flavanoids, saponin, and steroids were present. While phenol was absent in ethanolic extract . It may be due to poor solubility of this

phytochemical in ethanol. Alkaloid and steroids were absent in methanolic extract. Similar results were obtained by Mamta Arora *et al.*, (2013) who reported the presence of various phytoconstituents such as phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, fixed oils and fats.

Our results during phytochemical analysis of *Jatropha curcas* ethanolic and methanolic extract contains phytochemical such as flavanoids and phenol in higher amount. However, saponins was found to be absent in the extract made by using methanol (Table: 6). It may be due to poor solubility of this phytochemical in methanol These results are supported by work done by James *et al.*, (2011); Oseni and Alphonse (2011) and Oskoueian *et al.*, (2011). They reported aqueous leaf extract of *J. curcas* contains flavonoids, phenols, tannins and alkaloids.

The present study of phytochemical investigation of *Moringa oleifera seed* powder revealed the presence of alkaloids, flavanoids, phenol and steroids (Table: 6 & Plate: 4, 5). Saponin was absent in both ethanolic and methanolic extract. Whereas phenol in ethanolic extract was present in higher amount. This may be attributable to the higher solubility in ethanol. The observations made during the present study are in agreement with Nepolean *et al.*, (2009) but disagreed with the finding of saponins.

Our results indicated that alkaloids, flavanoids, phenol, saponin and steroids all components were shown their presence in both ethanolic and methanolic extract of *Strychnos potatorum* (Table: 6 & Plate: 4, 5). The results of the phytochemical screening of the

different extracts *Strychnos potatorum* is in accordance with the studies of Packialakshmi *et al.*, (2014) who reported the presence of alkaloids, flavanoids, phenol, saponin and steroids in seed extract of *Strychnos potatorum* .

These results are supported by work done by Mallikharjuna *et al.*, (2007); Venkatesh *et al.*, (2011); Srikanth Kagithoju. *et al.*,(2013) studied the phytochemical screening of *Strychnos potatorum* seed sample and described along with physical and chemical compound such as alkaloids, reducing sugar, phytosterol, fats, phenolic compounds and tannins.

The results obtained in the present study thus suggest that the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. Many studies have been conducted to establish the antimicrobial effect of the medicinal plants (Habsah *et al.*, 2000; Sudhakar *et al.*, 2006).

Antibacterial activity of *Cicer arietinum* seeds methanolic extract against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Plate: 7) indicate that the antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). Maximum inhibitory zone size was reported in erythromycin against all bacterial strain and minimum was in 50 mg/ml concentration of plant extract (Table: 7). As compared with

standard drugs, the results revealed that in the plant extracts show less antibacterial activity. The growth inhibition zone measured ranged from 2 to 32 mm for all the sensitive bacteria (Plate: 8.1, 8.2 & 8.3 and Figure: 1A).The efficacy of plant extracts evaluated as antimicrobial agents was dependent on the solvent of extraction.

According to Mamta Arora *et al.*, (2014) *Cicer arietinum* L. seed methanol and hydro alcoholic extracts shows more antimicrobial activity against gram negative bacteria (13mm) than gram positive bacteria (9mm).

The activity of *J. curcas* against *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* has also been reported by Kalimuthu *et al.* (2010) ; Kamal *et al.* (2011); Oseni and Alphonse (2011) they reported that the aqueous extract shows very little antimicrobial activity compared with the solvent extracts.

Results of present research indicates that antimicrobial activity of *Jatropha curcas* seeds methanolic extract shown (0 mm) zone size of *Bacillus subtilis* and *Pseudomonas aeruginosa* were reported in 50 and 100 mg/ml concentration. Maximum inhibitory zone size (23 mm) was reported in 200 mg/ml against *Escherichia coli* bacterial strain (Table: 8). The antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the plant extracts show more antibacterial activity. The growth inhibition zone measured ranged from 0 to 23 mm for all the sensitive bacteria. 50 mg/l concentration of extract less effective against all test organisms (Plate: 9.1, 9.2 & 9.3 and Figure: 1B). Similar findings were obtained

by Anwar and Rashid, (2007); Jamil *et al.* (2007); Lockett *et al.* (2000) who reported that the extracts of *Morinaga oleifera* showed antimicrobial activity.

Our results indicated that antimicrobial properties of *Moringa oliferea* methanolic extract increasing pattern of zone of inhibition was observed for *Bacillus subtilis* and *Escherichia coli but Pseudomonas aeruginosa* maximum zone size (15 mm) was reported in 200 mg/l of extract (Table: 9). The growth inhibition zone measured ranged from 0 to 18 mm for all the sensitive bacteria (Plate: 10.1, 10.2 & 10.3 and Figure: 2A). As compared with standard drugs, the results revealed that in the plant extracts show less antibacterial activity. 50 mg/l concentration of extract ineffective against all test organisms.

Antibacterial activity of *Strychnos potatorum* seeds methanolic extract against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* indicate that the antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the plant extracts show less antibacterial activity (Table: 10). The growth inhibition zone measured ranged from 4 to 20 mm for all the sensitive bacteria (Plate: 11.1, 11.2 & 11.3 and Figure: 2B). The results revealed that all plant extracts were potentially effective in suppressing microbial growth.

The results of the antibacterial activity of the *Strychnos potatorum* plant extract are in accordance with the studies of Mallikharjuna *et al.*, (2009) and Packialakshmi *et al.*, (2013) they

reported that *Strychnos potatorum* seed extract show antimicrobial activity against some pathogenic gram positive and gram negative bacteria.

Knowledge on the physico-chemical characters of the water source is very important because it may influence the immediate environment of aquatic, wet lands flora and human health. WHO (1971 & 1976) standards for drinking water specify that, water intended for human consumption must be free from organisms and from concentrations of chemical substances that may be a hazard to health. In addition, supplies of drinking water should be as pleasant to drink as circumstance permit.

Results of present research work indicate that water samples physical parameters were estimated in the form of colour, odor and clearity (Table: 11). In all samples control was very cloudy in clearity offensive in odor and dirty brown in colour. Whereas *Moringa oleifera* and *Strychnos potatorum* treated water was very clear, odorless and colourless. *Jatropha curcas* treated water was clear, slightly odorfull and light brown in colour. On the other hand sample *Cicer arietinum* treated water was cloudy clear, slightly odorfull and light brown in colour (Table: 11).

Colour in water may be caused by the presence of minerals such as iron and manganese or by substances of vegetable origin such as algae and weeds. Colour tests indicate the efficacy of the water treatment system. Odour and taste are associated with the presence of living microscopic organisms or decaying organic matter, industrial wastes containing ammonia, phenols, halogens, hydrocarbons.

According to WHO, (1984) the desirable pH of drinking water is 7 to 8.5, the pH has no direct adverse effect on health, but at the same time alters the taste of water. Higher pH reduces the germicidal potentiality of chlorine and induces the formation of toxic trihalomethanes (Trivedy and Goal, 1986).

Our results indicated that two different concentrations (50 mg/l and 100 mg/liter) were estimated on the behalf of pH before and after treatment of water sample. In all samples control shows maximum pH in both concentrations (8.22, 8.22). In concentration 50 mg/l of plant sample pH of *Strychnos potatorum* shown maximum pH (8.20) followed by equally *Moringa oleifera* and *Cicer arietinum* (8.15) and the minimum concentration was 8.11 of *Jatropha curcas* (Table: 12 & Figure: 4A).

On the other hand the concentration of plant sample 100 mg/l maximum pH was 7.95 of *Moringa oleifera* which was followed by *Strychnos potatorum* (7.70); *Jatropha curcas* (7.21) and *Cicer arietinum* (7.15) respectively (Table: 12 & Figure: 4B). This study has conclusively indicated that pH of water can be reduced considerably with the application of phytocoagulants.

Our results also shows similarly with the study of Eman *et al.*, (2010) that the pH of the treated wastewater decreased from after the addition of different coagulants.

Tasneembano Kazi and Arjun Virupakshi (2013) reported that *Cicer arietinum, Moringa oleifera* and *Cactus* used as locally available natural coagulants to reduce turbidity of tannery wastewater.

R.M.S. Radin Mohamed et *al.*, (2014) studied that the pH value of raw car wash waste water before treatment was in the range of 8.0 - 9.0. It was observed that after the treatment with alum, *Moringa Oleifera* and *Strychnos Potatorum* the pH reduced to the range between 6.5 and 7.0.

The optimum value of pH depends essentially on the properties of the water treated, type of coagulant used and its concentration (Abdul Aziz et al., 2007).

Turbidity refers to the cloudiness of a solution which is imparted by solid particles obstructing the transmittance of light through a water sample. Results of our present work indicate that after treatment turbidity was maximum in *Jatropha curcas* (12.6 NTU) and minimum in *Moringa oleifera* (7.4 NTU). In *Cicer arietinum* and *Strychnos potatorum* turbidity was estimated (8.4 NTU) and (7.8 NTU) respectively. Maximum turbidity was examined in control that was (54.0 NTU) (Table: 13 & Figure: 6A). Selected biocoagulants reduced turbidity ranged from 42.8% to 72.9% after treatment. This study has conclusively indicated that turbid water can be treated considerably with the application of phytocoagulants. It has been evident from our findings that *Moringa oleifera* was more effective as prime coagulant for turbidity removal in water purification. This act as green technology chemical over traditional chemicals of water purification.

Asrafuzzaman *et al* ., (2011) studied the reduction efficiencies of *Moringa* oleifera, *Dolichos lablab* and *Cicer* aretinum in treatment of synthetic water and reported that *Cicer aretinum* is most effective in reduction of turbidity.

Abidin Z.Z. (2011) reported that the *Jatropha* seed found to be an effective coagulant with more than 96% of turbidity removal at pH 1-3 and pH 11-12. The highest turbidity removal was recorded at pH 3 using a dosage of 120 mg/L.

Yasabie Abatneh *et al*., (2014) indicated that *Moringa oleifera*, *J. curcas* and *G. gum* reduce turbidity of water. The reduction efficiency is higher for more turbid waters. Turbidity reduction exceeding 90 % was achieved for all the three extracts on shallow well water with an initial turbidity of about 50 NTU.

The alkalinity natural or treated wastes is the capacity of some of its components to accept protons that is to be bind an equivalent amount of strong acid like hydroxyl ions and anions of weak acid (e.g. bicarbonates and carbonates). It is the therefore, a measure of the buffering capacity of the water (Train, 1978).

Our results indicated that comparative analysis of alkalinity of water before and after treatment with seed powder was observed in increasing order from *Cicer arietinum* to control. Maximum alkalinity was estimated (124 mg/l) for control and minimum was (98 mg/l) for sample *Cicer arietinum*. Whereas (100 mg/l, 116 mg/l, 120 mg/l) alkalinity were reported for *Jatropha curcas, Moringa oleifera* and

Strychnos potatorum respectively (Table: 14 & Figure: 6B). This study has conclusively indicated that alkalinity of treated water can be reduced considerably with the application of selected phytocoagulants. It has been evident from our findings that *Cicer arietinum* was more effective plant based coagulant for alkalinity removal in water purification.

Mangale Sapana M *et al.,* (2012) stated that alkalinity during the research work was observed to be 130mg/l for ground water. At various doses of *Moringa oleifera* seed powder, it was observed that the alkalinity reduced after the treatment at 50 mg/l dose. But at higher dose of 100 and 150 mg/l of *Moringa* seed, the alkalinity was slowly increased. The alkalinity was present in the range of 95 - 100 mg/l which was within limits of WHO standards.

Dissolved oxygen present in drinking water adds taste and it is a highly fluctuating factor in water. According to European Economic Community, the permissible standard of drinking water for dissolved oxygen in 5 mg/l to 7.3 mg/l.

Results of present research indicates DO of all samples were examined before and after treatment with seed powder. DO of untreated water was (0.2 mg/l). Maximum DO was reported in *Cicer arietinum* (4.5 mg/l) which is followed by *Moringa oleifera* (3.0 mg/l); *Strychnos potatorum* (2.9 mg/l) respectively. Minimum DO was (2.6 mg/l) shown by *Jatropha curcas* (Table: 15 & Figure: 5A). This study has conclusively indicated that DO of treated water can be

increased considerably with the application of selected phytocoagulants. It has been evident from our findings that DO levels are increased effectively by the application of *Cicer arietinum* based coagulant for water purification.

Drinking water usually has a BOD of less than 1 mg/l and water is considered to be fairly pure with BOD of 3 mg/ l and of doubtful purity when the BOD values reach 5mg/l (Rao, 1997).

Results of present work indicate that maximum BOD was estimated in control (70 mg/l). In various treated *Cicer arietinum* show maximum BOD (41 mg/l) followed *Moringa oleifera* (26 mg/l); *Strychnos potatorum* (25 mg/l) respectively. Minimum BOD was represented by *Jatropha curcas* (22 mg/l) (Table: 16 & Figure: 5B).Our study has conclusively indicated that BOD of treated water can be manipulated considerably with the application of selected phytocoagulants. Results indicated the use of natural coagulants derived from plants as an alternative to usage of chemical coagulants for water purification. The organic matter supported good microbial growth; the microorganism utilizes the oxygen and leads to high values of BOD, COD and low values of dissolved oxygen. Similar results were obtained by Chinedu *et al.*, (2011) and Shrivastava *et al.*, (2010).

GC-MS has a relatively broad coverage of non-volatile compound classes, mainly those involved in primary metabolism, including organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates within the polar phase (Roessner *et al.*, 2000; Schauer *et al.*, 2006), as well as lipophilic compounds such as

fatty acids, fatty alcohols, sterols, and aliphatics within the apolar phase (Fiehn *et al.*, 2000).

Enas J. Kadhim (2014) reported that GC-MS of *Moringa oleifera* the comparison of the mass spectrum with the NIST database library gave more than 90% match as well as a confirmatory compound structure match. Identified compounds may be used in drugs, pharmaceutical and therapeutic value.

GC-MS analysis of *Cicer arietinum* seeds methanolic extract revealed the presence of 28 bioactive compounds such as 2-Propyl-tetrahydropyran-3-ol/ N-Acetylmannosamine. 4-O-Methylmannose, 5-Hydrxoymethylfurfural, Linoleic acid, n-Hexadecanoic acid , Maltol , Piperidin-2-one-5-carboxylic acid along with other minor constituents (Table: 17 & Figure: 7). Present study results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory, flavor agent, antimicrobial activity. Based on the results plant powder contains chemical constituents of pharmacological, nutritional and phytocoagulants significance.

Results of present work the GC-MS analysis of *Jatropha curcas* seeds methanolic extract revealed the presence of 23 bioactive compounds such as Glycerin, 9,12-Octadecadienoic acid, Ethyl 2-acetylhexanoate, Methyl hexofuranoside, n-Hexadecanoic acid, 3-Hydroxy-4-methoxybenzoic acid along with other minor constituents (Table: 18 & Figure: 8). Present study results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory, antimicrobial, antifungal, antitumor activity. Based on the results

plant powder contains chemical constituents of pharmacological, nutritional and phytocoagulants significance.

GC-MS analysis of methanolic seed extract of *Moringa oleifera* revealed the presence of 21 bioactive compounds such as 1,2,3-propanetriol/ glycerol , Benzeneacetonitrile (RT: 13.1), Oleic acid , n-Hexadecanoic acid , momeinositol , D-Sorbitol , 2-octanon along with other minor constituents. Results confirmed the presence of potent biocoagulant compounds in the seed extract (Table: 19 & Figure: 9). The methanolic seed extract of *Moringa oleifera* proved to be reservoir of bioactive compounds. Present study results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory, anticancer, antimicrobial, antifungal, wound healing activity. Based on the results plant powder contains chemical constituents of pharmacological and phytocoagulants significance.

According to Adegbe A. A. *et al* (2016) *M. Oleifera* seed oil was analyzed by a combination of GC and GCMS. Twenty four constituents were identified. The major constituents were Oleic acid, Palmitic acid, 9-octadecenol and phenylbut-3-yne. The fixed oil of *M. Oleifera* seed is rich in fatty acids followed by hydrocarbons, others are aldehyde esters and oxygenated hydrocarbon.

GC-MS analysis of *Strychnos potatorum* seeds methanolic extract revealed the presence of 26 bioactive compounds such as Oleic acid, n-Hexadecanoic acid , Alpha amyrin, D-Allose , Nonanoic acid, Isosorbide , Tetradecanoic acid , Lupeol acetate along with other minor constituents (Table 20 & Figure: 10). The presence of phytocomponents reveals the importance of the plant as

medicinally used. Present study results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antifungal, appetite increasing, antitumor, antiproliferative activity. Based on the results plant powder contains chemical constituents of pharmacological and phytocoagulants significances.

Various ligands will select from literature survey and chemical compound will download from PubChem compound database reported by (Wang *et al.,* 2009). Various successful and research target will select from literature survey and TTD (Chen *et al.*, 2002). 3D structure of protein will download from the Protein Data Bank (PDB) (Abola *et al.,* 1996; Kouranov *et al.,* 2006). Then target will further analyze for active site details by using Rasmol (Abola *et al.,* 1996).

The compounds having drug like properties will select as ligands to carry out molecular docking studies in GOLD (Verdonk et al., 2003; Bharatham et al., 2007) software against the receptors, which will obtain from PDB (www.rcsb.org/pdb) (Abola *et al.,* 1996; Kouranov *et al.,* 2006). Scoring will use by Gold score (Jones *et al.*, 1997; Jacobsson, 2008) and Chem score method (Eldridge *et al.*, 1997; Jacobsson, 2008).

Our results indicated that computed properties of 9, 12-Octadecadienoic acid obtain from RASMOL and Pubchem contain number of hydrogen bond donor Count (1); Hydrogen bond acceptor count (2) (Table: 21 & Plate:12.2). The compounds will select as ligands to carry out molecular docking studies.

Our results indicated that that computed properties of Benzeneacetonitrile obtain from RASMOL and Pubchem shown absence of hydrogen bond donor but hydrogen bond acceptors were 2 (Table: 22& Plate:12.3). The compounds will select as ligands to carry out molecular docking studies.

Results of present work indicated that that computed properties of D- Allose obtain from RASMOL and Pubchem contain hydrogen bond donor count was 5 and Hydrogen bond acceptor count (6) (Table: 24 & Plate:12.4). The compounds will select as ligands to carry out molecular docking studies.

Result showed computed properties of Ethyl 2-acetylhexanoate obtain from RASMOL and Pubchem (Table: 25 & Plate:13.1). Absence of hydrogen bond donor but hydrogen bond acceptor were 3. The compounds will select as ligands to carry out molecular docking studies.

Present study revealed computed properties of Glycerin obtain from RASMOL and Pubchem (Table: 26 & Plate:13.2) that numbers of hydrogen bond donor Count were 3 and Hydrogen bond acceptor count were 3.

Our results indicated that Hexadecanoic acid contain number of hydrogen bond donor Count were 1 and Hydrogen bond acceptor count were 2 (Table: 27 & Plate:13.3). The compounds will select as ligands to carry out molecular docking studies.

Present study revealed computed properties of Linoleic Acid obtain using RASMOL and Pubchem shown presence of hydrogen

bond donor was 1 and hydrogen bond acceptor were 2 respectively (Table: 28 & Plate:13.4).

Present study revealed computed properties of Oleic Acid using RASMOL and Pubchem shown presence of hydrogen bond donor was 1 and hydrogen bond acceptor were 2 respectively (Table: 29 & Plate:14.1).

Our results indicated that computed properties of 2-Furaldehyde using RASMOL and Pubchem shown absence of hydrogen bond donor but presences of hydrogen bond acceptor were 2 (Table: 30 & Plate:14.2).

Computed properties of 2-O-Methylhexos reveled presence of hydrogen bond donor were 4 and hydrogen bond acceptor 6 respectively (Table: 31& Plate:14.3). The compounds will select as ligands to carry out molecular docking studies.

Capsules are known to be the preferred delivery method of medications and supplements. They are quick dissolving, fairly inexpensive and easy to fill for both home and commercial purpose. Empty capsule are available in two distinct categories that is traditional gelatin capsule and vegetarian verities. Gelatin is most common material with less expensive option (Plate: 15). Another factor to consider when choosing capsule was gelatin that gelatin capsule was only suitable for use with plant powder.

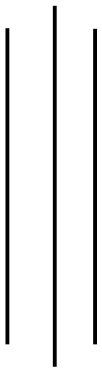
The findings of the present study further justify traditional uses of plant based biocoagulants for water purification. The use of natural coagulants from plant based source represent a vital development in

sustainable environmental technology as it focus mainly on the improvement of quality of life for communities. The bio coagulant process become more efficient and cost of treatment is reduced. Biocoagulants helpful in removal of turbidity, heavy metals, colour from wastewater. Hence, the present studies also justify the claimed use of these plants (*Cicer arietinum*, *Jatropha curcas*, *Moringa oleifera*, *Strychnos potatorum*) in the traditional water purification system. The study of qualitative phytochemical analysis of study plants seed extract revealed the presence of bioactive compounds such as terpenoids, alkaloids, flavonoids, phenols, steroids and saponins which were known to be antimicrobial in function.

The findings present study should stimulated the search for novel, natural product such as new antimicrobial agents derived from plants. Thus our finding regarding antibacterial potential of these studied plants may have small start and suggesting their utility in treatment of various human diseases caused by pathogenic bacteria.

Still there are not many reports available regarding GC-MS analysis, molecular modeling, dosage of seed powder for water purification and plant seed powder based capsule of studied plants.

CHAPTER – 6



FUTURISTIC APPROACH

futuristic approach

The usage of plant based natural coagulants represents a fundamental development in sustainable environmental technology for the improvement of quality of life for communities. In an era of increasing environmental concerns, water scarcity admits the draw backs of chemical coagulants and poor sanitary facilities in most low income earning countries, the need to further develop natural coagulants as alternative environmentally favorable water purifying chemicals is exigent. The usage of bio-coagulants derived from plant based sources represents a vital development in 'grassroots' sustainable environmental technology through cost effectiveness.

Design natural water purification techniques using plants extracts for bioremediation of turbid water. Application of this low-cost protocol will be recommended for simplified, point-of-use, low-risk water treatment where rural and peri-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water. The ultimate purpose of proposed research study is to come up with a compendium of plant coagulants that could be used as a technology that is cost effective and ecofriendly. It is felt that further research can be conducted by using the information described in this review as a platform to discover other plant species which are non-toxic and can be mass produced.

However there are many pressing issues that are hindering process development of these coagulants to be precise.

- Absence of mass plantation of the plants that affords bulk processing
- Perceived low-volume market and virtually non-existent supportive regulation that stipulates the quality of the processed coagulant extracts.
- The cost-effectiveness of using the natural coagulant as simple POU technology.
- The last factor is especially vital since it is normally difficult for regulatory authorities to endorse a product for sale to the general public.

It is felt that application of plant based coagulants is currently restricted to small-scale usage and academic research but it can benefit from fervent promotion and endorsement from relevant stakeholders, particularly the from the authorities. In technical terms, these natural coagulants are highly effectual for treatment of waters with low turbidity but may not be feasible in the case of wastewaters with extreme pH. As such, it is always prudent for water treatment practitioners to circumspectly select the most suitable natural coagulants and tailor them for specific proposes. Quite clearly it is felt that further research can be conducted by using the information described in this review as a platform to discover other plant species which are non-toxic and can be mass produced.

- To characterize the molecular structure, formula, weight, and charge of the isolated bioactive constituents.
- To carry out detailed studies on the bioactive constituents to determine possible positive synergistic effects in the combination of isolates on application in water (for potable use) and wastewater (industrial and municipal) treatment for water pollution control (surface and groundwater).
- To produce samples of the best combination of isolate of the bioactive constituents.
- To develop commercially viable, (recovery of value added products e.g. oil, biocompost, biofuel) sustainable, and efficient seed processing techniques.
- To develop user-friendly and appropriate technology techniques for application of processed seeds for water treatment and water pollution control in developing countries
- Development of economical and commercial processing techniques of plant based coagulants seeds i.e medium and high technology methods.
- Oil extraction (mechanical press, solvent extration, steam extraction, enzyme assisted extraction etc.) as value added product.
- Seed husk, pods etc. as raw material for biocoversion into biocompost, bioalcohol etc.

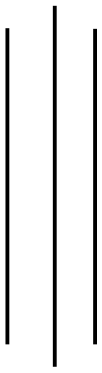
- Processing of cake for application in water treatment and water pollution control after oil extraction

Applications:

- Suitable for medium to large scale applications.
- Have high shelf life
- Large scale production for export to other countries.
- Efficient in the treatment of raw water with diverse characteristics.
- Low dosage applications.

Many useful byproducts e.g. oil, poultry and livestock feed, bio-fertilizer etc.

CHAPTER – 7



SUMMARY

Summary

Introduction:

Water is the most vital element among the natural resources. In many developing countries today access to clean and safe water is a crucial issue. The surface water becomes highly polluted due to indiscriminate discharge of untreated waste from tannery, textile, municipal waste into water bodies, poor drainage system. More than six million people die because of diarrhea which is caused by polluted water. Developing countries pay a high cost to import chemicals for water treatment. Water from all sources must have some form of purification before consumption. Various methods are used to make water safe to the consumer. The method employed depends on the character of the raw water. One of the problems with treatment of surface water is the large seasonal variation in turbidity. For the treatment of surface water, some traditional chemicals are used during the treatment of surface water at its various steps. Commonly used chemicals for various treatment units are synthetic organic and inorganic substances. In most of the cases, these are expensive since they are required in higher dose and do not shows cost effectiveness. Many of the chemicals are also associated with human health and environmental problems so, there raised a voice to develop cost-effective, easier and environmental friendly process of water clarification. In recent years there has been considerable interest in the development of usage of plant natural coagulants. These coagulants are biodegradable and are presumed to be safe for human health. In addition natural coagulants produce readily biodegradable and less voluminous sludge that amounts only 20– 30% that of alum

treated counterpart. The use of clean up technologies without producing other harmful waste products is required as best option using vegetation to remove, detoxify, or stabilize persistent pollutant is an accepted tool for cleaning of polluted soil and water.

The production of drinking water from most raw water sources involves coagulant use at a coagulation or flocculation stage to remove turbidity in the form of suspended and colloidal material. Many coagulants and flocculants are widely used in conventional water treatment processes. These materials can be classified into inorganic coagulants (e.g. aluminium and ferric salts) and synthetic organic polymers (e.g. polyacryl amide derivatives and polyethylene imine). Aluminium salts are cheap and are the most widely used coagulants in water and wastewater treatment all over the world. Regarding the application of synthetic polymers, the presence of residual monomers is undesirable because of their neurotoxicity and strong carcinogenic properties.

Natural coagulants have been used for domestic household for centuries in traditional water treatment in rural areas. Now a day, some reports describe natural coagulants from plants are used for natural water purification. The use of plant seed materials is receiving attention for their effectiveness in wastewater treatment. The technologies involved are economical, traditional and easy to implement and ideal for rural areas. The process being biological in nature does not generate any non-treatable wastes. These processes are easy to operate and require little or no maintenance. For the future development of the use of plant materials for wastewater treatment, other native plants and plant materials should be investigated as coagulants for color and turbidity removal.

Review of literature:

Phytoremediation is divided into different areas such as phytoextraction, rhizofiltration, phytostabiilzation, phytodegradation, rhizodegradation and phytovolatilization . Phytoextraction also called by the name of phytoaccumulation, refers to the uptake and translocation of metal contaminants in the soil by plant roots into the aerial parts of plants (Eaphan *et al*., 2005).

(Md. Asrafuzzaman *et al.,* 2011) reported that some locally available natural coagulants for example *Moringa oleifera, Cicer arietinum, Dolichos lablab* used for significant improvement in removing turbidity and total coliforms from synthetic raw water. Maximum turbidity reduction was found for highly turbid waters. After dosing, water-soluble extract of *Moringa oleifera, Cicer arietinum*, and *Dolichos lablab* reduced. It was also found that these natural coagulants reduced about 89–96% of total coliforms. (G.Muthuraman *et al.,* 2013) studied coagulation-flocculation followed by sedimentation and filtration is the most commonly used water treatment process in which turbidity or particles removal is strongly dependent on proper coagulant dosage, effect of pH ,effect of time, jar test and settling column tests were perform. (Tasneembano Kazi1 *et al.*, 2013) reported Natural coagulants are used as point-of- use technology in less-developed communities, since they are relatively cost-effective compared to chemical coagulants. Also they can be easily processed in usable form and biodegradable. *Cicer arietinum, Moringa oleifera*, and *Cactus* were used as locally available natural coagulants used to reduce turbidity and COD of tannery wastewater. (Daniyan S. Y. *et al.,* 2011) revealed

phytochemical analysis of the crude seed extracts of *Jatropha .curcas* and analysed the presence of alkaloid, glycosides, flavonoid and carbohydrate. The ability of the crude seed extracts of *Jatropha curcas* to inhibit bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial. The antimicrobial effects of the methanol, ethyl acetate and hexane extracts of *Jatropha curcas* seed. The ability of the crude seed extracts of *J. curcas* to inhibit bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. According to (Mangale S. M *et al.,* 2012) *Moringa oleifera* seeds acts as a natural coagulant, flocculent, absorbent for the treatment of drinking water. It reduces the total hardness, turbidity, acidity, alkalinity, chloride after the treatment. It also acts as a natural antimicrobial active against the micro-organisms which is present in the drinking water and decrease the number of bacteria. *Moringa oleifera* seed is not giving any toxic effect. It is eco-friendly and cheaper method of purification of water and therefore can be used in the rural areas where no facilities are available for the treatment of drinking water. (Sonal Choubey 2013) reported the performance of *Strychnos potatoram* seed extract as primary coagulant and compared with the performance of alum. *Strychnos potatoram* seed extract is effective as prime coagulant. The effectiveness of *Strychnos potatoram* in the removal of turbidity, total hardness, pH, COD, BOD and total dissolved solids (TDS) has been investigated. The results obtained from this study satisfy the drinking water standards prescribed by world health organization (WHO). *Strychnos potatoram* was found to be especially effective in reducing the parameters like turbidity, BOD, COD, hardness.

Obtained sludge cakes, with high content of proteins could be used as a fodder, but additional analyses should be conducted first. Phytochemical characterization using various solvent extracts was studied by (Neha grover *et al.*, 2013) indicate that various extracts of the leaf and flower of *Woodfordia fruticosa* were screened for the presence of steroids, reducing sugars, alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, glycosides and Vitamin by standard qualitative test procedures and further this study was extended by analyzing the potent bioactive compounds in the methanolic extract of *Woodfordia fruticosa* leaves using GC-MS analysis. In the qualitative phytochemical screening using various solvent extracts of plant, it was found that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. The GC-MS analysis revealed the presence of twenty one compounds in the methanolic leaf extract of *Woodfordia fruticosa*. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. (Lengauer and Rarey, 1996) Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. In the context of molecular modelling, docking means predicting the bioactive conformation of a molecule in the binding site of a target structure. (Blaney and Dixon, 1993) In essence, this is equivalent to finding the global free energy minimum of the system consisting of the ligand and the target. (Verkhivker *et al.*, 2000; Totrov and Abagyan, 1997)

Material and Methods:

(A) Phytochemical analysis:

Sample Collection: Riped fruits (pods) of *Cicer arietinum* (Fabacae), *Jatropha curcas* (Euphorbiacae), *Moringa oleifera* (Moringacae) *and Strychnos potatorum* (Loganiaceae) (Table-3) collected from seed suppliers JDG seed company Neemuch.

Sample Treatment**:** The seeds are peeled to obtain the nuts and dried in an oven for 1hr. Thereafter grind the dried seeds and sieved to mesh size of 150 pm.

Solvent Extraction: The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, sonication and supercritical fluid extraction. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Solvent extraction was done by using soxhlet extraction method.

Phytochemical analysis: Various extracts of the seed powder were screened for the presence of, alkaloids (Mayer's test), phenolics (Ferric chloride test), flavonoids (Aqueous sodium hydroxide test), steroids (Salkowski's test), saponins (Sodium bicarbontate test).

Detection of antimicrobial activity: Antibacterial assay was analysed by using agar well diffusion assay and observed inhibition zone formed around the well to detect antimicrobial activity.

(B) Biochemical analysis: Water quality was determined by estimating physical and chemical characteristics of water. Water sample was collected for near Chambal River by fabricated water sampler of 1L capacity and transported to lab where analysis was done during the period of 2 days. Preservation of waste water sample and methodology of analysis was referred from APHA-AWWA WPCF (1980). The physical parameters such as pH, turbidity were analyzed by using respectively pH meter and turbidity meter. While Alkalinity, Dissolved oxygen were determined.

(C) Phytochemical Analysis by Gas Chromatography-Mass Spectroscopy (GC-MS): Plant extracts was prepared by taking seed powder in stopper reagent bottle and mixed with solvent (Methanol) and kept for 12 hours in the dark, at room temperature with intermediate shaking. The screening of phytochemicals was carried out by taking 1 ml concentrated extract on Gas Chromatography Mass Spectroscopy (GC-MS - Model; QP 2010 series, Shimadzu, Tokyo, Japan) at Jawaharlal Nehru center for advance scientific research (JNCASR) Delhi.

(D) Molecular Docking: Molecular docking was used to evaluate the feasible binding geometries of a putative ligand with a target whose 3D structure is known. The binding geometries often called binding modes or poses include both the positioning of the ligand relative to the receptor (ligand configuration) and the conformational state of the ligand and the receptor. NCBI, PubChem,PubMed and Open Babel was used for molecular docking.

Observation and Results:

In present investigation various plant species has been selected as study material belonging to different families. These plants are *Cicer arietinum*, *Jatropha curcas*, *Moringa oleifera* and *Strychnos potatorum* (Table-3). Various secondary metabolites or phytochemical constituents like Alkaloids, Flavanoids, Phenol, Saponin, and Steroids were also assessed for different plant seed solvent extract. In *Cicer arietinum* all component present except phenol in ethanolic extract. Alkaloid and steroids were absent in methanolic extract. In *Jatropha curcas* Saponin in ethanolic and methanolic extract was absent but Flavanoids and Phenol were present in higher amount in methanolic extract. In *Moringa oleifera* Saponin was absent in both ethanolic and methanolic extract. Whereas Phenol in ethanolic extract was present in higher amount. In *Strychnos potatorum* all components were shown their presence in both ethanolic and methanolic extract (Table 6, Plate 4 & 5).

Water samples before and after treatment was estimated in the form of colour, odor and clearity. In all samples control was very cloudy in clearity offensive in odor and dirty brown in colour. Where as a *Moringa oleifera* and *Strychnos potatorum* was similar in clarity which was very clear, odorless in odor and totally colourless. *Jatropha curcas* was clear, slightly odorfull and light brown in colour. On the other hand *Cicer arietinum* was cloudy clear, slightly odorfull and light brown in colour (Table 5, Plate- 4).

Two different concentrations (50 mg/l and 100 mg/liter) were estimated on the behalf of pH before and after treatment of water

sample. In all samples control shows maximum pH in both concentrations (8.22, 8.22). In concentration 50 mg/l pH of *Strychnos potatorum* shown maximum pH (8.20) followed by equally *Moringa oleifera* and *Cicer arietinum* (8.15) and the minimum concentration was 8.11 of sample B.

On the other hand the concentration of 100 mg/l maximum pH was 7.95 of *Moringa oleifera* which was followed by sample *Strychnos potatorum* (7.70); *Jatropha curcas* (7.21) and *Cicer arietinum* (7.15) respectively (Table-12, Figure- 4B).

Comparative analysis of alkalinity of water before and after treatment with seed powder was observed in increasing order from *Cicer arietinum* to control. Maximum alkalinity was estimated (124) for control and minimum was (98) for *Cicer arietinum* (Table-14, Figure-6B).

Final turbidity was maximum in sample *Jatropha curcas*, (12.6) and minimum in sample *Moringa oleifera* (7.4) maximum turbidity was examined in control that was (54.0)

DO of all samples were examined before and after treatment with seed powder. DO of untreated water was 0.2. Maximum Do was reported in *Cicer arietinum* (4.5) which is followed *Moringa oleifera* and *Strychnos potatorum.* Minimum DO was 2.6 shown by *Jatropha curcas* (Table-15, Figure-5A).

Maximum BOD was estimated in control (70). In various treated *Cicer arietinum* show maximum BOD (41) followed by *Moringa oleifera* and *Strychnos potatorum* respectively.

Minimum BOD was represented by *Jatropha curcas* (22) (Table-16, Figure-5B).

% yield of secondary metabolite of each sample were estimated in ethanolic extract. For this purpose plant material of each sample was taken in the amount of 20 gm. For *Cicer arietinum* weight of extract was 1.12; yield was 5.6. For *Jatropha curcas* weight of extract was 0.86; yield was 4.3. For *Moringa oleifera* weight of extract was 1.81; yield was 9.0. For *Strychnos potatorum* weight of extract was 2.12; yield was 10.6. Two types of texture were observed that is oily and gummy. Likewise colour of extract Yellowish brown to brown colour was recorded.

Antimicrobial activity of *Cicer arietinum* maximum inhibitory zone size was reported in erythromycin against all bacterial strain and minimum was in 50 mg/ml concentration of extract (Table-7, Figure-1A).

Antimicrobial activity of *Jatropha curcas* methanolic extract shown (0 mm) zone size of *Bacillus subtilis* and *Pseudomonas aeruginosa* were reported in 50 and 100 mg/ml concentration (Table-8, Figure-1B). Maximum inhibitory zone size (23 mm) was reported in 200 mg/ml against *Escherichia coli* bacterial strain. Antimicrobial Properties of *M. oliferea* methanolic extract increasing pattern of zone of inhibition was observed for *Bacillus subtilis* and *Escherichia coli* but *Pseudomonas aeruginosa* maximum zone size (15 mm) was reported in 200 mg/l of extract. 50 mg/l concentration of extract ineffective against all test organisms (Table-9, Figure-2A). Antimicrobial Properties of *Strychnos potatorum* methanolic extract

increasing pattern of zone of inhibition was observed for *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*. Maximum zone size (20 mm) was reported of erythromycin (Table-10, Figure-2B).

The GC-MS analysis of *Cicer arietinum* seeds methanolic extract revealed the presence of various bioactive compounds such as 2-Propyl-tetrahydropyran-3-ol/ N-Acetylmannosamine, 4-O-Methylmannose, 5-Hydrxoymethylfurfural, Linoleic acid, n-Hexadecanoic acid along with other minor constituents were also present (Table-17, Figure-7). The GC-MS analysis of *Jatropha curcas* seeds methanolic extract revealed the presence of many bioactive compounds such as Glycerin, 9,12-Octadecadienoic acid, Ethyl 2-acetylhexanoate, Methyl hexofuranoside, n-Hexadecanoic acid along with other minor constituents were also present (Table-18, Figure-8). Methanolic Extract of Seeds of *Moringa oleifera* shown the presence of many bioactive compounds such as 1, 2, 3-propanetriol/ glycerol, Benzeneacetonitrile, Oleic acid, n-Hexadecanoic acid, momeinositol , D-Sorbitol along with other minor constituents were also present (Table-19, Figure-9). Results confirmed the presence of potent biocoagulant compounds in the seed extract. The GC-MS analysis Methanolic extract of seeds of *Strychnos potatorum* revealed the presence of many bioactive compounds such as Oleic acid, n- Hexadecanoic acid, Alpha amyrin, D-Allose, Nonanoic acid, Isosorbide along with other minor constituents (Table-20, Figure-10).

Properties of various bioactive compounds were obtained from RASMOL and Pubchem demonstrate structure, hydrogen bonds and

other hydrophobic interactions that stabilize the ligands at the target site and help alter binding affinity. Different chemical properties was shown by chemical constituent including number of hydrogen bond donor count, Hydrogen bond acceptor count and number of covalently bounded unit (Table 21-31).

Table: Show Result of some Phytochemical Parameters

Phyto Chemicals Para-meters	***Cicer arieti-num***	***Jatropha curcas***	***Moringa oleifera***	***Strychnos potatorum***	***Control***
Alkaloids	+	+	+	+	
Flavanoids	+	++	+	+	
Phenol	-	++	++	+	
Saponin	+	-	-	+	
Steroids	+	+	+	+	
Weight of extract	1.12 gm	0.86 gm	1.81 gm	2.12 gm	20 gm
pH	7.15	7.21	7.95	7.70	8.22
Alkalinity	98.0 ± 0.62	100.0 ± 0.51	116.0 ± 0.51	120.0 ± 0.43	124.0 ± 0.72
Turbidity	8.4 ± 0.83	12.6 ± 0.62	7.4 ± 0.29	7.8 ± 0.29	54.0
DO	4.5 ± 0.36	2.6 ± 0.49	3.0 ± 0.47	2.9 ± 0.43	2.9 ± 0.43
BOD	41 ± 1.3	22 ± 0.42	26 ± 0.53	25 ± 0.55	70 ± 0.46
Zone of inhibition (mm**)**	BS: 6 EC:14 PA:13	BS: 0 EC: 12 PA: 0	BS: 14 EC:12 PA:11	BS: 9 EC:11 PA:113	BS: 13 EC:20 PA:16

Conclusion:

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease. In recent years the interest for the study of the organic compounds from plants and their activity has increased.

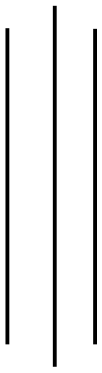
It has been evident from our findings that the usage of plant sbased natural coagulants represents a fundamental development in sustainable environmental technology for the improvement of quality of life for communities. In an era of increasing environmental concerns, water scarcity admits the draw backs of chemical coagulants and poor sanitary facilities in most low income earning countries, the need to further develop natural coagulants as alternative environmentally favorable water purifying chemicals is exigent. The usage of bio-coagulants derived from plant based sources represents a vital development in 'grassroots' sustainable environmental technology through cost effectiveness.

Design natural water purification techniques using plants extracts for bioremediation of turbid water. Application of this low-cost protocol will be recommended for simplified, point-of-use, low-risk water treatment where rural and peri-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water. The ultimate purpose of proposed research study is to come up with a compendium of plant coagulants that could be used as a technology that is cost effective and ecofriendly. It is felt that further research can be conducted by

using the information described in this review as a platform to discover other plant species which are non-toxic and can be mass produced.

The decisive purpose of proposed research study is to come up with a compendium of plant coagulants that could be used as a technology that is cost effective and ecofriendly. It is felt that further research can be conducted by using the information described in this review as a platform to discover other plant species which are non-toxic and can be mass produced. However, detailed studies are necessary to completely delineate the appropriate mechanisms like co- precipitation, co-flocculation, and self-agglomeration involved in the turbidity removal by various natural coagulants used, so that it can be applied on a large scale treatment basis. The results of these findings will lead to the development of household water treatment methods and a transfer of scientific knowledge to the rural people who are using natural coagulants. This work and our finding may have small start.

CHAPTER – 8



REFERENCES

RefeRences

Abbasi, S.S.; **Bhatia, K.K.S.; Kunhi, A.V.M. and Soni, R. (1996).** Studies on the Limnology of Kuttiadi Lake (North Kerla). *Eco. Evo. Cons.*, 2:17-27.

Abdul Aziz, H.; Alias, S.; Assari, F. and Adlan, M.N. (2007). The use of alum, ferric chloride and ferrous sulphate as coagulants in removing suspended solids, colour and COD from semi-aerobic landfill leachate at controlled pH, Waste Management Research. 25(6):556-565.

Adeniyi, B.A.; Aiyelaagbe,O.O.; Fatunsin, O.F. and Arimah, B.D. (2007). In vitro Antimicrobial Activity and Phytochemical Analysis of *Jatropha curcas* Roots. *International Journal of Phamacology*. 3(1):106-110.

Agarwal, D. and Agarwal, A.K. (2007). Performance and emissions characteristics of Jatropha oil (preheated and blends) in a direct injection compression ignition engine. *Applied Thermal Eng*. 27:2314-2323.

Aggarwal, B.B. and Shishodia, S. (2006). Molecular targets of dietry agents for prevention and therapy of cancer. *Biochem. Pharmacol.* 71: 1397-1421.

Ajboye, T. O.; Yakubu, M.T.; Salau, A.K.; Oladiji, A.T.; Akanji, M.A. and Okogun, J. I. (2010). Antioxidant and drug detoxification potentialof aqueous extract of *Annona senegalensis* leaves in carbon

tetrachloride-inducedhepatocellular damage. *Pharmaceutical Biology*. 48(12): 1361–1370.

Ajibad,L.T.; Fatoba, P.O.; Raheem, U.A. and Odunuga, B.A. (2005). Ethnomedicine and primary healthcare in Ilorin, Nigeria. *Ind. J. Trad. Knowl.* 4(2): 150-158.

Akbar, E.; Yaakob, Z.; Kamarudin, S.K.; Ismail M. and Salimon, J. (2009). Characteristic and Composition of *Jatropha curcas* oil seed from malaysia and its potential as biodiesel feedstock feedstock. *Eur. J. Sci. Res.* 29: 396-403.

Akujobi, C.O.; Anyanwu, B.N.; Onyeze, G.C. and Ibekwe, V.I. (2004). Antibacterial Activities and Preliminary Photochemical Screening of four Medicinal Plants. *Journal of Applied Sciences.* 7(3):4328-4338.

Alexandrov T.; Decker J.; Mertens B.; Deelder A.M.; Tollenaar RAEM; Maass P. and Thiele H. (2009). Biomarker discovery in MALDI-TOF serum protein profiles using discrete wavelet transformation. *Bioinformatics.* 25: 643.

Ali, G. H, El-Taweel, G. E. & Ali, M. A. (2004). The cytotoxicity and antimicrobial efficiency of *Moringa oleifera* seeds extracts. *International Journal of Environmental Studies.* 61(6):699-708.

Ali, N.A.; Ater, M.; Sunahara, G.I. and Robidous, P.Y. (2004). Phytotoxicity and bioaccumulation of copper and chromium using barley (*Hordeum vulgare* L.) in spiked artificial and natural forest soils. *Ecotox. and Environ. Saf.* 57: 363-374.

Alkorta, I. and Garbisu, C. (2001). Phytoremediation of organic contaminants in soils. *Biores. Tech*. 79: 273-276.

American Public Health Association (1989). Standard Methods for the Examination of Water and Wastewater 17th Edition, Washington, DC. 2-12.

Anderson, T.A.; Kruger, E.L. and Coats, J.R. (1994). Enhanced degradation of a mixture of three herbicides in the rhizosphere of a herbicids Tolerant Plant. *Chemosphere.* 28: 1551-1557.

Anith J.R.; Velliyu K.G.; Sangilimuthu A.Y. and Sudarsanam D. (2011). Antimicrobial activity of *Moringa Oleifera*a (Lam.) root extract. *J Pharm Res.* 4:1426–1427.

Anna, E.O.A. and Adedipe, N.O. (1996). Water quality monitoring and environmental status in Nigeria. *FEPA Monograph 6, FEPA, Abuja, Nigeria.* 239.

Antov, M.G.; Sciban, M.B. and Petrovic, N.J. (2009). Proteins from common bean (*Phaseolus vulgaris*) seed as a natural coagulant for potential application in water turbidity removal. *Biosource Technology*. 101: 2167-2172.

Anwar F, Rashid U (2007). Physico-chemical characteristics of *Moringa oleifera* a seeds and seed oil from a wild provenance of Pakistan. *Pak J Biol Sci.* 39:1443–1453.

Anwar F.; Latif S.; Ashraf M. and Gilani A.H. (2007). *Moringa oleiferaa*: a food plant with multiple medicinal uses. *Phytotherapy research*. 21:17–25.

Anwar, F.; Latif, S.; Ashraf, M. and Gilan, A.H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytother. Res.* 21:17-25.

Atawodi S.E. and Atawodi J.C. (2009). *Azadirachta indica* (Neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews.* 8:601–620.

Ayaz, S.C. and Akca, L. (2001). Treatment of waste water by natural systems. *Environ. Int.* 26: 189 195.

Babu R. and Chaudhuri M. (2005). Home water treatment by direct filtration with natural coagulant. *Journal of water and health.* 3:27–30.

Bahar B. and Altug T. (2009). Flavour characterization of sumach (*Rhus coriaria* L.) by means of GC/MS and sensory flavour profile analysis techniques. *Int J Food Prop*. 12: 379.

Balandrine M.F.; Kjocke A. and Watele, E. (1985). Natural Plant Chemicals: Sources of Industrial and Medical Material Science. 228:1154-1160.

Barroin, G. and Feuillade, M. (1986) Hydrogen peroxide as a potential algicide for oscillatoria rubescens D.C. *Water Research*. 20: 619-623.

Benesch J.L.P. (2009). Collisional activation of protein complexes: Picking up the pieces. *J Am Soc Mass Spectrom*. 20: 341.

Bereszczak J.Z. and Brancia F.L. (2009). Offline and online liquid chromatography mass spectrometry in quantitative proteomics. *Comb Chem High Throughput Scr.* 12: 185.

Bhargava, R.N.; Chandra, R. and Rai, V. (2008). Phytoextraction of trace elements and Physiological changes in Indian mustard plants (*Brassica nigra* L.) Grown in post methanated distillery effluent (PMDE) irrigated soil. *Biores. Techol.* 99:8316-8324.

Bhattacharjee, S. and Mukherjee, A.K. (2004). Heavy metal induced germination and early growth impairment in *Amaranthus lividus* L. Implications of oxidative membrane damage. *J. Pl. Biol*. 31(1): 1-11.

Birkemeyer, C.; Kolasa, A. and Kopka, J. (2003). Comprehensive chemical derivatization for gas chromatography-mass spectrometry-based multi-targeted profiling of the major phytohormones. *Journal of Chromatography*. A 993: 89-102.

Blaney J. and Dixon J. (1993). A good ligand is hard to find: Automated docking methods, Perspect. *Drug Disc. Des.* 1: 301-319.

Borris, R.P. (1996). Natural Products Research: Perspectives from A Major Pharmaceutical Company. *Journal of Ethnophamacology*. 51:29-38.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.

Brenner, K.P.; **Rankin, C.C.; Roybal, Y.R.J.; Stelma, G.N.; Scarpino, P.V. and Dufour, A.P. (1993).** New medium for the simultaneous detection of total *coliforms* and *Escherichia coli* in water. *Appl. Environ. Microbiol. 59*, 3534-3544.

Brix, H. (1994). Functions of macrophytes in constructed wetlands. *Water Sci. Technol*. 29, 71 78.

Broadley, M.R.; Willey, N.J. and Mead, A. (1999). A method to assess taxonomic variation in shoot casein concentration among flowering plants. *Environ. Poll*. 106: 341-349.

Broin M.; Santaella C.; Cuine S.; Kokou K.; Peltier G. and Joet T. (2002). Flocculent activity of a recombinant protein from *Moringa Oleifera*a Lam. seeds. *Applied Microbiology and Biotechnology.* 60:114–119.

Caceres A.; Cabrera O.; Morales O.; Mollinedo P. and Mendia P. (1991). Pharmacological properties of *Moringa Oleifera*a: Preliminary screening for antimicrobial activity. *J. Ethnopharmacol*. 33(3): 213–216.

Cantor K.P.; Lynch C.F.; Hildesheim M.E.; Dosemeci M.; Lubin J.; Alavanja M. and Craun G. (1998). Drinking Water Source and Chlorination Byproducts Risk of Bladder Cancer. *Epidemiology.* 9:21–28.

Chandrasekhar, S.V.A. (1997). Ecological studies of Saroonagar Lake, *Ecol. Env. Const.* 11: 35-39.

Chapman, D. and Kimstach, R. (1992). 'The selection of water quality variables', in D. Chapman (ed.), water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. *Chapter- 3, Chapman and Hall Ltd., London*.

Chaturvedi, S.; Sharma, P.K.; Garg, V.K. and Bansal, M. (2011). Role of Nutraceuticals in Health Promotion. *International Journal of PharmTech Research*. 3:442-448.

Cheremisinoff, N.P. (2002). Handbook of water and wastewater treatment technologies.

Choubey Sonal. (2013). Coagulation-Clarification of Turbid Colored Water by Natural Coagulant (*Strychnos potatoram*). *Journal of Applicable Chemistry*. 2 (1): 73-80.

Chuang, P.; Lee, C.; Chou, J.; Murugan, M.; Shief, B. and Chen, H. (2007). Antifungal activity of crude extracts and essential oil of *Moringa Oleifera*a Lam. *Bioresour. Technol*. 98 : 232-236.

Clark , J.A. and Pogel, J.E. (1977). Pollution indicators bacteria associated with municipal raw and drinking water supplies. *Can. J. Microbial* 23: 465-470.

Clijsters, H. and Van-Assche, F. (1985). Inhibition of photosynthesis by heavy metals. *Photosynth. Res*. 7: 31-40.

Cobbett C. and Goldsbrough P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu Rev Plant Biol*. 53:159-182.

Cowan M.M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Review.* 12(4):564-582.

Dalen M.B.; Pam, J.S. and Izang. (2009). Synergy between *Moringa Oleifera*a seed powder and alum in the purification of domestic water. *Science World Journal Vol* 4 :6-11.

Daniyan S.Y. ; Abalaka M.E.; Elemba O. M. and Aransiola S.A. (2011). In vitro antimicrobial activity and phytochemical screening of *jatropha curcas* seed extract. *IRJP*. 2 (7):60-64.

Devrimci H.A.; Yuksel A.M. and Sanin F.D. (2012). Algal alginate: A potential coagulant for drinking water treatment. *Desalination* 299:16–21.

Domingo J.L. (2006). Aluminum and other metals in Alzheimer's disease: a review of potential therapy with chelating agents. *Journal of Alzheimer's Disease.* 10:331–341.

Doppalapudi Sandeep; Sandya L.; Chandra Kalyan Reddy; Y, Nagarjuna. S.; Padmanabha Reddy. Y. and Saba Shafeen. (2012). Anti inflammatory activity of *Cicer arietinum* seed extracts. *Asian J Pharm Clin Res.* 5(1): 64-68.

Eaphan S. and Desouza, S.F. (2005). Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotech. Adv.* 23: 97-114.

Edeoga H.O.; Okwu D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 4: 685-688.

Eman, N.A.,Muyibi,S.A., Salleh, H.M., Salleh, M.R.M. and Alam, Md. Z.,(2010). "Moringa Oleifera seeds as natural coagulant for water treatment", Thirteenth International Water Technology (Conference), Hurghada, Egypt.

Eman, N.A.; Muyibi, S.A.; Salleh, H.M.; Salleh, M.R.M. and Alam, Md. Z. (2010). *Moringa Oleifera*a seeds as natural coagulant for water treatment. *Egypt.Environ Health*. 18:272-81.

Ezeamuzle I.C.; Ambedederomo A.W.; Shode F.O. and Ekwebelem S.C. (1996). Anti-inflammatory effects of *Moringa Oleifera*a root extract. *Int. J. Pharmacogncy*. 34: 207.

Fairless, D. (2007). Biofuel: The little shrub that could-maybe. *Nature*. 449: 652-655.

Faizi S.; Siddiqui B.S.; Saleem R.; Siddiqui S.; Aftab K. and Gilani A.H. (1995) Acetylated carbonate and hypotensive thiocarbamate glycosides from *Moringa Oleifera*a. *Phytochemistry*. 38:957.

Fakurazi S.; Hairuszah I. and Nanthini U. (2008). Prevents acetaminophen induced liver injury through restoration of glutathione level. *Food and Chemical Toxicology*. 46(8): 2611.

Farah, N.; **Zia, M.A.; Rehman, K. and Sheikh, M. (2002).** Quality characteristics and treatment of drinking water of Faisalabad city. *Int. J. Agric. Biol*., 3: 347–9.

Faruq, U.Z.; Adul-Malik and Dabai, Y.U. (2004). Antibacterial activity of crude anthraquinone extract of *senna italica* leaves. *Biosci. Res. Comm*.16: 7-13.

Ferreira, J.L.P.; Amaral, A.C.F.; de Araujo, R.B.; Carvalho J.R. and Proenca C.E.B. (2009). Pharmacognostical comparison of three species of Himatanthus. *Int. J. Bot*. 5: 171-175.

Fiehn, O. (2002). Metabolomics – the link between genotypes and phenotypes. *Plant Molecular Biology*. 48: 155-171.

Flaten, T.P. (2001). Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain Research Bulletin*. 55(2): 187-196.

Gambrell, R.P. and Patrick, W.J. (1988). 'Chemical and microbiological properties of anaerobic soils and sediments' in D.D. Hook and R.M.M. Crawford (eds.), *Plant Life in Anaerobic Environments*, pp. 375-423.

Gamedze, K.; Tevera, D.S. and Fadrian, A.O. (2012). Water quality assessment of various sources in rural areas in the Lowveld region of Swaziland. *Current Research Journal of Social Sciences*. 4(2): 99-102.

Ganesh, S. and Vennila, J.J. (2011). Phytochemical analysis of *Acanthus ilififolius* and *Avicennia officinalis* by GC-MS. *Res. J. Phytochem. Harborne, J.B.;* 1993. *Phytochemistry. Academic Press, London*, pp: 89-131.

Ghebremichael K.A.; Gunaratna K.R.; Henriksson H.; Brumer H. and Dalhammar G. (2005). A simple purification and activity assay of the coagulant protein from *Moringa Oleifera*a seed. *Water Research*. 39:2338–2344.

Ghebremichael, K.A.; Gunaratna, K.R. and Dalhammar, G. (2006). Single-step ion exchange purification of the coagulant protein from *Moringa Oleifera*a seed. *Applied microbiology and biotechnology*. 70(5): 526-532.

Ghosh, S.; Subudhi, E. and Nayak, S. (2008). Antimicrobial assay of Steviarebaudiana bertoni leaf extracts against 10 pathogens. *International Journal of Integrative Biology*. 2(1) : 27-31.

Giovanna Boschin; Anna Arnoldi. (2011). Legumes are valuable sources of tocopherols. *Food Chemistry*.127(3):1199-1203.

Giri, I.C.; Shamim, Q.M.D.; Safwan, A.K.; Jitendra, P.; Choudhary, R. and Singh, A. (2010). Short communication. Evaluation of anthelmintic activity of *Moringa Oleifera*a seeds. *Inter J. Pharma. Professional's Res. Vol* (88).

Girish K. and Shankara B. (2008). Neem–A Green Treasure. *Electronic journal of Biology*.4:102-111.

Gislene, N.G.F. (2000). Antibacterial activity of plant extracts and phytochemical on antibiotic resistance bacterial. *Brazilian Jou. of Microbiology*. 31: 247-256.

Goodacre, R.; Vaidyanathan, S.; Dunn, W.B.; Harrigan, G.G. and Kell, D.B. (2004). Metabolomics by numbers: acquiring and

understanding global metabolite data. *Trends in Biotechnology*. 22: 245-252.

Govindarajan R.; Vijayakumar M.; Singh M.; Rao C.H.V.; Shirwaikar A.; Rawat A.K.S. and Pushpangadan P. (2006). Antiulcer and antimicrobial activity of *Anogeissus latifolia, J. Ethnopharmacol*. 106: 57-61.

Grover Neha and Patni Vidhya (2013). Phytochemical characterization using various solvent extracts and GCMS analysis of methanolic extract of *Woodfordia fruticosa. IJPPS.* 5(4):291-295.

Guevara A.P.; Vargas C.; Sakurai H.; Fujiwara Y.; Hashimoto K. and Maoka T. (1991). An antitumor promoter from *Moringa Oleifera*a Lam. *Mutation Research*. 440: 181.

Gunaratna K.R.; Garcia B.; Andersson S. and Dalhammar G. (2007). Screening and evaluation of natural coagulants for water treatment. *Water science and technology: water supply.*7:19–25.

Gupta V.B.; Anitha S.; Hegde M.L.; Zecca L.; Garruto R.M.; Ravid R.; Shankar S.K.; Stein R.; Shanmugavelu P. and Jagannatha Rao K.S. (2005). Aluminium in Alzheimer's disease: *Cellular and molecular life sciences.* 62:143–158.

Habsah M.; Amran M.; Mackeen M.M.; Lajis N.H. and Kikuzaki H. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities, *J. Ethnopharmacol.*72: 403-410.

Hadacek, F. (2002). Secondary metabolites as plant traits: Current assessment and future perspectives. *Critical Reviews in Plant Sciences*. 21: 273-322.

Handreck, K. A. and Black, N.D. (1994). Growing media for ornamental plants and turf. *University of New South Wales Press, Sydney*.

Hasan, N.; **Mirani, Z.A. and Ismat, S. (2010).** Bacterial indicators of risk of disease from drinking water. *BALWOIS - Ohrid, Republic of Macedonia*.

Hassan, M.M.; Oyewale, A.O.; Amupitan, J.O.; Abdullahi M.S. and Okonkwo, B. (2004). Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of *Detarium microcapum. J. Chem. Soc. Nig*. 29: 26-29.

Hassan, S.W.; Ladan, M.J.; Dogondaji, R.A.; Umar R.A. and Bilbis L.S. (2007). Phytochemical and toxicological studies of aqueous leaves extracts of *Erythrophleum africanum. Pak. J. Biol. Sci*. 10: 3815-3821.

Heiras-Palazuelos M.J.; Ochoa-Lugo M.; Mora-Rochin S.; Gutiérrez-Dorado R.; Milan-Carrillo J.; Garzón-Tiznado J.A.; ReyesMoreno C. and Heiras-Palazuelos M.J. (2013) Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpeas (Cicer arietinum L.) cultivars. *International Journal of Food Science Nutrition*. 64(1):69-76.

Howard, A.G. (1998) "Aquatic Environmental Chemistry", *Oxford University Press, Oxford.*

Hutchinson, J. and Dalziel, J.M. (1958). Flora of West Tropical Africa, Vol. 1, Part II, Crown Agents for Oversea Governments and Administrations, London, UK.; pp: 298-756.

Ibrahim, H.; Sani, F.S.; Danladi B.H. and Ahmadu, A.A. (2007). Phytochemical and antisickling studies of the leaves of *Hymenocardia acida.* (Euphorbiaceae). *Pak. J. Biol. Sci*. 10: 788-791.

Igbinosa, O. O.; Igbinosa, E.O. and Aiyegoro, O.A. (2012). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology*. 3(2): 058-062.

Imasaka T.; Nakamura N.; Sakoda Y.; Yamaguchi S.; Watanabe-Ezoe Y.; Uchimura T. and Imasaka T. (2009). Data processing technique in gas chromatography/ time-of-flight mass spectrometry. Analyst**.** 134: 712.

Indu M. N.; Hatha A. A. M.; Abirosh C.; Harsha U.; Vivekanandan G. (2006). Antimicrobial activity of some of the South-Indian spice against serotypes of *Eschrichia coli, Salmonella, Listeria* monocytogenes and *Aeromonas hydrophila*. *Braz. J. Microbiol*. 37:153-158.

Irshad, M.; Ahmad, I.; Goel H.C. and Rizvi, M.M.A. (2010). Phytochemical screening and high performance TLC analysis of some cucurbits. *Res. J. Phytochem*. 4: 242-247.

Jahn S. (1988). Using Moringa Seeds as Coagulants in Developing Countries. *Journal American Water Works Association*. 80:43–50.

Jahn S.A.A. and Dirar H. (1979). Studies on Natural Water Coagulants in the Sudan, with Special Reference to *Moringa Oleifera*a Seeds. *WATER SA*. 5:90–106.

Jahn, S.A. (1988). Using Moringa Seeds as Coagulants in Developing Countries. *J. American Water Works Association*. 80(6) 43-50.

Jahn, S.A.A. **(1981).** Studies on natural water coagulants in the Sudan, with special reference to *Moringa oleifera* seeds. *Water SA* (L2): 90-97.

Jain, C.K.; Ali, I. and Sharma, M.K. (1998). Salinity modeling of ground water of a coastal region using best subset procedure. *IJEP*, 189(10):762-768.

James, O.; Unekwojo, E. G. and Ojochenemi, A. A. (2011). Assessment of Biological Activities: A Comparison of *Pergularia daemia* and *Jatropha curcas* Leaf Extracts. *British Biotechnology Journal*. 1(3). 85-100.

Jeon J.R.; Kim E.J.; Kim Y.M.; Murugesan K.; Kim J.H. and Chang Y.S. (2009). Use of grape seed and its natural polyphenol

extracts as a natural organic coagulant for removal of cationic dyes. *Chemosphere*. 77:1090–1098.

Kalimuthu, K.; Vijayakumar, S. and Senthilkumar, R. (2010). Antimicrobial activity of the biodiesel plant, *Jatropha curcas* L. *International Journal of Pharma and Bio Sciences*. 1(3):1-5.

Kamal, S.; Manmohan, S. and Birendra, S. (2011). A review on chemical and medicobiologicalapplications of *Jatropha curcas*. *International Research Journal of Pharmacy*: 61-66.

Kar A.; Choudhary B.K. and Bandyopadhyay N.G. (2003). Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J. *Ethnopharmacol*. 84: 105.

Kazi Tasneembano; Virupakshi Arjun. (2013). Treatment of tannery wastewater using Natural coagulants. *IJIRSET*. 2 (8): 215-221.

Kebreab A.G.; Gunaratna K.R.; Henriksson H.; Brumer H. and Dalhammar G.A. (2005). Simple purification and activity assay of the coagulant protein from *Moringa Oleifera*a seed. *Water Res.* 39:2338–2344.

Kitchen D.B.; Decornez H.; Furr J. R. and Bajorath J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov*. 3(11). 935-949.

Krishnan, P.R. and Paramathma, M. (2009). Potentials and *Jatropha* species wealth of India. *Curr. Sci.* 97: 1000-1004.

Kumar, A. and Sharma, S. (2008). An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. *Ind. Crops Prod*. 28: 1-10.

LeChevallier, M.W.; Evans, T.M. and Seidler R.J. (1981). Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. *Applied and environmental microbiology*.

Libanius, M. (**2008).** Fundamentals of quality and water treatment. 2nd ed. Campinas., Atom Ed, p 444.

Liston, P. and Maher, W. (1997). Water quality for maintenance of aquatic ecosystems: appropriate indicators and analysis, state of the environment Australia, technical paper series (Inland Waters). *Department of the Environment, Sport and Territories, Canberra*.

Liu, D.; Jiang, W.; Liu, Ch.; Xin, Ch. and Hou, W. (2000). Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard. *Biores. Tech.* 71: 227 273.

Lockett, C.T.; Calvet, C.C.; Grivetti, L.E. (2000). Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. *Int. J. Food. Sci. Nutr.* 51(3): 195-208.

Lombi, E.; Zhao, F.J.; Dunham, S.J. and McGrath, S.P. (2000). Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. *New Phytol*. 145:11-20.

Lowell, F.Fulie (2001). The Miracle Tree: The multiple Attributes of Moringa, Dakar, Senegal, CTA Postbus 380, 6700 A.J. Wageningen The Netherlands.

Lowry O.H.; Rosenbrough N.J.; Farr A.L. and Randall R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry.* 193:265–275.

M Madhu, V Neeraja (2016). Quantitative phytochemical analysis of some medicinal plant seed by using various organic solvents, Journal of Pharmacognosy and Phytochemistry. 5(2): 30-34.

Madusolomuo M.A.; Nadro M.S.; Wurocheke A.U. (1999). Antihepatotoxic properties of *Cassia sieberiana* in acetaminophen–treated rats. *Nig. J. Biochem. Mol. Biol*. 14: 21-25.

Maganga, F.P.; Butterworth, J.A. and Moriarty, P. (2002). Domestic water supply, competition for water resources and IWRM in Tanzania: a review and discussion paper. *Physics and Chemistry of the Earth*. 27: 919-926.

Mahato S. B.; Nandy A. K. and Roy G. (1992). Triterpenoid. *Phytochem*. 31: 2199-2249.

Mallikharjuna P.B. and Seetharam Y.N. (2009). In vitro antimicrobial activity of alkaloids fraction of *Strychnos potatorum*. *E J chem.*. 6(4): 1200-1204.

Mallikharjunah, P.B.; L.N. Rajanna, Y.N. Seetharam and Sharanabasappa, G.K. (2007). Phytochemical studies of *Strychnos potatorum* L..-A medicinal plant. *E-J. Chem*. 4: 510-518.

Mamta Arora; Satnam Singh and Parneet Kaur (2014). In-Vitro Antioxidant and Antimicrobial Activity Study on Selected Seeds of

Cicer Arietinum Linn. *Asian Journal of Biochemical and Pharmaceutical Research Issue*. 4 (4) :23-30.

Mangale S. M.; Chonde S. G.; Jadhav A. S. and Raut P. D. (2012). Study of *Moringa Oleifera*a (Drumstick) seed as natural Absorbent and Antimicrobial agent for River water treatment. *J. Nat. Prod. Plant Resour*. 2 (1):89-100.

Marobhe N.; Renman G. and Jacks G. (2007). The study of water supply and traditional water purification knowledge in selected rural villages in Tanzania. Indigenous knowledge systems and development: *Relevance for Africa*. 1:11– 120.

Marobhe, N.J.; Gunaratna, K.R. and Dalhammar, G. (2007). Simple and rapid method for purification and characterization of active coagulants from the seeds of *Vigna unguiculata* and *Parkinsonia aculeata*. *Environmental Technology*. 28: 671-681.

Marobhe, N.J.; Renman, G. and Jackson, M. (2007). Investigation of the performance of local plant materials for coagulation of turbid river water. *Journal of the Institution of Engineers Tanzania*. 8(3): 50-62.

Martinez-Herrera, J.; Siddhuraju, P.; Francis, G.; Davila-Ortiz G. and Becker, K. (2006). Chemical composition, toxic/ antimetabolic constituents and effect of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chem*. 96: 80-89.

Martyn C.N.; Osmond C.; Edwardson J.A.; Barker D.J.P.; Harris E.C. and Lacey R.F. (1989). Geographical relation between alzheimer's disease and aluminium in drinking water. *The Lancet.* 333:61–62.

McKay B.D. (1981). Practical graph isomorphism. Congressus Numerantium. 30:45–87.

Mckinney, M.L. and Schoch, R.M. (2003). Environmental science-systems and solutions. *Jones and Bartlett publishers, London*.

Md. Asrafuzzaman; Fakhruddin, A. N. M. and Md. Alamgir Hossain (2011). Reduction of turbidity of water using locally available natural coagulants. *ISRN Microbiology.* 2:31-36.

Meenakshi and Maheshwari, R.C. (2006). Fluoride in drinking water and its removal. *Journal of Hazardous Materials*. 137: 456- 463.

Mehta L.K.; Balaraman R.; Amin A.H.; Bafna, P.A.; Gulati O.D. (2003). Effect of fruits of *Moringa Oleifera*a on the lipid profile of normal and hypercholesterolaemic rabbits. J. *Ethnopharmacol*. 86:191 195.

Meyer, J. J. M.; Afolayan, A. J.; Taylor, M. B. and Erasmus, D. (1997). Antiviral activity ofgalangin from the aerial parts of *Helichrysum aureonitens. Journal of Ethanopharmacology*. 56: 165-169

Miller S.M.; Fugate E.J.; Craver V.O.; Smith J.A. and Zimmerman J.B. (2008). Toward Understanding the Efficacy and

Mechanism of *Opuntia* spp. as a Natural Coagulant for Potential Application in Water Treatment. *Environmental Science and Technology*. 42:4274–4279.

Min, B.R.; Pinchak, W.E.; Merkel, R.; Walker, S.; Tomita, G. and Anderson, R. C. (2008). Comparative antimicrobial activity of tannin extracts from perennial plants on mastitispathogens. *Scientific Research and Essay*. 3(2):66-73.

Mohapatra, M.; Anand, S.; Mishra, B.K.; Giles, D.E.; Singh, P.; (2009). Review of fluoride removal from drinking water. *Journal of Environmental Management.* 91: 67-77.

Morgan H.L. (1965). The Generation of a Unique Machine Description for Chemical Structures-A Technique Developed at Chemical Abstracts Service. *J Chem Docum*. 5:107–113.

Mueller A.; Duechting P. and Weiler, E.W. (2002). A multiplex GC-MS/MS technique for the sensitive and quantitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. *Planta (Berlin.*)**.** 216: 44-56.

Mujumdar, A.M.; Misar, A.V.; Salaskar, M.V and Upadhye, A.S. (2001). Antidiarrhoeal effect of an isolated fraction (J.C) of *Jatropha curcas* roots in mice. *Journal of National remedies.* 1:89-93.

Murakami A.; Kitazono Y.; Jiwajinda S. and Koshi Niaziminin K.A. (1998). Thiocarbamate from the leaves of *Moringa Oleifera*a holds a strict structural requirement for inhibition of tumour promoter induced Epstein Barr virus activation, Plantation. *Medical.* 64:319.

Muthuraman, G.; Sasikala, S.; Prakash, N. (2013). Proteins from Natural Coagulant for Potential Application of Turbidity Removal in Water Presidency College, Department of chemistry, Chennai. *International Journal of Engineering and Innovative Technology (IJEIT)*. 3(1):22-26.

Muyibi S. and Evison L. (1995). Optimizing Physical Parameters Affecting Coagulation of Turbid Water with *Moringa oleifera* Seeds. *Water Research* 29:2689–2695.

Muyibi S.A.;Wai K.T.; Idris A.; Johari M.M.N.M.; Mohammad T.A.; Ghazali A.H. and (2009). Evaluation on different forms of *Moringa Oleifera*a seeds dosing on sewage sludge conditioning. *Desalination and Water Treatmen.t* 10: 87–94.

Muyibi, A. S. & Evison, L. M. (1994). Remaoval of Total Hardness using *Moringa oleifera* seeds. *Water Resources.* 29:1099-1105.

Narayani, M.; Johnson, M.; Sivaraman, A. and Janakiraman, N. (2012). Phytochemical and Antibacterial Studies on *Jatropha curcas* L. *Journal of Chemical and Pharmaceutical Research.* 4(5): 2639- 2642.

Nascimento, G.; locatelli, J.; Freita, P.C. and Silva, G.L. (2000). Antibacterial Activities of Plant Extracts on Antibiotic Resistant Bacteria. *Journal of Microbiology*. 31(7):247-256.

Ndabigengesere, A. (1998). Quality of water treated by coagulation using *Moringa Oleifera*a seeds. Active Agents and Mechanism of

Coagulation of Turbid Waters Using *Moringa oleifera*. *Water Research*.29:703–710.

Ndabigengesere, A. and Narasiah, K.S. (1998). Quality of water treated by coagulation using *Moringa Oleifera*a seeds. *Water Research*. 32(3): 781-791.

Ndabigengesere, A.; Narasiah, K. S. and Talbot, B.G. (1994). Active agents and mechanism of coagulation of turbid waters using *Moringa Oleifera*a. *Water Research.* 29: 703-710.

Nickon, F.; Sand, Z.A.; Rehman, M.H. and Haque, M.E. (2003). In vitro antimicrobial activity of the compound isolated from chloroform extract of *M. oleifera* Lam. *Pak J. Biol. Sci*. 22: 1888-1890.

Nwaiwu, N.E. and Lingmu, B. (2011). Effect of Settling Time on Turbidity Removal Using *Moringa Oleifera*a Seed Powder. *Ozean Journal of Applied Sci*. 4(5):195-205.

Odum, E.P. (1971). *Fundamentals of ecological. 3rd Ed.* W.B. Saunserp Company Philadelphia, 578pp.

Oki, T. and Kanae, S. (2006). Global hydrological cycles and world water resources. *Science.* 313: 1068–1072.

Okuda, T. Baes, A. U. Nishitimas, W. & Okada, M. (2001). Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution. *Water.Resources.* 35(2): 405-410.

Okuda, T.; Baes, A.U.; Nishijima, W.; Okada, M. (2001). Coagulation Mechanism of Salt Solution-Extracted Active Component in *Moringa Oleifera*a seeds. *Water Research*. 35(3): 830 834.

Olafimihan, C.A. (2004). Effects of seasonal variation on the antibacterial activity of aqueous extract of *Azadirachta indica* fresh stem bark. *Biosci. Res. Commun*.16: 13-16.

Olawale-Abulude, F. (2007). Phytochemical screnning and mineral contents of leaves of some Nigerian woody plants. *Res. J. Phytochem.* 1: 33-39.

Oliveira-Filho De E C.; Lopes R.M. and Paumgartten, F.J.R. (2004). Comparative study on the susceptibility of freshwater species to copper-based pesticides. *Chemosphere.* 56:369 - 374.

Olowokudejo, J.D. (1993). Comparative epidermal morphology of West African species of *Jatropha* L. (Euphorbiaceae). *Bot. J. Linnean Soc*. 3: 139-154.

Olsen A. (1987). Low technology water purification by bentonite clay and *Moringa Oleifera*a seed flocculation as performed in Sudanese villages. *Water Research*. 21:517-522.

Omoregie, E.H. and Folashade, K.O. (2013). Broad Spectrum Antimicrobial Activity of Extracts of *Jatropha curcas*. *Journal of Applied Pharmaceutical Scienc.* 3(4):083-087.

Openshaw, K. (2000). A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biomass Bioenergy*. 19: 1-15.

Oseni, L.A. and Alphonse, P.K. (2011). Comparison of antibacterial properties of solvent extracts of different parts of *Jatropha curcas* (Linn). *International Journal of Pharmaceutical and Phytopharmacological Research*. 1(3): 117-123.

Oskoueian, E.; Abdullah, N.; Saad, W. Z.; Omar, A.; Ahmad, S.; Kuan, W. B.; Zolkifli, N. A.; Hendra,R. and Ho, Y.W. (2011). Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. *Journal of Medicinal Plants Research*. 5(1):49-57

Ozacar M. and Sengil A. (2000). Effectiveness of tannins obtained from Valonia as a coagulant aid for dewatering of sludge. *Water Research*. 34:1407–1412.

Ozacar M. and Sengil A. (2003). Evaluation of tannin biopolymer as a coagulant aid for coagulation of colloidal particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects 229:85–96.

Packialakshmi N.; Suganya C. and Guru V. (2014). Phytochemical and IR Spectrum analysis of *Strychnos potatorum* linn, *International Journal of Phytopharmacy*. 4(2): 63-66.

Packialakshmi, N.; Suganya, C. and Guru V. (2014). Studies on *Strychnos potatorum* seed and screening the water quality assessment of drinking water. *International Journal of Research in Pharma and Nano Sciences*. 3(5): 380 - 396.

Pal S.K. and Mukherjee, P.K. (1995). Studie *Moringa Oleifera*a leaf extract on gastric ulcer models in rats. *Phytotherapy Research*. 9: 463.

Pal S.K., Mukherjee P.K. and Saha B.P. (1995). Studies on the antiulcer activity of *Moringa Oleifera*a leaf extract. *Phytotherapy Research*. 9: 463.

Pal, S.K.; Mukherjee, P.K. and Saha, B.P. (1995). Studies on the antiulcer activity of M. oleifera leaf extract on gastric ulcer models in rats. *Phytother. Res.* 9: 463-465.

Parameters of water quality (2001): Interpretation and Standards Published by the Environmental Protection Agency, Ireland.

Parvathamma, S. and Shanthimma, C. (2001). Antimicrobial activity of *Mallugo ceriviana* Ser. *Ancient Science of Life*. Vol. XX (1+2).

Patil, P. R.; Badgujar, S. R. and Warke, A. M. (2001). Evaluation of ground water quality in Ganesh Colony area of Jalgaon City. *Oriental J Chem.*, 17(2), 283.

Prashant Tiwari; Bimlesh Kumar; Mandeep Kaur; Gurpreet Kaur and Harleen Kaur (2011). Phytochemical screening and Extraction: A Review. *International Pharmaceutica Sciencia*.1(1): 98-106.

Pritchard M.; Mkandawire T.; Edmondson A.; O'Neill J.G. and Kululanga G. (2009). Potential of using plant extracts for

purification of shallow well water in Malawi. *Journal of Physics and Chemistry of the Earth*. 34:799–805.

Purohit, M.G.; **Shanthaveerappa, B.K.; Badami, S. and Swamy, H.KS. (1995).** Antimicrobial activity of various extract of *Evolvulus alsinoids. Indian J. of Microb*. 35(1): 77-78.

Rachana, S.; Tarun, A.; Rinki, R.; Neha, A. and Meghna, R. (2012). Comparative Analysis of Antibacterial Activity of *Jatropha curcas* Fruit Parts. *Journal of Pharmaceutical and Biomedical Sciences*. 15(15):1-4.

Rafi Mohd, K.; Sirajudeen, K. and Shajahan, A. (2005). Antimicrobial activity of the leaf extracts of *Moringa concanensis. Advanced Plant Science.* 18(11): 893-894.

Raja, R. E.; Lydia, S.; Merlin, P. and Chritopher, G. (2002). Physico-Chemical analysis of some groundwater samples of Kotputli Town Jaipur, Rajasthan. *Indian J Environ Prot.* 22(2): 137.

Rajendran R.; Balachandar S.; Sudha S. and Muhammed Arshid. (2013). Natural coagulants an alternative to conventional methods of water purification. *International Journal of Pharmaceutical Research and Bioscience*. 2(1). 306-314.

Rajkumar, M. and Freitas, H. (2008). Effects of inoculation of plant- growth promoting bacteria on Ni uptake by Indian mustard. *Biores Technol*. 99: 3491-3498.

Rastogi Trapti. (2009). Comparative Studies on Anthelmintic Activty of *Moringa Oleifera*a and Vitex Negundo. *Asian J. Research Chem. vol*. 2(2).

Ren, W.; Qiao, Z.; Wang, H.; Zhu L. and Zhang, L. (2003). Flavonoids: Promising anticancer agents. *Med. Res. Rev*.; 23: 519-534.

Richter CA.(2009) Water: Methods and treatment technology. 1st ed. São Paulo. Ed Blucher, 333 p.

Roessner, U., Wagner, C., Kopka, J., Trethewey, R. N., and Willmitzer, L. (2000). Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *Plant J.* 23(1):131-142.

Romeo V.; Verzera A.; Zino M.; Condurso C. and Tripodi G. (2009). Headspace volatiles of *Vicia sativa* L. by solid-phase microextraction and gas chromatography/mass spectrometry. *J Essent Oil Res.* 21: 33.

Ruckmani, K.; Kavimani, S.; Anandan, R. and Jaykar, B. (1998). Effect of *Moringa Oleifera*a Lam on paracetamol-induced hepatotoxicity. *Ind. J. Pharmaceutical Sci.* 60: 33-35.

Saikia, D. and Khanuja, S.P.S. (2001). Comparative antimicrobialactivity of essential oils and constituents from distinct genotypes of Cymbopogon species. *Current Sc. Scientific Corresponding*. 80(10):1264-1266).

Samy R. P. and Ignacimuthu S. (2000). Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India, *J. Ethnopharmacol*. 69: 63-71.

Sanders R.J.; Ofman R.; Dekker C.; Kemp S. and Wanders R.J.A. (2009). Enzymatic diagnosis of Sjogren-Larsson syndrome using electrospray ionization mass spectrometry. *J Chromatogr B.* 877: 451.

Sanghi R.; Bhatttacharya B. and Singh V. (2002). *Cassia angustifolia* seed gum as an effective natural coagulant for decolourisation of dye solutions. *Green Chemistry*. 4:252–254.

Sanghi, R.; Dixit, A. and Guha, S. (2006). "Sequential batch culture studies for the decolorization of reactive dye by Coriolusversicolor", *Bioresour. Technol*. 97:396–400**.**

Sankar Narayan Sinha. (2012). Phytochemical analysis and antibacterial potential of *Moringa Oleifera*a L. *IJSID*. 2 (4): 401-407.

Sarin R.; Manvi M. and Sapna B. (2010). Evaluation of antibacterial potential of stem bark of *M. oleifera* Lam. *Bioscience.* 1:89–94.

Schauer, N. and Fernie, A. R. (2006). Plant metabolomics: towards biological function and mechanism.*Trends Plant Sci.* 11: 508-16.

Scoglio, M.E.; **Grillo, O.C.; Munao, F.; Dipietro, A. and Squeri, L. (1989).** Water quality and microbiological status of distribution system: Traditional parameters and emerging parameters. *Ann lg.* 1(5): 1243-1254.

Seifert M.H.; Kraus J. and Kramer B. (2007). Virtual high-throughput screening of molecular databases, *Current opinion in drug discovery and development*. 10 (3):298-307.

Sengupta M.E.M.; Keraita B.B.; Olsen A.A.; Boateng O.K.O.; Thamsborg S.M.S.; Pálsdóttir G.R.G. and Dalsgaard A.A. (2012). Use of *Moringa Oleifera*a seed extracts to reduce helminth egg numbers and turbidity in irrigation water. *Water Research*. 46: 3646–3656.

Sharma, A.; Saxena, S.; Rani, U.; Rajore, S. and Batra, A. (2010). Broad-spectrum antimicrobial properties of medicinally important plant *Jatropha curcas*. *Int Journal of Pharmaceutical Sciences Review and Research*. 4 (3): 11- 14.

Singh, R.P. and Mathur, P. (2005). Investigation of variations in physico-chemical characteristics of a fresh water reservoir of Ajmer city, Rajasthan. *Indian J. Environ. Sci.*, 9(1): 57-61.

Sinha, S.N. and Biswas, M. (2011). Effect of extracts from *Asparagus racemosus* Willd. Root against pathogenic bacteria. *Int. J. Applied Biol. Pharma. Technol*. 2(3): 312-314.

Skinner, F.A. (**1995)**. Antibiotics in: modern methods of plant analysis, Springer.

Sofowora, A. (1993). Medical Plants and Traditional Medicine in Africa (2nd Edition) Spectrum Books, Ibadan ,Nigeria**.** pp 9-25.

Sofowora, A., (1984). Medicinal Plants and Traditional Medicine in Africa, John Wiley and Sons, p. 142.

Srimurali, M.; Pragathi, A. and Karthikeyan, J. (1998). A study on removal of fluorides from drinking water by adsorption onto low cost materials. *Environmental Pollution*. 99: 285-289.

Stanley E. Manahan. (1993). "Fundamentals of Environmental Chemistry", *Lewis Publishers, USA*.

Stepanovic S.; Antic N.; Dakic I. and Svabic-vlahovic M. (2003). In vitro antimicrobial activity of propilis and antimicrobial drugs. *Microbiol. Res.*158: 353-357.

Stumm W. and Morgan, J.J. (1996). Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters, third edition. *Wiley-Interscience*. 1022 p.

Suarez M.; Entenza J.M.; Doerries C.; Meyer E.; Bourquin L.; Sutherland J.; Marison I.; Moreillon P. and Mermod N. (2003). Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. *Biotechnology and Bioengineering*. 81: 13–20.

Sunita, S. and Abhishek, S. (2008). A comparative evaluation of phytochemical fingerprints of Asteracantha longifolia Nees. Using HPTLC. *Asian J. Plant Sci.*7: 611-614.

Sutherland, J.P., Folkard, G. and Grant, W.D. (1990). Natural Coagulants for Appropriate Water Treatment: A novel approach.*Waterlines*, 8(4): 30 – 32.

Tahiliani P. and Kar A. (2004). Role of *Moringa Oleifera*a leaf extract in the regulation of thyroid hormone status in adult male and female rats, *Pharmacol. Resorce.* 70: 319.

Tambekar, D.H.; Bochare, V.G.; Gole, B.B. and Banginwar, Y.S. (2007). Bacteriological quality of groundwater in Amaravathi, India. *Poll. Res.*, 26(3): 473-475.

Thirunavukkarasu, P.; Ramanathan, T.; Ramkumar L. and Shanmugapriya, R. (2010). Anti ulcer effect of *Avicennia officinalis* leaves in albino rats. *World Applied Sci. J*. 9: 55-58.

Thongphasuk P.; Suttisri R.; Bavovada R. and Verpoorte R. (2004). Antioxidant lignin glucosides from *Strychnos vanprukii*. *Fitoterapia*. 75:623–8.

Tolonen A.; Turpeinen M. and Pelkonen A. (2009). Liquid chromatography-mass spectrometry in in vitro drug metabolite screening. *Drug Discov Today*. 14: 120.

Tona L.; Cimanga R.K.; Mesia K.; Musamba C.T. and de Bruyne T. (2004). In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the democratic republic of Congo. J. *Ethnopharm*. 93, 27-32.

Totrov M. and Abagyan R. (1997). Flexible protein-ligand docking by global energy optimization in internal coordinates, *Protein. Struct. Funct. Genet.* 29, 215-220.

Trease, G.E. and Evans, W.C. (1989). Parmacognosy Bailiere Tindal,London. 11th ed. 278-291.

Trease, G.E. and Evans, W.C. (1989). Trease and Evans: Pharmacognosy. 13th Edn.; Bailliere Tindale, London.

Trivedi, R.N.; **Singh, K.K. and Kumar, R. (2004).** The Earth elixir ground water in India. *Int. J. Mandel*, Vol. 21(3-4): 99-100.

Upadhyay, R.K. (2011). Plant natural products:Their pharmaceutical potential against diseaseand drug resistant microbial pathogens. Journal *of Pharmacy Research*. 4(4):1179-1185.

Van Buren, T.P. and Robinson, E.C. (1981). Formation of complexes between protein and tannic acid. *J. Agric*. *Food Chem*. 1: 77-82.

Varghese, A.; **Gavani, U.; Abraham, S.; Parambi, B.G.T.; Sathianarayanan; and Jose, A. (2009).** Phytochemical screening and antimicrobial investigation of *Typha angustifolia* Linn. *International Journal of Chemical Science*. 7 :1905-1910.

Venkatesh A. and Silambujanaki P. (2011). A Phyto-Pharmacological review on *Strychnos potatorum* linn. *Int. Res J Pharm. App Sci*.1(1): 109-114.

Verkhivker G.M.; Bouzida D.; Gehlhaar D.K.; Rejto P.A.; Arthurs S.; Colson A.B.; Freer S.T.; Larson V.; Luty B.A.; Marrone T. and Rose P.W. (2000). Deciphering common failures in molecular docking of ligand-protein Complexes, *J. Comput. Aided Mol. Des.* 14: 731-751.

Vieira A.; Vieira M.; Silva G. and Araújo Á. (2010). Use of *Moringa Oleifera*a seed as a natural adsorbent for wastewater treatment. *Water Air Soil Pollution.* 206:273–281.

Vijayaraghavan, G.; Sivakumar, T. and Vimal Kumar A. (2011). Application of plant based coagulants for waste water treatment. *IJAERS*. 1(1): 88-92.

W.H.O. **(2005).** World Health Report 2005: Make Every Mother and Child Count. Geneva: World Health Organization.

Waseem Ahmad; Shilpa Singh; Sanjay Kumar (2017). Phytochemical Screening and antimicrobial study of *Euphorbia hirta* extracts. *Journal of Medicinal Plants Studies.* 5(2): 183-186.

Watson, C. **and Cichra, C.E. (2006).** Department of fisheries and Aquatic Sciences Florida cooperatives extention services. Institute of food and agriculture sciences, University of Florida. *First edition; June 1990.*

Weckwerth, W. (2003). Metabolomics in systems biology. *Annu Rev Plant Biol.* 54: 669-689.

Weckwerth, W. (2008). Integration of metabolomics and proteomics in molecular plant physiology – coping with the complexity by data-dimensionality reduction. *Physiologia Plantarum*. 132: 176-189.

Weckwerth, W. and Morgenthal, K. (2005). Metabolomics: from pattern recognition to biological interpretation. *Drug Discovery Today*. 10: 1551-1558.

Weckwerth, W.; Wenzel, K. and Fiehn, O. (2004). Process for the integrated extraction identification, and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks. *Proteomics*. 4: 78-83.

Weininger D. (1988). SMILES, a chemical language and information system. *J Chem Inf Comput Sci.* 28:31–36.

WHO (World Health Organization), (2003). Emerging issues in water and infectious diseases. *World Health Organization (WHO).*

WHO, (1984). Guidelines for *water quality* (vol. 1) Recommendations *World Health Organization.*

WHO, (1996). Water and sanitation fact sheet. NII2.

WHO, (2003). Guidelines for Drinking Water Quality, pp. 81–7. *World Health Organization.*

WHO, **(2008).** Safer water, better health. Costs, benefits and sustainability of interventions to protect and promote health.

Wienkoop, S. and Weckwerth, W. (2006). Relative and absolute quantitative shotgun proteomics: targeting low-abundance proteins in *Arabidopsis thaliana*. *J Exp Bot.* 57: 1529-1535.

Wienkoop, S.; Morgenthal, K.; Wolschin, F.; Scholz, M.; Selbig, J. and Weckwerth, W. (2008). Integration of metabolomic and proteomic phenotypes: analysis of data covariance dissects starch and RFO metabolism from low and high temperature compensation response in Arabidopsis thaliana. *Mol Cell Proteomics*. 7: 1725-1736.

Wilson Rwai Waweru; Lawrence Obado Osuwat; Francis Karomo Wambugu (2017). Phytochemical analysis of selected indigenous medicinal plants used in Rwanda. *Journal of Pharmacognosy and Phytochemistry*. 6(1): 322-324.

Wilson S.A. and Andrews S.A. (2011). Impact of a natural coagulant pretreatment for colour removal on solar water disinfection (SODIS). Journal of Water, Sanitation and Hygiene for Development 1:57-67.

World Health Organization and United Nations Children's Fund (2000). Global Water Supply and Sanitation Assessment 2000 Report. 80 p.

World Health Organization. (2008). Guidelines for Drinking-water Quality: incorporating the first and second addenda, Volume 1, Recommendations, third edition. 515 p.

Wright J.; Gundry S. and Conroy R. (2004). Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. Tropical medicine and international health: *TM and IH*. 9:106–117.

Yangabi, K.A. (2004). Studies on the Potential Use of Medicinal Plants in Water and Wastewater Purification. Proceedings of an E-Seminar organized by the International Organization of Biotechnology.

Yarahmadi M.; Hossieni M. and Bina B. (2009). Application of *Moringa Oleifera* Seed Extract and Polyaluminum Chloride in Water Treatment. *World Applied Sciences Journal.* 7 (8): 962-967.

Yongabi K.A. (2010). Biocoagulants for Water and Waste Water Purification: a Review *International Review of Chemical Engineering* (I.RE.CH.E.). Vol. 2, N. 3.

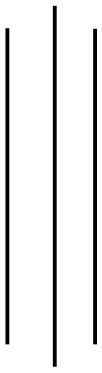
Yongabi, K.A.; Lewis, D.M. and Harris, P.L. (2011). Application of Phytodisinfectants in Water Purification in Rural Cameroon. African *Journal of Microbiology Res.* 5(6). 628-635.

Zhang J.; Jin Y.; Dong J.; Xiao Y.S.; Feng J.T.; Xue X.Y.; Zhang X.L. and Liang X.M. (2009). Systematic screening and characterization of tertiary and quaternary alkaloids from Corydalis yanhusuo using ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. Talanta 78: 513.

Zhang, J.; Livo, Y. and Yang, H. (2006). A Preliminary Study of Cactus as Coagulant in Water Treatment. Process. *Biochem*. 41(3) 730-733.

Zhao C.X.; Zeng Y.X.; Wan M.Z.; Li R.X.; Liang Y.Z.; Li C.Y.; Zeng Z.D. and Chau F.T. (2009). Comparative analysis of essential oils from eight herbal medicines with pungent flavor and cool nature by GC-MS and chemometric resolution methods. J Sep Sci. 32: 660.

CHAPTER – 9



LIST OF PUBLICATIONS

J. Microb. World 2013,
Microbiologists Society, ISSN – 0972 – 1487

Phytoremediation Enhancement of *Jatropha curcas* for Decontamination of Heavy Metals by Use of Biofertilizer

Satish Sharma[1],Anju Rani [1] Neerja Shrivastava,* Meenakshi Sharma[1]
*PG Department of Botany, Govt. College Kota
[1]Department of Basic and Applied Sciences Modi Institute of Management &Technology, Dadabari, Kota 324009
Email- ssat17@gmail.com

Abstract:

Heavy metal pollution becomes serious problem because it not only affects the production and quality of crops but also influences the quality of the atmosphere, soil texture and threatens health and life of animals and human beings. The phytoremediation investigations have been performed by using selected varieties of plant species to survive in phytotoxic environments that contain unusually high concentration of heavy metals. For effective phytoremediation process the plant species should be non edible and which can be grown abundantly in large scale on wastelands. Amendments such as biofertilizer play an important role in establishment of plant on contaminated land so the aim of the study was to evaluate the effect of *Azotobacter* biofertilizer in planting conditions of *Jatropha curcas* for reclamation of metal contaminated soils. Survival rate of plants in heavy metal contaminated soil increased with addition of amendments and metal contaminated soils could be suitably remediated by adapting appropriate phytoremediation measures.

Keywords: Biofertilizer, *Jatropha curcas*, Phytotoxic, Phytoremediation, *Azotobacter*

Introduction:

Natural environment pollution caused by heavy metals is a universal problem because these heavy metals are indestructible and most of them have toxic effects on living organisms when permissible concentration levels are exceed. Heavy metals frequently occurrences in contaminated soils are Cd, Cr, Pb, Zn, Fe and Cu (Akoto *et al.*, 2008). Heavy metal pollution becomes serious due to mining, mineral, smelting and tannery industries (Wang *et al.*, 2001). Recently, numerous efforts have been undertaken to find cost-effective technologies for remediation of heavy metal-contaminated soil (Chatterjee *et al.*, 2011). Therefore plants can be used to ameliorate heavy metal pollutants from the soil this cost effective approach is called phytoremediation which also referred as green solution (Butcher *et al.*, 2009).

The purpose is the remediation and reclamation of degraded lands or soils that contains unusually high concentration of heavy metals. For long term remediation, metal tolerance plant species are commonly used for revegetation of degraded lands (lan *et al.*, 1997). The physiochemical properties of heavy metals contaminated soil tends to inhibit plant growth (Sopper *et al.*, 1993). The quantity and activity of microorganisms represent sensitive indicators related to plant height, number of leaves, root length and biomass development processes. Therefore, the amendments such as organic materials and biofertilizer play an important role in establishment of plant on metal contaminated land.

For effective phytoremediation process the plant species should be non edible and which can be grown abundantly in large scale on

The Biosphere 5 (2) : 105-107, October 2013 ISSN 0975-3877
An International Biannual Journal of Life Sciences

SYNERGISTIC EFFECT OF *MORINGA OLEIFERA* SEED POWDER AND ALUM IN THE PURIFICATION OF DOMESTIC WATER : A NATURAL WATER PURIFICATION

NEERJA SHRIVASTAVA,* SATISH SHARMA, I RATI SHARMA, I MEENAKSHI SHARMA I
*PG Deaprtment of Botany, Govt. College Kota
[I]Department of Basic and Applied Sciences Modi Institute of Management Technology, Dadabari, Kota 324009

ABSTRACT

The high cost of treated water makes most people in the rural communities to resort to readily available sources which are normally of low quality exposing them to waterborne diseases. The seeds from *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities. Jar test on raw water sample displayed favorably characteristic properties in terms of colour, odour, turbidity, Alkalinity and total plate count in conformity with WHO standards at different concentration of alum and *Moringa* seeds. The optimum dose observed in the present study of 60:40 mg/l *Moringa oeifera* seeds powder reduces alum requirement as well as the cost of water treatment. The results indicate that *Moringa oleifera* has a double advantage compared to chemical alum because of the presence of phytochemicals which possess antimicrobial properties with potentials for conjugative use with alum for water purification in rural communities.

Key words : Synergistic effect, *Moringa oleifera*.

INTRODUCTION

Water is one of the fundamental requirements of life and undesired addition of chemical substances leads to its contamination and makes it unfit for human utility. Many industrial and power plants use rivers, streams and lakes to dispose of waste heat and also can have a disastrous effect on life in an aquatic ecosystem. The frequency of life threatening infections caused by consumption of untreated water has increased worldwide and is becoming an important cause of mortality in developing countries. In the recent years the use of various natural products has been widely investigated as an alternative for the currently expensive methods of water treatment. Some of the natural products can be effectively used as a low cost absorbent. *Moringa oleifera* seeds are also used as a primary coagulant in drinking water clarification and wastewater treatment due to the presence of a water-soluble cationic coagulant protein able to reduce turbidity of the water treated. Seeds are powdered and added to the water straight or after preparing crude extract (Ndabigengesere et al., 1995).*M. Oleifera* seed has also been found to have antibacterial activity (Shaikh PR, Bhosle, 2011) (Shaikh PR, Bhosle, 2011). The coagulant activity of *Moringa oleifera* seeds is widely known and applied in water treatment at household level in rural areas of developing countries. Coagulant recovery from waterworks sludge for re-use, though not a new concept remains a key option towards the reduction of chemical usage in the water industry. However there are constraints encountered in the use of chemical coagulants such as alum is the most commonly used coagulant in the developing countries, studies have linked it to the development of neurological disease due to the presence of aluminum ions in the drinking water (Jekel, 1991). Hence as a result of this consequence mentioned above, there is a need to develop alternative, cost effective and also environmentally friendly coagulants.

MATERIALS AND METHODS

Jar test :

moringa seeds and alum 1g each of powdered is dissolved in separate 100ml of distilled water as stock solutions. 200ml of raw water were measured and introduced into 7 beakers labeled 1-7 with designated dose blends of alum to *moringa* as: Jar 1=(2.0:0.0)ml, Jar 2=(1.6:0.4)ml, Jar 3=(1.2:0.8)ml, Jar 4=(0.8:1.2)ml, Jar 5=(0.4:1.6)ml, Jar 6=(0.0:2.0)ml, Jar 7(control)=(0:0). By using calibrated pipette each stock solution dosages of alum and *Moringa* solutions were added onto the water samples in the beakers. The sequence of addition is *Moringa* solution followed by Alum solution with stirring. The beakers are observed and evaluated for specific dosages and flock quality. The jar test mixer is turned off and the flock allowed settling in the beakers for 30mins and flock settling characteristics are observed.

ISSN 0970-3586

Ad. Plant Sci. 26 (II) 571 - 574, 2013

"Effect of Bioparameters in the Production of Lipase from *Streptomyces aurofaciens* and *Aspergillus niger*"

Anju Rani*, Vimla Meena, Satish Sharma, Apurva Shringi and Pooja Sharma
Department of Basic and Applied Sciences, Modi Institute of Management and Technology, Kota-324009
*Corresponding Author
E-mail : anjusingh.biotechnology@gmail.com Telephone : +91 9509054482

Lipase production both from *Streptomyces aurofaciens* and *Aspergillus niger* were tested using submerged fermentation (SmF) on a mineral culture medium and on basal medium. In the present study, different factors affect the activity of lipase were investigated. Olive oil was the best substrate for enhancing the enzyme activity. Due to high cost, instead of using olive oil sunflower oil and coconut oil were used as a substrate for production of lipase. Enzyme activity of lipase was determined by titrimetric method. In this study, olive oil was using as a standard, while sunflower oil and coconut oil as a test. The maximum lipase activity was achieved at 24 and 48 h of incubation period and the enzyme activity from *Streptomyces aurofaciens* was 47.61 and 52.82 U/ml and from *Aspergillus niger* was 26 and 26 U/ml by using sunflower oil and coconut oil as a substrate. The incubation period was very short for obtaining maximum lipase by using sunflower oil than coconut oil. No significant changes obtained in the pH ranges from 6 to 9.

Keywords : *Streptomyces aurofaciens, Aspergillus niger*, Sunflower oil, Coconut oil, Olive oil and Titrimetric method.

INTRODUCTION

Lipolytic enzymes are one of the most important groups of biocatalysts for biotechnological application (Jaeger and Eggert, 2002). Lipolytic enzyme includes esterase and lipases. Lipases (triacyl glycerol acyl hydrolase) catalyse the hydrolysis and the synthesis of ester formed from glycerol and long-chain fatty acids (Arpigny and Jaeger, 1999; Sharma *et al.*, 2001). Lipases are synthesized by micro-organisms which grow on fats or oils. Lipase is a potential biocatalysts employed in industries to hydrolyse fats and catalyse a number of useful reactions including esterification, transesterification and leather industries. Lipases posses the unique feature of acting at the phase.

Lipases secreted into the culture medium by many fungi and bacteria recently have attracted considerable attention owing to their biotechnological potential (Aires-Barros *et al.*, 1994, Mori *et al.*, 2009). Use of lipases in oleochemical processing saves energy and minimizes thermal degradation during alcoholysis, acidolysis, hydrolysis, and glycerolysis. Among the microbes fungi are widely recognized as the best sources of lipases. *Aspergillus niger* is among the well-known lipase producer used in many industrial applications (Macris *et al.*, 1996). The first commercial recombinant lipase 'Lipolase' which originated from the fungus *Thermomyces lanuginoscus* and was expressed in *Aspergillus oryzae*. In 1995, two bacterial lipases were introduced - 'Lumafest' and *Pseudomonas mendocina* and Lipoman from *P. alcaligenes* by Genencor International (Jaeger and Reetz, 1998). The enzyme produced by this organism is extracellular in nature. Their enzymes are mostly used in dairy industry (Arbige and Neubeck 1988). Fermentation studies carried out for the production of extracellular lipases by *Aspergillus niger* showed varied results among different strains of the fungus (Arbige and Neubeck 1988, Pal *et al.*, 1978). The organism was found to require an inducer for lipase synthesis (Pokorny *et al.*, 1994). Olive oil was found to stimulate lipase production the best. He also reported that lipase production by *Aspergillus niger* was enhanced by the presence of Mg^{2+}, metal ion. Lipase production both from *Streptomyces aurofaciens* and *Aspergillus niger* were tested using submerged fermentation (SmF) on a mineral culture medium and on basal medium.

In the present study a lipase production from *Streptomyces aurofaciens* and *Aspergillus niger* were determined by using olive oil, coconut oil and sunflower oil of its yield's also tabulated. Optimization of pH for biocatalyst was also analysed.

MATERIAL AND METHODS

Collection of Micro organisms and their maintenance : Two fungal species *Streptomyces aurofaciens* and *Aspergillus niger*

Dear **Dr. Neerja Shrivastava**

We are happy to inform you that **Thomson Reuters** has been accepted IJPSR for coverage in Web of Science - Emerging Sources Citation Index.
This is to acknowledge the receiving of your paper entitled '**PHYTOCHEMICAL CHARACTERIZATION AND GC-MS ANALYSIS OF METHANOLIC EXTRACT OF NATURAL WATER PURIFIER *CICER ARIETINUM* L..'**. Your article will be sent at the editorial board's desk for reviewing and after their comments it will be reverted back to you for correction if needed.

Kindly note your reference number- **IJPSR/RA-6588/05-16** and save this number for further correspondence. Selection of online publication of your manuscript totally depends upon the reviewing of the reviewer.

Selection of online publication of your manuscript totally depends upon the reviewing of the reviewer. You are requested to send the signed copyright form within 1-2 days. Please find Copy Right Form at our website www.ijpsr.com in **Instructions to Authors** section at end of the page.

Only after the copyright form is received duly signed by all author(s), your article will be forwarded for Reviewing Process to the Reviewer otherwise your article will not be considered for publication in IJPSR.

If you would like to receive **IJPSR updates**, you may follow us on **Facebook** http://www.facebook.com/IJPSR,**Twitter** http://twitter.com/IJPSR and **Linked in.**

Regards,
Mrs. Monika Sabharwal
Managing Editor
IJPSR [E-ISSN: 0975-8232; P-ISSN:2320-5148]
IJPSR Indexed with Embase (ELSEVIER)
IJPSR Indexed with PubMed Central
Projected Impact Factor (2014) : 1.54
DOI: 10.13040/IJPSR.0975-8232
Member of International Linking Association, Inc (PILA) - USA
www.ijpsr.com

Our other monthly publications
International Journal of Pharmacognosy (IJP)
E-ISSN: 2348-3962; P-ISSN: 2394-5583
www.ijpjournal.com
International Journal of Life Sciences and Review (IJLSR)
E-ISSN: 2394-9864
www.ijlsr.com

Appendix : I Drinking Water Standard Parameters

	Standards	Recommended Agency	Unit Weight
P^{H}	6.5 - 8.5	ICMR / BIS	0.2190
Total Alkalinity	120	ICMR	0.0155
Total Hardness	300	ICMR / BIS	0.0062
T.D.S.	500	ICR / BIS	0.0037
Calcium	75	ICMR / BIS	0.025
Magnesium	30	ICMR / BIS	0.062
Chloride	250	ICMR	0.0074
Nitrate	45	ICMR / BIS	0.0413
Sulphate	150	ICMR / BIS	0.0124
D.O.	5.0	ICMR / BIS	0..723
B.O.D.	5.0	ICMR	0.3723

⟹ All values in mg/L except pH

Appendix : II WHO Standards for Drinking water

S.No.	Parameter	WHO Standards
1.	Colour	acceptable
2.	Odour	unobjectionable
3.	Taste	agreeable
4.	Turbidity	5 NTU
5.	pH	7-8.5
6.	Electrical conductivity	1000 µmhos/cm
7.	Total Dissolved Solids	500
8.	Chloride	250
9.	Alkalinity	120
10.	Hardness	300
11.	Sulphate	200
12.	Nitrate	45
13.	Fluoride	1
14.	Dissolved Oxygen	5
15.	BOD	3

⟹ All values except Turbidity, pH and Electric Conductivity are expressed in mg/L