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Metabolites Detected in the Crude N-Hexane Extract of *Artemisia annua* Linn (Asteraceae) Cultivated in Langtang, Plateau State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author CEM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JIA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Artemisia annua was grown in and harvested from Langtang, Plateau State in Nigeria. The dried pulverized aerial part of *Artemisia annua* was extracted with n-hexane by percolation and concentrated in vaccum at 40℃. Analysis of crude n-hexan e extract by GC-MS revealed twelve major compounds, which were mostly terpenoids. These are Deoxyqinghaosu (6.85%), Arteannuic acid (3.99%), Caryophyllene oxide (4.49%), 9-Octadecenoic acid (25.54%), Camphor (2.93%), Borneol (1.48%), 5,5Dimethyl-6-methylenebicyclo[2,2,1]hept-2-yl acetate (1.18%), 2,6-dimethyl-3,5,7-octatriene-2-ol, E,E (1.48%), Carveyl acetate (0.52%), Spathulenol (1.57%), α-Cubebene (0.66%), β-Farnesene (0.57%). The result suggests that the *Artemisia annua* chemotypes cultivated in Langtang area of Plateau State, Nigeria, may be related to those grown in Ethiopia.

Keywords: Artemisia annua L.; metabolites; GC-MS; terpenoids.

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1. INTRODUCTION

Artemisia annua, also known as sweet wormwood, sweet annie, sweet sagewort or annual wormwood (English) and Qinghao (Chinese), is an annual aromatic herb belonging to the family Asteraceae. *Artemisia annua* is so named because it is almost the only member of the genus with an annual cycle [1]. *Artemisia annua* has fern-like leaves, bright yellow flowers and a camphor-like scent. It grows to about 2 m tall and has a single stem, alternating branches, and alternating leaves ranging from 2.5-5 cm in length. *Artemisia annua* is native to temperate Asia but naturalized throughout the world [2].

Artemisia annua has a long history of use in traditional Chinese medicine. The plant has been used for over 2,000 years in China, to treat symptoms associated with fever and malaria. The first mention of the name Qinghao is in a silk book excarvated from a tomb in 168 BC. In 340 AD, the first record of its application for treatment of fevers appeared in a medicinal book, ‘‘Handbook of Prescription for Emergency Treatment”. In 1596, the famous material medica “Ben Cao Gang Mu” described its use for treatment of chills and fevers caused by malaria. In 1967, the Chinese government began a systematic screen of plants used in traditional Chinese medicine with a specific focus on malaria treatment and in 1971; scientists demonstrated that the *Artemisia annua* plant extracts had antimalarial activity in primate models. In 1972, Chinese Scientists isolated and chemically characterized the active ingredient as *Artemisinin*. Currently, the war against malaria *has* revived the need for the plant, as it has been discovered to be very potent for the chemotherapy of malaria disease [3].

Besides artemisinin, the plant biosynthesizes and accumulates a great variety of gaseous secondary metabolites at ambient temperature, forming their essential oil. The investigation of these substances has allowed the identification of several sesquiterpenoids, flavonoids, cumarins, triterpenoids, steroids, phenolic compounds, purines, lipids and aliphatic compounds in samples coming from different parts of the world [4,5].

The phytochemistry of *Artemisia annua* is dominated by terpenoids (in particular sesquiterpene lactones), flavonoids, coumarines and other shikimate metabolites as is the case for many other plants in the Asteraceae family [6-8].

The sesquiterpene lactone, artemisinin is an important component of *Artemisia annua* because of its antimalarial property. Artemisinin is found in the leaves and flowers of *Artemisia annua*. Many of *Artemisia* species have been screened for the presence of artemisinin, but only *Artemisia annua* and to a lower extent *Artemisia apiacea* and *Artemisia lancea* were found to produce artemisinin [9,10]. Some co-metabolites such as Artemisitene, Artemisinic or Arteannuic acid, Arteannuin B, and Dihydroarteannuin have also been reported, and other compounds such as Dihydroartemisinin (Artenimol) and Deoxyartemisinin, [11-15].

Apart from artemisinin from *Artemisia annua*, essential oils are another important constituent of *Artemisia annua*. The essential oil of *Artemisia annua* was first studied as long ago as 1917 [6]. Depending on the geographical location, the oil yield in *Artemisia annua* ranges between 0.02-0.49% on fresh weight basis and 0.04-1.9% on dry weight basis [9].

The chemical constituents of *Artemisia annua* volatile oil vary from country to country (Table 1). For example the principal constituents of Chinese oil obtained through hydrodistillation were 63.9% Artemisia ketone, 7.5% Artemisia alcohol, 5.1% mercene, 4.7% α-guaine and 3.3% camphor. The Vietnamese oil majorly contained 21.8% camphor, 18.3% germacrene and 3.1% 1,8-cineol etc [8,7]. A study conducted in India also showed that *Artemisia* ketone (58.8%), camphor (15.8%), 1,8-cineol (2.2%), and gramicidin-D (2.4%) are the main components of the essential oil of *Artemisia annua* plant [16]. Verdian et al;(2008) in their study conducted in Iran showed that camphor (48%), 1,8-cineol (9.3%), camphene (6.98%), and spathulenol (4.89%) were identified as the prominent components [17]. Nibret and Michael (2010) reported deoxyqinghaosu (20.44%), Linolic acid (12.46%) and camphor (9.62%) as the principal volatile components of the dichloromethane extract of *Artemisia annua* from Ethiopia [18]. Ahmed (2008), identified 38 volatile constituents of essential oil of *Artemisia annua* from Ethiopia, in which camphor (43.84%) was reported as a major component of the essential oil of *Artemisia annua* obtained by hydrodistillation [19]. These differences may be ascribed to the existence of different *Artemisia annua* chemotypes [20].

The objective of this work is to identify a chemomarker if any, for *Artemisia annua L.* (Asteraceae) cultivated at University of Jos

Artemisia annua plantation situated at Gangnum in Langtang South Local Government of Plateau State (80 38'N, 90 48'E, 8.6330 N, 9.80 E), Nigeria by analysis and identification of the volatile constituents of the aerial parts of the plant using GC-MS.

2. MATERIALS AND METHODS

2.1 Plant Material

The dried aerial part (stem, leaves and flowers) of *Artemisia annua*, harvested at full flower stage from Langtang was supplied by the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos.

2.2 Extraction of Plant Material

The dried, pulverized aerial part of *Artemisia annua* (0.3 kg) was extracted with n-hexane (1.5 L), by percolation for 72 hrs. The procedure was performed at room temperature, with vigorous mechanical shaking occasionally. The extracts were filtered and evaporated under reduced pressure using rotary evaporator at 40℃. The crude n-hexane extract which is yellowish-green oily paste, weighed 7.10 g (2.37% yields). The crude n-hexane extract was subjected to GC-MS analysis, carried out at NARICT Zaria, Kaduna state, Nigeria.

2.3 Gas Chromatography – Mass Spectrometry (GC/MS) Analysis

The crude n-hexane extract was analyzed using GC–MS-QP2010 PLUS system (Shimadzu, Kyoto, Japan). With purge flow of 3 mL/min, the injector temperature was 250℃. Helium with constant flow of 1.5 mL/min served as carrier gas. The oven was programmed at the following rates; the initial temperature of the column was 80℃ (2 min hold) followed by 200℃ (4 min hold) and finally at 280℃ (5 min hold). The mass spectrometer conditions were as follows: electron impact ionization (EI); interface temperature, 250℃; ion source temperature, 200℃; the detector voltage, 1 kV; solvent delay, 1.5 min. All data were obtained by collecting the full-scan mass spectra within the scan range of m/z 30 – m/z 800 over 30 min.

3. RESULTS AND DISCUSSION

3.1 Result of GC-MS Analysis

The result of the GC-Ms analysis is presented in Table 1. Eighteen (18) compounds were identified belonging to different classes of terpenoids.

Table 1. Compounds in crude n-Hexane extract of *Artemisia annua* aerial part as suggested by GC-MS

Peak line	Compound	Retention time (Min.)	Peak area (%)	Compound type
1	Camphor	7.767	2.93	Monoterpene ketone
2	Borneol	8.216	1.48	Monoterpene alcohol
3	Bicyclo (2,2,1) hepta-3-methylene-2,2-dimethyl-5-ol	9.479	1.18	Monoterpene alcohol
4	2,6-dimethyl-3,5,7-octatriene-2-ol, E,E.	10.278	1.48	Monoterpene alcohol
5	Carveyl acetate	11.067	0.52	Monoterpene ester
6	α-Cubebene	11.286	0.65	Sesquiterpene hydrocarbon
8	β-Farnesene	12.282	0.57	Sesquiterpene hydrocarbon
10	Caryophyllene oxide	14.423	4.49	Sesequiterpene oxide
11	Spathulenol	15.833	1.57	Sesquiterpene alcohol
13	Arteannuic acid	18.689	3.99	Sesquiterpene acid
16	Deoxyqinghaosu (Deoxyartemisinin)	22.373	6.85	Sesquiterpene lactone
18	9-octadecenoic acid (E)	23.400	25.54	Unsaturated acid

3.2 Discussion

The extraction of the dried plant material (0.3kg) gave 7.1g of extract for n-hexane indicating a yield of 2.40%. The percolation method of plant extraction employed in this work is not only simple, but very robust and conservational especially for thermally unstable extracts. The yield of extract was quite adequate for the analysis carried out. Twenty (20) compounds were indicated in the gas phase chromatogram (Fig. 1) of crude n-hexane extract. A total of twelve (12) compounds (Table 1), representing 50.65% of the indicated constituents were successfully matched with library reference. These compounds were identified by comparing their spectral data and fragmentation pattern with data from NIST mass spectral library (2005).

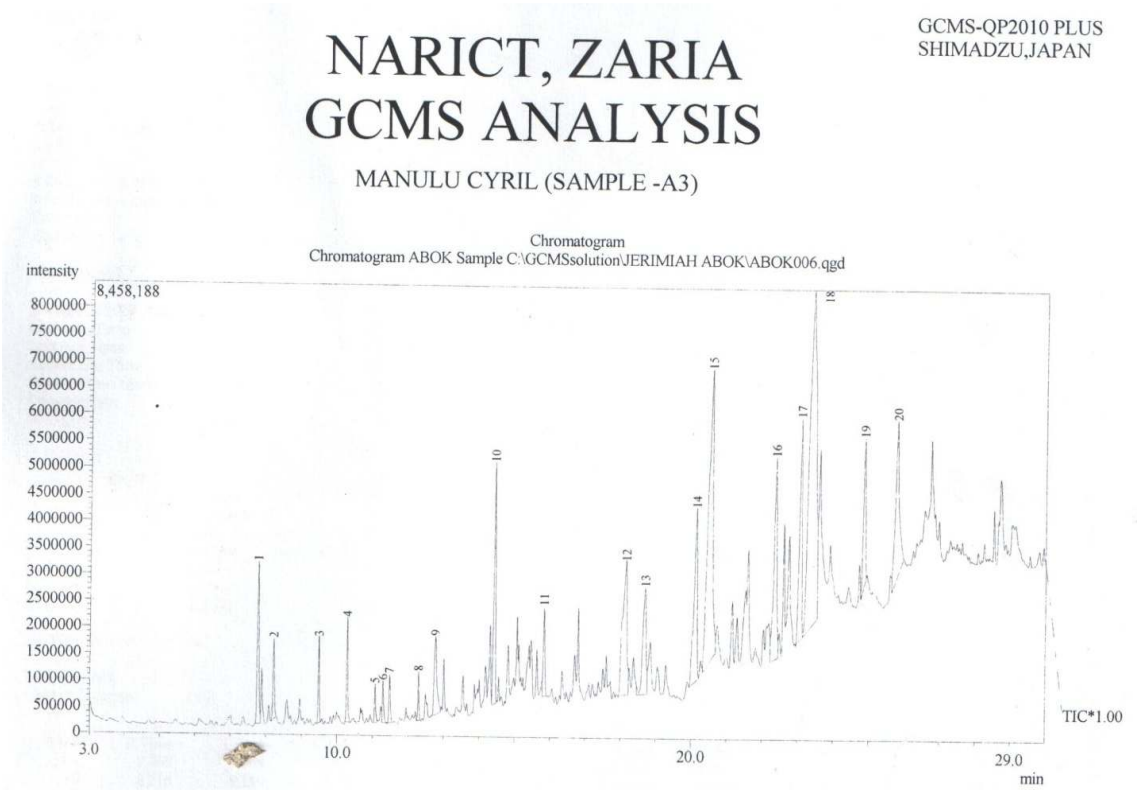

Fig. 1. GC-MS Chromatogram of n-Hexane extract of *Artemisia annua*

Table 2. Major volatile oil constituents in different samples of *Artemisia annua* [17-19]

Major volatile oil constituent	Origin of the plant				
	Ethiopia	China	Vietnam	Iran	India
Camphor	+	+	+	+	+
Mercene	–	+	–	–	–
Artemisia ketone	–	+	–	–	+
Artemisia alcohol	–	+	–	–	–
α-Guaine	–	+	–	–	–
Germacrene	–	–	+	–	–
1,8-cineol	–	–	+	+	+
Gramicidine-D	–	–	–	–	+
Camphene	–	–	–	+	–
Spathulenol	–	–	–	+	–
Deoxyqinghaosu	+	–	–	–	–
Linolic acid	+	–	–	–	–

Results from this study showed that the n-hexane extract of *Artemisia annua* cultivated in Plateau State of Nigeria contains camphor (2.93%), borneol (1.48%), 5,5Dimethyl-6-methylenebicyclo[2,2,1]hept-2-yl acetate (1.18%), 2,6-dimethyl-3,5,7-octatriene-2-ol, E,E. (1.48%), carveyl acetate (0.52%), α-cubebene (0.67), β-farnesene (0.57%), caryophyllene oxide (4.49%), spathulenol (1.57%), arteannuic acid (3.99%), deoxyqinghaosu (6.85%), 9-octadecenoic acid (E) (25.54%). These compounds are majorly terpenes. However, in this study, it was not possible to identify artemisinin from the GC-MS analysis, owing to the thermal degradation it must have undergone at the temperatures the column and the mass spectrometer was run. Ai et al. [21] reported that artemisinin was stable in neutral solvents or neat at up to 150℃ but extensive changes were however detected at 190℃ as a result of processes such as decomposition decomposing into three compounds at this temperature and rearrangements. Therefore, the presence of artemisinin is usually reported based on the thermal degradation products as opined by Ferreira et al. [22].

4. CONCLUSION

Thus, 9-octadecenoic acid (25.54%), Deoxyqinghaosu (6.85%), caryophyllene oxide (4.49%), arteannuic acid (3.99%) and camphor (2.93%) were found to be major constituents of the crude n-hexane extract of *Artemisia annua* plant grown in Langtang, Plateau state, Nigeria. This result further suggests that the *Artemisia annua* chemotypes cultivated in Langtang area of Plateau state, Nigeria, may be related to those grown in Ethiopia.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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