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Research Article

Journal of Pharmaceutical and Biological Research
www.pharmaresearchlibrary.com/jpbr
JPBR, 2013: Vol.1(1): 5-13

Antimicrobial Activity of Commonly Available Plants in Chennai for Human Pathogens

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Available Online: **27 November 2013**

ABSTRACT

Emergence of antibiotic resistance by bacteria commonly used for treatment of a variety of infectious diseases has alarmed the medical field. Plants are the major sources of therapeutic compounds that have been used in a variety of human ailments. A huge number of plants species of north –eastern region and Assam of India is known to have medicinal properties and used by tribes. The medicinal values of most of these are not yet evaluated scientifically for assessment of their potential as useful drugs. Destruction of forest and lack of proper documentation have made some of these species rare, threatened or endangered. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. Thus developing of modern biotechnology based conservation methodology like tissue culture, germplasm culture and screening of plant metabolites for discovering of potential drugs of pharmaceutical industry can be adopted for scientific exploration of these valuable bio-resources. Thus the present study was undertaken to study the Antimicrobial activity of the selected plants against three microorganisms for which the extraction was done with 3 different solvents. The Antimicrobial Activity Test was assessed with different solvents using Well Diffusion Method and Chloramphenicol and was compared with each other. The Phytochemical Test for each sample was evaluated to know the biochemical component present in the sample and finally Fourier transform spectroscopy test was conducted to qualitatively analyze the components present in each sample.

Keywords: Plants, Human ailments, Antimicrobial activity, Potential drugs, Pharmaceutical Industry

INTRODUCTION

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient (**Kaushik, P. and Dhiman, A. K. 2000**). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies **(Abramowics, M. 1990)**. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (**Mothana R.A. and Lindequist, U.2005)**. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency**.** Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin (**Cowan, M. M. 1999).**

1. Pomegranates
Kingdom - Plantae
Division – Magnoliophyta
Subclass – Rosidae
Order – Myrtales
Family – Lythraceae
Genus – Punica
Species – P.granatum
Botanical name – *Punica granatum*
Pomegranates are native to southeastern Europe and Asia and were grown in ancient Egypt, Babylon, India, and Iran. However, the best quality pomegranate fruits are produced in regions with cool winters and hot, dry summers. Few areas are too hot, and the pomegranate is more cold hardy (receives less damage) than citrus. Pomegranates vary in frost tolerance, but in some cases temperatures down to 10°F may not severely injure the plants. Several hundred hectares are cultivated in California and a small commercial industry existed in Florida during the 1800's. In the Indian Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years (**Camejo-Rodrigues, J**. ***et. al*., 2003)**.

2. Moringa oleifera
Kingdom- Plantae
Superdivision-Spermatophyta
Division – Magnoliophyta
Class - Dicotyledons
Subclass – Dilleniidae
Order – Capparales
Family – Moringaceae
Genus – Moringa
Species – *Moringa olifera* Lam (Horseradish tree)

Moringa oleifera (synonym: *Moringa pterygosperma*) is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. English common names include moringa, and drumstick tree, from the appearance of the long, slender, opical to warm temperate triangular seed pods, horseradish tree, from the taste of the roots which resembles horseradish, or ben oiltree, from the oil derived from the seeds. The tree itself is rather slender, with drooping branches that grow to approximately 10m in height. In cultivation, it is often cut back annually to 1–2 meters and allowed to regrow so the pods and leaves remain within arm's reach **(Gates, P. 2000)**. In developing countries, moringa has potential to improve nutrition, boost food security, foster rural development, and support sustainable landcare. It may be used as forage for livestock, a micronutrient liquid, a natural anti-helmintic and possible adjuvant. The leaves are the most nutritious part of the plant, being a significant source of vitamin B_6, vitamin C, provitamin A as beta-carotene, magnesium and protein, among other nutrients. The leaves of *Moringa oleifera* Lam. are eaten in African countries, such as Ghana, Ethiopia, Nigeria, East Africa and Malawi. Moringa tree is cultivated for foods and medicinal purposes. Moringa leaf is a natural anti-helmintic, antibiotic, detoxifer, outstanding immune builder used in some countries for the treatment of malnutrition and malaria. There are few natural plants on this world that can be legitimately called a “superfood.” One of the most powerful superfoods called *Moringa Oleiferahas* finally found its’ way into the Western World and some medical professionals are going so far as to call Moringa Oleifera the “Miracle Tree.”Since it is fairly new to the western world, not many people know about this amazing plant (**Rukangira, E. 2001)**.

3. Sweetlime
Kingdom: Plantae
Genus: Citrus
Species: *C. Limetta*

Citrus limetta is a species of citrus. Common names for varieties of this species include sweet limetta, Mediterranean sweet lemon, sweet lemon, and sweet lime.To prevent scurvy during the 19th century, British sailors were issued a daily allowance of citrus, such as lemon, and later switched to lime, which was not as effective at preventing scurvy but was easier to obtain on Britain's Caribbean colonies. It was later discovered that the greater effectiveness of lemons derived from the 4-fold higher quantities of vitamin C lemon juice contains compared to the West Indian limes used by the British. The composition of the citric fruits is generally composed of 90% terpenes,

5% oxygenated compounds, and less than 1% non-volatile compounds such as waxes and pigments D-Limonene, the most abundant terpene has antimicrobial properties, primarily the exhibition of antibacterial activity against Gram positive bacteria, and also increases the effectiveness of sodium benzoate as a preservative (**Santos, R. B.1985).**

Lime is a term referring to a citrus fruit which is typically round, green to yellow in colour, 3–6 cm in diameter, and containing sour and acidic pulp. Limes are a good source of vitamin C, and are often used to accent the flavours of foods and beverages. Citrus fruits are acidic fruits which contain healthy nutritional content that works wonders for the body. It acts as a fabulous source of vitamin C and a wide variety of essential nutrients required by the body. Fresh fruits and their hand-squeezed or industrially processed juices contain mostly flavanones and flavones. It is cultivated mainly for alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of lemon against clinically significant bacterial strains has been reported. Sweet limes are excellent source of free citric acid, natural sugar, vitamin C, calcium and phosphorus. They contain by far more vitamin C than the lemon. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, anticancer and antiviral activities. Literature suggests that the fruit of *C. aurantium* possess anti-anxiety activity and anti-obesity activity. Peel of *C. limonium* possesses cytotoxic and antimicrobial activity, fruit of *C.limonium* and *C.limetta* possesses anti-oxidative stress and anti-urinary lithogenesis (**Gautam, R *et. al*., 2007)**.

4. Cannonball Tree
Scientific name: *Couroupita guianensis*
Kingdom – Plantae
Phylum – Tracheophyta
Class- Magnoliopsida
Order – Lecythidales
Family – Lecythidaceae

The Cannonball tree is native to the tropical parts of South America and held in high regard by the shamans of the Amazon region. They call it “head of spirit” or Ayahuma. It is sacred to Hindus who call it Nagalingam, as it has what resembles the sacred serpent on the large Shiva lingam in the centre of the flower and there are other Shiva lingams around this. The flowers bloom for just one day but smell amazing when in bloom, like an expensive exotic perfume. In the Amazon all parts of the tree are used medicinally by the shamans who also eat the fruit, although this is not recommended for ordinary mortals who probably have allergic reactions to it. Peccaries eat it and disperse the seeds through their faeces. It can grow to heights of 115 feet**.** The effects of the Cannon ball tree in medical use are strong. As when using any natural medicine, the correct dosage is vital. In medicinal use, the flowers, leaves, bark and fruit flesh are used. The Cannonball Tree possesses antibiotic, antifungal, antiseptic and analgesic qualities. The trees are used to cure colds and stomach aches. Juice made from the leaves is used to cure skin diseases, and shamans of South America have even used tree parts for treating malaria. The inside of the fruit can disinfect wounds and young leaves ease toothache. The fruit emits an unpleasant odor and can be used as an insect repellent just by rubbing it to the skin or clothes. It's part of the family Lecythidaceae and grows up to 25 m (82 ft) in height. The "Cannonball Tree" is so called because of its brown cannon-ball-like fruits. Aubl. (Lecythidaceae) is commonly called Ayahuma and the Cannonball tree. It is distributed in the tropical regions of northern South America and Southern Caribbean. It has several medicinal properties. It is used to treat hypertension, tumours, pain, inflammatory processes, cold, stomach ache, skin diseases, malaria, wounds and toothache (**Farnsworth, N. R. 1988).**

5. Guava
Scientific name: *Psidium guajava*
Phylum: Plantae
Class: Dicotyledonae.
Family: Myrtaceae

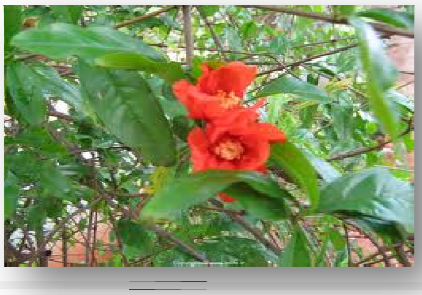
Guava originated in Latin America. It was introduced throughout the Pacific by Portuguese explorers and merchants during the 1600s. Seminole Indians grew guava trees in Florida as early as 1816. Since the 1950s, guavas– particularly the leaves – have been the subject for diverse research on their constituents, pharmacological properties and history in folk medicine. Most research, however, has been conducted on apple guava, with other species remaining unstudied. From preliminary medical research in laboratory models, extracts from apple guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain. Essential oils from guava leaves display anti-cancer activity in vitro. The guava is one of the most delicious fruits and is

known botanically as *Psidium gaujava*, and locally by different names such as Amrud, Peru, Piyara, Koyya, Sede Pandu etc. The fruit is one of the richest source of vitamin C (212 mg/100 g fruit). The quantity of vitamin C increases with the maturity of fruit. The fruit contains fairly large quantities of useful minerals like phosphorus (20-30 mg/100 g fruit) and calcium (20-210 mg/100 g fruit) depending on the variety.The seeds of guava fruits are very rich in orange-yellow colored aromatic oil (14%) that contains considerable amounts of iodine. Some of varieties produce fruits rich in potassium and carotene while others lack these useful constituents. Guava leaves and bark are used traditionally as a disinfectant and antiseptic for dressing wounds and sores. A decoction of Guava leaves is used for relieving tooth-ache and gum boils when used for gargling and also for Acute Diarrhea, Gastroenteritis, intestinal worm, dysmenorrhea, gastric disorders, Nausea and Vomiting, Vaginal discharges, Toothaches;Bad Breath; Bleeding gums; Mouth sores; sore throat and laryngitis, Cough, sore throat and laryngitis (**Evans *et. al*., 1986)**.

6. Indian Gooseberry

Kingdom	:	Plantae
Division	:	Flowering plant
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Phyllanthaceae
Tribe	:	Phyllantheae
Subtribe	:	Fluegginae
Genus	:	Phyllanthus
Species	:	P. emblica
Zoological name	:	Phyllanthus emblica

Also know as amalaki, dhatriphala in Sanskrit, Indian Gooseberry in English and *Emblica officinalis* scientifically, is the most widely used herb in the Ayurvedic system of medicine. It effectively controls digestive problems, strengthens heart, builds up and sustains defense mechanism, improves eye sight, imparts a natural glow to hair and body and a store house of Vitamin C, a powerful anti oxidant that prevents premature ageing. Amla fruit is acrid, cooling, refrigerant, diuretic, laxative, antipyretic, and aphrodisiac, tonic.It is the richest natural source of Vitamin C. In addition to Vitamin C, it also contains calcium, iron, protein and tannic acids, sugar, phosphorus, carbohydrates etc. The juice of fresh Amla fruit is given as tonic, diuretic and anti-bilious remedy. It is also helpful in burning sensation, over thirst, dyspepsia and other complaints of digestive system.The powder of the dried Amla fruit is an effective remedy of hyperacidity, ulcers and blood impurities. It is also used both internally and externally as a decoction and paste. Some of the common uses of Amla fruit are it strengthens the body, expel toxins from the body and improves defense mechanism of the body. Weakness of body, heart and mind are dispelled by taking fresh Amla juice in betwen meals. Massaging the head with amla oil induces sound sleep and is good for hair and prevents premature graying of hair. Its fruit is reputed to probably have the highest content of vitamin C compared with any other naturally occurring substances in nature. Active extracts of *P. emblica* have been shown to possess several pharmacological properties, e.g., analgesic, anti-inflammatory, antioxidant and chemoprotective activities. 2-4 drops of amla juice drops is inserted into each nostril to cure bleeding for nose. It helps in regulating blood sugar. It is very powerful, source anti-inflammatory herb, a wonderful antioxidant and a natural Source of Vitamin C. Amla helps scavenge free radicals. Amla is powerful food for the brain. Studies show that Amla helps lower cholesterol. Amla also helps maintain the functioning of the liver, increases hemoglobin, red blood cell count. It is useful for Cough, Bronchitis, and Asthma. Amla cleanses the mouth, strengthens the teeth. Its decoction is used in hyperacidity and with honey as an anti-helmintic. The presence of Amla results in an enhanced cell survival, decreased free radical production and higher antioxidant levels.There are various classic Ayurvedic preparations, such as Chyawanprash in which Amla is used as a chief ingredient. It help improve intelligence and memory power. Triphala and Brahmrasayana are other classic medicine in which Amla is being used since time immemorial (Figure 1).



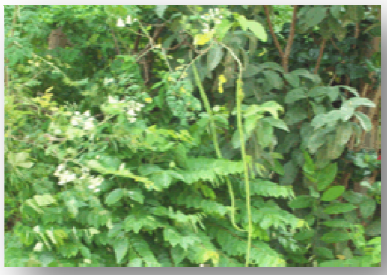


Figure 1: Samples taken for the Study

MATERIALS AND METHODOLOGY

The Antibacterial activity was carried out using Agar Diffusion Method followed by Phytochemical Analysis comprising of the following tests such as Tests for Tannins, Flavonoids, Quinone, Cellulose and Phenol. Since Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites, the following tests were carried out for the samples. The Plant samples were examined for Qualitative Analysis using Fourier Transform Infrared Spectrometer. FT-IR is most useful for identifying chemicals that are either organic or inorganic. The term Fourier Transform Infrared Spectroscopy (FT-IR) refers to a fairly recent development in the manner in which the data is collected and converted from an interference pattern to a spectrum. Today's FT-IR instruments are computerized which makes them faster and more sensitive than the older dispersive instruments. Our samples were analyzed using Perkin-Elmer Spectrum One FT-IR Spectrometer.

RESULTS AND DISCUSSION

In the above table the highest zone of inhibition was seen in the case of Aqueous extract of Drumstick leaves for which the zone of inhibition is 20mm against ***Vibrio cholera***. It is followed by Chloroform Extract of Drumstick leaves and pomegranate aqueous extract which gives 15mm (Table 1). Against ***Bacillus subtilis*** the highest zone of inhibition is observed in aqueous extraction and in Acetone Extraction (Table 2). Table 3 comprises of the highest zone inhibition observed in the case of Pomegranate Leaves of aqueous Extract about 16mm zone of inhibition against *Klebsiella pneumone. It is followed by* Drumstick leaves.

Table 1: Inhibition of Plant Extract on *Vibrio cholerae*

Sample used	Aqueous Extract(mm)	Chloroform Extract(mm)	Acetone Extract(mm)
Drumstick leaves	20	18	18
Pomegranate Leaves	15	14	13
Papaya Leaves	-	-	-
Guava Leaves	2	7	8
Cannon ball Leaves,	5	10	10
Gooseberry	-	-	-
Pomegranate stem	4	10	10

Table 2: Inhibition of Plant Extract on *Bacillus subtilis*

Sample used	Aqueous Extract (mm)	Chloroform Extract(mm)	Acetone Extract(mm)
Drumstick leaves	13	10	10
Pomegranate Leaves	5	6	8
Papaya Leaves	-	-	-
Guava Leaves	8	-	6
Cannon ball Leaves,	-	4	3
Gooseberry	5	6	7
Pomegranate stem	8	9	10

Table 3: Inhibition of Plant Extract on *Klebsiella pneumonia*

Sample used	Aqueous Extract(mm)	Chloroform Extract(mm)	Acetone Extract(mm)
Drumstick leaves	15	14	12
Pomegranate Leaves	16	15	13
Papaya Leaves	6	-	3
Guava Leaves	5	-	-
Cannon ball Leaves,	-	-	-
Gooseberry	6	-	10
Pomegranate stem	10	9	9

Zone of Inhibition Observed in Chromophenicol Disc Method

Here the Drumstick leaves of aqueous extract show maximum inhibition of about 20mm comparing with the chromophenicol disc. It is followed by Pomegranate (Table 4). Inhibition of Plant Extract on ***Bacillus subtilis*** revealed highest zone of inhibition for Drumstick leaves (Table 5). In Table 6 the highest zone of inhibition was observed in Pomegranate leaves followed by Drumstick leaves of Aqueous extract and chloroform extract of pomegranate leaves (Figures 6 – 10, Graphs 1 – 3).

Table.4: Inhibition of Plant Extract on *Vibrio cholerae* compared with the chromophenicol

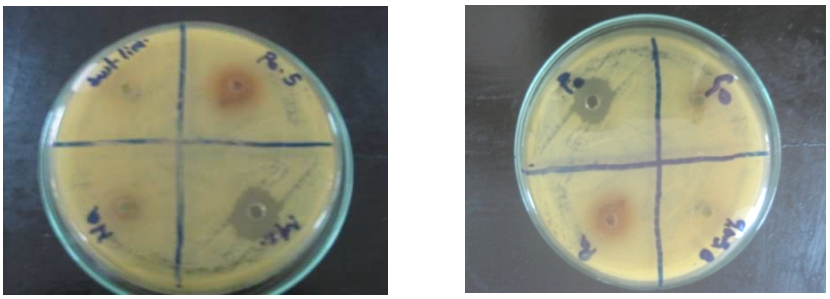
Sample used	Aqueous Extract(mm)		Chloroform Extract(mm)		Acetone Extract(mm)	
Drumstick leaves	20	14	18	14	18	14
Pomegranate Leaves	15	14	14	14	13	14
Papaya Leaves	-	14	-	14	-	14
Guava Leaves	2	14	7	14	8	14
Cannon ball Leaves,	5	14	10	14	10	14
Gooseberry	-	14	-	14	-	14
Pomegranate stem	4	14	10	14	10	14

Table.5: Inhibition of Plant Extract on *Bacillus subtilis* compared with the Chloramphenicol

Sample used	Aqueous Extract(mm)		Chloroform Extract(mm)		Acetone Extract(mm)	
Drumstick leaves	13	12	10	12	10	12
Pomegranate Leaves	5	12	6	12	8	12
Papaya Leaves	-	12	-	12	-	12
Guava Leaves	8	12	-	12	6	12
Cannon ball Leaves,	-	12	4	12	3	12
Gooseberry	5	12	6	12	7	12
Pomegranate stem	8	12	9	12	10	12

Table.6: Inhibition of Plant Extract on *Klebsiella pneumone* compared with Chloramphenicol

Sample used	Aqueous Extract(mm)		Chloroform Extract(mm)		Acetone Extract(mm)	
Drumstick leaves	15	14	14	14	12	14
Pomegranate Leaves	16	14	15	14	13	14
Papaya Leaves	6	14	-	14	3	14
Guava Leaves	5	14	-	14	-	14
Cannon ball Leaves,	-	14	-	14	-	14
Gooseberry	6	14	-	14	10	14
Pomegranate stem	10	14	9	14	9	14



A.Aqueous Extract **B.Aqueous Extract**

Figure.6: **Plates Showing Zone of Inhibition of Plant Extract against *Vibrio Cholorea***

Fig.7: Plates Showing Zone of Inhibition of Plant Extract against *Bacillus Subtillus.*

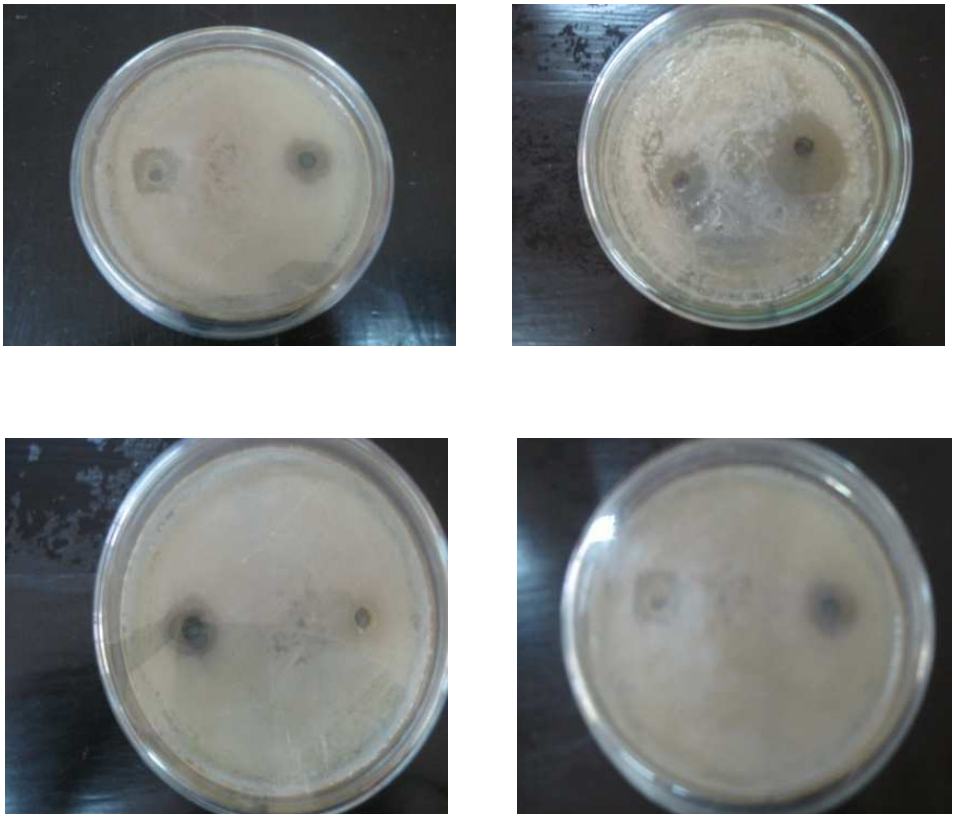


Fig.8: Plates Showing Zone of Inhibition of Plant Extract against *Klebsiella Pneumonia.*

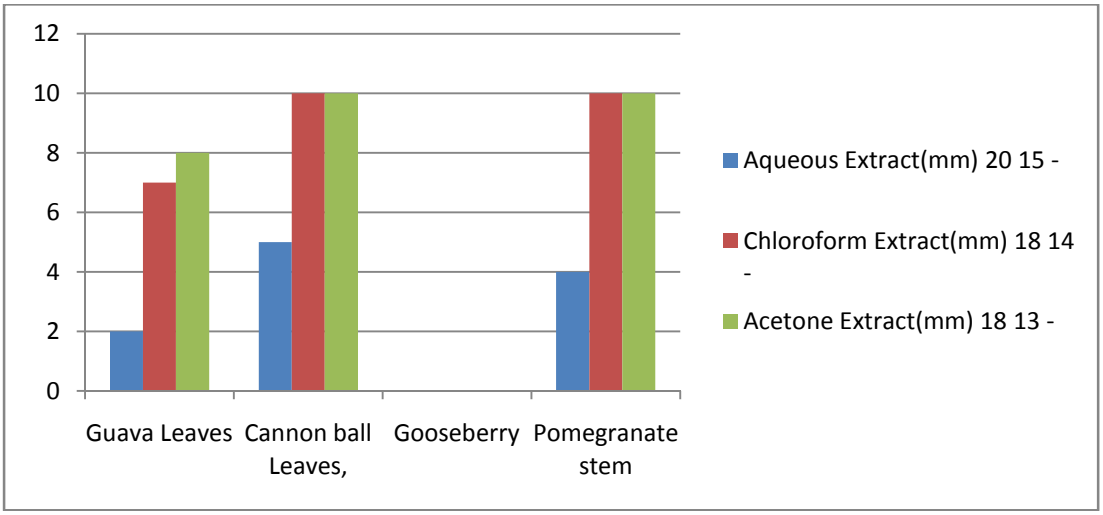
A. Aqueous Extract

B. Chloroform Extract

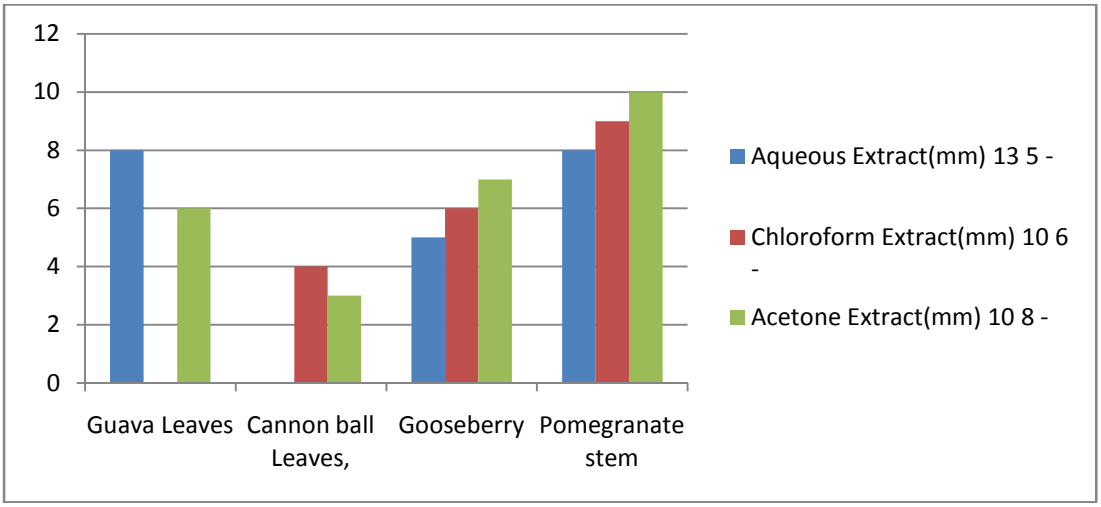
C. Acetone Extract

Figure.9: Plates Showing Zone of Inhibition of Plant Extract

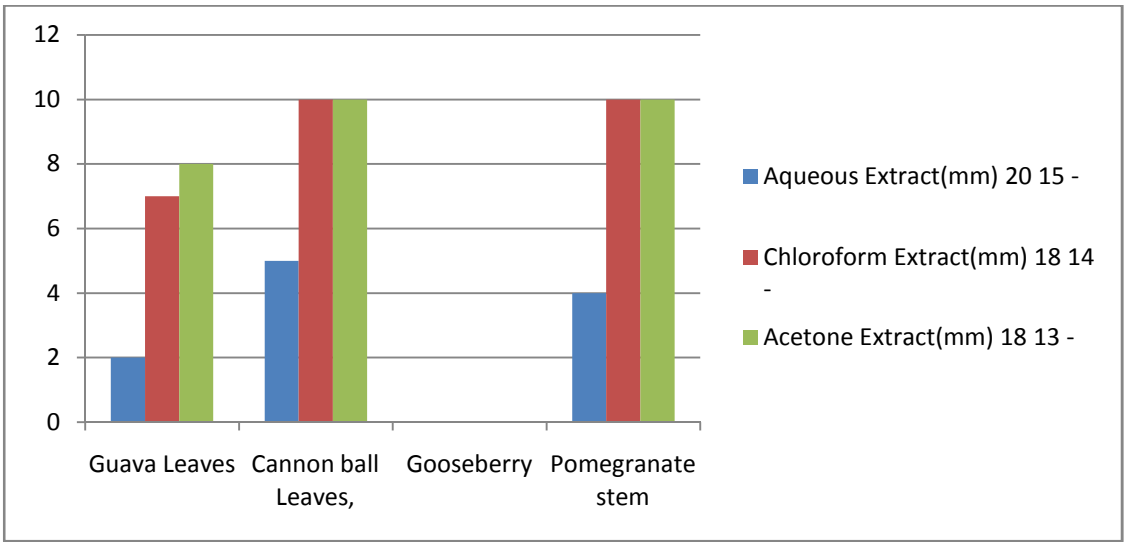
Figure.10: Control Plate of Disc Method for Studying Antimicrobial Activity of Plant Extract



Graph 1: Antimicrobial Activity of *Vibrio Cholorea*



Graph.2: Antimicrobial Activity of *Bacillus Subtillus*



Graph 3: Antimicrobial activity of *klebsiella pneumonia*.Phytochemical analysis

The Phytochemical Analysis reveals the presence of the below mentioned compounds (Table 7 & Figure 11).

Table 7: Phytochemical Result

Samples used	Phenol	Tannin	Flavanoids	Steroids	Quinone	Cellulose
Pomegranate	-	+	-	-	+	+
Guava	+	+	+	-	-	+
Cannon ball	+	-	-	-	+	+
Sweetlime	+	-	+	-	+	+
Drumstick	+	-	-	-	+	+
Gosberry	+	-	+	-	-	-
Papaya	-	-	-	-	+	+

PHENOL TANNIN FLAVANOIDS QUINONE CELLULOSE

Fig 11: Results of Phytochemical Analysis

IR Spectroscopy Studies

IR Spectroscopy studies reveal the intensity of the absorption of the infra red light by the samples taken for the present study (Figures 12 – 16).

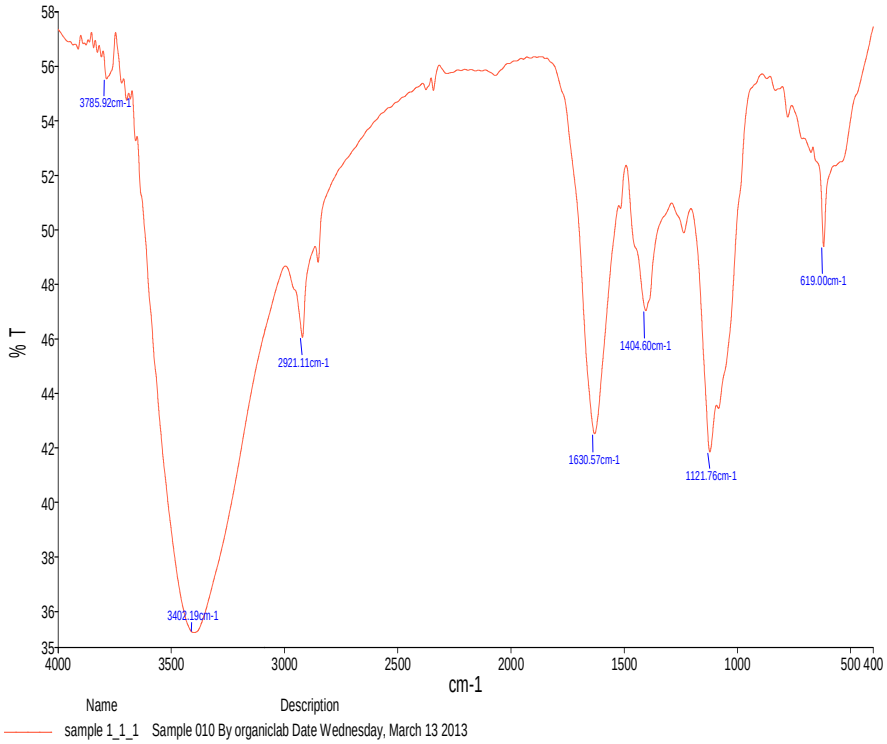


Figure.12: IR spectroscopy of Drumstick

Interpretation

The IR spectroscopy of Drumstick shows the range of
Phenols compound – O – H
Alkaline – CH3, CH2, CH
Amines – C – N

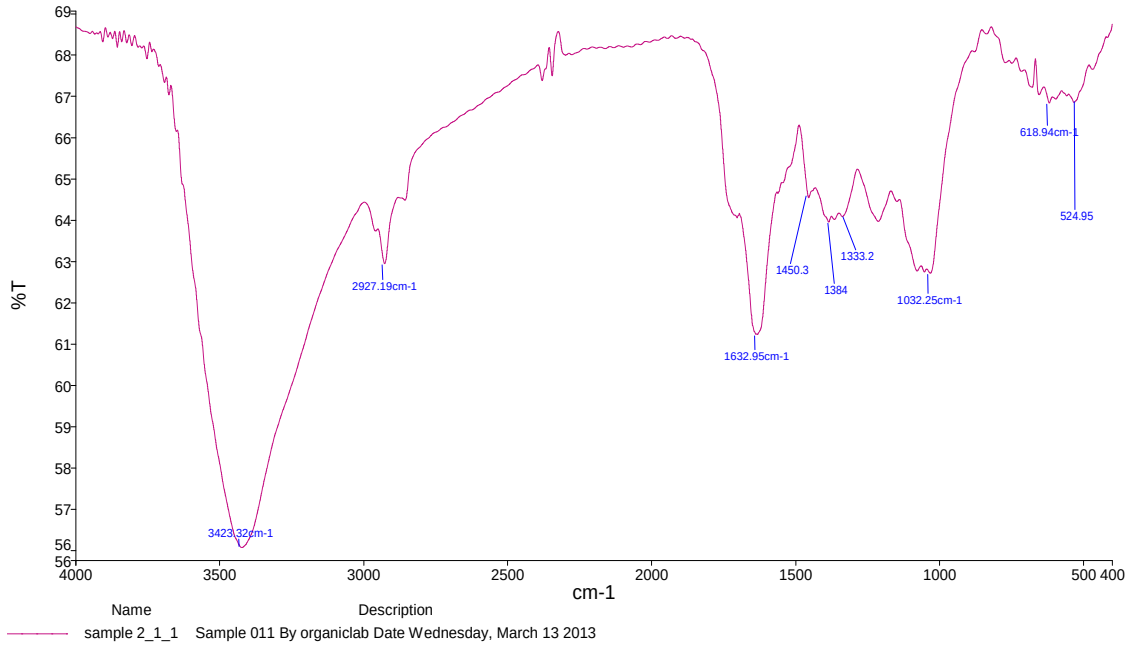


Figure.13: IR spectroscopy of Pomegranate

Interpretation

The IR spectroscopy of Pomegranate shows the range of
Phenols compound – N – H (1 amines), 2 bonds
Alkaline – CH3, CH2, CH
Amines – C - N

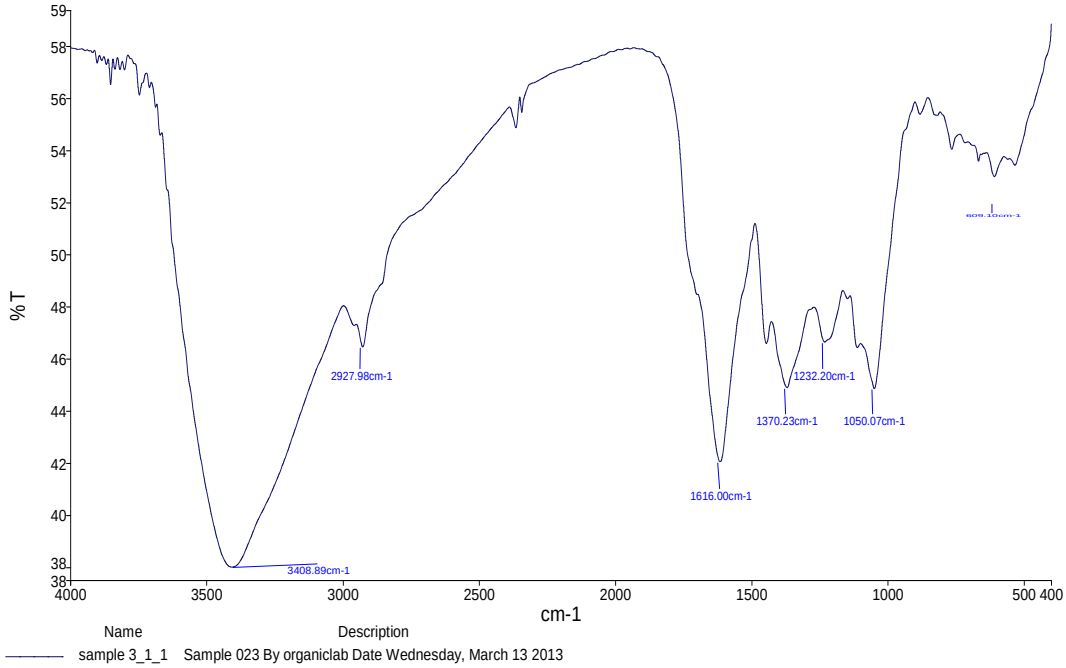


Figure.14: IR spectroscopy of Guava

Interpretation

The IR spectroscopy of Guava shows the range of
Phenols compound – O – H (H bond)
Alkaline – CH3, CH2, CH
Amines – N - H

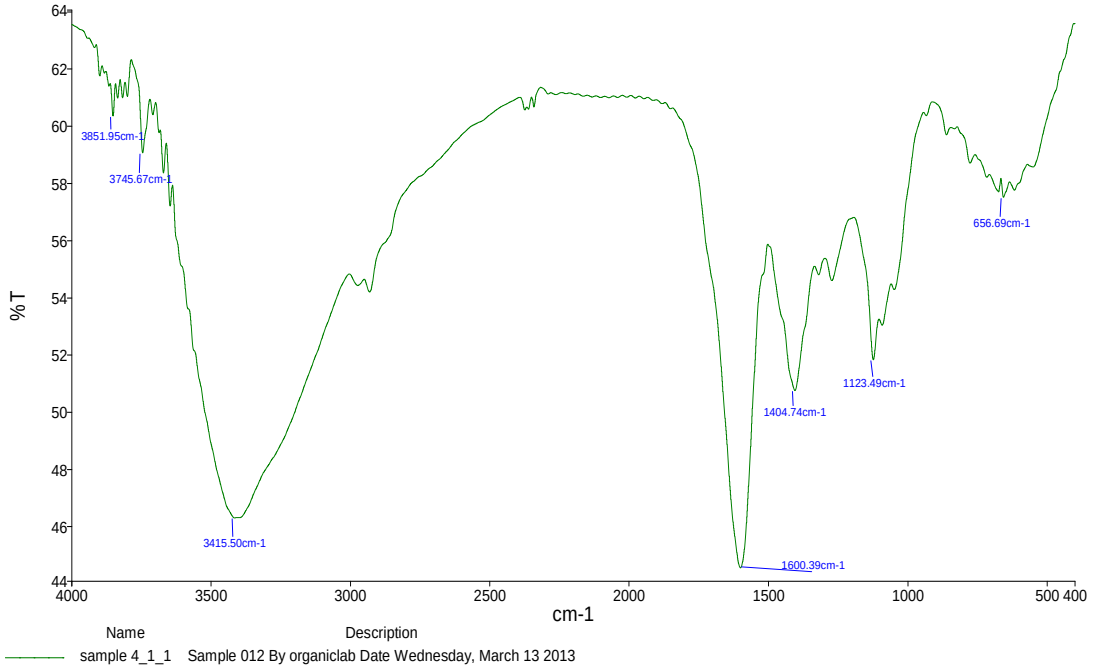


Figure.15: IR spectroscopy of Sweet Lime

Interpretation

The IR spectroscopy of Sweet lime shows the range of
Phenols compound – O - H
Alkaline – C - C
Amines – C - N

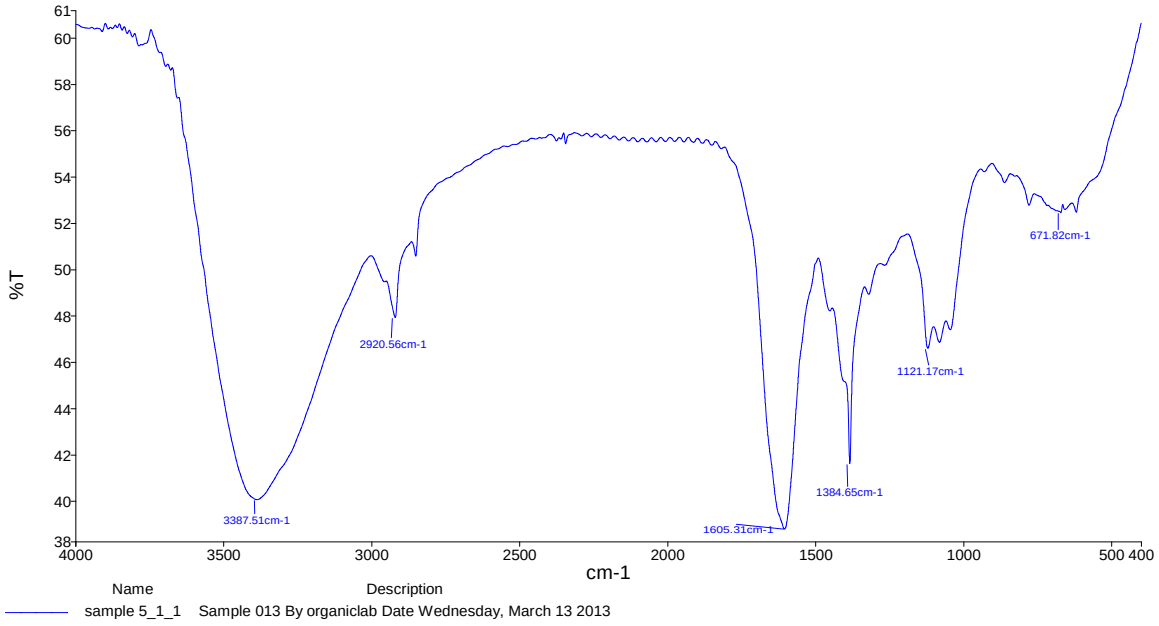


Figure.16: IR spectroscopy of Papaya Leaf

Interpretation

The IR spectroscopy of Papaya Leaf shows the range of
Phenols compound - 3387nm (3200-3550nm)
Alkaline - 2920nm (2850-3000nm)
Amines - 1121nm (1000-1250nm).

SUMMARY

The present study revealed that the selected plants did not only have a high antimicrobial activity but also good phytochemical property in the case of Drumstick Leaves and Pomegranate Leaves. The study also revealed the high antimicrobial activity of Plants against the selected microorganism which has a pathogenic manifestation causes in human as well as other animals and cattle. This can be cured easily by Herbal therapy. The present study clearly points out the antioxidant potential and antimicrobial potential of these plants thus emphasizing the importance in incorporating these plants as a regular component in the diet. These plants could also be exploited for commercial purification of specific antibiotics since they are available in abundance. "*You are what you Savour*" –this proverbial maxim forms the basis of 'phytotherapy' or treatment with plant extracts. The present study provides additional data for supporting the use of the experimented plants as natural antimicrobial agents. Popular knowledge of plants used by human is based on thousands of years of experience. By "trial and error", people learnt how to recognize and use plants, including those with a magic – religious function. Thus it can be concluded that Medicinal plants forms an indefinite base for healthy living and for the development of new and effective drugs.

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