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Research Article

ANTIBACTERIAL ACTIVITY OF *PONGAMIA PINNATA LINN* AND *MORINGA OLEIFERA LAM* FLOWER EXTRACTS AGAINST SELECTED BACTERIAL STRAINS AND THEIR COMPARATIVE EVALUATION

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ABSTRACT
Moringa oleifera Lam and *Pongamia pinnata* Linn are the two well known tropical trees with a rich world wide histroy of folklore medicine. Attempts to revisit the medicinal potential of these trees are the need of the hour with emerging drug resistance among common pathogens. In this regard few common pathogens have been considered for testing against the possible antibacterial activity of the concerned plants. The flower extracts of the plants were examined for antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia, Psuedomonas aeruginosa* and *Staphylococcus aureus* respectively. *P.pinnata* extract (aqueous, ethanol and fresh juice) seems to exhibit more effective antibacterial activity against *E.coli,* 65.25% more effectively than *M.oleifera* extracts and almost on-par with the control antibiotic. *S.aureus* also seems to exhibit more sensitivity to *P.pinnata* extracts, while better antibacterial activity against *K.pueumoniae* and *P.aeruginosa* could be seen with fresh juice extract of *P.pinnata* and solvent extract of *M.oleifera* respectively. The ethanol extract of the flowers were subjected to phytochemical analysis which indicated the presence of alkaloids, flavonoids and polyphenols in the extracts. The study asserts the prospective role of *Pongamia* and *Moringa* flower extracts as a source of natural and broad spectrum antibacterial compounds and opens up the need for further research on their use in the treatment of various bacterial infections and to discover the new bioactive compounds which can also be used for prophylactic treatment.
Keywords: Agar well diffusion; Antibacterial; Flower extract; Minimum Inhibitory Concentration (MIC).

INTRODUCTION

Finding the healing power in plants dates back to Vedic times. Having the divine origin Ayurveda, the science of life relies heavily on the plants for the therapeutic uses. There is a constant search for new compounds which have health benefits and in this regard the medicinal plants offer wide opportunities for scientific research as they are rich in phytochemicals such as flavonoids, lignin, phenolic acids and tannins. Priorities are given on the antimicrobial, antimutagenic and anticarcinogenic activity[1] of the phytochemical compounds.

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality especially in immune-compromised patients in developing countries[2]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds the urgency to the search for new infection-fighting strategies[3,4]. For a longtime, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties[5]. Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections[6,7]. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs[8]. It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials[9,10,11].

Moringa oleifera Lam. is considered a complete food it has an impressive range of medicinal uses with high nutritional value. Its multiple pharmaceutical effects are capitalized as therapeutic remedy for various diseases in traditional medicinal system[12].*Moringa oleifera* Lam. is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan[13] which is widely used for treating bacterial infection, fungal infection, antiinflammation, sexually-transmitted diseases, malnutrition and diarrhoea. *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumors[14].

Pongamia pinnata (Leguminosae) is widely distributed throughout the greater part of India, especially at low levels in wet places[15]. In traditional system of medicine leaves are used in eczema, scabies, leprosy, piles and ulcers. Leaves are also used in rheumatic pain[16]. From the phytochemical screening, presence of flavonoids from the chloroform extract and tannins from acetone extract has been reported[17]. In traditional system of medicines, such as Ayurveda and Unani, the *Pongamia pinnata* plant is used for anti-inflammatory[18],anti-plasmodial, anti-nonciceptive, anti-hyperglycaemics, anti-lipidoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic, CNS depressant activity[19]and antioxidant.

The literature survey showed that *M.oleifera* and *P. pinnata* is a potential medicinal plant. Flower is a part of a plant which naturally withers off on its own, which in turn becomes waste. Since, there is no report on antibacterial activity of flower extract of *M.oleifera* and *P.pinnata*; and

with the aim of bringing this into use; the present study is aimed at evaluating the efficacy of *M.oleifera* and *P. pinnata* flower extracts on bacterial pathogens.

MATERIALS AND METHODS

Collection of sample

The flowers of *Moringa oleifera* and *Pongamia pinnata* were collected freshly from reliable sources and it was cleaned, washed, shade dried and homogenized to a fine powdered and stored in airtight bottle.

Preparation of plant extracts

The flower extract was prepared by mixing 20gms of flower powder with 100ml ethanol and distilled water. Then the container was placed at room temperature for 3 days. The extracts were collected and evaporated on a water bath without shaking at atmospheric pressure and were concentrated by drying and final powder was preserved in air tight container to maintain its viability and stored at 4^0C**.**

For fresh juice preparation 25g of flowers were weighed and washed with distilled water to which 100ml of demineralized water was added and grinded, and then filtered.

For antimicrobial testing a 20% stock solution of each dry extract was prepared in pure Di Methyl Sulphoxide.

Phytochemical screening of the plant extracts

The alcoholic extracts were used for qualitative phytochemical screening for the identification of the different classes of active chemical constituents' viz. alkaloids, flavonoids and polyphenols, using standard prescribed methods[20,21,22].

Test Organisms

1. *Escherichia coli*
2. *Klebsiella pneumoniae*
3. *Pseudomonas aeruginosa*
4. *Staphylococcus aureus*

Screening of antimicrobial activity

Media for test organisms

Muller Hinton Agar (27 g) was added to100ml of sterile distilled water and autoclaved at 121^0C for 15 minutes at 15 lbs. 1.0 g of dextrose was added to 10 ml of sterile distilled water and steam sterilized for 15 minutes. After cooling both the content was mixed and poured into sterile petri plates and allowed to set at ambient temperature and used.

Antimicrobial activity by agar well diffusion method

Seeded broth (0.2ml) containing 10^7 test organisms were inoculated on the plates of solidified agar and spreaded uniformly. Wells of approximately 4mm in diameter and 2.5 mm deep were made on the surface of the solid medium using a sterile borer and filled with 20 µL of the plants extracts and plates were incubated at 37 ^{0}C about 24 hours[23]. Tetracycline was used as positive control and DMSO (100µl) was used as solvent control and incubated at 37 ^{0}C for 24 hours.

Minimal inhibitory concentration (MIC) evaluation

For the determination of MIC, which represents the minimum concentration that completely inhibits the growth of microorganisms; a micro-dilution broth susceptibility assay was used[24]. All tests were performed in nutrient broth (NB). Bacterial strains were cultured overnight at 37^0C in NB. The dried extracts were dissolved in DMSO at a concentration of 2560µg/ml and then further diluted with DMSO to obtain concentrations of 1280µg/ml, 640µg/ml, 320µg/ml and 160µg/ml. 1ml of varying concentrations of flower extracts were added in to the test tubes containing 9 ml of standardized suspension of tested bacteria (108 cfu ml^{-1}). The tubes were incubated at 37 °C for 24 h and positive controls were equally set up by using solvents and test organisms without extracts. The same test was performed simultaneously for the growth control and sterility control. Tetracycline was used as a reference compound for activity. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentrations[25].

RESULT & DISCUSSION

The antibacterial activity of ethanol, aqueous and fresh juice extracts was analyzed against selective human pathogenic strains of as *E.coli, K. pneumoniae, P.aeruginosa* and *S. aureus* by agar well diffusion method. The ethanol extract was subjected to phytochemical analysis which exhibited the presence of polyphenols, flavonoids and alkaloids (Table 1).

Table 1: Phytochemical components of Ethanolic extracts of *P.pinnata* and *M. oleifere*

S.No	Botanical Name	Phytochemicals identified		
		Polyphenols	Flavonoids	Alkaloids
1	*P.pinnata* Linn	+	+	+
2	*M. oleifera* Lam	+	+	+

The screening of antibacterial activity of various extracts on test cultures is tabulated (Table 2) and the results show varying degrees of antibacterial activities against the pathogens. Among the three different *M. oleifera* extracts used, the alcoholic extract showed maximum zone of inhibition(11mm) against *S.aureus* which was more than the activity of tetracycline followed by *K.pneumoniae* (10 mm), *P.aeruginosa* (9 mm) *and E.coli* (8.5 mm) where as no zone of inhibition was found with aqueous extract against *K.pneumoniae* and *P.aeruginosa*. Aqueous extract of *M.oleifera* showed better antibacterial activity against *E.coli* in comparison to ethanol extract and against *S.aureus* though the antibacterial activity was found, it was muted. The fresh juice extract of the *M.oleifera* showed positive antibacterial activity against all the four tested pathogens but the level of zone of inhibition clearly indicates the importance of solvent extraction and its role in increased antimicrobial activity. *Staphylococcus sp*. seems to be the most sensitive organism to the *M.oleifera* fresh juice and its various extracts, while *E.coli* growth is inhibited with aqueous extract.

Table 2: Antimicrobial activity of Aqueous, Ethanol and Fresh juice extract of medicinal plants against Test organisms

Plants	Extracts	Zone of Inhibition (mm)			
		E.coli	***K.pneumoniae***	***P.aeruginosa***	***S.aureus***
M. oleifera	Aqueous	9	ND	ND	6.5
	Ethanol	8.5	10	9	11
	Fresh Juice	6.5	4.0	5.2	6.8
P. pinnata	Aqueous	12.0	ND	ND	ND
	Ethanol	11.8	7.2	8.1	11.2
	Fresh Juice	13.0	8.0	7.8	10.0
Tetracycline		15.0	22.0	9.0	10.0

*ND – No Detection

In *P.pinnata,* among three extracts used the fresh juice extract showed maximum zone of inhibition (13mm) against *E.coli* followed by ethanolic extract against *E.coli* and *S.aureus*. Aqueous extract of *P.pinnata* showed no activity against *Klebsiella*, *Staphylococcus* and *Psuedomonas* which was also observed with aqueous extract of *M.oleifera* with *S.aureus* as an exception. One positive aspects of both the plant flower aqueous extract was that it was very effective against *E.coli*. *P.pinnata's* ethanolic extract showed better antibacterial activity against *P.aeruginosa* and *S.aureus*.

Table 3: Minimum Inhibitory Concentration

Test Organisms	Minimum Inhibitory Concentration (µg/ml)					
	Moringa oleifera			***Pongamia pinnata***		
	Aqueous	Ethanol	Fresh Juice	Aqueous	Ethanol	Fresh Juice
E.coli	32	64	128	16	16	16
K.pneumoniae	ND	64	256	ND	32	64
P.aeruginosa	ND	128	128	ND	64	128
S.aureus	64	32	64	ND	64	64

The results clearly indicate that the *P.pinnata* extract (aqueous, ethanol and fresh juice) seems to exhibit more effective antibacterial activity against *E.coli,* 65.25% more effectively than *M.oleifera* extracts (Table 3) and almost on-par with the control antibiotic. *S.aureus* also seems to exhibit more sensitivity to *P.pinnata* extracts, while better antibacterial activity against *K.pueumoniae* and *P.aeruginosa* could be seen with fresh juice extract of *P.pinnata* and solvent extract of *M.oleifera* respectively.

In conclusion the *P.pinnata* and *M.oleifera* solvent and fresh juice extracts show good prospectus for a successful isolation and characterization of an antibacterial compound and in this aspect their further purification and characterization will be carried out in future.

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