

RESEARCH ARTICLE

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Chemical Composition of *Ocimum americanum* In Sudan

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Abstract

Plants are one of the most important of medicines. Basil (*Ocimum americanum*) is one such plant which symbolize all that is extraordinary in nature because the entire whole plant has been used traditional medicine for household remedy against various human from antiquity. The genus *O.americanum* is known to include species accumulating essential oils. In this study the secondary metabolites of both flowers and leaves of *O.americanum* which occur in the central Sudan. The proximate analysis to samples showed the percentage of moisture content, total ash, crude fiber and fiber protein of the leave and flowers. The elemental analysis used Atomic Absorption Spectrometer (AAS) and flame emission for Ca, Fe, Na, K, Mg, Pb and Mn plant under study. Preliminary phytochemical screening for carbohydrate, reducing sugars, monosaccharide, tannins, saponins, flavonoids, terpenoids, steroids, alkaloids, protein, amino acids and anthraquinones were performed. Using Gas Chromatography–Mass Spectrometer (GC-MS) analysis of n-hexane extracts, 22 nature compounds were recorder, most of the identified compounds in the extracts were terponoids in *O.americanum* monoterpenes were found to be7.29% and sesquiterpenes (4.80%)whereas alcohols and fatty acids were found to be the major constituents (1-Octanol 39.09% and Octyl acetate 38.87%) respectively. The results obtained showed that *O.americanum* is a good source of food and therapeutic agents which could be of use food and pharmaceutical industries information about the chemical composition of the oil could then be useful in the classification of this plant.

Keywords: Basil (*Ocimum americanum*); Chemical Composition; GC/MS; North Sudan

INTRODUCTION

According to WHO (World Health Organization), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of spices, which include leaves, buds, bulbs, fruits, stem, rhizomes, bark and other plant parts, has been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices have been used during the middle ages for flavoring, food preservation, and/or medicinal purposes. Only a small percentage of plants species have been investigated phytochemically and the fraction submitted to biological screening is even Smaller [1].

The diversity of the climate of Sudan is responsible for its very rich flora. In most of the studies carried out in the Sudan, scientists have, concentrated on known medicinal plants that are used locally. These plants are grouped into three categories: first

medicinal plants that can be cultivated as field crops such as: Roselle (Karkadeh), second: Wild growing medical plant e.g.: Hargel, Colocynth, Hashab and Sunt. Third: imported medicinal plants e.g.: Dameseesa, and Chamomile [2]. Some compounds present in plant known as phytochemicals are valuable sources of food and medicine for the prevention of illness and maintenance of human health [3]. The family lamiaceae is widely distributed over the world. It comprises over 5,000 medicinal and aromatic plant species whose essential oils have multiple applications [4]-[5]. The genus *Ocimum* (Lamiaceae) comprises 160 species and is found throughout the tropical and sub-tropical regions of the world[6]. Several species of for the genus has commercial utility as a source of essential oil for the pharmaceutical, food, flavor and perfumery industries [7]. *Ocimum americanum* L. Linn commonly called as *Ocimum canum* belongs to the family lamiaceae (labiatae) [8]. The plant is a

pubescent erect much branched herb 15-60cm high with sub-quadrangular striate branches [9]. Leaves are elliptic-lanceolate, entire, glabrous and gland dotted strongly aromatic herb; branchlets puberulous, terete to four-angular [10]. Flowers are small, white, pink or purplish, in more or less closely set whorls in spiciform racemes [11]. Seeds are having nutlets with narrowly ellipsoid punctate black [12].

The main chemical constituents are volatile oils include methyl cinnamate, methyl heptenone, methyl nonyl ketone, d-camphor, citral, Ocimin, methyl chavicol, linalool, nevadensin, salvigenin, beta-sitosterol, betulinic, ursolic, oleanolic acids and flavonoids pectolinarigenin-7-methyl ether and nevadensin, polysaccharides composed of xylose, arabinose, rhamnose and galacturonic acids [13]. The main uses of *Ocimum americanum* are antimicrobial, antioxidant, antihelmintic and anti-diabetic [14].

The study to investigate the proximate composition and element analysis of *Ocimum americanum*, to carry out preliminary phytochemical screening of the organic constituents and characterize the chemical compounds for *Ocimum americanum* through Gas Chromatography-Mass Spectrometer (GC/MS) and hence to chemotaxonomically correlate them.

Materials and Methods

Plant material

The plant *Ocimum americanum* leaves and flowers were collected in April 2017 from Karkog region in Sennar state-Sudan. The plant material was taxonomically identified by Prof. Hatil Hashim El-kamali, University of Omdurman, Islamic Department of Botany. Dried plant 5 days in the herbarium. After dried plant samples were homogenized in pestle and mortar.

Proximate composition

Proximate composition was determined using the method described by AOAC [15] from the samples of investigated *Ocimum americanum*. The method was used to estimate moisture content, total ash, crude fibre and crude protein.

Elemental Composition

The Na and K were determined using flame emission photometer. The principle of this procedure relies on the fact that excitation of a metal in a flame gives rise to the emission of characteristic color. The intensity of the colour

emitted by the excitation of any given atom is a property of the specific element and the concentration of that element while others (Ca, Fe, Pb, Mn and Zn) were analyzed by Atomic Absorption Spectrometer (AAS). The principle of this procedure relies on the absorption property of the elements. The vapors of the elements containing free atoms absorb light having wavelength as that which the atoms of the elements are capable of emitting.

Phytochemical screening

The phytochemical screening of plant sample was adopted from previous work [16].

Preparation of Extract

50gm plant sample from *Ocimum americanum* were placed in 500ml conical flask and then 300ml 70% ethanol were added. The conical flask was placed in the water bath and was allowed to stand for 1 hour. The time was measured after boiling start (after appearance of the first bubble). The mixture (powder and ethanol) was filtered using filter paper while it is hot by using another 500 ml conical flask.

Qualitative phytochemical Analysis:

Phytochemical screening for the identification of major groups of chemical constituents using standard procedures [16-17]. The phytochemical components analyzed were, carbohydrates, reducing sugars, Monosaccharides, Protein, Amino acids, Tannins, Saponins, Flavonoids, Terpenoids and/or Steroids, Alkaloids, Anthraquinones.

Identification of the plant constituents by phytochemical tests:

Ethanol extract is subjected to various preliminary phytochemical analyses to test for present or absent of various phytoconstituents by the following tests.

Test for Carbohydrates (Molisch's Test):

To the extract 1ml of the Molisch's reagent was added then along the walls of the test tube carefully conc H₂SO₄ was added, formation of brown ring at the junction of two liquids was observed.

Test for reducing sugars (Fehling's Test):

The extract was taken in a test tube, and the 1ml of the Fehling's solution (A and B) was added and

boiled on the water bath. The solution was observed for the color change reaction.

Test for Monosaccharides (Barfoed's Test):

To the extract in a test tube 1ml of barfoed's reagent was added and boiled on the water bath. The solution was observed for colour change reaction.

Test for protein (Biuret Test):

To 0.5ml of the extract 2ml of Biured Reagent was added and the reaction mixture observed for the formation of violet colour solution.

Test for amino acids (Ninhydrin Test):

To 0.5ml of the extract 2ml of the Ninhydrin was added and heated few minutes and the reaction mixture was observed for the deep blue pale-yellow colouration.

Test for tannins (Ferric chloride):

0.5ml of the extract was boiled with 10ml of distilled water in a test tube and then, few drops of 0.1 % ferric chloride solution was added and the reaction mixture was observed for blue greenish black Colour change.

Test for saponins (Frothing Test):

0.5ml the extract was added to 5ml of distilled water in test tube. The solution was shaken vigorously and observed for the stable persistent forth. Frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for flavonoid:

To 0.5ml of the extract 5ml of distilled water was added and then apiece of magnesium ribbon and 2ml of concentrated HCL was added. The reaction mixture was observed for the pink or red colour solution.

Test for terpenoids and/or steroids:

To 0.5ml each of the extract 2ml of chloroform was added and then 3ml of the concentration H_2SO_4 was carefully added to for a layer. A reddish brawn coluration of the interface indicated the presence of tepenoid and steroids.

Test for alkaloid:

Three different Test were used for the identification of alkaloids.

Mayer Test:

To 0.5ml of the extract 2ml of Mayer's reagent (K_2HgI_4) was added and the reaction mixture was observed for formation of creamy white precipitate.

Wagner test:

To 0.5ml of the extract 2ml of wagner's reagent (dilute iodine solution) was added and the reaction mixture is observed for the formation of reddish-brown precipitate.

Dragendorffs

To 0.5ml of the extract 2ml of Dragendorff's reagent was added, the reddish-brown precipitate observed in the reaction mixure indicates the presence of alkaloids.

Test for anthraquinones (Borntrager's Test) :

To 0.5ml of the extract 5-10ml dilute HCL was added and boiled on water bath for 10 minutes and filtered then the filtrate was extracted with carbon tetra chloride and the equal amount of ammonia was added, after shaking the reaction mixture was observed for the formation of pink red colour in the ammonia layer.

Gas Chromatography Mass Spectrometer (GC/MS)

GC was equipped with MS (Shimatzo QP 2010 GC/MS Instrument equipped with reference libraries). Packed material for column were 50% phenyl and 50% methyl polysilosane , column length 30 meter , diameter 0.025 mm, the flow rate of helium as carrying gas was 1 ml/min. The temperature of program consisted of 60-270 C, at rate of 4 C/min. MS were taken at ionization voltage 70 UV. Library search was carried out using Wiley GC/MS library.

Identification of isolated compounds

The individual identifications were made by the comparison of fragmentation patterns with those found in the library of the mass spectrometer.

Result and Discussion

The result of proximate composition (Table 1) shows that the moisture content of the sample was flowers (5.53 ± 0.01) and leave (5.5 ± 0.1). This is

expected since the sample has been subjected to drying for five days to reduce the moisture content. High moisture content is an index of spoilage. The result also shows that the leaves of *O. americanum* contain higher level of ash content ($19.09 \pm 0.01\%$) than flowers ($8.87 \pm 0.05\%$). The ash content is a reflection of its mineral content. The ash content of the leaves compared to the flowers presents that the minerals are more concentrated in the leaves than in the flowers. The result also shows that the flowers have more crude fibre content with value ($29.23 \pm 0.02\%$) the leaves with value ($13.8 \pm 0.01\%$). Crude fibre provides roughages that aids digestion and reduces the accumulation of carcinogen in the body [18]. The protein content was flowers (10.15 ± 0.9) and leave (18.43 ± 0.31) the high protein content buttressed the use of the plant leave as flavor soup and spice meat. The basic function of protein in nutrition is to supply adequate amounts of required amino acids [19]. Protein deficiency causes growth retardation, muscle wasting, and abnormal swelling of the belly and collection of fluids in the body [20]. The concentration of calcium, iron, sodium, potassium, lead, manganese and zinc in plant sample obtained from the *Ocimum americanum* was performed using Atomic absorption (Table 2).

The minerals are very important in human nutrition. The results of the calcium content shows that the aerial parts of *O. americanum* (41.01 mg/100mg). Calcium is required in the body for human growth of bones and teeth [18]. Calcium (Ca) and potassium (K) are reported to be responsible for the repair of worn out cells, strong bones and teeth, building of red blood cells and for body mechanisms [21]. Also, Ca and K are essential for disease prevention and control and may therefore contribute to the medicinal influences of the plant [3]. Potassium (K) is needed for growth and transmission of the nervous system to transmit messages as well as regulating the contractions of muscles [22]. The results of sodium show (1.162 mg/100g) while the potassium content higher level of the (18.200) mg/100mg. The result of iron content shows the (1.596 mg/100g). The result of Pb shows that this element was not found. The result obtained shows the contain 0.122 mg/100g Zn. The concentration of calcium, iron, sodium, potassium, lead, manganese and zinc in

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Many phytochemicals found in plants are either the product of plant metabolism or synthesized for defence purposes. The phytochemicals are either useful or toxic to human body [23]. Saponins are steroidal glycosides with foaming characteristics and bitter taste that have beneficial effect on the blood cholesterol levels, fight cancer and help in health and the stimulation of immune system [24]. Saponins have hemolytic properties and are used as starting materials for the synthesis of steroidal drugs like corticosteroids, the sex hormone stimulants and contraceptives [3]. They are also used for the treatment of viral diseases [18]. Tannins are stringent bitter plant polyphenols that bind, precipitate and shrink proteins and various organic compounds. Tannins are known to have anti-viral, anti-tumor, anti-inflammatory and healing properties on wounds, kidney etc [17]-[18]. The *Ocimum americanum* extract, which was found to contain saponins, tannins, could be useful for medicine al purpose.

N-Hexane extract of *O.americanum* aerial parts was analyzed through GC/MS (Tables 4 and 5). A

total of twenty two compounds were identified from *O.americanum* aerial parts. Monoterpenes were found to be 7.94% and sesquiterpenes

(4.80%) whereas alcohols and fatty acids were found to be the major constituents (1-octanol 39.09% and Octyl acetate 38.87%), respectively.

Table 1. Proximate composition of the flowers and leaves of the *Ocimum americanu.L.* :

Part use	Moisture content	Total ash	Crude fiber	Crude protein
Flowers	5.53±0.1	8.87±0.05	29.23±0.2	10.15±0.9
Leaves	5.5±0.1	19.09±0.1	13.8±0.1	18.43±0.31

Mean ± Standard deviation of triplicate determinations.

Table 2. Element Composition (mg/100g) of Candidate *Ocimum americanum* using Atomic Absorption Spectrometer (AAS)

Minerals	Ca	Fe	Na	K	Pb	Mn	Zn
Mg/100g	41.01	1.596	1.162	18.200	ND	0.112	0.122

Mean ND=Not Detected

Table 3. phytochemical screening of ethanolic extracts of *Ocimum americanum*

Phytochemical screening	Regented use	<i>Ocimum americanu</i>	
		Leaves	Flowers
Carbohydrates	Moliches	+	+
Reducing sugars	Flehlings	+	+
Monosaccharides	Barfoeds	-	+
Proteins	Biuret	-	-
Amino acids	Ninhydrin	+	+
Tannins	Ferric Chloride	+	+
Saponins	Frothing	+	-
Flavonoids	magnesium +HCL	+	+
Terpenoids and steroids	Chloroform+H ₂ SO ₄ con	+	+
Alkaloids	Mayers Wagners Dragendorffs	-	-
Anthraquinones		-	-

Key : + = present ; - = Absent

Table 4. Chemical composition of n-hexane extract of *Ocimum americanum*:

NO	Compounds	RT	%	Formula
1	3-hydroxy-3-methyl pentanoic acid	4.711	0.91	C ₆ H ₁₂ O ₃
2	α-pinene	4.868	0.48	C ₁₀ H ₁₆
3	Hydroperoxide,1-methyl pentyl	5.271	0.21	C ₆ H ₁₄ O ₂
4	3-penten-2-one ,4-methyl	5.523	2.80	C ₆ H ₁₀ O
5	Octanal	6.135	1.23	C ₈ H ₁₆ O
6	Acetic acid, hexyl ester	6.323	0.41	C ₈ H ₁₆ O ₂
7	3-hepten-2-one	6.528	0.22	C ₇ H ₁₂ O
8	Cyclohexane,nitro	7.053	0.32	C ₆ H ₁₁ NO ₂
9	1-Octanol	7.537	39.09	C ₈ H ₁₈ O
10	Linalool	8.162	0.65	C ₁₀ H ₁₈ O
11	Octyl acetate	10.485	38.87	C ₁₀ H ₂₀ O ₂
12	Neral	11.185	2.43	C ₁₀ H ₁₆ O
13	Geranial	11.792	4.47	C ₁₀ H ₁₆ O

14	(E)- β -Farnesene	15.413	1.09	C ₁₅ H ₂₄
15	Humulene	15.540	0.91	C ₁₅ H ₂₄
16	β -Bisabolene	16.448	1.97	C ₁₅ H ₂₄
17	3,7,11,15-Tetramethyl-2-hexadecene	21.513	0.21	C ₂₀ H ₄₀
18	2-hexadecen-1-ol, 3,7,11,15-Tetramethyl	21.586	1.54	C ₂₀ H ₄₀ O
19	2-Hexyl-1-decanol	21.670	0.51	C ₁₆ H ₃₄ O
20	2-methyl-7-octadecyne	21.907	0.32	C ₁₉ H ₃₆
21	9-Eicosyne	22.140	0.58	C ₂₀ H ₃₈
22	Phytol	24.795	0.78	C ₂₀ H ₄₀ O

Table 5. Statistics chemical classes of n-hexane extract of *O.americanum* :

Compounds	No of compounds	Concentration%
Total monoterpene	3	7.29
Total sesquiterpene	5	4.80
Total oxygenated sesquiterpenes	1	0.51
Alkene derivative	2	1.75
Cyclic diterpene alcohol	1	0.78
Fatty acids	1	38.87
Ketone	2	1.45
Ester	1	0.21
Alcohol	1	39.74
Carboxylic acid	1	0.41
Hydrocarbone alkyne	1	2.80
Other	2	1.23
Total	22	99.91

Conclusion

The result of proximate composition and element analysis it is quite interesting that *Ocimum americanum* Ca and K higher. The findings of this study shows that the contained *Ocimum americanum* 1-Octanol and Octyl acetate

chemotaxonomy. The *Ocimum americanum* would serve as good sources of pharmaceutical drugs. The main identified constituents in *Ocimum americanum* n-hexane extract was 1-Octanol (39.09%), Octyl acetate (38.87%), Geranial (4.47%) and 3-penten-2-one,4-methyl (2.80%).

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