

Phytochemical Studies on Sudanese Rocket (*Eruca sativa*) Seeds and Oil constituents

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Abstract

This work as performed to investigates seed proximate composition, and oil content, oil physical, chemical properties, and constituents of *Eruca sativa*. The results of *Eruca sativa* seed chemical composition are; Oil content (20%), moisture content (3.64c), Crude protein (31.0), Crude fibers (20.376%), Total ash (4.33%) and Total carbohydrates (23.07). Physiochemicals of *Eruca sativa* oil are; relative density (90,77%), refractive index (1.469), relative viscosity (38cp), peroxide value (10), Acid value (2.24), Colour (red 5.4–yellow 3.1–blue 0.4), saponification value (165.495), unsaponification value (2.648%), iodine value (63.63). GC-MS analysis showed 28 oil constituents in *Eruca sativa* oil. Palmatic acid (hexadecanoic acid), Stearic acid (octadecanoic acid), Oleic acid (Octadecenoic acid), Linoleic acid as (Octadecadienoic acid), Erucic acid (Docosenoic acid).

Keywords: *Eruca sativa*; Glucosinolates

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Citation: Nail TNA, Ali MM, Salim ERA.

Phytochemical Studies on Sudanese Rocket
(*Eruca sativa*) Seeds and Oil constituents.
Am J Phytomedicine Clin Ther. 2017; 5:1.

Received: February 02, 2017; **Accepted:** February 08, 2017; **Published:** February 13, 2017

Introduction

The Brassicaceae family (formerly Cruciferae) consists of approximately 375 genera and 3200 species of plants, of which about 52 genera and 160 species are present in Australia [1]. The term 'canola' refers to those varieties of *B. napus* that meet specific standards on the levels of erucic acid and glucosinolates. Those cultivars must yield oil low in erucic acid (below 2%) and meal low in glucosinolates (total glucosinolates of 30 $\mu\text{mol/g}$ toasted oil free meal) [2] and are often referred to as "double low" varieties. *Eruca sativa* Miller belongs to the Brassicaceae family of plants, grown in parts of the Middle East, India and Pakistan as a minor oil crop and for the preparation of some traditional medicines and remedies [3]. It can be grown on marginal and barren land as well as in areas where rainfall and soil fertility are low. It is also known to be drought resistant and has some degree of salt tolerance [4]. *E. sativa* has powerful active components that might be effective in increasing human health and preventing cancer [5]. Antimicrobial activities of solvent extracts from leaves, roots and seed oil of *E. sativa* were reported on Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhi*, *Klebsiella pneumonia*) and Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*) bacteria [6]. The seed oil exhibited the highest inhibition rate for both Gram-positive and Gram-negative, though *K. pneumonia* and *S. epidermidis* was less sensitive. This inhibition was due to erucic and oleic acids as well as to some isothiocyanates like bis-isothiocyanatobutyl-disulphide. Another example of antimicrobial compound comes

from *D. tenuifolia* where erucin was found to be effective against pathogenic postharvest fungi [7]. Other studies revealed that the constituents of both *E. sativa* and *D. tenuifolia* seeds act as fumigants against the stored-product insects *O. surinamensis*, *R. dominica* and *S. oryzae* [8]. The industrial significance of oil is due to the presence of high content of erucic acid (EA) in *E. sativa*. Erucic acid is a predominant fatty acid there is an escalating global demand for the amide of erucic acid, namely erucamide, depending on its use in cosmetics, detergents, and polymer production. The seed could up to 25-35% oil and 37% of protein [9]. Its oil could possibly be comprised of some important fatty acids such as myristic acid, stearic acid, oleic acid, linoleic acid, linolenic, eicosenoic acid and erucic acid as it belongs to rapeseed group. The physicochemical properties of *E. sativa* oil showed low acid value of oil indicated that the triacylglycerols have not been hydrolyzed, which is an indication of good stability [10]. Mumtaz mentioned that refractive index of rocket oil is 1.484 [11] shows that the oil is thick. Hamid . who postulated that specific gravity at (30°C) is (0.8005 \pm 0.0) [12] while Mumtaz specify (0.92 \pm 0.03), since specific gravity of oils (vegetable) at any specific temperature when compared to water rises as the average molecular mass decreases. Obtained. Mumtaz et al. found that acid value of rocket is (0.86 mg g⁻¹) and postulate

that low acid value indicated that the triacylglycerols have not been hydrolyzed, which is an indication of good stability. Hamid mentioned that the saponification number of the *Eruca* oil found to be 174.43 g NaOH/100 g oil [12] but low than what was found by Mumtaz et al. (180.6 mg KOH g⁻¹). Mumtaz found that peroxide value of rocket oil is (8.5 meq kg⁻¹) and Saima et al. found that the peroxide value of *Eruca sativa* seed oil was 6.66 ± 1.527 meq²/kg [11,12]. Stearic acid which known as (octadecanoic acid) content in rocket oil as found by Saima et al. and Chakrabarti and Ahmad (30.8) [12,13]. Oleic acid which refer to as ((9Z)-Octadecenoic acid), ((Z)-Octadec-9-enoic acid), (*cis*-9-Octadecenoic acid or *cis*- Δ^9 -Octadecenoic acid) found as (17.8) as that found by Chakrabarti and Ahmad [13]. Linoleic acid which known as (*cis*, *cis*-9,12-Octadecadienoic acid) found as (1.44) by Chakrabarti and Ahmad [13]. Erucic acid which known as ((Z)-Docos-13-enoic acid) is (47.0) which found by Chakrabarti and Ahmad [13]. The objectives of this research study is to investigate phytochemical of *Eruca sativa* L. Proximate analysis of rocket vegetable seeds such as; moisture content, crude protein, crude fat, crude fiber, carbohydrates and ash content were determined and *Eruca sativa* L. oil content and its chemical, physical properties and oil constituents.

Materials and Methods

Materials

Source of materials: The *Eruca sativa* seeds were obtained from central Khartoum local market.

Methods

Moisture content: Moisture content was determined according to method described by AOAC (2006) method [14]

Crude oil: Total fat was determined by AOAC (2006) method [15].

Crude protein: Nitrogen content determinations were made on the sample by micro Kjeldahl technique following AOAC (2006) [16].

Crude fibre: Crude fibre was measured using Pearson method [17].

Ash content: Total ash was determined according to AOAC (2006) [18].

Physical properties of the oil

Specific gravity: Specific gravity was determined according to A.O.A.C (2000) [19].

Refractive index: The refractive index of the oil was determined by (AOAC, 1990) [20].

Determination of color: Colour was determined according to Hand book of Food Analysis [21].

Viscosity: Viscosity was determined according to Diamante and Lan [22].

Chemical properties of the oils

Acid value: Acid value was determined according to Handbook of Food Analysis [23].

Saponification value: Saponification value were determined according to ISO 3657: 2002 [24].

Un saponification value: Un saponification matters were determined according to British Standard [25].

Peroxide value: Peroxide value was determined according to ISO 3960: 2007 [26].

Iodine value: Iodine value was determined according to ISO 3961:1996 [27].

GC-MS spectrophotometry

Determination of fatty acids is by Official method 969.33 and 969.22 Fatty acids in oils and fats Preparation of methyl esters/ Gas chromatographic method (2000) [28].

Oil methylation: The oil sample (0.15-0.17 g) was taken in a test tube and 10 ml of n-heptane was added and then vortexed. Thereafter 4 ml of 3.5% methanolic KOH was added and vortexed again for 2 min. This solution was put in a water bath maintained at 70°C for 2 min. Thereafter the solution was vortexed 5 more times and the upper layer is drawn out in to a beaker and is evaporated till dried. Then 0.5 ml of n-heptane was added to the residue and mixed well. This constituted the fatty acid methyl esters extract for GC-MS analysis (**Appendix 1**).

Fatty acid content (FAC) profile: Oil composition was analysed by Gas-Liquid Chromatography with mass spectroscopy. Relative concentration of fatty acid (FA) from oil samples was measured as their corresponding methyl esters. One μ l of the extract prepared as above was injected in GC-MS instrument (SHIMADZU QP-2010) equipped with MS detector. And equipped with reference libraries (the instrument operation software was Real Time Analysis and for processing data using Postum Analysis). The column (0.10-0.25 mm) temperature was initially maintained at 140°C for 5 min, gradually increased to 180°C at 6°C/min, maintained for 2 min at 180°C, then further gradually increased to 240°C at 4°C/min and finally maintained for 15 min at 240°C. The carrier gas was helium at a flow rate of 1.21 ml/min. The injector and detector temperature were maintained at 230 and at 280°C, respectively and split ratio was 10:0. Fatty acid standards were procured from sigma.

Statistical analysis: Analyses of variance, followed by Duncan multiple range test with significance level $p \leq 0.05$ were performed on the data by Gomez and Gomez [29].

Results and Discussion

Chemical composition of *Eruca sativa* seeds

The results of chemical composition of *Eruca sativa* seed was illustrated in **Table 1**, which showed that oil content (20%). Seeds of *Eruca sativa* possess moisture content (3.64c), crude protein (31.0), crude fibers (20.376%), total ash (4.33%) and total carbohydrates (23.07). Oil content of Sudanese local cultivated *Eruca sativa* is lesser than what mentioned by Hamid who found that oil content in *Eruca sativa* is (29 ± 1.6). However, oil contents in seeds depend on many factors including maturity of the seed as well as degree of plant irrigation. Moisture content closed agreement with the results reported by Hamid (4.1%);

Table 1 Chemical composition of *Eruca sativa* seeds.

No.	Seed compositions	Results%
1	Oil content	20.0
2	moisture content	3.64
3	Crude protein (defatted sample)	32.0
4	Crude fibers (defatted sample)	17
5	Total ash	4.33
6	Total Carbohydrates	23.07

and lesser than Asmma 116 who found that *Eruca sativa* seeds contain moisture (6.6%) [30]. Protein content is relatively near what mentioned by Hamid who postulate that proximate analysis of *Eruca sativa* seeds indicates (30 ± 1.2) protein. Ash content of Sudanese local *Eruca sativa* is lesser than what found by Hamid (6.6%).

Physiochemical composition of *Eruca sativa* oil

The results of chemical composition of *Eruca sativa* oil was illustrated in **Table 2**, which showed that *Eruca sativa* oil physiochemicals are; relative density (90,77%), refractive index (1.469), relative viscosity (38cp), peroxide value (10), Acid value (2.24), Colour (red 5.4–yellow 3.1–blue 0.4), saponification value (165.495), unsaponification value (2.648%), iodine value (63.63). From the above results Sudanese *E. sativa* oil showed relative density (0.9077) which is greater than what found by Saima et al. who postulated that specific gravity at (30°C) is (0.8005 ± 0.0) [12] and Mumtaz et al. [11] (0.92 ± 0.03); since specific gravity of oils (vegetable) at any specific temperature when compared to water rises as the average molecular mass decreases. *Eruca* oil colour is (red-yellow-blue) (5.4–3.1–0.4) that revealed a dark yellow colour and coincides with hamid postulation who mentioned that rocket oil have color dark yellow state at room temperature Liquid. Sudanese *Eruca* oil refractive index value was 1.469; showed that the oil was less thick comparable to the refractive index of 1.484 obtained by Mumtaz et al. [11]. Acid value is (2.24) and this finding less than Mumtaz's (0.86 mg g⁻¹) who postulate that low acid value indicated that the triacylglycerols have not been hydrolyzed, which is an indication of good stability. And the oil shows moderate iodine value content (63.63) but lower than (106.20 g/100 g) obtained by Mumtaz et al. [11]. This indicates that Sudanese *Eruca* oil contain more saturated fatty acids than Indian one. Since the increment of iodine means more un saturated fatty acids were present in the oil. Saponification value is high (165.495) which is closed to saponification value of 168.1% reported by Picha et al. and Flanders and Abdul [30,31], a relatively low than Saima et al. [12] who mentioned that the saponification number of the *Eruca* oil found to be 174.43 g NaOH/100 g oil but low than what was found by Mumtaz et al. [11]. (180.6 mg KOHg⁻¹); Mumtaz et al. [11] postulated that high saponification value of the oil indicates a high content of triacyl glycerols demonstrating their potential to be used in the cosmetic and soap making industries. Saponification number is an indicator of the average molecular weight and chain length which is inversely proportional to the molecular weight of the lipid. Lower values of saponification prove that they have greater molecular mass comparing to that of common oils. This parameter is dependent upon the extent

of unsaturation which describes their iodine number along with the free fatty acid value. The *Eruca* seed oil had a They have intense applications in cosmetic and pharmaceutical industry, lubricants, food products and polymers as well. Peroxide value is (10%) which is higher than what was mentioned by Mumtaz et al. [11]. (8.5 meq kg⁻¹) and also greater than what mentioned by Saima et al. [12] who found that the peroxide value of *Eruca sativa* seed oil was found to be 6.66 ± 1.527 meq²/kg oil as given in order to determine quality of fats and oils, this parameter is highly significant because it suggests the oxidative constancy of the oil for the period of storage. Fat or oil which is processed from premium quality oil seed kernels produces the new peroxide values to visualize like the oil.

Oil constituents of *Eruca sativa* (rocket)

The GC-MS chromatography analysis of rocket oil constituents illustrated in **Table 3**. The results showed that there are 28 oil. When we compare the oil constituents present in Sudanese *Eruca sativa* (rocket) with others we found that Palmitic acid which known as (hexadecanoic acid) yielded 0.5 in this sample as a total some of three forms namely; (Hexadecenoic acid, methyl ester) (0.07), (n-propyl 9,12-hexadecadienoate) (0.39) and (9-hexadecenoic acid, methyl ester,(z)) (0.04) and it is low compare to Chakrabarti and Ahmad [13] and Mumtaz et al. [11] constituent present in *Eruca sativa* (rocket) oil. The following constituents are present (5-octadecenoic acid,methyl ester), (Cis-5-dodecenoic acid ,methyl ester); they postulate that Palmitic acid is yielded (2.80) in *Eruca sativa* (rocket). Stearic acid which known as (octadecanoic acid) found in the following forms (9, 12, 15-octadecatrienoic acid; methyl ester) (0.09), (, (9-octadecenoic acid (z)-, methyl ester) (23.26), (5-octadecenoic acid,methyl ester) (0.01) and totally yielded (23.36) and is lesser than what found by Chakrabarti and Ahmad [13] and Mumtaz et al. [11] (30.8). Oleic acid which refer to as ((9Z)-Octadecenoic acid), ((Z)-Octadec-9-enoic acid), (cis-9-Octadecenoic acid or cis-Δ⁹-Octadecenoic acid) found as (9-octadecenoic acid, methyl ester, (E)-) and yielded just (0.09) compare to (17.8) that found by Chakrabarti and Ahmad [13] and Mumtaz et al. [11]. Linoleic acid which known as (cis, cis-9,12-Octadecadienoic acid) found in the form (9,12-octadecadienoic acid (z ,z)- methyl) (9.92) and it is greater than (1.44) which was found by Chakrabarti and Ahmad [13] and Mumtaz et al. [11]. Erucic acid which known as ((Z)-Docos-13-enoic acid) found in the form (13-Docosenoic acid, methyl ester,(z)) and yielded (33.79) which is lower than (47.0) which found by Chakrabarti and Ahmad [13] and Mumtaz et al. [11] (**Appendix 2**).

Table 2 Physicochemicals of *Eruca sativa* oil.

No.	Physicochemicals	Results
1	Relative density	0.9077
2	Refractive index	1.469
3	Refractive viscosity	38cp
4	Peroxide value	10%
5	Acid value	2.24
6	Colour (red-yellow-blue)	(red 5.4–yellow 3.1–blue 0.4)
7	Saponification value	165.495
8	unsaponification value	2.648%
9	Iodine value	63.63

Table 3 Oil constituents of *Eruca satsaiva* rocket.

Peak	R. time	Area	Area	Name
1	14.504	27280	0.01	5-octadecenoic acid, methyl ester
2	14.607	17667	0.01	Cis-5-dodecenoic acid, methyl ester
3	14.769	88711	0.04	Pentadecanoic acid, methyl ester
4	15.499	141563	0.07	n-propyl 9,12-hexadecadienoate
5	15.565	306200	0.15	8,11,14-docosatrienoic acid, methyl ester
6	15.602	798515	0.39	9-hexadecenoic acid, methyl ester (z)
7	15.697	78922	0.04	11-Hexadecenoic acid, methyl ester
8	15.800	12302396	6.00	Hexadecenoic acid, methyl ester
9	16.567	170880	0.08	Cis-10-heptadecenoic acid, methyl ester
10	16.772	180230	0.09	Heptadecanoic acid, methyl ester
11	17.463	20333120	9.92	9,12-octadecadienoic acid (z, z)- methyl
12	17.529	47700159	23.26	9-octadecenoic acid(z)-, methyl ester
13	17.711	4726152	20.31	Methyl stearate
14	18.423	59659	0.03	Cis-10-Nonadecenoic acid, methyl ester
15	18.607	37751	0.02	Nonadecenoic acid, methyl ester
16	19.119	192523	0.09	9,12,15-octadecatrienoic acid; methyl ester
17	19.283	23957124	11.68	11-Eicosenoic acid, methyl ester
18	19.325	4760270	2.32	Cis-11-Eicosenoic acid, methyl ester
19	19.465	3838694	1.87	Methyl 18- methyl nonadecanoate
20	20.129	192110	0.09	9-octadecenoic acid, methyl ester,(E)-
21	20.293	105089	0.05	Heneicosanoic acid, methyl ester
22	20.377	90626	0.04	Phenol,2,2', methylenebis[6-(1,1-dimethyl
23	20.961	69285184	33.79	13-Docosenoic acid, methyl ester,(z)
24	21.089	3887641	1.90	Methyl 20-methyl-heneicosanoate
25	21.687	375488	0.18	Cis-13-Eicosenoic acid, methyl ester
26	21.851	226983	0.11	Tricosanoic acid, methyl ester
27	22.435	7863617	3.84	15-Tetracosenoic acid, methyl ester (z)-
28	22.586	1956519	0.95	Tetracosenoic acid, methyl ester
29		20503184	100.0	

Conclusion

Chemical composition of Rocket seeds showed high crude protein and moderate amount of crude oil. Oil physicochemical showed high refractive index, dark colour, low acid value and saponification value high peroxide and low iodine values that means the oil is more saturated therefore it can be use in medicine and other industrial purposes. GC-MS analysis showed 28 oil constituents, Palmatic acid, Stearic acid. Oleic acid, Linoleic

acid and Erucic acid. Erucic acid is the highest on among other fatty acids, therefore the oil is not edible.

Recommendations

Evaluation of different Sudanese localities Rocket seed and oils analysis are recommended. Evaluation of Rocket oil in different medicals industrial purposes. More cultural practices, harvest, postharvest are recommended to increase seeds and oil constituents for more economical uses in the different industries.

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