



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

Minbale Gashu*

Department of Chemistry, Debre Berhan University, College of Natural and Computational Science, Debre Berhan, Ethiopia

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 used chromatographic and spectroscopic techniques for separation and purification of the metabolites, and structure elucidation, respectively. Chemical study on *Artemisia annua* leaf, acclimatized in Ethiopia, verified the presence of new long chain alcohol (1-heptacosanol) along with the well-known scopoletin and artemisinin in it. The leaf essential oil isolated by hydrodistillation was also analyzed by GC-MS and found rich in camphor (81%).

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

1. Introduction

China based medicinal plant called *Artemisia annua* (*Asteraceae* family), nurtured in many countries including Ethiopia, is the basis of malaria drugs besides treating headaches, inflammation, fevers, and bleeding [1]. The plant leaf is rich, more than 600, in secondary metabolites dominated by coumarins, terpenoids and flavonoids [2,3]. The more bioactive terpenoid from the plant is Artemisinin (**1**) [3,4,5] which is potent in its antimalarial activity and in treating other parasitic diseases and infections [5]. Compounds like scopoletin (**2**), octacosanol, scopolin, and nonacosanol [3,6] were isolated from it. The leaf volatile oil from Bulgaria plant contained mainly α -humulene, α -copaene, α -cuvabene, artemisia ketone, α -selinene and camphor (**3**) [7]. The Chinese plant has largely artemisia ketone while that of Vietnamese contained mainly **3** and germacrene D [3,7,8]. Further phytochemical study on the leaf of the plant grown in Ethiopia was relevant for comparison. The focus 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 leaf volatile oil composition was also analyzed.

2. Materials and Methods

2.1. Material Preparation for Extraction

Leaf of the plant collected from AAU, Science faculty (Fig 1) identified by professional botanist in Ethiopian National Herbarium (S1220), was shade dried for a week. It was ground and stored for further use; of this leaves powder (100 g) was soaked in EtOH (95%) to afford extract (15 g). It was further fractionated into hex/CHCl₃ (1:1) (10 g) and MeOH (5 g), for phytochemical study.

2.2. Materials used

Solvents and chemicals, capillary melting point apparatus (Thomas Hoover), TLC (Merck) were used.

*Corresponding author: minbalegashu2012@gmail.com

.Received: May 16/2023 ,Revised: June 14/2023, Accepted: June 15/2023, Published: July 1/2023



Figure 1. *Artemisia annua* (Picture by author)

The UV (254 nm) and vanillin spray followed by heating for detection, Silica gel chromatography for separation, BUCHI rota vapor for solvent removal, and T 60 UV spectrophotometer, Bruker ACQ 400 AVANCE spectrometer and Perkin-Elmer BX IR Spectrometer were used for analysis.

2.3. Methods of Isolations and Structure Elucidations

The extract (8 g) on column was carried with pet. Ether: E. acetate: MeOH by polarity increment to get 20 solvent portions. Recrystallizations of Fr 5,8 and 14, respectively, afforded artemisinin (1), scopoletin (2) and 1-heptacosanol (4). The compounds obtained were characterized using NMR, IR, UV-VIS analysis and literature reports.

2.4. Essential Oil Isolation and Characterization

Hydrodistillation (2 h) using Clevenger apparatus was employed to isolate essential oil from dried leaf (50 g) and the components were identified by Agilent technologies, 7820A GC-MSD connected to a 5977E MSD with HP-88, 3000 cm x 25 cm x 250 mm thick film column coated with PDMS (100%). Sample (1 μ L) shoot was carried with He gas for 31.31 min. Retention indices compared with literature to identify and recorded mass spectra match with those in NIST 8 library of the GC-MSD data were made.

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).

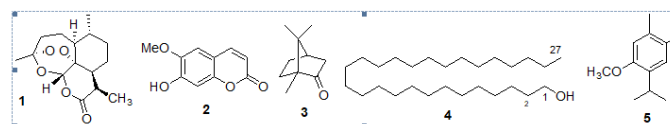


Figure 2. Structure of compounds from *A. annua*

Structures of isolated compounds are characterized as follows using their physical and spectroscopic data.

Compound 1

Compound 1 (200 mg) was found as colorless needle like structures (0.48 Rf in E. acetate:Hex (1:1); mp 141-142°C). The absence of UV-Vis absorption, and IR at (cm^{-1}) 2974/ 2849 (C-H) and 1739 (C=O) (Fig 2) were evident (Fig 3). ^{22}H from ^1H NMR spectrum observed at δ 1.23 (3H, *d*, *J*=7.2 Hz), 1.00 (3H, *d*, *J*=6.4 Hz) and 1.45 (3H, *s*) allocated to CH_3 at H-13, H-14 and H-15, respectively. Two doublets at tertiary carbon, 4 CH protons at δ 1.40 (H-1), 1.43 (H-10), 1.78 (H-7), 3.40 (H-11) and oxymethine at δ 5.89 (H-5), protons of 4CH_2 at δ 1.48 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) were observed.

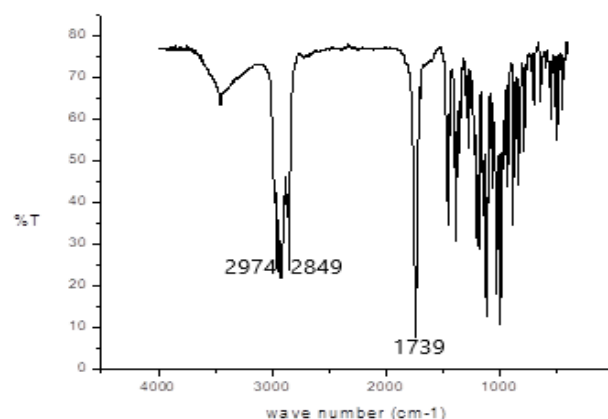


Fig. 3. FTIR spectrum of Compound 1

Fifteen carbon signals in ^{13}C NMR spectrum comprising 3CH_3 , 4CH_2 , 5 CH and 3 C were observed. The singlet CH_3 (δ 25.2) represent link to hetro-atom, and CH_3 (δ 12.6, 19.8) are connected to CH. Oxygenated signals (δ 93.7 (C-5), δ 105.4 (C-4), 79.5 (C-6) were seen. The above data including cyclic ester carbonyl signal (δ 172.1(C-12)) and CH peaks (δ 50.0 (C-1), 45.0 (C-7), 37.5 (C-10), 32.9 (C-11)) (Fig 3) were similar with that of artemisinin [4,9] (Fig 3,4). The endoperoxide oxygen bridge is evident and the compound is considered as main active principle of the plant.

Compound 2

An isolate (Compound 2, 20 mg) obtained as crystalline yellow solid (Rf 0.41 in Ethyl acetate:Hexane (1:1); mp 198-200 °C) later to dichloromethane recrystallization. The blue fluorescence under UV light (295 nm and 340 nm) showed its UV-activity. Benzenoid IR at (cm^{-1}) 3338 (O-H), 1702 (C=O) and 1614/1564 (Fig 4) and OCH_3 signal (δ 3.81 (3H, *s*, -OMe)) were demonstrated. The

M. Gashu

^1H NMR spectrum (DMSO- d_6) showed benzenoid singlets at $\delta 7.21$ (1H, s) and 6.78 (1H, s), and vinylic peaks at $\delta 6.21$ (1H, d , $J=9.6$ Hz) and 7.91 (1H, d , $J=9.6$ Hz). Ten carbon signals in ^{13}C NMR spectrum were observed, where aromatic CH signals resonate at $\delta 103.2$, 111.0, 112.1, 145.7, 151.7 and 145.0 and OMe signal at $\delta 56.5$. The carbonyl (lactone) signal at $\delta 161.2$ and 2 double bond signals at $\delta 110.0$ and 145.0 together with the above data represented a coumarin skeleton (Fig 5) and match with reported values of scopoletin (2) [10] (Table 1, Fig. 5,6).

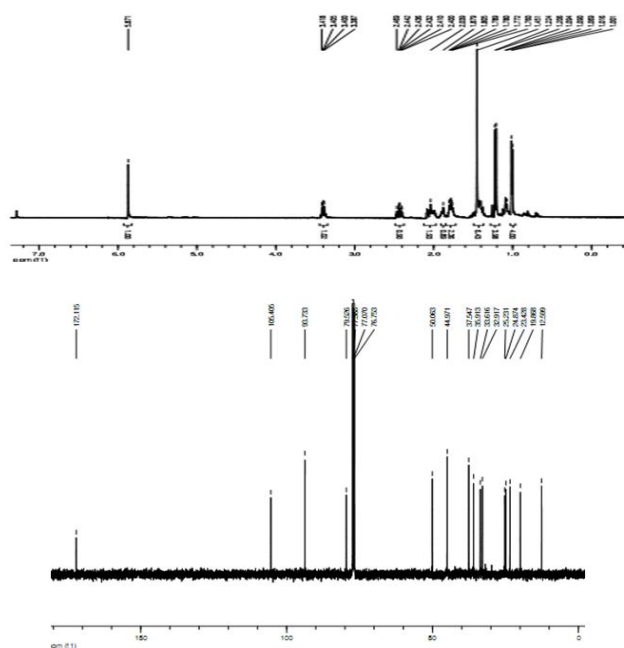


Fig. 4. Spectra of **1** ^1H -(top) and ^{13}C - NMR (bott)

Table 1. NMR data of **2** and scopoletin.

C n o	NMR of 2 , DMSO- d_6		scopoletin NMR, $\text{C}_3\text{D}_6\text{O}$	
	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

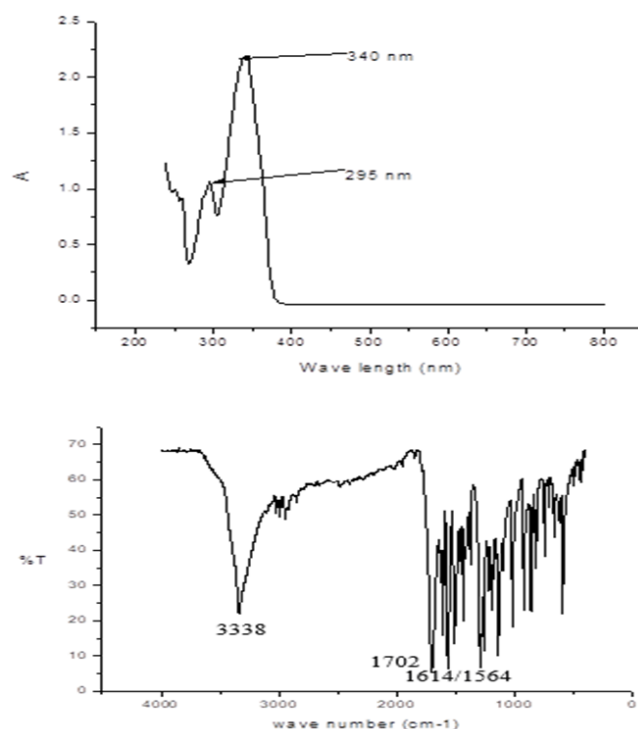


Fig. 5. UV-Vis (top) and FTIR (bott.) spectra of **2**

Compound 4

The bleached dust (R_f 0.50 in E. acetate:Hex (1:1); mp 73-75°C), labelled as Compound **4** (30 mg), was isolated from leaf extracts of *A. annua*. There was no maxima in UV-Vis spectrum (CHCl_3). IR at (cm^{-1}) 3336 ($-\text{OH}$) and 2923/2837 ($\text{C}-\text{H}$) (Fig 7) were seen. The triplet proton signal (δ 3.66 ($J = 6.4$ Hz)) was oxygen carbon connected CH_2 near to methylene. The signal at $\delta 1.60$ (quint, 2H), CH_2 , a carbon bordered by two CH_2 , and the proton signal at $\delta 1.86$ indicates alcohol function.

Broad intense characteristic signal for many overlapping CH_2 at $\delta 1.32$ (44H) and a signal at $\delta 29.6$ were supportive of each other. Triplet signal at $\delta 0.89$ (3H, $J = 6.7$ Hz) evidenced terminal CH_3 closer to CH_2 . The signal at $\delta 63.1$ was allocated to oxygen containing aliphatic CH_2 . The representative signal ($\delta 14.2$) of CH_3 at terminal position (Fig 8) and the whole data of **4** and reports of heptacosan-1-ol were in close agreement (Table 2) [11].

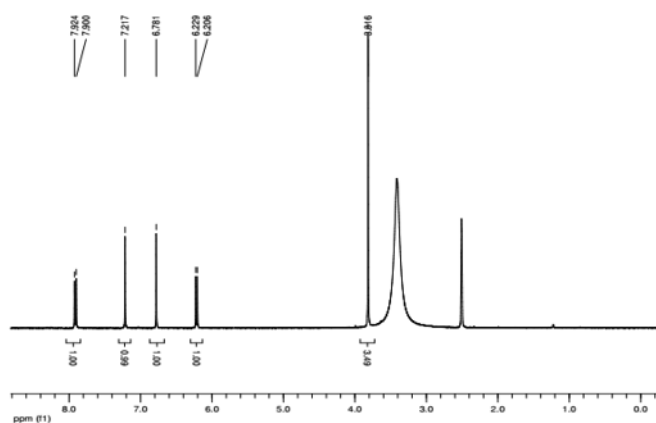


Figure 6. ^1H NMR (top) and ^{13}C NMR (bottom) spectra of **2**

Table 2. ^1H and ^{13}C -NMR spectral data for **4** related with 1-Heptacosanol reports (CDCl_3 , δ in ppm, J in Hz)

NMR data of 4		NMR data of 1-Heptacosanol	
^1H -NMR	^{13}C -	^1H -NMR	^{13}C -NMR
3.66 (2H, <i>t</i> , $J = 6.4$ Hz, H-1)	63.1	3.64 (2H, <i>t</i> , $J = 6.3$ Hz, H-1)	63.1 (C-1)
1.58 (2H, <i>m</i> , H-2)	32.8	1.57 (2H, <i>m</i> , H-2)	32.8 (C-2)
1.30 (48H, <i>br s</i> , H-3 to H-26)	31.9	1.25 (48H, <i>br s</i> , 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, <i>t</i> , $J = 6.7$ Hz, H-27)	14.2	0.88 (3H, <i>t</i> , $J = 6.6$ Hz, H-27)	14.1 (C-27)

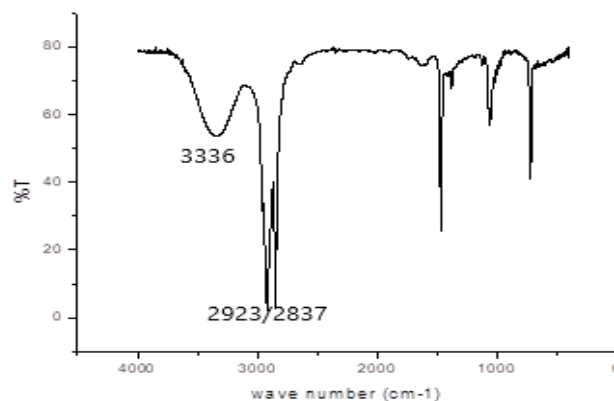


Fig. 7. FTIR Spectra of Compound **4**

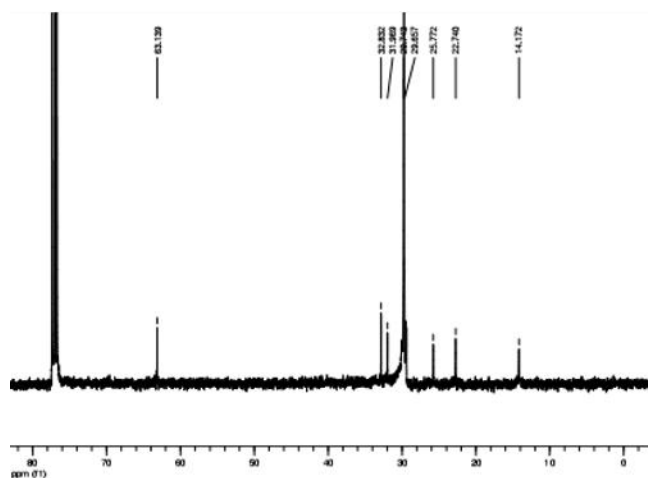
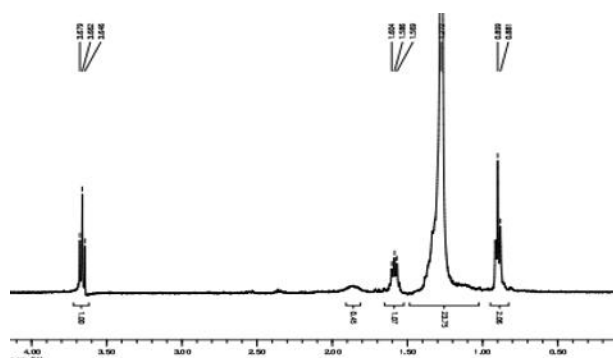


Fig. 8. ^1H - (top) and ^{13}C - NMR (bottom) Spectra of **4**

Essential oil analysis, GC-MS

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

Table 3. Chemical composition of *E.O*

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

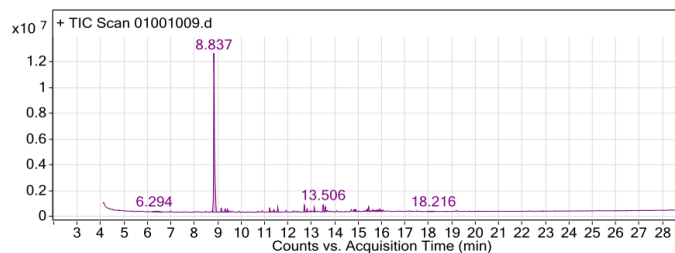


Figure 9. GC Chromatogram of the leaf essential oil

Conclusions

Phytochemical investigation of leaf extract of *Artemisia annua* afforded a new long chain alcohol called 1-heptacosanol, besides its common compounds, scopoletin and artemisinin. Camphor was also found the dominant component of its essential oil. The study shows that *Artemisia annua* grown in Ethiopia is rich in secondary metabolites and therefore, Ethiopia can be an alternative country to cultivate the plant for its medicinal value.

Acknowledgement

The author acknowledges Department of Chemistry (Addis Ababa University) for analysis

References

1. Klayman DL: *Artemisia annua*: from weed to respectable antimalarial plant, in Human Medicinal Agents from Plants, Kinghom AD and Balandrin MF, Eds. *American Chemical Society Symposium Series* 1993, 242–255.
2. Abou El-Hamd H, Mohamed Magdi A. El-Sayed, Mohamed E. Hegazy, Soleiman E Helaly, Abeer M. Esmail and Naglaa , Mohamed S: Chemical constituents and biological activities of *Artemisia herba-alba*. *Records of Natural Products* 2010, 4: 1-25.
3. Brown GD: The Biosynthesis of Artemisinin (Qinghaosu) and the Phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules* 2010, 15: 603-7698.
4. María José Abad , Luis Miguel Bedoya, Luis Apaza and Paulina Bermejo: The *Artemisia* L. Genus: A Review of Bioactive Essential Oils. *Molecules* 2012, 17: 2542-2566.
5. R. Tzenkova, Z, Kamenarska, A, Draganov, A. Atanassov: Composition of *Artemisia Annua* Essential Oil Obtained from Species Growing Wild in Bulgaria. *Biotechnology and Biotechnological Equipment* 2010, 24: 1833-1835.
6. Rubén S, Rimada, Walter O, Gatti, René Jeandupeux, Lázaro. R, Cafferata: Isolation, characterization of Artemisinin by NMR from Argentinean *Artemisia annua* L. *Bol Latinoam Caribe Plant Medicine and Aromatics* 2009, 8:275-281.
7. Garcia LC: A Review of *Artemisia annua* L. Its Genetics, Biochemical Characteristics, and Anti-Malarial Efficacy. *International Journal of Science and Technology* 2015, 5:38-46.
8. Brown GD, Sy L-K: *In vivo* transformations of dihydroartemisinin acid in *Artemisia annua* plants. *Tetrahedron* 2004, 60: 1139–1159.
9. Gábor Blaskó, Geoffrey A. Cordell, David C. Lankin: Definitive ^1H - and ^{13}C -NMR Assignments of Artemisinin (Qinghaosu). *Journal of Natural Products* 1988, 51(6):1273–1276.
10. Akhmad Darmawan, Soleh Kosela , Leonardus B.S. Kardono, Yana M. Syah: Scopoletin, a coumarin derivative compound isolated from *Macaran gagigantifolia* Merr. *Journal of Applied Pharmaceutical Science* 2012, 2(12):175-177.
11. Yen Chin Koay, Keng Chong Wong , Hasnah Osman ,Ibrahim M. S. Eldeen, Mohammad Zaini Asmawi: Chemical Constituents and Biological Activities of *Strobilanthes crispus* L. *Records of Natural Product*, 2013, 7:59-64.