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RESEARCH ARTICLE

Chemical and physical properties and gas chromatography mass spectrometer analysis of Ocimum Basilicum oil

Amar O. A. Salih *[1], Mahamoud Mohamed Ali mahmoud[2]

1.Minstriy of Higher Education and Scientific Research, Chemistry Department, Central Lab, PO.Box 7099, Khartoum, Sudan.

2.International University of Africa, College of pure and applied Science, Department of applied and industries Chemistry Department Khartoum, Sudan.

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Corresponding Author

Amar O. A. Salih

Abstract

The plants are one of the most important drugs of sources, including, for example, (sweet basil) is one of these plant tribes that symbolizes all that is extraordinary in nature, because the entire plant has been used by traditional medicine for the treatment of the family against various human diseases of antiquity. The iam of this paper is to review the literature on basil, samples were collected from different farms of Khartoum state, specifically the oil that is extracted by cold extraction with water (ocimum basilicum oil extracted by hydro steam distiliation) was to determine the percentage of oil1.37% and detected Chemical properties, which include iodine number and acidity and peroxide and ester value and physical properties, which include viscosity and refractive index, color and density and its compounds by gas chromatography instrument(GC/MS) with mass selective detector .

INTRODUCTION

Plants have been utilized as a source of medicine for thousands of Years and continue to play an important role globally in primary health care, mostly in developing countries (Balunas and Kinghorn, 2005). The use of medicinal plants is increasing because people believe they are safe for human consumption. There is also an increase in infectious diseases worldwide caused by both drug resistance; and lack of sufficient affordable medicine for people living in poor communities. The discovery of drugs from medicinal plants may be one of the solutions in the fight against infectious diseases. Thousands of natural products
are used in clinical trials and some are already confirmed useful in combating some of the diseases.

It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis[1].

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based traditional medicine systems continue to play an essential role in health care (Owolabi *et al*., 2007). India has several traditional medical systems, such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs.

According to the World Health Organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous, 2007a). Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo *et al*., 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the photochemical in them (Cowman, 1999; Adesokan *et al*., 2008). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000).

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive

compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important. The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities and narrow geographic ranges therefore they are more prone to extinction. Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes. Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector (Kala *et al*., 2006).

Material and methods

Materials

2.2. Source of leaves

The leave of ocimum basilcum plant were obtain in septm 2014 from farmer in Khartoum state (soba-shambat). The leave were first washed and shade dried. The pulp and stone were separated. were shade dried and powdered to be used in further analysis.

2.3. Chemicals and reagents

Chemicals and reagents used in this study were of analytical grade.

Methods

Steam distillation extraction:

In this laboratory exercise we will employ Steam Distillation to isolate an Essential Oil from part of leaves. This will involve distilling a mix of the plant material and Water to obtain the Oil, extracting the Oil from the Water, and then isolating the Oil from the extraction solvent. In the end, we will analyze the Oil's composition by separating its constituents using Gas Chromatography. Essential Oils are a mix of fragrant compounds common to a number of plants such as basil, Lavender, Pine, etc. which are isolated via steam distillation. Because these Oils were once considered to be the essence of the plant, they were initially sought as possible pharmaceuticals and are a part of

early medicine's contribution to modern chemistry. Today they are used as flavorings, perfumes and deodorants. Essential Oils are mixtures of organic compounds that are dominated by the Terpenes and the Terpenoids, oxygen containing derivatives of the terpenes. Terpenes themselves are a class of compounds built on the five carbon skeletal fragment of Isoprene[46].

Oil Extraction and determination of oil content

The oil content of the sample was determined according to using Soxhlet apparatus as follows:

40 grams of sample were taken and placed in a thimble. The thimble was covered using cotton wool. An empty, dry and clean round flask with a known weight was connected to siphoning apparatus and 200 ml water (with a boiling point of 50 to

60°C) were added. Extraction was carried out for 4 hr during which the solvent was distilled off. The round flask containing the extracted fat was weighted[47].The extracted oil was calculated and expressed as percentage according to the equation:

Physicals characteristics

2.4.3.1. Refractive index

The refractive index (RI) was determined by Abbe 60 refractmeter as described by the double prism was opened by means of screw head, few drops of oil were placed on the prism. The prism was closed firmly by tightening the screw head and the instrument was then left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument (32+2°C). The prisms were cleaned between reading by wiping of the oil with soft cloth, then with petroleum ether and then left to dry[48].

2.4.3.2. Color of oil

The color intensity of oils was recorded using a lovibont tintometer as units of red, yellow and blue where sample of oils were filtered through filter paper immediately before testing. An appropriate cell (2" cell) was filled with the oil and placed in tintometer near-by window for light. The instrument was switched on and looked through the eyepiece. The yellow colour was adjusted, and the slides were adjusted until a match color was obtained from acombination of red and blue. The values obtained by matching were recorded as red, yellow and blue[49].

2.4.3.3. Viscosity

The viscosity of the oil samples was recorded using an Ostwald U-Tube viscometer the viscometer was suspended in a constant temperature bath (32+2°C) so that the capillary was vertical. The instrument was filled to the mark at the top

of the lower reservoir with the oil by means of pipette inserted into the side arm. So that the tube above the mark was not wetted .The instrument was then left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument (32±2°C) By means of the pressure on the respective arm of the tube, the oil moved into the other arm so that the meniscus was 1cm above the mark at the top of the upper reservoir. The liquid was then allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at the bottom of the upper reservoir recorded[50].

2.4.3.4. Density

The oil density was determined using psycho-meter. An empty stopperad psycho-meter was weighed, filled with water and kept at constant temperature of 25^{o}C in a water bath for 30 min. The weight of water at 25°C was determined by subtracting weight of empty psycho-meter from its weight when filled with water. The end of time stopperad psycho-meter was adjust to proper level, dried with a cloth and weighted. In the same manner, the weight of the oil at 25°C was determined[51]. The density was calculated as follows:

2.2.5. Chemical characteristics of ocimum basilicum oil

2.2.5.1. Iodine value

The iodine value of the oil was determined approximately 0.2 g of oil was accurately weighed and placed in a dry and clean flask specially offered for the test. To 10 ml of chloroform was used for dissolving the oil, 25ml of pyridine sulphate dibromide solution was added and finally 20 ml of KI (0.1N) were added to the contents of the flask. The flask was then stoppered and the mixture was allowed to stand for 10 minutes in a dark place. The stopper and the side of the flask were rinsed with enough amount of distilled water, and the content of the flask were then shaken and titrated against 0.1N sodium thiosulphate solution using starch liquid as an indicator[52]. A blank determination was carried out simultaneously.

2.2.5.2. Peroxide value

The Peroxide value of the oil sample was determined, 1g of the oil was accurately weighed into 250ml conical flask. 30 ml of glacial acetic acid and chloroform (3:2) were added and the solution was swirled gently to dissolve the oil. A 0.5 ml of 0.1N KI was added to the flask, and the content of the flask were left to the stand for one minute before adding 30ml of distilled water. After a while, the contents were titrated with 0.01 N sodium thiosulphate until the yellow colour almost disappeared. The number of ml 0.01 N sodium thiosulphate required (a) were recorded. The same process was repeated for blank. The number of ml of 0.01 sodium thiosulphate required by the blank (b) was recorded[53].

2.2.5.3. Acid value

Acidity determination was carried out as Five grams (5 g) of oil were dissolved into solvent mixture of 75 ml of ethanol 96% and 75 ml of diethyl-ether, 1 ml of phenolphthalein was added and the solution was thereafter, titrated with **2.2.5.4. Saponification value**

The determination of the saponification value was carried out accurately weighted 2 gm of oil sample were introduced into a 200 ml conical flask. 25ml of 0.5 N alcoholic KOH solutions was added, and the contents of the flask were boiled under reflux for 1h with frequent rotation. 1ml of phenolphthalein indicator was added, while the solution was still hot, and the excess alkali was titrated with 0.5N HCl. The required volume of HCl (a) was noted. A blank was determined at the same time and condition and the required volume of the acid (b) was also recorded[55]. potassium hydroxide 0.1 N until a pink endpoint was reached[54]. The formula used to calculate acidity value was

GC-MS Analysis:

GC-MS analysis of the samples was carried out using instrument shimudzu QP2010, gas chromatography analyses. The GC-2010 with capillary column (D 30 m x 0.32 mm) Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 50°C and injection temp 300°C and injection mode split. With selective Detector mass with (ion source temp200°C and interface temp250°C solvent cut time 2.5 min, start time 3.00 min end time 27.00 ,mode scan event time 0.30 sec , scan speed 1666,start m/z 50.00 end m/z 500.00) Carrier gas: He Carrier gas linear velocity: 47cm/s Sample injection volume: 1 µL sample was injected with flow rate 1.67ml/ min GCMS-QP2010 total ion chromatogram (TIC) is also simple in the carrier gas constant linear velocity mode. Using the same type of column and setting the same carrier gas linear velocity values results in a virtually identical separation profile, the total running time for a sample is 27 min.

Parameter	Mean Value

Figure6: Gas Chromatography instrument

RESULTS AND DISCUSSION

3.1. Oil content:

Colour	Yellow - red 40 - 6.3
Refractive index	1.483
Relative Density	1.568 g/ml
Optical rotation	-6 degree
Viscosity	3.8

The extraction brought about yellowish red liquid with a strong odor, reminiscent of clove oil the essential oil in this study it was found 1.39% in the present study was comparable to report the yield of the basil essential oils from plants known in Serbia and Montego. Which it was High than a result reports by (bozin *et al.*, 2006) their found the yield of the essential oils from leave parts of *ocimum basilicum* by hydro distillation was 0.37 %. .

3.2. Physical properties characterization:

Table3: showed the physical properties of the ocimum basilicum oil

There are several standards which must be fulfilled to maintain the quality of *Ocimum basilicum* essential oil namely Appearance color , fragrance, transparent , fluid or pale yellow *Ocimum basilicum* distinctive flavor. The physical properties of *O. basilicum* essential oil obtained at 25^{o}C. The species of *O. basilicum* is the most cultivation in Sudan it is well known as Sweet basil which can be used as condiments and insect repellent (lwu, 1993; delille, 2007). The physical characteristics of *O. basilicum* oil are given in Table 1. It was found to be a red-yellowish liquid at ambient temperature and had a refractive index about 1.483. The density of the extracted oil was 1.568 and optical rotation (-6) values, in this study was refractive index lower than their result and density higher to their result and optical rotation higher than their result (WECECS, 2007., sanfransico USA) Their result refractive index(1.51)and optical rotation (-8.8) and density 0.952 , because it was different in geographic region and environment and seasonal. The value of viscosity of the oil in our study was lower (3.8 g-1.cm.s),

than their result (14.37 g-1.cm.s) reported their *Bowles* EJ (2003). Viscosity is a measure of resistance of a fluid to deform under shear stress[63].

3.3. Chemical properties of *Ocimum basilicum* leave oil

Table 4: showed the chemical properties of the ocimum basilicum oil

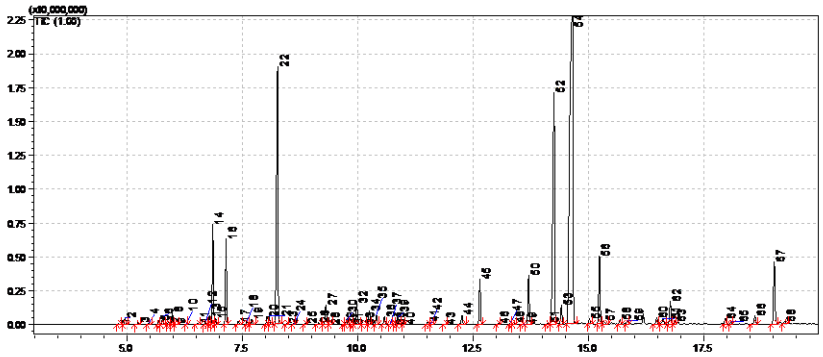
Parameter	**Mean Value**
Saponification value (mg KOH/ g oil)	0.570
Acid value (mg KOH/ g oil)	0.21
Ester	0.36
Iodine value (g I2/ 100g oil)	72.7
Peroxide value	4.9

3.3.1. Peroxide Value

one of the most widely used tests for oxidative rancidity in oils and fats, peroxide value is a measure of the concentration of peroxides and hydro peroxides formed in the initial stages of lipid oxidation. The peroxide value of *Ocimum basilicum* leave oil was in the range adopted as satisfactory (less than 1.0 mg/kg). In general, the lower the peroxide value, the better the quality of the oil, the more stable the oil (Borchani *et al.*, 2010). On the other hand the acid value was found to be very low (1.19) and this could be an indicative that the free fatty acid value is on the low side and thus the resulted value shows that this oil is stable (Elena and Yakov, 2005).

3.3.2. Iodine value:
The iodine value of the oil is 72.7 mg/g. The iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. The iodine value is high and this reflected the presence of sufficient amount of unsaturated fatty esters in the seed oil and this suggests that the oil will be susceptible to oxidative deterioration. The iodine value is a little high above 100 and this places the oil in the drying groups and the oil may find application as a raw material in industries for the manufacture of vegetable oil-based ice cream (Mohammed and Hamza, 2008).
3.3.3. **Saponification:**
Saponification value was 0.570(mg KOH/ g oil) The Saponification value was found to be moderately. saponification value is too high, the soap might contain too much alkali which may lead to its reaction with the skin. If the saponification value too small, the fatty acid salts may not to be sufficient enough to remove fat or oil. (Auwal *et al*., 2010).
3.3.4. Acid value
Acid value was 0.21 (mg KOH/ g oil) the acidity it filled in range (0.8-1.5) this result show that the acidity of *Ocimum basilicum* is low.
3.3.5. Ester value
The ester value of *Ocimum basilicum* was 0.36 it filled in range this result show that the ester of *Ocimum basilicum*

3.4. Thin layer chromatographic (TLC):
Table5: showed thin layer chromatographic profiles of *ocimum basilicum* oil plant as seen under spray reagent application and calculate Rf.



Peak	R. Time	Area	Area%	Name
1	4.816	117650	0.04	Alpha.-phellandrene
2	4.959	825396	0.27	Alpha.-pinene
3	5.248	291167	0.09	Camphene
4	5.470	159730	0.05	Benzaldehyde
5	5.695	552920	0.18	Bicyclo{3.1.0}hexane,4-methylene
6	6.577	1172827	0.38	Beta-pinene
7	5.899	294944	0.10	3-hebtanone,5-methyl
8	5.984	1543979	0.50	Beta-myrcene
9	6.043	86118	0,03	2-3-dehydro-1-8cineol
10	6.294	96713	0.03	1.5-cyclooctadiene, 3,8-dimethyl
11	6.540	21035	0.07	1,3-cyclohexadiene
12	6.702	378062	0.12	o-cymene
13	6.790	121961	0.39	D-limonene
14	6.862	127596	4.12	Eucalyptol
15	6.926	780958	0.25	Trans-beta-ocimene

16	7.149	108318	3.50	1,3,6-octatatriene,3,7-dimethyl
17	7.412	464193	0.15	Gama-terpinene
18	70601	412476	0.13	p-menth-8-en,steroisomer
19	7.7040	730020	0.24	Alpha-methyl-alpha-[4-methyl-3-penten
20	8.040	1193178	0.39	Trans-linalooloxide(furanoid)
21	8.081	1198580	0.39	l-fenchone
22	8.264	4503729	14.54	1,6-octadieno-3,7-dimethyl
23	8.461	125554	0.04	Hexanoic acid ,octenyl-3-2ester
24	8.619	107054	0.03	Bicycle[2.2.1}heptanol1,3,3-trimethyl
25	8.868	89517	0.03	2,4,6-octatriene,2,6-dimethyl
26	9.148	278211	0.09	2-dodecen-4-yne,(E)
27	9.310	288059	0.93	**(+)-2-Bornanone**
28	9.386	40593	0.01	Lilac aldehyde B
29	9.700	66190	0.02	Pinocarvone
30	9.748	39774	0.13	L-alpha-terpineol
31	9.868	47693	0.02	2H-pyran-3-ol,6-ethenytetraydro-2,2,6 trimethyl
32	0.979	30399	0.93	4-terpinenol
33	10.117	74812	0.02	Thymol
34	10.250	18816	0.61	Alpha-terpineol
35	10.401	13433	0.44	Estragole
36	10.580	11542	0.37	Formic acid, octyl ester
37	10.705	34047	0.01	Bicyclo[3.1.1]hept-3-en-2-one,4,6,6trimethyl
38	10.799	20522	0.01	Cyclopropane,2-(1,1-dimethyl-2-propenyl
39	10.870	10407	0.34	Fenchyl acetate
40	10.993	28609	0.01	2,6-octadien-1-ol,3,7-dimethyl-,(z)-
41	11.517	40432	0.13	Geraniol
42	11.588	62861	0.02	Carveol
43	11.881	63146	0.02	Citral
44	12.255	14265	0.46	Aceticacid,1,7,7-trimethylbicyclo{2.2.1}hept-2-yl ester
45	12.644	65834	2.13	1-3-penten-3-one,1-phenyl
46	13.047	19254	0.06	myrtenyl acetate
47	13.310	32053	0.10	Cyclohexane,1-ethenyl-1-methyl
48	13.371	25209	0.08	3-cyclohexene-1-methanol,6-methyl-
49	13.552	39868	0.01	Alpha –cubebene
50	13.699	77579	2.50	Eugenol
51	14.114	23713	0.08	4-hexen-1-ol,5-methyl-2-(1-methylethenyl)-,
52	14.249	47829	15.44	2-propenoic acid, 3-phenyl-,methylester
53	14.409	28104	0.91	1,5-cycloundecadiene,8,8-dimethyl-9-met
54	14.649	11610	37.48	Methyleugenol
55	15.233	10235	0.27	Caryophyllene

56	15.233	10235	3.30	Beta-curcumene
57	15.324	65411	0.21	Alpha-guaiene
58	15.673	72647	0.23	Humulene
59	15.835	52946	0.17	1H-Cyclopenta[1,3]cyclopropa[1,2]benzen
60	16.472	10279	0.33	1,5-cyclodecadiene,1,5-dimetyl-8-(1-methylhylidene)
61	16.610	10917	0.35	Azulene,1,2,3,5,6,7,8,8-octahydrodin
62	16.766	36267	1.17	Gama-muurolene
63	16.864	46294	0.15	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)
64	17.958	89612	0.29	Androstenediol
65	18.083	35899	0.12	Cycloheptane,4-methylene-1-methyl-2-
66	18.599	15113	0.49	Cubenol
67	19.018	10194	3.29	Tau-cadinol
68	19.250	52860	0.17	1,4-Methano-1H-indene,octahydro-1,7a-dimethyl-5-
		309780	100.00	

GC/MS analysis:

The essential oils of *Ocimum basilicum* were subjected to detailed GC-MS analysis in order to determine the components on their volatile constituents. The yield of oils ranged from 0.4- 1.3%. Exactly 68 compounds were identified in *Ocimum basilicum* (Table6) meanwhile 68 compounds. Essential oils found in *Ocimum basilicum* belong to a variety of groups including monoterpene hydrocarbon, oxygenated monoterpenes (e.g., 1, 8- cineole, L-camphor), sesquiterpene hydrocarbons (e.g., β-cubebene, γ-candinene) and aromatic compounds (e.g., methyl eugenol). From Table 5, the constituents from Ocimum Basilicum yielded methyl eugenol in abundance, supports previous findings proving that this plant belongs to the phenolic chemotype. The amount of methyl eugenol then increases gradually, until linalool was only present when methyl eugenol was relatively high. The constituents obtained from Ocimum basilicum were characterized by a high content of aromatic compounds, with methyl eugenol as the main constituent. As complementing the results with Ocimum Basilicum, a significant group such as monoterpene hydrocarbon (limonene), oxygenated monoterpene (cubenol), sesquiterpene hydrocarbons (e.g. α-Guaiene, β-curcumene), oxygenated sesquiterpenes (elemol) and aromatic compounds (e.g. , methyl eugenol). The methyl eugenol content is high amount of methyl eugenol and β-caryophyllene are consistently vice-versa[64].

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