A NEW SOURCE OF NATURAL ANTIOXIDANTS FROM TURKEY: *LINARIA GENISTIFOLIA* SUBSP. *GENISTIFOLIA*

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Abstract: The antioxidant properties of different solvent extracts (acetone, methanol and water) from *Linaria genistifolia* subsp. *genistifolia* were investigated. Antioxidant properties were evaluated by different methods including free radical scavenging (DPPH and ABTS), reducing power (CUPRAC and FRAP), phosphomolybdenum, β-carotene/linoleic acid and metal chelating assays. Also, total phenolic and flavonoid content were determined for each extracts. Generally, the acetone and methanol extracts exhibited strong antioxidant abilities with higher level of phenolics (40.17 mgGAEs/g for acetone and 28.18 mgGAEs/g for methanol). All extracts had remarkable inhibition abilities of linoleic acid oxidation (84.88% for acetone, 77.22% for methanol and 63.04 for water). The results indicate that *Linaria genistifolia* subsp. *genistifolia* could be considered as a source of natural antioxidant for preparing new food ingredients and pharmaceutical formulations.

Key words: Antioxidants, Phenolics, *Linaria*, Turkey

Introduction

Free radicals and their interactions in biology has become an area of intense interest. Free radicals have unpaired electron (s) in atomic orbital and thus they have high chemical reactivity. In this direction, a balance between free radicals and endogenous antioxidant system is necessary for normal physiological function. Overproduction of free radicals is known to be "oxidative stress" and is responsible for man chronic and degenerative diseases including cancer, cardiovascular diseases and Alzheimer's diseases (Devasagayam et al., 2004). At this point, dietary antioxidant can be assist in coping oxidative stress. For this purpose, many synthetic antioxidants are produce but they have unfavorable effects (carcinogenic or hepatotoxic) for human health (Carocho and Ferreira, 2013). Thus, the investigation of new, natural and safe sources of antioxidants is one of the most important subjects in the scientific area.

Linaria is an important genus belonging to the family Plantaginaceae (until recently included in the family Scrophulariaceae). The genus comprises about 200 species mainly distributed in Europe, Asia and North Africa (Handjieva et al. 1993), In Turkey, the genus is represented by 30 taxa (Davis, 1978). The *Linaria* species are traditionally used as several purposes such as tonic, antidiabetics and wound healing in different countries including Turkey (Akkol and Ercil, 2009; Cheiret et al., 2015). In this direction, many chemical and biological studies were performed on *Linaria* (Sokolowska-Wozniak et al. 2003; Tundis et al., 2005; Kouichi et al. 2011). However,

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to the best of our knowledge, the antioxidant properties of *Linaria genistifolia* subsp. *genistifolia* have not been reported. Therefore, this study was perform to determine the effect of different extraction solvents (acetone, methanol and water) on the total phenolics, flavonoids, antioxidant, potentials of *Linaria genistifolia* subsp. *genistifolia*.

Material and methods

Linaria genistifolia subsp. *genistifolia* was collected from Basarakavak-Altinapa road, Konya-Turkey when flowering season (July 2014). Taxonomic identification of the plant material was confirmed by the senior taxonomist Dr. Murad Aydin Sanda. The voucher specimen was deposited at the KNYA Herbarium of Department of Biology, Selcuk University, Konya-Turkey. The plant materials were dried at the room temperature. The dried aerial parts were ground to a fine powder using a laboratory mill. The powdered samples (10 g) were separately extracted with acetone and methanol in a Soxhlet apparatus for 6-8 h. The extracts concentrated under vacuum at 40 °C by using a rotary evaporator. To obtain water extracts, the powdered samples were boiled with 250 mL of distilled water for 30 min. The aqueous extracts were filtered and lyophilized (-80°C, 48 h). Extracts were stored at + 4°C in dark until use.

The total phenolics content was determined by reported method with slight modification and expressed as gallic acid equivalents (GAEs/g extract), while total flavonoids content was determined by reported method with slightly modification and expressed as rutin equivalents (REs/g extract) (Zengin et al., 2014).

The antioxidant activity was evaluated by phosphomolybdenum and β-carotene bleaching methods. Radical scavenging activities, measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS), were expressed as trolox equivalents (TEs/g extract). The reducing power measured using cupric ion reducing (CUPRAC) and ferric ion reducing antioxidant power (FRAP). Metal chelating activity on ferrous ions was expressed as EDTA equivalents (EDTAEs/g extract) (Zengin et al., 2014).

Results and discussion

Phenolic compounds are known to be the most effective biological agents including antioxidant and antimicrobial. The present study identified total phenolics and flavonoids contained extracts of *L. genistifolia* subsp. *genistifolia* using the Folin-Ciocalteu and $AlCl_3$ assays, respectively. The highest phenolics was found in acetone extract with 40.17 mgGAEs/g followed by methanol and water. Interestingly, the highest level of flavonoids was observed in methanol extract (78.67 mgREs/g) (Table 1). Similar observations were shown by several authors (Sowndhararajan and Kang, 2013; Hossain et al., 2014). Also, some authors suggested that methanol is a good solvent for flavonoids (Alam et al., 2010).

Free radical scavenging activities of the extracts were determined by DPPH and ABTS assays. DPPH is the most common radical for evaluating free radical scavenging activity. These assays also reflected electron-donation ability of the tested extracts. The results are given in Table 1. The methanol extract exhibited the highest scavenging

ability on both DPPH (35.80 mgTEs/g) and ABTS (61.23 mgTEs/g), followed by acetone and water. In accordance with our results, methanol extract obtained from different plants had remarkable free radical scavenging effects (Nagmoti et al., 2012; Pavithra and Vadivukkarasi, 2015)

Assay to measure reducing power of *Linaria* extracts may serve as a significant index of their potential antioxidant activity. For this purpose, CUPRAC and FRAP assay is used to evaluate reducing power of the studied extracts. Also, these assays are reflected to hydrogen-donating abilities of these extracts. As can be seen in Table 1, methanol and acetone extracts showed the most effective reducing ability, while water extract had the lowest the reducing effects. The methanol and acetone extracts containe the highest amount of phenolics. In this direction, the observed strong reducing abilities may be explained with the higher level of phenolics in these extracts. Similarly, many authors reported that a strong correlation between total phenolics and reducing abilities (Sarikurkcu et al., 2014; Liu et al., 2015).

Table 1. Antioxidant properties of Linaria genistifolia subsp. *genistifolia* (mean±SD)

Extracts	Total phenolics (mgGAEs/g extract)	Total flavonoids (mgREs/g extract)	Phosphomolybdenum (mmolTEs/g extract)	DPPH scavenging activity (mgTEs/g extract)	ABTS scavenging activity (mgTEs/g extract)	CUPRAC (mgTEs/g extract)	FRAP (mgTEs/g extract)	Metal chelating effects (mgEDTAEs/g extract)
Acetone	40.17±0.65	nd	1.92±0.14	25.93±1.82	42.62±1.52	87.38±2.69	35.27±1.42	36.65±2.00
Methanol	28.18±0.25	78.67±0.76	3.10±0.02	35.80±2.19	61.23±2.44	58.32±0.66	36.85±1.11	27.73±2.00
Water	19.07±0.53	9.90±0.25	2.01±0.02	20.62±0.79	52.51±1.31	43.00±1.07	34.13±0.55	25.98±0.43

GAE: gallic acid equivalents; RE: Rutin equivalents; TE: Trolox equivalents; EDTAE: EDTA equivalents; nd: not detected

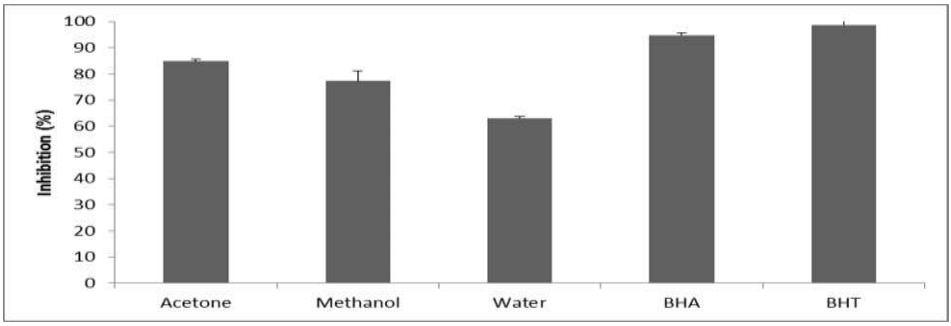

Figure 1. Inhibition values of the extracts and synthetic antioxidants (mean±SD; at 2 mg/ml concentration)

The phosphomolybdenum and β-carotene/linoleic acid assays are used to evaluate total antioxidant capacities of plant extracts. In phosphomolybdenum assays, Mo (VI) is reduced to Mo (V) and form a green colored phosphate/Mo (V) complex in the presence of antioxidants. The methanol extract show maximum activity (3.10 mmolTEs/g) while acetone extract was the lowest (1.92 mmolTEs/g). β-carotene/linoleic acid assay was perfomed to evaluate the ability of the extracts for linoleic acid oxidation. Inhibition

values (%) for extracts and synthetic antioxidants (BHA and BHT) are shown in Figure 1. The acetone extract was able to inhibit linoleic acid oxidation with 84.88% lower than that of BHA (94.83%) and BHT (98.69 %). The methanol extract ranked second in order of inhibition activity with 77.22 %. The observed highest activity for the acetone and methanol extracts may be explained with the higher levels of phenolics.

Iron is known as the most important pro-oxidant in lipid peroxidation. For this reason, the chelating ability can be considered as an important mechanism. We therefore assed the ferrous ion chelating capacity of the extracts by measuring Fe^{+2}-ferroxine test system. The results are expressed as EDTA equivalents. From Table 1, it is clear that chelating power of acetone extract were higher as compared to the other two extracts. The highest chelating ability for acetone extract may be result from the higher level of phenolics. Likewise, several authors were found that a linear correlation between metal chelating effect and total phenolic content (Ozsoy et al., 2009; Liu et al., 2015). However, Rice-Evans et al. (1996) was reported that metal chelating ability of phenolics is a minor role in their antioxidant effect.

Conclusion

The present study on different extracts *Linaria genistifolia* subsp. *genistifolia* indicate that acetone and methanol extracts are rich sources of antioxidant, with significant level of phenolics. The acetone and methanol extracts possess notable antioxidant properties including free radical scavenging, reducing power and metal chelating. Results of the present study suggested that the extracts from *L. genistifolia* subsp. *genistifolia* can be exploited as a source of natural antioxidants in the food and pharmaceutical area. Finally, further investigations are need to test in vivo biological effects of *L. genistifolia* subsp. *genistifolia* on human health.

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