Gezira J. of Engineering and applied Sciences
ISSN Linking: 1858-5698
http://journals.uofg.edu.sd/index.php/gjeas/

*

Determination of Physicochemical Properties and Fatty Acid Composition of Khella (Ammi visnaga L.) Seeds Oil using Gas Chromatography-Mass Spectrometry

Hadeel A. Noraldaum[1], Mohammed O. Babikir[2], Yasmin A. Aburigal[3]*

1,2. Faculty of Engineering and Technology, University of Gezira, Wad Medani, Sudan
3. Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan
*Corresponding author, Email: aburigalyasmin@gmail.com.

INFORMATION:

Submission:06/11/2022

Accepted: 30/03/2023

Publication: 11/12/2023

ABSTRACT

Ammi visnaga L. (Family Umbelliferae), also known as Khella Baldi or toothpick weed, is an annual herb indigenous to the Mediterranean region of North Africa, Asia, and Europe. The plant is known to have been used in traditional medicine a long time ago. Nowadays, it is used in modern medicine to treat many aliments such as renal colic and coronary insufficiency, and is used as an antioxidant, antifungal, and antibacterial, with a larvicidal effect on mosquito larvae. The aim of this work is to determine fatty acid composition and physio-chemical properties of oil extracted from the seeds of Ammi visnaga L. Amount of 75 g clean seeds were crushed and the oil was extracted by soxhlet using n-hexane as a solvent. The fixed oil from Ammi visnaga seeds oil was analyzed by gas chromatography coupled with mass spectrometer. The results obtained contain saturated as well as unsaturated fatty acids of A. visnaga seeds oil. From GC-MS analysis the main constituents of fatty acid oil were Betroselinic acid (62.01%) , Linoleic acid (8.9%), Palmitic acid (6.61%) and butyric acid (1.94%). The physio-chemical analyses of Ammi visnaga seeds oil were also measured according to the American Oil Chemists Society (AOCS) official methods,2003. Results showed that the percentage yield of the oil was 8.6% and the color of the oil was dark green. The density, moisture content and refractive index were 1.44 g/ml ,4.77%, 1.49 respectively. The acid value, saponification number, free fatty acids and peroxide values were in the order 9.61mgKOHg-1, 154.11mgKOHg-1, 6.768mgKOHg-1, 23.022 meqKg-1 respectively. The results revealed that Ammi visnaga oil can be used in cosmetic and pharmaceutical industries. The study recommend to use different solvents to extract oil from the seeds of Ammi visnaga L. and compare their result. Also study the effect of Ammi visnaga oil on microorganisms.

KEYWORDS:

Khella, toothpick weed, chemical composition, physiochemical properties

1. INTRODUCTION

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals , agrochemicals , flavors ,fragrances , colors and food additives. Medicinal plant is the natures gift to human beings to help them pursue a disease-free healthy life. Plants have been used as drugs by humans for thousands of years ago as a result of accumulated experience from the past generation [1].Today all the worlds cultures have an extensive knowledge of herbal in the developing countries, a large number of people depend on the traditional folk medicine as primary substances in their health care systems especially rural areas healers and patients from centuries. An estimated 35,000 to70,000 plant species are used for medicinal and therapeutic purposes in the world [1]. Khella belongs to the family Umbelliferae is a medicinal plant which is obtained from two species of Ammi for example, *Ammi majus* has been used medicinally in the Middle East for centuries in the treatment of leukoderma. *Ammi visnaga* is a perennial medicinal plant found mainly in the Mediterranean regions and also , Northern Africa and South west Asia, Pakistan as a wild medicinal plant.. It is called Khella in some Arab countries, tooth pick herb or Bishop's weed in England, Chile and Europe [2] .In Sudan, *Ammi visnaga* is well known , it is production is limited to areas in a Northern state as a winter crop [3] .The *Ammi visnaga* fruits contents furanochromones at least 1.0%γ- pyrone derivatives. Moreover it is included khellin was about (0.3-1.2%) visnagin about (0.05-0.3%), khellol, khellenin, khellinol, ammiol, visammiol, khellinone and visnaginone [4].The use of *A. visnaga* as an antidiabetic agent is considered famous in many countries [5]. The tea prepared from the crushed seeds is highly effective for the treatment of kidney stones [6]. The plant extract also showed a highly potent

diuretic activity which might be the possible mechanism of action of khellin extract. Beside this, the seeds are the main source of furocoumarins which is the reason for high demand of this valuable plant in pharmaceutical industry [7]. *A. visnaga* L., showing their effectiveness against various microorganisms such as Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae strains [8] however, they showed weak antifungal activities [9]. [10] identified components that strongly inhibited aflatoxin formation in toxigenic fungi, e.g., khellin, xanthotoxin, and bergapten [10]. Due to the increasing demand and safe usage of this herb either as crude or in purified extracts, hence considerable attention is now being paid to develop pharmaceutical based technology to enhance the seeds as well as secondary metabolites yields from *A. visnaga* to fulfill the requirement of the pharma sector [11] .

The objectives of this work are to extract oil from khella seeds, analyze physicochemical properties of khella oil and to Identify the chemical constituents of khella using Gas chromatography – Mass spectrum (GC- MS).

2.MATERIALS AND METHODS

A. Seeds Source

khella seeds were provided from the National Center for Research, Khartoum, Sudan.

B. Extraction of khella seed oil

The khella seeds were cleaned and ground into powder by laboratory blender to be ready for extraction and analysis. 75 gm of ground seeds were weighed into thimble and transferred to soxhlet apparatus, 600 ml of n-hexane was added into flask, the temperature was set at 60°C for 6 hours. Then oil separated from n-hexane by evaporated n-hexane at 70°C using rotary evaporator, the extracted oil was kept for analysis in glass containers.

C. Physicochemical analysis of khella seeds oil

The Physicochemical Analysis were identified at National Oil Seeds Processing Research Institute(NOPRI),University of Gezira ,Sudan. The evaluation of the following physicochemical properties of the extracted seed oil was determined according to the American Oil Chemists Society (AOCS) official methods,2003[12].

Oil content

The oil content was obtained by using soxhlet extraction method. Five grams of khella seed powder was taken into a thimble after grounding and drying in oven at 103 °C for one hour. A soxhlet apparatus and hexane as solvent were used. A round bottom flask containing known volume (250ml) of hexane was fixed to the end of the apparatus and a condenser was tightly fixed at the bottom end of the extractor in a heating mantle was used for six hours using n-hexane as a solvent, followed by solvent removal with a rotary evaporator. and the oil content was determined as the ratio of the weight of the extracted seed oil to the weight of the oilseed powder sample as described below.

$$\boldsymbol{oil\ content}(\%) = \frac{\boldsymbol{weight\ of\ oil \times 100}}{\boldsymbol{weight\ of\ sample}}$$

Moisture content

Seven grams of powdered seeds were put in dish and dried in vacuum oven at 105ºC for 3 hours, then the dish were transferred to desiccators to cool and reweighed the moisture content was calculated using the following equation:

$$\boldsymbol{moisture\ content}(\%) = \frac{(\boldsymbol{w1 - w2}) \times \boldsymbol{100}}{\boldsymbol{w0}}$$

Where :W0 = sample weight, W1 = weight of (sample +dish) before drying, W2 =weight of (sample +dish) after drying

Density

Density is the ratio between weight of oil to volume. Density of the sample was calculated by using the following formula.

$$\boldsymbol{D} = \frac{\boldsymbol{W1 - W2}}{\boldsymbol{V}}$$

Where: D: density of oil sample, W1:weight of bottle with oil, W2: weight of bottle without oil ,V: volume of oil sample.

Refractive index

The Refractive index of a substance is the ratio of the speed of light at a definite wavelength in vacuo to its speed in the medium. Few drops of the oil were placed between the prisms in such way that the space between the prism is completely filled and waited until the oil sample reached the temperature of prisms, refractive index was read by using ATACO digital refractometer model RX- 7000α- Japan.

Peroxide value

five grams of oil sample was placed in a 250 ml conical flask. A 20 ml of a mixture of 30 ml of acetic acid and 20ml of chloroform was added and shaken until the sample was dissolved. Then 0.5 ml of saturated potassium iodide solution was also added to it. The solution had been shaken for at least 1 minute. About 15 ml distilled water was added to the solution and. 2 ml of starch solution was then added and it was titrated carefully with 0.1 N Sodium thiosulphate solutions until the blue color just disappeared at the end point, The peroxide value expressed in mill equivalents of active oxygen per kilogram of the oil sample.

Oil + 2I $\rightarrow$ I_2

$S_4O_6 \pm 2I$ $\rightarrow$ $2S_2O_3 + I2$ -

$$\boldsymbol{pv} = \frac{(\boldsymbol{Vs - vb})\boldsymbol{N \times 1000}}{\boldsymbol{W}}$$

Where: Vs: is the volume in ml of sodium thiosulphate solution, Vb: is the volume, in ml, of the sodium thiosulphate solution used for the blank test, W: is the weight, in grams, of oil sample, N: is the normality of sodium thiosulphate solution.

Free fatty acids (FFA)

two grams of sample was weighted into flask; 10 ml of 98%ethanol was added. Then 1-3 drops of phenolphthalein indicator were added and the sample was heated for two minutes on the water bath and then, the sample was titration with sodium hydroxide 0.01N till the pink colour appeared.

The free fatty acids were calculated from the following relation:-

$$\boldsymbol{FFA}\%\ (\boldsymbol{as\ oleic}) = \frac{\boldsymbol{28.2 \times N \times V}}{\boldsymbol{W}}$$

Where: N: normality of NaOH solution,V: volume of NaOH solution required,W: weight of sample taken in g.

Acid value

The percentage of free fatty acids was converted to acid value (as oleic%) through multiplying. The percentage of free fatty acids by the factor 1.99 for KOH solution or 1.42 for NaOH solution.

$$\boldsymbol{Acid\ value} = \frac{\boldsymbol{F.F.A}\%(\boldsymbol{56.11})}{\boldsymbol{28.2}}$$

Saponification value

The saponification value is the number of milligrams of potassium hydroxide required to saponifiy 1 g of fat/oil under the conditions specified.. 0.5M KOH was prepared in 95 % ethanol, 5 grams of oil sample were weighed and 25 ml of 0.5 M KOH was added, 25 ml of the blank solution was also measured into a conical flask. The two samples were then connected to a reflux condenser and allowed to boil for one hour until the reflux was completed, 1 ml of phenolphthalein indicator was added to the mixture and the resulting mixture was titrated against 0.5 M HCL solution until the pink colour of the indicator just disappears. The volume of the HCL used to attain the end point was recorded, the blank determination was carried out using the same procedure described above until the pink colour of the indicator just disappears, then the volume of HCL used was noted.

The saponification value was determined using the relationship below:

$$\boldsymbol{SV} = \frac{\boldsymbol{56.1N(Vb - Vs)}}{\boldsymbol{w}}$$

Where: Vb: Volume of HCl used (in ml) for blanktitration, Vs: Volume of HCl used (in ml) for the sample of oil, N: is the normality of potassium hydroxid, W: is the weight, in grams of oil.

D. Fatty acid composition of khella seeds oil

Preparation of fatty acid methyl ester (FAME)

Two ml of khella seed oil were taken in a test tube, 7 ml of alcoholic NaOH were added (prepared by dissolving two grams of NaOH in 100 ml methanol) and 7 ml of alcoholic H2SO4 1% (prepared by mixing one ml conc H2SO4 + 99 ml methanol) and shaken by vortex for three minutes, all the contents were left overnight. Two ml of saturated NaCL, two ml of n-hexane were added, shaken for three minutes, n-hexane layer was collected. 5 µl of collected hexane layer were taken and diluted with 5ml diethyl ether, one gram of sodium sulphate was added as drying agent, filtered through syringe filter 0.45 µm and the filtrate was transferred directly to the GC-MS vial.

E. Determination of fatty acid composition of khella using GC-MS

Fatty acid composition was determined at Mohamed Ebaid Mubark Laboratories. The GC-MS analysis of the extracted oils was performed using GC-MS model (GC.MS-QP22010 Ultra), Shimadzu company, Japan with (Rtx-5MS) silica capillary column stationary phase, film thickness 0.25 µm, Length 30m and internal diameter 0.25 m. the GC oven initial temperature was 600C and increased to 3000C at a rate of 100C/min. Carrier gas was Helium at a flow rate of 1.61ml/min and the sample was injected in split mode. The GC coupled to mass selective detector transfer line heater maintained at 250^0C, and ion source temperature 200^0C. Identification of compounds was based on comparison of the relative retention time and mass spectral data and NIST mass spectral library of the GC-MS.

3. RESULTS AND DISCUSSION

Physicochemical analysis of khella seed oil. The physical properties of khella seed oil included moisture content, Refractive index, Density are presented in table (1).

Table 1. Physical Properties of khella seed oil

No.	Name of test	Result	FAO/WHO Standard
1.	Moisture content(%)	4.77	-
2.	Refractive index	1.49	1.4677-14705
3.	Density(g/ml)	0.97	0.9-1.16

The physical properties such as color, appearance, taste ,odor and solubility of the khella oil were determined by conventional methods. The oil was dark green in color. It had agreeable taste and spicy odor .It appeared as homogeneous, the khella seed oil is completely soluble in n-hexane.

The moisture content was 4.77% .The refractive index was 1.49 this value is higher than the refractive index specified by FAO/WHO (2009).The density of khella oil was 0.97g/ml and this value is in agreement with the FAO/WHO (2009) international standard**.**

Chemical Properties of khella seed oil

The chemical properties of khella seed oil included oil content ,saponification value ,peroxide value, acid value, free fatty acid are presented in table (2).

Table (2) Chemical Properties of khella seed oil

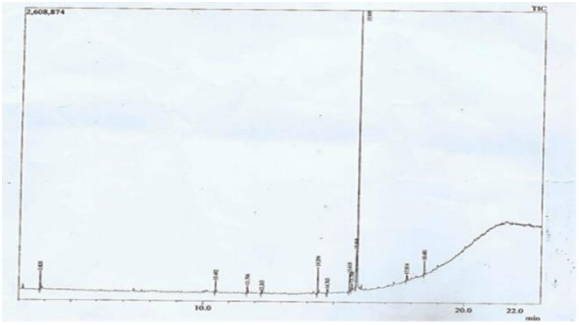
No.	The test	Result	FAO/WHO Standard
1	Oil content(%)	8.68	38-40
2	Saponification value (mg of KOH/g of oil)	154.11	181.4 ± 2.60
3	Free Fatty Acid (%)	6.768	5.78-7.28
4	Peroxide value (meqkg-1)	23.022	10
5	Acid value (mg/KOH/g)	9.61	4

The oil content of khella seed oil is 8.6%which is far lower than the oil content specified by FAO/WHO (2009) [13] but this value was almost in agreement with findings of [14] at 8.17% .The saponification value of the khella oil was 154.11, this value shows consistency with FAO/WHO (2009) standard. On the other hand, the free fatty acid of khella seed oil was 6.768 mgKOHg-1 which is within the range of the FAO/WHO (2009) standard. Peroxide value was 23.022 which is very higher than peroxide value specified by the FAO/WHO (2009). An acid value of 9.61 mg/ KOHg-1, this value is higher than the acid value specified by FAO/WHO (2009). This slight variation may be due to geographical location and environmental factor.

Fatty Acids Composition.

Table (3): The fatty acids of khella seed oil by GC-MS

No.	Fatty Acid	Retention time	Area %
1	Betroselinic acid C18:1	15.909	62.01
2	Linoleic acid C18:2	15.868	8.39
3	Palmitic acid C16:0	14.394	6.61
4	Butyric acid C12:3	11.706	1.94



Fig(1): GC-MS chromatogram of khella seed oil

The most abundant unsaturated fatty acid in khella seed oil was Betroselinic acid C18:1 (62.01%) followed by linoleic acid C18:2 (8.9%) while the least was butyric acid C12:3 (1.94%) ,saturated fatty acid was palmitic acid C16:2 (6.61%). [11] was determined the fatty acid of khella seed oil by gas chromatography-mass spectrometry(GC-MS)and found that the most abundant unsaturated fatty acid in khella seed oil was linoleic acid(11.39) which differs from our findings may be the area, nature of khella seed [11].There is another compound appeared from the GC-MC analysis called methoxsalen (1.99%) this compound use in pharmaceutical industries.

REFERENCES

[1] Al-Snafi A. E. 2013. Chemical constituents and pharmacological activities of *Ammi majus* and *Ammi visnaga*. A review. International Journal of Pharmacy and Industrial Research, 3(3): 257-26.

[2] Jaradat N.A. Abualhasan M. Al-Masri M. Johari A. Awad M.2015,Phytochemical Screening and In-vitro Evaluation of Antioxidant and Antimicrobial Activities of the Entire Khella Plant (*Ammi visnaga*.L.)A member of Palestinian Flora International Journal of Pharmacognosy and Phytochemical Research 7(1): 137-143.

[3] Khalid H. Abdalla W.E. Abdelgadir H. Opatz T. Efferth T.2012. Gems from Traditional North-African Medicine: Medicinal and Aromatic Plants from Sudan. Nat. Prod. Bioprospect. ; 2: 92 - 103.

[4] Batanoumy K.K. 2002. Wild Medical plant in Egypt. An inventory to support conservation and sustainable use. Designed by Arasia Lawrence company cited by (Ahmed et al;2009).

[5] Hassan J. Mhamed M. Mohammed E. 2002. Hypoglycemic effect of aqueous extract of Ammi visnaga in normal and streptozotocin-induced diabetic rats. J. Herb. Pharma. 43(2) :19– 29.

[6] Amina I. D. Magdi A. M . Wadah J. A. O. Haidar, A . Hassan S. K.2014. A Phytopharmacological Review on Four Antitumor Medicinal Plants Grown in Sudan American Journal of Pharm. Tech Research , 4(5):28-36.

[7] Ahmed S.A.R. 1989. Toxicity to goats of two plants. M.Sc. (Veterinary Science) thesis, University of Khartoum, Sudan .Pg 15-17.

[8] Soro K.N. Sabri L. Amalich S. Khabbal Y. Zair T 2015. Chemical composition of Moroccan *Ammi visnaga* L.(Lam.) and antibacterial activity of its essential oil against extended-spectrum beta-lactamase– producing and not producing bacteria. Phytotherapie , 13(8): 168– 175.

[9] Feirouz B. Salima K. 2014. Antibacterial activity and chemical composition of *Ammi visnaga* Essential oil collected from Boumerdes (Algeria) during three periods of the plant growth. J. Essent. Oil-Bear. Plants.5(17), 1317– 1328.

[10] Razzaghi-Abyaneh M. Shams-Ghahfarokhi M. Rezaee M.B. Sakuda S. 2010.Natural Aflatoxin Inhibitors from Medicinal Plants. In Mycotoxins in Food, Feed and Bioweapons; Rai, M., Varma, A., Eds.; Sprin\gerBerlin/Heidelberg, Germany: pp 329– 352.

[11] Riaz Ullah I. H. Jameel A. K. Naser M. A., Tayyeba S .2012. Investigation of fatty acid composition of Ammi visnaga seed oil bygaschromatography-mass spectrometry(GC-MS) . African Journal of Pharmacy and Pharmacology Vol. 6(47): pp. 3265-3267.

[12] AOCS. 2003. Official methods and recommended practicesof the American Oil Chemists Society. 6 th Edn., 2003,AOCS Press, Champaign.

[13] FAO/WHO 2009. Report on the 21st session of the Codex Alimentarius Committee on fats and oils. Kola Kinabalu, Malaysia.

[14] Hana, A.; Hiba, A. H; Nafesa, S. H. 2016. Extraction and analysis of Khella Seeds Oil. Sudan University of Science and Technology. M.Sc. Thesis: p22-23.