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Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil from Nigerian *Artemisia annua* L. at different growth stages

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Abstract: *Artemisia annua* is a reputable medicinal plant with long history of use as antimalarial and has characteris tic pleasant aroma. The aim of this study is to investigate the volatile oils from the aerial part of *Artemisia annua* fro m pre-flowering stage through to post-flowering growth stage using gas chromatography-mass spectrometry (GCMS). The colorless essential oils were obtained by hydrodistillation with yield ranging from 0.2% to 0.4% w/w. The maj or bioactive chemical compounds identified in the volatile oils at the various developmental stages were camphor (5. 67-16.84%), artemisia ketone (1.62-7.67%), eucalyptol (3.25-6.48%), arteannuic acid (1.36-4.27%), α-pinene (0.59- 3.62%), myrtenol (1.11-2.98%), caryophyllene (1.56-3.89%), copaene (0.68-1.72%), and deoxyartemisinin (0.19-0.6 4%). The volatile constituents of *Artemisia annua* were more at the post-flowering stage, the essential oil content inc reased with delay in harvest. From the results, the chemical composition of *Artemisia annua* volatile constituents var ied depending on the developmental stage.
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Keywords: *Artemisia annua*, volatile oils, camphor, artemisia ketone, arteannuic acid, deoxyartemisinin.

1. Introduction

Human society has always been in close contact with the environment and humans have obtained food, medicine, fiber and shelter from plant parts such as roots, stems, flowers, fruit and leaves (Jamshidi *et al.,* 2018; Ibrahim and Fagbohun, 2014). Natural products from plants, animals and minerals are used for treating diseases. Plants play important roles in the ecosystem, and diverse plant species from different families are distributed all over the world (Jamshidi *et al* 2018). Some plants are found in a particular region under specific environmental conditions.

The world health organization reported that about 80% of the world's populations in poor and less developed countries rely on traditional plant-based medicines for their primary health care requirements. Many diseases have been treated successfully with herbal medicines (Buba *et al.,* 2016). Medicinal plants are also used as trade commodities meeting the demand for the development of new drugs (Jamshidi *et al.,* 2018). Plants are also used as foods and food supplements and supply the body with nutrients and some important organic and inorganic chemical substances that help to boast immunity. Different plants contain different chemical compounds some of which are biologically active and used as effective agents against diseases (Oladeji, 2016).

All over the world, ethnopharmacology and drug discovery using plant derived natural products continue to attract attention. The rising cases of multi-drug resistance amongst pathogenic microbes are far greater than the increase in the arsenal of conventional drugs available to treat infections. Plants are sleeping giants of pharmaceutical industry (Itelima, 2017). Plants have shown their usefulness in the field of phytomedicine, pharmacognosy, and herbal science and pharmaceutical chemistry among others. Medicinal plants are use as stimulants, analgesic, anti-inflammatory, anti-convulsant, anti-microbial, anti-oxidant, anti-tumor, anti-malarial and many more (Izah *et al.,* 2018). One of such important medicinal plants reputed for its activity against *Plasmodium falciparum,* the parasite responsible for malaria, is *Artemisia annua*.

Artemisia annua is a globally reputable antimalarial plant with common names as sweet wormwood, annual wormwood, sweet annie or sweet sage wort (Smitha *et al.,* 2014). It is an annual plant (Das, 2009). It is native to Asia especially China but has been distributed all over the world including places such as Argentina, Bulgaria, France, Hungary, Italy,

Romania, Nigeria, Spain and USA (Das, 2009). It is an aromatic herb (Smitha *et al.,* 2014), and naturally occur as part of steppe vegetation at 1000m to 1500m above sea level (Shri, 2011). It is found in cool temperate and subtropical regions of the world (Garcia, 2015). *Artemisia annua* belongs to the family Asteraceae (Herman *et al.,* 2009). It is a large shrub of about 0.9m to 1.95m in height, single stemmed with alternate branches with leaves which are deeply dissected and has a length range of 2.5cm to 5cm (Itelima, 2017). The leaves and flowers both have 10-celled biseriatetrichomes and 5-cell filamentous trichomes each (Das, 2009). The nodding flower also known as capitula which is about 2mm to 3mm in diameter is greenish-yellow in color and enclosed by numerous imbricated bracets (Herman *et al.,* 2009). It is pollinated by wind and insect. It is extremely vigorous, essentially disease and pest free. It requires about 1000 hours of light annually (Nadali *et al.,* 2014). It produces best in open sunny positions on fertile sandy, loams and alluvial soils, neutral to slightly acidic with good moisture retention. It does not tolerate drought or water logging. It requires a minimum of six month for cultivation.

The fruit of *Artemisia annua* is an achene with a single seed inside which is approximately 1mm in length, it does not have a dormant phase (Nadali *et al.,* 2014). *Artemisia annua* is made up of volatile and non-volatile components (Smitha *et al.,* 2014); the volatile components are found at a concentration of 0.2% to 0.4% (Luz *et al.,* 2015) which includes sesquiterpenoids, flavonoids, coumarins, proteins and steroids. The main chemical component of *Artemisia annua* that is of global interest is artemisinin (Fioranelli, 2016).

Artemisinin is an endoperoxide sesquiterpene lactone. It is effective against drug resistant malaria parasite (Li *et al.,* 2017). The volatile oil of *Artemisia annua* displayed anti-malarial activity (Li *et al.,* 2017), anti-microbial activity, anti-oxidant activity (Lin *et al.,* 2017), anti-viral activity (Mehrangiz *et al.,* 2011), anti-inflammatory activity (Samira and Sepide, 2016), anti-cancer activity (Samira and Sepide, 2016) and anti-diabetic activity (Ogbonna *et al.,* 2017).

The phytochemistry of *Artemisia annua* had been studied extensively (Ajah and Eteng, 2010). The chemical composition of volatile oils in plants varies considerably based on the geographical location and developmental stages. Although the volatile oils constituent of *Artemisia annua* grown in different parts of the world and at different developmental stages have been studied (Danijela *et al.*, 2018), to the best of our knowledge, the comparative chemical and biological volatile oil constituent of *Artemisia annua* leaf at different stages of growth growing in Nigeria has not been reported. Therefore, this work aimed to investigate the essential oil constituents of the essential oil of *Artemisia annua* L. grown in Nigeria at different stages of growth.

2. Materials and Methods

Collection of plant sample

The aerial part of *Artemisia annua* were collected from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Industrial Area, Abuja, Nigeria, at different stages of growth. The plant was identified and authenticated by an expert at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Industrial Area Abuja, Nigeria. The plants were grown from the seed and transplanted on the 10th September 2018. First collection was on the 29th October (non-flowering), second collection was on 13th November (pre-flowering), third collection 26th November 2018 (onset of flowering), fourth collection 10th December 2018 (50% flowering), fifth collection 17th December 2018 (100% flowering) and sixth collection 8th January 2019 (post flowering). The samples were air-dried at 25 – 30°C for 7 days.

Isolation of Essential oil by Hydrodistillation

The air-dried aerial part of *Artemisia annua* were chopped into pieces and subjected to hydrodistillation for 4 hours using Clevenger type apparatus. The essential oils obtained were dried over anhydrous sodium sulphate and used immediately for GC-MS analysis. Essential oil yield percentage was calculated based on the dry weight of plant material and expressed as (% w/w). Yield ranging from 0.2% to 0.4% w/w of colorless essential oils were obtained for the different stages of growth investigated.

Gas Chromatography–Mass Spectrometry (GC-MS) analyses

The essential oils were analyzed by GC-MS using Shimadzu QP-2010plus GC with QP-2010 plus Mass Selective Detector [MSD, operated in the EI mode (electron energy=70 eV), scan range of 45-400 amu, and scan rate of 3.99 scans/sec], and Shimadzu GCMS solution software (version 2.53). The Gas chromatography column was HP-5MS fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium (99.99%) with flow rate of 1.61 mL/min. The oven temperature programming used was 60-180°C at a rate of 10°C/min, then held at 180°C for 2 min, followed by 18-280°C at a rate of 15°C/min, then again held at 280°C for 4 min. The injection port temperature was 250°C, ion source temperature 250°C, interface temperature 250°C while detector temperature was 280°C. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using autosampler and in the split mode with ratio of 10:90. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library. The

percentage of each component was reported as raw percentages based on the total ion current (Okhale *et al*., 2018).

3. Results and Discussion

Essential oil Content and Components

The *Artemisia annua* essential oil content ranged from 0.2% to 0.4% w/w for the different stages of growth investigated. The essential oil constituents identified in the dried *Artemisia annua* biomass and their percentage composition are shown in table 1.

Table 1: Percentage composition of essential oils of *Artemisia annua* at different stages of growth

SN	Name of Compound	RI	% Composition of each collection					
			1st	2nd	3rd	4th	5th	6th
1	Santolinatriene	907	-	-	-		-	0.9
2	Tricyclene	925	-	1.33	-	1.65	1.23	-
3	Artemisia triene	927	-	-	-	-	-	1.71
4	α-Pinene	935	2.87	1.47	0.59	3.62	1.87	1.66
5	Camphene	953	-	4.55	2.63	6.3	3.49	3.58
6	β-Pinene	981	-	-	1.33	-	0.6	-
7	3-Carene	1013	-	-	0.51	2.57	-	-
8	Eucalyptol (1,8-Cineole)	1032	4.16	5.26	3.25	6.48	1.76	6.1
9	o-Cymene(p-cymene reported)	1039	-	-	1.22	-	-	-
10	Artemisia ketone	1061	1.62	3.26	2.81	-	-	7.67
11	Nonanal	1105	-	-	-	-	-	2.03
12	Hotrienol	1107	-	-	-	-		0.63
13	α-Campholenal	1127	-	-	-	-	1.65	-
14	Camphenol	1135	-	-	-	-	-	1.56
15	Pinocarveol,	1140	-	-	2.32	-	1.47	-
16	Camphor	1144	6.3	15.62	5.67	15.81	16.84	11.06
17	Verbenol	1146	2.1	1.11	-	-	-	-
18	Citronellal	1153	0.88	-	-	-	-	-
19	m-Diisopropylbenzene	1155	-	-	-	-	0.67	-
20	1,3-Dimethyladamantane	1155	-	-	-	-	1.65	-
21	Borneol	1165	-	-	9.98	-	-	-
22	4-Terpineol	1175	1.25	1.52	1.24	1.39		1.5
23	Trans-2-Caren-4-ol	1180	2.41	1.95	2.48	1.95	3.17	1.89
25	α-Thujenal	1183	-	-	-	-	-	0.86
26	α-Terpineol	1192	-	1.24	1.3	-	2.5	1.64
27	Myrtenol	1202	2.98	1.11	1.3	1.32	1.2	1.6
28	α-Campholenal	1203	2.25	-	-	-	2.21	-
29	Trans-Carveol	1220	0.71	0.84	0.93	-	0.83	-
30	Bornylformate	1232	-	-	1.51	1.09	1.21	-
31	Cumin aldehyde	1238	-	-	-	-	-	0.62
32	Cis-3-Hexenyl valerate	1240	-	0.67	-	-	-	-
34	d-Carvone	1249	0.16	0.77	0.43	1.1	0.82	-
35	Lepalone 3-alcohol	1279	-	-	-	-	-	1.42
36	Bornyl acetate	1285	-	1.41	2.21	2.33	-	1.24
37	Lavandulyl acetate	1287	-	-	-	-	-	1.53
38	Thymol	1293	-	-	2.04	1.08	-	-
39	Cuminol	1295	-	-	-	-	0.66	0.87
40	Carvacrol	1301	-	0.66	-	-	-	-
41	2-Hydroxy-5-methylacetophenone	1317	-	-	-	-	-	1.21
42	Myrtenyl acetate	1332	-	-	-	-	0.84	-
43	3,5-Dimethyl-1-adamantanol	1338	-	-	-	-	-	0.91
44	trans-Carvyl acetate,	1341	1.46	-	1.02	0.86	2.23	2.16
45	Eugenol	1359	-	-	1.19	-	-	-

46	cis-Carvyl acetate,	1364	0.71	2.69	0.52	2.46	0.73	0.44
47	Ethyl 3-phenylpropanoate	1365	-	-	-	-	-	0.18
48	n-Decanoic acid	1373	-	-	-	-	-	0.45
49	Cyclosativene	1375	-	-	-	-	-	1.08
50	α-Copaene	1378	0.68	1.31	1.56	1.72	1.1	1.16
51	Benzyl isovalerate	1385	3.32	-	2.39	-	3.08	1.72
52	β-Cubebene	1390	-	-	3.57	-	3.27	2.24
53	β-Elemene	1390	-	1.46	-	-	-	-
54	Sativene	1395	-	-	-	-	-	0.6
55	Jasmone	1410	0.76	-	0.93	0.6	-	0.46
56	Isocaryophyllene	1410	-	-	-	0.54	0.94	-
57	α-Cedrene	1412	2.26	-	-	-	-	-
58	β-Gurjunene	1430	2.97	-	-	-	1.71	-
59	Thujopsene	1432	1.38	-	-	-	-	-
60	Calarene	1433	-	-	2.48	-	-	1.69
61	γ-elemene	1442	-	1.6	-	1.53	0.98	0.48
62	β-Farnesene	1452	1.45	2.15	1.23	2.7	1.85	2.09
63	α-Caryophyllene	1460	1.86	2.8	3.89	3.64	1.56	1.87
64	β-Caryophyllene	1467	-	1.11	-	1.32	-	0.61
65	γ-Muurolene	1473	-	-	-	-	-	0.58
66	γ-Muurolene	1475	-	-	0.55	-	-	0.66
67	β-Selinene	1492	2.58	2.78	2.27	3.15	2.33	1.33
68	β-Guaiene	1494	-	-	1.12	-	1	-
69	β-Acoradiene	1498	-	1.09	0.54	1.31	-	0.4
70	Trans-Chrysanthenyl acetate	1509	3.32	3.46	-	3.49	-	-
71	δ-Cadinene	1524	1.26	-	0.41	-	1.1	0.5
72	Calamenene	1528	-	-	-	-	-	0.62
73	Cadina-1(10),6,8-triene	1532	-	-	-	-	-	0.52
74	GermacreneD	1553	-	-	-	-	-	1.03
75	Nerolidol	1563	-	-	0.6	-	-	-
76	3-Hexenyl benzoate	1571	-	-	-	-	-	2.07
77	Arteannuic acid	1572	2.25	4.27	1.89	2.75	2.6	1.36
78	Caryophyllene oxide	1578	3.78	-	0.88	-	-	0.38
79	Spathulenol	1580	-	-	4.57	-	0.83	1.94
80	Isoaromadendrene epoxide	1585	2.08	-	-	-	1.95	-
81	Carotol	1594	-	-	-	-	-	1.89
82	Guaiol	1595	-	-	-	-	1.99	-
83	Cubenol	1640	-	2.74	-	1.42	-	2.24
84	α-Cadinol	1650	-	-	1.37	-	-	-
85	Aristolene epoxide	1652	-	-	-	1.57	-	0.73
86	α-Eudesmol	1652	3.35	-	-	-	-	-
87	α-Bisabolol	1683	1.25	-	-	-	-	-
88	Cedr-8-en-13-ol	1686	2.61	3.31	0.43	-	1.11	0.85
89	Farnesol	1723	-	-	-	-	-	0.47
90	7-Hexadecenal; Hexadecanol	1880	1.83	-	-	-	-	-
91	Palmitic acid	1951	-	1.98	-	-	1.18	1.08
92	Platambin-1,6-dione	1973	1.08	1.16	0.98	1.12	0.62	0.59
93	Deoxyartemisinin	1988	0.27	0.64	0.5	0.58	-	0.19
94	Scoparone	2031		2.22	-	-	-	-
95	1-Octadecanol	2083	0.16	-	-	-	-	-
96	Phytol	2123	0.33	1.01	0.87	-	-	0.38
97	Linoleic acid	2133	-	-	-	-	-	0.75
98	Longiverbenone	2147	2.44	-	-	-	-	-

RI: Retention indices relative to n-alkanes (C7-C40) on HP-5MS capillary column; -: Not detected.

The yield of the essential oils obtained at the different stages of growth ranging from non-flowering, pre-flowering, onset of flowering, 50% flowering, 100% flowering to post flowering was 0.2% - 0.4% w/w. From the GC-MS results, the compounds identified in the essential oil at all stages of growth consisted of α-pinene, eucalyptol, camphor, myrtenol, cis-carvyl acetate, copaene, caryophyllene, β-farnesene, β-Selinene, arteannuic acid, deoxyartemisinin and platambin-1,6-dione. The major essential oil constituents at the different growth stages were camphor (5.67%-16.84%), artemisia ketone (1.62%-7.67%), eucalyptol (3.25%-6.48%), myrtenol (1.11%-2.98%), arteannuic acid (1.36% -4.27%) and caryophyllene (1.56%-3.89%).

Camphor is a natural product found in plant and has a wide range of applications, such as in food flavorings, fumigants, perfumes, cosmetics, household cleaners, and topically applied analgesics. Camphor had activity against fungal infections and *Mycobacterium tuberculosis*. Camphor exhibited a number of biological properties such as insecticidal, antimicrobial, antiviral, anticoccidial, anti-nociceptive, anticancer and antitussive activities, in addition to its use as a skin penetration enhancer. α-pinene inhibited the growth of *Proteus mirabilis* (Letícia *et al.,* 2017).

Eucalyptol is a colourless liquid and occurs as natural organic compound used in food preparations. Eucalyptol showed activity against *Staphylococcus aureus* (Zoran *et al.,* 2000). Eucalyptol extended the lag phase of *S. typhimurium*, *E. coli* O157:H7 and *S. aureus* at the concentrations of 0.7%, 0.6% and 1%, respectively (Hatice and Ayse, 2014).

Myrtenol is a monoterpene with various pharmacological activities. Caryophyllene is a natural bicyclic sesquiterpenes. β-caryophyllene and β-caryophyllene oxide possessed significant anticancer activities, inhibiting growth and proliferation of numerous cancer cells (Fidyt *et al.,* 2016). Deoxyartemisinin also known as deoxyqinghaosu was detected at the different developmental stages of *Artemisia annua* investigated, except at the post-flowering stage. Deoxyartemisinin is a non perodixic derivative of artemisinin.

Artemisia ketone (28.30% -37.15%), camphor (18.00% -23.30%) and eucalyptol (9.00% -39%) were the main components of *Artemisia annua* in Turkey collected before flowering, 50% flowering, full-flowering and after flowering stages; the highest amount of the three components were obtained at full-flowering stage (Fidyt *et al.,* 2016). From the present study, the major volatile compounds in all the growth stages were camphor (5.67%-16.84%), artemisia ketone (1.62%-7.67%), eucalyptol (3.25%-6.48%), arteannuic acid (1.36% -4.27%), caryophyllene (1.56%-3.89%) and myrtenol (1.11%-2.98%). From the results, the chemical composition of *Artemisia annua* volatile constituents varied depending on the developmental stage.

Conclusion

From the present study, the major volatile compounds in all the growth stages were camphor (5.67%-16.84%), artemisia ketone (1.62%-7.67%), eucalyptol (3.25%-6.48%), arteannuic acid (1.36% -4.27%), caryophyllene (1.56%-3.89%) and myrtenol (1.11%-2.98%). The volatile constituents of *Artemisia annua* were more at the post-flowering stage, the essential oil content increased with delay in harvest. It can be concluded that the chemical component of essential oil from *Artemisia annua* vary depending on the growth stage.

Conflict of Interest

The authors declare no conflict of interest.

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