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# Isolation and Identification of Phytochemicals from *Xanthium strumarium*

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**Abstract:** *Xanthium Strumarium* L. (composite) is an annual herb found throughout India. The whole plant, specially root and fruit, is used as medicine. According to Ayurveda, *X. strumarium* is cooling, laxative, fattening, anthelmintic, alexiteric, tonic, digestive, antipyretic, and improves appetite, voice, complexion, and memory. It cures leucoderma, biliousness, epilepsy, salivation, fever and poisonous bites of insects. Xanthium species have been reported to possess an anti-inflammatory, analgesic, anti-ulcerogenic activity and antioxidant activity. The ethyl acetate fraction (8.03 g) and methanol extract (12.5 g) on repeated columns chromatography over Si-gel and sephadex LH20 afforded Caffeic acid, Xanthiazone, and Xanthiazone-(2-O-caffeoyl)-β-D-glucopyranoside, The identification of these compounds was made by concerted use of 1D- and 2D-NMR, Mass, UV and IR spectroscopy and chemical methods.
**Key words:***Xanthium Strumarium* L,Caffeic acid, Xanthiazone, Xanthiazone-(2-O-caffeoyl)-β-D-glucopyranoside.

## INTRODUCTION:

*Xanthium strumarium* L., Compositae is an annual herb with 2 strong hooked beaks [1]. It is a common weed found in India [2, 3]. The genus *Xanthium* includes 25 species, all of American origin *X. spinosum* Linn and *X. strumarium* Linn are used medicinally in Europe, North America and Brazil; *X. canadens* is used in North America and Brazil and *X. strumarium* Linn in China, India and Malaya [4]. Two species of *Xanthium*, *X. indicum* and *X. strumarium* have been reported in India. The whole plant, specially root and fruit, is used as medicine. According to Ayurveda, *X. strumarium* is cooling, laxative, fattening, anthelmintic, alexiteric, tonic, digestive, antipyretic, and improves appetite, voice, complexion, and memory. It cures leucoderma, biliousness, epilepsy, salivation, fever and poisonous bites of insects. The plant of *Xanthium* yields xanthinin which acts as a plant growth regulator. Antibacterial activity of xanthinin has also been reported. Seed yields semi-drying edible oil (30-35%) which resembles sunflower oil and used in bladder infection, herpes, and erysipelas. Cake can be used as manure whereas shell can be used as activated carbon [5, 6]. Xanthium species have been reported to possess an anti-inflammatory and analgesic [7], anti-ulcerogenic activity [8], and antioxidant [9] activity. Two sesquiterpene lactone glycosides and three kaurene glycosides closely related to carboxyatractyloside and atractyloside, together with the 3′, 4′-didesulphated-carboxyatractyloside and 3′, 4′-didesulphated-atractyloside, have been isolated from the aerial parts of *Xanthium spinosum* [16] along with xanthanol, isoxanthanol and their C-4 epimers [18]. Polyphenol 1,3,5-tri-*O*-caffeoylquinic acid, accompanied by 3,5-di-*O*-caffeoylquinic acid, has been isolated from the fruit of *Xanthium strumarium* [12]. Xanthanolide and

bis-norxanthanolide has been isolated from *Xanthium cavanillesii* [17].

The present investigation deals with the isolation and identification of Caffeic acid, Xanthiazone, Xanthiazone-(2-O-caffeoyl)-β-D-glucopyranoside from the ethyl acetate fraction (8.03 g) and methanol extract of whole plants of *Xanthium strumarium* on repeated column chromatography over Si-gel and Sephadex LH20.

## EXPERIMENTAL

$^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500 MHz and 125 MHz in $CDCl_3$ and $CD_3OD$ at $25^0$C with TMS as standard. Two-dimensional spectra were measured using standard methods of Bruker. The accuracy of the $^1$H and $^{13}$C chemical shifts were 0.01 ppm; $^1$H/$^1$H spin-spin coupling constants 0.2 Hz. IR spectra were recorded on a Shimadzu FTIR 8400S in KBr pellets. UV spectra were recorded in Beckman DU 700 UV spectrophotometer. Column chromatography (CC) was carried out on silica gel (kieselgel 60-120 and 70-230 mesh, Merck). TLC were conducted on Si-gel (E-Merck and BDH) coated on a thin glass plate (0.25mm thickness containing 13% $CaSO_4$ as binder). PC was carried out on Whatman filter paper N0.1 (descending) and spots were detected by spraying with aniline hydrogen phthalate (AHP) followed by heating. M. ps. were recorded in Boetius microscopic apparatus.

## PLANT MATERIAL

*Xanthium strumarium* was collected from Uttarkashi, Distt. Uttarkashi, Uttrakhand, in May, 2010, The plant species were identified by Dr. Sumer Chand, Systematic Botany Division, FRI, Dehradun, Uttarakhand. The voucher specimen (Hr. no. 83) was deposited in the herbarium of Department of Botany, Govt. P. G. College, Uttarkashi, Uttarakhand.

## EXTRACTION AND ISOLATION:

The air-dried and powdered plant of xanthium (1.5 kg) was exhaustively defatted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass extracted with 90% 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 $CHCl_3$: $H_2O$: MeOH (6:4:4) in a separatory funnel. The aqueous layer was concentrated under reduced pressure and then partitioned with ethyl acetate and 50% aqueous methanol. The ethyl acetate fraction was dried under reduced pressure to give EtOAc extract (8.03 g). The aqueous methanol extract was concentrated under reduced pressure to give methanol extract (12.5 g). The EtOAc extract (5.0 g) was column chromatographed over Si-gel using gradient elution using $CHCl_3$: MeOH with increasing MeOH content afforded two fractions. The fraction collected with $CHCl_3$: MeOH (9:3) were mixed dried and repeatedly column chromatographed over Si-gel and sephdex LH-20 eluted with 60% aqueous MeOH and with acetone afforded 1 (355 mg). The second fraction was column chromatographed on Si-gel with 50% aqueous MeOH and rechromatographed on Sephadex LH-20 with $CH_2Cl_2$-MeOH with increasing MeOH content afforded compound 2 (83 mg). The methanol extract (6.0 g) was column chromatographed over Si-gel successively eluted with $CHCl_3$ and $CHCl_3$-MeOH (2:1) afforded fraction A and B. Fraction A was concentration under reduced pressure, dried and digested with aqueous MeOH. The aqueous MeOH fraction on column chromatography on Si-gel eluted with 60% aq. MeOH afforded compound **2** (180 mg). Fraction B was subjected to column chromatography on Si-gel using $CH_2Cl_2$-MeOH solvent with increasing MeOH contents afforded a fraction. This fraction was subjects to preparative TLC on Si-gel 60 HPTLC (Merck) using developing system $CHCl_3$: MeOH: NH3 (80:20:3) afforded compound **3** (15 mg).

Compound (**1**): 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. UV ($\lambda_{max}$, MeOH): 240 (4.10), 280 (4.25), and 350, sh (3.61). IR ($V_{max}^{KBr}$) : $cm^{-1}$ 3300-2650, 1680, 1610, 1590, 1510, 1130, 760, 715, etc. $^1$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 (**2**): Colorless crystals (MeOH). M.P. 159-161˚C. Elemental Analysis: C=55.59%, H=5.34%, N=5.87%, S=13.29%, (calc. $C_{11}H_{13}O_3SN$); Molecular weight 239. FAB$^+$-MS : m/z 240 $[M]^+$, 203, 181, 153, 116, 109, 92, etc.. UV ($\lambda_{max}$, MeOH): 234, 278, 330, nm. IR ($V_{max}^{KBr}$) : $cm^{-1}$ 3440, 3310, 2925, 2851, 1655, 1627, 1600, 1430, 1210, 1150, 770, etc. $^1$H-NMR: (300 MHz, DMSO-$d_6$): δ 3.49 (2H, *s*, H-2), 1.36 (6H, *s*, $2CH_3$), 4.35 (2H, *s*, H-11), 6.40 (1H, *s*, H-6), 9.36 (1H, *s*, NH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 28.85 (C-2), 162.06 (C-3; -C=O), 175.40 (C-5, -

C=O), 120.10 (C-6), 170.56 (C-7), 41.97 (C-8), 26.95 (C-9, C-10, $2CH_3$), 59.70 (C-11), 141.64 (C-8a), 130.93 (C-4a).

Compound (**3**): Yellow crystals (MeOH), m.p. 187-189°C. Elemental Analysis: C=55.59%, H=5.34%, 1625, 1605, etc. $^1$H-NMR: (300 MHz, DMSO-$d_6$): δ 3.51 (2H, *s*, H-2), 6.61 (1H, *s*, H-6), 1.39 (6H, *s*, H-9, H-10, $2CH_3$), 4.50 (1H, *d*, *J*=15.0 Hz, H-11), 4.68 (1H, *d*, *J*=15.0 Hz, H-11), 9.30 (1H, *s*, NH), 4.36 (1H, *d*, *J*=7.0 Hz, H-1'), 4.67 (1H, *m*, H-2'), 3.45 (1H, *m*, H-3'), 3.15 (1H, *m*, H-4'), 3.45 (1H, *m*, H-5'), 3.35 (1H, *m*, H-6'), 7.05 (1H, *brs*, H-2"), 6.78 (1H, *d*, *J*=8.0 Hz, H-5"), 7.01 (1H, *d*, *J*=8.0 Hz, H-6"), 7.49 (1H, *d*, *J*=15.0 Hz, H-7"), 6.26 (1H, *d*, *J*=15.0 Hz, H-8"). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 28.85 (C-2), 162.27 (C-3; -C=O), 175.01 (C-5, -C=O), 121.79 (C-6), 164.09 (C-7), 41.47 (C-8), 26.75 (C-9), 26.34 (C-10), 65.72 (C-11), 141.01 (C-8a), 130.03 (C-4a), 101.92 (C-1'), 71.22 (C-2'), 73.55 (C-3'), 73.92 (C-4'), 74.22 (C-5'), 60.01 (C-6'), 125.34 (C-1"), 114.89 (C-2", 145.38 (C-3"), 148.76 (C-4"), 115.80 (C-5"), 121.22 (C-6"), 145.36 (C-7"), 113.73 (C-8"), 165.97 (C-9").

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 Xanthiazone (**2**) by comparison with authentic sample and the sugar was identified as β-D-glucose by paper chromatography.

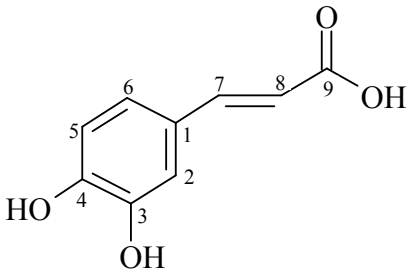
## RESULT AND DISCUSSION

### COMPOUND 1:

The elemental analysis of compound **1** 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 **1** 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 **1** displayed two ortho-coupled doublet (*J* = 8.0 Hz) each for 1H, at δ 6.76 and 7.01 and broad singlet for 1H 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 [10] 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*-disubstituted ethylene moiety in the molecule. The $^1$H and $^{13}$C chemical shifts of olefinic protons and carbons [δ 141.45 (C-7) and 127.51 (C-8)] were similar to those of *trans*-cinnamic acid [11].

The $^{13}$C-NMR spectrum of **1** 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 up field 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 145.29 (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 **1** was characterized as Caffeic acid.

### COMPOUND 2:

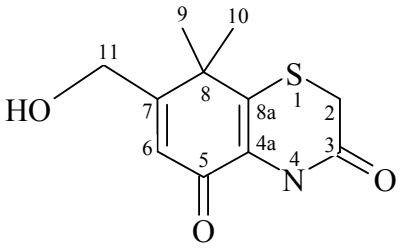
The positive ion FAB-MS of compound **2** afforded a molecular ion peak $[M+H]^+$ at m/z 240 indicated that the molecular formula of a compound is 239 which must contains an odd number of N-atoms. The elemental analysis of **2** corresponded to molecular formula $C_{11}H_{13}O_3SN$. The IR spectrum displayed an absorption band for hydroxyl group at 3440 $cm^{-1}$, carbonyl carbons at 1655, and 1627 $cm^{-1}$, and for olifinic bond at 1600 $cm^{-1}$. The UV spectrum exhibited absorption maxima at 244, 278, 330, nm supported the presence of carbonyl group as deduced by IR spectra.

The $^1$H-NMR spectrum of **2** exhibited five singlet signals at δ 1.36 for 6H, 3.49 for 2H, 4.35 for 2H, 6.40 for 1H and 9.36 for 1H. The $^{13}$C-NMR spectrum of compound **2** displayed presence of 10

carbon atoms. The singlet signal for six protons at 1.36 in $^1$H-NMR spectrum together with the carbon redonace at 26.96 suggested that the compound **2** contains 11 carbon atoms.

The $^1$H- and $^{13}$C-NMR data of compound **2** were quite similar to that of xanthiazone [12]. Previously isolated from fruits of *Xanthium strumarium*. The 1H proton signal exchangeable with $D_2O$ at δ 9.36 was assigned for proton attached at N-atom of heterocyclic ring. A singlet signal at δ 6.40 indicated presence of a tri-substituted olefinic bond in the molecule, which was confirmed from, the $^{13}$C chemical shift of proton carrying carbon of the double bonded carbon at δ 120.10 (C-6). The downfield chemical shift the disubstituted carbon of olefinic group at δ 170.56 (C-7) suggested its position β to carbonyl carbon (β-effect). The downfield chemical shift of methylene protons at δ 4.35 suggested that the hydroxyl group be attached to methylene carbon and were assigned for the hydroxymethyl substituted at C-7 whereas the upfield methylene singlet at δ 3.49 was assigned for the methylene group adjacent to carbonyl group. The $^{13}$C-chemical shifts at δ 175.40 suggested the presence of α,β-unsaturated carbonyl carbon and the $^{13}$C resonance at δ 162.06 indicated the presence of carbonyl carbon of secondary amide. The location of hydroxymethyl, and methyl groups were confirmed by comparing the $^1$H and $^{13}$C chemical shifts of compound **2** with that of reported [12].

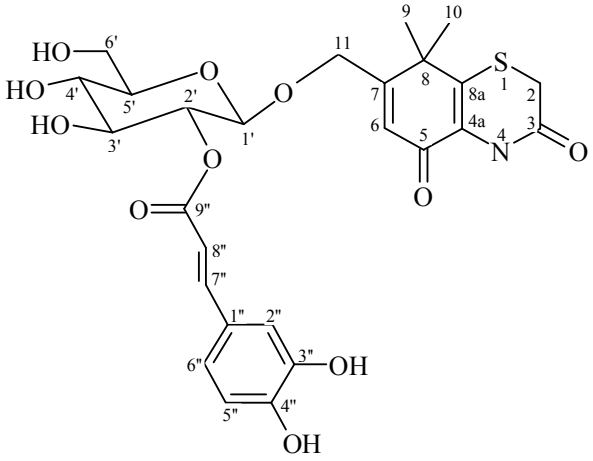
On the basis above discussed spectral data compound **2** was characterized as Xanthiazone; 7-Hydroxymethyl-8,8-dimethyl-4,8-dihydro-benzo[1,4] thiazine-3,5-dione.




**COMPOUND 3:**

The molecular weight of compound **3** was found to be 563 by positive-ion FAB mass spectrum which exhibited a molecular ion peak $[M+H]^+$ at m/z 564 (calc. for $C_{26}H_{29}O_{11}NS$) and $[M+Na]^+$ peak at m/z 586 (calc. for $C_{26}H_{29}O_{11}NSNa$). The IR spectrum displayed presence of an α,β-unsaturated carbonyl group at 1665 cm$^{-1}$, another carbonyl group at 1625 and an oleiifnic absorption band at 1605 cm$^{-1}$. The UV spectrum displayed an absorption maxima at 250, 280, 335 nm indicated presence of a carbonyl group, double bond and an aromatic ring in the molecule.

The $^1$H-NMR spectrum of **3** exhibited six signals in aromatic region, ten signals in aliphatic region and a singlet in the aromatic heterocyclic region. The $^{13}$C-NMR spectrum displayed twelve signals in aliphatic region and fourteen signals in the aromatic region. The DEPT spectrum showed presence of eleven methine, three methylene, two methyl and ten quaternary carbon atoms which accounts for twenty three protons. The one proton singlet at δ 9.30 was assigned for a proton attached to N-atom of heterocyclic ring. The remaining protons must present as hydroxyl groups.




In the aliphatic region of $^1$H-NMR spectrum a six protons singlet at δ 1.39, and two protons singlet at δ 3.51, were assigned for two methyl groups attached at C-8 and a methylene group adjacent to carbonyl group of Xanthiazone moiety [12]. In addition to these signals, two doublets ($J$ = 16.5 Hz), each for 1H at δ 4.50 and 4.68 was assigned for gem protons of oxymethyl group attached C-7 of Xanthiazone moiety. The $^1$H-NMR spectrum also displayed presence of a *trans*-disubstituted [δ 7.49 (1H, *d*, *J*=15.0 Hz, H-7"), 6.26 (1H, *d*, *J*=15.0 Hz, H-8") and a tri-substituted [δ 6.61 (1H, *s*, H-6)] double bond in the molecule. The $^1$H-NMR spectrum also displayed a broad singlet at δ 7.05 and two $A_2B_2$ type *ortho*-coupled doublets ($J$ = 8.0 Hz) indicated the presence of caffeoyl group in the molecule [13, 14]. A 1H doublet ($J$ = 7.0) δ 4.36 for anomeric proton along with other proton signals assignable to sugar moiety indicated presence of a β-sugar in the molecule. On acid hydrolysis compound **3** afforded a sugar which was confirmed as β-D-glucose by paper chromatography. In agreement with the above discussed $^1$H-NMR spectral data the $^{13}$C-NMR

spectrum displayed presence of a caffeoyl group, {δ 125.34 (C-1"), 114.89 (C-2"), 145.38 (C-3"), 148.76 (C-4"), 115.80 (C-5"), 121.22 (C-6"), δ 145.36 (C-7"), 113.73 (C-8") 165.97 (C-9")}. [7], anα,β-unsaturated carbonyl group at δ 175.01 (C-5, -C=O), and the olefinic carbons at δ 121.79 (C-6), 164.09 (C-7) of Xanthiazone moiety. In addition to these the $^{13}$C-NMR spectrum displayed chemical shift of anomeric carbon of β-D-glucose at δ 101.92 and other carbon resonances of sugar moiety. The downfield chemical shift of methylene carbon at δ 65.72 (C-11) and that of (C-1') of glucose at δ 101.92 suggested that the C-1' of glucose is attached at methylene carbon of Xanthiazone moiety. Furthermore the downfield chemical shift of C-2' carbon at δ 71.22 suggested that the caffeoyl group is attached at the C-2' of glucose.

On the basis of above discussed spectral data compound 3 was characterized as 7-hydroxymethyl-8,8-dimethyl-4,8-dihydroxybenzo[1,4] thiazine-3,5-dione-(2-O-caffeoyl)-β-D-glucopyranoside, which was further confirmed by comparing data with those of reported data [15].

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