

Research Article

Antioxidant and Antimicrobial Activities of Ethanol Extracts of *Cynara Scolymus* (*Cynarae folium*, Asteraceae Family)

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Abstract

Purpose: *Cynara scolymus* is a medicinal plant frequently used in traditional medicine for stomach and liver diseases. The purpose of the study was to identify the most suitable extraction solvent for maximum antioxidant and antimicrobial effect of fluidized bed extracts.

Methods: The extracts were obtained by conventional maceration and fluidized bed extraction using 25, 50, 75 and 97 % v/v ethanol as solvent. The antioxidant effect of the extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The quantities of phenols and flavonoids, and the reducing power of the extracts were also determined. The antimicrobial activity of the extracts was tested against different microbial strains using agar well diffusion method. The minimum inhibiting concentration (MIC) of the fluidized bed extract was also determined.

Results: The ethanol extract showed the highest antioxidant activity as well as yielded the largest quantity of polyphenolic compounds. For the extract obtained by fluidized bed technique, the optimum concentration of 10 mg/ml gave maximum antioxidant activity of 65.15 %. The MIC values obtained using the freeze-dried extract ranged from 5.0 mg/ml – 15.0 mg/ml. The extracts showed significant inhibitory activity against the tested strains of *Listeria innocua* CMGB 218, *Bacillus cereus* CMGB 215 with MIC of 5 mg/ml but showed MIC of 15 mg/ml for the other strains.

Conclusion: The results indicate that the freeze-dried extract from *Cynara scolymus* is capable of yielding nutritional supplements with antioxidant and antimicrobial activities.

Keywords: Artichoke, Freeze-drying, Fluidized bed extract, MIC

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INTRODUCTION

Artichoke (*Cynara scolymus*) is a species of perennial thistle, originating from the Mediterranean region, cultivated for the fleshy laminae in its bud as well as the edible thalami. It is a plant with large spiny leaves and a tall stem of up to 1.50 m. The leaves are used for medicinal purposes (*Folium Cynarae*) but must be picked when they are 30 - 35 cm long while the stalk and the main nerve are still tender; this makes it feasible for up to 4 - 5 harvests to be obtained per year.

The plant is used traditionally for the treatment of digestion-related illnesses, moderate hyperlipidemia as well as liver and bile diseases. It is thought that artichoke leaves are effective against liver and kidney diseases since they increase bile secretion and urine output, thus regulating the process of cholesterol formation and reducing blood sugar [1]. It is rich in cynarin and orthophenole constituents. Other phytochemicals present include cynaropicrin and sesquiterpene lactones which possess both hypoglycemic and hypolipidemic activities. It was reported that artichoke is rich in fiber and low in fat. Thus, its health benefits were due to its high fiber content [2-4].

The purpose of this study was to characterize antimicrobial and antioxidant activity of the freeze-dried ethanol extracts of artichoke. Another was to determine its total phenol and flavonoid contents.

EXPERIMENTAL

Plant material and its extraction

The dried leaves of *Cynara scolymus* was provided by Fares Bio Vital Laboratories, Orastie, România. A quantity of 20 g of the powdered leaves was subjected to extraction by percolation with ethanol (25, 50, 75 and 97 % v/v) for 2 h, 75 °C. In parallel, extraction by maceration with ultrapure water or 75 %

methanol was also carried out for 24 h at 150 rpm and 20 °C. Thereafter, the most effective alcohol extraction fluid was used to generate an extract using a fluidized bed extractor (fexIKA 200, IKA Labortechnik). The extract was freeze-dried (Martin Christ Christ Alpha 1-2 LD) to obtain the dry extract. The alcohol extracts were concentrated in a rotary evaporator (Buchi R 210) under vacuum at 40 °C, 175 mbar and 200 rpm. The elected concentrated solution was freeze-dried in a Martin Christ Christ Alpha 1-2 LD, to obtain the solid substance. The dried fractions were then re-dissolved in 80 % ethanol to yield solutions containing 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 mg of extract per ml [5,6].

Determination of total phenols content

Total phenolic content was determined using Folin-Ciocalteu reagent. Varying concentrations of the plant extract were separately mixed with 5 ml of Folin-Ciocalteu reagent (which had been diluted 1:10 with distilled water). Sodium carbonate (7.5 %, 4 ml) was added to the mixture, shaken for a few seconds, and then incubated for 30 min at 40 °C. The absorbance was read at 765 nm using a Helios spectrophotometer and against a reagent blank (ethanol:water, 50:50 v/v). The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of gallic acid in ethanol:water, 50:50 v/v. The total phenolic content was expressed in mg/g gallic acid equivalent [7,8].

Determination of total flavonoid content

A mixture consisting of 0.5 ml 2 % aluminium chloride (AlCl₃) in ethanol and 0.5 ml of plant extract was prepared. It was left for 60 min at room temperature and the absorbance measured at 420 nm and against a reagent blank (ethanol). The calibration curve was prepared by preparing quercetin solutions at concentrations ranging from 12.5 to 100 µg/ml in ethanol. The total flavonoid content was expressed as mg/ml equivalent to quercetin [9,10].

Determination of total antioxidant activity

The antioxidant activity of the extract was measured by determining the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capacity. The extract (100 μ l) was mixed with 3 ml of ethanol solution of 0.004 % DPPH and the absorbance was read at 517 nm 30 min later. Standard antioxidants (vitamin E and ascorbic acid, 1 mg/ml) were used for comparison as positive control. IC₅₀ value was calculated using the dose inhibition curve [5,11-13].

Reducing power assay

Various concentrations of the freeze-dried extract (2.5 ml) were separately mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Next, 2.5 ml of 10% trichloroacetic acid was added and the mixture centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionised water and 1 ml of 0.1 % of ferric chloride, and the absorbance measured at 700 nm: the higher the absorbance the higher the reducing power [11-13].

Preliminary assessment of antibacterial activity of extract

Escherichia coli CBAB 2, *Bacillus cereus* CMGB 215, *Listeria innocua* CMGB 218, *Candida sp.* ICCF 15, *Staphylococcus aureus* ATCC 6588, *Pseudomonas aeruginosa* ATCC 15442 and *Candida albicans* ATCC 20231 were used for the tests. For the aqueous and methanol extracts, each of the microbial strains was inoculated on a Petri plate into which LB /YPG-agar medium was previously poured. The plant extract (20 μ l) was added to the agar which was left to absorb the extract for 30 min. Thereafter, the plates were incubated at 28 to 30 °C for 24 h. The zone of inhibition was analyzed using a special Colony Quant software. [14,15].

Determination of minimum inhibitory concentration (MIC)

The standard agar dilution protocol with doubling dilution was used. The extract was incorporated into nutrient agar at concentrations ranging from 2.5 to 20 mg/ml. A control without the extract was also set up. Each of the test organisms (10 μ l), previously diluted to give 10⁶ CFU/ml, was inoculated in the plate. The plates were incubated at 30 °C for 24 h in the first instance, and for another 24 h, before the results were recorded after observing for growth. The minimum inhibitory concentration (MICs) of the extract for each test microorganism was taken as the agar plate having the lowest extract concentration without growth [15]. Ciprofloxacin and amoxycillin were used as standard antimicrobial agents and tested along with the extract.

Statistical analysis

All experiments were performed at least in triplicate and the results are presented as mean \pm SD (standard deviation). Statistical analysis was carried out using Statistica 6.0 (StatSoft Inc, Tulsa, USA). Pearson correlation test was conducted to determine correlations between the variables. Significant level was set at $p \leq 0.05$ [16].

RESULTS

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., non-fluidized method) contained high quantities of phenolic compounds. Maximum amount of phenolics was obtained using 75 % ethanol as the extraction solvent and corresponded to 50 mg/g equivalent of gallic acid. Phenolic content increased with increase in ethanol concentration except that at 97 % ethanol,

the phenolics dropped by 2.77 % compared to the extract obtained with 75 % ethanol. When 75 % methanol and water were used as extraction solvent, the decrease in phenol content (compared with 75 % ethanol extract) was 22.9 and 69.9 %, respectively. The least phenolic content resulted when 25 % ethanol was used as the extracting solvent, yielding 9.96 mg/g equivalent of gallic acid.

Flavonoid content

The total flavonoid content in the extracts varied between 104.4 mg equivalent of quercetin/ml for distilled water as the extracting solvent, to 313.65 mg/ml equivalent of quercetin for 75 % methanol. Flavonoid content also varied according to the solvent used and its ethanol concentration. The maximum was 271.5 mg/ml quercetin for 75 % ethanol (the highest for ethanol-based solvents), but this value was 13.43 % lower than that for methanol (Table 1).

Antioxidant activity

The extract obtained with 75 % ethanol demonstrated the highest inhibition of DPPH radicals. With regard to ethanol, the higher the ethanol content, the greater the inhibition capacity. Beyond an ethanol concentration of 75 %, the inhibition capacity of DPPH radicals dropped by approximately 3 % compared with 97 % ethanol.

Reducing power

Reducing power (Table 1) is a measure of antioxidant potential. Increase in ethanol concentration resulted in an increase in reducing power, except for 97 % ethanol, which showed a drop of 16.1 % in relation to 75 % ethanol. Methanol showed equivalent reducing power to ethanol when used at the same concentration. However, water continued to manifest as the weakest extraction solvent, with a decrease of 73.7 % compared to the highest values.

Antimicrobial activity

The results of the antimicrobial test are shown in Table 2. Ethanol (25 %) extract and that of 75 % methanol showed the weakest antimicrobial activity, except against the *Listeria innocua* CMGB 218, with an inhibition zone of at least 1.0 cm. The most efficient extract was the one obtained with 97 % ethanol, with a maximum inhibition zone of 1.7 cm against *Escherichia coli* CBAB 2 and *Listeria innocua* CMGB 218. In general, 75 % ethanol may be considered to be a good solvent, since extracts obtained with it were not only active against *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6588, but also against *Candida albicans* ATCC 20231, with an inhibition zone of 1.5 cm. *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6588 were not inhibited by the extract obtained with distilled water, 75 % methanol as well as by 25 and 50 % ethanol. The only exception was *Staphylococcus aureus* ATCC 6588 with an

Table 1: Total phenolic and flavonoid contents, as well as antioxidant activity and reducing power of extracts of *Cynara scolymus* obtained by simple extraction procedure

Solvent	Total phenolic content (mg/g gallic acid)	Flavonoid content (mg/ml quercetin)	Antioxidant activity (%)	Reducing power (OD 700 nm)
Ultrapure water	15.2±0.03	104.4±0.04	18.17±0.02	0.88±0.01
75% methanol	38.90±0.04	313.7±0.1	50.38±0.02	3.36±0.02
25% ethanol	9.96±0.04	106.2±0.0	31.29±0.04	2.37±0.02
50% ethanol	23.42±0.04	211.5±0.0	39.38±0.04	3.30±0.01
75% ethanol	50.50±0.00	271.5±0.0	50.38±0.04	3.36±0.04
97% ethanol	49.1±0.04	179.4±0.0	48.78±0.05	2.82±0.03

Table 2: Antimicrobial activity (cm, zone of inhibition) of extracts of *Cynara scolymus*

Extraction solvent	Microorganism						
	<i>Escherichia coli</i> CBAB 2	<i>Bacillus cereus</i> CMGB 215	<i>Listeria innocua</i> CMGB 218	<i>Candida specie</i> ICCF15	<i>Candida albicans</i> ATCC 20231	<i>Pseudomonas aeruginosa</i> ATCC 15442	<i>Staphylococcus aureus</i> ATCC 6588
Ultrapure water	0.80±0.02	-	0.90±0.03	0.80±0.01	-	-	-
75% methanol	-	1.00±0.03	1.00±0.02	-	1.00±0.00	-	0.80±0.01
25% ethanol	-	-	1.10±0.00	-	-	-	-
50% ethanol	-	0.90±0.20	1.50±0.03	0.80±0.04	0.50±0.04	-	-
75% ethanol	1.50±0.02	1.10±0.00	1.70±0.00	1.00±0.00	1.50±0.02	1.10±0.04	1.20±0.03
97% ethanol	1.70±0.03	1.50±0.04	1.70±0.02	1.20±0.04	1.00±0.02	0.70±0.05	1.10±0.04

Table 3: Antioxidant activity, total phenolic content and flavonoid content of various freeze-dried extract concentrations

Freeze-dried extract concentration	Antioxidant activity (%)	Total phenolic content (mg/g gallic acid)	Flavonoid content (mg/ml quercetin)
1	10.98±0.0	4.12±0.03	63.5±0.03
2.5	31.17±0.04	5.14±0.03	65.85±0.04
5	46.24±0.02	6.58±0.04	105±0.04
10	65.15±0.04	11.5±0.03	195.6±0.04
15	51.55±0.03	10.02±0.01	313.5±0.03
20	41.89±0.01	27.02±0.01	396.75±0.03
25	34.18±0.01	30.38±0.03	430.5±0.03

Note: The antioxidant activity of the positive controls - vitamin E and ascorbic acid (1 mg/ml each) - were 88.7±0.07 and 70.37±0.20 %, respectively

inhibition zone of 0.8 cm for 75 % methanol extract.

Antioxidant activity of freeze-dried extract

The antioxidant activity data for freeze-dried artichoke extract, which were obtained by the DPPH assay, are indicated in Table 3. Antioxidant activity increased with increase in extract concentration. The degree of discoloration of the extract indicates the potential for binding free radicals present by the freeze-dried extract. Extract concentrations > 10 mg/ml showed a

decrease in antioxidant activity. The decrease occurred gradually, by approximately 20 %, to a concentration of 50 %. At the said concentration, antioxidant activity was obtained representing approximately 77 % of ascorbic acid activity (84 %) and 73 % of α -tocopherol (88.5 %), at a concentration of 1 mg/ml. For this type of extract, the increase in concentration did not indicate a direct increase of antioxidant capacity. The IC₅₀ value of artichoke extract was of 5.9 mg/ml, which confirmed the presented data.

Table 4: Minimum inhibitory concentration (MIC) of freeze-dried artichoke extract, obtained by fluidized bed extraction, against some test microorganisms

Strain	MIC (mg/ml)	Ciprofloxacin (mg/ml)	Amoxicillin (mg/ml)
<i>Escherichia coli</i> CBAB 2	15	5	15
<i>Listeria innocua</i> CMGB 218	5	5	15
<i>Bacillus cereus</i> CMGB 215	5	5	15
<i>Staphylococcus aureus</i> ATCC 6588	5	5	7.5
<i>Pseudomonas aeruginosa</i> ATCC 15442	15	5	-
<i>Candida albicans</i> ATCC 20231	15	-	-
<i>Candida sp.</i> ICCF15	15	-	-

Total phenolic content varied between 2.12 and 30.38 mg/g equivalent of gallic acid and increased with increase in extract concentration (see Table 3). Similarly, flavonoids content also increased as extract concentration increased. The fluidized bed extract particularly showed high flavonoids content ranging from 63.5 to 430.5 mg/ml quercetin. Clearly, the use of fluidized bed extraction enhanced both the phenolic and flavonoid contents of the extract as well as its antioxidant activity.

Table 4 shows that the freeze-dried extract obtained by fluidized bed extraction had a powerful antimicrobial effect. The most sensitive strains were *Listeria innocua* CMGB 218 and *Bacillus cereus* CMGB 215 (MIC, 5 mg/ml). For all the other strains, including yeasts such as *Candida*, MIC was 15 mg/ml.

DISCUSSION

Polyphenolic compounds, often present in plant extracts, have various biological activities among which are antioxidant activity [17]. There is a direct relation between antioxidant activity and the reducing power. Furthermore, the direct relationship between reducing power and antioxidant activity has also been correlated with extract concentration and extraction method [6,16].

There was no direct relationship between phenolic content and antioxidant activity. Although, Yang *et al* [12] found such a direct relation but their work pertained to medicinal mushroom extracts. However, for medicinal

plant extracts, the studies of Bajpai *et al* [8] and Sengul *et al* [7] confirmed our own observations. These studies indicate that phenolics have varying antioxidant behavior which depends on the type of compound.

Enhancement of extraction from plant materials by the fluidized bed method indicate that the technique promotes the release of various phenolic compounds. The high flavonoid content of the fluidized bed extracts is significant in view of the claimed vasculoprotector and antiatherosclerotic effects of these compounds [4]. Thus artichoke extracts may be of use in reducing blood lipids, and when taken together with its antioxidant property, the extract could be potentially useful for cardiovascular protection. Based on the high levels obtained, it seems that a significant proportion of the pharmacological effect of the plant is due to flavonoids since the therapeutic effects of artichoke are ascribed to the presence of flavonoids.

CONCLUSION

The fluidized-bed ethanol artichoke extract showed varying antioxidant and antimicrobial activities. Although the values of these parameters were high, the fluctuation in antioxidant activity observed indicates that further studies are required to isolate the active principles of the extract. However, the extract's antimicrobial activity justifies its use in traditional medicine for the treatment of certain digestive diseases caused by pathogenic microbial strains.

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REFERENCES

1. Löhr G, Deters A, Hensel A. *In vitro* investigations of *Cynara scolymus* L. extract on cell physiology of HepG2 liver cells. *Braz J Pharm Sci* 2009; 45: 201-208.
2. Nazni P, Poongodi VT, Alagianambi P, Amirthaveni M. Hypoglycemic and Hypolipidemic Effect of *Cynara Scolymus* among Selected Type 2 Diabetic Individuals. *Paj J Nutr* 2009; 5: 147-151.
3. Gebhard R. Anticholestatic activity of flavonoids from artichoke (*Cynara scolymus* L.) and their metabolites. *Med Sci Mon* 2001; 7: 316-320.
4. Li H, Xia N, Brausch I, Yao Y, Ulrich F. Flavonoids from Artichoke (*Cynara scolymus* L.) Up-Regulate Endothelial-Type Nitric-Oxide Synthase Gene Expression in Human Endothelial Cells. *J Pharmacol Exp Ther* 2004; 310: 926-932.
5. Sreenivasan S, Ibrahim D, Mohd Kassim MJN. Free radical Scavenging Activity and Total Phenolic Compounds of *Gracilaria changi*. *Int J Nat Eng Sci* 2007; 1: 115-117.
6. Vamanu E, Vamanu A, Nita S. The obtaining of an antioxidant based on a *Rosmarinus officinalis* freeze-dried extract. *Int J Pharmacol* 2010; 6: 387 - 392.
7. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009; 22: 102-106.
8. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. *Int J Food Sci Nutr* 2005; 56: 287-291.
9. Sathishkumar T, Baskar R, Shanmugam S, Rajasekaran P, Sadasivam S, Manikandan V. Optimization of flavonoids extraction from the leaves of *Tabernaemontana heyneana* Wall. using L16 Orthogonal design. *Nature and Science* 2008; 6: 10-21.
10. Raquibul Hasan SM, Jamila M, Majumder MM, Raushanara A, Hossain MM, Mazumder MEH, Alam MA, Jahangir R, Rana MS, Arif M, Rahman S. Analgesic and Antioxidant Activity of the Hydromethanolic Extract of *Mikania scandens* (L.) Willd. Leaves. *Am J Pharm Toxicol* 2009; 13: 41-7.
11. Koksal E, Bursal E, Dikici E, Tozoglu F, Gulcin I. Antioxidant activity of *Melissa officinalis* leaves. *J Med Plants Res* 2011; 5: 217-222.
12. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. *Food Chem.* 2002; 77: 229-235.
13. Rajeshwar Y, Senthil Kumar GP, Gupta M, Mazumder UK. Studies on *in vitro* antioxidant activities of methanol extract of *Mucuna pruriens* (fabaceae) seeds. *Eur J Drug Res* 2005; 13: 31-39.
14. Seyyed MS, Niknejad M, Darabpoor I, Motamedi H. Antibacterial Activity of Hydroalcoholic Extract of *Callistemon citrinus* and *Albizia lebbek*. *Am J Appl Sci* 2010; 7: 13-16.
15. Oboh IE, Akerele JO, Obasuyi O. Antimicrobial activity of the ethanol extract of the aerial parts of *sida acuta burm.f.* (malvaceae). *Trop J Pharm Res* 2007; 6: 809-813.
16. Živković J, Zeković Z, Mujić I, Vidović S, Cvetković D, Lepojević Ž, Nikolić G, Trutić N. Scavenging Capacity of Superoxide Radical and Screening of Antimicrobial Activity of *Castanea sativa* Mill. Extracts. *Czech J Food Sci* 2010; 28: 61–68.
17. Hasan MdK, Kabir AKL, Mistry S. Chemical and Biological Investigation of Leaves of *Polygonum plebeju.*, *S J Pharm Sci* 2009; 2: 66-71.