p-Hydroxycinnamic acid Isolated from Stem bark of *Stereospermum chelonoides*

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Received on 30.04.2011. Accepted for publication on 16.09.2013

Abstract

***p*-Hydroxycinnamic acid was isolated from the chloroform extract of the fresh stem bark of Stereospermum chelonoides (Family: Bignoniaceae). The compound was subjected to antimicrobial screening and brine shrimp lethality bioassay. It showed poor inhibitory activity to microbial grwth while it showed significant cytotoxicity having LC_{50} 1.04 µg/ml.**

Keywords: *Stereospermum chelonoides*, Bignoniaceae, p-Hydroxycinnamic acid, Brine shrimp lethality bioassay, antimicrobial screening.

1. Introduction

Stereospermum chelonoides (Bengali name- Parul, Atkopali; Family- Bignoniaceae) is a large tree with pinnately compound leaves, flowers (with rose corolla having yellow lobes) in panicles and flattened seeds having membranous wings, grown in Chittagong and the northern districts **[1].** In fact decoction of root is antipyretic and useful in asthma, cough, and excessive thirst [**1, 2**] while decoction of the leaf and flowers is effective in chronic dyspepsia and also possesses antipyretic properties. On the other hand, the leaf juice boiled with oil is used in diseases of the ear, teeth and rheumatism. Moreover fruit juice is usually given to drink for dysentery**. [2]** However Lopachol contained in the root showed highly significant activity against Walker 256 carcinosarcoma**[3].** In addition brak contains a crystalline bitter substance. Leaves contain dinatin-7 glucuroniside and dinatin **[1, 2].**Besides β-sitosterol and n-triacontanol were isolated from root bark while root heartwood contains lapachol, dehydro-elapachone and dehydrotectol **[4].** Here, the isolation of a *p*-Hydroxycinnamic acid from the chloroform extract and its preliminary antimicrobial and cytotoxicity activities are reported.

2 Material And Methods

General experimental procedure.

The ^1H and ^{13}C NMR spectra were recorded by using a Bruker AMX-400 (400 MHz) and 100MHz instrument respectively. For NMR studies, deuterated methanol was used and the δ values for ^1H and ^{13}C spectra were referenced to the residual non-deuterated solvent signals.

Plant Material

Fresh stem bark of *Stereospermum chelonoides* was collected from Chittagong in the month of August. For confirmation, it was identified by the taxonomist of Bangladesh National Herbarium,Dhaka (DACB-25546).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.Furthermore a portion of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method **[5]** into *n*-hexane (1.5g), carbon tetrachloride (0.04 g), chloroform (2.54 g) & aqueous portion. The chloroform soluble fraction(2.54 g) was undergone by Vacuum Liquid Chromatography (VLC) over silica gel (70-230 mesh) using n-hexane, followed by mixtures of n-hexane and ethyl acetate ,then by ethyl acetate and finally ethyl acetate and methanol mixtures owing to increasing polarity to give 23 fractions, collecting in each 100mL. Moreover the 16th fraction of VLC afforded the compound (*p*-Hydroxycinnamic) by elution with 60% n-hexane in ethyl acetate obtaining as brown granules while it

appeared as a dark quenching spot on the TLC plate (Silica gel PF 254;R_f: 0.45; chloroform with 10% methanol) under UV light at 254nm. It is soluble in methanol and sparingly soluble in chloroform.

p-Hydroxycinnamic acid: brown granule; ^{1}H NMR (400 MHz, CD_3OD): δ 7.44 (2H, d, *J*=8.6Hz ,H-2,H-6), 6.81 (2H, d, *J*=8.6Hz ,H-3,H-5), 7.60 (1H,d,*J*=15.9Hz,H- 1′),6.28 (1H,d,*J*=15.9Hz,H- 2′)

Bioassays

The antimicrobial activity of the compound was determined by the disc diffusion method**[6, 7, 8].**The compound was dissolved separately in chloroform and applied to sterile filter paper disc at a concentration of 200 μg/ disc. Amoxicillin disc (30μg/disc) was used as standard in each study. DMSO solution of the compound was assayed for cytotoxicity against *Artemia salina* in a 1-day *in vivo* assay **[9, 10].**For the experiment 4 mg of the compound 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.

3. Results and Discussion

p-Hydroxycinnamic acid is isolated from the chloroform extract of the stem bark of *Stereospermum chelonoides* (Family: Bignoniaceae)The structure of the isolated compound was determined by ^{1}H and ^{13}C NMR data analysis as well as by comparison with previously reported values.

The ^{13}C NMR spectrum (100MHz, CD_3OD) of the compound displayed 9 carbon resonances, while the HSQC experiment indicated that 6 out of the 9 carbons were attached to protons. ^{1}H NMR (400 MHz, CD_3OD) and DEPT 135 (100 MHz, CD_3OD) spectra revealed the presence of 6 aromatic and/or olefinic methines.

The ^{13}C NMR, HSQC and HMBC chemical shifts are shown in Table-1 and Table-2 respectively. The identification of the compound and its ^{13}C assignments were established unambiguously by 2D experiments.

Analysis of one and two-dimensional NMR spectra including COSY, HSQC and HMBC led to the assignment of the structure as shown in the Figure. In this structure the C-2 and C-3, C-5 and C-6 and C- 1′ and C- 2′ skeletons were assigned by tracing of cross peaks in the COSY spectrum. A 1, 4-disubstituted benzene ring moiety was disclosed by HMBC correlations (H-2 & H-6 / C-1, C-4; H-3 & H-5/C-4, C-1). The presence of a 1.4-disubstituted benzene ring was further evidenced from the presence of two symmetric pairs of coupled two proton doublets at δ=6.81(J=8.6Hz, H-3 and H-5) and δ=7.44 (J=8.6Hz, H-2 and H-6) could be in the ^{1}H NMR spectrum. The resonance at δ = 6.28 and 7.60 ppm in the ^{1}H NMR and at δ = 115.6 and 146.6 ppm in the ^{13}C NMR spectra, in conjunction with the DEPT 135 spectrum, could be attributed to two olefine protons. The cross peak in the COSY spectrum between these two protons indicated that they are vicinal. The coupling constant *(J=15.9Hz)* of these olefin protons indicated their *(E)* configuration. The presence of two singles at δ =171.0 in ^{13}C NMR spectrum indicated the presence of a phenolic group and carboxylic group in the compound respectively.

HMBC correlation of H- 2′ with C-1 & C- 3′ indicated that the side chain containing the olefinic and carboxyl group was attached at C-1 of the benzene ring. On the basis of these spectral data, the compound was unambiguously identified as *p*-Hydroxycinnamic acid.**[11]**

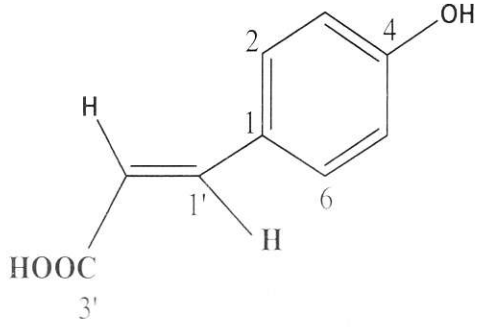

Table 1: ^{13}CNMR (100 MHz, CD_3OD) spectrum data the compound

Carbons	δ_c in ppm
C1	δ127.2
C2, C6	δ131.1
C3, C5	δ116.8
C4	δ161.1
C1	δ146.6
C2	δ115.6
C3	δ171.0

Table 2: HSQC and HMBC data for the compound.

PROTONS	COSY	HSQC	HMBC
H-2/H-6	δ6.81 (H-3/H-5)	δ131.1 (C-2/C-6)	δ115.6 (w) (C-2'), δ127.2(C-l), δ146.6(C-l'), δ161.1(C-4), δ171.0(w) (C-3').
H-3/H-5	δ7.44(H-2/H-6)	δ116.8(C-3/C-5)	δ115.6(C-2'), δ127.2(C-1), δ161.1(C-4).
H-1'	δ6.28(H-2')	δ146.6(C-l')	δ115.6(C-2'), δ131.1(C-6), δ171.0(C-3').
H-2'	δ7.60(H-1')	δ115.6(C-2')	δ127.2(C-l), δ171.0(C-3').

Table 3: Antimicrobial activity of *p*-Hydroxycinnamic acid (200 μg/disc) and amoxicillin (30 μg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)	
	p-hydroxycinnamic acid	AMOX.
Gram Positive		
Bacillus cereus	08	25
B. subtilis	07	22
Staphylococcus aureus	NA	20
Gram Negative		
Escherichia coli	8	22
Pseudomonas aeruginosa	9	25
Shigella boydii	7	20
Fungi		
Candida albicans	7	12
Aspergillus niger	10	10

AMOX: Amoxicillin **NA**: No Activity.

Table 4: LC_{50} data of *p*-Hydroxycinnamic acid and vincristine sulfate

Samples	LC_{50} (μg/ml)
VS	0.33
p-Hydroxycinnamic acid	1.04

The values of **LC_{50}** are expressed in **μg/ml**. **VS:** vincristine sulphate (Std.)

Acknowledgements

The authors wish to thank the Bangladesh Council of Scientific and Industrial Research under Ministry of Science and Information & Communication Technology, Government of the Peoples' Republic of Bangladesh.

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