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Antimicrobial Activity of Medicinal Plants against Human Pathogenic Bacteria

Chandra M

Department of Biosciences, Mangalagangothri, Mangalore University, Dakshina Kannada District, Karnataka–574199, India.

Abstract

An attempt was made to analyse the antimicrobial potential of 2 medicinally important plants viz., *Lagerstroemia indica* and *Annona reticulata* leaf extracts against human bacterial pathogens viz., *Klebsiella pneumoniae, Staphylococcus auerus, Salmonella typhi, Proteus vulgaris* and *Pseudomonas aeruginosa*. Antimicrobial study was carried out by disc diffusion method against the pathogens by using the methanol and aqueous extracts. The result of the present study showed the presence of wide spectrum of antibacterial activities against all the above bacterial pathogens studied. The maximum zone of inhibition observed in methanol extract compared to aqueous extract for each bacterium was as follows. *S. typhi* (12 mm), *K. Pneumoniae*(13 mm), *P. vulgaris* (20 mm), *P. aeruginosa* (12 mm) and *S. aureus* (12mm). The present study demonstrates that the selected medicinal plants are potentially good sources of antibacterial against the pathogens viz., *K. Pneumoniae, S. auerus, S. typhi, P. vulgaris* and *P. aeruginosa*. The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, deoxy sugars, saponins, phenolic compouns, and flavonoids which may contribute for the antimicrobial action of the above medicinal plants.

Keywords: Antibacterial, disc diffusion, human pathogens, medicinal plants.

1. Introduction

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.

A wide range of medicinal plant parts is used for extract as raw drugs and they posses varied medicinal properties (Srivastav *et. al.,* 1996). Medicinal plants are believed to be important source of new chemical substance s with potential therapeutic effects (Katrin Basha *et. al.*, 2011). The secondary metabolites of plants were found to be source of various phytochemicals that could be directly used as intermediates for the production of new drugs. Traditional medicine should be able to play an even greater role in the modern primary healthcare system of the developing countries. The natural medicines are believed to be more acceptable to the human body, when compare to modern synthetic drugs. Thus the most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate healthcare service to rural people (Ghani 1990). Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source, based on their use in traditional medicine. The plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of the plants further strengthens the search for pharmacological active components from plants (Egharevba and Kunle, 2010). Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agents, a systematic investigation was undertaken to screen the local flora for antibacterial activity of *Lagerstroemia indica* and .

The plant *L. indica* L. belongs to the family *Lythraceae* and commonly called Crape Myrtle. Leaves and flowers are considered hydragogue and drastic purgative. Bark is also considered stimulant and febrifuge. Roots are astringent and used as gargle. Seeds have narcotic properties (Yusuf *et al*, 2009). The *A. reticulate* belongs to the family *Annonaceae. A .reticulata* L. is a powerful astringent and given as tonic. The plant has been used as an anti-inflammatory agent in wound healing, anti-anxiety, anti-stress, anti-mutagenic, and spasmolytic agent.and spasmolytic activities (Rastogi and Mehrotra, 1993).

2. Materials and Methods

Fresh leaves of two different plants were collected from local area of the Mangalore University Campus. The leaves were washed thoroughly 2-3 times with running water and once with sterile water and then air dried on sterile blotter under shade.

2.1 Solvent Extraction

The leaves of two plants were shade dried and pulverized. 250 g of powdered material was packed in Soxhlet apparatus and subjected to continuous percolation for 8 h using 450 ml, methanol as solvent. The methanol extract was concentrated under vacuum and dried in a dessicator. Aqueous extract made by cold maceration method. About 50 g of powdered material mixed with 300 ml of distilled water and kept for 7 days at room temperature. The extract obtained from water was filtered through Whatmann filter paper No. 1 and residue water content was evaporated (4^0 C) with heating mantle.

The obtained extracts were stored in refrigerator and were dissociated in dimethyl sulfoxide for prior to use.

A total five microorganisms were used to assess the antibacterial properties, it include two gram–positive bacteria, *Staphylococcus aureus* and *Salmonella typhi;* three gram- negative bacteria, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. The microorganisms originally obtained from microbial collection centre, Institute of Microbial Technology, Chandigarh, India.

2.2. Phytochemical Screening

The phytochemical screening of methanol extract was done to identify the main groups of chemical constituents in methanol extracts *Lagerstroemia indica* and *Annona reticulata* by their color reaction (Evans, 1997).

2.3. Preparation of Inoculum

The microorganisms wee pre-cultured in nutrient broth overnight in a rotary shaker at 37^0 C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized Spectrophotometrically at 660 nm.

2.4. Antimicrobial Activity

The methanol leaf extracts of *L. indica* and *A. reticulata* were tested by disc diffusion method (Anonymous, 1996). Different concentration of the extracts (100 µg / ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method. 10ul with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extract were placed on test organism-seeded plates. Streptomycin sulphate (10 µg /ml) used as positive control and methanol solvent (100 µg /ml) used as negative control. The antibacterial assay plates were incubated at 37^0 C for 24h. The diameter of the inhibition zones were measured in milimeters (mm).

3. Results

3.1 Phytochemical Screeing

The extractive value of the methanol and aqueous extract was 14.52 % and 14. 48 % on dry weight basis. The methanol and aqueous extract of the plants revealed the following phytochemicals (Table. 1).

Table 1: Different group of phytochemicals present in methanolic and aqueous extract of L. indica and A. reticulata plant leaves

Sl. No.	Phytochemicals	*L. indica*		*A. reticulate*	
		methanol	**aqueous**	**methanol**	**aqueous**
1.	Tannin	+	+	+	+
2.	Saponin	+	+	+	+
3.	Terpenoid	+	+	+	+

4.	Deoxy sugars	+	+	+	+
5.	Phenolic compounds	+	+	+	+
6.	Flavonoid	+	+	+	+

3.2 Antimicrobial Activity

Results obtained in the present study relieved that the tested two medicinal plants extracts posses potential antibacterial activity against *S. aureus, S. typhi, K. pneumoniae, P. vulgaris, P. aeruginosa.* When tested by the disc diffusion method, the methanol leaf extracts of the plants viz., *L. indica* and *A. reticulata* showed significant activity against all the tested microorganisms when compared to aqueous extract (Table.2). The highest antibacterial activity recorded in *P. vulgaris* (20 mm) and *P. aeruginosa* (16mm) followed by *K. pnuemoniae* (13mm), *S. aureus* (12mm) and *S. typhi* (12mm) from the *L. indica* leaf extracts. Antibacterial activity of leaf extracts of *A. reticulata* was also significant against the tested microorganisms viz., *P.vulgais* (18mm), *P.aeruginosa* (16), *K.pneumoniae* (14mm), *S. aureus* (11mm) and *S. typhi* (10mm) compared to aqueous extract.

Table 2: Antibactrial activity of methanol and aqueous extract (100 µg /ml) of L. indica and A. reticulata by disc diffusion assay.

	Zone of inhibition* (in mm.)				
Human pathogenic bacteria	***L. indica***		***A. reticulata***		**Streptomycin sulphate (10µg/ml)**
	Methanol	Aqueous	Methanol	Aqueous	
S. aureus	12	8	11	10	22
S. typhi	12	6	10	7	19
K. pneumoniae	13	9	14	8	20
P. vulgaris	20	5	18	4	21
P. aeruginosa	16	7	16	6	20

*Values are the means of three replications.

4. Discussion

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism (Aqil *et. al.,* 2005) and many components of plants products have been shown to be specially targeted against resistant pathogenic bacteria (Nostro *et. al.*, 2006). The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India (Gopalakrishna Sarala *et. al*., 2010).Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. The present work was a pioneer to explore the antimicrobial property of L. *indica* and *A. reticulata*. Many reports are available on the antiviral, antibacterial antifungal, anthelmintic, antimolluscal and anti-

inflammatory properties of plants (Mahesh and Satish, 2008). The methanol leaf extract of *L. Indica* and *A. reticulata* showed the activity against all the five tested microorganisms but the activity was very significant against *P. vulgaris* and *P. aeruginosa* .The plant based products have been effectively proven for their utilization as source for antimicrobial compounds. For instance, methanol extracts of *A. ferox* and *W. somnifera* exhibited inhibitory activity against all strains of *N. gonorrhoea* (Kambizi and Afolayan, 2008). The methanol and aqueous extract of medicinal plants were significantly active against Staphylococcus species (Selvamohan *et. al*., 2012). The antimicrobial activities and phytochmical screening of *D. metel* leaf extracts showed significant antimicrobial activity and photochemical constituents showed most of the antibacterial activities (Bipul Biswas *et. al*., 2013). The present study shows that the methanolic and aqueous extract of leaves of *L. Indica* and *A. reticulata* have all the phytochemical constituent studied with negligible variation. Thus, the significant activity against *P. vulgaris* and *P. aeruginosa* may be due to their phytochemical or secondary metabolites.

5. Conclusion

It is concluded that this study would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

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