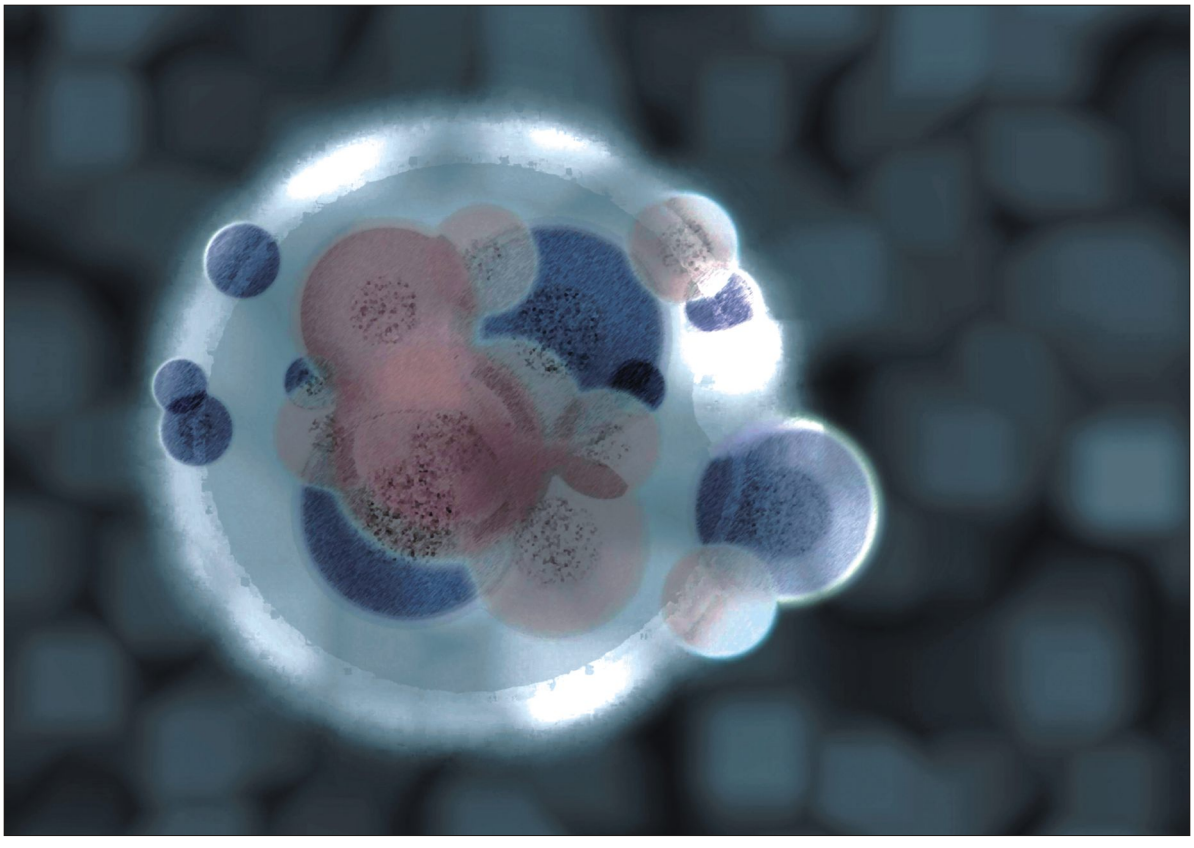

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Research Article
Roselle Seed as a Potential New Source of Healthy Edible Oil

[1]Sahar Youssef Al-Okbi, [2]Adel Gabr Abdel-Razek, [1]Shaimaa Elsayed Mohammed and [3]Mahmoud El-Sayed Ottai

[1]Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, Egypt
[2]Department of Fats and oils, National Research Centre, Dokki, Cairo, Egypt
[3]Department of Genetics and Cytology, National Research Centre, Dokki, Cairo, Egypt

Abstract

Background and Objective: Searching non-traditional sources of edible oil is crucial in Egypt. Roselle (*Hibiscus sabdariffa* L.) is a vital medicinal plant in Egypt, where all its parts are utilized except the seeds that are completely discarded. The aim of the present research was to study the possibility of consuming roselle seed oil (RSO) as edible oil. **Materials and Methods:** The composition of RSO from fatty acids, total phenolic, flavonoids and tocochromanols was assessed. *In vitro* antioxidant and ABTS scavenging activity and the oxidative stability of RSO were evaluated. Oral acute lethal toxicity test of RSO was studied in mice to assess its safety. Two experiments were carried out on rats, in the first experiment two balanced diets were fed to two groups of rats one contained 10% RSO while the other contained the same percentage of sunflower oil as reference oil for 4 weeks. At the end of the experiment, plasma lipid profile, malondialdehyde (MDA) and liver and kidney functions were assessed. In the second experiment, dyslipidemia was induced in rats then rats were fed either 10% RSO or 10% sunflower oil diet. At the end of the second experiment plasma glucose, lipid profile, interlukin 6 and MDA were assessed. Data were statistically analyzed using one-way analysis of variance ANOVA followed by Duncan's test. **Results:** The RSO showed to possess high safety and *in vitro* antioxidant activity. Major fatty acids were linoleic, oleic and palmitic. Total tocopherols and tocotrienols were 96.2 and 3.48 mg/100 g oil, respectively. Total phenolic and flavonoids contents were 56.31mg GAE and 4.99 mg catechin g^{-1} oil, respectively. Induction period of oxidative stability of RSO was 24.88 h. All assessed parameters of first rats experiment showed insignificant changes when rats fed on RSO diet was compared to those fed on sunflower oil diet. In the second experiment, significant improvements in all parameters were noticed when dyslipidemic rats were fed on either RSO or sunflower diet with superiority to RSO concerning MDA and interlukin 6. **Conclusion:** The RSO has high safety, oxidative stability and antioxidant activity and cardiovascular remedial effect. It is proposed that RSO is suitable as edible oil for human consumption.

Key words: Roselle seed oil, total phenolics, tocochromanols, oil safety, cardiovascular diseases

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Corresponding Author: Sahar Youssef Al-Okbi, Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, Egypt
Tel: +201003785152

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the near testament, when Egyptian cotton was the main summer crop in Egypt, Egyptians were producing the enough requirements of their edible oil from cotton seeds. After deterioration of cotton cultivation, Egypt's need to import edible oil has increased steadily, so that it imports now most of its actual needs (about 97%)[1]. It is warranted to find alternative sources to meet the requirement from edible oil.

Hibiscus sabdariffa L., also known as roselle, is one of the most important and famous medicinal plant in Egypt. It is used traditionally as hot and cold beverages and as a coloring agent in food industry. All roselle parts are useful and consumed, where the stem is exploited to produce fibers. The leaves are cooked like spinach in Africa and containing nutrients such as phosphorus, calcium, magnesium and potassium[2,3]. However, the economic part of roselle is the dry calyces which are rich in anthocyanin[4], amino acids, organic compounds and mineral salts[5] as well as vitamin C[6].

Roselle possesses different reported remedial effect due to presence of numerous phytochemicals. This supports the ethno medicinal use of roselle in promoting cardiovascular health and preventing hypertension, pyrexia, liver disorders and microorganism growth, as well as having a diuretic, digestive, anti-diabetic and sedative effect[7]. The red varieties of roselle have antioxidant and cyclo-oxygenase inhibitory activity[8].

However, only the seeds of roselle are completely neglected in Egypt and they are always discarded. The seed could represent a cheap source of edible oil since it contains a fixed oil ranged from 17-20% with characteristics similar to that of cotton seed oil where both are belonging to *Malvaceae* family[4,9]. Roselle is planted in an area of not less than 11114 acres in Egypt which could provide more than 2000 t of oil. This cultivated area encourages the production of roselle seed oil (RSO) economically in Egypt.

Before taking a decision for consuming RSO as edible oil, it is of much concern to study its safety and stability. To the best of our Knowledge, no safety records for such oil were reported. The aim of the present research was to conduct a biological and chemical study to assess the validity and safety of roselle seed oil for human consumption as a complementary source of edible oil.

MATERIALS AND METHODS

This study was accomplished in 2017.

Preparation of roselle seed oil by cold pressing: Roselle, *Hibiscus sabdariffa* L. (Family: *Malvaceae*), after being harvested the seeds were collected from the farms in Dandara Village, Qena Governorate, Egypt. The seeds (Fig. 1) were air dried, washed by running water then re-air dried. The seeds were squeezed by hydraulic pressure device to obtain the cold pressed crude oil. The obtained cold pressed oil was used in all the chemical and biological studies during the present research.

Assessment of crude oil content of roselle seeds by solvent extraction: The dry seed (100 g) was extracted by petroleum ether (of analytical grade) using Soxhlet apparatus (Bionic Scientific Technologies, Delhi, India) for 6 h. The crude oil extracted was concentrated in a rotary evaporator and dried by heating in a vacuum oven at 50°C for 1 h. The Percentage crude oil content was then determined gravimetrically[10].

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

Assessment of the composition of fatty acids in RSO by gas chromatography (GC): Methyl esters of fatty acids (FAME) were prepared according to AOCS official method and assessed by GC[11]. Diluted FAME were separated on a HP 5890 series II (Hewlett Packard, Palo Alto, USA) equipped with an innowax capillary column (30 m_0.20 mm_0.20 mm) and FID (FID). Hydrogen was used as the carrier gas at flow rate of 1.5 mL min^{-1}. The column temperature was isotherm (210°C). Detector and injector temperatures were set at 240°C. Fatty acids were identified by comparison of the retention times with authentic standards and the results were calculated as percentage of total fatty acids.

Oxidizability COX value was calculated as mentioned previously[12].

Determination of oxidative stability index: Oxidative stability of the oil was assessed[13] using an automated

Fig. 1: *Hibiscus sabdariffa* L. seed (Egyptian variety)

Metrohm Rancimat model 743 (Herisau, Switzerland) at 100±0.1°C and an air flow of 20 L h^{-1} to determine the induction period (IP) of the oil.

Assessment of total phenolic content (TPC) of RSO: The total phenolic assay was carried out using the Folin-Ciocalteu reagent[14]. One mg oil was mixed with 1 mL methanol to extract total phenolics then 500 µL of the extract was taken and added to 0.5 mL of the distilled water and 0.125 mL of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min before adding about 1.25 mL of 7% Na_2CO_3. The solution was adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of gallic acid (standard, 0-1000 µg mL^{-1}). Total phenolic content was estimated as mg gallic acid equivalents (GAE)/g oil through the calibration curve of gallic acid. The reaction was conducted in triplicate and results were averaged.

Determination of total flavonoid content (TFC) of RSO: The total flavonoid contents of RSO were determined by a modified colorimetric method using catechin as a standard[15]. Total flavonoids were extracted from RSO then the extract or standard solution (250 µL) was mixed with distilled water (1.25 mL) and 75 µL of 5% sodium nitrite ($NaNO_2$) solution followed by the addition of 150 µL of 10% aluminium chloride ($AlCl_3$) solution 5 min later. After 6 min, 0.5 mL of 1 M sodium hydroxide (NaOH) and 0.6 mL distilled water were added. The solutions were then mixed and absorbance was measured at 510 nm. The results were expressed as mg catechin/g of oil. The determination was performed in triplicate.

Determination of vitamin E (Tocochromanols): For the determination of tocochromanols (tocopherols and tocotrienols), about 250 mg of oil in 25 mL of n-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck Hitachi low pressure gradient system, fitted with aL-6000 pump, a Merck Hitachi F-1000 fluorescence spectrophotometer (detector wave lengths for excitation was 295 nm, for emission 330 nm) and a D-2500 integration system. The sample in the amount of 20 µL was injected with a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm-4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL min^{-1}. The mobile phase used was n-heptane/tert butyl methyl ether (99:1, v/v)[16].

***In vitro* antioxidant activity of RSO:** The antioxidant activity of RSO was determined by applying ferric thiocyanate (FTC) method[17-19]. The RSO (4 mg) or reference material [4 mg; butylated hydroxyl toluene (BHT)] were separately placed in screw cap tubes and mixed with 4 mL of absolute ethanol and 4.1 mL of 2.52% linoleic acid in absolute ethanol. An appropriate amount of 8 mL of 0.05 M phosphate buffer (pH 7.0) and 3.9 mL of distilled water were prepared and added to the previous mixture. The contents of each tube were mixed well and placed in an incubator at 40°C in the dark for a week. To 0.1 mL of this solution; 9.7 mL of 7.5% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. Precisely 3 min after an addition of 0.1 mL of 0.02 M ferrous chloride that is dissolved in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm. A control was run parallel to the test without the oil sample or reference. The antioxidant activity of RSO sample was carried out in triplicate.

Calculation of antioxidant activity[20]:

$$\text{Inhibition (\%)} = 100\text{-}[(A1\text{-}A0)\times 100]$$

where, A0 is the absorbance of the control and A1 is the absorbance of the sample.

ABTS radical scavenging assay: The ABTS radical scavenging assay[21] was based on that the pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen-donating antioxidants. Two stock solutions were prepared, the first is 7 mM ABTS solution and the second is 2.45 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. Before use, the solution was diluted with ethanol to obtain absorbance between 0.800 and 1.000 at 734 nm. This solution was mixed with the sample (25-100 µg mL^{-1}) or reference material (vitamin C). A control containing methanol and $ABTS^{\cdot+}$ solution was also run. The absorbance was read at 734 nm after 30 min incubation at 25°C. The percentage inhibition of free radical ABTS was calculated from the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}}\text{-Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\times 100$$

where, $Abs_{control}$ is the absorbance of control, Abs_{sample} is the absorbance of $ABTS^{++}$ + sample or reference material.

Animal experiments: Animal experiments in the present study were carried out according to the Medical Research Ethics Committee for institutional and national guide for the care and use of laboratory animals, National Research Centre, Cairo, Egypt.

Acute oral lethal toxicity test: Senile healthy albino mice (28 male and 28 female) of 23-25 g were used in the acute toxicity test[22]. Animals were obtained from the animal house of National Research Centre, Cairo, Egypt. Each group of mice (8 mice each: 4 male and 4 female) was kept in a stainless steel cage with free access to water and food. Mice were divided into seven groups (8 animals/group). The 24 h mortality counts (if any) among equal sized groups of mice receiving progressively increasing oral dose levels from 0.5-10 g RSO/kg mouse body weight were recorded.

Studying the effect of consumption of RSO in the diet on different biochemical and nutritional parameters in both normal healthy and dyslipidemic rats: Male albino rats weighing 100-120 g were obtained from animal house of National Research Centre, Cairo, Egypt. Rats were kept individually in stainless steel cages, water and food were given *ad libtium*. Two experiments were carried out at the same time. In the first experiment 12 rats were divided into two groups; each of 6 rats. Rats of the first group were fed on balanced diet containing 12% casein, 73.5% starch, 3.5% salt mixture, 1% vitamin mixture and 10% sunflower oil (Diet A). Rats of the second group were fed on a diet having the same composition except for replacing 10% sunflower oil by 10% RSO (Diet B). The experiment continued for 4 weeks. During the experiment body weight and food intake were recorded weekly. At the end of the study, total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. The growth curves of rats of the 2 groups were drawn. Rats were fasted and blood samples were obtained from eye vein orbital of anaesthetized rats and received in heparinized tubes. Plasma was separated by centrifugation at 3000 rpm (Hanil Science Industrial, Korea). Plasma lipid profile represented by total cholesterol (T.Ch), triglycerides (TG) and HDL-Ch were determined colorimetric[23-25]. Malondialdehyde (MDA) was assessed as an indicator of lipid peroxidation[26]. Liver function was evaluated through determination of aspartate transaminase (AST) and alanine transaminase (ALT) activity[27]. Kidney function was assessed through determination of plasma urea and creatinine[28,29]. Liver, kidney and heart were separated and weighed for calculating organ/body weight%. A second experiment was conducted parallel to the first experiment. In the second experiment, 18 rats of body weight ranging from 100-120 g were fed on dyslipidemic diet composed of 12% casein, 19.05% starch, 38.2% sucrose, 3.5% salt mixture, 1% vitamin mixture, 0.25% cholic acid, 1% cholesterol and 25% coconut oil for 4 weeks according to Mohamed *et al.*[30] study with some modifications. The modification resides in using 25% coconut oil in the present study instead of 20% on the expense of carbohydrate. Blood samples were obtained from 6 fasted rats for determination of plasma lipid profile and MDA using the aforementioned methods (to confirm the induction of dyslipidemia) and the rats were kept alive and continued feeding on dyslipidemic diet together with the other 12 rats. After a week rats were divided into three groups; each of 6 rats. Rats of the first group continued feeding on the same dyslipidemic diet while rats of the second and third groups were shifted to the aforementioned two balanced diets (A and B, respectively) and the feeding continued for three weeks. At the end of the second experiment, rats were fasted and plasma glucose was estimated[31]. Plasma lipid profile (T.Ch, TG and HDL-Ch) and MDA were determined by the above mentioned methods. Interlukin 6 was assessed using ELISA kit obtained from KOMA BIOTECH, Seoul, Korea. In the first and second experiments LDL-Ch was calculated[32]. Also HDL-Ch/T-Ch was calculated as a risk factor for cardiovascular diseases (CVD).

Statistical analysis: The results were expressed as the Mean±SE. Results of the biological experiments were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

RESULTS

Crude oil content of *Hibiscus sabdariffa* seeds obtained by solvent extraction was found to be 20%.

Fatty acids' composition of RSO: Fatty acids profile of roselle seed oil is illustrated in Table 1. The principal fatty acids present in RSO were linoleic (37.11%), oleic (33.08%), palmitic (17.19%) and stearic (7.96%). The percentage of saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were 27.49, 33.93 and 38.58%, respectively, which refers to a ratio of 0.82:1.02:1.16. Total unsaturated fatty acids were 72.51%. The ratio of PUFA/SFA and Cox value in roselle oil was calculated to be 1.4 and 4.47, respectively. Minor identified fatty acids were lauric, myrstic, palmitoleic, linolenic, arachidic and gondoic.

Table 1: Fatty acids' content of RSO as percentage of total fatty acids

Fatty acids	Total fatty acids (%)
Lauric (C12:0)	0.96
Myrstic (C14:0)	0.24
Palmitic(C16:0)	17.19
Palmitoleic (C16:1)	0.50
Stearic (C18:0)	7.96
Oleic (C18:1)	33.08
Linoleic (C18:2)	37.11
Linolenic (C18: 3)	1.47
Arachidic (C20:0)	1.14
Gondoic(C20:1)	0.35
Total saturated fatty acids	27.49
Total mono unsaturated fatty acids	33.93
Total poly unsaturated fatty acids	38.58
Total unsaturated fatty acids	72.51
PUFA/SFA	1.40
USFA/SFA	2.64
PUFA/MUFA	1.14
S:M:P	0.82:1.02:1.16
Cox value	4.47
Omega 6/omega 3	25.24

PUFA: Poly unsaturated fatty acids, SFA: Saturated fatty acids, USFA: Unsaturated fatty acids, S:M:P: The ratio between saturated, monounsaturated and polyunsaturated fatty acids

Table 2: Tocopherol (mg/100 g), tocotrienols (mg/100 g), total phenolic (mg GAE/g)and total flavonoids (mg catechin/g) contents of RSO

Parameters	Concentration
Alpha-Toc	27.54±0.17
Beta- Toc	0.20±0.00
Gamma- Toc	67.58±0.16
Delta- Toc	0.89±0.02
Total Toc	96.20±0.26
Alpha-T3	2.11±0.03
Beta-T3	0.52±0.01
Gamma-T3	0.85±0.02
Delta-T3	ND
Total T3	3.48±0.03
Total vitamin E	99.68±0.25
TPC	56.31±0.791
TFC	4.99±0.889

Toc: Tocopherol, T3: Tocotrienol, TPC: Total phenolic, TFC: Total flavonoids, values are Mean±SE

Oxidative stability: The Induction period of oxidative stability of roselle oil showed high value equal to 24.88 h.

Total phenolic and flavonoids: Total phenolic content was found to be 56.31±0.791 as mg GAE/g oil while TFC was 4.99±0.889 as mg catechin/g oil (Table 2).

Vitamin E: Tocochromanols contents of RSO are shown in Table 2. It could be noticed that RSO contains alpha, beta, gamma and delta tocopherols with the highest content of gamma tocopherol (67.58 mg±0.16/100 g oil). The oil also contained alpha, beta and gamma tocotrienols where alpha tocotrienol was the major (2.11 mg±0.03/100 g oil). Total tocopherol content was 96.2 mg±0.26/100 g oil while total tocotrienols concentration was 3.48 mg±0.03/100 g oil. Total vitamin E was accounted for 99.68 mg±0.25/100 g oil.

Table 3: $ABTS^{\cdot+}$ radical assay for roselle oil

Concentration of RSO and ascorbic acid (μg mL^{-1})	Inhibition of ABTS (%)	
	RSO	Ascorbic acid
25	16.53±0.62	87.06±2.31
50	35.66±0.63	88.97±0.22
75	62.81±0.34	91.78±0.27
100	68.40±0.66	92.65±0.28

Values are Mean±SE

Table 4: Nutritional parameters and organ/body weight% of rats fed on diet A and B (First experiment)

	Groups	
Parameters	Rats fed on diet A	Rats fed on diet B
Initial body weight (g)	109.83±5.42[a]	109.50±3.50[a]
Final body weight (g)	167.83±8.68[a]	168.67±8.57[a]
Body weight gain (g)	58.00±6.47[a]	59.17±5.82[a]
Total food intake (g)	455.83±14.97[a]	468.00±14.01[a]
Food intake g/day	16.28±0.53[a]	16.71±0.50[a]
Food efficiency ratio	0.13±0.01[a]	0.13±0.01[a]
Heart/ body weight%	0.39±0.02[a]	0.38±0.01[a]
Kidney/body weight%	0.69±0.03[a]	0.71±0.03[a]
Liver/body weight%	3.03±0.1[a]	3.53±0.08[a]

All values showed insignificant difference (similar superscripts in the same row), Diet A: Contained 10% sunflower oil, Diet B: Contained 10% roselle oil, data were expressed as Mean±SE

***In vitro* antioxidant activity and ABTS radical scavenging of RSO:** The antioxidant activity of RSO determined by iron thiocyanate method was 107±0.29% while that of BHT was 108.3±0.31%. The ABTS scavenging activity of RSO (Table 3) showed that the activity correlated to RSO concentration where increasing the concentration from 25-100 μg mL^{-1} elevated the % inhibition from 16.53±0.62 to 68.4±0.66, i.e., four times. On the other hand, the scavenging activity of vitamin C increased only from 87.06±2.31 to 92.65±0.28 when elevating the concentration from 25-100 μg mL^{-1}.

Animal experiments

Toxicity study: The results of the present study revealed the safe consumption of RSO. The highest safe dose level of the oil in the acute toxicity test was found to be 10 g kg^{-1} mouse body weight which was the highest tested dose.

Effect of RSO consumption in the diet on both normal and dyslipidemic rats: In the first experiment, nutritional parameters (final body weight, body weight gain, total food intake and food efficiency ratio) of rats fed on diet A and B for 4 weeks showed insignificant changes (Table 4). Growth

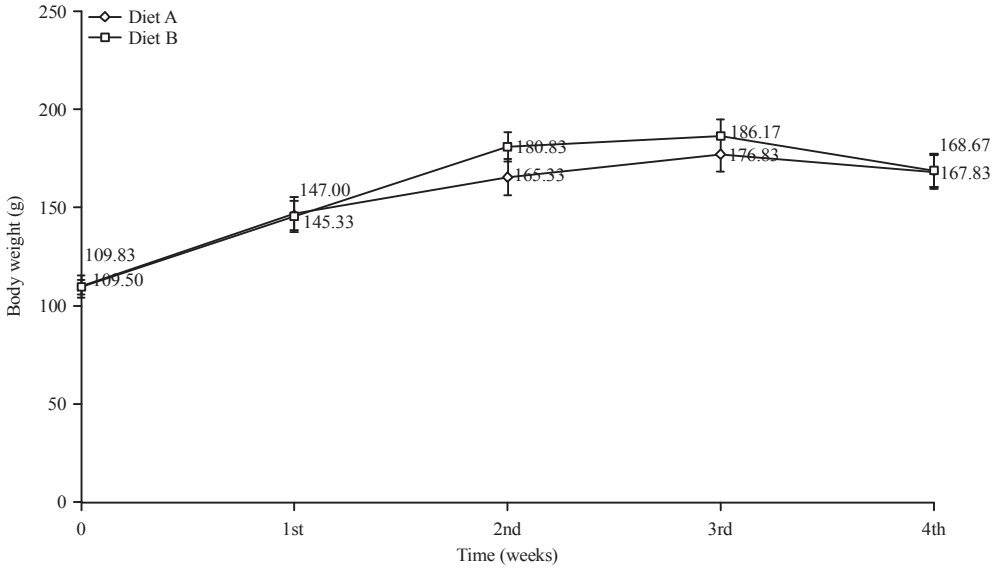

Fig. 2: Growth curves of rats fed on diets containing sunflower oil (Diet A) and roselle oil (Diet B)

Body weights showed insignificant changes on different time intervals when the rats fed on diet B were compared to those fed on diet A, values are Mean±SE

Table 5: Data of the first experiment compared to rats fed on dyslipidemic diet for 4 weeks

	Groups		
Plasma parameters	Healthy rats fed on diet A for 4 weeks	Healthy rats fed on diet B for 4 weeks	Rats fed on dyslipidemic diet for 4 weeks
TCh (mg dL^{-1})	73.98±0.63[a]	76.02±1.10[a]	107.07±0.95[b]
TG (mg dL^{-1})	64.58±1.41[a]	64.60±1.45[a]	96.30±3.93[b]
HDL-Ch (mg dL^{-1})	38.20±2.07[a]	38.60±1.92[a]	27.63±0.25[b]
LDL-Ch (mg dL^{-1})	22.87±2.75[a]	24.48±1.94[a]	60.17±1.37[b]
TCh/HDL-Ch	1.95±0.120[a]	2.00±0.089[a]	3.83±0.047[b]
MDA (nmol mL^{-1})	5.60±0.43[a]	5.82±0.49[a]	10.68±0.84[b]
ALT (IU L^{-1})	28.07±2.01[a]	28.00±2.53[a]	-
AST (IU L^{-1})	34.40±1.96[a]	34.00±1.26[a]	-
Creatinine (mg dL^{-1})	0.80±0.05[a]	0.84±0.05[a]	-
Urea (mg dL^{-1})	22.50±2.98[a]	20.32±0.46[b]	-

In the same row similar superscript letters mean insignificant difference while different letters mean significant difference, Diet A: Contained 10% sunflower oil, Diet B: Contained 10% roselle seed oil, data were expressed as Mean±SE

curves of rats fed on diet A and B are shown in Fig. 2. Body weights showed insignificant changes on different time intervals (1st, 2nd, 3rd and 4th weeks of the first experiment) when the rats fed on diet B were compared to those fed on diet A. No significant changes were noticed in liver, kidney and heart/body weight% on feeding RSO diet compared to sunflower diet.

Biochemical parameters of the first experiment (Table 5) showed insignificant changes in all determined plasma parameters when rats fed on balanced diet containing RSO were compared to those fed on balanced diet containing sunflower oil, except for urea that showed significant $p<0.05$ reduction on feeding diet containing RSO. In the second experiments rats fed on dyslipidemic diet for 4 weeks showed significant $p<0.05$ increase in plasma TCh, TG, LDL-Ch, MDA and TCh/HDL-Ch along with significant $p<0.05$ reduction in HDL-Ch compared to rats fed on balanced diet (A or B) for 4 weeks (from the first experiment) as illustrated in Table 5. This confirmed the induction of dyslipidemia in the second experiment after a feeding period of 4 weeks. Shifting diet from dyslipidemic to balanced A or B produced significant $p<0.05$ reduction in plasma TCh, TG, LDL-Ch and TCh/HDL-Ch as well as significant $p<0.05$ increase in HDL-Ch compared to rats fed on the dyslipidemic diet for 8 weeks (Table 6). Feeding diet A or B could also reduce lipid peroxidation represented by MDA, inflammatory biomarker (Int. 6) and plasma glucose compared to rats fed on the dyslipidemic diet for 8 weeks (Table 6). Rats fed on RSO diet

Table 6: Data of the second experiment after a feeding period of 8 weeks

Plasma parameters	Groups: Rats fed on dyslipidemic diet and shifted to diet A	Rats fed on dyslipidemic diet and shifted to diet B	Rats fed on dyslipidemic diet for 8 weeks
TCh (mg dL^{-1})	97.00±1.41[a]	95.73±2.33[a]	159.30±3.08[b]
TG (mg dL^{-1})	70.22±0.17[a]	73.05±1.62[a]	108.90±0.93[b]
HDL-Ch (mg dL^{-1})	42.57±0.52[a]	40.77±1.98[a]	22.80±1.53[b]
LDL-Ch (mg dL^{-1})	40.42±1.25[a]	39.70±2.36[a]	114.73±3.29[b]
TCh/HDL-Ch	2.28±0.037[a]	2.36±0.104[a]	7.18±0.595[b]
MDA(nmol mL^{-1})	10.27±0.06[a]	7.02±0.11[c]	15.92 ±0.21[b]
Glucose (mg dL^{-1})	61.05±1.55[a]	61.92±0.26[a]	85.88±4.23[b]
Interleukin-6 (pg mL^{-1})	415.00±52.96[a]	113.33±13.33[c]	1553.00±351.44[b]

In the same row similar superscript letters mean insignificant difference while different letters mean significant difference, Diet A: Contained 10% sunflower oil, Diet B: Contained 10% roselle oil, data were expressed as Mean±SE

produced significant $p<0.05$ reduction in both MDA and Int 6 compared to those fed on sunflower oil diet.

DISCUSSION

In Egypt there is a challenge in finding out new sources for edible oil since the majority of the Egyptian needs from edible oil are imported. Nowadays, one of the most commonly consumed oil in Egypt is sunflower. So the present research spots some lights on the possibility of consuming RSO as edible oil in comparison to sunflower oil as reference oil.

Roselle seed oil was reported previously to be 21.85% and 17.35% from the seed[33,34], while it was 20% in the present study. It was demonstrated that the most abundant fatty acids in RSO were C18:2 (39.31-44.72%), C18:1 (25.16-32.06%), C16:0 (18.52-20.84%), C18:0 (4.31-5.3-5.88%) and C19:1(1.7%) in addition of vernolic acid (3.52%) and dihydrosterculic acid (1.57)[33-35]. In another study[36], the major fatty acids were found to be oleic (37.92%), linoleic (35.01), palmitic (19.65) and stearic (6.07). Myristic (0.16), myristoleic (0.17), palmitoleic (0.56), arachidic (0.14) and eicosatrienoic (0.20) were minor fatty acids. Recently, Ali and Al-Anany[37] reported the presence of lauric (1.25%) and linolenic (0.4%) in addition to the above mentioned major fatty acids. The variability in oil percentage and fatty acids could be attributed to the method of extraction and or the geographic origin of the plant. The present results of fatty acids fall within the range of the aforementioned studies. The findings in the current study meet the requirements of the FAO and WHO[38] regarding the nutritional importance of the balance in fatty acid ratio (S:M:P). The current work showed roselle seed oil to belong to linoleic/oleic acid category indicating its usefulness to be utilized as a nutritional oil.

The reduction in the oxidizability Cox value was reported as a measure of oxidative stability of the oil[12]. Reduction in PUFA/SFA, PUSFA/MUSFA and USFA/SFA ratios also pointed to stability of oil against oxidation[39]. Cox value of RSO in the present study had the least value (4.47) compared to that previously mentioned for sunflower oil and soy bean oil (6.4 and 7.2, respectively)[40] referring to its superiority concerning resistance to oxidation. In the same aforementioned study[40], prepared blends from either sunflower oil or soy bean oil with palm super olein (1:1) aiming at improving stability gave similar Cox value to that of RSO. PUFA/SFA, USFA/SFA and PUSFA/MUSFA of RSO in the present study was of lower values than that of sunflower and soy bean oil as reported by Abdel-Razek *et al.*[40] pointed to the highest stability of RSO compared to both oils.

The ratio of SFA:MUFA:PUFA in most cooking oils does not reach the ideal ratios[40], however, this ratio was 0.82:1.02:1.16 in roselle seed oil in the present study which is almost near to the ideal ratio according to WHO (1:1:1). The balance in this ratio is related to generation of the best LDL/HDL ratio in blood and other health benefits. This balance is critical at any level of fat intake, therefore, the WHO recommends slightly less SFA and PUFA than MUFA in the balance. The unique balance of S:M:P in RSO makes it suitable as cooking and frying oil that could be utilized in food industry. The length of the IP is considered a relative measure of oils' stability. The IP of roselle oil in the present study was shown to be 24.88 h which is higher than that of soy bean oil (10.9) and sunflower oil (11.0) reported previously[40] indicating its high stability.

The presence of tocopherols and tocotrienols in RSO as seen in the present study renders the oil both high oxidative stability and health benefits as antioxidant. Gamma-tocopherol was the most considerable fraction (70% of total tocopherols), almost more than twice of alpha-tocopherol (28.6%), while beta-tocopherol was hardly detected. Alpha-tocotrienols represent the abundant fraction (61%) of the total tocotrienols followed by gamma and beta (24.4 and 14.9%, respectively). Gamma-tocopherol could delay

the oxidation of polyunsaturated fatty acids in oil seeds. Also, alpha and gamma-tocopherol have a special activity against free radicals and play many roles in the regulatory functions in the live cells[41,42]. So such content of tocopherol and tocotrienols gives the oil both antioxidant and anti-inflammatory activity. It was reported by Mohamed *et al*.[35] that RSO is a good source of antioxidants particularly gamma-tocopherol which agreed with the present results. However, these authors reported higher total tocopherols than that in the present study. The high content of antioxidants in RSO reflected in vitamin E, total phenolics and flavonoids that assayed in the present study could serve as natural preservative that protects the oil against oxidation or rancidity especially with the presence of total unsaturated fatty acids that reaches 72.51%.

The present research showed that feeding RSO diet produced no significant changes in the different assessed biochemical parameters and organ weights in normal rats when compared to sunflower oil. This clarified the safety of RSO consumption in normal condition where plasma lipids, liver and kidney function were in the normal level and even plasma urea showed significant $p<0.05$ reduction compared to sunflower diet indicating more improvement of kidney function. In India, a decoction of the seeds is used to relieve pain during urination[7] which pointed to its health benefits towards kidney disorder. The MDA that reflects the degree of lipid peroxidation and oxidative stress was in the normal level pointing to the normal antioxidant state on RSO consumption. Plasma TCh/HDL-Ch showed normal level indicating the safety of RSO consumption towards cardiovascular system since the elevation of this ratio is a risk factor towards cardiovascular diseases.

Feeding diet high in coconut oil and sucrose and containing cholesterol and cholic acid induced dyslipidemia when fed for 4 weeks in the present study. Dyslipidemia extremely increased when continued feeding the same diet for extra 4 weeks. Plasma MDA and TCh/HDL-Ch were significantly elevated after 4 weeks of consuming the dyslipidemic diet and was further increased by 49 and 87%, respectively after the next 4 weeks indicating elevation in oxidative stress and cardiovascular diseases risk, respectively. When shifting from feeding the dyslipidemic diet to balanced diet containing either sunflower or RSO (A and B diets, respectively) there was significant $p<0.05$ improvement in all plasma lipid parameters, TCh/HDL-Ch, MDA, glucose and the inflammatory marker Interleukin-6. This improvement pointed to the health benefit of consuming both oils in dyslipidemic subjects. The RSO diet consumption showed more improvement in MDA and interleukin-6 compared to sunflower diet reflecting the antioxidant and anti-inflammatory effect of RSO which may be related to the presence of phenolic compounds, flavonoids, tocopherols and tocotrienols in RSO seen in the present study and the previously reported phytosterols[43]. The *in vitro* antioxidant activity of RSO proved in the present study supported the reducing power towards both MDA and interleukin-6 in the animal experiment. The antioxidant activity of RSO was also confirmed by the ABTS radical scavenging activity in the current work and by the study of Mohd-Esa *et al*.[44] that related the antioxidant activity to strong scavenging activity of reactive oxygen species and was ascribed to the phenolic content of RSO. The anti-inflammatory effect of *Hibiscus sabdariffa* was reported to be mediated by reduction of interleukins and tumor necrosis factor-alpha[45] which agreed with the present study that showed reduction of Int.6 by RSO. *Hibiscus sabdariffa* flower aqueous extract could ameliorate diabetic nephropathy via improving oxidative status and regulating Akt/Bad/14-3-3γ signaling. Also it was capable of reducing the plasma levels of glucose, lipid peroxide and improving lipid profile in diabetic rats. The effect was attributed to phenolic compounds[46]. So, the presence of phenolic compounds in RSO could have a hand in the reduction of plasma glucose, MDA and improved dyslipidemia in the present study. Although many studies showed hepato and reno-protective, antioxidant, anti-diabetic, anti-inflammatory and hypolipidemic effect of polar extracts of roselle calyx and flower [45-51] however, scarce literature were only available concerning the remedial effect of RSO. In a previous study[37], RSO produced significant reduction in serum cholesterol, LDL-Ch and the atherogenic index compared to rats fed on coconut oil diet which agreed with the present study. The composition of fatty acids in RSO could influence plasma lipid profile, this includes the length of fatty acids and degree of unsaturation. The high percentage of oleic acid in RSO as shown in the present study could afford CVD protective effect. It was reported by Mohamed et al.[30] that plant extracts rich in oleic acid improved lipid profile and reduced oxidative stress in rats fed hyperlipidemic diets. Oleic acid showed previously to have beneficial effect in early events of atherosclerosis[52] since it decreased lipoprotein susceptibility to oxidation[53]. *In vitro* studies showed oleic acid to prevent endothelium activation by inhibiting nitric oxide production and the expression of adhesion molecules[54]. Also oleic acid reduces endothelial cell sensitivity to oxidants, creates a pro-oxidant environment by reducing reactive oxygen species[55]. The anti-atherogenic property of RSO shown by reduction of T-Ch/HDL-Ch in the present study might also be related to the high concentration of linoleic acid as

demonstrated previously[56]. It was reported by Nyam *et al.*[57] that RSO could be added to sunflower oil instead of synthetic antioxidant to prevent its auto-oxidation and to extend its shelf life. The same authors ascribed RSO antioxidant effect to the presence of phenolics, vitamin E and flavonoids which agreed with the present study.

The safety of RSO up to the highest tested dose in the acute toxicity test in the present study (10 g kg^{-1} mouse body weight) reflected its possible consumption as dietary oil. The dose of 10 g kg^{-1} mouse body weight corresponds to about 78 g/70 kg man body weight for human when the dose of mice was extrapolated to corresponding estimates in human adopting interspecies dosage conversion scheme[58] which reveals high safety of RSO. During the two rat experiments conducted in the present study, normal healthy and dyslipidemic rats were fed diet at 10% level of RSO for 4 weeks without showing any signs of toxicity or disturbance in either liver or kidney function or even death. If translating the 10% RSO in the diet to dose according to food intake and rat body weight, it could be accounted for about 12 g RSO/kg rat body weight that pointed to the highest safety on chronic administration. It is worthy to mention that no toxicity studies were found on RSO in the literature however some toxicity reports on polar extract (aqueous and alcoholic) of different parts of roselle other than the seeds showed a very low degree of toxicity at very high doses. Also, previous literature[6] did not reveal any case reports of adverse reaction following oral consumption of *Hibiscus sabdariffa* preparations. However, a single report has suggested that excessive doses of *Hibiscus sabdariffa* calyx extract for relatively long periods could have a deleterious effect on the testes of rats[59].

CONCLUSION AND FUTURE RECOMMENDATION

- RSO is rich in bioactive constituents represented by total phenolics, flavonoids and vitamin E especially gamma tocopherol with a unique balanced fatty acid ratio (S:M:P) refers to its possible direct human consumption
- RSO has high stability against oxidation as well as high *in vitro* antioxidant activity and high safety
- RSO is safe concerning liver and kidney functions as well as cardiovascular system and antioxidant/oxidant system when fed to normal rats
- RSO has a remedial effect towards cardiovascular diseases through reduction of inflammatory biomarker, lipid peroxidation and the atherogenic ratio (T.Ch/HDL-Ch)
- The stability and health benefits of RSO seem to be attributed to its bioactive constituents
- Within the extreme of the present study, it is proposed that RSO is suitable as edible oil for human consumption

Accordingly, roselle seed is recommended as a new source of edible and healthy oil. In this concern, it is important to pay more attention to pick the non-traditional sources to produce edible oil.

SIGNIFICANCE STATEMENTS

This study discovers new cheap source of edible oil that can be beneficial for human consumption and health. The present research studied for the first time the safety and stability of roselle seed oil as non-traditional source of edible oil from special point of view. This could represent a solution for the nutritional gap for edible oil in developing countries.

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