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# Comparative study on the volatile constituents of American and Pakistani almonds and their antioxidant activities.

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American almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. American] is always marketed with high price as compared to the Pakistani almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. Pakistan] due to its superior quality. To study a comparative account on their chemical composition, GC-MS analysis of the volatile fraction of *n*-hexane extracts of both was performed separately which led to the conclusion that former is enriched additionally with small amounts of some oxygenated constituents which are absent in the latter. These additional components might have been contributing for its better quality. Similarly, when the antioxidant activities of these extracts were compared by DPPH radical scavenging and phosphomolybdenum complex method, it was revealed that the former possesses slightly more potential as compared to the latter. The $IC_{50}$ of American almond extract was found to be 70±7 μL/mL while that of Pakistani almond extract was 108±19 μL/mL, respectively.

**Key words: American almond, Pakistani almond, Volatile fraction, GC-MS analysis, Antioxidant activities.**

The almond nut [*Prunus dulcis* (Mill.) D. A.Webb. cv. American; *Prunus dulcis* (Mill.) D. A.Webb. cv. Pakistan] is a species of *Purnus* belonging to the family Rosaceae. Twenty-six almond species form a distinct and easily identified taxonomic group in the world; therefore they are a potentially important source of new variation (Xu *et. al*. 2004). The global production of almonds is around 1.7 million metric tons with the California producing 80% of the world's almond. Lipid, the main storage component in almond seeds, constituting over 50% of the total weight of the seeds, is located as intracellular oil bodies. Proteins comprise about 22 to 25% of the seeds, while 11 to 12% is represented by dietary fiber (Mandalari *et. al.* 2008). The almond oil is extensively studied on account of its nutritional, industrial and medical importance. It is also used as excellent carrier oil for other materials. Furthermore, almond seeds are used both as a snack and as an ingredient in other food products. Because of the high content of monounsaturated fatty acids almond seeds can decrease the cholesterol levels and play an important role in prevention of cardiovascular pathologic conditions. Almond oil is widely used in many cosmetic formulations, because the beneficial action of almond oil on skin is known for centuries. Almond oil is a component of skin hydrating creams, anti-wrinkle and anti-ageing products (Malisiova *et. al.* 2004). Almonds provide protection against diabetes. They are also a good source of minerals and vitamin E, associated with promoting health and reducing the risk for chronic disease (Mandalari *et. al.* 2008).

Active oxygen and, in particular, free radicals are considered to induce oxidative damage in biomolecules and to play an important role in aging, cardiovascular diseases, cancer and inflammatory diseases. They are also well known to be major causers

of material degradation and food deterioration. Consequently, antioxidants are now known to be prospective protective or therapeutic agents. Natural ingredients are now-a-days very attractive to use for this purpose. At present, most of the natural antioxidants such as traditional nutrients, polyphenols, and flavonoids are obtained from plants (Liu *et. al.* 2004). Almond oil also possesses such properties (Ebringerova *et. al.* 2008). Here, we report the chemical composition of the volatile fraction of *n*-hexane extracts of American almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. American] and Pakistani almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. Pakistan], their DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activities and also their total antioxidant activities by Phosphomolybdenum method, separately.

## MATERIALS AND METHODS

**Plant Material:** American almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. American] and Pakistani almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. Pakistan] seeds were purchased from a local dry-fruit market on Mall road, Lahore, in March 2009, and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore.

**Extraction procedures:** The ground seeds (2 kg each) of American almond and Pakistani almond were exhaustively extracted with double-distilled *n*-hexane (2 L X 4) at room temperature, separately. The extracts were filtered through Whatman No. 1 filter papers thrice and it was distilled off completely till dryness using distillation assembly. The obtained distillate (volatile fraction) was used for the GC-MS analysis and antioxidant studies.

**Chemicals and standard:** $DPPH^{\cdot}$ (1,1-Diphenyl-2-picrylhydrazyl radical) and BHT (Butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and analytical grade *n*-hexane solvent, sulphuric acid, sodium phosphate and ammonium molybdate from Merck (Pvt.) Ltd. (Germany). Individual standards for GC-MS were purchased from Aldrich (Milwaukee, WI).

**GC-MS Analysis:** A Shimadzu 2010 (Shimadzu, Japan) gas chromatograph equipped with a split-splitless auto-injector model AOCi, an auto sampler model AOC-20s and a MS-QP 2010 (Shimadzu, Japan) series mass selective detector was used for the analysis of the extracts studied. A fused silica capillary column (J&W DB 5MS), 5% phenyl polysiloxane as non-polar stationary phase (30 m × 0.25 mm i.d.) and 0.25 µm film thickness, supplied by Agilent (Palo Alto, CA, USA) was used for the GC separation, with helium as carrier gas at a constant flow at 1.27 mL/min. The temperature program used was as follows: initial temperature, 40 $^{o}$C held for 5 min, then at the rate of 5 $^{o}$C /min to 180 $^{o}$C, and 3 $^{o}$C /min to 240 $^{o}$C and then maintaining this temperature for 5 min. The temperature of the injection port was 200 $^{o}$C and a 1 µL volume was injected in split mode with 90 % split ratio. Mass selective detector was operated in electron impact (EI) ionization mode with an ionizing energy of 70 eV, scanning from *m/z* 40 to 950 at 0.5 s per scan. The ion source temperature was 250 $^{o}$C and the MS transfer line temperature 205 $^{o}$C. The electron multiplier voltage (EM voltage) was maintained at 1000 V, and the solvent delay of 3.0 min was employed. All the compounds were identified by comparison of their GC retention data and mass spectra with those of pure and authentic samples, and also by comparing fragmentation patterns of mass spectra with those stored in the spectrometer data base and bibliography (Adams 1995).

**DPPH Radical Scavenging Activity:** The DPPH radical scavenging activity of volatile fraction of American almond and Pakistani almond was examined by comparison with that of known antioxidant, butylated hydroxytoluene (BHT) using the method of Lee *et al.* (1998). Briefly, various amounts of the volatile fraction of *n*-hexane extract (300 µL, 200 µL, 100 µL) were mixed with 3ml of chloroform solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for one an hour. Then Absorbance was measured at 517nm against chloroform as a blank in the spectrophotometer. Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula: Antiradical activity = $A_{control}$ - $A_{sample}$/ $A_{control}$ ×100

Each extract was assayed in triplicate and mean values were calculated.

**Total Antioxidant Activity:** The antioxidant activities of *n*-hexane extracts of American almond and Pakistani almond were evaluated by phosphomolybdenum complex formation method (Prieto *et. al.* 1999). Briefly, various amounts of volatile fraction (300 µL, 200 µL, 100 µL) were mixed with 4ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4mL of reagent solution. The vials were capped and incubated in water bath at 95 $^{o}$C for 90 minutes. After the samples had cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of butylated hydroxytoluene (BHT). All determinations were in triplicate and mean values were calculated.

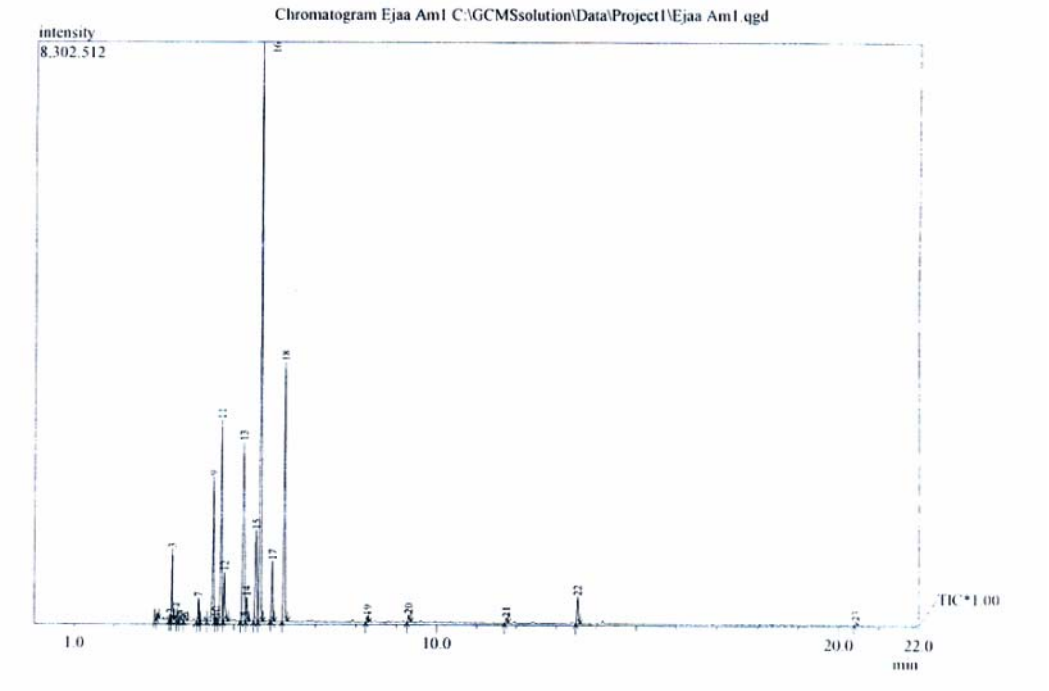
**Statistical Analysis:** All the measurements were done in triplicate and statistical analysis was performed by Microsoft excel 2003. Results are presented as average ± SEM.

## RESULTS & DISCUSSION

For studying a comparative account, the analysis of the volatile constituents of the *n*-hexane extract of the American almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. American] and Pakistani almond [*Prunus dulcis* (Mill.) D. A.Webb. cv. Pakistan] was performed separately on GC-MS and the names of identified compounds, their retention times and area percentages are shown in Table 1 and Table 2, respectively. American almond showed twenty-two constituents while Pakistani almond exhibited only eighteen compounds. When the profile of the former was compared with the latter, it was revealed that both have sixteen constituents in common, namely, -thujene (Zini *et. al.* 2001), -gurjunene (Batista-Pereira *et. al.* 2006), linalool, borneol (Angelini *et. al*. 2003), -thujone, viridiflorol (Kann, J. and Orav, A. 2001), verbenol, Myrtenol, *p*-cymen-8-ol (Buttery *et. al*. 2000), estragol (Kann J. and Orav A. 2001), geraniol (Sawai *et. al.* 2004), cedrol, carvactrol (Angelini *et. al*. 2003), isobutyl cyanide (Buttery *et. al*. 2000), *n*-octanol and benzyl alcohol (Sawai *et. al.* 2004). But the American almond was found to be additionally enriched with small amounts of some oxygenated constituents such as 1,8-Cineole (Angelini *et. al*. 2003), -Terpineol (Kann, J. and Orav, A. 2001) and nerolidol (Batista-Pereira *et. al.* 2006) along with hydrocarbons like camphene (Angelini *et. al*. 2003), sabinene (Kann J. and Orav A. 2001) and -myrcene (Zini *et. al.* 2001) which are absent in the Pakistani almond. However, nonanal (Sawai *et. al.* 2004) and globulol (Queiroga *et al.* 1990) were found only in Pakistani alomond. These additional components in American almond might have a contribution for its better quality. The GC chromatograph of both American almond and Pakistani almond are shown in Fig. 1 and Fig. 2, respectively. In a search for potential bioactive substances from plant origin we have studied the volatile fractions of American almond and Pakistani almond, for free radical scavenging activity using 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) and their total antioxidant activity by Phosphomolybdenum method and the results are shown in Table 3 and Table 4, respectively. The results showed that American almond, having some additional oxygenated constituents, possesses slightly more antioxidant activity as compared to that of Pakistani almond, although both exhibited a weak potential relative to butylated hydroxytoluene (standard). The $IC_{50}$ of American almond was calculated as 70 ± 7 µL/mL while that of Pakistani almond was found to be 108 ± 19 µL/mL, relative to butylated hydroxytoluene (BHT), having $IC_{50}$ of 12.1 ± 0.92 ug/mL. However, for both the fractions, greater was the concentration, greater was the antioxidant potential. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time. The addition of extracts to the DPPH solution caused a rapid decrease in the optical density at 517 nm. The degree of decoloration indicates the scavenging capacity of the extracts. Free radicals cause autoxidation of unsaturated lipids in food (Kaur, H. and Perkins, J. 1991). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity (Baumann *et. al.* 1979). Antioxidants cease the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups. Therefore forming a stable end-product does not permit further oxidation of the lipid (Sherwin 1978). The phosphomolybdenum method is based on the reduction of molybdenum (IV) to

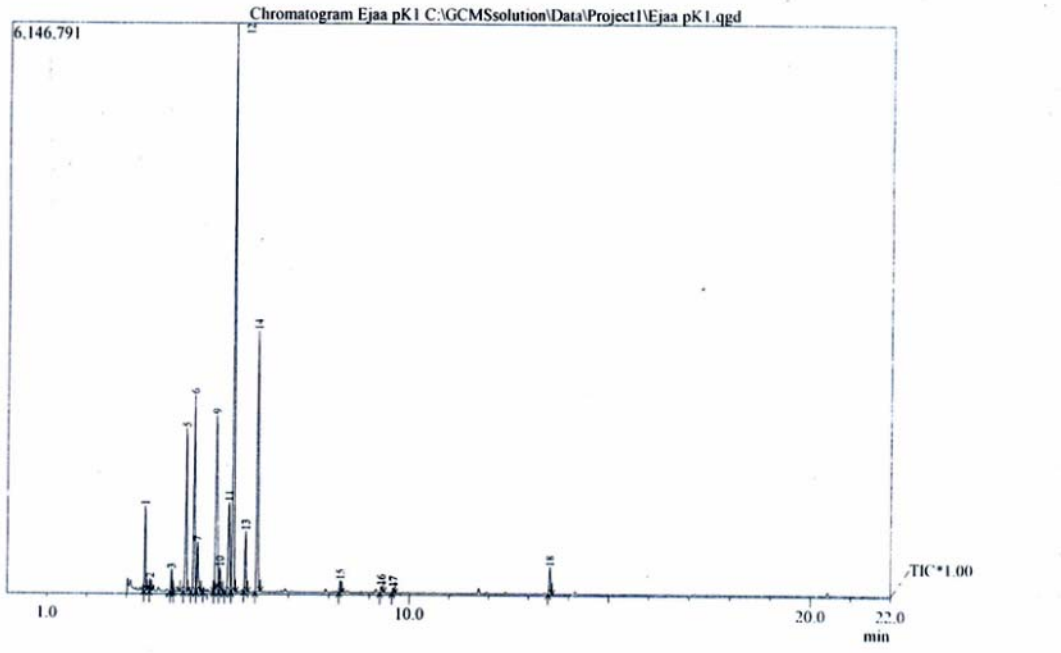
molybdenum (V) by the antioxidant compounds and the formation of green Mo (V) complex with a maximal absorption at 695 nm (Prieto *et. al.* 1999).

**Figure: 1.** Peak profile for the volatile fraction of American almond.




(Retention time on X-axis; intensity on Y-axis)

**Figure: 2.** Peak profile for the volatile fraction of Pakistani almond




(Retention time on X-axis; intensity on Y-axis)

**Table: 1. Constituents of volatile fraction of American almond with retention time and area%.**

| Peak No. | Retention Time | Compound Name | Area% |
|---|---|---|---|
| 1 | 3.102 | Camphene | 0.25 |
| 2 | 3.421 | Sabinene | 0.25 |
| 3 | 3.461 | $\alpha$-Thujene | 2.70 |
| 4 | 3.586 | $\alpha$-Gurjunene | 0.44 |
| 5 | 3.666 | $\beta$-myrcene | 0.23 |
| 6 | 3.789 | 1,8-Cineole | 0.23 |
| 7 | 4.120 | Linalool | 1.01 |
| 8 | 4.275 | Borneol | 0.26 |
| 9 | 4.477 | $\alpha$-Thujone | 7.68 |
| 10 | 4.555 | $\alpha$-Terpineol | 0.23 |
| 11 | 4.676 | Viridiflorol | 10.48 |
| 12 | 4.759 | Verbenol | 2.89 |
| 13 | 5.219 | Myrtenol | 13.54 |
| 14 | 5.312 | *p*-Cymen-8-ol | 1.48 |
| 15 | 5.532 | Estragol | 6.71 |
| 16 | 5.644 | Graniol | 31.21 |
| 17 | 5.939 | Cedrol | 3.29 |
| 18 | 6.229 | Carvactrol | 14.29 |
| 19 | 8.284 | Isobutyl cyanide | 0.40 |
| 20 | 9.305 | *n*-Octanol | 0.48 |
| 21 | 11.750 | Nerolidol | 0.33 |
| 22 | 13.539 | Benzyl alcohol | 1.62 |
| | | | 100 |

**Table: 2. Constituents of volatile fraction of Pakistani almond with retention time and area%.**

| Peak No. | Retention Time | Compound Name | Area% |
|---|---|---|---|
| 1 | 3.461 | $\alpha$-Thujene | 3.34 |
| 2 | 3.586 | $\alpha$-Gurjunene | 0.44 |
| 3 | 4.120 | Linalool | 0.98 |
| 4 | 4.275 | Borneol | 0.26 |
| 5 | 4.477 | $\alpha$-Thujone | 8.70 |
| 6 | 4.676 | Viridiflorol | 10.37 |
| 7 | 4.759 | Verbenol | 2.86 |
| 8 | 4.918 | Nonanal | 0.31 |
| 9 | 5.219 | Myrtenol | 13.36 |
| 10 | 5.312 | *p*-Cymen-8-ol | 1.51 |
| 11 | 5.532 | Estragol | 6.40 |
| 12 | 5.644 | Graniol | 31.04 |
| 13 | 5.939 | Cedrol | 3.20 |
| 14 | 6.229 | Carvactrol | 14.23 |
| 15 | 8.284 | Isobutyl cyanide | 0.70 |
| 16 | 9.305 | *n*-Octanol | 0.40 |
| 17 | 9.608 | Globulol | 0.31 |
| 18 | 13.539 | Benzyl alcohol | 1.60 |
| | | | 100.00 |

**Table: 3. Free radical scavenging activity of volatile fraction of American and Pakistani almond using 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH)**

| S. No. | Sample | Concentration in Assay (µL/3mL) | %age Scavenging of DPPH$^{\bullet}$ ± S.E.M.[a]) |
|---|---|---|---|
| 1 | American almond | 300 | 91.15 ± 0.83 |
| | | 200 | 91.15 ± 0.57 |
| | | 100 | 52.75 ± 1.41 |
| 2 | Pakistan almond | 300 | 90.99 ± 0.28 |
| | | 200 | 82.90 ± 1.43 |
| | | 100 | 42.39 ± 5.27 |
| 3 | BHT [b]) | 500 | 91.74 ± 0.62 |
| | | 100 | 85.11 ± 0.25 |
| | | 50 | 61.34 ± 0.06 |

[a] Standard mean error of three assays [b] Standard antioxidant

**Table: 4. Total antioxidant activity of volatile fractions of American and Pakistani almond by Phosphomolybdenum method (Absorbance at 695 nm).**

| S. No. | Sample | Concentration in Assay (µL/4mL) | Total Antioxidant Activity ± S.E.M.[a]) |
|---|---|---|---|
| 1 | American almond | 300 | 0.721 ± 0.01 |
| | | 200 | 0.621 ± 0.01 |
| | | 100 | 0.540 ± 0.03 |
| 2 | Pakistan almond | 300 | 0.850 ± 0.01 |
| | | 200 | 0.664 ± 0.02 |
| | | 100 | 0.556 ± 0.01 |
| 3 | BHT [b]) | 500 | 1.893 ± 0.01 |
| | | 100 | 1.760 ± 0.01 |
| | | 50 | 1.452 ± 0.01 |
| 4 | Blank | 75 | 0.102± 0.01 |

[a] Standard mean error of three assays [b] Standard antioxidant

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