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Phytochemical analysis of *Abutilon indicum*

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Abstract: *p*-β-D-Glucosyloxybenzoic acid, *p*-Hydroxybenzoic, Caffeic acid and have been isolated from the whole plant of *Abutilon indicum*. The concerted use of IR, UV, NMR spectroscopy and chemical methods allowed the identification of these compounds.
Key words: *Abutilon Indicum*, Malvaceae, *p*-β-D-Glucosyloxybenzoic acid, caffeic acid.

Introduction

Abutilon indicum (Malvaceae) is a hairy under-shrub with golden yellow flowers, found in hotter parts of India [1]. The plant is very much used in Siddha medicines. In fact, the root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men [2]. Methanol extract of *A. indicum* had some antimicrobial properties [3] A chemical compound, β-sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in *A. indicum* and a petroleum ether extract provided larvicidal properties against the mosquito larvae *Culex quinquefasciatus* [4]. The present investigation deals with the isolation and identification of *p*-β-D-Glucosyloxybenzoic acid, *p*-Hydroxybenzoic, Caffeic acid and have been isolated from the whole plant of *Abutilon indicum*.

Material and Method

Plant Material

Abutilon indicum were collected from Dehraadun, in Feb 2008. The plant species were identified by Dr. Sumer chand, Systematic Botany Division, FRI, Dehradun, Uttarakhand. The voucher specimen (Hr. no. 56) was deposited in the herbarium of Department of Botany, R.C.U. Govt. P. G. College, Uttarkashi, Uttarkashi, Uttarakhand.

Extraction and Isolation

The air-dried and powdered whole plant of *Abutilon indicum* (2.5 kg) was extracted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass extracted with 60% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O (6:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (8.35 g). The aqueous layer was extracted with BuOH (saturated with water). The BuOH layer was separated and concentrated under reduced pressure to give BuOH extract. The BuOH extract was further digested with MeOH:H_2O (8:2) and filtered. The filtrate was evaporated to dry under reduced pressure to give methanol extract. The chloroform extract, and

MeOH extracts were subjected to column chromatography over various adsorbents with various solvents in order to their increasing polarity.

The $CHCl_3$ extract (8.5 g) was subjected to CC over Sigel-G using gradient elution with C_6H_6-EtOAc (10:0 → 9:1) afforded various fractions having mixture of compounds. The C_6H_6-EtOAc (9:1) fraction (5.0 g) was subjected to CC over Si-gel using gradient elution with C_6H_6-EtOAc (98:2→9:1) afforded compound **1** (167 mg), and compound **2** (350 mg). The methanol extract (6.0 g) on repeated column chromatographed over Si-gel successively eluted with $CHCl_3$ and $CHCl_3$-MeOH with increasing contents of methanol afforded compound **3** (105mg).

Compound (1): white needles, m.p. 199-201^{0}C ($CHCl_3$-MeOH); Elemental Analysis: C=60.53%, H=4.25%, (calc. $C_7H_6O_3$); Molecular weight 138. UV (λ_{max}, MeOH): nm 230 (3.73).IR (V_{max}^{KBr}) : cm^{-1} 3400-3000, 1670, 1540, 1510, 1130, 760, 715, etc. ^{1}H-NMR: (300 MHz, Acetone-d_6): δ 7.83 (2H, *d*, *J* =8.5 Hz, H-2,6), 6.85 (2H, *d*, *J* = 8.5 Hz, H-3,5), 9.09 (4-OH). ^{13}C-NMR (75 MHz, Acetone-d_6): δ 122.39 (C-1), 132.69 (C-2,6), 116.02 (C-3,5), 162.76 (C-4), 167.81 (-C=O).

Compound (**2**) : Amorphous solid (MeOH); Elemental Analysis: C=60.32%, H=4.91%, (calc. $C_9H_8O_4$); Molecular weight 180; El-MS : m/z 180 $[M]^+$, 163, 135, 109, 92, 81, 75, 65, 45. IR (V_{max}^{KBr}) : cm^{-1} 3300-2650, 1680, 1610, 1590, 1510, 1130, 760, 715, etc. UV (λ_{max}, MeOH): 240 (4.10), 280 (4.25), and 350, sh (3.61). ^{1}H-NMR: (300 MHz, $CDCl_3$): δ 7.11 (1H, *s*, H-2), 6.76 (1H, *d*, *J* = 8.0 Hz, H-5), 7.01 (1H, *d*, *J* = 8.0 Hz, H-6), 7.50 (1H, *d*, *J* = 15.0 Hz, H-7), 6.28 (1H, *d*, *J* = 15.0 Hz, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 125.50 (C-1), 114.87 (C-2), 145.29 (C-3), 148.36 (C-4), 115.76 (C-5), 121.37 (C-6), 141.45 (C-7), 127.51 (C-8), 174.06 (C-9).

Compound (**3**): Colourless solid, m.p. 209-211^{0}C (MeOH); Elemental Analysis: C=51.97%, H=5.34%, (calc. $C_{13}H_{16}O_8$); Molecular weight 300. EI$^+$-MS : m/z 300 $[M+1]^+$, 283, 163, 128, 121, 110, 93, 65, 43,etc. IR (V_{max}^{KBr}) : cm^{-1} 3425, 2920, 2851, 1665, 1625, 1605, etc. UV (λ_{max}, MeOH): 234 nm. ^{1}H-NMR: (300 MHz, CD_3OD): δ 7.93 (2H, *d*, *J* = 8.4 Hz H-2,6), 6.86 (2H, *d*, *J* = 8.4 Hz H-3,5), 4.91 (1H, *d*, *J* = 6.3 Hz), 3.37 (1H, *m*, H-5'), 3.30 (1H, *m*, H-4'), 3.20 (1H, *t*, 6.9 Hz), 3.69 (1H, *dd*, 12.0 and 7.2 Hz, H-6'a)3.89 (1H, *dd*, 12.0 and 6.0 Hz, H-6'b). ^{13}C-NMR (75 MHz, CD_3OD): δ 130.26 (C-1), 132.20 (C-2,6), 115.34 (C-3,5), 166.86 (C-4), 99.89 (C-1'), 74.51 (C-2'), 78.14 (C-3'), 71.67 (C-4') 77.63 (C-5') 62.84 (C-6'), 168.01 (-C=O).

Acid hydrolysis of Compound 3: Compound **3** (10mg) was dissolved in 5% H_2SO_4 and refluxed on water bath for 3 hrs. The reaction mixture was cooled and poured on crushed ice and stand for 30 min. The precipitate was purified by re-crystallization from MeOH. The aglycone was identified as p-hydroxybenzoic acid (compound **1**) by comparison with authentic sample and the sugar was identified as D-glucose by paper chromatography.

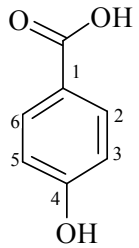
Results and Discussion

COMPOUND: 1

The elemental analysis of compound **1** corresponded to molecular formula $C_7H_6O_3$. The IR spectrum of **1** displayed characteristics absorption bands for hydroxyl group at 3452 cm^{-1} and 1670 for carbonyl carbon, indicating presence of a carboxylic group in the molecule. The UV spectrum displayed absorption maxima at 230 nm indicated the p-substituted aromatic ring in the molecule.

The ^{1}H-NMR spectrum of compound **1** exhibited two A_2B_2-type doublets (*J* = 8.5 Hz) in the aromatic region at **δ** 7.83 and 6.85 which revealed the presence of 1,4-disubstituted aromatic ring in the molecule. The ^{1}H-NMR spectrum also displayed a broad singlet for one proton at **δ** 9.09 indicated the presence of OH group. The presence of p-substituted aromatic ring was confirmed by the ^{13}C-chemical shifts of carbon resonances at **δ** 132.69 (C-2,6), 116.02 (C-3,5) (each of double intensity) in the ^{13}C-NMR spectrum of compound **1**, which fairly corresponded with those of hydrogen carrying carbons of p-cresol (**δ** 115.3, 130.2) [5]. The ^{13}C-chemical shift at **δ** 167.81 together with IR data confirmed that the compound contains a carboxylic group. The ^{13}C-chemical shifts at δ 122.39 and 162.76 assigned for C-1 and C-4 respectively together with the ^{13}C resonance of hydrogen carrying carbons of aromatic ring and the chemical shifts of protons confirmed that the –COOH and OH are at *para*-position to each other.

On the basis of above discussed spectral evidences compound **1** was characterized as *p*-hydroxybezoic acid. Co-IR, Co-TLC, and mmp with an authentic sample further confirmed the identity of the compound [6].

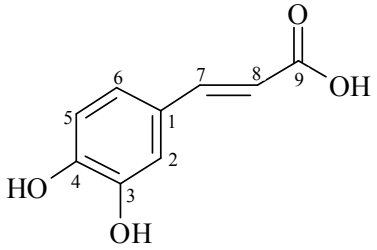


COMPOUND: 2

The elemental analysis of compound **2** corresponded to molecular formula $C_9H_8O_4$ that was substantiated by the molecular ion peak at m/z 180 in the EI positive mass spectrum. The IR spectrum of **2** exhibited broad absorption band at 3350-2600 cm^{-1} for OH group of carboxylic acid, an absorption band at 1680 for –C=O of carboxylic acid and an absorption band near 1610 cm^{-1} for -C=C- stretching.

The ^{1}H-NMR spectrum of **2** displayed two ortho-coupled doublet (J = 8.0 Hz) each for 1H, at δ 6.76 and 7.01 and broad singlet for ^{1}H at δ 7.11 in the aromatic region indicated the presence of a tri-substituted aromatic ring in the molecule. The chemical shifts of these signals indicated the presence of catechol moiety in the molecule [5] which was confirmed by ^{13}C-NMR chemical shifts of the hydrogen carrying carbon atoms at δ 114.87 (C-1), 115.76 (C-5) and 121.27 (C-6). The ^{1}H-NMR spectrum also displayed two doublets (J = 15.0 Hz), each for 1H, at δ 7.51 (H-7) and 6.28 (H-8). The large value of coupling constant indicated the presence of *trans*-disubsituted ethylene moiety in the molecule. The ^{1}H and ^{13}C chemical shifts of olifinic protons and carbons [δ 141.45 (C-7) and 127.51 (C-8)] were similar to those of *trans*-cinnamic acid [7].

The ^{13}C-NMR spectrum of **2** exhibited presence nine carbon atoms in the molecule. The ^{13}C chemical shifts of a carbon at δ 174.66 indicated the presence of carboxylic functional group in the molecule. The upfield chemical shifts of one of the etylenic carbon (C-8) and proton (H-1) indicated that the carboxylic group is located at C-8 position. The ^{13}C-chemical shifts of carbon atoms at δ 145.29 (C-3), 148.36 (C-4), indicated that the hydroxyl group are attached at C-3 and C-4 positions. The position of ethylene function was determined by chemical shift of C-1 carbon at δ 125.50 and the downfield chemical shifts of C-7 carbon and H-7 proton of ethylene moiety. On the basis of these spectral data compound **2** was characterized as caffeic acid.

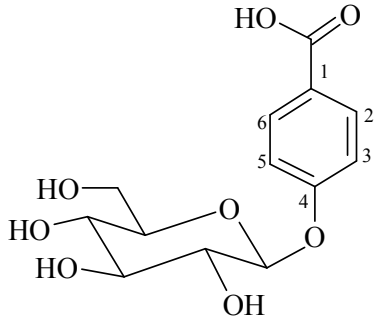


COMPOUND: 3

The molecular weight of compound **3** was found to be 300 by positive-ion FAB mass spectrum exhibited a molecular ion peak $[M+H]^+$ at m/z 301 (calcd. for $C_{13}H_{16}O_8$), which was substantiated from its elemental analysis. The IR spectrum displayed presence of a carbonyl group at 1665 cm^{-1}, and a hydroxyl group at 3435 cm^{-1}. The UV spectrum ddisplayed characteristic absorption maxima at 234 nm for p-substituted aromatic ring.

The ^{1}H-NMR spectrum of compound **3** showed two A_2B_2 type doublets (J = 8.4 Hz) in aromatic region at **δ** 7.93 (H-2,6) and 6.86 (H-3,5) indicated the presence of p-substituted aromatic ring in the molecule. The presence of p-substituted aromatic ring was confirmed by ^{13}C-NMR spectrum which displayed signals at **δ** 132.20 (C-2,6), 115.34 (C-3,5), each of double intensity for hydrogen carrying carbons, which confirmed that the aromatic ring contains oxygen function at *para*-position [5]. These observations was further confirmed by ^{13}C-chemical shifts of C-1 and C-4 atoms at **δ** 130.26, and 166.86 respectively. The ^{13}C-spectrum also displayed presence of carbonyl carbon of carboxylic group at **δ** 168.01, which was substantiated by IR absorption bands at 1665 (for –c=O) and 3435 cm^{-1} (for OH).

Compound **3** on acid hydrolysis afforded an aglycone identified as *p*-hydroxybenzoic acid by comparison of its physical data with compound **1**. The sugar was identified as D-glucose by PC with an authentic sugar. The presence of sugar in the molecule was displayed by ^{1}H-NMR spectrum which displayed a doublet (J = 6.3 Hz), at **δ** 4.91 along with the other signals assignable for sugar protons. The value of coupling constant is consistent with the anomeric proton of β-linked sugar [8] and thus confirmed the β-orientation of sugar moiety, which was further



confirmed by ^{13}C-NMR chemical shift of anomeric carbon at **δ** 99.89. The C-4 position of glucose was determined by ^{13}C-chemical shifts of C-4 at **δ** 166.86, which appear downfield as compared with the C-4 of compound **1** [**δ** 162.76 (C-4).

On the basis of these observations compound **3** was characterized as *p*-β-D-Glucosyloxybenzoic acid [9].

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