A Possibility of utilization of Mallow (Malva sylvestris) and Hummayd (Rumex vesicarius) leaves for improving serum lipid profiles in hypercholesterolemic rats

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Abstract

Mallow and hummayd leaves are Egyptian countryside common leafy vegetables. The aqueous extract of these leaves were used for estimating their total phenols and flavonoids contents and measuring the antioxidant activity. As well, mallow and hummayd leaves were cooked and mixed in ratios of 1:3, 1:2, 1:1, 2:1 or 3:1, respectively, and mixed with some natural additives for producing natural soup. The sensory evaluation of these products was carried out. Then, the biological assessment was done on rats fed a hypercholesterolemic diet to measure the effect of orally feeding mallow and hummayd leaves soup on the serum total fat and cholesterol levels. Also, body weight gain, food intake and food efficiency rate were measured.

Results showed that the total phenols and flavonoids content in the aqueous extract of the mallow leaves were 31.68 mg gallic acid equivalent (GAE) /g and 17.51mg quercetin acid equivalent (QE)/g, respectively. While the total phenols and flavonoids contents were 23.4 mg GAE/g and 12.30 mg QE/g, respectively, in the aqueous extracts of hummayd leaves which is directly proportional to the antioxidant activity at rates 45.24% of mallow leaves and 28.13% of hummayd leaves. Generally, the sensory evaluation of these products showed high degrees for sensory acceptance. The 3 mallow: 1 hummayd leaves soup had the highest score. The results of the biochemical assessment showed that there was a significant decrease in the incidence of all levels of total cholesterol, triglycerides, low-density lipoprotein and very low density, while it was noted a significant increase in the level of high-density lipoprotein with increasing doses intake of blend soup.

In general, the current study illustrated the importance of mallow and hummayd leaves consumption and their values in reducing the weight and improving lipid profile. The study recommends, also, the inclusion in nutrition education programs to illustrate the importance of green leafy vegetables especially mallow and hummayd for being cheap and easy obtain.

Introduction

Leafy vegetables are known for their high minerals content. In particular, they are of interest for their therapeutic value, as being rich source of antioxidant, vitamins and pigments (*Velvan et al., 2007 and Buyukokurglu et al., 2001*). In food application, impetus of exploring natural antioxidant has given more interest to eliminate the synthetic product/drug. Oxidative damage is a critical etiological factor implicated in several chronic diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative disease and also in the ageing process (*Schuler, 1990*).

Malva sylvestris belongs to the Malvaceae family. It is commonly known as common mellow in Europe (Mustafa and Ali 2011), khubaza in Egypt, tole in Iran, malva in Portugal, marva in Italy, and ebegümeci in Turkey (Tabaraki et al., 2012). Although it is found all around the world as a weed, its native regions are South Europe, North Africa, and

Southwest Asia. The plant preferably grows in damp places like near oceans, marshes, ditches, river banks, and meadows (*Razavi et al., 2011*). It has shown potential therapeutic effects against various diseases (*Jan et al., 2009; Pirbalouti et al., 2009 and Marouane et al., 2011*).

Rumex vesicarius, locally called sorrel, hummayd and hammad (sour-wort), is a wild leafy plant that belongs to the family of Polygonaceae (Mandaville, 1990). Hummayd is a shrub that grows in the central and northern regions of Egypt and Saudi Arabia (Tukan et al., 1998) and common in other parts of the world, e.g. in India (Rao et al., 2011). The raw leave may be used as a salad ingredient added during the preparation of (dried milk shared) to increase the acidity (Tukan et al., 1998). The whole plant is medicinally important and cures several diseases. The plant is stimulant, tonic and acts as an aphrodisiac agent (El-Bakry et al., 2011). The plants of rumex, a large genus containing about 250 species, are known for different pharmacological activities including antimicrobial, anti-inflammatory, anti-analgesic and antioxidant (Tavares et al., 2010 and Gautam et al., 2010). In folkloric medicine, the plants are used for the treatment of tumors, cancers and diseases related to liver and urinary/kidney functions (Kathi and Kemper 1999).

Free radicals are highly reactive compounds, they are chemical species associated with an odd or unpaired electron and can be formed when oxygen interacts with certain molecules. They are neutral, short lived, unstable and highly reactive to pair with the odd electron and finally achieve stable configuration. Once formed these highly reactive radicals can start a chain reaction they are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cells may poorly function or die if this occurs (*Ames et al., 1993*).

Although in dietary practice, healthy plant-based diets do not necessarily have to be low in fat. Instead, these diets should include unsaturated fats as the predominant form of dietary fat (e.g., fats from natural liquid vegetable oils and nuts), an abundance of fruit and green vegetables, and adequate n-3 fatty acids. Such diets, which also have many other health benefits, deserve more emphasis in dietary recommendations to prevent chronic diseases (*Suido et al., 2002*) Hyperlipidemia as a major risk factor of atherosclerosis is treated with different drugs. Concerning length of therapy and vast majority of side effects, herbal medication may be suitable for these drugs (*Kojuri et al., 2007*).

The current study aims to assess the mallow and hummayd leaves and their contents of total phenols and antioxidants activity. As well, production of mallow and hummayd leaves soup at different mixing levels, sensory evaluation and study the impact of the best treatment soup- obtained from sensory evaluation – on lipid profile in rats fed on hypercholesterolemic diet.

Materials and Method

Hummayd (*Rumex vesicarius*) and Mallow (*Malva sylvestris*) leaves were purchased from local market in Cairo. The plant was identified by Botany Department, Faculty of Agriculture, Menoufeya University and well preserved.
 Cholesterol (white crystalline powder), bile salt, casein, vitamins, minerals and cellulose were obtained from El-Gomhoriya Pharm. and Chem. Ind. Comp., Cairo, Egypt. Starch and corn oil were obtained from a local market.

Methods:-

Preparation of mallow and hummayd extracts:

Fresh mallow and hummayd leaves were dried and then powder-grinded. 25 g of each of the obtained dry powder was boiled separately in 100 ml of water for 10 min. and then filtered.

Chemical analysis:

- 1- Total phenols and flavonoids contents in each plant materials were determined by folin-ciocalteu reagent by *Arnous* et al., (2001) and Joyeux et al., (1995), respectively.
- 2- Antioxidant activity of mallow and hummayd leaves were determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method according to method of *Brand-Williams et al., (1995).*
- 3- Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were measured using an enzymatic colorimetric test (Boehringer Mannheim Kits) according to Allain et al., (1974), Fossati and Prencipe (1982) and Lopes-Virella et al., (1977) methods, respectively. Very low density lipoprotein cholesterol (VLDL-c) and Low density lipoprotein cholesterol (LDL-cholesterol) were calculated using Friedewalde equations (Friedewalde et al., 1972) as follows:

VLDL (mg/dl) = Triglycerides/5 LDL (mg/dl) = (Total cholesterol – HDL) – VLDL

HDL-C/TC and LDL-C/HDL-C ratios were calculated.

Preparation of mallow and hummayd leaves soup:

Tomatoes, onions, garlic were chopped and cooked with clear chicken soup in the cooking pot about 15 min. and covered. Then, the chopped mallow and hummayd leaves, salt, rice and cumin were added. All components were cover again and cooked about 15 min. The Ingredients of the prepared mallow and hummayd leaves soup were illustrated in table (1). An immersion blender was used. In the biological experiment, the puree was filtered to obtain clear vegetables soup.

Ingredients	The tested ratios of mallow : hummayd					
	0:0	3:1	2:1	1:1	1:2	1:3
Mallow (g)	-	112.5	100	75	50	37.5
Hummayd (g)	-	37.5	50	75	100	112.5
Clear chicken soup (ml)	400	250	250	250	250	250
Tomato (g)	80	80	80	80	80	80
Onion (g)	50	50	50	50	50	50
Butter (g)	5	5	5	5	5	5
Garlic (g)	5	5	5	5	5	5
Rice (g)	10	10	10	10	10	10
Salt (g)	2	2	2	2	2	2
Cumin (g)	3	3	3	3	3	3

 Table (1):

 The Ingredients of the prepared mallow and hummayd leaves soup.

Organoleptic evaluation:

The organoleptic characteristics of the stuffed vegetables leaves were done according to *Larmond (1970)*. Ten panelists were asked to evaluate the appearance, viscosity, odour, taste and colour attributes of the prepared soup using score of 10 for each character. The average score for each character was calculated

Experimental design:

Thirty male albino rats (weighing 125±10g) were housed in well-aerated wire cages. All animals were kept under a normal healthy condition and fed on basal diet for one week for adaptation. The basal diet consisted of casein (10%), cellulose (5%), corn oil (10%), salt mixture (4%), vitamin mixture (1%) and the remained amount was starch according to *Lane-Peter and Pearson (1971)*. After the adaptation period (7 days), they were divided into five groups, (6 rats in each group). The experimental animals were divided according the following scheme:

-The first group (negative control C-) was fed on basal diet only.

- The second group (positive control C+) was fed on hypercholesterolemic diet (basal diet mixed with 1% cholesterol, 0.25% bile salt and 15% beef tallow) in order to be hypercholesterolemia (*Abdel- Raheim et al., 1995*).

-The third group (T1) was fed on hypercholesterolemic diet and orally given 2ml of the prepared soup without hummayd and mallow leaves (1 ml at morning and 1ml at night).

- The fourth group (T2) was fed on hypercholesterolemic diet and orally given 2ml of the prepared (hummayd + mallow at rate 1:3 respectively) leaves soup (1 ml at morning and 1ml at night).

- The fifth group (T3) was fed on hypercholesterolmic diet orally given 4 ml of the prepared (hummayd + mallow at rate 1:3 respectively) leaves soup (2 ml at morning and 2ml at night).

During the experimental period (4 weeks) each rat was weighed every week and food consumption was recorded. The body weight gain (BWG) and food efficiency ratio (FER) were determined according to *Champman et al.,* (1959) by using the following formulas:

BWG = [final weight (g) – initial weight (g)]

FER = [Body weight gain (g)/food intake (g)] x 100

At the end of the experimental period, the tested rats were fasted over night before sacrificing and the blood was collected then centrifuged. The serum was separated and stored until analysis.

Statistical analysis:

Statistical analysis were performed by using computer program Statistical Package for Social Science (SPSS, 1998) and compared with each other using the suitable tests.

Results and Discussion

Total phenols and flavonoids contents:

Data presented in Table (2) observed that both plants extracts have high total phenolic and flavonoid contents. Mallow leaves aqueous extract have the higher phenolic value (31.68 mg GAE /g) and flavonoid (17.51 mg QE /g) contents, compared to hummayd (23.40 mg GAE /g and 12.30 mg QE /g, respectively). Several plants have been studied as sources of potentially safe natural antioxidants for the food industry and various compounds have been isolated with many of them being polyphenols. Previous results run parallel with *EI-Hawary et al., (2011)* and *Londonkar and Tukappa (2013)* reported that extract of *Rumex vesicarius* had high amount of phenolic compounds. Recent studies have shown, also, that many flavonoids and related polyphenol significantly contribute to the total antioxidant activity in fruits and vegetables *(Gil et al., 2000 and Marja et al., 1999).*

DPPH Free Radical Scavenging Assay:

DPPH is a free radical stable at room temperature and produces a purple color solution in water. It is reduced in the presence of antioxidant molecule, giving a rose to yellowish aqueous solution. One of the mechanisms involved in antioxidant activity assay is the ability of a molecule to donate a hydrogen atom to a radical and the propensity of the

hydrogen donation is the critical factor involved in free radical scavenging (*Jelili et al., 2011*). Also, Table (2) showed the free radical scavenging activity of mallow and hummayd leaves extracts were assessed by the DPPH test. Both samples showed good free radical scavenging activities. The extract of mallow leaves showed 45.24% free radical inhibition followed by hummayd which expressed 28.13% inhibition.

Sensory evaluation:

Table (3) shows the sensory evaluation for different rates for mallow and hummayd leaves soup. Generally, all samples were acceptable by the sensory evaluation panel. The 3 mallow: 1 hummayd leaves soup had the highest score (97.87±0.57) followed by 2 mallow: 1 hummayd leaves soup (96.15±0.78), while 1 mallow: 3 hummayd had the lowest score (92.65±0.35). This may attributed to the acidic taste of hummayd leaves *Tukan et al., (1998).* This led the author to use the mixture of 3mallow:1 hummayd in the experimental design.

Nutrition parameters:

Table (4) shows the mean values ± SD of body weight gain (BWG), food intake (FI) and food efficiency ratio (FER) (%) of rats as affected by different levels of mallow and hummayd contents of leaves soup. Results show that the highest mean value of body weight gain (BWG) (36.95±1.31g) was found in group positive control (C+), while the groups that received the tested soups had BWG average more close to that of the normal group (34.48±2.05, 32.60±1.26 and 29.94±2.15 g for groups T1, T2 and T3, respectively). Food intake showed an amount of 350.50±1.23g for the negative group while the control positive, T1, T2 and T3 groups recorded lower values were (294.75±2.70, 298.70±3.10, 309.50±2.35 and 322.25±2.98g), respectively.

Although positive control group demonstrated the highest values of weight BWG and FER, the other hypercholesterolemic groups that received the tested soups with different levels of mallow and hummayd were significantly lower values compared with those of both negative and positive groups. Also, results demonstrated that with increasing the food intake of mallow: hummayd soup, BWG and FER values were decreased. The effect of both mallow and hummayd addition was proportional to their level, which might be ascribed to the higher fiber content of these diets. These diets are in the same line with those of **Matos et al., (2005).** The efficiency of these treatments in reducing body weight gain and food efficiency ratio could be a potential for reducing body weight and thus treating obesity.

Serum lipid in hypercholesterolemic rats:

Data presented in Table (5) demonstrated that rats fed with high cholesterol diet for seven days exhibited a significant increase in TC, TG, and LDL-c and a significant decrease in HDL-c as compared to normal animals group. The oral administration of mallow and hummayd leaves soup at levels 1 and 2 ml twice daily caused a significant decrease in serum LDL-c, TG and TC levels with a significant increase in HLD-c (p<0.05) as compared to high cholesterol diet (positive) control group. The highest decrease level was shown in T3 group (rats fed on hypercholesterolemic diet and received orally 2ml mallow and hummayd of leaves soup twice daily), followed by T2 group (rats fed on hypercholesterolemic diet and received orally 1ml mallow and hummayd of leaves soup twice daily) with mean values of 113.73±2.19 mg/dl and 124.26±3.65 mg/dl, respectively, for total cholesterol and 121.21±1.99 mg/dl and 131.40±3.29 mg/dl, respectively, for triglyceride.

In the same Table, the presented data, also, showed that the positive control group had a significant decrease in serum HDL-c level of (31.27±4.89 mg/dl), while the negative group value was 45.32±3.25 mg/dl. Rats fed on hypercholesterolemic diet with orally mallow and hummayd of leaves soup at 1 and 2 ml daily caused a significant increase in serum HDL-c level at p<0.05 and p<0.01 (42.77±4.21 and 46.34±5.13 mg/dl, respectively) compared with the positive group. The results revealed that T2 and T3 groups had significant decrease in LDL-c and TG serum levels at p<0.001 as compared to the positive group. Generally, groups fed on different levels of mallow and hummayd of leaves soup twice daily showed a significantly decrease in serum LDL-c and VLDL-c levels compared to the positive control group. While soup without mallow and hummayd leaves (group T1) caused no significant decrease in serum VLDL-c,

triglycerides and total cholesterol levels (35.68±0.39, 178.41±2.81 and 153.57±3.21 mg/dl, respectively) compared to the positive control group.

This results agreed with that reported by several studies (*Suido et al., 2002* and *Corinna et al., 2005*), who reported that daily consumption of green vegetable beverage is useful in lowering serum TC and LDL-c levels in hypercholesterolemic rats. Evidence from prospective cohort studies indicates that a high consumption of plant-based foods such as fruits and green vegetables, nuts, and whole grains is associated with a significantly lower risk of coronary artery disease and stroke. The consumption of leaf vegetable, also, with a hypercholesterolemic diet improved the lipidemic profile and increased excretion of the total cholesterol end-products (*Ezz El-Arab, 2009*).

Effect of different dosages of mallow and hummayd leaves soup on risk ratios for rats fed on hypercholestrolemic diet was observed in Table (6). Results showed that the highest risk ratio (I) level for T3 group was 0.40 followed by T2 group being 0.34 compared to C+ group being 0.19. While, the risk ratio (II) results of T3 group recorded the lowest value was 0.93 followed by T2 being 1.29 compared to C+ group being 3.06.

Conclusion:

From previous results, it could be concluded that the mallow and hummayd soup improve lipid profiles in experimental model. Therefore, these leafy vegetables are recommended to be mentioned in nutrition education programs. Since it prevent the development of hypercholesterolemia and hyperlipidemia.

	Table	e (2):		
Total phen	ols and flavonoids contents and DPPH scaven	ging activities of Humn	nayd and Mallow leaves extract	s.
	T Phenols	Elavonoide		

	T.Phenols	Flavonoids	DPPH
	(mg GAE /g)	(mg QE /g)	(%)
Mallow	31.68	17.51	45.24
Hummayd	23.4	12.30	28.13

GAE: Gallic acid equivalent

QE: quercetin acid equivalent

Table (3): Sensory evaluation of different rates of mallow and hummayd leaves soup.

Mall.:Hum.	Color	Odor	Taste	viscosity	Appereance	Overall
	(20)	(20)	(20)	(20)	(20)	(100)
1:1	19.60±0.10	19.50±0. 10	17.00±0.20**	19.50±0.05	18.00±0.20*	93.77±0.14**
2:1	19.65±0.05	19.50±0. 10	18.50±0. 50*	19.50±0.10	19.00±0.11	96.15±0.78*
3:1	19.85±0. 10	19.50±0.10	19.50±0.25	19.55±0.05	19.46±0.11	97.87±0.57
1:2	19.50±0. 10	19.05±0.05	17.35±0.20**	19.70±0.10	18.00±0.10*	93.60±0.35**
1:3	19.00±0.20	19.05±0.05	16.00±0.20***	19.60±0.10	19.00±0.20	92.65±0.35***

Differences are significant at 5 % (P<0.05). ** Differences are significant at 1 % (P<0.01). *** Differences are significant at 0.1 % P<0.001)

Table (4):

Effect of different dosages of mallow and hummayd of leaves soup on body weight gain (BWG), food intake (FI) and food efficiency ratio (FER) for rats fed on hypercholestrolemic diet.

Groups	Body weight gain	(FI)	FER
	(g/28days)	(g/28 days)	(%)
Negative Control (-)	28.51±0.21	350.50±1.23	8.13±1.07
Positive Control (+)	36.95± 1.31	294.75±2.70	12.53±2.05
T1	34.48±2.05	298.70±3.10	11.54±1.30
T2	32.60±1.26	309.50±2.35	10.53±1.54
Т3	29.94±2.15	322.25±2.98	9.30±1.43

T1:1ml of soup without mallow and hummayd leaves twice daily.

T2:1ml mallow and hummayd of leaves soup twice daily.

T3:2ml mallow and hummayd of leaves soup twice daily.

Table (5):

Effect of different dosages of mallow and hummayd leaves soup on lipid profile for rats fed on hypercholestrolemic diet.

Experimental groups	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	Triglycerides)mg/dl(T.cholesterol)mg/dl(
Normal Control (-)	45.32±3.25	23.29±2.47	20.64±0.12	103.20±1.35	89.25± 2.15
Positive Control (+)	31.27±4.89	95.71±2.50	37.10±0.27	185.51± 3.58	164.08±2.10
T ¹	30.22±5.21	87.67±3.86*	35.68±0.39	178.41±2.81	153.57±3.21
T ²	42.77±4.21**	55.21±3.14***	26.28±0.25*	131.40±3.29***	124.26±3.65**
T ³	46.34±5.13***	43.15±2.07***	24.24±0.19***	121.21±1.99***	113.73±2.19***

* Differences are significant at 5 % (P<0.05). ** Differences are significant at 1 % (P<0.01). *** Differences are significant at 0.1 % (P<0.001

^{T1}:1ml of soup without mallow and hummayd leaves twice daily.

T²:1ml mallow and hummayd of leaves soup twice daily.

T³:2ml mallow and hummayd of leaves soup twice daily

Table (6):

Effect of different dosages of mallow and hummayd leaves soup on risk ratios for rats feed on hypercholestrolemic diet.

Groups	Risk ratio (I)	Risk ratio (II)
	(HDL/TC)	(LDL/HDL)
Control (-)	0.51	0.51
Control (+)	0.19	3.06
T ¹	0.20	2.90
T2	0.34	1.29
T ³	0.40	0.93

T¹:1ml of soup without mallow and hummayd leaves twice daily.

T²:1ml mallow and hummayd of leaves soup twice daily.

T³:2ml mallow and hummayd of leaves soup twice daily

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امكانية الاستفادة من أوراق الخبيزة والحميض في تحسين صورة دهون الدم بفئران التجارب المصابة بارتفاع الكوليستيرول

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الملخص العربى

تعتبر أوراق الخبيزة والحميض من أشهر الخضروات الورقية الشائعة بالريف المصرى. تم الحصول على المستخلص المائى لتلك الاوراق بغرض تقدير محتواها من الفينولات الكلية والفلافونيدات وقياس النشاط المضاد للأكسدة. كما تم طهى خليط من أوراق الخبيزة والحميض بنسب خلط ٢:١، ٢:١، ٢:١، ٢:١، على التوالى مع بعض الاضافات الغذائية لانتاج حساء طبيعى.وقد تم اجراء التقييم الحسى لتلك المنتجات ثم التقييم البيولوجي -لأفضل تلك النسب المقبولة حسيا- على فئران التجارب المغذاه على غذاء عالى الدهن (لاحداث الاصابة بارتفاع الدهون بالدم) لقياس مدى تأثير التغذية الفموية للحساء على مستويات الدهون الكلية والكوليسترول بأنواعه. كما تم قياس مقدار الزيادة في الوزن وكمية الغذاء المتناول بالاضافة الى تقدير معدل كفاءة الغذاء.

أظهرت النتائج أن محتوى الفينولات الكلية والفلافونيدات بالمستخلص المائى لأوراق الخبيزة يصل الى ٣١,٦٨ ملجم/ جم و ١٧,٥٠ ملجم/ جم على التوالى بينما سجل مقدار الفينولات الكلية والفلافونيدات ٢٣,٤٠ ملجم/جم و ١٢,٣٠ ملجم/جم على التوالى للمستخلص المائى لاوراق الحميض ، وهو ما انعكس طرديا على النشاط المضاد للأكسدة للمستخلص المائى لتلك الأوراق بنسب ٤٥,٢٤% لأوراق الخبيزة وبنسبة ٣٢,١٣٨ لأوراق الحميض. كما أوضحت نتائج التقييم الحسي بوجه عام ارتفاع درجات القبول الحسى لتلك المنتجات بمعدلات الاضافة المختلفة. وقد سجل خليط الحساء المكون بنسبة ٣ خبيزة: ١ حميض أعلى درجات القبول وهو ما تم استخدامه فى التقييم البيولوجى. بينما أظهرت نتائج التحليل الكيميائى لصور دهون الدم حدوث انخفاض معنوى فى كل من مستويات الكوليسترول الكلى والجليسريدات الثلاثية (TG) والليبوبروتينات المنخفضة الكثافة (LDL-c) والمنخفضة الكثافة بدرجة كبيرة جدا (VLDL-c)، بينما تلاحظ زيادة معنوية فى مستوى الليبوبروتينات عالية الكثافة (LDL-c) مع زيادة الجرعات المتناولة من الحساء. تلاحظ ايضا رحال (ال

خلصت الدراسة الى أن استهلاك أوراق الخبيزة والحميض يؤدى الى خفض الوزن وتحسين صورة دهون الدم وخفض معاملات الخطورة. توصى الدراسة باعداد برامج للتثقيف الغذائى يوضح من خلالها أهمية الخضروات الورقية وخاصة أوراق الخبيزة والحميض فى التغذية خاصة مع رخص ثمنها وسهولة الحصول عليها.