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ANTIBACTERIAL AND PHYTOCHEMICAL EVALUATION OF THREE MEDICINAL PLANTS OF BANGLADESH

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ABSTRACT

The present study was carried out to illustrate the relative evaluation of phytochemical constituents and antimicrobial activity of leaves of *Eclipta alba* and *Aphanamixis polystachya* and bark of *Premna integrifolia* in two different solvents. A preliminary phytochemical analysis was done and concluded that the presence of alkaloids, tannins, flavonoids and glycoside in both extracts of the above plants. The antimicrobial activity was tested using disc diffusion method against eight pathogenic bacteria using Kanamycin as antibiotic standards. The outcome showed that the antibacterial activity was effective in chloroform and ethyl acetate fractions of tested extracts. The MIC was determined in two different solvents of both extracts and observed that chloroform and ethyl acetate possess minimum inhibition in both extracts. From the current investigation, it can be concluded that *Eclipta alba, Aphanamixis polystachya* and *Premna integrifolia* crude extracts have quite effective antimicrobial activity and potent phytochemical constituents.

INTRODUCTION: The use of plants and plant products could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In Bangladesh, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times [1]. Herbal medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents [2]. Following the advent of modern medicine, herbal medicine suffered a set back, but during last two or three decades advances in phytochemistry and in identification in plant compounds effective against certain diseases have renewed the interest in herbal medicines [3].

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. This results in the need of higher dose use with increased risk of drug toxicity or consideration to change the regimen. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions.

This situation force scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [4]. The beneficial medicinal effects of plant materials typically results from combinations of secondary product present in plant.

In plants these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids which are capable of producing definite physiological action on body [1].

Based on this, an attempt has been made to assess Antibacterial activity and Minimum Inhibitory Concentration (MIC) of some locally used medicinal plants *Eclipta alba* (Family: Noctuoidea), *Aphanamixis polystachya* (Family: Meliaceae) and *Premna integrifolia* (Family:Verbenaceae). These plants grow wild and planted in many districts of Bangladesh [5] and almost all parts of these plants i.e. root, leaf, and bark have tremendous medicinal value and used in Bengali traditional medicine because of their anticancer, antimicrobial, anti-inflammatory and hepatoprotective properties [6-8].

The present study was performed to evaluate Antibacterial activity and Minimum Inhibitory Concentration (MIC) of chloroform and ethyl acetate extract of leaf of *Eclipta alba, leaf Aphanamixis polystachya* and bark of *Premna integrifolia* against some pathogenic Gram positive and Gram negative organisms.

MATERIALS AND METHODS:

Identification of Plant materials: The selected 3 plant materials used in this study were collected from various locations in Bangladesh and identified by DR. M.A. Razzaque shah PhD, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh.

Plant materials extraction and fractionation: The plants parts were first washed with water to remove adhering dirt & then cut into small pieces, sun dried for 4 days & finally dried at 45°C for 36 hrs in an electric oven. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinding machine & were stored in an airtight container for further use. Then, 200gm dried powders of leaf of *Eclipta alba,* leaf of *Aphanamixis polystachya* and bark of *Premna integrifolia* were soaked in 600ml of methanol in round bottom flasks for 7 days . The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then, it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated using rotary evaporator. The concentrated methanolic extracts of leaves of *Eclipta alba* (20 gm) and *Aphanamixis polystachya* (20 gm) and bark of *Premna integrifolia* (14 gm) were fractionated by the modified Kupchan partitioning method [9] into petroleum ether, chloroform and ethyl acetate.

The subsequent evaporation of solvents afforded petroleum ether (450 mg), chloroform (700 mg) and ethyl acetate (350 mg) from leaf extract of *Eclipta alba;* petroleum ether (350 mg), chloroform (500 mg) and ethyl acetate (450 mg) from leaf extract of *Aphanamixis polystachya* and petroleum ether (400 mg), chloroform (650 mg) and ethyl acetate (750 mg) from bark extract of *Premna integrifolia* respectively.

Phytochemicals Analysis: The phytochemical analysis of the extracts was carried out by the standard methods provided by Odebiyi and Ramstard [10] and Waterman [11].

Microorganisms and media: The test microorganisms used in this study were eight Gram positive and Gram negative bacterial strains such as *Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio parahemolyticus and Shigella dysenteriae*. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The bacterial isolates were first subcultured in a nutrient agar and incubated at 37°C for 18 h.

Assay for Antibacterial Activity:

Disc Diffusion Method: The antibacterial assay was performed by disc diffusion technique [12-13]. Disc diffusion technique is highly effective for rapidly growing microorganisms. The sample solution of the material to be tested was prepared by dissolving a definite amount of material in appropriate solvent to attain a concentration of 50mg/ml. 10 µl of such solution was applied on sterile disc (5mm diameter, filter paper) and allowed to dry off the solvent in an aseptic hood. Thus, such discs contain 500 µg of crude extracts Standard antibiotic discs (Kanamycin 30µg $disc^{-1}$) and blank discs (impregnated with solvents) were used as a positive and negative control.).

Briefly, in this study the test discs and standard disc were placed in a Petri dish seeded with particular bacteria and then left in a refrigerator at 4°C for 12-18 hrs in order to diffuse the material from the discs to the surrounds media in the Petri dishes. The Petri dishes were then incubated at 37°C for overnight to allow the bacterial growth. The antibacterial activity of chloroform and ethyl acetate extracts of leaves of *Eclipta alba* and *Aphanamixis polystachya* and bark of *Premna integrifolia* were then determined by measuring the respective zone of inhibition in mm.

Determination of Minimum Inhibitory Concentration: The effectiveness of antibacterial activity of the plant extracts was quantified using serial dilution technique according to Reiner [14]. The Minimum inhibitory concentrations (MICS) of all the experimental crude plant extracts of *Eclipta alba*, *Aphanamixis polystachya* and *Premna integrifolia* against *Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio parahemolyticus and Shigella dysenteriae* were determined by using different concentrations(64-512µg/ml). The sample solutions of all the extracts were prepared in dimethyl sulfoxide (DMSO) in such a way that the solutions had a concentration of l mg/ml.

In this technique a large no of sterilized test tubes were used and each of the test tube was filled with l ml of sterile nutrient broth media and graded doses of sample solution was added. Then these test tubes were inoculated with said organisms (inoculum contains cell/nil) followed by incubated at 37°C for 24 hours to allow growth of the bacteria used. Inhibition of growth observed in that test tube (the solution content was clear) which has lowest or minimum concentration of extract and above of which no growth observed too. This lowest or minimum concentration was considered as minimum inhibitory concentration (MIC). Another three test tubes containing medium, medium plus sample and medium plus inoculum were used as control. Bacterial growth observed only in test tube (solution content was cloudy) containing medium plus inoculum and the other two were clear that means no growth occurred.

RESULT: In the present study, chloroform and ethyl acetate extracts of 3 medicinal plants were examined for their phytochemical screening and antibacterial assay by Disc diffusion and Minimum inhibitory concentrations (MIC) techniques against 8 pathogenic bacteria.

Phytochemicals Analysis: The phytochemical screening of the various solvent extracts of *Eclipta alba, Aphanamixis polystachya* and *Premna integrifolia* were depicted in **Table 1**. The phytochemical results of leaves of *Eclipta alba, Aphanamixis polystachya* and bark of *Premna integrifolia* revealed the presence of alkaloids, tannins and phenolic compounds in all the extracts, flavonoids in chloroform and ethyl acetate extracts.

TABLE 1: PHYTOCHEMICAL SCREENING OF LEAVES EXTRACTS OF *ECLIPTA ALBA AND APHANAMIXIS POLYSTACHYA AND BARK EXTRACT OF PREMNA INTEGRIFOLIA*

Extracts	Phytochemical constituents			
	Alkaloids	Flavonoids	Tannins	*Glycoside*
Eclipta alba(leaf)				
Chloroform fraction	+	+	+	+
Ethyl acetate fraction	+	+	+	+
Aphanamixis polystachya (leaf)				
Chloroform fraction	+	+	+	+
Ethyl acetate fraction	+	+	+	+
Premna integrifolia(bark)				
Chloroform fraction	+	+	+	+
Ethyl acetate fraction	+	+	+	+

Phytochemical test: + positive,

Assay for Antibacterial Activity:

Disc Diffusion Method: The antibacterial activity of different solvent fractions of the three experimental plants was evaluated. **Table 2** exhibits the antibacterial activity (zone of inhibitions) of the 3 experimental crude plant extracts. All the extracts at a dose of 500µg/disc showed fair antibacterial activity against *Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio parahemolyticus and Shigella dysenteriae* with the zone of inhibition range from 7 to 16 mm.

TABLE 2: ANTIBACTERIAL SCREENING RESULTS OF CHLOROFORM AND ETHYL ACETATE EXTRACTS OF -LEAVES OF *ECLIPTA ALBA* AND *APHANAMIXIS POLYSTACHYAE* AND BARK OF *PREMNA INTEGRIFOLIA*

Bacterial type	Name of Bacteria	Diameter of zone of inhibition(in mm)					
		Leaf of *Eclipta alba*		leaf of *Aphanamixis polystachya*		Bark of *Premna integrifolia*	
		Chloroform extract	Ethyl acetate extract	Chloroform extract	Ethyl acetate extract	Chloroform extract	Ethyl acetate extract
Gram positive	*Bacillus subtilis*	12	10	12	8	11	15
	Bacillus megaterium	12	10	12	10	10	8
	Sarcina lutea	9	11	12	10	12	11
	Staphylococcus aureus	9	8	10	+	8	8
Gram negative	*Salmonella typhi*	13	10	11	7	12	16
	Escherichia coli	14	8	14	8	10	11
	Vibrio parahemolyticus	13	11	12	9	10	11
	Shigella dysenteriae	14	12	11	8	11	10

Here, '+' = Trace

Minimum Inhibitory Concentration (MIC): The MIC of the extracts against the tested bacterial organisms is shown in **Table 3**. Significant antibacterial effects, expressed as MIC, of crude extracts against the pathogenic organisms are presented in the concentration of 64 to 512 µg/ml.

TABLE 3: MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF CHLOROFORM AND ETHYL ACETATE EXTRACTS OF -LEAVES OF *ECLIPTA ALBA* AND *APHANAMIXIS POLYSTACHYAE* AND BARK OF *PREMNA INTEGRIFOLIA*

Bacterial type	Name of Bacteria	MIC values of Plant Extract (µg/ml)					
		Leaf of *Eclipta alba*		Leaf of *Aphanamixis polystachya*		Bark of *Premna serratifolia*	
		Chloroform extract	Ethyl acetate extract	Chloroform extract	Ethyl acetate extract	Chloroform extract	Ethyl acetate extract
Gram positive	*Bacillus subtilis*	256	128	128	256	256	128
	Bacillus megaterium	128	256	128	256	256	256
	Sarcina lutea	128	256	128	256	128	128
	Staphylococcus aureus	512	512	256	512	256	256
Gram negative	*Salmonella typhi*	128	256	128	256	128	128
	Escherichia coli	512	128	128	128	256	256
	Vibrio parahemolyticus	128	64	128	256	128	128
	Shigella dysenteriae	256	128	256	512	256	256

DISCUSSION: Based on the present study, we can consider the leaves and bark of understudied plants to be good sources of antimicrobial property. The bioactive compounds on the medicinal plants employed contain various secondary metabolites such as phenols, tannins, alkaloids, flavonoids, steroids and glycosides in appreciable quantities.

The effective inhibitory potency observed with the plants parts; proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates.

This observation as reported correlates with De and James [15] who emphasized that these compounds are known to show medicinal activity as well as exhibiting physiological activity. The effective inhibitory potency observed with the plants parts; proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates. Scientific search for new ways to treat various infections stimulates the exploration of new natural bioactive compounds as an alternative therapy. We aimed to screen medicinal plants with recognized antibiotic properties.

The plants were initially screened for their antibacterial properties against selected pathogenic bacteria. The results of zone of inhibitions are presented in Table 2. Among the scrutinized crude extracts the bark of *Premna integrifolia* almost showed moderate to good antibacterial activity against all the tested pathogenic microorganisms. The highest zone of inhibition was observed for ethyl acetate portion of bark of *Premna integrifolia* against *Salmonella typhi* (16 mm) followed by *Bacillus subtilis* (15 mm). The chloroform part of the above plant extract also gave moderate activity.

The lowest inhibition was observed against *Staphylococcus aureus* for both chloroform and ethyl acetate extracts of *Premna integrifolia* (8 mm).

Incase of leaves extracts of *Eclipta alba,* the chloroform fraction showed better antibacterial activity (9-14 mm) than ethyl acetate fraction (8-14 mm). The uppermost zone of inhibition was noticed against *Escherichia coli* and *Shigella dysenteriae* for chloroform part of *Eclipta alba.* The same extract exhibited sensible activity against *Salmonella typhi, Bacillus subtilis* and *Bacillus megaterium* (13-12 mm).

On the other hand the chloroform fraction of leaves of *Aphanamixis polystachya* showed almost good activity against all the tested pathogens (10-12 mm) but the ethyl acetate extract exhibited lower activity during screening test.

The MIC values of the studied plant extracts ranges from 64 to512µg/ml against the tested pathogenic bacteria indicates that the above three medicinal plants can be good sources for new antimicrobial agents. This perhaps helps to interpret that differences in inhibitory diameters (mm) could result in the same therapeutic potency when varied in concentrations, depending on the organism's susceptibility to the antibacterial components present in the extracts.

The presence and the phytochemical components of the studied plants, the inhibitory zones and the MIC concentrations at which values were effective on the tested organisms, highlights that there were variations in the antibacterial potency of the plants extracts. The variations in the sensitivity could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum's size [16].

It has been reported that antibiotics are not the only antibacterial agents and this study observed the effective potency of the studied plants extracts on the selected pathogenic bacterial isolate than some highly rated antibiotics (reference drug) in disease cure and prevention. One problem in the use of medicinal plants is the quantity desired to effect cure hence most times, medication is basically on unspecified quality of decoctions and infusions.

Irrespective of the plants parts in this study and methods of extraction (chloroform and ethyl acetate), a dosage of between 500 µg exhibited appreciable inhibitory values on the tested bacterial species.

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