Phytochemical and Biological Investigations of Sida Rhomboidea Linn.

A Chowdhury, M Ashraful Alam, M Rahman, M Rashid

Citation

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Abstract

Daucosterol (1) was isolated from the n-hexane soluble fraction of a methanol extract of the stems of Sida rhombifolia Linn. (Syn. S. rhomboidea Linn.). The n-hexane, carbon tetrachloride and dichloromethane soluble fractions of this methanol extract were subjected to antimicrobial and antioxidant screening as well as brine shrimp lethality bioassay. The dichloromethane and carbon tetrachloride soluble fractions showed moderate inhibitory activity to microbial growth while the n-hexane fraction showed highest cytotixicity having LC_{50} 0.84 µg/ml. The dichloromethane soluble fraction also revealed potent antioxidant activity with IC_{50} 20 µg/ ml.

INTRODUCTION

Sida rhombifolia Linn. (Syn. S. rhomboidea Linn., Family-Malvaceae, Bengali Name-Lal Berela) is a small erect undershrub, which grows all over Bangladesh [1]. The stems are used to treat rheumatism and tuberculosis [2]. It is also reputed as anthelmintic and sedative. The roots are used in epilepsy, neurological problem, and fever and heart diseases [3]. Fruits are as known to be antibacterial and antifungal. The whole plant in combination with other drugs is prescribed as an anitdote to snake and scorpion venom [4]. Previous phytochemical investigations of Sida species resulted in the isolation of stigmasterol, stigmatenol and -sitosterol. We, herein, report the preliminary antimicrobial, cytotoxic and antioxidant activities of the organic extractives as well as isolation of daucosterol from the n-hexane soluble fraction of S. rhomboidea for the first time.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURE

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

PLANT MATERIAL

Stems of S. rhomboidea were collected from Azimpur Govt. Quarter Area, Dhaka in the month of September 2007. A voucher specimen for this collection has been deposited in Bangladesh National Herbarium, Dhaka (accession no. -32897).

EXTRACTION AND ISOLATION

The powdered stems (600 g) of S. rhomboidea was soaked in 1.5 L methanol for 7 days with occassional shaking and stirring and filtered through a cotton plug followed by Whatman filter paper number-1. The extract was then concentrated by using a rotary evaporator at reduced temperature and pressure. A portion (5 g) of the concentrated methanol extract was fractioned by the modified Kupchan partitioning method [5] into n-hexane (1.0 g), carbon tetrachloride (0.7 g), dichloromethane (0.75 g) and aqueous soluble materials (2.0 g). The n-hexane soluble material was fractionated by column chromatography (CC) over silica gel (60-120 mesh) using n-hexane, ethyl acetate and methanol mixtures of increasing polarities to give 30 fractions each 5 ml. Preparative thin layer chromatography (stationary phase-silica gel F_{254}, mobile phase 20% ethyl acetate in toluene, thickness of plates 0.5 mm) of fraction n-hexane eluted with 20-30% ethyl acetate in n-hexane afforded compound 1.

BIOASSAYS

The antimicrobial activity of the extractives was determined by the disc diffusion method [6]. The samples were dissolved separately in chloroform or methanol and applied to sterile filter paper discs at a concentration of 400 µg/disc. Kanamycin disc (30 µg/disc) was used as standard.

For cytotoxicity assay DMSO solutions of the plant extracts

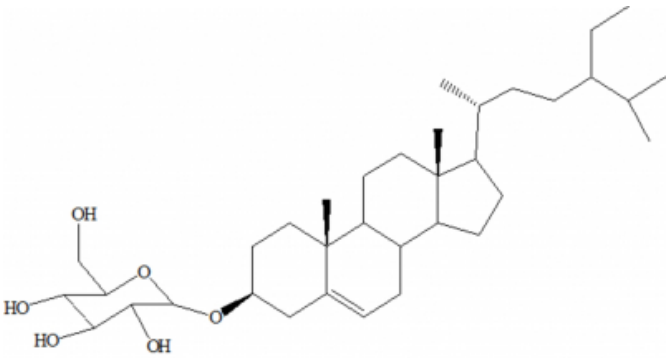
were applied against Artemia salina in a 1-day in vivo assay, the experimental details of which could be found elsewhere [7]. For the experiment 4 mg of each of the Kupchan fractions was dissolved in DMSO and test solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml were obtained by serial dilution technique. The median lethal concentration LC_{50} of the test samples after 24 hours of exposure was obtained by a plot of percentage of the shrimps killed against logarithm of the sample concentration. Here vincristine sulphate was used as the standard.

The free radical scavenging activity of the extractives on the stable radical 1, 1-dipheny1-2 picry1hydrazy1 (DPPH) were estimated by the method of Brand-Willimams [8]. Here 2.0 ml of a methanol solution of the samples at different concentration were mixed with 3.0 ml of DPPH methanol solution (20µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that produced by the standard antioxidant agents of tert-buty1 hydroxytoluene (BHT) and ascorbic acid (ASA) by UV spectrophotometer. Free radical scavenging activites of different partitionates of methanolic extract and standard tested by DPPH method. IC_{50} value of the standard BHT obtained 10 µg/ml.

RESULTS AND DISCUSSION

Compound 1 was isolated from the n-hexane soluble fraction of methanolic extract of the stems of S. rhomboidea by repeated chromatographic separation and purification over silica gel. The structure of the isolated compound was determined by comparison of ^{1}H NMR data with previously reported values [9] as well as co- TLC with authentic sample. Although it has previously been reported from many plants, this is the first report of its isolation from S. rhomboidea.

Figure 1



Daucosterol **(1)**

In the antimicrobial screening, the extractives as S. rhomboidea exhibited mild to moderate antimicrobial activity. The zone of inhibition produced by the carbon tetrachloride and dichloromethane soluble fractions of methanolic extract ranged from 8-13 and 8-12 mm respectively (Table -1). The n-hexane and aqueous soluble fractions did not show inhibit the growth of microorganisms.

Figure 2

Table 1. Antimicrobial activity of extractives at 400 µg/disc.

Test microorganisms	Diameter of zone of inhibition (mm)		
	CT	DCM	KAN
Gram positive bacteria			
Bacillus cereus	12	10	35
B. megaterium	9	10	36
B. subtilis	9	8	34
Staphylococcus aureus	8	10	34
Sarcina lutea	11	9	35
Gram negative bacteria			
Escherichia coli	10	8	36
Pseudomonas aeruginosa	8	9	36
Salmonella paratyphi	11	12	35
S. typhi	13	9	35
Shigella dysenteriae	8	10	32
S. boydii	8	9	33
Vibrio mimicus	10	9	37
V. parahemolipicus	11	8	31
Fungi			
Candida albicans	10	10	32
Aspergillus niger	10	10	32
Sacharomyces cerevacae	10	9	31

CT: carbon tetrachloride soluble fraction of the methanolic extract; DCM: dichloromethane soluble fraction of the methanolic extract; KAN: standard kanamycin disc (30µg/disc).

Following the procedure of Meyer [7], the lethality n-hexane (HX), carbon tetrachloride (CT), dichloromethane (DCM)

and aqueous (AQ) soluble fractions of the methanolic extract to brine shrimp was determined on. Table 2 shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC_{50} obtained from the best fit line slope were found to be 0.84, 5.89, 7.6 and 2.7 µg/ml for n-hexane, carbon tetrachloride, dichloromethane and aqueous soluble materials, respectively (Table -2). In comparison with the positive control (vincristine sulphate, LC_{50} 0.46µg/ml) the cytotoxicity exhibited by the n-hexane, methanolic aqueous, carbon tetrachloride fraction of methanolic extract was significant.

Figure 3

Table 2. LC data of test samples of

Sample	LC_{50} (µg/ml)
VS	0.46
HX	0.84
CT	5.8
DCM	7.6
AQ	2.7

¬¬¬¬VS: vincristine sulphate (std); HX: n-hexane soluble fraction of methanolic extract; CT: carbon tetrachloride soluble fraction of methanolic extract; DCM: dichloromethane soluble fraction of methanolic extract; AQ: aqueous soluble fraction of methanolic extract.

The antioxidant activity of various fractions of the methanolic extract was also determined. Table-3 shows antioxidant activity of the test samples. Here, tert butyl-1-hydroxy toluene (BHT) was used as positive control. The IC_{50} values for n-hexane, carbon tetrachloride, dichloromethane and methanolic aqueous were found to be 316, 217, 20 and 32 µg/ml, respectively. In comparison with positive control (BHT) the antioxidant activity exhibited by the dichloromethane and aqueous soluble fractions of methanolic extract was significant. The pure compound 1 was also tested for antioxidant activity, but no significant inhibitory capacity was found (data not shown in table-3).

Figure 4

Table 3. IC data of test samples of

Sample	IC_{50} (µg/ml)
BHT	9
HX	316
CT	217
DCM	20
AQ	32

BHT: tert butyl-1-hydroxy toluene (Std); HX: n-hexane soluble fraction of methanolic extract; CT: carbon tetrachloride soluble fraction of methanolic extract; DCM: dichloromethane soluble fraction of methanolic extract; AQ: aqueous soluble fraction of methanolic extract.

The results of antimicrobial, cytotoxic and antioxidant activities support the folk uses of S. rhomboidea in various diseases.

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Author Information

Akhtaruzaman Chowdhury, M.Sc.
Department of Chemistry Rajshahi University of Engineering and Technology Rajshahi Bangladesh

Md. Ashraful Alam, Ph.D.
Department of Chemistry Rajshahi University of Engineering and Technology Rajshahi Bangladesh

Mohammad Sharifur Rahman, M.Pharm
Department of Pharmaceutical Chemistry Faculty of Pharmacy University of Dhaka Bangladesh

Mohammad Abdur Rashid, Ph.D
Department of Pharmaceutical Chemistry Faculty of Pharmacy University of Dhaka Bangladesh