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Chemical Analysis of Aerial Parts of Justicia gendarussa

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Abstract: *Justicia. gendarussa* is an evergreen scented shrub, found throughout the greater part of India. The plant is considered febrifuge, emetic, and diaphoretic. It is used for the treatment of lunacy, debility and snakebite. It is also given for amenorrhea and stomach troubles. Chemical analysis of aerial parts of *Justicia gendarussa* afforded Compound 1, Compound 2 ,and Compound 3. Compound 1 was identified as β-sitosterol, Compound 2 was identified as β-Sitosterol-β-D-glycoside and Compound 3 was identified as aromadendrin.The structure of these compounds were determined by extensive of IR, UV, and NMR spectroscopy.
Key words: *Justicia gendarussa*, β-sitosterol, β-Sitosterol-β-D-glycoside and aromadendrin.

Introduction

Justicia gendarussa (Acanthaceae) is an evergreen scented shrub, 2-4 ft. high, found throughout the greater part of India and Andaman Islands. Leaves 2.5- 5.0 inch long, lanceolate or linear lanceolate, glabrous; flowers small, white with pink or purple spots inside, in terminal or axillary spikes; capsules 0.5 in. long, clavate, glabrous, containing four seeds.

Material and Method

Plant Material

The whole plant of *Justicia gendarussa* was collected from Botanical garden, Forest Research Institute, New Forest, Dehradun, in Jan, 2005. Dr. Sumer Chand, Systematic Botany Division, FRI, Dehradun, identified the plant species. The voucher specimen (Hr. no. 56) was deposited in the Department of Botany, R.H. Govt. (P. G.) College, Kashipur (U.S. Nagar), Uttarakhand.

Extraction and Isolation

The air-dried aerial parts (2.5 kg) were exhaustively extracted with light petroleum ether (60-80^0). The petroleum free mass further extracted with methanol. The methanol extract was concentrated under reduced pressure in vacuum and after drying, successively, chromatographed over silica-gel using $CHCl_3$ and MeOH as eluent with increasing polarity to get four fractions **A, B, C** and **D**. Fraction **A**, eluted with $CHCl_3$:MeOH (5:95) yielded compound **1** (81 mg), **2** (67 mg) and **3** (83 mg).

Result and Dissussion

COMPOUND: 1

It was crystallized from methanol as white needles, M.P. 135-137^{0}C.

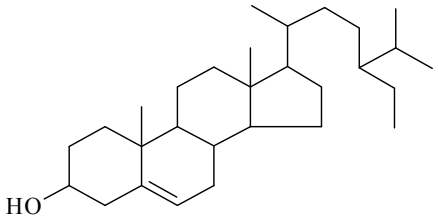
$[\alpha]_D^{20}$: -48.0^0 (MeOH)

Elemental Analysis: Found values C=84.1%, H=12.08%, required values for $C_{29}H_{50}O$; C=84.05%, H=12.07%, Molecular weight 414.

IR (ν_{max}^{KBr}): 3340, 2970, 2959, 2920, 1640, 1463 cm^{-1}.

Elemental analysis of compound **1** corresponded to the molecular formula $C_{29}H_{50}O$. It was found to be sterol as it responded positive to Liberman and Nollers' tests. It also shows positive TNM test for unsaturation. It was soluble in petroleum ether, benzene, chloroform and in hot methanol but insoluble in water and alkali. Its negative test with Molisch's reagent indicates the non-glycosidic nature of the compound. The IR spectrum of compound **1** showed characteristic absorption at 3340 cm^{-1} for OH group, 2970, 2959, 2920 for C-H stretching and 1640 cm-1 for C=C stretching.

On the basis of these experimental data compound **1** was identified as β-sitosterol. It was further confirmed by co-TLC, co-IR and MMP with that of the authentic sample [1]



COMPOUND: 2

It was crystallized from MeOH as white crystals, M.P. 289-291°C.

$[\alpha]_D^{25}$: -39 (c=0.9, pyridine).

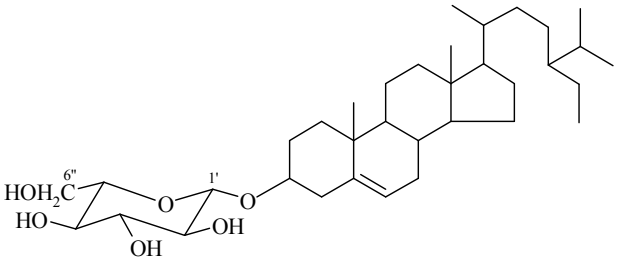
Elemental Analysis: Found values C=72.68%, H=10.2%; required values for $C_{35}H_{60}O_5$; C=72.97%, H=10.41%; Molecular weight 576.

IR (ν_{max}^{KBr}): cm^{-1} 3400, 1640, 878 and 780.

Hydrolysis of Compound 2: Hydrolysis of compound **2** was carried out by refluxing 5 mg of the compound with 5% HCl for 7 hr on water bath. The contents were cooled and extracted with chloroform where a crystalline compound as white needles was obtained. It was identified as β-sitosterol (compound **1**). The sugar was identified as glucose by PC.

Compound **2** gave positive Molisch's test and did not reduce Fehling,s solution. It also responded to LB test, characteristic for sterols and positive TNM test for unsaturation. The IR spectrum showed characteristic absorption at 3400 (OH stretching) and 1640 (C=C stretching), 878 and 780 cm^{-1} (of glycoside) [2]. On acid hydrolysis compound **2** afforded an aglycone (M.P. 135-136°C), identified as β-sitosterol by co-TLC, co-IR and MMP with an authentic sample. The sugar was identified as glucose by co-PC with an authentic sample. As the compound did not reduce Fehling solution, it indicated that the reducing group of sugar was involved in the glycosidic linkage.

On the basis of above observations compound **2** was identified as β-sitosterol-β-D-glycoside. It was further confirmed by co-TLC and co-lR and MMP



with an authentic sample.

Compound: 3

It was crystallized from MeOH as yellow crystalline solid M.P. 165^0C.

Elemental Analysis: Found values, C=52. 19%, H=5.13%, required values for $C_{15}H_{12}O_6$; C=52.33%, H=5.81%; Molecular weight 288.

MS-EI^+: m/z 288 $[M]^+$, 259, 165, 153, 134, 107,77 .

IR (ν_{max}^{KBr}): cm^{-1} 3480, 3260, 1650, 1600, 1500, 1420, 1350, 1280, 1220, etc.

^{1}H-NMR (400 MHz C_5D_5N): (Table1.1).

^{13}C-NMR (100 MHz C_5D_5N): (Table 5.1)

Compound **3** was obtained as yellow crystalline solid analyzed for $C_{15}H_{12}O_6$, which was substantiated by the presence of molecular ion peak at m/z 288 in the EI^+-mass spectrum (fig. 5.1). It gave olive green colour with $FeCl_3$ and responded positive to Shinoda's test, which indicated the presence of flavonoidal skeleton in the molecule [3]. The IR spectrum of compound **3** displayed two absorption bands at 3485 and 3260 cm^{-1} for chelated and non-chelated OH groups, respectively. The absorption bands at 1650 and 1600 cm^{-1} in IR spectrum indicated the presence of an α, β-unsaturated carbonyl group in the molecule. The IR spectrum also showed stretching of ether function at 1500, 1420 cm^{-1}.

The ^{1}H-NMR spectrum (table1.1) of compound **3** displayed presence of two *meta*-coupled doublets (J = 2.0 Hz, each for 1H, at δ 5.45 and 5.02), and two A_2B_2-type doublets (J = 8.5 Hz, each for 2H, at δ 7.73 and 7.23), in the aromatic region, suggesting the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl ring.

Table 1.1: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR data (in ppm relative to TMS) of compound 3 in C_5D_5N.

C/H	$^{\delta}$C	Multiplicity (DEPT)	$^{\delta}$H (J in Hz)	HMBC Correlation (H→→C)
2	84.5	CH	5.45, *d* (11.5)	C-3, C-4, C-11, C-12, C-16
3	73.2	CH	5.02, *d* (11.5)	C-2, C-4, C-11
4	198.7	C	--	--
5	165.0	C	--	--
6	97.4	CH	6.49, *d* (2.0)	C-5, C-7, C-8, C-10
7	168.7	C	--	--
8	96.2	CH	6.35, *d* (2.0)	C-6, C-7, C-9, C-10
9	163.8	C	--	--
10	101.6	C	--	--
11	128.8	C	--	--
12	130.2	CH	7.73, *d* (8.5)	C-2, C-14, C-16
13	116.2	CH	7.23, *d* (8.5)	C-11, C-14, C-15
14	159.5	C	--	--
15	116.2	CH	7.23, *d* (8.5)	C-11, C-13, 14
16	130.2	CH	7.73, *d* (8.5)	C-2, C-12, C-14

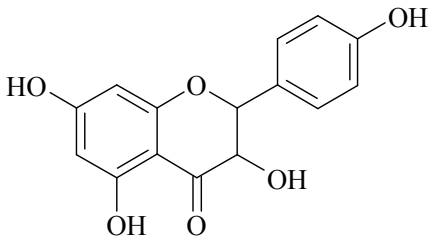
In the heterocyclic region of ^{1}H-NMR spectrum the appearance of two doublets (J = 11.5 Hz) at δ 5.45 and 5.02 were corroborated with the H-2 and H-3 protons of dihydroflavonols [4,5].

The 1,4-di-substituted phenyl ring as deduced by ^{1}H-NMR was determined to be *p*-hydroxyphenyl system from the ^{13}C-NMR chemical shifts (table 1.1) of carbon signals at δ 130.2 (C-12, 16) and 116.2 (C-13, 15), which fairly corresponded with those of hydrogen carrying carbons of *p*-cresol (δ 115.3, 130.2) [6]. The ^{13}C-NMR spectrum also showed presence of a carbonyl carbon δ 198.7 (C-4), a benzylic carbon at δ 84.5 (C-2) and oxygen bonded heterocyclic carbon at δ 73.2 (C-3).

In agreement with above discussed ^{1}H- and ^{13}C-NMR data the DEPT spectrum showed presence of eight methine (two of double intensity) and seven quaternary carbon atoms. The assignment of protonated carbon atoms and proton attached with them were made by HMQC experiment.

The crucial data for the structure determination were provided by HMBC spectrum (table 1.1) which displayed ^{1}H-^{13}C long-range correlation and thus the entire structure was mapped out.

On the basis of above discussed spectral data compound 3 was characterized as aromadendrin [7].



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