Asian Journal of Chemistry Vol. 18, No. 1 (2006), 207–211

Antimicrobial Activity of *Aristolochia bracteata*

K.M. ELIZABETH* and Ch. SURYANARAYANA RAJU
Department of Microbiology, College of Science
Gandhi Institute of Technology and Management, Visakhapatnam-530 045, India
E-mail: kmelizabeth@yahoo.com

Antimicrobial activity of *Aristolochia bracteata* was tested against several human pathogenic microorganisms by disc diffusion method. The methanol extract of fruits gave rise to three substances, namely, cold methanol extract, distilled methanol extract and residual methanol extract. Crude aqueous extract was also tried. The crude extract at a concentration of 4 mg/disc showed zones of inhibition ranging from 11–27 mm. This showed highest zone of inhibition against *S. aureus, i.e.*, 27 mm. The distilled methanol extract exhibited a range of 12–17 mm zones, cold extract 14–22.5 mm and the residual extract 15–22.5 mm. Methanol control showed zones ranging from 10–16 mm. The minimal inhibitory concentration of residual extract against these pathogens was determined which ranged from 50–800 μg/mL. The amylase and protease production was assessed in few strains and it was found that there was 78.2% reduction in the amylase activity of *Y. enterocolitica* and 100 and 60% reduction in amylase and protease production of *P. aeruginosa* following the treatment with residual extract of this fruit extract. From these observations it can be attributed that *A. bracteata* possesses strong antimicrobial activity.

Key Words: Antimicrobial activity, *A. bracteata*, Human pathogenic microorganisms.

INTRODUCTION

Aristolochia bracteolata (Aristolochiaceae) is synonymous with *A. bracteata, A. indica.* It is distributed throughout India. It is a shrubby, glabrous twiner, with long woody roots Fruits are hexagonal, septicidal, 6-valved capsules. The fruits taste bitter, acrid and stringent. They may be used as purgative, anthelmintic, against poisonous bites and stings, for bowel complaints, to reduce fever in children. Aristolochic acid, ceryl alcohol, β-sitosterol, KCl were isolated from leaves and in addition to these KNO_3 from roots was also isolated. Recently, magnoflorine and aristolochic acid-1 have been isolated from seeds[1]. Magnoflorine decreased arterial blood pressure in rabbits and induced hypothermia in mice. Aristolactam N-β-D-glucoside along with 3-β-hydroxyl stigmast-5-end-7-one from roots were isolated. LD.50 of aristolochic acid in mice was 14.32 mg/kg body weight. It did not prolong the life of tumour bearing mice or enhance immune system of mouse reticuloendothelial system, Recently, the methanol extract from roots of *A. bracteata* was tested against three microorganisms such as *E. coli, S. aureus* and *P. aeruginosa* and proved that it has replaced the synthetic preservatives of foods[2]. Several medicinal plants with different medicinal qualities and antimicrobial properties have been reported so far[3–10]. But the fruit extract of *A. bracteata* and its effect on different other microorganisms was not tested.

Therefore, an attempt was made to study the antimicrobial activity of *A. bracteata* fruit (methanol) on different human pathogenic microorganisms.

EXPERIMENTAL

In the present study, the fruits of *A. bracteata* were collected from Rajamandri, Andhra Pradesh, India, and were shade dried. Later the fruits were made into a fine powder (1/2 kg) and mixed in 2.5 L of methanol in a 5 L aspirator bottle. Two days later 20 mL of the surface layer was collected as cold methanol extract. Then the solution was taken in a distillation unit and distillation was performed. The distillate also contained the fruit extract instead of having only methanol, which was determined by the green colour and designated as distilled methanol extract in the present study which was tested. The residual extract (25 g) was in dark greenish black colour which was tested against the pathogens. The residual extract was diluted in methanol to give a concentration of 20 μg/μL and used as stock solution which was stored at 4°C, for all experiments.

Microorganisms: The following microbial cultures were obtained from MTCC, Chandigarh, India. *Staphylococcus aureus* (ATCC 23564), *Salmonella typhi* (ATCC 10749), *Salmonella typhimurium* (ATCC ATCC 23564), *Yersinia enterocolitica* (ATCC 9610), *Escherichia coli* (ATCC 8739), *E. coli* (isolated from drinking water, GITAM) *Pseudomonas aeruginosa* (ATCC 25619), *Candida albicans* (ATCC 2091), *Rhizopus* species and *Serratia marsescence.*

Microbial culture conditions: The bacterial cultures were maintained on nutrient agar slants or plates (peptone 0.5%, beef extract 0.3%, NaCl 0.5%, agar 2.0%) and fungal cultures on Sabouraud's agar slants or plates (mycological peptone 1%, dextrose 2–4%, agar 2%). Overnight cultures were used in all experiments by inoculating a single colony of each type of culture in respective 5 mL broth and incubating at 37°C for 18–24 h (bacteria) or at room temperature for 48 h (fungi).

Disc diffusion method: Nutrient agar plates or Sabouraud's agar plates were inoculated with 0.1 mL of each fresh culture containing 10^7 cells by spread plate method. Later, five sterile filter paper discs (5 mm) and reference antibiotic discs such as ampicillin (10 μg) and nystatin (100 units/disc) procured from Himedia, Mumbai, were placed in corresponding plates. On one filter paper disc, 4 mg of crude aqueous extract of fruit in 20 μL volume; on the 2nd disc, 20 μL of cold methanol extract; on 3rd disc, 400 μL of residual extract in 20 μL volume; on 4th disc, distilled methanol with fruit extract of 20 μL and finally on 5th disc, methanol control of 20 μL was dropped. Later one reference antibiotic or antifungal disc was placed in each corresponding plate and the plates were kept at room temperature for 1 h for the plant extracts to be diffused into the medium. Then the plates were incubated at 37°C for 18–24 h (bacteria) and at room temperature (29°C) for 48 h (fungi).

Determination of minimal inhibitory concentrations (MICs): The minimal inhibitory concentrations were determined by broth dilution technique. Duplicates of serial dilutions of respective broth and various concentrations of plant extracts were made in sterile test tubes for 1 mL. The controls did not receive any extract. 50 μL of culture containing 10^3 cells was added to each test tube.

The suspension was mixed well by rotating between the palms and poured on to respective agar plates which were later incubated in the incubator at 37°C for 18–24 h or at room temperature for 48 h in case of fungi. Later, the lowest concentration of the plant extract that inhibited the growth of microorganisms was noted as minimal inhibitory concentration by counting the colony forming units.

The amylase and protease production: The amylase and protease produced by some of these microorganisms were tested by cultivating the microorganisms in the presence (at respective MIC) and absence of this plant extract. These assays were performed according to the method of Sawhney and Singh[11].

RESULTS AND DISCUSSION

The results are tabulated in Tables 1–3. The crude extract (Table-1) showed zones of inhibition ranging from 12.0–27 mm with *S. aureus* forming the largest zone indicating that this organism was highly sensitive to *A. bracteata* fruit extract. Similarly, *Rhizopus* species was least sensitive. The cold methanol extract of this fruit extract yielded zones of inhibition ranging from 14.5–22.5 mm, *S. aureus* with largest zone and *Rhizopus* with minimal zone, indicating that cold methanol extract was highly inhibitory towards *S. aureus* and least to *Rhizopus*. The distilled methanol was strongly effective towards *Y. enterocolitica* (17 mm) and least effective against *E. coli*, the drinking water isolate and *S. typhimurium*. The residual extract of *A. bracteata* was strongly effective to *S. aureus* forming 22.5 mm zone with 400 μg extract/disc. It was least effective to *P. aeruginosa* (15 mm). The pure methanol (control) showed zones ranging from 10–16 mm indicating that *Y. enterocolitica* was highly sensitive to it and methanol was less effective to all microorganisms than all extracts of this plant tried. Penicillin (10 μg/disc) was highly effective against *S. aureus* as it formed 25 mm zone. These results indicate that cold extract of 20 μL/disc was strongly effective than residual extract, crude extract and distilled methanol extract. It should be noted that the amount of crude and residual extracts used were higher than the cold extract. The MICs (Table-2) of residual extract ranged from 50–800 μg/mL with *S. aureus* and *S. typhimurium* lowest MIC values, indicating that this extract was strongly inhibitory to these organisms and least effective to *C. albicans*. These results of the present study differ from the results of Nogi *et al.*[2] who observed high MIC values with methanol extract of roots of *A. bracteata* towards *S. aureus*, *P. aeruginosa* (300 μg/mL) and against *E. coli* it was 750 μg, indicating that the fruit extract was more strongly effective than methanol extract of root. The amylase production by *Y. enterocolitica* (Table-3) when cultured in the absence of residual extract was 660 μg/mL which was reduced to 140 μg/mL in the presence of residual extract of *A. bracteata*, indicating that this extract inhibited the amylase activity. Similarly, there was 100% inhibition of amylase activity in *P. aeruginosa* following the treatment with this plant extract. The protease production by *S. marsescence* was 550 μg tyrosine/mL/h which was inhibited by 21% and in *P. aeriuginosa* by 60%. These results clearly indicate that *A. bracteata* fruit extract possesses strong antimicrobial activity.

TABLE-1
ANTIMICROBIAL ACTIVITY OF *ARISTOLOCHIA BRACTEATA*

Organism	Zone of inhibition (mm)					
	Crude extract (4 mg/disc)	Cold extract (20 µL)	Distilled methanol (20 µL)	Residual extract (400 µg)	Pure methanol (control) (20 µL)	Penicillin reference (10 units/disc)
S. aureus	27.0	22.5	15.5	22.5	10.5	25.0
S. marsescence	15.0	17.5	15.0	16.5	11.0	20.0
Y. enterocolitica	18.5	20.0	17.0	16.0	16.0	12.0
P. aeruginosa	12.5	15.0	12.5	15.0	11.5	13.5
E. coli (UTI)	15.0	20.5	13.0	15.5	12.0	12.5
E. coli (W)	15.5	16.0	12.0	15.5	12.0	12.5
S. typhimurium	15.5	16.0	12.0	15.5	12.0	16.0
Rhizopus	12.0	14.5	15.5	19.5	12.0	17.0*
C. albicans	12.5	20.0	15.0	19.5	10.0	20.0*

*Nystatin 100 units/disc.

TABLE-2
MINIMAL INHIBITORY CONCENTRATIONS OF *A. BRACTEATA*

Organism	ug/mL
S. aureus	50.0
S. marsescence	100.0
Y. enterocolitica	200.0
P. aeruginosa	200.0
E. coli (UTI)	200.0
S. typhimurium	50.0
E. coli (drinking water isolate)	200.0
Rhizopus	100.0
C. albicans	800.0

TABLE-3
EFFECT OF *A. BRATEATA* ON AMYLASE AND PROTEASE PRODUCTION

Organism	Amylase without PE (µg/mL)	% inhibition with PE (amylase)	Protease without PE (µg/tyrosine/h)	% Inhibition with PE (protease)
Y. enterocolitica	660.0	78.2	ND	—
P. aeruginosa	20.0	100.0	250.0	60.0
S. marsescence	ND	ND	550.0	21 .0

ND = not determined.

ACKNOWLEDGEMENTS

One of the authors, Dr. K.M. Elizabeth, is grateful to UGC, New Delhi for financial help. The authors are thankful to GITAM management for providing laboratory facilities.

REFERENCES

1. R.P. Rastogi, B.N. Mehrotra, Compendium of Indian Medicinal Plants, Vol. 2, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi (1999).
2. P.S. Nogi, G. Anandaramakrishnan and G. Kyaprakasha, *J. Med. Food*, **6**, 401 (2003).
3. K.M. Elizabeth, *Asian J. Microbiol. Biotech. Environ. Sci.*, **4**, 509 (2002).
4. ———, *Indian J. Microbiol.*, **43**, 73 (2003).
5. A. Yogamoorthi and G. Srikala, *Asian J. Micro. Biotech. Environ. Sci.*, **6**, 259 (2004).
6. S. Ramasamy and A.C. Manoharan, *Asian J. Micro. Biotech. Environ. Sci.*, **6**, 209 (2004).
7. C. Perez, A.M. Agnese and J.L. Cabrera, *J. Ethano. Pharmcol.*, **66**, 91 (1999).
8. C.S. Vairappan, *Asian J. Micro. Biotech. Environ. Sci.*, **6**, 197 (2004).
9. R.J. Capon, E.L. Ghisalberti and R.P. Jefferies, *Aust. J. Chem.*, **34**, 1175 (1981).
10. A. Martins, L. Salgueiro, M.J. Gancalves, A. Proenca da Cunha, R. Vila, S. Canigueral, V. Mazzoni, F. Tomi and J. Kasanova, *Planta Med.*, **67**, 580 (2001).
11. S.K. Sawhney and R. Singh, Introductory Practical Biochemistry, Narosa Publishing House, New Delhi, pp. 452 (2002).

(*Reccived*: 20 December 2004; *Accpeted*: 17 September 2005) AJC- 4386

17th INTERNATIONAL MASS SPECTROMETRY CONFERENCE

27 AUGUST–1 SEPTEMBER 2006

PRAGUE, CZECH REPUBLIC

Contact:
Conference Secretariat, Institute of Microbiology
Videnska 1083, CZ-14220, Prague 4
Czech Republic.
E-mail: info@imsc2006.org
Web: www.imsc2006.org