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Evaluation of phenolic compounds and DPPH inhibition of two Iran endemic species from *vincetoxicum* genus

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Abstract

DPPH method is commonly method for determination of antioxidant and it is a free radical. It is possible to determine the antioxidant activity by studying the change of DPPH color. Plant phenols exhibit significant antioxidant properties with redox properties which allow them to act as reducing agents. Vincetoxicum belong to Apocynaceae family has 5 species in Iran and it was used to treat rupture, fever, scrofula and scabies in Chinese medicine. Two species from this genus is endemic to Asia such as *Vincetoxicum Pumilum* and *Vincetoxicum nigrum.* In this study, scavenging of DPPH radical and phenolic content of *Vincetoxicum Pumilum* and *Vincetoxicum nigrum* was studied. Methanol extracts of plant was prepared by maceration method and then their phenolic contents was evaluated by folin-ciocaltue method and radical scavenging was done with DPPH assay. Amount of phenolic content in *Vincetoxicum Pumilum* was 11725 µg/g dried extract and it was10150.5 µg/g dried extract for *Vincetoxicum nigrum*. In all of concentrations, *vincetoxicum pumilum* had higher power of scavenging the free radicals than *vincetoxicum nigrum* and *vincetoxicum pumilum* with high level of phenolic compounds had higher scavenging activity than *vincetoxicum nigrum*.

Key words : *Vincetoxicum Pumilum, Vincetoxicum nigrum,* DPPH, phenolic content.

INTRODUCTION

Antioxidants such as flavonoids, phenolics, terpenoids, flavonols, proanthocyanidins and tannins are found in various plant products [1]. DPPH (2,2-diphenyl-1-picrylhyndrazyl) method is commonly method for determination of antioxidant, because this method is simple, efficient and inexpensive [2]. DPPH is a free radical and it has a deep purple colour and a strong absorption in the 515520 nm and it can accept an electron or a hydrogen atom from the antioxidant scavenger molecule and reduced form of DPPH is yellow. It is possible to determine the antioxidant activity by studying the change of colour with spectrophotometer and the results are expressed as Efficient Concentration (EC_{50} or IC_{50}), and it is the amount of sample to decrease 50% of DPPH concentration [3].

Plant phenols exhibit significant antioxidant properties with redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [4]. There is a wide interest to finding natural sources that could replace synthetic antioxidants because of their toxicity such as butylated hydroxytoluene (BHT) which is used in food industry [5]. Vincetoxicum belong to Apocynaceae family, Asclepiadoideae subfamily [6]. From this genus, there is 5 species in Iran that they growh in zahedan, Khorasan and azarbaijan [7,8]. Formerly this herb was attributed to the genus Cynanchum and It is found in Europe, North America and Asia [9]. The rhizome leaves and dry seeds have been used for various medicinal purposes such as treat neurosis and malaria in folk medicine and it has diuretic, laxative and emetic effects. It was used to treat rupture, fever, scrofula and scabies in Chinese medicine [10]. This genus is containing glycosides, a toxic compound vincetoxin, alkaloid, triterpenes [11]. Total content of phenolics in methanol extracts *Vincetoxicum lutea L* was 86.0 mg gallic acid equivalents in 1 g of dry extract [12]. Two species from this genus is endemic to Asia such as *Vincetoxicum Pumilum* and *Vincetoxicum nigrum*. From *Vincetoxicum Pumilum* one Phenanthroindolizidine alkaloids was isolated [13]. In this study, scavenging of DPPH radical and phenolic content of *Vincetoxicum Pumilum* and *Vincetoxicum nigrum* was studied.

MATERIALS AND METHODS

Plant material

The Plant materials were collected in Jun 2014 from the North Khorasan Province Mountains of Iran. And they were identified from the Herbarium of research of natural products and medicinal plants. Voucher specimen (NMP 10/4-1) was for *Vincetoxicum Pumilum* and Voucher specimen for *Vincetoxicum nigrum* was NMP 8/3-2.

Preparation of plant extract

100 g of plants were macerated with methanol (MeOH) at room temperature. The whole extracts were filtered and solvent was evaporated under vacuum at 40 °C to afford extracts. Extracts were stored at 4 °C until analysis.

Diphenyl-2-picrylhydrazyl (DPPH) assay

The scavenging activity from methanol extracts of *Vincetoxicum Pumilum* and *Vincetoxicum nigrum* was done. This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine [14]. From each plant extract, some concentrations (0.6 .0.5, 0.4, 0.3, 0.2, 0.1, 0.05 and 0.01) mg/mL was made in methanol. 2.5 mL of sample solution of different concentrations was added to 1 mL of a 0.3 mM DPPH methanol solution. One mL DPPH solution with 2.5 mL of methanol was used as a negative control. The blank was methanol. The tubes were kept in room temperature for 30 minutes. The absorbance values were measured at 517 nm and % DPPH scavenging activity was earn by this equation:

% DPPH scavenging activity = [×100.

The tests were done in triplicate. The IC_{50} (The concentration of sample required to scavenge 50% of DPPH values) were

calculated by biodata fit online program [15].

Total phenolic Determination

The total phenolic content was determined by using Folin-Ciocalteu method. Briefly, 100 µL extract (1000 mg/L) was added with diluted Folin-Ciocalteu reagent (50:50, 100 µL) and Sodium carbonate (Na_2CO_3) (2%, 2 mL), 2 ml dionized water was added to each tube, then tubes were incubated for 30 min at room temperature. The absorbance was read at 720 nm. The analyses were performed in triplicates. The standard curve was prepared using solutions of Gallic acid in methanol. Total phenol values were expressed as Gallic acid equivalents (mg Gallic acid: (GA) per dry weight of extract) [16].

RESULTS

The yield of methanol extract of *Vincetoxicum Pumilum* was 9.6 % and 10.0 % for *Vincetoxicum nigrum.* The standard curve for gallic acid was demonstrated in Fig 1 and its equation was (y=0.005x+0.001, R^2= 0.995 .(Amount of phenolic content in *Vincetoxicum Pumilum* was 11725 µg/g dried extract and it was10150.5 µg/g dried extract for *Vincetoxicum nigrum*. The strength of scavenging of DPPH free radicals was evaluated by DPPH assay and in fig 2, the effect of two species concentrations on scavenging of free radicals was demonstrated in 517 nm and comparison of scavenging of DPPH free radicals by methanol extracts and positive controls (BHT and Vit C) was shown in Fig 3. The amount of IC_{50} was 0.21 mg/ml for *vincetoxicum pumilum* and it was 0.3 mg/ml for *vincetoxicum nigrum*. It was 0.003 mg/ml for BHT and 0.002 mg/ml for Vit C.

DISCUSSION

DPPH is free radical that used commonly in scavenging the free radicals. In this study, the ability of methanol extracts of two species in vincetoxicum genus was evaluated with DPPH assay and as shown in Fig 3, scavenging of DPPH was increased with increasing concentration of extracts and there was a good relationship between concentration and scavenging free radicals. In 0.6 mg/ml, scavenging free radicals in two plants was higher

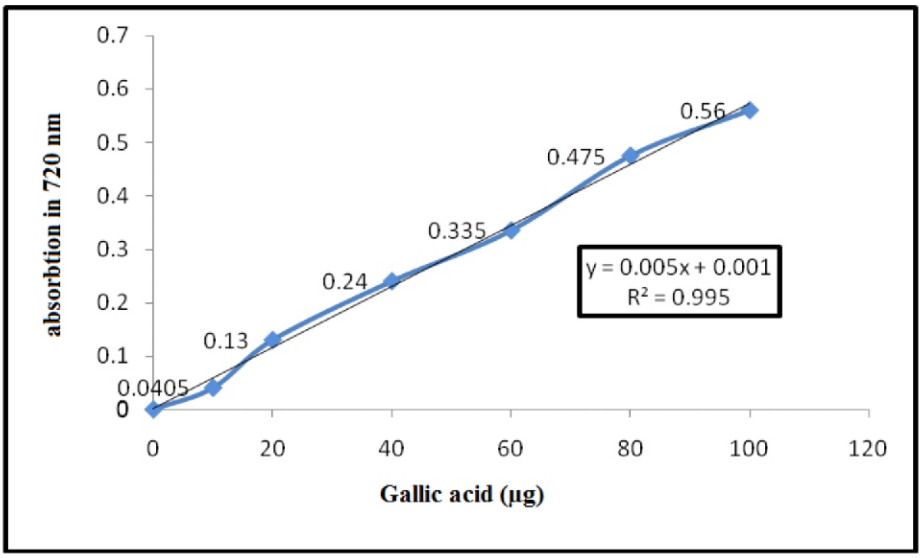

Fig. 1: Calibration of gallic acid for evaluation of phenolic content

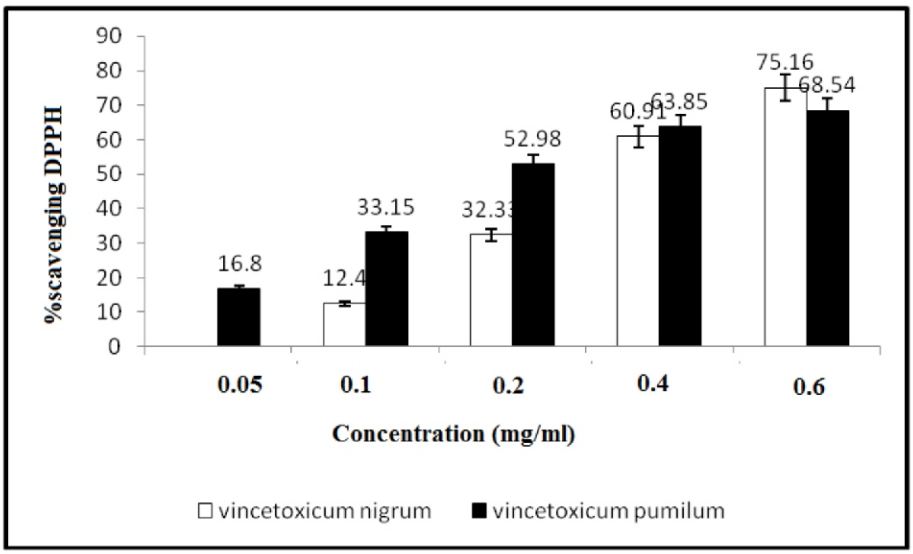

Fig. 2: The effect of concentration on scavenging free radical by *vincetoxicum nigrum* and *vincetoxicum pumilum*

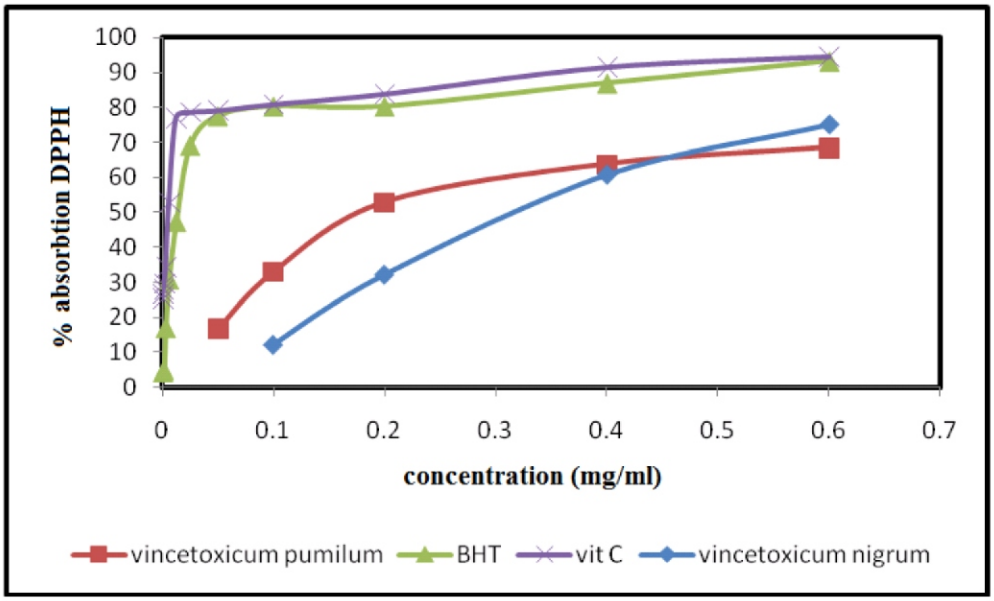

Fig. 3: Comparison of radical scavenging by methanol extracts of *vincetoxicum nigrum, vincetoxicum pumilum* and positive controls BHT and Vit C.

than 65% and in 0.05 mg/ml *vincetoxicum pumilum* can scavenge 16.8% of free radicals but *vincetoxicum nigrum* haven't any ability to scavenge the free radicals. In all of concentrations, *vincetoxicum pumilum* had higher power of scavenging the free radicals than *vincetoxicum nigrum* . Studies show that phenolic compounds had antioxidant activity because of their ability in scavenging the free radicals and this ability is depend on aromatic rings and hydroxyls groups [17]. Studies show that high levels of phenolic compounds is reason for high levels of antioxidant activity of extracts such as polar extracts and there is a positive relation between phenolic content and antioxidant activity [18] and also this relation was showed in this study, as can be seen *vincetoxicum pumilum* with high level of phenolic compounds had higher scavenging activity than *vincetoxicum nigrum*.

CONCLUSION

Results showed that Vincetoxicum pumilum had higher power of scavenging the free radicals than Vincetoxicum nigrum and vincetoxicum pumilum with high level of phenolic compounds had higher scavenging activity than Vincetoxicum nigrum. However, both species are good antioxidant sources and we think this herbal extract may be used and act as effective food preservative. Of course, it is necessary to assay the antimicrobial properties of these extract and to evaluate the effect in food systems to determine the sensory properties of final products.

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