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Antibacterial activity of some selected medicinal plants used by the Marma and Tripura indigenous communities of Bandarban and Chittagong districts of Bangladesh

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ABSTRACT

Thirty-one medicinal plants of 24 families, used by the indigenous people of Bangladesh for health care, were screened and bio-prospected for their antibacterial activity against eight pathogenic bacteria (Bacillus subtilis, B. megaterium, B. cereus, Staphylococcus aureus, Escherichia coli, Vibrio cholerae, Salmonella typhi and Shigella dysenteriae) using disc diffusion and agar cup assays. Twenty six plants showed positive antibacterial efficacy with Phyllanthus emblica, Terminalia chebula, Justicia adhatoda, Ocimin sanctum, Solanum torvum and Flemingia stricta depicting the most significant zone of inhibition. Maximum zone of inhibition (21mm) was recorded against V. cholera with the fruit extracts of Solanum torvum. The present study supports the traditional uses of medicinal plant by the indigenous communities for treating microbial infections and could constitute a potential source for the discovery of new antibiotics.

INTRODUCTION

Plants parts and their extracts have served as important ingredients for the preparation of traditional medicines and, constitute an important natural wealth of a country. There are substantial numbers of such medicinal plants in Bangladesh [6]. More than five thousand vascular plant species are present throughout the forests, hills, plains, crop fields, marshy lands and homegardens of Bangladesh [27], of which about 750 species have been reported to be used in traditional medicines to provide affordable health care to the millions of people in this country [11, 40, 41]. Some of these medicinal plants from Bangladesh are also being used in the preparation of Kabiraji, Hakimi, Unani, Ayurvedic, Homeopathic and Allopathic drug preparations [9, 20, 39]. There are about 35 smaller groups of Indigenous communities in Bangladesh (covering about 2% of the total population) that have been living in different pockets of the hilly zones and plain lands of the country. Among these, 12 communities live in Chittagong and Chittagong Hill Tracts districts of Bandarban, Khagrachari, and Rangamati like Bawm, Chak, Chakma, Khyang, Khumi, Lushai Marma, Mro, Pangkhoa, Tanchangya, Rakhaine and Tripura communities [31, 36]. These indigenous communities have vast traditional knowledge of herbal treatments [2, 30,

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31, 36, 41]. Of them, two indigenous communities namely Marma and Tripura have been selected for the present study. Different areas of Chittagong (Hazarikhil) and Bandarban (Lama, Sonaichuri) districts were surveyed to document ethno botanical and ethno microbial folklore information. The primary aim of this study was to validate the antimicrobial usage of some the of selected medicinal plants of thse regions. The gathered information on 31 such plant species was further tested scientifically against eight bacterial isolates using disc diffusion and agar cup assays. The data obtained is dicussed in the light of some earlier reported antimicrobial activities of these plants in Bangladesh [3, 4, 8, 14, 19, 30].

MATERIALS AND METHODS

Plant collection

Plant materials of 31 species (Table 1) were collected from various localities of Chittagong and Bandarban areas based on the reports of their uses by Tripura and Marma communities to treat bacterial infections. The botanical identity of each plant was confirmed before the preparation of their extract. All voucher specimens of the collected plants were preserved in the Chittagong University Herbarium (CTGUH) and allotted a Voucher number (Table-1, 2). The locations of the respective plants were recorded for further collection. All samples were kept in sterilized polythene bags, properly tied, labeled and brought to the laboratory for preparation of their extracts.

Preparation of extracts

Individual plants parts were cut into small pieces (1 mm size) and 5 g of each sample was taken in a test tube, mixed with 10 ml of 95% ethyl alcohol and kept for 48 hrs at normal room temperature. After 48 hrs the ethanolic extracts were filtered in a small vial and kept in refrigerator till further use.

Preparation of bacterial suspension

For preparing bacterial suspensions of the test organisms, one loop of each bacterial culture was transferred to 10 ml of sterilized distilled water and mixed well under aseptic condition. These bacterial suspensions were used in the sensitivity test.

Test organisms

In the present study, eight human pathogenic bacteria viz., *Bacillus subtilis* (BTCC 17), *B. megaterium* (BTCC 18), *B. cereus* (BTCC 19), *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC 25922), *Vibrio cholera* (AE 14748), *Salmonella typhi* (AE 14612) and *Shigella dysenteriae* (AE 14396) were used to screen the antibacterial activity of the plant extracts.

Antibacterial sensitivity analysis

The sensitivity spectrum analysis of plant extracts was done following Bauer *et al.* [7] for disc diffusion method, and Rao and Nigam [32] for the agar cup method. Briefly, for disc diffusion protocol, filter paper discs of 4 mm diameter were cut with the help of a cork borer and a scissors and sterilized

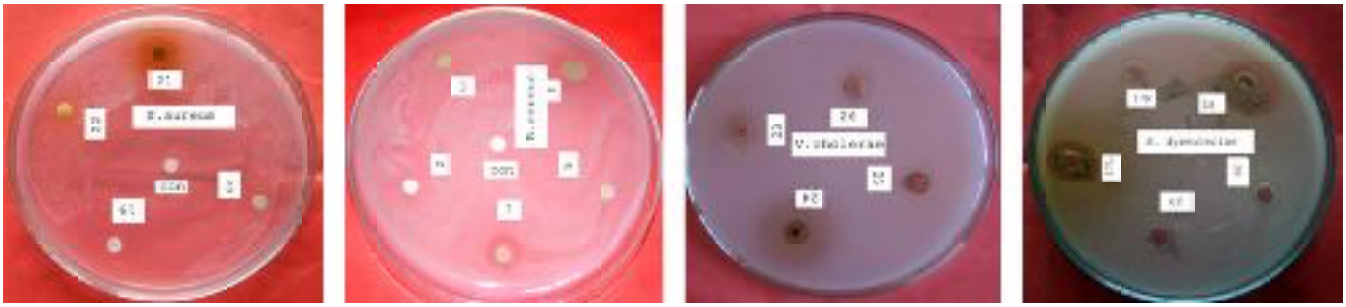

Figure 1: Zone of inibition produced by different plant extracts against selected bacteria. A-*Terminalia chebula* fruit extract against *S. aureus* (sample no. 21); B-*Embilica officinalis* fruit (sample No. 1) and *Eucalyptus globulus* leaf (sample No. 4) extracts against *B. cereus; C-solanum torbum* fruit (sample No. 23), *Flemigia stricta* leaf (sample No. 24) and *Passiflora foetida* whole plant (sample No. 25) extracts against *V. chlorerae*; D-*Adhatoda vasica* leaf & root (sample No.s 17L& 17R) and *Ocimum sanctum* leaf (sample No. 18) extracts against *S. dysenteriae*.

Table 1: Sensitivity spectrum analysis of plant extracts of 31 species of bangaldesh by disc diffusion assay

Voucher specimen no.	Sample number	Plant Species	Parts used	Test organism							
				Zone of inhibition in diameter (mm) (20µl extract/disc)*							
				Salmonella typhi	*Shigella dysenteriae*	*Escherichia coli*	*Vibrio cholerae*	*Bacillus megaterium*	*Bacillus cereus*	*Staphylococcus aureus*	*Bacillus subtilis*
M38,T99	1	*Phyllanthus emblica*	Fruit	10	10	-	13	9	12	10	10
M56,T79	2	*Allium sativum*	Bulb	10	9	-	17	15	-	-	-
M31, T87	3	*Calotropis gigantea*	Leaf	-	-	-	-	-	-	-	-
T100	4	*Eucalyptus globulus*	Leaf	12	-	-	-	10	11	5	8
M64,T107	5	*Kalanchoe pinnata*	Leaf	-	-	-	-	-	-	-	-
T122	6	*Terminalia arjuna*	Leaf	8	-	-	8	7	10		7
M 74, T80	7	*Amaranthus spinosus*	Root	-	-	-	-	-	-	-	-
T90	8	*Cassia fistula*	Leaf	-	-	-	-	8	-	10	-
M30	9	*Zingiber officinalis*	Modified stem	-	-	-	-	-	-	-	-
T94	10	*Curcuma longa*	Modified stem	5	-	-	-	-	-	4	-
T116	11	*Peperomia pellucida*	Leaf	-	-	-	-	-	-	-	-
T105	12L	*Hibiscus rosa- sinensis*	Leaf	-	-	-	-	-	-	-	-
T105	12F	*Hibiscus rosa- sinensis*	Flower	-	-	-	-	-	-	-	-
M57,T91	13	*Centella asiatica*	Whole plant		-	-		-	-		-
M65	14	*Euphorbia hirta*	Whole plant	-	-	-		10	9	10	-
T112	15	*Nigella sativa*	Fruit	-	-	-	-	-	15	-	-
M60,T88	16	*Senna alata*	Leaf	-	-	-	-	11	6	6	-
M63,T77	17L	*Justicia adhatoda*	Leaf	-	-	-	-	-	-	-	-
M63, T77	17R	*Justicia adhatoda*	Root	-	-	-	-	-	-	-	-
T113	18	*Ocimum sanctum*	Leaf	-	-	-	-	-	-	-	-
T83	19	*Areca catechu*	Root	-	-	-	-	-	-	-	-
M67,T106	20	*Jatropha curcas*	Stem	-	-	-	-	-	-	-	-
M46	21	*Terminalia chebula*	Fruit	13	18	-	10	8	11	13	13
M50, T 78	22	*Aegle marmelos*	Fruit	-	-	-	-	-	-	-	-
T121	23	*Solanum torvum*	Fruit	-	-	-	-	-	-	-	-
M16	24	*Flemingia stricta*	Leaf	-	-	-	-	-	-	-	-
M72	25	*Passiflora foetida*	Whole plant	-	-	-	-	-	-	-	-
T85	26	*Bacopa monniera*	Leaf	-	-	-	-	-	-	-	-
M61	27R	*Mimosa pudica*	Root	-	-	-	-	-	-	-	-
M61	27L	*Mimosa pudica*	Leaf	-	-	-	-	-	-	-	-
T84	28	*Asparagus racemosus*	Root	-	-	-	-	-	-	-	-
M48, T76	29	*Acorus calamus*	Root	-	-	-	-	-	-	-	-
M49	30	*Rauvolfia sarpentina*	Root	-	-	-	-	-	-	-	-
T120	31	*Santalum album*	Stem	-	-	-	-	-	-	-	-

Note : (-) denotes mean no ; T- Tripura; M- Marma;* paper disc (4mm in diameter) soaked with 20 ml ethanolic extract for testing

in autoclave. The discs were soaked with plant extract (20µl/discs). Extra plant extract was removed from the soaked filter paper disc. For performing the sensitivity spectrum analysis nutrient agar plate were seeded uniformly with the test organisms. The filter paper disc with definite solvent extracts was placed on the centre of the medium surface. A control plate was also maintained in each case without any test materials. The plates were incubated at 37°c for 24 hrs for the growth of bacteria. After incubation the plates were observed and zone of inhibition (mm) was

Table 2: Sensitivity spectrum analysis of plant extracts of 21 plant species of Bangladesh by agar cup method

Voucher specimen no	Sample no	Family/Species	Parts used	Test organism			
				Zone of inhibition in diameter (mm) (100µl/cup)			
				Shigella dysenteriae	*Escherichia coli*	*Vibrio cholerae*	*Bacillus cereus*
M31,T87	3	*Calotropis gigantean*	Leaf	-	-	-	-
M64,T107	5	*Kalanchoe pinnata*	Leaf	18	-	11	-
M74,T80	7	*Amaranthus spinosus*	Root	-	-	-	-
M30	9	*Zingiber officinalis*	Modified stem	15	-	-	12
T116	11	*Peperomia pellucid*	Leaf	14	-	8	-
T105	12F	*Hibiscus rosa- sinensis*	Flower	12	-	-	-
T105	12L	*Hibiscus rosa- sinensis*	Leaf	12	-	-	-
M57,T91	13	*Centella asiatica*	Whole plant	13	-	8	
M63,T77	17L	*Justicia adhatoda*	Leaf	20	18	10	11
M63,T77	17R	*Justicia adhatoda*	Root	14	10	-	-
T113	18	*Ocimum sanctum*	Leaf	20	14	18	20
T83	19	*Areca catechu*	Root	-	-	-	-
M67,T106	20	*Jatropha curcas*	Stem	12	12	-	-
M50,T78	22	*Aegle marmelos*	Fruit	15	11	10	-
T121	23	*Solanum torvum*	Fruit	15	08	21	10
M16	24	*Flemingia stricta*	Leaf	08	13	16	16
M72	25	*Passiflora foetida*	Whole plant	8	11	7	-
T85	26	*Bacopa monniera*	Leaf	14	16	15	
M61	27R	*Mimosa pudica*	Root	-	-	-	-
M61	27L	*Mimosa pudica*	Leaf	-	-	-	-
T84	28	*Asparagus racemosus*	Root	-	11	-	-
M48,T76	29	*Acorus calamus*	Root	-	09	-	-
M49	30	*Rauvolfia sarpentina*	Root	-	-	-	-
T120	31	*Santalum album*	Stem	12	14	8	

measured. In agar cup method a reservoir of plant extract was held in the nutrient agar plate, thereby establishing a diffusion gradient causing the extract to diffuse through the agar medium and thus exposing the microorganism to the extract. Zones of inhibition was formed when the organism is susceptible. 0.1 ml bacterial suspension was seeded in the sterile petri dishes having nutrient agar medium. Small circular depressions (10 mm) were made in the agar by inserting sterile corkborer to a depth of about 2mm, leaving a little cup in the agar to hold the extract to be tested. One drop of melted agar was placed at the bottom of each cup to form a thin layer of agar to seal off the bottom; 100µl of the extract was poured into each cups. The plates were incubated at 37º for 24 hrs and were examined for growth inhibition. The diameter of zone of inhibition surrounding the cup was measured.

RESULTS AND DISCUSSION

The present study has shown that ethanolic extracts of only 10 out of 31 plants that are used traditionally by the Marma and Tripura indigenous communities for the treatment of different bacterial infection showed positive activity against the tested bacteria in disc diffusion assays (Table 1). Fruit extract of *Terminalia chebula* and *Phyllanthus emblica* exhibited comparatively better antibacterial activity than other species and showed positive inhibitory activity against all tested pathogenic bacteria except *E. coli*. The ethanolic extracts of selected species exhibited zone of inhibitions from 4 to 18 mm in diameter. The largest zone of

inhibition (18 mm) was recorded against *S. dysenteriae* with the fruit extract of *Terminalia chebula* whereas the *Curcuma longa* showed the minimum activity (4 mm) against *S. aureus. The* overall observation indicated that the ethanolic extracts of *Nigella sativa, Euphorbia hirta, Senna alata, Cassia fistula, Allium sativum* and *Curcuma longa* have lesser impact on the in vitro growth of eight different human pathogenic bacteria than *Phyllanthus emblica, Eucalyptus globules, Terminalia arjuna* and *Terminalia chebula*. Most of the medicinal plants extracts did not show antibacterial activity in disc-diffusion method but a number of workers [5,13,16,18,21,22,25,28,35,37] have reported varying degree of inhibition against gram-positive and gram-negative bacteria from the same plant extract. This is probably due to the amount of alcoholic plant extract is increased (100µl/cup) in this method than the disc-diffusion method. In agar-cup method. In the present study, 21 species ware screened for their antibacterial activity against four human pathogenic bacteria (Table 2).

In agar-cup assays, fruit extract of *Solanum torvum* showed largest zone of inhibition (21 mm) against *V. cholera* (Table-2, Fig. 1). The collective analysis of antibacterial activity of ethanolic extract indicated that among the 21 medicinal plants used in the study *Justicia adhatoda, Ocimum sanctum, Solanum torvum* and *Flemingia stricta* have better efficacy against all the four species of pathogenic bacteria when compared to rest of the plant species. On the other hand, ethanolic extract of *Passiflora foetida, Bacopa monniera, Aegle marmelos* and *Santalum album* have showed positive activity against all tested pathogenic bacteria except *B. cereus*. The leaf extract of *Calotropis gigantea,* root extract of *Amaranthus spinosus*, root extract of *Areca catechu*, root and leaf extract of *Mimosa pudica* and root extract of *Rauvolfia sarpentina* did not exhibit any inhibitory activity against the test organisms. Similar antimicrobial efficiency of these plants was also observed by several earlier workers [1, 2, 10, 12, 17, 18, 23, 24, 25, 30, 33, 35, 37, 38].

From the above results it can be concluded that ethanolic extracts of different parts of plants used by Marma and Tripura communities of Bangladesh have strong antibacterial potentials against several pathogenic bacteria. The data presented here constitute a primary platform for further phytochemical and pharmacological investigation in thse plant species to develop novel clinically effective phytoceuticals to address the problem of multi-drug resitance.

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