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ISOLATION, PURIFICATION AND STRUCTURAL ELUCIDATION OF FLAVONOIDS FROM METHANOL EXTRACT OF ARIEL PARTS OF *DERRIS BREVIPES* (BENTH.) BAKER.

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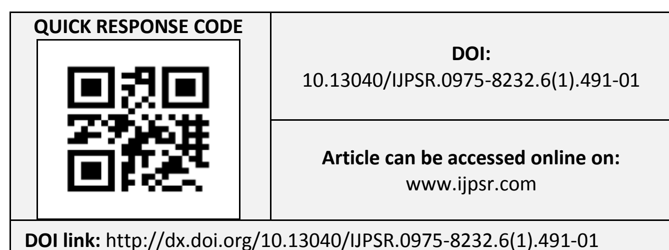
ABSTRACT: Two flavonoids were isolated by performing column chromatographic separation of methanol extract of ariel parts of *Derris brevipes* (Benth) Baker. Structural elucidation of purified compounds was done on the basis of spectral analysis using IR, ^{1}H NMR, ^{13}C NMR 2D NMR spectroscopy and Mass spectrometry. The structures of the flavonoids were elucidated as 2', 4'-dihydroxy-4-methoxy-3'-prenylchalcone (compound1) and Leutolin-3'-O-xilofuranose (compound 2).

INTRODUCTION: The genus *Derris* of the family Leguminosae, tribe Tephrosieae, subfamily Papilonoidae, has recieved much attention from phytochemical perspective as this species of this tribe is known for its large content of flavonoids [1]. *Derris* species are distributed over the tropical areas of asia and east africa. All species are woody perennials and mostly climbers. Abundant flavonoids and many other compounds of phytochemical interest are isolated from *Derris* plants and can be categorized into aurones, chalcones, coumarins, flavans, flavanones, flavones, glycosides, isoflavones, pterocarpans, quinoids, rotenoids, steroids, stilbenes and terpenoids.



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Many kinds of *Derris* flavonoids possess wide varieties of biological activities. *Derris* is popular ichthyotoxic [2], insecticide [3], pesticides [4], molluscide [5], antiviral [6] and have antibacterial [7] properties. *Derris* is also used as analgesic [8], antipyretic [9], for arthistic symptoms [10], antidysenteric [11], antidiuretic [12], antispasmodic [13], counter-irritant [14], for purification of blood [15], for treatment of leprosy [16], sore throat [17] and rheumatism [18].

Derris species from India are known for medicinal value and different parts of species have been used in folk medicine for bronchitis [19], cough, rheumatoid arthritis [20], diabetes and as anti- fertility agent [21]

Derris brevipes is a climbing shrub, distributed widely in India. Literature review revealed that chemical investigation of the aerial part of Derris brevipes has not been carried out. To discover naturally occurring bioactive compounds from Derris brevipes the methanol extract of ariel parts was column chromatographed. Column chromatographic separation resulted in flavonoids**.**

MATERIALS AND METHODS: Air-dried ariel parts of *Derris brevipes* (650 g), were powdered in a wiley mill and extracted with an order of solvents in increasing polarity petroleum ether, chloroform, methanol and water respectively. The extracts were filtered and subsequently concentrated by using rotary vacuum evaporator which afforded green solid for petroleum ether extract, dark green solid for chloroform extract brown solid for both methanol and aqueous extract.

Methanol extract was column chromatographed using a silica gel column having diameter of 50 mm and length 1 meter, initially with solvent chloroform followed by combination of chloroform and methanol. Polarity of solvent system was increased by gradually adding methanol in chloroform. Five parts of methanol were added to 95 parts of chloroform similarly polarity was increased by adding methanol in multiples of 5 to chloroform and reducing quantity of chloroform in proportion. Eluents of all solvent combinations were analysed on TLC and eluents which showed similar TLC patterns were mixed.

Further purification of isolated compounds was done by preparative thin layer chromatography. Compound 1 was crystallized from chloroform-methanol as yellow-orange needles, m.p. 157-158 ^{0}C. It gave a pale pink colour in the cyanidin test indicating it to be a flavonoid. Both compounds also gave positive Ferric chloride test indicating to be flavonoids.

RESULTS AND DISCUSSION: High resolution mass spectrometry indicated the molecular formula $C_{21}H_{22}O_4$ for compound1. IR spectrum shows the presence of conjugated carbonyl group at 1626 cm^{-1} and the presence of hydroxy group at 3365 cm^{-1}. ^{13}C NMR peak at δ 194.44 also confirmed the existence of carbonyl group. In ^{1}H NMR peaks at δ 7.65 (1H, d, J = 14.3 Hz) and 7.97 (1H d, J 15 Hz) which showed trans effect is characteristic pattern of chalcone a subtype of flavonoid. ^{13}C NMR peak at δ 191.54 represents carbonyl group of chalcone moiety. Mass spectrum at m/z 295 [M^+-43] shows the loss of CH moiety. Spectrum at m/z 283 [M^+-55] represents the loss of dimethyl allyl group. This can be deduced from ^{1}H NMR at δ 5.67 (1H, t, J = 7.1 Hz) representing an allyl proton, δ 1.71 (3H, s) and δ 1.81 (3H, s) indicating two methyl groups. Mass spectrum at m/z 77 indicates the presence of phenyl group and m/z 91 represents Ph CH_2 this can be confirmed by ^{1}H NMR at δ 3.52 (2H, d, J=7.1 Hz) indicating benzylic proton and at δ 7.0-8.2 representating protons on benzene ring. Molecular formula $C_{21}H_{22}O_4$ (molecular weight derived from MS data is 338).

^{1}H NMR signal at δ 3.57 (3H, s) indicates the methoxy group. Signal at δ 10.31 (1H broad singlet) shows hydroxy proton which can be confirmed by IR signal at 3365 cm^{-1}. Signal at δ 13.45 (1H, s) represents the hydroxy group chelated with carbonyl group. Signals at δ 7.69 (2H, d, J=8.8 Hz) and δ 7.21 (2H, d, J=8.8 Hz) indicate para substitution on ring of benzene. The two protons at δ 7.69 appear at lower field than two protons at δ 7.21 indicates that two protons at δ 7.21 locating next to quarternary carbon connecting with electron donating group which could be hydroxy group or methoxy group.

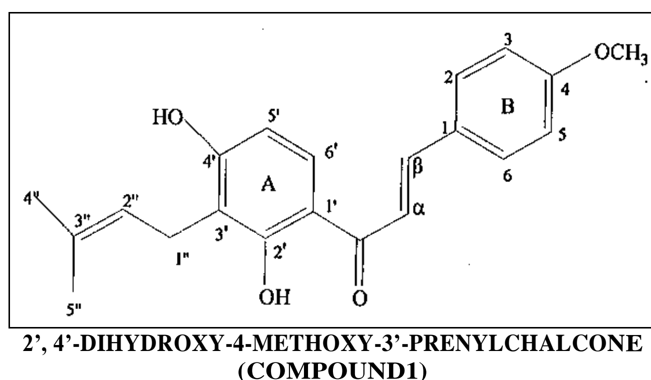
From COSY spectrum, two protons at δ 7.21 correlated to carbon at δ 115.763 while two protons at δ 7.85 correlate to carbon at δ 131.376. From HMBC, methoxy proton shows correlation with quarternary carbon at δ 163.762. Correlation of two protons at δ 7.21 and δ 7.65 with quarternary carbon at δ 165.831 is also shown in HMBC. Therefore, electron releasing group located on chalcone ring B should be methoxy group.

Mass spectrum at m/z 161 [M^+-177] is also confirmed the presence of methoxy group on ring B of chalcone. The rest substitution groups are hydroxy group, hydroxy chelated with carbonyl of chalcone and dimethylallyl group. These three substitution groups are located on ring A of chalcone. Chelated hydroxy at δ 13.45 (1H, s) is assigned to be located on position 2'. From ^{1}H NMR spectrum, there are two aromatic protons left. These two protons have correlation with each other shown by COSY spectrum.

This means these two protons are located on the adjacent carbons. Proton at δ 6.35 (1H, d, J = 9.0 Hz) is located on carbon at δ 109.615 and another

proton at δ 7.81 (1H, d, J = 9.0 Hz) is located on carbon at δ 132.115 as shown by HMQC. One proton at δ 7.81 appears at lower field than another proton at δ 6.35. This means proton at δ 7.81 is adjacent to electron withdrawing group which is carbonyl group of chalcone. Therefore proton at δ 7.81 is assigned as 6' proton and another adjacent proton at δ 6.35 is 5' proton. That 5' proton appears at higher field than other aromatic protons is due to the effect from electron releasing group.

Therefore, another hydroxy group δ 10.31 (1H, broad singlet) should locate at 4' position which is ortho position of 5' proton. The rest substitution group is dimethylallyl group which should locate on the 3' position.



2', 4'-DIHYDROXY-4-METHOXY-3'-PRENYLCHALCONE (COMPOUND1)

Compound 2 was isolated from column chromatographic fractions having solvent combination of chloroform: methanol in a proportion of 5:5 to 5:7 and was purified by Preparative thin layer chromatography. The absorption band at 3262 cm^{-1} is characteristic of OH stretching. Absorptions recorded at 1185 cm^{-1}, 1133 cm^{-1}, 1079 cm^{-1} and 1021 cm^{-1} are characteristic of the CO stretching. The absorption at 1651 cm^{-1} is characteristic carbonyl conjugate stretch. Analysis of the mass spectrum obtained from compound 2 shows that the compound has a molecular formula containing $C_{20}H_{18}O_{10}$.

The ^1H NMR spectrum shows peaks at δ 7.48 (m), δ 7.45 (m) and δ 6.91 (d) attributed to hydrogen atoms (H- 6 '), (H- 2') and (H- 5 ') respectively, of ring B of flavanoid moiety. The peak at δ 6.75 is assigned to hydrogen (H -3) of ring C of flavonoid moiety. The peaks at δ 6.80 (d) and 6.43 (d) were assigned to hydrogen atoms (H -8), and (H -6), respectively, of ring A. The peaks at δ 5.0 (d) to δ 2.5 are characteristic of glycoside residue. The ^{13}C and DEPT spectrum shows a signal at δ C 181.9 (C-4) assigned to the carbonyl carbon. Six peaks recorded at δ 164.5 (C -2), δ 162.7 (C- 7), δ 161.1 (C-5), δ 157.0 (C- 9) , δ 149.9 (C- 4 ') and δ 145.8 (C- 3 ') are assigned to oxygenated aromatic carbons .

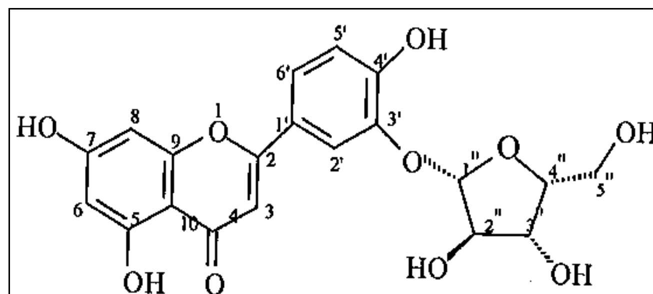
The peaks at δ 121.4 (C- 1 ') and δ 105.4 (C- 10) are of non- aromatic hydrogenated carbons. HSQC spectra shows the correlation signal of protons δ 7.48 (H-6 '), δ 7.45 (H-2'), δ 6.91 (H-5 '), δ 6.75 (H-3), δ 6.80 (H-8), and δ 6:43 (H-6) with peaks at δ 119.2 (C-6 '), δ 113.6 (C-2'), δ 116.0 (C-5 '), δ 103.1 (C-3), δ 94.6 (C-8) and δ 99.5 (C-6), respectively. The signal at δ 5.08 (H-1") correlates with that recorded in δ 100.3 (C-1") signal, this signal is of anomeric carbon and hydrogen respectively of glycoside residue. The peaks at δ 3.28 (H-4") δ 3.18 (H-3"), δ 3.16 (H-2") display correlation with the peaks at δ 69.2 (C-4"), δ 76.2 (C-3") and δ 72.9 (C-2"), respectively, and were assigned to atoms of mono-hydrogenated carbon glycoside residue.

Finally, the peaks recorded at δ 3.78 (H-5") is in correlation with δ 65.8 (C-5") signal assigned to the methylene carbon of the glycoside residue. As per the contour map HMBC shows correlations of the proton signal at δ 7.48 (H - 6 ') with the carbon signals at δ 164.5 (C -2), δ 149.9 (C- 4 ') and δ 113.6 (C- 2'). The hydrogen peak at δ 7.45 (H - 2 ') correlates with the carbon peaks at δ 164.5 (C -2), δ 149.9 (C- 4 '), δ 145.8 (C- 3') and δ 119.2 (C- 6 ').

The peak of proton at δ 6.91 (H- 5 ') correlates with the carbon peak at δ 119.2 (C- 6'), δ 145.8 (C- 3 ') and δ 121.4 (C- 1'). These HMBC correlations of the peaks δ 7:48 (H- 6 '), δ 7:45 (H- 2') and δ 6.91 (H- 5 ') correspond to an aromatic ring containing hydroxyl groups at positions meta and para to the substituent at C- 1 '. The peak at δ 6.75 (H- 3) shows HMBC correlation to the carbon peak at δ 181.9 (C-4), δ 164.5 (C-2), δ 121.4 (C- 1') and δ 105.4 (C- 10). The peak at δ 6.80 (H -8) shows HMBC correlations to the carbon signals at δ 162.7 (C- 7), δ 157.0 (C- 9), δ 105.4 (C -10) and δ 99.5 (C- 6). The peak at δ 6:43 (H -6) shows HMBC correlations with carbon signals at δ C162.7 (C- 7),

161.1 (C-5), 105.4 (C -10) and 94.6 (C- 8). The COSY contour map shows a strong correlation between the signals of proton at 7.48 (H-6 ') and 6.91 (H-5'), with the coupling constant (J = 8.4 Hz) which are characteristic vicinal hydrogen atoms in an aromatic ring. A weak correlation between the signals of protons δ 6.80 (H-8), δ 6.43 (H-6) (J = 2.0 Hz) is characteristic of hydrogen atoms in the meta position on the aromatic ring.

The COSY contour map also shows correlations between the signals of protons at 5.08 (H-1") is 3.16 (H-2") (J = 8.0 Hz) that is characteristic of hydrogen atoms in the positions anti-periplanar the glycoside ring. The NMR analyzes of compound 2 are consistent with the corresponding data of a derivative of luteolin.



LEUTOLIN-3'-O-XILOFURANOSE (COMPOUND 2)

TABLE 1: ^{1}H NMR AND ^{13}C NMR ASSIGNMENTS OF COMPOUND 1

Sr.No.	Position	Chemical shift ppm	
		^{1}H NMR	^{13}C NMR
1	1	-	127.694
2	2, 6	7.85 (2H, *d*, *J* = 8.8 Hz)	131.376
3	3, 5	7.01 (2H, *d*, *J* = 8.8 Hz)	114.834
4	4	-	161.846
5	1’	-	113.105
6	2’	-	163.995
7	3’	-	114.834
8	4’	-	162.8`12
9	5’	6.45 (1H, *d*, *J* = 9.0 Hz)	107.75
10	6’	8.05 (1H, *d*, *J* = 9.0 Hz)	130.35
11	1’’	3.22 (2H, *gem*, *J* = 7.1 Hz)	21.67
12	2’’	5.16 (1H, *t*, *J* = 7.1 Hz)	122.72
13	3’’	–	
14	4’’	1.70 (3H, *s*)	25.90
15	5’’	1.60 (3H, *s*)	18.12
16	α	7.75 (1H, *d*, *J* = 15.3 Hz)	118.8
17	β	7.83 (1H, *d*, *J* = 15.3 Hz)	144.0
18	4-OCH_3	3.81 (3H, *s*)	55.80
19	2’-OH	13.95 (1H, *s*)	-
20	4’-OH	10.60 (1H, *broad singlet*)	-
21	CO	-	192.14

TABLE 2: PROTON-PROTON CORRELATION IN NMR SPECTRUM OF COMPOUND 1 USING COSY TECHNIQUE AND THEIR ASSIGNMENTS.

S. No.	Proton position (chemical shift)	Proton position (chemical shift)
1	2, 6 (7.85)	3, 5 (7.01)
2	5’ (6.45)	6’ (8.05)
3	1’’ (3.22)	2’’ (5.16)
4	4’’ (1.70)	-
5	5’’ (1.60)	-
6	α (7.75)	β (7.83)
7	4-OCH_3	-
8	2’-OH	-
9	4’-OH	-

TABLE 3: HMBC CORRELATION IN NMR SPECTRA OF COMPOUND 1

Sr.No.	Proton position (chemical shift)	Carbon position (chemical shift)
1	2, 6 (7.85)	2, 6 (131.376), β (144.049), 4 (161.846)
2	3, 5 (7.01)	3, 5 (114.834), 1 (127.694), 4 (161.846)
3	5’ (6.45)	1’ (113.105), 4’ (162.812)
4	6’ (8.05)	1’ (113.105), 4’ (162.812), CO (192.144)
5	1’’ (3.22)	3’ (114.834), 2’’ (122.740), 3’’ (130.919)
6		4’ (162.812), 2’ (163.995)
7	2’’ (5.16)	5’’ (18.121), 4’’ (25.901)
8	α (7.75)	CO (192.144)
9	β (7.83)	CO (192.144)
10	4-OCH_3 (3.81)	4 (161.846)
11	2’-OH (13.95)	1’ (113.105), 2’ (163.995), CO (192.144)
12	4’’-CH_3 (1.70)	2’’ (122.740), 3’’ (130.919)
13	5’’-CH_3 (1.60)	2’’ (122.740), 3’’ (130.919)

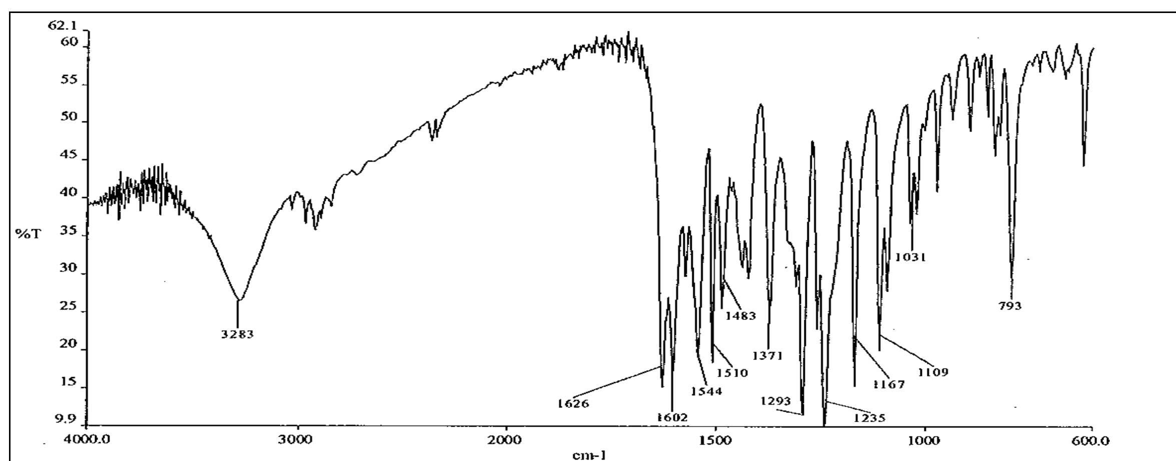


FIG.1: IR SPECTRUM OF COMPOUND 1

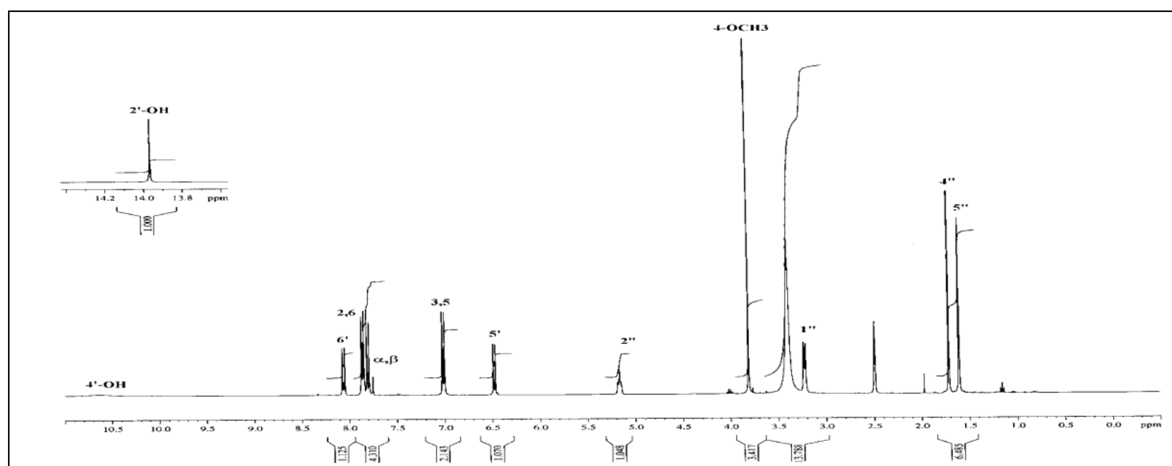


FIG. 2: ^{1}H NMR SPECTRUM OF COMPOUND 1

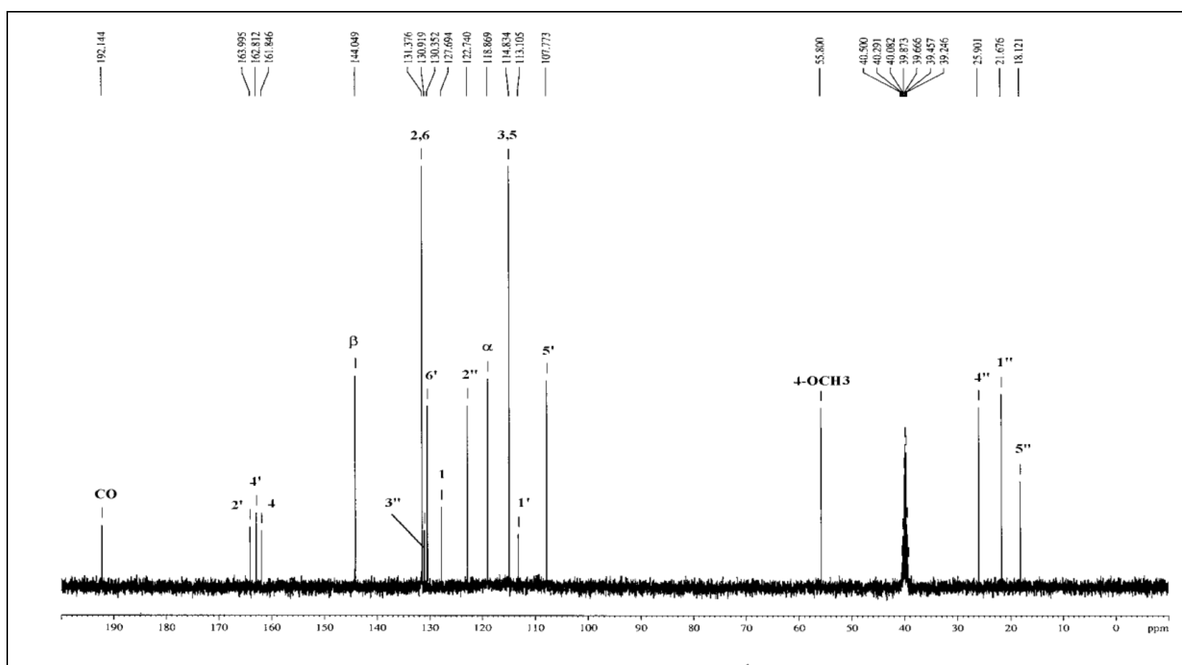


FIG.3: ^{13}C NMR OF COMPOUND 1

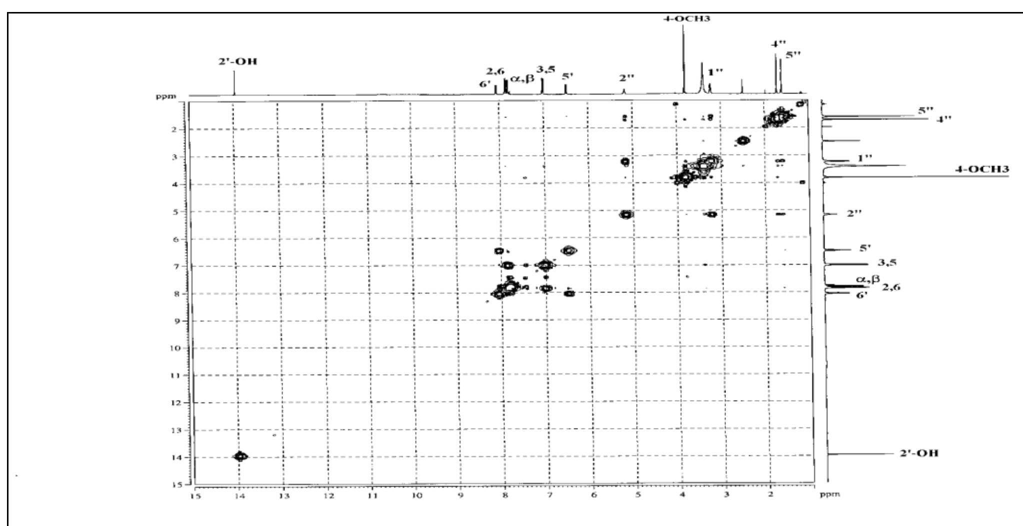


FIG. 4: COSY SPECTRUM OF COMPOUND 1

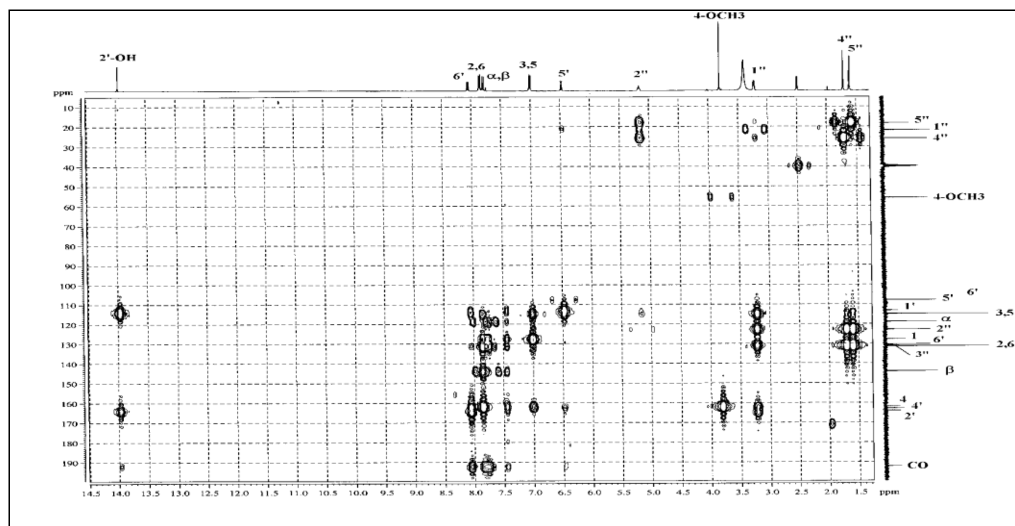


FIG.5: HMBC SPECTRUM OF COMPOUND 1

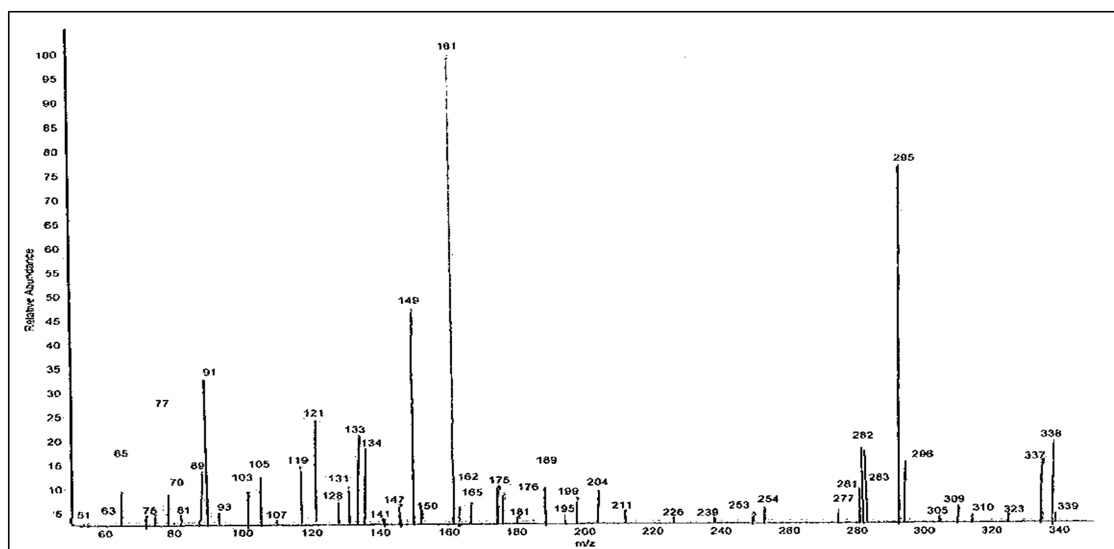


FIG.6: MASS SPECTRUM OF COMPOUND 1

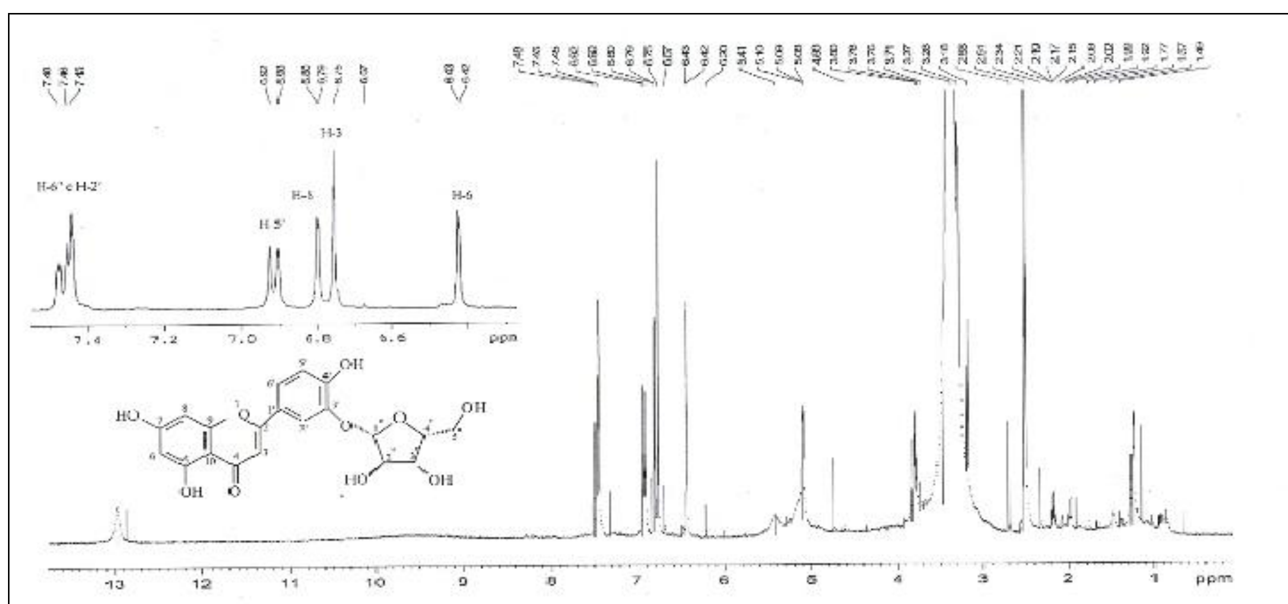


FIG.7: ^{1}H NMR SPECTRA OF (COMPOUND 2) LEUTOLIN-3-O-β-XILOFURANOSIDE

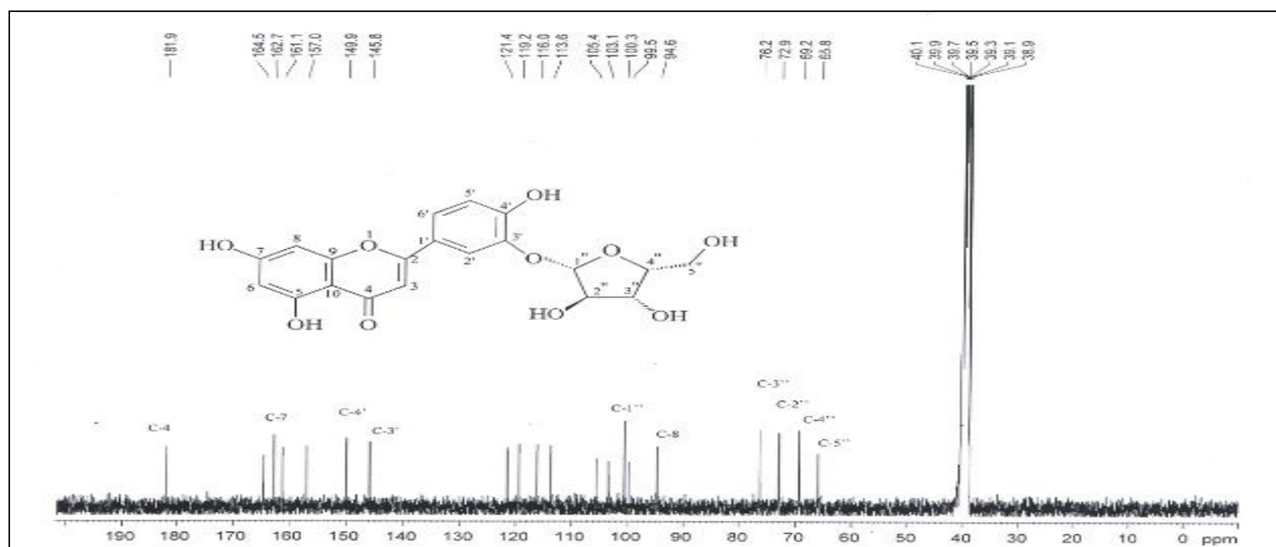


FIG. 8: ^{13}C CMR SPECTRUM OF (COMPOUND 2) LEUTOLIN-3-O-XILOFURANOSE

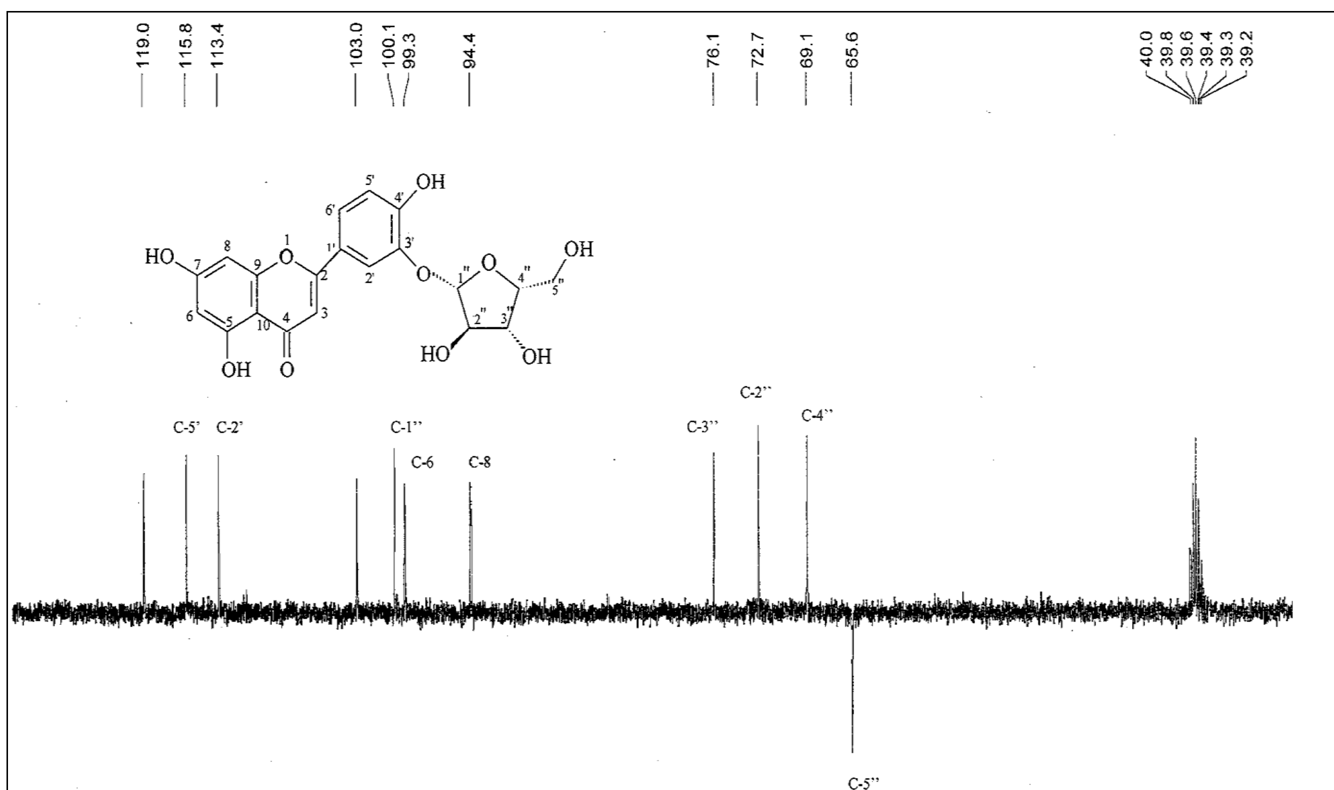


FIG.9: DEPT SPECTRA OF (COMPOUND 2) LEUTOLIN-3-O-XILOFURANOSE

FIG.10: COSY CORELATION SPECTRUM OF (COMPOUND 2) LEUTOLIN-3-O-XILOFURANOSE

FIG.11: HSQC SPECTRUM OF (COMPOUND 2) LEUTOLIN-3-O-XILOFURANOSE

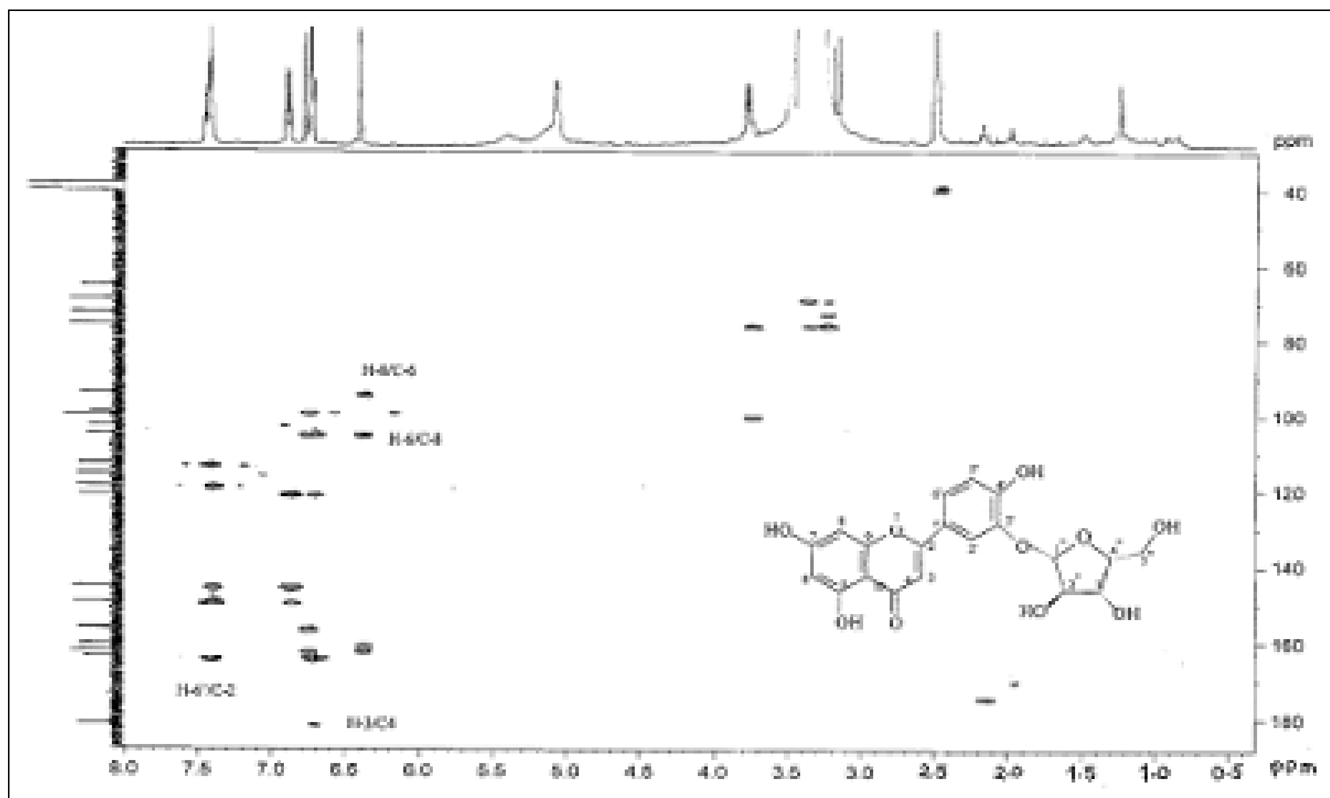


FIG.12: HMBC SPECTRUM OF (COMPOUND 2) LEUTOLIN-3-O-XILOFURANOSE

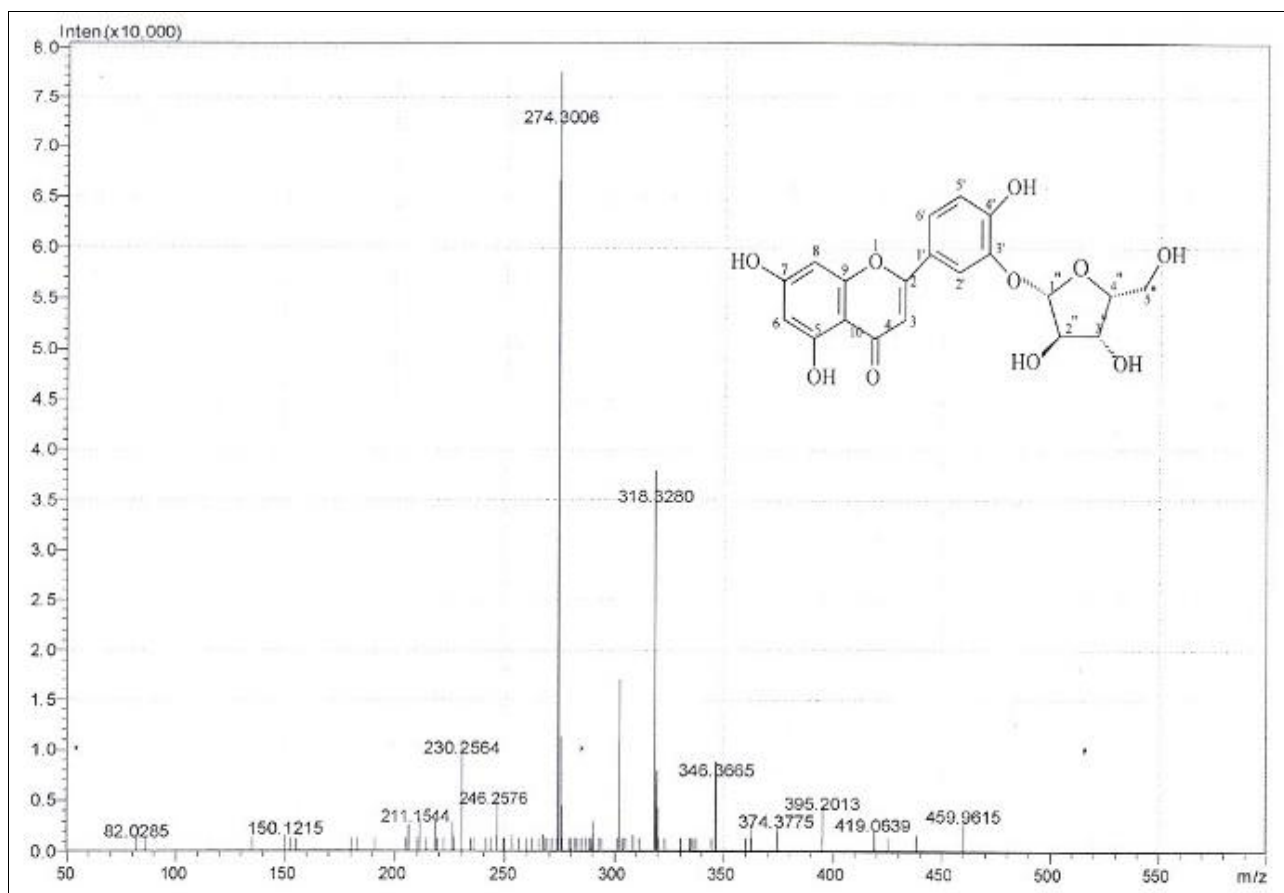


FIG.13: MASS SPECTRUM OF (COMPOUND 2) LEUTOLIN-*3-O*-XILOFURANOSE

CONCLUSION:

The results of IR, 1H, 13C NMR and 2D NMR spectroscopy for compound 1 were analysed and were consistent with the structure 2',4'-dihydroxy-4-methoxy-3'-prenyl chalcone. Mass spectrum and fragmentation patterns confirmed the structure of the compound as 2', 4'-dihydroxy-4-methoxy-3'-prenyl chalcone. Similarly for compound 2 spectroscopic data was consistent with the structure of Leutolin-3'-O-xilofuranose Mass spectrum and fragmentation patterns confirmed the structure of the compound as Leutolin-3'-O-xilofuranose.

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