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Short communications

Evaluation of the total antioxidant capacity and antioxidant compounds of different solvent extracts of Chilgoza pine nuts (*Pinus gerardiana*)

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ABSTRACT

The total antioxidant capacity of *Pinus gerardania*, or Chilgoza and the presence of antioxidant compounds which typically exist in pine nuts were quantified. Oxygen radical absorbance capacity assay, ferric reducing antioxidant power, and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) and 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation scavenging assays were conducted on dichloromethane, ethanol, ethyl acetate, hexane, methanol and water extracts of Chilgoza. The water and dichloromethane extracts displayed the highest antioxidant activity across all assays. Antioxidant compounds such as gallocatechin, catechin, lutein, lycopene, caretenoids and tocopherols were present in all extracts. Gallocatechin had the highest presence out of all the compounds. Its amounts present in the water and dichloromethane extracts were 64.9 and 56.9 μg per gram fresh weight (μg/g), respectively. The total carotenoid contents in the water and dichloromethane extracts were 14.72 and 14.26 μg/g, respectively, while the total tocopherol contents were 1.05 and 2.53 μg/g, respectively.

1. Introduction

Tree nuts have been identified as abundant sources of lipids that are rich in unsaturated fatty acids, antioxidants and phytosterols, as well as other health-promoting substances such as phospholipids (Miraliakbari & Shahidi, 2008a). *Pinus gerardania*, traditionally known as Chilgoza, is found in Khyber Pakhtunkhwa and Baluchistan Provinces of Pakistan as well as in the Northern Areas, Azad Jammu- Kashmir, eastern Afghanistan and northwest India. They are either eaten raw or roasted and are also included as an ingredient in a variety of traditional dishes, such as breads, candies, sauces and cakes, as well as in vegetable and meat dishes. In general, pine nuts are known to be a good source of nutrients. They contain approximately 50% fat, 30% protein, 10% carbohydrate, 4% ash and 6% moisture. They are also a rich source of unsaturated fatty acids (UFAs) where a previous study reported as 51% linoleic acid, and 37% oleic acid in a species of pine nuts which was known to contain the highest levels of UFAs (Destaillats, Cruz-Hernandez, Giuffrida, & Dionisi, 2010). Thus, pine nuts have a higher susceptibility to develop off-flavors and get rancid

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due to lipid oxidation (Kornsteiner, Wagner, & Elmadfa, 2006; Miraliakbari & Shahidi, 2008b). A previous study conducted by Miraliakbari and Shahidi (2008c) on the oxidative stability of different types of oils showed that pecan and pistachio oils were the most stable, whereas the oils of pine nuts and walnuts were the least stable. As a result of this phenomenon, pine nuts are generally expected to contain inherent antioxidants in order to reduce the rate of oxidation (Miraliakbari & Shahidi, 2008b). Overall, previous studies have shown nuts to have different types of antioxidants. For instance, almonds are known to contain flavonoids such as catechins, flavonols, and flavonones in their aglycone and glycoside form (Sang et al., 2002), while peanuts and pistachios contain flavonoids and have the highest concentration of resveratrol in comparison with the other nuts (Gentile et al., 2007). Walnuts contain a wide range of polyphenols and tocopherols (Anderson et al., 2001) and cashews have alkyl phenols as the principal antioxidant (Trevisan et al., 2006).

Only a few studies have been carried out to date on the antioxidant potential of Chilgoza and the measurement of its antioxidant compounds, although noticeable amounts of chiroinositol, pinitol and several glycosyl cyclitols (galactinol, galactopinitol A1, fagopyritol B1 and other glycosyl-inositols) have recently been reported in this type of nuts (Destaillats et al., 2010; Ruiz-Aceituno, Ramos, Martinez-Castro, & Sanz, 2012). In this study, the total antioxidant capacity of different solvent extracts of Chilgoza was examined using the oxygen radical absorbance capacity (ORAC) assay, ferric reducing antioxidant power (FRAP) and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) and 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical scavenging assays. In this aspect, a number of studies also present a measure of the total polyphenol content of food products in order to draw comparisons and to provide more detailed information about this sub-group of antioxidants (Halvorsen et al., 2002; Wu et al., 2004b). Polyphenolic compounds are known to be particularly important in the pathologies of heart disease, hypertension and age-related degeneration (Zern & Fernandez, 2005; Zhao, 2009). Thus, the total phenolics content of the various solvent extracts of Chilgoza were quantified in this study. Characterization and quantification of well-known antioxidant compounds such as gallocatechin, gallic acid, lycopene, α- and β-carotenes which were present in the various solvent extracts were carried out using high-performance liquid chromatography (HPLC) to provide a better identification as to the contributors of the observed antioxidant capacity.

2. Materials and methods

Pine nuts were purchased from Hangu, Khyber Pakhtoonkhwa, Pakistan. They were de-shelled and the kernel and brown skin were separated. Extracts of the de-shelled nuts were prepared as described in a later section. Anhydrous sodium carbonate, Folin–Ciocalteu's phenol reagent, KH_2PO_4 and K_2HPO_4, were obtained from Merck (Darmstadt, Germany). 4,6-Tripryridyl-s-triazine (TPTZ), gallic acid and trolox were purchased from Acros Organics (Morris plains, NJ, USA). 2, 2-Azobis (2-amidinopropane) dihydrochloride (AAPH), fluorescein disodium salt, vitamin C, neoxanthin, violaxanthin, lutein, zeaxanthin, lycopene, α- and β-carotene and meta-phosphoric acid were purchased from Sigma Chemicals (St. Louis, MO, USA). Sulfuric acid (95%) was obtained from BDH (London, UK). Tetrahydrofuran, n-hexane, methanol, n-butanol, ethyl acetate, acetone and acetonitrile (HPLC/Spectro grade) were purchased from Tedia (Fairfield, OH, USA). Absolute ethanol, butylated hydroxytoluene (BHT) and disodium sulfate (analytical grade) were obtained from Merck (Darmstadt, Germany). All other reagents, chemicals and HPLC standards used for the study were purchased from Sigma Chemicals (St. Louis, MO, USA).

2.1. *Preparation of Chilgoza extracts*

De-shelled Chilgoza samples were ground into a powder in a grinder (Philips HL 1606, Bangkok, Thailand) for 5 min at an ambient temperature of 22 ± 3 °C. The solvents used for subsequent extraction were dichloromethane (DCM), ethanol (EtOH), ethyl acetate (EtOAc), hexane (HEX), methanol (MeOH) and water. The powder (0.5–1.0 g, approximately 3–5 μm particle size) was extracted three times with each solvent. After the addition of solvent, the tube was vortexed for 30 s followed by sonication for 15 min with temperature maintained between 37 and 39 °C. The tube was shaken once in the middle of sonication to suspend the sample. After the sonication, the tube was allowed to cool to room temperature. The tube was then centrifuged and the supernatant was collected in a 25 mL volumetric flask and topped up to the mark with each solvent prior to conducting the various assays and analyses.

2.2. *Total phenolics content*

The total phenolics contents of the various solvent extracts were determined according to a modified version of the procedure described by Singleton and Rossi (1965) using the Folin and Ciocalteu's phenol reagent (Wijeratne, Abou-Zaid, & Shahidi, 2006). The value was determined using a standard curve prepared from gallic acid and expressed as milligram gallic acid equivalents per gram fresh weight (mg GAE/g) of sample.

2.3. *ORAC assay*

The assay was carried out according to the method by Prior et al. (2003) with a few modifications in 96-well microplate format using a Thermo Scientific Multiskan FC Microplate Reader. Fluorescein disodium was used for the kinetic monitoring of free radical quenching and AAPH was used as the free radical source. The excitation and emission wavelengths were 485 nm and 528–538 nm, respectively. The following components were added to a single well: (1) blank (phosphate buffered saline)/trolox standard/sample – 20 μL, (2) fluorescein working solution – 160 μL, and (3) AAPH – 20 μL. The reaction kinetics were monitored for 2 h at 37 °C, following which the area under the curve was used to calculate the ORAC value compared with those of the trolox standards. BHT was used as the positive control. Results were expressed as μmol trolox equivalents per gram fresh weight (μmol TE/g) of sample.

2.4. Determination of the DPPH radical scavenging activity

Extract concentrations of 62.5, 125, 250, 500 and 1000 mg/kg were prepared by dilution with 75 mM phosphate buffer (pH = 7.4). A 96-well microplate was used for the analysis where 140 μL of the respective extracts was pipetted along with 60 μL of 400 μM of DPPH (prepared in the 75 mM phosphate buffer solution). The blank wells consisted of 200 μL of the phosphate buffer solution, while the control wells consisted of 140 μL of the phosphate buffer solution and 60 μL of the DPPH solution. The microplate was incubated at 37 °C for 30 min and the absorbance was measured at 517 nm, using a Thermo Scientific Multiskan FC Microplate Reader. Each sample concentration was added in triplicate into the microplate. BHT was used as the positive control. The antioxidant activity was calculated as % DPPH radical scavenging activity, by substituting the absorbance values into the following equation:

$$\%\ \text{DPPH Radical Scavenging Activity} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Extract}} \times 100}{\text{Abs}_{\text{Control}}}$$

The % DPPH scavenging activity of 10 replicates of each sample was used to calculate the EC_{50} values in milligrams per kilogram fresh weight (mg/kg) of the samples.

2.5. FRAP assay

A modification of the FRAP assay of Benzie and Strain (1996) was carried out. Briefly, FRAP reagent was prepared from 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ made up in 40 mM HCl. All three solutions were mixed together in the ratio 10:1:1. The FRAP assay was performed by warming 1 mL of dH_2O to 37 °C before adding 25 μL of sample and 1 mL of reagent and incubating at 37 °C for 4 min. Absorbance at 593 nm was determined relative to a reagent blank also incubated at 37 °C. The total antioxidant capacity of samples was determined against a standard of known FRAP value, ferrous sulfate (1000 μM). BHT was used as the positive control.

2.6. ABTS•+ radical cation scavenging activity

Antioxidant activities of the samples were analyzed by investigating their ability to scavenge the ABTS•+ free radical using a methodology previously reported by Ozgen, Reese, Tulio, Scheerens, and Miller (2006). BHT was used as the positive control.

2.7. HPLC determination of the antioxidant compounds

A Shimadzu (Kyoto, Japan) HPLC system equipped with a diode array detector (SPDM10Avp) and a phenomenex Luna C-18(2) column (4.6 mm i.d. × 25 cm, 5 μm) was used for the quantification of the phenolic compounds of gallocatechin, gallic acid, catechin, vanillic acid, ellagic acid and quercetin and the epicatechin isomers of (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin-3-gallate (EGCG). A gradient profile using two solvents was applied at room temperature following the method by Wijeratne et al. (2006) with a few modifications. The solvents used were as follows: solvent A: 8% aqueous formic acid and solvent B: acetonitrile/methanol (10:90, v/v). A flow rate of 0.9 mL/min was maintained. The gradient was as follows: 0 min – 20% B; 7 min – 35% B; 14 min – 45% B; 21 min – 65% B; 25 min – 85% B; 32 min – 95% B. The wavelengths of the diode array detector were set at 260, 280 and 320 nm for monitoring of the phenolic compounds. HPLC analyses for the carotenoid compounds and tocopherols in the extracts were carried out using the same HPLC set-up according to the internal standard (IS) method by Lee, Su, and Ong (2004). For calibration, the method by Isabelle et al. (2010) was followed where 100 μL of the IS solution was mixed with 100 μL of standard mixtures of various concentrations. Stock solutions of each standard were prepared individually with relevant solvents as described by Lee, New, and Ong (2003). The concentrations of all the compounds in the extracts were quantified using standard curves and expressed as micrograms per gram fresh weight of powder (μg/g).

2.8. Statistical analysis

All data are presented as mean ± standard error mean (SEM) of at least three independent experiments ($n \geq 3$), where each experiment had a minimum of three replicates of each sample. For comparisons between samples, data were analyzed by ANOVA and Tukey's multiple comparison test (SPSS, version 17). A probability of 5% or less was accepted as statistically significant ($P < 0.05$).

3. Results

The total phenolics contents of the extracts and the results from the antioxidant capacity assays are shown in Fig. 1. The total phenolics contents of the extracts decreased in the order of water > DCM > EtOH > EtOAc > MeOH > HEX. The ABTS•+, FRAP and ORAC values of the extracts decreased in the same order as the total phenolics contents, while the DPPH EC_{50} values increased in the reversed order. The increases and differences of the various antioxidant values correlated with the total phenolics content, indicating the water extract to contain the highest amount of total phenolics and antioxidant potential of hexane being the lowest. Therefore, water was observed to be the best solvent for the extraction of the phenolic compounds from the Chilgoza powder with DCM coming a close second. All the antioxidant values were statistically significantly different ($P < 0.05$) than BHT, indicating that the antioxidant potential of the extracts were not as high as BHT. The correlation between the total phenolics content and the ORAC assay values ($R^2 = 0.985$) was higher than ABTS•+ ($R^2 = 0.854$), DPPH EC_{50} ($R^2 = 0.867$) and FRAP ($R^2 = 0.934$). The ORAC to total phenolics ratio for all extracts were in the range of 10–20. The high ratio might be due to the presence of non-phenolic antioxidants, or the presence of phenolics antioxidants having a strong radical scavenging activity (Wu et al., 2004a, 2004b). However, given the high R^2 value in relation to the ORAC values, the total phenolics content was observed to be a good indicator of the presence of antioxidant compounds for the Chilgoza extracts.

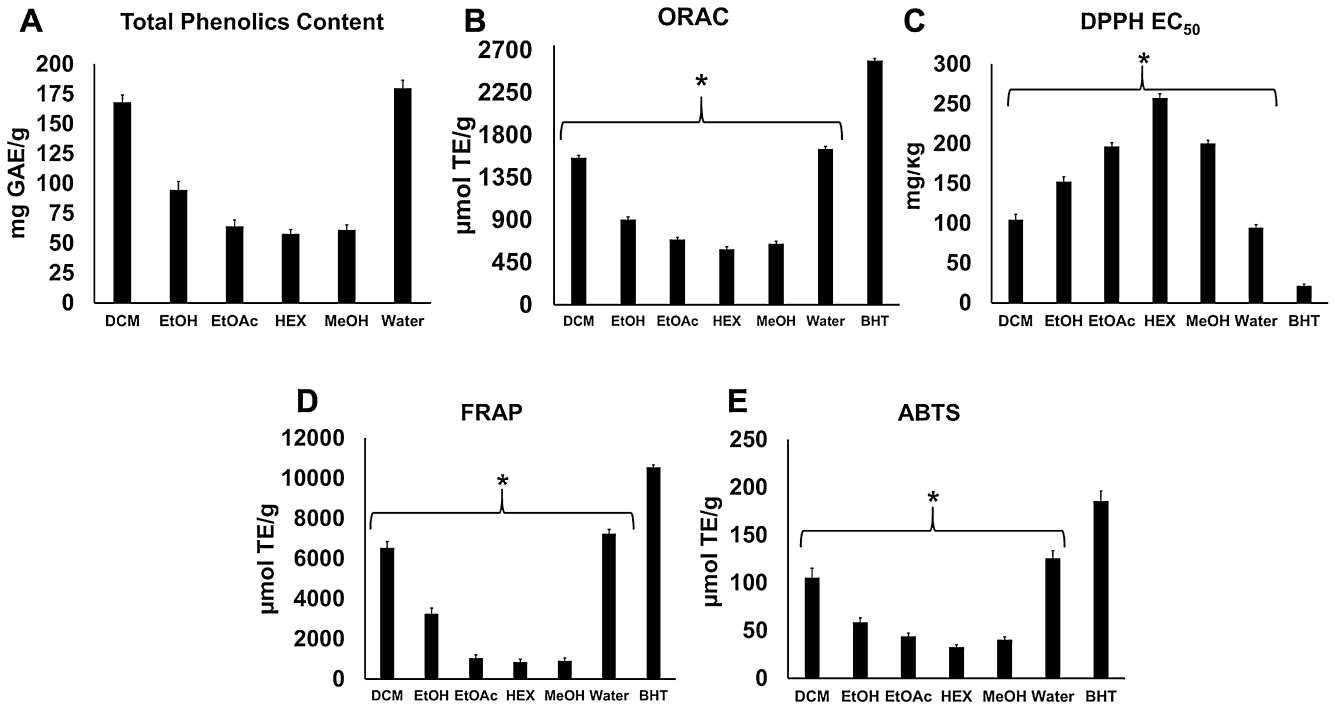

Fig. 1 – (A) Total phenolics contents, (B) ORAC, (C) DPPH EC_{50}, (D) FRAP and (E) ABTS radical scavenging activities of DCM, EtOH, EtOAc, HEX, MeOH and water extracts of Chilgoza. Results are expressed as mean ± SEM per gram fresh weight. *$P < 0.05$ *versus* the antioxidant values of BHT.

The list of phenolic compounds which were quantified to be present in the Chilgoza extracts are shown in Table 1. These values however, are tentatively determined and identified. Further complementing the total phenolics content values, the water extract was observed to contain the highest amount of phenolic compounds with DCM coming a close second. Gallocatechin was observed to have the highest presence in all six extracts, while gallic acid and ellagic acid were observed to have the next highest amounts in the extracts. The list of lipid-soluble antioxidants which were quantified to be present in all six Chilgoza extracts is shown in Table 2. As with Table 1, these values were also tentatively determined and identified. The amounts of neoxanthin, violaxanthin, lutein, zeaxanthin, lycopene, total carotenoids and total tocopherols varied greatly among all of the extracts. The total carotenoids were obtained from the sum of neoxanthin, violaxanthin, lutein, zeaxanthin and α- and β-carotene, while the total tocopherols content was obtained from the sum of α-, δ- and γ-tocophercol. However, the total carotenoid content and the total tocopherol content were the highest in DCM and water extracts, despite the lipophilic nature of these assayed antioxidants. No clear relationship was observed between the relationships of antioxidant parameters, total carotenoids and total tocopherol contents ($R^2 < 0.5$ for all). Overall, the hydrophilic antioxidant compounds were present in higher quantities than the lipophilic antioxidants in all of the extracts. Chemical structures of the major antioxidant compounds which had the highest overall presence are shown in Fig. 2.

Further complementing the results from Fig. 1, the results from Tables 1 and 2 ascertained water to be the better solvent for the extraction of antioxidant compounds from the Chilgoza powder. This observation was somewhat contradictory given the fat content of Chilgoza and therefore, the expectancy of a lipophilic solvent to be the better medium for the extraction of antioxidant compounds. However, it is also possible that despite the high content of fat, the overall antioxidant compounds in Chilgoza are more soluble in water, and therefore, water proved to be the better solvent for their extraction. Nevertheless, in comparing the amounts of phenolic compounds and the lipid-soluble antioxidant compounds, the former was present in higher quantities than the latter.

4. Discussion

This study mainly focused on quantifying the phenolic compounds and lipid-soluble antioxidants which are commonly found in nuts belonging to the *Pinus* family, and have been identified to be present in their extracts as per previous studies (Guri, Kefalas, & Roussis, 2006; Jerez, Pinelo, Sineiro, & Núñez, 2006; Miraliakbari & Shahidi, 2008b, 2008c). The compounds studied here are also well known for their antioxidant potential and therapeutic benefits (Baboota et al., 2013; Chang, 2013). Nevertheless, the possibility exists that novel antioxidant compounds may be present in the extracts which require extensive characterization procedures. Following this, whether these novel compounds possess any therapeutic potential also requires further elucidation through *in vitro* and *in vivo* studies. However, the existence of known antioxidant compounds with

Table 1 – Phenolic compounds present in the Chilgoza extracts.[a]

Solvent	Gallocatechin	EC	ECG	EGC	EGCG	Gallic acid	Catechin	Vanillic acid	Ellagic acid	Quercetin	Taxifolin	Protocatechuic acid	Syringic acid
DCM	56.9	14.5	15.9	18.5	17.2	25.8	35.8	20.9	15.9	26.8	1.6	1.6	0.9
EtOAc	26.9	9.7	8.5	10.2	9.6	15.7	29.8	15.4	14.5	16.9	1.2	1.7	1.1
EtOH	24.7	9.1	8.1	3.9	9.2	25.9	15.8	14.9	22.3	19.5	1.1	1.2	0.8
HEX	9.2	1.5	2.1	2.8	8.3	2.9	3.9	4.8	5.9	12.8	1.9	1.6	0.7
MeOH	12.8	5.4	5.2	2.9	5.6	9.6	5.5	6.2	5.8	3.9	1.5	1.2	0.9
Water	64.9	15.9	16.9	16.4	18.7	21.4	33.9	24.8	35.4	18.4	2.5	1.6	1.2
Lower detection limit	0.03	0.03	0.04	0.03	0.03	0.03	0.06	0.07	0.08	0.06	0.06	0.05	0.05
Lower quantification limit	0.06	0.06	0.08	0.07	0.07	0.07	0.13	0.15	0.16	0.12	0.12	0.10	0.10
Analytical CV	10%	10%	11%	12%	10%	11%	12%	10%	11%	10%	11%	11%	11%

[a] Data expressed as micrograms per gram fresh weight of powder (μg/g) and presented as mean.

Table 2 – Tocopherols and carotenoids present in the Chilgoza extracts.[a]

Solvent	Neoxanthin	Viola xanthin	Lutein	Zea xanthin	Lycopene	Carotene		Tocopherol			Total carotenoids[c]	Total tocopherols[d]
						α	β	α	δ	γ		
DCM	0.25	2.56	3.59	4.19	1.77	1.42	0.94	0.49	0.41	0.15	14.72	1.05
EtOAc	0.56	0.89	2.16	2.11	0.16	0.21	0.15	0.55	0.36	0.11	6.24	1.02
EtOH	0.12	1.67	1.49	0.58	1.48	ND[b]	0.33	ND	0.10	0.13	5.67	0.23
HEX	0.76	0.49	0.58	0.49	0.43	0.23	ND	0.39	0.17	0.14	12.98	0.70
MeOH	0.44	1.55	1.09	1.46	0.47	0.16	0.47	0.14	0.31	0.41	5.64	0.86
Water	0.12	0.46	0.49	3.59	4.10	2.94	2.56	1.43	0.88	0.22	14.26	2.53
Lower detection limit	0.02	0.02	0.05	0.05	0.05	0.01	0.04	0.01	0.02	0.02		
Lower quantification limit	0.05	0.07	0.13	0.12	0.15	0.04	0.12	0.03	0.05	0.05		
Analytical CV	12%	14%	11%	12%	13%	12%	10%	12%	11%	11%		

[a] Data expressed as micrograms per gram fresh weight of powder (μg/g) and presented as mean.
[b] ND: not detected.
[c] Total carotenoids obtained from the sum of neoxanthin, violaxanthin, lutein, zeaxanthin and α- and β-carotene expressed as micrograms of carotenoids per gram fresh weight of Chilgoza powder.
[d] Total tocopherols obtained from the sum of α-, δ- and γ-tocopherol expressed as micrograms of vitamin E per gram fresh weight of Chilgoza powder.

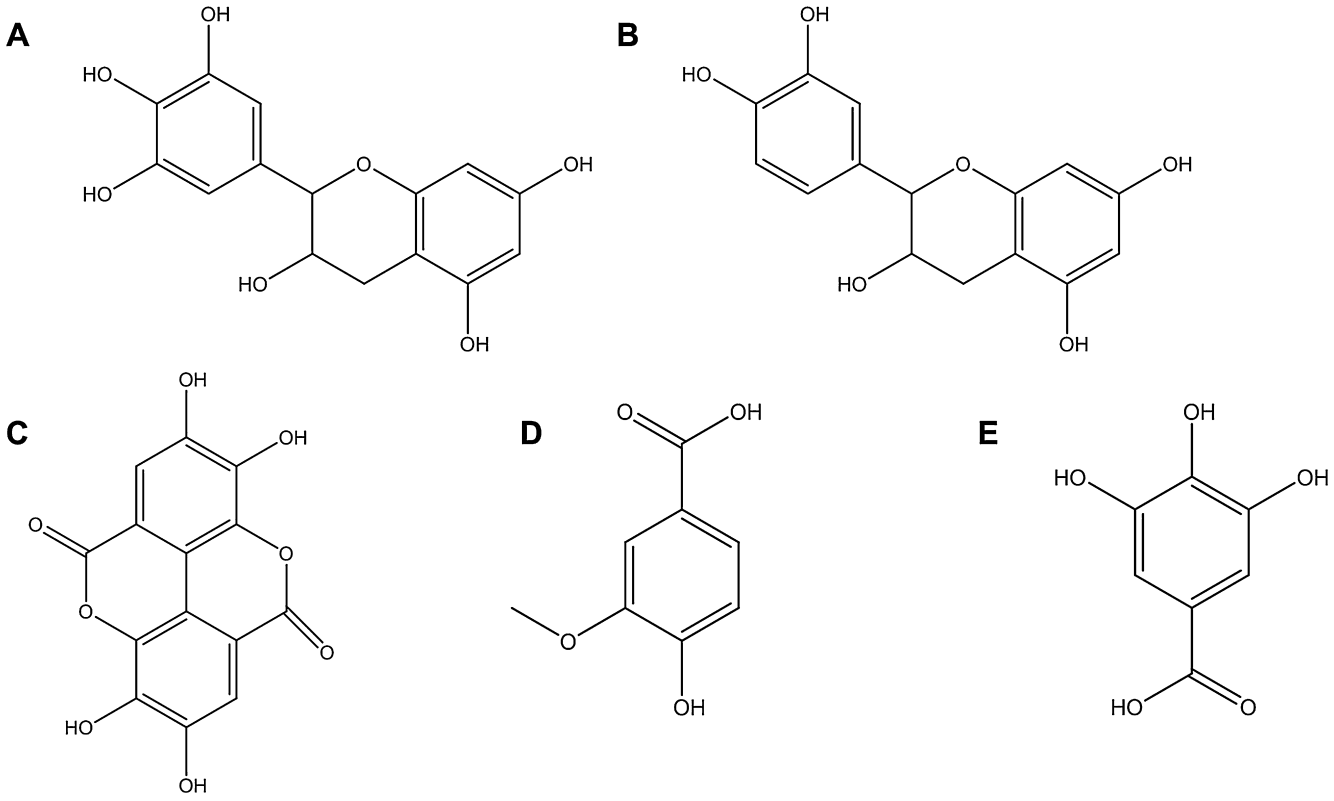

Fig. 2 – Chemical structures of the compounds which had the highest overall presence in all of the extracts: (A) gallocatechin, (B) catechin, (C) ellagic acid, (D) vanillic acid and (E) gallic acid.

established therapeutic properties in considerable amounts itself is a highlight of this study, given that many of the nuts in the *Pinus* family have not exhibited to contain these antioxidant compounds in these particular quantities (regardless of the type of solvent used for the extraction). The studies by Guri et al. (2006) and Jerez et al. (2006) are able to provide confirmation and evidence as to this claim, where the researches revealed a comparatively lesser amount of antioxidant compounds in other *Pinus* family members as compared with other categories of food products which are lesser known for their therapeutic potential.

Although nuts are typically known to contain a high fat content resulting in varied oxidative stabilities, since the word 'dietary fat' has become synonymous with obesity and heart disease, nuts are traditionally avoided by consumers in an attempt to lower blood cholesterol and the risk of coronary heart disease (CHD) (Alasalvar et al., 2003). However, there is increasing evidence suggesting that nuts may elicit cardioprotective effects (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2006; Shahidi & Miraliakbari, 2005). For instance, the Nurses Health Study has observed that those who consumed nuts five or more times a week were reported to have a 35% reduced risk of contracting CHD as compared with those who rarely consumed nuts (Hu et al., 1998). Similarly, in the Physicians Health Study (Albert, Gaziano, Willett, & Manson, 2002) and the Adventist Health Study (Fraser, Sabate, Beeson, & Strahan, 1992) a strong relationship was observed between the consumption of nuts and the reduction of the risk of CHD. Given that reactive oxygen species contribute to the pathogenesis of CHD, it is possible that the protective antioxidants existing in nuts such as Chilgoza could be attributed to their therapeutic potential in curbing the progression of the disease. Several types of nuts have been observed to contain antioxidant compounds which are well-known for their therapeutic benefits, thus being able to contribute to the reduction of diseases associated with reactive oxygen species and oxidative stress (John & Shahidi, 2010; Ryan et al., 2006).

Despite their association with therapeutic effects, the antioxidant content of plant material in general, could be affected by the cultivar, maturity level as well as growing conditions such as the location, soil state, climate, and agriculture practices (Lv et al., 2014; Wu et al., 2004a, 2004b). As a result, there could be a high variation of the antioxidant content within the same Chilgoza species. In addition, the measurement of the antioxidant potential of the various solvent extracts may be subjected to various interactions as well. It is possible that the phytochemicals contained in Chilgoza could work in synergy with other important nut constituents to promote antioxidant activities – a characteristic which has been highlighted in previous studies (Chen & Blumberg, 2008; Gawlik-Dziki, 2012).

Several methods have been used to assess the total antioxidant capacity of nuts in general. ORAC is based on the antioxidant's ability to react with or neutralize free radicals generated in the assay systems, whereas FRAP measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants. The total antioxidant capacity measure

was considered appropriate for assessing the cumulative antioxidant properties of plant foods (Pellegrini et al., 2006). However, the impossibility of comparing results obtained with different methodologies has seriously limited understanding of the role of total antioxidant capacity in disease prevention (Serafini, 2006). As a result, in the recent years, ambiguous results have been published about the possible effect of nuts on oxidative stress status (Mukuddem-Petersen, Oosthuizen, & Jerling, 2005). This was the rationale for further characterizing the antioxidant compounds present in the extracts. The antioxidants which were characterized to be present in Chilgoza have been identified to possess functional properties and health benefits. The proanthocyanidins which exist in the *Pinus* family are also typically found in fruits, cereals, legumes, wine, and chocolate. They affect nutritional value, appearance, taste, and texture of these foods and promote better health by preventing cardiovascular diseases, cancers, urinary tract infections, and other aging-related metabolic complications (Ou & Gu, 2014). β-Carotene is one of the most studied carotenoids, and is suggested to possess chemoprotective properties (Van Poppel, 1993). Lycopene – the major carotenoid in tomatoes – is a known antioxidant which has been observed to lower oxidative stress biomarkers in previous studies (Pinto, Rodriguez-Galdon, Cestero, & Macias, 2013). Different vitamin E isomers have unique bioactive properties unrelated to their antioxidant activity, although α- and γ-tocopherols have been shown to possess anti-inflammatory properties (Idris et al., 2014; Reiter, Jiang, & Christen, 2007).

5. Conclusions

The study was able to provide insights as to the solubility of antioxidant compounds present in Chilgoza in different types of solvents. Water was observed to be the better solvent for the efficient extraction of antioxidant compounds. Although the study only examined six pure solvents, their combinations may also be worthy of exploring for the efficacy of the extraction. Phenolic compounds and lipid-soluble antioxidants were observed to be present in the extracts in varying amounts. Overall, the amount of phenolic compounds was higher than the lipid-soluble antioxidant compounds in all the extracts. In addition, the total carotenoids content exceeded the total tocopherol contents, thus leading to the conclusion as the most abundant lipid-soluble antioxidant compounds present in Chilgoza. The bioactivity and the radical scavenging efficacy of the extracts in *in vitro* or *in vivo* systems are worthy exploration and could be considered as the next step following this screening process. Whether the identified antioxidant activities could be initiated in actual physiological conditions has always been the challenge since the chemical assays have not been essentially able to capture the true potential of antioxidants and plant-based food products containing antioxidant compounds. Thus, further studies on Chilgoza fully characterizing the antioxidant content, bioaccesibility, bioavailability, metabolism, and elimination in humans will be necessary in the future. The possible interactions between different nut antioxidant products and other important nut constituents which are able to promote antioxidant activities also need to be explored. Such studies may help elucidate the mechanisms explaining the effect of consuming nuts or their byproducts on oxidative stress status and their resulting or associated disease conditions in humans.

Conflicts of interest

The authors declare no conflicts of interest, financial or otherwise.

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