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Phenolic glycosides from roots of *Clerodendrum myricoides*

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Abstract

Clerodendrum myricoides is a plant traditionally used for treatment of rheumatism, asthma, inflammatory diseases, coughs, skin diseases, vermifuge, febrifuge and malaria. Phytochemical screening of the CH₂Cl₂/CH₃OH (1:1) and CH₃OH (100%) root extracts of the plant revealed the presence of phenolic compounds, steroids, flavonoids, saponins and terpenes. Chromatographic separation of CH₂Cl₂/CH₃OH (1:1) root extract of *C. myricoides* yielded a new phenylpropanoid glycoside (1) together with a known phenylpropanoid glycoside, verbascoside-6^{III}-apiose (2). The structures of these compounds were determined using spectroscopic techniques (UV-Vis, IR and NMR, 1D and 2D).

Keywords: *C. myricoides*, phenylpropanoid glycosides, flavanoides, steroids, terpenoids.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and a number of drugs have been isolated from natural sources. Higher plants as sources of medicinal compounds have continued to play a leading role [1]. *Clerodendrum myricoides* (Verbenaceae, fig. 1) is one of the traditional medicinal plants in Ethiopia known by the name 'Marasissa' (Afaan Oromoo), 'Algga' (Dawiro) [2] and 'Misirich' (Amharic) [3]. In Ethiopia, *C. myricoides* is widely distributed in the flora of Tigray, Gonder, Wollo, Shewa, Arsi, Welega, Illu Abba Boor, Kefa, Gamo Gofa, Sidamo and Harerge [4]. The bark of *C. myricoides* is used to treat abdominal pains, malaria and snake bites [4]. Root decoction is applied as antidotes in poisonings [5]. Bathing over the steam after boiling the leaves of *C. myricoides* is used for the treatment of epilepsy [6]. Roots and leaves of *C. myricoides* are used to treat gonorrhoea, rabies, measles, glandular TB, colic, eye disease, malaria, swellings in the body, wound dressings, hemorrhoids, asthma and as aphrodisiac [4, 7].

Earlier studies on the chemical constituents of the genus *Cleodendrum* indicated the presence of phenylpropanoids [8, 9], flavonoids [10], diterpenes [11], triterpenoids [12] and steroids [13]. As part of our ongoing study on Ethiopian medicinal plants, we hereby present a comprehensive phytochemical analysis on the roots of *C. myricoides*.



Fig 1: *C. myricoides* (Misirich) (picture taken by Habdolo.E, Dec, 2014)

2. Experimental Section

2.1 General experimental materials

UV-Vis spectrum was measured with GENESY's spectrometer (200-400 nm) in methanol at room temperature. Infrared (KBr pellet) spectrum was recorded on Perk-Elmer BX infrared spectrometer in the range 400-4000 cm^{-1} . Nuclear Magnetic Resonance (NMR) analysis was recorded on a Bruker Avance 400 MHz spectrometer with tetramethylsilane as internal standard. Structural assignments were done on the basis of COSY, gHMBC and gHMBC spectra. Thin Layer Chromatography (TLC) was done using silica gel 60 F254. Column chromatography was performed on silica gel 60 (60-100 mesh).

2.2 Plant material

The plant material was collected from Oromia Region, Arisi zone; Shashemene area located 248km from Addis Ababa, capital of Ethiopia. The collected plant specimen was identified and authenticated by botanist Reta Regasa, Department of Biology, Hawassa College of Teachers Education. Specimen was deposited at the herbarium of Hawassa College of Teacher Education, Hawassa, Ethiopia.

2.3 Extraction and Isolation

The collected specimens were dried and grounded into fine powder with the help of mortar and pestle. The grounded roots (500 g) were extracted by cold percolation with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (1:1) three times for 24 h while shaking at speed of 230 r/min and temperature controlled at 28.0°C. The marc left was further extracted with methanol as above. The extract was concentrated using rotary evaporator (40°C) to yield a brownish crude extract (46.8 g, 9.36%). The crude extract obtained was screened for the presence of secondary metabolites following the standard protocols given by Harborne [14]. The dried root extract (46.8 g) was subjected to column chromatography (260 g silica gel) with increasing gradient of ethyl acetate in *n*-hexane as eluent.

2.4 Phytochemical Screening test

Phytochemical screening test was carried out on the crude extract of $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (1:1) using standard procedures to identify the type of secondary metabolites present in crude extract.

2.4.1 Test for alkaloids

One mL of 1% HCl was added to 3 mL of the test extract in a test tube. The mixture was heated for 20 min, cooled and filtered. Then 1mL of the filtrate was tested with 0.5 mL Wagner's, Hager's and Mayer's reagents. Formation of reddish brown precipitate for Dragendorff's and Wagner's reagents, yellow precipitate for Hager's and cream precipitate for Mayer's indicated the presence of alkaloids [15].

2.4.2 Test for flavonoids

Flavonoids were determined by Mg-HCl reduction test. A piece of magnesium ribbon (powder) and 3 drops of conc. hydrochloric acid were added to 3mL of the test extract. A red coloration indicated the presence of flavonoids. Five milliliters of dilute ammonia solution was added to 5 mL of the aqueous filtrate of extract followed by the addition of 1mL concentrated H_2SO_4 . A yellow coloration indicated the presence of flavonoids. The yellow color disappeared on standing [16].

2.4.3 Test for phenols

The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.4.4 Test for glycosides

To 2 mL of extract 2 drops of Molisch's reagent was added and shaken well. Two mL of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates [17].

2.4.5 Test for terpenoids (Salkowski test)

About 5 mL of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was added. A reddish brown coloration at the interface confirmed the presence of terpenes [17].

2.4.6 Test for tannins

About 0.2 g of the dried powdered samples was boiled in 10 mL of distilled water in a test tube and then filtered. Addition of 0.1% FeCl_3 solution resulted in a characteristic blue, blue-black, green or blue-green color which confirmed the presence of tannins [17].

2.4.7 Test for saponins

About 0.2 g of powdered sample extract was boiled in 2 mL of distilled water on a water bath and filtered. A fraction of aqueous filtrate about 1 mL was mixed with 2 mL of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously. Formation of an emulsion confirms the presence of saponins [17].

3. Results and Discussion

Preliminary screening tests of the crude $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (1:1) revealed the presence of flavonoids, glycosides, terpenes, phenols, saponins, tannins and absence of alkaloids (Table 1). Column chromatographic separation of the root extract afforded two phenylpropanoid glycosides (**1**, **2**).

Table 1: Phytochemical screening of the roots of *C. myricoides*

Chemical component	Reagent used	Present(+)/Absent (-)
Alkaloids	Dragendorff's reagents	-
Flavonoids	Dilute ammonia solution	+
Phenols	FeCl_3	+
Glycosides	Conc HCl/ Molisch	+
Terpenoids	Chloroform & conc H_2SO_4	+
Tannins	FeCl_3	+
Saponins	Warring in water bath	+

Key + = present - = absent

Compound **1** was obtained as a yellowish amorphous powder (35mg) with R_f value of 0.42 (30% ethyl acetate in *n*-hexane as eluent). The UV-Vis spectrum indicated absorbance peaks λ_{max} at 286 nm and 332 nm attributed to $\pi-\pi^*$ transition of C=C double bond and transitions of lone pair of electrons $n-\pi^*$ respectively. The IR spectrum indicated vibrations at 3400 cm^{-1} (due to hydroxyl group), 2936 cm^{-1} (due to C-H stretching vibrations), 1640 cm^{-1} and 1708 cm^{-1} (due to α,β -unsaturated ester), and 1518 cm^{-1} (due to the presence of benzene ring). The ^1H NMR spectrum (DMSO- d_6 , Table 2) revealed the presence six aromatic protons with two sets of aromatic ABX

coupling system at $\delta 6.66$ ($d, J=2.0\text{Hz}$), $\delta 6.63$ ($d, J=8.1\text{Hz}$) and $\delta 6.52$ ($dd, J=8.1, 2.0\text{ Hz}$) for the 3,4-dihydroxy- β -phenylethoxyl moiety and $\delta 7.01$ ($d, J=2.0\text{ Hz}$), $\delta 6.73$ ($d, J=8.1\text{ Hz}$) and $\delta 6.90$ ($dd, J=8.1, 2.0\text{Hz}$) for the caffeoyl moiety. Vinyl protons of AB-type (trans-olefinic) were observed at $\delta 7.59$ ($d, J_{AB}=15.9\text{Hz}$, H-8^I) and $\delta 6.26$ ($d, J_{AB}=15.9\text{Hz}$, H-7^I). Four characteristic anomeric protons of sugar moieties observed at $\delta 4.38$ ($d, J=7.8\text{ Hz}$), $\delta 5.28$ ($d, J=1.5\text{ Hz}$), $\delta 5.17$ ($d, J=2.2\text{ Hz}$) and $\delta 5.22$ ($d, J=2.7\text{ Hz}$) are in agreement with a β -configuration of an anomeric proton. Signal at $\delta 1.05$ integrated for three protons is attributed to a methyl group (H-6^{III}) of rhamnose. The coupling constant value for anomeric proton at $\delta 5.28$ and that of $\delta 1.05$ ($d, J=6\text{Hz}$) is in conformity with the α -L-configuration of the rhamnopyranoside moiety. The downfield chemical shift value of H-3^{II} (at $\delta 3.83$) of glucose is due to glycosylation at the C-3^{II} carbon atom. The anomeric proton signals were consistent with the β -configuration of glucose, α - configuration of a rhamnose and β -configuration for both β -apioses.

The ¹³C NMR spectrum revealed tetraglycosidic nature exhibiting four anomeric carbon resonances at $\delta 104.5$ (β -glucose), 102.7 (δ -rhamnose), 111.5 (β -apiose) and 109.6 (β -apiose). Chemical shift values at $\delta 104.5$, $\delta 102.7$, $\delta 111.5$, and $\delta 109.6$ are attributed to anomeric sugar carbons of glucose (C-1^{II}), rhamnose (C-1^{III}) and apiose I and II (C-1^{IIII} and C-1^{IIIII}), respectively. The signals observed at $\delta 146.9$, $\delta 148.0$ and $\delta 144.7$ belong to C-3, C-1 and C-5 of aromatic nucleus. Signals at $\delta 115.2$ and $\delta 117.1$ indicate the presence of ester carbonyl carbon ($\delta 166.3$) of α and β conjugated system. Signals observed at $\delta 146.9$, $\delta 148.0$, $\delta 144.7$ and $\delta 146.2$ are attributed to oxygenated sp² quaternary carbons of a phenyl group at C-3, C-4, C-3^I and C-4^I, respectively. From the ¹H-¹H COSY spectrum the two doublet protons coupling each other at $\delta 7.59$ ($d, J=15.9, H-7^I$) and $\delta 6.26$ ($d, J=15.9, H-8^I$) show

HMBC correlation to ester carbonyl carbon at $\delta 168.3$ (C-9^I) suggesting these two protons belong to α and β protons of α, β -conjugated system of ester moiety. The ester linkage observed between C-4^{II} of the glucopyranose unit with the ester carbonyl carbon of the caffeoyl unit was confirmed on basis of the downfield chemical shift value of H-4^{II} signal ($\delta 4.93$) of glucose and HMBC cross-peak observed between H-4^{II} and the ester carbonyl carbon ($\delta 166.3$). A prominent HMBC correlation from the anomeric proton of glucose at $\delta 4.38$ to C-8 ($\delta 72.3$) of the 3,4-dihydroxy- β -phenylethoxyl moiety suggest that the glucose moiety is linked to C-8. The upfield chemical shift value of C-6^{II} at $\delta 62.3$ revealed the presence of a second β -apiose in this position. The HMBC coupling between H-1^{IIII} ($\delta 5.22$) of apiose and C-4^{III} of rhamnose showed that the site of the glycosylation of apiose to be C-4^{III} position of rhamnose. This was further supported by COSY correlation between H-1^{IIII} of apiose with H-4^{III} of rhamnose and correlation between the H-1^{IIII} of apiose with H-6^{II} of glucose.

The connectivity between the sugars was further established by the HMBC correlation between H-1^{III} ($\delta 5.28$) of the rhamnose and C-3^{II} ($\delta 80.5$) of the glucose indicating an interglycosidic between C-1^{III} (rha) and C-3^{II} (glu). Both isomers showed a correlation between H-1^{II} of the glucopyranose unit with C- α of the phenylethyl aglycone in the respective HMBCs confirming the position of the aglycone in the structure. The complete assignment of all proton and carbon resonances was based on the COSY, gHSQC, and gHMBC experiments. The compound was found to be a derivative of a known phenylpropanoid glycoside (Fig. 2), verbascoside, and the spectral data is in agreement with other related phenylpropanoid glycosides available in literature [20, 21].

Table 2: Complete NMR (DMSO-*d*₆, 400MHz) data of compound 1

Position	δ_c (ppm)	δ_H (ppm)	COSY	HMBC Correlation	
				² J	³ J
1	129.7		-	-	-
2	116.2	6.66 ($d, J=2.0$)	-	C-1,3	C-4,6,7
3	146.9		-	-	
4	144.7		-	-	
5	116.8	6.63 ($d, J=8.1$)	6	C-4,6	C-1,3
6	120.0	6.52 ($dd, J=2,8.0$)	5	C-1,5	C-2,4,7
α	35.5	2.75 ($t, J=7.3$)	8	C-8,1	C-2,6
β	72.3	3.96/3.7 (m)	7	C-7	C-1,1 ^{II}
Glucose					
1 ^{II}	104.5	4.38($J=7.8$)	2 ^{II}	C-2 ^{II}	C-8
2 ^{II}	73.3	3.41 ($dd, J=7.96/9.3$)	1 ^{II} , 3 ^{II}	C-1 ^{II} , 3 ^{II}	C-4 ^{II}
3 ^{II}	80.5	3.83 ($t, J=9.1$)	2 ^{II} , 4 ^{II}	C-2 ^{II} , 4 ^{II}	C-5 ^{II} , 1 ^{III}
4 ^{II}	69.2	4.93 ($t, J=9.5$)	3 ^{II} , 5 ^{II}	C-3 ^{II}	C-6 ^{II} , 9 ^I
5 ^{II}	72.1	3.59 (m)	4 ^{II} , 6 ^{II}	C-6 ^{II}	C-1 ^{II} , 3 ^{II}
6 ^{II}	62.3	3.54/3.66 ($dd, J=12.0/5.5$)	5 ^{II}	C-5 ^{II}	C-4 ^{II} , 1 ^{IIII}
Rhamnose					
1 ^{III}	102.7	5.28 ($d, J=1.5$)	2 ^{III}	C-2 ^{III}	C-3 ^{III} , 5 ^{III}
2 ^{III}	70.8	3.9 (s)	1 ^{III} , 3 ^{III}	C-1 ^{III} , 3 ^{III}	C-4 ^{III}
3 ^{III}	70.0	3.67 ($dd, 9.5/3.3$)	2 ^{III} , 4 ^{III}	C-2 ^{III} , 4 ^{III}	C-1 ^{III}
4 ^{III}	76.4	3.41 ($t, J=9.3$)	3 ^{III} , 5 ^{III}	C-3 ^{III} , 5 ^{III}	C-2 ^{III} , 6 ^{III} , 1 ^{IIII}
5 ^{III}	67.6	3.55 (m)	4 ^{III} , 6 ^{III}	C-4 ^{III} , 6 ^{III}	C-1 ^{III}
6 ^{III}	18.6	1.05 ($d, J=6.0$)	5 ^{III}	C-5 ^{III}	C-4 ^{III}
Apiose I					
1 ^{IIII}	111.5	5.17 ($d, J=2.2$)	2 ^{IIII}	C-2 ^{IIII}	C-3 ^{IIII} , 4 ^{IIII} , 4 ^{IIII}
2 ^{IIII}	74.7	3.9 ($d, J=1.8$)	1 ^{IIII}	C-1 ^{IIII} , 3 ^{IIII}	C-4 ^{IIII} , 5 ^{IIII}
3 ^{IIII}	74.8	-	-	-	
4 ^{IIII}	70.8	3.97 ($d, 9.9$), 3.70 ($d, 9.9$)	-	C-3 ^{IIII}	C-1 ^{IIII} , 2 ^{IIII} , 5 ^{IIII}
5 ^{IIII}	63.6	3.55 (s)	-	C-3 ^{IIII}	C-2 ^{IIII} , 4 ^{IIII}

Apiose II					
1 ^{IIII}	109.6	5.22 (d, J=2.7)	2 ^{IIII}	C-2 ^{IIII}	C-3 ^{IIII} , 4 ^{IIII} , 6 ^{II}
2 ^{IIII}	73.9	3.65	1 ^{IIII}	C-1 ^{IIII} , 3 ^{IIII}	C-4 ^{IIII} , 5 ^{IIII}
3 ^{IIII}	79.3	-	-	-	-
4 ^{IIII}	71.0	3.39/3.27 (d, J=11.6)	-	C-3 ^{IIII}	C-1 ^{IIII} , 2 ^{IIII} , 5 ^{IIII}
5 ^{IIII}	62.5	3.27 (d, 11.6)	-	C-3 ^{IIII}	C-2 ^{IIII} , 4 ^{IIII}
Acyl moiety					
1 ^I	125.9	-	-	-	-
2 ^I	115.2	7.01 (d, J=2.0)	-	C-1 ^I , 3 ^I	C-4 ^I , 6 ^I , 7 ^I
3 ^I	148.0	-	-	-	-
4 ^I	146.2	-	-	-	-
5 ^I	115.9	6.73 (d, J=8.1)	6 ^I	C-4 ^I	C-1 ^I , 3 ^I
6 ^I	122.0	6.90 (dd, J=2.8/8.1)	5 ^I	C-1 ^I	C-2 ^I , 4 ^I , 7 ^I
α	113.8	6.26 (d, J=15.9)	8 ^I	C-1 ^I , 8 ^I	C-2 ^I , 6 ^I , 9 ^I
β	149.0	7.59 (d, J=15.9)	7 ^I	C-7 ^I , 9 ^I	C-1 ^I
C=O	166.3	-	-	-	-

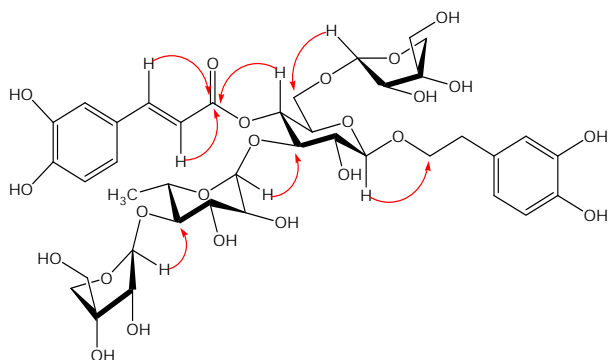


Fig 2: Important HMBC correlation of compound 1

Compound 2 was obtained as a yellowish amorphous powder (27mg) with R_f value of 0.68 under 30% ethyl acetate/*n*-hexane solvent system. The UV-Vis spectrum showed a similar pattern to that of compound 1 with λ_{max} at 286 nm and 332nm attributed to presence of Π - Π^* transition of C=C double bond and transitions of lone pair electrons n - π^* respectively. The IR spectrum indicated broad vibration at 3400cm^{-1} (due to hydroxyl group), vibrations at 2936cm^{-1} (due to C-H stretching), vibrations at 1640cm^{-1} (due to C=C), vibrations at 1708cm^{-1} (due to C=O) and vibrations at 1604cm^{-1} , 1518cm^{-1} and 1447cm^{-1} suggest the presence of benzene ring. The ^1H NMR spectrum (Table 3) exhibited characteristic signals arising from (E)-caffeic acid and 3, 4-dihydroxyphenethyl alcohol moiety together with signals for two *trans*-olefinic protons (AB system, $J_{AB} = 15.9$ Hz), a benzylic methylene proton (δ 2.79, 2H, t, $J = 7.5\text{Hz}$) and two non-equivalent protons (δ 4.05, 1H, *m* and 3.70, 1H, *m*).

Additional, three anomeric proton resonances were observed at δ 4.38 (d, $J=7.9$ Hz), 4.60 (d, $J=1.5$ Hz) and 5.17 (d, $J=2.7$ Hz), suggesting the presence of three sugar moiety. The anomeric proton signals were consistent with the β -configuration of glucose, α -configuration of rhamnose and β -configuration of an apiose. The ^{13}C NMR data of compound 2 confirmed triglycosidic nature exhibiting three anomeric carbon resonances at δ 104.4 (β -glucose), 101.7 (α -rhamnose) and 109.6 (β -apiose). The up field chemical shift value of C-6^{II} at δ 62.5 suggests the presence of a free OH group. The position of the caffeoyl group was confirmed to be at C-4^{II} of the glucose unit on the basis of the downfield chemical shift value of H-4^{II} (δ 4.97) and its gHMBC correlation with that of ester carbonyl carbon. HMBC correlation of the anomeric proton of glucose H-1^{II} at δ 4.38 to that of C- α atom of the 3, 4-dihydroxyphenethyl moiety (δ 72.3) also supports the 3, 4-dihydroxyphenethyl moiety is linked to C-1^{II} position. On the other hand, the downfield chemical shift value of H-3^{II} (δ 3.81) of glucose coupled with the HMBC correlation of H-1^{IIII} (δ 5.28) of rhamnose and C-3^{II} (δ 80.5) of glucose support that the rhamnose unit is linked to C-3^{II} position of glucose. Furthermore, the downfield chemical shift value of C-4^{IIII} (δ 80.0) of the rhamnose unit coupled with the gHMBC correlation between H-1^{IIII} (δ 5.17) of apiose and C-4^{IIII} of rhamnose showed that the site of the glycosidation of apiose is at C-4^{IIII} position of rhamnose. Consequently, the structure of compound 2 was determined to be 1-*O*-3,4-(dihydroxyphenyl)-ethyl- β -D-apiofuranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 3)-4-*O*-caffeoyl- β -D-glucopyranoside isolated previously from the aerial parts of *Phlomis samia*, *P. monocephala* and *P. carica* [18].

Table 3: NMR (DMSO- d_6 , 400MHz) data of compound 2

Position	$\delta_c(\text{ppm})$	$\delta_H(\text{ppm})$	Reference data ¹⁸	
			$\delta_H(\text{ppm})$ ¹⁸	$\delta_c(\text{ppm})$ ¹⁸
Aglycone				
1	129.7	-	-	131.4
2	116.2	6.66 (d, J=2.0)	6.69 (d, J=2.0)	116.6
3	145.4	-	-	146.0
4	144.0	-	-	142.0
5	116.8	6.63 (d, J=8.1)	6.68 (d, J=8.1)	117.1
6	120.0	6.52 (dd, J=2.8, 8.0)	6.56 (dd, J=8.1/2.0)	121.2
α	36.0	2.75 (t, J=7.3)	2.79 (t, J=7.5)	36.5
β	72.5	3.96/3.7(m)	4.05m, 3.07m	72.3
Glucose I				
1 ^{II}	104.4	4.28(J=7.9)	4.38 (d, J=7.9)	104.1
2 ^{II}	77.0	3.31 (dd, J=7.96/9.3)	3.41 (dd, J=7.9/9.3)	76.4
3 ^{II}	79.3	3.81 (t, J=9.1)	3.83 (t, J=9.1)	80.5

4 ^{II}	70.2	4.91 (t, <i>J</i> =9.5)	4.93 (t, <i>J</i> =9.5)	70.4
5 ^{II}	74.3	3.59(m)	3.55 ^(a)	75.9
6 ^{II}	62.5	3.54/3.66 (dd, <i>J</i> =12.0/5.5)	3.65 ^(a) 3.55 ^(a)	62.3
Rhamnose				
1 ^{III}	101.7	5.22(d, <i>J</i> =7.9)	5.28 (d, <i>J</i> =1.5)	102.1
2 ^{III}	73.3	3.16 (dd, <i>J</i> =7.9, 9.0)	3.90-3.85 ^(a)	72.4
3 ^{III}	71.5	3.35 (t, <i>J</i> =9.0)	3.67 (dd, <i>J</i> =9.5/3.3)	72.3
4 ^{III}	82.8	3.37 (t, <i>J</i> =9.0)	3.41 (t, <i>J</i> =9.3)	80.0
5 ^{III}	69.7	3.32(m)	3.55 m	68.8
6 ^{III}	24.6	1.87 (d, <i>J</i> =6.0)	1.12 (d, <i>J</i> =6.2)	18.7
Acyl moiety				
1 ^I	125.9	-	-	127.6
2 ^I	115.2	7.18 (d, <i>J</i> =2.0)	7.08 (d, <i>J</i> =2.0)	115.2
3 ^I	146.3	-	-	146.9
4 ^I	146.2	-	-	149.4
5 ^I	115.9	6.79 (d, <i>J</i> =8.0)	6.81 (d, <i>J</i> =8.2)	116.3
6 ^I	122.0	6.52 (dd, <i>J</i> =2.0/8.2)	6.98 (dd, <i>J</i> =8.2/2.0)	123.4
α	115.1	6.06 (d, <i>J</i> =15.9)	6.26 (d, <i>J</i> =15.9)	114.6
β	149.0	7.38 (d, <i>J</i> = 15.9)	7.59 (d, <i>J</i> =15.9)	148.0
C=O	168.3	-	-	168.2
Apiose moiety				
1 ^{III}	109.6	5.17(d, <i>J</i> =2.2)	5.22 (d, <i>J</i> =2.7)	111.4
2 ^{III}	77.0	3.90(d, <i>J</i> =1.8)	3.65 ^(a)	78.5
3 ^{III}	82.3	-	-	79.9
4 ^{III}	74.8	3.37 (d, <i>J</i> =11.3)	3.39 (d, <i>J</i> =11.6)	74.8
5 ^{III}	63.5	3.55 (d, <i>J</i> =10.9)	3.27 (d, <i>J</i> =11.6)	65.7

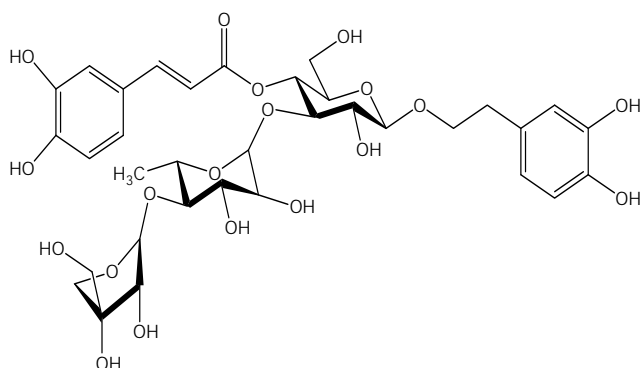


Fig 3: Structure of Verbascoside-6^{III}-apiose (2)

4. Conclusion

This work is the first attempt to phytochemically analyze the roots of *C. myricoides* from Ethiopian flora. Phytochemical screening of the roots revealed that the presence of glycosides, terpenoids, flavonoids, phenolics and tannins. Chromatographic separation of the CH₂Cl₂/CH₃OH (1:1) yielded two phenylpropanoid glycosides. This is the first report on the presence of such kind of phenylpropanoid derivatives from the genus *Clerodendrum* indigenous to Ethiopian flora. Phenylethanoid glycosides commonly occur in the order Lamiales, to which the Verbenaceae family belongs. The presence of these pharmacologically important secondary metabolites from root extracts sheds more light on the medicinal uses of the plant. The finding of this work is encouraging and indicates that the herb should be studied more extensively for its therapeutic benefits.

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