ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Evaluation of Egyptian Rocket seed oil as a source of essential fatty acids and its hypolipidemic effect in rats fed on high fat diet.

Medhat M. Abozid[1,2]; Yossuif A. Ashoush[1]; Abd elaziz A Sakr[1]; Khaled M. Taha[1] and Essoh Ayimba[2, 3] .

1 Biochemistry Department, Faculty of Agriculture, Minofiya University. Menofiya, Egypt.

2 Laboratoire de Procédés Biologiques, Génie Enzymatique et Microbien (ProBioGEM), IUT A/Polytech'Lille, Université de Lille, Science et Technologies Avenue Paul Langevin, F-12 59655 Villeneuve d'Ascq Cedex, France.

3 Centre National de Transfusion Sanguine (CNTS), Lomé, Togo, BP 20707.

Manuscript Info

Manuscript History:

Received: 15 May 2014
Final Accepted: 19 June 2014
Published Online: July 2014

Key words:
omega 3 fatty acids, rocket seed oil, hypolipdemic, Alpha-linolenic acid, Linolinc acid.

Corresponding Author

Medhat M. Abozid

Abstract

The seeds of rocket (Eruca Sativa) grown in Egypt contain about 35% total lipids (oil). The extracted oil has good physiochemical properties. The rocket seed oil, contain high content of unsaturated fatty acids 82.1%. Rocket seed oil has a high ratio of alpha lenolenic acid (19.34%) and the next is linoleic acid (13.67) also has a good amount from oleic acid (15.53). Most of the unsaponifiable matter is phytosterol (61.49%); beta-sitosterol is the main sterol because it has a high content (37.3%). There for the hypolipidemic effect of the rocket seed oil in the plasma of rats was studied , where as feeding on hyperlipidemic diet (group B) led to an acute increase in the plasma content of total lipids, triglycerides ,total cholesterol and LDL cholesterol, when compared with control (group A) . The supplementation of stomach tube dose (1 g /kg body weight) of rocket seed oil (group C) to rats fed on hyperlipidemic diet for a period of two months showed high effect as hypolipidemic agent. All lipid profile parameter except HDL – cholesterol decreased, when compared with the high fat diet (group B). We can conclude that rocket seed oil showed a good effect as hypolipidemic agent, and contained high amount of omeg-3 fatty acids, and omega-6fatty acids. We can recommend that this oil could be used as a high plant source of omeg-3, and omega-6 fatty acids in the human nutrition.

Introduction

The omega-3 and omega 6 fatty acids are considered 'essential', so that humans must ingest omega-3 and omega 6 fatty acids from their diet, since the essential fatty acids structure cannot be synthesized in humans **(Stoll et al., 1999)**. Alpha-linolenic acid (ALA) and alpha-linoleic acids (LA) are the major plant omega-3 and omega 6 fatty acids. Hyperlipidemia (mainly increased level of cholesterol or low density lipoprotein (LDL)-cholesterol) is an important risk factor in the initiation and progression of atherosclerotic lesions **(Harrison et al., 2003)**. The beneficial effects of (ALA) and (LA) on plasma lipid and lipoproteins are more controversial; it has been reported to decrease in total cholesterol (TC), LDL-cholesterol (LDL-C), and LDL-C/HDL-C **(Cunnane et al., 1995, Ashoush et al., 2009, Ramadan et al., 2011 and Abozid and Ayimba, 2014)**. Flaxseed oil is the richest natural source of this fatty acid, but alpha-linolenic acid is also present in large amounts in a variety of other plant oils **(Charles and Myers, 2000)**. **(Abd El-Hamid, 1999)** reported that rocket seed oil has a hypocholedterolemic effect; this may be due to the high percentage of unsaturated fatty acid (oleic 15.1%, alpha linolenic acid 11.11%, linoleic acid 8.3%). In a number of epidemiological studies, the consumption of food rich in unsaturated especially essential fatty acids were found to be correlated with reduced morbidity and mortality from the cardiovascular causes (**De lorgeril et al., 1999, Djousse et al., 2001, and Van schacky, 2003**). The aim of this study is the investigation of the Egyptian

rocket seed as new source of essential fatty acids, studying the physiochemical properties of rocket seed oil, type of fatty acids and unsaponifalable matter. And studying the hypolipidemic effect of this oil

3- Material and Methods

Rocket seeds were obtained from local market; identified by horticulture Dep., Faculty of Agric., Minufiya Univ. Cairo, Egypt.

Chemical reagents (Kits): Kits of total lipid, total cholesterol, HDL-Cholesterol, triglycerides, were obtained from Biodiagnostic Company, Cairo, Egypt.

Animals of biological experiment: Male Albino rats weighing 100-110 grams were obtained from Research Institute of Ophthalmology, Giza, Egypt. The rats were fed ad libitum on standard diet and water for 15 days as an adaptation period.

Extraction of oil from rocket seeds: The air-dried rocket seeds were pressed with laboratory type of Carver hydraulic press under 10.000 Ib/in^2 (psi) pressure for 1 h at room temperature according to the method of **Ustun et al. (1990).** The produced oil was filtrated and kept in dark bottles in the refrigerator until analysis.

Chemical analysis: The moisture, total lipid, crude protein, ash, were determined according to the method described in **A.O.A.C. (2000).** And total carbohydrate was determined by difference as the followed equation:

Total carbohydrates = 100 – (%Protein + %Lipids + %Ash + %Moisture).

Refractive index of the oil was determined according to the method described in **A.O.A.C. (2000)** using a refractometer.

Chemical properties of oil: Acid value, Iodine value, Saponification value, of the extracted rocket seeds oil, and flaxseed oil were determined according to the **A.O.A.C. (1995),** while Unsaponifiable matter of two oils were determined according to the **A.O.A.C. (2000).**

Identification of fatty acids by GLC: fatty acids of oils were converted to methyl esters by using sodium methoxide according to the method of **Hougen and Bodo (1973).** Fatty acid methyl esters were injected into gas liquid chromatography (GLC) apparatus. (Hewlett-Pakard 5890) under the following conditions:

1- Column: HP-5 M.S. (cross-linked 5% Phenyl Methyl Silicone) 30 m X 0.250 mm. 2- Carrier gas: Hellium, at flow rate 0.90 ml/min.

3- Injector Temp. Program: 170° C/1min (5° C/1min) to 280° C/1min.

4- Detector: Mass selective detector.

Separation of unsaponidiable matter: the unsaponifiable matters were separated from oils at room temperature according to the method described in **A.O.A.C. (2000).**

Identification of unsaponidiable matter fractions by GLC: the hydrocarbon and sterol compounds were identified using an Agilent Technologies gas chromatograph model 6890 N (Network GC system) equipped with a flame ionization detector under following condition:

1- Sample size: 1 µ l.

2- Oven: Initial temperature: 80 ° C. Initial time: 1 min. Rate: 8.

Final temperature: 250° C. Final time: 25 min.

3- Injection temperature: 250° C.

4- Column: capillary column HP-5. Length: 30m, diameter: 320 µm, film thickness: 0.25 µm. Flow: 3 ml/min. 5- Detector temperature: 300 ° C.

6- Carrier gas: N2: 30 ml/min. H2: 30 ml/min. Air: 300 ml/min.

Biological experimental: A total of 18 male albino rats were used in our study. The animal were divided into three groups each group include six rats. Diets and water were available ad libitum over the two months period. one group of each experiment continued feeding the standard diet was described by **Campbel (1961)** and saved as normal control (A).The other three groups of each experiment were allowed to feed high fat diet to induce hyperlipidemic through the feeding period. One of each experiment continued feeding high fat diet without any supplementation saved as high fat group (B) and the last group (C) was supplemented with stomach tube dose 1g/Kg body weight of rocket seed oil.

Blood sampling:

Blood samples were collected at zero time, and after 60 days in tubes contain heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 15 min. to obtain plasma, which was kept frozen until analysis.

Determination of biochemical parameters on plasma:

Plasma total lipid was determined according to the method described by **Frings and Dunn (1970)**. While Plasma triglycerides were determined according to the method described-by **Fossati and Prencipe (1982).** Plasma total cholesterol was determined according to the method described by **Richmond, (1973).** Plasma HDL - cholesterol was determined in the plasma according to the method of **Lopez et al. (1977).** All of these parameters were determined by kits were obtained from Biodiagnostic Company, Cairo, Egypt. Plasma LDL cholesterol was calculated according to the formula of **Lopez et al. (1977).**
LDL cholesterol = total cholesterol - HDL cholesterol.
Risk ratio was calculated according the formula of **Lopez et al., (1977).** Risk ratio = total cholesterol / HDL-cholesterol.

Statistical analysis:
Statistical analysis was done using analysis of variance (ANOVA), Least Significant Difference (LSD) were obtained to compare the means of treatments, followed by Duncan's new multiple range tests to assess differences between group's means. Differences of $P < 0.01$ were considered significant.

4- Results

Table (1): Component of standard and high fat diets:

Ingredients	Standard diet	High fat diet
Carbohydrates as starch	65%	55%
Protein as casein	20%	20%
Fats as corn oil	10%	5%
Sheep tail fat	0	15%
Vitamins Mixture	1%	1%
Salts mixture	4%	4%

Chemical composition of rocket seeds:
The obtained results in Table (2) indicate that: rocket seeds contain moisture 7.7%, total ash 5.98%, total lipid 35.3%, crude protein 24.5%, and total carbohydrate 26.52%.

Table (2): Chemical composition of rocket seeds.

Chemical composition	Rocket seeds
Moisture	7.7%
Ash	5.98%
Total lipid	35.3%
Crude protein	24.5%
Total carbohydrates	26.52%

Physiochemical properties of rocket and flaxseed oils:
Data in table (3) showed values of physiochemical properties:

Table (3) Physiochemical properties of rocketseed, and flaxseed oils.

Physiochemical properties	Rocket seed oil
Density	0.817
Refractive index	1.474
Acid value	1.35
Saponification value	228.05
Ester value	226.7
Iodine value	108.45
Total unsafalible matter %	1.14

Quantitative analysis of fatty acids in rocket seed oil.

Fatty acids were analyzed as methyl esters by gas-liquid chromatography, and the percentages of saturated and unsaturated fatty acids fatty acids in rocket seed oil are given in Table (4).

Table (4): Percentage concentration of the different fatty acids in rocket seed oil.

Fatty acids	Concentration %
	Rocket seed oil
Lauric acid (C_{12})	ND
Myristic acid (C_{14})	ND
Palmitic acid (C_{16})	6.38
Stearic acid (C_{18})	2.21
Oleic acid ($C_{18:1}$)	15.53
Linoleic acid ($C_{18:2}$)	13.67
Alpha-linolenic acid ($C_{18:3}$)	19.34
Arachidic acid (C_{20})	9.31
Erucic acid ($C_{22:1}$)	33.56
Total saturated fatty acids	17.48
Total monounsaturated fatty acids	49.09
Total polyunsaturated fatty acids	33.01
Omega 6 / Omega 3 ratio	0.71

ND: Non detected.

Analysis of rocket seed oil showed that alpha-linolenic acid reached 19.34% and oleic acid 15.53%, linoleic acid 13.67%. Total saturated fatty acids constitute 17.84% from the total oil, while monounsaturated fatty acids were 49.09% and polyunsaturated fatty acids were 33.01%. Rocket seed oil contains about 33.56% erucic acid. Erucic acid constitutes about 30-60% of total fatty acids in rapseed, mustard seed and wallflower seed oils, and up to 80% of nasturtium seed oil.

Quantitative analysis of unsaponifiable matters in rocket seed oil.

The unsaponifiable matters were analyzed by GLC and the percentages of sterols and hydrocarbons are given in Table (5). Rocket seed oil contains 38.51% hydrocarbons and 61.49% sterols. In rocket seed oil beta-sitosterol was the major sterols 37.3% followed by Campasterol 16.43% and beta-amyrine 4.04%.

Table (5): Concentration of unsaponifiable matters in rocket seed oil.

Unsaponifiable matters	Concentration %
	Rocket seed oil
C13	ND
C14	2.64
C15	1.84
C16	2.51
C17	0.88
C18	1.52
C19	0.48
C20	1.97
C21	5.6
C22	ND
C23	1.23
C24	1.18
C25	1.96
C26	4.75
C27	2.81
C28	0.57
C29	1.25
C30	3.02

C31	4.3
Total hydrocarbon	38.51
Alpha-amyrine	1.68
Cholesterol	ND
Campasterol	16.43
Stigmasterol	2.04
Beta-sitosterol	37.3
Beta-amyrine	4.04
Total sterols	61.49

ND: Non detected.

Studying the effect of Rocket seed, and Flaxseed, oils on animals feeding on high fat diet:

Data in table (6) showed values of plasma total lipid, triglycerides, and total cholesterol, on rats fed on normal, high fat diet, and high fat diet supplemented with rocket seed oil and all data described in the table.

Table (6): Effect of Rocket seed oil on plasma total lipid, triglycerides, and total cholesterol in animals feeding on high fat diet.

Group	Total lipid	Triglycerides	Total cholesterol
Standard diet (A)	178.3 ± 16.3 b	66 ± 1.4 c	58.3 ± 2.8 c
High fat diet (B)	333.3 ± 12 a	143.6 ± 20.9 a	93.1 ± 3.3 a
High fat diet + rocket seed oil 1g/Kg body weight (C)	230.7 ± 48.2 b	134.6 ± 13.5 b	80.7 ± 2.5 b

(a,b,c) means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at $p \leq 0.01$.

Each value represents a mean of (6) samples ± standard deviation.

Data in table (7) showed values of plasma HDL-cholesterol, LDL-cholesterol, and risk ratio, on rats fed on normal, high fat diet, and high fat diet supplemented with rocket seed and flaxseed oils and all data described in the table.

Table (7): Effect of rocket seed oil on plasma HDL-cholesterol, LDL-cholesterol, and risk ratio in animals feeding on high fat diet.

Group	HDL-cholesterol	LDL-cholesterol	Risk ratio
Standard diet (A)	40.2 ± 0.58 a	18.1 ± 2.9 c	1.45 ± 0.06 c
High fat diet (B)	33.6 ± 0.48 c	59.5 ± 6.8 a	2.77 ± 0.1 a
High fat diet + rocket seed oil 1g/Kg body weight (C)	37.6 ± 1.3 b	43.1 ± 4.6 b	2.146 ± 0.13 b

(a,b,c) means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at $p \leq 0.01$.

Each value represents a mean of (6) samples ± standard deviation

5- Discussion

From above results we can conclude that rocket seed oil which supplemented to rat's fed on high fat diet reduced all plasma lipid profile, rocket seed oil has excellent effect in reduce all lipid profile determined that's may be its contain the higher ratio of unsaturated fatty acids in general, and also contain high amount of plant sterols. We could suggest that this effect of this oil may be due to:

1- The presence of high percentage of polyunsaturated fatty acids.

The Rocket seed oil has high percentage of polyunsaturated fatty acids (33.01%) and also has a good percentage of alph-linolenic acid (19.43%). Long chain polyunsaturated fatty acids are predominantly oxidized by peroxisomes **(Reddy and Mannerts, 1990).** It is well documented that diets rich in long chain polyunsaturated fatty acids stimulate both gene expression and activation of enzymes involved in β- oxidation **(Jump and Clarke, 1999).** It has been proposed that polyunsaturated fatty acids cause a decrease in lipoproteins production. Polyunsaturated fatty

acids as compared to saturated fatty acids are less efficiently incorporated into triglycerides synthesized by the liver for export inform of VLDL **(Cortese et al., 1983).**
2- The presence of plant sterols. In rocket seed oil Beta-sitosterol was the major sterols 37.3% followed by Campasterol 16.43% and beta-amyrine 4.4%. It has been widely reported that phytosterol lower serum cholesterol level in animals and human **(Laraki et al., 1991; and Howard and Kritchevsky, 1997).** The hypocholesterolemic effect of phytosterol may be explained by two mechanisms, including inhibition of (a) cholesterol absorption and (b) hepatic cholesterol esterase **(Howard and Kritchevsky, 1997).** On the other hand plant sterols have been shown to lower LDL-cholesterol equivalently in hypercholesterolemic persons by suppressing cholesterol absorption **(Vanstone et al., 2002).** Plant sterols decreased the incorporation of dietary and biliary cholesterol into micells, this lowers cholesterol absorption. Cholesterol synthesis and LDL receptors activity increase, which ultimately leads to decreased serum LDL-cholesterol concentration **(De Jong et al., and Ostlund et al., 2003).**

6- Conclusion

We can concluded that rocket seed oil showed a good effect as hypolipidemic agent, and contained a high amount of unsaturated fatty acids, so we can recommend that rocket seed oil can be used after blending by other vegetables oils as high plant source of essential fatty acids in production of margarine shortening, salad oil and vegetable oil.

7- References

Abd El-Hamid, S.R., 1999.
Biochemical studies on some untraditional plant oils. M.Sc. Thesis. Biochemistry department. Faculty of Sciences. Ain-Shams University., pp 40.

Abozid M. M. and Ayimba E., 2014.
Effect of omega 3 fatty acids family in human health (Review). International Journal of Advanced Research, 2 (3): 202 – 211.

A.O.A.C. 1995.
Official Methods of Analysis of the Association of Official Analytical Chemists. Published by the A.O.A.C. International 16th Ed. Washington, D.C.

A.O.A.C. 2000.
Official Methods of Analysis of the Association of Official Analytical Chemists. Published by the A.O.A.C. International 17th Ed. Washington, D.C.

Ashosh; Y. A.,Taha; K. M., Sakr; A. A., and Abozid; M. M., 2009.
Egyptian purslane seed oil as a new source of omega-3 fatty acids and its hypolipidemic effect in rats fed on high fat diet. (Abstract) Published in"The 24th meeting of Saudi Biological Society, Biotechnology: Reality and Application, Madinah Almunawwarah at Taibah University, pp. 188. (7 – 9 April, 2009).

Campbell J. A., 1961.
Methodology of protein evaluation. RAG Nur. Doucum. 101: 37.

Charles, E., and Myers, J.R., 2000.
Alpha linolenic acid in flaxseed. Prostate Forum Rivanna Health Publications. The Prostate Forum February 2000 Rivanna Health Publecation (published August 2000) V5:2,7-8 SISSN:1523

Cortese, C., Levy, Y., and Janus, E.D., 1983.
Modes of action of lipid lowering diets in mans: studies of apolipoprotein B kinetics in relation to fat consumption and dietary fatty acids composition. Eur. J. Clin. Invest. 13: 79-85.

Cunnane, S.C., Gangali, S., and Menard, A.C., 1995.

High α-linolenic acid flaxseed (Linum usitatissimum). Some nutritional properties in humans. British Journal of Nutrition 69 (2), 443–453.

De Jong, A., Plat, J., and Mensink, R.P., 2003.
Metabolic effect of plant sterols and stanols. J. Nut. Biochem. 14, (7): 362-369.

De Lorgeril, M., Salan, P., Martin, J.L., Delay, I., and Mamelle, N., 1999.
Mediterranean diet, traditional risk factors, and rate of cardiovascular complications after myocardial infraction: final report of the Lyon Diet Heart Study. Circulation 99: 779-785.
Djousse, L., Pankow, J.S., Eckfeldt, J.H., and et al., 2001.
Relation between dietary linolenic and coronary artery disease in the National Heart, Lung, and Blood Institute Heart Study. Am.J.Chem.Nutr., 74:612-619.

Frings, C.S., and Dunn, R.T., 1970.
Colorimetric method for determination of total plasma lipids based on the sulphophospho-vanilin reaction. Am.J.Clin.Path. 53: 89-91.

Fossati, P., and Prencipe, L., 1982.
Colorimetric determination of plasma triglycerides. J. Clin. Chem., 28:2077.

Harrison, D., Kathy, K.G., Hornig, B., and Drexler, H., 2003.
Role of oxidative stress in atherosclerosis. American Journal of Cardiology 91, 7A–11A.

Hougen, F. W., and Bodo, V., 1973.
Extraction and methanolysis of oil from whole or crushed rapseed for fatty acid analysis. J. Am. Oil Chem. Soc., 50: 7, 230 – 234.

Howard, B.V., and Kritchevsky, D., 1997.
Phytochemical and cardiovascular disease. A statement for healthcare professionals from the American Heart Association. Circulation 95, (11): 2591-2593.

Jump, J.O., and Clarke, S.D., 1999.
Regulation of gene expression by dietary fat. Ann. Rev. Nutr. 19: 63-90.

Laraki, L., Pelletier, X., and Debry, G., 1991.
Effect of dietary cholesterol and phytosterol overload on wistar rat plasma lipids. Ann. Nut. Met. 35, (4): 221-225.

Lopez, M.F., Stone, S., Ellis, S., and Collwell, J.A., 1977.
Cholesterol determination in high density lipoproteins separated by three different methods. Clin. Chem., 23(5): 882-886.

Ostlund, R.E., Racette, S.B., and Stenson, W.F., 2003.
Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am. J. Clin. Nutr. 77 (6): 1385-1589.

Ramadan, M.F.; Zayed , R.; Abozid, M.M.; and Asker, M. M. S., 2011.
Apricot and pumpkin oils reduce plasma cholesterol and triacylglycerol concentrations in rats fed a high-fat diet. Grasas Y Aceites, 62 (4) 443 452.

Reddy, J.K., and Mannaerts, G.P.1990.
Peroxisomal lipid metabolism. Ann. Rev. Nutr. 14: 343-370.

Richmond, W., 1973.
Colorimetric determination of total cholesterol. Clin.Chem. 19: 1350.

Stoll, A. L., Locke, C. A., Marangell, L. B., and Severus, W. E., 1999.

Omega-3 fatty acids and bipolar disorder: a review. Prostaglandins, Leukotrienes and Essential Fatty Acids 60(5&6): 329-337.

Ustun, G.; Kent, L.; Cekin, N. and Civelekoglu, H. 1990.
Investigation of the technological properties of Nigella sativa (black cumin) seed oil. J.Am.Oil Chem. Soc., 67 (12): 958-960.

Vanstone, C.A., Raeini, S.M., Parsons, W.E., and Jones, P.J., 2002.
Unesterified plant sterols and stanols lower LDL-cholesterol concentration equivalently in hypercholesterolemic persons. Ann. J. Clin. Nutr. 76,(6): 1272-1278.

Van, C., Schacky, 2003.
The role of omega 3 **C.** fatty acids in cardiovascular disease. Curr. Atherosclerosis Rep., 5:139-145.