ANTIBACTERIAL ACTIVITY OF LEAF METHANOLIC EXTRACTS OF SOME IMPORTANT MEDICINAL PLANTS

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Summary

The present study aimed at evaluating the *in vitro* antimicrobial activity of methanolic extracts of some medicinal plants against *Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Salmonella Typhi*. The selection of methanolic extracts was based on the phytochemical screening for presence of secondary metabolites. The methanolic extract of *Alstonia scholaris* presented the highest anti-S. aureus activity and was effective against all bacterial strains tested.

Key words: Medicinal plants, antibacterial activity, Agar well diffusion

Introduction

Many higher plants accumulate extractable organic approaches, substances in quantities sufficient to be economically management of disease. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. In recent times, the rapid development of multiresistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents [1]. Antibiotic resistance has become a global concern [2]. The clinical efficiency of many antibiotics, in existence is being treated by the emergence of multi drug-resistant pathogen [3]. Throughout the history of mankind, many infectious diseases have been known to be treated with herbal remedies. The researchers are increasingly turning their attention to folk medicine. Continuous search leads into developing better drugs against microbial infections [4].

The current investigation aimed to explore scientifically the antibacterial potential of six medicinal plants used in folk medicine for the treatment of several ailments and hence, substantiate the folklore claims.

Methods

Plant material and preparation of the extract

Fresh leaves of six different plants viz., *Leucas aspera*, *Tabernaemontana coronariae*, *Adhatoda vasica*, *Mimosa pudica*, *Alstonia scholaris* and *Asparagus racemosus* free from disease were collected from local areas in Davanagere district, Karnataka. The plant material was shade dried and then powdered using a mechanical grinder. 100 grams of pulverized plant part was extracted successively in 500 ml of petroleum ether, ethyl acetate and methanol (LR grade, Merck, India) using Soxhlet apparatus. At the end of extraction, extracts were filtered under vacuum through a Whatman No. 1 filter paper and the process repeated until all soluble compounds had been extracted. The filtrate obtained was concentrated *in vacuo* using a Rotavapor (Buchi Flawil, Switzerland). The extracts were stored at 4 °C in air tight bottle until further use.

Phytochemical screening

The preliminary phytochemical analysis of petroleum ether, ethyl acetate, methanol extracts was carried out using the methods as described in Harborne (1984) [5]; Trease and Evans (1989) [6]; Kokate *et al*. (1998) [7]; Khandelwal (2005) [8].

The dry weight of the methanol extracts was obtained by allowing the solvent to evaporate and was used to determine concentration in mg/mL. (Methodology based on Betoni *et al*. [9]; Table 1). The extracts of the plants were screened for the antibacterial activity by the agar well diffusion method [10].

Table 1. Characteristics of the plant extracts

Scientific name	Common name	Part of the plant used	Extract dry weight (mg/ml)
Leucas aspera	Dronapushpi	Leaf	135.0
Tabernaemontana coronariae	Crape jasmine	Leaf	90.0
Adhatoda vasica	Malabar nut	Leaf	75.0
Mimosa pudica	Touch-me-not	Leaf	17.0
Alstonia scholaris	Dita bark	Leaf	70.0
Asparagus racemosus	Asparagus	Leaf	122.0

Microorganisms used:

The bacterial strains used in this study clinical isolates from different infection status of patients presenting symptoms of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella typhi* - associated diseases. The isolates were identified by a standard method [11]. The standard strains used were *P. aeruginosa* ATCC-20852, *K. pneumoniae* MTCC-618, *S. aureus* ATCC- 29737, *S.* typhi MTCC- 3214, *S. paratyphi* MTCC-735, *P. vulgaris* ATCC- 13315 and *C. albicans* ATCC-2091. The bacteria were maintained on agar slope at 4 °C and sub-cultured into nutrient broth by a picking-off technique [12] for 24 hrs before use.

Preparation of culture medium and inoculation

Nutrient agar (Hi Media, India) was used as the bacteriological medium. The media were sterilized by autoclaving at 120 °C for 20 minutes. Under aseptic conditions, in the laminar air flow, 15 ml of culture medium was dispensed into pre-sterilized petridishes to yield a uniform depth of 4 mm. After solidification of the medium, the microbial cultures were inoculated by spread plating technique.

Agar well diffusion

The extracts were dissolved in 10 % DMSO to a final concentration of 100 mg/ml. Pure DMSO was taken as the negative control and 50 mg/ml Ciprofloxacin as the positive control. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter. 10 mg /100µL of each extract were loaded in the corresponding wells. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar and then they were incubated at 37 °C for 18 h. Subsequently, the plates were examined for microbial growth inhibition and the inhibition zone diameter (IZD) measured to the nearest millimeter.

Statistical analysis

The results of the antibacterial study are expressed as mean ± SEM of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple pairwise comparison tests to assess the statistical significance. The data were considered significant at $P < 0.001$.

Results

The phytochemical profiles of various solvent extracts from plants used in this study are presented in Table 2. The analysis revealed the presence of alkaloids, flavonoids, triterpenoids, sterols, tannins and glycosides. In particular, methanol extracts showed positive for most of the secondary metabolites tested. Petroleum ether extracts showed mostly the presence of triterpenoids and sterols. The leaf methanol extracts were hence chosen for further evaluation of antibacterial activity.

Table 2. Phytochemical screening of different plant extracts

Plant	Extract	Plant constituent						
		Alkaloids	Flavanoids	Triterpenoids	Sterols	Tannins	Saponins	Glycosides
L. aspara	PE			+	+			
	EA		+		+	+		
	ME	+	+		+		+	+
T. coronariae	PE			+	+			
	EA		+			+		+
	ME	+	+			+	+	+
A. vasica	PE			+	+			
	EA		+			+		+
	ME	+	+		+		+	+
M. pudica	PE			+	+			
	EA					+		+
	ME	+	+			+		+
A. scholaris	PE			+	+			
	EA		+		+	+		+
	ME	+	+		+	+	+	+
A. racimosus	PE			+	+			
	EA				+		+	+
	ME	+	+		+		+	+

Abbreviations: PE, Petroleum ether extract; EA, ethyl acetate extract; ME, Methanol extract

Phytochemical test: - negative and + positive

In our study the results of the investigation showed that all the tested plant extracts were effective enough to execute potent antibacterial activity compared to the standard antibiotic ciprofloxacin and the values were significant at $P< 0.001$. The results are depicted in Table. 3.

Table 3. Antibacterial activity of selected medicinal plants against clinically isolated strains (Inhibition zone in mm)

Plant extract	Bacterial spp.			
	Pseudomonas aeruginosa	*Klebsiella pneumoniae*	*Staphyllococcus aureus*	*Salmonella typhi*
LAME	13.50±0.76*	13.17±0.44*	16.00±0.58*	13.50±0.58*
TCME	15.83±0.17*	15.00±0.29*	17.83±0.60	16.17±0.60*
AVME	17.17±0.44	17.00±0.29*	18.17±0.44*	17.50±0.76*
MPME	13.83±0.73*	14.33±1.20*	15.17±0.60*	14.27±0.74*
ASME	18.00±0.58*	17.33±0.73*	20.17±0.44*	17.87±0.13
ARME	14.67±0.88*	14.17±1.17*	16.00±0.87*	13.90±0.95*
Ciprofloxacin	23.83±0.73	23.17±0.60	24.83±0.44	21.90±0.59

The values are the mean of three experiments ± S.E.

*p< 0.001 vs. Standard antibiotic (Tukey's pairwise comparison test)

Abbreviations: LAME, *L. aspara* methanol extract; TCME, *T. coronariae* methanol extract; AVME, *A. vasica* methanol extract; MPME, *M. pudica* methanol extract; ASME, *A. scholaris* methanol extract; ARME, *A. racimosus*.

Among all the bacterial strains used, *S. aureus* (Gram positive bacteria), was observed to be more susceptible to the plant extracts with the inhibition zone ranging from 16.00±0.58 to 20.17±0.44. *K. pneumoniae* was least susceptible to the extracts except for *M. pudica* which was less effective against *P. aeruginosa* (13.83±0.73).

Among the plants screened, *A. scholaris* extract showed very good zone of inhibition with respect to the test strains. The values were significant except for *S. typhi* (17.87±0.13). *L. aspara* and *M. pudica* leaf extracts were less effective against both Gram positive and Gram negative bacteria (13.17±0.44 to 16.00±0.58 and 13.83±0.73 to 15.17±0.60). The values were moderate in case of *T. coronariae, A. vasica* and *A. racimosus.*

Discussion

Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent [9, 13]. It has already been established that crude extracts of some medicinal plants and some pure compounds from such plants can potentiate the activity of antibiotics *in vitro* [14, 15]. This search for antibiotic resistance modulators in plants represents a new dimension to addressing the problem of antibiotic resistance. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial

properties [16]. In the present work, all the plant extracts used were extracted from polar sovent and hence had exhibited appreciable activity against clinical strains. Our findings are in agreement with reports showing that polar extracts inhibited the growth of both Gram-positive and Gram-negative bacteria [17, 18]. The stronger extraction capacity of methanol could have produced a greater number of active constituents responsible for antimicrobial activity.

The results were more promising against the gram positive bacteria *S. aureus*. This could be attributed to the fact that the cell wall in Gram-positive bacteria has a single layer, whereas the Gram-negative cell wall is a multi-layered structure [13, 14], acting as a barrier to many environmental substances, including antibiotics [15].

Conclusion

Plant extracts have shown inhibitory effect on the growth of the bacteria studied, although of distinct forms. However, the activity level of the extracts may be more accurately evaluated in terms of MIC values as the zone of inhibition might be influenced by solubility and diffusion rate of the phytocompounds. In addition, *in vivo* studies are necessary to determine the toxicity of the active constituents, their side effects, circulating levels, pharmacokinetic properties and diffusion in different body sites. The antibacterial activities could be enhanced if the active components are purified and adequate dosage determined for proper administration. It is therefore recommended that the nature and the number of the active antibacterial principles involved in each plant extract be studied in detail.

References

[1] Rojas R. Bustamante B. Bauer Fernandez I. Alban J. Lock O. Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 2003; 88: 199-204.

[2] Westh H. Zinn C.S. Rosdahl V.T. Sarisa Study Group. An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. Microbiol Drug Resist 2004; 10(2): 169-176.

[3] Bandow J.E. Brotz H. Leichert L.I.O. Labischinski H. Hecker M. Proteomic approach to understanding antibiotic action. Antimicro Agents Chemotherap 2003; 47: 948-955.

[4] Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensm Wiss u Technol 2004; 37: 263–268

[5] Harborne J.J. Phytochemical Methods: *A guide to modern techniques of plant analysis* (2nd Edn), Chapman and Hall, New York, 1984; pp 85

[6] Trease G.E. Evans W.C. *Pharmacognosy* (13th Edn), ELBS Publication, Delhi, 1989; pp 171

[7] Kokate C.K. Purohit A.P. Gokhale S.B. *Pharmacognosy* (23rd Edn), Nirali Prakashan, Pune, 1998; pp 106-114.

[8] Khandelwal K.R. Practical Pharmacognosy: *Techniques and Experiments* (13th Edn), Nirali Prakashan, Pune, 2005; pp 149-156.

[9] Betoni J.E.C. Mantovani R.P. Barbosa L.N. Di-Stasi L.C. Fernandes A. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem Inst Oswaldo Cruz 2006; 101: 387-90

[10] Nair R. Kalariya T. Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 2005; 29: 41-47

[11] Cowan S.T. Steel S. *Manual for the Identification of Medica Bacteria*, Barrow GI, Feltham RKA (Eds), Cambridge University Press 1993; pp 32.

[12] Aneja K.R. *Experiments in Microbiology, Plant Pathology and Biotechnology* (4th Edn), New Age International Ltd., New Delhi, India, 2003; pp 196-197.

[13] Yao J. Moellering R. Antibacterial agents. In: Murray P. Baron E. Pfaller M. Tenover F. Yolken R. (Eds) *Manual of Clinical Microbiology*, ASM, Washington DC 1995; pp 1281-1290.

[14] Ozcelik S. *Genel Mikrobiyoloji*, Suleyman Demirel Universitesi Isparta Turkey 1998; pp 259 (in Turkish)

[15] Tortora G.J. Funke B.R. Case C.L. *Microbiology: An Introduction*, Benjamin Cummings San Francisco 2001; 88 pp

[16] Shibata H. Kondo K. Katsuyama R. Kawazoe K. Sato Y. Murakami K. Takaishi Y. Arakaki N. Higuti T. Alkyl Gallates, Intensifiers of ß-Lactam Susceptibility in Methicillin-Resistant *Staphylococcus aureus Antimicrob. Agents Chemother* 2005; 49(2): 549-555.

[17] Marquez B. Neuville L. Moreau N.J. Genet J.P. Santos A.F. Andrade M.C.C. Sant Ana AEG Multidrug resistance reversal agent from Jatropha elliptica Phytochem 2005; 66: 1804-1811.

[18] Smith E.C.J. Williamson E.M. Wareham N. Kaatz G.W. Gibbons S. Antibacterials and modulators of bacterial resistance from the immature cones of Chamaecyparis lawsoniana. Phytochem 2007; 68(2): 210-217.

[19] Lewis K. Ausubel F.M. Prospects for plant-derived antibacterials. Nat Biotechnol 2006; 24(12): 1504-1507.

[20] Karaman I. Sahin F. Gulluce M. Ogutcu H. Sngul M. Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J Ethnopharmacol 2003; 85: 231-235.

[21] Masika P.J. Afolayan A.J. Antimicrobial activity of some plants used for the treatment of livestock diseases in Eastern Cape, South Africa. J Ethnopharmacol 2002; 83: 129-134.