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ISOLATION OF ANTIMICROBIAL METABOLITES FROM A *STREPTOMYCES* STRAIN AND THEIR CYTOTOXICITY STUDY

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Abstract : Two antibiotics, designated as S_1 and S_2 were isolated from a strain of a *Streptomyces*. The antibiotics were extracted from the fermentation broth with ethyl acetate and were purified by preparative TLC and then by HPLC. Partial characterization of the antibiotics S_1 and S_2 was done by UV and IR spectroscopic methods. Strong antibacterial activity of the antibiotics S_1 and S_2 was observed against both gram-positive and gram-negative bacteria. The minimum inhibitory concentrations of the antibiotics against *Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Shigella shiga* were determined and found to 65, 65, 130 and 65 µgm/ml for S_1, 122.5,122.5,122.5 and 61.25 µgm/ml for S_2 respectively. In addition, the ethyl acetate extract and purified S_1 and S_2 showed positive results in the brine shrimp lethality bioassay. The LC_{50} values were 21.13, 54.95 and 94.41 µgm/ml for ethyl acetate extract, S_1 and S_2 respectively.

Key words : *Streptomyces*, Antibacterial activity.

Introduction

Since the discovery of penicillin in 1929, thousands of antibiotics have been developed from microorganisms or synthesized. Although a wide varieties of microorganisms are killed by antibiotics, the number of newer and resistant strains of bacteria are alarmingly increasing. Much of the problem is caused by irrational use of antibiotics. Century old diseases like tuberculosis, pneumonia and meningitis are fighting back furiously and huge number of bacteria are showing increasing resistance toward numerous antibiotics. That is why, researchers have been searching for newer and more potent agents with fresh efforts. But only a few of the antibiotics are suitable and available for treatment because of their range of activity and less toxicity. Considering these objects, we have isolated two antibiotics from a *Streptomyces* strain which showed marked antibacterial activity.

Materials and Methods

Collection and identification of the *Streptomyces* strain: The organism was isolated from a soil sample of Pabna and was identified as *Streptomyces* by crowded plate technique. (Hammond et al. 1978 & Masud, 1997) (Fig.1).

Fig. 1. Microscopic view of *Streptomyces* strain.

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Production, isolation and purification of antibiotics: The organism was grown in starch casein broth medium at pH 8 and at temperature 38^0C (0.50C) for 21 days. The culture filtrate (200 x 10) was extracted with ethyl acetate (30+20+10 ml ethyl acetate for each 100 ml filtrate). The ethyl acetate extract was evaporated in a rotary evaporator and a yellowish solid mass was obtained. Two fractions were purified by TLC and then by preparative TLC using chloroform: methanol (8:2) as the solvent system. The compounds were further purified by HPLC using methanol: water (70:30) for S_1 and (60:40) for S_2 as the developing solvent (Fig. 2 and 3).

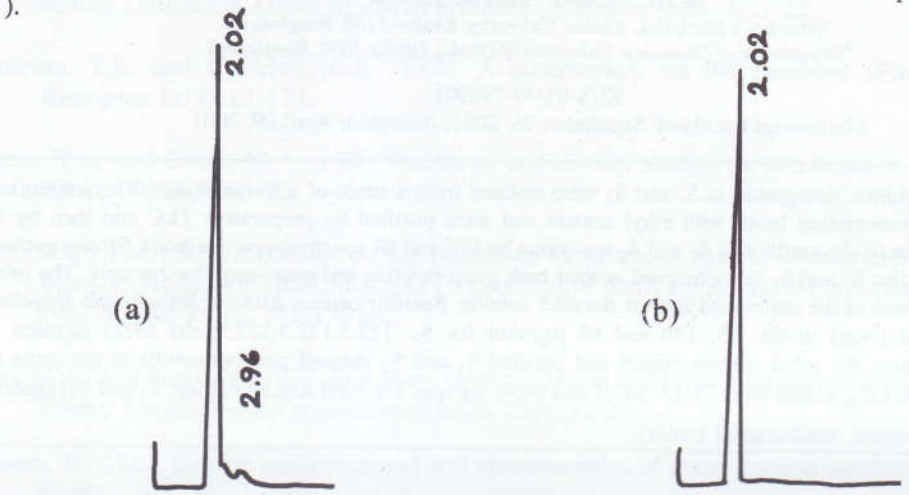

Fig. 2. (a) HPLC trace of compound S_1 before purification (b) HPLC trace of compound S_1 after purification.

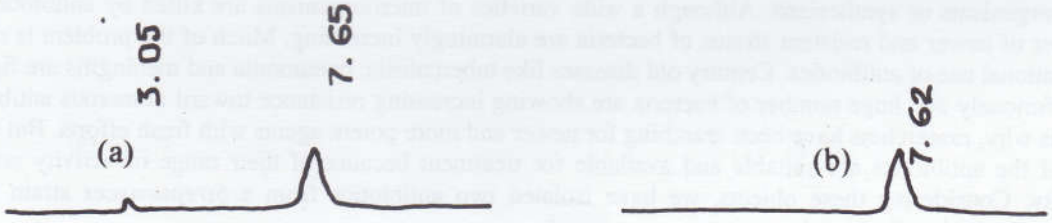

Fig. 3. (a) HPLC trace of compound S_2 before purification (b) HPLC trace of compound S_2 after purification.

Identification of S_1 and S_2: These compounds were partially identified on the basis of their Ultraviolet (UV) and Infrared (IR) spectral data.

Antibacterial screening: The antibacterial activity of the ethyl acetate extract, compound S_1 and S_2 were observed against 7 gram-positive and 9 gram-negative bacteria at a concentration of 200 µgm/disc by disc-diffusion technique (Barry, 1980; Bauer et al., 1966; Berghe et al., 1991 & Rios et al., 1988). Kanamycin 30 µgm/disc was used as standard.

Determination of MIC values: The minimum inhibitory concentration (MIC) of both S_1 and S_2 were determined against *Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Shigella shiga* by serial dilution method (Reiner, 1982).

Determination of cytotoxic activity of the ethyl acetate extract, S_1 and S_2: The cytotoxic activity of the metabolites were evaluated by brine shrimp lethality bioassay (Masud, 1997; Meyer et al., 1982 & Persoone, 1980).

Results and Discussion

During the course of investigation, an organism was isolated from a soil sample of Pabna. The microorganism was identified as a strain of *Streptomyces* because of the following characteristics:
Vegetative and aero hyphae were present, aerial mycelia were long and branched, spores were round, the spores were in a straight chains of about 7-10 in number, sporulation begins at the top of the aerial hyphae, the spores were smooth. It was concluded from the above mentioned morphological observations that the organism belonged to the following classification:
Order-*Actinomycetes*, Family-*Streptomycetaceae*, Genus-*Streptomyces*.

The culture filtrate of the *Streptomyces* strain afforded two antibiotics designated as S_1 and S_2. They were partially characterized on the basis of their UV and IR spectral data. S_1 and S_2 showed absorption maximum

at 302 nm and 351 nm respectively indicative of unsaturation in the molecule. IR spectrum of S_1 displayed peaks at 3300 cm^{-1}, 2920 cm^{-1}, 1725 cm^{-1}, 1650 cm^{-1}, 1460 cm^{-1}, 1375 cm^{-1}, 1345 cm^{-1}, 1275 cm^{-1}, 840 cm^{-1} and S_2 displayed peaks at 3320 cm^{-1}, 2920 cm^{-1}, 1710 cm^{-1}, 1620 cm^{-1}, 1450 cm^{-1}, 1190 cm^{-1}, 1040 cm^{-1}, 930 cm^{-1} which indicate the presence of different groups like -CO, -OH, -NH, -CN, -COOR, -CONH2 etc.
All of these compounds including ethyl acetate extract exhibited a significant antibacterial activity against both gram-positive and gram-negative bacteria at a concentration of 200 µgm/disc (Table 1). The antibacterial activity of these compounds was compared with that exhibited by a standard broad-spectrum antibiotic, Kanamycin. The zone of inhibition produced by ethyl acetate extract, S_1 and S_2 were 17-29 mm, 18-31 mm, 12-26 mm respectively. On the other hand, zone of inhibition produced by Kanamycin were 6-14 mm at a concentration of 30 µgm/disc. It is appeared from that table that the activity of these compounds were comparable with that of Kanamycin.

Table 1: Antibacterial activity of the ethyl acetate extract, compound S_1, compound S_2 and Kanamycin standard

Test bacteria	Diameter of zone of inhibition (mm)			
	Ethyl acetateextract 200µgm /disc	S_1 200µgm /disc	S_2 200µgm /disc	Kanamycin 30µgm /disc
Gram- positive				
Bacillus subtilis	28	31	13	10
Bacillus cereus	27	30	14	6
Bacillus polymyxa	24	22	16	9
Bacillus megaterium	-	-	-	10
Sarcina lutea	24	-	-	9
Staphylococcus aureus	25	30	12	8
Streptococcusβ haemolyticus	24	-	-	10
Gram-negative				
Escherichia coli	29	25	26	12
Salmonella typhi A	28	26	15	14
Salmonella typhi B	24	22	12	10
Shigella shiga	25	20	15	8
Shigella sonnei	28	21	22	10
Shigella boydii	27	18	20	11
Shigella flexneri	22	-	-	10
Shigella dysenteriae	21	-	-	8
Pseudomonas aeroginosa	17	-	-	7

The MIC values of S_1 and S_2 were determined against *Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Shigella shiga* and were found to be 61.25-130 µgm/ml (Table 2).

Table 2: Determination of minimum inhibitory concentration of the isolated antibiotics

Antibiotics	**Minimum Inhibitory Concentration (µgm/ml)**			
	Bacillus subtilis	*Bacillus cereus*	*Escherichia coli*	*Shigella shiga*
S_1	65	65	130	65
S_2	122.5	122.5	122.5	61.25

The cytotoxicity of the crude ethyl acetate extract and the isolated antibiotics were determined by brine shrimp lethality bioassay. The LC_{50} values are 21.13 µgm/ml for ethyl acetate extract, 54.95 µgm/ml for S_1 and 94.41 µgm/ml for S_2. The cytotoxic effect of the crude extract was higher than that of the antibiotics S_1 and S_2 . The higher cytotoxicity of crude extract may be due to synergistic effect of S_1 and S_2. Another explanation may be that compounds other than S_1 and S_2 may be present in the extract which we could not trace out.

From the experiment it can be demonstrated that *Streptomyces* strain which was isolated from the soil sample of Pabna possesses potent antimicrobial metabolites.

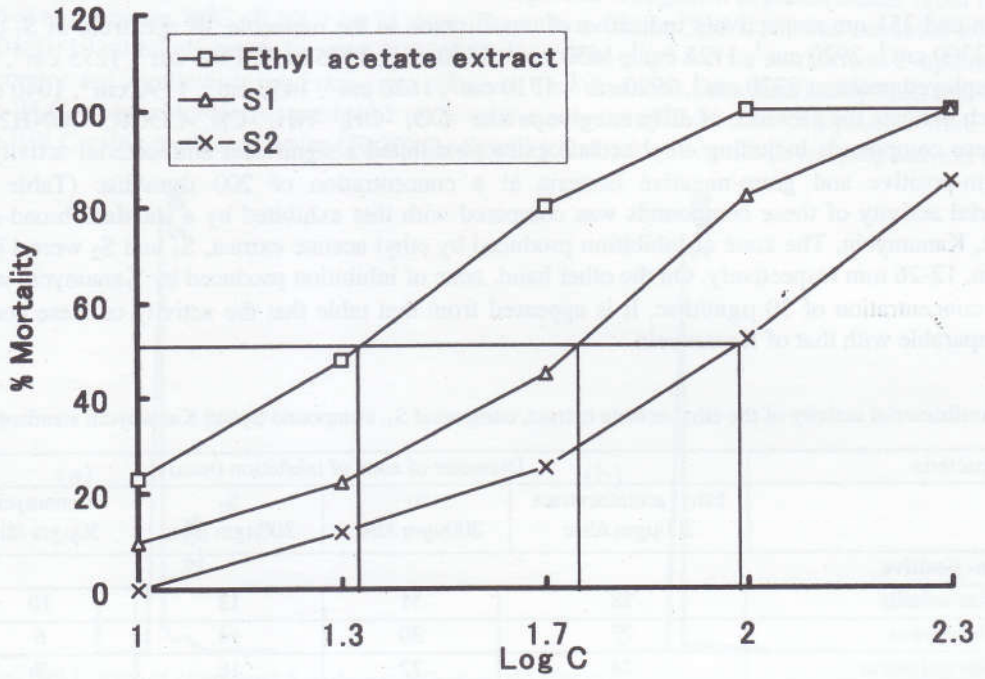

Fig. 4. Brine Shrimp Lethality Bioassay.

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