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Investigation on In-Vitro Cytotoxic, Antibacterial and Phytochemical Screening of Ethyl Acetate Extract of *Geodorum densiflorum* (Lam.) Schltr.

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The purpose of the study is to evaluate the antimicrobial, cytoxic activity and phytochemical screening of ethyl acetate extract of Geodorum densiflorum. Antimicrobial screening of Ethyl acetic extract of Geodorum densiflorum was done by Disc diffusion method. The ethyl acetic extract of Geodorum densiflorum showed activity against both Gram-positive and Gram-negative organisms. The zone of inhibition was range from 10-15 mm in diameter at a concentration of 500µg/disc. A large zone of inhibition was observed 15 mm against Klebsiella spp. The cytotoxic activities of crude extract was determined using Brine shrimp lethality Bioassay and compared to Vincristine sulfate (with LC50 of 0.52µg/ml), ethyl acetate extract demonstrated a significant cytotoxic activity (having LC50 value of 2.23µg/ml). Qualitative Phytochemical screening is carried out by general test. The Phytochemical screening was revealed that the presence of carbohydrate, alkaloids, flavonoids, saponins, tannins. The study confirms the antimicrobial activity and Cytotoxicity of the ethyl acetic extract of Geodorum densiflorum and is therefore, a potential drug that requires further studies and development.

Keyword: *Geodorum densiflorum*, cytotoxic, antibacterial, and phytochemical.

INTRODUCTION: "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs."[1]

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This definition of Medicinal Plant has been formulated by WHO (World Health Organization). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now

been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties.

A number of plants have been used in traditional medicine for many years due to their antimicrobial properties.[2] Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body.[3] The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds. These phytochemicals are toxic to microbial cells.

In order to choose a promising plant for activity–guided isolation, plant materials are extracted and evaluated biologically. The brine shrimp lethality bioassay presents a simple technique for identification of potential plants for isolation of cytotoxic compounds.[4] This procedure has proven to be useful, rapid and inexpensive. The activity of the extracts is manifested as toxicity to the brine shrimps and the median lethal concentration, LC_{50} (concentration of the extract that kills 50% of the shrimps), can be estimated. This calculation uses the method of linear regression analysis of the recorded percentage death of shrimps at 95% confidence level.[4, 5]

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses. Medicinal plants have a long-standing history in many locations in Bangladesh and continue to provide useful and applicable tools for treating ailments. Nevertheless, little scientific research was done to investigate the plants *Geodorum densiflorum.*

The activities have been selected because of their great medicinal relevance. Within the recent years, infections have increased to a great extent and resistance against antibiotics becomes an ever-increasing therapeutic problem .[6] Because natural products of higher plants may give a new source of antimicrobial agents, as well as anticancer agents, many research groups are now engaged in medicinal plants research. In present study focus in vitro evaluation of antimicrobial activity and cytotoxicity of the separated fractions of ethyl acetate, extracts from the root parts of *Geodorum densiflorum* are bioassay against pathogenic microorganism and with the help of Brine Shrimp.

Materials and Methods

Plant materials

The roots portion of Geodorum densiflorum was collected from the local market of Savar, Bangladesh. A sample representing the collection has been deposited in the Bangladesh national Herberium (Accession Number DACB 34377).

Antimicrobial screening

The antibacterial assay was performed by disc diffusion technique.[7] Disc diffusion technique is highly effective for rapidly growing microorganisms. The microorganisms were collected as pure cultures from the Microbiology Lab, International Islamic University Chittagong, Bangladesh. 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. To compare the activity with standard antibiotics, Kanamycin (30 µg/ disc) was used.

The extract of *Saccharum spontaneum* was tested against four Gram- positive and six Gram negative *(Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Klebsiella spp, Sarcina lutea, Shigella sonnei , Bacillus mageterium,*

Proteus species, Shigella dysenteriae, Pseudomonas aeruginos and Vibrio cholerae) bacteria. 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 activities of the extracts were then determined by measuring the respective zone of inhibition in mm.

Cytotoxicity test

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds.[8, 9] Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to mature shrimp called nauplii. The brine shrimp lethality bioassay was performed to predict the cytotoxic activity[8, 12] of the Geodorum densiflorum. For experiment, The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations – 5µg/ml,10µg/ml, 20µg/ml, 40µg/ml, and 80µg/ml.

A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulphate was used as positive control.[10.11] then matured shrimps were applied to each of all experimental vials and control vial. After 24 hrs, the vials were inspected using a magnifying glass and the number of survived naupili in each vials was counted. The mortality end point of this bioassay was defined as the absence of control forward motion during 30s observation.[13] from this data the percent of lethality of the brine shrimp naupili for each concentration and control was calculated. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality[14] was plotted on the graph paper and the values of LC_{50} were calculated Using Microsoft excel 2007.

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).[2, 15, 16]

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Teat for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample , [2, 16] 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of

ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steriods: Two ml of acetic anhydride was added to 0.5 gm ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Results

Result of antimicrobial screening

The results of the ***in vitro*** antibacterial activity of the ethyl acetate extract of *Geodorum densiflorum* (500µg/disc) determined by diameters of inhibition zones are presented in Table 1. These results indicated that the diameters of inhibition zones varied from 10 - 15 mm. Among the test bacteria the growth of ***Klebsiella spp*** **(15mm)** Was strongly inhibited. Kanamycin used as a standard antibiotic at the concentration of 30 µg/disc exhibited higher diameters of inhibition than other extracts. These results revealed that the diameters of inhibition zones increased with the concentration of extract.

Table -1 Antibacterial effect of crude extract.

Test organisms	Diameter of zone of inhibition	
	Kanamycin (30µg/disc)	Ethyl acetate extract (500µg/disc
Gram positive		
Proteus species	32	11
Staphylococcus aureus	30	10
Bacillus mageterium	30	10
Bacillus subtilis	30	11
Gram negative		
Salmonella typhi	28	10
Vibrio cholerae.	35	13
Klebsiella spp	30	15
Pseudomonas aerugenosa	30	11
Shigella sonnei.	30	12
Shigella dysentriae	32	11

Result of Brine Shrimp Lethality Bioassay

The Ethyl acetic extract of Geodorum densiflorum possesses cytotoxic activity. The LC_{50} values obtained from brine shrimp lethality bioassay was 2.23µg/ml whereas Vincristine sulfate showed 0.52µg/ml.

Results of Phytochemical screening

The findings revealed that extract from *Geodorum densiflorum* contain phytochemicals which offer an enormouspotential as bio control of these pathogens and source of antimicrobial agents of therapeutic importance. The phytochemical analysis revealed the presence of saponins, alkaloids, flavonoids, steroids, resin as shown in Table 6. The results showed that the extracts from *Geodorum densiflorum* have antimicrobial activity against all the isolates tested even at lower concentration.

Table -2 Cytotoxicity effect of crude extract

Concentration in µg/ml	% Mortality	Log C	LC_{50} (µg/ml)	Conc. (µg/ml)	% Mortality	Log C	LC_{50} (µg/ml)
400 µg/ml	100	2.602		40	100	1.602	
200 µg/ml	100	2.301		20	100	1.301	
100 µg/ml	100	2.000		10	100	1.000	
50 µg/ml	100	1.698	2.23	5	90	0.699	0.52
25 µg/ml	90	1.398		2.50	80	0.398	
12.5 µg/ml	80	1.097		1.25	60	0.097	
6.25 µg/ml	70	0.796		0.63	50	-0.201	
3.125	70	0.494		0.31	40	-0.509	

Table 6. Phytochemical Screening of ethyl acetate extract of *Geodorum densiflorum*.

Carbohydrate	Glycosides	Saponins	Alkaloids	Flavonoids	Steroids	Tannins	Resins	Glucosides
+	-	+	+	+	+	-	+	-

Discussion and Conclusion

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization.[17] Although a significant number of studies have been used to obtain purified plant chemical, 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.[18] In the present investigation, the active phytocomponents of *Geodorum densiflorum* was studied and further the antimicrobial activity and Cytotoxicity of the plant extract was also tested to understand the most effective activity. The antimicrobial activity of the plant extract was tested against ten potentially pathogenic microorganisms *by using disc diffusion method* at different concentrations of the extract to understand the most effective activity. The maximum zone of inhibition was obtained for *Klebsiella spp(15mm) Vibrio cholera, Sarcina lutea (13mm), Shigella sonne (11.25)* at a concentration of 500µg/disc. The Ethyl acetic extract of *Geodorum densiflorum* possesses cytotoxic activity. LC_{50} was found at the dose of 2.23µg/ml. The antimicrobial and cytotoxic activity might be due to the presence of saponins, alkaloids, flavonoids which was confirmed by the Phytochemical screening.

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethno medical preparations and prescriptions of

plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethno botany and other biological actions for drug discovery.

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