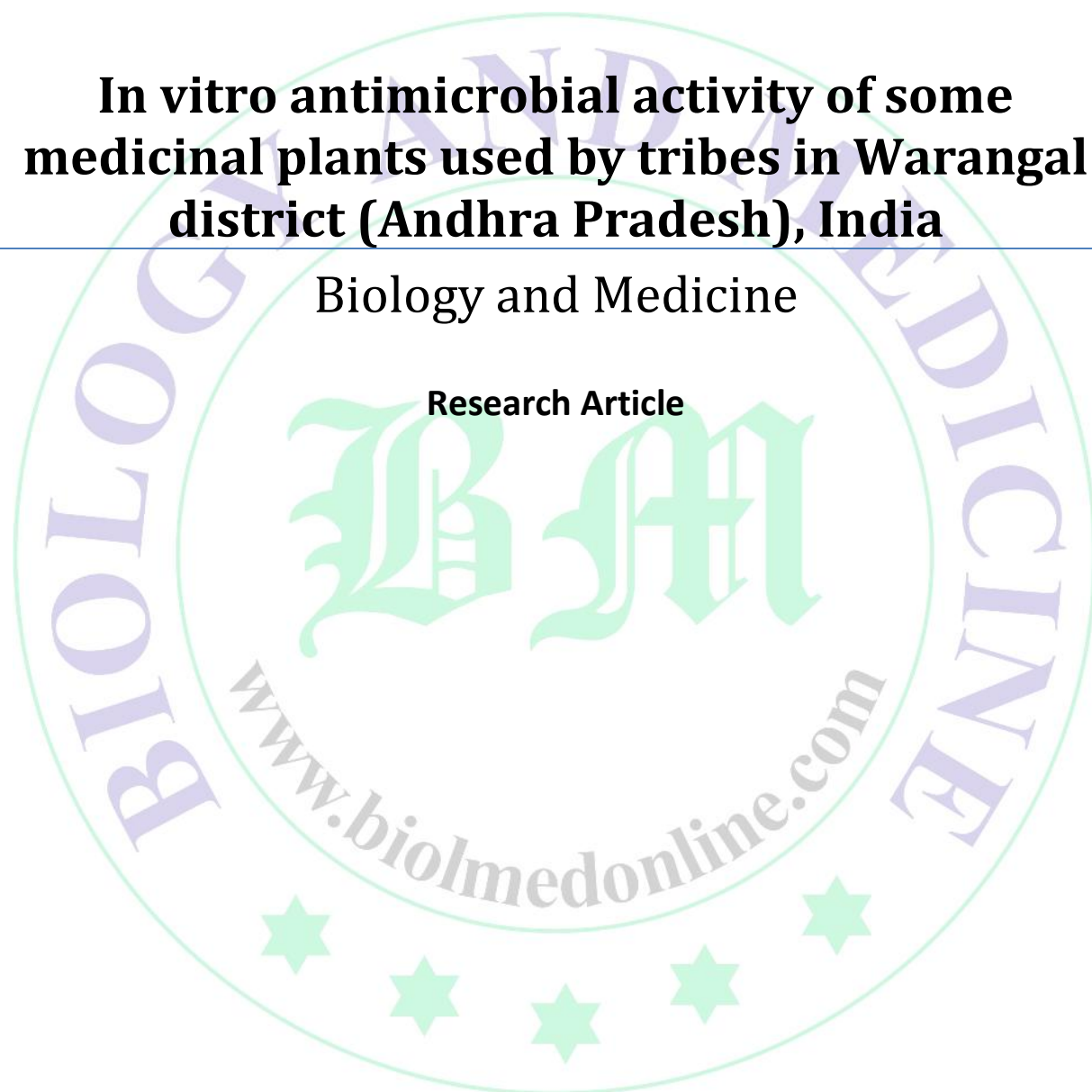


In vitro antimicrobial activity of some medicinal plants used by tribes in Warangal district (Andhra Pradesh), India

Biology and Medicine

Research Article



***In vitro* antimicrobial activity of some medicinal plants used by tribes in Warangal district (Andhra Pradesh), India**

L Venkanna, M Estari*

Metabolic Disorders and Infectious Diseases Research Lab, Department of Zoology, Kakatiya University, Warangal, Andhra Pradesh, India.

*Corresponding Author: estari08@gmail.com

Accepted: 14th Jun 2012, Published: 1st Jul 2012

Abstract

India has rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha, and Unani besides folklore practices. The aim of the present study was to evaluate the antimicrobial activity of different plant extracts. The antimicrobial activities of some plant species (*Phyllanthus emblica*, *Tinospora cordifolia*, *Eclipta alba*, and *Cassia occidentalis*) extracts were evaluated against four bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*) by the disc diffusion method. *Phyllanthus emblica* and *T. cordifolia* had better activity against all the tested organisms compared to other plant extract fractions. Aqueous fraction of *C. occidentalis* and *P. emblica* showed high activity against *P. aeruginosa* and *S. aureus* bacteria. *n*-Hexane fraction of *T. cordifolia* showed high activity against *E. coli* (162 ml/g), *P. aeruginosa* (162 ml/g), and *S. aureus* (162 ml/g) bacteria.

Keywords: Antimicrobial activity; methanol extract; MIC; total activity; disc diffusion method.

Introduction

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semisynthetic derivatives of natural products and are used in the traditional systems of medicine. Thus, it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test, and a sustainable number of new antibiotics introduced the efforts for exploring leads from Ayurveda, the traditional system of medicine in India (Santos *et al.*, 1995; Rodriguez-Fragoso *et al.*, 2008) and South Africa (Street *et al.*, 2008). Ayurvedic system of medicine has its long history of therapeutic potential. The use of both plant extracts and phytochemicals with known antimicrobial properties is of great significance. 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 (Rojas *et al.*, 2003). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds that are a part of the essential oils (Jansen *et al.*, 1987) as well as tannin (Saxena *et al.*, 1994).

In the past few years, a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz *et al.*, 1995; Nascimento *et al.*, 1990; Drewes *et al.*, 2006). Initially, four plant species (*Phyllanthus emblica*, *Tinospora cordifolia*, *Eclipta alba*, and *Cassia occidentalis*) were selected for preliminary screening. All the four plant species were part of the selection based on the knowledge of traditional healers. Fruits of *P. emblica* and leaves of remaining three plants were used for extraction in this study. The aim of the present study was to evaluate the antimicrobial activity of the abovementioned plant extracts.

Materials and Methods

Preparation of plant extracts

All the plant materials (leaves of *E. alba*, *T. cordifolia*, and *C. occidentalis* and fruits of *P. emblica*) were collected from Parvatagiri Village of Torur Mandal, Warangal district, Andhra Pradesh. Voucher specimens were prepared and identified at the Department of Botany, Kakatiya University, Warangal. Four plants were collected and maintained at room temperature for 2 weeks to dry. Samples were chopped into smaller pieces and then ground into powder and extracted by Soxhlet techniques with methanol. Obtained methanolic crude extracts of the four plants were then fractionated successively using solvents of increasing polarity such as *n*-hexane fraction (HXF), carbon tetrachloride

fraction (CTF), chloroform fraction (CFF), and aqueous fraction (AQF). All the fractions were evaporated to dryness using rotary evaporator at a temperature of 39°C.

Bacterial species

Bacterial species selected for the study were the four pathogens, namely, two Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli*. All the cultures were maintained on Mueller--Hinton agar at 40°C. The cells were inoculated and incubated at 37°C in broth for 12 hours before the screening procedure.

Antimicrobial activity

The serial microplate dilution method developed by Eloff (1998) was used to determine the minimum inhibitory concentration (MIC) for plant extracts using tetrazolium violet reduction as an indicator of growth. Briefly, residues of the different extracts were redissolved in methanol to a concentration of 1 mg/ml. For each of the four bacteria used, 100 µl of each plant extract tested were two-fold serially diluted with 100 µl sterile distilled water in a sterile 96-well microtiter plates. A similar two-fold serial dilution of gentamicin (0.1mg/ml) was used as a positive control against each bacterium. One hundred microliters of each bacterial culture were added to each well. The plates were covered and incubated overnight at 37°C. To indicate bacterial growth, 40 µl of 0.2 mg/ml *p*-iodonitrotetrazolium violet were added to each well and the plates were incubated at 37°C for 30 minutes. Bacterial growth in the wells was indicated by red color, whereas clear wells indicated inhibition of the bacterial growth by the plant extracts.

Total activity test

Total activity indicates the degree to which the active compound in 1 g of plant material can be diluted and still inhibit the growth of the tested bacterial microorganisms (Eloff, 2004). This was calculated as follows:
 Total activity (ml/g) = Quantity of material extracted from 1 g of plant material (mg)/MIC (mg/ml).

Results and Discussion

Percentage yield of the different plant extracts

The yield of methanol crude extract of *P. emblica*, *E. alba*, *T. cordifolia*, and *C. occidentalis* was 75, 82, 45, and 38 g, respectively. The highest percentage yield of

the four plant extracts screened was obtained from *E. alba* (16.4%), with the lowest from *C. occidentalis* (7.6%). The percentage yield of the above mentioned plants' crude methanol extracts was shown in Figure 1.

Antimicrobial activity

The MIC values and total activity of the four fractions of methanol crude extract of different plants against all the tested bacteria are presented in Table 1. *Phyllanthus emblica* and *T. cordifolia* had better activity against all the tested organisms compared to other plant extract fractions. MIC value as low as 0.08 mg/ml was obtained from *P. emblica* against *P. aeruginosa* and *S. aureus*. Aqueous fraction of *T. cordifolia* had MIC value of 0.08 mg/ml against *S. aureus* only. Aqueous fractions of *C. occidentalis* and *E. alba* also had noteworthy MIC values of 0.08 mg/ml and 0.63 mg/ml against *S. aureus* and *E. faecalis*, respectively.

Total activity

The highest total activity obtained on *C. occidentalis* (295 ml/g) and *P. emblica* (200 ml/g) is presented in Table 2. *Phyllanthus emblica* and *C. occidentalis* had consistent total activity against *P. aeruginosa* and *S. aureus* bacteria. Other plant species had a moderate total activity against more than these two bacterial. The lowest (11 ml/g) total activities were obtained from *E. alba* HXF fraction against *E. faecalis* (Ef), *P. aeruginosa* (pa), and *S. aureus* (Sa) bacteria.

Aqueous fraction of *C. occidentalis* and *P. emblica* showed high activity against *P. aeruginosa* and *S. aureus* bacteria. *n*-Hexane fraction of *T. cordifolia* showed high activity against *E. coli* (162 ml/g), *P. aeruginosa* (162 ml/g), and *S. aureus* (162 ml/g) bacteria. Carbon tetrachloride fraction of *E. alba* showed high total activity against *E. coli* (146 ml/mg) and *E. faecalis* (146 ml/g) bacteria. Chloroform fraction of *T. cordifolia* showed high total activity against *E. coli* (160 ml/g), *E. faecalis* (160 ml/g), and *P. aeruginosa* (160 ml/g) bacteria.

Conclusion

The highest percentage yield of the four plant extracts screened was obtained from *E. alba* (16.4%). With only exception of *E. alba*, the other three plant species, i.e., *P. emblica*, *C. occidentalis*, and *T. cordifolia*, that were screened had reasonable activity of the tested bacterial species. *Phyllanthus emblica* and *C. occidentalis* had the most consistent MIC values with an overall average of 0.13 and 0.08 mg/ml, respectively. The highest total

activity was obtained with *C. occidentalis* and *P. emblica* fractions.

Conflict of Interests

Authors have no conflicting interests.

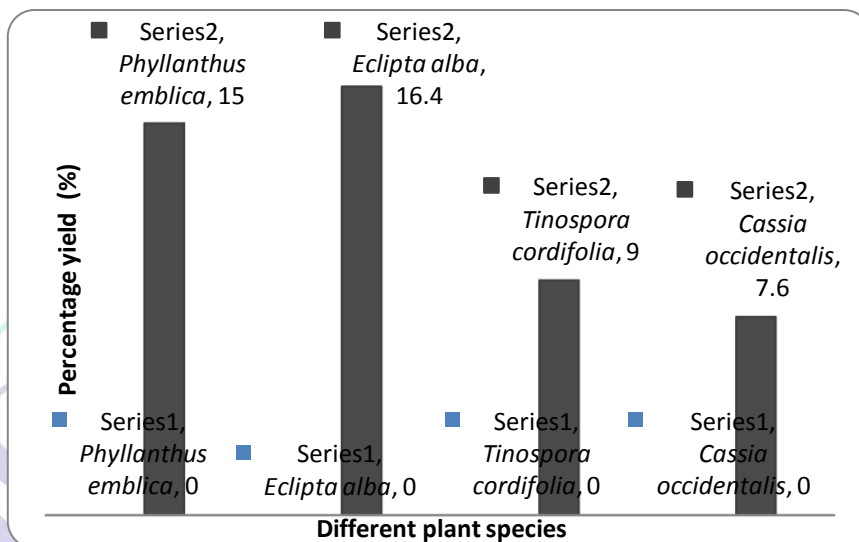


Figure 1: Percentage yield of four plant extracts (*P. emblica*, *E. alba*, *T. cordifolia*, and *C. occidentalis*) extracted with methanol.

Table 1: MIC values (mg/ml) of the four plant extract fractions of the study, *P. emblica*, *E. alba*, *T. cordifolia*, and *C. occidentalis*, against four bacteria, *E. coli* (*Ec*), *E. faecalis* (*Ef*), *P. aeruginosa* (*Pa*), and *S. aureus* (*Sa*). Gentamicin was used as a positive control.

Plant Species	<i>Ec</i>	<i>Ef</i>	<i>Pa</i>	<i>Sa</i>
<i>P. emblica</i>				
HXF	0.63	0.31	0.08	0.15
CTF	1.25	1.25	0.15	0.15
CFF	1.25	0.63	0.16	0.08
AQF	0.31	0.15	0.08	0.08
<i>E. alba</i>				
HXF	0.15	0.63	0.63	0.63
CTF	0.15	0.15	0.31	0.63
CFF	1.25	0.62	1.25	1.25
AQF	0.63	0.63	1.25	0.63
<i>T. cordifolia</i>				
HXF	0.08	0.15	0.08	0.08
CTF	0.15	0.08	0.08	0.15
CFF	0.15	0.15	0.15	0.30
AQF	0.25	0.15	0.15	0.08
<i>C. occidentalis</i>				
HXF	0.63	0.63	1.25	1.25
CTF	0.63	0.63	0.08	0.08
CFF	0.30	0.63	0.15	0.08
AQF	0.15	0.08	0.15	0.08
Gentamicin (µg/ml)	8.00	1.60	0.20	0.30

Table 2: Total activity (ml/g) of four plant extract fractions of this study, *P. emblica*, *E. alba*, *T. cordifolia*, and *C. occidentalis*, against four bacteria, *E. coli* (*Ec*), *E. faecalis* (*Ef*), *P. aeruginosa* (*Pa*), and *S. aureus* (*Sa*).

Plant Species	<i>Ec</i>	<i>Ef</i>	<i>Pa</i>	<i>Sa</i>
<i>P. emblica</i>				
HXF	27	55	216	108
CTF	22	22	186	186
CFF	12	25	100	200
AQF	25	53	100	100
<i>E. alba</i>				
HXF	48	11	11	11
CTF	146	146	70	34
CFF	11	22	11	11
AQF	15	15	8	15
<i>T. cordifolia</i>				
HXF	162	86	162	162
CTF	44	82	82	44
CFF	160	160	160	80
AQF	35	58	58	110
<i>C. occidentalis</i>				
HXF	37	37	17	17
CTF	24	24	196	196
CFF	78	37	157	295
AQF	104	196	104	196

References

Alonso-Paz E, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, *et al.*, 1995. Screening of Uruguayan medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology*, 45: 67–70.

Drewes S, Horn M, Khan F, 2006. The chemistry of plants and pharmacology of medicinal plants. In *Commercialising Medicinal Plants – A Southern African Guide*. Ed., Diederichs N, Stellenbosch: Sun Press, pp. 87–96.

Eloff JN, 1998. A sensitive and quick method to determine the minimum inhibitory concentration of plant extracts for bacterial. *Planta Medica*, 60: 1–8.

Eloff JN, 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. *Phytomedicine*, 11: 372–377.

Jansen AM, Cheffer JJC, Svendsen AB, 1987. Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspects of the test methods. *Planta Medica*, 40: 395–398.

Nascimento SC, Chiappeta A, Lima RM, 1990. Antimicrobial and cytotoxic activities in plants from Pernambuco, Brazil. *Fitoterapia*, 61: 353–355.

Rodriguez-Fragoso L, Reyes-Esparza J, Burchiel S, Torres E, 2008. Risks and benefits of commonly used herbal medicines in Mexico. *Toxicology and Applied Pharmacology*, 227: 125–135.

Rojas R, Bustamante B, Bauer J, 2003. Antimicrobial activity of selected Peruvian medicinal plants. *Journal of Ethnopharmacology*, 88: 199–204.

Saxena G, McCutcheon AR, Farmer S, Towers GHN, Hancock REW, 1994. Antimicrobial constituents of *Rhus glabra*. *Journal of Ethnopharmacology*, 42: 95–99.

Street RA, Stirk WA, Van Staden J, 2008. South African traditional medicinal plant trade-challenges in regulating quality, safety and efficacy. *Journal of Ethnopharmacology*, 119: 705–710.