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Isolation and Characterization of Chemical Compounds from *Artemisia annua* Leaf Grown in Ethiopia

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Abstract

Artemisia annua is usually known as sweet wormwood which is native to China, and it is now cultivated in different countries like Ethiopia for its medicinal use. In Chinese traditional medicine the plant is known for the treatment of fevers, inflammation, headaches, bleeding, and malaria. The leaf of this medicinal plant is an important source of the known antimalarial marker compound named as Artemisinin. Additional phytochemical study on the leaf of the plant grown in Ethiopia was of interest for comparison. The study uses chromatographic and spectroscopic techniques for separation and purification of the metabolites, and structure elucidation, respectively. Chemical study on *Artemisia annua* leaf, acclimatized in Ethiopia, resulted in the isolation and characterization of its new long chain alcohol called 1-heptacosanol along with the known metabolites such as scopoletin and artemisinin. The leaf essential oil isolated by hydrodistilation was also analyzed by GC-MS and found rich in camphor (81%).

Keywords: Artemisia annua, 1-heptacosanol, camphor, scopoletin, Ethiopia

1. Introduction

Artemisia annua (Asteraceae) is originated in China, and now it is cultivated in many parts of the world including Ethiopia. In China *A. annua* is used for the treatment of fevers, inflammation, headaches, bleeding, and as a source of medicine for treating malaria [1]. About 600 secondary metabolites were reported from *A. annua* leaf in the literature. Chemical investigations showed the dominant nature of terpenoids, coumarins and flavonoids which are largely responsible for the importance of this plant in medicine [2,3]. Artemisinin (**1**) is found more bioactive than other terpenioids isolated from it [3,4,5]. In addition to its potent antimalarial activity, it is effective in treating other parasitic diseases, some viral infections and as allelopathic herbicide [5]. Besides scopoletin (**2**), scopolin, octacosanol and nonacosanol [3,6] were also reported from it. A study on leaf essential oil of *A. annua* obtained from Bulgaria yielded mainly α-humulene, α-cuvebene, α-copaene, α-selinene, artemisia ketone and camphor (**3**) [7]. The Chinese variety reportedly contained predominantly artemisia ketone while the Vietnamese oil was dominated by **3** and germacrene D [3,7,8]. Further phytochemical study on the leaf of the plant grown in Ethiopia was of interest for comparison. The aim of this study was isolations and structural elucidations of compounds found in *Artemisia annua* leaf collected from Ethiopia. In the course of the study the essential oil isolated from its leaf was also characterized.

2. Materials and Methods

2.1. Plant Material collection, Sample Preparation and Extraction

Leaf of the plant was collected from Addis Ababa University, Science faculty (Fig 1) and shade dried for a week. Its identification was made by a professional botanist and its specimen was stored in Ethiopian National Herbarium (S1220). It was ground and stored for further use; of this leaves powder 100 g was macerated with ethanol (95%) to afford 15 g crude

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extract for phytochemical study. The plant extract was further fractionated into two portions using hexane/ chloroform (1:1) (10 g) and methanol (5 g), and subjected to chemical study.

Figure 1. Artemisia annua (Picture by author)

2.2. Chemicals and Equipment

All chemicals and solvents used were of analytical grade. Melting point was determined using Thomas Hoover capillary melting point apparatus. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF254 (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm) and spraying with vanillin followed by heating with hot air gun. Column chromatography was performed using silica gel (70-230 mesh) Merck. Samples were applied on column by either adsorbing on silica gel. Solvent was freed using rota vapor BUCHI, RE 111. The UV-Vis spectral measurements were done using UV-Vis on T 60 U spectrophotometer (PG instruments, UK) equipped with deuterium and tungsten lamps. NMR spectral measurements were done on Bruker ACQ 400 AVANCE spectrometer operating at 400 MHz. The IR spectra were recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr.

2.3. Methods of Isolations and Structure Elucidations

Separation was made using combination of chromatographic techniques (Column chromatography using column packed with silica gel and Thin Layer Chromatography). About 8 g of the extract applied on column packed with silica gel (70-230 mesh) was eluted with petroleum ether: EtOAc: MeOH of increasing polarity to afford 20 fractions. Three compounds namely artemisinin (**1**), scopoletin (**2**) and 1-heptacosanol (**4**) were isolated and purified by recrystallizations from Fr 5,8 and 14, respectively. The structures of purified compounds were elucidated using the data generated by appropriate spectroscopic methods such as NMR, UV-VIS, IR, and comparison with the literature.

2.4. Essential Oil Isolation and Characterization

Dried leaf (50 g) essential oil was isolated by hydrodistillation (2 h) using Clevenger apparatus and the essential oil components were separated and identified on GC-MSD system (Agilent technologies 7820A GC system) coupled to a mass detector (5977E MSD, USA) and HP-88 column (30 m x 0.25 mm x 0.25 µm film thickness) coated with 100% poly (dimethylsiloxane). Samples (1 µL) were injected and Helium was used as carrier gas. The total run time was set to 31.31 min. The identification of the constituents was based on a comparison of their retention indices relative to those of the literature, and further identification was made by matching their recorded mass spectra with those stored in the mass spectral library (NIST 8) of the GC–MSD data system.

3. Results and Discussion

Nonpolar-solvent (hexane/chloroform (1:1)) fractionated ethanol crude extract of powdered *Artemisia annua* leaf was subjected to phytochemical study. These chemical investigations using chromatographic methods resulted in the isolations of two known compounds *artemisinin* (**1**) and scopoletin (**2**), and one new long chain alcohol called 1-heptacosanol (**4**) (Fig. 2).

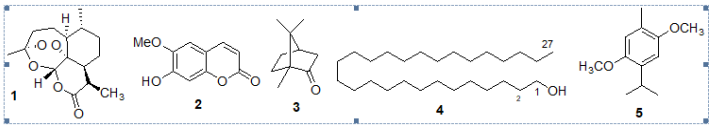

Figure 2. Chemical structure of some compounds isolated from *Artemisia annua*

The structures of the isolated compounds are characterized as follows using their physical and spectroscopic data.

Compound 1

Compound **1** (200 mg) was isolated as colorless needles (Rf 0.48 in EtOAc:Hex (1:1); mp 141-142°C) from leaf extracts of *A. annua*. The UV-Vis spectrum showed no absorption maxima. Strong IR absorption bands were observed at 2974/ 2849 cm^{-1} (C-H) and 1739 cm^{-1} (C=O) (Fig 2). ^1H NMR spectrum was integrating for twenty two hydrogens. Signals at δ1.23 (3H, *d*, *J*=7.2Hz), 1.00 (3H, *d*, *J*=6.4Hz) and 1.45 (3H, s) were observed and assigned to methyl protons corresponding to H-13, H-14 and H-15, respectively. Two of them were found as doublet which showed their presence at tertiary carbon. In the spectrum four multiplets at δ1.40 (H-1), 1.43 (H-10), 1.78 (H-7) and 3.40 (H-11) due to methine protons and singlet at δ5.89 (H-5) owing to oxymethine proton were observed. Methylene protons were also evident as multiplet at δ1.48 and 2.05 (H-2), 2.08 and 2.43 (H-3),

1.07 and 1.90 (H-8) and 1.07 and 1.78 (H-9). There was no exocyclic double bond in the molecule (Fig 3).

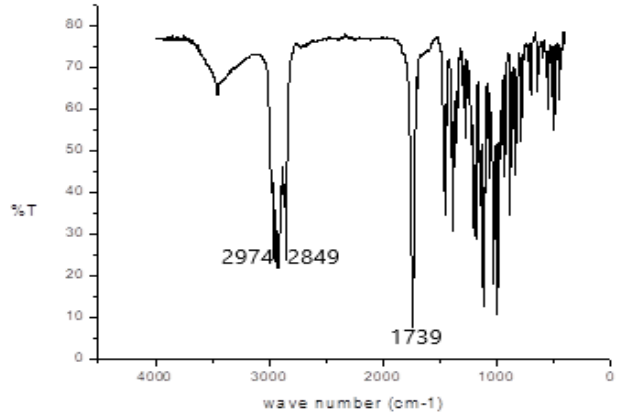

Fig. 3. FTIR spectrum of Compound 1

The ^{13}C NMR spectrum showed fifteen carbon signals comprising three methyl, four methylene, five methine and three quaternary carbons as identified by its DEPT-135 spectrum. One of the methyl carbon signals found deshielded and singlet (δ25.2) representing its bonding closer to hetroatom. The other methyl carbons resonated at δ12.6 and 19.8 were bonded to tertiary carbon atoms. A tertiary carbon signal at δ93.7 (C-5), and quaternary carbon signals at δ105.4 (C-4) and 79.5 (C-6) were also distinguished as oxygenated.

Lactone carbonyl signal was also showed at δ172.1(C-12) besides methine carbon signals at δ50.0 (C-1), 45.0 (C-7), 37.5 (C-10) and 32.9 (C-11) (Fig 3). The data was comparable with reported values in the literature for artemisinin [4,9] (Table 1, Fig 3,4). This sesquiterpene lactone has an endoperoxide oxygen bridge and it is also considered as the major active principle of the plant.

Table 1. ^{1}H and ^{13}C NMR spectral data of Artemisinin **1** compared with Literature ($CDCl_3$, δ in ppm, *J* in Hz).

C no	NMR data of 1		NMR data of Artemisinin	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	1.40 (1H,m)	50.0	1.37 (1H,m)	49.90
2	2.05 (1H,*m*),1.48 (1H,m)	24.9	2.01 (1H,*m*),1.47 (1H,m)	24.79
3	2.43 (1H,m),2.08(1H, m)	35.9	2.43(1H,*m*), 2.05(1H,*m*)	35.77
4		105.4		105.22
5	5.89 (1H, s)	93.7	5.87 (1H, s)	93.62
6		79.5		79.38
7	1.78 (1H,*m*)	45.0	1.75(1H,*m*)	44.80
8	1.90(1H,*m*),1.07 (1H,m)	23.4	1.87(1H,*m*), 1.12 (1H,m)	23.32
9	1.07 (1H,*m*),1.78(1H. m)	33.6	1.08 (1H, *m*), 1.79 (1H,*m*)	33.45
10	1.43(1H,*m*)	37.5	1.42 (1H,*m*),	37.42
11	3.40(1H,m)	32.9	3.40 (1H,*m*)	32.78
12		172.1		171.92
13	1.23(3H,*d*,J=7.2)	12.6	1.21 (3H,d,J=7.2)	12.47
14	1.00(3H,d,J=6.4)	19.8	0.99 (3H,d,J=6.4)	19.74
15	1.45(3H,*s*)	25.2	1.44(3H,s)	25.10

Compound 2

Compound **2** (20 mg) was isolated as yellow crystalline solid (Rf 0.41 in EtOAc:Hex (1:1); mp 198-200^{0}C) after recrystallizations in dichloromethane. It showed blue fluorescence under UV light. It was UV-active and showed two absorption maxima at 295 nm and 340 nm. The IR spectrum showed absorption bands at 3338 cm^{-1} (O-H), 1702 cm^{-1} (C=O) and 1614/1564 cm^{-1} (benzene) (Fig 4). The ^{1}H NMR spectrum (DMSO-d6) showed methoxy proton signal at δ3.81 (3H, s,-OMe). It also displayed singlets in aromatic region at δ7.21 (1H,s) and 6.78 (1H,s), and vinylic proton signals at δ 6.21(1H, *d*, *J*=9.6HZ) and 7.91 (1H, *d*, *J*=9.6HZ). The phenolic proton was not observed in ^{1}H NMR whereas the IR spectrum did it. The ^{13}C NMR spectrum showed ten carbon signals of which resonances at δ103.2, 111.0, 112.1, 145.7, 151.7 and 145.0 represented aromatic methine carbons and signal at δ56.5 showed methoxy carbon. Together with lactone carbonyl signal at δ161.2 and two olefinic carbon signals at δ110.0 and 145.0 the data showed a coumarin type skeleton (Fig 5). The overall data was found matching with literature values of scopoletin (**2**) [10] (Table 2, Fig. 5,6).

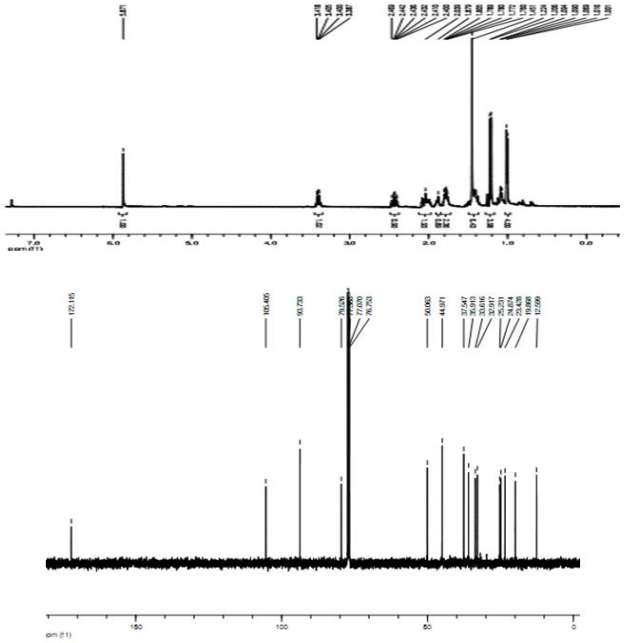

Fig. 4. ^{1}H NMR (top) and ^{13}C NMR (bottom) Spectra of Compound 1

Table 2. ^{1}H and ^{13}C NMR data of **2** compared with literature value of scopoletin (DMSO-d6, δ in ppm, J in Hz).

C no	NMR data of 404, DMSO-d6		NMR data of scopoletin, C_3D_6O	
	δH	δC	δH	δC
1	-	161.2	-	161.5
2	6.21 (1H,*d*,J= 9.6)	110.0	6.21 (1H,d,J=9.3)	107.6
3	7.91 (1H,*d*,J=9.6)	145.0	7.92 (1H,d,*J*=9.3)	143.4
4	-	111.0	-	111.5
5	7.21 (1H,s)	112.1	7.21 (1H,s)	113.4
6	-	145.7	-	144.1
7	-	151.7	-	150.3
8	6.78 (1H,s)	103.2	6.78 (1H,s)	103.2
9	-	150.0	-	149.8
10	3.81(3H,s, OCH_3)	56.5	3.87(3H,s,OCH_3)	56.5

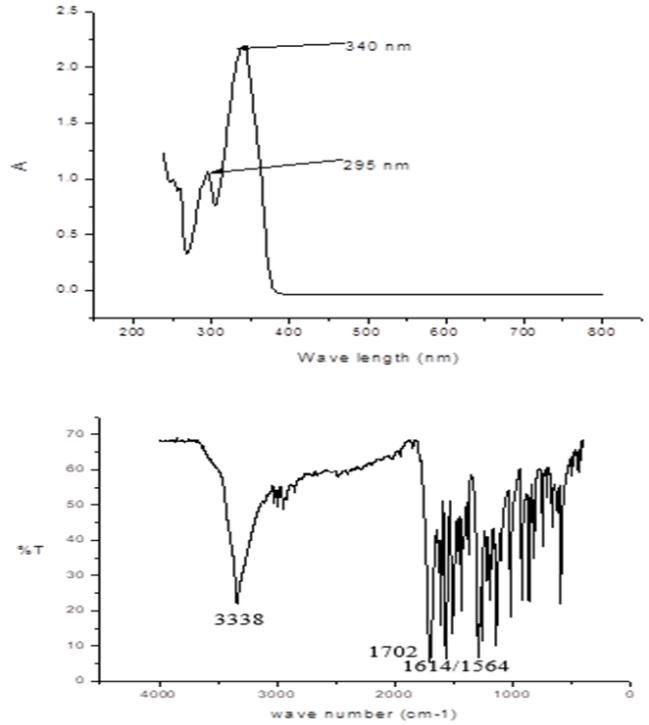

Fig. 5. UV-Vis (top) and FTIR (bottom) spectra of Compound 2

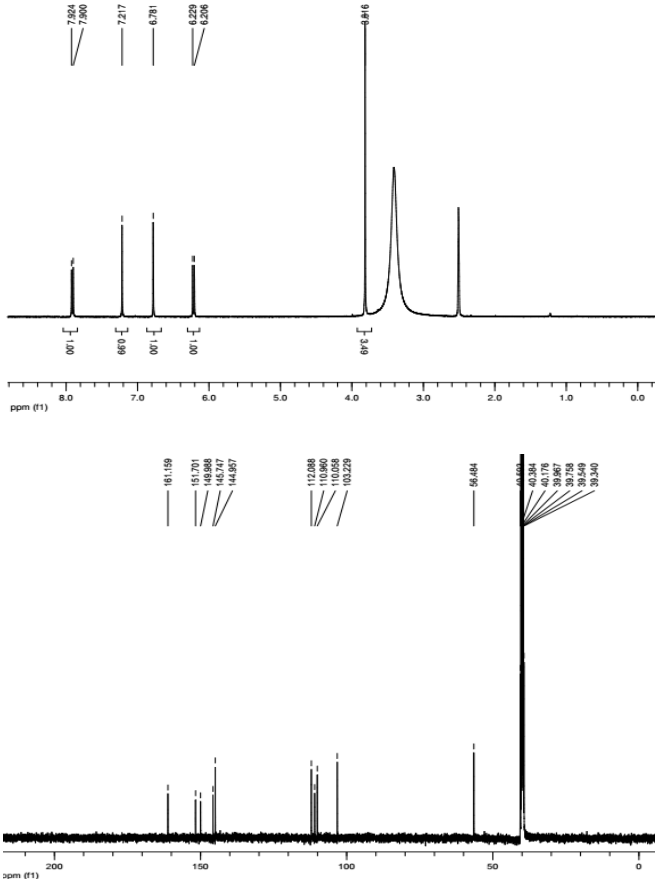

Figure 6. ^{1}H NMR (top) and ^{13}C NMR (bottom) Spectra of Compound 2

Compound 4

Compound **4** (30 mg) was isolated as white powder (Rf 0.50 in EtOAc:Hex (1:1); mp 73-75°C) from leaf extracts of *A. annua*. The UV-Vis spectrum (in $CHCl_3$) showed no absorption maxima in the region. The IR spectrum displayed absorption bands at 3336 cm^{-1} (-OH) and 2923/2837 cm^{-1} (C-H) (Fig 7). The ^{1}H-NMR spectrum showed a triplet at δ 3.66 (J = 6.4 Hz) assigned to methylene protons on oxygenated carbon adjacent to methylene proton. The quintet at δ1.60 integrated for two protons was due to methylene protons on carbon flanked between two methylene groups. A proton resonance at δ1.86 showed the presence of alcohol function. A broad intense singlet at δ1.32 (44H) is characteristic signal for many overlapping methylene protons which was supported by appearance of an intense carbon signal at δ 29.6 in the ^{13}C-NMR spectrum. An up field triplet at δ 0.89 (3H, J = 6.7 Hz) was an evidence for the presence of terminal methyl group closer to methylene protons. In the ^{13}C-NMR spectrum the down field signal at δ 63.1 corresponds to an oxygenated aliphatic methylene carbon. The carbon resonance at δ 14.2 is characteristic signal for terminal methyl group (Fig 8). Thus, comparison of the NMR spectral data of compound **4** with the reported value for heptacosan-1-ol was in close agreement (Table 3) [11]. It was isolated from *Citrullus colocynthis*, *Buddleja crispa* and *Crataegus monogyna.*

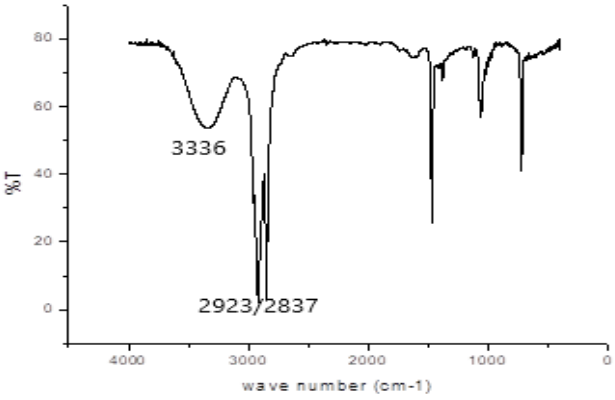

Fig. 7. FTIR Spectra of Compound 4

Table 3. The ^{1}H and ^{13}C-NMR spectral data for **4** compared with literature report ($CDCl_3$, δ in ppm, J in Hz)

NMR data of 4		NMR data of 1-Heptacosanol	
^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
3.66 (2H, *t*, *J* = 6.4 Hz, H-1)	63.1	3.64 (2H, *t*, *J* = 6.3 Hz, H-1)	63.1 (C-1)
1.58 (2H, *m*, H-2)	32.8	1.57 (2H, *m*, H-2)	32.8 (C-2)
1.30(48H, *br s*, H-3 to H-26)	31.9	1.25(48H, *br s*, H-3 to H-26)	31.9 (C-3)
	30.0-29.4		29.7-29.3 (C4-24)
	25.8		25.7 (C-25)
	22.7		22.6 (C-26)
0.90(3H, t, *J* = 6.7Hz, H-27)	14.2	0.88 (3H, *t*, *J* = 6.6 Hz, H-27)	14.1(C-27)

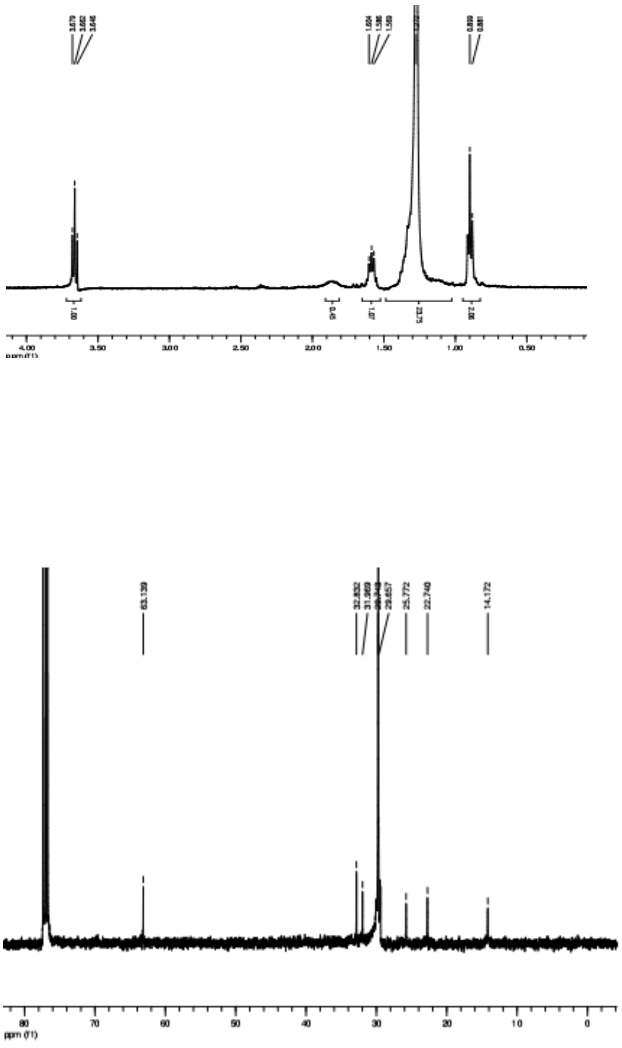

Fig. 8. ^{1}H NMR (top) and ^{13}C NMR (bottom) Spectra of Compound 4

3.1 GC-MS analysis of Leaf essential Oils from *Artemisia annua*

Hydrodistillation of powdered leaf (50 g) resulted in 100 mg (0.2%) essential oil and its GC-MS analysis showed camphor 3 (81%) as major component (Fig 9) as described elsewhere [4,5] followed by 2,5-dimethoxy-4-isopropyltoluene (2.3%) 5 (Table 4).

Table 4. Chemical composition of Essential oil of *A. annua* leaf

Peak no	Compound name	Chemical structure	Retention time (min)	% comp
1	Camphor	3	8.837	80.90
2	2,5-Dimethoxy-4-isopropyltoluene	5	12.710	2.30

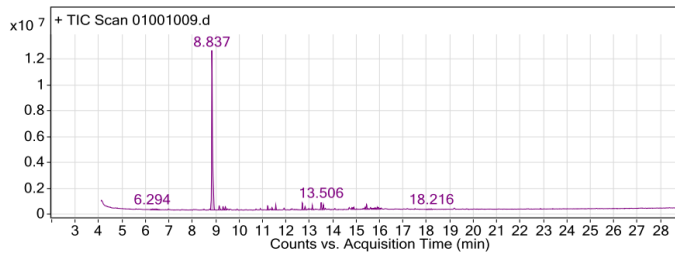

Figure 9. GC Chromatogram of the Artemisia annua leaf essential oil

Conclusions

Phytochemical study of the ethanol (95%) crude extract obtained from the leaf of the known medicinal plant, *Artemisa annua*, afforded a new long chain alcohol called 1-heptacosanol, besides the common compounds found in it, scopoletin and artemisinin. Camphor was also found the dominant component of the leaf essential oil of the plant grown in Ethiopia. The leaf of the plant grown in Ethiopia is rich in secondary metabolites.

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