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A significant by-product of the industrial processing of pistachio: Shell skin! (RP-HPLC analysis, antioxidant and enzyme inhibitory activities of the methanol extracts of *Pistacia vera* L. shell skins cultivated in Gaziantep-Turkey)

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Running Title Header: Phytochemistry and biological activity of pistachio skin

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Abstract

The aim of this study is to evaluate the antioxidant and enzyme inhibitory activities of the methanol extracts of immature and mature shell skins of *Pistacia vera* L. As well as the biological activity tests, phytochemical compositions of the extracts were also investigated. Total phenolic and flavonoid contents were determined in addition to the amounts of gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, caffeic acid, (-)-epicatechin, syringic acid, *p*-coumaric acid, hesperidin, quercetin, kaempferol, and apigenin. Immature shell skin was found rich in both phenolic and flavonoid compounds (52.29 mg gallic acid equivalent (GAEs) per g extract and 16.78 mg rutin equivalent (REs) per g extract, respectively). In immature shell skin, amounts of protocatechuic acid (4335 µg per g extract), *p*-hydroxybenzoic acid (12925 µg per g extract), *p*-coumaric acid (120 µg per g extract), quercetin (620 µg per g extract), and apigenin (190 µg per g extract) were higher than that of the mature one. In parallel to these findings, immature shell skin exhibited higher antioxidant activity in all test systems than that of the mature one. The samples did not show any inhibitory activity on butyrylcholinesterase and α -glucosidase. Mature shell skin exhibited considerable inhibitory activity on acetylcholinesterase (2.15 mg galantamine equivalent (GALAEs) per g extract). Tyrosinase inhibitory activity of the mature shell skin was also found as 3.14 mg kojic acid equivalent (KAEs) per g extract. Immature shell skin remained inactive on this enzyme. The samples also showed remarkable inhibitory activity on α -amylase.

Keywords: *Pistacia vera* L.; Shell skin; Antioxidant activity; Enzyme inhibitory activity; Phenolics; Flavonoids

1. Introduction

With the development of chromatography techniques, scientists have begun to focus on the isolation of biologically active secondary metabolites produced by plants, which are the major sources of bioactive substances. As well known, the prices of medical services and pharmaceutical preparations are very high and people all over the world do not benefit equally from these services ¹. Therefore, people try to meet their therapeutic requirements from the medicinal plants before getting a medical help.

Plants synthesize a wide variety of phenolic compounds as following: phenolic acids, flavonoids, vitamins, carotenoids, anthocyanins, alkaloids, etc. Particularly phenolic acids and flavonoids have remarkable biological abilities such as antioxidant, antimicrobial, antiviral, photo protective etc. ^{2, 3}. Plants and plant parts rich in natural antioxidants have been proven to have strong protective activity against the destructive effects of oxidative stress ⁴⁻⁶.

Pistachio (*Pistacia vera* L.), a member of the Anacardiaceae family, is native of aride zones of Central and west Asia and distributed throughout the Mediterranean basin ⁷. According to the geographical literature, Turkey is known as the gene center of pistachio. The main pistachio producers in the northern hemisphere are Iran, United States and Turkey. In Turkey, pistachio is cultivated mainly in Southeast Anatolian region (especially in Gaziantep, Şanlıurfa, Adıyaman, Kahramanmaraş, and Siirt locations). It is mainly cultivated in arid, rocky and sloping lands that are not conducive to the growth of other crops ^{8,9}.

According to the results of several studies, pistachio have been proven to have various groups of valuable phytochemicals such as anthocyanins, flavan-3-ols,

proanthocyanidins, flavonols, isoflavons, flavanones, stilbenes and phenolic acids ¹⁰. These phytochemicals have excellent biological activities ¹¹. For example; anthocyanins, which are among the main constituents of the shell skin of pistachio, have been shown to possess antioxidant, anti-inflammatory, anticarcinogenic ^{12, 13} and antiangiogenic activities ¹⁴. Catechins present in the same tissue of pistachio have been shown to decrease the oxidation of low-density lipoproteins (LDL) ¹⁵⁻¹⁷ and thus prevent cardiovascular diseases ¹⁸. In addition to these findings, isoflavones have been proven to act as partial agonists on estrogen receptors ¹⁹.

Approximately 60-70% of pistachio is consumed as nuts and the rest are used in dessert, cake, ice cream and confectionery industries worldwide. Pistachio cultivated in Turkey is especially preferred on world markets due to its aroma, color and taste. In Turkey, the average annual production volume of pistachio is approximately 100.000 tons. The average of 2500-3000 tons of pistachio produced in Turkey is exported annually and approximately one million dollars revenue is earned from this export ^{8,9}.

In Turkey, during the industrial processing of pistachio, approximately 3% of total production (an average of 3500-4000 tons annually) is arisen as waste product that is known as pistachio shell skin (or external skin) (Figures 1 and 2). This material can lead to environmental pollution when released into the environment in uncontrolled conditions. Due to its high content of phenolic compounds, shell skin of pistachio is likely to exhibit excellent biological activities and can be used as an alternative source of biologically active compounds ^{8,9}.

The aim of this study is to evaluate the antioxidant and enzyme inhibitory activities of the methanol extracts of immature and mature shell skins of *Pistacia vera* L. As well as the biological activity tests, phytochemical compositions of the extracts were

also investigated. Total phenolic and flavonoid contents were determined in addition to the amounts of gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, caffeic acid, (-)-epicatechin, syringic acid, *p*-coumaric acid, hesperidin, quercetin, kaempferol, and apigenin. By this study, we hope that pistachio shell skin, which is an important byproduct of pistachio industry in the world, can be used as an alternative source in pharmacology industry for developing and producing new and/or alternative therapeutic agents.

2. Materials and Methods

2.1. Chemicals

All the standard compounds including phenolics and other standards were supplied from Sigma-Aldrich and their purities were all over 97%.

2.2. Preparation of extracts

Samples (15 g) were macerated with 300 mL of methanol at room temperature for 24 h. Methanol was then removed with a rotary evaporator at 40 °C. The extracts were stored at +4 °C until analyzed. Yields of the methanol extracts from mature and immature shell skins of *P. vera* were determined as 36.11 and 12.23% (w/w), respectively.

2.3. Phytochemical analyses

2.3.1. Quantification of phenolic compounds by RP-HPLC

Phenolic compounds were evaluated by reversed-phase highperformance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Kyoto, Japan). Detection and quantification were carried out with a LC-10ADvp pump, a Diode Array Detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations

were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm x4.6 mm length, 5 µm particle size). The eluates were detected at 278 nm. The mobile phases were A: 3.0% acetic acid in distilled water and B: methanol. For analysis, the samples were dissolved in methanol, and 20 µL of this solution was injected into the column. The elution gradient applied at a flow rate of 0.8 mL/min was: 93%A/7%B in 0.1 min, 72%A/28%B in 20 min, 75%A/25%B in 8 min, 70%A/30%B in 7 min and same gradient for 15 min, 67%A/33%B in 10 min, 58%A/42%B in 2 min, 50%A/50%B in 8 min, 30%A/70%B in 3 min, 20%A/80%B in 2 min 100%B in 5 min until the end of the run. Phenolic compositions of the extracts were determined according to the method of Sarikurkcu, et al. ²⁰. Gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (-)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, luteolin, kaempferol and apigenin were used as standards. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as microgram per gram of extract using external calibration curves, which were obtained for each phenolic standard.

2.3.2. Total phenolic and flavonoid contents

Total phenolic and flavonoid contents of the samples were determined by employing the methods given in the literature ²¹.

2.4. Antioxidant activity

2.4.1. DPPH free radical scavenging activity

The effects of the samples on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated according to the procedure in literature ²².

2.4.2. Reducing power

The reducing power was investigated using cupric ion reducing (CUPRAC) ²³ and ferric reducing antioxidant power (FRAP) ²⁴ methods, as previously described in the literature.

2.4.3. Metal chelating activity on ferrous ions

Metal chelating activity on ferrous ions was evaluated by the method described by Aktumsek, et al. ²⁴.

2.4.4. Total antioxidant activity by phosphomolybdenum method

Total antioxidant activities of the samples were evaluated by phosphomolybdenum method ²¹.

2.5. Enzyme inhibitory activity

Enzyme inhibitory activities the samples were determined using cholinesterase (ChE), α -amylase, α -glucosidase and tyrosinase enzymes by employing the methods given in the literature ²¹.

2.6. Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviations (mean \pm SD). Statistical differences between the extracts were analyzed using Student *t*-test ($\alpha=0.01$). All the analyses were carried out by using SPSS v22.0 software.

3. Results and discussion

3.1. Phytochemical composition

Total phenolic and flavonoid compositions of the methanol extracts of immature and mature shell skins of *P. vera* were evaluated in order to see the correlation between biological activity potential and phytochemical composition (Table 1). Additionally,

amounts of twelve different phytochemicals were also determined by using RP-HPLC analysis (Table 1).

As can be seen from the table, methanol extract of the immature shell skin was found rich in both phenolic and flavonoid compounds (52.29 mg GAEs per g extract and 16.78 mg REs per g extract, respectively). It is quite interesting to point out that, amount of total flavonoids was found approximately five folds higher in immature shell skin extract than that of the mature one.

In addition to the qualitative analysis, amounts of Gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (-)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, luteolin, kaempferol and apigenin were also determined quantitatively by RP-HPLC analysis (Figures 3 and 4). In immature shell skin extract, amounts of protocatechuic acid (4335 µg per g extract), *p*-hydroxybenzoic acid (12925 µg per g extract), *p*-coumaric acid (120 µg per g extract), quercetin (620 µg per g extract), and apigenin (190 µg per g extract) were higher than that of the mature one (Table 1). Additionally, immature shell skin contained (+)-catechin and kaempferol at 1240 and 135 µg per g extract concentrations, respectively. However, these two compounds could not be detected in the mature shell skin. As we understood from this finding, some phytochemicals might be eliminated during the maturation process of tissue by turning into other compounds which are not biologically active as the compounds of immature tissue. On the other hand, gallic acid (13205 µg per g extract), caffeic acid (155 µg per g extract), (-)-epicatechin (980 µg per g extract), syringic acid (80 µg per g extract), and

hesperidin (120 µg per g extract) were found at higher concentrations in mature shell skin extract than that of the immature one.

As can be seen from the biological activity section of this paper, in general, immature extract exhibited higher biological activity potential than that of the mature one. Data obtained from the phytochemical analyses significantly supported the biological activity patterns of shell skin samples.

As far as our literature survey could ascertain, several studies have been carried out on the phenolic profile of the skin tissue of pistachio. But all these studies have mainly concerned on the internal skin ^{7, 25, 26}. Therefore, phenolic profile data presented for the both immature and mature shell skins (or external skin) of pistachio could be assumed as the first report for the literature.

3.2. Antioxidant activity

Antioxidant activities of the methanol extracts of immature and mature shell skins of *P. vera* were evaluated by five different test systems named as DPPH free radical scavenging, reducing power (CUPRAC and FRAP assays), metal chelating, and phosphomolybdenum assays.

Results obtained from DPPH free radical scavenging effect assay are presented in Table 2. According to data presented in the table, radical scavenging potential of the immature shell skin was found superior to that of the mature one (171.35 mg TEs per g extract). Results obtained from the shell skin samples were found different from the statistical point of view ($p < 0.01$).

DPPH free radical scavenging potential of the gums ²⁷ and seeds ²⁸ of *P. vera* have previously been reported elsewhere. None of these reports concentrated on the radical scavenging potential of the shell skin of pistachio.

Reducing power potentials of the methanol extracts of immature and mature shell skins of *P. vera* were screened by using CUPRAC and FRAP assays (Table 2). As can be seen from the table, immature shell skin extract showed higher reducing power than that of the mature shell skin extract. Activity values of the immature extract were found as 267.93 and 255.23 mg TEs per g extract in CUPRAC and FRAP systems, respectively. Ferric reducing antioxidant potential of the immature shell skin was found approximately two folds greater than that of the mature one in FRAP assay. Results of the shell skin samples obtained from these two test systems were found different from the statistical point of view ($p < 0.01$).

Metal chelating activities of the methanol extracts of the mature and immature shell skins of *P. vera* were also evaluated on ferrous ions (Table 2). Unlike the results of other test systems, both samples showed almost equal activities in this assay (16.10 and 17.54 mg EDTAEs per g extract, respectively). Metal chelating activities of the samples were found similar from the statistical point of view ($p > 0.01$).

Finally, antioxidant activities of the samples were also studied by using phosphomolybdenum assay (Table 2). As observed in the results of previous tests presented here, phosphomolybdenum assay was also resulted in the superiority of the methanol extract of immature shell skin of which antioxidant activity was measured as 2.31 mmol TEs per g extract. Results of the mature and immature shell skins were found different from the statistical point of view ($p < 0.01$).

Antioxidant activity of pistachio nuts have widely been studied by many research groups. According to Tomaino, et al. ⁷, pistachio nuts are among the rich sources of phenolic compounds and have recently been ranked among the first 50 food products highest in antioxidant potential. On the other hand, some researchers have especially

been concentrated on the internal skin of pistachio rather than the nuts or have compared skin with nut in terms of their phytochemical profile and/or biological activity potential since the internal skin has much more valuable phenolic compounds than that of the nuts ^{7, 25}. But, antioxidant activity of the shell skin (or external skin) of pistachio has not previously been reported. Therefore, data presented under this section could be assumed as the first report for the literature.

3.3. Enzyme inhibitory activity

Recently, the inhibition of key enzymes is considered as one of the most effective theory for the treatment in Alzheimer Diseases, Diabetes mellitus and skin disorders. In this direction, many synthetic inhibitors have been produced (galanthamine, acarbose or kojic acid), but they have adverse effects such as gastrointestinal disturbances and liver damage ^{29, 30}. Thus, there is an increasing interest in finding natural enzyme inhibitors from plant materials in order to replace synthetic ones. From this point, inhibitory activities of the methanol extracts of mature and immature shell skins of *P. vera* were evaluated on acetylcholinesterase, butyrylcholinesterase, α -amylase, α -glucosidase, and tyrosinase, respectively (Table 3).

As can be seen from the table, the samples did not show any inhibitory activity on butyrylcholinesterase and α -glucosidase. Mature shell skin of *P. vera* exhibited higher inhibitory activity on acetylcholinesterase than that of the immature one (2.15 mg GALAEs per g extract). Tyrosinase inhibitory activity of the mature shell skin was also found as 3.14 mg KAEs per g extract. However, immature shell skin remained inactive on this enzyme.

The samples also showed inhibitory activity on α -amylase. According to data presented in the Table 3, inhibitory activities of the mature and immature shell skins of *P. vera* were found as 3.72 and 4.91 mg ACEs per g extract, respectively.

As far as our literature survey could ascertain, butyrylcholinesterase, α -amylase, α -glucosidase, and tyrosinase inhibitory activities of *P. vera* has not previously been reported elsewhere. Therefore, data presented on these enzymes could be assumed as the first report for the literature.

On the other hand, acetylcholinesterase inhibitory activity of *P. vera* hydrolysates obtained by gastrointestinal enzymes have been studied by Li, et al. ³¹. According to this study, *in vitro* acetylcholinesterase inhibitory activities of the hydrolysates prepared by pepsin and tyripsin digestion were measured as 0.87 mg per ml (IC₅₀). As can be seen from the details of above-mentioned paper, target sample is the nuts of *P. vera* and the shell skin was left out of the scope. Therefore, current study can also be accepted as the first report on the acetylcholinesterase inhibitory activity of the shell skin of *P. vera*.

4. Conclusions

As can be seen from the results presented here, in general, immature shell skin of pistachio exhibited greater activity than that of the mature one. This finding is also supported with the results of both qualitative and quantitative chromatographic analyses and statistical outputs. Moreover, it could be assumed that, some other phytochemicals, which are not detected chromatographically, are likely to contribute the biological activity of this material. Pistachio nuts having immature shell skin, in particular, are used to produce the world-famous “baklava”, which is a kind of sweet pastry with pistachio nuts. According to the results of our experiments, immature shell

skin of pistachio arisen from the industrial processing of pistachio nuts exhibited remarkable antioxidant and enzyme inhibitory activity and therefore can be considered as a new source of valuable secondary metabolites instead of releasing environment in uncontrolled conditions.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. S. Tagboto and S. Townson, *Advances in Parasitology*, 2001, 50, 199-295.
2. M. P. Kahkonen, A. I. Hopia, H. J. Vuorela, J. P. Rauha, K. Pihlaja, T. S. Kujala and M. Heinonen, *J Agr Food Chem*, 1999, 47, 3954-3962.
3. E. J. Lien, S. J. Ren, H. Y. H. Bui and R. B. Wang, *Free Radical Bio Med*, 1999, 26, 285-294.
4. A. M. Faure, J. Werder and L. Nystrom, *Food Chem*, 2013, 141, 589-596.
5. A. J. Javan and M. J. Javan, *Food Chem*, 2014, 165, 451-459.
6. S. Keser, *Nat Prod Res*, 2014, 28, 1789-1794.
7. A. Tomaino, M. Martorana, T. Arcoraci, D. Monteleone, C. Giovino and A. Saija, *Biochimie*, 2010, 92, 1115-1122.
8. Gaziantep Chamber of Commerce, 2013. Report of the development of pistachio industry: analysis of current situation and development strategies.
URL:<http://www.ika.org.tr/upload/yazilar/TRC112DFD2024-Antepfisiği-Sektorunun-Gelistirilmesi-Projesi-552654.pdf>
9. Pistachio Report, 2011. Ministry of Customs and Trade of Turkey.
URL:<http://koopgtb.gov.tr/data/53319b03487c8eb1e43d728e/2011%20Yılı%20Antep%Fıstığı%20Raporu>
10. F. Lau, B. Shukitt-Hale and J. Joseph, *Nutrition*, 2006, 22, 295-302.
11. A. Agouni, A. H. Lagrue-Lak-Hal, H. A. Mostefai, A. Tesse, P. Mulder, P. Rouet, F. Desmoulin, C. Heymes, M. C. Martinez and R. Andriantsitohaina, *PloS one*, 2009, 4, e5557.
12. D.-X. Hou, T. Yanagita, T. Uto, S. Masuzaki and M. Fujii, *Biochemical Pharmacology*, 2005, 70, 417-425.
13. G. J. Mazza, *Annali dell'Istituto superiore di sanita*, 2007, 43, 369-374.
14. L. S. Wang and G. D. Stoner, *Cancer Lett*, 2008, 269, 281-290.
15. K. J. Anderson, S. S. Teuber, A. Gobeille, P. Cremin, A. L. Waterhouse and F. M. Steinberg, *J Nutr*, 2001, 131, 2837-2842.
16. C. Y. Chen, P. E. Milbury, K. Lapsley and J. B. Blumberg, *J Nutr*, 2005, 135, 1366-1373.
17. A. Basu and E. A. Lucas, *Nutr Rev*, 2007, 65, 361-375.
18. P. Velayutham, A. Babu and D. M. Liu, *Curr Med Chem*, 2008, 15, 1840-1850.

19. S. Basaria, A. Wisniewski, K. Dupree, T. Bruno, M. Y. Song, F. Yao, A. Ojumu, M. John and A. S. Dobs, *J Endocrinol Invest*, 2009, 32, 150-155.
20. C. Sarikurkcu, M. C. Uren, B. Tepe, M. Cengiz and M. S. Kocak, *Ind Crop Prod*, 2014, 62, 333-340.
21. G. Zengin, C. Sarikurkcu, A. Aktumsek, R. Ceylan and O. Ceylan, *Ind Crop Prod*, 2014, 53, 244-251.
22. C. Sarikurkcu, *Afr J Biotechnol*, 2011, 10, 831-839.
23. R. Apak, K. Guclu, M. Ozyurek, S. E. Karademir and E. Ercag, *Int J Food Sci Nutr*, 2006, 57, 292-304.
24. A. Aktumsek, G. Zengin, G. O. Guler, Y. S. Cakmak and A. Duran, *Food Chem Toxicol*, 2013, 55, 290-296.
25. M. Martorana, T. Arcoraci, L. Rizza, M. Cristani, F. P. Bonina, A. Saija, D. Trombetta and A. Tomaino, *Fitoterapia*, 2013, 85, 41-48.
26. N. P. Seeram, Y. J. Zhang, S. M. Henning, R. Lee, Y. T. Niu, G. Lin and D. Heber, *J Agr Food Chem*, 2006, 54, 7036-7040.
27. M. H. Sehitoglu, H. Han, P. Kalin, I. Gulcin, A. Ozkan and H. Y. Aboul-Enein, *J Enzym Inhib Med Ch*, 2015, 30, 264-269.
28. F. G. Kirbaslar, G. Turker, Z. Ozsoy-Gunes, M. Unal, B. Dulger, E. Ertas and B. Kizilkaya, *Rec Nat Prod*, 2012, 6, 339-349.
29. C. L. Burnett, W. F. Bergfeld, D. V. Belsito, R. A. Hill, C. D. Klaassen, D. C. Liebler, J. G. Marks, R. C. Shank, T. J. Slaga, P. W. Snyder and F. A. Andersen, *Int J Toxicol*, 2010, 29, 244s-273s.
30. Y. I. Kwon, E. Apostolidis and K. Shetty, *Bioresource Technol*, 2008, 99, 2981-2988.
31. P. Li, J. Jia, M. Fang, L. Zhang, M. Guo, J. Xie, Y. Xia, L. Zhou and D. Wei, *Process Biochemistry*, 2014, 49, 898-904.

Table 1

Phenolic components, total phenolics and total flavonoids of the mature and immature shell skins of *P. vera* (mean \pm SD)* and analytical characteristics used for determination of phenolics.

No	Retention time (min)	Phenolic components	Concentration (μg per g extract)		Analytical characteristics			
			Mature shell skin	Immature shell skin	Linear range (mg per L)	R ²	LOD (mg per L)**	LOQ (mg per L)**
1	5.2	Gallic acid	13205 \pm 396 ^a	9115 \pm 403 ^b	0.20-25.0	0.9993	0.075	0.227
2	8.7	Protocatechuic acid	1000 \pm 35 ^b	4335 \pm 375 ^a	0.20-25.0	0.9991	0.086	0.260
3	12.3	(+)-Catechin	nd****	1240 \pm 49	0.90-113	0.9988	0.172	0.522
4	13.5	<i>p</i> -Hydroxybenzoic acid	7400 \pm 212 ^b	12925 \pm 247 ^a	0.20-25.0	0.9994	0.007	0.020
5	15.1	Chlorogenic acid	nd	nd	0.35-45.0	0.9988	0.080	0.241
6	17.6	Caffeic acid	155 \pm 11 ^a	125 \pm 11 ^a	0.16-21.0	0.9993	0.054	0.162
7	19.1	(-)-Epicatechin	980 \pm 42 ^a	630 \pm 42 ^a	0.50-66.0	0.9990	0.170	0.514
8	19.9	Syringic acid	80 \pm 1 ^a	70 \pm 1 ^b	0.05-12.0	0.9995	0.030	0.090
9	20.8	Vanillin	nd	nd	0.08-10.0	0.9995	0.020	0.060
10	24.5	<i>p</i> -Coumaric acid	70 \pm 4 ^b	120 \pm 5 ^a	0.04-6.0	0.9996	0.066	0.199
11	27.8	Ferulic acid	nd	nd	0.12-17.0	0.9993	0.004	0.011
12	29.2	Sinapic acid	nd	nd	0.12-17.0	0.9993	0.017	0.053
13	33.8	Benzoic acid	nd	nd	0.85-55.0	0.9998	0.111	0.335
14	39.4	<i>o</i> -Coumaric acid	nd	nd	0.24-32.0	0.9988	0.023	0.069
15	44.1	Rutin	nd	nd	0.40-56.0	0.9989	1.113	3.373
16	49.7	Hesperidin	120 \pm 3 ^a	95 \pm 3 ^a	0.43-55.0	0.9992	1.080	3.280
17	54.9	Rosmarinic acid	nd	nd	0.02-7.0	0.9998	0.148	0.447
18	57.3	Eriodictyol	nd	nd	0.33-21.0	0.9998	0.140	0.410
19	65.9	<i>trans</i> -Cinnamic acid	nd	nd	0.02-7.0	0.9998	0.148	0.447
20	71.4	Quercetin	255 \pm 11 ^b	620 \pm 11 ^a	0.40-55.0	0.9999	0.013	0.040
21	74.3	Luteolin	nd	nd	0.13-17.0	0.9999	0.020	0.060
22	76.8	Kaempferol	nd	135 \pm 11	0.05-15.0	0.9996	0.021	0.062
23	77.2	Apigenin	145 \pm 5 ^a	190 \pm 5 ^a	0.17-11.0	0.9997	0.034	0.104
Total phenolics (mg GAEs per g extract)*****			33.65 \pm 1.75 ^b	52.29 \pm 0.84 ^a				
Total flavonoids (mg REs per g extract)*****			3.59 \pm 0.07 ^b	16.78 \pm 0.10 ^a				

* Data marked with different subscripts within the same row indicate significant difference statistically ($p < 0.01$).

** LOD, limit of detection;

*** LOQ, limit of quantification;

**** nd, not detected;

*****GAEs, gallic acid equivalents;

*****REs, rutin equivalents

Table 2

Radical scavenging (DPPH), reducing power (CUPRAC and FRAP), metal chelating and antioxidant (by phosphomolybdenum method) activities of the mature and immature shell skins of *P. vera* (mean \pm SD)*

Assays	Mature shell skin	Immature shell skin
DPPH (mg TEs per g extract)**	137.16 \pm 3.50 ^b	171.35 \pm 6.60 ^a
CUPRAC (mg TEs per g extract)**	175.58 \pm 1.91 ^b	267.93 \pm 13.81 ^a
FRAP (mg TEs per g extract)**	140.08 \pm 15.35 ^b	255.23 \pm 7.39 ^a
Chelating effect (mg EDTAEs per g extract)***	16.10 \pm 0.70 ^a	17.54 \pm 0.30 ^a
Phosphomolybdenum (mmol TEs per g extract)**	1.35 \pm 0.07 ^b	2.31 \pm 0.01 ^a

* Data marked with different subscripts within the same row indicate significant difference statistically ($p < 0.01$).

** TEs, trolox equivalents.

*** EDTAEs, disodium edetate equivalents.

Table 3Enzyme inhibitory activities of the mature and immature shell skins of *P. vera* (mean \pm SD)*

Assays	Mature shell skin	Immature shell skin
Acetyl cholinesterase (mg GALAEs per g extract)**	2.15 \pm 0.05 ^a	1.49 \pm 0.03 ^b
Butyryl cholinesterase (mg GALAEs per g extract)**	na	na
α -Amylase (mg ACEs per g extract)***	3.72 \pm 0.15 ^b	4.91 \pm 0.15 ^a
α -Glucosidase (mg ACEs per g extract)***	na****	na
Tyrosinase (mg KAEs per g extract)****	31.14 \pm 2.33	na

* Data marked with different subscripts within the same row indicate significant difference statistically ($p < 0.01$).

** GALAEs, galanthamine equivalents.

*** ACEs, acarbose equivalents.

**** KAEs, kojic acid equivalents.

***** na, not active



Figure 1. External and internal skins of *P. vera*

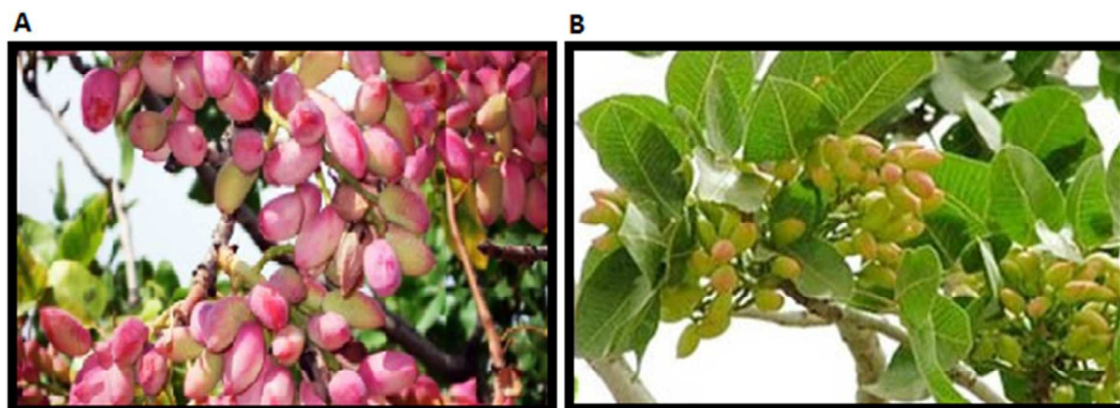


Figure 2. Mature (A-pink colored) and immature (B-green colored) shell skins of *P. vera*

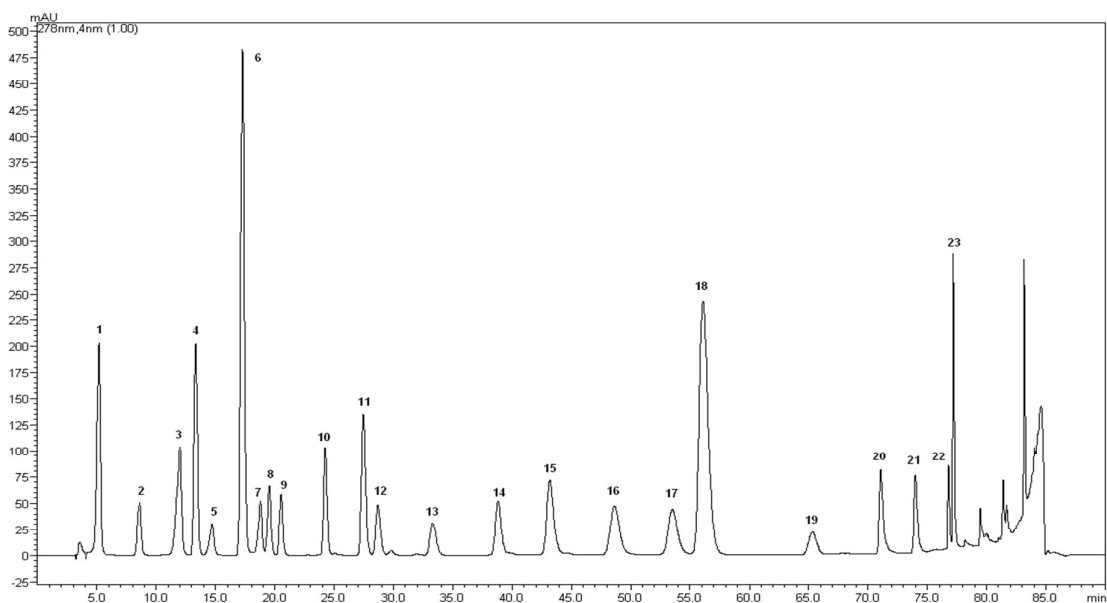


Figure 3. Chromatographic profile of the chemical standards (1. Gallic acid, 2. Protocatechuic acid, 3. (+)-Catechin, 4. *p*-Hydroxybenzoic acid, 5. Chlorogenic acid, 6. Caffeic acid, 7. (-)-Epicatechin, 8. Syringic acid, 9. Vanillin, 10. *p*-Coumaric acid, 11. Ferulic acid, 12. Sinapinic acid, 13. Benzoic acid, 14. *o*-Coumaric acid, 15. Rutin, 16. Hesperidin, 17. Rosmarinic acid, 18. Eriodictyol, 19. *trans*-Cinnamic acid, 20. Quercetin, 21. Luteolin, 22. Kaempferol, 23. Apigenin)

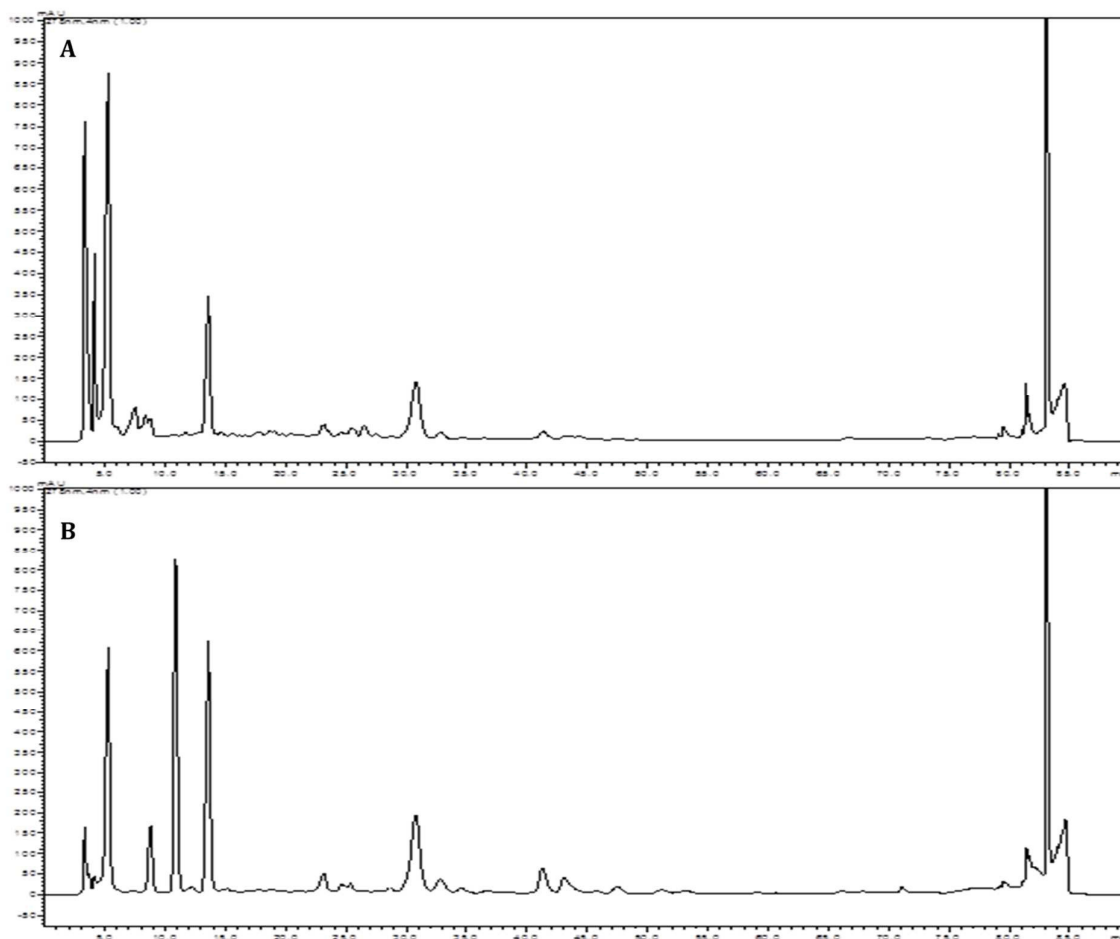


Figure 4. HPLC chromatograms of the methanol extracts of the mature (A) and immature (B) shell skins of *P. vera*.