Egypt. J. of Appl. Sci., 31 (11) 2016 *62-75*

UTILIZATION OF ARTICHOKE LEAVES AS HEALTHY DRINK

Amani A.A. Metwalli ; Samia A. Al-Askalany and M.S. Negm
Food Tech. Res. Inst., Agric. Res. Center., Giza, Egypt.

ABSTRACT

Artichoke is a traditionally consumed vegetable in many countries. The agro-industrial by-products of artichoke about 80% of the plant biomass. Artichoke leaves dried by two different ways. From the results obtained in this study, it could be noticed that, artichoke is being a rich source in polyphenols and flavonoid compounds. The sensory evaluation of artichoke drink showed that, hot air dried leaves displayed higher values of various quality attributes than microwave dried leaves. Artichoke leaves extract (ALE) is effective at lowering LDL-C concentration, and improve HDL-C levels. Moreover, ALE can utilize (oven dried leaves) as a healthy drink with good acceptability.

INTRODUCTION

Economic analysis showed that production of one product was expensive, therefore, production of numerous products from the same crop is a must **(Johansson *et al.*, 2015).**

Artichoke (*Cynara scolymus* L.) is a traditionally consumed vegetable in many countries. It is widely cultivated in the Mediterranean regions **(Fratianni *et al.*, 2007).** Artichoke can be eaten as a fresh, canned or frozen vegetable **(Costabile *et al.*, 2010)**. Artichoke is one of the world's oldest medicinal plants. It has been known by the ancient Egyptians **(Xia *et al.*, 2014).** It has been used in folk medicine since Roman times (**Pandino *et al.*, 2011b**). Artichoke has long been used as a treatment for liver ailments, it may regenerate liver tissue **(El Sohaimy, 2013)**. It is considered a healthy food due to its composition. It contains a high proportion of phenolics **(Fratianni *et al.*, 2007)**.

The agro-industrial by-products of artichokes such as leaves, external bracts and stems which amount to about 80% of the plant biomass and could be a hopeful and cheap source of health-promoting polyphenolic compounds (**Pandino *et al.*, 2011a).** There is a relationship between antioxidant activity and phenolic compounds as reported by **Michiels *et al.*, (2012).** Artichoke is a good source of antioxidants **(Lutz *et al.*, 2011 and Metwally *et al.*, 2011).** It has cynarin and silymarin, these have ability to lower cholesterol, increase bile production, protect and support liver function, and impede gallstones. Artichoke leaf extract can reduce the symptoms of irritable bowel syndrome and improve fat digestion **(Bundy *et al.*, 2004).** Artichoke has cholerectic, hypocholesterolemic, hypolipidemic properties **(Wider *et al.*, 2009 and**

Küskü-Kiraz *et al.*, 2010), hepatoprotective (**Kucukgergin *et al.*, 2010)**, antifungal **(Lopez-Molina *et al.*, 2005)** and antimicrobial activities **(Zhu *et al.*, 2004).**

Chlorogenic acid is the major bioactive component of the artichoke and associated with its anti-inflammatory, antiallergenic, anti-thrombotic, cardio-protective, vaso-dilatatory and hepatoprotective activity **(Pandino *et al.*, 2012 and Abu-Reidah *et al.*, 2013).** Many of these properties are because of antioxidant activity of phenolic compounds **(Yuan *et al.*, 2012 and Garbetta *et al.*, 2014).** Because of the pharmacological and nutritional properties of the artichoke have greatly increased the demand of global artichoke and consequently its production **(Guida *et al.*, 2013)**

Nowadays, they are considered as functional foods, so, a wider use of artichoke is expected. **(Christaki *et al.*, 2012)**

The aim of our investigation is using artichoke leaves which are rich in bioactive compounds, particularly, polyphenolic compounds as healthy drink

MATERIALS AND METHODS

Materials:

1- Artichoke leaves were obtained from food factory as by-products. The leaves were washed, dried by two different ways of drying, ground and kept in polyethylene bags at 4^{o}C until used.
2- Chemicals used in this study were purchased from El-Gomhoria Company, Egypt.

Drying methods

1- Combined microwave-air oven (MW-AO).The microwave (MW) treatment was performed in a domestic digital microwave oven (LG model MH7948AS, LG Electronic. Ltd., Changwon, Korea) with technical features of 220 V, 50 Hz, 1000 W and a frequency of 2450 MHz. Leaves were applied to MW power at 800 W for 120 secs. MW process was stopped 5 seconds every 30 sec during the treatment to release the entire moisture as intermittent action. For combined microwave-air oven (MW-AO), the air oven was adjusted at 40°C for four hours to the MW pretreated samples.
2- Classical air oven drying at 50°C (48 h). The dried materials were ground and kept in polyethylene bags at 4^{o}C until used.

Extraction, fractionation and identification of phenolic compounds.

Four g of dried artichoke leaves were homogenized with a homogenized IKA RW20 Germany and extracted by stirring with 100 ml aqueous methanol (60%, v/v) for 1 h at ambient temperature according to the method described by **Schutz *et al.*, (2004)**. After filtration through a filter paper, the extracts were evaporated to dryness in vacuo at 30°C.

The residue was dissolved in methanol to yield a concentration (w/v) of 10 mg/mL. The polyphenolic compounds of artichoke extracts were fractionated and identified for phenolic compounds by HPLC, according to the method described by **Pinto *et al.*, (2008)**. Identification of individual phenolic compounds was performed on Hewlett- Packard HPLC (Model 1100), using a hypersil C18 reversed- phase column (25 × 4.6 mm) with 5µm particle size. Injection was done by means of a Rheodyne injection value (Model 7125) with 50µl fixed loop.

Preparation of artichoke leaves drink

The artichoke leaves drink was prepared by adding 1000 ml of boiling water to 15 g of leaves and left for 5 min. then, Poured in cups

Sensory evaluation of artichoke leaves drink

Sensory evaluation of artichoke leaves drink were evaluated by ten panelists for various quality attributes such as appearance, taste, color and flavor, on a 1 to 10 hedonic scale.

Biological experiment:

Thirty male albino rats (140–145 g) were housed individually in wire cages (duration of the experiment 56 days), and fed on basal diet for 7 days to acclimate them to our facility, according to **AOAC, 2000**, Salt mixture and vitamin mixture were prepared as described in **AOAC, 1990**. Rats were divided into five groups of 6 each. One group was fed on basal diet only (control 1). The diet of other groups was supplemented with cholesterol (1%) and bile salts (0.25%) for 7 days **(Zamora *et al.*, 1991)**. One group was kept as control 2, (fed on hypercholesterolemic-inducing diet only). The remaining three groups were fed on cholesterol diet and artichoke leaves extract (ALE) at 1.5%, 3% and 4.5% levels till the end of the experiment. All the rats were administrated orally (by stomach tube) with ALE. ALE prepared as described by **Abdel Magied *et al.*, 2016.**

Serum triglyceride **(Fossati and Principe 1982)**, total cholesterol **(Richmond, 1973 and Allain *et al.*, 1974)**, HDL-cholesterol **(Burstein *et al.*, 1970 and Lopes-Virella *et al.*, 1977)** were measured by enzymic-colorimetric procedures. LDL-cholesterol concentration was calculated as the difference between total and HDL- cholesterol **(Friedewald *et al.*, 1972)**. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activities **(Reitman and Frankel 1957)**, and creatinine **(Schirmeister, 1964)** were determined colorimetrically.

Statistical analysis.

The data were statistically analyzed using the analysis of variance as out lined by **Snedecor and Cochran, (1980).**

RESULTS AND DISCUSSIONS

Identification of phenolic compounds for dried artichoke leaves.

Data given in Table (1) represent values of phenolic compounds (mg/100g) for dried artichoke leaves (AL) in relation to drying treatment. From artichoke dried leaves 24 phenolic acids were HPLC detected: Gallic, pyrogallol, 4-Amino-benzoic, Protocatchuic, Catechin, Chlorogenic, Catechol, Epicatechein, Caffeine, P-OH-benzoic, Caffeic, Vanillic, P-Coumaric, Ferulic, Iso-Ferulic, Resveratrol, Ellagic, E-vanillic, Alph-Coumaric, Benzoic, 3,4,5 methoxy, Coumarin, Salycillic, cinnamic. Among phenolic compounds, Chlorogenic acid was the most abundant followed by pyrogallol and Ellagic. Obviously, the content of chlorogenic, pyrogallol and Ellagic were very high in leaves of artichoke treated with MW-AO (321.70, 226 and 105.8 mg/100g respectively), while lower contents were observed in conventional air oven dried leaves (196.3, 70.33 and 52.8 mg/100g respectively). Artichoke is being a rich source in polyphenols. The results are in agreement with (**Pandino *et al.*, 2011b; Pandino *et al.*, 2012 and Abu-Reidah *et al.*, 2013)**

Table (1): Phenolic acids contents (mg/ 100g) in artichoke dried leaves in relation to drying treatment.

Phenolic acids	MW + Oven dried leaves	Oven dried leaves
Gallic	14.20	6.31
Pyrogallol	226.00	70.33
4-Amino-benzoic	1.20	0.80
Protocatchuic	9.10	7.28
Catechin	29.23	9.50
Chlorogenic	321.70	196.30
Catechol	94.90	74.20
Epicatechein	7.50	7.27
Caffeine	2.30	1.40
P-OH-benzoic	13.20	8.70
Caffeic	2.40	0.60
Vanillic	20.83	16.56
P-Coumaric	27.52	23.62
Ferulic	5.60	6.10
Iso-Ferulic	30.10	7.20
Resveratrol	2.50	2.40
Ellagic	105.80	52.80
E-vanillic	62.32	41.68
Alph-Coumaric	1.10	1.77
Benzoic	82.70	24.90
3,4,5 methoxy	2.80	0.20
Coumarin	2.00	0.60
Salycillic	5.20	2.52
Cinnamic	1.40	0.35
Total Phenolic acids	1071.60	563.40

Identification of flavonoid compounds for dried artichoke leaves.

Flavonoids possess hypocholesterolemic and antioxidant properties via reduction in de novo cholesterol synthesis and free radical scavenging activity **(Bundy *et al.*, 2008)**.

Data given in Table (2) demonstrate values of flavonoid compounds for dried artichoke leaves (mg/100g) in relation to drying treatment. 11 flavonoid compounds for artichoke leaves were identified. The flavonoids content, calculated as the sum of individual flavonoid compounds after HPLC separation (Table 2). The total flavonoid compounds identified in leaves treated with microwave recorded the highest amount (1020.04 mg/100 g). Increasing the drying time led to decrease the amount of flavonoids in leaves (202.15 mg/100g).

The Naringin was the most abundant compound recorded in MW-AO artichoke leaves (457.92 mg/100 g) followed by Hisperidin (227.3 mg/100 g) and Luteolin (153.95 mg/100 g). On the other hand, the classical air dried artichoke leaves showed less amounts for the three compounds (64.00, 49.06 and 44.15 mg/100 g for Naringin, Luteolin and Hisperidin respectively). The results are in agreement with (**Lattanzio *et al.*, 2009)** who reported that, the flavonoid such as the flavones (e.g., luteolin) have been identified in artichoke tissues, and also, in agreement with **(Pandino *et al.*, 2011 a)** who reported that, flavones, are not found in many food plants, artichoke represents a significant source of this substance and its conjugates

Table (2): Flavonoids contents (mg/ 100g) in artichoke dried leaves in relation to drying treatment.

Flavonoids	MW + Oven dried leaves	Oven dried leaves
Luteolin	**153.95**	**49.06**
Naringin	**457.92**	**64.00**
Rutin	**19.02**	**0.57**
Hisperidin	**227.30**	**44.15**
Rosmarinic	**2.69**	**0.46**
Quercetrin	**94.36**	**15.38**
Quercetin	**10.92**	**4.78**
Naringenin	**19.74**	**14.84**
Hespertin	**16.37**	**4.69**
Kaempferol	**9.23**	**1.30**
Apegnin	**8.54**	**2.92**
Total flavonoid compounds	**1020.04**	**202.15**

The microwave dried leaves exhibited higher values of all phenolic and flavonoid compounds than the hot air dried samples. Drying temperature and time are critical parameters for significant change during

drying. Therefore, lower degradation of microwave dried leaves may be due to the substantial reduction in drying time. These results in agreement with **Vadivambal and Jayas (2007)**, who stated that a combined microwave and hot air treatment had minimized the reduction of polyphenol compounds in a short time and the adverse effect of heat was minor in the samples treated with this combined method when compared to classical hot air dried samples. Moreover, chlorogenic acid and luteolin may impede atherosclerosis (**Brown and Rice-Evans, 1998)**. Subsequently, artichoke leaf extracts show hypocholesterolemic activity.

Sensory evaluation of artichoke leaves drink in relation to drying treatment.

Sensory evaluation of artichoke leaves drink were evaluated in relation to drying treatment by ten panelists for various quality attributes such as appearance, color, taste and flavor, on a 1 to 10 hedonic scale. Results are illustrate in Table (3). Data revealed that, the drink made of microwave dried leaves displayed lower values of various quality attributes than the drink made of hot air dried leaves. These significant differences, may be because of the higher phenolic compounds content in microwave dried leaves than hot air dried leaves. This would decidedly encourage the utilization of ALE (oven dried leaves) as a healthy drink.

Table (3): Sensory evaluation of dried artichoke leaves drink after drying treatment in Oven and MW+Oven

characteristic	MW+Oven dried leaves	Oven dried leaves
Appearance	7.00[b]	10.0[a]
Color	7.00[b]	9.00[a]
Taste	7.50[b]	9.70[a]
Flavor	8.50[b]	9.00[a]
Overall acceptability	7.50[b]	9.43[a]

Values followed by the same letter in rows are not significantly different at $p \leq 0.05$

Effect of artichoke leaves extract (ALE) supplementing the hyper-cholesterolemia-inducing diet on serum triglyceride concentration and lipoprotein profile.

Data given in Table (4) elucidate values of serum triglyceride and the lipoprotein profile in rats consuming various diets for 56 days. It is clear that, the level of triglycerides in serum blood of control 1 (rats fed basal diet) was 50.52 mg/dl, TC, LDL-C and HDL-C for this group of rats showed concentrations of 95.02, 40.94 and 54.08 mg/dl, respectively. Control 2 (rats fed hypercholesterolemic diet) showed higher TAG (115.22 mg/dl) concentration. Total cholesterol and LDL-C levels

increased simultaneously as well, being 236.05 and 203.9 mg/dl, respectively. HDL-C was recorded lower concentration (32.15 mg/dl) for the control 2. Results displayed that, supplementation of diet with 1% cholesterol and 0.25% bile salts was significant effective in upraise serum cholesterol levels in the rats. From the above-mentioned results it could be also observed that, elevated TAG level and lower HDL-C was associated with a raise in LDL-C and TC.

Table (4): Serum triglycerides and the lipoprotein profile in rats consuming different diets for 56 days.

Blood parameter	Animal group diets				
	Control (1)	Control (2)	1	2	3
TAG	50.52^{de}	115.22^{a}	87.10^{b}	70.15^{c}	52.09^{d}
TC	95.02^{e}	236.05^{a}	172.72^{b}	139.86^{c}	109.02^{d}
LDL-C	40.94^{e}	203.9^{a}	136.45^{b}	99.57^{c}	61.72^{d}
HDL-C	54.08^{a}	32.15^{e}	36.27^{d}	40.29^{c}	47.30^{b}
TC/ HDL-C ratio	1.76	7.34	4.76	3.47	2.31
LDL-C / HDL-C ratio	0.76	6.34	3.76	2.47	1.31

Values followed by the same letter in rows are not significantly different at $p \leq 0.05$
(TAG)=Total triglycerides (mg/dl)
(TC)=Total cholesterol (mg/dl)
(LDL-C)=Low-density lipoprotein (mg/dl)
(HDL-C)=High-density lipoprotein (mg/dl)
(1)=animals fed on cholesterol diet+1.5% ALE
(2)=animals fed on cholesterol diet+3% ALE
(3)=animals fed on cholesterol diet+4.5% ALE

TC and LDL-C levels were increased as a result of dietary cholesterol raises. The ratios of TC/HDL-C and LDL-C/HDL-C were studied for all rats fed on the different diets. Data explain that, the TC/HDL-C ratio increased to be 7.34 for the control 2 as a result of addition cholesterol and bile salts to the basal diet. The ratio of TC to HDL-C to be desirable less than 4.0 and high risk of heart disease above 6.0 **(Baur, 1995)**. The ratio of LDL-C to HDL-C was higher on control 2 (6.34). On the other hand, supplementing the diet with ALE showed significant reductions in TAG concentration and the lipoprotein profile. The different levels of ALE were varied in their effects on blood parameters. As elucidated by results, given the hyper-diet containing 1.5%, 3% and 4.5% ALE to the groups resulted in lowering cholesterol levels. Adding ALE at 4.5% level to the hyper-diet was more effective to diminish the different blood parameters concentrations, than the other supplementing levels. Cholesterol concentrations in rats fed on the diet

with ALE were significantly less than in rats fed on the control 2. TAG and TC were decreased as compared with the control 2. Also, HDL was increased while LDL was decreased in rats. From results, it is clear that, diet with ALE was able to impede an increase in serum cholesterol levels, even though the large quantity of cholesterol in the diet. Results show the efficacy of high phenolic compounds content diet (with ALE) to provide a favorable lipoprotein cholesterol profile. Our results are in agreement with those reported by **(Küskü-Kiraz *et al.*, 2010; Qiang *et al.*, 2012 and Rondanelli *et al.*, 2013).**

Effect of artichoke leaves extract (ALE) supplementing the hyper-cholesterolemia-inducing diet on serum ALT and AST activities and creatinine.

The data in Table (5) illustrate differences in ALT activity values of rats fed on the different diet meanwhile an experimental period of 56 days. The values ranged between 21.98 IU/L for control 1 and 33.02 IU/L for the control 2. Values for rats fed on the three other groups recorded 30.05, 28.39 and 27.02 IU/L for 1.5%, 3% and 4.5% ALE supplemented diets, respectively. Thus, data for ALT activities in rats fed on different diets were reflecting no volume of cellular damage. AST for rats on control 1 recorded 38.85 IU/L. An increasing was noticed in rats of control 2 (90.01 IU/L) compared to the control 1. ALE supplementations led to significantly lesser values of AST activities, with regard to the control 2.

Table (5): Serum ALT and AST activities and creatinine in rats fed on different diets for 56 days .

Dietary group	ALT (IU/L)	AST (IU/L)	AST/ ALT	Creatinine (mg/dl)
Control (1)	21.98^{e}	38.85^{e}	1.77	0.42^{c}
Control (2)	33.02^{a}	90.01^{a}	2.73	0.76^{a}
1	30.05^{b}	78.01^{b}	2.59	0.65^{b}
2	28.39^{c}	70.51^{c}	2.48	0.62^{b}
3	27.02^{d}	63.20^{d}	2.34	0.58^{bc}

Values followed by the same letter in columns are not significantly different at p ≤0.05
(1)=animals fed on cholesterol diet+1.5% ALE
(2)=animals fed on cholesterol diet+3% ALE
(3)=animals fed on cholesterol diet+4.5% ALE

The results explain that, rats fed on hypercholesterolemic diet have relative increase in serum creatinine (0.76 mg/dl) regard to the control 1 (0.42 mg/dl). Supplementing the high-cholesterol diet with 1.5%, 3% and 4.5% ALE led to significant reduction in creatinine values (0.65, 0.62 and 0.58 mg/dl, respectively) regard to the control 2 and the

differences in those three values were not comparatively high. Our results are in agreement with those reported by **(Kucukgergin *et al.*, 2010)** who reported that artichoke leaf extracts decreased serum lipids, as well as hepatic and cardiac oxidative stress in rats fed on high cholesterol diet.

According to the **European Medicines Agency (2011)** the pharmaceutical forms of artichoke are acceptable with respect to clinical safety. In the coming days, a wider use of artichoke is prospective, since this crop might be utilized in many new applications, particularly those that can redeem the increasing consumer's demands for natural products and functional foods **(Christaki *et al.*, 2012).**

CONCLUSION

Reviewing above-mentioned results obtained in this study, it could be concluded that, ALE consider an excellent source of phenolic compounds (1071.6 and 563.4 mg/ 100g for microwave and hot air dried leaves respectively), and flavonoid compounds (1020.04 and 202.15 mg/ 100g for microwave and hot air dried leaves respectively). ALE showing the effectiveness of high phenolic content diet (with ALE) to provide an favorable lipoprotein cholesterol profile. Thus, ALE is effective at lowering LDL-C concentration, and improve HDL-C levels. Moreover, ALE can utilize (oven dried leaves) as a healthy drink with good acceptability.

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استخدام اوراق الخرشوف كمشروب صحى

أمانى عبد الرحمن على متولى – سامية على العسقلانى – محمد صلاح نجم

معهد بحوث وتكنولوجيا الأغذية – مركز البحوث الزراعية – جيزة

الخرشوف يستخدم كخضار فى بلاد كثيرة. و تمثل مخلفات تصنيعة حوالى 80% من وزن النبات.فى هذه الدراسة تم تجفيف اوراق الخرشوف بطريقتين مختلفتين و اظهرت النتائج ان الاوراق مصدر غنى بالمركبات الفينولية و الفلافونويدات. و قد تم عمل مشروب صحى من اوراق الخرشوف المجففة و اظهر التقييم الحسى للمشروب ان التجفيف بالفرن العادى اعطى صفات حسية و درجة قبول اعلى للمشروب عنه فى التجفيف بالميكروويف.

تمت دراسة تاثير مستخلص اوراق الخرشوف على كوليستيرول الدم فى الفئران و اوضحت النتائج فعالية المستخلص فى خفض LDL , TC, TG كما ادى الى تحسن الكوليستيرول الجيد HDL. وتوصى الدراسة بالاستفادة من اوراق الخرشوف المجففة بالفرن كمشروب صحى و خافض للكوليستيرول LDL.