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Anthraquinones from the Roots of Aloe Gilbertii and Aloe Eleganis

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Abstract
Aloe gilbertii and Aloe eleganis are endemic to Ethiopia, and Northern Ethiopia and Eritrea, respectively, where the leaf latex and root are traditionally used for the treatment of malaria, hepatomegaly, splenomegaly, diabetics and abdominal pain, leishmanaiasis and wound healing. Phytochemical screening tests of the CH_2Cl_2/CH_3OH (1:1) root extract of *Aloe gilbertii* revealed the presence of alkaloids, anthraquinones, terpenoids, flavonoids and the absence of tannins, saponins and steroids while CH_2Cl_2/CH_3OH (1:1) root extract of *Aloe Elegans* revealed the presence of anthraquinones, terpenoids, phenols, saponins, tannins, glycosides whereas flavonoids, alkaloids and steroids are absent. Chromatographic separation of CH_2Cl_2/CH_3OH (1:1) root extract of *Aloe gilbertii* yielded geranyl benzoate derivative (**1**) and 8-methoxy chrysopanol (**2**) whereas aloesaponarin I (**3**) and aloesaponol I(**4**) were isolated from roots of *Aloe eleganis*. Complete characterizations of the isolated compounds were done with the help of spectroscopic techniques (UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, gHMBC and gHSQC).
Keywords: *Aloe gilbertii*, *Aloe eleganis*, Anthraquinones, Phytochemical screening

INTRODUCTION

Medicinal plants have a long history of use in most communities and according to the World Health Organization (WHO), more than 3.5 billion people in the developing world rely on medicinal plants in one way or another as components of their primary health care. The vast majority of people (70-80%) in Africa consult Traditional Medical Practitioners (TMPs) for their primary healthcare (1).

Aloe gilberti (Fig 1) occurs around Sidamo, Central part of Ethiopia and Gamo Gofa floristic regions and it is a succulent shrub, grouped together with other shrubby (caulescent) Aloes such as *A. calidophila* and *A. megalacantha* in the south and eastern Ethiopia, respectively. *Aloe elegans*, known by the name "arei" (Tigrigna) (Fig 2.), is succulent herb, grows in rocky slopes, mostly on sandstone or limestone, in areas of evergreen bushland or wooded grassland between 1500 and 2400 m in Tigray, Wello, Gojam and Shewa floristic regions in Ethiopia and in Eritrea (3). In an ongoing project to study the chemical constituents of Ethiopia, we hereby report a comprehensive phytochemcial analysis of the roots of *Aloe gilberti* and *Aloe elegans*.

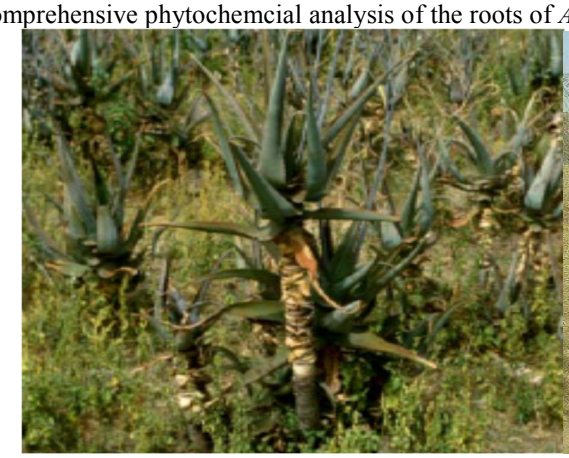

Fig.1. Picture of *A. gilbertii*

Fig.2. Picture of *A. elegans*

MATERIALS AND METHODS

Instrumentation

Column chromatographic separation was carried out on silica gel (230-400 mesh size, Merck). Thin layer chromatography was done on silica gel 60 F-254, 0.2 mm thick layer on aluminum sheets for detection of spots. The UV-Vis spectrum was recorded on UNICAM UV-300 double beam spectrophotometer using $CHCl_3$ as internal standard. The IR absorption spectrum was determined by Shimadzu 440 instrument using KBr disk in the range of 500-4000cm^{-1}. The ^{1}H NMR, ^{13}C NMR, DEPT-135, gHSQC, and gHMBC spectra were recorded using

Bruker Avance 400MHz spectrometer using TMS as internal standard. Chemical shift values for all NMR data are reported in parts per million (ppm) relative to internal standard. All the chemicals used were analytical grade.

Plant material collection and authentication

The root part of the *Aloe Gilbertii* was collected from Alamura Hill, Morocho on the road to Billate 4-5 km from Hawassa town and Alaba Mountain slopes of Southern Ethiopia. The plant material was authenticated by botanist Professor Fikre Dessalegn, Department of Biology, Hawassa University. The root part of *Aloe elegans* was collected from Adwa Woreda, Axum zone, Tigray Region, Ethiopia that is 1112 km from Addis Ababa. The plant material was authenticated by botanist Professor Sebsebe Demissew, Department of Biology, Addis Ababa University. The root was dried, powdered to suitable size and made ready for solvent extraction.

Extraction

The roots of *Aloe gilbertii* (360g) and *Aloe elegan* (500 g) were soaked sequentially with CH_2Cl_2/CH_3OH (1:1) for 3x24hrs with occasional shaking. The extract was filtered and concentrated using rotary evaporator at 40^oC to give brown crude (32.6g, 9.09% yield) and black crude (40.43g, 8.086% yield), respectively.

Isolation of compounds

The crude extract of *Aloe gilbertii* (15g) was subjected to silica gel column chromatographic separation (140g silica gel) and eluted with increasing gradient of acetone in chloroform. A total of 38 fractions (each 50 mL) were collected. The constituent profile of each fraction was monitored by TLC (5% acetone in chloroform) and visualized under UV-Vis light (λ_{max} 254 and 366nm). Fractions 8-14 were combined based on their TLC profile and was further purified by column chromatography (eluent; increasing gradient of acetone in chloroform). Fractions 15-23 were combined and purified by PTLC to give compound **1** (148mg). Fractions 5-7 (one major spot) were combined, dried and washed successively with *n*-hexane to give compound **2** (15mg).

The crude extract of *Aloe elegan* (20g) was subjected to silica gel column chromatographic separation (140g silica gel) and eluted with increasing gradient of Ethyl acetate in *n*-hexane. A total of 42 fractions were collected. Fractions 1-10 were colorless, fractions 11- 16 were orange, fractions 17-22 were reddish brown, fractions 23-31 were red and fractions 32-42 were light red. Fractions 1-10 were discarded because their TLC showed impurities. Fractions 11-16 revealed three spots, deep orange, yellow and red. Fractions 17-22 showed three spots, two common with fractions 11-16 (eluent: 80:20 *n*-hexane/ethyl acetate). Due to their common spots, fractions 11-16, 17-23 and 23-31 were combined together and subjected to further fractionation by 20%, 30%, 40% and 50% of ethyl acetate in *n*-hexane solvent system. A total of 20 fractions were collected, monitored by TLC. Fraction 4 (20% of ethyl acetate in n-hexane) gave a red precipitate showing red spot under UV light with R_f value of 0.85. This precipitate was separated and washed with n-hexane repeatedly and yielded compound **3** (12mg). Fractions 9-10 (30% of ethyl acetate in n-hexane) gave colorless precipitate which fluorescence under UV light having the R_f value of 0.35 and afforded compound **4** (17mg).

Preliminary phytochemical screening

Phytochemical screening tests were carried out on the CH_2Cl_2:CH_3OH (1:1) extract using the following literature protocols (4-7).

Test for alkaloids **(Dragendroff's Test)**

The crude extract (300mg) was mixed with 2mL of concentrated hydrochloric acid. The mixture was then filtered and mixed with small amount of amyl alcohol at room temperature. Few drops of dragendroff's reagent (Solution of Potassium Bismuth Iodide) was added to the acid layer and a reddish brown precipitate was observed (4).

Test for tannins (Gelatin Test)

Small quantity of the extract was mixed with water and heated on water bath. To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins (4).

Detection of phenols

The extract (500mg) was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds (5-7).

Test for anthraquinones

About 0.5g of the methanol extract was boiled with concentrated hydrochloric acid for few minutes in water bath and filtered. The filtrate was allowed to cool and equal volume of $CHCl_3$ was added to it. Few drops of ammonia were added to the mixture and heated in water bath. Formation of rose-pink color was inspected (5-7).

Test for saponins (Froth Test)

The crude extract (0.1g) was dissolved in 20ml of water shaken in a graduated cylinder for 15 minutes. Formation

of 1cm layer of foam indicates the presence of saponins (7).

Test of terpenes
Methanol extract (0.25g) was mixed with 2mL of CHCl3 and 30mL of concentrated H2SO4 was added carefully to form a layer. Reddish-brown coloration of the interface was inspected (8).

Test for flavonoids (Alkaline Reagent Test)
Few drops of sodium hydroxide solution was added to the extract and formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (7-9).

Tests for steroids (Liebermann-Burchard test)
Each extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated H_2SO_4 (2 mL) was added alongside of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids (8-9).

Result and Discussion
Preliminary phytochemical screening
Preliminary phytochemical screening of CH_2Cl_2:CH_3OH (1:1) extract revealed the presence of alkaloids, anthraquinones, terpenoids, flavonoids and the absence of tannins, saponins and steroids (table 1).
Table 1. Phytochemical screening tests of the CH_2Cl_2:CH_3OH (1:1) extract of *A. gilbertii and A.elegan*

S.No	Test	*A. gilbertii*	*A. elegans*
1	Alkaloids	+	-
3	Saponins	-	+
4	Phenols	+	+
5	Tannins	-	+
6	Flavanoids	+	-
7	Anthraquinones	+	+
8	Terpenes	+	+
9	Steroids	-	-

(+): presence of constituent; (-): absence of constituent

Compound 1 was isolated as a yellow powder with R_f value of (0.45) in (5% acetone in chloroform). A broad IR spectrum revealed absorption band at 3150-3650cm^{-1} for the hydroxyl group and absorption bands at 1690cm^{-1}, 1450cm^{-1} and 1250cm^{-1} are attributed to carbonyl group of an ester moiety, olefinic system and C-O part of ester moiety, respectively. The absorption peaks at 700cm^{-1}, 2922.92cm^{-1} and 3010cm^{-1} suggest CH_2 rocking, sp^3 C-H stretching and sp^2 C-H stretching vibrations, respectively. The UV spectrum showed characteristic absorption for ester carbonyl moiety and alkene double bond at λ_{max} 263 and 210, respectively.

The ^{1}H NMR spectrum revealed the existence of AA'XX' spin system suggesting a 1,4-di-substituted artomatic ring [δ_H 7.55 (2H, dd) and δ_H 7.6 (2H, dd)]. The peaks at δ_H 4.35(2H,t) and δ_H 4.2 (2H, s) suggest the presence of two oxymethylene peaks attributed to methylens C-2' and C-1''. The presence of an olefinic proton at δ_H 5.2 (1H, t, H-6'') coupled with two symmetrical methylsgroups at δ_H 1.71 (3H,s, H-8'',9''), four methylenes (C-1'',2'', 4'', 5'') suggest a geranyl moiety. The ^{13}C NMR spectrum revealed a total of 15 carbon peaks of these the presence of ester moiety at δc 167, a 1,4-disubstitued phenyl ring (δc 132.4, 130.9 and 128.8 and 155.0), two oxymethylenes (δc 65.6 (C-1''), δc 71.8 (C-2'), one of them is part of the geranyl moiety, are all clearly evident. The gHSQC spectrum showed ^{1}J correlations between H2'↔C2', H1''↔C1'', H5''↔C5'' and H6''↔C6'', The gHMBC correlation showed ^{2}J and ^{3}J correlations between H2'→C4, H2,6→H-1', H1''→C1',2'',3'' and H6''→C5'',8'',9'' suggesting that the oxymethylene is located at C-4 position of phenyl ring whereas the ester geranyl moiety is located at para position to the oxymethylene (C-1). Moreover, all spectral pattern is in good agreement that the geranyl moiety is directly connected to the ester. Thus, based on the above spectral data the compound **1** was proposed to be a geranyl benzoate derivative shown below.

Table 2: NMR data of compound **1** (400MHz, DMSO-d_6)

Position	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)	DEPT-135 (δ in ppm)	HSQC	HMBC
1	-	128.86	-		
2	7.6(2H, dd)	132.39	-		
2'	4.2(2H, d)	71.81	71.81	H2'↔C2'	H2'→C4
3	7.55(2H,dd)	130.93	-		
4	-	155	-		
5	7.55(2H, dd)	130.93	-		
6	7.6 (2H,d*d)*	132.39	-		
1'	-	167	-		
1''	4.35 (2H,*t,)*	65.59	65.59	H1''↔C1''	H1''→C1',2'', 3''
2''	1.71(2H, nd)	30.57	30.57		
3''	1.69(1H, nd)	31.95	-		H3''→C2'', 4''
4''	1.70(2H,q)	29.71	29.71	H4''↔C4''	
5''	1.79(2H,nd)	38	-	H5''↔C5''	
6''	5.2(2H, nd)	128.72	-	H6''↔C6''	H6''→C5'',8'', 9''
7''	-	132.51	-		
8''	1.71(3H, s)	22.5	-		H8''→C6'',7''
9''	-	27.27	-		
10''	1.06(3H,nd)	19.7	-		

Compound 2 is a yellow solid with $R_f$value of 0.57 in n-hexane/ethylacetae (7:3) solvent system. The IR spectrum showed broad and weak absorption approximately at 3400 cm^{-1} attributed to hydroxyl group. The strong absorption band at approximately 2920 and 2850cm^{-1}revealed the presence of aliphatic C-H stretching vibration. The strong stretching vibration at 1615 cm^{-1} showed the presence of phenyl group. The absorption bands at 1715 cm^{-1} and 1665 cm^{-1} indicate the absorption of the un-chelated and chelated carbonyl carbon, respectively. The ^{1}H NMR spectrums revealed a singlet signal at δ12.90 (1H, s) indicate the presence of chelated hydroxyl group at C-1 peri to carbonyl C-9. A triplet peak at δ 7.64 coupled with a doublet proton at δ δ7.64 indicates the presence of an ABX aromatic pattern on one ring of anthraquinone skeleton. Two protons showed a weak coupling not well resolved multiplicity (close to broad singlet) at δ7.32 and 7.28 attributed to aromatic protons at C-4 and C-2 of ring A. A singlet peak at δ 4.08 (s, 3H) and δ2.99 (s, 3H) each integrated to three protons indicates the presence of methoxy group at C-8 position, peri to carbonyl C-9, and a methyl at C-3 position, as expected biogenetically. The chemical shift of the methoxy group was desheiled to 4.1 due to the peri effect with the carbonyl carbon C-9. Based on close comparison with literature (11**)**, the structure of the compound was found to be a close derivative of chrysopanol, 8-methoxychrysophanol (**2**) Thus, based on the above spectral data, compound **2** was found to be 1-hydroxy-8-methoxy-3-methylanthracene-9,10-dione, known as 8-methoxychrysophanol (**2**).

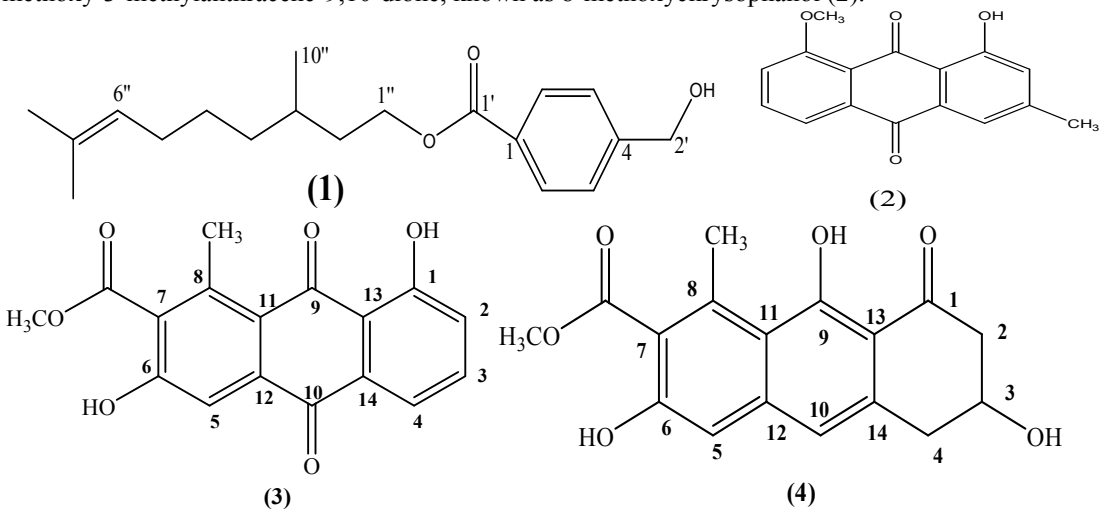

Fig 2: Structure of compounds isolated from the roots of CH_2Cl_2/CH_3OH (1:1) extract of *A. gilbertii* and *A. elegan*

Table 3. Comparison of the observed ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data of 8-methoxy chrysopnaol (**2**) with the reported value of chrysophanol

Position	^{1}H NMR of 3 (in ppm)	Chrysophanol (11)
1-OH	12.9 s	12.03 *s*
2-H	7.28 *brs*	7.11 *br s*
3-CH_3	2.90 *s*	2.47 s
4-H	7.32 *brs*	7.30, *dd*, *J*=1.2
5-H	7.78 *dd*(*J*=7.5, 1.2)	7.83 Hz
6-H	7.66 *dd*	7.66, *t*
7-H	7.78 *dd*(*J*=8.0, 1.2)	7.81 *dd*, *J*=1.1, 7.5 Hz
8-OH	-	12.13 *s*
8-OCH_3	4.10 *s*	

Compound 3 was isolated as a red precipitate having R_f value of 0.85 (30% ethyl acetate in *n*-hexane). The UV spectrum showed absorption at λ_{max} (in ethanol) 282 and 410 nm attributed to an anthraquinone chromophore (18). In the IR spectrum (Appendix 2), a broad absorption band at 3488 cm^{-1} and absorption band at 1740 cm^{-1} indicated the presence of OH and stretching of unsaturated carbonyl group, respectively. Intense peaks at 2933 cm^{-1} is indicative of C-H stretching and medium peaks at1640 and 1385 cm^{-1} correspond to aromatic ring C=C bond stretch and inplane O-H bend.

The ^{1}H NMR spectrum ($CDCl_3$, 400MHz) showed the presence of two broad singlets at δ 2.95 and 4.12 which correspond to protons of CH_3 and OCH_3. There are four sets of aromatic protons of which three of them (δ 7.32 (*dd*, 8.4, 1.2 Hz), δ 7.64 (t), δ 7.79 (dd, 7.4, 1.2 Hz)) belong to ring A (H-2, H-3 and H-4, respectively) having ABX multiplicity pattern with ortho-para and one meta coupling and a singlet resonated at δ 7.80 (H-5). Furthermore, peaks at δc 10.45 and 12.95 shows a hydroxyl group peri to carbonyl carbon, which assigned to carbon atoms at δc 162.47(C-6), and 163.43(C-1), respectively.

The ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) showed seventeen carbon signals, two of which are carbonyl carbons at δc 189.6 and 182.1 corresponding to (C-10) and (C-9) typical of anthraquinone skeleton. The peaks at δ 170.5 and 53.2 reveal the presence of methyl ester group attached to C-7. The DEPT-135 indicated that there are four aromatic methines at δc 115.1(C-2), 118.9(C-4), 125.0(C-5), 135.8(C-3), two methyls (one methyl connected to aromatic ring having δc 21.8 and methyl of ester at δc 53.2) and eleven quaternary carbons (Table 4). Thus, based on the above spectral data and comparison with literature the structure of compound **3** was proposed to be similar with the structure of aloesaponarin I (19) (Figure 3). It was reported that this compound is found in *Aloe turkanensis* and *Aloe secondiflora* (20).

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$, 400MHz) data of compound **3**

Position	Compound **3**			Literature data (19-20)	
	δ_H	δ_C	δ_C (DEPT-135)	δ_H	δ_C
1		163.4			163.5
2	7.32 (*dd*,8.4,1.2Hz)	115.1	CH (115.1)	7.31 (dd,8.4,1.4Hz)	115.3
3	7.64 (t)	135.8	CH (135.8)	7.62 (t)	125.3
4	7.79 (dd,7.4,1.2Hz)	118.9	CH (118.9)	7.77 (dd,8.0,1.2Hz)	119.2
5	7.81 (s)	125.0	CH (125.0)	7.80 (s)	124.7
6		162.5			162.7
7		121.2			
8		117.5			
9		189.6			189.8
10		182.1			182.4
11		132.6			132.8
12		124.5			136.0
13		138.8			138.9
14		147.9			148.1
-COO		170.5			170.7
-OCH_3	4.12 (s)	53.2	CH_3 (53.2)	4.06 (s)	53.4
-CH_3	2.95 (s)	21.8	CH_3 (21.8)	2.95 (s)	22.1
6-0H	10.45 (s)			10.41 (s)	
1-OH	12.93 (s)			12.91 (s)	

Compound 4 was obtained as colorless precipitate with R_f value of 0.35 in ethyl acetate: *n*-hexane (3:7). The ^{1}H NMR spectrum (DMSO-d_6, 400MHz) revealed a deshielded singlet proton at δ_H 15.25 (1H, s) due to the proton

of the hydroxyl group at C-9 and two singlet aromatic protons at δ_H 6.90 (1H, s, H-10) and δ_H 6.95 (1H, s, H-5). It also showed peak at δ_H 4.25 (oxygenated methine at C-3), a singlet at δ_H 2.51 (methyl group at C-8) and another singlet at δ_H 3.85 (methyl ester) and peaks at δ_H 2.70 (1H, *dd*, H-2), δ 2.95 (1H, *dd*, H-2); and δ_H 3.15 (1H, *dd*, H-4), δ_H 2.88 (1H, *dd*, H-4) indicate the protons of methylenes.

The ^{13}C NMR spectrum (DMSO-d_6, 400MHz) showed a total of seventeen different carbons; oxygenated quaternary carbons at δ_C 156.2 (C-6) two carbonyl carbons at δc 204.1 (C-1) and 168.7 (ester carbonyl carbon), methyl at δc 21.1, two methylenes at δc 38.1 (C-4) and 46.9 (C-2), methyl of an ester at δc 52.6, and oxygenated methine at δc 64.9 (C-3). Moreover, the DEPT-135 spectrum displayed five upward peaks at δc 21.1, 52.6, 64.9, 108.1 and 117.1 and two downward peaks at δc 38.1 and δ 46.9 attributed to two methylenes. Based on spectroscopic evidence compound 4 in in good agreement with compound reported in literature as 3,6,9-trihydroxy-8-methyl-1-oxo-5,6,7,8-tetrahydroanthracene-2-carboxylic acid methyl ester known by trivial name aloesaponol I (Figure 4). This compound was previously reported from *Aloe saponaria*, *Aloe turkanensis and Aloe secondiflora* (12-15).

Table 2. ^{1}H and ^{13}C NMR (DMSO-d_6, 400MHz) data of compound **4**

Position	Compound **4**			Literature data (12-15)	
	δ_H	δ_C	δ C (DEPT-135)	δ_H	δ_C
1		204.1			203.7
2	2.70 (dd, 5.5Hz) 2.95 (dd,17.3Hz)	46.9	CH_2 (46.9)	2.70 (dd, 5.4 Hz) 2.96 (dd,17.1 Hz)	46.4
3	4.25 (m)	64.9	CH (64.9)	4.24(m)	64.4
4	2.88 (dd,15.4Hz) 3.15 (dd,15.5Hz)	38.1	CH_2 (38.1)	2.90 (dd,15.6 Hz) 3.14 (dd,15.8 Hz)	37.5
5	6.95 (s)	117.1	CH (117.1)	6.95 (s)	116.6
6		156.2			155.1
7		141.4			140.8
8		137.7			137.2
9		166.5			165.9
10	6.90 (s)	108.1	CH (108.1)	6.92 (s)	107.5
11		126.1			125.4
12		115.8			-
13		137.1			136.6
14		110.7			110.2
-COO		168.7		-	168.2
-OCH_3	3.85 (s)	52.6	CH_3 (52.6)	3.83 (s)	52.1
8 -CH_3	2.51 (s)	21.1	CH_3 (21.1)	2.70 (s)	20.8
9-0H	15.27 (s)			15.27 (s)	

Conclusion

This work is one of the few attempts to analyze the chemical constituents of polar extracts of the roots of *A. gilberti,* indigenous to Ethiopia, and *A. elegan,* indigenous to Northern Ethiopia and Eritrea. Phytochemical screening tests of the crude CH_2Cl_2/CH_3OH (1:1) root extract of *Aloe gilbertii* revealed the presence of alkaloids, anthraquinones, terpenoids, flavonoids and the absence of tannins, saponins and steroids. while CH_2Cl_2/CH_3OH (1:1) root extract of *Aloe elegans* revealed the presence of anthraquinones, terpenoids, phenols, saponins, tannins, glycosides and the absence of flavonoids, alkaloids and steroids. Chromatographic separation of CH_2Cl_2/CH_3OH (1:1) root extract of *Aloe gilbertii* yielded geranyl benzoate derivative (**1**) and 8-methoxy chrysopanol (**2**) whereas *A. elegan* yielded aloesaponarin I(**3**) and aloesaponol I (**4**).In agreement with the previous study, the wide traditional use of the plant may be attributed to its rich anthraquinones and phenolic compounds constituents. Thus, further work is recommended on this endemic plant so as to validate the traditional use and identify more bioactive secondary metabolites in support of its traditional use.

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