

In-vitro antibacterial effect of selected medicinal plant extracts

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ABSTRACT

The antibacterial activities of five important plant species were selected and tested in turbidometric methods in a microplate reader. The various solvent (methanol, ethyl acetate, and chloroform) extracts of *Morus Alba*. L (leaves), *Mangifera indica*. (L.) (leaves) *Eugenia jambolana*. L (Fruits), *Albizia lebbeck*. L (bark), *Gymnema sylvestris* R. br (leaves) and isolated gymnemic acid were evaluated for antibacterial activity against *B. cereus*, *E.coli*, *P. mirabilis* *P. aeruginosa* *Staphylococcus aureus* and *Salmonella typhi*. Methanolic extracts was showed significant activity against all the tested bacteria followed by ethyl acetate and chloroform extracts.

Albizia lebbeck was observed 66%, *Eugenia jambolana* 56.12%, *Gymnema sylvestris* 59.21% and gymnemic acid 58.33% antibacterial activity against *E.coli*. *Albizia lebbeck* showed 67.36% against *Bacillus cereus*, 55% against *Proteus mirabilis* and it was less significant in *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. In case of *Salmonella typhi*, the tested plant extracts activity were not significant. Gymnemic acid was showed 68.33% against *Staphylococcus aureus*,

Comparison of the inhibitory activity of the plant extracts with gentamicin (positive reference standard) revealed that methanol extracts of *Morus Alba* (68.25%), *Eugenia jambolana* (66.33%), *Gymnema sylvestris* (65.33%) and gymnemic acid (68.33%) were significantly higher than that of the antibiotics tested against *Staphylococcus aureus*. The described method is a rapid, homogeneous, cost effective and easy-to-perform system for screening of new potential antimicrobial agents in drug discovery.

Keywords: Antibacterial activities, Different solvent extracts, Five plant species.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Jamine, et al., 2007). About three quarter of the world's population relies on plants and plant extracts for their

healthcare. India represented by rich culture, traditions and natural biodiversity, offers a unique opportunity for drug discovery researchers (Jachak, 2007). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Elizabeth, 2005).

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes (Iwu, 1999).

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno medicinal plants in India. Interest in a large number of traditional natural products has increased.

The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products. The turbidometric method

In the present work a few selected medicinal flora were screened for potential antibacterial activity by turbidometric methods.

Screening of medicinal plants:

Mangifera indica (Family: Anacardiaceae) - It is native to tropical Asia and has been cultivated in the Indian subcontinent for over 4000 years and is now found naturalized in most tropical countries. Most parts of the tree (Fruit, seeds, pulp, stem bark, roots and leaves) are used medicinally.

Gymnema sylvestre R.Br.-It is an herb native to the tropical forest of southern and central India. *Gymnema sylvestre* (also called gurmar), a woody plant that grows wild in India, has a long history of use in Ayurvedic medicine. The leaves of *G. sylvestre* contain glycosides, the peptide gurmarin, gymnemic acids, saponins, stigmasterol, quercitol, and several amino acid derivatives.

Morus Alba- In traditional medicine, the fruit & leaves are used to treat prematurely grey hair to tonify the blood and treat constipation and diabetes. Hypoglycemic and antioxidant potency of some phenolic compounds (Flavonoids, stilbenes and 2-arylbenzofurans) have been reported from *Morus Alba*. Besides, *Morus Alba* has been known to show antiviral and antimicrobial effect.

Albizzia lebeck- *Albizzia* has a long history of use in Ayurvedic herbal medicine for the treatment of allergic disorders. *Albizzia lebeck* has been extensively studied for anti-allergic, anti-fungal, anti-inflammatory, anti-microbial, cardio-tonic, hypocholesterolemic and antidiabetic

Eugenia jambolana-The ripe purple berries of the native Indian plant *Eugenia jambolana* L, known as Jamun. The fruit is oblong, ovoid, starts green and turns pink to shining crimson black as it matures. The Fruits is used in various alternative healing systems like Ayurveda (to control diabetes), Unani and Chinese medicine for digestive ailments.

MATERIALS AND METHODS

Chemicals used: DMSO chemical was purchased from M/s Sigma, St Louis, USA. Gentamicin was purchased from Himedia Laboratories, Mumbai, India. Gymnemic acid isolated & characterized by solvents

Solvent extraction: Test plants were collected from Bangalore and other parts of Karnataka, India and the plant materials were authenticated. The powdered plant materials (leaves of *Morus Alba*, *Mangifera indica*, *Gymnema sylvestris*, fruits of *Eugenia jambolana* and bark of *Albizia lebbeck*) of 600g each were extracted separately with methanol, ethyl acetate & chloroform using soxhlet apparatus. The resulting extracts were evaporated in vacuum and finally lyophilized into solid mass devoid of solvent (Yield – 10.75%, 11.58 %, 10.28 %, 9.65 % and 10.47% respectively) and stored in desiccators until further use.

Human pathogenic microorganisms: The microbial strains used in this study were *E.coli* (Uropathogen), *Salmonella typhi* (NCTC 8393), *Staphylococcus aureus* (ATCC 9144) and *Pseudomonas aeruginosa* (ATCC 25619) procured from MTCC, Chandigarh, India.

Antibacterial assay: The assays were carried out in standard, sterile 96-well micro plates (*Nunc A/S*, Denmark). The assay was started by adding 200 μ L of bacterial suspension and 50 μ L of sample solution (or corresponding solvent into control wells) into the wells.

The antibacterial activity of the compounds was determined by following the bacterial growth as absorbance of the suspension on Hidex plate reader at $\lambda = 620$ nm. To obtain growth curve of test microorganism, the samples were collected at each hour and the organism reached the stationary phase in six hours of incubation [37°C]. The antimicrobial activity was calculated by using the following equation:

$$C = 100 - (A/B) \times 100,$$

Where C is the inhibition in %, A absorbance in the sample wells ($n = 3$) and B absorbance in the control wells ($n = 3$)

RESULTS

Different solvent extracts of *Morus Alba*. L (leaves), *Mangifera indica*. (L.) (leaves) *Eugenia jambolana*. L (Fruits), *Albizia lebbeck*. L (bark), *Gymnema sylvestris* R. br (leaves) and isolated gymnemic acid were tested turbidometric methods for antibacterial activity.

A rapid and simple micro dilution technique on 96-well micro plate based on turbidimetry was optimized and validated for screening of antimicrobial activity against *B. cereus*, *E.coli*, *P. mirabilis* *P. aeruginosa* *Staphylococcus aureus* and *Salmonella typhi* are presented in table 1.

On the other hand, we found the simple absorbance measurement of bacterial suspension to be a reliable, easy, fast and reproducible measure of growth.

Albizia lebbeck was observed 66% antibacterial activity against *E.coli*, 67.36% against *Bacillus cereus*, 55% against *Proteus mirabilis* in turbidometric methods and it was less significant in *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. Gymnemic acid was showed 68.33% against *Staphylococcus aureus*, but the activity against *E.coli* and *Proteus mirabilis* were similar compared to positive control.

Among methanol, Ethyl acetate & Chloroform extracts, methanol extracts recorded significant antibacterial activity in turbidometric method. Comparison of the inhibitory activity of the extracts with the antibiotics gentamicin,

positive control revealed that methanol extracts of *Morus Alba*, *Eugenia jambolana*, *Gymnema sylvestris* and gymnemic acid were significantly higher than that of the antibiotics tested against *Staphylococcus aureus*. *Staphylococcus aureus* was found highly susceptible to methanolic extracts of all tested plant, where as *Proteus mirabilis* and *salmonella typhi* was less susceptible.

DISCUSSION

The main features pursued when selecting and developing screening methods are simplicity, robustness, and cost efficiency, together with relevance and reproducibility of the produced data. For the screening of antimicrobial activity, several alternative methods for assessing bacterial growth have been described. On the other hand, we found the simple absorbance measurement of bacterial suspension to be a reliable and reproducible measure of growth.

Since several *Staphylococcus* strains are reported to express drug resistance and natural plants were shown to be good anti- *staphylococcus* activity. In the present study, among extraction assayed, the methanolic extract of leaves showed significant inhibition against *Staphylococcus aureus*

Two important pathogens viz., *E. coli* and *Ps. Aeruginosa* frequently associated with infant bacteria is highly susceptible to methanolic extract of *Albizzia lebbeck* compared to positive reference standard, gentamicin. Gymnemic acid was also showed antibacterial activity against *E.coli*. The present study records the scientific validation of this plant for use as an anti-infective agent. *Gymnema sylvestris* & *Albizzia lebbeck* showed significant inhibitory activities as compared to gentamicin towards *bacillus cereus*. Holtparmacological investigation and suggests antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens

The current study showed significant *in vitro* antibacterial activity of selected medicinal plants against various pathogens. In conclusion, the present results showed a process of optimizing and validating an antimicrobial activity assay for screening purposes. The presented assay is a simple, homogeneous and low-budget way of measuring inhibition of bacterial growth of highly relevant erythromycin-resistant bacterial strains and can be carried out with standard laboratory equipment. The most active extracts can be subjected to isolation of the therapeutic and carry out further pharmacological evaluation.

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TABLE - 1: Antibacterial activity by turbidometric method of selected medicinal plant extracts against pathogenic bacteria							
Plant materials	Solvent extracts/ Dissolved	Percentage of antibacterial activity					
		<i>E. cereus</i>	<i>E. coli</i>	(mean \pm SD)		<i>S. aureus</i>	<i>S. typhi</i>
				<i>P. mirabilis</i>	<i>P. aeruginosa</i>		
<i>Morus alba</i>	Methanol	34.56 \pm 0.27	16.22 \pm 0.47	23.70 \pm 0.39	45.38 \pm 0.3	68.25 \pm 0.28	53.00 \pm 0.37
Leaves	Ethyl acetate	25.00 \pm 0.23	10.50 \pm 0.24	13.83 \pm 0.36	10.50 \pm 0.24	13.33 \pm 0.27	20.50 \pm 0.24
	Chloroform	20.66 \pm 0.12	8.33 \pm 0.27	10.67 \pm 0.27	16.17 \pm 0.14	11.33 \pm 0.29	26.17 \pm 0.14
<i>Mangifera indica</i>	Methanol	33.57 \pm 0.40	39.22 \pm 0.44	29.44 \pm 0.35	38.22 \pm 0.41	49.33 \pm 0.27	31.12 \pm 0.41
Leaves	Ethyl acetate	27.12 \pm 0.42	10.50 \pm 0.28	12.23 \pm 0.39	19.50 \pm 0.24	23.23 \pm 0.29	21.50 \pm 0.24
	Chloroform	21.66 \pm 0.14	28.29 \pm 0.29	18.67 \pm 0.29	26.17 \pm 0.19	32.39 \pm 0.26	15.17 \pm 0.16
<i>Eugenia jambolana</i>	Methanol	33.55 \pm 0.40	56.12 \pm 0.47	35.11 \pm 0.37	53.22 \pm 0.25	66.33 \pm 0.36	33.22 \pm 0.28
Fruits	Ethyl acetate	35.11 \pm 0.50	45.50 \pm 0.29	32.83 \pm 0.36	21.50 \pm 0.29	23.33 \pm 0.37	21.50 \pm 0.24
	Chloroform	19.11 \pm 0.32	18.33 \pm 0.37	29.67 \pm 0.36	36.11 \pm 0.28	28.33 \pm 0.29	15.17 \pm 0.24
<i>Albizia lebbek</i>	Methanol	67.36 \pm 0.40	66.00 \pm 0.47	55.00 \pm 0.39	48.10 \pm 0.48	55.33 \pm 0.27	55.22 \pm 0.47
Bark	Ethyl acetate	29.11 \pm 0.46	36.50 \pm 0.34	13.81 \pm 0.36	20.50 \pm 0.24	28.33 \pm 0.37	20.50 \pm 0.29
	Chloroform	26.66 \pm 0.42	29.23 \pm 0.37	28.67 \pm 0.27	36.17 \pm 0.2	38.33 \pm 0.47	26.17 \pm 0.34
<i>Gymnema sylvestris</i>	Methanol	62.33 \pm 0.44	59.21 \pm 0.47	35.00 \pm 0.39	46.00 \pm 0.36	65.33 \pm 0.37	48.22 \pm 0.50
Leaves	Ethyl acetate	37.33 \pm 0.40	46.50 \pm 0.24	22.83 \pm 0.33	31.50 \pm 0.38	33.33 \pm 0.27	35.50 \pm 0.34
	Chloroform	17.33 \pm 0.40	40.23 \pm 0.37	29.67 \pm 0.37	28.17 \pm 0.35	38.33 \pm 0.27	36.17 \pm 0.4
Gymnemic acid (50 μl)	DMSO	50.66 \pm 0.22	58.33 \pm 0.27	48.67 \pm 0.27	46.17 \pm 0.34	68.33 \pm 0.27	56.17 \pm 0.34
Gentamicin* (50 μl)	DMSO	60.66 \pm 0.12	58.33 \pm 0.27	48.67 \pm 0.27	66.17 \pm 0.14	60.33 \pm 0.27	75.17 \pm 0.14
* Positive control							