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Nutritional and Antioxidant Properties of Dehydrated Whole Lime (*Citrus latifolia*) and Shallot (*Allium cepa* var. aggregatum), Two Popular Ingredients Used in Iran

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ABSTRACT

Introduction: Two flavouring ingredients largely used in Iran, namely, dehydrated whole lime (*Citrus latifolia*) and shallot (*Allium cepa* var. aggregatum) powders were analysed for nutritional composition and antioxidant properties. **Methods:** The dehydrated spice powders were analysed for nutritional composition using standard AOAC techniques. Antioxidant components and activity were determined in aqueous and solvent extracts. Reducing power, free radical scavenging activity and total antioxidant activity were used for antioxidant assays. **Results:** Nutritional composition of lime and shallot were found to have the following values per 100g of dry sample respectively: protein: 5.36g & 7.81g; fat: 4.32g & 0.41g; vitamin C: 88.19mg & 13.06mg; carotenoids: 77.20mg & 68.17mg and calcium:- 569mg & 75.22mg. Both samples had a very high content of soluble dietary fibre (33.97-42.53%) while lime showed a higher insoluble fibre content (30.27%). Both shallot and lime extracts showed higher antioxidant components in 100°C water extract than all other extracts. Total antioxidant activity in water extract of shallot ranged from 648,525 - 772,059 µmol/g. Comparative values for whole lime were 88,225 - 90,588 µmol/g. Solvent extracts had lesser total antioxidant activity. Whole lime exhibited high antioxidant activity with reducing power and free radical scavenging activity in comparison to shallot, which was much lower and did not exhibit any activity in methanolic and ethanolic extracts. The antioxidant components showed high correlation with water extracts for both spices. **Conclusion:** Both shallot and lime were found to have essential nutrients and exhibited antioxidant potential as evidenced by different assays.

Key words: Antioxidant components, free radical scavenging activity, nutrients, reducing power, total antioxidant activity

INTRODUCTION

Spices are defined as "aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition. They are true to name, and from them no portion of any volatile oil or other flavouring principle has been removed" (USDA, 2002). Some spices, such as saffron, paprika and turmeric, are used for colour as well as flavour and when used as ingredients in foods are designated as "spice and colouring." Most spices are derived from bark (e.g, cinnamon), fruit (e.g, red and

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black pepper), seed (e.g, nutmeg) and roots (e.g, ginger). On the other hand, many herbs used in cooking for similar purposes are typically leaves and stem.

Spice usage varies among different populations. Oriental countries use a large amount of pungent spices compared to western countries. Population-wise, average dietary intake of common spices has been estimated at 0.5 g/person per day in Europe and 1.0 g/person per day in New Zealand (Fowles, Mitchell & McGrath, 2001). In contrast, in the Indian subcontinent, turmeric consumption alone has been estimated at 1.5 g/person/day and average total spice consumption is 10-12g/person /day (Sharma *et al.*, 2001). Spices are used not only for culinary purposes but for medicinal uses as well. For centuries, people have not only recognised their inherent value, but also possible toxicity, if any, of phytochemicals in relation to human health. At present, the role of spices in the prevention of cancer is also being emphasised (Lampe, 2003).

Free radicals have been associated with many diseases such as diabetes, ageing, neurodegenerative diseases, atherosclerosis, carcinogenesis, inflammation and metabolic disorders. Foods rich in antioxidants exhibit free radical scavenging activity which are linked to reduction of oxidative stress and play a crucial role in the prevention of many diseases (Fan *et al.*, 2006; Sultana & Anwar, 2008). There is scientific evidence that dietary antioxidants such as polyphenols, carotenoids and vitamin C and E play an important role in human health (Liu, 2003; Medoua, Egal & Oldwage-Theron, 2009). There are several studies reporting the presence of antioxidant components and activity in foods (Wojdylo, Oszmianski & Czemerys, 2007; Lako *et al.*, 2007; Podsedek, 2007).

Antioxidants are also widely employed to increase the shelf-life of lipid and lipid containing food products and to reduce wastage by inhibiting and delaying oxidation. There are two kinds of antioxidants: natural and synthetic antioxidants. There is a move to restrict the use of synthetic antioxidants due to their carcinogenicity (Diaz-Reinoso *et al.*, 2006; Zia-ur-Rehman, 2006; Nanditha & Prabhasankar 2009). Vitamin C, carotenoids and tocopherols are the most important natural antioxidants which are also used in industry to improve shelf life of products. Other than these antioxidants, fibre, polyphenols, flavonoids, conjugated isomers of linoleic acid, epigallocatechin, soya protein, isoflavones, selenium, chlorophyllin, glutathione, protease inhibitors, sulphides, and catechin are natural antioxidants which are found in plants.

Whole dry lime and dry shallot powder are commonly used spices in Iran as flavoring ingredients and antioxidant properties of these have not been reported earlier. Hence, the objective of the present study was to analyse the nutritional composition, antioxidant components and antioxidant activities of these two common spices.

METHODS

Plant material

Whole fresh Persian lime (*Citrus latifolia*) and shallot (*Allium cepa* var. aggregatum) were procured from the local market of Iran, cleaned, dried, powdered and stored in airtight containers under refrigerator conditions till further use.

Chemical reagents and solvents

The chemicals used for the study were procured from Qualigen Company Mumbai, India, Himedia Company, Mumbai, India and Sigma Company, USA. They were all of analytical grade. Glass double distilled water, methanol, ethanol, acetone, 80% methanol and 80% ethanol were used for extraction. All analysis were carried out in triplicate. For measurement of antioxidant activities, a minimum of four replicates were used for all determinations.

Sample preparation for determination of antioxidant activity

Two hundred and fifty mg sample portions were mixed with 25ml of extraction media and extracted for 3 h, centrifuged at 4000 rpm for 20 min, and passed through filter paper (Whatman No.1) to obtain a clear extract. Water extracts were taken at room and boiling temperature (30°C and 100°C respectively). Only fresh extracts were used for all measurements.

Nutritional composition

Standard techniques were used for all measurements and a brief description is provided below. The methodologies used for determination of nutritional composition were as follows - moisture by oven drying method, total protein by Kjeldahl distillation and subsequent conversion of nitrogen to protein using a factor of 6.25, fat by Soxhlet distillation using petroleum ether for repeated extraction, ash by incinerating the sample in a muffle furnace and weighing the residue, total carotenoids, iron and phosphorus by colorimetric estimation, calcium and vitamin C by titremetric procedures (Horwitz & Latimer, 2005). Dietary fibre was estimated by enzymatic-gravimetric method of Asp *et al.* (1983).

For analysis of trace minerals, the ash solutions were prepared by wet digestion (Ranganna, 2002) and analysed for zinc, copper, chromium and manganese using atomic absorption spectrophotometer (AAS) (GBC Scientific equipment, Australia). Instrumental para-meters such as resonant wavelength, slit width and air-acetylene flow rate that are appropriate for each element were selected (AOAC, 2000). A calibration curve (concen-tration vs. absorbance) for each mineral to be determined was prepared using a range of working standards. The concentration of metals in ash solutions of samples as well as in blank solutions were read from the calibration curve and the concentration in the test sample calculated taking into account the dilutions and the weight of the sample taken.

Antioxidant components

Total polyphenol content was estimated using Folin-Ciocalteu (FC) assay which is widely used in routine analysis and expressed in mg of tannic acid equivalents (TAE) /100 g of sample (Matthaus, 2002). The total flavonoid content was determined using the Dowd method and expressed as g of quercetin equivalents /100g of sample (Arvouet-Grand *et al.*, 1994). Colorimetric estimation of tannins was based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution (Ranganna, 2002). Total tannin content was expressed as mg tannic acid equivalent /100 g of sample.

Determination of antioxidant activity of different extracts

Radical scavenging activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl)

Effect of different extracts on DPPH free radical was measured according to Lee, Park & Choi (1996). Positive control (standard) was prepared by mixing 4.0 ml of ascorbic acid (0.05 mg/ml) and 1.0 ml of DPPH (0.4 mg/ml) for water extract, and negative control (blank) was prepared by mixing extract base (water/methanol/ethanol/acetone) with 1.0 ml of DPPH. Four different concentrations of extract were mixed with 4.0 ml of DPPH, mixed well after making up the volume and left to stand at room temperature in a dark place for 30 min. Absorbance was read using a spectrophotometer at 520 nm. The ability of extract to scavenge DPPH was calculated using the following equation.

$$\text{Radical scavenging activity \%} = \frac{\text{(Control OD-Sample OD)}}{\text{(Control OD)}}$$

Reducing power

A spectrophotometric method was used for the measurement of reducing power.

Different concentrations of extracts were mixed with each 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (10 mg ml-1). The mixture was incubated at 50°C for 20 min, cooled, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), allowed to stand for 10 min and the absorbance read at 700 nm (Oyaizu, 1986).

Total antioxidant activity by phosphomolybdenum method A 0.1 ml of extract (10mg/ml) was mixed with reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in 100 ml), tubes capped and incubated in boiling water bath at 95°C for 90 min, cooled and absorbance read at 695 nm against blank. Water soluble antioxidant capacity was expressed as equivalent of ascorbic acid (µmol/g of sample) (Prieto, Pineda & Aguilar, 1999).

Statistical analysis

Data were expressed as mean ± SD in all experiments. Data were subjected to the analysis of variance ($P<0.05$) using the computer programme Excel 2007.

RESULTS AND DISCUSSION

Nutritional composition

Whole dry lime powder is used as an acidulant whereas shallot is favoured for its strong delightful flavour. Both are used for meat and vegetable curries and in rice dishes. Nutritional composition of whole dry lime and dry shallot is presented in Table 1. Dry lime had a higher range of moisture content than shallot. The other constituents are also given on dry weight basis for a valid comparison. Shallot had a higher content of protein (7.81%) when compared to lime (5.36%), while the fat content was too low in shallot with 0.41%. Lime comparatively had higher fat content (4.325), which could be because of the peel potion being a part of it. Lime peel is known to be rich in essential oils. There was a wide difference in insoluble fibre content in both samples with lime having 30.27% and shallot having a much lower value of 6.48%. The soluble fibre, however, was in a similar range for both samples. In general, the peel portion of fruits and vegetables is high in insoluble dietary fibre content as reported earlier. The insoluble fibre content of three varieties of banana peel ranged from 35.6-49.9%; whereas for three vegetable peels, it ranged from 41.5-53.8% (Shyamala & Prakash, 2012; Shyamala & Prakash, 2014). Fresh lime is known as a rich source of Vitamin C and even in dry form, it retained considerable amounts of the vitamin. The carotenoids content for both were in similar range, and can be considered a good source too. Lime had a higher ash content reflecting in turn a high calcium content (455mg%), whereas all the trace minerals were much higher in shallot. The overall nutritional composition of both spices indicated that they could contribute a fair amount of essential nutrients to the daily diets, even if used in small amounts.

Antioxidant components

Antioxidant components of samples were estimated in seven different extracting media and are presented in Table 2. Antioxidant activity of plant extract is usually linked to their phenolic content. Hydrogen donating characteristics of the phenolic compounds is responsible for the inhibition of free radical induced lipid peroxidation. Phenolic compounds are high level antioxidants because of their ability to scavenge free radicals and give oxygen species such as a single oxygen, superoxide free radicals and hydroxyl radicals (Calucci *et al.*, 2003), though, it is well established that non phenolic antioxidants might also contribute to the antioxidant activity of plant extracts (Hassimoto, Genovese &

Table 1. Nutritional composition of shallot and whole dry lime (per 100g)

Constituents	*Lime*	*Shallot*	*Constituents*	*Lime*	*Shallot*
Moisture (g)	20.06 ± 0.03	11.98 ± 0.50	Ash (g)	5.01 ± 0.11 (6.27)	2.73 ± 0.06 (3.10)
Protein (g)	4.28 ± 0.001 (5.36)	6.87 ± 0.31 (7.81)	Calcium (mg)	455.0 ± 6.8 (569.0)	66.21 ± 1.4 (75.22)
Fat (g)	3.45 ± 0.1 (4.32)	0.36 ± 0.1 (0.41)	Phosphorous (mg)	126.0 ± 5.7 (158)	206.0 ± 6.3 (234)
Insoluble fibre (g)	24.2 ± 0.05 (30.27)	5.7 ± 0.00 (6.48)	Iron (mg)	4.0 ± 2.1 (5.0)	8.0 ± 3.0 (9.09)
Soluble fibre (g)	34.0 ± 0.2 (42.53)	29.9 ± 0.64 (33.97)	Zinc (mg)	0.65 ± 0.07 (0.81)	2.62 ± 0.03 (2.98)
Carbohydrate (g) By difference	9.00 ± 0.08 (11.25)	42.46 ± 0.33 (48.23)	Copper (mg)	0.44 ± 0.00 (0.55)	1.57 ± 0.04 (1.78)
Vitamin C (mg)	70.5 ± 0.41 (88.19)	11.5 ± 0.07 (13.06)	Manganese (mg)	0.115 ± 0.00 (0.144)	0.47 ± 0.31 (0.53)
Total carotenoids (mg)	61.71 ± 0.23 (77.20)	60.00 ± 0.12 (68.17)	Chromium (µg)	ND	3.0 ± 0.35 (3.40)

Note: Figures in parenthesis indicate values on dry weight basis. ND: not detected

Table 2. Antioxidant components and total antioxidant activity of shallot and whole dry lime in different extracts

Constituents	*Water (100°C)*	*Water (30°C)*	*Methanol*	*Ethanol*	*80% Methanol*	*80% Ethanol*	*Acetone*
Total polyphenols (mg/100g)							
Shallot	100±3.5	99±4.1	ND	20±1.9	90±2.6	60±4.4	25±2.7
Whole Lime	480±7.9	420±11.2	140±8.3	170±5.5	360±8.1	460±7.4	50±2.1
Tannins (mg/100g)							
Shallot	225±7.8	212±3.9	104±4.1	89±2.7	195±6.1	174±3.0	65±1.2
Whole Lime	900±5.2	856±4.8	601±9.0	531±14.6	795±8.9	731±11.0	271±13.3
Flavonoids (g/100g)							
Shallot	2.14 ±0.010	1.80 ±0.003	0.512 ±0.004	0.478 ±0.004	0.694 ± 0.006	0.717 ±0.003	0.553 ±0.030
Whole Lime	5.72±0.6	2.42±0.07	2.14±0.10	1.81±0.09	4.6±0.50	3.2±0.01	1.7±0.02
Total antioxidant activity (µmol/g)							
Shallot	90,588 ±25	88,225 ±94	73,235 ±33	51,470 ±28	64,411 ±45	39,110 ±32	1,753 ±19
Whole Lime	7,72,059 ±42	6,48,525 ±56	64,705 ±38	ND ±0	1,90,073 ±69	58,829 ±61	3,250 ±29

Lajolo, 2005). In our study, polyphenols estimation revealed the highest level in hot water extracted lime. Since the lime sample was taken with the peel which is a good source of polyphenols, it showed a high polyphenol content of 480mg/100g. Similar values were also reported by Ahmed & Rocha (2009) for their lime sample (404mg/100g). Shallot had a much lower levels of polyphenols in different extracts. In general, water media extracted more polyphenols than solvents.

Tannin content was also estimated in different extracts in both samples and the highest values were seen in 100°C followed by 30°C water extracts. Since tannin is a water soluble component, the values were less than in other solvent extracts. Between samples, whole dry lime showed higher tannins than shallot. Similar results were obtained for flavonoids. Both samples exhibited the highest flavonoid content in aqueous extracts and it was higher for lime. The acetonic extracts showed the least amount of flavonoids compared to other extracting media.

Antioxidant activity

The antioxidant activity of the samples was measured by three methods and the results obtained were correlated with the antioxidant components. The data is presented in Table 2 (total antioxidant activity),Figure1Aand1B(reducingpower), Figure 2A and 2B (free radical scavenging activity) and Table 2 (correlation between antioxidant components and activity). Total antioxidant activity measured as µmol ascorbic acid/g of sample was very high for water extracts of shallot, that is, 772,059 and 648,525 µmol/g of the samples respectively, at 100°C and 30°C (Table 2). In solvent extracts, lesser activity was noted. In pure ethanol extract antioxidant activity could not be detected at all. In contrast, lime showed only 11.7 - 13.6% of activity seen in shallot in water extracts, though pure ethanol and methanol had slightly higher values.

Total antioxidant activity was highly correlated with all the antioxidant components extracted in water. The solvent extracts also had a positive correlation, which was higher for shallot and slightly less for lime. In lime, tannins were more associated with a higher R value (0.740) than polyphenols and tannins (Table 3).

Reducing power of shallot and lime illustrated in Figures 1A and 1B show that in this assay, shallot exhibited lesser activity than lime with no activity seen in methanol and ethanol extracts at all. The concentration of samples used for the assay was similar for both samples (2000ppm - 8000ppm). This was in contrast to the results obtained using total antioxidant assay as that indicated a high level of activity for shallot. It is well known that for measurement of antioxidant activity, a combination of assays are to be used as there are differences in the responses of a sample with each assay. Lime showed highest activity in 80% methanolic extract with slightly less values for 100°C water extract. These were followed by 80% ethanol, 30°C water, methanol and ethanol. Acetone showed the least activity. The reaction was dose dependent as with increasing concentration, a higher absorbance was noted for all samples proportionally. The reducing power activity showed a high association with antioxidant components in water extracts, though for shallot, there was negative correlation between solvent extracts and antioxidant components. In lime, a strong association was seen both for water as well as solvent extracts (Table 2). Many of the antioxidant components are known to occur with dietary fibre and they are released by enzymic digestion in the intestine (Siddique & Prakash, 2014). Since these spices are rich in dietary fibre, they also carried a higher level of antioxidant constituents exhibiting an association between the components and activities.

Free radical scavenging activity estimated by DPPH method also indicated a very high level of activity by lime in

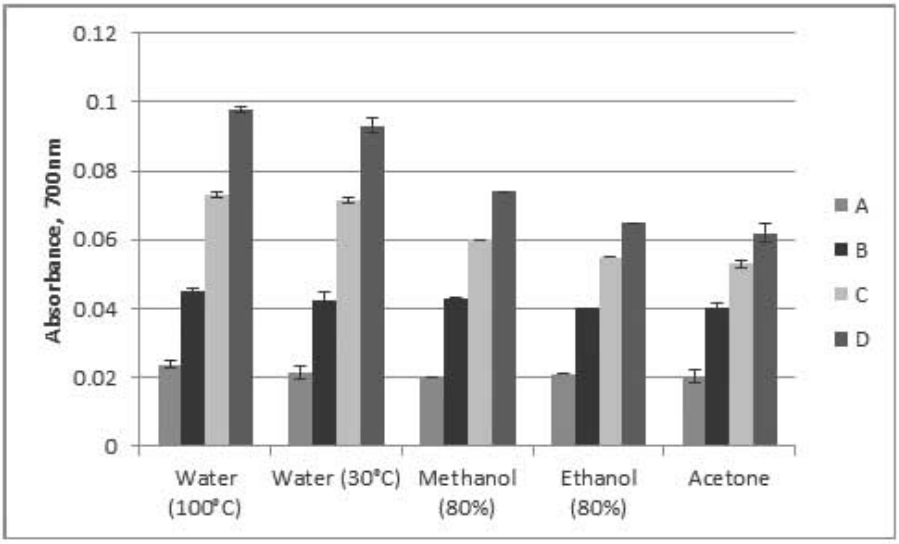

Figure 1A. Reducing power of shallot in different extracts Concentration: A.2000ppm, B. 4000ppm, C.6000ppm, D.8000ppm]

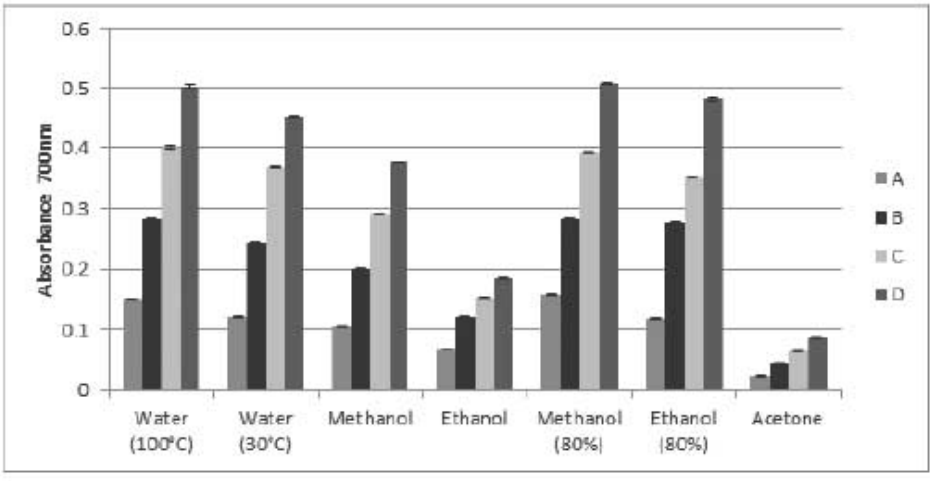

Figure 1B. Reducing power of lime in different extracts Concentration: A.2000ppm, B. 4000ppm, C.6000ppm, D.8000ppm

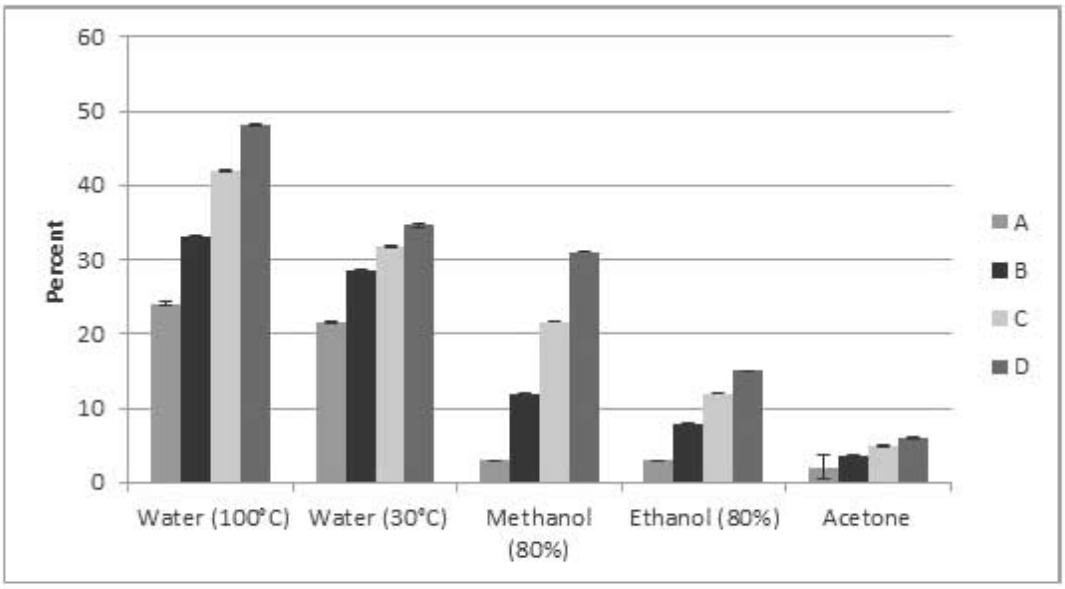

Figure 2A. Radical scavenging activity of shallot in different extracts
Concentration: All extracts: A: 5.0, B: 10.0, C: 15.0 and D: 20.0 mg of sample

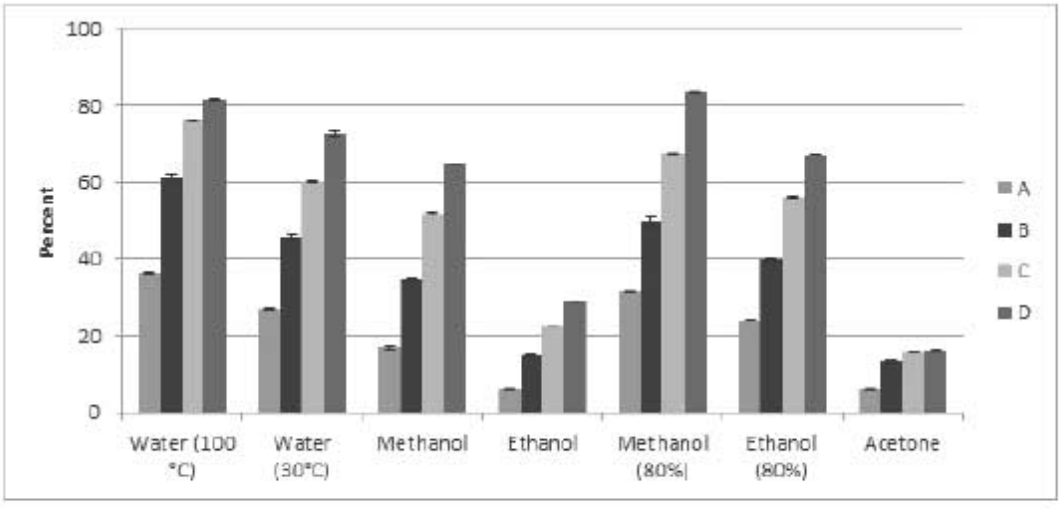

Figure 2B. Radical scavenging activity of lime in different extracts
Concentration: All extracts: A: 2.5, B: 5.0, C: 7.5 and D: 10 mg of sample

Table 3. Correlations between antioxidant components and antioxidant activity of shallot and whole dry lime in different extracts (R Value)

Antioxidant Components	*Solvent Extract*		
	DPPH	*Reducing Power*	*TAA*
Dry Shallot			
Flavonoids	0.860	-0.195	0.654
Polyphenols	0.970	-0.218	0.758
Tannins	0.860	-0.600	0.838
Whole dry Lime			
Flavonoids	0.843	0.836	0.408
Polyphenols	0.746	0.851	0.318
Tannins	0.931	0.954	0.740

comparison to shallot. In lime the highest activity was seen in 80% methanolic extract followed by 100°C and 30°C water extracts and least activity was seen in acetonic extract. Shallot showed highest activity in 100°C water extract and did not show any activity in methanolic and ethanolic extracts despite using a much higher concentration of sample than lime (Figure 2A and 2B). As seen in other assays, DPPH assay also showed strong correlation with all antioxidant components for water extracts of samples. For the solvent extract, a positive correlation was seen for both samples which was also in the higher range indicating that the antioxidant activities could be attributed to the presence of these components. A higher extent of antioxidant activities have also been observed in water extracts of heat treated spices in other studies (Nikousaleh & Prakash, 2008; Nikousaleh & Prakash, 2009).

CONCLUSION

The results of the study bring out the nutritional and antioxidant potential of shallot and lime, the two spices used in Iranian cuisine as flavouring ingredients. Though spices are used in limited quantities, they can contribute small amounts of essential nutrients and have varying degrees of antioxidant activities, which could be beneficial physiologically. Both spices are rich sources of antioxidant components, the presence of which could be associated with antioxidant activities.

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