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**IN VITRO ANTIOXIDANT ACTIVITY AND PHENOLIC PROFILE OF
*HYOSCYAMUS NIGER***

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ABSTRACT

Nowadays, it is observed that many of the diseases are due to the Free radicals and they induce damages to biomolecules and the uses of many synthetic drugs that scavenge free radicals but they have adverse side effects. Therefore, there have been increased interests to find natural antioxidant compounds to help oxidative damages to human. So the aim of this study was to evaluate the antioxidant activity and phenolic compounds in aerial parts of *Hyoscyamus niger*. *Hyoscyamus niger* is from Solanaceae family and it is has been used for diarrhea, stomach pain, Parkinsonism and hysteric patients. The total phenolic content was determined using Folin-Ciocalteu phenol reagent method and antioxidant activities was evaluated with 2 methods DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Ferric reducing antioxidant power (FRAP) assays. Efficient Concentration (EC50) for methanol extract was 377 ± 1.21 $\mu\text{g/mL}$ and it was 21 ± 0.68 and 4.8 ± 0.32 $\mu\text{g/mL}$ for butylated hydroxytoluene (BHT) and ascorbic acid. The results of the present study apparently indicated that methanol extract of *Hyoscyamus niger* may constitute a suitable source of phenolic and could be used as natural antioxidants in food industries.

Keywords: *Hyoscyamus niger*, Antioxidant, Total Phenolic Content, DPPH, FRAP

INTRODUCTION

Nowadays, it is observed that many of the diseases are due to the Free radicals and they induce damages to biomolecules and promote serious health problems [1]. The uses of many synthetic drugs that scavenge free radicals but they have adverse side effects [2]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are being widely used as food additives to prevent oxidative deterioration of food products but they have been reported the toxicity concern [3]. Medicinal and aromatic plants are known to produce a variety of antioxidants. Therefore, there have been increased interests to find natural antioxidant compounds to help oxidative damages to human [4]. Therefore, the use of natural antioxidant has obtained much attention from consumers because they are considered safer than synthetic antioxidants. Recently, there has been a worldwide trend towards the use of natural antioxidants present in different parts of plants due to their phytochemical constituents [5, 6]. Antioxidants such as phenolics, flavonoids, terpenoids, proanthocyanidins and tannins are found in various plant products [7]. Antioxidant activities are known to increase proportionally to the polyphenol content, mainly due to their redox properties [8]. Phenolic compounds are secondary plant

metabolites that play a key role in fruits, vegetables and other plants [9]. Among the diverse roles of polyphenols, they protect cell constituents against destructive oxidative damage, thus limiting the risk of various degenerative diseases associated with oxidative stress and thus tending to be potent free radical scavengers. Their ability to act as antioxidants is due to their chemical structure and ability to accept electrons, thus delocalizing the unpaired electron within the aromatic structure [10]. Plant phenols exhibit significant antioxidant, antiviral, anti-inflammatory and antibiotic properties [11]. Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [12]. There is an interest in extracting of these compounds. However, there are several methods for the extraction of polyphenols from plant materials and they vary in solvents and conditions used. Therefore, the extraction method is essential for estimation of antioxidant content [13]. Several different methods have been used for measuring antioxidant activity [14]. *Hyoscyamus niger* is from Solanaceae family [15], it is widely distributed in Europe and Asia and 18 species have been reported from Flora Iranica [16]. In Iranian medicine, this plant has been used for

diarrhea, stomach pain, Parkinsonism and hysteric patients [16]. Major chemical compounds are extracted from aerial parts of *Hyoscyamus niger* are hyoscyamine and scopolamine (tropane alkaloids), hyoscine, apohyoscine, Daturamine, skimmianine, tropine and belladonines [17, 18]. The aim of this study was to evaluate the antioxidant activity and phenolic compounds in aerial parts of *Hyoscyamus niger*.

2. MATERIALS AND METHODS

2.1 Materials

Methanol (CHROMASOLV, $\geq 99.9\%$, Sigma-Aldrich), Folin-Ciocalteu reagent (F9252, Sigma-Aldrich), Na₂CO₃ (451614, anhydrous powder, 99.999%, Sigma-Aldrich), gallic acid (91215, Fluka), 2,2-diphenyl-1-picrylhydrazyl (257621, Sigma-Aldrich), ascorbic acid (A1300000, European Pharmacopoeia (EP) Reference Standard, Fluka), BHT (W218405 ≤ 99 , Sigma-Aldrich), TPTZ (T1253 for spectrophotometric, $\geq 98\%$, Sigma), FeSO₄.7H₂O (21542, $\geq 99.0\%$, Sigma-Aldrich).

2.2 Plant materials

The aerial parts of *Hyoscyamus niger* were collected from North khorasan mountains in Jun 2014, and was identified in Herbarium of natural products & medicinal plants research center. The leaves of plant were

air-dried at room temperature and were stored for later analysis.

2.3. Plant extraction

Dry powdered (100 g) of *Hyoscyamus niger* was macerated by methanol in 48 h and then extractive solution concentrated at 40 °C under reduced pressure to dryness [19]. The yield of extraction was 6.87%.

2.4. Determination of total phenolic content

The total phenolic content in methanol extract of *Hyoscyamus niger* was determined using Folin-Ciocalteu phenol reagent method [20]. Briefly, 100 μ L of extract (1000 mg/L) was added with diluted Folin-Ciocalteu reagent (50%, 100 μ L). After 1 min of reaction, Sodium carbonate (Na₂CO₃) (2%, 2 mL) and 2.8 ml H₂O were added to tube, then the tube was vortexed and incubated for 60 min at room temperature. The absorbance was read at 720 nm using UV-Vis spectrophotometer. The analysis was performed in triplicates. The standard curve was prepared using 0 to 100 micrograms of gallic acid. Total phenol value was expressed as gallic acid equivalent (mg GA /g of dry extract), which is a common reference compound for phenolic compounds [20].

2.5. Antioxidant activity

2.5.1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the extracts was evaluated by the method of Sharma et al. [21]. DPPH was prepared to 0.16 mM with methanol. Extracts were dissolved in methanol to several concentrations (8, 4, 2, 1, 0.5, 0.25 mg/ml) and 0.1 mL of the solution and 0.1 ml of DPPH were mixed and were kept in the dark for 30 min and the absorbance was read at 517 nm. The experiment was carried out in triplicate. The percentage of radical scavenging activity was calculated using Eq.1.

$$\% \text{ DPPH scavenging activity} = \frac{A_{517} \text{ of control} - A_{517} \text{ of sample}}{A_{517} \text{ of sample}} \quad (1)$$

Methanol was used as control, ascorbic acid and butylated hydroxy toluene (BHT) used as positive controls. AI was calculated as IC_{50} values were calculated using Graph Pad Prism software, version 5.01 [21].

2.5.2. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of the extracts was measured by method of Xu et al [22]. The FRAP reagent was contained from 1 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM in 40 mM HCl), 1 mL of $FeCl_3 \cdot 6H_2O$

(20 mM) and 10 mL of acetate buffer with pH 3.6 (0.3 M). Three mL from FRAP reagent mixed with 100 μ l of each sample and then they were incubated at 37 °C for 10 min in a water bath. After incubation, the absorbance was measured at 593 nm. Aqueous solutions of $FeSO_4 \cdot 7H_2O$ in the range of 0-1 mM, were used for calibration curve. FRAP values were expressed as mean \pm standard error (SE) mmol Fe (II) per gram.

3. RESULTS

In this study, phenolic content was determined by Folin-Ciocalteu method. The regression equation for determination of total phenolic contents was: $Y=0.0096X-0.0073$ ($R^2=0.996$) and gallic acid was used for standard curve. The total phenolic content of methanol extract is shown in Table 1. In the present study, using two different methods; DPPH and FRAP, it was found that methanol extract of plant possess antioxidant properties (Table 1). The result of DPPH reduction is shown in Table 1. Equation of FRAP for standard solutions was: $y= 0.350x+ 0.012$ ($R^2 =0.992$). The result of the FRAP assay is reported in Table 1.

Table 1. Antioxidant properties and phenolic contents from methanol extract of *Hyoscyamus niger*

Sample	Total phenolic (Gallic acid equivalents mg/g of dry extract)	EC_{50} (μ g/mL)	FRAP value (mmol Fe^{2+} /g dry extract)
Methanol extract	5833.33 \pm 0.4	377 \pm 1.21	287.5 \pm 3.64
BHT		21 \pm 0.68	
Ascorbic acid		4.8 \pm 0.32	

4. DISCUSSION

Antioxidant activity seems to be dependent on the phenolic structure and the phenolic compounds influence the antioxidant activity [23]. solvent polarity will play an important role in extraction of phenolic compounds and in other studies, the methanol was better solvent than the others in extracting phenolic compounds due to their polarity and good solubility of them [24]. So, the polar extracts had more phenolics than the non-polar extracts [25]. Radical scavenging activity is related to the nature of phytochemicals and their hydrogen donating activity [26]. The antioxidant activity is generally attributed to phenolic compounds in plant extracts [27]. The antioxidant activity of phenolic compounds is due to their chemical structures, which allow them to act as reducing agents, hydrogen donors and singlet quenchers [23]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is simple, efficient and inexpensive and it is one of the most commonly methods [28, 29]. DPPH is a stable free radical which has a deep purple color and it has strong absorption band in 517 nm. In the presence of antioxidant compounds, DPPH can accept an electron or a hydrogen atom from them, to be converted to a more stable DPPH molecule. The reduced form of DPPH is yellow; it is possible to determine

the antioxidant activity by studying the change of color with spectrophotometer. The greater the free radical scavenging activity of an antioxidant compound is the more reduction for absorbance of DPPH and the less purple color in the sample. The results are normally expressed as Efficient Concentration (EC_{50}), which is defined as the amount of sample can decrease the DPPH concentration by 50%. The parameter EC_{50} was introduced by Brand- Williams et al [30] and it is very useful for comparing results because it is independent of the sample concentration. In this method, it is well-known that the lower EC_{50} has the higher antiradical activity [31]. The result obtained in this study clearly demonstrated that the methanol extract of *Hyoscyamus niger* is appropriate antioxidant. As shown in Fig 1, the scavenging activity from methanol extract of plant was concentration-dependent.

In the current investigation, vitamin C had the highest radical scavenging activities and its EC_{50} was lower than BHT and methanol extract ($4.8 \pm 0.32 \mu\text{g/mL}$). The FRAP assay is a simple method for measuring the antioxidant activity in a sample by oxidation-reduction potential. In this method, antioxidants react with the ferric tripyridyltriazine complex and produce the intense blue color ferrous tripyridyltriazine complex [32]. The antioxidant activity was

expressed as the concentrations of antioxidants having a ferric reducing ability equivalent to that of 1 mM of FeSO₄.

Antioxidants reduce Fe (III)-TPTZ to form a blue colored Fe (II)-TPTZ complex with increase in the absorbance at 593 nm [32].

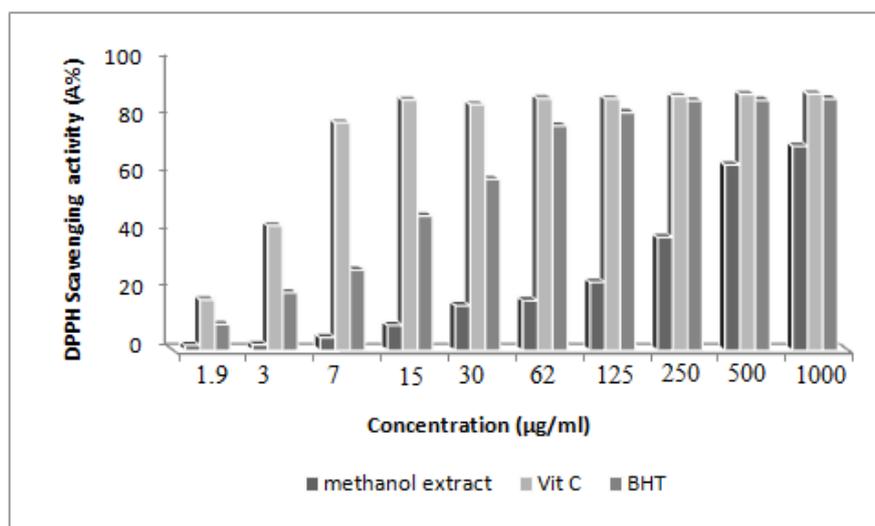


Fig 1: The effect of concentration in scavenging of DPPH

5. CONCLUSION

The results of the present study apparently indicated that methanol extract of *Hyoscyamus niger* may constitute a suitable source of phenolic and could be used as natural antioxidants in food industries.

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