Gezira J. of Agricultural Science
ISSN Linking: 1858-8735
http://journals.uofg.edu.sd/index.php/gjas/

Extraction, Refining and Characterization of the Fixed Oil of Basil (Ocimumbasilicum L.) Seeds

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INFORMATIONs
Submission: 22/09/2020
Accepted: 05/07/2021
Publication: 04/08/2021

ABSTRACT

Basil is considered one of the important medicinal and aromatic plants in Sudan and worldwide. It is very rich sources of phytochemicals which have vital properties affecting human health in reduce risks and diseases. The present study aimed to evaluate the physicochemical characteristics of basil seeds and the oil extracted from them. This included four accessions of basil grown in National Oil Seed Processing Research Institute (NOPRI) farm-University of Gezira- Sudan: (two local accessions of NOPRI and Umteraibat, and two accessions (Egyptian and Maldivian) obtained from Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. Themethods used the American Oil Chemists Society (AOCS) official methods. The percentage of moisture content (4-5), oil content (19-28%), crude protein (15-20%), crude fiber (30-36%)and ash (5-8%)for basil seeds. The physical properties of crude oil were specific gravity at room temperature (0.92-0.93) and refractive index (1.4809-1.4823) at 20°C. The Lovibond readings for crude oil color were in the range (0.6-2.5) for the red color and (18-70) for the yellow color, while for the refined oil the red color was (0.2-0.5) and yellow was (2.5-6.2). Chemical properties were: free fatty acids for crude oil (0.2-0.76%) and (0.04-0.06%) for refined oil; peroxide and saponification values and the unsaponifiable matter were (5.7-24.9) ml.eqv.\kg, (195-198) mg KOH/g and (1.6-2.2%) respectively. The fatty acid composition was analyzed using GC-MS, and the percentage of the unsaturated fatty acids oleic, linoleic and linolenic were (0.78-2.13), (22.91-40.39%)and (22.83-39.32%)respectively; the most abundant saturated fatty acids were palmitic and stearic at (14.7-16.67%) and (9.18-11.57%) respectively.

1. INTRODUCTION

There is an increasing trend for using medicinal and aromatic plants in Africa as the population grows and pressure on medicinal plant resources will become greater than ever (Prins*et al.*, 2010). Medicinal and aromatic plants are very important in our life. They are used as sources of drugs, flavours, colouringagents, tonic plants, diuretics, digestive plants, sedatives, pesticides and in the cosmetic industries (Abuzaid, 2000).

Sudan, is a large tropical sub-Saharan country approaching 1.7 million square kilometers in area, has always been considered a treasure house of valuable medicinal and aromatic plants species due to its characteristic geographical position (Gamal, 2004). Basil (*Ocimumbasilicum* L.), locally known as Rehan, is one of these medicinal plants. It is a perennial herb belonging to the family Lamiaceae, and is considered as an important source of essential and fixed oils (Aburigal, 2017). Basil grows in the wild and is also cultivated in Northern and Central Sudan (El Ghazali*et al.*, 2004). Fresh leaves have been normally used in natural or can extract a valuable essential oil from basil, used in the manufacture of perfumes and flavors for food beverages (Marotti*et al.*, 1996). In essential oil containing plants the chemicals are sometimes more important characteristics than morphological features. Therefore, the chemo-type has to be determined before the plant is used for industrial purposes (Grayer *et al.*, 1996; Paton and Putievsky, 1996).The chemical composition of basil essential oil, similarly to other oil plants, depends on genetic, ontogenetic, and environmental factors (Nurzynska*et al.*, 2012; Aburigal, 2017). Chemical variability among the fixed oils of Sudanese basil accessions was extremely broad. The main fatty acids of basil seed oil are linolenic (49–75%), linoleic (12–32%), oleic (6–10%), palmitic acid (5–13%) and stearic acid (2–3%) (Noure*t al.*, 2009). The oils were rich in triacylglycerols (94–98%) and contained 1–3% of monoacylglycerols and diacylglycerols. The unsaturated fatty acids of fixed basil oil averaged between 85.6 and 88.1%, including α-linolenic (49.3–52.4%), linoleic (23.6–26%), and oleic acids (10.3–12.3%). The most abundant saturated fatty acids were palmatic (8.0–9.2%) and stearic (3.6–3.8%) (Kakaraparthi*et al.*, 2015).

Most of the previous research carried out in basils focuses on the essential oils characterization and evaluation of their biological activities, however, few studies addressed the potential of fixed oil in industry and pharmaceutics. This present research aims to study the chemical constituents of the Sudanese wild basils and compare it with introduced ones to diversify their uses as bioactive ingredients that promote health and reduce the risk of disease.

2. MATERIALS AND METHODS

Materials

Seeds Source

Sweet basil seeds were obtained from four sources; two from Sudan (NOPRI and Umteraibat, Gezira State) and were collected from the wild and the other two from Egypt and Maldives and were provided from the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

Planting and Harvesting

Planting of seed were conducted at the field of the National Oil Seed Processing Research Institute (NOPRI), May 2017. The experiment was arranged in randomized complete block design (RCBD) with three replicates. The seeds were harvested after fruiting stage; October 2017.The collected seeds were cleaned and stored for further analysis.

Solvent Extraction

The basil seeds were cleaned dried at room temperature and crushed using mortar and pestle. The seed fixed oil was extracted via Soxhlet (MS-EAM, Favorit) for six hours using hexane as a solvent, after complete extraction, the solvent were evaporated via rotary evaporator (Heidolph Instruments GmbH and Co.KG91126 Schwabach), and further drying under open air in a dark area at room temperature. The oil percentages were calculated on dry weight basis.

Mechanical Pressing

Basil seeds were crushed by a table top screw pressing machine (OEKO TEC- IBG MONFORTS, type CA 59G, 2006, Machine No.20201550 Germany).

Oil Content

Oil content was determined according to American Oil Chemists' Society (AOCS) official method (Aa 4 – 38), (Ab 3-49) and (Am 2-93), revised (2000). 10 g of basil seeds sample were taken into a thimble of soxhlet apparatus after drying in oven 105°C for three hours. The extraction was continued for 6 hr using *n*-hexane as a solvent, followed by solvent removal with rotary evaporator. The oil content was evaluated as the ratio of the weight of the extracted seed oil to the weight of the basil seed powder sample as described below.

$$\text{Oil content (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

Proximate Analysis and Physicochemical Characteristics of Basil Seeds

Proximate analysis and physicochemical characteristics of basil seeds were determined according to AOCS (2003) methods.

Fatty acid Composition

The fatty acid composition analysis was carried out at the University of Medical Sciences and Technology, Khartoum, Sudan. To identify the constituents of the fixed oils extracted from 4 accessions, GC-MS Instrument (QP 22010 Ultra), equipped with a capillary column (0.25 diameter; film thickness 0.25 µm) was used. The carrier gas used was helium (99.99%). Fatty acid composition of the basil seed oil was determined by identifying comparison of their retention time (RT) with the mass spectral library of the GC-MS data software system (NIST library). The total running time for a sample was 25 min.

Oil Refining

The refining process was carried out according to (Chow, 2008). Crude basil seed oil was acid-degummed by pretreatment the oil with 0.3% w/w of phosphoric acid (85% concentration) at 70^{o}C for 10 min. Then, distilled water (3% w/w) was added into the oil and stirred in a heated water bath at 70^{o}C for 30 min. After cooling the mixture, the gums were removed from the degummed oil by centrifugation. A stoichiometric quantity of sodium hydroxide solution (16^{o}Baume) with an excess level of 0.5% was added into the degummed oil to neutralize the free fatty acids. The soap stock was removed by centrifugation. The neutralized oil was washed three times with distilled water to remove the residual soap in the oil. The neutralized oil was bleached with 1.2% w/w of bleaching earth at 95^{o}C for 30min. The bleached oil was followed by the final deodorization step performed using a lab-scale glass deodorizer at 200^{o}C for 1 h.

3. RESULTS AND DISCUSSION

Proximate Analysis of Basil Seeds

The proximate composition (moisture, oil content, ash, crude protein, and crude fiber) of basil seed accessions are presented in table 1.

Table 1. Proximate Analysis of Basil Seeds

Sample Name	Moisture Content%	Oil Content%	Crude Protein %	Crude Fiber %	Ash Content %
Egyptian Basil	4.68	25.36	18.83	32.37	5.92
Maldivian Basil	4.97	23.64	15.38	29.87	5.61
Wild Sudanese Basil (NOPRI)	4.41	27.60	19.71	33.51	7.60
Wild Sudanese Basil (Umteraibat)	4.60	19.00	19.75	35.98	5.08

Moisture Content

The moisture content of the four basil seed accessions (NOPRI and Umteraibat) was 4.68%, 4.97%, 4.41 % and 4.6% respectively, ranged between 4.41-4.97%. The moisture content of basil seeds reported in the literature varied between 5.20% ± 0.042% (Sarfraz*et al.*, 2011).

Oil Content

The oil content of basil seed accessions ranged from 19to 27.6%. Wild Sudanese basil seed (NOPRI) showed the highest oil content, but wild Sudanese basil seed (Umteraibat) was the lowest compared to the

other introduced samples (25.36 for Egyptian Basil and 23.64 for Maldivian Basil). A similar result was previously reported by Noure*t al.* (2009) who stated that the oil content of 14 accessions of basil varied between 8.8-30%. Kakaraparthi*et al*. (2015) reported that the oil content of basil was range between 12.4 to 21.6%.

Crude Protein

Two Sudanese basil accessions (NOPRI and Umteraibat) showed similar results as 19.71% and 19.75% respectively. Maldivian basil seed had the lowest crude protein content as 15.38%. The Crude protein of the four basil seed accessions was higher than the findings of Sarfraz*et al.* (2011) who reported that the crude protein of basil was 11.4 ± 0.325 %.

Crude Fiber

Wild Sudanese basil seed (Umteraibat) showed the highest crude fiber content as 35.98%, while for Egyptian basil seed it was 32.37% and for NOPRI basil seed was 33.51%. Maldivian basil seed fiber (29.87%) was the lowest, and this could be due to the environmental and other factors according to Nurzynska*et al.* (2012).

Ash Content

The highest ash content was found in NOPRI basil seed. There were no real differences in the ash content of three basil accessions (Egyptian, Maldivian, Umteraibat) which were 5.92%, 5.61%, 5.08% respectively. Sarfraz*et al.* (2011) reported that the ash content of basil was 6.3 ± 0.247%.

Physical Characteristics of Basil Seed Oil

Physical properties of basil seeds oil such as specific gravity, refractive index and color were estimated. The results are presented in table 2.

Table 2. Physical Characteristics of Basil Seed Oil

Sample Name	Specific gravity	Refractive Index	Color	
Egyptian Basil	0.93	1.4816 at 20°C	Red:	0.6
			Yellow:	19
Maldivian Basil	0.93	1.4813 at 20°C	Red:	0.7
			Yellow:	18
Wild Sudanese Basil (NOPRI)	0.92	1.4809 at 20°C	Red:	0.9
			Yellow:	24
Wild Sudanese Basil (Umteraibat)	0.93	1.4823 at 20°C	Red:	2.5
			Yellow:	70

Specific Gravity

The specific gravity of basil seed accessions was found to be 0.92-0.93 at 25°C. These results are in accordance with Giri (1998) who determined the specific gravity of basil as 0.92 at 32°C.

Refractive Index

There were no real differences in refractive index value of the four basil seed accessions at 20°C. These results are in agreement with the findings of Angers *et al.* (1996) who reported that the refractive index of basil seed oil ranged from 1.479 to 1.481.

Color

The color determined for basil seed accessions shows match 0.6, 0.7, 0.9, 2.5 red and 19,18,24,70 yellow respectively. Giri (1998) reported a match of 0.9-3.9 red and 20-23 yellow.

Chemical Characteristics of Basil Seed Oil

The chemical characteristics of the four basil seed oil accessions include free fatty acids F.F.As, acid value (AV), peroxide value (PV), saponification value (SV), unsaponifiable matter and antioxidant activity and are shown in table 3.

Table 3. Chemical Characteristics of Basil Seed Oil

Parameters	Sample name			
	Egyptian Basil	**Maldivian Basil**	**Wild Sudanese Basil (NOPRI)**	**Wild Sudanese Basil(Umteraibat)**
Free Fatty Acids %	0.21	0.20	0.30	0.76
Acid Value mg NaOH\g	0.30	0.28	0.43	1.08
P.V (meq\ kg)	16.1	17.4	05.7	24.9
S.V (mg KOH\g)	196	196	198	195
Unsaponifiable Matter %	2.0	2.0	1.6	2.2
Antioxidant Activity %	75±0.04	84±0.07	76±0.03	76±0.09

Free Fatty Acids (FFA)

The results show that the free fatty acids of the four basil seed accessions (Egyptian, Maldivian NOPRI and Umteraibat) were 0.21, 0.2, 0.3, and 0.76 % respectively. Giri (1998) reported that the free fatty acids of basil were 0.68%.

Acid Value

The acid value was obtained by calculated equation of multiplying the free fatty acids value by the factor 1.42, were: Egyptian basil seed oil (0.3), Maldivian basil seed oil (0.28) and wild Sudanese basil seed oil (NOPRI) (0.43). Wild Sudanese basil seed oil (Umteraibat) (1.08) was the highest. Giri (1998) reported that the acid value of basil was 1.25 mg NaOH\g.

Peroxide Value

The peroxide values of Egyptian basil seed oil, Maldivian basil seed oil, wild Sudanese basil seed oil (NOPRI) and wild Sudanese basil seed oil (Umteraibat) were 16.1, 17.4, 5.7 and 24.9 ml.eqv.\kg respectively. The lowest peroxide value was recorded in the wild Sudanese basil seed oil (NOPRI). These values are lower those reported by Giri (1998) as (54 ml.eqv.\ kg).

Saponification Value

The highest saponification value was found in wild Sudanese basil (NOPRI, 198) and the lowest saponification value was found in wild Sudanese basil seed oil (Umteraibat, 195). The results of this study are in accordance with Angers *et al.* (1996) who reported that the saponification value of basil was between 199 to 200 mg KOH\g,oil.

Unsaponifiable Matter

The highest unsaponifiable matter was found in the wild Sudanese basil seed oil (Umteraibat, 2.2) and the lowest was found in the wild Sudanese basil seed oil (NOPRI, 1.6). Giri (1998) reported that the unsaponifiable matter of basil was 1.4% which is lower than the results of the current study.

Chemical Composition of Basil Extracted Seed Oil

The chemical compounds of the extracted seed oil for the four accessions were determined by analyzing methyl esters using GC-MS. The identification of the chemical compounds was based on comparison of their retention times and mass spectra with those reported in the NIST and further confirmed by the reported pattern. From the most prominent peaks identified, corresponding fatty acids, retention times, and measured peak areas are summarized in Table (4). A total of 26, 20, 20, and 21 fatty acids, constituting 99% of the oil, were identified in the seed oil of Umteraibat, Egyptian, Maldivian, and NOPRI accessions, respectively. Squalene and 7-Tetradecenal were identified in all oil samples. There were 20 saturated, 5 monounsaturated, and 4 polyunsaturated fatty acids (Table 4). The chemical composition of the total methyl esters of fatty acids from the seed oils of four basil accessions showed to have the same retention time and very similar profile. The fatty acids palmitic, linoleic, linolenic, and stearic acids were identified in all oils as major compounds. Umteraibat oil was found to contain high levels of linoleic (40.39%) followed by lenolenic (22.83%), while the major saturated fatty acids were palmitic (16.33%) and stearic acid (9.94%). The NOPRI accession had the highest concentration of lenolenic (39.82%), palmitic (16.67 %), and stearic acid (11.57), whereas the linoleic acid was the lowest (22.91%) among the other accessions.

Table 4. Retention time and fatty acids area percentage in basil seed oil (GC.MS) of basil Accessions:

	Accession Name		**Umteraibat**	**Egyptian**	**Maldivian**	**NOPRI**
No	**Identified Compounds**	RT*	Area%	Area%	Area%	Area%
1	Myristic acid	13.5	0.16	0.10	0.10	0.21
2	Pentadecanoic acid, ME*	14.6	0.09	0.04	0.05	0.09
3	7-Hexadecenoic acid, ME, (Z)-	15.4	0.10	0.05	0.07	0.04
4	Palmitoleic acid, ME, (Z)-	15.4	0.65	0.34	0.52	0.59
5	Palmitic acid, ME	15.6	**16.33**	**14.70**	**15.13**	**16.67**
6	Hexadecanoic acid, 14-methyl-, ME	16.3	0.93	0.47	0.79	0.80
7	cis-10-Heptadecenoic acid, ME	16.4	0.17	0.12	0.19	0.12
8	Margaric acid, ME	16.6	0.46	0.27	0.47	0.43
9	Linoleic acid, ME	17.3	**40.39**	**32.42**	**36.72**	**22.91**
10	Oleic acid, ME	17.4	0.91	2.13	0.78	1.31
11	Linolenic acid	17.4	**22.83**	**36.57**	**29.78**	**39.82**
12	Methyl stearate	17.6	**9.94**	**9.81**	**10.58**	**11.57**
13	gamma.-Linolenic acid, ME	19.0	0.72	0.29	0.42	0.47
14	7-Tetradecenal, (Z)-	19.0	0.57	0.30	0.43	0.53
15	cis-11-Eicosenoic acid, ME	19.1	1.14	0.38	0.58	1.01
16	Arachidic acid, ME	19.3	1.53	0.89	1.43	1.57
17	Heneicosanoic acid, ME	19.9	0.34	0.29	0.39	0.35
18	Behenic acid, ME	20.9	0.29	0.15	0.27	0.23
19	Tricosanoic acid, ME	21.5	0.13	0.08	0.16	0.10
20	Tetratetracontane	22.4	0.09	0.09	0.17	0.16
21	Squalene	23.2	0.48	0.14	0.18	0.22
22	Octadecanoic acid, 17-methyl-, ME	18.2	-	0.37	0.60	0.65
23	Cerotic acid, ME	21.5	0.15	-	-	0.15
24	Margaric acid, 15-methyl-, ME	18.2	0.66	-	-	-
25	Lignoceric acid, ME	22.4	0.24	-	-	-
26	Tetrapentacontane	23.6	0.27	-	-	-
27	.gamma.-Tocopherol	24.9	0.08	-	-	-
28	Hexatriacontane	25.0	0.28	-	-	-
29	Methyl 18-methylicosanoate	20.1	0.05	-	-	-
30	Tetradecanoic acid, 12-methyl-, ME	14.3	0.02	-	-	-
31	8,11,14-Docosatrienoic acid, ME	18.0	-	-	0.19	-

*RT is the Retention time, Area% is the concentration of fatty acid in the seed oil as %.ME is the Methyl Ester.

The two Sudanese accessions revealed higher concentration of the major fatty acids than the Egyptian and Maldivian accessions. The concentration of oleic and arachidic, and eicosenoic acid varied between 0.78-2.13%, 0.89-1.57%, and 0.38-1.14%, respectively. Overall, the concentration of unsaturated fatty acids

averaged 68.5%, whereas the average of saturated fatty acids was 30.5% (Fig. 1). Comparing the identified fatty acid profile of the seed oil in this study with those previously reported, we found that the major fatty acids and their concentrations in the extracted oil of the four investigated basils were approximately similar and in the concentration range that reported locally and worldwide (Giri, 1998; Noure*t al.*, 2009). The results reported by Nour and others (2009) revealed that the major fatty acids of the oil extracted from seed of 14 accessions investigated under Sudan conditions are similar with what we identified with slight difference in the concentrations. The basil seed constitutes an edible and drying oil with fatty acid profile similar to linseed (Angers *et al.*, 1996), and could be processed in the same way as linseed oil. Linoleic acid (omega-6) is the principal polyunsaturated fatty acid in most western diets. Squalene is a polyunsaturated hydrocarbon, C30H50, with a low-density compound and important biological properties (O'Brien, 2009). The GC-MS percentage of squalene varied between 0.13 to 0.42% (1300 to 4200 mg/kg oil). The wild Sudanese accession (Umteraibat) had the highest percentage (Table 4). Virgin olive oil is a major source of phytosqualene, with a content ranging from 800 to 1200 mg/kg depending on the olive cultivar (De Leonardis*et al.*, 1998). According to promising results from recent studies, squalene can be used as anticancer, antioxidant, detoxifier, drug carrier and skin hydrating and play a crucial rule in steroid synthesis in human (Kim and Karadeniz, 2012).

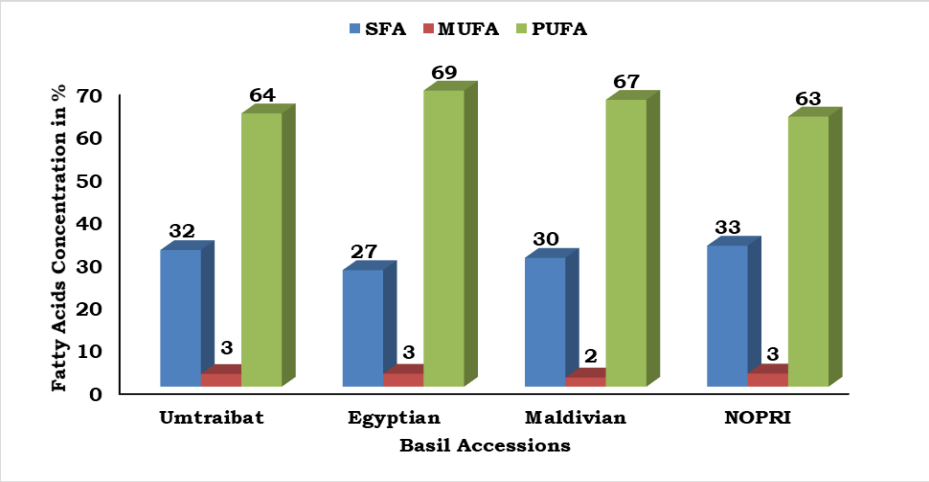

Figure 1. Total Saturated (SFA), Monounsaturated (MUFA) and Polyunsaturated Fatty Acids (PUFA)

Refining Basil Seed Oil

The FFA% and color for the four basil seed accessions (Egyptian, Maldivian, Umteraibat and NOPRI) were shown in table (5).The free fatty acids within the ranged (0-0.05) % reported by (Gupta, 2017). There is significant different in the color between crude and refined oil, due to remove of color compounds during refining process (degumming, reutilization and bleaching).

Table 5. Refined and Crude Basil Seed Oil

Sample Name	Refined Oil		Crude Oil	
	F.F.A %	Color	F.F.A %	Color
Egyptian Basil	0.05	Red: 0.2 Yellow: 2.5	0.21	Red: 0.6 Yellow: 19
Maldivian Basil	0.04	Red: 0.4 Yellow: 3.4	0.20	Red: 0.7 Yellow: 18
Wild Sudanese Basil(Umteraibat)	0.06	Red: 0.5 Yellow: 6.2	0.76	Red: 2.5 Yellow: 70

CONCLUSIONS

The following conclusions can be drawn from this study:

It can be concluded that the environmental factors during growth of basil and its variety have a significant effect on the content (extraction yield) and the qualitative as well as quantitative profile of basil essential oils. The wild Sudanese basil seed oil (NOPRI) had the highest oil content (27.6%). High Linolenic acid (omega-3) was found in wild Sudanese basil seed (NOPRI) (Area, 39.82%) and high Linoleic acid (omega-6) was found in Umteraibat accession (Area, 40.39%) in GC-MS. Basil seed oil was well refined and characterized.

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