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INVESTIGATION ON ANTIMICROBIAL ACTIVITIES OF THE PLANT *SWERTIA CHIRATA* HAM.

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Abstract: Biological activities of fresh stem of the plant *Swertia chirata* Ham. (F. Gentianaceae) extracted in rectified spirit is reported here. The crude rectified spirit extract was fractionated by using standard chromatographic techniques on alumina, which gave six fractions (A, B, C, D, E & F). When subjected to column chromatographic analysis on neutral alumina, fraction D yielded a pure compound tentatively known as AJ-1 that have melting point of 178° C. AJ-1 was screened for its antibacterial activities against 12 pathogenic bacteria, 6 Gram positive and 6 Gram negative, by disc diffusion method at a concentration of 200 µg/disc. The results obtained were compared with those for a standard antibiotic Kanamycin. AJ-1 showed significant activity against *Bacillus megaterium* (11 mm), *Bacillus subtilis* (9 mm), *Salmonella typhi-A* (10 mm), *Shigella flexeneriae* (10 mm) and *Klebsiella* sp (11 mm) but a little activity against *Staphylococcus aureus*. The Minimum Inhibitory Concentrations (MIC) of AJ-1 determined against *Bacillus megaterium* and *Salmonella typhi-A* were 128 µg/ml and 128 µg/ml, respectively when tested in a nutrient broth medium. AJ-1 also showed significant activity against the brine shrimp (*Artemia salina*) nauplii (LC_{50} value of 9.34 µg/ml), in which the mortality rate increased with the increasing concentration of the compound, suggesting a positive correlation between brine shrimp toxicity and cytotoxicity.

Key words: Biological activity, ***Swertia*** *chirata*, Gentianaceae, pathogenic bacteria

mvivsk: GB M‡elYvq RxevYi wei"‡× †iKwUdvBW w¯úwiU Øviv wb®wkZ wPiZvi (†RbwUqvb¨wm cwievif³) cwi"Qb Kv‡Ûi KvhKwiZv eYbv Kiv n‡q‡Q| wPiZvi cwi"Qb KvÛ Acwi‡kwaZ †iKwUdvBW w¯úwiU G·Uv± A¨vjwgbv Kjv‡g cgvb †µvg¨v‡UvMwdK c×wZ‡Z c_K K‡i cvß QqwU As‡ki (dvKkb A, B, C, D, E I F) g‡a¨ dvKkb D †K wbi‡c¶ A¨vjwgbv Kjv‡g Kjvg †µvg¨v‡UvMwd Øviv we‡klb K‡i 178^0 †m Mjbv¼ wewkó †hŠM AJ-1 Drcb nq| †hŠM AJ-1 Gi KvhKwiZv wbi|c‡Yi Rb¨ 200 µg/disc NbgvÎv wW¯‹wWdDkb c×wZ‡Z 6 wU Mvg-cwRwUf I 6 wU Mvg †b‡MwUf †gvU 12 wU c¨v‡_v‡RwbK e¨vK‡Uwiqvi Dci cix¶v-wbix¶v Kiv n‡q‡Q| cvß djvdj cgvY GwUev‡qwUK K¨vbvgvBwmb c`Ë djvd‡ji ms‡M Zjbv Kiv nq| AJ-1 e¨wmjvm †gMv‡Uwiqvg (11 wg.wg.), e¨wmjvm mvewUwjm (9 wg.wg.), m¨vj‡gv‡bjv UvBwd-G (10 wg.wg.), wm‡Rjv ‡d‡R‡bwiqv (10 wg.wg.) I †Kewmjv †¯ú. (11 wg.wg.)-Gi Dci D‡jL‡hvM¨ KvhKwiZv c`kY K‡i, wKš÷¨wd‡jv‡KvKvm Awiqvm-Gi wei"‡× Zvi mvgvb¨ KvhKwiZv Av‡Q| e¨wmjvm †gMv‡Uwiqvg I m¨vj‡gv‡bjv UvBwd-Gi Dci AJ-1 Gi b¨bZg cwZ‡iva NbgvÎv wbi|cb Kiv n‡q‡Q hv wbDwU‡qvU e_ wgwWqv‡g cix¶v K‡i h_vµ‡g 128 µg/ml I 128 µg/ml cvIqv †M‡Q| AJ-1 Gi ewBb wk¤ú b¨vcjxi Dc‡iI D‡jL‡hvM¨ KvhKwiZv (9.34 µg/ml) †Z LC_{50} Zvi D"P mvB‡UvUwi·K cwZwµqv wb‡`k K‡i hvi g‡a¨ AJ-1 Gi NbgvÎv ewi×i ms‡M ms‡M ewaZ gZ¨nvi ewBb wk¤ú Uwi·wmwU I mvB‡UvUwi·wmwU-Gi g‡a¨ GKwU abvZ‡K mgm¤úK we`¨gv‡bi wb‡`kK|

Introduction

The plant S*wertia chirata* Ham. (F. Gentianaceae) is a tropical family of small trees and herb. It consists of 180 species of which 8-10 species exist in Indian subcontinent (Hooker, 1885). This plant is indigenous to Himalayas at altitudes above 4000 ft from Kashmir, Nepal to Bhutan (Bhattacharjee, 1980). All members of this family are used as medicinal plants. The main chemical constituents of this plant are two bitter principles, *viz*, ophelic acid, an amorphous bitter principle and chiratin, a yellow bitter glucoside. The plant also contains resins, tannin, gum, carbonates, phosphates and 4-6% ash, lime and magnesia (Nadkarni, 1976; Chopra, 1982). A number of workers have shown that the plant contains bitter glucosidal components, chiratin and amarogentin, swerchirin, gentiopicrin, phytosterd and also a number of acid, yellow crystalline phenols and saccharine (Korte, 1955; Dalall and Shah, 1956; Korte and Schicke, 1956). The present work describes the biological activity of the various fractions of the crude as well as pure extracts of the whole plant.

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Materials and Methods

Collection of the plant: Dried stems of *S. chirata* (locally known as Chirata) were collected from Bhangura Kabiraji Shop at Rajshahi Shaheb Bazar in January, 2006. Identity of the plant was confirmed by A.T.M. Nadiruzzaman, Professor, Department of Botany, University of Rajshahi.

Preparation of plant materials: The stems along with the leaves of the plant were cut into small pieces by a sharp knife and dried in the sun for 72 hrs. This was further dried in the oven for 24 hrs at a temperature below 40^{o}C. About 650g of the dried plant materials were weighed by an electric balance and grinded with a grinding machine.

Extraction of plant materials: The powdered materials were taken in a clean flat bottomed glass container (2.5L) and macerated with sufficient amount of rectified spirit and with occasional shaking. After 15 days the solvent was

decanted and filtered by Tincture filter press (Karl Kolb, Scientific-Technical Supplies, Frankfurt, Germany); and then filtered through fresh cotton. The filtrate thus obtained was taken in a beaker.

Evaporation of the solvent: The solvent of the extract was evaporated under temperature and pressure to obtain a gummy mass, which was preserved in a refrigerator at 4^{o}C for chemical investigation.

Isolation of the compounds: Rectified spirit extract of the plant was a crude product that contained a mixture of compounds. Thin layer chromatographic (TLC) examination of the extract under petroleum ether: ethyl acetate (1:2) system showed 3 spots having R_f values of 0.1, 0.54 and 0.73, respectively. After isolating, different fractions were obtained. The fractions were combined on the basis of their preliminary TLC examination. Each examination gave combined fractions designated as A, B, C, D, E and F, each one was evaporated to dryness under reduced pressure. The residue (110mg) from Fraction D was subjected to a small chromatographic column on alumina using petroleum ether: ethyl acetate (1:1). The eluants were colleted and evaporated to get two compounds designated as AJ-1 (45mg) and AJ-2 (8mg). The residues from the remaining fractions (fraction A, B, C, E, and F) did not give any spot and tailed badly and therefore was not worked out further. The compound AJ-1 was crystalline while the compound AJ-2 was amorphous. AJ-2 being insufficient in quantities was not considered for further investigation.

Bioassays of the crude and pure extracts: Brine shrimp lethality bioassay is a recent development in the bioassay for bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (*e.g*. anticancer, antiviral, pesticidal, AIDS etc.) of the compounds. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simple pharmacology at a higher dose. Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, antimicrobial and pharmacological activities natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. Here in vivo lethality of a simple zoological organism (brine shrimp nauplii) is used as a convenient monitor for screening a fractionation in the discovery of new bioactive natural products. Generally the median effective dose (ED_{50}) values for cytotoxicities are one tenth (1/10) of median lethal dose (LC_{50}) values in the brine shrimp test.

Results and Discussion

Antibacterial activity of test sample: The antibacterial activities of the crude extract and the pure compound AJ-1 were tested. The results obtained are shown in Table 1. The inhibitory activities are shown in Fig.1.

Antibacterial activity of rectified spirit extract: Antibacterial activity of rectified spirit extract was tested against eight bacteria at concentrations of 30µg/disc and 90µg/disc. Standard antibiotic disc of chloramphenicol (30µg/disc) was used for comparison. The results obtained were shown in Table 1 and Fig. 1. The produced zone of inhibition for rectified spirit extract against *Staphylococcus aureus*, *Bacillus megaterium* and *Escherichia coli* were 9mm, 8mm and 8mm at 30µg/disc dose respectively. At 90 µg/disc dose, the produced zone of inhibition against the same bacteria was 16mm, 13mm and 12mm respectively. It was evident that the antibacterial activity of rectified spirit extract against the above bacteria showed decrease dose dependency.

Table 1. *In vitro* antibacterial activity test of the crude extract of *S. chirata* and its pure compound AJ-1

Test organisms	Crude extract		Pure Compound AJ-1 (200µg/disc)	Standard Chloramphenicol (30µg/disc)
	30µg	90µg		
Staphylococcus aureus	09	16	-	33
Bacillus subtilis	07	11	9	35
B. megaterium	08	13	11	29
Sarcina lutea	-	-	-	27
Salmonella typhi-A	08	12	10	23
Shigella sonnei	-	-	-	35
S. shiga	-	-	-	26
Escherichia coli	08	12	-	34
S. flexeneriae	07	11	10	30
Klebsiella sp.	09	13	11	32

Values indicate zone of inhibition (diameter in mm)

Antibacterial activity of AJ-1

The pure compound AJ-1 was screened for their antibacterial activities against 12 pathogenic bacteria, 6 Gram-positive and 6 Gram-negative, by disc diffusion method at a concentration of 200µg/disc. The results

obtained were compared with those for a standard antibiotic Kanamycin. AJ-1 showed significant activity against *Bacillus megaterium* (11mm), *Bacillus subtilis* (9mm), *Salmonella typhi-A* (10mm), *Shigella flexeneriae* (10mm) and *Klebsiella* sp. (11mm). A little activity of AJ-1 against *Staphylo coccus aureus*, *Sarcina lutea* and *Shigella dysenteriae* was observed.

Minimum inhibitory concentration (MIC) of the test sample

Minimum Inhibitory Concentration (MIC) of rectified spirit extract was determined by serial dilution technique (Roland 1982) against two Gram-positive bacteria *Staphylococcus aureus* and *Sarcina lutea* and two Gram-negative bacteria *Escherichia coli* and *Salmonella typhi-A*. The MIC value of AJ-1 was determined against a Gram- positive bacterium (*Bacillus megaterium)* and a Gram-negative bacterium (*Salmonella typhi-A*) These values are shown in Table 2.

MIC of the crude extract

For the Rectified spirit extract the growth was observed in the test tube containing 128 µg/ml of extract against *Escherichia coli, Sarcina lutea, Bacillus megaterium* and *Salmonella typhi-A* and for *Staphylococcus aureus* in the test tube containing 64µg/ml of rectified spirit extract.

So the MIC values of Rectified spirit extract for *Sarcina lutea, Escherichia coli, Bacillus megaterium* and *Salmonela typhi-A* were 128µg/ml and for *Staphylococcus aureus* was 64µg/ml (Table 2).

MIC of AJ-1

The Minimum Inhibitory Concentrations of the pure compounds AJ-1 was determined against *Bacillus megaterium* and *Salmonella typhi-A*. The MIC of the pure compound AJ-1 against *Bacillus megaterium* and *Salmonella typhi-A* were 128µg/ml and 128µg/ml respectively when tested in nutrient broth medium. The results are shown in Table 2.

Table 2. Minimum Inhibitory Concentration (µg/ml) values of rectified spirit extract of *S. chirata* and its pure compound AJ-1.

Bacterial strains	Rectified spirit extract (µg/ml)	Pure compound AJ-1
Gram positive		
Staphylococcus aureus	64	-
Sarcina lutea	128	-
Bacillus megaterium	128	128
Gram negative		
Escherichia coli	128	-
Salmonella typhi-A	128	128

Values indicate zone of inhibition (diameter in mm)

Brine shrimp mortality of test sample

Brine shrimp mortality test is a recent development in the bioassay for the bioactive compounds (McLaughlin 1990). Bioactive compounds are almost always toxic in high dose. There is a positive correlation between brine shrimp toxicity and cytotoxicity (Persoon 1980; Mayer *et al.* 1982; McLaughlin and Anderson 1988). The crude rectified spirit extract and pure compound AJ-1 showed positive result in brine shrimp lethality bioassay. The results were shown in Table 3.

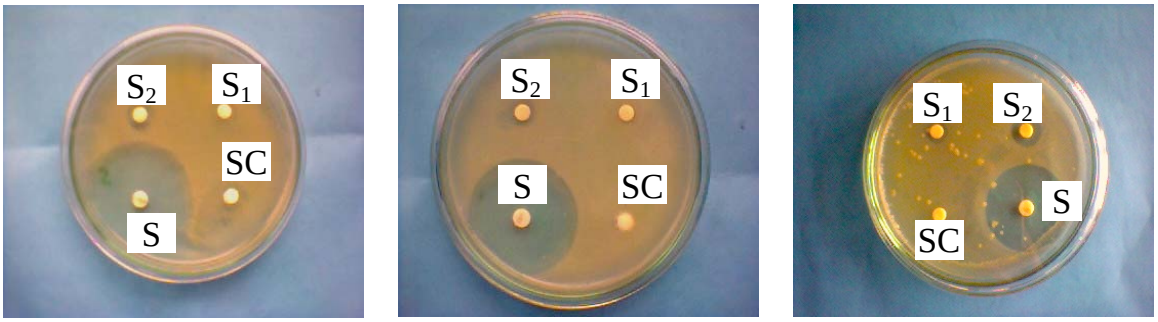

Fig. 1. Antibacterial activity of the crude extract of *S. chirata* against *B. megaterium* (A) *E. coli* (B) and *S. aureus* (C) where S = Standard (Chloramphenicol 30µg/disc); SC = Solvent control; S_1 = 30µg/disc and S_2 = 90µg/disc.

Brine shrimp mortality of rectified spirit extract

To determine the cytotoxic effect of rectified spirit extract, the lethal concentration, LC_{50} (concentration at which 50% mortality of the nauplii occurred) was measured and found to be 60.25µg/ml. This was obtained from a plot of percentage of mortality versus log of concentration on the graph, which produced approximate linear correlation between them (Fig. 2). The result of brine shrimp lethality of rectified spirit extract was given in the Table 3.

Table 3. Results of the rectified spirit extract of S. chirata on brine shrimp lethality bioassay.

Samples	Concentration µg/ml	Log C	Mortality (%) after 24 hrs*	LC_{50} µg/ml
Crude extract	10	1.00	10	60.25
	20	1.30	20	
	45	1.65	40	
	80	1.90	60	
	120	2.07	80	
Compound AJ-1	0	0.00	0	12.58
	5	0.69	20	
	10	1.00	40	
	20	1.30	70	
	40	1.60	90	
	80	1.90	100	

*10 nauplii used per concentration.

Brine shrimp mortality by AJ-1

To determine the cytotoxic effect of pure compound AJ-1 medium lethal concentration (LC_{50}) of brine shrimp lethality was measured and it was found to be 9.34µg/ml, which was obtained from a plot of percentage of mortality versus log of concentration (µg/ml) on the graph. This afforded an approximate linear correlation between them (Fig. 2). The results of brine shrimp mortality of pure compound AJ-1 are shown in the Table 3.

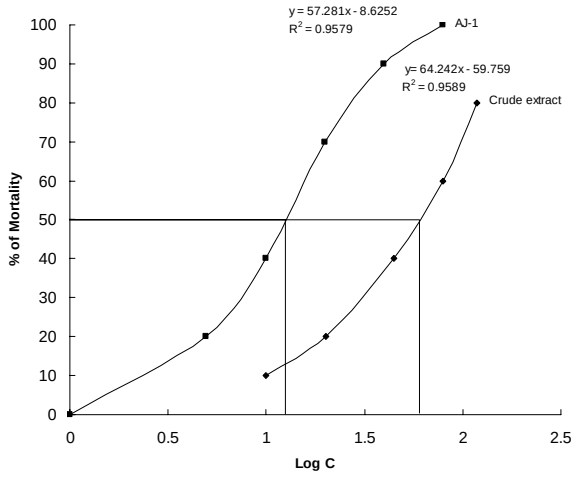

Fig. 2. Determination of LC_{50} of rectified spirit extracts of *S. chirata* and its pure compound AJ-1 against the brine shrimp (*Artemia salina*) nauplii

The pure compound AJ-1 was found to show significant activity against the brine shrimp nauplii. In this bioassay, the mortality rate of brine shrimp is found to increase with the increasing concentration of the compound. So it was observed that there existed a positive correlation between brine shrimp toxicity and cytotoxicity. The very low value of LC_{50} (12.58µg/ml) indicated the high cytotoxic effect of the pure compound AJ-1.

Conclusion

The rectified spirit extract and the pure compound of the medicinal plant *S. chirata* demonstrate appreciable antibacterial as well as biological activities. Further work is being continued for the identification of the isolated pure compound AJ-1.

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