Indian Journal of Natural Products and Resources
Vol. 3(4), December 2012, pp. 589-593

SHORT COMMUNICATION

Antimicrobial efficacy of *Bridelia retusa* (Linn.) Spreng. and *Asclepias curassavica* Linn.

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Received 27 July 2011; Accepted 22 October 2012

The *in vitro* bioassay of the bark extracts of *Bridelia retusa* Spreng. and root extracts of *Asclepias curassavica* Linn. was done by cold percolation and Soxhlet method against four bacterial species, viz. *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris* and two fungal species *Candida albicans* and *Aspergillus niger*. The MIC and MBC values for the extracts obtained by both the methods were tested separately. The acetone extract of *B. retusa* showed MIC values ranging from 25, 42.5 to 85 mg/mL against the tested strains where as the bactericidal and fungicidal concentrations were found to be ≥170 mg/mL. The MIC value for root extract of *A. curassavica* was 3.06 mg/mL and the bactericidal concentration was found to be 100 mg/mL. The present work is an attempt to justify that the method of extraction influences the efficacy of the plant extracts.

Keywords: *Asclepias curassavica*, *Bridelia retusa*, Ethnomedicine, Antimicrobial, Cold percolation, Soxhlet extraction.

IPC code; Int. cl. (2011.01)—A61K 36/00.

Introduction

Medicinal and aromatic plants are gift of nature and are being used against various infections and diseases in the world since ages[1]. *Bridelia retusa* Spreng. of family Euphorbiaceae is distributed throughout the warmer parts of India. In Karnataka it is mainly distributed in deciduous to semi evergreen forests. It is a small moderate sized deciduous tree. In Ayurveda the bark of the plant is used for the removal of urinary concretions[2]. Paste of the bark is applied to wounds and bark juice is taken internally for snake bite. Traditional practitioners use the stem bark to treat dysentery, diarrhoea and diabetes. *Asclepias curassavica* Linn. of Asclepiadaceae, a plant introduced from the West Indies, found throughout the tropics, is a common weed in wet places in Karnataka. Root of this plant is regarded as purgative, astringent and also a remedy for gonorrhea[3]. On the basis of the ethnomedicinal information available, the two plants were selected to find out their antimicrobial efficacy[3-5].

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Materials and Methods

Collection of plant material: Bark of *B. retusa* and roots of *A. curassavica* were collected from the Dharwad district of Karnataka, India. The plants were identified with the help of Flora of Presidency of Bombay, Flora of British India, Flora of Madras and Flora of Karnataka. Both the plant materials were powdered using electric grinder[6-9].

Extraction of the plant material

Soxhlet method: The powdered plant material were extracted using four different solvents, viz. chloroform, acetone, ethanol and water in order of their increasing dielectric constants[10].

***Cold percolation method*:** This is similar to the traditional method of extraction used by herbalists throughout the world. A known amount of dried material (5 g/50 mL) was soaked in the desired solvent and kept for continuous shaking for nearly 48 h. This was followed by filtration and evaporation of excess solvent without applying heat. Each time before extracting with the next solvent, the material was air dried at room temperature[10] and the same method was repeated for next solvent[11].

***Preparation of extracts*:** In both the methods, extracts were weighed and re-dissolved in Dimethyl formamide (DMF) to obtain the extract solution. Three different concentrations, 25, 50 and 100 mg/mL were selected for screening purpose.

Microbial Cultures: The bacterial strains *Staphylococcus aureus* (MTCC 737), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688) and *Proteus vulgaris* (MTCC 1771) and a fungal strain *Candida albicans* (MTCC 183) were procured from the MTCC (Microbial Type Culture and Collection) Chandigarh, India. Another fungal strain *Aspergillus niger* was obtained from the stock culture maintained in the Mycology Laboratory, Department of Botany, Karnatak University, Dharwad.

Antimicrobial activity: The antimicrobial activity was studied by disc diffusion method[12] and inoculum suspensions of all the strains were prepared. For all the bacterial strains and the fungal strain, *C. albicans* peptone water was selected as the growth medium and sterilized distilled water was taken as the growth medium for the fungus *A. niger*. Nutrient agar (Hi-media) was selected as the bacterial medium and Potato Dextrose Agar (Hi-media) as fungal medium. Twenty milliliter of the sterilized medium was poured in the pre-autoclaved petri plates and allowed to solidify. The 12 h culture broth was swabbed on the agar surface. Sterile discs impregnated with 10 µL of the extract were placed on the media and gently pressed down to ensure contact with the medium. Then the plates with bacterial strains were incubated at 37°C for 24 h and 48 h for fungi. The zone of inhibition was noted. Streptomycin was used as standard for bacteria and Nystatin was used as standard for fungi.

Determination of Minimum Inhibitory Concentration (MIC)

Serial tube dilution method[13]: The MIC values were determined by serial tube dilution method. The concentrations of the extracts range in the decreasing order from 170 mg/mL to 0.33 mg/mL in DMF against the inhibitory microorganism.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)[14]

The tubes that showed no visible growth were streaked on fresh nutrient agar plates (for bacteria) and on PDA plates (for fungi). The plates were incubated at 37°C for 24 h and examined for growth. MBC or MFC was regarded as the lowest concentrations of the extracts that prevent the growth of the bacterial or fungal colony on solid medium.

Statistical Analysis

In order to determine whether there is a statistically significant difference among the results obtained from antimicrobial effect of the tested plants, variance analyses were carried out using SPSS 16.0 software package. Values of $P<0.05$ were considered as significantly different. The results were subjected to analysis of variance (ANOVA) and mean values were separated according to Duncan's multiple range test at $P=0.05$ level[15].

Results

Results presented in the Tables 1-4 show the effect of plant extracts on the growth of microorganisms in the form of inhibition zones measured in mm. The acetone and ethanol extracts of *B. retusa* were active against the Gram negative strains *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. These extracts also showed good activity against the fungal strain *Candida albicans*. The cold percolation extracts of acetone and ethanol showed inhibitory activity against the bacteria *P. aeruginosa* and *P. vulgaris* but did not show any activity against the bacteria, *K. pneumoniae.* Further, cold percolation extract of acetone showed good activity against the

Table 1—Antimicrobial activity of bark extract of *Bridelia retusa* Spreng. (Soxhlet method)

Extracts	Con. (mg/ml)	Zone of inhibition (mm)					
		K. pneumoniae	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*	*A. niger*
Chloroform	25	-	-	-	-	-	-
	50	7.3±0.33^{b}	-	-	-	-	-
	100	8.3±0.33^{a}	-	-	-	9.6±0.33^{a}	-
Acetone	25	9.3±0.33^{c}	-	10.3±0.3^{b}	8.6±0.66^{b}	9.6±0.33^{c}	-
	50	10.6±0.33^{b}	-	11.3±0.3^{b}	10.3±0.33^{a}	11.1±0.1^{b}	-
	100	13.0±0.00^{a}	-	12.6±0.33^{a}	11.3±0.33^{a}	12.8±0.16^{a}	-
Ethanol	25	-	-	-	7.6±0.33^{c}	-	-
	50	-	-	8.3±0.33^{b}	9.0±0.00^{b}	9.3±0.33^{b}	-
	100	9.3±0.25^{a}	-	10.1±0.16^{a}	10.1±0.16^{a}	10.5±0.28^{a}	-
Water	25	7.16±0.33^{b}	7.3±0.33^{b}	-	-	-	-
	50	8.3±0.33^{b}	9.5±0.28^{a}	-	-	-	-
	100	10.5±0.00^{a}	10.5±0.28^{a}	-	9.0±0.00^{a}	7.3±0.33^{a}	-
Standard value*		25±0.0 mm	18±0.0 mm	28±0.00 mm	22±0.00 mm	31±0.0 mm	21±0.0 mm

*Streptomycin (10 µL/disc) for bacteria and Nystatin (100 units/disc) for fungi. Data given are mean of three replicates ± standard error. Mean values followed by the same letter are not significantly different according to DMRT at $p=0.05$

Table 2—Antimicrobial activity of bark extract of *Bridelia retusa* Spreng. (Cold percolation method)

Extracts	Con. (mg/mL)	Zone of inhibition (mm)					
		K. pneumoniae	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*	*A. niger*
Acetone	25	-	10.3±0.33[c]	10.3±0.33[c]	13.0±0.00[c]	10.3±0.3[b]	-
	50	-	13.1±0.1[b]	11.5±0.3[b]	14.3±0.33[b]	11.3±0.3[b]	-
	100	-	18.1±0.16[a]	13.3±0.33[a]	15.8±0.44[a]	12.3±0.33[a]	-
Ethanol	25	-	-	-	8.3±0.33[b]	-	-
	50	-	-	7.6±0.33[b]	9.3±0.33[a]	9.0±0.00[b]	-
	100	-	9.5±0.28[a]	10.3±0.33[a]	10.0±0.00[a]	10.1±0.16[a]	-
Standard value*		25±0.0 mm	18±0.0 mm	28±0.0 mm	22±0.0 mm	31±0.0 mm	21±0.0 mm

*Streptomycin (10 µl/disc) for bacteria and Nystatin (100 units/disc) for fungi.
Data given are mean of three replicates ± standard error. Mean values followed by the same letter are not significantly different according to DMRT at *P*=0.05

Table 3—Antimicrobial activity of root extract of *Asclepias curassavica* Linn. (Soxhlet method)

Extracts	Con. (mg/ml)	Zone of inhibition (mm)					
		K. pneumoniae	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*	*A. niger*
Chloroform	25	7.0±0.00[b]	-	-	12.6±0.33[c]	-	-
	50	7.1±0.16[b]	-	-	15.5±0.28[b]	-	-
	100	8.3±0.33[a]	-	7.0±0.00[a]	17.8±0.44[a]	-	-
Water	25	-	-	-	-	8.6±0.33[b]	-
	50	8.1±0.16[a]	-	-	-	9.1±0.16[b]	-
	100	8.3±0.33[a]	-	-	-	10.0±0.00[a]	-
Standard value*		25±0.0 mm	18±0.0 mm	28±0.0 mm	22±0.0 mm	31±0.0 mm	21±0.0 mm

*Streptomycin (10 µL/disc) for bacteria and Nystatin (100 units/disc) for fungi.
Data given are mean of three replicates ± standard error. Mean values followed by the same letter are not significantly different according to DMRT at *P*=0.05

Table 4—Antimicrobial activity of root extract of *Asclepias curassavica* Linn. (Cold percolation method)

Extracts	Conc. (mg/ml)	Zone of inhibition (mm)					
		K. pneumoniae	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*	*A. niger*
Chloroform	25	-	-	-	-	9.0±0.00[c]	-
	50	-	-	7.3±0.33[b]	-	10.1±0.1[b]	-
	100	-	-	8.0±0.00[a]	7.0±0.00[a]	11.5±0.28[a]	-
Water	25	-	-	-	7.3±0.33[c]	10.0±0.0[b]	-
	50	-	-	-	8.1±0.16[b]	10.3±0.3[b]	-
	100	-	-	-	9.1±0.16[a]	11.3±0.33[a]	-
Standard values*		25±0.00 mm	18±0.0 mm	28±0.0 mm	22±0.0 mm	31±0.0 mm	21±0.0 mm

*Streptomycin (10 µL/disc) for bacteria and Nystatin (100 units/disc) for fungi.
Data given are mean of three replicates ± standard error. Mean values followed by the same letter are not significantly different according to DMRT at *P*=0.05

Gram positive strain *Staphylococcus aureus* but the ethanol extract was slightly active against this strain. Similar to Soxhlet extracts the cold percolation extracts of acetone and ethanol showed good activity against the fungus *C. albicans*.

The chloroform extract of *Asclepias curassavica* obtained by Soxhlet method showed good activity against the Gram negative bacteria *P. aeruginosa* and the highest zone of inhibition obtained was 17.8 mm. This extract showed moderate inhibition of 8.3 mm

for another Gram negative strain *K. pneumoniae* but did not show any antifungal activity. The water extract showed growth inhibition of 8.3 mm for *K. pneumoniae* and 10.0 mm for the fungus *C. albicans.* Both chloroform and water extracts obtained by cold percolation method were moderately active against the bacterial strain *P. aeruginosa* and the fungal strain *C. albicans.* The MIC, MBC and MFC values for both plant extracts were tested for the values exceeding 12 mm. From the MIC tubes, inoculations were made on the agar plates to test the bactericidal and fungicidal concentrations. The acetone extract of *B. retusa* showed MIC values ranging from 25, 42.5 to 85mg/mL against the tested strains *S. aureus, P. aeruginosa, P. vulgaris, C. albicans* and *K. pneumoniae,* respectively where as the bactericidal and fungicidal concentrations were found to be ≥170 mg/mL. The MIC value for root extract of *A. curassavica* for *P. aeruginosa* was 3.06 mg/mL and the bactericidal concentration was found to be 100 mg/mL.

Discussion

The results clearly indicate that both *B. retusa* and *A. curassavica* possess thermolabile and thermostable compounds. The cold percolation method is beneficial for such thermolabile compounds and is similar to the method followed by traditional practitioners. The bark of *B. retusa* is mainly experimented for its other pharmacological activities like antioxidant[16], immunomodulatory[17], analgesic and anti-inflammatory[18], hypoglycemic[19], antioxidant, hepatoprotective and nephroprotective[20, 21], total phenolic and antioxidant[22]. Little work has been reported on the antimicrobial property of the bark of *B. retusa,* where the extract was active against the strains *S. aureus*, *Bacillus subtilis*, *E. coli* and *C. albicans*[23]. Isolated antifungal constituents were active against *Cladosporium cladosporioides*[24]. The plant, *A. curassavica* extract was found to be active for its antifungal nature[25-27]. Recently antibacterial property for *Clavibacter michiganense*, *Pseudomonas solanacearum*, *E. coli* and antifungal property for *Aspergillus niger*, *Helminthosporium oryzae* and *Fusarium oxysporum* has been reported[28-30].

Conclusion

The results indicated that the bacterial strains were more susceptible than the fungal strains. Most of the pathogenic strains are developing resistance against antibiotics and also introduction of synthetic drugs are responsible for various side effects. Now-a-days herbal drugs are being introduced to find out remedy for most of the diseases and can be used as a substitute for the synthetic drugs. The present result showed that the extracts of the screened plants possess some chemical components which can act against both bacteria and fungi. Antimicrobial properties of these plants can be exploited further in the preparation of natural therapeutic agents against pathogenic bacteria.

Acknowledgements

The authors are thankful to the UGC-SAP, New Delhi, India for their financial assistance and Karnatak University, Dharwad for facilities.

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