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Phytochemical Investigation of the root of *Moringa oleifera*

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ABSTRACT

The phytochemical investigation of *Moringa oleifera* leads to isolation and purification of a new compound , 6-ethyl-5-hydroxy-8-methoxy flavone-7-*O*-α-L(–)- rhamnopyranoside-4'-*O*-β-D(+)-glucopyranoside from ethyl alcohol extract of the root and the structure of this isolated compound was established by chemical and UV,IR,^{1}H NMR,^{13}C NMR and Mass spectral data. *Moringa oleifera (Moringaceae)* commonly called "Sahjna" is widely cultivated in Asia, Africa and other parts of the world. Almost every parts of the plant show pharmacological properties .

1. Introduction

Plants are the rich source of bioactive compounds that brings out many beneficial health effects on human beings. The different parts of a plant such as leaves, stems, fruits, flowers, seeds, roots may contains a large numbers of phytochemicals. From the ancient time, it has been proved that plant kingdom has a therapeutic importance[1]. *Moringa oleifera* is a small deciduous tree which grows up to height up to height of 10-12 m[2]. It is known by different names as miracle vegetable, ben oil tree, drumstick, Mother' best friend, segva, etc.[3]. It is widely distributed in western and Himalayan regions, Asia, Africa and Arabia[4,5] . Almost every part of *Moringa oleifera* is used for edible purpose[6-9]. The different parts of this tree shows pharmacological properties like antifungal[10], antioxidant[11,12], antihypertensive[13]. The seeds are also act as natural coagulant, which helps in coagulating the suspended mud and turbidity and shows disinfectant effect against the pathogens[14]. The present study involves the isolation and characterization of a new flavone glycoside, compound 1 (6-ethyl-5-hydroxy-8-methoxy flavone-7-*O*-α-L(–)- rhamnopyranoside-4'-*O*-β-D(+)- glucopyranoside) from the root of *Moringa oleifera*.

2. Experimental

General Experimental procedure:

All the solvents used were of analytical grade. Melting points were determined by open glass capillary method and are uncorrected. The IR spectra were recorded in KBr on Perkin

Elmer 157 spectrometer. UV spectra were recorded on Beckmans-DK2 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL JNM- A 500 spectrophotometer in $CDCl_3$ using TMS as an internal standard at 400 MHz and 100 MHz respectively. The mass spectra on JEOL JMS- D 300 mass spectrometer.

Plant material:

The root of *Moinga oleifera* was collected from Phaphamau, Allahabad and the tree was identifying by Botanical Survey of India (BSI), Allahabad.

Extraction, Fractionation and Isolation:

The shadow dried powdered root (4 kg) was extracted with 95% hot ethanol in soxhlet apparatus for12 h. This extract was concentrated under reduced pressure by using rotatory evaporator and then poured in to ice cold distilled water with constant stirring, a dark brown insoluble residue and a light brown aqueous solution were obtained which were separated by filtrations.

The water insoluble fraction was loaded over a sintered column and eluted with different organic solvents with increasing polarity and from chloroform: ethyl acetate (8:2,v/v) the aforesaid compound is obtained. The compound was separated by column chromatography and purified by preparative TLC.

Study of compound 1*:*

The Compound was light yellow coloured and recrystallized from methanol. Molecular formula: $C_{30}H_{36}O_{15}$, m.p. 187^{o}C, m/z: 636[$M^{+\cdot}$], 327, 300, 297; found (%): C, 56.90, H, 5.82; Calcd. (%): C, 56.93, H, 5.69; UV(MeOH) λ_{max} (nm): 332, 276; (MeOH/NaOMe) 397, 276; (MeOH /fused NaOAc) 332, 291; (MeOH /$AlCl_3$/HCl) 332, 296; IR λ_{max} (KBr): 3450, 2870, 1660, 1590, 1190; ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 6.21 (1H, s, H-3), 7.56 (1H, dd, *J* = 9.0 and 2.5 Hz, H-2'), 7.68 (1H, dd, *J* = 7.0 and 2.5 Hz, H-3'), 7.6 (1H, dd, *J* = 9.0 and 2.5 Hz, H-5'), 7.50 (1H, dd, *J* = 9.0 and 2.5, H-6'), 5.356 (1H, d, *J* = 7.5 Hz), 5.343

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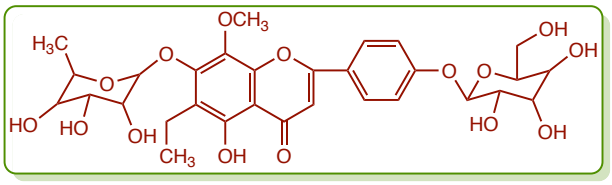
(1H, d, J = 7.5 Hz), 1.20 (d, 3H, rham. -CH_3), 3.03- 3.73 (5H, m, rham.), 2.53-3.57 (6H, m, glu. Protons) ; ^{13}CNMR (100 MHz, $CDCl_3$) δ (ppm): 153.2 (C-2, s), 133.5 (C-3, d), 147.4 (C-4, s), 161.2 (C-5, s), 165.0 (C-6, s), 168.0 (C-7, s), 140.2 (C-8, s), 166.3 (C-9, s), 108.4 (C-10, s), 134.2 (C-1', s), 133.9 (C-2', d), 115.0 (C-3', d), 147.2 (C-4', s), 115.3 (C-5', d), 120.0 (C-6', d), 61.2 (-OCH_3), 97.9 (C-1", d), 71.8 (C-2", d), 69.8 (C-3", d), 70.3 (C-4" d), 63.4 (C-5", d), 65.2 (C-6", q), 95.5 (C-1'", d), 71.6 (C-2'", d), 71.3 (C-3'", d), 66.6 (C-4'", d), 70.2 (C-5'", d), 61.8 (C-6'", t).

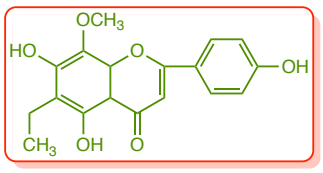
Acid hydrolysis of compound 1:

The compound (0.06 g) was refluxed with 50 mL of 7% ethanolic sulphuric acid on a water bath for 4 hr. The reaction mixture was then concentrated, cooled and poured into ice cold water. Aglycone was extracted in ether, concentrated and the residue was purified by column chromatography over silica gel using $CHCl_3$: Ethyl acetate (8:2, v/v) to give compound **2** (aglycone).



Compound 1: 6-ethyl-5-hydroxy-8-methoxy flavone-7-*O*-*α*-L(–)-rhamnopyranoside-4'-*O*-*β*-D(+)- glucopyranoside

The compound **2** was identified as 6-ethyl-5,7,4'-trihydroxy-8-methoxy flavone by comparison with its known spectral referred data. The aqueous hydro lysate was neutralize with $BaCO_3$ and $BaSO_4$ and filtered off. The filtrate was concentrated and chromatographed on paper using n-BuOH:AcOH:H_2O (4:1:5, v/v), aniline hydrogen phthalate as detecting agent, sugars were identified as D-glucose (R_f = 0.20) and L-rhamnose (R_f = 0.18).



Compound 2: 6-ethyl-5,7,4'-trihydroxy-8-methoxy flavone

Study of compound 2 :

Mol. formula: $C_{18}H_{16}O_6$, m.p. 145°C; Found (%): C, 62.02; H, 3.58, Calcd. (%) : C, 62.97, H, 3.50; IR λ_{max} (KBr): 3450, 2870, 1660, 1590, 1190; ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 6.21 (1H, s H-3), 7.56 (1H, dd, J = 9.0 and 2.5 Hz, H-2'), 7.68 (1H, dd, J = 9.0 and 2.5 Hz, H-3'), 7.6 (1H, dd, J = 9.0 and 2.5 Hz, H-5'), 7.50 (1H, dd, J = 9.0 and 2.5, H-6'); ^{13}CNMR (100 MHz, $CDCl_3$) δ (ppm): 153.2 (C-2, s), 133.5 (C-3, d), 147.4 (C-4, s), 161.2 (C-5, s), 165.0 (C-6, s), 162.5 (H-7, s), 140.2 (C-8, s), 166.3 (C-9, s), 108.4 (C-10, s), 134.2 (C- 1', s), 133.9 (C-2', d) 115.0 (C-3', d), 142.3 (C-4', s), 115.3 (C-5', d), 120.0 (C-6', d), 61.2 (-OCH_3); m/z: 328 $[M]^{+\cdot}$, 327, 300, 297, 211, 210, 121, 118.

3. Results and discussion

The ethanolic extract of this plant afforded a flavone glycoside 1, mol. Formula: $C_{30}H_{36}O_{15}$; m.p. 187°C, m/z: 636[$M^{+\cdot}$], 327, 300, 297. It gave Molisch's test showing the presence of sugar moiety. However, it neither reduced Fehling's solution nor gave a characteristic colour with Aniline Hydrogen Phthalate (AHP) reagent[2], indicating that reducing group of sugar was not free and involved in glycosidic linkage. The Flavone nature of the compound was confirmed by its absorption maxima at 332 (Band I) and 276 (Band II) in UV spectrum. The site of glycosidation was found to be at C-7 on the basis of UV shift with NaOAc and ^{13}C NMR of the glycoside as compared with that of the aglycone. The glycoside showed the down field signal of C-7 at δ 168 ppm which appeared at δ 162.5 ppm in the aglycone similarly the presence of another glycosidic linkage at C-4' was also confirmed by compairing the ^{13}C NMR spectra of the glycoside with aglycone. The C-4' of the glycoside in ^{13}C NMR spectra appeared at lower field δ 147.4 ppm which appeared at δ 142.3 ppm in aglycone. These confirmed the attachment of sugars at C-7 and C-4' position.

The ^{1}H NMR spectrum showed the presence of five protons in the aromatic region, indicating that compound is penta-substituted flavone. IR spectrum showed an absorption peak of hydroxy group at 3450 cm^{-1} and two absorption bands at 1650 and 1590 cm^{-1} for an *α, β*-unsaturated ketone (C_4 - of flavone). The absorption peaks at 2870 and 1190 cm^{-1} showed the presence of methoxy group and absorption peak of ethyl group at 2872 cm^{-1}. The presence of glucose and rhamnose were supported by anomeric proton signals in ^{1}H NMR at δ 5.343 (1H, d, J = 7.5 Hz) and 5.359 (1H, d, J = 7.6 Hz) respectively. The signals in the ^{1}H NMR of the glycoside at δ 5.359 (1H, d, J = 1.5 Hz) suggested *β*-linkage of D-glucose with the aglycone and δ 5.343 (1H, d, J = 7.5 Hz) suggested *α*-linkage of L-rhamnose. The ^{1}H NMR spectrum of compound showed signal for glucose proton at δ 3.57 (6H, m) and at δ 3.03 (5H, m) and δ 1.20 (3H, d, rham. -CH_3) for rhamnose sugar.

It was found that in the aglycone ^{1}H NMR exhibited one three proton triplet at δ 1.8 and two proton quartet at δ 2.5 which showed the presence of ethyl group. The presence of an ethyl group was further confirmed by δ 166.4 for C-6 in ^{13}C NMR spectra.

In case of aglycone (compound 2) the position of hydroxy and methoxy were confirmed by the^{1}H NMR and ^{13}C NMR spectra. The position of hydroxy and methoxy groups were explained on the basis of ^{13}C NMR spectrum which showed singlet at δ 161.2, 168 and 147.4 ppm corresponding to C-5, C-7 and C-4' for carbon atoms containing hydroxy groups and quartet at δ 61.2 ppm and singlet at 140.2 was assigned the presence of one methoxy group.

^{1}H NMR showed a singlet at δ 6.21 (1H, s) ppm indicated proton at C -3. As no vacant position was available in the ring A, so all the four remaining protons were present in the ring B. Signals at δ 7.68 (1H, dd, J_{ortho} = 9.0 Hz and J_{meta} = 2.5 Hz), 7.62 (1H, dd, J_{ortho} = 9.0 Hz and J_{meta} = 2.5 Hz), 7.56 (1H, dd, J_{ortho} = 9.0 and J_{meta} = 2.5 Hz), 7.50 (1H, dd, J_{ortho} = 9.0 and J_{meta} = 2.5 Hz) were assigned for C-3', C-5', C-2', C-6' respectively. One proton singlet at δ 13.2 due to chelated hydroxy group.

Enzymatic hydrolysis of compound 1:

Compound 1 (15mg) was dissolved in MeOH (20 mL) and hydrolysed with same volume of almond emulsion. The reaction mixture was kept at room temperature for 3 days and filtered. The residue is identified as 6-ethyl-5,7,4'-trihydroxy-8-methoxy flavone.

The hydrolysate was concentrated and subjected to paper chromatography technique using BAW (4:1:5, v/v), that shows the presence of D-glucose (R_f = 0.20) and L-rhamnose (R_f = 0.18).

4. Conclusion

Medicinal plants owe their therapeutic importance to active principles. *Moringa oleifera* is one of the most prominent medicinal plant and from the root of this plant we have isolated a new flavone glycoside, 6-ethyl-5-hydroxy-8-methoxy flavone-7-*O*-α-L(–)- rhamnopyranoside-4'-*O*-β-D(+)- glucopyranoside.

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