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Study on phytochemical constituents and antibacterial activity of *Mimusops elengi* L.

S. Jamuna Devi*, L. Prince

PG and Research Department of Microbiology, Maruthupandiyar College, Thanjavur (Affiliated to Bharathidasan University, Tiruchirappalli), Tamilnadu, India

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Abstract

Mimusops elengi L. is an important aromatic herb of the family Sapotaceae which is routinely grown as a traditional medicinal herb in India. In this phytochemical compounds investigation terpenoids, steroids, flavonoids, tannins, phlobatannins and cardiac glycosides showed positive results and saponin showed negative results in aqueous extract. Phytochemical constituents were analysed by FT-IR Spectroscopic method. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. Soxhlet apparatus were used for extracting antibacterial active compounds from the plant leaves powered. The discs were prepared and immersed in various solvent extracts. Antibacterial activity was analysed against some clinical pathogens such as *Alcaligenes faecalis, Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes*. Chloroform and methanol extracts were most effective followed by other water extract. *Streptococcus pyogenes* and *Escherichia coli* were more sensitive for Chloroform and methanol extract of leaves of the tested plants. Aqueous extracts low inhibition against the tested organism compared to other test plant extracts.

* **Corresponding Author:** S. Jamuna Devi ✉ jamuna2803@gmail.com

Introduction

Health is the real wealth of nation. Nature has provided all necessary things for survival. Medicinal plants are nature's best gift to cure a number of diseases for men and women. These medicinal plants are most valuable natural resources. It is necessary to identify those medicinal plants. Rapid urbanization is resulting in the loss of many important medicinal plants. Nowadays using antibiotics to subside infection produces adverse toxicity to host organs tissue and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonize with herbs. Herbal molecules are sage will overcome the resistance produced by the pathogens since they are in combined form or in pooled form of more than one molecules in the protoplasm of the plant cell. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Diallo *et al.,* 1997).

Mimusops elengi L. is an important aromatic herb of the family Sapotaceae which is routinely grown as a traditional medicinal herb in India. Plant extracts of *Mimusops elengi* L. exhibited high amounts of polyphenols and higher antioxidant activity (Bharat and Parabia, 2010). The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhea and fever (Warrier *et al.,* 1995). The uses of medicinal plants in traditional medicine are widespread and still serve as leads for the development of novel pharmacological agents. Many such medicinal plants have hepatoprotective, neuroprotective, anti-inflammatory and also antioxidant or radical-scavenging properties (Perry *et al.,* 1999; Lin and Huang, 2000).

In Southern-Western Ghats of India (Tamil Nadu) an ethnobotanical survey was carried out to collect information on the use of medicinal plants. The aqueous extract of *Mimusops elengi* L. is used traditionally certain in India as remedy against cough, intestinal disorder and bacterial infection. Numbers of studies have been conducted in different countries to prove such efficiency. Therefore, I have chosen the study on phytochemical constituents and antibacterial activity of *Mimusops elengi* L. Thus the objective of this work was to evaluate the potential of plant extracts and phytochemical constituents on standard microorganism's strains.

Materials and methods

The medicinal plant *Mimusops elengi* L. (leaves) was collected from Perungalur in Pudukkottai Dist, Tamil Nadu, India. The plants were identified based on the morphological characteristics. The leaves were air-dried. After drying at 37°C for 24 hrs the plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) made for the laboratory. Exposure to direct sunlight was avoided to prevent the loss of active components. The powdered medicinal plant material was taken and subjected to successive solvent extraction. The extraction was carried out with the following solvents in the increasing order of polarity such aqueous, chloroform and methanol extracts. Twenty grams of powdered plant materials mixed with 100ml of various solvents (Distilled water, Chloroform and Methanol solution) (Alade and Irobi, 1993). The plant extracts were prepared by using soxhlet apparatus, collected and stored in a vial for further studies. Standard procedures were followed to identify the chemical constituents in the extract of leaves of *Mimusops elengi* L. as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Plant sample extraction 2 g of air dried powder of leaf sample was extracted with 50 ml of aqueous, chloroform and methanol with gentle stirring for 72 h respectively. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through Whatmann No. 1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4°C before use. The extracts were examined under visible

and UV light for proximate analysis. For UV-VIS and FT-IR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

FT-IR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.* (1966). Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile Petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each Petri plates and also placed control and standard (Ofloxacin) discs. The plates were incubated at 37°C for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm. The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar (1984).

Results and discussion

In the analysis of Tannin compounds brownish green colour developed to indicate the presence of tannin. Similarly, based on the presence or absence of colour change indicate positive and negative results. In this investigation terpenoids, steroids, flavonoids, tannins, phlobatannins and cardiac glycosides showed positive results and saponin showed negative results in aqueous extract (Table 1). Many of them are known to have different therapeutic values. Tannins possess antibacterial, antiviral, moluscicidal and antitumoral properties (Scalbert, 1991). While steroids, also present in *Mimusops elengi* L. (leaves) is recognized to have anticancer, antiviral and antihemorrhagic properties (Simoes *et al.,* 2002).

Table 1. Preliminary phytochemical study of *Mimusops elengi* L. (leaves)

Phytochemicals	Aqueous extract	Chloroform extract	Methanol extract
Cardiac glycosides	Positive	Positive	Positive
Terpenoids	Positive	Positive	Positive
Steroids	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive
Tannins	Positive	Positive	Positive
Phlobatannins	Positive	Positive	Positive
Saponins	Negative	Negative	Positive

+ indicates the presence and – indicates the absence of the chemical constituents.

Hamburger and Hostettmann, (1991) reported that the total number of plant chemicals may exceed 400,000 and out of it more than 10,000 are secondary metabolites whose major role in plant is defensive in nature. Thus, plant based secondary metabolites, which have defensive role may be exploited for the management of storage pest. However, the most species of higher plants have never been described surveyed. Their chemical or biologically active constituent which is potential to be used as new sources of commercially valuable pesticides remain to be discovered (Balandrin *et al.*, 1985). This is mainly due to the lack of information on the screening/evaluation of diverse plants for their antibacterial potential.

Phytochemical constituents were analysed by FT-IR Spectroscopic method. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. Aqueous extract FT-IR spectrum profile was illustrated in the Table 2, 3, and 4. The FT-IR spectrum confirmed the presence of Alcohols, Phenols, Alkanes, Primary amines, Aromatics, Aliphatic amines and Nitro compounds. Chloroform extract FT-IR spectrum confirmed the presence of Phenols, Primary amines, Aromatics, Aliphatic

amines, Alkanes and Aromatics. Methanol extract FT-IR spectrum confirmed the presence of Phenols, Primary amines, Aromatics, Amines Alkanes and Aliphatic amines.

Table 2. FT-IR peak values and functional groups of *Mimusops elengi* L. (leaves) aqueous extracts

Peak values	Functional groups
3950.22	Unknown
3873.06	Alcohols
3695.61, 3657.04	Phenols
3525.88, 3417.86, 3232.70	Alkanes
2947.23, 2831.50, 2708.06	Primary amines
2314.58, 2044.54	Aromatics
1759.08	Aliphatic amines
1489.05, 1319.31	Nitro compounds
1172.72, 1026.13, 748.38	Alkanes

Table 3. FT-IR peak values and functional groups of *Mimusops elengi* L. (leaves) chloroform extracts

Peak values	Functional groups
3896.21	Unknown
3695.61, 3657.04	Phenols
2970.38	Primary amines
2314.58, 2175.70	Aromatics
1859.38, 1813.09, 1759.08	Aliphatic amines
1504.48	Amines
1373.32, 1311.59, 1226.73, 1172.72	Alkanes
1095.57	Aromatics

Table 4. FT-IR peak values and functional groups of *Mimusops elengi* L. (leaves) methanol extracts

Peak values	Functional groups
3417.86, 3240.41	Phenols
2954.95, 2839.22, 2708.06	Primary amines
2306.86, 2175.70, 2044.54	Aromatics
1998.25, 1759.08, 1666.50	Aldehydes
1597.06	Amines
1496.76, 1381.03, 1311.59	Alkanes
1219.01, 1172.72, 1095.57	Alphatic amines

On the basis of the results obtained in this present investigation, it is concluded that the extract of *Mimusops elengi* L. leaves had significant *in vitro* antibacterial activity (Table 5).

The present study *Staphylococcus pyogenes* showed the most susceptibility to the extract. In contrast, *Alcaligenes faecalis* was the least susceptible bacterium. This may be due to the fact that *Staphylococcus pyogenes* has intrinsic resistance from a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (MDRs). The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Britto, 2001). In the present work chloroform and methanol extracts of *Plectranthus amboinicus* extract showed higher activity to the test bacteria such as *Staphylococcus pyogenes, Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus*. Aqueous extracts of low inhibition against the tested organisms compared to other test plant extracts. The results showed that the inhibition diameters ranging from 16 mm to 23 mm in diameter. The results were present in Plate-III, Table 2 and Fig. 1. The present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators (Mohanasundari *et al.,* 2007).

These results are consistent with others *in vitro* experimental findings. Oliveira *et al.* (2005) and Nogueira *et al.* (2008) have reported the antimicrobial effects of leaves from *Mimusops elengi* L. in the form of essential oil and crude extracts, respectively. Moreover, some studies demonstrated absence of toxicity *in vitro* and *in vivo* (Castillo and Gonzalez, 1999), which makes the use of this species very safe in the treatment of multi-resistant infections.

Traditional healers have long used plants to prevent or cure infectious conditions. Western medicine is trying to authenticate their efficacy. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Currently, one-quarter to one-half of all pharmaceuticals dispensed in the US are of higher-plant origin; very few are sourced for use as antimicrobials, since people have relied on bacterial and fungal sources for these activities. Since the advent of antibiotics in the 1950`s the use of plant

derivatives as antimicrobials has been virtually nonexistent (Cowan, 1999). Natural-product chemists and microbiologists alike feel that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Borris, 1996).

Similarly supported by (Annie Pritima and Selvaraj Pandian, 2008) has been studied the antimicrobial activity, against RTI causing microbes. *Candida krusei* showed the highest zone of inhibition of growth, followed by *Candida albicans, Proteus mirablis, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae* and the least inhibition was observed for *Neisseria gonohorreae*. *Coleus aromaticus* exhibits an effective antifungal and marked antibacterial activity.

The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Britto, 2001).

Table 2. Assay of antibacterial activity of *Mimusops elengi* L. (leaves)

Name of the organisms	Zone of inhibition (mm in diameter) (M±SD)				
	Standard Of*	Control	Aqueous extract	Chloroform extract	Methanol extract
Alcaligenes faecalis	25	-	-	8±0.38	8±0.54
Escherichia coli	11	-	-	8±0.29	14±0.66
Klebsiella pneumoniae	17	-	-	10±0.47	8±0.64
Staphylococcus aureus	25	-	-	10±0.64	9±0.48
Staphylococcus pyogenes	24	-	-	13±0.53	10±0.40

Of* - Ofloxacin (disc 20mg); M –Mean; SD – Standard deviation

Results of present research indicated were supported by the work done by various works. Alcoholic leaf extract was found to have antibacterial effect against the pathogen by (Gehlot and Bohra, 2000). Several workers have reported that many plants possess antimicrobial properties including the parts which include; flower, bark, stem, leaf, etc. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (Bushra Beegum and Ganga Devi, 2003).

Many substances may be antimicrobial, but only a few of them will be potential therapeutic agents for the simple reason that mammalian cells are more sensitive to chemical inhibition than microbial cells (Sivakumar and Alagesaboopathi, 2006). Moreover, emphasized the need for toxicity testing of drugs derived from medicinal plants because the crude products obtained from such cheaper sources are often associated with a large number of compounds that have discomforting abilities. Hence, the herbal drugs have to be subjected to extensive pharmacological, toxicological and clinical tests to confirm the prescribed status. Thus the ethnobotanical approach will be like a search for molecular diversity subjecting a wide variety of new molecules from plant sources and testing them with as many different tests as possible (Muhammad and Muhammad, 2005).

Conclusion

From this study, it is clear that *Mimusops elengi* L. (leaves) indeed exhibits an anti-bacterial activity. More research needs to be done to unravel the inhibitory effect of this plant. Since this herb had been used for ages traditionally and effectively, it is presumed that side effects should be less. Use of herbs by Indian (south) community is a well-known fact; there is a treasure of herbs that we use daily in our food or in other forms customarily, even without knowing their medicinal benefits. Such use of plant material has always been a tradition, mostly community based that is passed on from one generation to another. In general, lesser known or lesser used herbs and plant materials have to be researched further to study their medicinal properties

especially their antibiotic nature. This will enable the use of our own local, rich plant heritage as effective medicines with probably fewer side effects.

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