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Biological Activities of Phenolics in Different Parts of Local Cultivar of Globe Artichoke (*Cynara cardunculus*, var. *scolymus* L.) †

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Abstract: Different parts of *Cynara cardunculus*, var. *scolymus* L. have been used in traditional medicine to treat various disorders and as coagulant in cheese making. In this work, phenolics from different parts of glob artichoke of the local cultivar "Violet d'Alger" (outer and inner bracts, stem, choke and heart) were extracted by Soxhlet method and partially purified. Extraction yield and purification yield were determined and phenolic compounds were analyzed by Folin-Ciocalteu method. Thin-layer chromatography was performed and the antioxidant activity by 2,2-diphenyl 1-picrylhydrazyl (DPPH·) scavenging assay was achieved. Antibacterial and antifungal activities were estimated against the following bacteria and fungi: *Bacillus subtilis*, *Geobacillus stearothermophilus*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus fumigatus*, and *Candida albicans*. Results showed that all extracts had considerable amounts of phenolics with a concentration-dependent antioxidant activity and an effectiveness against bacterial and fungal strains. Among the different parts of globe artichoke, choke exhibited the highest phenolic content, antioxidant activity and antimicrobial effect.

Keywords: *Cynara cardunculus*; var. *scolymus* L.; "Violet d'Alger", phenolics; antioxidant activity; antimicrobial activity

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1. Introduction

Glob artichoke (*Cynara scolymus* L.), belonging to the family of Asteraceae (Compositae), is an herbaceous perennial crop, widely cultivated in the Mediterranean area [1]. Artichoke is not only a good food, known for its pleasant bitter taste [2], but it has been known since the middle ages for their medicinal proprieties [3]. Leaf extracts are used in phyto-medicine for their hepathoprotective effect, anticholestatic activity, bile expelling, and protection against atherosclerosis and for their antimicrobial and antioxidant actions [3–5]. These medicinal properties are related to their phenolic compounds [6], mainly composed of mono- and dicaffeoylquinic acids and flavonoids such as apigenin and luteolin glycosides [7–10]. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituent, and range from simple phenolic molecules to highly polymerized compounds [5]. The chemical activities of polyphenols in terms of their reducing properties, as hydrogen or electron-donating agents, predict their potential effect as free-radical scavengers [11,12]. The phenolic compounds prevent selectively the growth of pathogenic microbes and the level of inhibition was related to the chemical structure of the phenolic compounds and the bacterial species [13,14].

The aim of the present study was to determine the total polyphenol contents in the different parts of globe artichoke (outer and inner bracts, stem, choke and heart) and their antioxidant, antibacterial and antifungal activities.

2. Materials and Methods

2.1. Plant Sample Preparation

Cynara scolymus flowering heads, Violet d'Alger cultivar, were obtained from a garden in Boumerdès department, Algeria. The different parts (outer and inner bracts, stem, choke and heart) were washed and shade-dried for 20 days. The samples were grinded using a coffee grinder and stored in glass containers.

2.2. Extract Preparation

Thirty grams of powdered *Cynara scolymus* sample were extracted for 6 h by Soxhlet apparatus using 300 mL of 70% *v*/*v* ethanol. The extract was filtered and the solvent was evaporated to dryness using a rotary evaporator (BÜCHI). The yield (%) of evaporated dried extracts was calculated as 100DWE/DWS, where DWE was the dry weight of the extract after solvent evaporation and DWS was the dry weight of the sample. The obtained ethanolic extracts were partially purified by liquid-liquid extraction using petroleum ether to eliminate pigments and lipids and then chloroform for further purification. The obtained aqueous extracts were extracted three times by ethyl acetate (100 mL), to which we add 20% of ammonium sulfate and 2% of metaphosphoric acid. Organic phases were regrouped and dried using a sufficient quantity of sodium sulfate anhydrous. The solvent was evaporated using a rotary evaporator (BÜCHI) at 50 °C and the residue was stored in a glass vial at 4 °C.

2.3. Phenolic Content

Total phenolic contents were determined by the Folin–Ciocalteu's method previously described by Boizot and Charpentier [15]. Total phenolic content was expressed as milligram gallic acid equivalents per gram of dry plant extract (mg GAE/g DE) through the calibration curve of gallic acid.

2.4. Antioxidant Activity

The antioxidant activity of *Cynara scolymus* extracts was evaluated by the DPPH. scavenging assay [16]. 2.7 mL of DPPH solution (6×10^{-5} mol/L) were added to 0.3 mL of extract. After 60 min, incubation in the dark, the absorbance was measured at 517 nm (spectrophotometer UV-visible, Perkin Elmer). Methanol was used as a blank and DPPH. solution was used as a control. The percentage of DPPH radical inhibition was determined using the equation: DPPH. inhibition% = $100 \times (A_{control} - A_{sample})/A_{control}$ where, $A_{control}$ was the absorbance of the DPPH. solution and A_{sample} was the absorbance of the extract in different concentrations. The antioxidant activity was expressed as IC50 (concentration in mg/mL of the extracts required to inhibit 50% of the DPPH· radical formation). The IC50 values were calculated from the linear regression between the percentage of inhibition and the concentrations of extracts. Quercetin was used as standard.

2.5. Thin-Layer Chromatography (TLC)

The TLC silica gel plates with fluorescent indicator (20 × 20 cm, 60 F_{254}) were used as stationary phase. The following solvents were screened to determine the best separation compound for the TLC technique: (1) Chloroform/ethyl acetate/formic acid (5/4/1, *v*/*v*/*v*). 2) n-butanol/ acetic acid/water (4/1/5, *v*/*v*/*v*). (3) Acetone/ water (5/5, *v*/*v*). Five µL of each extract and standard (gallic acid, tannic acid, quercetin, and catechol) were added by syringe to a different TLC plate and this latter was placed in the glass tank previously saturated with the solvents. The TLC plates were dried by oven at 105 °C for 20 min. Substances were identified using UV detection at 254 and 366 nm. For visualization, plates

were sprayed with the following reagents: (1) $FeCl_3$ (1%), $K_3Fe(CN_6)$ (10%) to detect phenolic compounds. (2) Methanolic aluminum chloride $AlCl_3$ (1%) solution to detect flavonoids. (3) sulfuric vanillin (0.5%) to detect terpenoids, phenylpropane derivatives and phenols. DPPH. Solution (2.5 mg/100 mL) to detect antioxidant compounds [17].

2.6. Antimicrobial Activity

The antimicrobial activity of outer and inner bracts, stem, choke and heart of globe artichoke was determined by the agar disk diffusion assay [18] against four bacteria: *Bacillus subtilis* (1.10649 Merck KGaA), *Geobacillus stearothermophilus* (1.11499 Merck KGaA), *Staphylococcus aureus* and *Escherichia coli* and two fungi: *Aspergillus fumigatus* and *Candida albicans*. Initially, the extracts were dissolved in DMSO and filtered throw a 0.45mm Millipore filter. Bacterial and yeast inoculum suspensions, adjusted to contain 10^7 CFU/mL of bacteria and 10^6 CFU/mL of yeast, were prepared and spread on Mueller-Hinton agar medium and Sabouraud respectively. Filter paper disks of 13 mm of diameter containing 30 µL of each extract were placed on the inoculated Petri dishes. Negative control was performed using DMSO solvent employed to dissolve the different extracts. Petri dishes were then incubated during 24 h at 30, 37 and 55 °C for bacterial strains and 48 h at 30 °C for fungi. Antimicrobial activity was assessed by measuring the inhibition zone (mm) against the studied microorganisms, including disc diameter.

2.7. Statistical Analysis

Data were expressed as mean ± standard errors (SD). A one-way analysis of variance (ANOVA) using Minitab 15 statistical program was achieved to determine the significant difference with $p < 0.05$ level.

3. Results and Discussion

3.1. Extraction Yield and Total Phenolic Contents

The extraction yield of *Cynara scolymus* flowering heads depended on the studied parts (Table 1). The highest yield was registered in bracts and choke 29.21 and 28.03% respectively. The bracts' extract yield in our study is 2.5 to 20.9 folds higher than those of water, methanol, ethanol, and acetone extracts registered by Peschel et al. [19]. In addition, Falleh et al. [5] showed a very low *Cynara cardunculus* flowers' extract yield (7.56%). Thus, the extraction yield depends on the studied plant, the nature and the physicochemical characteristics of the used solvents and in particular their polarity [20]. Other parameters can influence the extract yield such as plant part, temperature, pH and time of sample contact with solvent.

Table 1. Extraction yield (%) and total phenolic contents (mg GAE/g DW) of artichoke extracts.

Parts		Bracts	Choke	Stem	Heart
Extraction yield (%)		29.21	28.03	8.8	12.19
Total phenolic contents (mg GAE/g DW)	Crude extracts	12.45	65.16	46.33	32.04
	Partially purified extracts	4.12	10.76	7.22	4.8
Purification yield (%)		33.09	16.51	15.58	14.98

Total phenolic contents in different parts of globe artichoke are shown in Table 1. Choke followed by stem crude extracts contained the highest total phenolic contents (65.16 and 46.33 mg GAE/g DW respectively). In our study bract's phenolic content (12.45 mg GAE/g DW) is lower than those reported by Peschel et al. [19] (36.65 to 102.33 mg GAE/g DW). Lattanzio et al. [21] found that artichoke's outer bracts and heart are a good source of phenolic compounds (275.76 and 1028.98 mg E caffeic acid/100 g FW). According to these authors, artichoke by-products (offshoots, leafs and external bracts of artichoke

heads), unused for human nutrition, are rich in phenolic compounds, especially chlorogenic acid and 1,5-*O*-dicaffeoylquinic, 3,5-*O*-dicaffeoylquinic and 3,4-*O*-dicaffeoylquinic acids.

The purification decreased significantly ($p < 0.05$) the total phenolic content in globe artichoke extracts. The statistical analysis showed a negative correlation between the initial total phenolic content in the crude extracts and the purification yield. Where the bracts, which had the lowest phenolic content (12.45 mg GAE/g DW), registered the highest purification yield (33.09%) (Table 1).

3.2. Antioxidant Activity

According to the data of the antioxidant activity shown in Figure 1, the IC50 values of globe artichoke organs ranged from 0.025 to 0.031 mg/mL, where choke partially purified extract exhibited the highest antioxidant capacity. Scavenging of DPPH. radical was concentration-dependent ($p < 0.05$) but not part dependent ($p > 0.05$). DPPH. radical's quenching activity of heart in our study was clearly strangest than that registered (69.91%) by Lutz et al. [22]. On the other hand, Peschel et al. [19] showed an antioxidant activity of 41.82% at 0.01 mg/mL for bract extract. In addition, the antioxidant activity of choke extract was higher than that found by Falleh et al. [5] (64.4% at 2 mg/mL) in *Cynara cardunculus* flowers. This difference shown in antioxidant activity seems to be in relation to the species, the part of the plant and the concentration used.

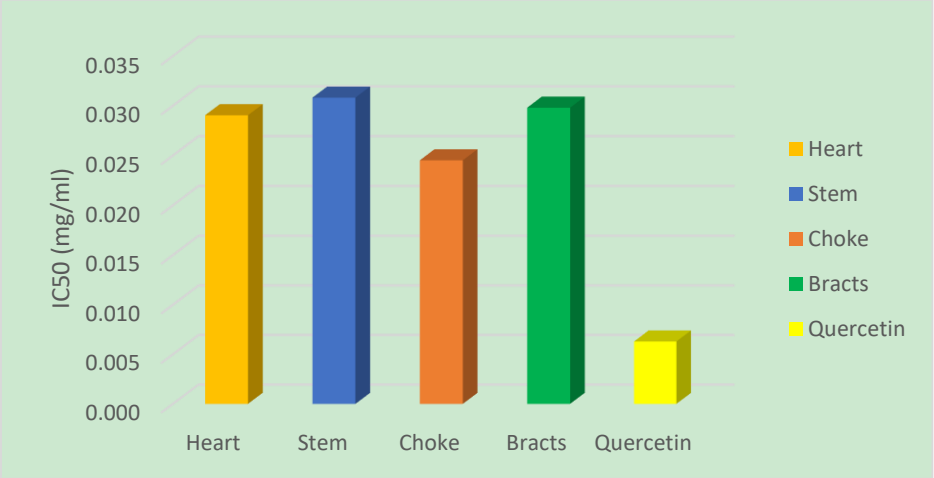

Figure 1. Antioxidant activity of *Cynara scolymus* extracts IC50 (mg per mL).

Quercetin, used as standard, expressed the strongest activity (0.006 mg/mL) as compared to globe artichoke organs. Quercetin, thanks to its antioxidant activity, was used successfully to stabilize the lipids of the meat [23]. A positive correlation has been observed ($r = 0.66$) between total phenolic content and antioxidant activity. This is confirmed by Lutz et al. [22] and Falleh et al. [5] and opposed by Peschel et al. [19].

3.3. Thin-Layer Chromatography (TLC)

Chloroform/ ethyl acetate/ formic acid (5/4/1, *v*/*v*/*v*) was the best mobile phase used to separate and identify the phenolic compounds in the samples of globe artichoke. It was noticed the presence of different phenolic compounds, with antioxidant activity, from phenolic acid, flavonoid, and tannin classes. Gallic acid, quercetin, catechol and tannic acid were identified in all the samples (data not shown).

3.4. Antimicrobial Activity

Data from Table 2 showed that choke extract expressed the highest inhibitory effect against the tested bacterial strains (1.60 ± 0.14 to 5.60 ± 0.14 cm), whereas heart extract

exhibited a moderate antibacterial activity and it was ineffective against *Staphylococcus aureus* and *Escherichia coli*. The tested extracts showed higher antibacterial potency then commercial Spiromycin.

Table 2. Inhibitory effect of partially purified extracts of globe artichoke (cm) against bacterial strains.

Bacterial Strains / Parts	*Geobacillus stearothermophilus*	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
Bracts	4.65 ± 0.49	5.15 ± 0.21	2.65 ± 0.21	1.85 ± 0.21
Choke	5.20 ± 0.28	5.60 ± 0.14	2.85 ± 0.21	1.60 ± 0.14
Stem	3.85 ± 0.07	5.35 ± 0.21	2.25 ± 0.21	2.05 ± 0.07
Heart	1.85 ± 0.21	3.65 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
Spiromycin	3.5	2.5	ND	ND

Data are reported as means ± SD of three measurements. ND: Not determined.

The inhibitory diameters differed significantly ($p < 0.05$) according to the bacterial strains. *Bacillus subtilis* appeared to be the most sensitive to the purified extracts of the various artichokes' parts with a maximum diameter of 5.6 cm followed by *Geobacillus stearothermophilus,* which recorded a maximum diameter of 5.2 cm. On the other hand, *Escherichia coli* followed by *Staphylococcus aureus* were found to be the most resistant species. Mossi and Echeverrigaray [24] found that *Cynara scolymus* leaf extract completely inhibited the growth, with a bactericidal effect, of *Staphylococcus aureus* and *Bacillus subtilis*. In their study on the antibacterial effect of the *Cynara cardunculus* leaves, Falleh and *al*. [5] reported also a greater inhibiting effect on *Staphylococcus aureus* (2.57 cm of diameter). These authors found that their extracts had an antibacterial effect against Gram (+) and Gram (-) bacteria, which is in agreement with our results. Choke partially purified extract had the most effective effect against fungal strains (2.6 cm for *Aspergillus fumigatus* and 1.95 cm for *Candida albicans*). However, heart extract expressed a low activity against *Candida albicans* (1.45 cm) and it was ineffective against *Aspergillus fumigatus* (Table 3).

Table 3. Inhibitory effect of partially purified extracts of globe artichoke (cm) against fungal strains.

Fungal Strains / Parts	*Aspergillus fumigatus*	*Candida albicans*
Bracts	1.85 ± 0.71	1.70 ± 0.14
Choke	2.60 ± 0.14	1.95 ± 0.07
Stem	1.80 ± 0.28	1.75 ± 0.07
Heart	0.00 ± 0.00	1.45 ± 0.07
E. conazole	3	0

Data are reported as means ± SD of three measurements.

In their study about *Cynara scolymus* leaves, Zhu et al. [25] found that artichoke polyphenols have a high antifungal activity. The targets of polyphenols, according to a study on *Candida albicans* realized by Boochird and Flegel [26] are the cellular wall, the cytoplasmic membrane and the cytoplasm, thus their effects on these three sites depend on the concentration used. Antifungal activity was not Fungal strains-dependent ($p > 0.05$).

4. Conclusions

Our results confirm that different parts (Bracts, choke, stem and heart) of globe artichoke (*Cynara scolymus*) showed high levels of phenolic contents and good antioxidant and antimicrobial activities. Artichoke heart and by-products appeared as a good source of health-promoting polyphenols.

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