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# Evaluation of Antibacterial Potential of Leaf extracts of *Mimusops elengi*

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## Abstract

*Different solvent extracts of the leaves of Mimusops elengi were prepared and screened for their antibacterial activity against six different bacterial strains including both gram negative strains such as Escherichia coli (MTCC 40), Pseudomonas aeruginosa (MTCC 424), Proteus vulgaris (MTCC 426) and gram positive strains such as Streptococcus pneumoniae (MTCC 237) and Staphylococcus aureus (MTCC 87), Staphylococcus epidermidis (MTCC 2639). The extracts were evaluated at 200 and 300mg/ml by using cup-plate method. All the tested extracts exhibited significant antibacterial activity in a dose dependent manner. Among the various extracts of Mimusops elengi leaves, methanol extract exhibited high inhibitory zone followed by ethanol, chloroform and petroleum ether. Petroleum ether extract was found to be ineffective at 200 mg/ml against all the test organisms. Thus, this in vitro study supports its traditional application as a preventive remedy for the treatment microbial diseases.*

**Keywords:** Mimusops elengi, Leaves, Bacterial strains, Cup-plate method.

## Introduction

The practices of traditional medicine are based on hundreds of years of belief and observations and analysis, which helps in the development of modern medicine. Today, there is widespread interest in herbal drugs. This interest is primarily based upon the belief that herbal medicines are safe, inexpensive and have less adverse effects. The world health organization (WHO) estimates that two third of the world population still depends upon traditional medicines for the treatment of various types of diseases. One such important traditional medicinal plant is *Mimusops elengi* Linn. belongs to sapotaceae family popularly known as bakula or Spanish cherry or bullet wood and it is well known for its ayurvedic medicine. It is a small to large tree found all over India. It is also cultivated in gardens as an ornamental plant. It has been used in the indigenous system of medicine for the treatment and cure of various types of ailments. All parts of the plant have medicinal properties[1]. The bark is acrid and sweet; cooling, cardiotonic, alexipharmic, stomachic, anthelmintic, astringent; cures biliousness and diseases of the gum and teeth[2]. The flowers are sweet, acrid, oleagenous; cooling, astringent to the bowels; good for the teeth, causes flatulence. They are used as an expectorant; cures biliousness, liver complaints, diseases of the nose, headache. The smoke of the flower is used for treatment of asthma[3]. The seeds are used to fix loose teeth; as an errhine cures nasal congestion and also useful in case of headache[4]. The root is sweet and sour; aphrodisiac, diuretic, cardiotonic, stomachic, astringent to the bowels; good for gonorrhoea. The root is used as a gargle for strengthening the gums[1]. The fruits are sweet and sour, aphrodisiac, diuretic, astringent to the bowels, good in case of gonorrhoea. The pulp of the ripe fruit is sweetish and astringent and has been successfully used to cure chronic dysentery[1,5]. The leaves are traditionally used in treatment of different types of diseases such as fever, postural eruptions of skin, ulcer, headache, dental diseases, bacterial diseases[6]. The leaves were found to have antioxidant, cytotoxic, analgesic, wound healing and antipyretic activities[3,7].

## Material and Methods

**Collection and Authentification of the plant material:** Fresh leaves of *Mimusops elengi* L. (*Sapotaceae*) was collected from Berhampur (District of Odisha). The plant material was identified and authenticated by Dr. Sujata Mahapatra, HOD Department of Botany and Biotechnology, Khallikote (Auto) College, Berhampur, Odisha, India. Before use it the leaves were checked for contamination, sand and no microbial growth. The leaves were washed thoroughly two to three times with running tap water and once with sterile distilled water. The leaf material was then air dried under shade. After complete drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable airtight container for further use.

**Preparation of the extracts:** Extraction is the common process for separation of active constituents by the use of different solvents. First powdered material of the plant was subjected to successive soxhlet extraction with the help of petroleum ether. The marcs left after petroleum ether extraction were extracted with chloroform followed by methanol and ethanol until the solvent became colourless. The extracts obtained were further evaporated to dryness under vacuum and stored in the refrigerator for further use. All the extracts were encoded as: PELM - Petroleum ether extract of leaves of *Mimusops elengi*, CELM- Chloroform extract of leaves of *Mimusops elengi*, MELM - Methanol extract of leaves of *Mimusops elengi* and EELM - Ethanol extract of leaves of *Mimusops elengi*.

**Phytochemical tests:** The crude extracts were subjected to preliminary phytochemical screening. Then, the extracts were used for pharmacological screening[8] to find out their possible antibacterial potency.

**Antibacterial study:** The extracts obtained were screened *in vitro* for their antibacterial activity against both gram positive and gram negative bacterial strains by cup plate method using nutrient agar as the medium[9]. The bacterial strains were procured from the Microbial Type Culture Collection, Department of Microbiology, M. K. C. G. Medical College, Berhampur, Odisha, India. The bacterial strains used for the determination of antimicrobial activity are *Escherichia coli (MTCC 40)*, *Staphylococcus aureus (MTCC 87)*, *Staphylococcus epidermidis (MTCC 2639)*, *Pseudomonas aeruginosa (MTCC 424)*, *Streptococcus pneumoniae (MTCC 237)* and *Proteus vulgaris (MTCC 426)*. The solutions of the extracts were prepared at 200 and 300mg/ml in dimethyl sulfoxide (DMSO). After 24 hour of incubation at 37$^{o}$C, the zone of inhibition formed was measured in millimeter against the standard drug tetracycline and the data were presented in table 2.

## Results and Discussion

Plant products have been the part of phytomedicine since ancient times. They can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active constituents[10]. Basic knowledge about the chemical constituents of plants is highly essential because such information will be helpful for the synthesis of complex chemical substances. The preliminary qualitative phytochemical screening of the crude powder of the plant was done to assess the presence of bioactive components. The presence of alkaloids, carbohydrates, glycosides, tannins and phenolic compounds, steroids, saponins and flavonoids were determined and were included in table 1.

To test the antibacterial activity of the extracts different methods are available in the literature. One of the most commonly followed methods is cup-plate method. This method is based on the diffusion of the test compounds from a cavity through the solidified agar layer taken in petriplate, to such an extent that the growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing the test compound. Among the various extracts of *Mimusops elengi* leaves, methanol extract exhibited high inhibitory zone followed by ethanol, chloroform and petroleum ether. The extracts that showed the zone of inhibition more than or equal to 21mm, in between 15-20.9 mm and less than 15 mm were considered as "most effective", "moderately effective" and "mild effective" respectively. The methanol extract of *Mimusops elengi* was found to be most effective against *P. vulgaris*, *E. coli*, *S. aureus* and moderately effective against *P. aeruginosa* and *S. pneumoniae* at the concentration of 300mg/ml. At the concentration of 200mg/ml the methanol extract was found to be moderately effective against *P. vulgaris* and *E. coli* and mild effective against all other test organisms. At the concentration of 300mg/ml the ethanol extract was found to be moderately effective against *E. coli* and mild effective against all other test organisms whereas it was found to be mild effective at the concentration of 200mg/ml against the all test organisms. The chloroform extract exhibited moderate effect against *P. vulgaris*, *E. coli*, *S. aureus* and mild effect against other test organisms at 300mg/ml. Similarly at 200mg/ml the chloroform extract showed mild effect against the test organisms. In case of petroleum ether extract the test organisms were found to be ineffective at 200mg/ml and at 300mg/ml the extract exhibited mild effect against the test organisms, shown in table 2.

**Figure-1**
**Photograph showing extraction of leaves of *Mimusops elengi* using soxhlet apparatus**

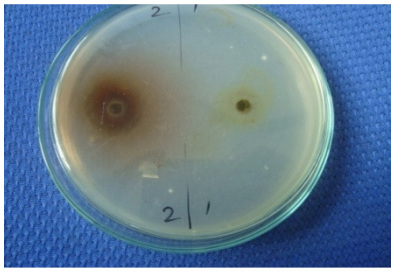

**Figure-2**
**Photograph showing the zone of inhibition of methanol extract of leaves of *Mimusops elengi* at 200mg/ml (1) and 300mg/ml (2) against *P. aeruginosa***

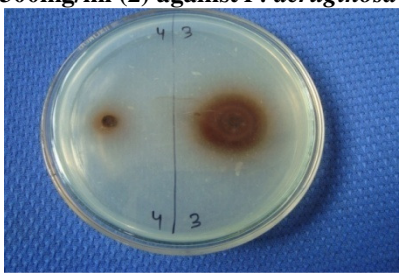

**Figure-3**
**Photograph showing the zone of inhibition of methanol extract of leaves of *Mimusops elengi* at 200mg/ml (4) and 300mg/ml (3) against *S. aureus***

**Table-1**
**Preliminary phytochemical screening of different extracts of the leaves of *Mimusops elengi***

| Test | PELM | MELM | EELM | CELM |
|---|---|---|---|---|
| **Test for Alkaloids** | | | | |
| a) Dragendroff's test | + | + | + | + |
| b) Mayer's test | + | + | + | + |
| c) Hager's test | + | + | + | + |
| d) Wagner's test | + | + | + | + |
| **Test for Carbohydrates** | | | | |
| a) Molisch's test | + | + | + | + |
| b) Iodine test | - | + | - | - |
| c) Fehling's test | + | + | + | + |
| d) Benedict's test | + | + | + | + |
| e) Barfoed's test | - | - | - | + |
| f) Selwinoff's test | - | + | + | - |
| **Test for Glycosides** | | | | |
| a) Legal's test | + | + | + | + |
| b) Baljet's test | + | + | - | + |
| c) Keller Killiani test | - | + | - | - |
| d) Libermann's test | - | - | + | + |
| **Test for Proteins** | | | | |
| a) Biuret test | - | - | - | - |
| b) Ninhydrin test | - | - | - | - |
| c) Millon's test | - | - | - | - |
| d) Xanthoprotein test | - | - | - | - |
| e) Precipitation test | - | - | - | - |
| **Test for Tannins and Phenolic compounds** | | | | |
| a) Ferric chloride | + | + | - | + |
| b) Lead acetate | - | - | + | + |
| c) Gelatin | - | + | + | + |
| d) Bromine water | - | + | + | + |
| e) Acetic acid | + | + | + | - |
| f) $KMnO_4$ | + | + | + | - |
| **Test for Steroids and Sterols** | | | | |
| a) Salkowski Reaction | - | + | + | - |
| b) Libermann-Burchard Reaction | + | + | - | - |
| c) Libermann reaction | + | + | + | + |
| **Test for Saponins** | | | | |
| a) Foam test | + | + | + | - |
| b) Haemolytic test | - | + | - | - |
| **Test for Flavonoids** | | | | |
| a) Shinoda test | + | + | - | - |
| b) Lead acetate test | - | + | + | + |
| c) Sodium hydroxide test | + | + | - | + |
| d) Ferric chloride test | - | + | + | - |

**Table-2**
**Antibacterial activity of solvents extracts of leaves of *M. elengi***

| Types of extract | Conc (mg/ml) | *Diameter of zone of inhibition (mm)* | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***P. vulgaris*** | ***P. aeruginosa*** | ***E. coli*** | ***S. aureus*** | ***S. epidermidis*** | ***S. pneumoniae*** |
| PELM | 200 | - | - | - | - | - | - |
| | 300 | 9.33 ± 0.57 | 12.00 ± 1.00 | 11.00 ± 1.00 | 9.66 ± 0.57 | 07.66 ± 0.57 | 09.33 ± 1.52 |
| MELM | 200 | 17.00 ± 1.00 | 14.00 ± 1.00 | 17.00 ± 1.00 | 11.66 ± 1.15 | 09.33 ± 0.57 | 13.66 ± 1.52 |
| | 300 | 21.00 ± 1.00 | 18.66 ± 1.52 | 28.33 ± 1.52 | 24.66 ± 1.15 | 14.33 ± 1.52 | 20.66 ± 0.57 |
| EELM | 200 | 13.33 ± 0.57 | 15.00 ± 1.00 | 13.00 ± 1.00 | 13.33 ± 0.57 | 11.66 ± 0.57 | 12.33 ± 0.57 |
| | 300 | 20.00 ± 1.00 | 17.00 ± 1.00 | 24.00 ± 2.00 | 18.33 ± 1.52 | 16.00 ± 1.00 | 15.33 ± 1.52 |
| CELM | 200 | 12.33 ± 0.57 | 09.66 ± 0.57 | 13.33 ± 0.57 | 11.66 ± 1.15 | 09.33 ± 0.57 | 11.66 ± 0.57 |
| | 300 | 17.00 ± 1.00 | 14.00 ± 1.00 | 17.00 ± 1.00 | 16.33 ± 0.57 | 14.66 ± 0.57 | 13.33 ± 1.00 |
| Control | | - | - | - | | - | - |
| Tetracycline(25mg) | | 28.33 ± 0.57 | 24.66 ± 0.57 | 31.66 ± 0.57 | 26.66 ± 1.52 | 23.00 ± 1.00 | 23.33 ± 0.57 |

Results were expressed as Mean ± S.D. (n = 3), "-" indicates no zone of inhibition.

## Conclusion

*Mimusops elengi* is a valuable plant source for traditional drug preparations. The results obtained from this study showed that the different solvent extracts of the leaves of *Mimusops elengi* was found to be effective against both gram positive and gram negative bacterial strains. Among these the methanol extract was more effective against the test organisms, may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids etc. This study may be a lead for further ethnopharmacognostic investigation to identify new antibacterial compounds with therapeutic benefits.

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