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Der Pharmacia Sinica, 2013, 4(3):75-79

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Phytochemical and antibacterial screening of root extracts of *Abroma augusta* Linn

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ABSTRACT

Phytochemical studies on the roots of Abroma augusta (A.augusta) revealed the presence of alkaloids, carbohydrates, steroids, tannins and saponins. The petroleum ether, benzene, acetone, chloroform, alcohol and aqueous extracts of roots of A. augusta were screened for antibacterial activity against Escherichia coli (Clinical isolates), Staphylococcus epidermis (MTCC 435), Micrococcus luteus (MTCC 106), Pseudomonas aeruginosa (MTCC 424), B.cereus (MTCC 645), B.pumilus (Clinical isolates), and B.subtilis (MTCC 441). The results of the study showed that petroleum ether extract of roots of A.augusta demonstrated the best antibacterial activity.

Key words: *Abroma augusta*, medicinal plants, antibacterial activity

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INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased [1]. The problem of microbial resistance has become a global issue of concern, as about 70 % of the bacteria that cause infections in hospitals are resistant to at least one of the antibiotic most commonly used for treatment [2, 3].

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to even broad spectrum antibiotics raises the specter of untreatable bacterial infections and add urgency to the search for new infection-fighting strategies [4]. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural [3].

A renewed interest has occurred in the last few decades to search for phytochemical of native and naturalized plants for pharmaceutical and nutritional purposes with the recognition that plant-derived products have great potential as sources of pharmaceuticals [5]. Medicinal plants have been used for ages not only in the treatment of diseases but they also have provided a source of inspiration for novel drug compounds [6, 7].

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Worldwide, infectious disease are the major cause of death accounting for approximately one-

half of all deaths in tropical countries [8]. A major part of total population in developing countries still uses traditional folk medicine obtained from plant resources [9].

In recent years, herbal medicines have increasingly been using to treat infections difficult to manage [10]. According to the report of World Health Organization (WHO), 80% of the world population relies mainly on traditional therapies which involve the use of plant extracts or their active constituents [11]. Plants are known to produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria [12, 13].

Abroma augusta Linn (Sterculiaceae), commonly known as Ulatkambal (Bengali and Hindi) is a large spreading bushy shrub with fibrous bark and irritant hairs, widely distributed (native or cultivated) throughout the hotter part of India- U.P., Sikkim (3000 ft.), Khasia hills (4000 ft.) and Assam. The fresh viscid sap of the root bark is considered to be a valuable emmenagogue and uterine tonic. It is also used in dysmenorrhoea [14]. Some preliminary studies also reported that *A.augusta* possesses not only antihyperglycemic but also hypolipidemic effect [15]. No pharmacological study has been reported on *A.augusta* for its antibacterial activity, so this study was designed to evaluate the phytochemical and antibacterial properties of *A.augusta*, in order to establish it's another therapeutic property in folkloric use.

MATERIALS AND METHODS

Plant material

The roots of the plants were collected from local herbal garden of Dehradun in the month of May-June 2009. The plant was confirmed and taxonomically authenticated at the herbarium of the Forest Research Institute (FRI), Dehradun, (Uttarakhand), and a voucher specimen (Accession no. 157029) was deposited there for future reference.

Chemicals

All the chemicals used in the experiment were of analytical grade and obtained from Himedia laboratories Pvt. Ltd. Mumbai, India. Amoxicillin was obtained as a gift sample from Ranbaxy Laboratory Ltd., Dewas (M.P.)

Preparation of plant extracts

The roots of the plant were air dried under a shade, coarsely powdered in a grinder. Powdered dried part was extracted using soxhlet apparatus successively with petroleum ether, benzene, chloroform, acetone, ethanol and water. The various extracts were concentrated in vacuo at 40^{o} C using a rota vapor [16].

Phytochemical screening

The crude plant extracts were screened for phytochemical characteristics [17]. The various chemical constituents, extractive values, ash values and moisture content of the crude extracts were determined [18].

Antibacterial activity screening

The crude extracts of roots of *A.augusta* were screened for antibacterial activity against *Escherichia coli, Staphylococcus epidermis, Micrococus luteus, Pseudomonas aeruginosa, B.cereus, B. pumilus, B.subtilis*, collected from Microbiology department of Shri Guru Ram Rai Institute of Technology and Science, Dehradun. An inoculum of each bacterial strain was suspended in 5 ml of Mueller Hinton broth (MHB) and incubated overnight at 37ºC. The overnight cultures were diluted with Mueller Hinton broth and adjusted to give a concentration of bacterial cells equivalent to a McFarland 0.5 standard prior to the bacterial testing [19].

Antibacterial activity of the crude extracts was investigated against bacterial strains by the paper disk diffusion technique [20] using Mueller-Hinton agar (Hi Media, India) plates. The Mueller-Hinton agar plates were swabbed with inoculum which had turbidity equal to a 0.5 Mcfarland standard (1.5×10^{8} CFU/mL). The extract was dissolved in DMSO (dimethylsulphoxide) to make a 1 gm/ml solution and then filtered. Paper discs (6 mm diameter) soaked (50 µl) in single concentration (500 mg/ml, 250 mg/ml, 125 mg/ml) of each extract were placed on Mueller Hinton agar medium previously inoculated with a bacterial suspension. All plates were incubated at 37^{o}C for 24 hr and the diameter of zones of inhibition was subsequently measured using zone reader and was compared to that of standard antibiotic, Amoxicillin (5 mg/ml) [21]. For negative control DMSO paper discs were used, prepared by dipping the disc into the 10% DMSO. The plates were incubated at 37^{o}C for 24 hrs and examined for zone of inhibition of growth and there was no inhibition to the growth of the microorganism all through it.

RESULTS

Preliminary phytochemical analysis

Preliminary qualitative tests of various extracts of *A.augusta* showed presence of alkaloids, carbohydrates, steroids, tannins and saponins. Extractive values of different extracts were 0.96% w/w (petroleum ether extract), 0.45%w/w (benzene extract), 0.86% w/w (chloroform extract), 0.98% w/w (acetone extract), 2.01% w/w (alcohol extract) and 11.67% w/w (aqueous extract). Loss on drying was 5.3% w/w and different ash values were 4.85% w/w (total ash), 1.55% w/w (acid insoluble ash) and 2.56% w/w (water soluble ash). (**Table: 1 and 2)**

Table: 1 Preliminary qualitative test of different extracts of roots of *Abroma augusta*

Phytoconstituents/solvents	PEE	BE	ACE	CE	AlcE	AqE
Alkaloids	-	+	-	+	+	+
Carbohydrates	-	-	+	+	+	-
Fats and oils	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Gums and mucilage	-	-	-	-	-	+
Proteins	-	-	-	-	-	-
Steroids	+	+	-	-	-	-
Tannins	+	-	+	-	+	+
Saponins	+	-	-	-	-	+

'+' = Present, '-' = Absent,
PEE = Petroleum ether extract, BE= Benzene extract, ACE = Acetone extract
CE = Chloroform extract, AlcE = Alcohol extract, AqE = Aqueous extract

Table: 2 Physiochemical analysis of root extract of *Abroma augusta*

S. No.	Parameters	Observation
1.	**Extractive Values**	
	a)Petroleum Ether	0.96% w/w
	b) Benzene	0.45%w/w
	c)Chloroform	0.86% w/w
	d) Acetone	0.98% w/w
	e)Alcohol	2.01% w/w
	f) Aqueous	11.67% w/w
2	**Loss on drying**	5.3% w/w
3	**Ash values**	
	a) Total ash	4.85% w/w
	b) Acid insoluble ash	1.55% w/w
	c) Water soluble ash	2.56% w/w

Antibacterial screening

In the present investigation antibacterial activity of various extracts of roots of *A.augusta* was assessed by measuring zone of inhibition. The results of experiment revealed that among all the extracts, the petroleum ether extract showed the best activity followed by acetone extract. Upon statistical analysis, the pet. ether extract showed no significant difference with the control group (amoxicillin 5 mg/ml) against *E.coli* (ZI=16.3), *Staphylococccus epidermis* (ZI=14), *Pseudomonas aeruginosa* (ZI=16.5), *Bacillus cereus* (ZI= 16) and *Bacillus subtilis* (ZI=16) at dose of 500 mg/ml. Pet. ether extract also exhibited no significant difference with control group against *B. pumilis* at dose of 250 mg/ml and 500 mg/ml (ZI=14.8 and 17.4 respectively). The extract showed significant difference with control group against *Micrococcus luteus* at all dose levels.

Least activity against all the microorganism was observed with benzene extract at dose of 250 and 500 mg/ml, whereas no activity was observed with aqueous extract against all the microorganisms except slight inhibition of *E.coli*. The results of the study showed that the pet. ether extract of the drug is most active against *B.pumilus* even at low dose when compared to other microorganisms **(Table-3)**.

Table: 3 Antibacterial activity of root extracts of *Abroma augusta*

Microorganisms	Mean Zone of inhibition (mm)																		Amoxycillin (5 mg/ml)
	125 mg/ml						250mg/ml						500mg/ml						
	P	B	A	C	E	Aq.	P	B	A	C	E	Aq.	P	B	A	C	E	Aq.	
E. coli (clinical isolates)	08 ±0.6	0.0 ±0.0	06 ±0.5	5.3 ±0.1	5.0 ±0.3	3.5 ± 0.5	12.4 ±0.3	2.5 ±0.6	10.3 ±0.3	7.6 ±0.6	6.8 ±0.6	4.0 ±0.5	16.3* ±0.4	4.3 ±0.1	12.0 ±0.3	8.6 ±0.5	8.4 ±0.8	4.5 ±0.6	16.5 ±0.5
S. epidermis (MTCC 435)	09 ±0.2	0 ±0	8 ±0.1	5 ±0.2	5 ±0.3	0 ±0	12.5 ±0.1	1 ±0.1	10 ±0.2	6 ±0.5	7 ±1	0 ±0	14* ±0.6	2 ±0.12	12 ±0.4	5 ±0.4	11 ±0.6	0 ±0	15 ±0.5
M. luteus (MTCC 106)	6 ±0.3	0 ±0	6 ±1	5.5 ±0.6	8.3 ±0.4	0 ±0	12.5 ±0.6	0 ±0	7 ±0.9	8 ±0.2	8 ±0.5	0 ±0	15 ±0.5	3 ±0.1	13 ±0.7	10 ±0.5	9 ±0.2	0 ±0	18.5 ±0.5
Pseudomonas aeruginosa (MTCC 424)	7 ±0.5	0 ± 0	6 ± 0.3	5 ± 0.5	3 ± 0.4	0 ±0	13.6 ± 0.5	1 ±0	8 ±1	6.6 ±0.8	5 ±0.2	0 ±0	16.5* ±0.6	2 ±0	11 ±0.3	6 ±0.2	7.2 ± 0.4	0 ±0	17.5 0.4
B. pumilus (Clinical isolates)	7 ±0.4	0 ± 0	8 ±0.4	6 ± 0.1	6 ±0.4	0 ±0	14.8* ± 0.3	2 ±0.1	10 ±0.12	9 ±0.6	10.5 ± 0.4	0 ±0	17.4* ± 0.5	2 ±0.3	13 ±0.3	12 ±0.81	10 ±0.4	0 ±0	16 ±1
B. cereus (MTCC 645)	6.5 ±0.4	0 ±0	6 ±0.5	5.5 ±0.3	2 ± 0.3	0 ± 0	13 ±0.6	5 ±0.1	7 ±0.3	8.5 ±0.5	3 ±0.1	0 ±0	16* ±0.6	6 ±0.5	12.2 ± 0.5	10 ± 0.4	5.5 ±0.3	0 ± 0	15.3 ±0.5
B. subtilis (MTCC 44)	7.5 ±0.3	0 ± 0	6 ±1	4.5 ±0.3	5 ±0.3	0 ±0	13.6 ±0.3	2 ±0.5	9 ±1	8.5 ±0.7	7 ± 0.3	0 ±0	16.0* ±0.6	3 ±0.1	13 ±0.2	9.0 ± 0.4	10 ±0.2	0 ±0	17 ±1

** no significant difference when compared to active control group*

Zone of inhibition (mm) are average of triplicate experiments. Disc diameter = 6 mm

P: petroleum ether, B: benzene, A: acetone, C: chloroform, E: ethanol, Aq.: aqueous

DISCUSSION

Infectious diseases are the world's leading cause of premature deaths. In recent years, drug resistance to human pathogenic bacteria has commonly been reported from all over the world. Even though pharmaceutical companies produce number of new antibacterial drugs, but gradual resistance to these drugs has increased which is matter of global concern besides synthetic drugs are normally associated with side effects (hypersensitive, immune suppression etc). Use of phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments [22]. Present study is an effort towards this direction. The results of the study indicated that the roots of *A. augusta* possess antibacterial activity against various pathogens. Phytochemical analysis of the plant extract demonstrated the presence of alkaloids, tannins, steroids and saponins which are reported to have significant antimicrobial activity. Alkaloid, tannins, saponins and steroids have varying pharmacological effects on animals and scientists have shown that these metabolites play defensive roles in the plants producing them. From this work *A. augusta* is found to possess wide range of antibacterial activity against various microorganisms like *S. aureus*, *P.aeruginosa*, *E.coli*, *B.cereus* etc. These bacteria are known to cause infections and other ailments. Therefore the results justify the use of roots of *A. augusta* in the treatment of bacterial infection.

CONCLUSION

From the above results we can conclude that extracts of *A.augusta* has remarkable antibacterial activity and need further investigation to determine the active constituents responsible for antibacterial activity.

REFERENCES

[1] G. F. N. Gislene, L. Juliana, C. F. Paulo, L. S. Giuliana, *Braz J Microbiol*, **2000**, 31, 247-256.
[2] R. Bisht, A. Katiyar, R. Singh, P. Mittal, *Asian J Pharm Clin Res*, **2009**, 2 (2), 34-39.
[3] G.G.F. Nascimento, J. Locatelli, P.C. Freitas, G.L. Silva, *Braz J Microbiol*, **2000**, 31, 247-256.
[4] D. Janovska, K. Kubikova, L. Kokoska, *Czech J Food Sci*, **2003**, 21(3), 107–110.
[5] R.B. Joy, L.W. Donald, C.S. Craig, L.K. Kendra, R.G. Fulcher, J.E. Nancy, D.B. David, F.B. Russell, *J Med Plants Res*, **2009**, 3(10), 707-718.
[6] D.M. Eisenberg, R.B. Davis, S.L. Ettner, *JAMA*, **1998**, 280, 1569-75.
[7] T.F. Nuriye, T.C. Yeliz, Y.C. Ahmet, O. Duzgun, T. Esra, D. Belma, T. Necla, *Fitoterapia*, **2009**, (80), 48–50.
[8] M.I. Maurice, R.D. Angela, O.O. Chris,. New antimicrobial of plants origin, ASHS Press, Alexandria, VA, **1999**, 457- 462.
[9] M.P. Panthi, R.P. Chaudhary, *Scientific World*, **2006**, 4(4), 16-21.
[10] R.D. Lin, Y.P. Chin, M.H. Lee, *Phytother Res*, **2005**,(19), 612.11
[11] S. Gur, D.T. Balik, N. Gur, *World J Agric Sci*, **2006**, 2(4), 439-442.
[12] A. Basile, S. Giordano, J.A. López-Sáez, R.C. Cobianchi, *Phytochemistry*,**1999** (52):1479.
[13] G.A. Pesewu, R.R Cutler, D.P. Humber, *J Ethnopharmacol*., **2008**, 116, 102.
[14] K.R. Kirtikar, B.D. Basu, Indian Medicinal Plants, Sudhindra Nath Basu, Bahadur Ganj, Allahabad, **1918**, 210-211.
[15] M.H. Eshrat,. *Indian J Clin Biochem*, **2003**, 18 (2), 54-63.
[16] I.G. Ndukwe, J.D. Bello I.A., J.I. Okoh, *Afri J Biotech*, **2006**, 5(19), 1789-1791.
[17] G.E. Trease, W.C. Evans, Textbook of Pharmacognosy, 12th Edition, Balliere, Tindall, London, **1983**, 57-59 and 343-383.
[18] P.K. Mukharjee, Quality control of herbal drugs-an approach to evaluation of botanicals (Business Horizons Pharmaceutical Publication, New Delhi, **2002**) 183-197.
[19] A. Samie, C.L. Obi, P.O. Bessong, L. Namrita, *Afri J Biotech*, **2005**, 4(12), 1443-1451.
[20] A.W. Bauer, E. Kirby, E.M. Sherris, M.Turk, *Am J Clin Path*, **1966**, 45, 493-496.
[21] J. Parekh, S. Chanda. *Braz J Microbiol*, **2007**, 38, 204-207.
[22] G. Singh, P. Kumar, A. Jindal. *Int J Pharm Sci*, 4(3),159-162.