IJPSR (2016), Vol. 7, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 September, 2015; received in revised form, 22 February, 2016; accepted, 16 March, 2016; published 01 April, 2016

PHYTO-CHEMICAL CONSTITUENTS FROM STEM OF *TARAXACUM OFFICINALE* WEBER

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Key words:

Taraxacum Officinal weber., Compositae, Stems, Flavone glycoside

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ABSTRACT: A new flavone glycoside 1 m. p. 223-225ºC, m. f. $C_{34}H_{42}O_{20}$, [M+] 770 (FABMS) has been isolated from the stems of *Taraxacum Officinal* weber along with one known compound Taxifolin. A compound 1 was characterized as 6, 4′-dimethoxy-5, 7-dihydroxy-flavone- 7 - O - α - L-rhamnopyranosyl-(1→4) - O – β - D xylopyranosyl-(1→4)-O-β-D-glucopyranoside by various chemical degradations and spectral analysis.

INTRODUCTION:

Taraxacum officinale (L.) Weber [1-3] belongs to family Compositae. It is commonly known as **'**Kanphool or Kukraundha**'** in Hindi. It is found in Himalayas and the Khasi Hills of Meghalaya, Mishmi Hills of Arunachal Pradesh and hills of South India at 3000-5000m and Gujarat. It used in the treatment of kidney and liver problems. It is also use full in chronic diseases of the digestive organs especially hepatic affections, an jaundice. It is used to make dandelion wine and the greens are used in salad. Earlier workers [4-6] have reported various constituents from this plant. In the present paper, we report the isolation and structural elucidation of a new flavone glycoside 6, 4'-dimethoxy - 5, 7 - dihydroxy-flavone-7-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-O-β-D- glucopyranoside (1) along with one known compound Taxifolin (2) from methanolic extract of the stems of this plant.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(4).1761-64

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (4).1761-64

MATERIAL AND METHODS:

General Experimental Procedure: All the melting points were determined on a thermoelectrically melting point apparatus and are uncorrected. UV Spectra were determined in MeOH and Mass Spectra on a Jeol SX-102 (FABMS) mass spectrometer. IR Spectra were obtained on a Shimadzu FTIR-8400 spectrometer. ^{1}H-NMR Spectra were recorded at 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard. ^{13}C-NMR Spectra were recorded at 75 MHz spectrometer using $CDCl_3$. Thin Layer Chromatography on silica gel G and column chromatography on silica gel were used.

Plant Material: The stems of *Taraxacum officinal weber*. were collected 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 and powdered stems (6.00 kg) of the plant were extracted with

rectified spirit in Soxhlet extractor for seven days. The stems were successively extracted with methanol for two days. The MeOH soluble fraction of the plant was concentrated under reduced pressure to yield a brown viscous mass (3.0 gm) which was subjected to TLC examination over silica gel-G using nBAW (4:1:5) as solvent and I_2 vapors as visualizing agent, showed four spots, indicating it to be mixture of two compounds A and B. These compounds were separated and purified by column chromatography over silica gel using $CHCl_3$: MeOH in various proportions. After removal of the solvent and crystallization from ether, above eluates yielded compound **1**(1.26 gm.), compound and **2** (0.85 gm.) respectively.

Study of Compound 1: It had m. p. 222-224ºC, m. f. $C_{34}H_{42}O_{20}$, [M^+] 770 (FABMS) found (%); C 53.04, H 5.49, calcd for m.f. $C_{34}H_{42}O_{20}$, (%); C 52.98, H 5.45, UV MeOH λ_{max} ; 273, 322 (+$AlCl_3$), 302, 350 (+$AlCl_3$ / HCl), 284, 354 (+NaOAC); 247, 286 (+NaOMe); 283, 324 nm. IR (KBr) ν_{max} 3484, 3210, 2930, 1738, 1650, 1610, 1432, 1325, 1085, 810cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.02 (1H, s, H-3), 7.31 (2H, d, J 8.4 Hz, H-2′, 6′), 6.81 (2H, d, J 8.4, Hz, H-3′, 5′), 11.85 (1H, s, OH-5), 5.81 (1H, d, J 7.2 Hz, H-1′′), 4.42 (1H, dd, J 8.4, 7.51, Hz, H-2′′), 4.30 (1H, dd, J 8.2, 8.1 Hz, H-3′′), 3.9 (1H, dd, J 8.2, 8.3 Hz, H-4′′), 3.97 (1H, dd, J 8.0, 6.4 Hz, H-5′′), 4.20 (2H, dd, J 6.12, 10.2 Hz, H-6′′), 5 (1H, d, J 6.4 Hz, H-1′′′), 3.78-3.90 (3H, m, H-2′′′, H-3′′′, H-4′′′), 4.28 (2H, dd, J 6.12, 11.5 Hz, H-5′′′), 5.28(1H, s, H-1′′′′), 3.96-4.59 (4H, m, H-2′′′′, 3′′′′, 4′′′′, 5′′′′), 1.17 (3H, d, J 5.3 Hz, Rham-6′′′′), ^{13}C-NMR (75 MHz, $CDCl_3$) δ; 162.4 (C-2), 107.9 (C-3), 175.7 (C-4), 159.4 (C-5), 133.4 (C-6), 162.6 (C-7), 95.0 (C-8), 154.4 (C-9), 103.6 (C-10), 120.9 (C-1′), 128.6(C-2′), 115.3 (C-3′), 162.9 (C-4′), 117.0 (C-5′), 128.5 (C-6′), 102.01 (C-1′′), 84.6 (C-2′′), 78.5 (C-3′′), 70.8 (C-4′′), 76.2 (C-5′′), 65.3 (C-6′′), 105.9 (C-1′′′), 76.8 (C-2′′′), 78.2 (C-3′′′), 73.5 (C-4′′′), 75.3 (C-5′′′), 99.4 (C-1′′′′), 85.0 (C-2′′′′), 75.6 (C-3′′′′), 70.1 (C-4′′′′), 76.0 (C-5′′′′), 17.68 (C-6′′′′).

Acid hydrolysis of Compound 1: Compound 1 (50 mg) was dissolved in methanol (15 ml) and refluxed with 10 ml of 10% H_2SO_4 on water bath for 7-10 hrs. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$:MeOH (4:6) to give compound **1-A** which was identified as 6, 4′-dimethoxy- 5, 7-dihydroxy-flavone by comparison of its spectral data with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent and aniline hydrogen phthalate as detecting agent which confirmed the presence of D-glucose (R_f 0.19), D-xylose (R_f 0.26), and L-rhamnose (R_f 0.36).

Permethylation of Compound 1: Compound **1** (40 mg) was refluxed with MeI (5 ml) and Ag_2O (30 mg) in DMF (20 ml) for two days and then filtered. The filtrate was hydrolysed with 10% ethanolic H_2SO_4 for 7 hrs to give methylated aglycone, identified as 7-hydroxy- 5, 6, 4′trimethoxy flavone and methylated sugars which were identified as 2, 3, 4-tri-O-methyl-L-rhamnose, 2, 3-di-O-methyl-D-xylose and 2, 3, 6-tri-O-methyl-D-glucose.

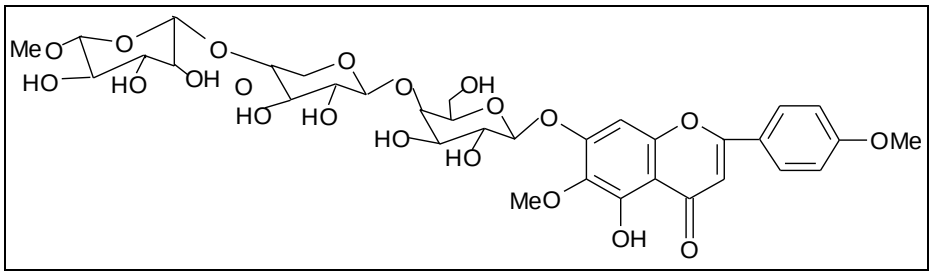
Enzymatic hydrolysis of Compound 1: The compound 1 (25 mg) was dissolved in MeOH (10 ml) and hydrolysed with an equal volume of taka diastase enzyme. The reaction mixture was allowed to stay at room temperature for five days and filtered. The proaglycone and hydrolysate were studied separately.

The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying reagent, showed the presence of L-rhamnose (R_f 0.36). The proaglycone was dissolved in MeOH (20 ml) and hydrolysed with equal volume of almond emulsin at room temperature as usual procedure, yielded aglycone identified as 6, 4′-dimethoxy- 5, 7-dihydroxy flavone and sugars were identified as D-glucose (R_f 0.19) and D-xylose (R_f 0.26) (Co-PC).

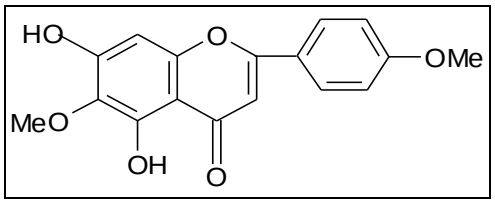
Study of Compound 1-A: It had m. f. $C_{17}H_{14}O_6$, m. p. 264-265^{0}C, [M^+] 314 (FABMS), found (%); C 61.83, H. 4.26, calcd for m. f. $C_{17}H_{14}O_6$, (%) C

61.81, H 4.24. UV MeOH λ_{max}; 280, 362, (+$AlCl_3$); 308, 352, (+$AlCl_3$ / HCl); 285, 355 (+NaOAc); 248, 286, (+NaOMe); 283, 334 nm. IR (KBr) ν_{max} ; 3250, 2955, 1714, 1635, 1600, 1540, 1428, 1370, 1255, 1082, 810 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.21 (1H, s, H-3), 7.33 (2H, d, J 8.5 Hz, H-2′, 6′), 7.11 (2H, d, J 8.5, Hz, H-3′, 5′), 12.63 (s, OH-5), 3.91 (3H, s, OMe-6), 4.13 (3H, s, OMe-4′). ^{13}C-NMR (75 MHz, $CDCl_3$); δ 161.4 (C-2), 106.8 (C-3), 176.5(C-4), 152.2 (C-5), 131.6 (C-6), 162.3 (C-7), 94.6 (C-8), 154.5 (C-9), 103.9 (C-10), 120.4 (C-1′), 128.6 (C-2′), 117.0 (C-3′), 152.0 (C-4′), 116.1 (C-5′), 128.7(C-6′).

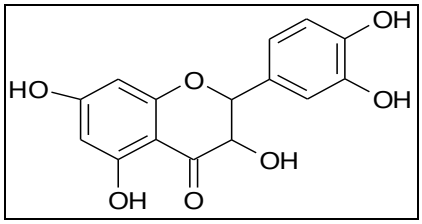
RESULTS AND DISCUSSION: Compound **1** showed it gave yellow colour with 10% methanolic sulfuric acid and pink colour with Shinoda confirmed the presence of flavone and also showed positive Molisch test with formation of violet ring. along with one known compound Taxifolin (**2**) by comparisons of their spectral data. Compound **1** had m. f. $C_{34}H_{42}O_{20}$, m. p. 222-224^{o}C, [M^+] 770 (FABMS). It gave Molisch [7] and Schinoda test [8] showing its flavonoidal glycosidic nature. The compound also responded to neutral ferric chloride test. The UV and IR spectral data also shows the nature of flavone. The IR spectrum showed strong absorptions at 3484 (-OH), 2930 (-CH saturated), 1650 (>C=O), 1610 (aromatic ring). Two bathochromic shifts of 26 nm and 44 nm in bands I on addition of $AlCl_3$ and $AlCl_3$ + HCl relative to methanol confirm the presence of -OH group at C-5 and C-7position. A bathochromic shift of 15 nm in bands I with NaOMe showed the presence of -OMe group at C-4' position in compound A [9, 10]. In ^{1}H-NMR spectrum of compound A, two singlets at δ 7.33 and δ 6.80 were assigned to H-2′, 6′ and H-3′, 5′ respectively. The anomeric proton signals at δ 5.80 (1H, d, J 7.2 Hz, H-1′′), δ 5. 02 (1H, d, J 6.4 Hz, H-1′′′) and δ 5.23 (1H, s, H-1′′′′) were assigned to H-1′′ of D glucose, H-1′′′ of D-xylose, H-1′′′′ of L-rhamnose.



COMPOUND A



Compound 1-A



Compound 2

FIG.1: COMPOUNDS ISOLATED FROM *TARAXACUM OFFICINAL WEBER*.

Acid hydrolysis of compound **1** with ethanolic 10% H_2SO_4 yielded aglycone **1-A**, m.p. 264-265^{0}C, m. f. $C_{17}H_{14}O_6$, [M^+] 314 (FABMS) which was identified as 6, 4′-dimethoxy-5, 7-dihydroxy-flavone by comparison its spectral data with reported literature values [11-12]. The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was

concentrated and subjected to TLC and paper chromatography examination[13-14] and the sugars were identified as D-glucose (R_f 0.19), D-xylose (R_f 0.26), and L-rhamnose (R_f 0.36) (Co-PC, Co-TLC). Periodate oxidation[15-16] of compound **1** confirmed that all sugars were present in the pyranose form. The glycosidic linkage is located at 7-position in aglycone [17].

The position of sugar moieties in the compound **1** were determined by permethylation[18-19] followed by acid hydrolysis which yielded methylated aglycone identified as 7-hydroxy- 5, 6, 4′-trimethoxy-flavone which confirmed that hydroxy group at C-7 position of the aglycone were involved in glycosidation. The methylated sugars which were identified as 2, 3, 6-tri-O-methyl-D-glucose, 2, 3- di-O-methyl-D-xylose and 2, 3, 4-tri-O-methyl-L-rhamnose according to Petek indicating that the C-1′′′′ of L-rhamnose was linked to C-4′′′ position of xylose and C-1′′′ of D-xylose was attached with C-4′′ of D-glucose and C-1′′ of D-glucose was attached to the C-7 position of aglycone and also showed the interlinkage (1→4) between D-xylose and D-galactose. That was further confirmed by their ^{13}C-NMR spectral data. Enzymatic hydrolysis [20] of compound **1** with takadiastase enzyme liberated L-rhamnose (R_f 0.37) and proaglycone identified as 6, 4′-dimethoxy- 5, 7-trihydroxy - flavone -7 - O- β - D - xylopyranosyl-(1→4)-O-β-D-glucopyranoside that confirmed the presence of α-linkage between L-rhamnose and C-7 position of aglycone. Proaglycone on further hydrolyzed with almond emulsin liberated D-glucose (R_f 0.19), D-xylose (R_f 0.26) suggesting the presence of β-linkage between D-xylose and D-glucose as well as D-glucose and aglycone. On the basis of above evidences the structure of compound **1** was characterized as 5, 7 -dihydroxy-6, 4′-dimethoxy-flavone - 7 - O - α - L- rhamnopyranosyl - (1→4) - O - β - D – xylopyranosyl - (1→4)-O-β-D- glucopyranoside.

Compound **2.** light It was powdered, analyzed for, m. p. 215-217 ^{0}C, m. f. $C_{15}H_{12}O_7$, [M^+] 304 (EIMS) and identified as salvigenin by comparison of its spectral data with reported literature values [21].

ACKNOWLEDGEMENT: Authors are grateful to Head, CDRI Lucknow for recording various spectral and elemental analys. One of the author (S. Khan) is grateful to Head Department of Chemistry, Dr. H. S. Gour University, Sagar (M.P.) India, for providing necessary laboratory facilities.

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How to cite this article:
Khan S, Duby K, Jain R and Vishwakarma UK: Phyto-Chemical Constituents from Stem of *Taraxacum officinale* weber. Int J Pharm Sci Res 2016; 7(4): 1761-64.doi: 10.13040/IJPSR.0975-8232.7(4).1761-64.