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Original Article

Comparison of the Antioxidant Activity of Volatile Compounds of Traditional Herbal Waters Per Serving Cup

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Abstract

Herbal water is referred to the liquid obtained from the distillation of medicinal plants. Different parts of plants, such as flowers, fruits, leaves, seeds and roots have long been used to produce herbal waters. Herbal waters are used as dietary supplements and alternative medicine and are commonly used for flavoring in baking. Previous studies focused on the non-volatile constituents of herbs. However, plants also contain numerous volatile chemicals. Therefore, antioxidant capacities of 20 Iranian herbal waters were assessed by FRAP, DPPH and TEAC assays, and their total phenolic contents measured by Folin–Ciocalteu assay. These herbal waters exhibited a broad range of antioxidant activities, varying from 0.18 to 3.20 mmol Fe2+/l in the FRAP assay, 91.43-94.99% inhibition in the DPPH assay, 19.27-28.79 mg trolox equivalent/ml in the TEAC assay and 2.90-132.51 mg gallic acid equivalent/l in total phenolic content. Classification of Iranian herbal water was performed by cluster analysis and principal component analysis and four groups were recognized based on antioxidant activity and total phenolic content. Rose, thyme, summer savory and mint herbal waters were screened as the highest antioxidant activity and total phenolic content.

Keywords : Antioxidant, Cluster analysis, Herbal water, Principal component analysis, Total phenolics, Volatile compounds.

1. Introduction

Free radicals and active oxygen and nitrogen species oxidize biological molecules such as lipids, proteins, DNA. Free radicals are constantly generated in the body as a result of oxidative metabolism. The human body has an antioxidant defence system. Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defence system, which results in functional tissue damage [1]. Oxidative stress has been implicated in the etiology of many diseases such as: heart diseases, autism, cancer, stroke, diabetes, Alzheimer's dementia, Parkinson's disease, aging, arthritis and muscular degeneration [2, 3]. The balance between antioxidation and oxidation is believed to be critical in maintaining a healthy biological system.

Plants produce a wide range of redox active secondary metabolites such as ascorbic acid, carotenoids, polyphenols and enzymes with antioxidant activity, which protect the cells from oxidative damage. Herbs have

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Table 1. Names and medicinal functions of herbal waters.

	Scientific Name	Common Name	use
1	*Chamaemelum nobilis*	Chamomile	Anti spasmodic, carminative, appetizer
2	*Cichorium intybus*	Chicory	Stomach tonic, control of fever, control of liver and renal diseases
3	*Cuminum cyminum*	Cumin	Anti-obesity, lowering blood fat, Food digester
4	*Anethum graveolens*	Dill	Fragrant, energetic, digestive, stomach tonic, diuretic, lowering blood lipids
5	*Foeniculum vulgare*	Fenel	Diuretic, anti-aspasm, carminative, cardiac and gastric tonic, increase mother 's milk.
6	*Trigonella foenum- graecum*	Fenugreek	energetic and relieve anemia, anti-diabetes, lowering blood sugar
7	*Thymus vuldaris*	Thyme	Food digester, anti-menstrual pain
8	*Urtica dioica*	Nettle	Diuretic, lower blood sugar and pressure, increase milk, anti-diabetes
9	*Lavandula vera*	Lavender	Fragrant, tonic, sedative, carminative, reducing cough, diuretic, relief of stomachache
10	*Glycyrrhiza glabra*	Licorice	Anti-inflammatory, anti-infection and colds, aid for healing stomach and duodenal ulcers
11	*Olea europaea*	Olive Leaf	Diuretic, lower the blood sugar, tonic
12	*Mentha piperita*	Mint	Stomach nourishing, Carminative, anti-diarrhea,
13	*Salix aegyptiaca*	Pussy	Heart and stomach tonic, sedatives and laxatives, hematopoietic, counter headache and muscular pains
14	*Rosa damascene*	Rose	nerves and heart tonic, suppler skin
15	*Citrus aurantium*	Sour orange	Fragrant, nerve and heart tonic, sedative, carminative
16	*Satureja hortensis*	Summer savory	Digester, treatment gout and diarrhea and rickets
17	*Artemisia dracunculus*	Tarragon	Anti-rheumatism, appetizer, improve blood circulation
18	*Juglans regia*	Walnut leaf	Treatment of joint and gout inflamation, anti-diabetes
19	*Salix babylonica*	White willow	Anti-pyretic, blood purifier, anti-rheumatism,
20	*Achillea millefolium*	Yarrow	Anti-inflammatory, anti-spasmodic, lower blood pressure, anti-infection

been used for a large of purposes including medicine, nutrition, flavorings, beverages and cosmetics. Today, over 7000 herbs have been employed by 80% of the world's population for primary health care needs [4]. Traditional medicinal products constitute multi-billion-dollars industries worldwide.

Herbal water is referred to the liquid obtained from the distillation of medicinal plants. It can also be called aqueous herbal extract which is a water based preparation of a plant containing the biologically active portion of the plant without its cellular residue. Different parts of plants, such as flowers, fruits, leaves, seeds and roots have long been used to produce herbal waters. Herbal waters are used as dietary supplements and alternative medicine and are commonly used for flavoring in baking. Many Middle Eastern pastries and desserts include floral waters and can also be added to savory dishes.

Floral waters are waters which have been made from various flowers. Some common floral waters are made with flowers like lavender, rose, orange blossom, chamomile and rosemary. These products are often available at markets and they can also be ordered directly from the companies. One of the famous products is rose water or "Golab" which is less familiar to most westerners. Rose water is made using damask roses. These were first grown in Iran and Bulgaria, but are now frequently found in Spain, Italy and France. However, the Middle Eastern countries remain some of the largest producers of rose water because of the availability of damasks.

When a specific herb is brought to the factory, it initially goes into the sorting pro-

cess. If the plant is fresh, it is washed out and transferred to the steel distillation cauldron. For dried herbs, scrubbing or grinding may be needed before transferring to the cauldron. After adding water, the cauldron is heated and the vapor is passed through the distillation device. The final product is a colorless liquid which is transferred to preservation storage. After filtration and pasteurization procedures, the herbal water is packed and stored.

Previous studies focused on the non-volatile constituents of herbs [5-9]. In these works, aqueous or methanolic extracts were used. However, plants also contain numerous volatile chemicals, which have been widely used in both folk medicine and aromatherapies. In the present study, antioxidant activities of traditional herbal waters were compared per serving cup.

2. Materials and Methods

2.1. Chemical

2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), Folin Ciocalteu (FC) reagent and gallic acid were purchased from Merck. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich.

2.2. Herbal Waters Sample

The herbal water products of three famous factrories were purchased in October and November 2010 in Ilam and Isfahan markets, Iran. Three samples of each herbal water with different production dates were bought. The scientific names of the herbs, common name and medicinal functions are detailes in table 1.

2.3. Total Phenolic Content

The total phenolic content (TPC) of the herbal waters was determined using the Folin- Ciocalteu reagent [10]. Eight mililiters of sample were mixed with 5 ml Folin-Ciocalteau reagent and 1.5 ml 15% Na_2CO_3 solution. The reaction mixture was left to stand for 20 min. Finally, the absorbance was measured at 765 nm, using a UV-visible spectrophotometer. A calibration curve was prepared, using a standard solution of gallic acid. TPC was expressed as mg gallic acid equivalents per liter of herbal water.

2.4. Ferric Reducing Antioxidant Power (FRAP) Assay

A modifed method of FRAP assay was used [11]. A working FRAP solution was prepared from acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily. Six militers of sample were added to 1.5 ml of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The standard curve was constructed using iron (II) sulfate solution, and the results were expressed as mmol Fe (II) per liter of herbal water. All measurements were repeated in triplicate.

2.5. Trolox Equivalent Antioxidant Capacity (TEAC) Assay

This assay is based on the ability of a compound to scavenge the stable ABTS radicals [12]. The stock solutions included 7.0 mM ABTS solution (A) and 140 mM potassium persulphate ($K_2S_2O_8$) solution (B). For ABTS radicals production, the working solution was prepared by mixing 5.0 ml of A and 88 µl of B, and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol in order to obtain an absorbance in the inspection range of UV-Vis spectrophotometer at 734 nm. Fresh ABTS solution was prepared for each assay. Sample (4 ml) was mixed with 2.0 ml of ABTS solution and the mixture was left at room temperature for 1.0 h in dark. The absorbance was then measured at 734 nm. The activity was expressed as mg Trolox equivalent in ml of herbal water. All measurements were repeated in triplicate.

Table 2. Mean antioxidant activity and total phenolic contents of 20 Iranian herbal waters.

	Name	FRAP mmol Fe^{2+}/l	DPPH %Inhibition	TEAC mg Trolox/ml	TPC mg GAE/l
1	Chamomile	0.54±0.10	91.73±2.40	21.57±0.47	8.04±7.34
2	Chicory	1.19±1.48	92.76±2.45	19.27±2.52	9.36±4.04
3	Cumin	0.90±0.40	94.00±0.43	23.21±0.89	20.24±15.15
4	Dill	0.89±0.33	93.88±0.65	21.52±1.09	20.26±4.20
5	Fenel	0.63±0.47	93.32±0.90	21.66±3.46	7.35±7.20
6	Fenugreek	0.18±0.14	92.45±1.01	21.70±2.52	3.68±3.52
7	Thyme	2.34±1.28	94.83±1.01	28.79±1.13	132.51±78.23
8	Nettle	0.76±0.70	93.65±2.22	20.23±0.65	5.63±0.68
9	Lavender	0.38±0.38	93.66±0.67	20.43±1.08	10.68±9.32
10	Licorice	0.34±0.21	91.43±1.32	19.90±2.41	3.23±0.36
11	Olive Leaf	0.38±0.23	93.55±1.44	20.86±0.55	3.93±2.14
12	Mint	0.66±0.26	94.99±0.99	22.35±2.16	11.43±0.51
13	Pussy	0.34±0.17	93.78±2.50	20.51±0.36	2.90±1.21
14	Rose	3.20±1.15	94.63±0.96	23.65±1.74	11.72±5.02
15	Sour orange	0.95±0.73	93.26±1.33	20.78±1.61	5.59±1.38
16	Summer savory	1.73±1.07	94.39±0.58	26.15±3.90	84.56±80.66
17	Tarragon	1.48±1.49	93.11±1.02	22.08±0.49	11.41±4.86
18	Walnut leaf	0.95±0.31	94.09±1.51	19.49±2.12	4.66±0.72
19	White willow	0.77±0.68	93.68±1.56	21.94±0.02	3.65±1.33
20	Yarrow	0.45±0.56	93.37±0.56	20.41±1.47	8.12±4.53

FRAP, Ferric reducing antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; GAE, gallic acid equivalents.

2.6. DPPH Radical Scavenging Activity Assay

For DPPH assay the modifed method of Brand-Williams *et al*. was used [13]. Radical scavenging activity of herb waters against stable DPPH radical was determined spectrophotometrically. When DPPH radical reacts with an antioxidant compound it donates hydrogen and reduced. The color change was from deep-violet to light-yellow. Fresh DPPH methanolic solution (A=1.71 ± 0.010) was prepared before every measurement. Nine mililiters of herbal water were mixed with 1 ml of a 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 515 nm. Antiradical activity (%) was calculated according to the following equation:

$$\text{Antiradical activity \%} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

where Abs control is the absorbance of fresh DPPH solution.

2.7. Statistical Analysis

Pearson correlation coefficients were calculated to obtain the possible correlation among the different herbal water antioxidants. Principal component analysis (PCA) was performed to assess the correspondences among the herbal waters. Hierarchical cluster analysis (HCA) was used to determine groupings among the herbal waters. These products were classified according to antioxidant capacity and total phenolics content. The data were standardized (z-scores) and the Euclidean distance was used as a similarity measurement. Ward's method was used to obtain hierarchical associations. All calculations were performed using Minitab software (version 14.1, Minitab Inc., State College).

Table 3. Antioxidant activity and total phenolic contents of 20 Iranian herbal waters.

Herbal water	Serving size	Description of serving (per day)	mg phenolics/serving
Chamomile	1/2 cup	2	0.40
Chicory	1 cup	2	0.94
Cumin	1/2 cup	3	1.01
Dill	1 cup	2	2.03
Fenel	1/2 cup	3	0.37
Fenugreek	1/2 cup	3	0.18
Thyme	1/2 cup	3	6.62
Nettle	2/3 cup	2	0.38
Lavender	1/2 cup	3	0.53
Licorice	1/2 cup	2	0.16
Olive Leaf	1 cup	3	0.39
Mint	1/2 cup	2	0.57
Pussy	1/2 cup	3	0.14
Rose	1/2 cup	2	0.59
Sour orange	1/2 cup	2	0.28
Summer savory	1/2 cup	2	4.23
Tarragon	1/2 cup	3	0.57
Walnut leaf	2/3 cup	2	0.31
White willow	2/3 cup	3	0.24
Yarrow	1/2 cup	3	0.41

3. Results and Discussion

Herbal waters have long been consumed in Iran, mainly due to the medical and pharmaceutical properties. The manufacture of herbal waters is growing every year in significant amounts by factories, workshops and private gardens.

The results of three *in vitro* total antioxidant capacity assays (FRAP, DPPH and TEAC) and phenolic contents of twenty herbal waters were systematically assessed. Mean values of the herbal water products of three famous factrories are given in table 2. The antioxidant capacities are influenced by many factors, which can not be fully described with one single method. Therefore, it has been recommended that at least two methods be used due to the differences between the test systems investigated [14]. All the three assays of antioxidant capacity used in this study are spectrophotometry-based methods. However, it is not surprising to find the differences in the order of top samples with highest antioxidant capacity among the assays. These assays have different mechanism of action and different reaction conditions. Therefore, chemical compounds react differently.

The total antioxidant capacities of the herbal waters determined by FRAP method, a simple, speedy, inexpensive and reproducible method which can be applied to the assay of antioxidants in botanicals. Comparing all the herbal waters, the FRAP values indicated large difference in antioxidant activity. The values varied from 0.18 mmol Fe2+ per liter herbal water (fenugreek) to 3.20 (rose). In order to evaluate the antioxidant capacity of the herbal waters, two methods based on the DPPH and ABTS radicals were performed. DPPH assay values showed a variation from 91.43% inhibition (licorice) to 94.99 (mint). The TEAC values of ABTS radical scavenging exhibited variation from 19.27 mg Trolox per milliliter of herbal water (chicory) to 28.79 (thyme).

The total phenolic contents (TPC) of these samples were estimated using the Folin-Cio-

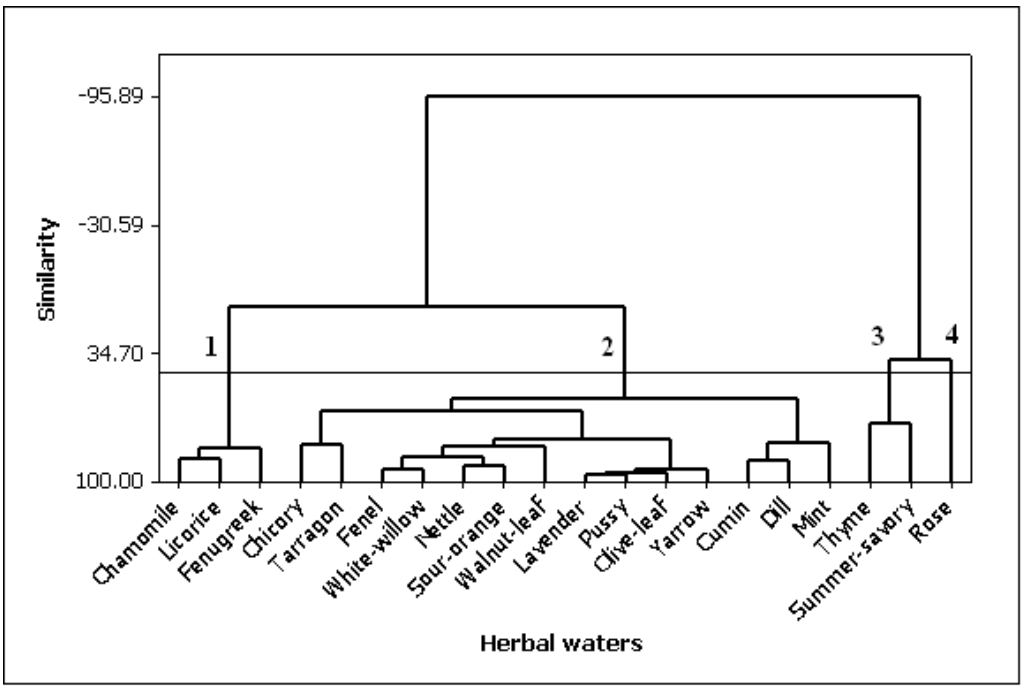

Figure 1. Dendrogram from a Ward linkage cluster analysis of Iranian herbal waters. Solid line defines "phenon line", which is chosen to differentiate groups or subgroups.

calteu colorimetric method. It was found that TPC of the herbal waters also showed significant variation, ranging from 2.90 mg gallic acid equivalents per liter of sample (pussy) to 132.51 (thyme). There was about 50-fold difference in TPC values of herbal waters. Some of the herbal waters with the strongest antioxidant capacity and the highest phenolic content were screened for their phenolic profiles, such as thyme, mint and rose. We have also compared the total phenolic content of herbal waters per serving cup (table 3). The polyphenol composition of the herbal waters varies greatly, and therefore the protective role of dietary antioxidants requires characterization of compounds and their absorption, tissue distribution, metabolism and biological actions. The data will provide some useful information for people's healthy dietary and the potential application of herbal waters.

Pearson's correlation analysis was used to determine relationships between antioxidant activity and total phenolic content. The r-values between ABTS and TPC assays, ABTS and DPPH assays, and ABTS and FRAP were 0.895, 0.526 and 0.650, respectively. Previous studies have also found that phenolic compounds are major antioxidant constituents in selected herbs, vegetables and fruits, and direct relationships between their antioxidant activity and total phenolic content.

The cluster analysis is an efficient means of establishing associations on the basis of nearness criteria between objects. The cluster analysis was carried out by the Ward linkage method for agglomeration and the Euclidean distance as the criterion of proximity (figure 1). The resulting dendrogram has four major groups based on a similarity. The first association consist of three herbwaters (chamomile, licorice and fenugreek) with low antioxidant activity and total phenolic content. The second group arise from the breaking down of the large association, which included the majority of herbwaters with moderate antioxidant activity and total phenolic content.The third group is composed of thyme and summer savory with highest TEAC and TPC values. The fourth group was comprised of rose water with highest FRAP values.

Principal component analysis (PCA) is among the most versatile of all chemometric

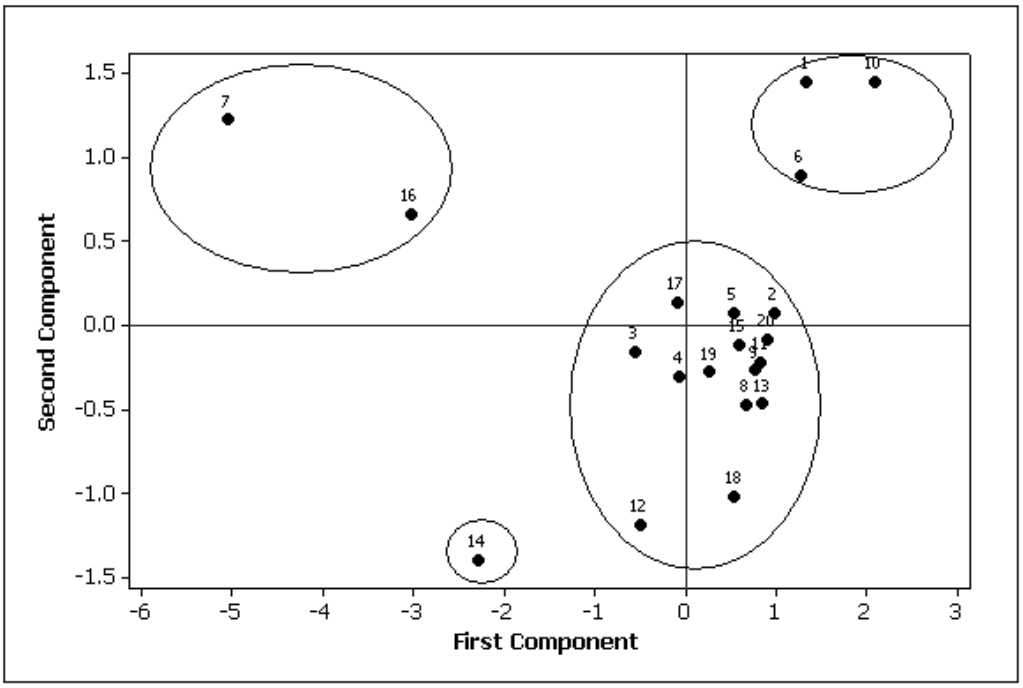

Figure 2. Principal component analysis of antioxidant capacitiy of Iranian herbal waters.

methods. It seeks to maximize the variance information present in a data set in as few new dimensions as is possible. The mean element of this approach consists of the construction of a small set of new orthogonal variables drived from a linear combination of the original ones. Figure 2 shows the projections of different herbal waters on the reduced space determined by the first two rotated principal components. The first principal components accounted for 70.4% of the total variance (eigenvalue ≥1). PCA results agree with those obtained by the cluster analysis.

4. Conclusion

Herbal waters have been used to treat human diseases in the East for centuries. People are becoming increasingly interested in medicinal plants because of their good therapeutic performance and low toxicity. Evaluation of herbal waters is useful for understanding their functionality and chemical constituents, and also supports the view that they can be potential sources of potent natural antioxidants. This study has revealed that a wide range of total antioxidant capacities and phenolic contents exist among the 20 herbal waters assayed. A significant correlation coeffiocient (0.895) was found between antioxidant capacity and phenolic content, indicating the phenolic compounds are a major contributer to antioxidant activity in the herbal waters. Classification of herbal waters was performed by cluster analysis and principal component analysis and four groups were recognized based on antioxidant activity and total phenolic content. Some of the herbal waters with the strongest phenolic content were thyme, mint and rose. Such products would merit further investigation to determine the specific volatile antioxidants and their biological functions.

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