academicJournals

Vol. 8(7), pp. 697-701, 12 February, 2014
DOI: 10.5897/AJMR2013.6192
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antimicrobial activities of *Delonix elata* (Bojer ex Hook.) Raf. and *Spathodea campanulata* P. Beauv.

M. Vijayasanthi* and V. Kannan

Post Graduate and Research Department of Botany, National College (Autonomous), Tiruchirappalli – 620 001, Tamilnadu, India.

Accepted 12 December, 2013

Methanol and aqueous extracts of *Delonix elata* (Bojer ex Hook.) Raf. (Fabaceae) and *Spathodea campanulata* P. Beauv. (Bignoniaceae) were evaluated for their antimicrobial activities against nine bacterial species: *Bacillus cereus, Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Proteus vulgaris* and *Shigella flexneri* and two fungal species: *Aspergillus niger* and *Candida albicans*. The susceptibility of the microorganism to the extracts of these plants was compared with each other and with selected antibiotics. All these plants were effective against three or more of the pathogenic microorganisms. This *in vitro* study corroborated the antimicrobial activity of the selected plants used in folklore medicine.

Key words: Medicinal plants, infectious diseases, solvents, disc diffusion methods, microbial pathogens.

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Yadev and Khan, 2012). The increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity. The use of medicinal plants as a source of relief from illness can be traced back to over five million years' written documents of the early civilization in China, India and the near East; it is doubtless an art as old as mankind.

Spathodea campanulata P. Beauv. is a species belonging to the Bignoniaceae family, native of equatorial Africa. It is commonly found and planted in the coffee estates of Munnar, South Tamilnadu and is denoted by the name Malaria Maram (tree). Its flowers and stem bark extracts have shown molluscicidal activity and also employed in diuretic, anti-inflammatory treatments. The leaves are used for kidney diseases, urethra inflamemations and as an antidote against animal poisons. The stem bark preparations are used for enemas, fungus skin diseases, herpes, stomach aches and diarrhea (Adriana et al., 2007; Mendes et al., 1986). Hypoglycemic, anti-HIV and anti-malarial activities were also observed in the stem bark extracts (Niyonzima et al., 1999; Makinde et al., 1988).

Delonix elata L. (Family: Fabaceae) is a deciduous tree of about 2.5-15 m height; it has a spreading, rather rounded crown, crooked poor stem form and drooping branches. The plant is traditionally used for the treatment of abdominal pains, rheumatism and flatulence. The stem bark of this plant is considered as good febrifuge and is much appreciated as an antiperiodic and anti-inflammatory (Abd El and Hegazi, 2011). We report here the results of the antimicrobial properties of extracts from the leaves of *D. elata* and *S. campanulata*.

*Corresponding author. E-mail: bmynbayeva@gmail.com. Tel: +7(701)9531372 or +7(777)3792917.

MATERIALS AND METHODS

Collection of plant samples

Fresh plant leaves were collected randomly from the gardens and villages of Kovilpatti taluk, Tamilnadu from natural stands. The botanical identity of these plants was confirmed by Dr. V. Sampath Kumar, Scientist- C, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu. The voucher specimens are deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamilnadu, India.

Preparation of extracts

Aqueous extraction

One hundred gram of dried powder was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through eight layers of muslin cloth and centrifuged at 5000 rpm and 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to one-fifth of the original volume.

Solvent extraction

100g of dried plant powdered samples was extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4°C in airtight bottles for further studies.

Antimicrobial activity

Microorganisms

Microorganisms were obtained from the Microbial Type Culture Collection centre (MTCC), Chandigarh, India. Amongst eleven microorganisms investigated, nine were bacterial strains viz., *Staphylococcus aureus* MTCC 3160, *Bacillus cereus* MTCC 442, *Streptococcus pneumonia* MTCC 655, *Escherichia coli* MTCC 598, *Pseudomonas aeruginosa* MTCC 42642 *Klebsiella pneumoniae* MTCC 7407, *Salmonella typhi* MTCC 3917, *Proteus vulgaris* MTCC 742 and *Shigella flexneri* MTCC 1457, while the other two were fungal strains viz. *Aspergillus niger* MTCC 2546 and *Candida albicans* MTCC 183. All the bacterial strains were maintained on nutrient while fungi were maintained on potato dextrose agar slants.

Disc diffusion method

Antimicrobial activity was carried out by the disc diffusion method. The antimicrobial assays of aqueous and methanolic extracts were performed according to the method of Bauer et al. (1966). Each plant extract was tested at two different concentrations (100 and 200 µg/ml) to see their inhibitory effects against microbial pathogens. Sterile paper discs (6 mm in diameter) prepared from Whatman No. 1 filter paper was impregnated with drug, containing solution placed on the inoculated agar. The inoculated plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms. The potato dextrose agar plates were inoculated each with fungal culture by point (10 days old cultures) inoculation. The filter paper discs loaded with 100 and 200 µg/ml concentrations of the extracts were placed on test organism-seeded plates. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm (Taylor et al., 1995). Chloramphenicol and Fluconazole are used as standard antibiotics.

Minimum inhibitory concentration (MIC)

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacterium. Different concentrations of plant extracts ranging from 0.125 to 8 $\mu g/ml^{-1}$ were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculums of respective bacteria (10^5 CFU ml-1) and kept at 37°C for 24 h. The test tube containing the lowest concentration of extract which showed reduction in turbidity when compared with control was regarded as MIC of that extract (Muhamed et al., 2011).

Total activity (TA) determination

Total activity is the volume at which test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g (Sharma and Kumar, 2009).

AI = Activity Index (IZ developed by extract/IZ developed by standard).

RESULTS AND DISCUSSION

Methanol and aqueous extracts of two different plant species were examined for their antimicrobial activity against the isolated human pathogens and the results are in Tables 1 to 6. Both crude methanol and aqueous extracts of *D. elata* exhibited varying degrees of antimicrobial activities against the test organisms. The 200 µg/ml crude methanol extract showed higher inhibition zone than crude aqueous extract against *S. aureus, S. pneumoniae, B. cereus* and *S. flexneri*, respectively (Table 1). Similarly, 200 µg/ml methanol extract of *S. campanulata* exhibited inhibition zone of 18 mm (AI = 0.666) for *S. pneumoniae* and 14 mm (AI = 0.615) for *S. aureus,* respectively.

The aqueous extract showed highest inhibition zone of 9 mm in (AI = 0.346) for *S. aureus* and 8 mm (AI = 0.296) for *E. coli* (Tables 2 and 6). Antibiotics chloramphenicol and fluconazole have shown greater inhibition zone diameter than that of plant extracts. It had the inhibition zone in the range of 15 to 28 mm. There sre no fungal activities in both plant species. Methanol extract of *D. elata* showed least MIC value that is, 0.125 µg/ml (MBC = 0.250 µg/ml) against S*. aureus* while aqueous extract had moderate activity at 4 µg/ml (MBC = 4 µg/ml) concentration (Table 3). Similarly, the *S. campanulata* methanol extract was found to be highly effective as it has shown very low MIC value (0.125 µg/ml) against *S. aureus* (Table 4). The total activity was highest for methanol extracts of both plants (3.6 and 4.0 ml/g) against *S. aureus* (Tables 5 and 6). Our results support this view as methanol extracts had comparatively more inhibition action

Table 1. Antimicrobial activity of crude extracts of *D. elata* (Bojer ex Hook.) Raf.

Name of the strain	Zone of Inhibition (mm)				
	Methanol (µg/ml)		Aqueous (µg/ml)		Chloramphenicol
	100	200	100	200	
Staphylococcus aureus	10	16	-	10	26
Streptococcus pneumoniae	11	14	-	8	21
Bacillus cereus	-	13	-	-	19
Escherichia coli	-	11	-	-	27
Pseudomonas aeruginosa	8	10	-	-	18
Klbseillae pneumoniea	-	9	-	8	22
Salmonella typhi	10	12	-	-	29
Proteus vulgaris	-	10	-	-	20
Shigella flexneri	-	13	-	-	28
Antifungal activity					**Fluconazole**
Candida albicans	-	-	-	-	15
Aspergillus niger	-	-	-	-	17

Table 2. Antimicrobial activity of crude extracts of *S. campanulata* P. Beauv.

Name of the strain	Zone of Inhibition (mm)				
	Methanol (µg/ml)		Aqueous (µg/ml)		Chloramphenicol
	100	200	100	200	
Staphylococcus aureus	10	14	-	9	26
Streptococcus pneumoniae	10	18	-	-	21
Bacillus cereus	-	-	-	-	19
Escherichia coli	-	12	-	8	27
Pseudomonas aeruginosa	8	11	-	-	18
Klbseillae pneumoniea	-	-	-	-	22
Salmonella typhi	-	10	-	-	29
Proteus vulgaris	-	-	-	-	20
Shigella flexneri	-	12	-	-	28
Antifungal activity					**Fluconazole**
Candida albicans	-	-	-	-	15
Aspergillus niger	-	-	-	-	17

Table 3. The MIC index of methanol and aqueous extracts of *D. elata* (Bojer ex Hook.) Raf.

Name of the strain	Methanol			Aqueous		
	MIC (µg /ml)	MBC (µg /ml)	MIC_{index}	MIC (µg /ml)	MBC (µg /ml)	MIC_{index}
S. aureus	0.125	0.250	2	4	4	1
S. pneumoniae	0.250	0.500	2	-	-	-
B. cereus	1	0.500	0.5	-	-	-
E. coli	1	0.500	0.5	-	-	-
P. aeruginosa	0.500	2	4	-	-	-
K. pneumoniae	2	4	2	-	-	-
S. typhi	1	2	1	-	-	-
P. vulgaris	2	1	0.5	-	-	-
S. flexneri	2	0.500	0.25	-	-	-
C. albicans	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Table 4. The MIC index of methanol and aqueous extracts of *S. campanulata* P. Beauv.

Name of the strain	Methanol			Aqueous		
	MIC (µg /ml)	MBC (µg /ml)	MIC_{index}	MIC (µg /ml)	MBC (µg /ml)	MIC_{index}
S. aureus	0.125	0.250	2	4	4	1
S. pneumoniae	0.250	0.500	2	-	-	-
B. cereus	-	-	-	-	-	-
E. coli	0.500	0.500	1	-	-	-
P. aeruginosa	1	2	1	-	-	-
K. pneumoniae	-	-	-	-	-	-
S. typhi	2	2	1	-	-	-
P. vulgaris	-	-	-	-	-	-
S. flexneri	0.500	0.500	1	-	-	-
C.albicans	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Table 5. Activity index and total activity of crude extracts of *D. elata* (Bojer ex Hook.) Raf.

Name of the strain	Methanol			Aqueous		
	Activity index		Total activity (ml/g)	Activity index		Total activity (ml/g)
	100	200		100	200	
S. aureus	0.384	0.615	3.6	-	0.384	0.1
S. pneumoniae	0.523	0.666	1.8	-	0.380	-
B. cereus	-	0.684	0.45	-	-	-
E. coli	-	0.407	0.45	-	-	-
P. aeruginosa	0.444	0.555	0.9	-	-	-
K. pneumoniae	-	0.409	0.225	-	0.363	-
S. typhi	0.344	0.413	0.45	-	-	-
P. vulgaris	-	0.500	0.225	-	-	-
S. flexneri	-	0.464	0.225	-	-	-
C.albicans	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Table 6. Activity index and Total activity of crude extracts of *Spathodea campanulata* P. Beauv

Name of the strain	Methanol			Aqueous		
	Activity index		Total activity (ml/g)	Activity index		Total activity (ml/g)
	100	200		100	200	
S.aureus	0.384	0.538	4	-	0.346	0.1
St. pneumoniae	0.476	0.857	2	-	-	-
B. cereus	-	-	-	-	-	-
E.coli		0.444	1	-	0.296	-
P.aeruginosa	0.444	0.611	0.5	-	-	-
K. pneumoniae	-	-	-	-	-	-
S. typhi	-	0.344	0.25	-	-	-
P.vulgaris	-	-	-	-	-	-
S. flexneri	-	0.428	1	-	-	-
C.albicans	-	-	-	-	-	-
A.niger	-	-	-	-	-	-

than aqueous extracts (Hugo et al., 2005).

Several workers have reported that many plants possess antimicrobial properties including the parts, that is, flower, bark, stem, leaf, etc (Doss et al., 2009a, b; Sasikumar et al., 2006; Venkataswamy et al., 2010). Recently, a number of plants have been reported for antimicrobial properties across the world. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (Palombo and Semple, 2001). In the present study, the methanol extracts of the selected plants also showed zone of inhibition against the isolated human pathogens with varied diameter. In conclusion, the methanol extracts of both plants possess broad spectrum of antibacterial activity against the test bacteria species. The results obtained from this work give high hope for the development of new antibacterial agents.

REFERENCES

Abd El G, Hegazi M (2011). In vitro studies on *Delonix elata* L.-an endangered medicinal plant. World Appl. Sci. J. 14(5):679-686

Adriana P, Jurandir PP, Dalva TF, Noemia KI, Raimundo PF (2007). Iridoid glucose and antifungal phenolic compounds from *Spathodea campanulata* roots, Cien. Agrar, 28:251-256.

Bauer AW, Kirby WMW, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45:494-496.

Doss A, Dhanabalan R, Sasikumar JM, Palaniswamy M, GeethaV, Ayyappa DM (2009b). Antibacterial activity of twelve medicinal plants from Western Ghats. Pharmacology 1:83-89.

Doss A, Muhammed MH, Dhanabalan R (2009a). Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian J. Sci. Technol. 2(2):41-43.

Hugo JB, Anneleen K, Anders B, William RM, Inga H, Jolanta JL (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96:461-469.

Makinde JM, Amusan OOG, Adesogan EK (1988). The antimalarial activity of Spathodea campanulata stems bark extract on *Plasmodium berghei berghei* in mice. Plant Med. 54(2):122-25.

Mendes NM, Souza CP, Araujo N, Pereira JP, Katz N (1986). Atividade moluscicida de alguns produtos naturais sobre Biomphalaria Memorias do Instituto Oswaldo Cruz, Rio de Janeiro. 81:87-91.

Muhamed MH, Doss A, Dhanabalan R, Venkataswamy R (2011). Activity of some selected medicinal plants against Bovine Mastitis Pathogens. J. Anim. Vet. Adv. 6:738-741.

Niyonzima G, Laekeman G, Witvrouw M, VanPoel B, Pieters L, Paper D, Clercq E, Franz G, Vlietinck AJ (1999). Hypoglycemic, anticomplement and anti-HIV activities of *Spathodea campanulata* stem bark. Phytomedicine. 6(1):45-49.

Palombo EA, Semple SJ (2001). Antibacterial activity of traditional medicinal plants. J. Ethnopharmacol. 77:151-157.

Sasikumar JM, Pichai Anthoni Doss A, Doss A (2006). Antibacterial activity of *Eupatorium gladulosum*. Fitoterapia. 76(2):240-243.

Sharma B, Kumar P (2009). Extraction and Pharmacological Evaluation of Some Extracts of Tridax procumbens and Capparis deciduas. Int. J. Appl. Res. Nat. Prod. 1(4):5-12.

Taylor RSL, Manandhar NP, Hudson JB, Towers GHN (1995). Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethanopharmacol. 546:153-159.

Venkataswamy R, Muhamed Mubarack H, Doss A, Lakshmi Devi J, Sukumar M (2010). Preliminary phytochemical screening and Antimicrobial studies of *Lantana indica* Roxb. Indian J. Pharm. Sci. 72(2):66-68.

Yadav M, Khan KK (2012). Investigations of antibacterial activity of some ethnomedicinal plants against certain pathogenic bacterial strains. Indian J. Sci. 1(2):57-59.