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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF MIMUSOPS ELENGI L. FLOWERS AND TRICHOSANTHES CUCUMERINA L. FRUITS FROM SOUTH INDIA

L. JOJI REDDY[1] AND BEENA JOSE[1]*

[1]Department of Biotechnology, Loyola Academy Degree & P.G. College, Alwal, Secunderabad, Andhra Pradesh, 500010, [1]*Department of Chemistry, Vimala College, Thrissur, Kerala, 680009, India. Email: drbeenajose@rediffmail.com

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ABSTRACT

The antibacterial activity of *Mimusops elengi* (Sapotaceae) flowers and *Trichosanthes cucumerina* (Cucurbitaceae) fruits were evaluated. The antibacterial activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of the flowers of *Mimusops elengi* (Sapotaceae) and fruits of *Trichosanthes cucumerina* (Cucurbitaceae) were screened against various pathogenic Gram positive and Gram negative bacterial strains viz. *Bacillus cereus* MTCC-1305, *Enterobacter faecalis* MTCC-5112, *Salmonella paratyphi* MTCC-735, *Staphylococcus aureus* MTCC-96, *Escherichia coli* MTCC-729, *Proteus vulgaris* MTCC-426, *Klebsiella pneumoniae* MTCC-109, *Pseudomonas aeruginosa* MTCC-647 and *Serratia marcescens* MTCC-86 by 'agar well diffusion' method. The methanol extract of *Mimusops elengi* flower and *Trichosanthes cucumerina* fruit exhibited pronounced activity on all organisms tested and their activity is quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions. The antimicrobial potency of these plant extracts is due to the presence of phenolic compounds, flavonoids, carotenoids and terpenoids. The study shows that the methanolic extract of *Mimusops elengi* flower and *Trichosanthes cucumerina* fruit can be used as a potential external antiseptic and can be incorporated into drug formulations.

Keywords: *Mimusops elengi*, *Trichosanthes cucumerina*, Antibacterial activity, Agar well diffusion method, Standard antibiotics, Drug formulation.

INTRODUCTION

The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many herbs on earth which lies unexplored in the field of medicine or Science. One such plant is *Mimusops elengi* (Sapotaceae). *Mimusops elengi* L. commonly known as Bakul is a small to large evergreen tree found all over the different parts of India. It has been used in the indigenous system of medicine for the treatment of various ailments. The bark is acrid, sweet, cooling, cardiotonic, alexipharmic, stomachic, anthelmintic, astringent, cures biliousness also used in fever, ulcers, diseases of the gums and teeth [1]. Charaka prescribed extract of the bark and root with honey or in a medicated ghee in helminthiasis and fevers. Sushruta gave flowers internally in coughs and bilious derangement, as an ingredient of medicinal liquor in diseases of the urinary tract. The bark powder is an ingredient in a number of commercial tooth powders, prescribed in disease of gums and teeth (ododentopathy). An infusion of the bark, in folk medicine, is prescribed internally in diseases of the bladder and urethra. Leaves may be boiled and applied to the head as a cold compress for headache and juice of the leaves squeezed into the eye for sore eyes.[1] The *Mimusops elengi* have been evaluated for its antiulcer, anthelmintic, antihypertensive, antibacterial, antihyperlipidemic activities [2, 3]. The aqueous extracts of *Mimusops elengi* L. showed a significant diuretic activity [4].The volatile organic matter from the bark of *Mimusops elengi* L. was analyzed by Anjali Ruikar and co-workers [5].

The bark, flowers and fruits of *Mimusops elengi* L. are acrid, astringent, cooling and anthelmintic. Bark is used as a tonic [6], febrifuge, as a gargle for odontopathy, inflammation and bleeding of gums. Powder of dried flower is a brain tonic and is useful as snuff to relieve cephalalgia. Young twigs are used for cleaning teeth. It is antipyretic and increases fertility in women. It is also useful in urethrorrhoea, cystorrhoea, diarrhea and dysentery. Flowers are used for preparing lotion for wounds and ulcers. Unripe fruit is used as masticatory and helps to fix loose teeth. Seeds are used for preparing suppositories in cases of constipation especially in children. Ripe fruit pulp is useful in chronic dysentery. Leaves are used in snake bite[6]. Comparative analysis of the fragrance obtained from *Mimusops elengi* flowers by different methods viz. water soluble volatiles, hexane extract and liquid CO_2 extract was done by Prasant et al [7]. The extracts of *Mimusops elengi* L. bark, fruit and seed were evaluated for antibacterial activity by using spectrophotomertic method against gram positive and gram negative strains [8].

Trichosanthes cucumerina, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to its good nutritional value. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti inflammatory, larvicidal effects [9].

Trichosanthes cucumerina is used in the treatment of headache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrhoea, haematuria and skin allergy. *T. curcumineria* is used as an abortifacient, vermifuge, refrigerant, purgative, malaria, laxative, hem agglutinant, emetic, cathartic, bronchitis and anthelmintic [10]. A novel isoflavone glucoside, 5, 6, 6'-trimethoxy- 3', 4'-methylenedioxyisoflavone 7-O-beta- D- (2''-O-p-coumaroyl glucopyranoside) has been characterized from the seeds of *Trichosanthes* [11]. The positive effects of the plant are due to the carotenoids, flavonoids, lycopene, phenolics and ß-carotene present in it [12]. The seed is said to be cooling. The dried seeds are used for its anthelmintic and anti-diarrhoeal properties. Seeds have anti-bacterial, anti-spasmodic and insecticidal properties. Hot aqueous extract of root tubers of *Trichosanthes cucumerina* exhibited significant anti-inflammatory activity [13]. The root extract of *Trichosanthes cucumerina* L. and the fruit juice tested cytotoxicity against four human breast cancer cell lines and lung cancer cell lines and one colon cancer cell line. The root extract inhibited more strongly than the fruit juice [14]. Crude ethanolic extract of *Trichosanthes cucumerina* showed significant blood glucose lowering activity in alloxan diabetic albino rats [15]. The acetone extract of leaves of *Trichosanthes cucumerina* showed moderate larvicidal effects [16]. Hot water extract of aerial parts of *Trichosanthes cucurmerina* has noted to improve glucose tolerance and tissue glycogen in non insulin dependent diabetes mellitus induced rats. Studies showed that the drug possess antidiabetic activity with volume improvement in oral glucose tolerance and glucose uptake in peripheral tissues [17].

Literature survey showed no reports on the antibacterial activity of the flowers of *Mimusops elengi* and fuits of *Trichosanthes cucumerina.* The present study was therefore envisaged to evaluate the antibacterial activities of the flower extracts of *Mimusops elengi* and fruit extracts of *Trichosanthes cucumerina* so as to discover the medicinal potential of these plant extracts.

MATERIALS AND METHODS

Plant material

Flowers of *Mimusops elengi* and fruits of *Trichosanthes cucumerina* were collected from Kerala, South India and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Preparation of plant extracts

Fifty grams of each of powered plant material were extracted successively with 150mL of petroleum ether (60-80^{0}C), chloroform, ethyl acetate and methanol as solvents for 24 hours by Soxhlet equipment [18]. The resultant extracts were combined and combined extract was filtered and concentrated under a vacuum to obtain a semi-solid mass.

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-96) and *Enterobacter faecalis* (MTCC-5112) and Gram-negative bacteria such as *Salmonella paratyphi* (MTCC-735), *Escherichia coli* (MTCC-729), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647) *Proteus vulgaris* (MTCC-426) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^{0}C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10mL of nutrient broth and was incubated at 37^{0}C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^{0}C was distributed by pipette (20mL) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1mL of bacterial suspension was taken and poured into Petri plates containing 20mL nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar diffusion method is used for the antimicrobial evaluations. The crude extracts were dissolved in sufficient amount of the respective solvents, so that each 10µL of solutions contained 100µg of the test materials for antibacterial tests. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50µL of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the flowers of *Mimusops elengi* and fruits of *Trichosanthes cucumerina* were added in each well (500µg/well). Wells introduced with 50µL of pure petroleum ether, chloroform, ethyl acetate and methanol served as negative controls. The plates were incubated at 37^{0}C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm [19]. All the assays were performed in triplicate and expressed as average values.

The antibacterial spectra of the flower extracts of *Mimusops elengi* and fruit extracts of *Trichosanthes cucumerina*, showing the zone of inhibition in millimeters, against Gram-positive bacteria such as *Staphylococcus aureus, Bacillus cereus* and *Enterobacter faecalis* and Gram-negative bacteria such as *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Salmonella paratyphi* and *Serratia marcescens* are summarized in table 1. In addition, the inhibition zones formed by standard antibiotics and those of negative controls are listed in table 2.

Table 1: Antibacterial activity of the flower extracts of *Mimusops elengi* and fruit extracts of *Trichosanthes cucumerina*

Microorganisms	Diameter of inhibition zones (mm/50µL)							
	M. elengi				*T. cucumerina*			
	A	B	C	D	A	B	C	D
1. *Bacillus cereus*	28	16	15	14	20	18	16	14
2. *Enterobacter faecalis*	30	18	15	11	26	20	16	14
3. *Salmonella paratyphi*	26	16	14	12	24	20	16	11
4. *Staphylococcus aureus*	30	15	13	11	24	20	18	16
5. *Escherichia coli*	26	16	14	12	20	16	14	12
6. *Proteus vulgaris*	28	15	13	11	24	22	17	14
7. *Klebsiella pneumoniae*	25	14	12	10	18	16	14	12
8. *Pseudomonas aeruginosa*	30	22	20	16	24	22	18	15
9. *Serratia marcescens*	25	14	12	11	18	16	13	11

A: methanol extract; B: ethyl acetate extract; C: chloroform extract; D: petroleum ether extract

Table 2: Inhibition zones formed by the standard antibiotics and negative controls

Microorganisms	Diameter of inhibition zones (mm/50µL)				
	Tob	Gen	Oflo	Cip	Control
	10µg	10µg	10µg	10µg	A, B, C, D
1. *Bacillus cereus*	28	32	34	30	--
2. *Enterobacter faecalis*	26	32	32	26	--
3. *Salmonella paratyphi*	25	30	28	30	--
4. *Staphylococcus aureus*	26	28	24	24	--
5. *Escherichia coli*	30	36	32	34	--
6. *Proteus vulgaris*	26	30	24	32	--
7. *Klebsiella pneumoniae*	26	32	32	36	--
8. *Pseudomonas aeruginosa*	26	24	32	28	--
9.*Serratia marcescens*	24	32	30	30	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

RESULTS AND DISCUSSION

As can be seen from table 1, the methanolic flower extract of *Mimusops elengi* showed pronounced antibacterial activity against all the microorganisms tested (25-30mm/50µL inhibition zone). Ethyl acetate (14-22mm/50µL inhibition zone) and chloroform (12-20mm/50µL inhibition zone) extracts of the flowers were also found to be active against all the tested microorganisms such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens.* The petroleum ether extract of the flower exhibited appreciable activity against *Pseudomonas aeruginosa* (16mm/50µL inhibition zone).

Methanolic fruit extract of *Trichosanthes cucumerina* displayed remarkable activity against all the tested microorganisms (18-26mm/50µL inhibition zone). The fruit ethyl acetate extract was also found to be effective on all test bacteria (16-22mm/50µL inhibition zone). *Trichosanthes cucumerina* fruit chloroform extract showed appreciable activity on *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa* (16-18mm/50µL inhibition zone). The fruit petroleum ether extract inhibited the growth of *Staphylococcus aureus* (16mm/50µL inhibition zone) and *Pseudomonas aeruginosa* (15mm/50µL inhibition zone) to a considerable extent.

The results obtained were compared with standard antibiotics and it was observed that *Mimusops elengi* flower methanol extract at a concentration of 500µg was more active against *Enterobacter faecalis* and *Staphylococcus aureus* than tobramycin and ciprofloxacin (10µg each). The activity of flower extract (500µg) against *Pseudomonas aeruginosa* was higher than that of the tested standard antibiotics except ofloxacin (10µg). The activity of the flower methanol extract of *Mimusops elengi* against other microorganisms was comparable with that of standard antibiotic tobramycin (10µg). The fruit methanol extract of *Trichosanthes cucumerina* showed inhibitory effect against *Enterobacter faecalis* comparable with that of the standard antibiotic tobramycin.

Out of the eight herbal extracts examined for antibacterial activity, flower methanol extract of *Mimusops elengi* showed highest activity against all microorganisms and its activity is quite comparable with that of the standard antibiotics. The antimicrobial potency of methanol extract of *Mimusops elengi* is due to the presence of terpenoids [7], flavonoids and phenolic compounds [20] and that of *Trichosanthes cucumerina* is due to the presence of phenolic compounds, flavonoids and carotenoids [12, 21]. These natural products might be acting synergistically or individually to produce antibacterial effects. It is interesting to note that even crude extract of these plants showed prominent activity against various pathogenic bacteria where modern therapy has failed. The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts.

CONCLUSION

Amongst the herbal extracts of *Mimusops elengi* flowers and *Trichosanthes cucumerina* fruits examined for antibacterial activity, methanol extracts of *Mimusops elengi* flower and Trichosanthes *cucumerina* fruits showed significant activity against the different strains of bacteria. The activities of these extracts are found to be quiet comparable with the standard antibiotics screened under similar conditions. So these extracts can be used as an external antiseptic in prevention and treatment of bacterial infections. The incorporation of these extracts into the drug formulations is also recommended.

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