한국환경과학회지 제17권(제1호), 19~27, 2008
Journal of the Environmental Sciences

Total Phenolic Compounds and Flavonoids in the Parts of Artichoke (*Cynara scolymus* L.) in Viet Nam

Bui Ha Thu Thi and Moon-Ki Park

Department of Environmental Engineering and Architecture, Daegu Haany University, Gyeongbuk 712-240, Korea

(Manuscript received 28 December, 2007; accepted 15 January, 2008)

Abstract

Artichoke extracts are widely used alone or in association with other herbs for embittering alcoholic and soft drinks and to prepare herbal teas or herbal medicinal products in Viet Nam. The objective of this paper was a screening of flavonoids and total phenolic compounds content in the parts of artichoke *(Cynara scolymus* L.) as flowers, leaves, roots, trunks, stumps. The total phenolic compounds and flavonoids in the parts of artichoke were extracted among 3 extraction methods as methanol extraction (EM1), mixing methanol and water method (EM2) and water extraction method (EM3). Total phenolic compounds and flavonoids were determined by UV/VIS, HPLC techniques. The apigenin 7-O-glucosides, cynarin, narirutin, gallic acid, caffeic acid were found as the main flavonoids constituents in all parts of artichoke. It showed that value of total phenolic compounds and flavonoids by EM3 were higher than that of total phenolic compounds and flavonoids by EM1 and EM2. Furthermore, the results of this study revealed that total phenolic compounds and flavonoids, obtained by these convenient extraction methods, may show the quick efficacy of artichoke in all respects of their quality and quantity.

Key Words : Artichoke, Total phenolic compounds, Flavonoids, Cynarin, Narirutin, Apigenin 7-O-glucoside, Caffeic acid, Gallic acid, Extraction method

1. Introduction

The globe artichoke has become important as a medicinal herb in recent years. The main pharmacologically active constituents are thought to be the phenolic acids and flavonoids. One study using artichoke leaf juice showed it improved endothelial reactivity, most likely by its antioxidant constituents[1]. Polyphenolic compounds, present mainly in the leaves rather than in the artichoke flower, have been documented as the active principles or this plant. The results from several clinical investigations showed the artichoke extracts in the treatment or hepatobiliary and digestive complaints, such as loss or appetite, nausea, and abdominal pain[2].

Flavonoids are found in most plant material. The most important dietary sources are fruits, tea, soy bean. Artichoke, green and black tea contains about 25% flavonoids. Some of the activities attributed to flavonoids include: antiallergic, anticancer, antioxidant, antiinflammatory and antiviral. The flavonoids are known for its ability to relieve fever, eczema, sinusitis and asthma[3].

2. Materials and Methods

2.1. Materials and regents

The parts of the body of artichoke were collected at market in DaLat, Viet Nam. The flowers, leaves, trunks, roots, stumps of artichoke were dried and cleaned, then they were kept in sealed bags at room temperature for further extraction.

Corresponding Author : Department of Herbal Pharmaceutical Engineering, Daegu Haany University, Gyeongbuk 712-240, Korea
Phone: +82-53-819-1420
E-mail: moonki@dhu.ac.kr

Methanol (MeOH), acetic acid (AA), acetonitrile (ACN) was obtained from Daejung Chemicals & Metals Co., ltd. Folin-Ciocalteu's reagent (2N) from Sigma Chemical Co., U.S. Sodium carbonate Na_2O_3 from Sigma Chemical Co., U.S. Caffeic acid, gallic acid, apigenin 7-O-glucoside, cynarin, narirutin were purchased from Sigma Chemical Co., U.S.

2.2. Methods for extraction

This method describes the container preparation, field sampling and field extraction preservation procedure to be used in conjunction with the analysis of plants samples for flavonoids. Plants extract also include herb extract. Regarding herb extract and essential oils, we are following information for your reference. Extracts are obtained by compressing herbs typically with a hydraulic press while soaking them in alcohol or water. The alcohol or water is allowed to evaporate the remaining substance is a concentrated extract. Extractions are the most common forms of herbal remedies found on the shelf of health food stores in the U.S. They are also the most effective form of herbal substance used as a remedy. Alcohol free extracts a desirable to individuals who are recovering alcoholics, concerned about being exposed to alcohol and/or the alcohol solution to which the extract is packaged. Alcohol free extracts are also considered to be more desirable because of quality. Since extracts are highly concentrated amounts of the chemical composition, care should be taken to avoid over use.

The Hot Water Extraction (HWE) method is a method used in chemistry for extraction and for "steam cleaning". The pressurised hot water extraction (PHWE) process uses a combination of high water pressure for agitation, and hot water to increase reaction rate[4].

Extraction method 1 (EM1): Artichoke samples collected for flavonoids and total phenolic compounds analysis must be cut into many small pieces.

Each parts of artichoke 5 g was extracted by 100 ml MeOH. This experiment run 3 times and one times was taken 3 days by dissolubility.

Extraction method 2 (EM2): Ground samples of artichoke heads and pomace were extracted with 60% aqueous methanol as described previously without further modification[5]. But this study was modified that the combination methanol and water were in the ratio of 60% methanol to 40% water in condition of soaking temperature (25°C), time (25 min) in ultrasonic bath. The 5 g of each samples was extracted by 100 ml ($MeOH:H_2O$, 60:40), this solution guaranteed the maximum solubility for analyses were prepared by dissolubility for all the samples. Then, all the samples applied a laboratory ultrasonic bath for 25 minutes in laboratory temperature.

Extraction method 3 (EM3): This is an easy way to take good natural compounds from many kinds of teas and herbs in general and artichoke in particular[6].

Put 5 g material with 350 ml distilled water into stainless spot and boiled for 1 hour.

2.3. Preparation for total phenolic compounds analysis

From each 0.25 ml calibration solution, sample, or blank into 25 ml volumetric flask and to each add 5-10 ml water, and then add 1.25 ml of the Folin-Ciocalteu reagent, and mix well. Wait for between 30 seconds and 8 minutes, and then add 3.75 ml of the sodium carbonate solution (20%), and shake to mix. Leave the solutions at 20°C for 2 hours and determine the absorbance of each solution at 760nm against the blank (the "sample free" solution) and plot absorbance concentration.

Preparation for total phenolic compounds analyzed by UV/VIS spectrophotometer (Optizen 2120 UV).

2.4. Preparation for HPLC analysis

About 5 mg of each standard compound was accurately weighed and placed into a 25 ml volumetric flask. 15 ml of 60% methanol were added, and the solutions sonicated for 15 minutes. The flasks were allowed to cool to room temperature and filled to full volume with 60% methanol solution. 5 ml of the above solution were transferred to a new 25 ml volumetric flask and diluted to the full volume using 60% methanol. Calibration curves were established on five data points covering concentration 100, 50, 25, 12.5, 6.25 mg/l and 1 μl aliquots were used for HPLC analysis.

Flavonoids analyses were carried out using the Ultra Performance Liquid Chromatography of Waters. UPLC stability indicating assay. UPLC conditions: Column:

2.1× 30 mm 1.7 μm Acqity Beh C18 at 30°C. UV detection at 273 nm and 40 pts/s. A: - 0.1% AA/ACN, B: 0.1 AA% / Water (Acetonitrile (ACN), Acetic Acid (AA). Gradient of liquid phase of HPLC was showed in Table 1.

All data given here mean values ± standard deviation of two independent experiments (n=2).

3. Results and Discussion

3.1. Total phenolic compounds in the parts of artichoke

The major class of natural compounds which are common to antioxidative stress is phenolics. Since most of the parts of artichoke have phenolics compounds. Table 2 lists 15 samples of species with higher concentration of phenolic compounds. All of the samples contained remarkable total phenolics values of mg gallic acid equivalent per 100 g dry weight (DW).

Table 1. Gradient of liquid phase of HPLC

	Time (min.)	Flow rate (ml/min.)	%A	%B
1	Initial	0.4	99.0	1.0
2	1.00	0.4	98.0	2.0
3	6.00	0.4	80.0	20.
4	8.00	0.4	70.0	30.0
5	9.00	0.4	50.0	50.0
6	11.00	0.4	80.0	20.0
7	13.00	0.4	99.0	1.0

Table 2. Total phenolic compounds in the parts of arichoke in Viet Nam. 1: EM1, 2: EM2, 3: EM3

Sample names	Total phenolics (mg/100 g dry weight)
Leaves 1	456.00±0.3
Leaves 2	490.85±0.7
Leaves 3	568.12±0.9
Flowers 1	398.04±0.2
Flowers 2	412.30±0.1
Flowers 3	425.69±0.4
Roots 1	356.23±0.6
Roots 2	401.30±0.7
Roots 3	412.36±0.3
Trunks 1	302.42±1.1
Trunks 2	318.96±0.4
Trunks 3	320.85±0.5
Stumps 1	246.65±0.3
Stumps 2	287.25±0.8
Stumps 3	299.68±0.7

We measured that the total phenolic compounds in the parts of artichoke were ranged from 246 (in the stumps) to 568 mg/100 g DW (in the leaves) by EM1, while their total phenolic compounds varied from 287.25 (in the stumps) to 490.85 mg/100 g DW (in the leaves) by EM2 and total phenolic compounds by EM3 were 299.68 (in the stumps) to 568.12 mg/100 g DW.

Among these, total phenolics compounds were the highest (568.00 mg/100 g DW) in the leaves by EM3 and lowest (246 mg/100 g DW) in the stumps by EM1 (Fig. 1). Total phenolics compounds concentration in the flowers is the second, in the roots was the third and then in trunks, respectively (Fig. 2). However, scientists usually focus on natural compounds in the leaves of artichoke and people also use the leaves, flowers of artichoke as tea or food. And, sometimes they take full advantage of other parts as the roots, trunks, stumps etc, because they have also good natural compounds but the concentration is less than its in the leaves and flowers.

Among all extraction methods, the EM3 (100% H_2O) had total phenolic compounds to be higher than which of by the EM1 and EM2. The concentration of total phenolic compounds in the leaves was the highest in the parts of artichoke. By EM3, total phenolic compounds was 568.120 mg/100 g DW, was 490.85

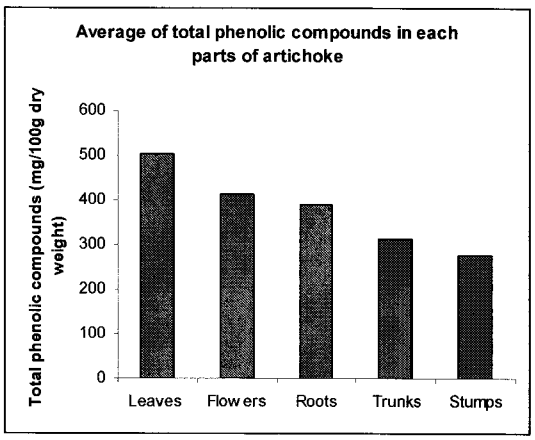

Fig. 1. Total phenolic compounds in the parts of arichoke in Viet Nam.

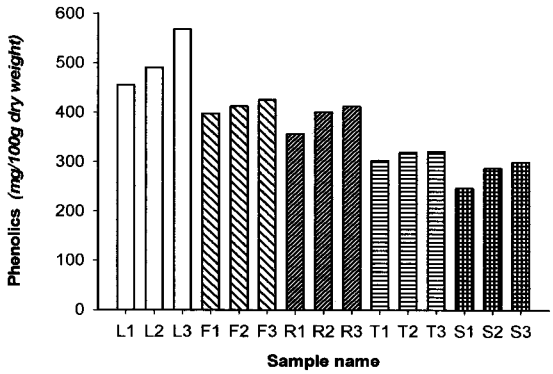

Fig. 2. Comparison of total phenolic compounds among 3 extraction methods, L: leaves, F: flowers, R: roots, T: trunks, S: stumps, 1: EM1, 2: EM2, 3: EM3.

mg/100 g DW by EM2 and 456.00 mg/100 g DW by EM1. Similarly, total phenolic compounds by EM3 in the flowers, roots, trunks and stumps were higher than by other extraction methods. The EM3 was applied because a recent paper report[6] data on the biological activity of aqueous artichoke leaves extracts, often with no or scanty information regarding their composition.

Since most medicinal herbs are prepared for consumption of herbal tea or soup, the aqueous extraction applied in this study was necessary. The difference total phenolic compounds content in the parts of artichoke by 3 extraction methods was recognized that total phenolic compounds contented aqueous extracts were higher than those of methanol extracts (Table 2). Hot water (100°C) extraction was a useful method with extracting efficiency for total phenolic content, as compared with methanol extract and mixing of methanol and water. The value of total phenolic compounds in the parts of artichoke between aqueous, mixing of methanol and water was descending order, respectively, indicating that these two extraction methods were basically consistent.

For both of EM1 and EM2, the total phenolic compounds showed comparable values to those by EM3 (Fig. 2). The highest amounts of total phenolic compounds were obtained for the sample prepared only with water (100%H_2O). That was the reason for using all of parts of artichoke by boiling way to drink and eat because this is an easy way to take good natural compounds from many kinds of teas and herbs in general and artichoke in particular.

3.2. Major flavonoids in the parts of artichoke

In recent years, there has been an increasing trend towards the exploration of safer and effective antioxidants and functional ingredients from natural dietary sources like fruits, vegetables, oilseeds, cereals, grains and herbs[7]. Most of these antioxidants are believed to play a potential role to interfere/retard the process of lipid oxidation by reacting with free radicals, chelating catalytic metals and scavenging oxygen in lipid based food products and biological systems[8]. The antioxidative effect of fruits and vegetables is mainly due to the occurrence of phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes[9]. Some antioxidant compounds like ascorbic acid and carotenoids are very sensitive to heat and storage and lost during different vegetable processing steps[10]. However, flavonoids and some phenolic compounds are quite stable at high temperature and over long periods of storage[11].

The effects of different extraction methods on the flavonoids in the parts of artichoke were assessed by HPLC. The concentration of some flavonoids was measured by concentration of each flavonoids/100 g dry weight. Generally, the concentration was decreased by boiling, mixing of methanol and water, methanol soaking. The results of the present research showed that all the methods affected the flavonoids properties of the parts of artichoke. Thus, an appropriate method might be sought for the processing of such herbs to retain their natural chemical components at maximum level.

Cynarin (1,3-dicaffeoylquinic acid)

One of a major flavonoids compound in globe artichoke are the cynarin. Extracts containing cynarin (1, 3-dicaffeoylquinic acid) have effects on hepatobiliary diseases, hyperlipidaemia and cholesterol metabolism (Fig. 3).

It was present in substantial quantities: By EM1, the concentration of cynarin from 2.23 (in the roots) - 16.13 (in the stumps) mg/100 g DW (Fig. 4). By EM2, the concentration of cynarin was the lowest in trunks (4.19

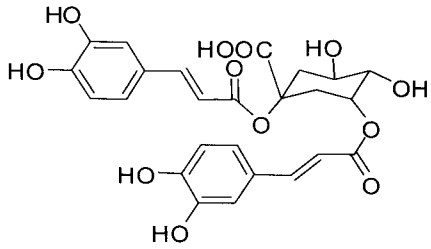

Fig. 3. Molecular structure of cynarin.

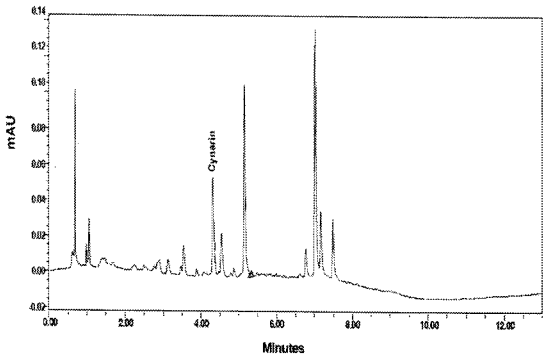

Fig. 4. A chromatogram of cynarin in the stumps of artichoke by EM1.

mg/100 g DW) and the highest in the leaves (8.23 mg/100 g DW). By EM3, the concentration of cynarin in the parts was higher than two other extraction methods, from 4.32 mg/100 g DW (in the trunks) to 9.95 mg/100 g DW (in the roots). The leaves of artichoke contented phenolic compounds in general and flavonoids in particular and the concentration of phenolic compounds in leaves was the highest in artichoke. However, for each extraction method, the concentration of cynarin in each part was different. Flavonoids may be was lost directly and not correlated with the increases in temperature, time and evaporation during soaking procedure (Fig. 5).

Narirutin (naringenin-7-rutinoside)

Artichoke can be a valuable source of narirutin (Fig. 6). The levels of the narirutin were evaluated highly in the trunks, roots and leaves as 43.22, 36.42, 37.78 mg/100 g DW by EM2, more than in the flowers and the stumps as 24.44, 29.7 mg/100 g DW, respectively. The highest narirutin content was detected in the trunks (43.22 mg/100 g DW) by EM2 (Fig. 7), while

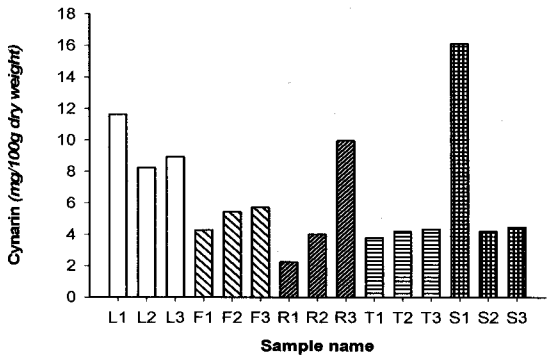

Fig. 5. Comparison of cynarin concentrations among 3 extraction methods and parts of artichoke, L: leaves, F: flowers, R: roots, T: trunks, S: stumps, 1: EM1, 2: EM2, 3: EM3.

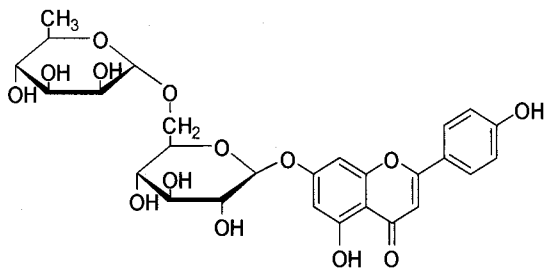

Fig. 6. Molecular Structure of narirutin.

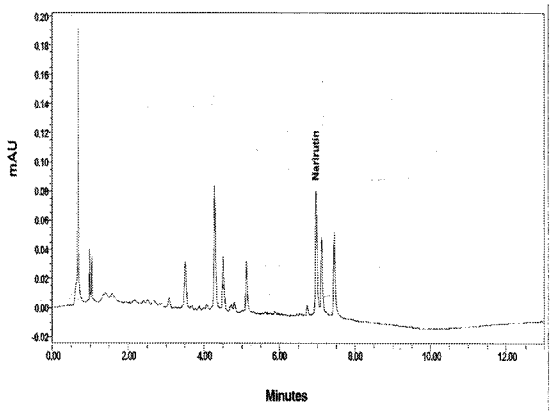

Fig. 7. A chromatogram of narirutin in the trunks of artichoke by EM2.

low levels were found in the flowers by EM1 and leaves by EM3.

In this study narirutin extraction conditions were optimized to maximize the narirutin by EM2 while mini-

mized by EM1. Soaking temperature, time, and evaporation of methanol may be affected directly the concentration of narirutin in particular and flavonoids in general. Thus, the concentration of narirutin in the parts of artichoke by EM1 as a whole was lower than other 2 extract methods. Consequently, the higher the temperature rises by EM3 and the longer the time takes by EM1, the greater the yield of narirutin was not extracted than by EM2. The present study indicated that the EM2 was found to be optimum conditions of narirutin in parts of artichoke. The characterization of narirutin extracted from parts of artichoke was performed using HPLC (Fig. 8).

Apigenin 7-O-glucoside

The present study was conducted to determine phenolic compounds and flavonoids as apigenin 7-O-glucoside (Fig. 9) in the parts of artichoke in Viet Nam. Apigenin 7-O-glucoside concentration ranged between 6.02 (by EM1 in the leaves) and 107.12 mg/100 g DW (by EM3 in the flowers). By EM1, the concentration of apigenin 7-O-glucoside was lower than other extract methods from 6.02 mg/100 g DW in the leaves to 24.6 mg/100 g DW in the flowers. By EM2, the concentration of apigenin 7-O-glucoside was higher than by EM1 but lower than by EM3, from 9.62 mg/100 g DW in the roots to 82.85 mg/100 g DW in the flowers. By EM3, the concentration of apigenin 7-O-glucoside was the highest in 3 extraction methods, from 21.96 mg/100 g DW in the leaves to 107.12 mg/100 g DW in the flowers. The leaves were found to be superior to the trunks, roots, stumps with regard to phenolic accumulation for all compounds tested while the flowers accumulated the highest levels of apigenin 7-O-glucoside (Fig. 10). Apigenin 7-O-glucoside contented in all the parts of artichoke and reached in the flowers. However, the content in the parts was the highest by EM3. The present findings might be useful to obtain increased concentration of these natural compounds (Fig. 11).

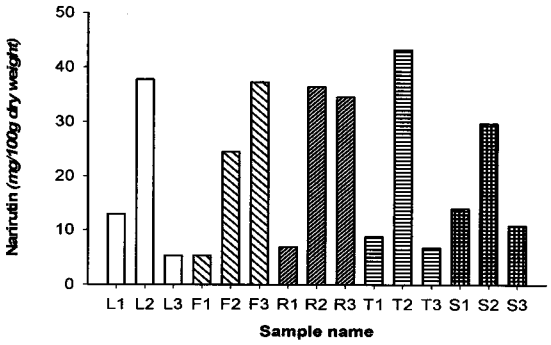

Fig. 8. Comparison of narirutin concentrations among 3 extraction methods and parts of artichoke, L: leaves, F: flowers, R: roots, T: trunks, S: stumps, 1: EM1, 2: EM2, 3: EM3.

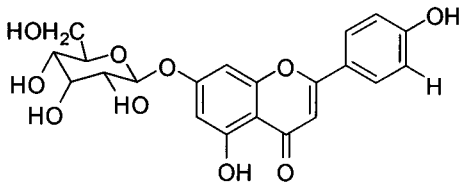

Fig. 9. Molecular structure of apigenin 7-O-glucoside.

Differences in the contents in the parts of artichoke, apigenin 7-O-glucoside was not due to the presence of individual substances but only due to their amount. Comparing the ratio of simple and derived apigenin 7-O-glucoside forms parts and extraction methods were found that apigenin 7-O-glucoside were well balanced among 3 extraction methods but different in the parts of artichoke.

Caffeic acid (3,4-Dihydroxycinnamic acid)

Caffeic acid (Fig. 12) is a natural ingredient not only in coffee beans but also in apples, bell peppers, pears, and other crops. For artichoke, caffeic acid had

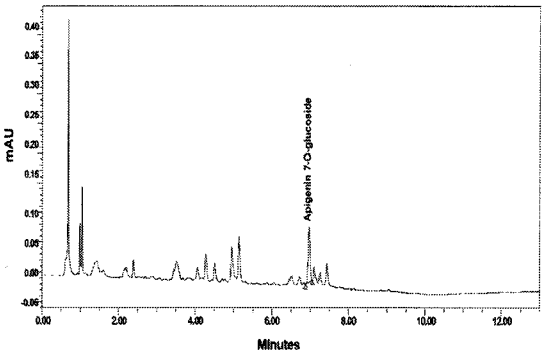

Fig. 10. A chromatogram of apigenin 7-O-glucoside in the flowers of artichoke by EM3.

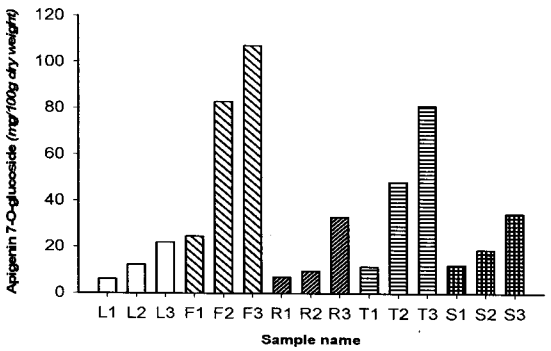

Fig. 11. Comparison of apigenin 7-O-glucoside concentrations among 3 extraction methods and the parts of artichoke, L: leaves, F: flowers, R: roots, T: trunks, S: stumps, 1: EM1, 2: EM2, 3: EM3.

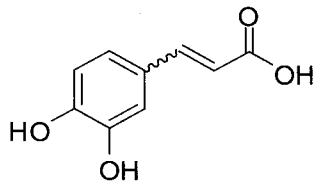

Fig. 12. Molecular structure of caffeic acid.

also the concentration in lower than other kind of plants. Caffeic acid concentration in the leaves was not higher than other parts, and the highest in the flowers (42.29 mg/100 g DW) by EM1. Caffeic acid by EM3 was comparatively less significant than that of methanol extraction (EM1). Caffeic acid concentration in the flowers and trunks by EM1 were higher than EM2 and EM3. But, caffeic acid concentration in the leaves and stumps by EM1, EM2 and EM3 were the same in concentration. Caffeic acid in the roots was not detected by EM1 and EM3 and a little concentration by EM2 (Fig. 14).

Gallic acid (3,4,5-Trihydroxybenzoic acid)

In the group of phenolic acids, the highest amounts were exhibited in the case of chlorogenic acid, followed by gallic acid (Fig. 15), with trace amounts of syringic acid. The amounts of gallic acid were noted in the artichoke, too. Previous findings also indicated that the leaves of globe artichoke have higher polyphenolic content than the flowers. When the activity

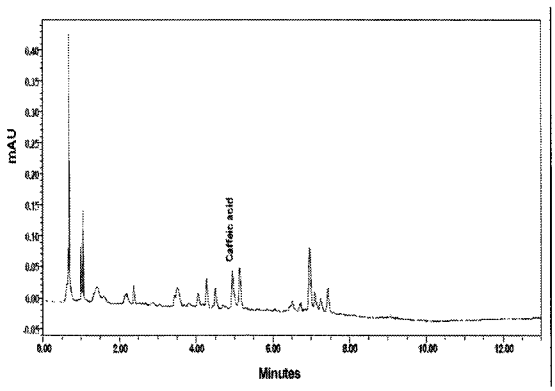

Fig. 13. A chromatogram of caffeic acid in the flowers of artichoke by EM1.

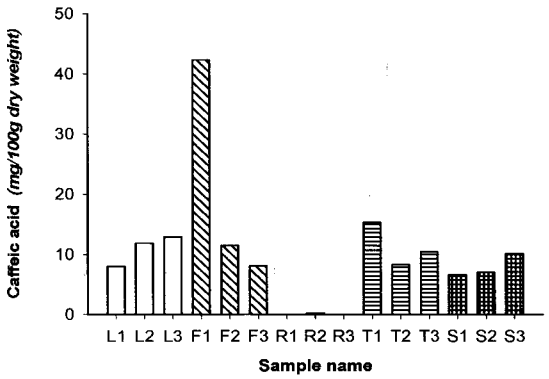

Fig. 14. Comparison of caffeic acid concentrations among 3 extraction methods and parts of artichoke, L: leaves, F: flowers, R: roots, T: trunks, S: stumps, 1: EM1, 2: EM2, 3: EM3.

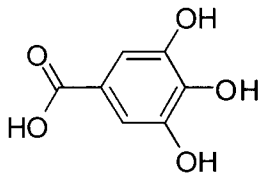

Fig. 15. Molecular structure of gallic acid.

of all the isolated compounds was compared, it was found that gallic acid showed the lowest in five major flavonoids in this study.

The analysis revealed that total polyphenols in the leaves were higher than in flowers. However, the concentration of gallic acid was small. It was non-detected

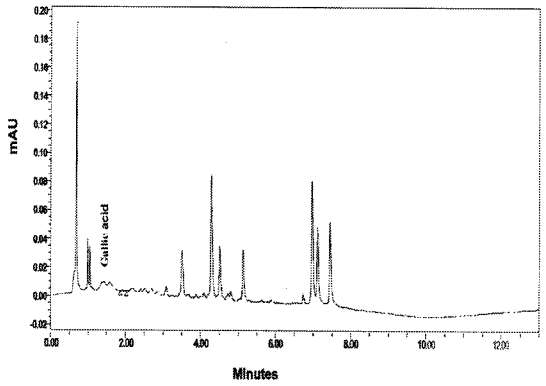

Fig. 16. A chromatogram of gallic acid in the trunks of artichoke by EM3.

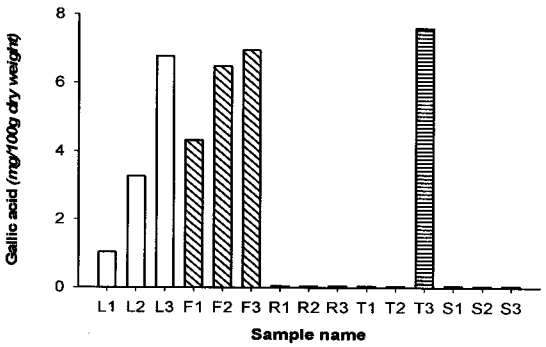

Fig. 17. Comparison of gallic acid concentrations among 3 extraction methods and parts of artichoke, L: leaves, F: flowers, R: roots, T: trunks, S: stumps, 1: EM1, 2: EM2, 3: EM3.

in most of all roots, trunks, stumps and had small amounts in leaves and flowers. The highest values were recorded in the trunks by EM3 (Fig. 16), and similar value recorded in the flowers and leaves by 3 extraction methods. That may be explained by strong structure of the trunks and EM3 by which trunks were boiled for 1 hour, had productivity to be higher than other two methods EM2 and EM1 (Fig. 17).

4. Conclusions

It has been well known that artichoke is rich in polyphenol compounds such as flavonoids. The leaves had the highest concentration in artichoke (about > 500 mg/100 g DW) and the flowers and roots had also high.

Individual flavonoids varied among species. This study was determined by five major flavonoids in artichoke: cynarin, narirutin, apigenin 7-O-glucoside, caffeic acid, gallic acid. The parts of artichoke showed a diverse flavonoid profile. The flavonoids have a wide variety of clinical applications. This information should prove to be useful to the nutraceutical industry in identifying alternative sources of flavonoids.

These extraction methods described the container preparation, field sampling and field extraction, preservation procedure to be used in conjunction with the analysis of plants and herbs samples for flavonoids in particular and phenolic compounds in general. The EM3 had higher concentration of phenolic compounds than other extraction methods. EM2 was fairly good method for extraction that is showed in this study and some other studies also. The present study indicated that the combination of methanol and water was found to be optimum conditions of natural chemicals in the parts of artichoke. EM1 needed time (9 days) and MeOH solvent and flavonoids may be lost directly and correlated with the increases in temperature, time, and evaporation during soaking procedure.

These results are for the further basic study about medical properties of artichoke as teas and foods.

Acknowledgments

The work was supported by the Regional Innovation Center Program of the Ministry of Commerce, Industry and Energy through the Research Center for Biomedical Resources of Oriental Medicine at Daegu Haany University, Korea.

References

1) Lupattelli G., 2004, Artichoke juice improves endothelial function in hyperlipeia, Life Sci., 76.
2) Wegener T., Fintelmann V., 1999, Pharmacological properties and therapeutic profile of artichoke (Cynara scolymus L.), Wien Med. Wochenschr, 149, 241- 247.
3) Erdman Jr J. W., Balentine D., Arab L., Beecher G., Dwyer J. T., Folts J., Harnly J., Hollman P., Keen C. L., Mazza G., Messina M., Scalbert A., Vita J., Williamson G., Burrowes J., 2005, Flavonoids and

Heart Health, Proceedings of the ILSI North America Flavonoids Workshop, DC1-4, Journal of Nutrition, 137(3), 718-737.

4) Hyotylainen T., Andersson T., Hartonen K., Kuosmanen K., Riekkola M. L., 2000, Pressurized hot water extraction coupled on-line with LC-GC: Determination of polyaromatic hydrocarbons in sediment, Anal. Chem., 72 (14), 3070-3076.
5) Wang M., Simon J., Aviles E., He I., Zheng F. K., Zheng Q., Tadmor Y., 2003, Analysis of antioxidative phenolic compounds in artichoke (Cynara scolymus L.), J. Agric. Food Chem., 51, 601- 608.
6) Bushra S., Farooq A., Shahid I., 2006, Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan, International J. of Food Science and Technology, 40, 1-8.
7) Son Y., Kim J., Lim J. C., Chung Y., Chung G. H., Lee J. C., 2003, Ripe fruits of Solanum nigrum L., inhibit cell growth and induces apoptosis in MCF-7 cells, Food Chemistry and Toxicology, 41, 1421-1428.
8) De Gaulejac N. S., Glories Y., Vivas N., 1999, Free radical scavenging effect of anthocyanins in red wines, Food Research International, 32(5), 327-333.
9) Siddhuraju P., Becker K., 2003, Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.), World Rev. Nutr. Diet, 91, 41-59.
10) Zhang D., Hamauzu Y., 2004, Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking, Food Chemistry, 88, 503-509.
11) Vallejo F., Tomas-Barberan F., Garcia-Viguera C., 2003, Health compounds in broccoli as influenced by refrigerated transport and retail sale period, Journal of Agriculture and Food, 51(10), 3029-3034.