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## ANTIOXIDANT ACTIVITY OF *PISTACIA KHINJUK* SUPPORTED BY PHYTOCHEMICAL INVESTIGATION

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**Abstract:** *Pistacia khinjuk* is one of the fifteen known species of *Pistacia* belonging to Anacardiaceae family. Keeping in view the possible therapeutic utility of this genus and the lack of literature on this plant, this study involves phytochemical investigation of *P. khinjuk* and its antioxidant activity. The phytochemical investigation was conducted on crude methanolic extract and its fractions namely, n-hexane, chloroform, n-butanol, ethyl acetate and aqueous. Total phenolic contents and flavonoids were also determined by phosphomolybdenum and ferric thiocyanate method in crude extract and its fractions. The results of phytochemical investigation indicated the presence of alkaloids, tannins, flavonoids, saponins, triterpenoids, cardiac glycosides, carbohydrates, proteins and sterols in the crude extract of *P. khinjuk*. Crude extract and its fractions exhibited remarkable antioxidant activity. This study showed that the crude extract and its fractions have potent antioxidant activity, among all ethyl acetate showed  $1.109 \pm 0.029$  the highest activity. This research concluded that crude extract of *P. khinjuk* and its fractions contained phenolic and flavonoid compounds that show significant antioxidant activity.

**Keywords:** *Pistacia khinjuk*, antioxidant, phenols, flavonoid

Medicinal plants always exhibited a potential role in the development of different drugs. Many drugs of known therapeutic effects have been obtained from different plant species. Anacardiaceae family consisted of around 800 species with almost 82 known genera. *P. khinjuk*, a potential plant of genus *Pistacia* exhibited remarkable medicinal potential in folkloric tales. It is well known with different names, like Mastic, Mastix, Ushgai, Gulgnoor, Bzuzgai, Baneh, Guan, Kelkhong and Gazwan, representing diversity of the plant in different regions (1). Its natural habitat is Egypt, Syria, Turkey, Iraq, Iran, Afghanistan and Pakistan. Pakistan, being a fertile land, facilitated the growth of such species, a major population found in Balochistan, Khyber, Chitral and Gilgit. The trees of *Pistacia khinjuk* are smaller than the other species found in Pakistan. The trees are different from *Pistacia atlantica* due to fewer leaves which are larger in size and lathery. Fruit clusters are broader and up to 14–22 cm long. They grow on

a lower branch which adds more breadth to the appearance. The fruits showed color tones of blue and black. The seeds are yellowish and ripe between August and September. However, at altitudes around 2000 m, the time extends to October. The nuts of this species are too small and therefore mostly used as rootstock for a better edible variety *Pistacia vera*.

It has been considered as an edible species and being used by the people of Balochistan province (1). Flavonoids and glycosides fractions were extracted and purified by Kawashty (2). A unique flavonoid composition has been exhibited by *P. khinjuk*, which was attributed to myricetin-3-glucoside, myricetin-3-galactoside and myricetin-3-rutinoside. Several flavonoids including gallic acid, methyl gallate, myricetin-3-O- $\beta$ -D-galactopyranoside (hyperin), myricetin-3-O-rhamnoside (myricitrin), 1,6-digalloyl- $\beta$ -D-glucose, 1,4-digalloyl- $\beta$ -D-glucopyranoside, and 2,3-di-O-galloyl-( $\alpha/\beta$ )-4C1-glucopyranose (nilocitin) were isolated

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from aqueous methanolic extracts of *P. khinjuk* (3). Antimicrobial activity revealed the presence of essential oils such as  $\alpha$ -pinene,  $\beta$ -pinene, myrcene,  $\beta$ -caryophyllene, garmacrene B and spathulenol showing antimicrobial activity against bacteria and fungi (4). Resin extracted from plant has been used to treat indigestion and toothache. Folkloric tales of Bakhtiari confirmed the use of *P. khinjuk* as tonic and astringent (5). Resin of the plant known as "Gulgul" exhibited anti-inflammatory activity, which is being used by the natives of Balchistan for the treatment of eye infections. Galls have been reported to exhibit several biological activities. The folkloric usage suggested potential antibacterial, anti-inflammatory and antioxidant activity. The antioxidant and antimicrobial activity in the genus of *Pistacia* showed comparable efficacy to antibiotics. The *in vivo* experiments revealed potential of methanolic extract of *P. khinjuk* to be used as a wound healer (6).

The study aimed at the determination and validation of ash values, phytochemical investigation, phenolic and flavonoid content estimation and evaluation of antioxidant activity through different methods (phosphomolybdenum and ferric thiocyanate).

## MATERIALS AND METHODS

### Collection and identification of plant material

Fresh plant material of *P. khinjuk* was collected from hilly areas of Quetta in July 2012. The plant material was authenticated and the voucher specimen No. GC.HERB.BOT.1910 was deposited in the Herbarium of The Department of Botany, Government College University, Lahore. Plant was dried in the shade at room temperature. The dried plant material was pulverized to a fine powder in a special herbal grinder with temperature not exceed-

ing 40°C. Plant powder was then stored in an air tight container.

### Methods

One kg powdered *P. khinjuk* was macerated thrice with 5 L of methanol in round bottom flask for 15 days. Filtered through Whatman filter paper, the filtrate was evaporated in rotary evaporator to obtain the crude methanolic extract (105.68 g). Fractionation process was done by dissolving methanolic extract (75 g) in 250 mL of distilled water. The solvents used for fractionation were n-hexane, chloroform, ethyl acetate, n-butanol and water. After fractionation, the amount of extracts obtained were n-hexane 16.11 g, chloroform 15.17 g, ethyl acetate 17.36 g, n-butanol 12.35 g and aqueous fraction 14.01 g. These fractions were subjected to determination of phytoconstituents, total phenolic content, flavonoid content and *in vitro* antioxidant activity.

### Determination of total phenolic contents in the plant extracts

The concentration of phenolics in plant extracts was determined using spectrophotometric method according to the method of Singleton (7). Methanolic solution of the extract/fractions in the concentration of 1 mg/mL was used for the analysis. The reaction mixture was made by mixing 1 mL of methanolic solution of extract/fractions and 9 mL of distilled water. One mL of Folin-Ciocalteu's reagent was then added and the mixture was shaken. After 5 min, 10 mL of 7% Na<sub>2</sub>CO<sub>3</sub> was added and the resulting solution was diluted to 25 mL with distilled water and mixed. Distilled water was used for the preparation of blank solution. Standard solutions of gallic acid were prepared in the concentration range of 10, 20, 40, 80, 100 and 120  $\mu$ g/mL to draw a calibration curve. The samples, blank and standard solutions were incubated

Table 1. Absorbance of gallic acid and quercetin standards at  $\lambda_{\text{max}} = 750$  nm and  $\lambda_{\text{max}} = 415$  nm, respectively.

Gallic acid and quercetin conc. ( $\mu$ g / mL)	Absorbance of gallic acid	Absorbance of quercetin
10	0.082 $\pm$ 0.006	0.022 $\pm$ 0.002
20	0.122 $\pm$ 0.003	0.047 $\pm$ 0.001
40	0.259 $\pm$ 0.002	0.098 $\pm$ 0.002
80	0.537 $\pm$ 0.002	0.227 $\pm$ 0.002
100	0.668 $\pm$ 0.001	0.365 $\pm$ 0.002

Each value is calculated by taking the means  $\pm$  S.D. of 3 absorbances

Table 2. Total phenolic content, flavonoid contents, total antioxidant activity and lipid peroxidation inhibition values of the crude methanolic extracts and its fractions of *Pistacia khinjuk*.

Extract / fraction	Phenolic content (mg GA /g)	Flavonoid content (mg QE/g)	Total antioxidant activity	Inhibition of lipid peroxidation (%)
Crude methanol extract	260.741 ± 1.322	191.305 ± 0.976	0.754 ± 0.006	49.680 ± 0.445
<i>n</i> -Hexane fraction	43.537 ± 0.599	53.885 ± 1.688	0.38 ± 0.035	12.670 ± 0.854
Chloroform fraction	94.667 ± 1.528	84.674 ± 5.100	0.40 ± 0.005	20.823 ± 0.211
Aqueous fraction	255.648 ± 1.083	182.810 ± 1.556	0.523 ± 0.008	45.564 ± 1.293
BHT	---	---	1.202 ± 0.002	62.870 ± 0.211

Each value is expressed as the means ± S.D. (n = 3) of 3 separate determinations.

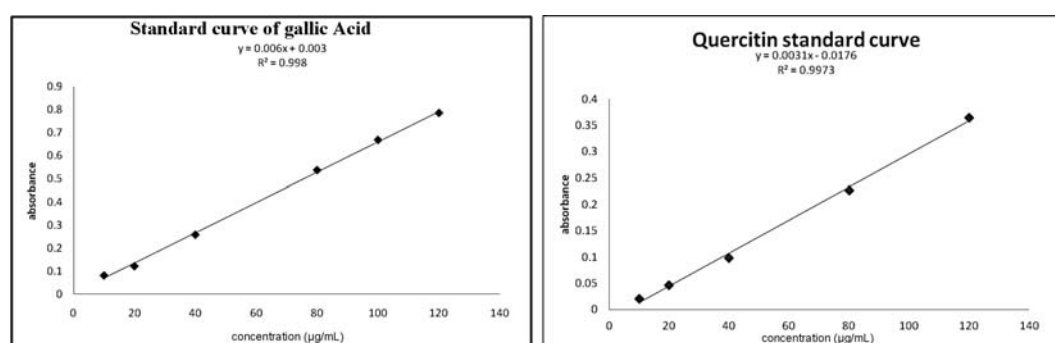


Figure 1. Calibration curve of gallic acid and quercetin (standard)

at room temperature for 90 min. The absorbance value was determined by using a spectrophotometer at  $\lambda_{\text{max}} = 750$  nm. All the experiment was conducted in triplicate. The concentration of phenolics was read ( $\mu\text{g/mL}$ ) from the calibration curve and the content of phenolics in extract/fractions was expressed in terms of gallic acid equivalent (mg of GA/g of extract). The standard curve of gallic acid is shown in Figure 1 and the results are given in Table 1.

#### Determination of flavonoid concentrations in the plant extracts

Spectrophotometric method was used to determine flavonoids concentration of plant extract (8). The sample contained 1 mL of methanol solution of the extract/fractions in the concentration of 1 mg/mL, quercetin was used as standard in the concentration of 1 mg/mL. It was further diluted to 10, 20, 40, 80, and 120  $\mu\text{g/mL}$  to draw a calibration curve. The diluted solution and the samples (200  $\mu\text{L}$ ) were taken in a test tube with micro pipette and mixed with aluminium chloride (100  $\mu\text{L}$ ), 1 M potassium acetate (100  $\mu\text{L}$ ) and 4.6 mL of water. The samples were left for 45 min at room temperature. The absorbance was determined using spec-

trophotometer at  $\lambda_{\text{max}} = 415$  nm. All the experiments were conducted in triplicate. The concentration of flavonoids were observed ( $\mu\text{g/mL}$ ) from the calibration curve. The content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QE/g of extract). The standard curve of quercetin is given in Figure 1 and results are given in Table 1.

#### Antioxidant activity

##### Phosphomolybdenum method

The total antioxidant activities of fractions of plant were calculated by phosphomolybdenum complex formation method (9). Five hundred  $\mu\text{g/mL}$  of each crude methanolic extract and its fractions were mixed with 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. Blank solution contained 4 mL of reagent solution. The vials were capped and were left in water bath at 95°C for 90 min. After getting the samples cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was measured relative to that of butylated hydroxytoluene (BHT). All determinations were assayed in triplicate and mean values were calculated. The results are given in Table 2.

### *Ferric thiocyanate (FTC) assay*

The antioxidant activities of the different fractions of the plant on the inhibition of linoleic acid peroxidation were assayed by using thiocyanate method (10). One tenth mL of each of sample solutions (0.5 mg / mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.02 M, pH 7.0). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween 20 as emulsifier and 50.0 mL of phosphate buffer (0.02 M, pH 7.0). The reaction mixture was kept for 5 days at 40°C. The mixture without extract was used as the control measure. The 0.1 mL aliquot of the mixture was taken and mixed with 5.0 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid and allowed to stand at room temperature. After 3 min, the addition of ferrous chloride to the reaction mixture was performed. The absorbance was recorded at 500 nm. The antioxidant activity was expressed as:

$$\text{Inhibition of lipid peroxidation (\%)} = \frac{[1 - (A \text{ sample}) / (A \text{ control})] \times 100}{\text{where } A = \text{absorbance}}$$

where A = absorbance

The antioxidant activity of BHT as reference standard was assayed for comparison. The results are given in Table 2.

### Statistical analysis

The data statistically analyzed using SPSS version 20. Student's t-test and one way analysis of variance (ANOVA) followed by *post-hoc* Tukey's

test were employed as appropriate. All the data were expressed as the mean  $\pm$  SEM.

## RESULTS AND DISCUSSION

Flavonoids and phenolic contents, known as secondary metabolites are considered important since they are involved in performing various defense functions in plant. However, light, humidity and temperature contributed to the synthesis of these compounds. It has been reported that phenolic compounds show antioxidant activity and play a crucial role in its variation (11). Since phenolic compounds inhibit free radicals, therefore they showed antioxidant activity. They are also found to have prevention capability of decomposing hydroperoxides into free radicals.

In this study, the phenolic and flavonoid concentrations have been identified in crude methanolic extract and other fractions and their relationship with antioxidant activity has been reported. Table 2 showed phenolic content concentration in the crude methanolic extract and fractions expressed as milligrams of gallic acid equivalents per gram of dry weight. The methanol fraction showed maximum concentration of phenolic content ( $260.741 \pm 1.32$  mg/g). It was followed by aqueous extract. Chloroform soluble fraction (and n-hexane soluble fraction contained  $94.667 \pm 1.53$  mg/g and  $43.537 \pm 0.59$  mg/g, respectively.

Table 2 also reports flavonoids concentration in the crude methanolic extract and fractions

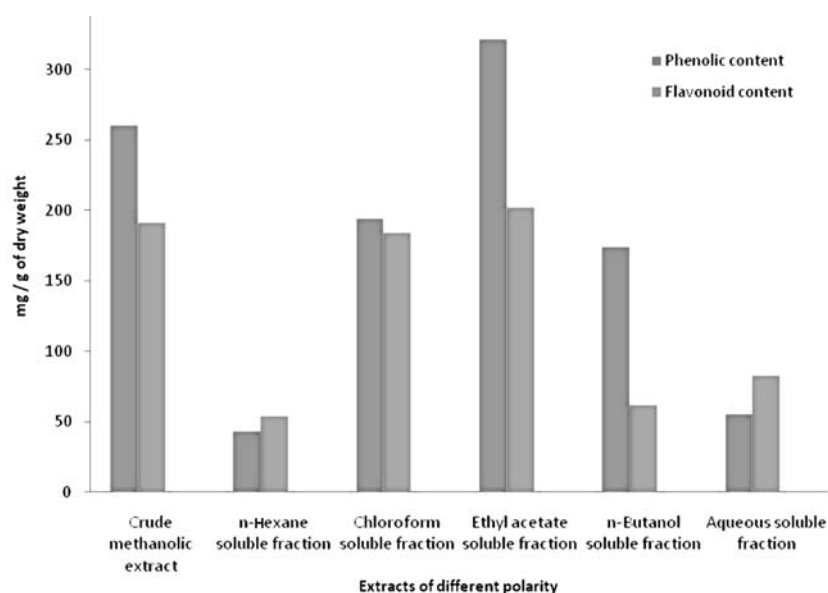


Figure 2. Comparison of phenolic and flavonoid content of the crude methanolic extracts and its fractions of *Pistacia khinjuk*

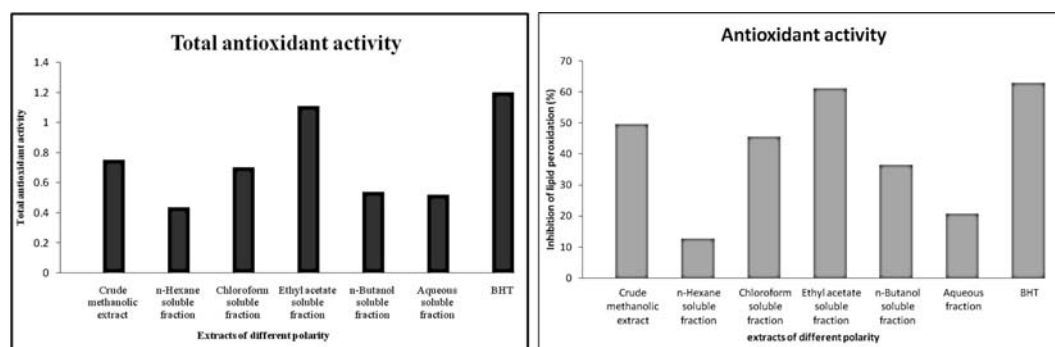


Figure 3. Total antioxidant activity and lipid peroxidation inhibition in the crude methanolic extracts and its fractions of *Pistacia khinjuk* as compared with standard BHT

expressed as milligrams of quercetin equivalents per gram of dry weight. The methanol fraction showed maximum concentration of flavonoid content ( $191.3 \pm 0.98$  mg/g). It was followed by aqueous extract. Chloroform soluble fraction and n-hexane soluble fraction contained  $84.67 \pm 5.1$  mg/g and  $53.89 \pm 1.69$  mg/g, respectively.

A study on the antioxidant activity of flavonoids by Hernández (12) stated that flavonoids are considered mainly for their health promoting activity, they outperform vitamin C and vitamin E in terms of antioxidant activity because they have a stronger capacity to donate electrons.

Rice Evans suggested that for the role of flavonoids as antioxidants, it is imperative to understand the mechanics of their chemical absorption and this should be done by drawing inferences from the relationship of *in vivo* and *in vitro* experimentation (13). This will help in predicting how flavonoids will cross blood-brain barrier and target tissues. Flavonoids have shown to exert antioxidant activity *in vitro* but their complete function is not clarified using *in vivo* experiments. They can be powerful enzyme inhibitors and inducers, can play a desirable role in cell division and possess antioxidant and anti-inflammatory activities. Flavonoids are also studied as contributors to redox regulation parallel to their antioxidant activity; they are capable for reducing the cell ageing process by coupling with intracellular reductant network. It yet remains to establish the factors that influence the absorption of flavonoids in intestines (13). The antioxidant activity of ethanolic extract of *Pistacia khinjuk* has been reported by Shojaei (14) using DPPH method but this study has focused on the antioxidant activity of methanolic extract of *Pistacia khinjuk* and its fractions using phosphomolybdenum and ferric thiocyanate (FTC) methods which further validate the claim made by Shojaei.

Phosphomolybdenum method was used to determine the total antioxidant activity of the studied fractions. In this method a reduction of molybdenum VI to molybdenum V takes place by antioxidants and green phosphate Mo (V) complex forms at acidic pH. The structure of the antioxidant determines electron transfer. The total antioxidant activities of these fractions were compared with the standard antioxidant BHT and the results are shown in Table 2.

The results indicated that methanolic fraction had the highest total antioxidant activity ( $0.754 \pm 0.006$ ). The aqueous extract showed the antioxidant activity after the methanolic fraction. The antioxidant activity of the various plant fractions was decreased in the following order: methanolic fraction > aqueous fraction ( $0.523 \pm 0.008$ ) chloroform soluble fraction ( $0.40 \pm 0.005$ ) > n-hexane soluble fraction ( $0.38 \pm 0.035$ ). BHT showed total antioxidant activity  $1.202 \pm 0.002$ .

Antioxidant activity of these fractions by using the ferric thiocyanate (FTC) method have also been investigated. Peroxidation of lipids occurs both *in vivo* and *in vitro* and causes cytotoxic and reactive product outcomes. These products are known to affect the cell and can give rise to damaged or modified DNA. The FTC method is used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate, a reddish pigment (15).

It was observed that the absorbance value was lower than for negative controls (without plant extracts) in all fractions in the end, which means the presence of antioxidant activity. Significantly lower absorbance as compared to control was observed, which indicate that these fractions have greater

antioxidant activities. The fractions which showed greater values of percent inhibition of lipid peroxidation might contain primary antioxidant compounds, which are able to react aggressively with free radicals, particularly hydroxyl radicals, thereby terminating the radical-chained reaction and retarding the formation of hydroperoxides (16)

The highest percentage of inhibition of lipid peroxidation was exhibited by methanolic fraction ( $49.68\% \pm 0.445$ ), the crude aqueous extract showed ( $45.564 \pm 1.293$ ), Chloroform soluble fraction showed  $20.823 \pm 0.211$ , while *n*-hexane soluble fraction had the lowest percentage of inhibition of lipid peroxidation ( $12.67\% \pm 0.85$ ). The inhibition of lipid peroxidation by BHT (standard) was  $62.87\% \pm 0.21$ .

The importance of the antioxidant activity of *Pistacia khinjuk* cannot be ignored as a remedy for various diseases caused by free radical formation. Free radicals are formed as a consequence of weak chemical bonding as the number of electrons required to keep an atom stable changes (17). The resultant molecules that lose an electron become free radicals and as a chain reaction they cause instability in other molecules eventually damaging the cell. Studies have shown that the free radicals can also damage the DNA of a cell. The process logically causes an oxidative stress that becomes an onset for various disorders such as Parkinson's disease, Alzheimer's disease and cardiac malfunctioning including heart failure and myocardial infarction (18). Given the natural chemical orientation of the body, free radicals are naturally handled but their excess creates a problem for which antioxidants become a necessary remedy since they neutralize free radicals by donating electrons. It also showed notable wound healing capacity and this was probably due to the presence of flavonoids which are noteworthy for their scavenging activity against free radicals (19). In a study conducted by Shojaei (14) on the antioxidant and antimicrobial activity of ethanolic extract of *P. khinjuk*, the plant showed antioxidant activity determined by DPPH radical scavenging activity.

## CONCLUSION

It is concluded from the results that *Pistacia khinjuk* showed flavonoid and phenol contents

which are responsible for its antioxidant activity. So this plant seems to be a potential candidate for further study by pharmaceutical industry since it showed antioxidant activity which can be compared to other species of *Pistacia* and various available drugs that promise health benefits.

## REFERENCES

1. Al-Saghir M.G., Porter D.M.: Am. J. Plant Sci. 3, 12 (2012).
2. Kwashty S.A.: Biochem. Syst. Ecol. 28, 915 (2000).
3. Esmat A., Al-Abbasi F.A., Algandaby, M.M., Moussa A.Y., Labib R.M. et al.: J. Med. Food 15, 278 (2012).
4. Taran M., Sharifi M., Azizi E., Khan A.M.: J. Med. Plants Res. 9, 81 (2010).
5. Ghasemi A., Aghae K.: Elect. J. Biol. 7, 67 (2011).
6. Tohidi M., Khayami M., Nejati V., Meftahizade H.: J. Med. Plants Res. 5, 4310 (2011).
7. Singleton V.L., Rossi J.A.: Am. J. Enol. Vitic. 16, 144 (1965).
8. Chang C.C., Yang M.H., Wen H.M., Chern J.C.: J. Food Drug Anal. 10, 178 (2002).
9. Prieto P., Pineda M., Aguilar M.: Anal. Biochem. 269, 337 (1999).
10. Valentao P., Fernandes E., Carvalho F., Andrade P.B., Seabra R.M. et al.: J. Agric. Food Chem. 50, 4989 (2002).
11. Cai Y., Luo Q., Sun M., Corke H.: Life Sci. 74, 2157. (2004).
12. Hernández I., Alegre L., Breusegem, F., Munne-Bosch S.: Trends Plant Sci. 14, 125 (2009).
13. Rice-Evans C.: Curr. Med. Chem. 8, 797 (2001).
14. Shojaei A., Javidnia K., Miri R.: Eur. J. Pharmacol. 668, 43 (2011).
15. Aqil F.A.I.: Turk J. Biol. 30, 177 (2006).
16. Ismail H.: Food Chem. 119, 643 (2010).
17. McCord J.M.: N. Engl. J. Med. 312, 159 (1985).
18. Mecocci P., MacGarvey U., Beal M.F.: Ann. Neurol. 36, 747 (2004).
19. Heim K.E., Anthony B., Dennis J.: J. Nutr. Biochem. 13, 572

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