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Investigations on DPPH scavenging capacity before and after UV-irradiation of aqueous root extract of *Glycyrrhiza glabra*

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ABSTRACT

Stable radical 1, 1-dipheny-2-picrylhydrazyl (DPPH) is widely used at *in vitro* models to investigate antioxidant and radical scavenging abilities of natural extracts. This work presents comparative study on DPPH radical scavenging capacity before and after UV irradiation of aqueous extract of *Glycyrrhiza Glabra,* a plant species belonging to the Indian flora. DPPH scavenging activities of different extract concentrations (*at different incubation time intervals*) were analyzed and compared by *in vitro* spectrophotometry and electron paramagnetic resonance (EPR) spectroscopy. 9.93% and 16.79%, DPPH scavenging activities before and after UV irradiation respectively were found by spectrophotometry. By the EPR spectroscopy study statistical significant increase in DPPH radical scavenging for the *Glycyrrhiza Glabra* extracts was established after UV irradiation (78.39± 0.001%) comparing to the non irradiated samples (14.02± 0.02).

Keywords: *Glycyrrhiza Glabra*; DPPH scavenging capacity; Spectrophotometrically and EPR investigations;

Introduction

Plant roots are valuable sources of useful different chemicals and chemical compositions, such as secondary metabolites and enzymes, which are used by the chemical and pharmaceutical industries, for food and others (Shabani et al., 2009). *Glycyrrhiza glabra* (belongs to genus Glycyrrhiza; licorice and Sweet wood) is a common herb of Indian region. The roots of *Glycyrrhiza glabra* contain many compounds, as triterpene saponins (including glycyrrhizin), phenolic compounds, flavonoid content (*includes liquiritin, isoliquiritin*) and isoflavones (*glabridin and hispaglabridins A and B*) with strong antioxidant activity (Karami et al., 2013) and can reduced 1,1-diphenyl-2-picrylhydrazyl

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(DPPH) radicals. Earlier reports showed that the derivatives of Glycyrrhiza glabra (isoflavones) have protective role against oxidative stress (Haraguchi *et al.*, 2002). Oxidative stress is a result of an imbalance between reactive oxygen species (ROS) formation and the capacity of antioxidant protective role (Bjelakovic et al., 2007). In recent years, it has been well established that certain novel plant compounds or plant combinations, possess strong therapeutic antioxidant efficacy and playing role as a radical scavengers, increased the activity of antioxidant enzyme defense system in the cellular environment and thus ultimately reduces negative consequences of chemotherapy- and or radiotherapy-induced oxidative stress (Arora et al., 2005). Moreover, Electron Paramagnetic Resonance spectroscopy is a highly sensitive techniques utilized for investigation of radical structures (Halliwell and Gutteridge, 1985) free radical scavenging capacity (Raffi at al., 2000) and antioxidant activity of naturally isolated products before and after UV- or gamma irradiation (Grigorov et al., 2012). The aim of present study, using spectrophotometry *in vitro* and direct EPR spectroscopy, was to investigate and compare the DPPH radical scavenging capacity and reduction capability before and after UV irradiation of Indian aqueous roots extract of *Glycyrrhiza glabra* for their potential as antioxidants.

Material and Methods

Plant extract

The air dried roots of *Glycyrrhiza glabra* were made into a coarse powder and after dissolved in 2l/ distilled water was subjected to cold maceration process for 24h. The water extract was filtered through muslin cloth and the filtrate was evaporated under reduced pressure and vacuum dried. The extract was providing from INMAS, India as reference.

Samples preparation

1 mg/ml of samples of aqueous roots extract of *Glycyrrhiza glabra* in powder was stirred in 1 ml of d. water for 30 min at room temperature. Solutions with different concentrations of extract (0.1- 0.0125 %) were sonicated for 2 min (Sonicator Water bath Elmasonic PH750 EL) and after that were added to the ethanol solution of DPPH (200 µM) and their free radical scavenging capacities were determined and compared.

Chemicals and UV irradiation

1, 1-dipheniyl-2-picrylhydrazyl (DPPH), Tris-HCl buffer (pH=7.9) were purchased from Sigma Chemicals (St. Louis, MO, USA). Deionized and distillated water was used for all experiments. Other chemicals used were analytical or HPLC grade. Samples were irradiated using a UV–VIS Transilluminator-4000 (Stratagene, USA) in the wavelength range from 290 nm to 320 nm for 2 h in dark.

Direct EPR spectroscopy study on root extract of Glycyrrhiza glabra in powdered and aqueous solution form before and after UV irradiation

For all EPR measurements an X-band EMXmicro, EPR spectrometer (Bruker, Germany) equipped with standard Resonator was used. Experiments were performed in triplicate at room temperature (18-230C) and relative humidity 40%. Spectral processing (g-value calculation) was performed using Bruker WIN-EPR and SimFonia software.

Spectra of Glycyrrhiza glabra in powder and aqueous solution form were recorded at following EPR settings: for the powdered form– gain 2 x 103, microwave power 0.645 mW, center field 3514 G, time constant 327.680 ms, sweep time 61.440 s, modulation amplitude, 12.00 G, 1 scan per sample and for the solution form– gain 1 x 105, microwave power 6.494 mW, center field 3514 G, time constant 163.840 ms, sweep time 16.384 s, modulation amplitude 12.00 G, 1 scans per sample. The settings for the UV-irradiated samples were the same, only the modulation amplitude was reduced to 1.00 G.

In vitro EPR spectroscopy study on DPPH radical scavenging capacity of aqueous root extract of Glycyrrhiza glabra

Radical scavenging capacity of extract was determined according to Bernardo dos Santos et al., 2009 with modifications described by Zheleva et al., 2011. Briefly, to 250 µl ethanol solution of DPPH (200 mM) was added 10 µl non- and UV- irradiated samples of Glycyrrhiza Glabra (0.1% -0.0125%) dissolved in water. After 2 min incubation in dark the mixture was transferred to a capillary tube. The control sample contained 250 µl ethanol solution of DPPH plus 10 µl of distillate water. The percent of the DPPH radicals scavenged by sample was calculated according to the equation:

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Scavenged DPPH radical (%) = [(Io- I) / Io] x 100%
where – Io was integral intensity of the DPPH signal of the control sample and I was integral intensity of the DPPH signal after addition of extract to the control sample.

In vitro spectrophotometry study on DPPH scavenging activity of aqueous root extract of Glycyrrhiza glabra

DPPH radical scavenging activity was performed according to Brand-Williams et al., 1995 with some modifications. 0.3 mL of extract before and after UV irradiation was added to 0.1 mL/1 M Tris-HCl buffer (pH=7.9) mixed with 0.6 ml of 80 mM DPPH ethanol solution and after incubation for 20 min at room temperature its absorbance at 517 nm was measured. The experiments were carried out in triplicate. Percent of the DPPH radicals scavenged by the studied concentration was calculated according to equation:

Scavenged DPPH radical (%) = $[(A_0 - A_S)/A_0] \times 100$
where - A_0 was absorption of the control sample at 517 nm and A_S was absorption of the stadied sample at 517 nm.

In vitro EPR spectroscopy study on the effect of incubation time on DPPH radical scavenging capacity of Glycyrrhiza glabra extract in solution form before and after UV irradiation

After addition of 10 µl of non- and UV- irradiated samples of extract (0.1%) to 250 µl ethanol solution of DPPH the mixtures were incubated in the dark for 10, 20 and 30 min at room temperature and their radical scavenging capacities were determined and compared.

Statistical analysis

Statistical analysis was performed with Statistica 6.1, StaSoft, Inc. and results were expressed as means±standard error (SE). Statistical significance was determined by the Student's t-test. A value of p= 0.05 was considered statistically significant.

Results

Results from direct EPR spectroscopy study on the aqueous root extract of Glycyrrhiza glabra in powder form before and after irradiation are presented on Figure 1 A, 1B. As is seen in the both studied samples (before and after UV irradiation) were registered EPR spectra which are similar in their shape but the intensities of the corresponding peaks were different. Almost the same EPR spectra were recorded in aqueous root extract of Glycyrrhiza glabra in solution form before and after UV irradiation (result is not shown).

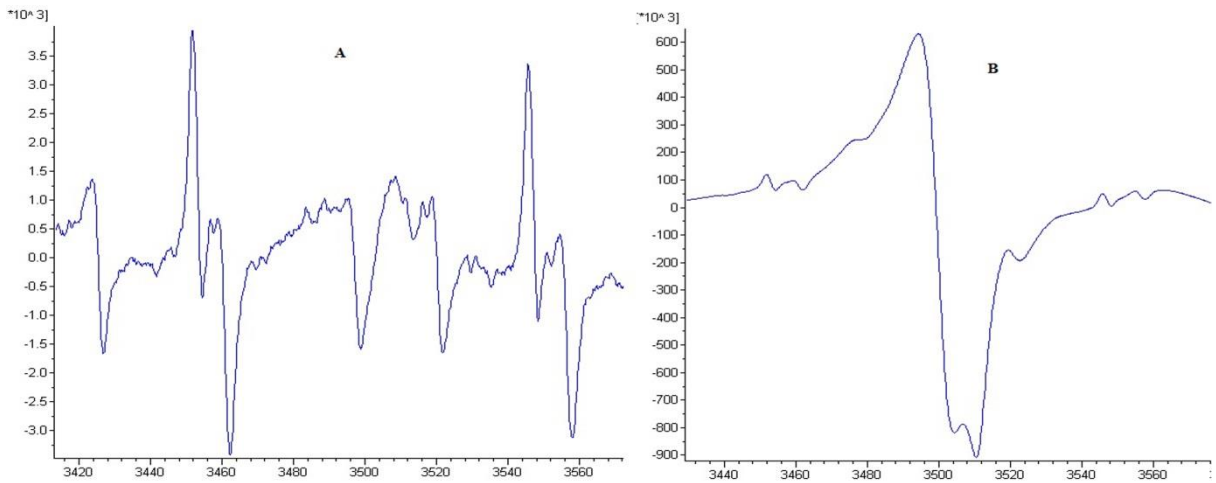

Figure 1. *EPR spectra of Glycyrrhiza glabra root extract in powdered form, before (A) and after UV irradiation (B).*

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Result from EPR spectroscopy study on DPPH radical scavenging capacity of Glycyrrhiza glabra root extract (0.1% - 0.0125%) before and after UV irradiation is shown on Figure 2.

When the concentration of the two studied extract samples decreased, the percent of the scavenged DPPH radicals also decreased in a linear dependence (from 14.02 ± 0.04% at 0.1% to 6.39±0.01% at 0.0125% for non irradiated sample and from 78.39±0.007% at 0.1% to 28.10±0.02% at 0.0125% for UV irradiated sample).

Results obtained by the spectrophotometry method are presented on Figure 3 and as is shown they positively correlated with those established by the EPR method (see Figure 2).

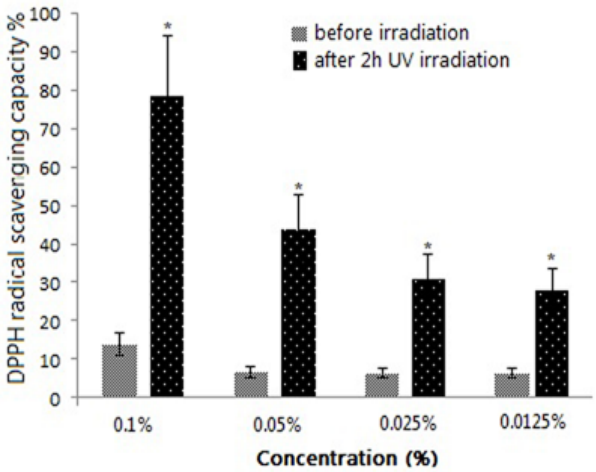

Figure 2. *Percent of DPPH scavenged radicals (before and after UV irradiation) by root extract of Glycyrrhiza glabra determined by EPR method.*

It is also should be noted that in each study concentration the percent of scavenging activity determined by EPR spectroscopy was higher than that determined by spectrophotometry.

The effect of the incubation time on DPPH scavenging capacity of the extract is presented on Figure 4. 20 min after incubation was found with higher scavenging activity comparing to that measured after 10 min incubation. Moreover, DPPH scavenging activity measured at 30th min was almost the same as that at the 20th min.

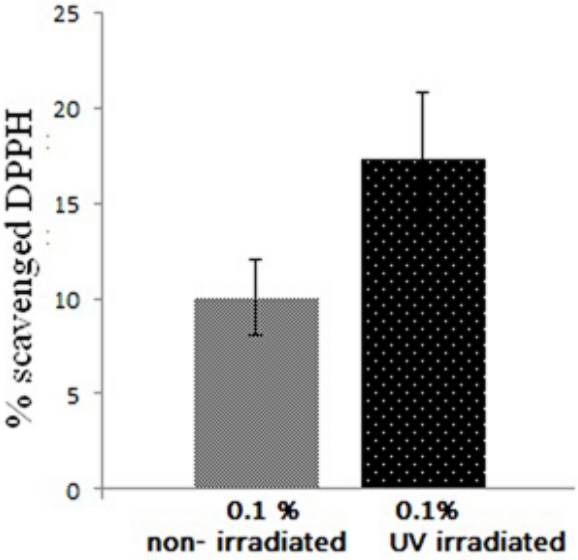

Figure 3. *Percent of DPPH scavenged radicals (before and after UV irradiation) by root extract of Glycyrrhiza glabra determined by spectrophotometry method.*

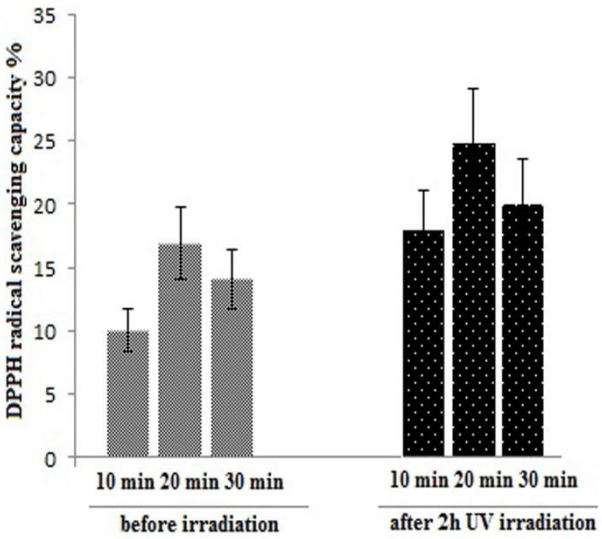

Figure 4. *Percent of DPPH radicals scavenged (before and after UV irradiation) by root extract of Glycyrrhiza glabra determined by EPR method, after different times of incubation.*

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Discussion

Recently, in a number of studies and review papers were reported data about the phytochemical constituent, pharmacological activities and medicinal uses of aqueous and alcoholic root extracts of Glycyrrhiza glabra (Roshan et al., 2012; Cheel et al., 2013; Parvaiz et al., 2014; Mohammed, 2014). Sharma and co-workers have performed a phytochemical screening of hydro-methanolic extracts of Glycyrrhiza glabra (growing in India). They found this extract was rich in saponins, flavonoids, alkaloids, steroids, terpenoids, tannins and glycosides (Sharma et al., 2013). High content of phenolic and flavonoid compounds was also reported in aqueous and ethanolic root extracts of Glycyrrhiza glabra (Mohammed, 2014). Recently by direct ERP spectroscopy we have studied extracts isolated from Piptadeniastrum africanum and Haberlea rhodopensis plant species. Based on the presence of different phenols, flavonoids and tannins, the radicals registered in these extracts were ascribed as semiquinone radical structures (Grigorov et al., 2014). Since, in this study calculated g values of the two peaks of the EPR spectrum registered in UV irradiated sample (g= 2.01062±0.00020 G and g= 2. 00536±0.00020 G) were almost the same as those previously reported (g = 2.01043±0.00003 G and g= 2.00550±0.00010 G, Grigorov et al., 2014) and that in aqueous root extracts of Glycyrrhiza glabra was found a rich content of flavonoids and tannins (Sharma et al., 2013) we assumed formation of semiquinone radical structures during UV treatment of the studied extract.

Different spectrophotometric and EPR methods using DPPH stable radical have been developed for evaluation antioxidant or free radical scavenging activity of natural or synthetic substances (Emaru et al., 1991; Anesini et al., 1993; Perez et al., 2007; Cämmerer & Kroh, 2006; Wolniak et al., 2007; Kim et al., 2010; Alvarez-Parrilla, et al., 2011). EPR spectroscopy method possesses some advantages over the spectrophotometry because the first method measures directly the scavenged DPPH radicals, only while optical spectroscopy besides the appearance of absorption due to the other compounds presenting in the extract sample there is also an increment at 320 nm due to generation of corresponding hydrazide as a final product of DPPH radical reduction (Hashen, 2007; Bernardo dos Santos et al., 2009). Based on the foregoing, we consider that the results obtained for DPPH radical scavenging activity determined by EPR spectroscopy are more real than those by spectrophotometry

Considerably higher DPPH activity found for UV–irradiated extract can be explained by formation of semiquinone radical structures (confirmed by direct EPR spectroscopy of the extract) which are additionally involved in the reaction with DPPH radical.

Wolniak et al., 2007 was found that the incubation time might affect the radical scavenging abilities of the naturally isolated antioxidant products. By the 20th min the effect of incubation time was partially confirmed but from the 20th to 30th min DPPH scavenging activity of the Glycyrrhiza glabra extracts virtually no change (Figure 4).

Conclusion

At this stage of the research it can be concluded that after UV irradiation Glycyrrhiza glabra aqueous root extract possesses well expressed DPPH scavenging activity and might find application in cosmetics as an UV– protector. Further studies on ROS scavenging activity of this extract are in progress in our laboratory.

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