Jundishapur Journal of Natural Pharmaceutical Products 2010; 5(1): 1-5

Jundishapur Journal of Natural Pharmaceutical Products

INVESTIGATION OF ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF VARIOUS FRACTIONS OF AERIAL PARTS OF *PIMPINELLA BARBATA* (DC.) BOISS

Namjooyan F[1,2], Azemi ME[1,2*], Rahmanian VR[3]

[1]*Department of Pharmacognosy, School of Pharmacy, Judishapur University of Medical Sciences, Ahvaz, Iran.*

[2]*Medicinal Plant Research Center, Judishapur University of Medical Sciences, Ahvaz, Iran.*

[3]*School of Pharmacy, Judishapur University of Medical Sciences, Ahvaz, Iran.*

Received: 7 March 2010 ***Accepted: 1 November2010***

Abstract

Free radicals play an important role in degenerative diseases. They can also induce nutrition and medicines deterioration. Fortunately, formation of free radical is controlled by a variety of systems which are called "antioxidants". Herbal medicines have been focused as new sources of antioxidants with limited complications. *Pimpinella barbata* (DC.)Boiss is an annual plant from Apiaceae family. In this study, DPPH radical scavenging assay and FRAP method were used for assaying the antioxidant activity and Folin-Ciocalteu, for quantitative determination of total phenolic content of methanolic, n-hexane, and dichloromethane extract of *P.barbata*.

The antioxidant power in DPPH assay and total phenolics in Folin-Ciocalteu method were evaluated as shown in decreasing order: Methanolic extract > dichloromethane extract > n-hexane extract.

In FRAP assay, dichloromethane extract was the most potent while the methanolic extract was the weakest one. In both DPPH assay and Folin-Ciocalteu method, methanolic extract exhibited the highest activity and the most phenolic content respectively while in FRAP test dicholoromethane was shown the highest activity. These results are in agreement with other studies and showed that different tests do not necessarily confirm each other.

Key word:

Free radicals, *Pimpinell barbata* (DC.)Boiss, FRAP, DPPH, Total phenolic content.

Introduction

Free radicals play an important role in degenerative diseases like cancer, cataract, immune system weakness and brain problems(1,2). Free radicals can also induce nutrition and medicine deterioration(3). Fortunately, formation of free radicals is controlled by a variety of systems which called "antioxidant". Antioxidants are defined as compounds which can reduce oxidation rate considerably(4). When availability to antioxidants depot in our body cells decrease or in the cases of oxidants attack (like smoking, air pollution, inflammation, and ischemia) free radicals induce oxidative damages(5).

Herbal medicines have been focused as a new source of antioxidants with limited complications. A group of well known plants which are used as spices and condiments belong to the Apiaceae family. The plants such as coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* L.), cow parsnip (*Heracleum persicum* L.), anise (*Pimpinella anisum* L.), are usually used to improve either the flavor or taste (6).

*E-mail: M.E.Azemi@ajums.ac.ir

Pimpinella barbata is an annual plant which grows in Khouzestan, Iran (7).
Although the measurement of the antioxidant capacity with various in vitro methods is a matter of growing interest, there is lack of official standard method for evaluation of antioxidant activity, thus it is recommended that each evaluation made with various oxidation conditions and different methods of measurement(8).
The aim of the present study was to evaluate the antioxidant activity and measure total phenolic content of n-hexane, dichloromethane, and methanolic extract of *P.barbata* by 1,1-diphenyl-2-picrylhydrazyl(DPPH), ferric-reducing antioxidant power(FRAP) and Folin-Ciocalteu methods which is based on different reaction mechanisms.

Materials and methods

Plant materials and chemicals
All the chemicals were of analytical grade. Solvents were purchased from Merck (Darmstadt, Germany). DPPH radical was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Plants were collected from Ramhormoz region, Iran in April 2008. The plants were identified at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Ahvaz, Iran where the voucher specimens were preserved.

Extraction and fractionation procedure
The aerial parts of plant(85g) were dried and powdered and were extracted with 80% methanol-water(80:20) for 6 hours by using soxhelet. After filtration and evaporation of the solvent under reduced pressure, the crude extract of *Pimpinella barbata* was partitioned successively with n-hexane, dicholoromethane.

DPPH radical scavenging test
The antioxidant capacity of the extracts (IC_{50}) were estimated and compared with ascorbic acid (positive control) using the stable DPPH radical. Briefly, a 23mg/ml solution of DPPH in methanol was prepared and its absorbance was measured at 517nm. DPPH, a purple-colored, stable free radical is reduced to the yellow-colored diphenylpicrylhydrazine when antioxidants are added. All samples were analyzed in triplets. The 50 µL of different concentrations of positive standard or the samples added and the absorption were measured after 4 minutes in a spectrophotometer.
The capability of scavenging DPPH radicals was calculated by the following equation:

Scavenging effect % = [1-(ABS. sample/ABS. control)]

DPPH solution was used as a blank (9,10). The Scavenging effect % (S.C%) was plotted against the sample concentrations and a logarithmic regression curve was established in order to calculate the IC_{50} value (mg/mL) which is the concentration of the extract that inhibited DPPH by 50% .

Measurement of total phenolic content
From the extracted material 500 mg of the extracts were dissolved in methanol to obtain the 50 mg/ml concentration which is the most concentrated solution. The concentration of 25, 12.5, 6.25 and 3.125 mg/ml were prepared by serial dilution for each extract. In this method gallic acid was used as standard. Briefly, 0.1ml of sample or the standard was added to sodium bicarbonate 2%w/v and then incubated for 2 minutes and then 0.1 ml from Folin- ciocaltue reagent was added and incubation was proceed for 30 minutes. At the end, the absorption of each concentration was measured at 750 nm (9).

Folin-Ciocalteu
Briefly, an aliquot (0.1 mL) of appropriately diluted extracts or Gallic acid were mixed with 2mL of sodium bicarbonate 2% and incubated in darkness for 2 minutes.Then, 0.1 ml of

Folin–Ciocalteu reagent was added and incubated in darkness for 30 minutes. The absorbance of the reaction mixtures was measured at 750nm. Gallic acid (GA) was used to prepare standard curve (0–50 mg/L). Measurements of every sample were taken in triplicate and the results
were expressed as milligram Gallic acid equivalents (GAE)/g dried weight of plant material (11).

Ferric-Reducing Antioxidant Power (FRAP) assay

The antioxidant capacity of plant extracts was performed according to the method of Benzie and Strain (12) with some modifications. The FRAP reagent included 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3$ solution and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10(v/v). Then, 50 µL of each diluted extracts were mixed with 3 mL of freshly prepared FRAP reagent and the reaction mixtures were incubated at 37 °C for 30 min.

Absorbance at 593 nm was determined against distilled water blank. Aqueous solutions of ferrous sulfate (100–2000 µM) were used for calibration. Triplicate measurements were taken and the FRAP values were expressed as mmol of Fe(II)/g dry weight of plant powder(13).

Results and discussion

The antioxidant power in DPPH assay and Folin-Ciocalteu method were evaluated by decending order as following: Methanolic extract > dichloromethane extract > n-hexane extract.

In FRAP test, dichloromethane extract was the most potent extract while the methanolic extract was the weakest one (Table 1).

After analysis of datas, we could find a nearly good correlation between Scavenging effect % (S.C%) and total phenolic contents for methanolic and n-hexane extracts ($p > 0.05$). The correlation between Scavenging effect % (S.C%) and total phenolic contents for methanolic and n-hexane extracts of Pinpinella barbata is show in Fig. 1.

However, there is no correlation between S.C% and total phenolic content in dicholoromethane extract. This result is compatible with other studies in which the results of total phenolic content did not support by other methods (6, 14, 15).

Eugenol could react with DPPH and this would result in falsely low readings for antioxidant capacity of samples containing eugenol and other phenols having a similar structure. On the other hand, it had been shown that eugenol was the main constituent in volatile oil of *P.barbata* (9, 16). Eugenol compounds form complexes with reduced metals and this could produce some errors in FRAP results (16).

In FRAP test, the absorbances are measured in the wavelength of 593_{nm} which is selected according to the maximum absorbance for Ferrous and TPTZ complex. Therefore, the probable presence of compounds which have any absorbance in this wavelength can affect the results of FRAP test. This can be also considered for other methods.

Table 1: Total phenolic content and antioxidant capacity of different extracts of *Pinpinella barbata* by different methods

Methods / Fractions	DPPH (IC_{50}) mg/ml	FRAP (EC_1) mmol Fe^{2+}/ L	Folin-Ciocaltue mgG.A/100g
CH_3OH	1.14	2.37	0.220
CH_2CL_2	1.87	1.18	0.015
n-hexane	5.20	1.305	0.014

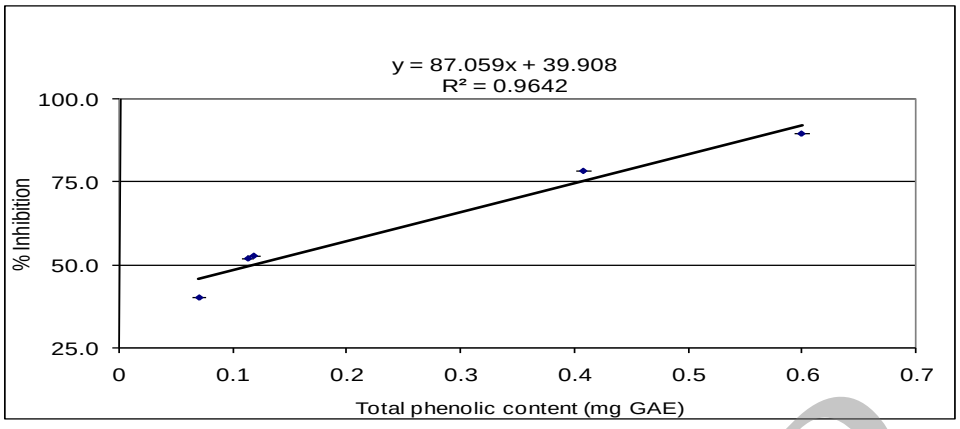

Fig. 1: Relationship between inhibition % of DPPH radical(or S.C%) and total phenolic content (mgG.A/100g) in the same concentrations of methanolic extract of *Pinpinella barbata.*

The extracts are complex mixtures of many compounds which can show different results by different methods due to number of phenolic groups, their locations and the effect of these compounds on each other. At last, we can affirm that different methods in antioxidant activity screening can not necessarily confirm each other.

Acknowledgment

This work was extracted from pharm. D. thesis and financially supported by Judishapur University of Medical Sciences, Ahvaz, Iran(Grant No. 88049).

References

1. Sies H, Stahl W, Sundquist AR, Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. Ann. N. Y. Acad. Sci. 1992; Sep 30; 669:7-20.
2. Lim YY, Murtijaya J, Antioxidant properties of *Phyllantus amurus* extract as affected by different drying method .L.W.T.; 2007.40:1664-1669.
3. Maryadele J, Neil O, The Merck index Editorial staff. USA: Merck. 2001; 9820, 3362.
4. Jacob R; The integrated Antioxidant System. Nur. Res. 1995; 15(5), 755-766.
5. Haliwell B, Chirico S, Lipid peroxidation: its mechanism, measurement and significance. J. Clin. Nut. 1993; 57: 715 – 725.
6. Nickavar B, Abolhasani FS, Screening of Antioxidant Properties of Seven Umbelliferae Fruits from IRAN. Pak. J. Pharm. Sci., 2009; Vol.22, No.1, 30-35.
7. Mozaffarian V., Flora of Khuzistane. Research center of Natural Resource and Husbandry of Khuzistane, 1999; Vol. 1.
8. Frankel EN, Meyer AS, The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants, Journal Science Food Agriculture. 2000; 80, 1925–1941.
9. Dejian H, Boxin O, Ronald L, The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 2005; 53: 1841-1856.
10. Li-Chang LU, Yue-Wen C, Cheng-Chun C, Antibacterial and DPPH Free Radical-scavenging Activities of the Ethanol Extract of *Propolis* Collected in Taiwan. Journal of Food and Drug Analysis, 2003; 11(4), 277-282.
11. Woraratphoku J, Intarapichet KO, Indrapichate A, Phenolic compounds and antioxidative properties of

selected Wines from the northeast of Thailand. Food Chemistry. 2007; 104, 1485-1490.
12. Benzie IFF, Strain JJ, The ferric reducing ability of plasma(FRAP**)** as a measure of "antioxidant power": The FRAP assay, Analytical Biochemistry1996.; 239 (1),70–76.
13. Heinonen M, Lehtonen PJ, Hopia A, Antioxidative activity of berry and fruit wines and liquor. Journal of Agricultural Food Chemistry, 1998. 46, 25-31.
14. Haiying L, Nongxue Q, Huihuang D, Ruiqi Y, Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Research International, 2008; 41(4), 363-370.
15. Nickavar B Kamalinejad M, Haj-yahha M, Shafaghi B, Comparison of the free radical scavenging activity of six Iranian *Achillea* species. Pharm. Biol. 2006; 44: 208-212.
16. Fakhari AR, *Sonboli A,* (2006). Essential Oil Composition of Pimpinella barbata (DC.) Boiss. From Iran; Journal of Essential Oil Research, 2006, 18(6), 679-681.
17. Murakami KIM, Yoshino M: Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. Food Chem Toxicol. 2005 Mar; 43(3):461-6.