Study the Physico-Chemical Properties and Antihyperlipidemic Activities of Garden Cress Seed Oil

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Abstract: Garden cress (*Lepidium sativum*) is an annual herb, grown mainly for its seeds. Physico-chemical of oil, fatty acid composition tocopherols and total phenolic of garden cress seed oil were studied. Also, the objective of the current investigation was to evaluate the effects of feeding on garden cress oils with different levels 25, 50 and 75% on serum lipid levels in Albino rats. Thirty adult albino male rats were classified into two groups. One was fed on standard diet and kept as control group. The second of rats were fed on standard diet with cholesterol for 3 weeks to be hypercholesterolemic, and then classified into 4 groups. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG), were determined. Also, serum alanine and aspartate aminotransferase (ALT and AST) were calculated. The results showed that linolenic acid (34.8%) was the major fatty acid in garden cress seed oil followed by oleic acid (21.9%), linoleic acid (11.4%). A qualitative estimation of tocopherols in garden cress oil showed the presence of alfa(α), gama(γ) and sigma (ϛ) - tocopherol, were 957, 668 and 535 ug/g oil, respectively. The experimental animal fed with Supplemented diet of rats with garden cress seed oil significant decrease in triglycerides, total cholesterol, LDL-c, VLDL-c and (AST and ALT) enzyme compared to positive control. However, The HDL levels were marginally enhanced in the rats that were fed on different levels from garden cress oil. Therefore, the diets rich in garden cress oil have more favorable effects on the blood lipid profile and plasma lipoproteins and can be recommended for patients with dyslipidemia diseases.

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1. Introduction

Hypercholesterolemia and its associated cardiovascular diseases (CVD) represent one of the greatest worldwide economic, social and medical challenges that we are currently facing (**Olshansky *et al.*, 2005**). The relationship between plasma lipid and lipoprotein concentrations and the risk of developing cardiovascular diseases (CVD) on the basis of dietary fat type is well documented (**Krauss *et al.*, 2001**). A high plasma concentration of total cholesterol, triglycerides, and LDL cholesterol and a low plasma concentration of HDL cholesterol are considered important risk factors for the development of coronary diseases (**Kannel *et al.*, 1979**), and these plasma indexes or biomarkers must be jointly considered in the assessment of risk for human populations (American Heart Association scientific cholesterol levels, 2004). Cress (*Lepidium sativum* L.), otherwise known as garden cress, garden cress pepper weed or garden pepperwort, is a fast growing annual herb belonging to the *Brassicaceae* family that is native to Egypt and west Asia but is widely cultivated in temperate climates throughout the world for various culinary and medicinal uses (**Gokavi *et al.*, 2004**).

Garden cress seeds are brownish red in color and oval in shape. Morphologically, garden cress seeds resemble that of an oil seed with the dicotyledonous endosperm accounting to 80 - 85% of the seed matter, the seed coat account for 12 - 17% and the embryo for 2 - 3% of the seeds. The garden cress seeds are bitter, thermogenic, depurative, rubefacient, galactogogue, tonic, aphrodisiac, ophthalmic, antiscorbutic, antihistaminic and diuretic. They are useful in the treatment of asthma, coughs with expectoration, poultices for sprains, leprosy, skin disease, dysentery, diarrhoea, splenomegaly, dyspepsia, lumbago, leucorrhoea, scurvy and seminal weakness (**Archana and Anita, 2006**).

Garden cress seeds are found to contain 18–24% of fat (**Gopalan and Rama Shastri 2004**) of which ~34% of total fatty acids is Linolenic acid ALA **(Mathews, *et al.*, 1993 and Sumangala, *et al.*, 2004)**. It contains good amount of lignans (29.4%) and antioxidants. Garden cress oil has Linoleic acid: Linolenic acid (LA: ALA) ratio in the range of 1:4–2:3, which could give it nutritional advantages over the currently available ALA-rich plant oils in altering the n- 6/n-3 ratio in vivo. **Gaafar *et al.* (2013)** reported that *L. sativum* seeds with high nutritional value can be exploited as a functional food ingredient. Garden cress meal and protein isolate can be used as source of minerals and protein rich in essential amino

acids. Also, they reported that the bioavailability of meal and protein isolate and the possibility of using the GCS as a functional food ingredient apart from using it as a source of dietary fiber.

The objective of the present study is to determine the physicochemical properties, fatty acid content and antioxidant activity of oil from garden cress seed. Therefore, in the present study, garden cress oil (*Lepidium sativum*) was screened for their *in vitro* antioxidant activity. Also, evaluate the possible beneficial effect of antioxidant on Hypercholesterolemia using an experimental model induced by high fat diet.

2. Material and Methods

2.1. Materials

Garden cress seed (*Lepidium sativum* L.) were obtained from Harraz Spices and Herbs Co. Cairo, Egypt. A diet containing 10% corn oil was used as the control. All reagents and chemicals used in this work were of analytical grade.

2.2. Methods

Garden cress oil extraction

Garden cress seeds (*Lepidium sativum* L.) were crushed and pressed using a hydraulics laboratory press model. Anhydrous sodium sulphate was added to the extracted oil and allowed to stand for 30 min to remove excess residual moisture. The resulting dry oil was centrifuged and filtered through Whitman filter paper No.1 and kept in a brown glass bottle at 4 ± 0.5 °C.

Physico-chemical properties of garden cress oil.

Refractive index, color, acid value, peroxide value and iodine value were determined according to (A. O. A. C. 2005). Oxidative stability of oil (garden cress) was determined by the Rancimat method at 100±2°C according to **Mendez *et al*., (1996).**

Fatty acid compositions of garden cress oil.

Fatty acid compositions of garden cress was analyzed on the gas liquid chromatography (GLC). The oil was etherified before GLC analysis using the method described by **Stahle, 1967**.

Determination of Tocopherols. Garden cress oil (1.0 g) was saponified with 4 ml of 5% ethanolic pyrogallol (w/v), 1 ml KOH (100%) and boiled in a water bath for 3 min. Samples were then cooled, 30 ml distilled water was added and the mixture was extracted three times with diethyl ether. The combined extracts were washed with water to neutralise and remove fatty acid soaps. The extract was dried with anhydrous sodium sulphate and

evaporated to dryness under a vacuum at 40 °C. The residue was dissolved in 1.0 ml ethanol and 4.0 ml of benzene and dried under a stream of nitrogen. The residue was dissolved in 1.0 ml of ethanol and used for identification and quantification of tocopherol isomers by an HPLC method (**Hatman and Kayden 1979**) using a Agilent 1100 Series HPLC system equipped with a fluorescence detector and Phenominix C18, column (250 9 4.60 mm, 5 lm). The excitation wavelength used was 290 nm and the emission wavelength was 330 nm. An isocratic elution program was employed using a mobile phase containing methanol. The flow rate was 1.0 ml/min. The individual tocopherol homologues were calculated based on the calibration curve of standards of α , δ and γ tocopherols.

Determination of total phenolic.

Total phenols in garden cress oil were determined calorimetrically at 725nm With the Folin–Ciocalteau reagent as previously done by **Gutfinger, 1981**.

Experimental animal.

Male albino rats of Wister strain weighing approximately 145 ± 6 g were used. A total of thirty Albinos male rats were raised in the animal house of Ophthalmology Research Institute, Giza, Egypt. The animals were fed with a basal diet for 7 days as an adaptation period. The basal diet was formulated according to **AIN (1993)** and consisted of casein (12%), corn oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%) and starch (68%); Water was available *ad libitum*. The rats were divided into five groups and each group comprised six rats. The first group presents the control rats and the rats of four groups were allowed to feed on hypercholestrolemic diet (2% cholesterol + 0.2 bile salts) to induce hypercholesterolemia through the feeding period. Groups were given diet containing 25, 50 and 75% garden cress oil, respectively. Blood samples were taken at the start and the end of experiment (4 weeks). The blood samples were obtained from orbital plexus venus by means of fine capillary glass tubes according to the method described by **Schermer (1967)**. The blood samples were placed in dray and clean centrifuge tubes and allowed to clot for 1 - 2 h at room temperature. Serum was removed using a Pasteur pipette and centrifuged for 20 min at 1100 x g. The clean supernatant sera were then kept frozen until analysis.

Measurements of biochemical variables

The levels of serum cholesterol, low and high density lipoproteins, and total triglycerides were determined according to the methods outlined by **Roeschlau *et al.* (1974), Assmann (1979) Levy (1981) and Fossati and Prencipe (1982).** Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the methods described by **Bergmyer and Harder (1986) and Kachmar and Moss (1976)**, respectively. Each determination was carried out in triplicate and the mean values are presented in the text.

3. Results and Discussion

Physicochemical properties of garden cress oil (GCO):

Physico-chemical parameters provide important information regarding storage, structural stability and quality of seed oils. The physico-chemical properties of the investigated oils (Table 1) are in agreement with those reported earlier for *L. sativum* (**Diwakar *et al*., 2009**). Regarding the physico-chemical properties of garden cress oil, the obtained data indicated that the refractive index of oil was 1.475. The color of oil is an important feature which often determines the consumers' acceptability of the product. The oil color observed for garden cress oil is dirty yellow. Oil color is mainly due to the presence of some pigments like chlorophyll and carotenoids which are unintentionally co-extracted during the oil extraction process (**Diwakar *et al.*, 2010**). The results indicated that the free fatty acid as (% oleic acid) of garden cress oil was lower (0.29 %). The peroxide value of garden cress oil is lower with value 2.06 meq. Peroxide / Kg oil. The iodine value of garden cress oil is higher (120.60g of I_2 absorbed/100g oil). Meanwhile, the saponification number of garden cress oil was (179.5 mg KOH/g oil). Oxidative stability of garden cress on 100°C using Rancimat method was 30.00 hours. These characters as whole indicate the increase of shelf life of garden cress oil. **Diwakar (2010)** reported that the oil of garden cress is fairly stable at refrigerated temperatures and the natural antioxidants tocopherol and carotenoids present in it protect the oil from rancidity.

Table (1) Physicochemical properties of Garden cress oil.

Ingredients	Garden cress oil
Refractive Index	1.475± 0.08
Color at 35 yellow red	3.20±0.13
Peroxide Value (meq. peroxide/kg oil)	2.06±0.08
Free Fatty Acids (% oleic)	0.29±0.02
Saponification value (mg KOH/g oil)	179.5±3.4
Iodine value (g of I_2 absorbed/100 g oil)	120.6±2.8
Oxidative stability (hrs)	30.0

Values are given as means of three replicates ± standard deviation.

Fatty acids content of Garden cress oil (GCO):

Data given in Table (2) show the fatty acid composition of garden cress seed oil. From the obtained results it could be observed that the major fatty acids in garden cress oil were linolenic acid $C_{18:3}$ (34.8%), oleic acid $C_{18:1}$ (21.9%), linoleic acid $C_{18:2}$ (11.4%), Eicosanoic acid $C_{20:1}$ (11.5%) and palmitic acid $C_{16:0}$ (10.6%). The fatty acid composition of GCO in this study is in agreement with data reported by (**Sumangala**, ***et al.*, 2004**). Garden cress seed oil analysis indicated that the most abundant saturated fatty acid is palmitic acid, whereas the main unsaturated fatty acids present are linolenic, oleic and linoleic acids. The saturated fatty acids (SFA) content was as low as 16.5% and the unsaturated fatty acids (UFA) content was as high as 83.5% in GCO. Our results agree with **White (2007)** he reported that garden cress seed oil contained 15.6% SFA, 37.6% MUFA and 46.8% PUFA unlike flax seed oil (**White, 2007**) which contains predominantly ω -3 PUFA (52.7%). Hence, flax seed oil is highly unstable and categorised as a drying oil. Erucic acid content in garden cress oil was less than 5% and conforms to the WHO norms for erucic acid content in edible oils.

Table (2) Fatty acids content of Garden cress oil

Fatty acids	%
Myristic (14:0)	0.4
Palmitic acid (16:0)	10.6
Stearic acid (18:0)	2.6
Oleic acid (18:1)	21.9
Linoleic acid (18:2)	11.4
Linolenic acid (18:3)	34.8
Arachidic acid (20: 0)	2.9
Eicosanoic acid (20:1)	11.5
Erucic acid (22:1)	3.6
Saturated fatty acid	16.5
Unsaturated fatty acids	83.5
UFA/SFA (P/S) ratio	5.1: 1

Tocopherol and total phenolic content in garden cress oil:

Tocopherols are natural antioxidants that inhibit oil oxidation. Tocopherols act as biological scavengers of free radicals and could prevent diseases, besides possessing an important nutritional function for human beings as a source of vitamin E (**Brigelius-Flohe *et al.*, 2002**). A qualitative estimation of tocopherols in garden cress oil showed the presence of α, δ and γ -tocopherol, respectively (Table 3). The α -tocopherol is found to be the highest amount tocopherol in GCO followed by γ and δ, respectively. δ-tocopherol content in vegetable oil is positively correlated with the amount of α-linolenic present in it (**Kamal-Eldin and Anderson, 1997**). Tocopherols are naturally occurring constituents found in vegetable oils in varying amounts. The presence of these compounds is important in relation to oil stability and nutritional labeling. Besides, consumption of these oils is recommended thanks to their beneficial effects on health (**Gliszczynska-Swigło and Sikorska, 2004**).

The study of the phenols in vegetables is great interest, owing to the qualitative and quantitative differences appearing as a function of the species, cultivar and degree of ripening. Good amount of phenolic was estimated in garden cress oil (Table 3), wherein the level of total phenols, as determined by

Folin–Ciocalteu method, was 36.41 mg/100 g oil as gallic acid equivalents.

Table (3) Tocopherols and total phenolic contents of garden cress seed oil.

Ingredients	garden cress oil
Tocopherols (ug/g oil):	
α – tocopherol	957
δ – tocopherol	535
γ- tocopherol	668
Total phenolic compounds (mg GAE/100g oil)	36.41

GAE: gallic acid equivalents

Effect of feed on garden cress oil on Triglycerides (TG) and total cholesterol (TC)

Triglycerides in the blood tends to damage vascular endothelial cells, leading to heart disease. A high fat diet produces an increase in TG levels due to lipoprotein lipase TG hydrolysis, so that the accumulation in the liver becomes more evident (**Feoli *et al*., 2003**). In contrast, the effect of PUFA can be attributed to a reduction in the hepatic synthesis of fatty acid, which decreases the concentration of TG in the liver. The data in Table (4) show the levels of serum TG of hyperlipidemic rats fed with supplemented by garden cress oil. Supplementation of rats diet with garden cress oil at levels 25, 50 and 75% resulted in a significant ($p \leq 0.05$) decrease in triglycerides and total cholesterol. Triglycerides were reduced by 29.26, 38.05 and 52.66%. After 4 weeks, the data revealed that all groups showed a decrease in serum TG in comparison with the positive control group. These effects might be due to high plasma lipoprotein lipase activity, an enzyme involved in hydrolysis of

plasma VLDL triacylglycerols. Our data agree well with **Diwakar *et al.* (2008)** rats fed with 10% garden cress oil showed lowered serum triglycerides by 40.4% compared to sun flower oil fed group.

The data show that control group had the lowest serum TC compared with the HCD group. Feeding rats with garden cress oil (75%) caused a decrease in total cholesterol of -43.36% while group 3, which was fed 25% garden cress oil, showed a decrease of -20.66% compared with the positive control group. The rise in cholesterol in plasma may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism, as evidenced by a reduction in bile acid production and turnover of bile acids. The metabolism of free and ester cholesterol is impaired in liver, spleen and thymus tissue, and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats (**Choi *et al*., 2001; Feoli *et al*., 2003**).

Table (4). Effect of different levels of garden cress oil on lipids profile including TC and TG of experimental rats.

	Cholesterol (TC)			Triglycerides (TG)		
	0 week	4 weeks	% changes	0 week	4 weeks	% changes
Normal Control $^-$	85.6±4.23^b	88.3±3.59^a	3.15	103.6±4.26^b	102.5±4.52^e	-1.06
HCD Control $^+$	187.3±9.87^a	207.5±11.21^d	10.87	235.1±8.69^a	271.3±9.85^a	15.39
HCD +25% GCO	184.9±8.42^a	146.7±7.46^c	-20.66	239.6±9.06^a	169.5±6.96^b	-29.26
HCD+50% GCO	186.1±9.16^a	121.8±6.55^c	-34.55	236.8±10.65^a	146.7±8.43^c	-38.05
HCD +75% GCO	185.2±8.88^a	104.9±6.43^b	-43.36	238.5±9.97^a	112.9±7.86^d	-52.66

Means within the same column with different letters are significantly different ($P < 0.05$).
GCO, refers to garden cress oil; HCD, refers to high cholesterol diet.

Effect of feed on garden cress oil on Low and high density lipoprotein cholesterol (HDL- and LDL-cholesterol)

Table (5) showed that the control (+ve) group showed a significant increase in serum LDL-c and VLDL-c ($p< 0.05$) but significant decrease in HDL-c ($p <0.05$) in comparison with control (-ve). On the other hand, the rats fed with different levels of garden cress oil showed a significant decrease in LDL-c and VLDL-c level ($p < 0.05$) compared to control (+ve) group. After 4 weeks, data showed that LDL levels were significantly decreased in all treatments compared to the control groups. Low density lipoprotein was ($p \leq 0.05$) reduced in rats supplemented with GCO at level 25, 50 and 75% by 31.08, 49.56 and 59.09%, respectively. These results suggest that garden cress oil may be preventative against atherosclerosis. Increased plasma low density lipoprotein (LDL) concentration is associated with the susceptibility to developing atherosclerosis (**Penumathsa *et al*., 2007**).

After 28 days of the feeding experiment it was generally noted that the levels of HDL increased in both groups compared with the control groups. The highest increase in HDL was measured in normal group (46.8 mg/dl) followed by rats group fed on 50% garden cress oil was increased in these rats by 40.42 – 34.95%. HDL has been indicated as a positive factor in determining the development of atherosclerosis (**Miller and Miller, 1997**). Supplementation with garden cress oil increased the rate of HDL-cholesterol. Garden cress seeds oil have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), flavonoids, and amino acids like glutamine, cysteine,

and glycine. The tannin and flavonoids may have antioxidant activity whenever glutamate, cysteine, glycine are intermediates for synthesis of the endogenous antioxidant glutathione (**Hamer and Steptoe, 2006**). Feeding rats with 10% Garden cress seed oil lowered Very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) levels decreased by 9.45% in serum of 10% garden cress oil fed rats, while HDL remained unchanged among *Lepidium sativum* oil fed rats (**Diwakar, *et al.*, 2008**).

Table (5). Effect of different levels of garden cress oil on lipoproteins including HDL-C, LDL-C and VLDL-C in experimental rats.

	High Density Lipoproteins HDL-C (mg/dl)			Low Density Lipoproteins LDL-C(mg/dl)			Very Low Density Lipoproteins VLDL-C(mg/dl)		
	0 week	4 weeks	% changes	0 week	4 weeks	% changes	0 week	4 weeks	% changes
Normal Control	33.4±1.6^{a}	46.9±2.6^{a}	40.42	31.4±1.5^{b}	30.9±2.1^{e}	-1.59	20.7±1.2^{b}	20.5±1.6^{d}	-0.97
HCD Control	34.4±2.1^{a}	34.6±2.7^{c}	0.58	105.8±4.6^{a}	181.6±5.2^{a}	71.64	47.0±3.2^{a}	54.2±2.4^{a}	15.32
HCD+25% GCO	31.7±1.8^{a}	40.3±2.4^{b}	27.13	105.2±5.1^{a}	72.5±4.7^{b}	-31.08	47.9±2.8^{a}	33.9±1.5^{b}	-29.23
HCD+50% GCO	30.9±2.5^{a}	41.7±2.8^{b}	34.95	102.7±4.9^{a}	51.8±4.5^{c}	-49.56	47.3±2.9^{a}	29.3±1.6^{c}	-38.05
HCD+75% GCO	33.0±1.9^{a}	39.8±2.6^{bc}	20.61	103.9±5.3^{a}	42.5±4.9^{d}	-59.09	47.7±3.1^{a}	22.5±1.0^{cd}	-52.83

Means within the same column with different letters are significantly different ($p < 0.05$).
GCO, refers to garden cress oil; HCD, refers to high cholesterol diet.

Effect of fed on garden cress oil on liver functions

From data presented in Table (6), it could be noticed that hypercholesterolemic control rat groups showed a significant increase in serum alanine and aspartate aminotransferase (ALT & AST) at compared to control (-ve) group. The experimental rat groups fed with garden cress oil showed a significant decrease in serum alanine and aspartate aminotransferase enzymes in comparison with control (+ve) group. The highest level of ALT was 86.27 U/L in the positive control group, supplementation with GCO at levels 25 and 50% reduced AST enzyme by 26.64 and 42.12%. However, the level of 75% from garden cress seed oil reduced it by 40.62%. The increase in serum ALT activity indicates liver cell necrosis and hepatic injury. Treatment with different levels of garden cress oil induced a decrease in the high activity of ALT. Supplemented rats with 50% GCO were more effective ($p > 0.05$) in reducing ALT enzyme than those supplemented with 25 and 75%. AST and ALT as shown in Table 6 agrees with those of **Daher *et al.* (2006).** ALT and AST are closely correlated in most cases of liver diseases. Excessive storage of fat in the liver effects on liver functions and increases the susceptibility to free radical attack in hyporcholesterolemic rats resulting in liver damage as described by **Tahri *et al.* (2000) and Yadav *et al.* (2009)**.

Table (6). Effect of different levels of garden cress oil on liver functions including AST and ALT on experimental rats.

	Aspartate aminotransferase (AST)			Alanine aminotransferase (ALT)		
	0 week	4 weeks	% changes	0 week	4 weeks	% changes
Normal Control $^{-}$	24.36±2.34^{b}	23.94±3.46^{d}	-1.71	19.64±2.44^{b}	18.26±2.15^{d}	-7.02
HCD Control $^{+}$	85.64±6.71^{a}	91.61±7.35^{a}	6.97	72.35±5.71^{a}	86.27±5.96^{a}	-19.24
HCD +25% GCO	87.58±8.05^{a}	64.25±3.47^{b}	-26.64	71.89±6.07^{a}	59.24±5.34^{b}	-17.59
HCD+50% GCO	86.97±7.66^{a}	50.34±3.02^{c}	-42.12	73.54±5.62^{a}	44.39±3.22^{c}	-39.64
HCD +75% GCO	86.34±7.13^{a}	51.27±2.73^{c}	-40.62	74.25±5.89^{a}	45.46±4.15^{c}	-38.77

Means within the same column with different letters are significantly different ($p < 0.05$).
GCO, refers to garden cress oil; HCD, refers to high cholesterol diet.

Conclusion:

In this study, garden cress oil can be considered as a potential, alternate and non-conventional seed oil for ω-3 fatty acids and can be blended with suitable edible oils or in the form of a supplement to increase ω-3 fatty acids in functional foods. The results of *in vivo* experiment indicate that administration of garden cress has a profound influence on the metabolism of lipids in rats fed high cholesterol diet. It could be suggested that the use of garden cress oil by patients suffering from coronary atherosclerosis would prevent the development of this disease.

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