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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF SOME TERPENOIDS AND FLAVONOIDS FROM ROOTS OF BERGENIA LIGULATA (SAXIFRAGACEAE)

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Abstract:

*For the first time five compounds have been isolated from the roots of **Bergenia ligulate**. Lanost-7(8)-en-3β-formyl-6β-acetoxy-11β,15β-diol-26-heptanoxy-27-oic acid, Urs-12(13)-en-3β-22α-diol-15β-formyl-21β-glucoside, 9,11-Seco-lanost-20(22)-en-3β-formyl-18-phenoxyoate-12β-D-glucose, Sumatrol, and 5`-Methyleriodictyol-3`-O-β-D-galactopyranosyl(1→4)-α-L-rhamno- pyranose. Their structures were elucidated by chemical and physical data (IR, UV, ^{1}H-NMR, ^{13}C-NMR and Mass spectra).*

Keywords: *Bergenia ligulata, Roots, Terpenoids, Flavonoids, Trihydroxybenzoic acid and Chemical Structure.*

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INTRODUCTION:

Bergenia species are evergreen herb belonging to the family saxifragaceae. The rhizomes of these plants are used in the indigenous system of medicines. The family includes about 80 genera and 1250 species worldwide. Most members of the Saxifragaceae family are herbs, and usually have a flower cluster held well above the basal whorl of leaves. Many of its members grow in rocky places. The fruit is a capsule with a lot of seeds [1].

Bergenia ligulata syn. Saxifraga ligulata is being widely accepted under this name. the use of various names attributed to it, viz., Pashanbheda, Pashana, Zakhmehayat, Asmaribheda, Ashmabhid, Ashmabhed, Nagabhid, Upalbhedak, Parwatbhed and Shilabhed (dissolving or piercing stones or slabs) etc.13 It belongs to family saxifragaceae. Its medicinally used part is rhizome. The rhizome and other parts of B. ligulata is used in urinary bladder stone, antilithic activity diuretic activity, anti-bradykinin activity, antiviral activity, antipyretic activity, antibacterial, anti-inflammatory, hepatoprotective activity, insecticidal activity, α-glucosidase activity and all these activities of the plant is due to presence of its constituents like; β-Sitosterol, Tannic acid, Stigmesterol, Gallic acid, Bergenin, (+)- Afzelechin, (+)-afzelechin, (+)-afzelechin tetraccetate, (+)-5,7,4'-trimethoxyafzelechin, (+)-tetramethoxyazelechin, (+)-3-acetyl-5,7,4'-trimethoxyafzelechin [1].

Previous chemical investigations of the plant have indicated the presence of β–sitosterol, β-sitosterol-D-glucoside, bergenin, p-hydroxybenzoyl bergenin [2], 11-O-galloylbergenin [3], afzelechin [4], norbergenin, catechin, gallic acid, arbutin [5], (+)-(6S)-Parasorbic acid, isovaleric acid, 1,8-cineole, (Z)-asarone, and terpinen-4-ol [6]. Medicinal importance and scanty work on this plant accelerates our interest to carry out the excellent investigations of the plant **B. ligulata**. The present discussion deals with the isolation and characterization of the following five compounds from the roots of

B. ligulata.

1. Lanost-7(8)-en-3β-formyl-6β-acetoxy-11β,15β-diol-26-heptanoxy-27-oic acid.
2. Urs-12(13)-en-3β-22α-diol-15β-formyl-21β-glucoside.
3. 9,11-Seco-lanost-20(22)-en-3β-formyl-18-phenoxyoate-12β-D-glucose.
4. Sumatrol.
5. 5`-Methyleriodictyol-3`-O-β-D-galactopyranosyl(1→4)-α-L-rhamno-pyranose.

MATERIAL AND METHODS:

The dried and powdered roots (2.0 kg) of B. ligulata, procured from Saudi Arabia, were consecutively extracted with the following solvents and yielded the indicated fractions.

Two extractions (24 hours, periods) with light petroleum (60-80ºC) (2x4l) yielded fraction `A` (60 + 15 gm). Two continuous extractions (24 hours, periods) with hot chloroform yielded fraction `B` (80+10 gm). Finally, the roots were extracted with hot acetone, yielded fraction `C` followed by methanol, fraction `D`.

Fractions `A` and `B` on TLC examinations in different solvent systems showed a number of compounds with the same R_f-values, in varying concentrations. The two fractions were therefore mixed together. The combined material was chromatographed over silica gel column using successively petroleum ether, petroleum ether-chloroform mixtures, chloroform, chloroform-methanol mixtures and methanol as eluting solvents. Appropriate fractions (IR spectra and TLC) were combined. Repeated column chromatography of the fractions followed by fractional crystallizations afforded four crystalline TLC homogenous substances, labeled as compounds **(1), (2), (3) and (4).**

The acetone extract gave a positive test for flavonoids [7-8]. TLC examination of the extract in different solvent systems, showed the presence of only one major compound along with some minor impurities. Repeated column chromatography over silica gel and fractional crystallization failed to purify the compound, therefore, a recourse was taken to the preparative TLC using EtOAc-EtMeCO-AcOH-H_2O (5:3:1:1) as a solvent system. A TLC homogenous, light-yellow substance was obtained, labeled as compound **(5)**.

RESULT AND DISCUSSION:

Compound (1)

Compound **(1)** was obtained as colourless amorphous powder from petroleum ether-chloroform (9:1) mixture. It was crystallized by chloroform-methanol (200 mg), m.p.161-62ºC. It gave effervescence with sodium bicarbonate solution and responded positively to Liebermann-Burchard test [8-9]. Its ir spectrum showed the characteristic absorptions for

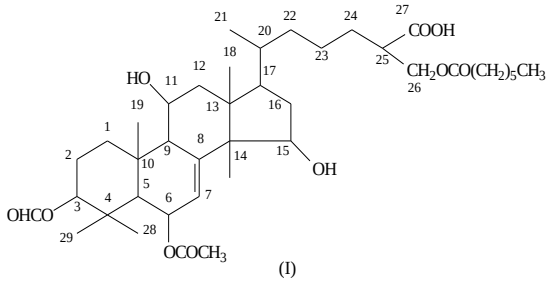
hydroxyls (3425 cm^{-1}), ester group (1734 cm^{-1}) and unsaturation (1595 cm^{-1}). Its molecular weight was established as 704 $[M]^+$ relating to $C_{40}H_{64}O_{10}$ on the basis of positive ion FAB mass spectrum.

The ^{1}H-nmr spectrum of compound **(1)** displayed a one-proton downfield singlet at δ 8.55 assigned to the formate proton. A one-proton multiplet at δ 5.31 was ascribed to H-7. Two one-proton each doublets with coupling interaction of 6.5 Hz at δ 4.13 and 4.08 were associated with the C-26 ester substituted methylene group. The H-6, H-3α and H-11α carbinolic protons appeared at δ 4.05 (d, J= 5.5 Hz), 3.65 (m) and 3.57 (m), respectively. The methyl signals resonated as broad singlets at δ 1.28 (Me-30), 1.18 (Me-28), 1.15 (Me-29), 0.96 (Me-19), 0.83 (Me-18) and as a doublet at δ0.88 (J= 6.0 Hz, Me-21). The signals in the range δ1.23-2.80 were due to the remaining methine and methylene groups.

The mass spectrum exhibited typical ion fragments at m/z 661 $[M\text{-}Ac]^+$, 390 [661-$C_{15}H_{27}O_4$, SC, 271]$^+$, 591 $[M\text{-}C_6H_{13}CO]^+$, 320 [591-SC]$^+$, 686 $[M\text{-}H_2O]^+$, 675 $[M\text{-}CHO]^+$, 128 [ion a]$^+$, 576 [ion b]$^+$, 558 [ion b-H_2O]$^+$, 445 [558-$C_6H_{13}CO$]$^+$, 532 [ion b-CO_2]$^+$, 305 [ion b-SC]$^+$, 287 [305-H_2O]$^+$, 269 [287-H_2O]$^+$, 168 [ion c]$^+$, 139 [ion c-CHO]$^+$, and 153 [ion c-Me]$^+$, suggesting the lanostenic type nature of the molecule possessing a formyl group in ring-A, which was placed at C-3 on the basis of biogenetic ground. It also possessed an acetoxy and two hydroxyl groups and a C_8-saturated side chain with a carboxylic and a heptanoyl groups. The existence of acetoxyl group at C-6 was deduced from the ion peaks of m/z 240 [ion d]$^+$, 211 [ion d-CHO]$^+$, 197 [ion d-$COCH_3$]$^+$, 308 [ion g]$^+$, 279 [ion g-CHO]$^+$, 265 [ion g-Ac]$^+$ and 278 [ion g-CHOH]$^+$. The ion peaks at m/z 463 [ion e]$^+$, 445 [ion e-H_2O]$^+$, 426 [ion f]$^+$, 355 [ion h]$^+$, 325 [ion h-CHOH]$^+$, 340 [ion h-Me]$^+$, 311 [ion h-CO_2]$^+$, 242 [ion h-$C_6H_{13}CO$]$^+$, 393 [ion i]$^+$, 379 [ion i-CH_2]$^+$ and 349 [379-CHOH]$^+$ reflected the presence of the hydroxyl groups at C-11 and C-15. (Scheme-I).

The Compound **(1)** on methylation with diazomethane yielded a monomethyl ester **(1)** (Me). Acetylation of **(1)** with acetic anhydride-pyridine formed a diacetyl product, **(1)** (Ac).

On the basis of above spectral studies Compound **(1)** was identified as Lanost-7(8)-en-3β-formyl-6β-acetoxy-11β,15β-diol-26-heptanoxy-27-oic acid (I).



(I)

Compound (2)

Compound **(2)** was obtained as crystalline solid from petroleum ether-chloroform (7:3) eluants. It was crystallized by chloroform-methanol mixture as white needles (230 mg), m.p. 190-91°C. It gave a positive Liebermann-Burchard [8-9] test and exhibited strong ir bands at 3425 (hydroxyl-group), 1735 (ester-group) and 1605 cm^{-1} (unsaturation). It had a molecular ion peak in its positive ion FAB mass spectrum at m/z 664 corresponding to a pentacyclic triterpenic glycoside, $C_{37}H_{60}O_{10}$. It indicated seven degrees of double bonds.

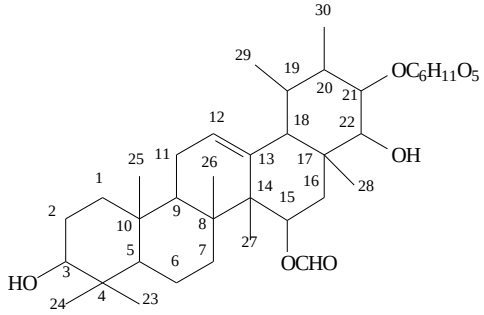
The ^{1}H-nmr spectrum of compound **(2)** displayed a one-proton downfield singlet at δ 8.50 assigned to formate group, a one-proton triplet at δ 5.30 (J=5.5 Hz) due to H-12, a one-proton doublet at δ 3.85 (J= 5.5 Hz) for H-22, two one-proton each broad singlets at δ4.83 and 3.80 associated with H-1` anomeric and H-21 methine protons, a one-proton broad multiplet at δ 4.30 due to H-15 methine proton and a one double doublet at δ 3.53 for carbinol placed at C-3 on the basis of biogenetic analogy and its coupling interactions of 4.5 and 8.5 Hz indicated its α-orientation. The glucose protons appeared as broad singlets at δ4.13 (1H, H-6`a), 4.06 (1H, H-6`b) and 3.60 (4H, H-2`, H-3`, H-4`, H-5`). The eight methyl signals, all located on saturated carbons, resonated at δ1.20(Me-24, Me-25), 1.16 (Me-27), 1.13 (Me-23), 1.08 (d, J=6.6 Hz, Me-29), 0.86 (d, J=6.5 Hz, Me-30), 0.80 (Me-28), and 0.76 (Me-26).

The presence of a doublet at δ 2.30 (J= 9.0 Hz), attributed to H-18, indicated the compound to be related to the ursane series. The remaining methine and methylene groups appeared in the range of δ 1.56-2.80.

The mass spectrum of compound **(2)** demonstrated the existence of characteristic ion fragments at m/z 207 [ion a]$^+$ and 444 [ion b]$^+$ formed due to retro-Diels-Alder fragmentation pattern of Δ^{12}-amyrene series [10]. The ion fragments at m/z 192 [ion a-Me]$^+$, 189 [ion a-H_2O]$^+$, 398 [ion b-HOCHO]$^+$, 426 [ion b-H_2O]$^+$, 415[ion b-CHO]$^+$, 397 [415-H_2O]$^+$, 281 [ion b-$C_6H_{11}O_5$]$^+$, 263 [281-H_2O]$^+$ and 235 [281-HCOOH]$^+$ supported the presence of hydroxyl group in rings A/B and formate, hydroxyl and glucoside groups in the rings C/D. The ion peaks at m/z 444[ion c]$^+$, 278 [ion d]$^+$, 426 [ion c-H_2O]$^+$, 414 [ion c-CHOH]$^+$, 416 [ion c-CH_3CH]$^+$, 386 [416-CHOH]$^+$, 368 [386-H_2O]$^+$, 250 [ion d-$CHCH_3$]$^+$, 98 [ion d-$C_6H_{12}O_6$]$^+$ attested the location of glucoside unit at C-21 and hydroxyl group at C-22. The existence of formate group at C-15 was deduced from the ion peaks appearing at m/z 390 [ion f]$^+$, 346 [ion e]$^+$, 318 [ion f-$CH_2CHOCHO$]$^+$, 328 [ion e-H_2O]$^+$, 138 [318-$C_6H_{12}O_6$]$^+$, 332 [ion e-CH_2]$^+$, 314 [332-H_2O]$^+$, 332 [ion f-CHOCHO]$^+$, 152 [332-$C_6H_{12}O_6$]$^+$, 152 [ion f-CHOCHO-$C_6H_{12}O_6$]$^+$, 274 [ion e-CH_2CHOCHO]$^+$, 259 [274-Me]$^+$, 256 [274-H_2O]$^+$, and 210 [ion f-$C_6H_{12}O_6$]$^+$, 328 [ion e-H_2O], (Scheme-II).

More compelling evidence for the structure of compound **(2)** was provided by chemical reactions. Treatment of compound **(2)** with acetic anhydride and pyridine afforded a peracetylated product **(2)** (Ac). Acid hydrolysis of compound **(2)** yielded the aglycone, **(2)** (Agl), and glucose which was identified by co-chromatography with an authentic sample of glucose.

On the basis of above spectral studies the **(2)** was identified as Urs-12(13)-en-3β, 22α-diol-15β-formyl-21β-glucoside (II).



(II)

Compound (3)

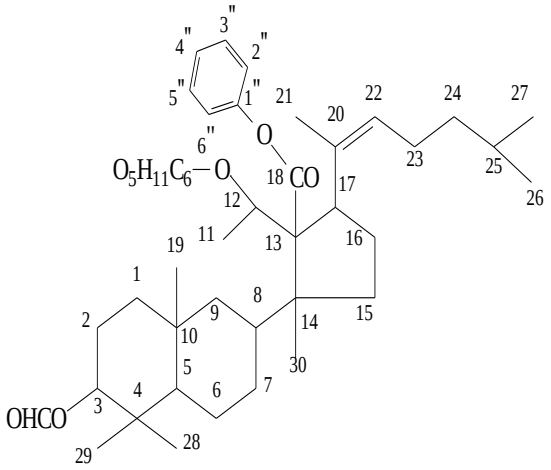
Compound **(3)** was eluted from the column by petroleum ether-chloroform (3:1) mixture as amorphous mass. It was crystallized from methanol (180 mg) m.p. 210-11ºC. It gave positive tests of triterpenoids [8-9] and glycosides [11]. Its ir spectrum demonstrated the presence of hydroxyl groups (3435 cm^{-1}), ester (1735, 1725 cm^{-1}) and unsaturation (1600 cm^{-1}). It had molecular ion peak in its positive ion FAB mass spectrum at m/z 742 [M]$^+$, corresponding to $C_{43}H_{66}O_{10}$.

The ^{1}H-nmr spectrum of compound (3) displayed a one-proton downfield singlet at δ 8.43 assignable to OCHO and three aromatic protons as multiplets at δ 7.07 (2H, H-2``, H-6``), 6.95 (2H, H-3``, H-5``) and 6.70 (1H, H-4``). A one-proton broad singlet at δ 5.26 was attributed to H-22 vinylic proton. The carbinolic protons appearing as a double doublet at δ 4.36 (J= 4.5, 8.5 Hz), and as a multiplet at δ 4.15 were ascribed to H-3β and H-12. The protons of glucose moiety resonated at δ 4.87 (d, J= 6.0 Hz, H-1`), 4.05 (d, J= 6.0 Hz, H-6`a), 4.00 (d, J= 6.0 Hz, H-6`b), 3.96 (m, H-5`), 3.53 (m, H-4`) and 3.40 (m, H-2`, H-3`). A three-protons broad singlet at δ 1.80 was attributed to C-21 methyl group attached to C-20 olefinic carbon. The signals for three-protons each at δ 1.08, 1.02, 0.80 and 0.76 were associated with the tertiary Me-29, Me-19, Me-28 and Me-30 respectively. The secondary Me-26 and Me-27 appeared as doublets with coupling interactions of 6.5 Hz at δ 0.97 and 0.87. The signals in the range δ 1.15-2.71 were accounted to the remaining methylene and methine functionalities.

In its mass spectrum, the ion fragments at m/z 727 [M-Me]$^+$, 631 [M-C_8H_{15}, SC]$^+$, 579 [M-$C_6H_{11}O_5$]$^+$, 562 [M-$C_6H_{12}O_6$]$^+$, 111 [C_8H_{15}, SC]$^+$, 163 [$C_6H_{11}O_5$]$^+$, 180 [$C_6H_{12}O_6$]$^+$, 93 [C_6H_5O]$^+$ and 410 [562-$C_{11}H_{20}$]$^+$ suggested that the compound belonged to lanostene-type triterpene which possessed C_8H_{15} an unsaturated side chain, a glucose moiety, a formyl group and a phenolic ester. The ion peaks at m/z 128 [ion a]$^+$, 113 [ion a-Me]$^+$, 99 [ion a-CHO]$^+$, 168 [ion b]$^+$, 139 [ion b-CHO]$^+$, 223 [ion c]$^+$, 194 [ion c-CHO]$^+$ and 177 [ion c-HCOOH]$^+$, indicated the presence of formyl group in ring-A which was placed at C-3 on biogenetic grounds. The ion fragments at m/z 519 [ion d]$^+$, 408 [ion d-SC]$^+$, 426 [ion d-C_6H_5O]$^+$, 356 [ion d-$C_6H_{11}O_5$]$^+$, 339 [ion d-$C_6H_{12}O_6$]$^+$, 207 [$CH(CH_3)OC_6H_{11}O_5$]$^+$, 312 [ion d-207]$^+$, 219 [312-C_6H_5O]$^+$ and 297 [312-Me]$^+$ supported the C_9-C_{11} seco-nature of ring-C and the existence of phenoxy group at C-18 and glucosyl moiety at C-12. (Scheme-III).

Acid hydrolysis of compound **(3)** yielded an aglycone, **(3)** (Agl), and a sugar moiety which was identified as D-glucose by comparing it with an authentic sample of glucose on TLC.

On the basis of above spectral studies the **(3)** was identified as 9,11-Seco-lanost-20(22)-en-3β-formyl-18-phenoxyoate-12β-D-glucose (III).



(III)

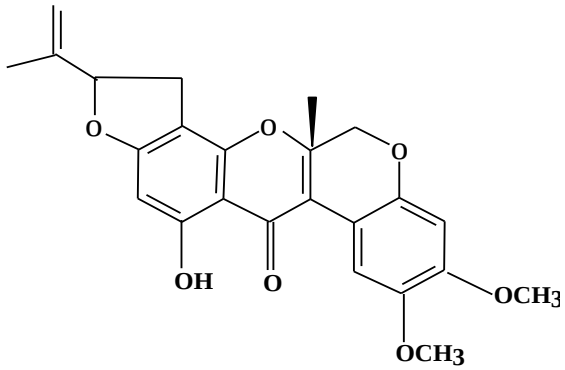
Compound (4)

Compound **(4)** was eluted with chloroform-methanol (8:2) mixture. Recovery of the solvent left a residu, which was crystallized from acetone as colourless needles (750 mg), m.p. 190-92 ºC. It was sparingly soluble in methyl alcohol, acetic acid and dilute aq. sodium hydroxide, but readily dissolved in chloroform. With ferric chloride it gave a deep brown colouration tinged with green. The analytical results obtained for **(4)** gave the molecular formula as $C_{23}H_{20}O_5(OMe)_2$. A purple colouration with Durham test [12] and a green one in the Rogers and Calamari test [13] indicated that the compound might be rotenoid.

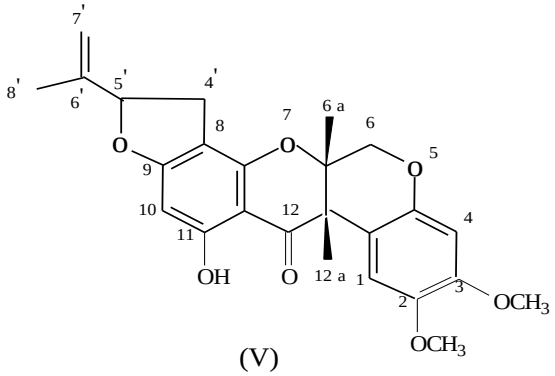
The formation of an oxime, m.p. 245-46 ºC established the presence of an active carbonyl group. This fact, in conjunction with the strong ferric reaction indicated the presence of a phenolic group in the ortho position to the carbonyl group.

Treatment of compound (4) with iodine and sodium acetate and elimination of iodine from the product by means of zinc dust and acetic acid according to the standard procedure yielded a colourless compound, m.p. 190-92ºC, which like the parent compound was optically active, almost insoluble in dilute aq. NaOH and gave a strong ferric reaction. It was identified as dehydrosumatrol [14] (IV) by melting and mixed melting point with an authentic sample.

The above observations showed that the **(4)** is sumatrol (V). Further confirmation to its identity was furnished by spectral studies, as the authentic sample of sumatrol could not be obtained.



(IV)



(V)

The ^{1}H-nmr spectrum revealed a vinyl methyl at δ 1.75, two methoxyls at δ 3.79 and 3.82, three aromatic protons at δ 6.0, 6.46 and 6.86, all of which appeared as singlets besides signals for nine other protons between δ 2.5-5.2. A careful study of the last-mentioned region showed that three of these formed a ABX system centered at δ 2.8 (dd), 2.3 (dd) and 5.2 (t) attributed to the dihydrofuran ring protons. The two broad singlets at δ 4.95 and 5.08 could be assigned to olefinic protons of ($CH_3C=CH_2$) unit consistent with the presence of a vinyl methyl. Four other signals at δ 3.8 (d, J= 4.0Hz), 4.18 (d, J= 12.0Hz), 4.6 (dd, J= 12.0Hz, 4.0Hz) and 4.9 (t, J= 4.0Hz) were reminiscent of rotenoid skeleton. The presence of a chelated hydroxyl was indicated by a low field signals at δ 12.5 the data suggested the structure (V).

The ^{13}C-nmr spectrum further confirmed the above structure. The assignments of individual carbon atoms are given in Table-1.

The lower field position of the carbonyl at 194 is suggestive of a reduced pyrone ring system. The two aliphatic carbons of this ring appearing at δ 44 and 72 (both doublets in off resonance) is indicative of an elaboration of isoflavanone chromophore to rotenoid type skeleton. The presence of isopropenyl dihydrofuran ring system is also substantiated by signals at δ 17 (CH_3), 30 (CH_2), 88 (oxygenated carbon), 112 (terminal methylene) and 143 (quarternary olefinic carbon). There were three protonated aromatic carbon resonances at δ92, 101 and 110 of which the first can be assigned to phloroglucinol ring methine carbon. There were two methoxyl carbon resonances near δ 56. The remaining signals were other aromatic quaternary carbons falling in two classes, three nonoxy- genated 101.4, 104.3 and 104.8 and six oxygenated 144, 147, 149.9, 156, 166 and 169. Thus these data also lead to the same conclusion derived from ^{1}H-nmr and confirmed that **(4)** is sumatrol (V).

Table-1; ^{13}C-nmr spectral data of compound (4)

Assignment	Signals
C-1	110
C-2	144
C-3	149.9
C-4	101
C-4a	147
C-6	66
C-6a	72
C-7a	156
C-8	101.4
C-9	166
C-10	92
C-11	169
C-11a	104.3
C-12	194
C-12a	44
C-4`	30
C-5`	88
C-6`	143
C-7`	112
C-8`	17
2 x OCH_3	56

Compound (5)

Compound **(5)** was obtained as light-yellow solid from chloroform-methanol (3:1) mixture. The solid on TLC examination showed a major spot with some minor impurities. It was therefore, further purified by preparative TLC (ethyl acetate-ethyl methyl ketone-acetic acid-water (5:3:1:1)). A light-yellow substance was obtained, labelled as compound **(5)** and crystallized from ethanol as pale-yellow needles (350 mg), m.p. >250°C. It responded positively to Shinoda's test [7-8], Molisch test [15-16] and the uv spectrum showed λ_{max} in methanol at 275 nm (Band II). Analysis of functional groups revealed the presence of phenolic OH (3450 cm^{-1}), α, β-unsaturated ketonic C=O (1680 cm^{-1}) and a complex aromatic substitution pattern (1500, 1365, 1140, 800 cm^{-1}) besides a strong band at 2950 cm^{-1}. The colour reaction, ir, uv spectral data with diagnostic shift reagents [17] coupled with appearance of a double doublet (C_2-1H, δ5.2, J=5.0 and 10.0 Hz) and a multiplet (C_3-2H, δ 2.95-3.20) in the ^{1}H-nmr spectrum confirmed the presence of flavanone skeleton bearing hydroxyl groups at 4`, 5, 7-positions.

Acid hydrolysis with 0.2 N hydrochloric acid gave an aglycone and equimolar quantity of galactose and rhamnose, identified by paper chromatography. The aglycone showed characteristic features of 3′, 4′-dihydroxyflavanone as it rapidly decomposed in the presence of NaOAc, in uv spectrum (absent in glycoside), thus suggesting that sugars may be attached to 3′-position of the aglycone [17]. The formation of tetraacetate derivative and the presence of one C-methyl group (^{1}H-nmr signal at δ 2.40 corresponding to 3H of C-Me) was in agreement with the structure of the aglycone as 3′,4′,5,7-tetrahydroxy-5`-methyl-flavanone (5′-methyleriodictyol) (VI) corresponding to molecular formula $C_{16}H_{14}O_6$.

On acetylation with acetic anhydride and pyridine, **(5)** formed a crystalline nonacetate **(5)** (AC), m.p. 85-86 °C. The ^{1}H-nmr spectrum of **(5)** (Ac) (Table-2) showed multiplets at δ 2.01-2.48 integrating for 30 protons due to one C-methyl group and nine acetoxyls (3 aromatic and 6 aliphatic). A quartet centered at δ2.95-3.20 is assigned to C-3 methylene and double doublet at δ 5.20 (J=5.0 and 10.0 Hz) identified the C-2 proton. The 5,7-disubstitution was demonstrated by the presence of two meta coupled doublets at δ 6.82 and 7.28 assigned to C-6 and C-8 protons which have shifted considerably downfield due to derivatisation. A doublet at δ7.75 is ascribed to 2′, 6′-protons of the ring-B. The mass spectrum of **(5)** (Ac) fully supported the structure of compound **(5)** as it clearly exhibited M^+ at m/z 428 (M-Gly) in accordance with the flavanone containing three acetoxyl and one C-methyl substituents. The subsequent removal of three acetoxyls gave fragment ions at m/z 386, 344 and 302. The fragment at m/z 302 was observed as the base peak as it corresponds to the aglycone. Other major fragments were observed at m/z 286, 283, 273, 245, 236, 153, 137 and 111 (Scheme-IV).

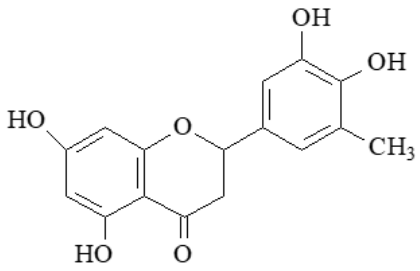
Table-2: ^{1}H-nmr data of (5) (Ac), (values on δ-scale)

Assignments	No. of protons	Signals
H-2′,6′	2	7.75 (d, J=9.0Hz)
H-8	1	7.28 (d, J=2.5Hz)
H-6	1	6.82 (d, J=2.5Hz)
H-2	1	5.20 (dd, J_1=10.0Hz, J_2=5.0Hz)
H-3, 3	2	2.95-3.20 (q, J_1=12.0Hz, J_2=4.0Hz, J_3=17.0Hz)
6 Aliphatic acetoxyls		
3 Aromatic acetoxyls	30	2.01-2.48 (m)
C-methyl group		

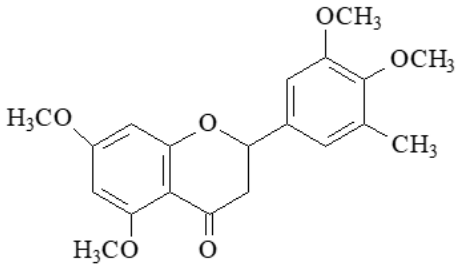
s = singlet, d=doublet, q=quartet, m=multiplet. Spectrum run in $CDCl_3$ at 300 MHz, using TMS as internal standard.

Kuhn methylation [18] of the glycoside followed by acid hydrolysis gave 2,3-di-O-methyl-rhamnose [19],2,3,4,6-tetra-O-methyl-D-galactose [20] indicating the disaccharide to be galactosyl (1→4) rhamnose and an aglycone characterized as 5′-methyl-4′,5,7-tri-O-methyleriodctylo (VII) by its spectral studies. The sugar moieties were found to be attached to position-3′ by the formation of this partial methyl ether as well as by the comparison of uv spectrum of the aglycone and the glycoside in the presence of NaOMe. Further evidence for the vicinal dihydroxyl system in the aglycone was obtained by the conversion to diphenylmeth- ylenedioxy derivative with diphenyldichloromethane [21]. The glycoside however failed to form this derivative thus finally confirming the sugar linkage at 3′-position. Emulsion hydrolysis of the glycoside gave only galactose in aqueous hydrolysate showing it to be terminal and β-linked. Quantitative estimation of sugars and periodate oxidation showed two moles of sugars per mole of the aglycone and both the sugars to be in pyranose form.

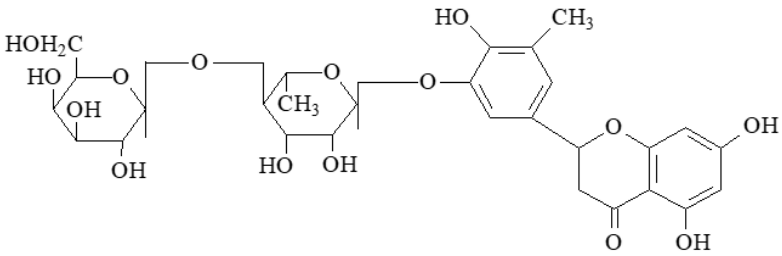
On the basis of these results, the flavanone glycoside has been identified as 5′-methyleriodictyol-3′-O-β-D-galactopyranosyl(1→4)-α-L-rhamnopyranose (VIII).



(VI)



(VII)



(VIII)

IV. EXPERIMENTAL:

General

The melting points were taken on a Kofler block and are uncorrected. All ultraviolet spectra were measured on Beckmann Model DU and Pye Unicam PU-8800 spectrophotometers in methanol / ethanol. Infrared spectra were taken on Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The mass and ^{1}H-nmr spectra were obtained from different institutes in the country and outside. The mass spectra were mostly measured in E.I. mode on Jeol D-300 while, the ^{1}H-nmr spectra were usually recorded on Varian EM-360 L (60 MHz), 270 MHz, JEOL 4H-100 MHz, Perkin Elmer R-32 (90 MHz), Brucker dpx 200 MHz, DRX 300 MHz and WM 400 MHz in $CDCl_3$ / DMSO-d_6 using TMS as internal standard.

The silica gel used for different chromatographic purposes, was obtained from E. Merck (India), E. Merck (Germany) and SRL (India). TLC solvent systems used were benzene-pyridine-formic acid (BPF, 36:9:5), toluene – ethylformate - formic acid (TEF, 5:4:1), ethylacetale-ethylmethylketone-acetic acid-water (EtOAc-EtMeCO-AcOH-H_2O, 5:3:1:1; 20:3:1:1; 30:3:1:1), ethylacetate-methanol-water (EtOAc-MeO -H_2O, 8:1:1), petrol-benzene (2:8) n-butanol-acetic acid-water (BAW, 4:1:5), n-butanol-water-ethanol (BEW, 60:28.5:16.5).
Alcoholic ferric chloride, iodine vapours and aniline hydrogen phthalate solutions were used as spray reagents for visualization of spots on TLC and on paper chromatograms.

Plant material

Biological source: Bergenia ligulate
Family: Saxifragaceae
Part used; Roots
Place of collection: Saudi Arabia
Identified by: Prof. Wazahat Hussain, Department of Botany, A.M.U., Aligarh, India.
Voucher specimen: Has been deposited at Department of Botany, A.M.U., Aligarh.

Extraction and isolation

The dried and powdered roots (2.0 kg) of **B. ligulata**, were consecutively extracted with the following solvents and yielded the indicated fractions.

Two extractions (24 hours, periods) with light petroleum (60-80ºC) (2x4l) yielded fraction `A` (60 + 15 gm). Two continuous extractions (24 hours, periods) with hot chloroform yielded fraction `B` (80+10 gm). Finally, the roots were extracted with hot acetone, yielded fraction `C` followed by methanol, fraction `D`.

Fractions `A` and `B` on TLC examinations in different solvent systems showed a number of compounds with the same R_f-values, in varying concentrations. The two fractions were therefore mixed together. The combined material was chromatographed over silica gel column using successively petroleum ether, petroleum ether-chloroform mixtures, chloroform, chloroform-methanol mixtures and methanol as eluting solvents. Appropriate fractions (IR spectra and TLC) were combined. Repeated column chromatography of the fractions followed by fractional crystallizations afforded four crystalline TLC homogenous substances, labeled as compounds **(1), (2), (3) and (4).**

The acetone extract gave a positive test for flavonoids[6]. TLC examination of the extract in different solvent systems, showed the presence of only one major compound along with some minor impurities. Repeated column chromatography over silica gel and fractional crystallization failed to purify the compound, therefore, a recourse was taken to the preparative TLC using EtOAc-EtMeCO-AcOH-H_2O (5:3:1:1) as a solvent system. A TLC homogenous, light yellow substance was obtained, labeled as compound **(5)**.

Compound (1)

The compound **(1)** was eluted from the column by petroleum ether-chloroform (9:1). It was crystallized by chloroform-methanol as colourles amorphous powder (200 mg), p.m. 161-62ºC. Purified by preparative TLC, (n-hexane-petroleum ether, 3:1).

UV λ_{max} (MeOH) nm
208

IR, υ^{KBr}_{max} cm^{-1}
3425, 2925, 2860, 1734, 1595, 1561, 1441, 1367, 1254, 1240, 1180, 1112, 1066, 1044, 965, 815, 745.

^{1}H-nmr (300 MHz, $CDCl_3$ + MeOD), values on δ-scale
δ 8.55 (1H, br s. OCHO), 5.31 (1H, m, H-7), 4.13 (1H, d, J=6.5, Hz, H-26`a), 4.08 (1H, d, J=6.5 Hz, H-26`b), 4.05 (1H, d, J=5.5 Hz, H-6), 3.65 (1H, m, H-3α), 3.57 (1H, m, H-11α), 3.50 (1H, m, H-15α), 2.80 (2H, m, H_2-1), 2.31 (5H, m, H_2-2`, H_2-16, H-5), 2.17 (1H, m, H-17), 2.10 (4H, m, H_2-16, H_2-24), 1.93 (3H, m, $COCH_3$), 1.77 (1H, d, J= 5.5, H-9), 1.65 (5H, m, H_2-22, H_2-23, H-20), 1.28 (3H, br s, Me-30), 1.23 (10H, br s,

5x CH_2), 1.18 (3H, br s, Me-28), 1.15 (3H, br s, Me-29), 0.96 (3H, br s, Me-19), 0.88 (3H, d, J= 6.0 Hz, Me-21), 0.83 (3H, br s, Me-18).

FABMS m/z (rel. int.)
704 $[M]^+$ (3.3), 686 (3.2), 675 (2.0), 663(11.9), 661 (3.8), 647 (8.7), 591 (2.5), 576 (1.7), 558 (1.7), 532 (4.7), 463 (1.7), 445 (2.6), 427 (2.9), 426 (2.1), 408 (3.6), 396 (3.9), 393 (3.1), 379 (3.5), 364 (2.3), 355 (4.5), 350 (3.2), 349 (2.3), 340 (4.9), 338 (4.4), 325 (5.7), 323 (4.9), 320 (4.8), 311 (8.2), 308 (7.9), 305 (6.2), 287 (3.3), 279 (7.8), 278 (3.9), 271 (4.2), 269 (4.0), 265 (8.8), 253 (5.8), 242 (5.6), 240 (8.2), 225 (5.7), 211 (6.8), 210 (2.8), 207 (16.3), 197 (6.1), 192 (11.1), 173 (9.3), 168 (13.2), 155 (13.9), 153 (11.0), 139 (8.3), 128 (21.7), 119 (30.8), 117 (25.9), 109 (41.5), 107 (39.1), 105 (59.0), 99 (10.3), 97 (46.2), 95 (94.3), 93 (100).

Methylation of compound (1)
The compound **(1)** (50 mg) was dissolved in ether (100 ml). Diazomethane was passed throughout the solution till it's saturated. The flask was corked and kept overnight at room temperature. The ether was evaporated and the residue was washed well with sodium bicarbonate solution and then with water. It was dried and purified on silica gel column using the chloroform as the eluants.

The methylated product was crystallized from chloroform-methanol mixture as colourless needles (35 mg), m.p. 114-15 °C.
Analysed for $C_{41}H_{66}O_{10}$
Calcd.: C, 68.52; H, 9.19
Found: C, 68.49; H, 9.16

Acetylation of compound (1)
Crystalline **(1)** (25 mg) was treated with a acetic anhydride (1 ml) and dry pyridine (0.5 ml). The mixture was allowed to stand overnight at room temperature (22-27ºC) and then heated on water bath for two hours. The reaction mixture was cooled at room temperature and poured on crushed ice. The solid was separated, filtered, washed with water and dried over sodium sulphate. It was crystallized from chloroform-methanol mixture as white-needles, m.p. 110 ºC.
Analysed for $C_{44}H_{69}O_{12}$
Calcd.: C, 66.92; H, 8.74
Found: C, 66.89; H, 8.79

Compound (2)
Elution of the column with petroleum ether-chloroform (7:3) gave **(2)**. It was crystallized from chloroform-methanol as white needles (230 mg), m.p. 190-91ºC.

UV λ_{max} (MeOH) nm
210

IR, υ^{KBr}_{max} cm^{-1}
3425, 2925, 2820, 1735, 1605, 1562, 1449, 1375, 1255, 1100, 820.

^{1}H-nmr (300 MHz, $CDCl_3$ + MeOD) values of δ-scale
δ 8.50 (1H, s, OCHO), 5.30 (1H, t, J= 5.5 Hz, H-12), 4.83 (1H, br s, H-1`), 4.30 (1H, br m, H-15), 4.13 (1H, br s, H-6`a), 4.06 (1H, br s, H-6`b), 3.85 (1H, d, J= 5.5, H-22), 3.80 (1H, br s, H-21), 3.60 (4H, br s, H-2`, H-3`, H-4`, H-5`), 3.53 (1H, dd, J= 4.5, 8.5 Hz, H-3α), 2.80 (2H, m, CH_2-1), 2.53 (2H, m, CH_2-16), 2.30 (1H, d, J= 9.0 Hz, H-18), 2.16 (3H, m, CH_2, CH), 2.03 (5H, m, 2x CH_2, CH), 1.90 (3H, br s, CH, CH_2), 1.80 (1H, d, J= 5.5 Hz, CH-5),1.56 (3H, m, CH_2, CH), 1.20 (6H, br s, Me-24, Me-25), 1.16 (3H, br s, Me-27), 1.13 (3H, br s, Me-23), 1.08 (3H, d, J= 6.6 Hz, Me-29), 0.86 (3H, d, J= 6.5 Hz, Me-30), 0.80 (3H, br s, Me-28), 0.76 (3H, br s, Me-26).

FABMS m/z (rel. int.)
664 $[M]^+$ (1.4), 646 (1.0), 618 (1.8), 533 (1.4), 503 (2.1), 460 (23.9), 458 (3.7), 444 (1.2), 426 (1.5), 416 (1.4), 415 (3.6), 413 (3.6), 398 (6.2), 398 (1.1), 397 (2.3), 390 (1.3), 386 (3.0), 368 (3.8), 355 (14.0), 346 (1.2), 340 (11.2), 334 (1.3), 332 (1.3), 328 (2.8), 324 (1.9), 318 (1.7), 314 (1.4), 281 (33.8), 278 (8.7), 274 (2.0), 266 (14.7), 264 (10.5), 263 (4.0), 259 (2.4), 256 (3.6), 250 (5.1), 246 (3.0), 235 (3.9), 220 (43.1), 210 (3.4), 207 (43.9), 192 (12.3), 191 (14.6), 189 (10.0), 176 (11.0), 163(5.8), 152 (5.6), 146 (100), 145 (13.4),138 (3.9), 133 (23.3), 131 (17.4), 123 (12.4), 117 (16.3), 111 (14.7), 107 (20.3), 105 (28.0), 101 (14.3), 98 (42.4), 93 (30.0).

Acetylation of compound (2)
Crystalline **(2)** (50 mg) was treated with acetic anhydride (1 ml) and dry pyridine (0.5 ml). The mixture was allowed to stand at room temperature (22-27°C) and then heated on water bath for 2 hours. The solid product obtained, after usual work-up, was crystallized from chloroform-methanol as white needle-shaped crystals. **(2)** (Ac), m.p.125 °C.

IR, υ^{KBr}_{max} cm^{-1}
1735, 1725, 1720.

Acid hydrolysis of compound (2)
The glycoside **(2)** (100 mg) was dissolved in 80% methyl alcohol and 20 ml of 0.6N hydrochloric acid was added to it. The mixture was refluxed over a water bath for three hours. After leaving overnight, the aglycone thus separated out was filtered, washed well with water and dried. The crude product was crystallized from methanol as white needles, m.p. 230-31 °C.
Analysed for $C_{31}H_{50}O_5$
Calcd.: C,74.10; H, 9.96
Found: C, 73.97; H, 9.94

Chromatographic identification of sugar
The acidic filtrate left after filtering the aglycone was extracted with ether to ensure the complete removal of any residual aglycone. The clear filtrate thus obtained was concentrated to a syrup in vacuum over KOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on a Whatmann No.1 filter paper using butanol: acetic acid: water (4:1:5) and n-butanol: water: ethanol (60:25.8:16.5) as solvent mixtures, using descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05°C showed the presence of glucose only.

Compound (3)
The compound **(3)** was eluted from the column by petroleum ether-chloroform (3:1) mixture. It was crystallized from methanol (180 mg), m.p. 210-1°C. Purified by preparative silica-gel TLC, R_f = 0.3 (n-hexan-petroleum ether, 3:1).

UV λ_{max} (MeOH) nm
222, 275.

IR, υ^{KBr}_{max} cm^{-1}
3435, 2900, 2855, 1735, 1725, 1600, 1563, 1441, 1412, 1377, 1265, 1250, 1155, 935.

^{1}H-nmr (300 MHz, $CDCl_3$ + MeOD), values on δ-scale
δ 8.43 (1H, br s. OCHO), 7.07 (2H, m, H-2``, H-6``), 6.95 (2H, m, H-3``, H-5``), 6.70 (1H, m, H-4``), 5.26 (1H, br s, H-22), 4.87 (1H, d, J=6.0 Hz, H-1`), 4.36 (1H, dd, J=4.5, 8.5 Hz, H-3β), 4.15 (1H, m, H-12), 4.05 (1H, d, J=6.0 Hz, H-6`a), 4.00 (1H, d, J= 6.0 Hz, H-6`b), 3.96 (1H, m, H-5`), 3.53 (1H, m, H-4`), 3.40 (2H, m, H-2`, H-3`), 2.71 (2H, m, H_2-1), 2.30 (1H, m, H-5), 2.17 (1H, m, H-8), 2.13 (1H, m, H-22), 2.07 (2H, m, H_2-7), 1.87 (2H, m, H_2-15), 1.80 (3H, br s, Me-21), 1.67 (1H, m, H-17), 1.60 (1H, m, H-25), 1.50 (4H, m, H_2-2, H_2-16), 1.30 (3H, d, J= 6.0 Hz, Me-11), 1.15 (8H, br s, H_2-6, H_2-9, H_2-23, H_2-24), 1.08 (3H, br s, Me-29), 1.02 (3H, br s, Me-19), 0.97 (3H, d, J=6.5 Hz, Me-26), 0.87 (3H, d, J= 6.5 Hz, Me-27), 0.80 (3H, br s, Me-28), 0.76 (3H, br s, Me-30).

FABMS m/z (rel. int.)
742 $[M]^+$ (2.8), 727 (3.7), 705 (11.0), 663 (16.3), 647 (13.1), 631 (3.1), 594 (15.4), 579 (2.2), 562 (2.2), 519 (2.9), 445 (100), 426 (3.3), 414 (3.9), 410 (2.2), 408 (2.6), 356 (4.5), 339 (5.0), 312 (8.2), 297 (9.3), 282 (10.7), 266 (12.0), 223 (9.8), 219 (5.6), 208 (12.3), 207 (12.2), 194 (105), 193 (9.3), 180 (14.4), 179 (18.2), 177 (17.1), 168 (10.0), 163 (28.2), 153 (12.5), 146 (29.7), 141 (11.4), 139 (20.4), 128 (36.3), 115 (62.1), 113 (3.7), 111 (3.7), 105 (65.9), 99 (5.4), 93 (21.1), 91 (99.1).

Hydrolysis of compound (3)
The glycoside, **(3)** (80 mg) was dissolved in 80% methyl alcohol and 30 ml of 0.6N hydrochloric acid was added to it. After usual work-up the aglycone (30 mg) obtained was crystallized by petrol-chloroform mixture, m.p. 250-51 °C.

The sugar was identified as glucose by co-chromatography with an authentic sample of glucose.
Analysed for $C_{37}H_{56}O_5$
Calcd.: C, 76.55; H, 9.65
Found: C, 76.52; H, 9.62

Compound (4)
Elution of the column with chloroform-methanol (8:2) gave **(4)** (750 mg). The compound was sparingly soluble in methyl alcohol and in dilute aq. Sodium hydroxide. It gave colourless needles m.p. 190-92 °C from acetone. With alcoholic ferric chloride it gave a deep brown colour.
Analysed for $C_{23}H_{20}O_5(OMe)_2$:
Calcd.: C, 67.31; H, 5.36; OMe, 15.12.
Found: C, 67.19; H, 5.4; OMe, 16.1.

UV λ_{max} (EtOH) nm
237, 275, 305, 335.

IR ν^{mull}_{max} (cm^{-1})
1686, 1631, 1587, 1546, 1504 and 937.

^{1}H-nmr ($CDCl_3$), values on δ scale
1.75 (vinyl methyls), 3.79, 3.82 (s, 2 x OCH_3), 6.0, 6.46 and 6.86 (singlet for three aromatic protons), 2.8 (dd), 2.3 (dd) and 5.2 (t), 4.95 and 5.08 (H_3C-C=CH_2); 3.8 (d, J= 4.0 Hz), 4.18 (d, J= 12.0 Hz), 4.6 (dd, J= 12.0 and 4.0 Hz) and 4.9 (t, J= 4.0 Hz), 12.0 (s, OH).

^{13}C-nmr data of compound (4), values on δ scale
17 (C-8`), 30 (C-4`), 44 (C-12a), 56 (2 x OCH_3), 66 (C-6), 76 (C-5a), 88 (C-5`), 92 (C-10), 101 (C-4), 101.4 (C-8), 104.3 (C-11a), 110 (C-1), 112(C-7`), 143 (C-6`), 144 (C-2), 147 (C-4a), 149.9 (C-3), 156(C-7a), 166 (C-9), 169(C-11), 194 (C12).

Oxime
A mixture of **(4)** (250 mg), hydroxyl amine hydrochloride (250 mg) and dry pyridine (2.5 ml) was heated on the water bath for twenty hours, cooled and poured on ice. After being kept for one hour, the resulting oxime was collected, washed, dried and crystallised from alcohol as colourless needles m.p. 245-46ºC.
Analysed for $C_{23}H_{23}O_7N$
Calcd.: C, 64.94; H, 5.41; N, 3.29.
Found: C, 65.17; H, 5.46; N, 3.45.

Dehydrosumatrol
Iodine (400 mg), dissolved in a little alcohol, was added in the course of 5-10 minutes to a solution of sumatrol **(4)** (300 mg) in boiling alcohol (50 ml), containing sodium acetate (1 gm), the mixture then boiled for two hours, after the addition of greater part of the iodine (about 360 mg) the solution retained a permanent brown colour. Next day the resulting crystalline iodo-derivative (150 mg) was collected, washed and dried, a further quantity of (300 mg) of crude iodo-compound was obtained when the alcoholic filtrate was concentrated and treated with water. The crystalline product gave a deep green ferric reaction and on being heated decomposed at about 200ºC.

A mixture of iodo-derivative (150 mg), acetic acid (3 ml) and zinc dust (270 mg) was refluxed for two hours, after one and half hours more zinc (125 mg) was added. The hot solution was filtered and the zinc washed with boiling acetic acid (2 ml). On cooling the combined solution deposited the dehydro-compound (100 mg) which separated from chloroform-methyl alcohol in tiny, pale yellow prisms, m.p. 190-92ºC. With alcoholic ferric chloride it gave a deep green colour.
Analysed for $C_{23}H_{20}O_7$
Calcd: C, 67.64; H, 4.90.
Found: C, 67.45; H, 4.88.

Compound (5)
Elution of the column with chloroform-methanol (3:1) mixture gave **(5)**. It was crystallized from ethanol as pale-yellow needles m.p.> 250°C.
Analysed for $C_{28}H_{34}O_{15}$
Calcd.: C, 55.08; H, 5.57
Found: C, 54.68; H, 5.49

UV data λ_{max} nm

MeOH	275, 326 sh
$AlCl_3$/HCl	299, 370
NaOAc	272 sh, 309
NaOMe	274, 310

IR, υ^{KBr}_{max} cm^{-1}
3450 (OH), 1680(C=O), 2950, 1500, 1365, 1205, 1140, 800.

Acetylation of compound (5)
Compound **(5)** (40 mg) was heated with pyridine (1.5 ml) and acetic anhydride (3 ml) on a water bath for 2 hours. After usual work-up the crude product was crystallized from chloroform-methanol as colourless needles, m.p. 85-86 °C.

^{1}H-nmr ($CDCl_3$) on δ-scale
7.75 (2H, d, J= 9.0 Hz, H-2′, 6′), 7.28 (1H, d, J = 2.5 Hz, H-8), 6.82 (1H, d, J=2.5 Hz, H-6), 5.20 (1H, q, J=5.0 Hz, 10.0 Hz, H-2), 3.20(2H, m, H-3,3), 2.01-2.48(30H, m, 9xOAc, C-Me), 2.95-3.20 (2H, q, J=12.0 Hz, 4.0 Hz, 17.0 Hz, H-3).

Mass m/z (rel. int.)
[M^+] Absent, 428 [M - Gly], 386 [428-CH_2=C=O],• 344 [386-CH_2=C=O], 302 [344-CH_2=C=O, agl], 286, 283, 273, 245, 236, 153, 137, 111.

Acid hydrolysis on compound (5)
Compound **(5)** (100 mg) was hydrolysed by heating it with 0.2 N HCl over a water bath for 45 minutes. The solid obtained was crystallized with methanol as yellow silky needles, m. p. 245-246°C.
Analysed for $C_{16}H_{14}O_6$:
Calcd.: C, 63.57; H, 4.63
Found: C, 63.06, H, 4.90

Acetylation of compound (5) (Agl)
The aglycone (30mg) was acetylated by the method described earlier. The crude product on crystallization from ethanol gave white shining needles m.p. 135-36 °C.
Analysed for $C_{24}H_{22}O_{10}$:
Calcd.: C, 51.06; H, 4.68
Found: C, 51.26, H, 4.78

Emulsin hydrolysis
Compound **(5)** (5 mg) was hydrolysed with emulsion prepared from almond at 30-40 °C for 70 hours. Liberation of galactose in the hydrolysate was confirmed by paper chromatography.

Identification of sugars
The neutral aq. hydrolysate on paper chromatographic examination as described earlier showed the presence of galactose and rhamnose.

GLC of sugars
The neutral aqueous layer obtained after the hydrolysis of the glycoside **(5)** was extracted with ethyl acetate and evaporated to dryness. The aqueous residue (4mg) was dissolved in dry pyridine and trimethylsilyl ether, prepared by addition of hexamethyldisilazane (1ml) and trimethyl chlorosilazane (0.5 ml). The mixture was separated on a column of 3% OV-I on silanized chromosorb W operated at 180 °C, helium flow rate at 35 ml/minute. The R_t(minute) observed for investigated TMSi derivatives correspond to α- and β-rahmnose 0.21 and 0.28, α- and β-galactose 0.57 and 0.69. The observed R_t-values are in agreement with those of authentic samples of rhamnose and galactose.

Methylation of the glycoside followed by acid hydrolysis
The glycoside **(5)** (40 mg) in dimethyl formamide (1 ml) was treated with CH_3I (2 ml) and Ag_2O (30 mg) and kept for sixty hours at room temperature. The mixture was filtered and the residue washed with little dimethyl formamide. The filtrate was evaporated to dryness and the residue was treated with ethyl alcohol (25 ml). The syrup obtained after removal of ethyl alcohol was hydrolysed with 0.3 N HCl (4 hours). Work-up in the usual manner afforded 5′-(methyl-4′, 5, 7-tri-O-methyleriodic-toyl, 2, 3-di-O-methyl-L-rhamnose and 2, 3, 4, 6-tetra-O-methyl-D-galactose.

Diphenyl methylenedioxy derivatives
5′-methyleriodictoy (25 mg), dichlorodiphenyl methane (0.25 ml) were heated on a metal bath at 185°C for 5 minutes. The mixture in benzene was passed through small column of silica gel and the product crystallized from ethanol as straw-colored needles (15 mg), m.p. 254 °C.
Analysed for $C_{29}H_{24}O_6$:
Calcd.: C, 74.35; H, 5.12
Found: C, 74.48, H, 5.21

Estimation of sugars:
The glycoside **(5)** (30 mg) was hydrolysed by refluxing it with 2% H_2SO_4 for two hours. After cooling overnight, the aglycone was filtered and dried. The ratio of the aglycone to the glycoside was found to be 44.6% indicating the presence of 2 moles of sugar per mole of the aglycone. Somogyis copper-micro method gave the value (1.64 cc) which also corresponds to two moles of sugar per mole of the aglycone.

IV. CONCLUSION:

known constituents have been isolated from roots of **Bergenia ligulata** for the first time. In the present study can be categorized under the Terpenoids, Flavonoids and Trihydroxy benzoic acid. The constituents named as Lanost-7(8)-en-3β-formyl-6β-acetoxy-11β,15β-diol-26-heptanoxy-27-oic acid, Urs-12(13)-en-3β-22α-diol-15β-formyl-21β-glucoside, 9,11-Seco-lanost-20(22)-en-3β-formyl-18-phenoxyoate-12β-D-glucose, Sumatrol and 5`-Methyleriodictyol-3`-O-β-D-galactopyranosyl(1→4)-α-L-rhamno- pyranose. The work was carried out by means of various physical (solvent extraction, radial chromatography) and spectral techniques.

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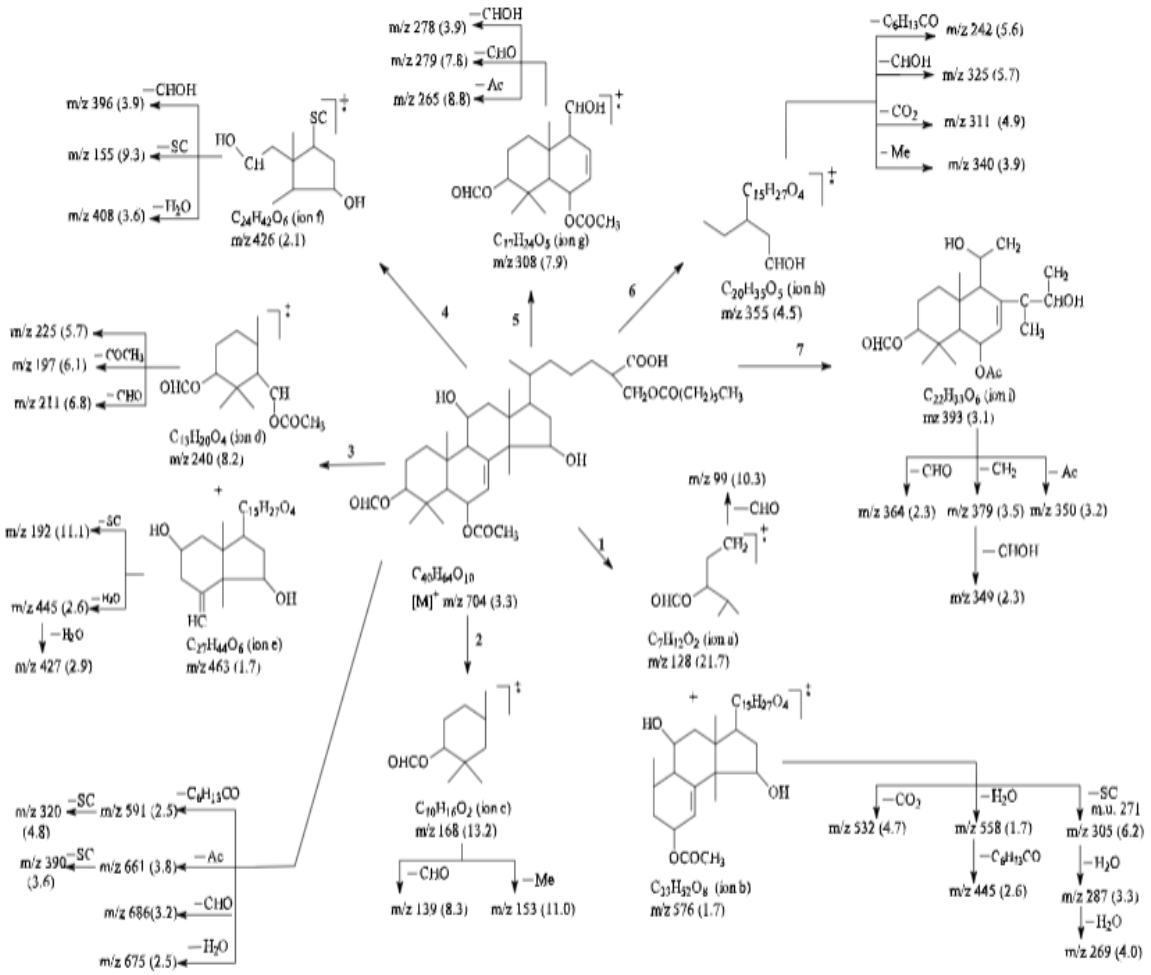
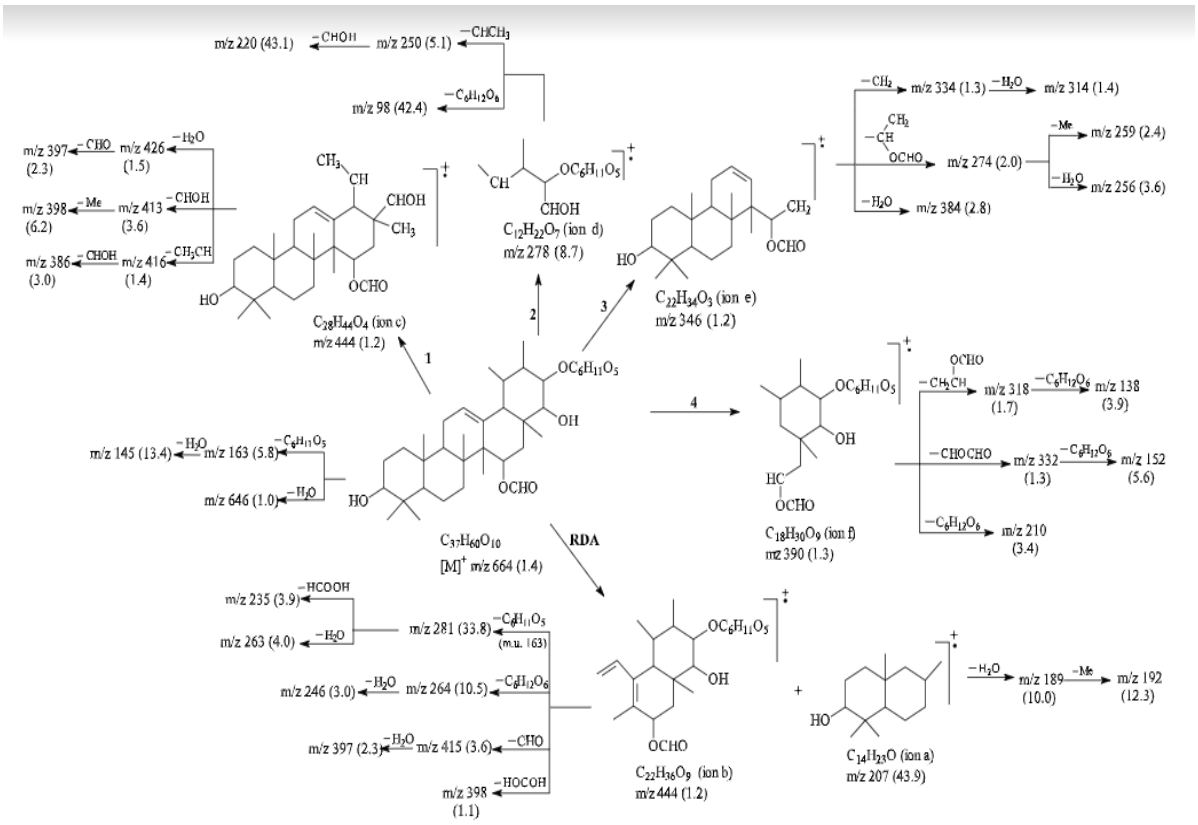
REFERENCES:

[1]. Ruby K, Rajani C, Swapnil S, Jaya D. Polypharmacological Activities of Bergenia Species. *International Journal of Pharmaceutical Sciences Review and Research*. (**2012**); *13(1)*: 100-110.
[2]. Sadat A, Uddin G, Alam M, Ahmad As, Siddiqui BS. Structure activity relationship of bergenin, p-hydroxybenzoyl bergenin, 11-O-galloylbergenin as potent antioxidant and urease

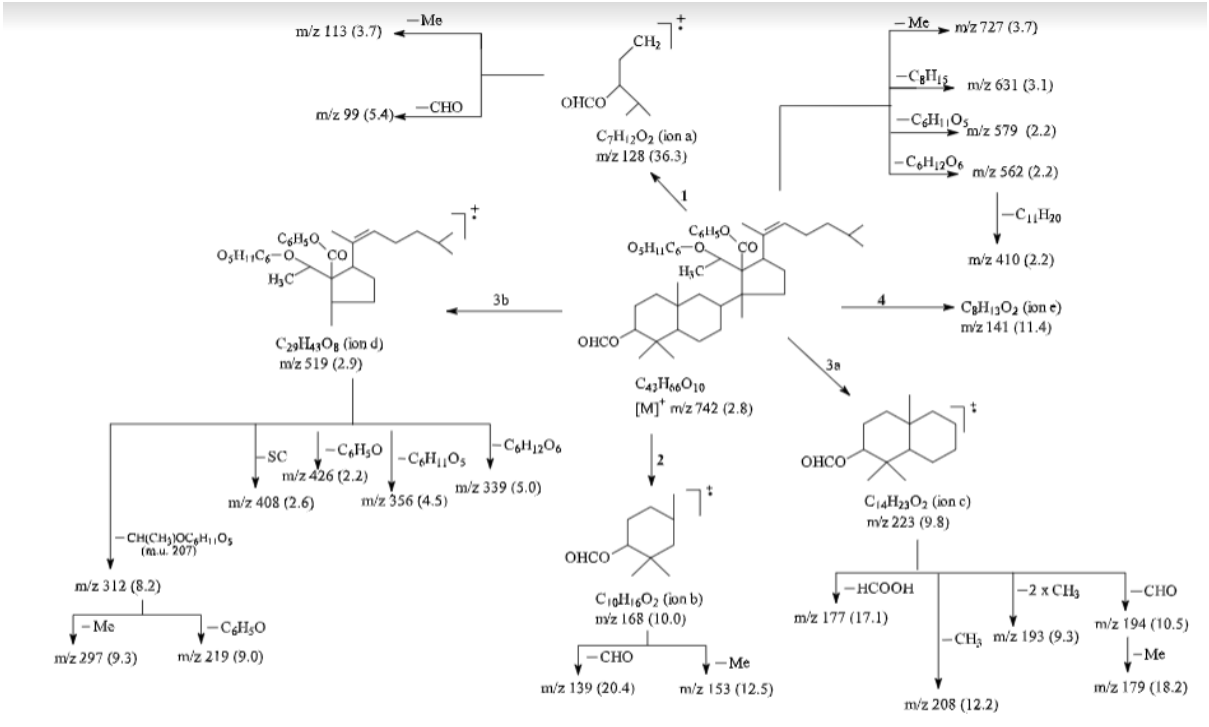
inhibitor isolated from Bergenia ligulate. Natural product research. (2015); 29(24): 2291-4.
[3]. Uddin G, Sadat A, Siddiqui BS. Comparative Antioxidant and Antiplasmodial Activities of 11-O-Galloylbergenin and Bergenin Isolated from Bergenia ligulate. World Applied Sciences Journal. (2013); 27(8): 977-981. DOI:10.5829/idosi.wasj.2013.27.08.1083
[4]. Saijyo J, Suzuki Y, Okuno Y, Yamaki H, Suzuki T, Miyazawa M. a-Glucosidase inhibitor from Bergenia ligulate. Journal of Oleo Science. (2008); 57(8): 431-435. DOI:10.5650/jos.57.431.
[5]. Hendrychova H, Tumova L. Bergenia genus - composition and biological activity. Ceska a Slovenska Farmacie. (2012); 61(5): 203-209.
[6]. Kashima Y, Yamaki H, Suzuki T, Miyazawa M. Insecticidal Effect and Chemical Composition of the Volatile Oil from Bergenia ligulate. Journal of Agricultural and Food Chemistry. (2011); 59(13): 7114-7119. DOI:10.1021/jf201401j
[7]. Nayaka HB, Londonkar RL, Umesh MK, Tukappa A. Antibacterial attributes of apigenin, isolated from Portulaca oleracea L. International journal of bacteriology. (2014); Volume 2014: |Article ID 175851 | https://doi.org/10.1155/2014/175851
[8]. Hasan M.H. M. Extraction, Isolation and Characterization of Valuable Worked on Acacia Tortilis. Egyptian Journal of Chemistry. (2021); Articles in Press- DOI: 10.21608/EJCHEM.2021.75122.3688
[9]. Cheok C. Y, Salman H. A. K, Sulaiman R. Extraction and quantification of saponins: A review. Food Research International. (2014); 59: 16-40.
[10]. Hasan M.H. M. A Review on Chemical Constituents of Acacia Tortilis (Leguminosae). IOSR Journal of Pharmacy. (2021); 11(3): Series. I, 10-21.
[11]. Wilson K, Walker J. Principles and Techniques of Practical Biochemistry (5th Ed.). E.J. Wood Press. (2006); https://doi.org/10.1002/bmb.2002.494030030062
[12]. Satoshi T. A Journey of Twenty-Five Years through the Ecological Biochemistry of Flavonoids. Bioscience, Biotechnology, and Biochemistry. (2007); 71(6): 1387-1404. https://doi.org/10.1271/bbb.70028.
[13]. Jih Ru Hwu, Ke-Yung King. Versatile reagent ceric ammonium nitrate in modern chemical synthesis. Current Science. (2001); 81(8): 1043-1053.
[14]. Oyvind M A, Kenneth R M. Flavonoids (Chemistry, Biochemistry and Applications). CRC Press. (2005); Boca Raton, DOI https://doi.org/10.1201/9781420039443
[15]. Zakia, Khanam; Farook, Adam; Ompal, 0Singh; Jamal Ahmad. A Novel Acylated Flavonoidic Glycoside from The Wood of Cultivated Acacia Nilotica (L.) Willd. Ex. Delile. Bioresource. (2011); 6(3).
[16]. Hasan, M.H. M. Flavonoid Glycosides from Acacia Tortilis. Merit Research Journal of Medicine and Medical Sciences. (2020); 8(10): 575-580.
[17]. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids, Springer. (2012); New York.
[18]. Ionel C. Per-O-methylation reaction for structural analysis of carbohydrates by mass spectrometry. Analytica Chimica Acta. (2006); 576(2): 147-155.
[19]. John A. Adamovics. Chromatographic analysis of pharmaceuticals. Publisher: [Place of publication not identified]: Routledge. (2017).
[20]. Lederer E, Lederer M. Chromatography. 115, Elsevier, London (1955).
[21]. Chihiro I, Masataka I, Naoki K, Harukuni T, Tetsurou H, Hoyoku N, Hiroshi F. Chemical Constituents of Millettia taiwaniana: Structure Elucidation of Five New Isoflavonoids and Their Cancer Chemopreventive Activity. J. Nat. Prod. (2004); 67(7): 1125–1130. https://doi.org/10.1021/np030554q.


m/z 278 (3.9) −CHOH
m/z 279 (7.8) −CHO
m/z 265 (8.8) −Ac
m/z 396 (3.9) −CHOH
m/z 155 (9.3) −SC
m/z 408 (3.6) −H2O
C24H42O6 (ion f)
m/z 426 (2.1)
C17H24O5 (ion g)
m/z 308 (7.9)
−C6H13CO m/z 242 (5.6)
−CHOH m/z 325 (5.7)
−CO2 m/z 311 (4.9)
−Me m/z 340 (3.9)
C15H27O4
C20H35O5 (ion h)
m/z 355 (4.5)
m/z 225 (5.7)
m/z 197 (6.1) −COCH3
m/z 211 (6.8) −CHO
C13H20O4 (ion d)
m/z 240 (8.2)
C40H64O10
[M]+ m/z 704 (3.3)
C22H33O6 (ion i)
m/z 393 (3.1)
m/z 364 (2.3) −CHO
m/z 379 (3.5) −CH2
m/z 350 (3.2) −Ac
m/z 349 (2.3) −CHOH
m/z 192 (11.1) −SC
m/z 445 (2.6) −H2O
m/z 427 (2.9) −H2O
C21H44O6 (ion e)
m/z 463 (1.7)
m/z 99 (10.3) −CHO
C7H12O2 (ion a)
m/z 128 (21.7)
C10H16O2 (ion c)
m/z 168 (13.2)
m/z 139 (8.3) −CHO
m/z 153 (11.0) −Me
m/z 320 (4.8) −SC
m/z 390 (3.6) −SC
m/z 591 (2.5) −C6H13CO
m/z 661 (3.8) −Ac
m/z 686 (3.2) −CHO
m/z 675 (2.5) −H2O
C33H52O8 (ion b)
m/z 576 (1.7)
m/z 532 (4.7) −CO2
m/z 558 (1.7) −H2O
m/z 445 (2.6) −C6H13CO
m/z 305 (6.2) −SC m.u. 271
m/z 287 (3.3) −H2O
m/z 269 (4.0) −H2O

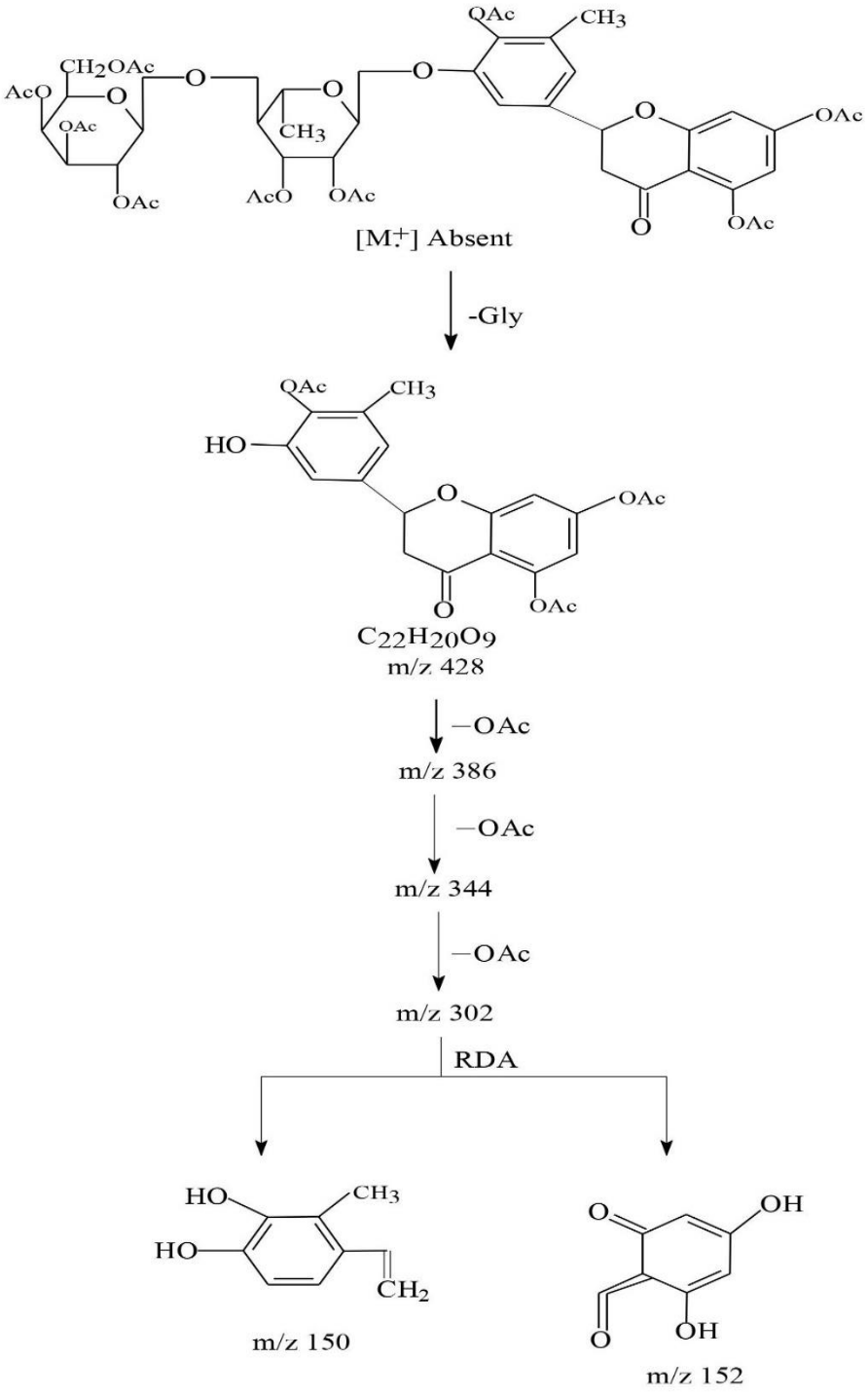
Scheme - I



Scheme - II



Scheme - III


[M.+] Absent
-Gly
C22H20O9
m/z 428
—OAc
m/z 386
—OAc
m/z 344
—OAc
m/z 302
RDA
m/z 150
m/z 152

Scheme-IV