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FLAVONE AND FLAVONE GLYCOSIDE FROM *CICHORIUM INTYBUS* LINN.

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ABSTRACT

Cichorium intybus Linn (Compositae) contains UV-absorbing metabolites. Studies on methanol extract of *Cichorium intybus* Linn by means of HPLC-UV, NMR, HPLC-MS resulted in isolation and identification of three previously unknown flavones glycosides: 5, 6, 7, 3′, 4′, 7′-hexahydroxy flavones-7-O-β-D-glucopyranoside (**1**), 3, 5, 7 – trihydroxy-3′,4′-dimethoxy-flavone-7-O-β-D-galactopyranosyl-(1→4)-β-D-xylopyranosyl-3-O-α-L-rhamnopyranoside (**2**), 3, 5, 7-trihydroxy-6, 4′-dimethoxy flavones (**3**) also isolated were three known flavones, luteolin (**4**), ladanetin (**5**) and spicoside (**6**).

INTRODUCTION: *Cichorium intybus* Linn (Compositae), is commonly known as "Kasani"[1] in Hindi. It is found wild in Punjab and Andhra Pradesh. This plant is also cultivated in Bihar, Punjab, Himachal Pradesh, Assam, Maharashtra, Gujarat, Tamil Nadu, Orissa, Andhra Pradesh and Kerala.

It is an erect, usually rough and more or less glandular, perennial herb; Juice milky; stems 0.3-0.9 m., angled grooved; branched tough, rigid, spreading. Radical and lower leaves 7.5-15 cm. pinnatifidlobes toothed, pointing downwards; upper leaves alternate, small, entire. Heads ligulate 2.5-3.8 cm. diam., terminal and solitary or axillary's and clustered, sessile or on short, thick stalks. Flowers bright blue; pappus of, or 2 series of short, blunt erect scales; ligules very long, spreading, toothed; style-arms long. Achene's smooth, angled, crowned with the ring of pappus scales.

Its sweet variety is cultivated. The plant is a good tonic [2]; provides cooling; useful in thirst, headache, opthalmia, and relief in throat inflammation, enlargement of the spleen, fever, vomiting, and diarrhea. The root is the best part of the plant; good stomachic and diuretic; enriches and purifies the blood; lessens inflammation and pain in the joints. Wild like variety- The plant is also a tonic, emmenagogue, alexiteric; astringent to the bowels; cures asthma, biliousness, inflammation; enriches the blood (Yunani)**.** Flowers and leaves contain escutetin and its glycoside. Roots gave flavonoids, catechol tannins, unsaturated sterols and triterpenoids. Recently (in 1998), researcher isolated a phenolic compound, esculetin, from the roots and confirmed hepato-protective activity in mice against paracetamol & Carbontetra-chloride induced hepatic damage.

The production of UV-absorbing compounds in submerged plants has been known for decades (Yadava R.N. et.al, 2010). Based on the observations that the plant does not appear to contain photo protection compounds, whereas the shallow-water *Cichorium intybus* Linn. adjust to alterations in UV exposure (Durako *et al*.,). We reasoned that it may utilize natural sunscreens as a means of photo protection.

To our knowledge, the chemical constituents of *Cichorium intybus* Linn., have not been investigated before, and here we report on the isolation and structural identification that likely play a UV-protecting role in the plant. We also qualitatively compared the flavones and flavones glycoside contents in *Cichorium intybus* Linn. Roots versus the below-ground rhizome and leaves provide more evidence for their UV-Protecting role in this sandy cost region. Earlier workers [3-6] have reported various chemical constituents from this plant. In this paper, we have reported the isolation and structure elucidation of three new flavone glycosides along with three known compounds from methanolic extract of the leaves of this plant.

METERIAL AND METHODS:

General Experimental Procedure: All the m.p. was determined on a thermoelectrically melting point apparatus and are uncorrected. All solvents employed for chromatography were HPLC grade and purchased from Burdick & Jackson Company. The IR spectra were recorded on Shimadzu 8201 PC spectrometer in KBr pellets. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker DRX-300 spectrometer at 300 MHz using $CDCl_3$ as solvent using TMS as internal standard; UV spectra were recorded on a molecular device Flexstation 3 instrument coupled with softmax pro software, and Mass spectra on Jeol-SX-102 (FAB) mass spectrometer.

Plant Material: The leaves of the plant were collected locally around Sagar region and were taxonomically authenticated by the Department of Botany, Dr.H.S.Gour Central University, Sagar (M.P.), India. A voucher specimen has been deposited in the Natural Products Laboratory, Department Of Chemistry, Dr. H. S. Gour Central University, Sagar (M.P.), India.

Extraction and Isolation: Air-dried powdered leaves (4 kg) of the plant were extracted with 95% methanol in a Soxhlet apparatus for 48 hours. The methanolic extract of the leaves of this plant was concentrated under reduced pressure and partitioned with pet-ether (40-60^{o}C), chloroform, ethyl acetate, acetone and ethanol. The methanol soluble fraction was concentrated under reduced pressure to yield brown viscous mass (1.80gm), which was subjected to PC and TLC examination, indicating it to be a mixture of more than six compounds 1, 2, 3, 4, 5 and 6. These compounds were separated and purified by column chromatography and HPLC techniques. The quantity of the compound 7th and other was found in very small quantity, therefore it was rejected.

RESULT AND DISCUSSION: HPLC analysis of methanol extracts of the whole plant of *Cichorium intybus* Linn. Showed the presence of a number of flavonoids, by the different tests [7], which we readily recognized by the especially characteristic strong absorption at 342-350 nm (Lu and Foo, 2000) observed by the diode array UV detector. The profile of the flavonoid components in the methanol extract of *Cichorium intybus* Linn [8-9], at the UV wavelength of 342 nm is shown in its UV spectra [10-11]. Compounds 1-6 were isolated and purified by means of combiflash chromatography and HPLC methods [12]. Spectroscopic analyses of 1-6 by NMR (^{1}H-NMR and ^{13}C-NMR), IR and LC-MS analysis established that 1-3 are previously unknown flavone glycosides and 4-6 are known flavones.

Compound 1 obtained as a pale yellow solid. The UV spectral data assigned three absorption bands at 224, 285 and 343 nm, indicating it might be 6-hydroxylated flavone derivative [13]. The molecular formula was established as $C_{21}H_{20}O_{13}$ based on the HRMS ESI* data ($[M+1]^{+}$] 481.0979) and NMR data. The LC-MS ESI* showed a fragment ion at m/z 319 [M-162], confirming as a glycoside composed of a hexahydroxy flavone aglycone and a sugar moiety with a molecular weight of 162. The LC-MS ESI* data also showed a fragment ion at m/z 151, corresponding to a trihydroxylated C ring moiety $C_6H_5O_3$ containing C-2 and C-3, in an established fragmentation pattern for flavone glycosides.

The ^{1}H-NMR spectrum [14] of compound showed four aromatic protons appearing as three singlet's at δ 6.59 (1H,s), 6.95 (1H,s) and 7.00 (2H,s) and compound their corresponding carbon signals appear at δ 103.5, 95.2 and 106.4 respectively. Above combined information it was possible to deduce that the aglycone moiety contained five phenolic groups at C-5, C-6, C-3, C-4 and C-5 and that the sugar moiety was connected to the flavone aglycone through an ether link at C-7 position. The ^{1}H-NMR spectrum contained an anomeric proton at 5.05 (1H, d, 7.4) and another six oxy-methine protons in the range δ 3.4- 4.0 ppm.

The sugar moiety was readily determined as a glucopyranose based on the ^{1}H-^{1}H COSY data, and by comparing the proton and carbon chemical shifts with published data. The coupling constant of the anomeric proton (7.4 Hz), indicated the β configuration for the glucopyranose moiety. Thus the structure of **compound (1)** was unambiguously determined as 5, 6, 3′, 4′, 5′-pentahydroxy flavone-7-O-β-D-glucoside (**1**), a previously unknown flavone glycoside.

Compound (2) has m.f. $C_{34}H_{46}O_{20}$, m.p. 252-254^{o}C, $[M]^{+}$ 774, (FABMS). Its IR spectra showed absorption bands at 3445, 3015, 2969, 2888, 1650, 1620, 1485, 1262, 1075 and 860cm^{-1}. The band in UV spectrum at 271 nm, and 354 nm showed its flavonoidal skelton. Two singlets at δ 6.85 and δ 6.81 were assigned to H-6 and H-8, respectively. In ^{13}C-NMR spectra, the two signals at δ 157.73 and 156.19 confirmed the presence of –OMe group at C-3′ and C-4′ position[15]. The anomeric proton signals at δ 5.31 (1H, d, *J* 7.8 Hz), 5.35 (1H, d, *J* 7.5 Hz) and 5.76 (1H, d, *J* 7.5 Hz) were assigned for H-1′′, H-1′′′, and H-1′′′′of L-rhamnose, D-xylose and D-galactose respectively.

Characteristic ion peaks appeared at m/z 774 $[M]^{+}$, 610 [M^{+} D-galactose], 476 $[M]^{+}$ [D-galactose-D-xylose] and 328 $[M]^{+}$ [D-galactose-D-xylose] [L-rhamnose], [aglycone] were obtained by subsequent loses from the molecular ion of each molecule of D-galactose, D-xylose and L-rhamnose suggesting D-galactose as terminal sugar at C-7 position and D-xylose was linked to aglycone at C-7 position. L-rhamnose attached at C-3 position of aglycone.

The position of sugar moieties in **compound (2)** was determined by permethylation followed by acid hydrolysis which yielded methylated aglycone identified as 3, 7 –dihydroxy- 5, 3′, 4′-trimethoxy-flavone, showed that glycosylation were identified as C-3 and C-7 position of the flavone and sugars were identified as 2, 3,- 4 –tri-O-methyl-L-rhamnose (R_G 1.01), 2, 3-di-O-methyl-D-xylose (R_G 0.74) and 2, 3, 4, 6 -tetra-O-methyl-D-galactose (R_G 0.88), which showed that C-1′′′′ of D-galactose was linked with C-4′′′ of D-xylose, C-1′′′ of D-xylose attached with C-7 position of aglycone and C-1′′ of L-rhamnose was attached with C-3 position of the aglycone. The inter linkages (1→4) was found between D- galactose and D-xylose.

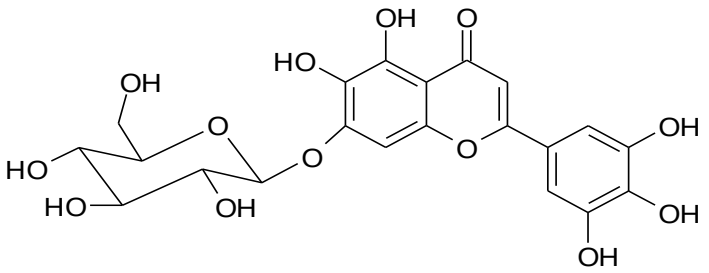
From above evidences new **compound (1)** identified as 3′, 4′, 5′-pentahydroxy flavone-7-O-β-D-glucoside (**1**). Whereas, the structure of **compound** (**2**) was characterized as 3, 5, 7 –trihydroxy- 3′, 4′-dimethoxy-flavone-7-O- β -D-galactopyranosyl-(1→4)-O-β-D-xylopyranosyl-3-O-α-L-rhamnopyranoside[16].

On the basis of ^{1}H-NMR, ^{13}C-NMR, UV, Mass, HPLC and various chemical reaction the compound **3** has been identified as 3, 5, 7-trihydroxy-6, 4′-dimethoxy flavone [17].

The glycoside **compound** (**4**) Spicoside is pale yellow powders [18]. Their structure was determined as spicoside [C30H26O15; M^{+}H m/z 625] (Albach e al., 2003) on the basis of ^{1}H and ^{13}C- NMR spectroscopic analysis and comparison with published NMR data.

The structure of **compound (5**) and **compound (6**) were determined as the flavonoids ladanetin[19] (Arisawa et al., 1970) and luteolin[20] (Lu et al., 1980), respectively.

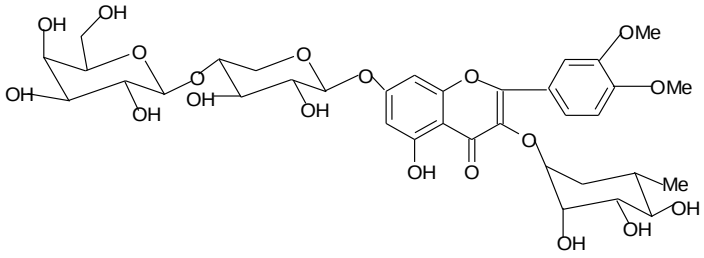
Compoumd (1): 5, 6, 7, 3′, 4′, 7′-hexahydroxy flavones-7-O-β-D-glucopyranoside. Yellow solid (0.9 mg); UV 285 and 343; IR ν_{max}^{KBr} Cm^{-1} 3382, 2931, 1672, 1202, 1122; HRMS ESI* m/z 481.0981 $[M+H]^{+}$ (Calcd. for $C_{21}H_{21}O_{13}$, m/z 481.0984). ^{1}H-NMR (500 MHz, DMSO-d6) δ 6.59 (1H, s, H-3), 6.94 (1H, s, H-8), 7.0 (2H, s, H-2′ and 6′), 5.05(1H, d, 7.4, H-1″), 3.39 (1H, m, H-2″), 3.38 (1H, m, H-3″), 3.24 (1H, m, H-4″), 3.49 (1H, m, H-5″), 3.76 (1H, m, H-6″ a), 3.54 (1H, m, H-6″ b), 12.76 (1H, brs., 5-OH), 8.66 (1H, brs., 6-OH); 13C-NMR (500 MHz, DMSO-d6, Partial observed by HSQC) δ 103.4 (C-3), 95.2 (C-8), 106.4 (C-2′ and C-6′), 102.3 (C-1″), 74.3 (C-2″), 76.6 (C-3″), 70.7 (C-4″), 78.8 (C-5″), 61.4 (C-6″).



COMPOUND **1**

5, 6, 7, 3′, 4′, 7′-hexahydroxy flavones-7-O-β-D-glucopyranoside

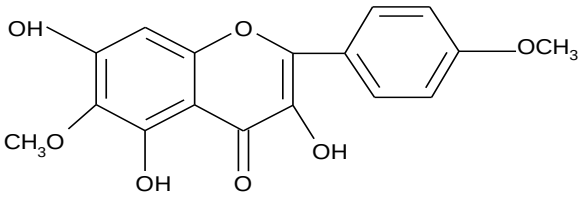
Compound (2) : 3, 5, 7 – trihydroxy-3', 4'-dimethoxy-flavone- 7- O- β- D- galactopyranosyl- (1→4)- β- D-xylopyranosyl-3-O-α-L-rhamnopyranoside: It was crystallized from acetone to yield 0.85gm. It has m.p. 252-254°C, m.f. $C_{34}H_{46}O_{20}$, $[M]^+$ 774, Found (%) C, 52.71; H, 5.94; Calcd. for m.f. $C_{34}H_{46}O_{20}$ C, 52.43, H 5.20; UV: λ_{max} (MeOH) (nm) 245, 268, 334, (+$AlCl_3$-HCl) 253, 368; (+NaOAc) 273, 345; IR: ν^{KBr}_{max} (cm^{-1}); 3445, 3015, 2969, 2888, 1650, 1620, 1485, 1262, 1075, 860 cm^{-1}. ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm); 6.85 (1H, d, *J* 2.1 Hz, H-6), 6.81 (d, *J* 2.3 Hz, H-8), 11.93 (1H, s, -OH-5), 7.72 (1H, d, *J* 2.2 Hz, H-2'), 7.35 (1H, d, *J* 8.5 Hz, H-5'), 7.86(1H, d, *J* 8.5 Hz, H-6'), 3.72 (3H, s, -OCH_3-3'), 3.70 (s, -OCH_3-4'), 5.31 (1H, d, *J* 7.8 Hz, H-1''), 4.53 (1H, dd, *J* 2.2, 10.2 Hz, H-2''), 3.99 (1H, dd, *J* 3.6, 9.5 Hz, H-3''), 4.55 (1H, dd, *J* 3.2, 8.8 Hz, H-4''), 4.52 (1H, m, H-5''), 2.18 (3H, s, Me-6''), 5.35 (1H, d, *J* 7.5 Hz, H-1'''), 4.57 (1H, dd , *J* 3.7, 10.2 Hz, H-2'''), 4.61 (1 H, dd, *J* 2.7, 10.1 Hz, H-3'''), 4.5 (1H, m, H-4'''), 4.78 (2H, d, *J* 6.9 Hz, H-5'''), 5.76 (1H, d, *J* 7.5, H-1''''), 4.33 (1H, dd, *J* 7.5, 9.0 Hz, H-2''''), 4.32 (1H, dd, *J* 3.6, 9.7 Hz, H-3''''), 3.60 (1H, dd, *J* 3.0, 9.0 Hz, H-4''''), 4.45 (1H, m, H-5''''), 4.41 (3H, m, H-6''''). ^{13}C-NMR: (300 MHz, $CDCl_3$) δ (ppm); 148.84 (C-2), 164.70 (C-3), 136.6 (C-4), 103.46 (C-4a), 91.5 (C-5), 97.15 (C-6), 168.20 (C-7), 97.5 (C-8), 147.8 (C-8a), 123.37 (C-1'), 112.15 (C-2'), 157.73 (C-3'), 156.19 (C-4'), 141.55 (C-5'), 126.00 (C-6'), 58.51 (-OCH_3-3'), 61.22 (-OCH_3-4'), 101.21 (C-1''), 71.0 (C-2''), 69.0 (C-3''), 61.70 (C-4''), 68.30(C-5''), 18.10 (C-6''); 107.20 (C-1'''), 80.33 (C-2'''), 172.10 (C-3'''), 75.4 (C-4'''), 66.2 (C-5'''), 88.1 (C-1''''), 77.7 (C-2''''), 71.0 (C-3''''), 76.40 (C-4''''), 68.89 (C-5''''), 101.00 (C-6''''). $[M]^+$ 774 (FABMS).



COMPOUND 2

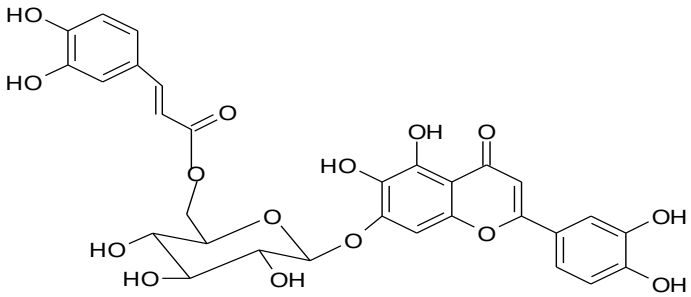
3, 5, 7 – trihydroxy- 3', 4'- dimethoxy-flavone- 7- O- β- D- galactopyranosyl- (1→4)- β- D- xylopyranosyl- 3- O- α- L- rhamnopyranoside

Compound (3): 3, 5, 7-trihydroxy-6, 4'-dimethoxy flavone: It has m.f. $C_{17}H_{14}O_7$, m.p. 211-213°C, $[M]^+$ 330 (EIMS); found (%) C, 61.81; H, 4.24; Calcd. for m.f. $C_{17}H_{14}O_7$, (%); C 61.97, H 4.65. UV: λ_{max} (MeOH) (nm) 274, 344; IR: ν^{KBr}_{max} (cm^{-1}); 3350, 1645, 1610, 1275, 875 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm); 8.16 (2H, d, *J* 8.5 Hz, H-2', H-6'), 6.98 (2H, d, *J* 8.0 Hz, H-3',H-5'), 6.64 (1H, s, H-8), 3.97 (3H, s, OMe-6'), 3.78 (3H, s, OMe-4'). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 158.6 (C-2), 135.8 (C-3), 176.2 (C-4), 106.4 (C-4a), 153.4 (C-5), 132.5 (C-6), 56.5 (OMe-6), 150.8 (C-7), 91.5 (C-8), 160.8 (C-8a), 122.2 (C-1'), 130.4(C-2' and C-6'), 115.8 (C- 3'), 162.8 (C-4'), 55.8 (4'-OMe).



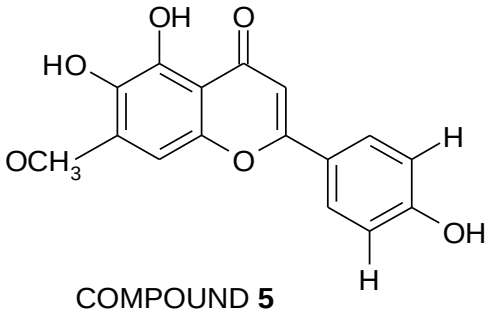
COMPOUND 3

3, 5, 7-trihydroxy-6, 4'-dimethoxy flavones



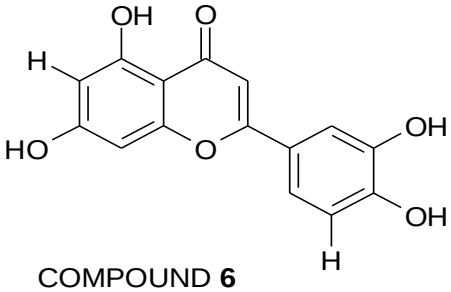
COMPOUND 4

Spicoside



COMPOUND **5**

Ladanetin



COMPOUND **6**

Luteolin

CONCLUSION: This paper represents the firstly chemical investigation of the flavonoids in *Cichorium intybus* Linn., herb as flavone glycoside and flavone was observed in the HPLC-UV chromatogram of the methanol extract. Apart from a few very minor components, the remaining compounds in the methanol extract of *Cichorium intybus* Linn., were isolated, and several new derivatives have been identified. The distribution of these flavonoids compounds throughout the plant was also examined. To this end, leaves and combined rhizomes and roots were separated and extracted with methanol, and the extracts analyzed separately by HPLC-UV-MS.

The various chromatograms obtained in above practical. In simple comparison it can be seen that here are differences in the distribution of these metabolites. The flavonoid glycosides **compound 1 and 2** are the most abundant flavonoid derivatives in the leaves, and roots.

The flavone **compound 3** second most abundant components in the leaf, whereas in the roots. **Compound 4, 5 and 6** are the next most abundant components, perhaps suggesting that the flavonoids are transported within the cell as glycoside derivatives. **Compound** (**1**): 3′, 4′, 5′-pentahydroxy flavone-7-O-β-D-glucoside, **Compound (2):** 3,5,7–trihydroxy-3′,4′-dimethoxy-flavone-7-O-β-D-galactopyranosyl-(1→4)-β-D-xylopyranosyl-3-O-α-L-rhamnopyranoside, **Compound (3)**: 3,5,7-trihydroxy-6,4’-dimethoxy flavone, **Compound (4)**: spicoside, **Compound (5**): ladanetin and **Compound (6):** luteolin

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