

Chemical Composition, Antioxidant and Antimicrobial Potential of Artichoke

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Abstract: Artichoke can be eaten as a fresh, canned or frozen vegetable for its health benefits due to the high content of polyphenols. The aim of this study is to elucidate the chemical composition, antioxidant and antimicrobial activity of globe artichoke (*Cynara cardunculus L*) and baby anzio artichoke (*Cynara scolymus*). The results of this investigation revealed that, the globe artichoke showed a moisture, protein and carbohydrate content higher than baby anzio. On the other hand the baby anzio showed a lipid content higher than globe artichoke. Baby anzio extract showed a higher phenolic compounds than globe artichoke extract. The baby anzio methanol extract showed the antioxidant activity higher than globe artichoke (IC₅₀ of baby anzio extract lower than IC₅₀ of globe artichoke extract). At the same time the baby anzio extract exhibited more toxicity and inhibition zone diameter against 5 pathogenic bacterial strains than globe artichoke extract. This study confirmed that the two varieties of artichoke exhibited moderate functional properties like antioxidant and antimicrobial activity. Subsequently, baby anzio artichoke is more effective and powerful in antioxidant and antimicrobial activity.

Keywords: Anti-oxidant activity, anti-bacterial activity, artichoke, chemical composition, phenolic content.

INTRODUCTION

Artichoke belonging to the family Asteraceae is an herbaceous perennial crop [1]. Recently, a renewed and growing interest in the artichoke with a focus on new uses as a functional food has been observed [2]. Artichoke is widely cultivated for its large immature inflorescences, called capitula or heads. With edible fleshy leaves (bracts) and receptacle, which represent an important component of the Mediterranean diet, it is a rich source of bioactive phenolic compounds [3]. Artichoke can be eaten as a fresh, canned or frozen vegetable [2, 4]. Since Roman times, this plant has been used in folk medicine for its health benefits which are mainly due to the high content of phenolic compounds and inulin [5, 2, 6]. Phenolic compounds are very important substances for human nutrition since they are involved in the prevention of cancer Cardiovascular diseases; osteoporosis, diabetes mellitus and neurodegenerative diseases [7]. In addition, leaves, rich in phenolic compounds [8], are used in herbal medicine and have been recognized since ancient times for their beneficial and therapeutic effects. Extracts from artichoke have been used for hepato-protection [9]. Among the common edible plants, artichoke is a rich source of dietary anti-oxidants; therefore it could be used in phytopharmaceutical applications [2, 10]. The edible parts of

the artichoke plants are the large immature flowers, harvested in the early stages of their development, which represent about 30-40% of its fresh weight, depending on the variety and harvesting time. Since only the central portion of the capitula is consumed, the ratio of the edible fraction to the total biomass produced by the plant is very low, ranging from 15 to 20% of total biomass. This ratio further decreases, if the contribution to the total biomass represented by odd shoots that are often removed as part of common cultural procedures, is also considered [3]. The pharmacological properties of artichoke flower heads are well documented in several in-vivo and in-vitro studies for the treatment of hepato-biliary dysfunction, dyspeptic syndromes, gastric diseases, as well as for inhibition of cholesterol biosynthesis and low density lipoproteins (LDL) oxidative agents responsible for arteriosclerosis and coronary heart disease [2, 10]. Artichoke leaf extracts decreased serum lipids, as well as hepatic and cardiac oxidative stress in rats fed on high cholesterol diet [11]. Wild artichoke extracts fed to aged rats seemed to exert cardioprotective effects [11]. The classic green globe artichoke variety (*Cynara cardunculus L*), sometimes called just the globe, has a buttery-tasting heart and bottom and an ample amount of meat at the base of the petals. This artichoke, which ranges in size from three to five inches in diameter and was traditionally cultivated as a perennial, was originally brought to California from Italy but is similar in shape and flavour to the French camus de bretagne, a summer choke grown in Brittany. Light red and only roughly one inch in diameter when fully grown, the purple baby anzio (*Cynara scolymus*) is a relative of the romanesco artichoke of the Lazio region of Italy. Like many baby artichokes, baby

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anzios can be cooked and eaten whole. The aim of this study is to explore the chemical composition of different varieties of artichoke (Classic globe and baby anzio) and evaluate its potentiality as antioxidant and antimicrobial substance, to develop our knowledge about chemical and functional properties of artichoke.

MATERIALS AND METHODS

Plant Materials

The fresh artichoke Globe (*Cynara cardunculus L*) and Baby anzio (*Cynara scolymus*) plants were collected by us from the farm of the Ministry of Agriculture, Alexandria, Egypt and transferred to the laboratory under cooling condition in ice box with ice to keep the temperature under 10°C to avoid any lose of the active ingredients. The plant materials were washed with tap and distilled water, then stored in the polyethylene bags at -80°C for further analysis [12]. All experiments were carried out in triplicate.

Methanol Extract Preparation

The crude methanol extract was prepared by maceration and crush of the frozen sample in a porcelain mortar (50 g sample) with 1000 ml methanol (1:20 w/v). Mixture transferred to 2 L glass beaker, covered with aluminum foil or parafilm to prevent the evaporation of the solvent and stirred for 20 min at room temperature. The extract was filtered with filter paper (Wattman No.1) and evaporated to dryness under vacuum. The dried extract was stored at (-80°C) for further analysis.

The Yield of extraction was calculated as follows:

$$\% \text{ Yield} = (DW_e / DW_s) \times 100$$

DW_e is the weight of extract after evaporation of solvent, and DW_s is the dry weight of sample.

Moisture Content

These methods were based on measuring the mass of water in a known mass of sample. The moisture content was determined by measuring the mass of food before and after the water is removed by drying according the standard method [13].

$$\% \text{Moisture} = \frac{M_{\text{INITIAL}} - M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100$$

Here, M_{INITIAL} and M_{DRIED} are the mass of the sample before and after drying, respectively.

Total Lipid Content

Total lipid was determined according to [14, 15]. Homogenized tissue (10 g) was progressively added to small amounts of a chloroform/methanol 2:1 (v/v) mixture (up to 200 ml), with vigorous shaking, and then the extraction was carried out for further 2 h, using an electromagnetic stirrer. The mixture was filtered, re-washed with fresh solvent and pressed. Fifty milliliters of 0.88% potassium chloride were added and the mixture was shaken. The aqueous layer was removed by aspiration and the washing procedure was repeated. Adding anhydrous sodium sulphate, then filtered

again before the solvent was removed using a rotary evaporator, then dried the extract. The extract was then placed in a desiccators overnight and weighed

Total Carbohydrate Content

Chemical analysis for the determination of total carbohydrate was adapted from the phenol-Sulphuric acid method as described by [16]. One gram (1 g) of dried sample was mixed with 1 ml phenol solution (5% w/v) followed by the addition of 5 ml concentrated Sulphuric acid. The sample was left at room temperature for 30 min prior to measuring absorbance at 485 nm using a spectrophotometer (Ultrospec 2000, Amersham Pharmacia Biotech, Piscataway, NJ, USA). The total amount of carbohydrate was determined based on a standard calibration curve. The calibration curve was generated by serial concentrations of glucose (0, 25, 50, 75, 100, 125, 150, 175, 200 µg/ml). The concentration of sugar referred to original dried sample. All analyses using the phenol-Sulphuric acid method were performed in triplicate.

Total Protein Content

Determination of total nitrogen (N) was conducted using the Kjeldahl procedure according to Nelson and Sommers method [17]. 1 g sample as weighed on Wattman No. 1 filter paper circles and filter paper was folded around and putted in Kjeldahl flask. One blank with the samples was run. 2 boiling chips were added to every flask and 30 ml of Sulphuric acid were added to flask. The sample digested in the digestion Kjeldahl unit for 2 hrs then removed from digestion unit and capped immediately with rubber stoppers and let to cool for 30 min. For distillation 50 ml of boric acid was added to 500 ml flask for sample and blank and put in the Kjeldahl distillation unit and the distillation was run out with sodium hydroxide. After distillation the titration was carried out with 0.1 N Sulphuric acid. The total nitrogen was calculated as follow

$$\% \text{N} = (T - B) \times N \times 1.401$$

where: T = mL of sample titrated, B = mL of blank titrated, N = acid normality

The % of protein = % N x 6.25

Total Phenolic Compounds

The total phenolic compounds assay was carried out using the Folin-Ciocalteu reagent, following the method of [18], and based on the reduction of a phosphomolibdate-phosphomolebdate complex by phenolics to blue reaction products. 1mg extract was dissolved in 1ml methanol and 500 µl of dissolved sample was taken and added to 0.5 ml of distilled water and 0.125 ml of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before addition of 1.25 ml of 7% Na₂CO₃. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/mL). Total phenolic content was estimated as µg Gallic acid equivalents (GAE)/mg of dry weight.

DPPH Radical Scavenging activity

The Diphenyl Picrylhydrazyl (DPPH) Radical scavenging activity was estimated according to [19]. The dried plant extract was diluted in pure methanol at different concentrations ranging from 1-to 200- μ g/ml, and then 2 ml were added to 0.5 ml of a 0.2 mM DPPH methanol solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. For each dilution, the DPPH scavenging activity was calculated as $(A_0 - A_1) / A_0 \times 100$, where A_0 is the absorbance of the control after 30 min incubation, and A_1 is the absorbance of sample after 30 min incubation. The antiradical activity was finally expressed as IC_{50} (μ g/ml), the extract concentration required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher anti-oxidant activity of the plant extract. All results compared with the same serial concentration of Ascorbic acid as known antioxidant agent.

Anti-Microbial Activity

The antimicrobial activities against 5 types of bacterial strains were obtained from Cairo-MIRCEN (Microbiological Resource Center) Faculty of Agriculture, Ain Shams University, (*Proteus vulgaris* ATCC6830, *Escherichia coli* 0-143, *Staphylococcus aureus* 0006, *Klebsiella pneumonia* 8961 and *Bacillus subtilis* ATC6633) were studied. The determination of the minimal inhibitory concentration (MIC) was achieved by an adaption of the agar streak dilution method based on radial diffusion [20, 21]. Suspensions of the microorganism were prepared to contain approximately 180 cfu/ml and the plates containing agar medium were incubated (100 μ l spread on the surface). Different concentrations of extract were placed in the hole (3 mm depth, 4 mm diameter) made in the center of the agar. Under the same conditions, different concentrations of Ampicillin were used as standard antibiotic. The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 hr.

Statistical Analysis

Statistical analysis was carried out by Student T-Test

program. All experiments were carried out in triplicate and the results were presented as mean \pm SD and $p \leq 0.05$.

RESULTS

Chemical Analysis

The results of chemical analysis of artichoke are reported in Table 1. The results of moisture analysis showed that, Moisture content of globe artichoke was (72.53 ± 0.24) whereas the moisture content of baby anzio artichoke was (65.24 ± 0.15) . The same trend was recorded with protein $(14.54 \pm 0.12; 12.39 \pm 0.18)$ and carbohydrate $(73.65 \pm 0.13; 56.72 \pm 0.23)$ contents for globe artichoke and Baby anzio artichoke respectively. The total lipid of globe artichoke was (02.36 ± 0.07) and for baby anzio was (03.78 ± 0.14) . From the obtained results we noted that the protein and carbohydrate content in globe artichoke significantly higher than baby anzio species but less in lipid content.

Total Phenolic Compounds (TPC)

Methanol extract of artichoke was prepared to determine the total phenolic content. The yield of extract obtained from two species of dry artichoke (globe and baby anzio) materials were $(17.5\% \pm 0.14g)$ for globe artichoke and $25.4\% \pm 0.21$ for baby anzio as shown in Table 2. The total phenolic content (TPC) in methanol extract was determined and the results revealed that, the TPC in globe artichoke and baby anzio species were $(30.70 \pm 1.87$ and 38.31 ± 0.96 mg GAE/g dry sample) respectively Table 2. These results revealed that the baby anzio species contained TPC 1.25 times more than globe variety.

DPPH-Free Radical-scavenging Activity

DPPH-Free radical-scavenging activity assay was performed to determine antioxidant activity in the two examined varieties of artichoke methanol extract. The obtained results presented that, IC_{50} of globe artichoke extract was 75 μ g/ml that gave 55.12 ± 0.31 percent inhibition, and 50 μ g/ml for baby anzio gave the 49.52 ± 0.16 percent inhibition. Whereas; IC_{50} of ascorbic acid was less than 10 μ g/ml was gave 58.41 ± 0.16 percent inhibition

Table 1. Chemical composition of artichoke. The values is the mean of three replicates of the sample \pm SD.

General Analysis	Classic Glob	Baby Anzio
Total Moisture	72.53 \pm 0.24	65.24 \pm 0.15
Total Protein	14.54 \pm 0.12	12.39 \pm 0.18
Total Carbohydrate	73.65 \pm 0.13	56.72 \pm 0.23
Total Lipid	02.36 \pm 0.07	03.78 \pm 0.14

Table 2. The yield and total phenolic compounds of artichoke.

	Classic Globe	Baby Anzio
Yield	17.5% \pm 0.14	25.4 \pm 0.21%
Total Phenolic compounds (mg GAE/g DW)	30.70 \pm 1.87	38.31 \pm 0.96

Table 3. The ascorbic acid as standard showed the higher antioxidant activities than the two varieties of artichoke extract especially at low concentrations. The baby anzio species showed significant antioxidant activity higher than globe artichoke variety ($p \leq 0.05$).

Anti-microbial Activity

Results of the evaluation of antibacterial activity of methanol extract of artichoke showed a significant toxicity against *Proteus vulgaris* ATCC6830, *Escherichia coli* 0-143, *Staphylococcus aureus* 0006, *Klebsiella pneumonia* 8961 and *Bacillus subtilis* ATC6633. The MIC of methanol extract of globe artichoke variety was 100 mg/ml but for baby anzio variety was 75 mg/ml. The artichoke methanol extract exhibited different inhibition zones against different bacterial strains as shown in Table 4. The toxicity was higher against *Escherichia coli* (2.76 ± 0.21 cm) and *Proteus vulgaris* (2.63 ± 0.15 cm), than against *Staphylococcus aureus* (1.75 ± 0.13), *Klebsiella pneumonia* (1.60 ± 0.14) and *Bacillus subtilis* (1.86 ± 0.23) for globe artichoke extract. At the same time, the baby anzio species showed higher toxicity against *Escherichia coli* (3.54 ± 0.25), *Proteus vulgaris* (3.42 ± 0.32) and *Staphylococcus aureus* (3.45 ± 0.19) than that against *Klebsiella pneumonia* (2.12 ± 0.16) and *Bacillus subtilis* (2.67 ± 0.26). Methanol extract of baby anzio was more

effective against five tested bacterial strains than globe artichoke extract ($p \leq 0.05$). The ampicillin as positive control showed a higher toxicity against five examined bacterial strains than both globe and baby anzio extracts Table 4.

Processing the data, we found a significant positive correlation between the concentration of total phenolics in artichoke and antioxidant activity and antibacterial activity.

DISCUSSION

Chemical Analysis

The general chemical analysis revealed that, globe artichoke containing water more than baby anzio, this may attributed to the leaves of globe artichoke species thicker than of baby anzio species and retains a large amount of water in tissues. The protein and carbohydrate contents in globe variety are higher than baby anzio ($p \leq 0.05$); on the other hand, the lipid contents in baby anzio species are greater than globe species. These differences in the chemical composition of two species of artichoke might be attributed to the essence of the plant species and the growth conditions of the plant. These findings agree with Lutz *et al.* 2011 [22] in their study of chemical composition of mature artichoke. The results of chemical analysis revealed that; artichoke might be considered a good source for protein and carbohydrate.

Table 3. Antioxidant activity of artichoke ($p \leq 0.05$). The values mentioned are the means of triplicates \pm SD.

Conc. μ g/ml \ % Inhibition	Globe Artichoke Extract	Baby Anzio Extract	Ascorbic Acid
10	17.41 \pm 0.26	20.34 \pm 0.23	58.41 \pm 0.16
25	25.45 \pm 0.41	36.23 \pm 0.21	65.92 \pm 0.22
50	31.92 \pm 0.32	49.52 \pm 0.16	76.61 \pm 0.08
75	55.12 \pm 0.31	62.19 \pm 0.19	82.17 \pm 0.13
100	68.36 \pm 0.38	74.86 \pm 0.21	88.81 \pm 0.15
150	72.96 \pm 0.29	79.78 \pm 0.14	92.65 \pm 0.10
175	77.96 \pm 0.19	86.43 \pm 0.17	95.80 \pm 0.13
200	80.55 \pm 0.14	87.57 \pm 0.24	97.25 \pm 0.18

Table 4. Antibacterial activity of methanol extract of artichoke (MIC=100mg/ml for globe artichoke, MIC= 75mg/ml for baby anzio) ($p \leq 0.05$). *(IZD) = Inhibition zone diameter. (-) = No inhibition zone detected. The values mentioned are the means of triplicates \pm SD.

Bacterial Strain	Artichoke Methanol Extract (IZD* cm)		Ampicillin (IZD* cm)	Methanol (IZD* cm)
	Globe	Baby anzio		
<i>Proteus vulgaris</i>	2.63 \pm 0.15	3.42 \pm 0.32	3.63 \pm 0.06	-
<i>Escherichia coli</i>	2.76 \pm 0.21	3.54 \pm 0.25	3.82 \pm 0.07	-
<i>Staphylococcus aureus</i>	1.75 \pm 0.13	3.45 \pm 0.19	2.56 \pm 0.03	-
<i>Klebsiella pneumonia</i>	1.60 \pm 0.14	2.12 \pm 0.16	2.85 \pm 0.03	-
<i>Bacillus subtilis</i>	1.86 \pm 0.23	2.67 \pm 0.26	3.65 \pm 0.07	-

Total Phenolic Content

The baby anzio variety gave an extraction yield more than globe one. The less yield of globe artichoke may be due to its high content of water. In the same time, the baby anzio gave TPC 1.25 times more than globe artichoke in methanol extraction. It may be due to the nature of variety and biosynthesis pathway and accumulation of the phenolic compounds in the plant.

Antioxidant Activity

The baby anzio species showed antioxidant activity higher than the globe species ($p \leq 0.05$). It may be due to the higher phenolic content of baby anzio than that in globe artichoke. These results agree with [23], who reported the aqueous methanol extract of artichoke contains a unique polyphenolic compounds having a strong antioxidant activities. These results show that, the artichoke containing a reasonable level of antioxidant ingredients that can play an important role in the potentiality of artichoke as a good nutrition source.

Antimicrobial Activity

The baby anzio species delivered a broad antimicrobial activity than the globe one ($p \leq 0.05$). In the same time both species showed reasonable antimicrobial activities against five pathogenic strains, (*Proteus vulgaris* ATCC6830, *Escherichia coli* 0-143, *Staphylococcus aureus* 0006, *Klebsiella pneumonia* 8961 and *Bacillus subtilis* ATC6633), but methanol extract showed the most toxicity against *Escherichia coli* 0-143 and *Proteus vulgaris* ATCC6830. On the other hand *Staphylococcus aureus* 0006 and *Klebsiella pneumonia* 8961 were the most resistant to methanol extract of artichoke. The mechanism of antibacterial may be due to diffusion of the active compounds from the site of application to the site of action where it can exert its toxicity [24, 25]. This indicates that these extracts may be used as an antibacterial agent with reasonable safety margins to inhibit bacterial growth. The known anti-bacterial mechanism associated to each class of chemical to which the isolated compounds belong, may explain the antibacterial potency of the crude extract. The anti-bacterial activity of artichoke extract might be due to the presence of phenolic compound and the ability of phenolic compounds to bind to bacterial cell walls [26] and inhibit the microbial growth. The high effect of baby anzio extract may be attributed to the high content of phenolic compounds.

CONCLUSION

The aim of this study is to explore the chemical composition of globe (*Cynara cardunculus* L) and Baby anzio (*Cynara scolymus*) artichoke species and elucidate their antioxidant and antimicrobial activities to understand the nutritional potential of artichoke. Artichoke chemical analysis brings to light that artichoke contains a reasonable amount of essential nutritional ingredients like protein, carbohydrate and fibers that can play the key role of the metabolic process and has health benefits in human nutrition. Methanol extracts of globe and baby anzio artichoke showed a considerable antioxidant ingredients and antimicrobial

activity. The baby anzio artichoke showed a higher antioxidant and antimicrobial activity than globe artichoke extract. This study revealed that there is a relationship between phenolic compounds and the potentiality of artichoke as antioxidant and antimicrobial. These findings revealed that, phenolic compounds are a key factor in the anti-oxidant activity of artichoke extract. Finally, we can conclude that; the artichoke plant since they may have a health benefit due to the high level of phenolic compounds and may be used to extract and formulate effective food supplements and additives.

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