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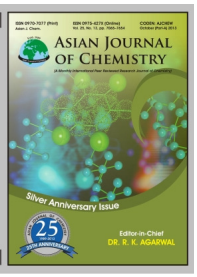

A New Flavonol Glycoside from *Kalanchoe pinnata* Leaves

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A new flavonol glycoside *i.e.*, 7-O-methylkaempferol-3-O-α-L-rhamno-pyranosyl-(1→6)-O-β-D-galactopyranoside (**1**) together with a known flavonol glycoside; kaempferol-14-O-α-L-rhamnopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside (**2**) were isolated from methanolic fraction of leaves of *Kalanchoe pinnata*. Their structures were determined with the help of chemical method and concerted use of 1D and 2D NMR spectroscopy.

Key Words: *Kalanchoe pinnata*, *Crassulaceae*, Flavonol glycoside.

INTRODUCTION

Kalanchoe pinnata (Crassulaceae) is a glabrous herb 0.3-1.2 m high is believed to be a native of tropical Africa, naturalized throughout the tropics of the world. The leaves slightly toasted are used as an application to wounds, bruises, boils and bites of venomous insects. Juice of leaves is used in dysentery. The leaves are used to cure sore eyes, burn and also applied to corns[1].

Kalanchoe pinnata is rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids[2-4]. We report herein the isolation and characterization of a new flavonol glycoside *viz.*, 7-O-methylkaempferol-3-O-α-L-rhamno-pyranosyl-(1→6)-O-β-D-galactopyranoside (**1**) together with a known flavonol glycoside; kaempferol-14-O-α-L-rhamnopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside (**2**) from methanolic fraction of leaves of *Kalanchoe pinnata.*

EXPERIMENTAL

General experimental procedure: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500 and 125 MHz in C_5D_5N at 25 ºC with TMS as standard. Two dimensional spectra were measured using standard methods of Bruker. 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 (0.25 mm thickness containing 13 % $CaSO_4$ as binder). Paper chromatography was carried out on Whatman filter paper No. 1 (descending) and spots were detected by spraying with aniline hydrogen phthalate (AHP) followed by heating. Melting points were recorded in Boetius microscopic apparatus. Optical rotations were recorded in methanol on Jasco DIP-140 digital polarimeter.

Kalanchoe pinnata leaves were collected from Tiosa, Amravati district (Maharashtra, India) during the month of November 2008 and was identified by botanist and authenticated by plant taxonomist Dr. Rajshekharan. A voucher specimen (SLT-Med. Plant. -820) was deposited in the S.L.T. Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur (Chhattisgarh, India).

Extraction and isolation: The air-dried and powdered leaves (1.5 kg) were exhaustively defatted with light petroleum ether (60-80º). The petroleum free mass extracted with 70 % 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 (6:2:4). The chloroform layer was separated out and the aqueous layer was concentrated under reduced pressure and then partitioned with ethyl acetate and 50 % aqueous methanol. The aqueous methanolic extract was concentrated under reduced pressure to give methanolic extract (12.5 g). The methanolic extract (10 g) was column chromatographed over Si-gel successively eluted with $CHCl_3$ and $CHCl_3$-MeOH (2:1) afforded fractions A and B. Fraction A was concentrated under reduced pressure, dried and subjected to column chromatography over Si-gel

eluted with aqueous MeOH (2:1) which afforded several fractions. First few fractions were mixed, dried and digested with aqueous MeOH. The aqueous MeOH fraction on concentration gave compound **1** (31 mg). Fraction B was subjected to column chromatography over Si-gel eluted with aqueous $CHCl_3$:MeOH:H_2O (6:3:2) mixture of two compounds. The residue was further separated by preparative TLC on Si-gel 60 HPTLC (Merck) developed with $CHCl_3$:MeOH (98:2) and $CHCl_3$:MeOH (8:2) afforded compound **1** (17 mg) and **2** (51 mg) respectively (Fig. 1).

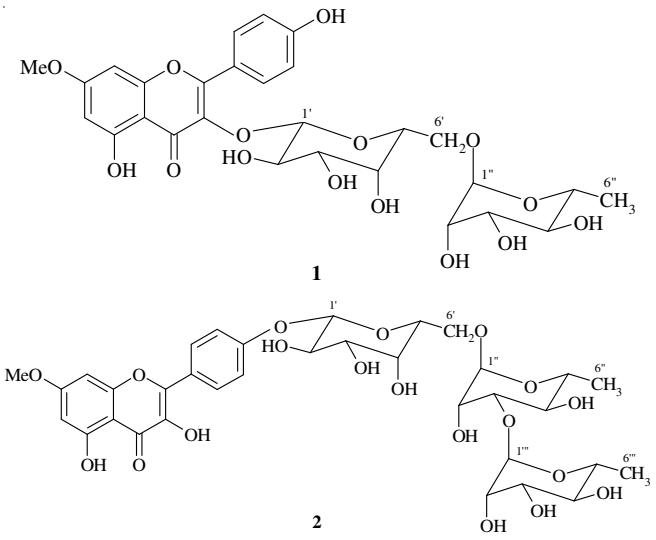

Fig. 1. Structures of compound **1** and **2**

7-O-Methylkaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside (1): Yellow solid (aqueous MeOH); m.p. 215-217 ºC; $[\alpha]_D^{23}$: -32.41 (c = 0.40, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3430 (OH), 2904, 1660 (C=O), 1590, 1490, 1350, 1215, 1170, 1140 *etc.*; UV (λ_{max} MeOH): nm (log ∈) 266.5 (4.04), 350.4 (3.98), (+NaOAc) 266.2, 258.4, (+$AlCl_3$) 274.8, 303.0, 355.1, 396.0; (+)-FAB-MS: *m/z* 631 $[M + Na]^+$, 609 $[M + H]^+$, 460, 307, 154, 137, 89, 39; Elemental analysis (%): C = 55.34, H = 5.15, calc. (%) for $C_{28}H_{32}O_{15}$; m.w. 608; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) (Table-1).

Kaempferol-14-O-α-L-rhamnopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside (2): Yellow needles (MeOH), m.p. 205-207 ºC; $[\alpha]_D^{23}$: -87.9 (c = 0.88, H_2O); IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2925, 1656 (C=O), 1620, 1520, 1365, 1215, 1170, 1140 *etc.*; UV (λ_{max}MeOH): nm (log ∈) 267.5 (4.24), 318.4 (4.05), 366.0 (4.23); (+NaOMe) 279.2, 413.5, (+NaoAc) 275.3, 399.8, (+$AlCl_3$) 270.8, 304.8, 344.1, 418.0; (+)-FAB-MS: *m/z* 763 $[M + Na]^+$, 741 $[M + H]^+$, 765, 599, 446, 315, 275, 154, 137, 89, 39; Elemental analysis (%): C = 53.84, H = 5.49, calcd. (%) for $C_{33}H_{41}O_{19}$; m.w. 740: ^1H (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) (Table-1).

Acid hydrolysis of compound 1: 5 mg was refluxed with 3 % aqueous HCl (10 mL) at 80°C for 3 h. After cooling, the reaction mixture was neutralized with $AgNO_3$. The filtrate was extracted with EtOAc and the organic solution containing the aglycone was crystallized from $CHCl_3$ as yellow needles (m.p. 226-227 ºC). The aglycone was identified as kaempferol-7-O-methyl ether by comparison with authentic sample. The aqueous layer after concentration under reduced pressure was subjected to PC using BuOH:AcOH-H_2O (5:1:4) with authentic sugars. The R_f values of sugars were identical with those of D-galactose and L-rhamnose. The ratio of galactose and rhamnose was found to be 1:1 by photocolorimetry using light of 420 nm wavelengths.

TABLE-1
^1H NMR (500 MHZ) AND ^{13}C NMR (125 Mhz)
DATA OF COMPOUND **1** AND **2** IN C_5D_5N

C/H	**1**		**2**	
	δ_C	δ_H (*J* in Hz)	δ_C	$^{\delta}H$ (*J* in Hz)
2	148.3[s]	–	146.3	–
3	135.9[s]	–	138.2	–
4	178.9[s]	–	177.8	–
5	162.3[s]	–	162.4	–
6	98.6[d]	6.61, d (2.1)	98.7	6.70, d (2.0)
7	165.9[s]	–	165.8	–
8	92.6[d]	6.65, d (2.1)	93.1	6.95, d (2.0)
9	157.4[s]	–	157.4	–
10	106.2[s]	–	106.2	–
11	123.8[s]	–	125.2	–
12	132.1[d]	8.51, d (8.5)	132.1	8.53, d (8.3)
13	116.2[d]	7.23, d (8.5)	116.0	7.52, d (8.3)
14	161.9[s]	–	159.6	–
15	116.2[d]	7.23, d (8.5)	116.0	7.52, d (8.5)
16	132.1[d]	8.51, d (8.5)	132.1	8.53, d (8.5)
1’	104.2[d]	6.03, d (7.9)	104.1	6.05, d (7.6)
2’	73.1[d]	4.75, dd (7.9, 9.4)	73.1	4.77, dd (7.6, 9.5)
3’	75.2[d]	4.29, dd (9.4, 3.3)	75.2	4.29, dd (9.5, 3.2)
4’	69.7[d]	4.41[a]	69.7	4.42[a]
5’	75.2[d]	4.17[a]	75.2	4.18[a]
6’a	67.0[t]	4.03, dd (8.8, 6.4)	67.0	4.05, dd (9.0, 6.4)
b		4.43[a]		4.43[a]
1”	102.1[d]	5.23, brs	102.1	5.23, brs
2”	71.8[d]	4.57, brd (3.9)	71.8	4.61, brd (4.0)
3”	72.6[d]	4.49, brd (8.8)	79.9	4.49, brd (9.0)
4”	72.7[d]	4.29[a]	72.7	4.30[a]
5”	69.9[d]	4.23, brd (4.9)	69.9	4.25, brd (4.9)
6”	18.5[q]	1.47, d (5.8)	18.5	1.46, d (5.8)
1”’	–	–	104.8	5.92, brs
2”’	–	–	72.4	4.72, brs
3”’	–	–	72.7	4.54, brd (3.0)
4”’	–	–	74.2	4.26, brd (9.4)
5”’	–	–	69.9	4.53, brd (6.0)
6”’	–	–	18.7	1.56, dd (6.1)
-OCH_3	55.9[q]	3.75, s	–	–

RESULTS AND DISCUSSION

The methanolic fraction of leaves of *Kalanchoe pinnata*. on column chromatography over Si-gel eluted with various solvents afforded a new flavonol glycoside 7-O-methyl-kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyrano-side (**1**) and known flovonol glycoside kaempferol-14-O-α-L-rhamnopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside (**2**). Compound **2** was identified by comparison of its physical and spectral data with the literature values[5]. Compound **1** was obtained as yellow crystalline solid from aqueous methanol.

Its elemental analysis corresponded to molecular formula $C_{28}H_{32}O_{15}$ which was substantiated by the presence of $[M + Na]^+$ and $[M + H]^+$, ion peak at *m/z* 631 and 609 in the positive FAB-mass spectrum. Its IR spectrum showed characteristic absorption at 3430 cm^{-1} for -OH group and at 1660 cm^{-1} for carbonyl group. Its alcoholic solution gave pinkish colour when treated with few drops 2N-HCl and Mg and/or Zn turnings indicated flavonoidal nature of the molecule[6].

^1H NMR spectrum (Table-1) of **1** displayed presence of two *ortho*-coupled and two *meta*-coupled doublet in the aromatic region while in aliphatic region it showed a signal for methoxy protons, one methyl resonance and two signals for anomeric protons of sugars along with signals assignable for sugar protons. ^{13}C NMR spectrum (Table-1) showed fourteen carbon atoms (two of double intensity) in aromatic region, eleven carbon atoms in aliphatic region and a carbonyl carbon. The multiplicity of carbon signals was determined by DEPT spectrum, which showed presence of two methyl, one methylene, sixteen methine (two of double intensity) and nine quaternary carbon atoms. The assignment of proton and carbon signals was made by ^1H ^1H COSY and heteronuclear multiple quantum correlation (HMQC) spectroscopy.

^1H and ^{13}C NMR data (Table-1) of **1** were strongly reminiscent of that reported for Kaempferol-7-O-methyl ether[7] and two hexose sugar moieities. The presence of two *meta*-coupled doublet (J = 2.1 Hz) each for 1H at δ 6.61 and 6.65 and two *ortho*-coupled A_2B_2-type doublets (J = 8.5 Hz) at δ 8.51 and 7.23 in the aromatic region of ^1H NMR spectrum, suggested that a *tetra*-substituted and a 1,4-disubstituted phenyl ring was present in the molecule. The 1,4-disubstituted phenyl ring was confirmed to be *p*-hydroxyphenyl system by the ^{13}C-chemical shift of the carbon signals at δ 132.1 (C-12, 16) and δ 116.2 (C-13, 15)[8] whereas the ^{13}C resonances of carbon signals at 162.3 (C-5) and 165.9 (C-7) were corroborated with the 5,7-dihyhydroxy ring-A of flavonoids. The ^{13}C NMR spectrum also showed the presence of C=O group at δ 178.9, a benzylic carbon (C-2) at δ 158.3 and one oxygen bonded ethylenic carbon (C-3) at δ 135.9. The ^1H NMR spectrum of **1** displayed a singlet for 3H at δ 3.75 indicated presence of metoxy group, which was substantiated by the ^{13}C chemical shifts of carbon atom at δ 55.9. The downfield ^{13}C chemical shifts of aromatic carbon atoms at δ 162.3 (C-5), 165.9 (C-7) and 161.9 (C-14) as compared to ^{13}C-chemical shifts of other aromatic carbon atoms confirmed the presence of a flavonoidal skeleton. These ^{13}C-chemical shifts suggested that C-5, C-7 and C-14 carbon atoms are substituted with phenolic functions. The position of methoxy group was ascertained at C-7 by HMBC experiment which showed long range correlation between methoxyl protons and carbon atom resonated at δ 165.9 (C-7).

^1H NMR spectrum of **1** also showed a doublet (d, J = 7.9 Hz) at δ 6.03 and a broad singlet at δ 5.23 corroborated with the anomeric protons of sugar moieties[9] along with other proton signals assignable for sugar suggested the presence of two sugar moieties in the molecule. The presence of two sugar moieties was confirmed by the ^{13}C NMR chemical shifts of two anomeric carbon signals (δ 104.2 and 102.1) and three terminal carbons of sugars (δ 67.0 and 18.5) along with carbon resonances assigned for sugar moiety[9]. On acid hydrolysis **1** gave kaempferol-7-O-methyl ether as the aglycone and the component sugars were identified as D-galactose and L-rhamnose by paper chromatography. The ratio of galactose and rhamnose was found to be 1:1 by photo-colorimetry method using light of 420 nm wavelength[10]. The β-configuration of galactose and α-configuration of rhamnoses were determined by the magnitude of coupling constant of H-1' of galactose (J = 7.9 Hz) and a broad singlets at δ 5.23 assigned for H-1" of rhamnose.

A comparison of the UV *maxima* of **1** with those of kaempferol in MeOH solution and on the addition of NaOAc and $AlCl_3$ suggested that the C-3 hydroxyl group of kaempferol was substituted by the sugar moiety[11]. The up-field ^{13}C chemical shift (by 1.5 ppm) of C-3 carbon (at δ 135.9) and the downfield shift (by 10.0 ppm) of C-2 carbon (δ 158.3) than that of C-2 (δ 148.3) and C-3 (δ 137.4) of kaempferol-7-O-methyl ether[7] provides evidences for C-3 position of sugar moiety. Moreover, the C-3 position of sugar moiety was confirmed by HMBC experiment, which showed long-range correlation between anomeric hydrogen (H-1') of galactose with C-3 carbon of kaempferol.

The points of linkages within the sugars in **1** were established by the chemical shifts of carbon atoms and HMBC experiment. The chemical shift of C-6' carbon of galactose was observed downfield at δ 67.0 than was expected. These observations suggested that the rhamnose moiety is attached to the C-6' of galactose unit[5,10,12]. The HMBC spectrum showed long-range correlation between anomeric hydrogen H-1' with C-3 carbon of kaempferol-7-O-methyl ether, H-1" of inner rhamnose with C-6' of galactose. From these findings it was clear that the rhamnose was linked at hydroxyl group on C-6' of galactose, while the galactose was attached to the C-3 hydroxyl group of kaempferol-7-O-methyl ether.

The above discussed spectral and chemical evidences led to the identification of a new flavonol glycoside characterized as 7-O-methylkaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside.

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