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Chemical constituents of *Solanum xanthocarpum*

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ABSTRACT

Solanum xanthocarpum is a spiny diffuse herb and used in medicine in various forms, such as decoction, electuary, ghrita, etc. Its roots are one of the constituents of well known Ayurvedic preparation "Dasmula Ashva". Phytochemical investigation of the ethyl acetate extract of the roots of *Solanum xanthocarpum growing in Ramnagar region led to the isolation of caffeic acid and oleanolic acid using different chromatographic methods (i.e. paper, thin layer, and column chromatography). The structure of these compounds was determined by extensive IR, UV, and NMR spectroscopy.*

Key words: *Solanum xanthocarpum*, caffeic acid, oleanolic acid.

INTRODUCTION

Kantkari (*Solanum xanthocarpum*) is a very spiny diffuse herb up to 1.2m tall, commonly found throughout India[1, 2]. The plant which is also known as Choti Katheri, Bhutkatya or Bhumiringani in Hindi is used in medicine in various forms, such as decoction, electuary, ghrita, etc. A decoction of the root is given with the addition of long pepper and honey, in cough and catarrh, and with rock salt and assafoetida in spasmodic cough. The dried whole plant shows significant improvement in some respiratory diseases like bronchial asthma[3, 4]. Its roots are one of the constituents of well known Ayurvedic preparation "Dasmula Ashva[5]. It forms a constituent of herbal cough remedy (Koflet) reported to promote expectoration.

Vasocin (National Institute of Ayurvedic Medicine) contains Solanum xanthocarpum[6]. Previous phytochemical studies on the genus *Solanum* showed the presence of alkaloids[7], flavonoids[8], steroidal glycoside[9] and steroidal saponins[10]. It was disclosed by the literature survey of *Solanum*

xanthocarpum that its roots were not chemically analyzed. The isolation, structure elucidation and characterization of new bioactive constituents from this plant were carried out by modern sophisticated techniques such as modern chromatographic methods, NMR and mass spectrometry.

EXPERIMENTAL SECTION

Solanum xanthocarpum was collected from the river bank of kosi and plains of Ramnagar region and was identified by Prof. Deep Mehrotra of Department of Botany, R. H. Govt. P. G. College Kashipur. A voucher specimen was deposited in the Herbarium of Department of Botany, R. H. Govt. P. G. College, Kashipur (U. S. Nagar), Uttarakhand.

Melting points were recorded in BOETIUS microscopic melting point apparatus. UV and IR spectra were obtained on BECKMAN DU-64 spectrophotometer and SP-3-200 PYE UNICAM and FT-IR-8100 Shimadzu spectrophotometer as KBr palettes respectively. ^{1}H and ^{13}C NMR were recorded on BRUKER DRX-300 (300 MHz for ^{1}H and 75 MHz for ^{13}C NMR) with $CDCl_3$ & D_2O solvents. Mass spectra were obtained using JEOL-Accu TOF JMS- T 100 LC Mass spectrometer having a DART (Direct Analysis in Real Time) source or [Jeol Sx-102 (FAB)] mass spectrometer. Column chromatography and analytical TLC were carried out using Silica gel G.

Extraction of Plant Materials

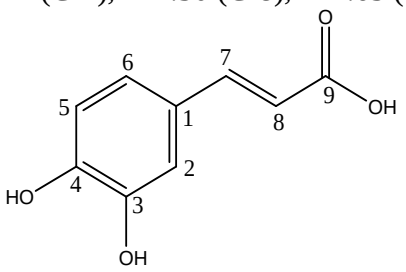
The air dried and powdered root (630 gm.) were extracted with 95% ethanol for twelve hours and the resulting extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was partitioned with petroleum ether, followed by ethyl acetate and n-butanol and finally the residual extract upon concentration to give petroleum ether, ethyl acetate, 1- butanol and aqueous fractions respectively. The crude compounds were present in the ethyl acetate fraction. Ethyl acetate fraction was subjected to column chromatography.

Isolation of caffeic acid (I)

Ethyl acetate fraction was subjected to column chromatography on silica gel using petroleum ether-acetone (100:0~0:100) as the eluting solvent to give twenty five fraction (A_1-A_{25}). Fraction (A_6-A_{10}) contained the major compound with a few minor constituents. The fractions (A_6-A_{10}) were combined and then subjected to another column chromatography, using $CHCl_3$: MeOH (1:1~0:1). Ten 25 ml fractions were collected with fraction 5-8 containing the desired compound (50mg). The compound obtained was yellow-brown powder with a melting point of 137-140 ^{0}C. The ^{1}H and ^{13}C-NMR data of the compound were consistent with the reported data of caffeic acid[11, 12, 13].

Elemental Analysis: Found values C= 60.11%, H= 4.42%, required values for $C_9H_8O_4$; C= 60.00%, H= 4.44%, Molecular weight 180.
MS- FAB^{+}: m/z 180.08[M^{+}], 163.06, 135.08, 109.08, 92.06, 80.09, 75.07, 65.07;
IR (V_{max}^{KBr}): cm^{-1} 3368.42, 2650.20, 1680.87, 1610.25, 1593.15, 1510.30, 1123.18, 760.98, 715.34 etc.
UV (λ_{max},MeOH): 240 (4.18), 280 (4.17) and 350, sh (3.60);

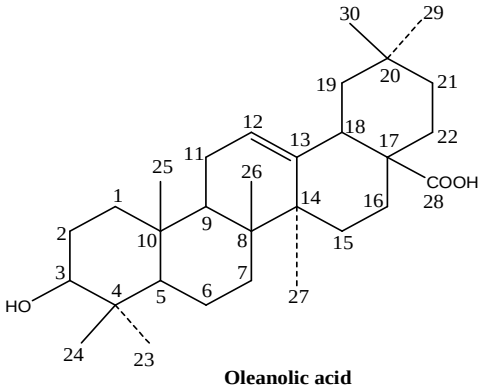
^{1}H NMR (300 MHz, D_2O): δ, (ppm) 7.511 (1H, d, J= 15.0 Hz, H-7), 7.101 (1H, s, H-2), 7.029 (1H, d, J= 8.0 Hz, H-6), 6.807 (1H, d, J= 8.0 Hz, H-5), 6.351 (1H, d, J= 15.0 Hz, H-8);
^{13}C NMR (75 MHz, $CDCl_3$): δ, (ppm) 125.42 (C-1), 114.86 (C-2), 145.21 (C-3), 148.35 (C-4), 115.75 (C-5), 121.35 (C-6), 141.41 (C-7), 127.50 (C-8), 174.65 (C-9);



Caffeic acid
3, 4-Dihydroxy-cinnamic acid

Isolation of oleanolic acid (II)

Compound II was isolated from ethyl acetate extract. The ethyl acetate extract was subjected to column chromatographed over Silica gel using gradient elution with $CHCl_3$: MeOH (10:0~9:1) to get various fractions. The $CHCl_3$: MeOH (95:5) fraction was subjected to column chromatographed over Silica gel using gradient elution with $CHCl_3$: MeOH (98:2~90:10) afforded various fractions. The fractions obtained $CHCl_3$: MeOH (95:5) were mixed and evaporated to dryness. The extract obtained was further subjected to column chromatographed over Silica gel eluted with $CHCl_3$: MeOH (95:5) afforded compound (II). It was crystallized from MeOH as white needles, melting point 305-306 ^{0}C.



Oleanolic acid
3β-Hydroxyolean-12-en-28-oic acid

Elemental Analysis: Found values C= 78.58%, H= 10.51%, required values for $C_{30}H_{48}O_3$; C= 78.94%, H= 10.52%, Molecular weight 456.
MS-FAB$^+$: m/z 456 [M] $^+$, 443, 411, 391, 324, 307, 289, 248, 203, 189, 133, 107;
IR (V_{max}^{KBr}): cm^{-1} 3400.15, 3123.24, 2920.34, 2880.25, 1690.17, 1440.37, 1373.31, 1360.15, 1450.13;
^{1}H NMR (300 MHz, D_2O): δ, (ppm) 5.39 (1H, t, J= 4.0 Hz, H-12), 4.97(1H, t, J= 5.2 Hz, H-3), 2.80 (2H, dd, J= 2.0 and 11.6 Hz), 2.41 (2H, dd, J= 4.0 and 8.0 Hz), 1.99 (2H, m), 1.35 (3H, s), 0.99, 0.80, 0.68 (each 3H, s)

^{13}C NMR (75 MHz, D_2O): δ, (ppm) 33.81 (C-1), 81.22 (C-3), 33.92 (C-4), 56.12 (C-5), 31.52 (C-7), 40.12 (C-8), 47.61 (C-9), 123.51 (C-12), 143.62 (C-13), 41.51 (C-14), 46.56 (C-17), 40.25 (C-18), 45.55 (C-19), 30.11 (C-20), 33.92 (C-21), 32.51 (C-22), 184.42 (C-28), 33.81 (C-29), 24.03 (C-30);

RESULTS AND DISCUSSION

Extraction of roots of *Solanum xanthocarpum* followed by extensive chromatographic techniques resulted in the isolation of caffeic acid and oleanolic acid. Caffeic acid (I) was isolated from the crude ethyl acetate extract *of Solanum xanthocarpum*. The compound has melting point of 137-140 ^{0}C. The elemental analysis of (I) corresponded to molecular formula $C_9H_8O_4$ that was substantiated by the molecular ion peak at m/z 180.08 in FAB positive mass spectrum. The IR spectrum of (I) exhibited broad absorption and 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 (I) displayed two ortho – coupled doublet (J=8.0 H_z) each for 1H, at δ 6.807 and 7.029 and broad singlet for 1H at δ 7.101 in the aromatic region indicated the presence of a trisubstituted aromatic ring in the molecule. The chemical shifts of these signals indicated the presence of catechol moiety in the molecule, which was confirmed by ^{13}C-NMR chemical shifts of the Hydrogen carrying Carbon atoms at δ 114.86(C-2), 115.75 (C-5) and 121.35 (C-6). The ^{1}H-NMR spectrum also displayed two doublets (J=15.0 Hz), each for 1H, at δ 7.511 (H-7) and 6.351 (H-8). The large value of coupling constant indicated the presence of Trans – disubstituted ethylene moiety in the molecule. The ^{1}H and ^{13}C chemical shifts of olefinic protons and carbons [δ 141.41 (C-7) and 127.50 (C-8)] were similar to those of Trans – cinnamic acid[13]. The ^{13}C-NMR spectrum of (I) exhibited presence of nine carbon atoms in the molecule. The ^{13}C chemical shifts of a carbon at δ 174.65 indicated the presence of carboxylic functional group in the molecule. The upfield chemical shifts of one of the ethylenic 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 145.21 (C-3), 148.35 (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 125.42 and the downfield chemical shifts of C-7 carbon and H-7 proton of ethylene moiety. On the basis of these spectral data compound (I) was characterized as caffeic acid.

The second compound, oleanolic acid (II) was isolated from the ethyl acetate extract of *Solanum xanthocarpum*. The compound has melting point of 305-306^{o}C. The molecular formula of (II) was determined to be $C_{30}H_{48}O_3$, by elemental analysis, which corresponded to the molecular weight 456. The molecular weight determined by elemental analysis was substantiated by FAB-mass spectrum, which displayed molecular ion peak $[M]^+$ at m/z 456. It gave positive Liberman and Noller test and developed yellow colour with TNM indicating triterpenoid nature of the molecule. It did not respond Molisch's test showing non-glycosidic nature of the molecule.

The IR spectrum of compound (II) exhibit characteristic absorption band for –OH function at 3400.15 cm^{-1}, for OH of carboxylic function at 3123.24 cm^{-1}, C-H stretching at 2920.34 cm^{-1} and 2880.25cm^{-1}, for carboxylic function at 1690.17 cm^{-1} and presence of double bond at 1450.13 cm^{-1}. The ^{1}H-NMR spectrum of compound (II) exhibit presence of methyl groups at δ 0.99, 0.80,

0.68 and 1.35 and a characteristic olefinic proton of C12-C13 double bonded pentacyclic triterpenoid at δ 5.39 (1H, t, J=4.0 Hz, H-12).

The location of the double bond at C12-C13 position was supported by the diagnostic mass spectral fragmentation pattern[14] exhibited by compound (II)forming ion peak at m/z 289. The fragment ion peaks at m/z 248 $[C_{16}H_{24}O_2]^+$, 203 $[C_{15}H_{23}]^+$ derived from D, E rings via Retro-Diels-Alder fission indicating that compound (II) possesses one hydroxyl group in the A, B - ring and a carboxyl group in D, E – ring on the Olean-12-ene-skeleton[15].The ^{1}H-NMR spectrum also showed a downfield signal for oxygenated methine proton at δ 4.97 (1H, t, J= 5.2Hz), which was assigned for H-3 proton. The ^{13}C-NMR spectrum of (II) revealed presence of signals due to an oxygenated carbon signal at δ 81.22 (C-3), one tri-substituted double bond at δ 123.51 (C-12) and 143.62 (C-13) and one carboxyl group at 184.42 (C-28). Moreover ^{13}C-NMR signals due to C-18-C-22 [40.25(C-18), 45.55(C-19), 30.11(C-20), 33.92(C-21), and 32.51(C-22)] suggested that (II) was an olean-12-en derivative[16]. It forms mono acetate with acetic anhydride-pyridine suggesting the presence of one hydroxyl group in the molecule.

On the basis of above spectral and chemical evidences compound II was identified as oleanolic acid. The identity of the compound was finally determined by CO-TLC and MMP with an authentic sample and by comparison of ^{13}C-chemical shifts with the reported data[17].

A base peak at m/z 248 is typical for α or β type triterpenes. In order distinguish between the two, carbon 13 values of the compound was examined. The fundamental difference between the two triterpenes is at C-29 and C-30 of the ring E. In α type triterpene (ursolic acid), both the methyl groups at ring E are secondary whereas in β type triterpene (oleanolic acid), both the methyl groups are tertiary. By comparing the carbon -13 values of the compound against that of α and β type triterpenes, the compound was found to show a closer resemblance to the β type. The interpretation is further supported by the ^{1}H-NMR spectra of the compound. A quartet at 2.13 ppm with a J value of 11.3 Hz was indicated coupling between a single proton at C-18 and two protons at C-19. This quartet only appears if the compound is of the β-type triterpene (where only two protons are attached to C-19). On the other hand if the compound is of α-type, a doublet will be appeared (because the two groups attached to C-19 are hydrogen and methyl and the coupling between a single proton at C-18 and at C-19 would give a doublet[18].

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