PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF WATER TREATED WITH SELECTED SEED EXTRACTS

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Researcher

J.RENUKA, M.Sc., M.Phil.,

(Reg.No.P3301)

Research Supervisor

Dr. J.KARUNYAL M.Sc., B.ed., Ph.D.,

Co Guide

Dr. R. SHENBAGARATHAI M.Sc., M.Phil., Ph.D.,

MADURAI KAMARAJ UNIVERSITY

MADURAI - 625 021

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INTRODUCTION

"Water is the driving force of nature" Leonardo da Vinci

Water is the most essential natural resource for sustainability of life on earth. It is the chemical substance which is essential for every living organism to survive on this planet to perform normal function. About 70% of the human body weight is made up of water. Therefore it plays an important role in the structure and function of the human body. The inadequate supply of treated water in urban settlements has resulted in lack of drinking water. **(Eze and Ananso – 2014).** Water covers 71% of the Earth's surface, mostly in oceans and other large water bodies, with 1.6% of Water resources all over the world are threatened not only by over exploitation and poor management but also by ecological degradation. **(Rajendran et*al* 2013)**

Household water treatment and safe storage (HWTS) are an important public health intervention to improve the quality of drinking-water and reduce diarrhoeal disease, particularly among those who rely on water from unimproved sources, and in some cases, unsafe or unreliable piped water supplies. Further, safe drinking water is an immediate priority in most emergencies, and HWTS can be an effective emergency response intervention. Household water treatment applications are any of a range of technologies, devices or methods employed for the purposes of treating water at the household level or at the point.

Ground water is one of the potable water resources available for both domestic and industrial purposes. In Tamil Nadu, hard rock formations occupy 73% of the total geographical area and the remaining area is occupied by sedimentary rocks. Ground water is extracted from these rocks by means of dug/tube/bore wells. Ground water pollution has been reported throughout the developing countries and it is a major threat to the environment. **(Aneez Ebrahim *et al* 2011).**

Pure uncontaminated water does not occur in nature. Water pollution is any undesirable change in the state of water, contaminated with harmful substances. It is the second most important environmental issue next to air pollution. Any change in the physical, chemical and biological properties of water that has a harmful effect on living things is termed as 'water pollution **(WHO 1997, Balachandra *et al* 2013).** Surface water contains inorganic and organic compounds of natural origin as suspended matter and dissolved substances. In most cases, water in river and lake is contaminated by waste, sewage, chemicals, hydrocarbons, medicine, hormones, antibiotics, bacteria, viruses, fertilizers, plant-protective agents, etc. and their decay products.

Sources such as dams, dug outs, streams, rivers, and lakes. Water from these sources is usually turbid and contaminated with microorganisms that cause many diseases including guinea worm and bilharzia. Waterborne diseases are one of the main problems in developing countries; about 1.6 million people are compelled to use contaminated water and more than a million people (of which two million are children) die from diarrhea each year. **(Balachandra *et al* 2013).**

The ground water pollution is mainly due to high TDS content. TDS can be explained as the total amount of mobile charged ions, including minerals, salts or metals dissolved in a given volume of water, expressed in units of mg per unit volume of water (mg/L), also referred to as parts per million (ppm). TDS is directly related to the purity of water and the quality of water purification systems. It affects everything that consumes, lives in, or uses water, whether organic or inorganic, whether for better or for worse. Dissolved solids come from organic sources such as leaves, silt, plankton, and industrial waste and sewage, runoff from urban areas, road salts used on street during the winter, fertilizers and pesticides used on lawns and farms. (**Aneez and Mohamed Ali – 2011**).Various problems due to impure water in developing countries. Large seasonal variation in raw water quality e.g. turbidity, Water treatment chemicals are imported with scarce foreign currency, High cost of water treatment chemicals which constitute in between 35% to70% of recurrent expenditure, Inadequate water treatment, Inadequate laboratory facilities to monitor process performances required to operate the plants, Inadequate funding, low revenue base, Water supply considered as a social commodity rather than an economic resource inadequate skilled manpower, maintenance schedules, technology, Inadequate supply to meet growing demand, under dosing of chemicals leading supply of poor quality water. (**Renuka, Binaye and Jadhav – 2013)**

Traditional water treatment methods

The earliest recorded attempts to find or generate pure water dates back to 2000 B.C. Early Sanskrit writings outlined methods for purifying water .These methods ranged from boiling or placing hot metal instruments in water before drinking it to filtering that water through crude sand or charcoal filters. **(Sathish, Selvaganesapandian and Arul Amuthan, 2012).** Filtration through winnowing sieve (used widely in Mali)**,** Filtration through cloth (commonly used in villages in India, Mali and the southern part of Nigeria, Filtration through clay vessels (used in Egypt), Clarification and filtration through plant material (commonly used in Tamil Nadu and Kerala, India), Jempeng stone filter method (used in Bali, Indonesia).

Chemical Treatment of water

Water is treated conventionally in different parts of the world to ensure good water quality and providing safe drinking water. This reduces the widespread of water borne diseases and combating its effects. The treatment processes include coagulation, flocculation, sedimentation, filtration and disinfection. The difficulty for adoption of conventional water treatment technologies in developing countries is as a result of the high cost and scarcity chemical coagulants and disinfectants**. (Eze and Ananov – 2014).**This pressure on the nations over – burdened financial resources since they are imported there by making treated water very expensive in most developing countries and beyond the reach of most rural folks. Hence, they resort to sources such as dams, dug outs, streams, rivers and lakes. Natural purification effects within filter layers and in the subsurface are caused mainly by filtration, sedimentation, precipitation, oxidation-reduction, sorption-desorption, ion-exchange and biodegradation.

Problems/challenges faced using artificial Coagulants

Many coagulants are widely used in conventional water-treatment process for turbidity removal during potable water production. These coagulants may be classified as inorganic, synthetic organic polymer, and natural polymer. These coagulants are used for various purposes depending on the chemical characteristics of the water to be treated. Aluminum salts are by far the most widely used coagulant in water and wastewater treatment. There is also the problem of reaction of alum with natural alkalinity present in water leading to reduction of pH and a low efficiency in coagulation of cold waters.

Disinfection by products DBPS may also be associated with cardiovascular disease, cancer and birth defects. The emergence of chlorine resistant strains of organisms such a *Cryptosporidium oo*cysts, strains of *Salmonella* species, *Entamoeba*cysts, *Mycobacterium* species, *Escherichia coli* and *Helicobacter pylori* have also been reported.These limitations make it essential for cost-effective, simple and environmentally friendly drinking water purification methods to be introduced in developing countries especially in rural areas in Nigeria.(**Eze,** and **Ananaso – 2014).**

Health hazards due to chemicals in water treatment

Earlier research findings **Crapper *et al* (1973) and Miller *et al* (1984)** showed that the chemicals used for water purification can cause serious health hazards if an error occurs in their administration during the treatment process. These reports suggested that a high level of aluminum in the brain is a risk factor for Alzheimer's disease and similar health related problems associated with residual alum in treated waters, beside production of large sludge volumes, non-biodegradable after treatment and therefore poses disposal problems leading to increase cost of treatment. (**Letterman and Driscoll 1988, Mallevialle *et al* 1984, Miller *et al 1984***) have also, studies have raised doubts about the advisability of introducing aluminum into the environment by the continuous use of aluminum sulphate as a coagulant in water treatment. (alum), the widely used water coagulant in addition to other effects, generate acidic water which is unsafe for pregnant women and causes predementia.

Alternative approach to purify polluted water (Natural Coagulants)

Natural polyelectrolytes of plant origin have been used for many centuries in developing countries for clarifying turbid water **Schulz and Okun, (1984).** For home water treatment, the materials have to be used in the form of powder or paste, 90% of which consists of substances other than the polyelectrolytes. Even under such conditions, a few plant seeds make effective coagulants**. (Jahn, 1988).**

At present, a number of effective coagulants have been identified of plant origin. Of the large number of plant materials that have been used over the years, the seeds from *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment, especially in rural communities**.** In addition, indigenous knowledge indicates that there are several plant species that can be used as a coagulant and disinfectant.

Natural coagulants have been reported to have several advantages compared to Alum. Natural coagulants produce much lower sludge volume, the natural alkalinity is not consumed during the treatment process, they are biodegradable, safe to human health, cost effective since they can be locally grown and have a wider effective dosage range for flocculation of various colloidal suspensions. (**Yasabie *et al* 2014**).

The performance of plant extracts such as Nirmali tree (*Strychnous potatorum*), Tamarind tree (*Tamarindus indica*) , Guar plant (*Cyamopsisp soraloides*) ,Red Sorella plant (*Hibisicus sabdariffa*) ,Fenugreek (*Trigonella foenum*) , *Drum stick (Moringa. oleifera, M. concanensis*) , Barbados nut(*Jatropha curcas)* , King Tuber Mushroom *(Pleurotus tuberrregium sclerotium)* ,Okra (*Abelmoschus esculentus*) , Red bean (*Vigna ungularis*) ,Red maize (Zea mays) ,*Cactus(Cactus latifera)* ,Mesquite(*Prosopis juliflora)* ,*Sugar apples(Annona squamosa*) , *Fig (Ficus racemosa)* ,Lentils (*Lens esculenta)* have been used to treat raw water with turbidity that ranged from 50 to 7500 NTU. (**Yasabie *et al* 2014**). Using the right plants to purify and filter water will clean and remove heavy metals and harmful bacteria, Heavy metals, Harmful bacteria, Parasites, Radioactive isotopes (porous carbon and green coal work much better then plants), Chemicals, Cysts(with time the cysts tract themselves to the plant roots with prolonged exposure this method is not 100%)

The present study is aimed at examining the water samples before and after treatment with 40 different plant species and the purity of water has been examined following scientific techniques and the results is presented in the thesis.

OBJECTIVES OF THE STUDY

- To Identify and collect water samples from different water sources such as sea, river, lake, sewage. dam, spring and well.
- To investigate the quality of the collected water samples by examining the physico – chemical parameters and bacteriological analyses.
- To isolate bacteria from different water samples
- To screen forty different plant species showing clarification of water in terms of turbidity and bacterial count reduction.
- To identify the plant species which exhibit maximum reduction in the turbidity and bacterial count.
- To analyse the physico – chemical parameters of selected water samples before and after treatment with pulverized plant material.
- To examine the antibacterial effects of the plant extract against the bacteria isolated from water with pulverized plant material.
- To scientifically validate the antibacterial activity of the plant extract on antibacterial activity when subjected to various temperatures and pH.
- To scientifically validate the antibacterial activity of the plant extracts using a standard bactrerial culture MTCC, *Escherichia coli* 739 was also used.
- To identify the phytochemical properties in the plants.
- To analyze the toxic elements present in the selected plant material in order to exclude the possibility of introducing these toxic elements during water treatment by Atomic Absorption spectroscopy.
- To identify the active principle(s) responsible for the antibacterial activity of the selected plant extracts by subjecting them to HPLC and Gas chromatography Mass Spectrometry. (GCMS)

METHODOLOGY

Collection of Water Samples

Water sources for the collection of water samples were selected based on the proximity of the source from the Institution and the feasibility. Water samples were collected aseptically in sterile plastic containers (1litre capacity) from the selected location and transferred to the laboratory for the investigation. The method given by Cheesbrough**. (2006)** for water sample collection was adopted. Experiments on coagulation studies were carried out using natural and artificially prepared turbid water using china clay. A conventional jar test was used for the experiments to coagulate the above water samples.

Synthetic turbid water for the jar tests was prepared by using china clay and tap water. A 3% W/V suspension of clay was prepared and stirred for one hour to achieve the uniform distribution of the clay particles (90–120) NTU. Then it was allowed to settle for 24 hours for the complete hydration of the particles. After the settlement of particles the suspension was used for the coagulation study. Alum (A) solution was prepared by dissolving 1gm of alum (AL2 (SO4)3) in distilled water (pH – 7 – 7.2) and the solution volume was increased to 1L. Each 1ml of prepared stock solution was equal to 10mg/L, when added to 1L artificial water to be tested. (APHA, 1998).

Collection of Plants Samples & Identification of Plants

The plant samples used in the study were obtained from different locations in and around Madurai. The fresh plants samples were collected individually in open jute bags for proper air circulation. The collected plants samples were photographed on the same day presented in the results and discussion.

Clarification Study Using Pulverized Samples

Turbidity of the water was measured at 560nm using a colorimeter before and after treatment with seed powder and the results was tabulated**.** Turbid water without pulverized plant served as a control**,** the results are presented in the thesis.

Analysis of Physico-Chemical Parameters of Turbid Water before and after Treatment

A number of physicochemical parameters of the water samples were determined before and after treatment with individual plants for 40 selected plant species. The analysis was performed in the district water testing laboratory of Tamilnadu Water and Drainage Board (TWAD) in Mattudhavani, Madurai.

Bacteriological Examination

- Total count - Standard plate count per ml
- Total coliform per 100 ml – Most Probable Number (MPN)Technique
- Fecal coli form per 100 ml – Most Probable Number (MPN)Technique
- Fecal Streptococcus per 100 ml – Most Probable Number (MPN) Technique.

Were investigated following, (Gunasekaran, Rajamanickam).

All analyses were done and compared with BIS STANDARDS and the results were tabulated. The isolated bacteria were identified following Cheeseburg 2006.

Analysis of antibacterial activity of seed extract

Type culture of *Escherichia coli* MTCC 739, was obtained from (MTCC) Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India was used for the investigation along with the bacterial isolates. Disc diffusion and well diffusion methods were followed for the antibacterial study .The methanolic and aqueous extract of the seeds were used for the investigation.

Qualitative phytochemical screening of *Artocarpus heterophyllus* and *Guazuma tomentosa* seeds

The plant extracts were subjected to qualitative phytochemical screening for the identification of various classes of chemical constituents alkaloids, glycosides, terpenoids, steroids, flavonoids, saponin, and tannin using the standard method described by Trease and Evans (1989) and Harborne (1973) & Packialakshmi**., *et al*.,2014.**

Heavy Metal Analysis (Atomic Absorption Spectroscopy)

The selected 2 plant materials collected were subjected for heavy metal analysis usinf atomic absorption spectroscopy (AAS). The samples were digested with triacid mixture. (Concentrated Nitric acid, sulphuric acid and perchloric acid) were added together at the ratio 9:2:1.

Separation and Identification of Active Principle(S)

In order to identify the active principle(s), from the two plant species *Artocarpus heterophyllus* and *Guazuma tomentosa* , the methanolic extracts were subjected to High Performance Liquid Chromatography (HPLC) and Gas Chromatography –Mass Spectrometry (GCMS) analysis were followed.

FINDINGS

Different water sources available in and around Madurai are Sea, River, Lake, Dam, spring, well and industry water. Water from some of the above sources cannot be utilized unless and until further treatment. Fourteen water samples were collected from different places in and around Madurai for the analysis as shown in Table: 1

Table 1: List of water samples from various sources

S.No	Sample	Type of Water	Location
1.	S1	Sea Water	Rameswaram
2.	S2	Sea Water	Tiruchendur
3.	R1	River Water	Vaigai
4.	R2	River Water	Cauveri
5.	SW1	Sewage Water	Goripalayam, Madurai
6.	SW2	Sewage Water	Karimedu, Madurai
7.	L1	Lake Water	Thiruparankundrum, Madurai
8.	D1	Dam Water	Sathiyar Dam
9.	D2	Dam Water	Vaigai Dam
10.	SP 1	Spring Water	Sholavandan
11.	SP 2	Spring Water	Pattiveeranpatti
12.	W1	Well water	Thethur
13.	W2	Well Water	Madurai
14.	I1	Industry Water	Allanganallur sugar factory, Madurai

Forty different plant species were selected for the screening of clarification property of water using turbidity measurement at 560nm and the lists of plants are given in Table 2

Table: 2 List of the plant species used for the study

S.No	**Vernacular Name**	**Botanical Name**	**Part of Plant Used**	**Collected Area**
1.	Vannimaram	*Albizia lebbeck (L)*	Seed	Pudur Market, Madurai
2.	Jack fruit	*Artocarpous heterophyllus(Lam)*	Seeds	Pudur Market, Madurai
3.	Custard Apple	*Anona squamosa(L)*	Seeds	Mahathma Gandhi Nagar, Madurai
4.	Groundnut	*Arachis hypogea (L)*	Seeds	Village -Therkkutheru
5.	Cluster beans	*Cyamopsis tetragonolobo(L)*	Seeds	Village -Therkkutheru
6.	Bitter Orange	*Citrus medica(L)*	Seeds	Solai alagu puram, Madurai
7.	Papaya	*Carica papaya(L)*	Seeds	Local Market –Karimadu
8.	Chickpea	*Cicer arietinum(L)*	Seeds	Local Market, Madurai
9.	Water melon	*Citrullus lanatus(Thunb)*	Seeds	South Gate Market, Madurai
10.	Indian Cherry	*Eugenia Jambolana(Lam)*	Seeds	Pudur Market, Madurai
11.	Sacred fig	*Ficus religiosa(L)*	Seed	Villapuram, Madurai
12.	Katurudraksham	*Guazuma tomentosa (Lam)*	Seed	American College Campus
13.	Henna	*Lawsonia inermis(L)*	Seed	Ellis Nagar, Madurai
14.	Barbados nut	*Jatropha curcas(L)*	Seed	Village- Therkkutheru
15.	Sapota	*Manilkara zapota(L)*	Seed	American College Campus
16.	Mango	*Mangifera indica(L)*	Seed	South Gate Market,

				Madurai
17.	Banana	*Musa acuminate(Colla)*	Peel	South Gate Market, Madurai
18.	Cassava	*Manihot esculenta (Crantz)*	Root	Local Market,Madurai
19.	Pongam	*Millettia pinnata(L)*	Seed	American College campus
20.	Horse raddish tree	*Moringa oleifera (Lam)*	Seed	South Gate market, Madurai
21.	Curry Leaves	*Murraya koengii(L) Sprengel*	Seeds	Jaihindupuram, Madurai
22.	Nutmeg tree	*Myristica fragrans(Houtt)*	Seeds	Villapuram Market, Madurai
23.	Cactus	*Opuntia ficus-indica (L.)Mill*	Leaves	Village- Therkkutheru
24.	Copper pod	*Peltophorum ferrugineum((Decne.) Benth.*)	Seeds	American College campus
25.	Mesquite	*Prosopis juliflora(L) panigrahi*	Seeds	Village - Therkkutheru
26.	Amla	*Phyllanthus emblica(L)*	Seeds	Village- Therkkutheru
27.	Guava	*Psidium guajava*	Seeds	Village- Therkkutheru
28.	Apple	*Pyrus malus (L)*	Seeds	Pudur, Madurai
29.	Kodikka	*Pithesellobium dulce(Roxb.)Benth*	Seeds	Pudur Market, Madurai
30.	*Thorn apple*	*Solanum incanum(L.)*	Leaves	Village- Therkkutheru
31.	Sugar cane	*Saccharum officinarum(L.)*	Leaves	Nel Pettai, Market
32.	Bhel fruit	*Strychnos potatorum(Linn.)*	Seeds	American College Campus
33.	Myrobalan	*Terminalia chebula(Retz)*	Seeds	American College Campus

34.	Teak	*Tectona grandis(L.)f*	seed	American College Campus
35.	Fenugreek	*Trigonella foenum(L)*	seeds	Pudur Market, Madurai.
36.	Tamarind	*Tamarindus indica(L)1753*	Seeds	American College campus
37.	Jujube red date	*Ziziphus jujuba (Mill)*	Seed	Village- Therkkutheru
38.	Grape	*Vitis vinifera(L.)*	Seed	Pudur market, Madurai
39.	Cow pea	*Vigna unguiculata(L.) Walp*	seeds	Village- Therkkutheru
40.	Corn	*Zea mays(L.)*	Leaves	Village – Therkkutheru
41.	Chemical Control	*Alum*	Powder	Local shop, Madurai

- The inclusion of the selected plant powder caused a drastic reduction in the turbidity of natural and synthetic water samples. Forty different plant species were screened for their clarification property in terms of turbidity at 560nm before and after treatment. (the results are not shown).The results showed that among the tested 40 plants 12 plants exhibited more coagulation properties and thereby showed more clarification property. The name of the plant species are as follows.1. *Zea mays, 2. Guazuma tomentosa, 3. Trigonella foenum, 4. Artocarpus heterophyllus, 5. Carica papaya, 6. Cicer arietinum, 7. Peltophorum ferrugineum, 8. Phyllanthus emblica, 9. Eugenia Jambolana, 10. Moringa oleifera Lam, 11. Terminalia chebula, 12. Strychnous potatorum,* Alumwas used as control (13). Clarity of water was examined by measuring turbidityof water at 560nm. When the seed extract was included the turbidity showed a drastic decrease in case of *Artocarpus heterophyllus* within a day. Turbid water treated with *Peltophorum ferrugineum* and *Phyllanthus emblica* took a week for clarification.
- There is about a fourfold reduction in turbidity of water after treatment with *Artocarpus heterophyllus, Guazuma tomentosa, Zea mays*after one day. The degree of water clarification in terms of turbidity reduction in case of all the tested water samples could be represented as*Artocarpus heterophyllus*> *Guazuma tomentosa*> *Moringa oleifera Lam*> *Strychnous potatorum*> *Carica papaya*> *Zea mays*> *Trigonella foenum*>

Phyllanthus emblica> *Cicer arietinum*> *Peltophorum ferrugineum*> *Eugenia Jambolana*> *Terminalia chebula* .

- The inclusion of plant extracts significantly reduced the turbidity of water and there by showed clarification of water. The physicochemical analysis of water was carried out before and after treatment using all the forty different plant species. The mean values of physico – chemical parameters of the fourteen water samples from different location before and after treatment were presented in the thesis.
- The total coliform, fecal coliform and fecal streptococcus were enumerated for all the water samples and the results showed drastic reduction in number after treatment.
- The purification potential of 12 plant seeds in 14 water samples were accessed by analyzing the microbiological qualities of water samples before and after treatment and the results showed reduction are presented in the thesis.
- Bacteria isolated from the sewage water were characterized and identified using colony morphology, microscopic and biochemical examination. The isolated bacteria were identified as *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia and Staphylococcus aureus* .
- In order to quantitate the antibacterial activity, extract included microbial assay was carried out. The nutrient broths with and without the plant extracts were inoculated with the isolated bacterial strains (*Escherichia coli, Bacillus subtiils, Klebsiella pneumonia and Staphylococcus aureus*). When growth in terms of turbidity at 560nm was measured it was inhibited in the plant extract included medium as compared to the control.

- All the plant extracts (methanolic) showed antibacterial activity against the tested bacteria at the neutral pH (natural /biological pH). However, when the pH was altered pH 2, 6,8,12 there was no antibacterial activity in case of *Guazuma tomentosa*, *Peltophorum ferrugineum and Phyllanthus emblica*. When the pH was altered (pH 2, 6, 8, 12) the antibacterial activity was decreased in case of *Artocarpus heterophyllus* against*Klebsiella pneumonia*, *Bacillus subtilis* and *Staphylococcus aureus.*
- When the *Guazuma tomentosa* extract was subjected to various temperatures 4.C, 30.C, 50.C, 100.C & 121.C the results showed that the diameter of the inhibitory zone

decreased in *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* as compared to the room temperature. However when the *Artocarpus heterophyllus* extract was subjected to various temperatures 4.C, 30.C, 50.C, 100.C & 121.C. Th result showed that the diameter of the inhibitory zone decreased in case of *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* when compared to the room temperature 30.C. But the extract remained stable at 121.C.

- In order to scientifically validate our results a standard culture *Escherichia coli* MTCC 739 was also obtained from MTCC, Chandigarh. The pulverized seeds of *Artocarpus heterophyllus and Guazuma tomentosa* were extracted with water and methanol. The aqueous extract showed no inhibitory zones around the wells loaded with the extract on the bacterial lawn *Escherichia coli* MTCC 739. The boiled aqueous extract also exhibited no inhibitory zones. However, when the methanolic extract was analyzed for its antibacterial activity by well diffusion studies, it exhibited inhibitory antibacterial activity by forming inhibitory zones on the bacterial lawn around the well.

- Phytochemical analysis of methanolic and aqueous extracts of the selected plants showed various phytochemicals such as tannins, proteins, phenols and steroids, alkaloids, flavanoids, tannins, coumarin, phytosterol, oils and fats, gums and mucilage, carbohydrate in case of *Artocarpus heterophyllus*. *Guazuma tomentosa* showed the presence of tannins, steroids. tannins, proteins, phenols and steroids, alkaloids and flavanoids

- The results of elemental analysis of four mineral elements obtained by the comparator method of **Atomic Absorption Spectroscopy** (AAS) techniques of plants seed powder revealed that the concentrations of heavy metals under study were below the permissible value of plant mg/kg as recommended by WHO.

- Analysis of active principle(S) using HPLC revealed the presence of six compounds six compounds showing the retention time ranging from 1.680-7.610 in case of *Guazuma tomentosa* and in case of *Artocarpus heterophyllus* twelve compounds with the retention time ranging from 0.910-12.363. The compounds are suggested to be alkaloids which are responsible for the antibacterial activity. Butylated hydroxytoluene (BHT) was used as standard.

- The methanolic seed extract of *Artocarpus heterophllus was* subjected to GCMS analysis and the results showed largest chromatographic peaks and other minor peaks were also identified and presented in the thesis.

- The methanolic seed extract of *Guazuma tomentosa* showed the largest peak, with the retention time of 21.32min was identified as n-Hexadecanoic acid, (MW: 256.4241). However, other peaks with various retention times (minor peaks) and the respective compounds were also observed and presented in the thesis.

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