NJESR/ July-2023/Volume-5/Issue-7 **E-ISSN-2582-5836**

DOI-10.53571/NJESR.2023.5.7.60-65

Two Malonated Flavonol Glycosides from the Flowers of Prunus Napualensis Steud

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(Received:15June2023/Revised:30June2023/Accepted:12July2023/Published:22July2023)

Abstract:

20% HOAc Cellulose column chromatography fractionation of BuOH soluble derived from aqueous methanolic extract of the flowers of ***Prunus napualensis*** afforded two malonylated flavonol glycosides, Kaempferol-3-O-β -[(6-malonyl) sophoroside] –7-O-β -D-glucoside and Syringetin-3-O-β-[(6-malonyl)sophoroside]-7-O-β-D-glucoside and were identified by chromatographic properties, acid and alkali hydrolytic methods, H_2O_2 oxidation, enzymatic hydrolysis, UV, ^{1}HNMR and MS Studies.

Looking on the significances of bioflavonoids as a dietary components of human and animal food and feed and in the field of medicines, the flowers of a food plant, ***Prunus napualensis*** was investigated for antioxidative flavonoids.

Keywords: Prunes Napualensis, Glycosides, Methonalic Extract, Enzymatic Hydrolysis, Biflavonoids

Introduction :

Prunus napualensis steud., family Rosaceae, is a higher flowering plant. It is a wild as well as cultivated, food and fodder plant of Kumaun Himalaya. It has widely been reported from an altitude varies from 1200m to 3000m from the hills of central Himalayas. Literature survey revealed that the plant is still awaited for chemical investigation although other species of the ***genus Prunus*** have widely been reported for flavonoidal constituents **(Olzewska and Wolbis, 2001; Yoshikawa et al. 2002; Williams and Grayer, 2004 and Levy et al, 2004).** Present chemical investigation reveals the presence of two malonylated flavonol glycosides from BuOH fraction of ***Prunus napualensis.***

Material And Methods:

Prunus napualensis steud. (Fam. Rosaceae), an endemic plants of Kumaun Himalaya, was collected from various sites of Kumaun hills and identified by Prof. P.C. Pandey, Department of Botany, Kumaun University Campus, Almora (Uttarakhand).

About 2 Kg air dried and powdered white flowers of the plant was collected from Kumaun hills. The air dried and powdered sample of the flower was extracted sequentially with 80% Aq. MeOH and 50% Aq. MeOH for six days. Both the extracts were combined and evaporated to dryness under reduced pressure at 40 °C in Rotary evaporator. The residue was partitioned between CH_2Cl_2-H_2O (1:1). After the separation of CH_2Cl_2 Soluble, the H_2O layer was further partitioned with BuOH. The BuOH soluble was concentrated and residue was adsorbed on cellulose CC and eluted initially with H_2O then increasing polarity with acetic acid. On eluting CC with 20% HOAc, a dark purple or violet fluorescent band was observed on CC with UV light. It was eluted and collected separately by monitoring CC with UV light.The eluent was evaporated to dryness and residue was dissolve in 70% Aq. MeOH. It was chromatographed on whatman No. 3 PC using BAW(n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as a developing solvent. The dried and developed PC was inspected with UV light (360 nm). Two violet fluorescent bands were observed on PC at R*f*, 35 and 30 and each band was eluted with 70% Aq. EtOH separately. The eluent of faster moving band, representing compound (A) and slower moving band, representing compound (B), were finally purified on sephadex LH-20 CC separately using 30% MeOH and 40% MeOH.

Result and Discussion:

COMPOUND (A): ESI-MS of the compound gave m/z at 881 $[M+Na]^+$ and ESI-MS/MS of m/z at 859 $[M+H]^+$ calculated for $C_{36}H_{42}O_{24}$. Methanolic solution of the compound gave positive tests for $FeCl_3$, Mg+HCl and α-naphthol, indicated a flavonoidal glycosidic compound. Flavone appeared as a violet spot on PC under UV light and changed to Yellow-green with NH_3 Vapours, indicating the presence of free 5-and 4'- hydroxyl groups. When cellulose TLC plate was sprayed with Naturstoffreagenz A (NA), the spot turned yellow, indicating a free 4'- hydroxyl but no ortho-dihydroxyl group in the B-ring. Acid hydrolysis of the compound with 2N-HCl, gave an aglycone, Kaempferol (CoPC) and a sugar moiety, glucose (CoPC). Alkali hydrolysis of the compound gave a flavonoidal compound (A-1) and malonic acid (CoPC).

UV spectra of compound (A-1) in MeOH and shifts obtained with diagnostic reagents indicated it has free hydroxyls at C-4', and C-5 **(Table 1):**

Table 1: UV spectra of compound (A-1) in MeOH and shifts obtained with diagnostic reagents indicated it has free hydroxyls at C-4', and C-5

MeOH	-	259sh, 267, 346
NaOMe	-	245,273,352sh, 387(int)
NaOAc	-	257sh,267,395
NaOAc+H_3BO_3	-	267,352
$AlCl_3$	-	258sh,300,355,398
$AlCl_3$+HCl	-	255sh,275,299,349,395

It also gave positive tests for sugar. Acid hydrolysis of the compound gave Kaempferol and Glucose(CoPC). H_2O_2 oxidation of the compound (A-1) gave Kaempferol-7-O-glucoside(CoPC) and sophorose sugar(CoPC). Thus, the compound (A-1) could be identified as Kaempferol-3-O-sophoroside-7-O-β -D-glucoside.

Thus, the structure of compound (A) was calculated as A-1+ malonic acid. With the assignments of hydrolysed products, Kaempferol-3-O-sophoroside-7-O-β-D-glucoside (A-1) and malonic acid from compound (A), now only problem is to be justify the position attachment of malonic acid in the glucose moiety.

It can be decided by ^{1}HNMR spectral studies of compound (A)

TABLE 2: ^{1}HNMR (DMSO-d_6) OF COMPOUND (A)

S.No.	Shift	Multiplicity J=Hz	Proton identification
1.	8.08	2H,d, J=9Hz	H-2' and H-6'
2.	6.93	2H,d,J=9Hz	H-3' and H-5'
3.	6.82	1H,d, J=2.0Hz	H-8
4.	6.54	1H,d, J=2.0Hz	H-6
5.	5.46	1H,d,J=7.5Hz	H-1"
6.	5.10	1H,d,J=7.5Hz	H-1"'
7.	4.82	1H,d,J=7.0Hz	H-1"''
8.	4.26	1H,d,J=1.8Hz	H-6(a)
9.	4.13	1H,d,J=5.0Hz	H-6(b)
10.	3.43	1H,d,J=12.0Hz	H-5
11.	4.0-3.1	M	Rest of sugar protons

A methylene protons sharp singlet appeared at 3.80. A downfield shift of H-6(a) and H-6(b) at 4.26 indicated that malonyl group is attached at C-6 of glucose moiety of primary sugar of sophorose unit.

Thus, the compound(A) was identified as [Kaempferol-3-O-β-(6-malonyl)-sophoroside]-7-O-β-D-glucoside.

COMPOUND(B): Methanolic solution of the compound gave positive tests for Mg+HCl, $FeCl_3$ and α-naphthol indicated a flavonoidal glycosidic compound. It appeared as a violet fluorescent spot on PC under UV light and changed to yellow-green with NH_3 vapours and NA reagent, indicating the presence of free hydroxyls at C-4' and C-5 ***(Mabry et al.,1970).*** Acid hydrolysis of the compound with 2N HCl gave a dull yellow UV fluorescent flavonol, B(a) and a sugar, glucose (CoPC). The aglycone, B(a) was identified as a myricetin-3', 5-dimethyl ether or syringetin by ^{1}HNMR studies: (DMSO-d_6, 400 MHz), ^{1}HNMR of aglycone gave two meta coupled doublets each with J=2.0Hz, at 6.23 and 6.47, representing H-6 and H-8 of A-ring. A two proton singlet appeared at δ 7.60 indicating symmetrically substituted B-ring or similar substituents are present at C-3' and C-5' of the B-ring. A sharp singlet for 6H (2 x OMe) appeared at 3.95, indicating presence of methoxyl substituents at C-3' and C-5'. Thus, the compound B(a) was identified as myricetin-3', 5'-dimethyl ether or syringetin. When aglycone was hydrolysed with HI by standard method it gave myricetin (CoPC.)

Alkali hydrolysis of the compound (B) gave a flavonol 3,7-di-O-substituted glycoside (B-1) and a malonic acid (CoPC). Acid hydrolysis of compound (B-1) gave an aglycone syringetin (CoPC) and sugar,glucose (CoPC).. The ^{1}HNMR of compound (B-1) was found similar to the compound (A-1) in sugar region. Thus, the compound (B-1) was identified as syringetin –3-O-sophoroside-7-O-β-D-glucoside. It was finally confirmed by H_2O_2 oxidation as it has produced syringetin-7-O-β-D glycoside and sophorose (CoPC). The ^{1}HNMR of the compound (B) was found similar to the compound (A) in sugar region. Thus, the compound (B) was identified as syringetin –3-O-β – [(6-malonyl)] sophoroside-7-O-β-D-glucoside.

Antioxidant activity: The antioxidant activity of compound (A) and (B) were observed against methanolic solution of DPPH by UV-VIS spectrophotometer at 517nm.The intensity peaks of DPPH was highly reduced by the methanolic solution of compound (A) compared to compound(B).

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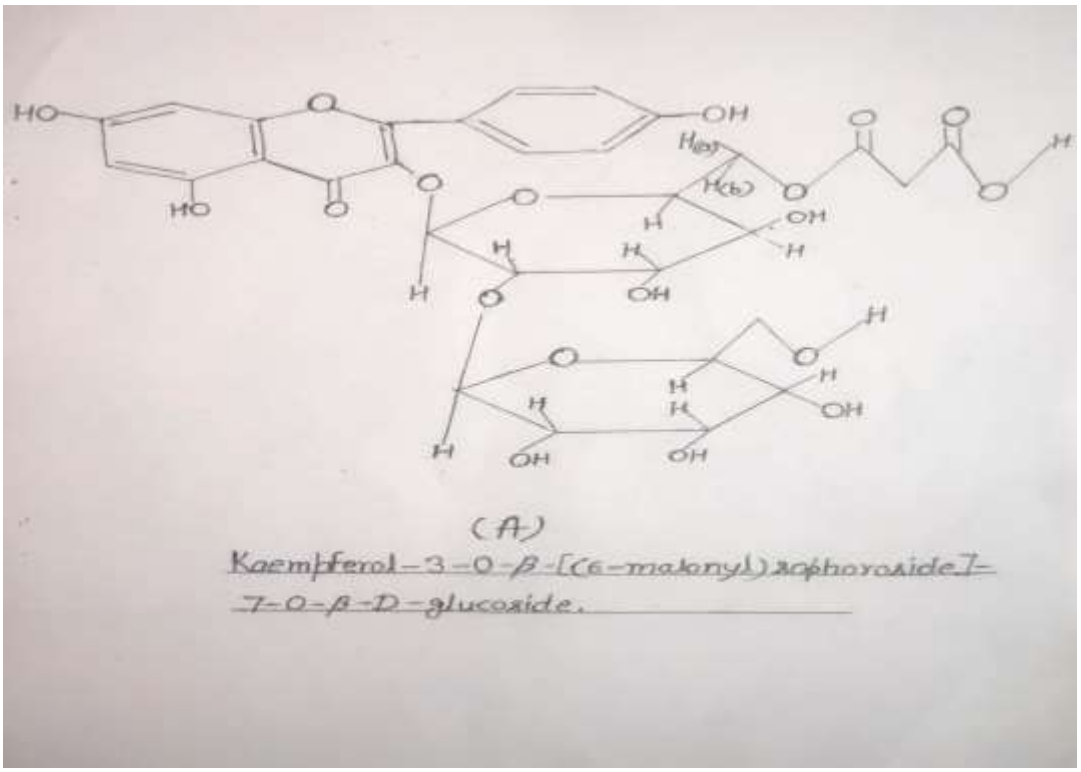
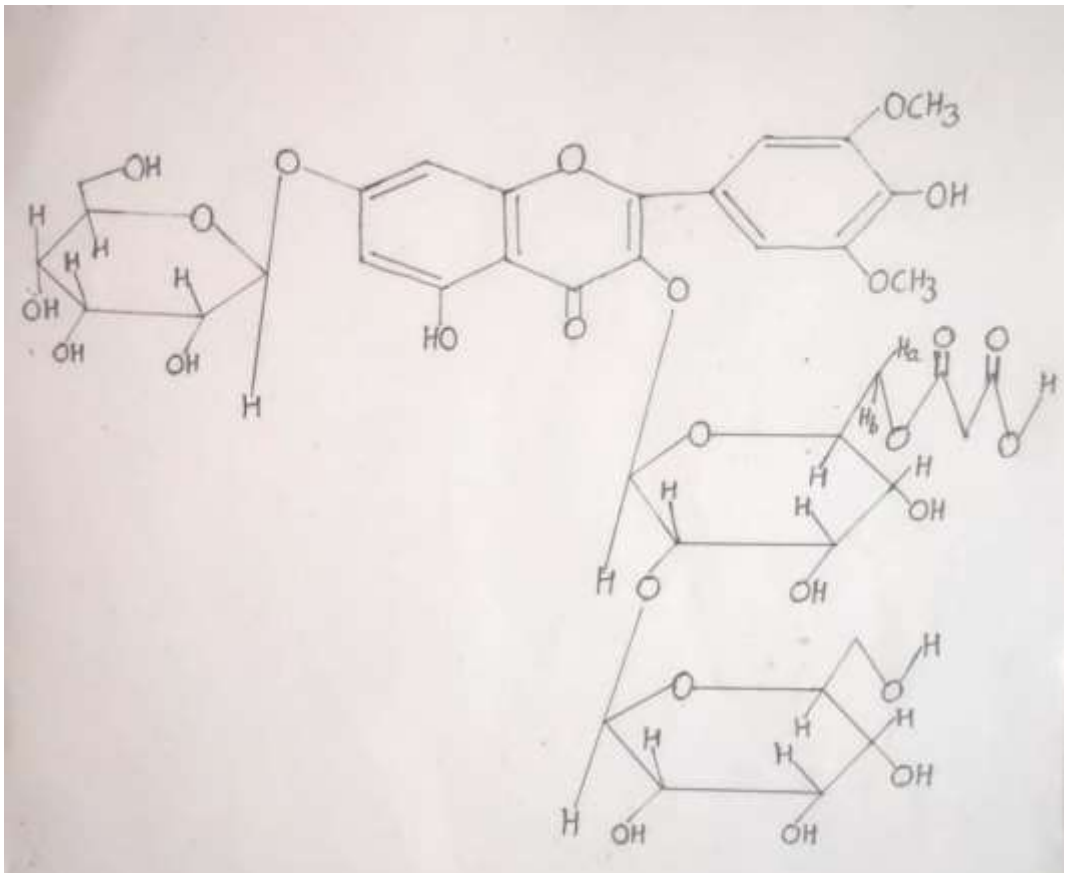


(A)

Kaempferol-3-O-β-[(6-malonyl)sophoroside]-7-O-β-D-glucoside.



(B)

Syringetin-3-O-β-[(6-malonyl)sophoroside]-7-O-β-D-glucoside