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Evaluation of antioxidant potentials of extracts of cotton thistle (*Onopordum leptolepis* DC.) obtained by various solvents

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Abstract

Medicinal plants are valuable natural resources that recently have interested in developed countries and are considered as primary material to turn to drugs well thought-out safe for humans. Antioxidants may be defined as components that effectively prevent of reactive oxygen species (ROS) and result to reducing damage or cell death, cardiovascular diseases and cancers. The objective of this study was to investigate the antioxidant potential of extracts prepared by methanol (M), methanol 70% (H.A), hexane (Hex), and chloroform (Chl) from vegetative parts and flowers of *Onopordum leptolepis* (*O. leptolepis*). Antioxidant potential of the extracts was measured by ferric reducing antioxidant potential (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical methods. Results showed that the flowers had antioxidant activity lower than that of vegetative parts. The results also demonstrated that H.A was the best solvent to extract higher antioxidant components from flowers and vegetative parts of *O. leptolepis* in both of methods (DPPH and FRAP). However, the lowest antioxidant activity was related to extracts obtained by Hex. Moreover, the scavenging activity of DPPH free-radical increased by increasing concentration of *O. leptolepis* plant extracts from 100 to 750µg/ml in all of solvents used in this study.

Keywords: *Onopordon leptolepis,* Solvent extraction, antioxidant activity, DPPH radical scavenging, Ferric reducing activity.

Nomenclature:

BHT	Butylated hydroxytolune	Hex	Hexane
BHA	Butylated hydroxyanisole	H.A	Methanol 70%
TBHQ	Tertiary butylhydroquinone	Q	Quercetin
TPTZ	Tripyridyl-S-Triazine	DPPH	1,1-diphenyl-2-picrylhydrazyl radical
Chl	Chloroform	FRAP	Ferric reducing antioxidant potential
M	Methanol	ROS	Reactive oxygen species

Introduction

Onopordon (*Onopordon leptolepis DC.*) is a perennial plant belonging to *Asteraceae* family. There are seven species of this genus growing in semi-arid of Iran. The pink flowers, coarse inflorescence, spiny leaves and shoots, faveolate receptacle, and smoth achen, glabrous and almost tetrachen are typical characteristics of this genus (Drake, 2009). Onopordon is a valuable medicinal plant that is widely used in traditional medicine in Europe. The application of Onopordon as food is limited and its main importance is due to medicine utilization. Because of having flavonoid compositions, Onopordon is an important plant. The extract of this plant due to having antioxidant properties and anti-tumor effects was used as an excellent protective agent in formulation of drugs caring the skin (Joudi and Habibi, 2010). Most studies in biology and medicine fields are dedicated to radicals such as ROS (reactive oxygen species) (Mousavizadeh and Sedaghathoor, 2011). Many organisms for normal metabolic processes of cell such as phagocytosis, Disinflation, cell division, and collagen synthesis need to ROS (Culter, 1995; Poli and Parola, 1997). Nevertheless, considerable evidence presently exist claim that ROS cause oxidative damages on biomolecules (especially in proteins, lipids, and DNA). This in turn result to different clinical disorders including cardiovascular disease, aging and neurologic injuries such as Alzeimer disease, Parkinson disease, genetic mutations, and cancer (Cox and Cohen, 1996; Finkel and Holbrook, 2000). Living organisms have created antioxidant complex network to Neutralize ROS that are harmful to human life (Halliwell and Gutteridge, 1998; Yu et al., 2004; Pourtaghi et al., 2011). Several enzyme systems exist that can detoxify free radicals, for instance, superoxide dismutase containing copper and zinc, catalyze conversion superoxide anion to hydrogen peroxide and follow that hydrogen peroxide convert to water and oxygen by enzymes such as catalase and glutathione peroxides and is removed from the body environment. Furthermore, some antioxidant components found in foods can serve as a barrier against the damages arising from free radicals through preventing of radicals formation, absorb or accelerating destruction of free radicals (Ames, 1998; Abdollahi et al.,

2005). Antioxidants may be described as components that effectively and by different mechanisms prevent reaction of free radicals in form of active oxygen and nitrogen with biomolecules including proteins, amino acids, lipids, and DNA and result to reducing damage or cell death, cardiovascular diseases and cancers (Sharififar et al., 2007). Besides their role in biological systems, in foods which rich in unsaturated fats prohibit of nutritional quality and safety reduction, bad taste and colorless created by toxic compound. Antioxidants are classified in two natural and chemical groups. The later that are widely used in food industry, involve butylated hydroxytolune (BHT), butylated hydroxyanisole (BHA), TBHQ, and propel galat that carcinogenic and the negative effects of these complexes on human safety have been revealed (Kahl and Kappus, 1993). Hence, currently utilization of the widespread group of medicinal plants and theirs aromatic compounds has noticed by scientist as natural sources having Antioxidant property (Kulisic et al., 2004). Therefore, the objective of this study was to determine antioxidant activities of *Onopordum leptolepis DC.* extracts obtained from the different solvents in order to find new potential sources of natural antioxidants.

Results

DPPH assay of O. leptolepis vegetative bodies and flowers extracts antioxidant activity

Results obtained from antioxidant activity evaluation of vegetative bodies extracts by different solvents using DPPH free-radical reduction method in four different concentrations (100µg/ml, 250 µg/ml, 500 µg/ml, and 750 µg/ml) have been shown in Fig. 1. As depicted in this figure, the extract obtained by H.A solvent possessed the highest antioxidant activity, with 45.5-74.7% of inhibition of DPPH radical. The M-extract had the second highest activity (23.4-49.87% of inhibition of DPPH radical), followed by the Chl extract (13.11-17.88% of DPPH radical inhibition). Among the four studied solvents, Hex extract of vegetative parts showed the lowest scavenging activity compared to quercetin (as standard blank) in all of the concentrations (9.5-14.4%). Results obtained of antioxidant activity evaluation of flowers extracts by DPPH free-radical reduction method in four different concentrations (100µg/ml, 250 µg/ml, 500 µg/ml, and 750 µg/ml) have been shown in Fig. 2. Among the tested extracts, Hex (7.77-10.16%) and Chl (11.12-15.34%) extracts of flowers did poorly in scavenging activity as compared to quercetin and was not affected by increasing concentrations. Conversely, H.A had high efficiency in DPPH radical scavenging (40.5-53.76%) and with increasing concentration of the extract, yhe antioxidant activity increasedas concentration was more, the antioxidant activity made better too. Similar to vegetative parts, the M-extract was useful in DPPH radical scavenging activity and its antioxidant activity also developed by increasing concentration. Generally, the antioxidant activity of flower was less in comparison to vegetative parts.

FRAP antioxidant activity of flower and vegetative bodies extracts of O. Leptolepis

FRAP antioxidant activity of flower and vegetative bodies extracts of *O. leptolepis* (250 µg/ml) are presented in Figs. 3 and 4. Based on the results observed (Figs.3 and 4), as DPPH method, the highest antioxidant activity among tested extracts was observed in H.A with 285.05 and 246 mmol Fe^{2+}/L for extracts of flower and vegetative parts, respectively. The M-extract compared to Chl and Hex extracts had higher activity. FRAP antioxidant activity of the obtained extracts flower and vegetative bodies obtained from Chl was 120 and 181.71 mmol Fe^{2+}/L, respectively, while the corresponding values for Hex were 115 and 174.49 mmol Fe^{2+}/L, respectively. Thus, Chl and Hex extracts had activity approximately the same as each other (Fig. 3). Furthermore, the antioxidant activity of flower reported lower than vegetative parts.

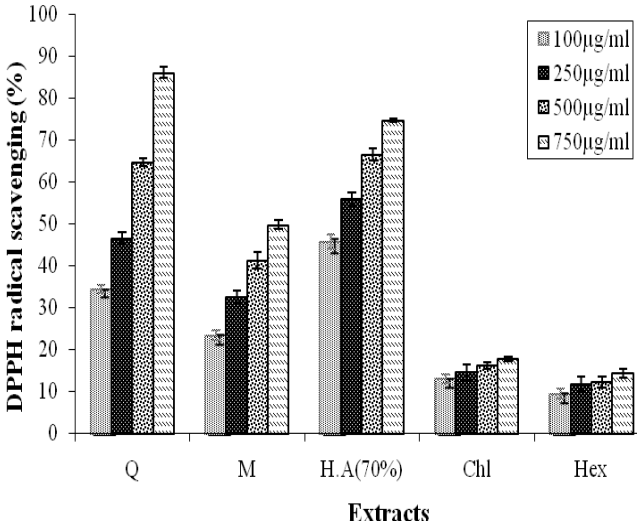

Fig 1. Comparison of DPPH free radical scavenging of different concentrations of O. *Leptolepis* vegetative bodies extracts.

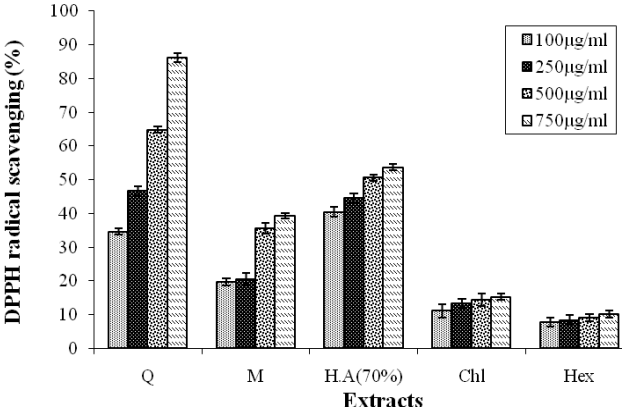

Fig 2. Comparison of DPPH free radical scavenging of different concentrations flower.

Discussion

Natural antioxidants are able to remove free radicals prior to disorder in oxidation chain reactions of membrane or parts of cell involved lipid. Collecting the reactor radical species has a significant effect on stability of vulnerable cell compounds and provides bodies' cell and tissue of safety. As a matter of fact, on occasion perform of antioxidants to prohibit of oxidization reactions guarantee the present security. Presently, medicinal plants consider as a one of the major sources of antioxidants and have superior position clinical researches (Sharififar et al., 2007). DPPH scavenging activity by different concentrations of flower and vegetative parts

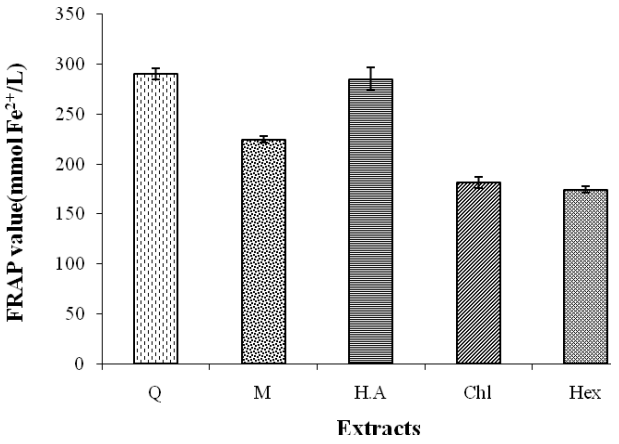

Fig 3. FRAP antioxidant potential assay of vegetative parts extract of O. *Leptolepis.*

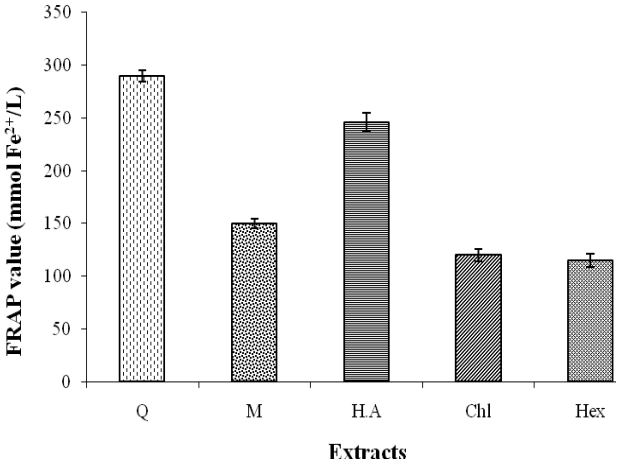

Fig 4. FRAP antioxidant potential assay of flower parts extract of O. *Leptolepis.*

extracts of O. *leptolepis* have been shown in Figs. 1 and 2. The percentage of scavenging activity by non- polar extracts (Hex and Chl) of vegetative parts and flower of O. *leptolepis* were insignificant compared to quercetin. Whilst, H.A and M extracts were efficiency successful in removal DPPH free radical in term of concentration. This fact is probably due to polar nature of antioxidant components in the studied plant parts of O. *leptolepis*. It seems that hydrophilic compounds are responsible for antioxidant activity because of high antioxidant ability of polar extracts than non polar one (Nazanin and Hassan, 2009). Also, Dehghan et al. (2007) showed that the extracts obtained by polar solvents specially methanol showed the greatest activity against DPPH values.

The FRAP values also depicted for extracts obtained by the different solvents from flower and vegetative parts of O. *leptolepis* in Figs. 3 and 4. As showed in Figs. 3 and 4, Hex and Chl extracts had approximately the same potentials to reducing Fe^{3+} to Fe^{2+}. Also, the FRAP value of H.A extracts indicated the highest among all solvents. These results showed that antioxidant activity of extracts increased in term of solvent applied to extraction had high polarity. In other words, the polar extracts showed the maximum reduction activity. Studies have demonstrated that most phenol compounds as strong antioxidant sources having ability to quench free radicals in plant extracts obtained by polar solvents (Benzie and Strain, 1996; Nazanin and Hassan, 2009). Therefore, the high antioxidant ability in extracts obtained by polar solvents can be attributed to the existence of phenol structures (Nazanin and Hassan, 2009). Dehghan et al. (2007) also found that the higher antioxidant potential of the methanol extract of *Ferula szovitsiana* indicates that most of the active constituents of this plant are polar phenolic components. At low concentrations of phenol compounds or other antioxidants, the breakdown of chain reactions of free radicals is determined as main mechanism and it appears that phenol consider compounds of plants being very active and as a result these compound undertake this work (Bektas et al., 2006). Results entirely displayed that M-extracts of vegetative parts and flower of O. *leptolepis* in both DPPH and FRAP had high antioxidant properties, but the flower extracts exhibited low antioxidant activity in comparison to vegetative parts.

Materials and methods

Plant material and chemicals

The plants (*Onopordum leptolepis* DC.) were collected from surrounding a village named Benis located in shabestar city (by the longitude of 45˚05′ E, latitude of N 38˚42′ and 1413 m in elevation) Tabriz state, Iran. The plant was identified and authenticated by the herbarium of Tabriz University Faculty, and then plants dried in vitro and was prepared for extraction (Nazanin and Hassan, 2009).

Chemicals and solvents used in this study were included 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, chloriderich acid, sodium acetate, acetic acid glacial, ferric chloride, ferrous sulfate, Tripyridyl-S-Triazine (TPTZ), querstine, hexane, methanol, chloroform. All the chemicals including solvents were of analytical grade and were supplied from Merck Co, from German.

Preparation of extract

After drying the plant under laboratory conditions, the vegetative bodies and flowers of *O. leptolepis* were separately ground into powder and prepared for extraction. Presently, several methods are applied for extraction from plants selected soaking one among them. Four extracts including methanol (M), methanol 70% (H.A), hexane (Hex), and chloroform (Chl) were prepared of the vegetative bodies and flowers of *O. leptolepis* and were soaked for 3 days at 4 °C.

Evaluation of antioxidant activity

DPPH free-radical scavenging activity assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is an unstable free radical that can receive an electron or hydrogen radical, and becomes a steady state. The presence of antioxidant compounds having DPPH free-radical scavenging determine by monitoring continuously the decrease of absorption at 517 nm. (Jimenez-Escrig et al., 2000). As a freshly prepared DPPH solution shows a deep purple color with absorption maximum at 517 nm. This color gradually disappears when an antioxidant is present in the medium. Therefore, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule)

and convert them to a colorless/bleached product, resulting in a decrease in absorbance at 517 nm. To determine the scavenging activity of extracts, four different concentrations (100µg/ml, 250 µg/ml, 500 µg/ml, and 750 µg/ml) obtained from plant extracts of *O. leptolepis* were added to DPPH solution (2 ml). Then absorbance value at 517 nm was measured after 5 min of mixing using a UV-visible spectrophotometer (DR/4000U-HACH, USA). Quercetin as an antioxidant combination was used as standard blank of this experiment. The assays were carried out in triplicate. The percentage inhibition of DPPH by antioxidant compounds of *O. leptolepis* plant extracts was calculated according to the following equation:

$$\%\,\text{Inhibition of DPPH} = (1 - A_c / A_d) \times 100$$

where A_c is the absorbance of the solution when the extract has been added at a particular level, and A_d is the absorbance of the DPPH solution.

Ferric reducing antioxidant potential (FRAP) method

The method was conducted in buffer containing

This method is based on made of a buffer contained TPTZ (Tripyridyl-S-Triazine), ferric chloride, and acetate buffer. Antioxidants having an ability to reducing Fe^{3+} to Fe^{2+}, cause conversion of colorless complex of TPTZ-Fe^{3+} to blue complex of Fe^{+2} TPTZ-Fe which its severity is measured at 593 nm (Iris et al., 1999; Benzie and Strain,1996). Briefly, 250 µg/ml of plant different extracts were taken of vegetative bodies and flowers and then 2 ml of FRAP solution contained 10 Mm TPTZ (was prepared in 40 mM of HCl), 20 Mm of $FeCl_2$ and 300 Mm of estate buffer (PH =3.6), was added to final volume. The resulting sample was then left to stand at 37°C for 10 min and color intensity was detected at 593nm against a blank. To draw standard curve in FRAP method different concentrations of ferrous sulfate (125µM, 250 µM, 500 µM, 1000 µM) were used and the antioxidant activity of extracts were presented in term of mM of Fe^{2+}. Quercetin as a standard blank was applied in this experiment.

Conclusion

The objective of this investigation was to determine anti-oxidative and radical scavenging activity of organic extracts of the flowers and vegetative parts of O. *leptolepis* with evaluation of FRAP and DPPH radical scavenging activities. Generally, non polar solvents had lower antioxidant activity than that of polar solvents such M and H.A. In addition, the extracts of vegetative parts were more antioxidant activity than the extracts obtained from flowers of O. *leptolepis.* According to these findings, the H.A-extract obtained from vegetative bodies had the highest antioxidant potential in both of methods (DPPH and FRAP). In conclusion, to use of this plant in food and medicinal industry with regard to its high antioxidant potentials, it is suggested that complement experiments to accomplish on extracts of vegetative parts and flower of *O. leptolepis*.

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