J. bio-sci. 18: 128-133, 2010 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index

ANTIBACTERIAL ACTIVITY IN THE LEAVES OF SEVEN BITTER MEDICINAL PLANTS OF BANGLADESH

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Abstract

Context: Development of resistance in human pathogens against conventional antibiotic necessitates searching indigenous medicinal plants having antibacterial property. Seven medicinal plants used actively in folklore, ayurvedic and traditional system of medicine were selected for the evaluation of their antimicrobial activity for this study.

Objectives: Evaluation of the effectiveness of some medicinal plant extracts against four Gram-positive and five Gram-negative bacteria.

Materials and Methods: The antibacterial activity of the crude ethanolic extracts obtained from the leaves of seven medicinal plants; viz., *Andrographis paniculata*, *Catharanthus roseus*, *Adhatoda vasica*, *Vitex vegundo*, *Aloe vera*, *Flacortia ramontchi* and *Nyctanthes arbortristis* were tested against nine bacteria at concentrations of 300-, 400- and 500 μg/ml. Standard antibiotic disc kanamycin (30μg/ml) was used for comparison. The minimum inhibitory concentration (MIC) of ethanolic extracts of the leaves of these medicinal plants were determined by testing the extracts on four Gram-positive and five Gram-negative bacteria by serial tube dilution method.

Results: All the extracts have notable antimicrobial activities against the test organisms. The ethanolic extracts of the leaves showed the highest antimicrobial activities against *Bacillus megaterium* and *Shigella dysenteriae* for *An. paniculata, Ad. vasica* and *Al. vera; Bacillus subtilis* and *Salmonella typhi* for *C. roseus* and *N. arbortristis*; *Staphylociccus aureus* and *Salmonella typhi* for *V. vegundo*; and *Bacillus subtilis* and *Shigella sonnei* for *F. ramontchi* respectively. The extract of the plants had MIC values ranging from 32 to 128 mg/ml. All plant extracts showed no MIC against *Shigella shiga* and against *Sarcina lutea* only *C. roseus* showed MIC 128 mg/ml.

Conclusion: The results revealed that the ethanolic extracts of the plants under present investigation have notable antimicrobial activities.

Keywords: medicinal plants, antimicrobial screening, MIC, bacteria.

Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Srivastava *et al.* 1996).

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel 1991). Although a significant number of studies have been used to obtain purified phytochemicals, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veeramuthu *et al*. 2006). The greater susceptibility of gram-positive bacteria to plant extracts has been previously reported in South American (Paz *et al*. 1995), African (Kudi *et al.* 1999, Vlietinck *et al*. 1995) and Australian (Palombo and Semple 2001) medicinal plant extracts. Susceptibility differences between gram-positive and gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics (Tortora *et al*. 2001).

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Andrographis paniculata (Burm.f.) Wall. ex Nees., also known commonly as 'King of Bitters' (fam, Acanthaceae) extract is traditionally used as a medicine to treat different diseases in India, China and Southeast Asia including Bangladesh. In Scandinavian countries, it is commonly used to prevent and treat common cold (Cacer *et al*. 1997). *Catharanthus roseus* (L.) G.Don, or *Vinca rosea* L. (fam. Apocynaceae) is a commonly found herb in Bangladesh and is traditionally used in diabetis. Its alkaloids have hypotensive, seductive and also anti-cancerous property. Ethanol extract of leaf possess wound-healing activity too (Shivananda 2006). *Adhatoda vasica* Nees (fam. Acanthaceae) is a primary herb of the ayurvedic system used in the treatment of coughs, bronchitis, asthma and symptoms of common cold (Karthikeyan *et al.* 2009). *Vitex negundo* Linn. (fam. Verbenaceae) is a large aromatic shrub distributed throughout the greater part of Indian subcontinent up to an altitude of 1500 m in the outer Himalayas. Almost all parts of the herb are useful as a drug but the leaves and roots are most important and sold as drugs (Kirtikar and Basu 1933). *Aloe vera* (L.) Burm. f. or *Aloe indica* Royle (fam. Liliaceae) is a succulent with its origin in African continent. Its thick leaves contain the water supply for the plant to survive long periods of drought. *Flacourtia ramontchi* L.'Herit (fam. Flacourtiaceae), commonly called Governor's Plum or Madaraskara Plum in English, is a native of tropical Africa and Asia and its leaf is used in inflammation, jaundice and as blood purifier (Kirtikar and Basu 1933). *Nyctanthes arbortristis* Linn. (fam. Oleaceae) commonly known as Night Jasmine is one of the well known medicinal plants. Different parts of *N. arbortristis* are known to possess various ailments by rural mainly tribal people along with its use in Ayurveda, Sidha and Unani systems of medicines. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic (Nadkarni 1982).

Inspite of having the reported usefulness of the extracts of these plants in folk-medicine, no such works on their antimicrobiological investigations have so far been reported in the literature. Our present investigation deals with the extraction and microbiological investigations of the extracts of the seven bitter medicinal plants of Bangladesh.

Materials and Methods

Plant samples and extraction: Leaves of seven medicinal plants viz, *An. paniculata*, *C. roseus*, *Ad. vasica*, *V. vegundo*, *Al. vera*, *F. ramontchi* and *N. arbortristis* under present investigation were collected dried and separately grinded with a grinding machine to get powered materials. The powdered leaves were separately extracted with ethanol and the extracts were concentrated under reduced pressure.

Source of test microorganisms: Four Gram-positive bacteria (*Bacillus megaterium, Staphylococcus aureus, Bacillus subtilis, Sarcina lutea*) and five Gram-negative bacteria (*Shigella shiga, Salmonila typhi-A, Escherichia coli, Shigella sonnei, Shigella dysenteiae*) were collected from the Department of Pharmacy and the Department of Biochemistry and Molecular Biology, University of Rajshahi for determination of antimicrobial activity.

Preparation of fresh culture, standard discs and test samples: Slants were prepared by dispersing about 5 ml of nutrient agar media in each test tube. The test tubes were sterilized in an autoclave at 121^{o}C under a pressure of 15 lb/sq. inch. for 15 min. The sterilized test tubes were held in inclined position for solidification. The test tubes were then incubated at 37.5°C. The test organisms were transferred from the culture medium to the slants. These were then incubated at 37.5°C for 24 h. These fresh cultures were used within 2-3 days.

Antibacterial activity: Antibacterial activity of the ethanolic extracts were evaluated by the disc diffusion method (Bauer *et. al.* 1966). The extracts were compared using kanamycin (30 μg/disc) as standard disc. Filter paper along with residual solvent was used as negative control. Crude ethanolic extracts of the leaves of above medicinal plants were dissolved separately in methanol to produce solutions having concentrations of 300-, 400- and 500 μg/μl. Fresh culture of the test organisms in the slants were transferred into the plates

of different pretridishes. Sample discs and standard disc were placed on the solidified agar plates. These were kept at 4°C in a refrigerator for 24 h in order to diffuse the extracts and antibiotics in the culture medium. The plates were incubated at 37.5°C for 24 h. The antimicrobial activities of the crude ethanolic extracts were estimated by measuring the diameter (mm) of the zone of inhibition of the organism. In each case, this was compared to that of standard sample.

Determination of minimum inhibitory concentrations (MIC): The above mentioned bacteria were utilized for determining the MIC of the ethanolic extracts. Nutrient broth medium and culture media were prepared following the standard methods (Reiner 1982). Kanamycin (30 μg/disc) was used as standards disc. Crude ethanolic extracts of the leaves of the above medicinal plants were transferred in separate vials containing 2% DMSO solution (2 ml). This was mixed well to achieve sample solutions having concentration 1024 μg/ml. Nine sterilized test tubes containing 1-, 2-, 4-, 8-, 16-, 32-, 64-, 128- and 256 μg/ml sample solutions were prepared by serial dilution. Three test tubes containing media (C_M), media plus sample (C_{MS}) and media plus inoculums (C_{MI}) were also maintained. Diluted inoculums (10 μl) was added to each of the nine test tubes and mixed well. One ml of the sample was added to C_{MS} and mixed well. 10 μl of inoculums was added to C_{MI} to observe the growth of the organisms in the media. C_M containing media was used the check the sterility of the solution. The test tubes were incubated at 37.5°C for 24 h. The lowest concentration of the extracts which inhibited microbial growth was recorded as the MIC.

Results

The diameters of the zones of inhibition derived by ethanolic extract of the leaves of seven plants are presented in Tables 1 and 2. *An. paniculata* shows maximum activity against *B. megaterium* and *Shigella dysenteriae*. No activity of the extract on *Sarcina lutea, Shigella shiga*, *Shigella sonnei* was experienced. The zones of inhibition produced by *C. roseus* were *Staphylociccus aureus*, *B. subtilis*, *Sarcina lutea, Salmonella typhi, E. coli* and *Shigella dysenteria* and the maximum activity was against *B. subtilis* and *Salmonella typhi*. Activity of the extract against *B. megaterium, Shigella shiga* and *Shigella sonnei* was not found. *Ad. vasica* shows the maximum activity against *B. megaterium* and *Shigella dysenteriae*. The extract had no or little activity against *Sarcina lutea*, *Shigella shiga* and *Salmonella typhi*. *V. vegundo* showed the maximum activity on *Staphylociccus aureus* and *Salmonella typhi*. The extract had a little activity on *Sarcina lutea*, *Shigella shiga* and *Shigella sonnei*. *A. vera* had the maximum activity against *B. megaterium* and *E. coli*. The extract showed no activity on *Sarcina lutea*, *Shigella shiga* and *Shigella sonnei*. *F. ramontchi* showed maximum activity against *B. subtilis* and *Shigella sonnei*. The activity of the extract against *Sarcina lutea*, *Shigella shiga* and *Salmonella typhi* was not found. *N. arbortristis* was very active against *B. subtilis* and *Salmonella typhi*. The activity of the extract against *B. megaterium*, *Sarcina lutea* and *Shigella shiga* was not found.

The MIC values of ethanolic extracts of the leaves of the above medicinal plants are shown in Table 3. It is evident from the table that all extracts have notable antimicrobial activities against the test organisms. The extract of the plants had MIC values ranging from 32 to 128 mg/ml. All plant extracts showed no MIC against *Shigella shiga* and against *Sarcina lutea* only *C. roseus* showed MIC 128 mg/ml

Discussion

Sule *et al.* (2010) evaluated non-polar (dichloromethane) and polar (MeOH and aqueous) extracts of *An. paniculata* (whole plant) for *in vitro* antibacterial activity against 12 skin disease causing bacterial strains using the disc diffusion method at three concentrations; 1000, 500, and 250 μg/disc respectively and found significant antibacterial activities against both the Gram-positive and Gram-negative bacterial strains tested. Diterpenoids and flavonoids are the main chemical constituents of *An. paniculata* which are believed to be responsible for the most biological activities of this plant (Tang and Eisenbrand 1992). *C. roseus* has a variety of medicinal properties, such as antibacterial (Carew and Patterson 1970, Raza *et al*. 2009),

Table 1. Antimicrobial activities of ethanolic extracts of *Andrographis paniculata*, *Catharanthus roseus*, *Adhatoda vasica* and *Vitex vegundo* leaves and standard Kanamycin (30 μg/disc)

Bacteria	Diameter of zone of inhibition (mm)															
	Andrographis paniculata				*Catharanthus roseus*				*Adhatoda vasica*				*Vitex vegundo*			
	Extract μg/ml			Kana-mycin μg/ml	Extract μg/ml			Kana-mycin μg/ml	Extract μg/ml			Kana-mycin μg/ml	Extract μg/ml			Kana-mycin μg/ml
	300	400	500	30	300	400	500	30	300	400	500		300	400	500	30
Gram-positive																
Bacillus megaterium	11	12	13	18	-	-	-	23	13	14	16	24	13	15	16	20
Staphylococcus aureus	10	11	12	23	15	17	19	30	11	12	14	31	15	17	18	31
Bacillus subtilis	09	10	12	17	16	18	19	24	12	13	14	27	15	16	18	24
Sarcina lutea	-	-	-	18	13	16	17	20	-	-	-	21	-	-	-	21
Gram-negative																
Shigella shiga	-	-	-	19	-	-	-	22	-	-	-	22	-	-	-	20
Salmonila typhi-A	11	12	16	24	14	16	18	25	-	-	-	29	13	14	17	26
Escherichia coli	10	12	14	23	13	15	19	23	12	15	17	23	12	14	18	23
Shigella sonnei	-	-	-	16	-	-	-	21	13	14	16	26	-	-	-	20
Shigella dysenteiae	12	14	18	20	11	13	16	23	15	16	18	21	11	13	14	19

Table 2. Antimicrobial activities of ethanolic extracts of *Aleo vera*, *Flacortia ramontchi* and *Nyctanthes arbortristis* leaves and standard Kanamycin (30 μg/disc)

Bacteria	Diameter of zone of inhibition (mm)											
	Aleo vera				*Flacortia ramontchi*				*Nyctanthes arbortristis*			
	Extract μg/ml			Kanamycin μg/ml	Extract μg/ml			Kanamycin μg/ml	Extract μg/ml			Kanamycin μg/ml
	300	400	500	30	300	400	500	30	300	400	500	
Gram-positive												
Bacillus megaterium	12	12	15	19	12	13	13	24	-	-	-	20
Staphylococcus aureus	09	12	13	27	13	14	15	28	21	23	25	27
Bacillus subtilis	10	13	14	17	13	15	17	30	23	26	28	31
Sarcina lutea	-	-	-	20	-	-	-	22	-	-	-	17
Gram-negative												
Shigella shiga	-	-	-	19	-	-	-	21	-	-	-	19
Salmonila typhi-A	12	14	15	30	-	-	-	30	20	24	28	29
Escherichia coli	13	14	16	23	14	15	18	19	17	18	24	25
Shigella sonnei	-	-	-	17	14	16	19	23	16	20	26	29
Shigella dysenteiae	14	16	19	19	12	14	15	20	17	19	21	22

Table 3. Minimum inhibitory concentration (μg/ml) of the crude ethanolic extracts.

Bacteria	*Andrographis paniculata*	*Catharanthus roseus*	*Adhatoda vasica*	*Vitex vegundo*	*Aleo vera*	*Flacortia ramontchi*	*Nyctanthes arbortristis*
Gram-positive							
Bacillus megaterium	32	-	32	64	32	128	-
Staphylococcus aureus	128	64	128	32	128	64	64
Bacillus subtilis	128	32	128	64	128	32	32
Sarcina lutea	-	128	-	-	-	-	-
Gram-negative							
Shigella shiga	-	-	-	-	-	-	-
Salmonila typhi-A	64	32	-	32	64	-	32
Escherichia coli	64	64	64	128	32	64	128
Shigella sonnei	-	-	64	128	-	32	128
Shigella dysenteiae	32	128	32	128	128	128	64

antifungal (Jaleel *et al.* 2007) and antiviral (Farnsworth *et al*. 1968). The alkaloids from *C. roseus* are famous for their anticancer activity (El-Sayed and Cordell 1981). Studies revealed its wound healing action in the rats (Nayak and Pinto-Pereira 2006). One of the isolated endophytes produced potential antimicrobial activity against some selected human pathogenic bacteria and a yeast (Roy and Banerjee 2010).

The previous results of the phytochemical analysis of *Ad. vasica* show that phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars are present in the leaves. It has also been shown that tannins are biologically active, against *E. coli Staphylococcus aureus*, *Salmonila paratyphi* and *Candida albicans* (Nair and Chandra 2004). The Preliminary phytochemical screening of successive extracts indicated presence of lipids, flavonoids, saponins, alkaloids, tannins, carbohydrates, terpenoids, and steroids in *F. ramontchi* leaves (Hardik *et al.* 2010). Antimicrobial susceptibility test showed that both the gel and the leaf of *Al. vera* inhibited the growth of *S. aureus* (Agarry *et al.* 2005). Methanol extract of *F. ramontchi* leaves possess broad-spectrum antimicrobial activity at concentration 10000 µg/ml whereas hydromethanolic and chloroform extracts having more or less antimicrobial activity (Lalsare 2011). Leaves extracts of *N. arbortristis* was found to have antimicrobial activity (Khandelwal *et al.* 1999). The chloroform extract of this plant was found to have both antibacterial and antifungal activity whereas the petroleum ether and ethanol extracts possess only antibacterial activity (Manisha *et al*. 2009, Verma *et al*. 2011).

Different extracts of *Vitex negundo* leaves were investigated by Aswar *et al*. (2009) for its antimicrobial and antifungal activity on five bacterial species and three fungal species. Among all extracts water-ethanol (50:50) extract showed maximum anti microbial and water extract showed maximum antifungal activity against all tested species. Most of the bacterial pathogens like *Salmonella paratyphi, Klebsiella pneumonia, Vibrio choera, Streptococcus mutans* and *E. coli* were found to be susceptible in leaf extracts of the *Vitex negundo* (Rose and Cathrin 2011). The present results supports the above findings.

Conclusion

From the foregoing evidences, we can conclude that the ethanolic extracts of the plants under present investigation possess appreciable antibacterial activities. The MIC values of the extracts have remarkable significance about the therapeutic effects of the active principles associated with the leaves of the plants.

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