# EFFECT OF EXTRACTION ON PHENOILC CONTENT, SILYMARIN AND ANTIRADICAL ACTIVITIES OF ARTICHOKE LEAVES AND ROOTS

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**Abstract**

Artichoke is a source of biophenols that exhibit lots of pharmacological activities. The aim of the present study is to determine the effect of different extraction methods on the phenolic, and silymarin contents as well as antiradical activities of artichoke leaves and roots. First, the total phenol content was determined by the Folin–Ciocalteu procedure; the gallic acid equivalent values ranged from 16.42-30.28 mg/g. Generally, different leave extracts showed higher contents of phenol than the root extracts**.** It was found that the methanolic extract of the leaves exhibited the most value of phenolic content (30.28 mg/g) while the root infusion showed the least value (16.42 mg/g). The analysis of silymarin and silybin compounds from each extract was carried out by reversed phase high performance liquid chromatography (RP-HPLC). The infusion extract of leaves showed also the higher content of silymarin than that of root extracts, and the infusion of the leaves exhibited the higher value while the water extract of roots exhibited the least one. Antiradical activities were evaluated using 1, 1-Diphenyl-2-picrylhydrazyl ($DPPH^0$) free radical and $\beta$-carotene bleaching methods. Good correlation ($R^2$ =0.9752) was found between phenolic content and antioxidant activity. The

minimum $EC_{50}$ was obtained in the leaves methanol extract that showing value of 16.48 ug/g under the same conditions.

According to these results, the use of artichoke extracts is suggested from industrial waste as possible ingredients to functionalize foodstuffs (to decrease lipid peroxidation and to increase health-promoting properties) and as a potential source for pharmacological industries.

**Keywords:** Artichoke leaves and roots, total phenol, silymarin, antiradical activities

**Introduction**

The packing houses and food-processing industry dealing with vegetables produce large amounts of wastes and residues (leaves, stems, wastewaters, *etc*.). Sometimes, these wastes can reach ~ 60% of harvested vegetal as in the case of the industrial manipulation of artichoke. In Egypt there is an annual production of nearly 202458 MT of artichoke (FAO, 2012). In fact, the artichoke-based industry yielded ~8098 tons of waste. These residues are very perishable products that are difficult to manage because of environmental problems in the industries.

Different studies about artichoke have demonstrated their health-protective potential, especially their hepatoprotective (Gebhardt, 1997; Clifford, 2000) anticarcinogenic (Wang *et al*., 2003), and hypocholesterolemic (Clifford and Walker, 1987; Englisch *et al.*, 2000) activities. Artichoke leaf extract (ALE; *Cynara scolymus* L.) has been shown to reduce symptom severity in a subset of patients with dyspepsia identified as suffering from Irritable bowel syndrome IBS (Walker *et al*., 2001).

Gebhardt (1997) found that artichoke leaf extract significantly prevented oxidative damage to hepatocyte membranes exposed to tertiary butyl hydroperoxide (t-BHP) and that chlorogenic acid and cynarin were the main contributors to this strong antioxidant effect. The study of Karin, 1999 demonstrated a pronounced antioxidant potential by artichoke leaf extract.

Silymarin is also used for the treatment of *Amanita phalloides* mushroom poisoning (Vogel *et al*., 1984). *In vitro* and animal studies have demonstrated the hepatoprotective properties of silymarin or silybin (a mixture of SBA and SBB) (Hoofnagle, 2005; Crocenzi and Roma, 2006). Several clinical trials have shown an excellent safety profile for silymarin in humans (Fraschini *et al*., 2002; Ball and Kowdley, 2005; Dryden *et al*., 2006). However, the clinical efficacy and dose-exposure relationships in humans remain unclear, because of the small number of participants and the lack of information on the exposure levels of the

major silymarin flavonolignans with administration of standardized dosage regimens (Jacobs *et al*., 2002; Mayer *et al*., 2005; Rambaldi *et al*., 2005).

The aim of the present study is to determine the effect of different extraction methods on the phenolic content, silymarin, silybin of artichoke leaves and roots. In addition, the *in vitro* antiradical activities of these extracts will be assayed in order to discuss the potential role of artichoke waste as a source of health-promoting phenolics associated with their antioxidant activity.

**Experiment**

**Plant**

Artichoke (*Cynara scolymus* L. family Compositae) leaves and roots are the residue from fresh handling processing of artichoke hearts. Artichoke was purchased from local market (Cairo, Egypt). Artichoke wastes used in the present study were designated infusion, water and methanol extraction.

**Extraction Protocols**

**Infusion:** 100 g of artichoke wastes (fresh leaves and roots) were extracted with boiling water for 10 minutes. The filtrate was evaporated under reduced pressure at 55 °C.

**Methanol Extract**. 100 g of artichoke waste either leaves or roots (fresh weight) were extracted with cold methanol for 5 days. The extract was filtered, and the methanol was removed under reduced pressure at 40 °C.

**Water extract:** A quantity of 100 g of either leaves or roots was subjected to extraction by maceration with cold water for 5 days. The filtrate was evaporated under reduced pressure at 55 °C.

**Chemicals**

Folin–ciocalteu**,** gallic acid**,** 1, 1-diphenyl 1-2-picrylhydrazyl ($DPPH^0$)**,** BHA and TBHQ were purchased from sigma. Methanol was of HPLC grade. $\beta$ -carotene, chloroform, linoleic acid, Silymarin and silybin were purchased from Sigma-Aldrich (St. Louis, MO)**.**

**Determination of total phenolic content (TPC) assay**

The total phenolic content (TPC) was determined in all the extracts and fractions following the Folin-Ciocalteu method (Singleton and Rossi, 1965). The reaction mixture was composed of 0.1 ml extract (1 or 10 mg/ml, depending on the activity), 7.9 ml distilled water, 0.5 ml of Folin–Ciocalteu reagent, and 1.5 ml of a 20% sodium carbonate anhydrous solution (added 2 min after the Folin–Ciocalteu reagent). After initial mixing the opaque flasks were allowed to stand for 2 h. The optical density of the blue-coloured samples was measured at 765 nm. The total phenolic content was determined as gallic acid equivalents (GAE) and

values are expressed as mg of gallic acid/g of extract (in GAE). Results were the mean ± SD of 3 determinations of each extract.

**Determination of total antiradical activities**

**Evaluation of the 1,1-diphenyl-2-picrylhydrazyl ($DPPH^0$) radical-scavenging activity (RSA)**

The antioxidant activity of the extract was measured as described by Gebhard (2001) using the 1, 1–diphenyl– 2–picrylhydrazyl ($DPPH^0$) free radical scavenging capacity. The extract was mixed with 3 ml of methanol solution of 0.004 % DPPH and the absorbance was read at 517 nm 30 min later.

The radical scavenging activity of the samples (antioxidant activity) was expressed as percent inhibition of $DPPH^0$ radical as following:

$$\% \text{ Inhibition} = [(A_{control} - A_{treatment})/A_{control})] \text{ X } 100$$

Where: $A_{control:}$ is the absorbance of the control; $A_{treatment}$: is the absorbance of the treatments.

Standard antioxidants (BHA and TBHQ, 200 ppm) were used for comparison as positive control. $IC_{50}$ value was calculated using the dose inhibition curve**.**

**Antioxidant activity with the *β*-carotene bleaching method**

The antioxidant activity of extracts was evaluated using *β*-carotene-linoleic acid (Kabouche *et al.*, 2007). A stock solution of *β*-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of *β*-carotene was dissolved in 1 mL of chloroform (HPLC grade); 25 uL of linoleic acid and 200 mL of tween 40 were added as emulsifier because *β*-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water was added with vigorous shaking for 30 min; 2500 uL of this reaction mixture was dispersed to test tubes, and 150, 250 and 350 uL portions of extracts were added. The emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHA, TBHQ and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Tests were carried out in triplicate.

Inhibition of coloration of *β*-carotene in percentage (I%) was calculated as:

$$\text{I\% inhibition} = [(A_{blank} - A_{sample})]/ A_{blank}] \times 100$$

Where $A_{blank}$ is the absorbance of the control reaction (containing of the reagents except the test compound) and $A_{sample}$ is the absorbance of the test compound.

**Extraction of silymarin and silybin for HPLC analysis**

Appropriate weights of the grinding plant transferred to a 25-ml volumetric flask and then mixed with 20 ml of MeOH. The mixture was sonicated for 15 min at room temperature

and diluted to 25 ml with MeOH. The mixture was filtered by a Millex-HX Nylon syringe filter (0.45 um, 25 mm; Millipore, Bedford, MA) to remove any particles. The first 5 ml of the filtrates was discarded, and the following filtrates were collected. Appropriate aliquots of the filtrates were diluted with 50% MeOH. and analyzed by the HPLC.

**Silymarin and silybin Standards Preparation**

Accurately weight 20 mg of standards silymarin and silybin dissolved in 30 mL HPLC methanol, sonicate for 30 min and adjust the volume to 25 mL. Standard and samples were dissolved in the mobile phase.

**Standard curve estimation**

Six concentrations of silymarin and silybin standards solution were prepared: 5, 10, 20, 40, 80, and 100 ppm. The solutions were injected into injector once for each and the area under curve was recorded and measured for the correlation coefficient in linear regression equation ($Y = ax + b$).

**HPLC Conditions**

Each extract was analyzed using HPLC chromatography equipped with a UV detector Agilent 1100. HPLC separation was performed on a Pursuit $C_{18}$ column at room temperature. The mobile phase was MeOH-0.1% glacial acetic acid (pH 2.8) (46:54, v/v). The elution has been made in an isocratic mode at a flow-rate 1 ml /min and the detection at 288 nm (Zhiming *et al.*, 2008).

**Statistical analysis**

All experiments were performed at least in triplicate and the results are presented as mean ± SD (standard deviation). Statistical analysis was carried out using SPSS.14.One-way analysis of variance (ANOVA) and least significant difference (LSD) was performed to determine any significant difference among various treatments and also were used to compare between means. Significant level was set at $p \leq 0.05$ (Živkovic *et al*., 2010).

**Results and Discussion**

**Phenolic content**

The total phenolic content of the extracts from medicinal plant species varies according to the solvent used and its alcohol concentration. Alcohol extracts of artichoke obtained by simple extraction (*i.e.*, nonfluidized method) contained high quantities of phenolic compounds. Maximum amount of total phenolics **(Table 1)** was obtained from leaves using methanol as the extraction solvent and corresponded to 30.28 mg/g equivalent of gallic acid.

**Table (1):** Phenolic content (mg/g) and $IC_{50}$ of artichoke leaves and roots under different extraction method

| Extraction | Total phenolic content (mg/g) | $IC_{50}$ (ug/g) | |
|---|---|---|---|
| | | DPPH$^0$ | $\beta$-carotene |
| **Water** | | | |
| Leaves | 20.00±0.06$^{a*}$ | 30.34±0.05 | 34.3±0.12 |
| Root | 18.19±0.06$^{b}$ | 46.38±0.07$^{a}$ | 52.8±0.18 |
| **Infusion** | | | |
| Leaves | 20.81±0.11$^{a}$ | 21.07±0.06 | 24.6±0.09 |
| Root | 16.42±0.05 | 39.82±0.09 | 41.7±0.13 |
| **Methanol** | | | |
| Leaves | 30.28±0.012 | 16.48±0.03 | 19.7±0.08 |
| Root | 18.63±0.07$^{b}$ | 29.24±0.04$^{a}$ | 32.6±0.14 |

*: Values are expressed as mean ± SD;
Values with the same letter within the same column are not significant ($P \leq 0.05$)

There was no direct relationship between phenolic content and antioxidant activity. Although, Yang et al. (2002) found such a direct relation but their work pertained to medicinal mushroom extracts. However, for medicinal plant extracts, the studies of Bajpai et al. (2005) and Sengul et al. (2009) confirmed our own observations. These studies indicate that phenolics have varying antioxidant behaviour which depends on the type of compound.

**Antiradical activities**

The antioxidant activity data for different artichoke extracts, which were obtained by the DPPH$^0$ and $\beta$-carotene bleaching assayas, are indicated in **Fig.1**. While, the antioxidant activity based on DPPH$^0$ was measured at three different levels; 200, 300 and 400 µL, the levels in $\beta$-carotene assay were 150,250 and 350 uL. It was found that there is a proportional relation between the antioxidant activity and the extract concentrations. The degree of discoloration of the extract indicates the potential for binding free radicals present by the various extracts. These results are in agreement with those obtained by Emanuel et al. (2011). The highest antioxidant activity was observed in the methanol extract of the leaves while the least one was detected in the water extract of the roots.

The free radical scavenging and antilipoperoxidation properties of silybin glycosides (silybin galactoside, glucoside, lactoside and maltoside) were investigated (Kosina *et al*., 2002). These glycosides were found to be weaker electron donors than silybin, but were more potent scavengers of the 1, 1-diphenyl-2-picrylhydrazyl and the 2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)-derived radicals. These glycosides were more efficient than silybin in preventing *tert*-butylhydroperoxide-induced lipoperoxidation of rat liver mitochondrial membranes and damage of rat erythrocytes and primary hepatocytes in culture.

Gonzalez-Correa et al. (2002) investigated the effects of silymarin MZ-80 on hepatic oxidative stress in rats with biliary obstruction. Their studies were designed to evaluate the effects of three pharmaceutical forms of silymarin (silymarin MZ-80, silybinin-B-cyclodextrin and silybinin) on the oxidative status of the liver *in vitro* and after oral administration to rats with extrahepatic biliary obstruction (EBO). All three compounds inhibited the production of thiobarbituric acid reactive substance (TBARS) *in vitro,* as well as in EBO rats. Silymarin MZ-80 was most effective in increasing GSH peroxidase and GSH transferase activities and the GSH level. This study suggested the antilipoperoxidation activity of these three silymarin derivatives, from which silymarin MZ-80 also enhanced the GSH antioxidant system**.**

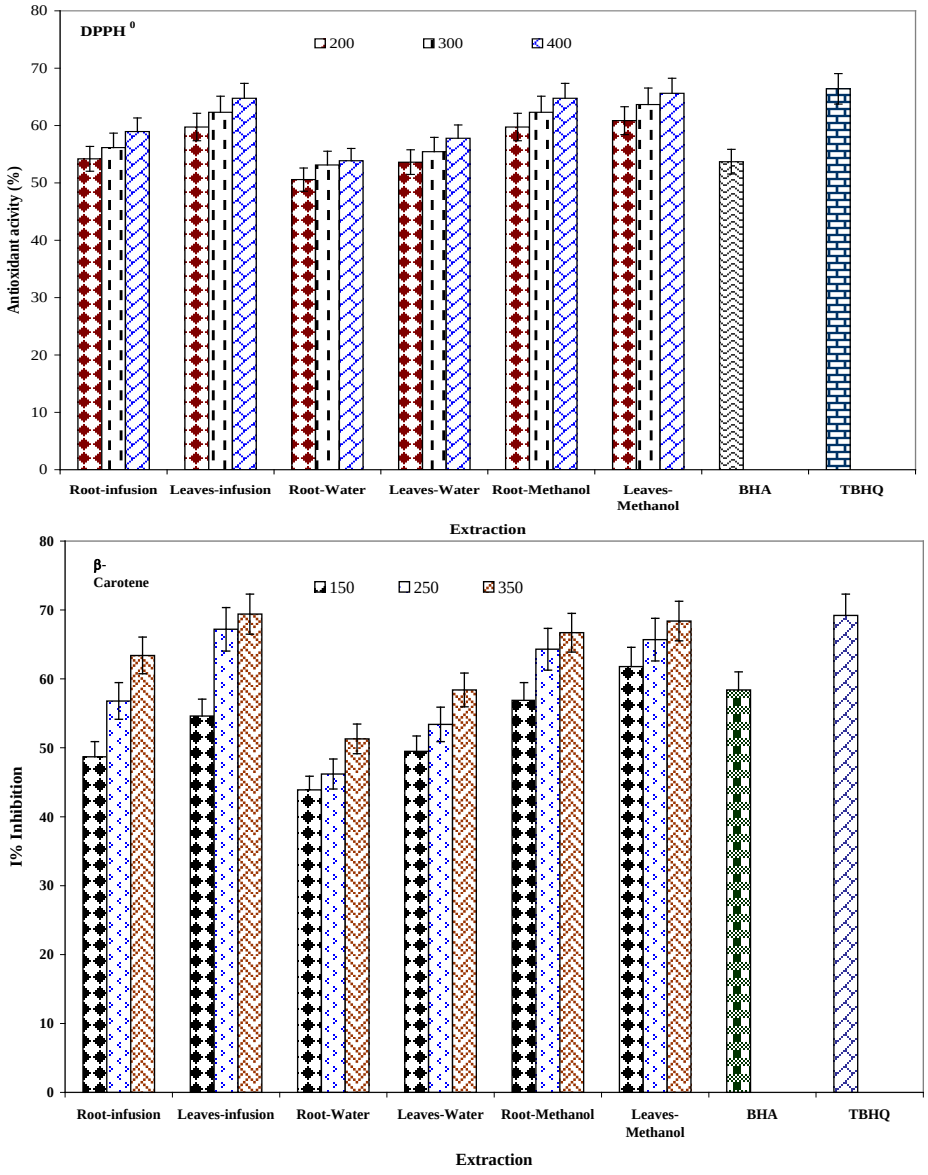


**Fig. (1)** Antiradical scavenging activity of different extraction of artichoke leaves and roots as measured by $DPPH^0$ (A) and $\beta$-carotene (B) assays

**Silymarin and silybin analysis by HPLC**

**Table 2** shows that the silymarin and silybin content obtained by various extraction methods as determined by RP-HPLC. The highest content of silymarin was detected in the leaves extracted by infusion (25.6 ug/g) while the water extract of roots showed the least value (13.2 ug/g). The same results were detected for silybin (infusion of leaves; 5.2 ug/g and water extract of roots; 2.9 ug/g)

**Table (2)** Effect of extraction method on silymarin and silybin concentrations (ug/g) of artichoke leaves and roots.

| **Extraction** | **Water** | | **Infusion** | | **Methanol** | |
|---|---|---|---|---|---|---|
| | leaves | Roots | leaves | Roots | leaves | Roots |
| Silymarin | 14.7 | 13.2 | 25.6 | 18.3 | 23.8 | 19.6 |
| Silybin | 3.4 | 2.9 | 5.2 | 3.5 | 4.1 | 3.9 |

The use of hot water as an extraction solvent for milk thistle at temperatures above $100^0$C was explored by Duan et al. (2004) they observed that the maximum extraction yield of each of the silymarin compounds and taxifolin did not increase with rising temperatures, most probably because of the degradation of the compounds. However, the time required for the yields of the compounds to reach their maximum was reduced from 200 to 55 minutes when the extraction temperature was increased from 100 to $140^0$C. Severe degradation of silymarin compounds was observed, with first-order degradation kinetics at 140 $^0$C (Duan *et al*., 2004).

The obtained results are in agreement with Alvarez-Baretto et al. (2003) who show this preferential extraction effect in studying the extraction of silymarin compounds and taxifolin over a temperature range of 50– 100°C. As expected, the maxima were reached faster as the temperature was increased and pseudo equilibrium was approached.

It is known that extraction efficiency increases with temperature. Richter et al. (1996) theorized that this is owing to enhanced solubilization, higher diffusivity of solutes, and disruption of the strong solute-matrix interaction caused by Van der Waals forces, hydrogen bonding, and dipole forces of solute molecules and active sites on the matrix at increased temperature. According to these theories, the yields of the silymarin compounds should have increased with temperature. Thus, temperature had a positive effect on extraction yield; however, it was perhaps circumvented by a temperature-induced degradation.

**Conclusion**

The artichoke different extracts of leaves and roots showed varying concentrations of phenolic content, silymarin and silybin as well as antioxidant activities. Although the values of these parameters were high, the fluctuation in antioxidant activity observed indicates that

further studies are required to explore the effect of thermal preparation as microwave or freeze-drying on these parameters.

More investigations are highly recommended to elucidate the potential use of artichoke wastes as a rich source of natural antioxidant phenolics. In this way the "functionalization" of foodstuffs by using artichoke wastes extracts should be taken into account. To this purpose, sensory modification of foodstuffs, as well as the stability and activity of artichoke extracts within food matrices, should be investigated. In addition, toxicological studies should be also carried out to ascertain the boundary between health-beneficial effects and risk damage.

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