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IN VITRO EVALUATION OF *MIMUSOPS ELENGI* L. PLANT EXTRACT FOR ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS

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ABSTRACT

In vitro evaluation of antibacterial activity of aqueous and solvent extract of *Mimusops elengi* L.(*Sapotaceae*) was investigated against five pathogenic bacteria viz., *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi , Vibrio cholera* and *Streptococcus pneumonia* at 10,20,30,40 and 50 µl concentration. Among the five pathogen tested, *Streptococcus pneumonia* and *E.coli* showed a maximum inhibition of 26.9 mm and 24.4 mm at 50µl concentration compared to standard antibiotics Gentamicin, Tetracycline and Streptomycin. In solvent extract at 10 to 50µl concentration, methanol and ethanol recorded a maximum inhibition of 32.2 mm and 31.3 mm against *Streptococcus pneumonia* and *E.coli.* Phytochemical analysis of *M. elengi* revealed the presence of Tanins, Alkaloids, Saponins, Cardiac glycosides, Steroids, Flavonoids and Reducing sugar.

Keywords: *Mimusops elengi*, Phytochemical, Antibacterial, Aqueous extract, Solvent extract.

INTRODUCTION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value (Adegoke *et al.*, 2009). Plants are used in modern medicine where they occupy a very significant place as raw material for important drugs (Audu *et al.*, 2007). Plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. Extreme interest in plants with

microbicidal activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms (Nagendra *et al*., 2010). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Plants specifically herbal medicines have received much attention as source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and for environment (Abdul *et al*., 2010.) Plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances; most drugs of plant origin used by medical practitioners are in the form of extract of the whole plant material or part of it. In view of this, local medicinal plants, which show suitable biological effect, could be standardized and similarly utilized. Some of the effects elaborated by extract of plants used in traditional medicine include antiviral, antitumor, antimicrobial, insecticide and central nervous system effect (Audu *et al*., 2007). The use of phytochemicals as natural antimicrobial agents commonly called "biocides" is gaining popularity. There is growing interest in correlating phytochemicals constituents of plant with its pharmacological activity. There has been growing interest in the investigation of the natural products from plants for the discovery of new antibacterial agent. It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents (Nagendra *et al*., 2010). In the present study, aqueous and solvent extracts of leaf of *M. elengi*, belongs to family *Sapotaceae* were evaluated phytochemically and tested against five different human pathogens to identify the potentiality of antibacterial activity.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Mimusops elengi* L. (*Sapotaceae*) free from diseases were collected from Mysore. The leaves were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, leaf material was then air dried on a sterile blotter under shade and used for extraction.

Phytochemical Analysis

Tannins

200 mg of leaves of *M.elengi* was macerated in 10ml of distilled water. 2 ml of filtrate was treated with 2 ml of $FeCl_3$ (Ayoolla *et al*., 2008).

Alkaloids

200 mg of leaves of *M. elengi* was macerated in 10ml of methanol and filtered. 2 ml of filtrate was treated with 1% HCl and steam, filtered. To 1ml of obtained filtrate add 6 drops of Mayor‟s or Wagner‟s reagent and dragendroff reagent (Ayoolla *et al*., 2008).

Saponins

Exactly 0.5 ml of filtrate was added to 5 ml of distilled water and warm (Frothing test) (Ayoolla *et al*., 2008).

Cardiac glycosides

2 ml of filtrate was added to 1 ml of glacial acetic acid, 1 ml of $FeCl_3$ and 1 ml of concentrated H_2SO_4 (Liebermann-Burchard reaction) (Ayoolla *et al*., 2008).

Flavonoids

200 mg of leaves of *M. elengi* was macerated in 10 ml of ethanol, filtered. 2 ml of filtrate was treated with 2 ml of conc. HCl and 2 ml of magnesium (Ayoolla *et al*., 2008).

Reducing sugar

0.5 ml of extract was added to 1ml of water and 5-8 drops of hot Fehling‟s solution (Ayoolla *et al*., 2008).

Extraction

Aqueous extraction

50 grams of thoroughly washed leaves of *M. elengi* were macerated with 50 ml of sterile distilled water in a waring blender (waring International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 gram for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120^{o}C for 30 minutes. The extract was preserved aseptically in a brown bottle at 5^{o} C until further use (Gupta *et al.*, 1996; Pinto *et al.*, 1998).

Solvent extraction

Thoroughly washed leaves of *M. elengi* were dried in shade for five days and then powdered with the help of Waring blender. 25 grams of shade dried powder was filled in the thimble and extracted successively with petroleum ether, toluene, chloroform, methanol and ethanol in a Soxhlet extractor for 48 hours. Solvent extracts were concentrated under reduced pressure. After complete evaporation, 1gram of each concentrated solvent extracts were dissolved in 9 ml of methanol and used for antibacterial assay.

Test Pathogens

In vitro antibacterial activity was examined for aqueous and solvent extracts. Four Gram negative human bacteria viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholera*. One Gram positive bacteria *Streptococcus pneumonia* was obtained from Kumar hospital, Bangalore. All Gram negative bacteria were grown in Mac Conkey agar medium and Gram positive bacteria in blood agar medium and maintained at 4^{o}C until further use.

Antibacterial Assay

*Preparation of standard culture inoculums of test organism***:** Three or four isolated colonies of all the test Gram negative bacterial species were inoculated in the 2 ml Mac Conkey broth and Gram positive bacteria to nutrient broth and incubated at 37^{o}C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Aqueous Extract

Agar cup diffusion method

Agar cup diffusion method described by Joshi *et al*., 2009 was employed. An overnight culture of *E. coli, P. aeruginosa, S. typhi, V. cholera* and *S. pneumonia* was standardized to contain approx.107cfu/ml and inoculated into 20 ml of Mac Conkey broth. The culture medium was allowed to set. Thereafter, all the inoculum was swabbed over the surface of the Mac Conkey agar medium for Gram negative bacteria and blood agar medium for Gram positive bacteria plate using sterile cotton swab. Using a sterile cork borer of 5 mm diameter, five wells were made in solidified sterile Mac Conkey agar medium and blood agar medium plate (one in the centre and four wells at the corner). The agar plugs were removed with a flamed and cooled wire loop. Then 10,20,30,40 and 50µl of aqueous extract of *M.elengi* leaves were placed in the wells made in inoculated plates. The treatment also includes 50 µl of sterilized distilled water as control. All the plates were incubated for 24 hours at 37^{o}C and zone of inhibition if any around the well were measured in millimeter (mm). For each treatment ten replicates were maintained. The same procedure were followed for standard antibiotics Gentamicin (25mg), Tetracycline (25mg) and Streptomycin (25mg) to compare the efficacy of plant extract against test organisms.

Solvent Extract

One gram of different solvent extract of *M. elengi* leaves were dissolved in 9 ml of methanol. The sterile Mac Conkey agar medium

(for Gram negative bacteria) and Blood agar medium (For Gram positive medium) in petri dishes was uniformly smeared with test culture. 5 mm wells were made in each petri dish to which 10,20,30,40 and 50 µl of different solvent extracts dissolved in methanol were added. For each treatment ten replicates were maintained. Respective solvents served as control. Standard antibiotics viz., Gentamicin (25mg), Tetracycline (25mg) and Streptomycin (25mg) was used to compare the efficacy of solvent extract against test organisms.

RESULTS

Phytochemical Analysis

Among the seven phytochemical constituents tested, *M.elengi* showed the presence of tannins, alkaloids, cardiac glycosides, steroids, flavonoids, reducing sugar and saponins when subjected to standard conformation test (Table1).

Antibacterial Assay

Aqueous extract

Among the five pathogen tested at 10,20,30,40 and 50 µl concentration, *S.pneumoniae* recorded a maximum inhibition of 26.9 mm at 50 µl concentration. At 10, 20, 30 and 40 µl concentration tested, it showed 8.9, 12.6, 18.9 and 22.3 mm zone of inhibition respectively. Standard antibiotics gentamicin, tetracycline and streptomycin recorded 35.7, 35.5 and 34.2 mm inhibition respectively. *S. pneumoniae* is followed by *E.coli* and recorded a significant inhibition of 24.4 mm at 50 µl and 15.4 mm at 10 µl concentration. Standard antibiotics Gentamicin, Tetracycline and Streptomycin recorded 36.0, 33.2 and 30.9 mm inhibition respectively. *P.aeruginosa* and *V.cholerae* recorded a moderate activity of 20.8 mm and 21.2 mm at 50 µl concentration and 17.8 and 16.7 mm at 40 µl concentration. Least activity was observed in *S.typhi* and recorded 15.4 and 10.9 mm at 50 and 40 µl concentrations (Table 2).

Solvent extract

Antibacterial activity of five solvent extract was also tested *in vitro*. Among the five solvents tested petroleum ether, toluene and chloroform extract did not showed any inhibition against all the pathogens. Maximum and highly significant activity was observed in methanol and ethanol extract tested at 10, 20, 30, 40 and 50 µl concentration. *S. pneumoniae* recorded maximum inhibition of 32.2 and 30.9 mm at 50 µl concentration of methanol and ethanol extract. Standard antibiotics Gentamicin, Tetracycline and Streptomycin showed 35.7, 35.5 and 34.2 mm inhibition respectively. In *E.coli* at 50 µl concentration, methanol and ethanol extract showed highly significant activity and recorded 31.3 mm and 28.3 mm inhibition. *E.coli* is followed by *P. aeruginosa* and showed a moderate inhibition of 30.9 and 25.7 mm inhibition at 50µl concentration in methanol and ethanol. Least inhibition was observed in *V. cholerae* and *S. typhi* and recorded 29.4 mm and 25.0 mm in methanol and 20.9 and 20.8mm in ethanol extract. The zone of inhibition goes on increasing as the concentration of methanol and ethanol extract increased from 10 to 50 µl in all the bacteria tested (Table 3).

DISCUSSION

There are many reports available that plants have been evaluated *in vitro* for their antibacterial potency against some important human pathogenic fungi (Hiremath *et al.*, 1993; Srivastava and Lal, 1997; Adelakun *et al.*, 2001; Verma and Dohroo, 2003; Singh and Singh, 2005; Patni *et al.*, 2005). In the present investigation the leaves of *M.elengi* which ecologically synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, saponins, steroids, flavonoids and reducing sugar were screened. Aqueous and solvent extracts of leaves were also subjected to check its potency. Higher plants have been used traditionally in folk medicine showing inhibition

against bacteria, fungi and yeasts. It was well known that *E. coli* which causes Urinary tract infections (UTI), *diarrhea* and *Meningitis* in infants and *S. pneumonia* which causes acute bacterial pneumonia and meningitis in adults, otitis media and sinusitis in children were recognized as a highly potent pathogen. In addition, *S. typhi, V. cholera* and *P. aeruginosa* were causing a life threatening diseases in humans. To manage these diseases the easiest way is to go for synthetic antibiotic which has its own side effects. Our aim in this work is to test the medicinal plants which is ecologically safe and does not have any side effects. There are many reports available that medicinal plants contain many phytochemical constituents which show strong antibacterial activity. A further study is required to isolate a bioactive compound and to identify the bioactive principal of *M.elengi* plant.

The aqueous extract of *M.elengi* showed a strong antibacterial activity and recorded 26.9 mm and 24.4 mm inhibition against *E. coli* and *S. pneumonia* at 50 µl concentration. It also showed a significant activity against *P. aeruginosa*, *V. cholera* and *S. typhi*. We have noticed that as the concentration of aqueous extract increases the zone of inhibition also increases.

Among the different solvent extract tested, methanol and ethanol extract showed a highly significant activity against all the pathogen tested. In methanol extract, *E. coli* and *S. pneumonia* pathogen were completely inhibited at 50 µl which is equal to the inhibition compared to synthetic antibiotic gentamicin, tetracycline and streptomycin. It was also noticed that the other three pathogens also showed inhibition at 10, 20, 30 and 40 µl concentration. From this investigation it was observed that *M.elengi* is a strong and highly potent plant which shows antibacterial activity and need further investigation to isolate bioactive principles which is highly specific to human pathogenic bacteria.

CONCLUSION

Medicinal plants which posses medicinal properties by synthesizing tannins, alkaloids, saponins, cardiac glycosides, steroids, flavonoids and reducing sugar should investigated *in vitro* against various pathogens. In the present study, it was reported that aqueous and solvent extract of *M.elengi* showed a very strong antibacterial activity at different concentration. A further investigation is necessary to isolate the bioactive compound responsible for antibacterial activity and further phytochemical analysis of various components of plant is necessary which can inhibit many human pathogens.

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Table 1: Phytochemical screening of *M.elengi* leaves

Phytochemicals	Test	Reactions	Present/Absent
Tannins	Ferric chloride test	Blue-black precipitate indicated the presence of Tannins	Present
Alkaloids	Mayer‟s test Dragendroff‟s test Wagner‟s reagent	Creamish precipitate brownish-red precipitate orange precipitate indicated the presence of respective alkaloids	Present
Saponins	Frothing test	Frothing persistence on warming indicated presence of saponins	Present
Cardiac glycosides	Keller-Kiliani test	Green-blue colour indicated the presence of cardiac glycosides	Present
Steroids	Liebermann-Burchard reaction	Blue-green ring indicated the presence of terpenoids	Present
Flavonoids	NaOH test	Ribbon pink-tomato red color indicated the presence of flavonoids	Present
Reducing sugar	Fehling‟s test	Brick red precipitate	Present

Table 2: Antibacterial activity of aqueous leaf extract of *M.elengi*

Bacteria	Zone of inhibition(mm)							
	10 µl	20 µl	30 µl	40 µl	50 µl	Gentamicin (25mg)	Tetracycline (25mg)	Streptomycin (25mg)
E.coli	15.4^{a} ±0.5	18.7^{b} ±0.1	20.2^{c} ±0.5	22.5^{d} ±0.3	24.4^{e} ±0.1	36.0^{c} ±0.1	33.2^{b} ±0.4	30.9^{a} ±0.2
P.aeruginosa	7.9^{a} ± 0.1	10.8^{b} ±0.1	13.7^{c} ±0.5	17.8^{d} ±0.3	20.8^{e} ±0.1	32.4^{b} ±0.1	33.3^{c} ±0.4	27.8^{a} ±0.1
S.typhi	3.8^{a} ±0.5	6.9^{b} ±0.1	8.9^{c} ±0.5	10.9^{d} ±0.3	15.4^{e} ±0.1	34.8^{c} ±0.1	30.0^{a} ±0.4	32.9^{b} ±0.2
S.pneumoniae	8.9^{a} ±0.2	12.6^{b} ±0.1	18.9^{c} ±0.5	22.3^{d} ±0.5	26.9^{e} ±0.2	35.7^{c} ±0.1	35.5^{b} ±0.2	34.2^{a} ±0.2
V.cholerae	5.5^{a} ±0.3	8.9^{b} ±0.1	11.3^{c} ±0.5	16.7^{d} ±0.5	21.2^{e} ±0.1	34.8^{c} ±0.1	30.6^{b} ±0.2	30.0^{a} ±0.1

- Values are the mean of three replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey‟s HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 3: Antibacterial activity of solvents extracts of leaf of *M. elengi*

Solvent extract	Concentration	Zone of inhibition (mm)				
		Microorganisms				
		E. coli	*P. aeruginosa*	*S. typhi*	*S. pneumoniae*	*V. cholerae*
Petroleum ether extract	10 µl	-	-	-	-	-
	20 µl	-	-	-	-	-
	30 µl	-	-	-	-	-
	40 µl	-	-	-	-	-
	50 µl	-	-	-	-	-
Toluene extract	10 µl	-	-	-	-	-
	20 µl	-	-	-	-	-
	30 µl	-	-	-	-	-
	40 µl	-	-	-	-	-
	50 µl	-	-	-	-	-
Chloroform extract	10 µl	-	-	-	-	-
	20 µl	-	-	-	-	-
	30 µl	-	-	-	-	-
	40 µl	-	-	-	-	-
	50 µl	-	-	-	-	-
Methanol extract	10 µl	10.2^{a} ±0.5	8.3^{a} ±0.1	6.9^{a}±0.1	10.9^{a}±0.1	7.7^{a}±0.1
	20 µl	16.7^{b}±0.1	14.3^{b}±0.2	10.9^{b}±0.1	13.9^{b}±0.1	10.0^{b}±0.1
	30 µl	22.2^{c}±0.1	20.4^{c}±0.1	16.9^{c}±0.1	18.9^{c}±0.1	15.6^{c}±0.5
	40 µl	25.6^{d}±0.5	27.3^{d}±0.1	20.4^{d}±0.1	25.8^{d}±0.2	22.9^{d}±0.5
	50 µl	31.3^{e}±0.1	30.9^{e}±0.5	25.0^{e}±0.1	32.2^{e}±0.1	29.4^{e}±0.5
Ethanol extract	10 µl	7.5^{a}±0.1	5.5^{a}±0.1	5.8^{a}±0.1	8.1^{a}±0.2	5.8^{a}±0.3
	20 µl	10.4^{b}±0.1	9.7^{b}±0.1	9.8^{b}±0.1	12.9^{b}±0.3	8.0^{b}±0.4
	30 µl	15.6^{c}±0.1	13.9^{c}±0.2	13.6^{c}±0.1	15.4^{c}±0.1	13.2^{c}±0.2
	40 µl	22.9^{d}±0.1	18.5^{d}±0.1	17.2^{d}±0.1	21.8^{d}±0.2	17.9^{d}±0.3
	50 µl	28.3^{e}±0.0	25.7^{e}±0.2	20.9^{e}±0.1	24.9^{e}±0.3	20.9^{e}±0.2
Gentamicin	25 mg	36.0^{c}±0.3	32.4^{b}±0.1	34.8^{c}±0.1	35.7^{c}±0.3	34.8^{c}±03
Tetracycline	25 mg	33.2^{b}±0.1	33.3^{c}±0.1	30.0^{a}±0.3	35.5^{b}±0.1	30.6^{b}±0.2

Streptomycin	25 mg	30.9[a]±0.2	27.8[a]±0.1	32.9[b]±0.1	34.2[a]±0.2	30.0[a]±0.1

- Values are the mean of three replicates, ±standard error
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey"s HSD
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

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