Journal of Chemical Health Risks 3(2): 27-34, 2013
ISSN:2251-6719

Study of Antioxidant and Free Radical Scavenging Activities of *Cotoneaster medicus* and *Glycyrrhiza glabra* Plants

M. M. Heravi[1,], S. Rodi[1], P. Ardalan[2]*

[1] Department of Chemistry , Mashhad Branch , Islamic Azad university, Mashhad, Iran

[2] Young Researchers Club, Mashhad Branch, Islamic Azad University, Mashhad, Iran

(Received: 10 April 2013 Accepted: 10 July 2013)

Abstract: Extracts of *Cotoneaster medicus*, *Glycyrrhiza glabra*, as endemic plants of Iran, along with mixture of them were investigated for their antioxidant activities using 2, 2- diphenyl-1-picrylhidrazyl (DPPH) reagent. UV-Vis spectrophotometry method was used to evaluate the ability of Cotoneaster and Glycyrrhiza *glabra* antioxidant to scavenge DPPH radical. The kinetic parameters such as rate constant and activation energy in experimental conditions were calculated. The rate constants of the H atom abstraction by DPPH (k_1), in the presence of *C. medicus* and *G. glabra* antioxidant were obtained under pseudo-first-order conditions at different temperatures. The order in DPPH radical-scavenging was: mixture of *C. medicus* and *G. glabra* > *C. medicus* > *G. glabra* plants. The numerical values of activation energy were found to be 45.84 $kJ.mol^{-1}$for *G. glabra* and 62.02$kJ.mol^{-1}$ for *C. medicus*.

Keywords: Antioxidant activity, DPPH, *Cotoneaster medicus*, *Glycyrrhiza glabra*, Rate constant , Activation energy

INTRODUCTION

Free radicals, which are generated in several biochemical reactions in the body, have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases [1-3]. It is commonly accepted that, in a situation of oxidative stress, reactive oxygen containing species, such as superoxide ($O^{\cdot}_2$), hydroxyl ($OH^{\cdot}$) and peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$) radicals, are generated. The reactive oxygen containing species plays an important role related to the degenerative or pathological processes of various serious diseases, such as aging [4], cancer, coronary heart disease, Alzheimer's disease [5,6], neurodegenerative disorders, atherosclerosis, cataracts, and inflammation [7]. Although these free radicals can be scavenged by the *in vivo* produced antioxidant compounds, the endogenous antioxidants are insufficient to completely remove them and maintain a balance. As a result, dietary antioxidants are required to counteract excess free radicals [8-11]. The genus *C. medicus* is a shrub member of subtribe Pyrinae (formerly Maloideae), Family Rosaceae [12]. *Cotoneaster* consists of approximately 260 species in temperate regions of the northern hemisphere [13] of which 19 occur in most regions of Iran, but its main distribution range includes Alborz Mts. and elevations in northwest Iran (Azerbaijan province). Among these species, three ones (*C. assadii*, *C esfandiarii* and *C. persicus*) are endemic to Iran. *G. glabra*, also known as licorice and sweetwood, is native to the Mediterranean and certain areas of Asia such

Corresponding Author: M. M. Heravi, Department of Chemistry , Mashhad Branch , Islamic Azad university, Mashhad, Iran E-mail: drmh45@yahoo.com

as Iran. Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. In modern medicine, licorice extracts are often used as a flavoring agent to mask bitter taste in food preparations, and as an expectorant in cough and cold preparations [14]**.** The aim of the research was to study the antioxidant activities of *C. medicus* and *G. glabra* extract by using DPPH radical method.

MATERIAL AND METHOD

Chemical

Ethanol, hydrochloric acid, acetonitrile and 2,2-diphenyl-1-picryl hydrazyl (DPPH), were purchased from Sigma Chemicals Co (USA). Stock solutions of samples were prepared by dissolving DPPH in acetonitril as solvent. Other concentrations of DPPH were prepared by diluting of stock solutions.

Plant Material

For preparation of alcoholic extract five gram portions of *C. medicus* powder were added to a mixture of 85 mL of ethanol (95%) and 15 mL HCl (1.5 M). The mixture was stirred at a speed of 1000 rpm at room temperature for 30 min and then it was filtered. The same procedure was performed for *G. glabra* plant**.**

Apparatus

Spectral data were recorded for the different extracts (*C. medicus*, *G. glabra* plant and mixture of them) using a double beam spectrophotometer (Perkin Elmer Lambda 25) over the range 400 - 800 nm in quartz cuvettes at different temperatures.

UV- Vis spectra

The stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) was used for the determination of free radical-scavenging activities of the *C. medicus* and *G. glabra* extracts. The H-transfer reactions from an antioxidant to DPPH were monitored using the UV–Vis spectrophotometer (scheme 2) [15]. In a typical procedure, 2 mL of a freshly prepared 10^{-4} M solution of DPPH in acetonitrile was placed in the spectrophotometer cell. Then, 0.1–0.4 µL of a freshly prepared alcoholic extracts of the antioxidants were added. All the spectra were recorded at 517 nm [16, 17]

The inhibition percentage was calculated according to the following equation:

$$\%Inhibition = \frac{(A_0 - A_\infty)}{A_0} \times 100 \qquad (1)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_∞ was the absorbance in the presence of the extract at end time.

The second-order rate constant (k_2) was determined with the anti-radical compound [Antioxidant] in large excess as compared with the radical compound [DPPH], forcing the reaction to behave as first order in DPPH as follows [18].

$$-\frac{d[DPPH]}{dt} = k_1[DPPH] \quad (2)$$

Where

$$k_1 = k_2[Antioxidant] \qquad (3)$$

[Antioxidant] is assumed to remain constant throughout the reaction and can be modified to obtain different k_1 values. Therefore, DPPH was depleted from the medium under pseudo-first-order conditions following the equation:

$$[DPPH] = [DPPH]_0 . e^{k_1 t} \qquad (4)$$

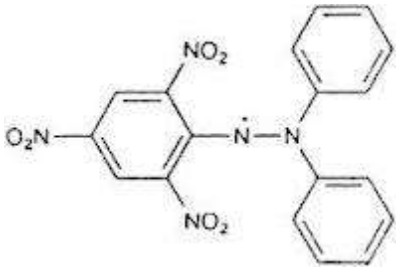
Where [DPPH] is the radical concentration at any time (t), $[DPPH]_0$ is the radical concentration at time zero, and k_1 is the pseudo-first-order rate constant. This

constant (k_1) is linearly dependent on the concentration of the antioxidant. The rate constants were determined by plotting $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time and the respective calculated rate constants listed in table 1.

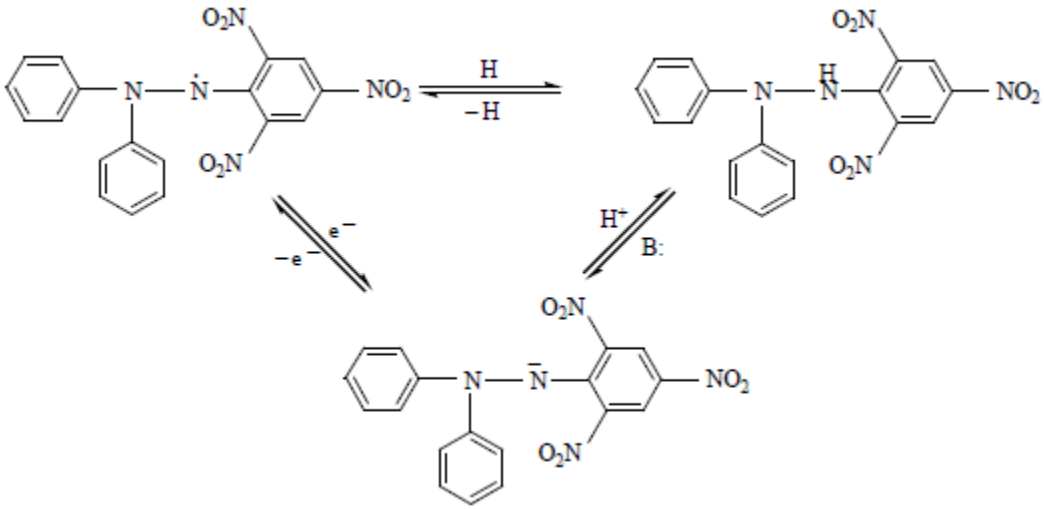
Kinetic studies were conducted by measuring the disappearance of DPPH in acetonitrile at a wavelength of 517 nm [19] under pseudo-first-order conditions. The pseudo first order rate constants were determined by plotting $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time.

The activation energy for DPPH scavenging in the presence of *G. glabra* and *C. medicus* was calculated from the angular coefficient of the plot of $\ln(k)$ versus 1/T as shown in Figure 3.

Scheme1. Chemical structure of DPPH



Scheme 2. The schematic H-transfer equilibria for DPPH radical. the reduced product of DPPH· resulting from hydrogen atom transfer (HAT) or electron transfer, which is the main mechanism of the reaction between DPPH and free radicals.



RESULTS AND DISCUSSION

In our study methanolic extract of *G. glabra* and *C. medicus* plants were evaluated for antioxidants activity by using the DPPH model. The method was based on reduction of DPPH solution in the presence of hydrogen donating antioxidant due to formation of non-radical form namely DPPH-H through the reaction. The extract was able to reduce stable radical DPPH to the yellow colored *Diphenylpicrylhydrazyl*.

The scavenging activity against the DPPH radical was determined by measuring the absorbance at

517 nm until the reaction reached the steady state (Figures 1 and 2). In DPPH model, methanol extracts of *G. glabra* and *C. medicus* showed a significant dose-dependent reduction of DPPH radicals. The inhibition values show in table 1. From the results it was clear that the mixture of *G. glabra* and *C. medicus* plants have greater activity than individual components. Furthermore, the individual extracts of *G. glabra* and *C. medicus* plants have similar antioxidant activity.

Table 2 shows that the rate constants for G. *glabra* and *C. medicus* antioxidants were increased by increasing of temperature and *C. medicus* extracts showed the highest rate constant for DPPH radical scavenging at 307K.

Moreover, the activation energy for DPPH scavenging in the presence of *G. glabra* and *C. medicus* plants, as powerful antioxidant, was calculated from the angular coefficient of the plot of ln□*k* □ versus 1/T as shown in Figure 3. The values found for activation energy were Ea = 45.84kJ.mol^{-1} and Ea = 62.02 kJ.mol^{-1} for *G. glabra* and *C. medicus*, respectively. Table 2 also represents that these radical scavenging reactions have positive activation energies.

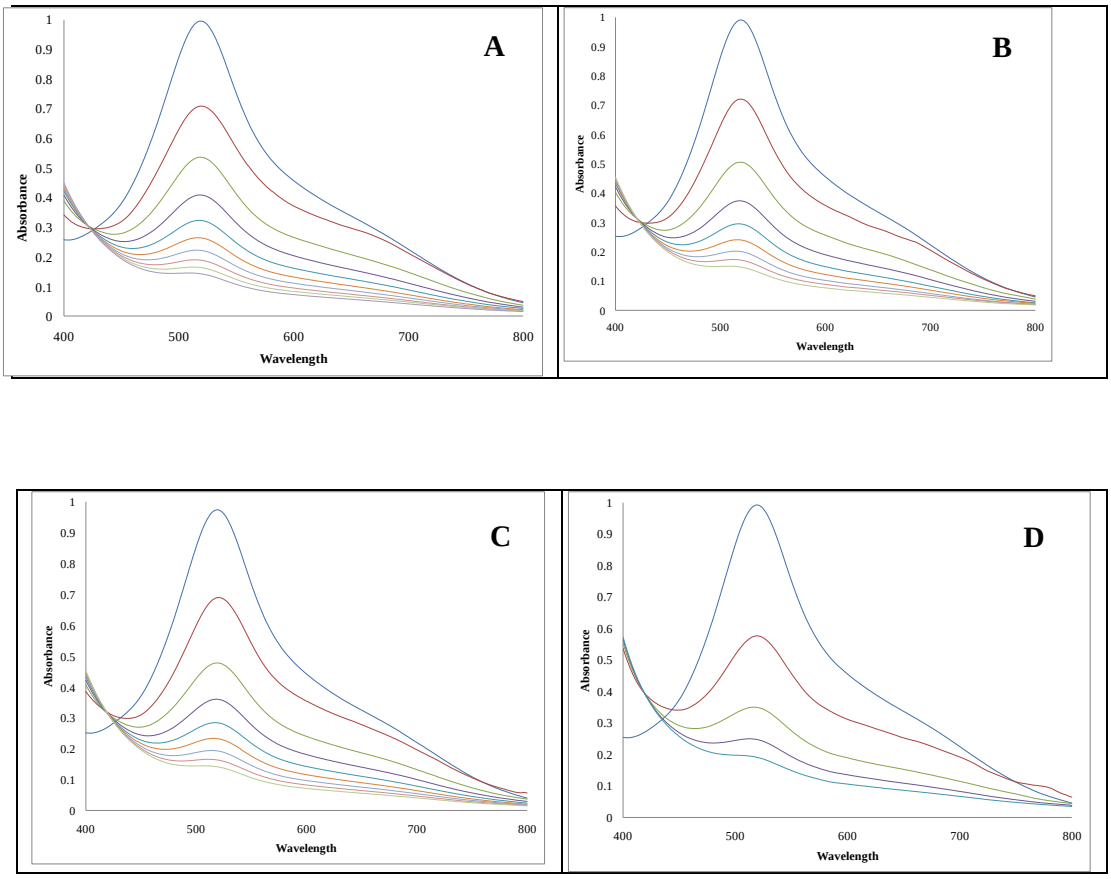


Figure 1.UV-Vis spectra of DPPH in the presence of A) 0.1 µL B) 0.2 µL, C) 0.3 µL, D) 0.4 µL extracts from *G. glabra* plant.

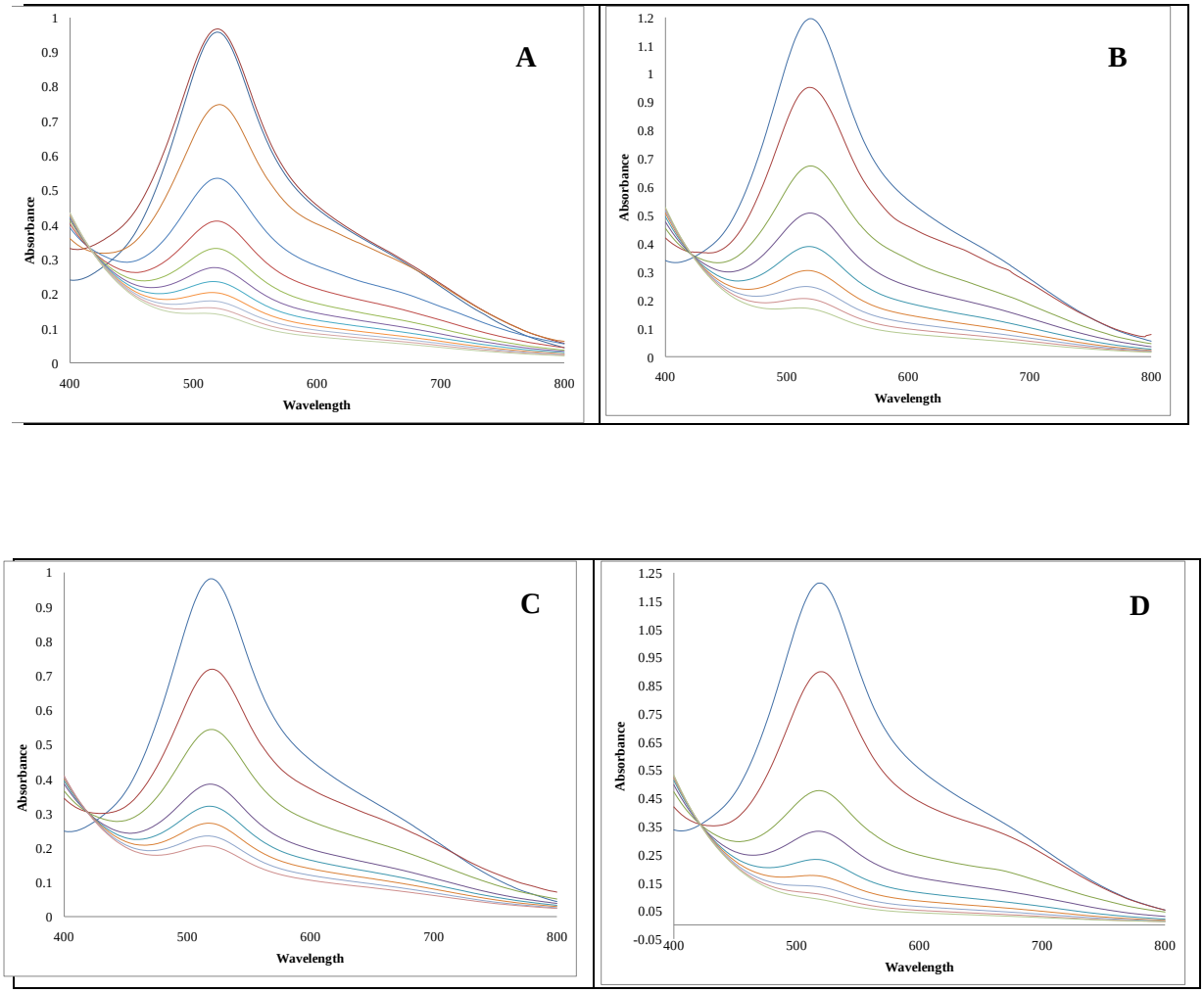


Figure 2. UV-Vis spectra of DPPH in the presence of A) 0.1 µL B) 0.2 µL, C) 0.3 µL, D) 0.4 µL extracts from *C. medicus* plant.

Table1**.** Calculated rate constants in the pretense of different amounts of extracts from *G. glabra* and *C. medicus* at 298K.

Antioxidant(µl)	***Glycyrrhiza glabra***				***Cotoneaster medicus***				**Mixture of *Glycyrrhiza glabra and Cotoneaster medicus***		
	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1 Glyc, 0.1 Coton	0.2 Glyc, 0.2 Coton	0.3 Glyc, 0.3 Coton
$k_1(s^{-1})$	0.402	0.459	0.470	0.904	0.404	0.520	0.583	0.638	1.016	1.529	1.631
% Inhibition	85	86	89	91	85	86	88	92	84	88	94

Table2. Calculated rate constants in the pretense of 0.2 µL extracts from *Glycyrrhiza glabra* or *Cotoneaster medicus* at different temperatures.

Antioxidant	**$k_1(s^{-1})$**				**Activation Energy (kJ/K.mol)**
	298K	301K	304K	307K	
Glycyrrhiza glabra	0.365	0.544	0.739	0.983	45.84
Cotoneaster medicus	0.520	0.608	1.156	1.459	62.02

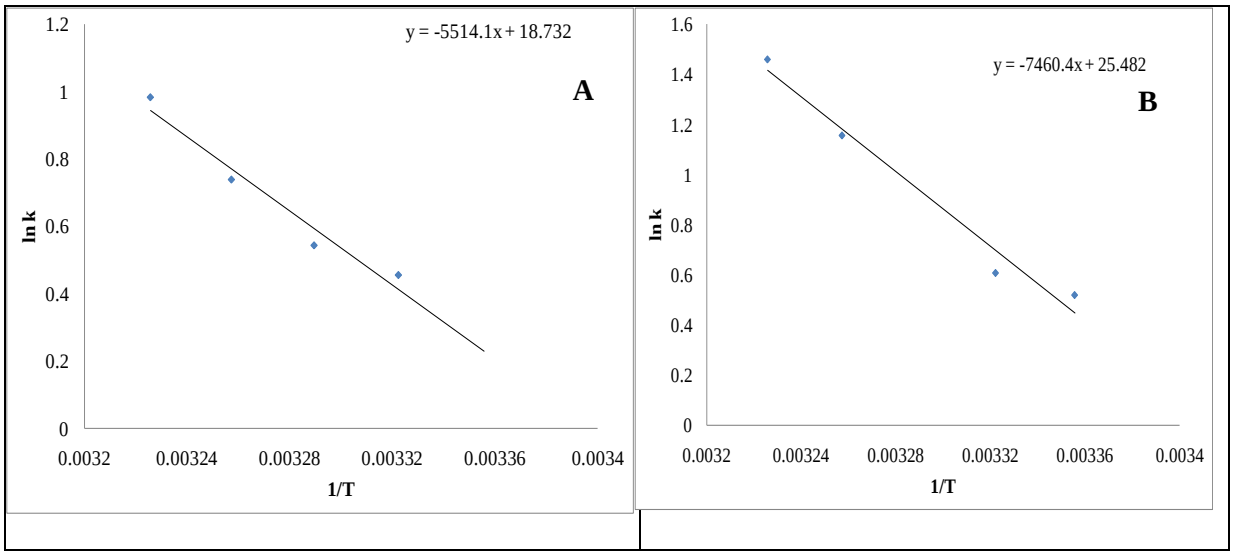


Figure 3. Plot of Arrhenius equation for DPPH scavenging in the presence of 0.2 µL of (A) *Glycyrrhiza glabra* (B) *Cotoneaster medicus*.

CONCLUSION

In this study extracts of *C. medicus* and *G. glabra* plants were investigated for their antioxidant activity using the DPPH reagent. In DPPH model, methanol extracts of *G. glabra* and *C. medicus* showed a significant dose-dependent reduction of DPPH radicals. *G. glabra* showed % inhibition value of 91 and *C. medicus* showed % inhibition value of 92. Therefore extracts of *C. medicus* and *G. glabra* showed similar radical scavenging and reducing power ability. The present investigations revealed that mixture of *G. glabra* and *C. medicus* plants have greater activity than individual components.

REFERENCES

1. Al-Dabbas M., Suganuma T., Kitahara K., X.Hou D., 2006. antioxidant and antibacterial activities of *Varthemia iphionoides* Boiss. Extracts. J Ethnopharmacol.108: 287-293.

2. Tsa R., Deng Z., 2004. Separation procedures for naturally occurring antioxidant phytochemicals. J. Chromatogr. B 812: 85-99.

3. Hayet E., Maha M., Samia A., Mata M., Gros P., Raida H., Ali M. M., Mohamed A. S., Gutmann L., Mighri Z., Mahjoub A., 2008. Antimicrobial, antioxidant, and antiviral activities of Retama raetam (Forssk.) Webb flowers growing in Tunisia.World J. Microbiol. Biotechnol. 24: 2933-2940.

4. Burns J., Gardner P.T., Matthews D., Duthie G.G., Lean M.E., Crozier A., 2001. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. J.
Agric. Food Chem. 49: 5797-5808.

5. Smith M. A., Perry G., Richey P.L., Sayre L.M., Anderson V.E., Beal M.F., 1996. Oxidative damage in Alzheimer's. Nature 382**:** 120-121.

6. Diaz M., Frei B., Vita J.A., Keaney J.F., 1997. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med. 337: 408-416.

7. Aruoma O. I., 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Oil Chem.Soc. 75: 199-212.

8. Lim Y. Y., Murtijaya J., 2007. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT-Food Sci. Technol. 40:1664-1669.

9. Scalbert A., Manach C., Morand C., Remesy C., 2005. Dietary Polyphenols and the Prevention of Diseases. Crit. Rev. Food Sci. Nutr. 45: 287-306.

10. Wang K., Pan Y., Wang H., Zhang Y., Lei Q., Zhu Z., 2010. Med. Chem. Res.
19: 166-176.

11. Gulcin I., Elias R., Gepdiremen A., Taoubi K., Koksal E., 2009. Antioxidant secoiridoids from fringe tree (Chionanthus virginicus L.).Wood Sci. Technol. 43: 195-212.

12. Dickinson T. A., Arsenault M. P., 2007. Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): limited resolution of a complex evolutionary history. -Pl. Syst. Evol. 266: 119 – 145.

13. Mabberley D. J., 1997. The Plant Book, second edition. -Cambridge University Press.

14. Tamir S., Eizenberg M., Somjen D., 2001. Estrogenlike activity of glabrene and other constituents isolated from licorice root. J Steroid Biochem Mol Bio .78:291-298.

15. Yang Me D., Wang Me Q., Ke Be L., Jiang Be J., Ying T., 2007. Antioxidant activities of various extracts of lotus (Nelumbo nuficera Gaertn) rhizome. Asia Pacific Journal of Clinical Nutrition, 16: 158-163.

16. Ilhami G., Ercan B., Hilal S., Ehitog Lu., Mine B., Ahmet C. G., 2010. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food and Chemical Toxicology, 48; 2227–2238.

17. Jing D., Wangyuan C., Guangzhong Y., 2011. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chemistry. 125 ; 1430-1435

18. Bolvar A., Evalos C. C., Luiscisneros Z., 2003. Stoichiometric and Kinetic Studies of Phenolic Antioxidants from Andean Purple Corn and Red-Fleshed Sweetpotato. journal of Agriculcural and food chemistry,51;3313−3319.

19. Hristea E., Caproiu M., Pencu G., Hillebrand M., Constantinescu T., Alexandru T., 2006. Reaction of 2,2-Diphenyl-1-picrylhydrazyl with HO·, O2·–, HO– ,and HOO– Radicals and Anions. Int. J. Mol. Sci. 7: 130-143.