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ANTIOXIDANT ACTIVITY, TOTAL FLAVONOID, TOTAL PHENOLIC AND ANTHOCYANIN CONTENTS OF *CYNARA SCOLYMUS L.* LEAVES AND *TRIGONELLA FOENUM-GRAECUM L.* SEEDS

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Abstract. *In the present comparative study we are analyzing the antioxidant activities, total flavonoid, total phenolic and total anthocyanin contents of artichoke (Cynara scolymus L.) and fenugreek (Trigonella foenum-graceum L.) extracts. Aqueous and alcoholic extracts were made from equal amounts of artichoke leaves and fenugreek seeds. The obtained results are indicating that the aqueous artichoke and fenugreek extracts antioxidant capacities are similar, while the corresponding alcoholic extracts antioxidant capacities are greatly reduced. The total phenolic content of alcoholic artichoke and fenugreek extracts was much greater than in case of the corresponding aqueous extracts. The anthocyanin type of flavonoids were not detected in any of our extracts made of artichoke leaves and fenugreek seeds. In case of artichoke leaf or fenugreek seed extracts, the total flavonoid and total phenolic contents, presumably, have very little if any involvement in the antioxidant capacities of artichoke and fenugreek, respectively.*

Keywords: artichoke, fenugreek, DPPH, flavonoid, polyphenols, anthocyanin

1. Introduction

Artichoke

Teas and extracts made from the leaves of the artichoke plant are a traditional tonic for the liver and gallbladder [1, 2]. Both laboratory and clinical studies strongly suggest artichoke leaf extract to be a good therapeutic option in dyspeptic syndrome (irritable stomach, nervous gastropathy, flatulence, irritable colon, functional biliary tract disease), [2]. In Europe the plant is widely used to treat

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arteriosclerosis and lower cholesterol. Several studies have hinted at the ability of artichoke extract to lower lipid levels. It works by affecting cholesterol synthesis in the liver at several points in the synthetic pathway and by increasing the elimination of cholesterol [1]. The plant chemical constituent cynarin acts as lipolysis-inhibitor in much the same manner as nicotinic acid (niacin), without the gastric upset and flushing that niacin supplements could cause. The diuretic properties of the artichoke are also useful in kidney disease [2].

Fenugreek

Fenugreek (*Trigonella foenum-graecum*) is a medicinal food plant that has the beneficial effect of lowering blood sugar. The bitter seeds called "methi" in India, are used as a condiment, and the leaves are used in teas. Fenugreek contains potent antioxidants that have beneficial effects on the liver and pancreas, making it useful in the treatment of diabetes, high cholesterol, and digestive disorders [3, 4]. The hard, brown, red and yellow seeds are the part used medicinally and in cooking. Traditional uses included bronchial problems, tuberculosis, gout, general body pain, swollen glands, skin problems and low libido.

2. Materials and methods

The artichoke dried leaves were produced by the TTDR 2000 Ltd., Hungary. 10 g of sample was extracted for 5 min with 200 ml of boiling distilled water (Aqueous Artichoke extract, AqA). It was allowed to cool to room temperature (RT), filtered and stored in refrigerator. Another 50 g of dried artichoke leaves were extracted twice with 500 ml aqueous 50% ethanol solution by stirring for 4 hrs on a magnetic stirrer (Alcoholic Artichoke extract, AlA). This extract was filtered, evaporated under vacuum and stored in refrigerator

The fenugreek seeds were produced by TRIGONELLA MED. LTD., Mosonmagyaróvár, Hungary. Fenugreek seeds were dried at 40 °C and powdered. 15 g of the powder was extracted with 300 ml of boiling distilled water for 5 min. The mixture was allowed to cool at RT, filtered and stored in refrigerator (Aqueous Fenugreek extract, AqF). Other 15 g of powdered fenugreek seeds were refluxed on a magnetic stirrer with 300 ml aqueous 80% ethanol for 3 hrs (Alcoholic Fenugreek extract, AlF). The mixture was filtered and the filtrate was concentrated by rotary evaporator and stored in refrigerator.

2.1. Determination of antioxidant capacity by the DPPH method

The DPPH (2,2- Diphenyl-1-picrylhydrazyl, Sigma-Aldrich) method was used to determine the antioxidant capacity of artichoke and fenugreek extracts. The method is based on the scavenging of DPPH radical through the action of an antioxidant that decolourizes the DPPH solution. The procedure was described by

Molyneux (2004) [5]. 200 µl of extract were added to 300 µl of methanol and 2400 µl DPPH solution (22.6 µg/ml). The mixture was left to stand at room temperature for 30 minutes in the dark and filtered (0.45 µm) before the absorbance was measured at 517 nm. (UV/VIS Spectrometer, Perkin Elmer Lambda 35). All the measurements were performed in triplicate. The results were expressed as ascorbic acid equivalents by means of the dose-response calibration curve. The sample concentration providing 50 % inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration. Each extract was measured in triplicate.

2.2. Quantification of total flavonoids

The total flavonoid content of the extract was determined by the aluminium chloride colorimetric method following the procedure described by Dae-Ok Kim et al. (2003) [6]. We have made a reagent solution containing 5 ml (10g/ml) of aluminium chloride, 5 ml of KOAc (potassium acetate, 1M/l), 75 ml of methanol and 140 ml of distilled water (AS). 0.5 ml of the extract was mixed with 4.5 ml of AS, filtered (0.45 µm) and the absorbance was determined at 415 nm in the spectrophotometer (Perkin Elmer Lambda 35). The concentrations were calculated based on the equation obtained from the standard rutin curve (10, 40, 70, 100 µg/ml). Results were expressed as mg rutin equivalents. Each extract was measured in triplicate.

2.3. Determination of total phenolic content

Total phenolic content was determined using the method developed by Singleton and Rossi (1965) [7]. Total phenolic content was estimated by the Folin-Ciocalteu colorimetric method, using gallic acid as a standard phenolic compound. 200 µl of extracts were added to 3000 µl of distilled water, 500 µl of Folin-Ciocalteu reagent and 2000 µl of sodium carbonate solution (15g/100ml), and the mixture was allowed to stand for 20 min at RT. 4300 µl of distilled water was added and the absorbance was measured at 765 nm after 1 hour incubation period with the spectrophotometer (Perkin Elmer Lambda 35). The total flavonoid contents were calculated from a calibration curve with 50, 100, 300, 500, 750 µg/ml points and the results were expressed as mg gallic acid equivalent per g dry weight. Each extract was measured in triplicate.

2.4. Determination of total anthocyanins

The total anthocyanin (TA) content was determined using an earlier described method (Lee et al., 2005) [8]. It detects the total monomeric anthocyanin concentration by the pH differential method. The method is a rapid and simple

spectrophotometric application based on the anthocyanin structural transformation that occurs upon a change in pH (colored at pH 1.0 and colorless at pH 4.5). The standard solution was prepared by weighing 82.2 mg of cyanidin-3-glucoside chloride, dissolving in distilled water and diluting to a final volume of 1 L in a volumetric flask. 0.3 ml of extract was added to 2 ml of buffer (pH=1 or pH=4.5) and 1.7 ml of distilled water. Samples were filtered (0.45 µm), and absorbance recorded using a Perkin Elmer Lambda 35 spectrophotometer at wavelengths of 520 and 700 nm, for solutions at pH 1.0 and pH 4.5, respectively. Results were expressed as cyanidin-3-glucoside (% w/w) equivalents. Total anthocyanin contents were calculated as follows:

$$TA_{(mg/L)} = \left(\frac{A * MW * DF * 10^3}{\varepsilon} \right) \div l \qquad (2)$$

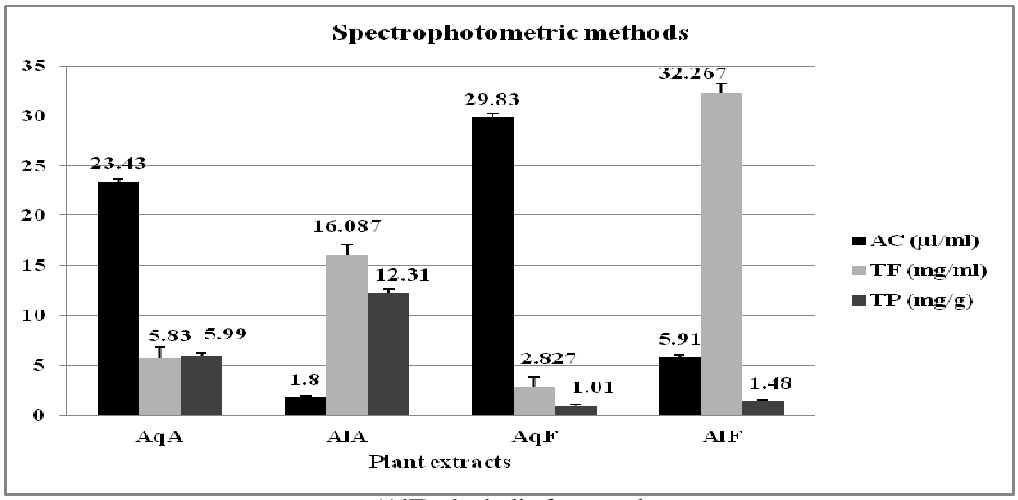
where A = (A_{520nm} – A_{700nm})pH 1.0 – (A_{520nm} – A_{700nm})pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; l = path length in cm; ε = 26 900 molar extinction coefficient, in L* mol^{-1} * cm^{-1} , for cyd-3-glu; and 10^3 = factor for conversion from g to mg. Each extract was measured in triplicate.

3. Results and discussions

The artichoke and fenugreek are considered to have important health promoting effects, and many of such claims were related to their antioxidant properties that seemed to be correlated with the total flavonoid, total phenolic and anthocyanin content. Therefore we proposed to analyze the artichoke and fenugreek antioxidant activity, total flavonoid, total phenolic and anthocyanin contents. All the experiments were carried out as we described in Materials and methods, and the obtained results are presented in Table 1 and Figure 1.

Table 1) Means and standard error of antioxidant capacity, flavonoid-, phenolic- and anthocyanin content of artichoke and fenugreek extracts (n=3)

Spectrophotometric methods	**Aqueous artichoke (AqA)**	**Alcoholic artichoke (AlA)**	**Aqueous fenugreek (AqF)**	**Alcoholic fenugreek (AlF)**
Average antioxidant capacity (µl/ml)	23.43±0.25	1.80±0.16	29.83±0.41	5.91±0.18
Average total flavonoid content (mg/ml)	5.830±0.31	16.087±0.39	2.827±0.22	32.267±0.61
Average total phenolic content (mg/g)	5.99±0.36	12.31±0.34	1.01±0.15	1.48±0.12
Average total anthocyanin content (mg/l)	0	0	0	0



extract, (AlF) alcoholic fenugreek extract

From the obtained data we can conclude that the aqueous artichoke and fenugreek extracts antioxidant capacities were relatively high, while the alcoholic artichoke and fenugreek extracts antioxidant capacities were markedly reduced as compared to their corresponding aqueous counterparts. The aqueous extract compared to the alcoholic extract showed an approximately 16 times greater antioxidant capacity in case of artichoke. In case of fenugreek the aqueous extract showed a 5 time greater antioxidant capacity than the alcoholic extract.

The total flavonoid content of artichoke and fenugreek alcoholic extracts exceeded substantially the flavonoid content seen in both plants derived aqueous extracts. Accordingly, the artichoke specific alcoholic extract total flavonoid content appears 3 times greater as compared to the aqueous extract. The fenugreek total flavonoid content of the alcoholic extracts looks almost 12 times greater than the aqueous extract related values. Moreover, if the two plants specific alcoholic extracts are compared, it looks obvious that the fenugreek values are two times greater than the artichoke values for total flavonoid content suggesting that the fenugreek seeds contain twice as much flavonoid than the artichoke. Surprisingly, when the aqueous extracts of artichoke and fenugreek are compared, the artichoke extract specific total flavonoid content was approximately double to that seen in case of fenugreek extract.

The total phenolic content of artichoke alcoholic extract exceeded more than 2 times the values observed for the artichoke aqueous extract suggesting that the alcoholic artichoke extract contains twice as many phenolic compounds like the aqueous artichoke extract. However, the aqueous and alcoholic fenugreek extracts seemed to contain similar amount of phenolic compounds.

Surprisingly we were unable to detect anthocyanin in any of our artichoke and fenugreek extracts.

Conclusions

(1).The aqueous artichoke and fenugreek extracts antioxidant capacities are almost the same, and the corresponding alcoholic extracts are showing law values suggesting that the alcoholic extracts antioxidant capacities are greatly reduced.

(2). The total phenolic content of alcoholic artichoke and fenugreek extracts was much greater than in case of the corresponding aqueous extracts,

(3). It seems that anthocyanin type of flavonoids are not present in artichoke leaves and fenugreek seeds.

(4). In case of artichoke leaf or fenugreek seed extracts, the total flavonoid and total phenolic contents, presumably, have very little if any involvement in the antioxidant capacities of artichoke and fenugreek, respectively.

(5). Both artichoke leaves and fenugreek seeds should have antioxidants other than those that were assessed. It remains to get identified the antioxidant properties ensuring compounds in the case of alcoholic extracts.

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R E F E R E N C E S

[1] K. Kraft, *Phytomedicine* **4**, 369 (1997).

[2] R. Mabey, *The New Age Herbalist* (Simon & Schuster, New York, 1988).

[3] J. A. Duke, *The Green Pharmacy* (Rodale Press Emmaus, Pennsylvania, 1997).

[4] J. Barnes and L. A. Anderson, *Herbal Medicines* (Pharmaceutical Press, London, 2007).

[5] P. Molyneux, *Songklanakarin J. Sci. and Technol*. **26**, 211 (2004).

[6] D. O. Kim et al., *J. Agric. Food Chem*. **51**, 6509 (2003).

[7] V.L. Singleton and J.A. Rossi, Amer. *J.* Enol *Viticult.* **16**, 144 (1965).

[8] J. Lee et al., *J. AOAC Int.* **88,** 1269 (2005).