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CHEMICAL CONSTITUENTS AND PHYTOCHEMICAL STUDIES OF EPAZOTE (*Chenopodium ambrosioides*) LINN GROWN IN NORTH CENTRAL NIGERIA

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ABSTRACT

The essential oil from the leaves of C.ambrosioides of North Central Nigeria was obtained by hydro-distillation using Clevenger apparatus. The oil was analysed by a combination of GC and GCMS. Nineteen components amounting to 98.37% of the total oil were identified .The main constituents of the North Central Nigeria grown C.ambrosioides are 2-Carene(17.80%),2-bornene(14.79%),p-Cymene(12.93%),α-Terpinene(8.98%), Cyclohexene-4-methyl-3-(1-methylethylidene)(7.94%),α-Terpinolene(7.90%),γ-Terpinene (6.94%).The oil was found to be a yellow liquid, the obtained yield is 1.35%w/w based on the dry weight. The preliminary phytochemicalinvestigation showed that C.ambrosioides methanolic leaf extract contains some secondary metabolites such as Flavonoids, Terpenoids, Steroids, Alkaloids and Saponins. The result justified the use of C.ambrosioides in treating various infectious diseases.

KEYWORDS: *Volatile constituents, Phytochemical screening, Epazote, hydro distillation, Chenopodium ambrosioides.*

RESUMO

O óleo essencial de folhas de C. ambrosioides da parte Norte-Central da Nigéria foi obtido por hidrodestilação usando o aparelho de Clevenger. O óleo foi analisado com uma combinação de cromatografia gasosa e cromatografia gasosa e espectrometria de massa. Foram identificados 19 componentes correspondendo à 98,37% do total. Os constituintes principais de C. ambrosioides foram careno (17,80%), 2-borneno (14,79%), p-cimeno (12,93%), alfa-terpineno (7,90%), ciclohexeno-4-metil-3(1-metiletilideno) (7,94%), alfa-terpinoleno (7,90%) e gama-terpineno (6,94%). O óleo é um líquido amarelo e corresponde à 1,35% por peso do peso seco. Estudos preliminares fitoquímicos indicaram que as folhas contém flavonoides, terpenoides, sterois, alcalóides e saponinas e justificam o uso de C. ambrosioides no tratamento de doenças infecciosas.

PALAVRAS CHAVE: Chenopodium ambrosioides, Óleo essencial,Hidrodestilação, Estudos fitoquímicos, Constituintes voláteis

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INTRODUCTION

Higher and aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast. Biologically active compounds from natural sources have always been of great interest to scientists [1].The essential oils known as volatile oils evaporate in contact with air and possess a pleasant fragrance. Chemically essential oils are very complex. They are found in many different species of plants of various families. All aromatic plants contain essential oils. Generally the oils are secreted in the oil glands [2]

According to WHO, about three quarter of the world population relies upon traditional remedies [mainly herbs] for the health care of its people. In fact plants are the oldest friends of mankind. They not only provide food and shelter but also serve the humanity to cure different ailments [3].

The plant family Chenopodiaceae is a large family comprising about 102 genera and 1400 species [4]. The genus Chenopodium include varieties of weedy herbs (more than 200 species) native to Europe, Asia and both North and South America [5]. Chenopodium ambrosioides Linn also called (Epazote, Mexican tea, Wormseed e.t.c) is a widespread species native to tropical America [6]. It is annual or short-lived perennial herbs that have been used for centuries as condiment, traditional purgative for intestinal worms and many other medicinal purposes [7-9]. The leaves and seeds are green when fresh. The plant is sometimes cultivated principally for its medicinal use in West Africa as purgative, for treating sores, edema and flavouring purposes [10]. They are employed for different ailments in Africa. The importance of Chenopodium species is due to their wide variety of medicinal properties [11].

C.ambrosioides is called Ewe-imi, Asin and Arunpale in some parts of Nigeria [12]. The whole plant leaves are used as anthelmintics, emollient and to treat rheumatism and tumour in parts of the country [13]. It has also been employed by empirical herbalist and healers against intestinal parasites (especially small tapeworms and roundworms) throughout Latin America; as well as West Indies [14]. As a result of its effect against intestinal parasites,traditional healersin North central Nigeria administer about 2-3 gm of the dried powdered leaves of C.ambrosioides mixed with pap to treat hookworm infection and hookworm inflammatory diseases, the decoction is also used in this region as wash for various skin diseases, to treat fever and as a fumigant against mosquitoes.

The biological activity of C.ambrosioides has been shown to affect viruses [15], bacteria [16], fungi [17], nematodes [18], and insects [19]. Furthermore the plant is traditionally and widely used as antiparasitic, anti-inflammatory and antibiotics which efficacy has been scientifically proven [20].

Chemical composition of the essential oil of C.ambrosioides from different parts of the world has been widely studied such as from Brazil [21], Cuba [22], Mexico [23], Cameroon [24], Nigeria [25], Rwanda [26], China [27,28], and India [29,30]. Ascaridole and Isoascaridole that were found in the Brazil grown C.ambrosioides [31] were absent in the North Central Nigeria grown C.ambrosioides, also α-Terpinene that constituted the major component in the Nigeria grown C.ambrosioides by[Gbolade et al,32] was just (8.98%) in the North Central Nigeria grown C.ambrosioides, 2-carene was found to be the major constituent.

Phytochemical are bioactive compounds found in plants they are non-nutritive compound (secondary metabolites) that contributed to flavour, colour [33, 34]. They are needed by plant for purpose such as disease, pathogen defence and control.The aim of this study was to identify the volatile constituents of the leaf oil of C.ambrosioides grown in North Central Nigeria by using modern GC/MS machine [SHIMADZU GCMS-QP2010 PLUS], as well as to find out the phytochemical components in the methanol extracts of C.ambrosioides grown in this region.

MATERIALS AND METHODS

Collection of plant leaves and identification

Fresh matured leaves of the plant were collected in May 2014 from a village in Anyigba of Kogi State, North Central Nigeria. They were identified by Dr Aina of the Department of Botany, Kogi State University, Anyigba. Voucher specimens are deposited in the herbarium of the Faculty of Bbiological Sciences, Kogi State University, Anyigba, Nigeria

Isolation of the volatile oil

The method employed for the extraction of the volatile oil of the leaf of Chenopodium ambrosioides was hydro distillation method according to European Pharmacopoeia (2008). Oils were collected and kept in the refrigerator without further treatment before GC-MS analysis.

Preparation of plant extracts of methanol

The collected fresh matured leaves of the plant of C.ambrosioides were air dried usually at room temperature in a well aerated room; the air dried leaves were grinded into coarse powdered using a Thomas Willey machine. The aqueous extract was prepared by soaking 200g

of the dried powdered samples in 400ml of methanol for 24 hrs. The extracts were filtered using Whatman filtered paper No 42 (125mm).

Screening of Phytochemical Components

To identify the phytochemical derivatives in the methanolic extract, standard phytochemicals screening was performed [35-37].Alkaloid test was performed by Meyer's tests, flavonoid and tannins by ferric chloride test, terpenoid and sterols by Salkowski's test, saponin by frothing test and anthraquinones by Borntrager's test.

Identification Test

The following tests were carried out so as to detect active chemical constituents such as alkaloids, tannins, flavonoids, anthraquinones, steroids, terpenoids and saponin.

Test for Alkaloids

1cm^3 of 1%HCl was added to 3cm^3 of the extract in a test tube. The extract was treated with a few drops of Meyer's reagent. A creamy white precipitate was observed indicating the presence of alkaloid.

Test for Tannins

About 0.5g of the dried powdered samples was boiled in20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue back colouration. There was no brownish green or blue colouration observed.

Test for Saponin

About 2g of the powdered samples was boiled in 20ml of distilled water in a water bath and filtered.10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

About 0.5g of the extract was boiled with 5.0ml of distilled water and then filtered .To 2.0ml of this filtrate; a few drops of 10% ferric chloride solution were added. A greenish blue or violet colouration indicated the presence of a phenolic hydroxyl group.

Test for Terpenoids

5ml of the extract was mixed in2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Sterols

A few milligrams of the plant extract were dissolved in2ml of chloroform and then 2ml of concentrated H_2SO_4 was added from the side of the test tube. The test tube was shaken for a few minutes. Red colour development in the chloroform layer indicated the presence of sterols.

Test for Anthraquinones

An aliquot of 0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour change. There was no colour change.

2.2 Gas chromatography/mass spectrometry analysis

The chemical composition of the essential oil was analysed using GC/MS technique. The mass spectrometer was SHIMADZU GCMS-QP2010 Plus (Shimadzu Corporation, Japan) in the electron impact (EI) ionization mode (70eV) and HP-5MS (bonded 0.25μm) Capillary column (restek, Bellefonte, PA). Injector and Detector temperature were set at 250°C. The oven temperature was held at 60°C for 30minutes, then programmed to 240°C at rate of samples (1/100 in hexane v/v) of 1.0ml were injected automatically. The linear velocity of the column was 36.8cm/sec, each peak was then analysed and assigned a number in the order that it was detected. The identification of the components was based on comparison of their mass spectra with those of NIST library mass spectra database and literature.

Table 1. Volatile Constituents of the Leaf Oil of Chenopodium Ambrosioides.

S/N	RI	COMPONENT	% COMPOSTION
1	932	2-Bornene	14.79
2	948	IR-α-pinene	1.82
3	998	α-Terpinene	8.98
4	1001	2- Carene	17.8
5	1010	3,7,7-trimethyl-1,3,5-cycloheptatriene.	7.94
6	1023	4-methyl-3-(1-methylidene)-1-cyclo hexane	4.31
7	1042	ρcymene	12.93
8	1052	α-Terpinolene	7.90
9	1094	Nona-3,5-diene-2-ol.	1.88
10	1119	*m–Xylene*	4.61
11	1143	α-Terpineol	0.50
12	1179	γ-Terpinene	6.94
13	1195	Decyn-2-ol	1.94
14	1248	1,2:4,5:9,10-Triepoxydecane	1.88
15	1763	Z-10-pentadecen-1-ol	0.57
16	2007	9-Octadecenal	0.64
17	2085	16-Octadecenoic acid, methyl ester	1.44
18	2175	Oleic acid	1.14
19	2682	15-Tetracosenoic acid	0.36
TOTAL IDENTIFIED			98.37%

Note; RI Means Retention Index

RESULTS AND DISCUSSION

The hydro distillation of the leaf oil of *C.ambrosioides* yielded 1.35% w/w essential oil. Nineteencomponents amounting to 98.37% were identified in the leaf oil. The identified components, their retention index and percentage composition of each component is given in table1 above. Most of the components being monoterpenes and oxygenated monoterpenes. The major components found in the essential oil of *C.ambrosioides were* 2-*Carene (17.8%), 2-Bornene (14.79%), p-Cymene (12.93%), α-Terpinene (8.98%), Cyclohexene-4-methyl-3-(1-methylethylidene) (7.94%),α-Terpinolene (7.90%) and γ-Terpinene (6.94%).*

Constituents present in significant amount are *m-Xylene (4.61%), 3, 7, 7-Trimethyl-1, 3, 5-cycloheptatriene (4.01%), Decyn-2-ol (1.94%), Nona-3, 5-dien-2-ol, IR-α-Pinene (1.82%) e.t.c.* It is apparent that the main compounds characterizing the leaf oil are qualitatively and quantitatively different. It is also worth mentioning that compounds like *m-Xylene, nona-3,5-dien-2-ol, decyn-2-ol, 3,7,7-trimethyl-1,3,5-cycloheptatriene and 2-bornene* which are detected in the North Central Region of Nigeria samples have not been reported previously as part of the constituents of the volatile oil of *C.ambrosioides*.

The composition of the essential oil of *C.ambrosioides* has been the subject of several studies and data from literature shows that it has no constancy neither with respect to the

relative humidity, irradiance, photoperiod and the method of extraction, location, plant cultivation techniques, soil structure and climate heavily influence the composition and quality of essential oil. [38].These factors have been used to ascertain why exact specification of the component of essential oils is not acceptable.

The percentage constituents obtained for this sample shows that there is a marked difference in the earlier report given on *C.ambrosioides* leaf by different authors [39, 40& 32].The report shows that *C.ambrosioides*leaf was composed of α-terpinene as the major constituent. However, the major component of the North Central Region of Nigeria*C.ambrosioides* oilwas found to be 2-carene with17.8 percentage. Ascaridole was not detected in the North Central Nigeria oil of *C.ambrosioides.*

The phytochemical screening of the methanolic extract was carried out in order to analyse the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids, sterols e.t.c] by utilizing standard methods [35-37]. Table 2 shows the results of the preliminary phytochemicals analysis. This study has revealed the presences of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoid, flavonoid, alkaloid, sterols, were present in *C.ambrosioides* leaves[Table: 2.] Plants rich in terpenoid have been reported to have anti inflammatory, antimalaria, antiviral, inhibition of cholesterol synthesis and antibacterial [41], while those rich in alkaloid are used in medicine to reduce headache and fever. These are attributed for antibacterial and analgesic properties [42]. Epidemiologic studies recommended that coronary heart diseases are opposed by dietary flavonoids. [42].

Table 2. Phytochemical Constituents of the Leaf of Chenopodium Ambrosioides.

Phytochemicals	**Test**	**Me**
Alkaloids	Meyer's Test	+
Tannins	Ferric Chloride Test	–
Saponin	Frothing Test	+
Flavonoids	Ferric Chloride test	+
Terpenoids	Salkowski Test	+
Anthraquinones	Borntrager's Test	–
Sterols	Salkowski Test	+

Key: (+) means Present; (-) means absent;Me meansMethanolic Extract

CONCLUSION

The results obtained in this study showed that *C.ambrosioides* possesess essential oil in the leaves of the plant and that their oil compositions were quantitatively and qualitatively different. This study represents the first to the best of my knowledge analysis of the leaves volatile constituents of *C.ambrosioides* by using a modern GCMS machine [SHIMADZU GCMS QP-2010 PLUS]. The phytochemical analysis showed the presence of effective biological compounds like alkaloids, steroids, flavonoids saponin and terpenoid in C.*ambrosioides* leaves thus providing knowledge of the phytochemical metabolites. Observation drawn from this experiment shows clearly that the leaves of *C.ambrosioides* contain phytochemical constituents. However the present investigation showed that the studied plant is a potential good source of traditional medicine and because it's a medicinal plant, it has commercial interest in both research institutes and pharmaceuticals company for the manufacture of new drugs in the treating of various diseases.

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