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Biological Screening of Some Sewage Microbes in Bangladesh

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Abstract: The aim of present study was to investigate the bioactivity (antibacterial, antifungal and cytotoxicity etc.) of the ethylacetate extract isolated from *Streptomyces* sp. *Aspergillus* sp. and *Bacillus* sp. from sewage of different regions of Bangladesh. The ethylacetate extract of *Streptomyces* sp., *Aspergillus* sp. and *Bacillus* sp. shown modest antibacterial and antifungal activities at the concentration of 200 μg disc^{-1}. The maximum antibacterial and antifungal activities were shown by the *Streptomyces* sp. The minimum inhibitory concentration of the microbial extracts was between 64 to 128 μg mL^{-1} against test organisms. Brine srimp lethality bioassay was carried out for cytotoxicity measurement of the extracts and the LC_{50} values were calculated after probit transformation of the resulting mortality data. All the ethyl acetate extract showed lower cytotoxicity properties (*Streptomyces* sp. 42.37 μg mL^{-1}, *Aspergillus* sp. 52.25 μg mL^{-1} and *Bacillus* sp. 47.51 μg mL^{-1}) compared with the reference standard Bleomycin (0.41 μg mL^{-1}) and Galic acid (4.53 μg mL^{-1}).

Key words: Antibacterial, antifungal, cytotoxicity (LC_{50}), gram positive bacteria

INTRODUCTION

Microorganisms have historically provided a rich source of structurally diverse, biologically active metabolites[1] and sewage is the home of microbes. People consider it as a dirty place, which contain only harmful microbes but it was reported that harmful microbes as well as friendly microbes are also present in the sewage[2]. The environment i.e. temperature, pH and humidity etc. of sewage of Bangladesh is very much optimum for microbial growth. So there is a great to search newer and safer bioactive compounds from microorganisms isolated from sewage. Though a great number of bioactive compounds have been discovered only a few of them are useful and rest of them is toxic to human and animals or not highly effective against diseases caused by bacteria, fungi, viruses, insects etc. Moreover microbial resistance to bioactive compounds is a threat to public health throughout the world[3,4]. Due to indiscriminate use of bioactive compounds and other unknown reasons, the pathogenic organisms are gaining resistance to existing bioactive compounds[5]. The microbial infections are a major worldwide health problem, particularly in less developed countries where these diseases also cause a substantial economic burden. The global prevalence of human microbial infections already exceeds 60% and is increasing. The challenge of developing modern biology for the twenty first century needs a systemic research at least for the welfare of humanity. The great development of chemotherapy in the last few decades has led to a marked decrease in the rate of death caused by infectious diseases. In recent years, attempt has been made by the scientists to investigate the indigenous bioactive compounds from microbes of choice of infectious diseases for mitigation for suffering of the vast masses of humanity.

In the continuation of this research for bioactive compounds we have done primary screening of antimicrobial activity and subsequently by isolating crude compounds from sewage microbes' throughout Bangladesh and other bioactivity test such as antifungal and cytotoxicity study for the primary basis of biological activity.

MATERIALS AND METHODS

Organism collection, pure culture and isolation of bioactive compounds: Sewage samples were collected from wastages of drains from the different regions of Bangladesh such as Natore, Rangpur, Pabna, Bogra and Rajshahi and then by serial dilution technique[6] pure culture of the organisms were collected. After optimization of growth conditions such as temperature, pH, carbon sources, nitrogen sources, media, they were largely cultured in CDA (acidic), CDA (alkaline) and NB media. By using ethylacetate as solvent bioactive compounds were isolated and were dried under normal conditions. The ethylacetate extract of compounds were used in the following bioactivity tests to observe the activity of the isolated crude compounds.

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Antibacterial screening: *In vitro* antibacterial activity is generally performed by disc diffusion methods[7,8] for the primary selection of compounds as therapeutic agents. In this method activity of the test compounds are expressed by measuring the diameter of zone of inhibition. Generally the more susceptible the organisms the bigger the zone of inhibition. The antibacterial activity of the ethylacetate extract of the compounds isolated from *Streptomyces* sp., *Aspergillus* sp. and *Bacillus* sp. were determined at a concentration of 30 and 200 μg disc^{-1} against six gram positive and nine gram negative bacteria. The diameter of the zone of inhibition produced by the crude extracts were compared with the standard antibiotic kanamycin 30 μg disc^{-1}. The experiment was performed three times to minimize the errors.

Minimum Inhibitory Concentration (MIC) determination: MIC of the compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. MIC of the crude extracts was determined against six pathogenic bacteria by serial dilution technique[6]. The result was compared with the standard antibiotic kanamycin. The media used in this respect was nutrient agar (DIFCO).

Antifungal assay: The antifungal activity of the crude extracts was tested against seven pathogenic fungi at a concentration of 50 and 200 μg disc^{-1} for each. The media used in this respect was Potato Dextrose Agar (PDA). The activity was determined after 72 h of incubation at room temperature. For a better correlation of the antifungal activities Nystatin 50 μg disc^{-1} was used as a standard.

Cytotoxicity study of the crude extracts: Brine shrimp lethality bioassay is a recent development in the assay procedure of bioactive compound which indicates cytotoxicity as well as a wide range of pharmacological activities (such as anticancer, antiviral, insecticidal, pesticidal and AIDS etc) of the compounds. Here *in vivo* lethality test was carried out by using brine shrimp nauplii eggs (*Artemia salina* L.). Eggs were hatched after 48 h in 3.8% NaCl solution (Sea water) and after two days hatching, the nauplii were ready for experiment. Standard solution of the extract was prepared whose concentration was 5 μg mL^{-1} (3 mg extract was dissolved in 0.6 mL of DMSO). From the stock solution 5, 10, 20, 40 and 80 μL were placed in 5 different vials and the volume was made upto 5 mL with NaCl (3.8%) solution. Thus the final concentration of the sample in the vials become 5, 10, 20, 40 and 80 μg mL^{-1}, respectively. Then 10 brine shrimp nauplii were placed in each vial. For the control of each vial, one vial containing equal volume of DMSO and NaCl solution upto 5 mL. After 24 h each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. From the data % of mortality was calculated and plotted against Log dose (logC). From the graph LC_{50} of the extract was determined.

RESULTS AND DISCUSSION

Antibacterial activity: The ethylacetate extracts of the three organisms shown moderate antibacterial activities at the concentration of 30 μg disc^{-1} with respect to the standard antibiotic Kanamycin but showed remarkable activities at the high concentration of 200 μg disc^{-1}. Among the three sewage dwelling microbes, *Streptomyces* sp. showed comparatively better activity against *Shigella shiga* and the lowest activity was observed in case of *Baclilus* sp. against *Sarcina lutea*. Organism *Aspergillus* sp. showed moderate activity, (Table 1, Fig. 1B and C)

Table 1: *In vitro* antibacterial activity of the ethylacetate extract from *Streptomyces* sp., *Aspergillus* sp., *Bacillus* sp. and standard Kanamycin

	Determination of zone of inhibition (in mm)						
	Streptomyces sp. (μg disc^{-1})		*Aspergillus* sp. (μg disc^{-1})		*Bacillus* sp. (μg disc^{-1})		Kanamycin (μg disc^{-1})
Test organisms	30	200	30	200	30	200	30
Gram positive bacteria							
Staphylococcus aureus	15	23	13	21	11	21	30
Streptococcus- β-haemolyticus	13	19	12	20	12	20	29
Bacillus megaterium	14	22	12	20	09	18	31
Bacillus subtilis	15	24	13	23	00	17	29
Sarcina lutea	14	20	12	18	10	16	32
Bacillus cereus	14	22	12	19	11	17	30
Gram negative bacteria							
Salmonella typhi	14	20	13	17	12	19	31
Shigella dysenteriae	16	24	12	18	11	22	30
Shigella shiga	17	26	12	18	9	20	29
Shigalla flexneri	14	24	13	19	11	23	30
Shigella sonnei	13	22	11	18	00	20	30
Shigella boydii	13	20	12	20	09	18	31
Escherrichia colli	14	25	12	18	00	19	32
Pseudomonous aeruginosa	13	20	13	17	10	18	30
Klebsiella sp.	14	20	11	17	10	17	31

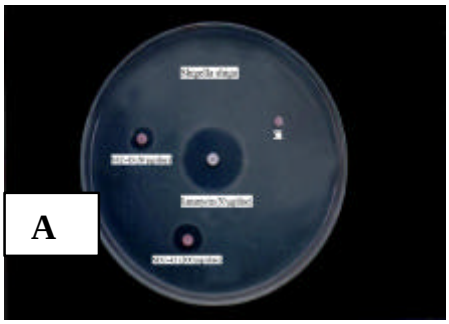

Fig. 1A: Effect of the ethyl acetate extracts from *Streptomyces* sp. against *Shigella shiga*

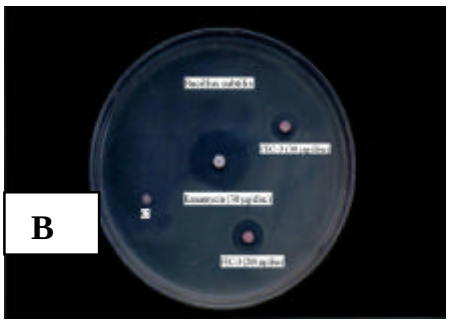

Fig. 1B: Effect of the ethyl acetate extracts from *Aspergillus* sp. against *Bacillus subtilis*

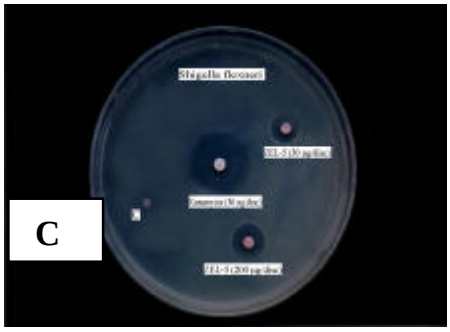

Fig. 1C: Effect of the ethyl acetate extracts from *Bacillus* sp. against *Shigella flexneir*

Table 2: Minimum Inhibitory Concentration (MIC) values of the ethylacetate extract of *Streptomyces* sp., *Aspergillus* sp., *Bacillus* sp. and standard Kanamycin

	Minimum inhibitory concentration (μg disc^{-1})			
Test organisms	*Streptomyces* sp.	*Aspergillus* sp.	*Bacillus* sp.	Kanamycin
Shigella shiga	64	128	128	4
Escherrichia coli	64	128	64	5
Shigelle flexneri	128	64	64	4
Staphyococeus aureus	128	128	128	4
Bacillus subtilis	128	64	128	5
Streptococcus-β- haemolyticus	128	64	128	4

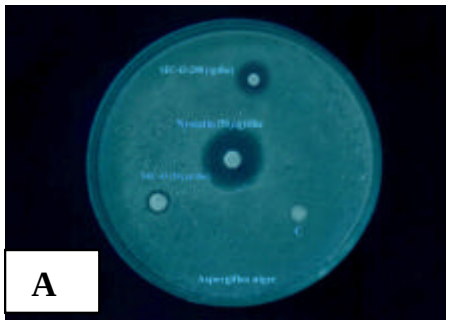

Fig. 2A: Effect of ethyl acetate extract from *Streptomyces* sp. (50 and 100 μg disc^{-1}) and standard Nystatin, (50 μg disc^{-1}) against *Aspergillus niger*

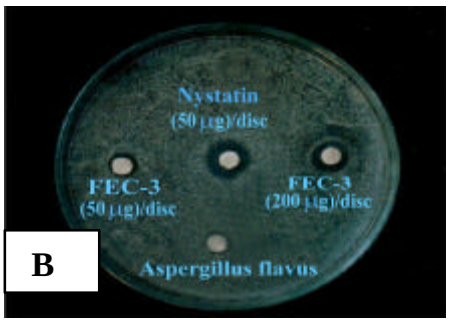

Fig. 2B: Effect of ethyl acetate extract from *Aspergillus* sp. (50 and 200 μg disc^{-1}) and Nystatin, standard (50 μg disc^{-1}) against *Aspergillus flavus*

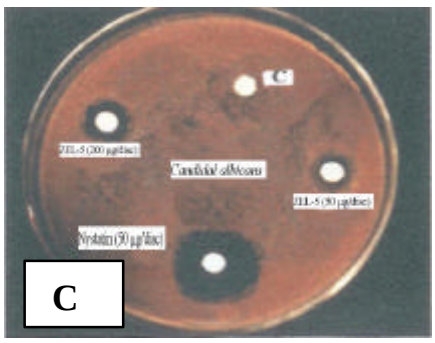

Fig. 2C: Effect of ethyl acetate extract from *Bacillus* sp. (50 and 200 μg disc^{-1}) and Nystatin, standard (50 μg disc^{-1}) against *Candidal albicans*

the more antibacterial activity of the organism *Streptomyces* sp. may be due to the *Streptomyces*. A compilation of the microbial sources of antibiotics discovered in USA and Japan between 1953 to 1970 reveals that approximately 85% of the antibiotics are obtained from Actonomycetes, 11% from fungi and 4%

Table 3: *In vitro* antifungal activity of the ethylacetate extracts from *Streptomyces* sp., *Aspergillus* sp., *Bacillus* sp. and standard Nystatin

	Diameter of zone of inhibition (mm)						
	Streptomyces sp. (μg disc^{-1})		*Aspergillus* sp. (μg disc^{-1})		*Bacillus* sp. (μg disc^{-1})		Nystatin (μg disc^{-1})
Test organisms	50	200	50	200	50	200	50
Candida albicans	8	12	-	11	-	09	29
Aspergillus fumigatus	-	09	-	10	-	10	32
Aspergillus flavus	-	10	-	11	7	10	28
Aspergillus nigar	-	11	-	10	-	11	30
Fusarium sp.	-	10	-	10	-	11	30
Penicillium sp	-	11	-	10	-	10	29
Vasien fectum	-	11	-	11	-	10	30

from bacteria[6]. Among the actinomyceties, the majority of known antibiotics are produced by *Streptomyces*[9]. In the following years, the production of antibiotics by *Streptomyces* is well reputed[10-12] and some of these are used in chemotherapeutics. Further studies were needed to explore the mechanism of antibacterial activity of these sewage dwelling microbes. Many authors also reported antibacterial activity of microbial extracts[13-17].

Minimum Inhibitory Concentration (MIC): The MIC value of the ethylacetate extract from *Streptomyces* sp. against *Shigella shiga, Escharichia coli, Shigela flexneri, Staphylococcus aureus, Bacillus subtilis* and *Streptococcus β-haemolyticus* were 64, 64, 128, 1128, 128 and 128 μg mL^{-1}, respectively (Table 2); that for ethylacetate extract from *Aspergillus* sp. were 128,128,64,128,64 and 128 μg mL^{-1}, respectively; and that for ethylacetate extract of *Bacillus* sp. were 128, 64, 64, 128, 128 and 128 μg mL^{-1}, respectively. From the MIC result we can conclude that the ethylacetate extract of sewage dwelling microbes are significantly less active compared with the standard kanamycin which give MIC values between 4 to 5 μg mL^{-1}.

Antifungal activity: Ethylacetate extract from *Streotomycies* sp. *showed* comperatively better antifungal activity than the other extracts from *Aspergillus* sp. and *Bacillus* sp. with comparison of the standard antifungal agent Nystatin (Table 3, Fig. 2A, B and C). The antifungal activity of microbes also reported by many researchers[18-20].

Catotoxicity study: In the brine shrimp lethality bioassay, the ethylacetate extracts from sewage microbes showed positive result indicating that the compounds of ethylacetates are biologically active. The mortality rate of brine shrimp nauplii was found to be increased with the increase in concentration of the sample. The median lethal concentration (LC_{50}) values of ethylacetate extracts from *Streptomyces* sp, *Aspergillus* sp, *Bacillus* sp. and standard Bleomycine and Galic acid were found to be 42.37, 51.25, 47.51 and 0.41 and 4.53 μg mL^{-1}, respectively

Table 4: The results of cytotoxic effect of ethylacetate extract from *Streptomyces* sp., *Aspergillus* sp., *Bacillus* sp. and Standard Bleomycin and Galic acid

Test samples	LC_{50} (ppm)	90% confidence limit (ppm)		Regression equation	χ^2
		Lower	Upper		
Streptomyces sp	42.37	28.52	62.93	Y=2.54+1..51 X	044
Aspergillus sp	51.25	33.80	77.69	Y=2.30+1.59 X	127
Bacillus sp	47.51	30.60	73.77	Y=2.60+1.43 X	037
Standard Bleomycin	0.41	00.276	0.62	Y=3.16+2.99 X	062
Standard Galic acid	4.53	03.33	6.15	Y=3.93+1.62 X	125

(Table 4). The lowest LC_{50} values was found in case of *Streptomyces* sp., which is indicative of its higher cytotoxicity than other sewage microbes. The highest LC_{50} values was found in case of *Aspergillus* sp. The moderate LC_{50} values were found in case of *Bacillus* sp. Many researchers[21-23] explored the cytotoxic properties of microbes. Present results suggested the cytotoxicity of previously reported findings.

Lastly it may be concluded that various bioactive compounds can be explored from *Streptomyces* sp., *Aspergillus* sp. and *Bacillus* sp. by screening of sewage an abundant source of microbes. The activity profile of these isolated ethylacetate extracts from the above three sewage dwelling microbes reveals that *Streptomyces* sp. of sewage may be considered as potential led compounds for the development of new bioactive compounds.

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