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Investigating The Antibacterial Activity And Phytochemical Screening Using Methanolic Leaf Extract Of Mimusops Elengi

Dr. M.Nithiya soundari[1] and M.Arputha Bibiana[2]

PG Department of Microbiology, New Prince Shri Bhavani Arts and Science College, Medavakkam, India.

Abstract

The plant Mimusops elengi commonly known as 'Spanish cherry' is found mostly in South Asia. The stem, barks, leaves and fruits of the plant are used in the preparation of various Ayurvedic medications to treat some ailments. The present study involves the preparation of methanolic extract using the M. elengi leaves by soxhlet extraction method. The crude extracts were further subjected to preliminary phytochemical testing which reveals that the methanolic extract contains flavonoids,saponins,alkaloids,carbohydrates,fats, fatty acids and sterols. Methanolic leaf extract using Mimusops elengi were prepared and screened for their antibacterial activity against four different clinical samples including E.coli, Pseudomonas sps, Vibrio sps and Salmonella sps. The minimum inhibitory concentration was done for the methanolic extract against the selected bacterial strains. The zone of inhibition was measured and tabulated. Among these four strains, the higher inhibitory activity was shown in E.coli, Salmonella sps and Vibrio sps then the Pseudomonas sps. In this in vitro study revealed that the plant mediated drug formulation may produce a higher activity in response to treat various diseases.

Key Words: Antibacterial activity, phytochemical screening, well diffusion method and methanolic extract

INTRODUCTION

Plants are considered as chemical factories which biosynthesize a variety of chemical compounds such as alkaloids, glycosides, saponins, resins, lactones and oils which act on human body in different ways. The biological properties of the medicinal plants are due to presence of specific phytochemicals synthesized in different parts. These phytochemicals can be valuable in maintenance of health in humans and other animals. The promising results from researches on various properties of the medicinal plants have forced scientists to search for plant derived drugs for treatment of different diseases. Therefore, there has been growing interest among scientists to isolate and study the pharmacological properties of the phytochemicals. Herbal medicines are employed to cure a wide variety of health related problems ranging from treatment of common colds to treatment of cancer.

Medicinal plants are used world-wide in the treatment of many diseases. They predate the development of many modern medications as well. One such medicinal plant is Mimusops elengi (belongs to the family Sapotaceae) which is commonly found in the southern and the eastern countries of Asia. Almost all parts of the plant have some medicinal properties to offer [1]. The plant is commonly called as 'Spanish Cherry' or 'Bullet Wood' but in India it is very popularly known as 'Maulsari' or 'Bakul' [2].

Several natural plant-based products are widely used for diverse therapeutic applications owing to their safe and potential medicinal properties [3, 4]. Recently available studies offer essential data that herbal products may comprise akin antimicrobial potential to reputable chemotherapeutics. The World Health Organization guidelines define herbal medicines as finished, labeled medicinal products containing an active ingredient, i.e., obtained from the aerial or underground parts of botanicals or other plant materials or their combination [5]. Specifically, since ancient days, Mimusops elengi Lin, a wild plant distributed in tropical and subtropical regions belonging to the family Sapotaceae, has been known for its myriad of medicinal values [6].

In Ayurveda, M. elengi has been reported to be used for arresting bleeding gums [7]. The use of unripe fruit and seed for fixing loose teeth is documented [8]. Herbal mouth rinse derived from M. elengi bark aqueous extract acts as a potent plaque inhibitor and anti-inflammatory agent in gingivitis [9]. Chloroform extract of M. elengi bark exhibited prominent anti-bacterial activity in dental patients by the ditch plate technique [10]. Whereas, ethanolic extracts of bark, leaves and seeds M. elengi are reported to be anti-bacterial agents against some pathogens [11].

The leaf is enriched with flavonoids, phenolics, sterols and carbohydrates. The plant possesses anti diuretic and astringent properties [12]. The various parts of the plant are also used to strengthen the teeth and deal with various gum related problems. The bark confers wound washing properties and a solution containing bark extract is used to heal sore eyes. Plant extracts can be used to treat gastric problems as well. Extract of flowers are used against heart diseases and act as anti diuretic in polyuria and antitoxin [13]. The snuff made from the dried and powdered flowers used in a disease called Ahwa, which is seen in Bengal, in which strong fever, headache and pain in the neck, shoulders and other parts of the body occurs [14]. The objective of the present study is to optimize and determine the antibacterial against selected bacterial strains and phytochemical properties of methanolic extract of the leaves of Mimusops elengi.

MATERIALS AND METHODS

A. Preparation of plant material

The fresh Mimusops elengi leaves were collected from Adambakkam, Chennai and India. The leaves were dried for one week at sunlight. Then the leaves were powdered and stored in a sterile plastic bag for further study.

B. Preparation of methanolic Extract

The processed leaf powder was weighed about 25 gms and it was packed in the soxhlet apparatus and then extracted with methanol. The aliquots of extracts were collected and passed through Whatmann No.1 filter paper. Then the solvent was completely evaporated into dryness at 60°C by vacuum distillation. The crude extract was subjected to various phytochemical analysis and antibacterial activity.

C. Fluorescence Analysis

The fluorescence properties of the powdered sample was carried out by using various reagents and under long UV (365nm) and short UV (254nm) [15].

D. Preliminary Phytochemical screening

Crude extracts of M. elengi was dissolved in distilled water (ethanol and aqueous) whereas petroleum ether and ethyl acetate extracts were dissolved in respective solvents itself and subjected to the following phytochemical tests [15-17].

Test for Phenolics and Tannins Ferric Chloride Test

To 2ml of extract solution, few drops of neutral FeCl3 solution were added. Formation of dark green colour indicates the presence of phenolics.

Lead Acetate test

To 2ml of extract solution few drops of 10% of lead acetate were added. White precipitate appeared which confirms the presence of phenolic compounds.

Test for Flavonoids (Alkaline reagent test)

To 2ml of extract solution few drops of 10% NH4OH was added. Formation of yellow fluorescence indicates the presence of flavonoids.

Shinoda test (Magnesium Hydrochloride reduction test)

Extract (10mg) was dissolved in 5ml of alcohol. To this solution, magnesium ribbon and concentrated hydrochloric acid were added. The mixture is heated in a water bath. Magenta colour appeared which proves the presence of flavonoids.

Test for Carbohydrates

Molische's test

2 drops of alcoholic solution of 1-naphthol was added to 2 ml of extract solution. Conc.H_2S0_4 (1ml) was added slowly along the sides of the test tube and allowed it to stand for a few seconds. Formation of violet ring indicates the presence of carbohydrates.

Fehling's Test

Few drops of Fehling solution A and Fehling solution B was added to 2 ml of extract solution and boiled the mixture for 5 minutes. Appearance of a red precipitate indicates the presence of carbohydrates.

Benedict's test

Few drops of Benedict's reagent was added to 2 ml of extract solution. The mixture was boiled for 2 minutes. A brownish red color precipitate appeared which confirms the presence of carbohydrates.

Test for Sterols

Salkowski test

About 2 ml of extract solution was mixed with 2ml of $CHCl_3$ and 3 ml of conc.H_2S0_4. A reddish brown coloration of the interphase indicates the presence of sterol.

Libermann Burchards test

Extract (5mg) was dissolved in a minimum of chloroform; to this 2ml acetic anhydride and two drops of concentrated sulphuric acid was added. Array of colours indicates the presence of phytosterols. Detection of proteins and amino acids

Biuret test

To the extract of M. elengi copper sulphate solution (2%) was mixed. To the above solution 1 ml of ethanol and excess of potassium hydroxide pellets were added. Formation of pink color indicates the presence of proteins.

Test for saponin

Foam test

The extract solution was shaken in a graduated cylinder for 10 minutes. Layer of foam appeared which indicates the presence of saponins.

Detection of Alkaloids

Mayer's test

Alkaloids will give a cream colored precipitate with Mayer's reagent (Potassium mercuric iodide solution). No characteristic change observed, which confirms the absence of alkaloids in the M. elengi extracts.

Detection of Fats and Oils

Saponification test

Alcoholic KOH solution (0.5 N) was mixed with constant stirring to the extracts and a drop of phenolphthalein was added. Formation of soap indicates the presence of fixed oils and fats.

ANTIBACTERIAL ACTIVITY

The Antibacterial activities of methanolic leaf extract was determined by well diffusion method using Muller Hinton Agar .The bacterial strains selected for the present study were procured from the clinical laboratory. The bacterial strains used for the determination of antimicrobial activity are Escherichia coli, Salmonella Sps, Pseudomonas Sps and Vibrio Sps. The solutions of the extracts were prepared at 200 and 300mg/ml in dimethyl sulfoxide (DMSO). Various concentrations of methanolic extracts (10 µl, 20µl, 30 µl and 40 µl) were taken and placed in to the well. After 24 hour of incubation at 37°C, the zone of inhibition formed was measured in millimeter [18].

RESULT AND DISCUSSION

Phytochemical Screening

The ethyl acetate, hexane, methanol, and ethanol M. elengi extracts had antibacterial activity against the dental caries causing bacteria Streptococcus mutans isolated from the patients [19]. The acetone, petroleum ether, methanol and water extracts of M. elengi bark have been tested for their antibacterial activity against dental infection causing bacteria: Staph. aureus, Str. mutans, Str. salivarius, Str. sanguinis, and fungus (Candida albicans) by well diffusion method [20,21]. M. elengi leaf extracts had antibacterial activity against Bacillus subtilis and Trichoderma viride [22]. In the current study,the preliminary qualitative phytochemical screening of the methanolic leaf extract of M. elengi 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 and figure 1&2.

Table 1 : Phytochemical Screening of Methanolic Leaf Extract

S.NO	TESTS	RESULT
Test for Alkaloids	Mayer's test	+
Test for Carbohydrates	Molisch's test	+
	Fehling's test	+
	Benedict's test	+

Test for Tannins and Phenolic compounds	Ferric chloride	+
	Lead acetate	+
Test for Saponins	Foam test	+
Test for Flavonoids	Shinoda test	+
	Sodium hydroxide test	+
	Ferric chloride test	+
Test for Steroids and Sterols	Libermann-Burchard Reaction	+
Test for Proteins	Biuret test	+
Test for Glycosides	Libermann's test	+
Test for Fats and Oils	Saponification Test	+

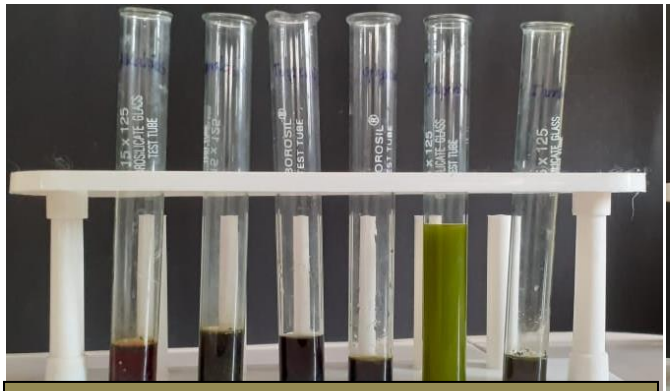

Figure 1: Phytochemical Screening of Methanolic Leaf Extract

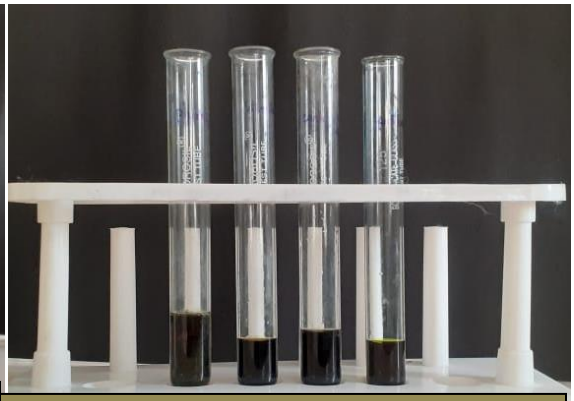

Figure 2: Phytochemical Screening of MLE

Antibacterial Activity

The results indicate that the extracts of M. elengi have antibacterial potential and can be used in the treatment of infectious diseases caused by resistant microorganisms. The extracts of different parts of M. elengi inhibited the growth of microorganisms with various degrees. The bark extracts showed significant antibacterial activities in similar fashion while the seed and fruit extracts were almost inactive against all tested organisms. The minimum inhibitory concentration (MIC) of all active extracts of M. elengi was calculated using macro dilution method. The ethyl acetate extract showed the highest percentage age inhibition (74.5 % age, MIC = 0.6 mg/ml) against B. subtilis. The aqueous methanol (2:8) extract also showed

significant results with 84.9 age inhibition (MIC = 0.9 mg/ml) against N. asteroids, comparable to standard antibacterial drugs (streptomycin and ampicillin).

The results also confirmed that the gram-positive bacterial strains were more susceptible to the plants extracts as compared to gram negative bacteria. This is in agreement with the fact that grampositive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier [23]. Antibacterial activity of the seed extract of M. elengi has also been reported recently [24]. In the present study, the methanolic leaf extract was showed higher degree of inhibitory against E.coli, Vibrio sps and Salmonella sps. Whereas the little inhibitory effect was showed against Pseudomonas sps. The antibacterial activity of methanolic leaf extract against various clinical samples with different concentrations were included in the Table.2.

Table.2 : Zone of Inhibition against Methanolic Leaf Extract (mm)				
Clinical Samples	**10µl**	**20 µl**	**30 µl**	**40 µl**
E.coli	7	10	13	16
Vibrio sps	4	7	10	13
Pseudomonas sps	2	4	5	6
Salmonella sps	5	8	11	14

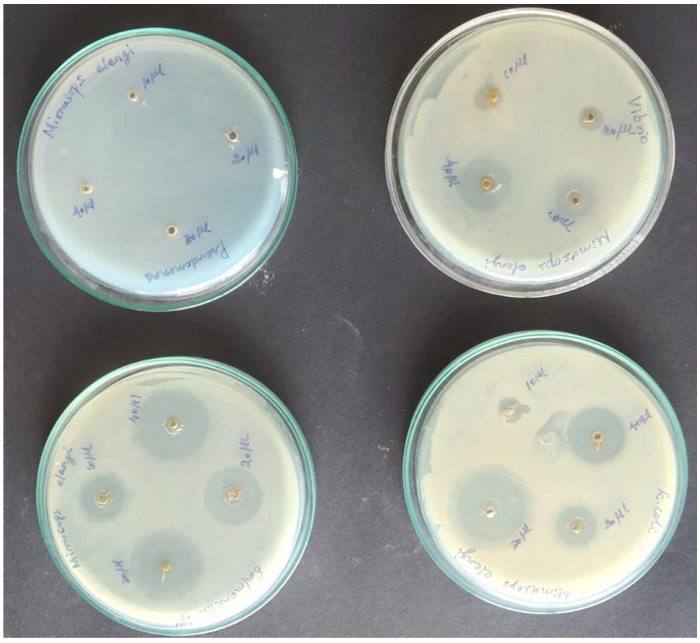

Figure 3: Antibacterial activity of methanolic leaf extract of M. elengi

CONCLUSION

Mimusops elengi is a valuable plant source for traditional drug preparations. The results obtained from this study showed that the methanolic leaf extract of Mimusops elengi was found to be effective against selected clinical samples. 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. M. elengi (Bakul) is one of the most important medicinal plants used in preparations of Ayurveda because of having a number of medicinal properties. It is the source of a variety of biologically active phytoconstituents which are responsible for antimicrobial, antioxidant, anti hyperglycemic, anticancer and protective effects on various vital organs such as nerves, heart, kidney and liver. The present study may be a lead for further ethnopharmacognostic investigation to identify new antibacterial drugs with more therapeutic benefits.

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