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Chemical and Biological Investigations of *Stereospermum chelonoides*

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Abstract

β-sitosterol is isolated from the dichloromethane soluble material of the methanol extract of the stem bark of ***Stereospermum chelonoides*** (Family: Bignoniaceae). The crude extract of ethyl acetate, dichloromethane extract and pure compound isolated from dichloromethane extract were screened for their antimicrobial activity against a wide range of gram-positive bacteria, gram-negative bacteria and fungi by disc diffusion method and brine shrimp lethality bioassay.The dichloromethane extract showed moderate to high inhibitory activity to microbial growth while the ethyl acetate extract showed strongest cytotoxicity having **LC_{50} 0.57µg/ml.**

Key words: *Stereospermum chelonoides,* Bignoniaceae, β-sitosterol,Brine shrimp lethality bioassay,Antimicrobial.

I. Introduction

Stereospermum chelonoides (Bengali name- Parul, Atkopali; Family- Bignoniaceae) is a middle-sized tree having young shoots covered with viscid pubescence, leaflets 3-5 pair, elliptic, shortly acuminate, often serrulate, blade 3-6 inch long, rough on the upper[1] which are available in subhimalayan tract and outer hills, ascending to 4000 ft. from the Jamuna east-wards, rare between Jamuna and Jhelam, rajputana, Singbhum, Central India, Western Peninsula, Burma, Upper and Lower, often Sal and English Forest and also in Bangladesh, it is grown in Chittagong and the northern districts.[2] The root is useful in asthma, cough, chronic dyspepsia; disease of ear, teeth and rheumatism.The bark is used in asthma, carminative, diuretic and cardiac tonic; clinically used in general debility, dyspepsia and flatulence.The fruits are good in diseases of the heart, throat, piles and bronchitis. [3]

Though there are about 120 genera and more than 850 species in the family Bignoniaceae, chemical investigations have been very limited with only a few genera, notably, *Macfadyena, Catalpa, Jacaranda, Tecoma, Tabebuia, Oroxylum* and *Crescentia* have been examined widely.[4] Literature review revealed that this plant contains lapachol (2-hydroxy-3-(3-methyl-2-butenyl)-1,4-napthaquinone[5], tannins, flavonoids, alkaloids and quinines.[6,7] Among these chemical constituents flavonoids are the most prevalent ones. Here, the preliminary antimicrobial and cytotoxicity activities of the organic extractives and the isolation of a **β-sitosterol** from the dichloromethane soluble material of the methanol extract are reported.

II. Materials and Methods

General Experimental Procedure

The ^{1}H NMR spectra were recorded by using a Bruker AMX-400 (400 MHz) instrument. For NMR studies deuterated chloroform was used and the δ values for ^{1}H spectra were referenced to the residual nondeuterated solvent signals.

Plant Material

Fresh stem bark of *Stereospermum chelonoides* was collected from Chittagong in the month of August 2007. It was identified by Bangladesh National Herbarium, Dhaka. A voucher specimen has been deposited in the Bangladesh National Herbarium, Dhaka (DACB-25546), for the collection. The bark was at first sun dried for five consecutive days. Finally the dried crispy bark was ground into a coarse powder using a grinding machine.

Extraction and Isolation

The powdered stem bark (533 g) of ***S. chelonoides*** was soaked in 2.5 L methanol for 15 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator. A portion of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method[8] into *n*-hexane, carbon tetrachloride, chloroform, dichloromethane & ethyl acetate. The dichloromethane soluble fraction(1.6gm) was fractionated by column chromatography (CC) over silica gel (70-230 mesh) using n-hexane, dichloromethane and methanol mixtures of increasing polarities to give 44 fractions, collecting each 50 ml. Preparative thin layer chromatography (stationary phase- silica gel F_{254}, mobile phase - 65 % dichloromethane in n-hexane, thickness of plates-0.5 mm) of fractions 09 afforded compound (***β*-sitosterol).**

***β*-sitosterol: white crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.52(1H, m,H-3),5.34 (1H, m, H-6), 1.00 (3H, s, 10-C*H*$_3$), 0.67 (3H,s, 13-C*H*$_3$), 0.91 (3H, d , *J* = 6.4, 20-C*H*$_3$), 0.81 (3H, d , *J* = 7.4, 25-C*H*$_3$), 0.84(3H, d , *J* = 7.4, 25-C*H*$_3$), 0.86 (3H, *J* = 6.5 Hz, 28-C*H*$_3$).**

Bioassays

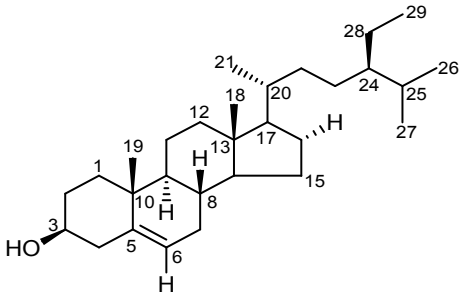
The antimicrobial activity of the crude extracts was determined by the disc diffusion method.[9, 10, 11] The samples were dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 500 µg/ disc.Amoxicillin disc (30µg/disc) was used as standard in each study. DMSO solutions of the plant extracts were assayed for cytotoxicity against *Artemia salina* in a 1-day

in vivo assay.[12] For the experiment 4 mg of each of the Kupchan fractions was dissolved in DMSO. Solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781µg/ml were obtained by serial dilution technique. The median lethal concentration LC_{50} of the test samples after 24 hrs was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. Here vincristine sulphate was used as a standard.

III. Results and Discussion

β-sitosterol was isolated from the dichloromethane soluble fractions of a methanolic extract of the stem bark of repeated chromatographic separation and purification over silica gel. The structure of the isolated compound was determined by ^{1}H NMR data analysis as well as by comparison with previously reported values.[13]

The ^{1}H NMR spectrums (400 MHz, $CDCl_3$) of **β-sitosterol** showed two one-proton multiplets at δ 3.52 and δ 5.34 typical for H-3 and H-6 of a steroidal nucleus. The spectrum further revealed two singlets at δ 0.67 and δ 1.00 each integrating for three protons, assignable to two tertiary methyl groups at C-13 and C-10 respectively. The ^{1}H NMR spectrum also showed two doublets centered at δ 0.81 (J = 7.4 Hz) and 0.84 (J = 7.4 Hz) which could be attributed to two methyl group at C-25. The doublet at δ 0.91 (J = 6.4 Hz) was demonstrative of a methyl group at C-20. On the other hand, a triplet integrating for three protons at δ 0.86 (J = 6.5 Hz) indicated the presence of a methyl group at C-28.



β-sitosterol

These NMR spectral features are characteristics of a steroidal carbon skeleton of *β*-sitosterol. Finally, the structure of was identified as ***β*-sitosterol** by comparing its reported ^{1}H NMR data.[13]

Following the procedure of Meyer [11, 12], the lethality of crude extract of ethyl acetate and dichloromethane extract were screened by brine shrimp lethality bioassay for probable cytotoxic activity. The LC_{50} obtained from the best-fit line slope were found to be 1.2µg/ml, 0.57µg/ml and 2.18 for ethyl acetate,dichloromethane extract and pure compound (**β-sitosterol**) respectively cited in table-2.In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by dichloromethane extract was significant.

The results of antimicrobial and cytotoxicity screening were found to be consistent with the folk uses of ***S. chelonoides*** by local people.

Table. 1. Antimicrobial activity of *S. chelonoides* extracts (500 µg/disc) and Amoxicillin(30 µg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)		
	DCM	EA	AMOX.
Gram Positive			
Bacillus cereus	12	NA	NA
B. megaterium	12	NA	33
Sarcina lutea	11	07	10
Staphylococcus aureus	12	08	28
Gram Negative			
Escherichia coli	13	NA	20
Pseudomonas aeruginosa	13	07	10
Salmonella paratyphi	12	NA	15
Shigella boydii	12	07	NA
Shigella dysenteriae	10	06	20
Vibrio parahemolyticus	13	07	12
S. typhi	12	NA	NA
Vibrio mimicus	12	NA	30
Fungi			
Candida albicans	11	08	12
Aspergillus niger	13	07	10
Sacharomyces cerevacae	13	07	NA

DCM: dichloromethane extract; **EA**: ethyl acetate extract; **AMOX**: Amoxicillin **NA**: No Activity.

Table. 2. LC_{50} data of *S. chelonoides* extracts and vincristine sulfate.

Samples	LC_{50} (µg/ml)
VS	0.33
DCM	1.2
EA	0.57
Pure Compound (**β-sitosterol**)	2.18

The values of LC_{50} are expressed in **µg/ml**. **VS:** vincristine sulphate (Std.); **DCM**: dichloromethane extract; **EA**: ethyl acetate extract

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