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Phenolic Compounds from Artichoke (*Cynara scolymus* L.) By-products and their Antimicrobial Activities.

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Abstract

Cynara scolymus L. is a medicinal plant frequently used in traditional medicine for liver diseases. The aim of this study is to evaluate the antimicrobial activity of the different parts of Artichoke. Maximum antimicrobial activity was observed with methanolic extract of bound phenols for (bract and heart) against Gram negative bacteria. The MIC values for bound phenols from heart were (63µg/ml). Whereas, the bound phenols for bracts was ranged from (312 and 486µg/ml) against Gram negative and (486µg/ml) against Gram positive. Free phenols of the heart of artichoke showed the lowest MIC (204 to 206) µg/mL for microorganism Gram positive and Gram negative. Therefore, this study indicate that the free phenolic extract from bracts of *Cynara scolymus* L. might be of interest within the developing market of nutritional ingredients and is capable of yielding nutritional supplements with antimicrobial activities.

Key words: artichoke wastes, antibacterial effects

1. Introduction

Artichoke (*Cynara scolymus* L.) represents an important component of the Mediterranean diet. Artichoke is a rich source of minerals, a low amount of lipids, dietary fibre and a high proportion of phenolics (Llorach *et al*., 2002 &Fratianni *et al.,* 2007). Nutritional and pharmaceutical properties of both artichoke bracts and heart are showed high levels of polyphenolic compounds and inulin (Luttanizio *et al*. 2009). In Egypt there is an annual production of nearly 202458 MT of artichoke (FAO, 2012).

The manipulation of food processing wastes is now becoming a very serious environmental issue. Peels and leaves are often the waste part of various fruits. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their unknown benefit of commercial application.

The artichoke (*Cynara scolymus* L.) canning industry generates large amounts of agricultural waste, represent (about 80–85% of the total biomass of the plant) consisting mainly of the leaves, Jimenez-Escrig *et al*., (2003) stems and the external parts of the flowers (bracts) which are not suitable for human consumption and could be used as a source of inulin, phenolics, and should be considered as a raw material for the production of food additives and nutraceuticals.

Recent studies on artichoke demonstrated their health-protective potential, especially their hepatoprotective (Gebhardt, 1997 ; Aktay *et al.,* 2000) anticarcinogenic (Wang *et al*., 2003), and hypocholesterolemic activities (Lupattelli *et al*., 2004), antimicrobial (Zhu *et al.,* 2004). The spoilage and poisoning of foods by microorganisms is a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available. Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve a sufficiently long shelf life of foods and a high degree of safety with respect to food borne pathogenic microorganisms. In nature, there are a large number of different types of antimicrobial compounds that play an important role in the natural defence of all kinds of living organisms (Varmanu *et al*., 2011).

The aim of the present study is to evaluate the potential role of artichoke and artichoke by-products as a source of health-promoting phenolics associated with their antimicrobial activities.

2. Materials and Methods

2.1 Sampling extraction of free and bound phenolic compounds

Phenolic compounds were extracted into free and bound phenolics according to the methods of Adom & Liu (2002) ; Sosulski, *et al*. (1982), respectively, with a slight modification. Free phenolic compounds of flours (1 g) were extracted with 10 ml of 80% chilled ethanol for 20 min with continuous shaking. After centrifugation at 2500g for 10 min, the supernatant was collected. The residue was re-extracted twice with 10 ml of 80% chilled

ethanol under the same conditions. All supernatants were combined and evaporated to dryness under reduced pressure. Then the concentrated slurry was dissolved with methanol to a final volume of 10 ml. The free phenolic compounds were then stored at – 40 °C until use. The residue from the extraction of free phenolic compound was hydrolyzed directly with 20 ml of 2 N NaOH for 90 min with continuous shaking at 60 °C (Yeh, *et al*. 1980). The hydrolysate was acidified to pH 2 (6 N HCl) and centrifuged to separate cloudy precipitate. The clear supernatant was extracted five times with hexane at a hexane to water phase ratio of 1:1 to remove free fatty acids and other lipid contaminants. The liberated phenolic acids were then extracted six times with ethyl acetate at a solvent to water phase ratio of 1:1. The ethyl acetate extracts were evaporated to dryness and then bound phenolic compounds were dissolved and filled up to 10 ml of methanol and stored at (– 40)°C until use.

2.2 Total phenolic content (TPC)

The total phenolic content (TPC) of free and bond phenolic extracts of different parts of artichoke was spectrophotometrically determined by Folin Ciocalteu reagent assay using Gallic acid for the preparation of calibration curve (20 – 120 mg/l) according to (Singleton *et al*.1965). A suitable aliquot (1 ml) of each extract or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu`s phenol reagent was added to the mixture and shaken. After 5 min. 10 ml of 7 % Na_2CO_3 solution were added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min. at room temperature, the absorbance was determined at 750 nm with Spectrophotometer (Unicum UV 300) against prepared reagent as blank. Total phenolic contents in samples were expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analysed in triplicates.

2.3 Total flavonoid content (TFC)

Total flavonoid content (TFC) of free and bond phenolic extracts of different parts of artichoke was spectrophotometrically determined by the aluminium chloride method using quercetin as a standard (Zhishen *et al*., 1999). One ml of extract or standard solution (quercetin, 20–120 mg/l) was added to 10 ml volumetric flask, containing 4 ml of distilled water. To the flask 0.3 ml 5 % $NaNO_2$ was added and after 5 min 0.3 ml 10 % Al Cl_3 was added. At 6th min, 2 ml 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample were expressed as mg quercetin equivalents (QE)/ g fresh weight. Samples were analyzed in triplicates.

2.4 Antimicrobial activity

The antimicrobial activities were carried out according to the conventional disk diffusion test (Greenwood, 1983) using cultures of *Bacillus subtilis* NRRL B-94, *Escherichia coli* NRRL B-3703, *Psedumonas aeruginosa* NRRL, *Staphylococcus aureus* NRRL, *Aspargillus niger* NRRL313, and *Candida albicans* NRRL477. The bacterial strains were cultured on nutrient medium, while the fungi and yeast strains were cultured on malt medium & yeast medium, respectively. Broth media included the same contents except for agar. For bacteria and yeast, the broth media were incubated for 24 h. As fungus, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation. For preparation of plate inoculation, 0.5 ml of inoculate were added to 50 ml of agar media (50 °C) and mixed by simple inversion. The agar was poured into 120 mm Petri dishes and allowed to cool to room temperature. The sterile filter paper disk (2mm in diameter) was saturated by sample. The saturated filter paper steel to evaporate solvent and fixed on the surface of agar. The microbial growth inhibition zone was measured after incubation at 30 °C at the appearance of the clear microbial free inhibition zones, beginning within 24 h for yeast, 24–48 h for bacteria and 48–72 h for fungus. Both antimicrobial activities could be calculated as a mean of three replicates.

2.4.1 Minimal inhibitory concentration (MIC)

The culture medium (25 ml) was poured into Petri dishes (9 cm in diameter) and maintained at 45 ºC until the samples were incorporated into the agar. The samples were added as 50, 100, 200, 300, 400, 500 and600 µg/ml. The different microbial strains were layered by using an automatic micropipette to place 30 µL over the surface of the solidified culture medium containing a sample. After the microorganisms were absorbed into the agar, the plates were incubated at 30°C for 24-48 h. MIC was determined as the lowest concentration of artichoke extracts inhibiting the visible growth of each organism on the agar plate.

2.5 Statistical Analysis

The statistical analysis was performed according to Snedecor & Cochran (1989) for comparison between different mean values, LSD test at 5% level was used (Duncan 1995). Differences were considered significant when $p \leq 0.05$.

3. Results and Discussion

3.1 Phenolics and Flavonoids contents

The artichoke (*Cynara scolymus* L.) canning industry generates large amounts of agricultural waste, represent (about 80–85% of the total biomass of the plant) consisting mainly of the leaves, stems and the external parts of the flowers (bracts) which are not suitable for human consumption and could be used as a source of inulin, phenolics, and should be considered as a raw material for the production of food additives and nutraceuticals. Data presented in (Table 1) indicated that the artichoke (bracts) showed a higher content of total free phenolic compounds FTPC (14.16 mg/gDW) followed by the artichoke (heart) which contained only 9.06 mg/gDW.On the other hand both inner and outer parts of artichoke showed the lower content of the bound phenolic compounds (5.35 and 4.2 mg/gDW, respectively).

Previous studies showed that the artichoke (bracts) contained higher amount of phenolic compounds along with higher amount of minerals and dietary fibres.As a role of phenolics, the artichoke (bracts) should posses higher free radicals scavenging activity than the artichoke (heart), These results were supported by the results of (Perez-Garcia *et al*., 2000 who found that nutritional and pharmaceutical properties of both heart and bracts are linked to their special chemical composition which includes high levels of polyphenolic and inulin which possess potential antioxidant activity (Perez-Garcia *et al*. 2000).

Lattanzio *et al*., (2009) mentioned that the therapeutic properties have been ascribed to the cynarin (1,3-Odicaffeoylquinic acid)content of these extracts. in various pharmacological test systems, artichoke bracts extracts have exhibited hepatoprotective, anticarcinogenic, antioxidative, antivacterial and anti-HIV activities.

Similar results found by Sallam *et al*., (2005 & 2008) who found that artichoke by-product contained (8.1 mg Tannic acid/g DW). Lattanzio *et al*., (2009) found that by-product of artichoke are very rich in phenolic compounds and hence can regarded as a functional food.

In addition, the total flavonoids contents (TFC) of free and bound phenolic extracts of artichoke bracts and heart are shown in (Table 1). The flavonoid content of the free phenolic extracts was higher than that the bound phenolic extracts.

The total flavonoids concentrations of free phenolic in artichoke bracts extracts was significantly higher (9.85 mg/g DW) when compared to artichoke heart (5.91 mg/g DW).

In conclusion, among the two fractions of artichoke the (bract and heart) was found to contain the highest content of flavonoids and phenolic of free phenolic extract. Significantly different was observed in the total flavonoids of the different parts of artichoke.

Table 1. Total phenolic and total flavonoids content of free and bound methanolic extracts of different parts of artichoke.

Extracts		TPC mg/g	TFC mg/g
Heart	Free phenolic extract	$9.06^{c} \pm 0.06$	$5.91^{c} \pm 0.12$
	Bound phenols extract	$5.35^{b} \pm 0.08$	$4.17^{b} \pm 0.15$
Bract	Free phenols extract	$14.16^{d} \pm 0.08$	$9.85^{d} \pm 0.12$
	Bound phenols extract	$4.20^{a} \pm 0.07$	$2.06^{a} \pm 0.11$
LSD at 0.05		0.14	0.23

All values are the mean of three replicates $\pm$ SD. All values with the same letters are not significantly different at $p \geq 0.05$

3.2 Antimicrobial activity

The spoilage and poisoning of foods by microorganisms is a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available. Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve a sufficiently long shelf life of foods and a high degree of safety with respect to food borne pathogenic microorganisms. In nature, there are a large number of different types of antimicrobial compounds that play an important role in the natural defence of all kinds of living organisms. Data presented in (Table 2) show the antimicrobial activity of different types of penolics methanolic extracts (free and bound) of artichoke bracts and heart against different species of Gram positive (*Bacilluss ubtilis*and *Staphylococcus aureus)* Gram negative

(*Escherichia coli* and *P. aeruginosa*) bacteria and the fungus (*Aspergillus niger*, and *Candida albicans).* The antimicrobial effects of both extracts of artichoke presented variable inhibition effects against pathogenic bacteria, and fungus. The antimicrobial activity of bound phenolics methanol extracts from artichoke bracts are found to be very effective in inhibiting the growth of all the tested bacteria giving a range of 21.70–27.55mm inhibition zone diameter (Table 2).

Table 2. Antimicrobial activities of free and bound methanolic extracts of different parts of artichoke

Extracts		Diameter of inhibition zone (mm)					
		Bacteria				Fungus	
		E. coli	*P. aeruginosa*	*St. aureus*	*B. subtilis*	*A. niger*	*C. abbicans*
Heart	Free phenolic extract	13.5	8.55	8.8	8.45	12.7	11.25
	Bound phenols extract	24.4	19.85	21.75	20.3	18.85	21.2
Bract	Free phenols extract	8.2	7.45	9.45	9.3	11.55	9.85
	Bound phenols extract	27.55	21.7	24.5	25.35	24.2	22.7

The maximum inhibitory effect of bound phenolics was observed on *E. Coli* (27.55 *B. subtilis (25.35)* and *St. Aureus* (24.50), meanwhile, it was moderate against *P. aeruginosa* (24.50) more than those of free phenolic extracts against pathogenic bacteria from different parts of artichoke (Heart and bract). The antibacterial activities against both gram positive and gram negative bacteria may indicate the presence of broad spectra antibiotic compounds or simply metabolic toxins in plant extracts (Moniharapon & Hashinaga, 2004). Total phenolics generally possess antimicrobial activities which provide chemical barriers for invading microorganisms. On the other hand, flavonoids inhibit bacterial growth by inhibition of DNA, cytoplasmic membrane function and energy metabolism. Gallic acid and pyrogallol as phenolic compounds, daidzein as isoflavonoid and rutin and myricetin as flavonoids are effective as antibacterial agent (Dorman & Deans, 2004).

Similar pattern was observed with fungus growth. The bound phenolic extract of the bracts and heart of artichoke exhibit antifungal activities as indicated in Table (2). Such observation was supported by Irobi &Adedayo (1999), who found that polar solvent extract has high antifungal activity against a wide range of fungal isolates including *Aspergillus niger and Candida albicans*. Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nuclide acids (Oyaizu *et al*. 2003). The results of the present study are in agreement with Zhu *et al*., (2004 & 2005) who found that the the *n*-butanol extract of artichoke leaf extract exhibited the most significant activities against seven bacteria species, four yeasts and four molds. Antimicrobial activities may be attributed to the presence of phenolic compounds chlorogenic acid, four caffeoylquinic acid, cynarin, and four flavonoids, luteeolin-7-rutinoside, cyaaroside, apigenin-7-rutinoside.

3.3 Minimum inhibition concentration (MIC)

MIC results are given in (Table 3).The bound phenolic extract from the heart was the most effective one against bacteria 0.63–0.83µg/ml followed by bound phenolic extract of bract 102–136µg/ml. The testing antibacterial activity of bound phenolic extracts from bract of artichoke exhibited that *E. Coli* were the most sensitive bacteria to the bound phenols heart extract (MIC = 63µg/ml).The variation in the effectiveness of different extracts against different strains may depend on the differences in the cell wall permeability of these microbes (Dorman &Deans, 2004).

The antimicrobial activities of free and bound phenolic methanolic extracts in different parts of artichoke can be related to their content of flavonoids and phenols which have been found effective antimicrobial substances against a wide array of microorganisms *in vitro* (Varmanu *et al*. 2011). The activity of free and pound phenolic compounds extracts is probably due to their ability to complex with extracellular and soluble proteins and too complex with bacterial cell walls (Dorman & Deans, 2004). The Minimum inhibitory concentrations (MIC) of different types of phenolic extract from different parts of artichoke against *Aspergillus flavus and Aspergillus Niger* are shown in Table (3).

Table 3. Minimum inhibition concentration (MIC) of free and bound phenolic methanolic extracts of different parts of artichoke.

Extract		MIC (µg/ml)					
		Bacteria				Fungus	
		E. coli	*P. aeruginosa*	*St. aureus*	*B. subtilis*	*A. niger*	*C. abbicans*
Heart	Free phenolic extract	204	306	306	306	306	306
	Bound phenols extract	63	63	84	84	105	105
Bract	Free phenols extract	312	468	468	468	468	468
	Bound phenols extract	102	102	102	136	136	136

The bound extract exhibited fungi toxicity against both species; however, the toxicity was higher against *A. niger* and *C. Abbicans.* Both fungi are pre-harvest pathogens of several important food crops. Both fungi produce aflatoxins, which are a potent hepatotoxic and carcinogen.

4. Conclusion

The results obtained from this study indicated that presence of various phenolics and flavonoids, together with other metabolites that are probably involved in the antibacterial, activities, support the traditional medicinal use of artichoke by-products and provide grounds for further establishing its use as a functional food. All the extracts showed very good activities that are comparable with the commercial antibiotics.

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