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Chemical constituents from the leaves of *Sarcococca saligna*

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Abstract- From the Methanolic extract of the leaves of *Sarcococca saligna* 7, 4’-dihydroxy-6,3’-dimethoxy flavanone and 3, 5, 7-trihydroxy- 6, 8- dimethoxy flavanone have been isolated. The structures of the isolated compounds were identified with the help of chemical and spectral studies.

Key words: *Sarcococca saligna*, Buxaceae, flavanone glycosides.

Introduction

Sarcococca saligna is an evergreen shrub with a slender, arching habit, known for its narrow, willow-like leaves and fragrant, creamy-white flowers in late winter. The flowers are followed by small, purple berries, and the plant is shade-tolerant, making it ideal for shady spots, low hedges, or as a filler plant. The leaves of *Sarcococca saligna* are used as laxative, blood purifier and muscular analgesic[1-2].The species is native to parts of the Indian subcontinent and East Asia, specifically found in areas such as the Himalayas, Pakistan, Nepal, China, and Taiwan. It typically grows in the undergrowth of subtropical and temperate forests at elevations between 1200 and 3500 meters.

The members of Sarcococca plants are used as TCM and traditional folk medicine for the treatment of stomach pain, rheumatism, swollen sore throat and traumatic injury. The ethanolic extract of *S. saligna indicate anti*-bacterial activity against several human pathogenic bacteria and anti-fungal against Aspergillus species[3-4]. The extract of the plant is also used to increase the antifungal activity of fluconazole against resistant *Aspergillus* species. The present paper deals with isolation and identification of secondary metabolites from the methnolic extract of the leaves of the plant.

Material and Methods

Melting points were recorded on a permit melting point apparatus. UV spectra on perkin-Elmer, Lambda-25 spectrometer (methanol as solvent), IR spectra recorded on Perkin-Elmer, spectrum RX I FT-IR spectrometer (KBr discs). NMR spectra were obtained on JEOL NMR spectrometer (300 MHz for ^{1}H and 125 MHz for ^{13}C, DMSO-d_6 as solvent, TMS as internal standard). LCMS spectra were recorded on Finniagan MAT spectrometer (CA, USA, xcalibur ver-2 software). Column chromatography was performed on silica gel (Merck 60-120 mesh, 15×100 cm). Thin layer chromatography was carried out on silica gel (Merck 10-40 µm) precoated plates were visualized by spraying with 7% H_2SO_4 as a universal spray reagent.

Plant Material

Fresh flowers (10kg) of*Sarcococca saligna* were collected from Nandanagar Chamoli (Garhwal) UK., during March 2025 and identified by Taxonomist (Department of Botany H.N.B. Garhwal University Srinagar).

Extraction and Isolation

Air dried powdered leaves were extracted exhaustively with 95% ethanol at 30-50°C (for 15 hours, 3 times) on a heating mantle. The solvent was distilled on water bath and evaporation of solvent under reduced pressure afforded (250g) crude extract, which was subjected to column chromatography on silica gel column using solvent gradient systems in the order of increasing polarity $CHCl_3$: MeOH (97:3-85:25). The fractions obtained from column were collected (100ml) and combined on the basis of TLC analysis. The $CHCl_3$: MeOH (97:3-85:25) afforded R-1 and R-2. These fractions were concentrated at room temperature.

R-1 -White amorphous powder (0.252 g); mp. 120-123 °C; M.F. $C_{17}H_{16}O_6$; IR: 3560, 1650, 1715, 1725 cm^{-1}; ^{1}H, ^{13}C and 2D NMR data: Table 4.1; LCMS(m/z): 316 $[M]^+$, 286$[M-OCH_3]$; 270 $[M-OCH_3+OH]$; 240$[M-2\times OCH_3+OH]$; 224 $[M-2\times OCH_3+2\times OH]$; 148 $[M-2\times OCH_3+2\times OH+\text{benzyl}]$.

Table-1^{13}C (125 MHz), ^{1}H (300 MHz) NMR and HMBC data of R-1 in DMSO d^6:

Position C/H	$\delta_C$ppm	$\delta_H$ppmJ(Hz)	HMBC
2	78.90	5.46(dd,12.0,4.0)	C-3,C-2’,C-6’
3	42.36	2.73(dd,16.0,4.0)	-
		3.23(dd,12.0,16.0)	-
4	197.27	-	-
4a	103.09	-	-
5	112.89	7.50(d,9.0)	-
6	146.95	-	-
7	167.89	-	-
8	98.09	6.80(d,2.0)	C-8a
8a	163.25	-	-
1’	135.43	-	-
2’	114.35	6.94 (d,2.0)	C-6’,C-3’,C-2
3’	163.67	-	-
4’	149.03	-	-
5’	107.05	7.02(d, 7.0)	-
6’	119.29	6.90 (dd,7.0,2.0)	-
OCH_3-3’	56.14	3.38s	C-2’,C-3’
OCH_3-6	56.63	3.28s	C-5, C-6
OH-4’	-	9.20s	C-4’
OH-7	-	12.09s	C-7

R-2 - White amorphous powder, m.p.272-274°C; M.F. $C_{17}H_{16}O_7$; IR:

3410, 1650, 1715 and 1725 cm^{-1}; ^{1}H, ^{13}C and 2D NMR data: Table 2; LCMS (m/z): 332 [M+H]$^{+}$, 302 [M-OCH_3], 286 [M-OCH_3+OH], 256 [M-2×CH_3+OH], 240 [M-2×OCH_3+2×OH], 224 [M-2xOCH_3+3×OH] and148 [M-2×OCH_3+3×OH+benzyl].

Results and Discussion

R-1 - It was crystallized from chloroform-methanol as white amorphous powder; m.p. 120-123°C. It gave green colour with alcoholic ferric chloride indicating the presence of phenolic hydroxyl group. An absorption maximum at 287 nm in the UV spectrum was indicative of flavanone derivative. The UV absorption band at 287 (bathochromic shift 10 nm) indicated substitution at 6 and 7 position in ring A [5-6]. After addition of NaOMe in methanolic solution of R-1, a bathochromic shift of 15 and 10 nm in band I and II was observed of reduced intensity indicated substitution at C-3' and C-4' positions, whereas bathochromic shift of 14 nm after the addition of NaOAc indicated hydroxyl group present at C-7. The IR spectrum exhibited bands at 3560 cm^{-1} characteristics of hydroxyl group, 1650 cm^{-1} for aromatic ring and a band at 1715 and 1725 cm^{-1} was clearly expressive for the stretching in a carbonyl group. The LCMS of compound showed molecular ion peak at m/z 316 [M^{+}], other fragmentation peaks observed at m/z 286[M-OCH_3]; 270 [M-OCH_3 +OH]; 240[M-2×OCH_3+OH]; 224 [M-2×OCH_3+2×OH]; 148 [M-2×OCH_3+2×OH+benzyl] and the two prominent Retro-Diels-Alder fragmentation peaks at m/z 162 and 154 respectively. These peaks were indicative of the nature and number of the substituents in the ring A and B (monohydroxy and monomethoxy). The ^{1}H- NMR spectrum showed one proton signal at δ 12.09 confirming the presence of a hydroxyl group at C-7. Two double doublets at δ 2.73 (dd, J=16.0, 4.0 Hz) and δ 3.23 (dd, J=12.0, 16.0 Hz) a proton as double doublet at δ 5.46 (J=12.0, 4.0 Hz, H-2) as expected for the presence of C-3 and C-2 protons of flavanone were also observed in the ^{1}H-NMR spectrum. The ^{13}C NMR spectrum of the compound showed that the C-2 and C-3 atoms appeared at δ 78.90 and 42.36. Two proton signals for one proton each which appeared at δ 7.02 (d, J=7.0 Hz) and δ 6.90 (dd, J=7.0, 2.0 Hz) were assigned to the H-5' and H-6' while a doublet at δ 6.94 (1H, J=2.0Hz) was also attributed to H-2'. A singlet integrating of one proton at δ 9.20 was assigned to the H-4' proton of ring B. ^{13}C-NMR spectrum further indicated that the carbon of carbonyl function at C-4 appeared at δ197.27. In HMBC spectrum H-3 proton showed the correlations with C-2 and C-4. The H-8 proton showed its multiple cross peaks with C-7, C-8 and C-4a carbon atoms while the hydroxyl function attached to C-7 also showed its correlations with C-5, C-6 and C-8 in HMBC spectrum. All these confirmed by ^{1}H-^{13}C and HMBC spectral data. On the basis of spectral data (IR, UV, ^{1}H, ^{13}C, 2D-NMR and MS) and the data reported in the literature [7], it was characterized as **7,4'-dihydroxy-6,3'-dimethoxyflavanone** (Fig.1).

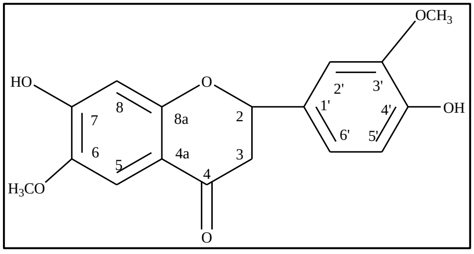

Figure-1

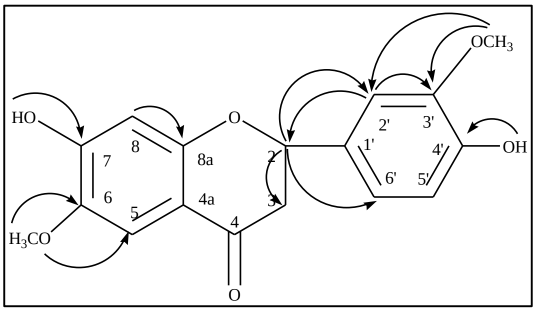

Figure- 1a. ^{1}H-^{13}C HMBC correlation of R-1

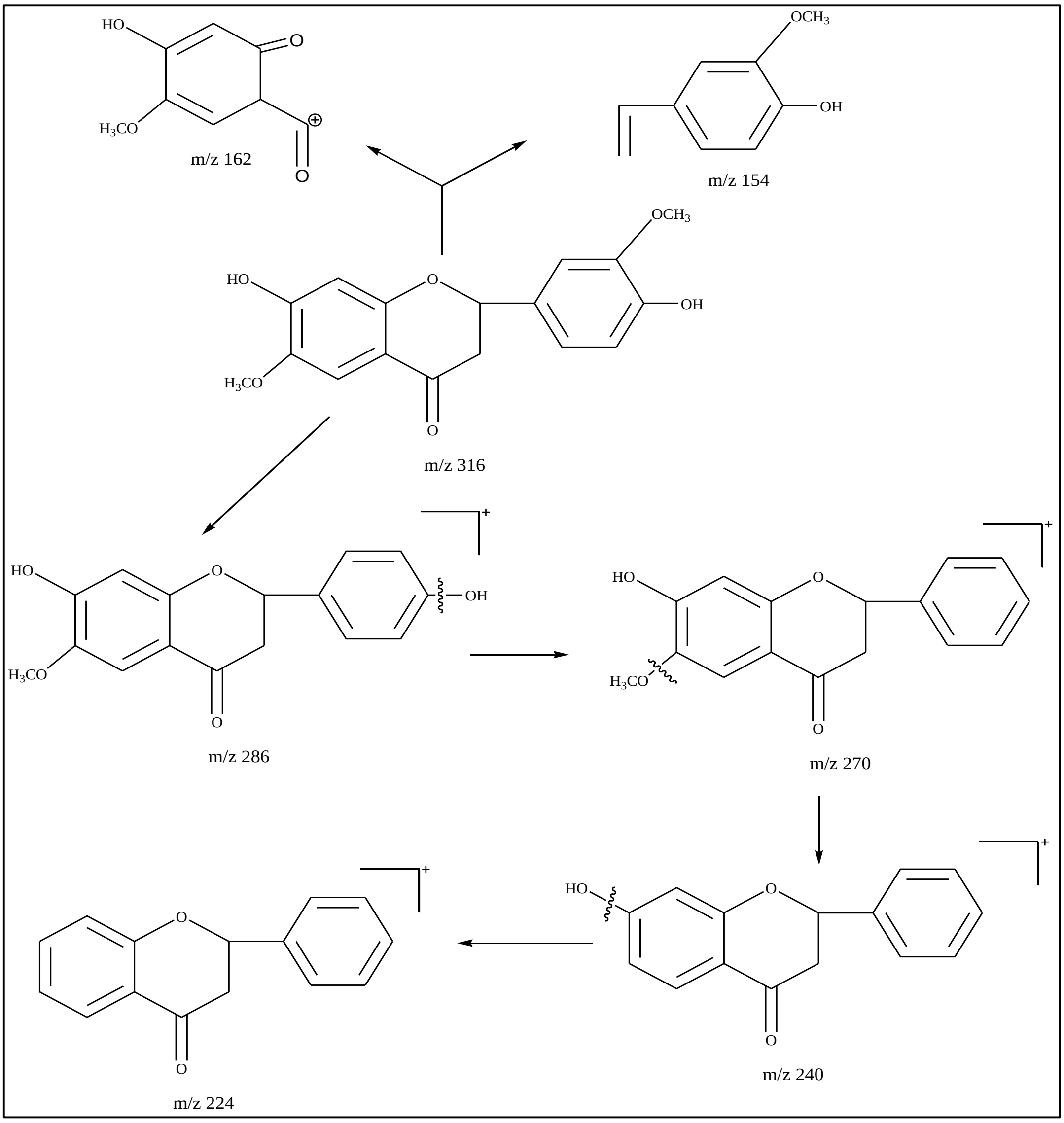

Figure-1b. Proposed mass fragmentation of R-2

Compound R-2 was crystallized as white amorphous powder from $CHCl_3$: MeOH, m.p. 272-274°C. Its molecular formula deduced as $C_{17}H_{16}O_7$ by molecular ion peak at m/z (332.304) in its LCMS. Bathochromic shift of band I by 27 nm in the UV spectrum R-2 in the presence of $AlCl_3$/HCl confirmed the presence of hydroxyl function at C-5 and other at C-3[8]. The C-6 and C-8 position was blocked by methoxyl function was also evidenced from negative response towards gossepetone test, by the parent compound as well as the characteristic mass spectral fragmentation pattern[9]. Batho-chromic shift of 14 nm after the addition of NaOAc indicated free hydroxyl group present at C-7. The IR spectrum exhibited bands at 3410 cm^{-1} characteristics of hydroxyl group, 1650 cm^{-1} for aromatic ring and a band at 1715 and 1725 cm^{-1} was clearly expressive for the stretching in a carbonyl group. The appearance of intense green colour with ferric chloride imparted by the parent compound, locates one of the hydroxyl groups at C-5 position as also revealed from its IR and ^{1}H-NMR spectra. In the LCMS spectrum, the molecular ion peak was observed at m/z 332[M^+] and other fragmentation peaks were observed at 302 [M-OCH_3], 286 [M-OCH_3+OH], 256 [M-2×CH_3+OH], 240 [M-2×OCH_3+2×OH], 224 [M-2×OCH_3+3×OH] and148 [M-2×OCH_3+3×OH+benzyl]. These peaks are indicative of the nature and number of the substituents in the ring A (dihydroxy and dimethoxy) and ring C (monohydroxy). The ^{1}H-NMR spectrum showed one proton signal at δ 12.08 confirming the presence of a hydroxyl group at C-7. Two double doublets at δ 2.73 (dd, J=16.0, 4.0Hz) and δ 5.47 (J=12.0, 4.0 Hz, H-2) as expected for the presence of C-3 and C-2 protons of flavanone were also observed in the ^{1}H-NMR spectrum.

^{1}H NMR spectrum furnished three doublets at δ_H 7.53 (2H, dd,J=8.0, 2.0 Hz, H-2',6'), δ_H 7.41 (2H, d,J=8.0, 2.5Hz, H-3',5') and δ_H 7,39 (1H, d,J=8.0Hz, H-4'). Two singlets each for three protons at δ3.78 (3H) and δ3.65 (3H) specified the presence of two methoxyl groups assigned for H-6 and H-8 which were further authenticated by their ^{13}C-chemical shifts at δ56.11 and δ56.37[3]. A singlet integrating of one proton at δ 9.20 was assigned to the H-5 and other at δ6.44(1H, s) due to free phenolic hydroxyl group of ring A. The downfield singlet at δ167.89 and 197.23 in the ^{13}C NMR spectrum were assigned to C-5 and C-4. All these confirmed by ^{1}H-^{13}C and HMBC spectral data (**Table-2**). On the basis of spectral data (IR UV, ^{1}H, ^{13}C, 2D-NMR and MS) and the data reported from literature. The structure of R-2 was characterized as **3,5,7-tri-hydroxy-6,8-dimethoxyflavanone** (**Figure-2**).

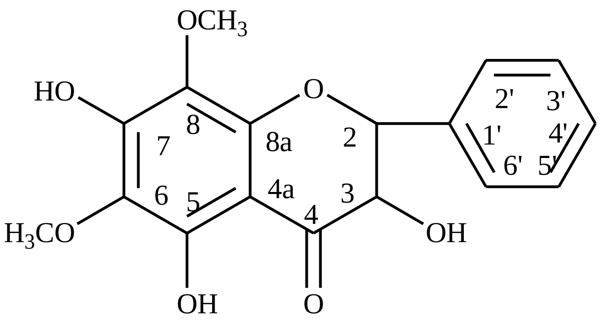

Figure-2

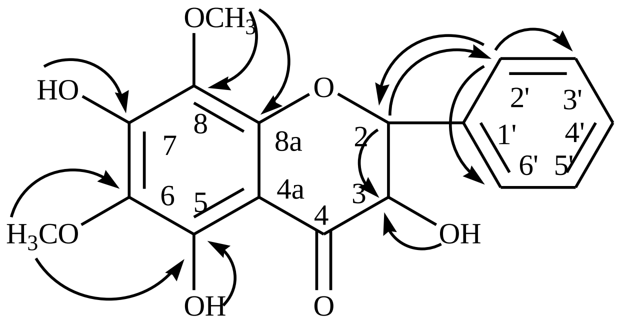

Figure- 2a

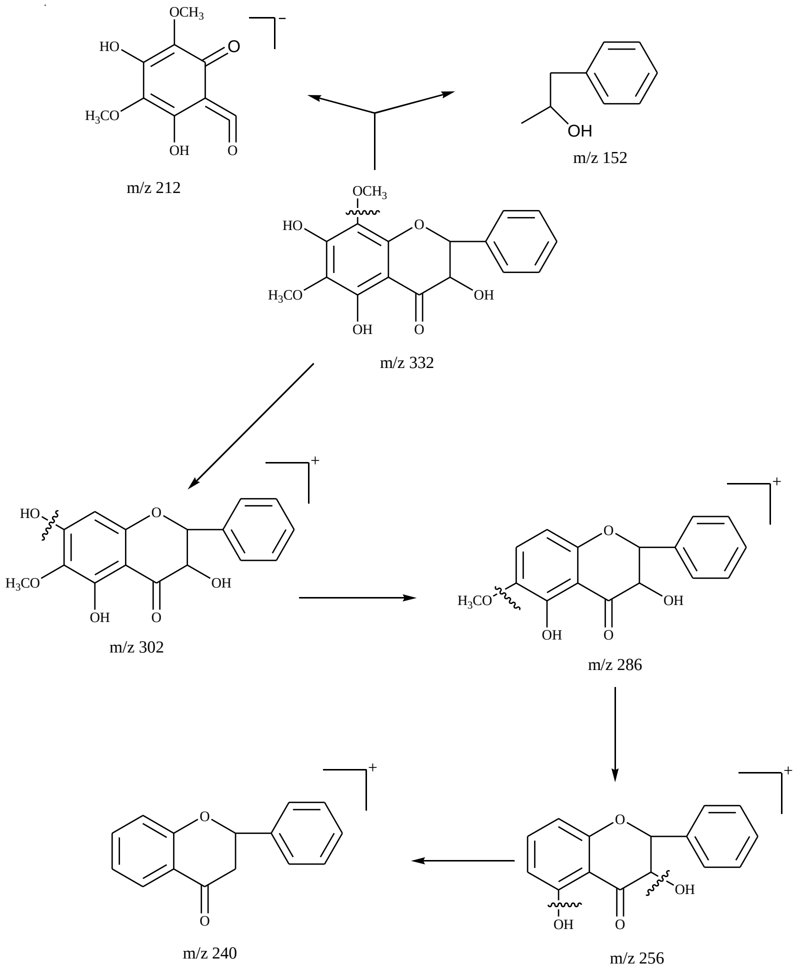

Figure- 2b. Proposed mass fragmentation of R-2

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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