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Antioxidant Behavior of Hyoscyamus Niger Having Narcotic Effect on Heavy Metal Reduction and Radical Scavenging

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ABSTRACT

Medicinal plants with various healing effects are essential for alternative therapy, and adverse environmental conditions in daily life such as stress and heavy metals are some of the main causes of oxidative stress associated with excessive free radical concentration in cells. In this context, the regulation of radical concentration is essential to protect the cell and cellular metabolic pathways against neurodegenerative diseases and cancer. Due to the antiradical and antioxidant effects of medicinal plants, they are important actors and indispensable candidates in alternative therapy. *Hyoscyamus niger* (*H niger*) having a narcotic effect is a member of the Solanaceae family and is used in the treatment of various diseases besides as used as a painkiller. In this study, it was aimed to partially clarify whether the positive effect of *H niger* in diseases is parallel with its antioxidant properties. For this purpose, *H niger* was collected from Patnos/Ağrı province at 1635 m height and the several biological properties of ethanol extract prepared from the leaves of *H niger* were evaluated to improve the moderate literature of *H niger*. First, the heavy metal reduction tests (cupric ions (Cu^{2+}) reduction capacity by CUPRAC method and ferric ions (Fe^{3+}) reduction capacity by FRAP method) and DPPH radical scavenging activity were performed, and second, total antioxidant activity was determined to verify the results of heavy metal reduction and radical scavenging effects.

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1. INTRODUCTION

Plants have been the focus of humanity for centuries due to the healing effects in various diseases. In the recent past, awareness about the side effects of synthetic medicines in society increased the importance of the plants that have medicinal effects, and the tendency towards alternative therapy. In our daily life, adverse environmental conditions such as stress and heavy metals affect us in many ways. A certain level of reactive oxygen species (ROS) concentration in a healthy cell is essential for several mechanisms carried by signal transduction such as apoptosis, cell growth and cell division (Budak et al. 2014c; Song and Zhang 2019). Consequently, it is well known that excessive free radical concentration leads to various neurodegenerative diseases such as Parkinson's Alzheimer, and cancer by affecting some special proteins, lipids, and nucleic acids (Budak et al. 2014b; Ceylan et al. 2019). Heavy metals that we are exposed to in daily life may cause the excessive production of free radicals and cellular toxicity (Ceylan et al. 2019). Therefore, the elimination of heavy metals is of great importance and antioxidant molecules in plants such as flavonoids, terpenoids, and phenolics are important candidates for this. As a matter of fact, plants known as the sources of exogenous antioxidants play a protective role in a cell against neurodegenerative diseases and cancer by regulating cellular ROS concentration (Budak et al. 2014a; Kausar et al. 2018).

The members of the Solanaceae family are widely used in medicine due to their moderate therapeutic effects and these effects have been attributed to herbal flavonoids, terpenoids, phenolics, and narcotic properties in previous studies (Ghorbanpour et al. 2013; Al-Snafi 2018). *H niger* is one of the members of the Solanaceae family and it is a herbaceous plant with soft hairy. *H niger*, known as infidel poppy, grows in almost all regions of Anatolia and mostly lives up to two years. The leaves, roots, and seeds of *H niger* are used in the treatment of various diseases (Liberman 1994) and they are known to have antispasmodic, sedative, and analgesic properties. (Dulger and Dulger 2015; Behravan et al. 2017). *H niger* is also known to be a natural painkiller, and its overdose has been reported to have side effects such as confusion, dilated pupils, restlessness, vomiting, respiratory arrest, and hypertension (Liberman 1994). These effects are attributed to the tropane alkaloids, and scopolamine content (Al-Snafi 2018). In contrast to these negative effects, *H niger* has been reported the use in treatment of bone disorders, rheumatism, toothache, asthma, cough, nerve diseases, stomach, and the pains of kidney stone due to its narcotic effect (Wickens 2005; Kumar 2017; Unterladstetter 2018). The seeds of *H niger* have been reported to have antimicrobial, antidiarrhoeal, antispasmodic, hypotensive, anticonvulsant, anti-inflammatory, analgesic, and antipyretic activities, (SajeliBegum 2010) and it is stated that the methanol extract of *Hyoscyamus albus* leaves has high antioxidant properties (Al-Snafi 2018). Another study reported that the antioxidant enzyme activities of *H niger* extract increased under Ni stress but decreased when pretreated with arginine (Nasibi et al. 2013).

It is a known fact that plants may have different biological effects depending on the geography or species and environmental conditions in which they grow. This suggests that the biological effects of *H niger* may be beneficial despite undesirable side effects. The mentioned properties and the anti-inflammatory effects revealed that the biological properties of *H niger* should be investigated. For this reason, *H niger* was collected from Patnos/Ağrı province at 1635 m height and *in vitro* antioxidant properties of ethanol extract prepared from *H niger* leaves were investigated using different methods. In this context, Cu^{2+} reduction capacity by CUPRAC method and Fe^{3+} reduction capacity by FRAP method, and DPPH radical scavenging activity were investigated. In addition to this, total antioxidant activity of the ethanol extract was determined to verify the results of heavy metal reduction and radical scavenging.

2. MATERIALS AND METHOD

2.1. Plants and Extract Preparation

H niger was collected in Patnos/Ağrı province at 1635 m height and dried. The dried plant was crushed twice with the help of liquid nitrogen and mixed with ethanol (1:10, respectively). After the incubation by stirring for one day at room temperature. Ethanol was removed using the evaporator device. Sample obtained from the evaporator and standards were prepared at different concentrations (10 µg/mL, 20 µg/mL, and 30 µg/mL). Butylated hydroxyanisole (BHA), Trolox (Trx), and Pyrogallol (Prg) were used as standard.

2.2. Antioxidant methods

2.2.1. Ferric iron (Fe^{3+}) reduction test by FRAP

The iron reduction capacity of the extract was determined by the FRAP method (Oyaizu 1986). 2.5 mL of phosphate buffer (pH: 6.6) and $[K_3Fe(CN)_6]$ and 1 mL of distilled water were transferred into the test tubes, respectively. Then, samples were added and incubated for 20 min at 50 °C. After the incubation, 2.5 mL of trichloracetic acid (TCA) was added, vortexed, and centrifuged briefly. An ideal volume from upper phase of sample and standards was taken and transferred to another tubes. Distilled water at the same volume and $FeCl_3$ solution as much as 20% of distilled water was added on. The absorbance values of the sample and standards were read spectrophotometrically at 700 nm.

2.2.2. Cupric ion (Cu^{2+}) reduction test by CUPRAC

Cu^{2+} reduction capacity was determined by the CUPRAC method (Apak et al. 2006). According to this, 1 mL of $CuCl_2$ solution was transferred into test tubes and neocuprine solution and acetate buffer at the same volumes (pH: 6.5) were added on. Then, different concentrations of extract were added and incubated for 30 min. After the incubation, absorbance values were read spectrophotometrically at 450 nm.

2.2.3. DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging effect was tested using minimal modification of the method of Blois (1958). Using a sufficient amount of ethanol, samples were formed at the desired concentration, and then, DPPH radical solution was transferred into the tubes as much as 1/3 of ethanol volume. After the

incubation for 30 min, absorbance volumes were read spectrophotometrically at 517 nm. A mixture of DPPH radical solution and ethanol (1:3) was used as a control sample.

2.2.4. Total antioxidant activity by ferric thiocyanate method

Total antioxidant activity was evaluated by the method of Mitsuda (1966). According to this, Using a sufficient amount of phosphate buffer (pH: 7,4), samples were prepared at the desired concentration and the same amount of linoleic acid was transferred into test tubes and samples were incubated to see maximum total antioxidant activity. At intervals of 9 hours, 20 µL of samples were mixed with 940 µL of ethanol and 20 µL of $FeCl_2$ and NH_4SCN solutions. Absorbance volumes of samples were recorded at 500 nm. The test was terminated when the control absorbance reached the highest value.

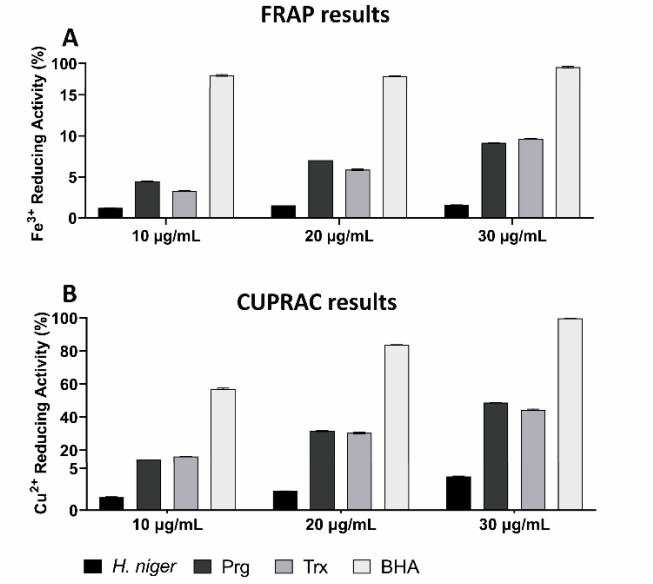

Figure 1. A) % Fe^{3+} reduction capacities of ethanol extract and standards at different concentrations **B)** % Cu^{2+} reduction capacities of ethanol extract and standards at different concentrations

3. RESULT AND DISCUSSION

Antioxidant contents of plants are important candidates for free radical scavenging and heavy metal reduction reactions. This study was performed to determine some biological activities of *H niger* which is a member of the Solanaceae family and increasing activities were detected in all test results as depend on sample volumes. Although the Fe^{3+} reduction activity of *H niger* ethanol extract was relatively similar to Trolox and Pyrogallol standards at low concentrations, the result of the extract was significantly lower than BHA (Figure 1A). According to the increase of sample concentration, the means of FRAP results are as follows; 1.22%, 1.51%, and 1.57%.

Cu^{2+} reduction results of the extract were also found to be quite low compared to the standards. (Figure 1B). According to the increase of sample concentration, the means of CUPRAC results are as follows; 1.56%, 2.30%, and 4.02%. *H niger* with a narcotic effect is used as a natural painkiller but, it is known to have toxic effects due to tropane alkaloids (SajeliBegum 2010; Al-Snafi 2018). The reason why the ethanol extract shows poor heavy metal reducing activity may be due to alkaloids in plant structure, which also known as the cause of its side effects.

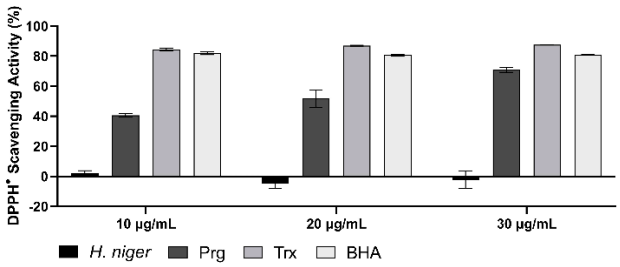

Figure 2. % DPPH radical scavenging activities of ethanol extract and standards at different concentrations.

In the DPPH radical scavenging results, *H niger* ethanol extract was also found to have quite low radical scavenging activity compared to the standards. Depending on the increase of extract concentration, the means of results are as follows; 2%, -4.62%, -2.22%. As can be seen from negative values at high concentrations, radical generation may be taking place unlike radical scavenging in low concentrations (Figure 2). This effect in low concentrations supports the therapeutic effect of *H niger*. Moreover, the reason for radical production at high concentrations may be due to molecules in plant structure such as tropane and scopolamine.

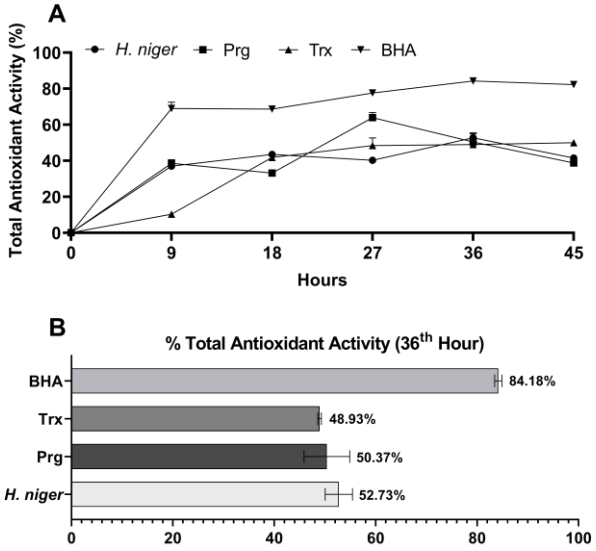

Figure 3. A) % total antioxidant activities of 30µg/mL of ethanol extract and standards at 9 hours intervals **B**) % total antioxidant activities of 30µg/mL of ethanol extract and standards at the 36th hours.

In order to confirm the results of heavy metal reduction and DPPH radical scavenging effect, the total antioxidant activity of the extract was determined at intervals of 9 hours, and the highest activity was detected at the 36th hour. In addition, it was determined that the highest level of pyrogallol standard activity was detected at 27th hour (Figure 3A). This result may indicate that the reaction rate of pyrogallol is higher than other standards and ethanol extract. Although the total antioxidant activity is similar to the activities of pyrogallol and Trolox standards at the 36th hour, it is considerably lower than BHA activity. A study reported that the methanol extract of *Hyoscyamus Albus* leaf has high antioxidant and antiradical properties and showed high metal chelating activity (Al-Snafi 2018). These results do not match our study results. The reason for this may be the use of different plant species, environmental conditions, and/or geography where the plant grows. It is also clear that total antioxidant activity of *H niger* extract was similar to the standard activities as in the literature. However, radical scavenging and metal reduction activities of the extract were found to be rather poor. This result indicates that the ethanol extract of *H niger* has an unsuitable antioxidant content for metal reduction and radical scavenging.

4. CONCLUSION

Although the antioxidant activity of *H niger* ethanol extract was quite similar to the standards as in the literature, its radical scavenging and heavy metal reduction activities were found to be rather poor. The reason for this may be due to the alkaloids in plant structure, which are known as the cause of its side effects. Negative results indicating the radical generation were obtained at high concentrations in contrast to radical scavenging. This result supports the causes of side effects reported in previous studies. Although the results obtained in all steps of the study are highly similar, it is clear that the antioxidant effect is better than the radical scavenging and heavy metal reduction effects.

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