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Scopoletin Isolated from Stem Bark of *Bursera serrata* Wall. With Antimicrobial and Cytotoxic Activities of the Crude Extract

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Abstract

Scopoletin (1) was isolated from the dichloromethane soluble fraction of the Stem bark of ***Bursera serrata*** (Family:Burseraceae).The dichloromethane extract was subjected to antimicrobial screening and brine shrimp lethality bioassay while it showed mild to poor inhibitory activity to microbial growth and also showed significant cytotoxicity having **LC_{50} 3.1 μg/ml.**

Key words: *Bursera serrata*, Burseraceae, Scopoletin, Brine shrimp lethality bioassay, antimicrobial screening.

I. Introduction

Bursera serrata **(**Bengali name-Hajna; Family- Burseraceae**)** is an evergreen resinous tree and it grows up to 15m in height and 30-40cm in diameter. Leaves are imparipinnate, up to 30cm long, petiole is about 1cm long, leaflets are 4-6 pairs, opposite or nearly so up to 8cm × 3cm, ovate-lancelet, entire or sometimes serrated-crenate, pubescent or glabrous. The pedicel are greatly thickened and elongated.Flowers are very small, hermophodite, publescent externally.It grows in Chittagong, Mymensing, Rangamati,Sylhet, Assam, Rajmahal hills.[1] Very few phytochemical investigations with this plant have been done so far but some of the investigations led to the isolation of terpenoids, coumarin, flavanoid, steroid and essential oils.[2]

II. Materials and Methods

General experimental procedure

The ^{1}H NMR spectra were recorded 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

The plant of ***Bursera serrata*** was collected from National Botanical Garden. It has been given for identification in Bangladesh National Herbarium, Dhaka. A voucher specimen **(DACB-30733)** has been deposited in the Bangladesh National Herbarium, Dhaka for the collection**.** The stem bark is firstly separated from the stem and cut into small pieces and then dried in air for several days. The pieces were then dried in oven for 24 hours at considerably low temperature to affect grinding. The oven dried leaves were then ground into a coarse powder using a grinding machine.

Extraction and Isolation

The air dried and powdered plant material (500 gm) was extracted in a soxhlet apparatus separately and successively with petroleum ether (60^0-80^0C), dichloromethane (CH_2Cl_2) and methanol. The three extracts were filtered individually and concentrated to a small volume using a conventional distillation set and then a rotary evaporator (Buchi) under reduced pressure. The extract was filtered through fresh cotton bed and finally Whatman No.1 filters paper. The filtrate was concentrated with a rotary evaporator at low temperature (40-50^0C) and reduced pressure. The weight of the dichloromethane crude extract was 1.7 gm. The dichloromethane soluble fraction(1.7gm) was fractionated by column chromatography (CC) over silica gel (70-230 mesh) using petroleum ether, followed by mixtures of petroleum ether and chloroform and then chloroform and finally by chloroform and methanol (The polarity was gradually increased by adding increasing proportions of chloroform and methanol) to give 09 fractions, collecting in each 100ml beakers. Depending on the TLC behavior, a portion of fraction-6(solvent system- chloroform: methane=95:5) weighing 98mg was subjected to column chromatography for fractionation by column chromatography (CC) over silica gel (70-230 mesh) eluting n-hexane, followed by mixtures of n-hexane and ethyl acetate and then ethyl acetate and finally ethyl acetate and methanol according to increasing of polarity to give 73 fractions, collecting in each 50ml and 10ml beakers. The mixture of fractions 18 to 32 was subjected to preparative thin layer chromatography (stationary phase- silica gel $F_{254,}$ mobile phase – n-hexane: ethyl acetate =70:30 with 3 drops of acetic acid, thickness of plate-0.25 mm). From the developed plate a band was scrapped and eluted with dichloromethane and ethyl acetate (1:1).Evaporation of solvent afforded **compound (1).**

Scopoletin (1): white gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.6 (1H, d,J = 9.46 Hz, H-4), 6.83 (1H, s, H-8), 6.91 (1H, s, H-5), 6.27 (1H, d,J = 9.46 Hz, H-3), 3.94 (3H,s, OMe-6).

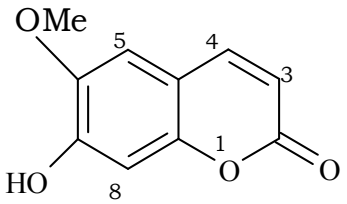
Bioassays. The antimicrobial activity of the crude extracts was determined by the disc diffusion method [3, 4, 5].The extracts were dissolved separately in chloroform and applied

to sterile filter paper discs at a concentration of 500 ug/ disc. Amoxcillin (30 µg/disc) was used as standard in each study. For cytotoxicity screening DMSO solutions of the compounds were applied against Artemia salina for 24 hours in a simplified in vivo simplified assay.[5, 6] In this experiment, the extracts were dissolved in DMSO and by serial dilution technique, solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml were obtained. Then each of these test solutions was added to test tubes containing 10 shrimps in simulated brine water (5 ml).After 24hrs,the lethal concentration(LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. Vincristine sulphate was used as positive control in this assay to compare the cytotoxicity of the extracts.

III. Results and Discussion

Compound(1) was isolated from the dichloromethane soluble fraction of the Stem bark of ***Bursera serrata*** (Family:Burseraceae) by repeated chromatographic separation and purification over silica gel.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **compound (1)** displayed signals characteristics of a 6, 7-dioxygenated coumarin. The spectrum revealed two doublets at δ 6.27 (^{1}H, *d, J=9.46 Hz)* and δ 7.6 (^{1}H, *d, J=9.46 Hz)* characteristic of H-3 and H-4 protons respectively of the pyrone ring of a coumarin. The presence of two aromatic proton singlets at δ 6.91 and δ 6.83 were attributable to H-5 and H-8 respectively. In this spectrum a three-proton singlet at δ 3.94 was assigned for a methoxyl group at C-6.



Compound (2)

On this basis, **compound (1)** was identified as **Scopoletin** the identity of this compound as **Scopoletin** was further substantiated by comparison of its spectral data with previously reported values. [7, 8, 9]

In the antimicrobial screening, the dichloromethane extract of the ***Bursera serrata*** exhibited poor antimicrobial activity against most of the test organisms cited in table-1.The zone of inhibition produced by the dichloromethane extract ranged from 07 – 8 mm respectively at a concentration of 500 µg/disc.

Following the procedure of Meyer [6], the lethality of dichloromethane extract was screened by brine shrimp lethality bioassay for probable cytotoxic activity. The LC_{50} obtained from the best-fit line slope were found to be 3.1µg/ml for dichloromethane extract cited in table-2.In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by dichloromethane and compound (1) was promising and the dichloromethane extract might have antitumour or pesticidal compounds as its **LC_{50} is 3.1**.

Table. 1. Antimicrobial activity of *Bursera serrata* extract (500 µg/disc) and Amoxycillin (30 µg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)	
	DCM	**Amoxcillin**
Gram Positive		
Bacillus cereus	07	33
B. megaterium	07	35
B. subtilis	NA	NA
Staphylococcus aureus	07	33
Sarcina lutea	07	12
Gram Negative		
Escherichia coli	07	20
S. typhi	07	14
Shigella boydii	08	NA
Vibrio mimicus	08	25
Pseudomonas aeruginosa	07	10
Salmonella paratyphi	08	15
Shigella dysenteriae	07	20
Vibrio parahemolyticus	08	12
Fungi		
Candida albicans	07	NA
Aspergillus niger	NA	NA
Sacharomyces cerevacae	10	15

DCM: dichloromethane extract; **NA**: No Activity.

Table. 2. LC_{50} data of *Bursera serrata* extract and vincristine sulfate.

Samples	LC_{50} (µg/ml)
VS	0.33
DCM	3.1
Pure Compound	3.16

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