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ANTIBACTERIAL PROPERTIES OF MAJOR ETHNOMEDICINAL PLANTS USED BY THE LOCAL PEOPLE OF BRAHMANBARIA, BANGLADESH

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Abstract

Antibiotics resistant pathogenic bacteria are one of the emerging challenges of 21st century. Recently, researchers are looking for antibacterials (therapeutic agents) from ethnomedicinal indigenous plants to be considered new sources to meet the emerging challenges. These antibacterials could be used as an alternative to conventional antibiotics against infectious human diseases. The antibacterial properties of ethnomedicinal plants used by the local people of Brahmanbaria were determined. Ethnomedicinal data was collected in between June 2015 and June 2017 from 467 local people using mainly key informant's interviews followed by group discussions, filed interviews and plant interviews. A total of 243 ethnomedicinal plants were recorded. In order to identify the most important ethnomedicinal plants Factor of informant consensus (F_{ic}) was calculated and 8 medicinal plants were identified based on the higher consensus of informants. These are *Litseaglutinosa* (Lour.), Robinson, *Scopariadulcis* (L.), *Dalbergiasissoo* (Miq.), *Clerodendrumviscosum* (Vent.), *Holarrhena antidysenterica* (L.) Wall.*ex*Decne., *Phyllanthus reticulatus* (Poir.), *Paederia foetida* (L.) and *Stephania japonica* (Thunb.) Miers. Both the aqueous and ethanol extracts of leaves were screened for antibacterial activity against 8 clinical strains through disc diffusion assays. The experimental results revealed that maximum (85%) plant extracts were potentially effective in inhibiting growth of pathogenic bacteria with variable potency. *Litseaglutinosa* was the most effective plant species retarding microbial growth of eight tested pathogenic bacteria. This plant can be evaluated for production of potential herbal antimicrobials alternative to antibiotics.

Introduction

Antibiotics resistant to bacteria are of one of the new challenges in the medical sector. This became bioburden for medical scientists, doctors and bio-researchers. To overcome this situation, scientists should find antimicrobials from alternative ,,sources and the ethnomedicinal indigenous plants may be the possible sources. About 80% of the world's populations used to use traditional medicine extracted from plants (Owolabi *et al*. 2007). It is estimated that about 75% of plants are used globally as traditional medicinal plants (Tomoko *et al*. 2002). Global prevalence of infectious diseases caused by bacteria is a major public health concern (Zhang *et al*. 2006). Recent emergence of antibiotic resistance and related toxicity issues limit the use of antibiotics (Eggleston *et al*. 2010). Research on finding antimicrobials from plants against antibiotics resistant human pathogens is given priority for its safety and efficacy (Alviano and Alviano 2009). Ethnobotanical records suggest that plants are natural source of antimicrobial drugs that controlling some infectious diseases globally (Hostettmann and Hamburger 1991). Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases (Malini*et al*. 2013). Crude antimicrobial drugs have been extracted from a large number of plants in different location around the world (Dranghon 2004), but the potential higher plants as source for new drugs is still unexplored (Mahesh and Satish 2008). In Bangladesh, plant based antibacterial

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activities started at 1986 and few works have been done (Hoque *et al.* 1986, Chowdhury *et al.* 2011, Haque *et al.* 2014, Hossain *et al.* 2014, Tahia *et al.* 2016). None of these works were carried out antibacterial activity of ethnomedicinal plants by consensus analysis in a particular area. Selection of important taxa using ethno-directed method is essential because of time does not allow to evaluate all existing medicinal plants scientifically from a particular area (Canalesa *et al*. 2005). Therefore, the present study was undertaken to find antibacterial properties of ethnomedicinal plant species selected based on consensus analysis of local people used for bacterial diseases management from Brahmanbaria district.

Materials and Methods

Brahmanbaria is a district in east-central Bangladesh lies between 23°57′10″ and 23°.95′28″ N latitude and between 91°07′00″ and 91°11′67″E longitudes (Musa and Mahmud 1998). The district is geographically characterized by low-lying land with small hills and hillocks of red soil. Total population of Brahmanbaria is 2,840,498 and density 1,500/km2. This area is located near the Tripura border of India, so cultural knowledge of two countries cross connected by this area. It also comprises heterogeneous habitat including foothills, wetlands, floodplain, and homestead. Railway and junction transition area lies between Bangladesh and India. So, biodiversity of this area is quite high (Haque *et al.* 2017).

Ethnobotanical study was carried out following the standard guidelines for ethnobotanical survey (Alexiades 1996, Martin 1995). Ethnomedicinal plant selected for antibacterial activity using informant consensus factor (Fic), Fidelity level (Fl) and Citation frequency (Cf) (Heinrich *et al*. 1998). These are the most cited species claimed by the local people of Brahmanbaria district. Among them *Litseaglutinosa*, *Scopariadulcis*, *Dalbergia sissoo*, *Clerodendrum viscosum*, *Holarrhena antidysenterica*, *Phyllanthus reticulatus*, *Paederia foetida* and *Stephania japonica* were selected for the evaluation of antibacterial activity based on highest consent values to find their ethnomedicinal uses (Fig. 1). The leaves were collected from study area, taken out in the taxonomy laboratory, University of Dhaka, Identifications of ethnomedicinal plants species were confirmed using standard literature (Prain 1903). Voucher specimens were deposited at Dhaka University Salar Khan Herbarium, Department of Botany, University of Dhaka.

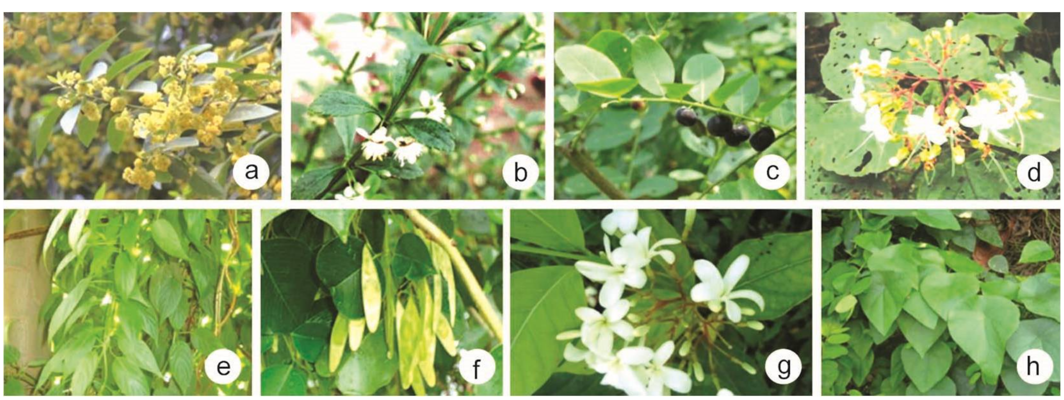

Fig. 1. Selected ethnomedicinal plants for antibacterial activity. (a) *Litseaglutinosa* (Lour.) Robinson, (b) *Scoparia dulcis* L. (c) *Phyllanthus reticulatus* Poir. (d) *Clerodendrum viscosum* Vent. (e) *Paederia foetida* L. (f) *Dalbergia sissoo* Miq. (g) *Holarrhena antidysenterica* (L.) Wall. ex Decne. (h) *Stephania japonica* (Thunb.) Miers.

The selected plant samples (leaves) were cleaned, air dried and ground to a fine powder using a grinding machine (Jaipen prince mixed grinder). The powder was stored in tightly closed glass containers in the dark at room temperature (Elisha *et al*. 2017).

Fifty grams of leaves powder were successively extracted with distilled water and ethanol. Both extracts were filtered successively. Semi-solid mass of both solvent extracts were obtained by evaporating solvents with vacuum rotary evaporator and the mass was preserved aseptically at 4^0C for further study (Pratiwi and Sukandar 2016).

There were eight test organisms used for antibacterial activity test. The test organisms such as *Pseudomonas aeruginosa* (DMCH-1), *Escherichia coli* (DMCH-5), *Staphylococcus aureus* (DMCH-18), *Bacillus cereus* (S- 01), *Bacillus subtilis*(S-02), *Salmonella paratyphi* (S-03), *Shigella dysenterae* (S-05) were obtained from the, Department of Microbiology, Pharmaceutical Chemistry, University of Dhaka and Dhaka Medical College. Stock cultures were maintained on Mueller Hinton agar medium in a refrigerator at 4^0C.

The antibacterial activities of the plant extracts were carried out using Kirby Bauer disc diffusion method (Bauer *et al*. 1966). The test organisms were inoculated in 10 ml of Muller Hinton broth and incubated at 37°C for 24 hrs and these plates were seeded with the test organisms growing in Muller Hinton broth. The extracts were made solution at a concentration of 4mg/ml. Ten sterile discs 8 mm in diameter (ADVANTEC, Disc for antibiotic Assay; Toyo Roshi Kaisha, Ltd. Japan) were impregnated with 100 µl of each of the above extract solution and the discs were dried for 10 min in a sterile petridish. Disc impregnated with ethanol and distilled water was used as control. These plates were then kept at 4°C for 4 hrs to allow maximum diffusion of samples. The plates were then incubated at 37°C for 24 hrs. Clear zone around the disc is developed due to inhibition of the test organism, suggesting antibacterial activity of the extract imployed. The diameter of zone of inhibition was measured in millimeter with mm scale. The experiment was carried out three times and the average zone of inhibition was calculated. The experimental results were expressed as mean ± standard deviation (SD) of the three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA). P values less than 0.001 were considered statistically significant. To determine the possible statistical correlation, using two-way ANOVA method of statistical analysis for the data obtained from the research work. Microsoft Excel 2010 statistical package was used for all analyses (JMP software, version 4.0.0).

Results and Discussion

A total of 243 medicinal plants under 88 families have been recorded from 467 local people using mainly key informant's interview and also followed by group discussions, filed interviews and plant interviews. In order to identify the most important ethnomedicinal plants in the study area, Factor of informant consensus (F_{ic}), Fidelity level (Fl) and Citation frequency (Cf) of medicinal plants have been calculated. Maximum F_{ic} value was found in case of Diarrhoea and dysentery category. This disease category was treated by 51 ethnomedicinal plants by the local people of Brahmanbaria district. Among them eight medicinal plants have been selected for antibacterial activity based on the consensus values (Table1). These were *Clerodendrum viscosum, Dalbergia sissoo, Holarrhena antidysenterica, Litsea glutinosa, Paederia foetida, Phyllanthus reticulatus, Scoparia dulcis, Stephania japonica.* For all such plants leaves were the leading part used to treat diseases.

Antibacterial activity of aqueous and ethanol extracts of all the selected plant extracts were analyzed using the Analysis of Variance (ANOVA) single factor statistical tool which indicated that there was a significant difference in the sensitivity of the tested microorganisms to the various extracts. In the present study, leaf extract has been assessed for their antibacterial activity. Results showed that the highest zone of inhibition was prominent for the positive control (Ciprofloxacin (30 µg/disc) ranging from (24-28mm). No zone of inhibition found for negative control (water and Ethanol).

Table 1. Selected indigenous ethnomedicinal plant species based on highest consent values.

Plants name and voucher number	Citation frequency (Cf)	Fidelity level (Fl)	Formularies found in current study
Litsea glutinosa (Lour.) Robinson, TH- 08	39.2%	91.28%	Leaves were mashed with water than 150 ml taken morning & evening until the disease is cured
Scoparia dulcis L., TH-172	14%	100%	Decoction of leaves were taken
Holarrhena antidysenterica (L.) Wall. ex Decne., TH-148	12.50%	100%	Juice of fresh leaves were taken at morning for seven days
Clerodendrum viscosum Vent., TH-153	12.20%	91.93%	Two spoonful extract of leaves taken twice daily
Dalbergia sissoo Miq, TH-104	11.20%	100%	One or two spoonful juice taken twice daily for seven days
Phyllanthus reticulatus Poir., TH-61	10%	76%	One spoonful of leave extracts were taken
Paederia foetida L., TH-30	8.2%	100%	Paste taken internally with or without boiled rice
Stephania japonica (Thunb.) Miers., TH-60	6.3%	64%	Grinded & mixed with water which is taken internally twice a day for 7 days

Most of the plant extracts showed clear zone of inhibition accept S*tephania japonica* at 400µg/disc of crude extracts. Out of eight selected plants, maximum zone of inhibition was found for aqueous leaf extract from *Litsea glutinosa* against *Escherichia coli* (21.6 ± 0.33mm) at 400 µg/disc and minimum zone of inhibitions found for aqueous leave extract from *Wrightia antidysenterica* (Synonym *Holarrhena antidysenterica*) against *Bacillus subtilis* (9.4±1.05mm). *Litsea glutinosa* was the most effective in retarding microbial growth followed by *Dalbergia sissoo, Scoparia dulcis, Holarrhena antidysenterica* against 3 and *Clerodendrum viscosum* against 2 tested bacterial strains. *Paederia foetida* and *Phyllanthus reticulatus* were effective only against *Escherichia coli* and *Staphylococcus aureus*, respectively and *Stephania japonica* did not show any antibacterial activity (Table 2).

The antibacterial activity of eight selected ethnomedicinal plant against *Escherichia coli* statistically correlate against citation frequency of eight ethnomedicinal plants. Result showed significant correlation between most cited ethnomedicinal plants and antibacterial activity. Both the aqueous and ethanol extract of *Litsea glutinosa* showed positive correlation against *Escherichia coli* (Fig. 2 and 3).

In the present study *Litsea glutinosa* was found to occupy the highest antibacterial activity (against 5 bacterial strains) of the total selected plants, aqueous extract displayed a result compared to ethanol against both Gram positive, Gram negative bacteria and this could have attributed to its potent extraction capacity. Haque *et al*. (2014) reported more or less same result from the bark and leaf extracts where best result was found against *Staphylococcus aureus* and *Escherichia coli*. Hosamath (2011) reported the petroleum ether extract, ethanolic extract and aqueous extracts of the *Litsea glutinosa* bark have the antibacterial against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphii* and *Pseudomonas aeroginosa*. Antibacterial activity against *Bacillus cereus* and *Shigella dysenteriae* has not yet been reported. Aqueous extracts showed more antibacterial activity than ethanolic extracts.

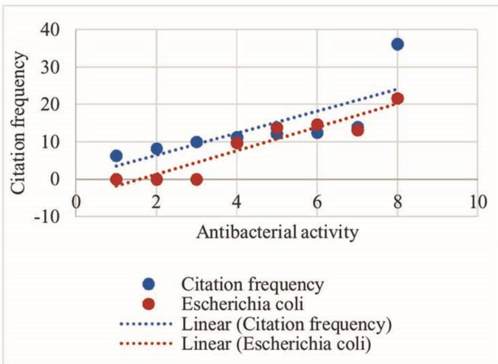

Fig. 2. Correlation between citation frequency of eight ethnomedicinal plants for aqueous extracts and antibacterial activities (r = 0.7027, P = 0021, n=8)

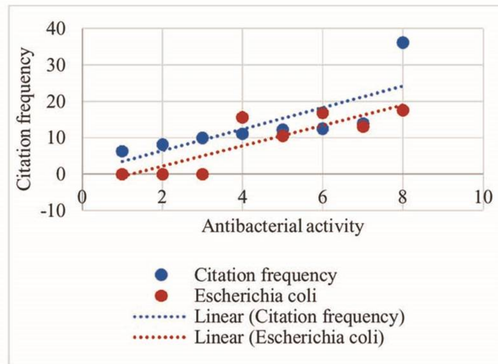

Fig. 3. Correlation between citation frequency of eight ethnomedicinal plants for ethanolic extracts and antibacterial activities (r = 0.8039, P = 0.007, n=8)

The crude extracts of *Dalbergia sissoo* showed significantly highest zone of inhibition against *Bacillus cereus* followed by *E. coli* and *S. aureus.* Behera *et al.* (2013) reported that the hexane extracts of *Dalbergia sissoo* exhibited no activity against *S. aureus* at 40 µl but showed good activity against *E. coli.* Subedi *et al.* (2017) also reported the antibacterial activity of methanol extracts of leaves of *D. sissoo* was found to be more as compared to other extracts*. Staphylococcus aureus* was found to be more susceptible to extracts of *D. sissoo* than *E. coli.*

Scopria dulcis possesses a good antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* which was significantly highest ($p < 0.001$) than *B. cereus.* The antibacterial activity of the different concentrations of different extracts of *Scoparia dulcis* against *Klebsiella pneumonae* and *E. coli*. Wankhar *et al*. (2015) reported the antimicrobial activity of the plant were tested by broth dilution method ranging from 8 to 256 mg/ml. Thus, justifying its efficacy as a potential broad-spectrum antimicrobial agent which matched the present result.

In the present study *Holarrhena antidysenterica* showed more antibacterial activity than *Clerodendrum viscosum*in. Niraj and Varsha (2015) reported the antibacterial activity of extracts of seed of *Hollarhena antidysenterica.* Extracts of leaves were tested for antibacterial activity and results obtained that the ethanolic and aqueous extracts showed mean inhibition zone against *Escherichia coli* followed by *Staphylococcus aureus* and *Bacillus subtilis* which was not co-related with previous studies (Kaundal and Sagar 2016). In case of *Clerodendrum viscosum* leaf extract showed activity against *Escherichia coli* and *Shigella boydii.* This observations are more or less similar to the previous studies (Ashoor *et al.* 2018).

Phyllanthus reticulatus and *Paederia foetida* showed less antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, respectively. Antibacterial activity against *Pseudomonas aeruginosa* did not matched with present study. Morshed *et al*. (2012) showed that the methanol extracts of whole plants of *Paederia foetida* possess no antibacterial activity but, the n-hexane, chloroform and ethyl acetate fractions of the plant have moderate to less antibacterial functions against some strains which support the present result.

Table 2. Mean diameter of inhibition zones (mm) with standard error of mean (SEM) as shown by tested plant extracts.

Plant species	Solvent	*Bacillus cereus*	*Bacillus subtilis*	*Staphylococcus aureus*	*Esherichia coli*	*Shigella dysenteriae*	*Pseudomonas aeruginosa*	*Shigella boydii*	*Salmonella paratyphii*
Litsea glutinosa	Aqueous	14.2±0.64	-	16.8±0.29	21.6± 0.33	12.9±0.37	-	10.7±0.88	-
	Ethanol	10.6±1.05	-	17.6± 0.56	18 ± 0.14	12.5±0.37	-	10.3±0.88	-
Dalbergia sissoo	Aqueous	14.6±0.23	-	10.2 ± 1.02	9.7± 0.57	-	-	-	-
	Ethanol	15.4±0.38	-	11.6±0.22	15.6± 0.58	-	-	-	-
Scoparia dulcis	Aqueous	12 ± 0.57	-	14.4±0.22	13.2± 0.22	-	-	-	-
	Ethanol	9.7 ± 0.55	-	14.9 ± 0.87	13.1± 0.21	-	-	-	-
Holarrhena antidysenterica	Aqueous	-	9.9 ± 0.23	12.2 ± 0.43	14.7± 0.55	-	-	-	-
	Ethanol	-	10.1±0.27	13.5 ± 0.73	16.1 ±0.44	-	-	-	-
Clerodendrum viscosum	Aqueous	-	-	-	13.8± 0.56	-	-	13.1±0.38	-
	Ethanol	-	-	-	10.6± 0.22	-	-	11.3±0.78	-
Phaederia foetida	Aqueous	-	-	-	12.5± 0.44	-	-	-	-
	Ethanol	-	-	-	10.5± 0.35	-	-	-	-
Phyllanthus reticulatus	Aqueous	-	12.5±0.36	-	-	-	-	-	-
	Ethanol	-	12.9±0.45	-	-	-	-	-	-
Stephania japonica	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
+ve control	Ciprofloxacin	28.2±0.41	24 ± 0.14	25.5 ± 0.22	25.2± 0.26	23.5±0.27	27.6 ± 0.45	27.1±0.15	**25.5 ±0.26**
-ve control	Ethanol	-	-	-	-	-	-	-	-

It was found that about 85% of the selected plants showed antibacterial activity. This result is an evidence of the effectiveness of the ethno-directed method. The information of the uses of the leaves of *Litsea glutinosa, Dalbergia sissoo, Scoparia dulcis, Holarrhena antidysenterica* and *Clerodendrum viscosum* provided by the people of Brahmanbaria is an agreement of the present results found in antibacterial activities. It was observed that there is a positive co-relation between the citation frequency and antibacterial activity of ethnomedicinal plants in the study area. Important species obtained by quantitative analysis showed highest antibacterial activity which scientifically supports the traditional uses of ethnomedicinal plants in the study area. Further and more specific studies, *in vivo*, are recommended to determine the efficacy of these ethnomedicinal plants. Ethnomedicinal plants with high antibacterial activities are always vulnerable to extinction in nature because of over harvesting. Therefore, appropriate policy should be taken to conserve the species in the study area.

Litsea glutinosa plant species could be the potential source of new antibacterial agents. The present analysis of ethnomedicinal data of the district have been brought into focus a good number of economic potential ethnomedicinal plants those need to be cared for commercialization and conservation. The ethnomedicinal plants showed antibacterial activity that could be subjected to over harvesting needed to be under conservation programs in the district. Appropriate policy should be taken and applied in ethnomedicinal plants management for sustainable local resource conservation, economic growth, primary healthcare, community development and drug discovery research.

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