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Antibacterial Activity of Some New Compounds of Sarcococca saligna

Tahira Moeen Khan, Ismat Naeem

Department of Chemistry, Lahore College for Women University, Jail Road, Lahore, Pakistan

Abstract: ***Pharmacological studies of S. saligna revealed that the crude ethanolic extracts of S. saligna (aerial parts) and roots were cytotoxic and antibacterial but produced no platelet aggregation induced by ADP. Antibacterial activity of four alkaloids (compounds) was determined for the first time against 11 bacterial strains .Compound 1 (Sracosalgmine) showed moderate activity against Shigella boydii, Klebsiella pneumoniae. Compound 2 (Sracosalgminol) showed less activity with Staphylococuss aureus and Streptococuss pyrogens. Compound 3 (sracosalgnenone) and Compound 4 (14-15 dehydrosarcovagine-D) were inactive against all bacterial strains.***

Keywords: Sarcococca saligna, Buxaceae, steroidal alkaloid, antibacterial alkaloid, Sracosalgmine, Sracosalgminol, Sracosalgnenone, 14-15 dehydrosarcovagine-D

1. Introduction

Sarcococca saligna Muel (syn. *Sarcococca pruniformis* Lindl.) is an ever green shrub abundantly found in northwest regions of Pakistan.[1]. Family Buxaceae has high contents of steroidal alkaloids[2].It has different genera i.e Pachysandra, *Sarcococca*, simmonsia and Buxus. Genus *Sarcococca* and *Pachysandra* has simple pregnane type of alkaloids, structurally they are very close to steroidal alkaloids of *Apocynaceae*[3]. The *Pachysadra* alkaloids have been found to be active against gastric ulcers in mice [4]. A crystalline base isolated form leaves of *S. saligna* was tested for biological activity and its effect on neuromuscular transmissions were found to be remarkable [5]. The extracts of various species of this genus have been used for the treatment of a variety of ailment and skin diseases[6].The increasing recognition and importance of fungal and bacterial infections, the difficulties encountered in their treatment and the increase in resistance to antimicrobials have stimulated the research for therapeutic alternatives especially from natural products.

2. Literature Survey

The leaves and the aerial parts of *S. saligna is a source of a* number of steroidal alkaloids, some of them showing biological activity [7][5][8][9][10][11][12][13][14][15][16]. The cytoxicity of crude methanolic extract of *S. saligna* (aerial parts was tested by Brine Shrimp method) [17] and found to be toxic in concentration 1000 µg/µl. The Crude ethanolic extract of *S. saligna* produced no platelets aggregation induced by ADP.Agar well diffusion method [18] was used to test antibacterial activity of crude ethanolic extract of *S. saligna* and isolated pure alkaloids against *Pseudomonas pseudomalliae, Shigella boydii, Staphylococcus aureus, Escherichia coli, Bascillus subtilis and Corynebacterium diphtheriae* bacterial strains (**Table**).

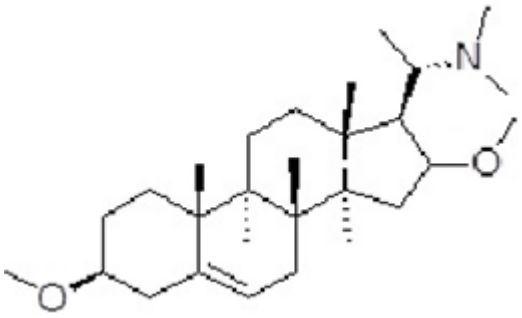
3. Materials and Method

General experimental procedure:IRspectra:JASCO 302-A spectrophotometer; UV spectra :Hitachi U3200 spectrophotometer EI, FD and HREI MS:JMS 11×100(with data system) and JMS-DA 500 mass spectrometers; ^{1}H and ^{13}C NMR spectra :Bruker NMR spectrometer at 500 and 125 MHz, respectively at room temperature; chemical shift values(δ)in ppm, coupling constants (J)in Hz. Standard pulse sequences were used for COSY,HOHAHA,DEPT,HMQC and HMBC experiments.

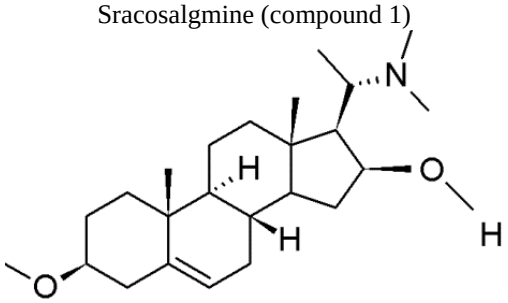
Chromatographic conditions: TLC (pre coated silica G-25plates UV254);CC:Silica gel,230-400 mesh.Detection of the spots:254 and 336 nm by UV and Dragendorff's spray reagent.

Plant Material: Aerial parts of *Sarcococca saligna* Muel.(40kg) were collected from Kuldana Murree Hills, Pakistan, in October 2004.

Extraction and Isolation: The ethanolic extract of the air dried plant (20kg) was evaporated to a gum (2.1kg) and extracted with petroleum ether to remove non polar constituents. Total alkaloids (900g) were obtained by extraction into 10% acetic acid. Partial separation of the alkaloids were achieved by extraction with $CHCL_3$ at different pH values (3.5,8.5).The fraction obtained at pH 3.5 (80g) was subjected to C.C on silica gel and eluted with $CHCL_3$ and then with $CHCL_3$-MeoH to obtain several fractions.



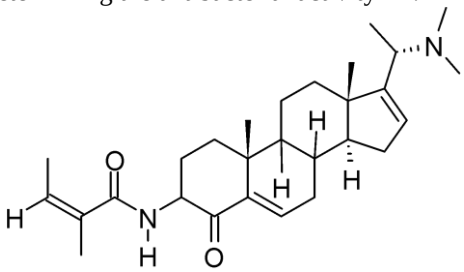
Sracosalgmine (compound 1)

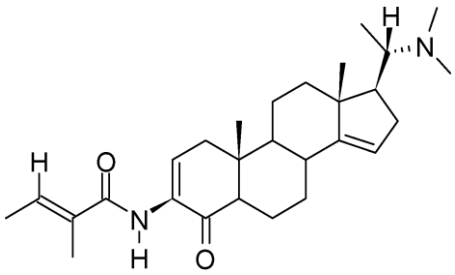


Sracosalgminol (compound 2)

Bioassay

Toxicity was determined by brine shrimp method. Bactericidal activity was determined by agar well diffusion method. This test was performed by spreading 18-24 hour old pathogenic bacterial cultures containing approximately.10^4-10^6 colony forming units (CFU/ml) on the surface of nutrient a gar (Bio M Laboratories, USA BMO 13-62-N) plates. Wells were dugged in the media with the help of sterile metallic borer. Test samples of different concentrations prepared in dimethylsulfoxide (DMSO Merck) were added in their respective wells. Pure DMSO was used as control. Other wells were supplemented with reference compounds i.e. amoxillin. 3 H_2O and cephalexin- Na^+, serving as positive control when determining the antibacterial activity [18].





Sracosalgnenone (compound 3)14-15
Dehydrosarcovagine-D (compound 4)

For determination of antibacterial activity microbial strains of *Staphylococcus aureus* ATCC 25923 (*S.aureus*). *Escherichia coli* (ATCC 2592) (*E.Coli*), *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Streptococcus pneumoniae* (ATCC 49619) (*S. pneumoniae*) *Bacillus subtilus* (ATCC 6051) (*B. subtilus*) and *Sarcinalutae* (ATCC 9341) (*S. lutae*), were obtained from Peeds Microbiology Laboratory Mayo Hospital, Lahore. These microbial strains are already identified from National Institute of Health, Islamabad and DTL, drug testing Laboratory, Lahore.

Table 1: Antibacterial Activity of Methanol Extract and pure isolated Compounds (**1- 4)** of *S. saligna*

Sr. No	Bacterium	Amp	Tob (20µg/ µL)	Crude extract	Compounds 25µg/ml		
					1	**2**	**3/4**
		Zone of inhibition (mm)					
1.	*S.aureus*	16±0.1	17±0.1	-	6±0.1	8±0.1	-
2.	*C.diphtheriae*	17±0.2	16±0.1	8±0.0	-	6±0.0	-
3.	*S. pyrogenes*	17±0.2	18±0.2	10±0.1	-	8±0.0	-
4.	*P. mirabilis*	17±0.2	18±0.3	-	-	--	
5.	*Shigella boydii*	18±0.3	16±0.1	-	9±0.1	6±0.1	-
6.	*P.aeruginosa*	17±0.1	17±0.1	8±0.1	8±0.1	--	-
7.	*S. flexnariae*	16±0.1	17±0.1	-	-	--	-
8.	*S. typhi*	14±0.1	15±0.1	-	7±0.1	--	-
9.	*K. pneumonia*	16±0.1	17±0.1	-	9±0.2	6±0.1	-
10.	*E.coli*	19±0.1	20±0.2	7±0.0	7±0.0	7±0.1	-
11.	*B.subtilis*	18±0.1	19±0.1	7±0.1	-	--	-

Very active>10 Moderately active<10 Least active<9
Good activity >13, Average activity<13, Least activity<10

4. Result and Discussion

The crude ethanolic extract of *S. saligna* on purification yielded pure alkaloids **1-4** which were evaluated for antibacterial activity against eleven strains of bacteria namely *Staphylococcus aureus, Cornybacterium-diphtheriae, Streptococcus pyrogenes, Proteus mirabilis, Shigella boydii, Pseudomonas aeruginosa, Shigella flexnariae, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli and Bacillus subtilis* (**Table**) by the Agar well diffusion method [18]. The crude ethanolic extract of *S. saligna* was active against *S. pyrogenes*. The plant extract, however, was least active against *C. diphtheriae, P. aeruginosa, E. coli, B. subtilis.* MIC was determined according to the method described in literature [19].

Comparative study of antibiotics (Ampicillin and Tobramycin) with 3 new alkaloids and one known alkaloid *was done.* The activity of Compound **1** and **2** were found to be active against almost all bacterial strains, while the compound **3** and **4** was inactive. Compound **1** showed moderate activity against *Shigella boydii, Klebsiella pneumoniae* (**Table**) . *It was found that first two compounds were not very* effective antibacterial agent against *some bacterial strains* in concentrations of 25µg/ml. For the inhibition of *S. aureus, Pseudomonas aeruginosa, Salmonella typhi , Escherichia coli* bacterial strains 100 ⌈g/ml of compound **1** was needed (**Table**). Compound **2** showed least activity against *S. aureus, S. pyrogenes, Escherichia coli, C. diphtheriae S. boydii,* and

K. pneumaoniae (**Table**). For *S.aureus* and *S.pyrogens* 100 µg of compound **2** was needed while compounds **3** and **4** were inactive against all bacterial strains.

5. Acknowledgement

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