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Original article

Evaluation of total antioxidant status, total oxidant status and oxidative stress index of some economically important plants from Turkey

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Summary. This study aims to develop antioxidant properties of thirty four economically important plants of Asteraceae and Lamiaceae families, from Turkey. Analyses were done with reliable measurement research kits. Total antioxidant status, total oxidant status were determined with a novel method using available enzyme-linked immuno assay kit (TOS Cat. No: RL0024, TAS Cat. No: RL0017).Total antioxidant (TAS) level of studied Asteraceae and Lamiaceae taxa were detected as 71.61±37.05 mmol/L and 36.97±9.6 mmol/L, respectively. In addition, TAS level of Asteraceae taxa was found to be higher ($p<0.38$) than Lamiaceae taxa. When compared total oxidant (TOS) level of Asteraceae (3.02±0.66) and Lamiaceae taxa (5.26±1.06), no significant ($p=0.08$) differences were observed. In the similar way OSI levels in the Asteraceae (12.52±3.63 AU) Lamiaceae families (30.8±8.4 AU) were found to be no significant difference ($p=0.05$). The present study concluded that antioxidative feature of studied plant taxa is remarkable. So studied plant taxa are emerges a natural source of antioxidants and could be a good experimental system for further researches.

Key words: antioxidant, asteraceae, lamiaceae, oxidative stres, oxidant, plant

Introduction

Turkey is regarded as an significant gene-centre for the Lamiaceae family; most aromatic and medicinal plants are in the Lamiaceae family, are used as herbal tea, herbs, spices, folk medicines and a source of fragrance in Turkey (1, 2). Moreover, most of Lamiaceae taxa have great importance due to their economic values; this family is represented about 258 genera and 3500 species in the world (3); and it is represented about 46 genera and 763 taxa exists in Flora of Turkey (4-6). Asteraceae also is one of the largest plant family and many genera and species have worldwide distribution comprising many useful plants and it contains about 25.000 taxa in the World (7). Many members of Asteraceae have traditionally been used in balsams, cosmetics, dyes, insecticides, anti-helmintic for migraine, neuralgia, rheumatism and they are important for medicinal, ornamental, economic purposes (8-12). Free radicals play significant role in the development of tissue damage in various human diseases such as cancer, aging, neurodegenerative disease, malaria, arteriosclerosis and pathological events in living organisms (13). Antioxidants may have an important role in the prevention of these diseases; so there is an increasing interest antioxidant compounds derived from plants (14). Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids (15). In addition, antioxidants are added to nutrients to prohibit deterioration in their taste, colour and smell (16); the fruits, vegetables and aromatic plants includes such components more and supplementation of human diet with herbs, containing especially high amounts of compounds capable of deactivating free radicals (16, 17). In Lamiaceae and Asteraceae there are many medicinal and aromatic

plants and they are promising and diverse sources of natural antioxidants. Therefore, a great number of different plant taxa and aromatic herbs have been investigated for their antioxidant activity. Some *Salvia*, *Origanum* and *Thymus* taxa have rich essential oil content and have been found to be very effective with regard to natural antioxidants (18-20). Antioxidants are classified into two major categories, natural and synthetic; natural antioxidants are expensive, so the use of synthetic antioxidants are more common. Some synthetic antioxidants, are used in foods and have many side and toxic effects such as mutagenesis carcinogenic in human beings (21). So, researchers have focused their researches on plant-derived natural antioxidants (22); natural antioxidants are safe and also bioactive; among the various natural products, phenolic compounds have anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities (23, 24).

In this study, 34 wild plant taxa of Asteraceae (13 taxa) and Lamiaceae (21 taxa) from Turkey were studied. With this research we aimed to determine with in vitro analyses average total antioxidant status, total oxidant status and oxidative stress index of selected economically important plants, that might be helpful potential usefulness, biological activities and other phytochemical studies of these plants.

Material and Methods

Collection of plant materials

Studied plants and voucher specimens number (parentheses) of plant samples are like; *Inula oculus-christii* (5342), *Inula graveolens* (5944), *Achillea vermicularis* (5184), *Serratula cerinthifolia* (4981), *Centaurea depressa* (5122), *Taraxacum montanum* (5516), *Anthemis tinctoria* var. *tinctoria* (5884), *Senecio vernalis* (5534), *Tanacetum parthenium* (5840), *Tanacetum heterotomum* (5138), *Tanacetum abrotanifolium* (5246), *Tanacetum densum* subsp. *amani* (5194), *Tanacetum zahlbruckneri* (5671); *Stachys ramosissima* var. *ramosissima* (5305), *Stachys mardinensis* (5165), *Stachys lavandulifolia* var. *lavandulifolia* (5843), *Nepeta fissa* (5347), *Nepeta nuda* subsp. *lydiae* (5760),

Scutellaria orientalis subsp. *bicolor* (5348), *Prunella vulgaris* (5204), *Lycopus europaeus* (5520), *Thymus kotschyanus* var. *kotschyanus* (4992), *Marrubium astracanicum* subsp. *astracanicum* (5074), *Sideritis taurica* (5649), *Sideritis montana* subsp. *montana* (5076), *Teucrium chamaedrys* (5725), *Ziziphora taurica* (4862), *Ziziphora capitata* (4717), *Origanum acutidens* (4985), *Origanum vulgare* subsp. *gracile* (5368), *Salvia brachyantha* (5681), *Salvia aethiopis* (4780), *Salvia multicaulis* (5527), *Salvia trichoclada* (4938). All plant taxa were collected between May-July months of 2014-2015 years by O.Kilic from vicinity of Dikme Village (Bingöl-Turkey), plants are collected around geographical coordination, $38^0\ 54'\ 56.16''$ K and $40^0\ 18'\ 36.40''$ D., an altitude of 1500-1800 meters. Plants identificatied by plant taxonomist O. Kilic and voucher specimens are deposited in the Park-Garden Plants Department of Technical Vocational college (Bingol University). Studied plants are listed in Table 1, 2. Dried leaves of plants were used for analysis.

Preparation of the plant extracts

The plate was then read at 530 (TOS) and 660 (TAS) nm with a Spectramax Plus384 plate reader (Molecular Devices LLC, Sunnyvale, CA). Sample preparation: dried leaves of plant materials (1 g) were cut into small pieces and homogenized with 10 ml water. The sonicated homogenate was filtered through four layers and centrifuged at 1000 g for 10 min. The supernatant was collected and used directly for the TAS, TOS (24). The method applied to determine TAS and TOS in studied plant materials were valid for this research.

Total antioxidant and oxidant status

Erel method has been used to determine total antioxidant (TAS) levels. Biochemical analyses of total antioxidant status of plant samples were evaluated using commercially available kits (Rel Assay, Diagnostics Turkey). The new automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation by antioxidants. In this assay, antioxidative effect of the sample over the potent free radical reactions started by the produced hydroxyl radical is calculated. The assay has excellent precision values and obtained results were expressed as mmol Trolox equivalent/L (25). Total antioxidant

Table 1. Antioxidative-oxidative and oxidative stress parameters of studied Asteraceae taxa

Plant Taxa	TAS (mmol/L)	TOS (μmol/L)	OSI (AU)
Inula oculus christii	19.94	1.34	6.72
Inula graveolens	17.6	2.09	11.9
Achillea vermicularis	461.48	2	0.43
Serratula cerinthifolia	17.96	1.4	7.79
Centaurea depressa	35.06	1.44	4.1
Taraxacum montanum	73.47	3.33	4.53
Anthemis tinctoria var. tinctoria	18.01	1.12	6.26
Senecio vernalis	7.15	2.65	37.12
Tanacetum parthenium	142.48	7.24	5.08
Tanacetum heterotomum	26.38	1.49	5.6
Tanacetum abrotanifolium	142.48	7.24	5.08
Tanacetum densum subsp. *amani*	22.98	7.69	33.45
Tanacetum zahlbruckneri	16.69	4.56	27.32

Table 2. Antioxidative-oxidative and oxidative stress parameters of studied Lamiaceae taxa

Plant Taxa	TAS (mmol/L)	TOS (μmol/L)	OSI (AU)
Stachys ramosissima var. *ramosissima*	11.77	4.05	34.45
Stachys mardinensis	23.74	14.45	60.86
Stachys lavandulifolia var. *lavandulifolia*	20.09	1.57	7.81
Nepete fissa	14.66	5.77	39.36
Nepeta nuda subsp. *lydiae*	19.18	9	46.96
Scutellaria orientalis subsp. *bicolor*	11.21	13.46	120.08
Prunella vulgaris	7.15	2.3	32.17
Lycopus europaeus	9.94	2.8	5.99
Thymus kotschyanus var. *kotschyanus*	46.68	1.98	4.25
Marrubium astracanicum subsp. *astracanicum*	14.15	8.7	61.45
Sideritis taurica	173.27	2.78	1.6
Sideritis montana subsp. *montana*	138.92	17.98	12.94
Teucrium chamaedrys	31.71	2.16	6.83
Ziziphora taurica	49.21	2.74	5.57
Ziziphora capitata	*79.61*	*3.65*	*4.59*
Origanum acutidens	*10.7*	*2.74*	*25.64*
Origanum vulgare subsp. *gracile*	*5.48*	*7.78*	*142*
Salvia brachyantha	*46.58*	*1.19*	*2.57*
Salvia aethiopis	*14.86*	*3.26*	*21.96*
Salvia multicaulis	*11.01*	*0.56*	*5.08*
Salvia trichoclada	*36.53*	*1.72*	*4.72*

status was calculated according to the following formula; TAS: ((ΔAbs H_2O) _ (ΔAbs Sample)) / ((ΔAbs H_2O) _ (ΔAbs Standart)). Erel method has been used to measurement of plant exstract total oxidant status levels (Erel, 2005). Total antioxidant status of natural bee products were measured using commercially available kits (Rel Assay Diagnostics, Turkey). In the assay, ferrous ion solution, which is present in the Reagent 1, is mixed with hydrogen peroxide, which ispresent in Reagent 2. Oxidants present in the sample

oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the samples. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (26). Total oxidant status value was calculated according to the following formula; TOS: (ΔAbsSample)/(ΔbsStandard) X Conc. of standard

Oxidative stress index

The oxidative stress index (OSI) of plant samples were determined with the ratio of TOS to TAS. The OSI value was calculated according to the following formula; OSI (arbitrary unit)=TOS (μmol H_2O_2 equivalent/L)/TAS (mmol Trolox equivalent/L)×100 (25). Values are expressed as means±SEM. The Kolmogorov– Smirnov Z test showed that the data were normally distributed. Unpaired t-test used to assess between-group data. A mean difference was significant at the 0.05 level.

Results and Discussion

Total antioxidant (TAS) level of studied Asteraceae and Lamiaceae taxa were detected as 71.61±37.05 mmol/L and 36.97±9.6 mmol/L, respectively. In addition, TAS level of Asteraceae family was found to be higher ($p<0.38$) than Lamiaceae taxa. When compared total oxidant (TOS) level of Asteraceae (3.02±0.66) and Lamiaceae taxa (5.26±1.06), no significant ($p=0.08$) difference was observed. Antioxidant compounds found in plants can be isolated using different polarity and different solvents; in extraction process water, ethanol, methanol and acetone solvents are more uses (27). In this study, water was used as solvent for the extracts. A number of methods has been proposed to determine antioxidant activity. In this study, total antioxidant status, total oxidant status and oxidative stress index were determined. There is no accepted reference method to determined oxidant status, but in the devised method of Erel (2004), dianisidine is used instead of benzoate and the suppression of oxidation reaction by the sample monitored by following the change of absorbance of the dianisidyl radical instead of the measurement of the release of thiobarbituric acid reactive substances which are released from the oxidized benzoate. By using this method, the steps in the process were decreased, the assay period was shortened, the requirement of boiling of sample was eliminated, and fully automated measurement was easily performed by using an automated analyzer (26). In this research, our results have demonstrated that Lamiaceae family's TAS levels, were lower than Asteraceae family's TAS levels.

Achillea vermicularis showed the highest activity; various species of the *Achillea* are used in wound healing; abdominal pain; stomachache; symptomatic relief of colds, ulcer, and diarrhea; as diuretic; appetizer; carminative; insecticidal agent and ethnomedicinal uses (28-31). Some veterinary use and antioxidant effect of *Achillea* taxa were also reported for many other regions in the world (32). Besides medical applications, plants are used as spices and additives in food products, while essential oil and extracts of some species are used for preparation of digestive teas, cosmetic products and used in gardening or as cutflowers (33). The genus *Achillea* is rich in terpenoids and flavonoids, which are possible bioactive compounds and monoterpenes were reported to be the major constituents essential oil of the genus (34). In studied Asteraceae family, *Tanacetum densum* subsp. *amani* showed the highest TAS activity and *Senecio vernalis* showed the highest OSI activity (Table 1). The OSI reflects the redox balance between oxidation and antioxidation as determined from the total antioxidant status and total oxidant status (35). *Tanacetum* spp. are rich in essential oils, sesquiterpene lactones and bitter substances and they have antiinflammatory, antibacterial and antihistaminic activities; in moderate doses, tansy essential oils are stomachic, cordial effect and used as a food additive (36, 37). Some *Tanacetum* taxa have been cultured in gardens and used in salads, omelets, cakes, dyes, medicines, cosmetics and preservatives as herbal cure; in addition some members of *Tanacetum* have also been used as antioxidant,

antiinflammatory, antibacterial and antifungal activities (38). Literature reports on the phytochemistry of *Senecio* species shows a large variety of pyrrolizidine alkaloids, sesquiterpenoids, diterpenoids, triterpenoids, shikimic acid cacalolide derivatives and essential oils (39); furthermore, biological activities such as antibacterial molluscicidal, antimicrobial, cytotoxic activities and biosynthesis of algal pheromones have been reported for these plants (40). Also in traditional medicine, the use of *Senecio* species for bronchitis, digestive, asthma and eczema have been reported (41). Moreover, the genus *Senecio* contains species that are highly toxic, while others are used in traditional medicine as antiemetic, antiinflammatory, vasodilator and for the treatment of wounds (42). *Inula* is a large genus and it comprises several species of reputed medicinal value (43).

In Lamiaceae family, *Sideritis montana* subsp. *montana* showed the highest TAS and TOS activity; and *Scutellaria orientalis* subsp. *bicolor* showed the highest OSI activity (Table 2); *Sideritis* has an important place among the other Lamiaceae genera because of the high percentage of endemism and used as herbal tea and folk medicine in Turkey (44). Many *Sideritis* taxa and their chemical constituents have been reported to have analgesic, anti-inflammatory, antiulcer, antioxidant, antimicrobial effects (45); in addition infusion aerial parts of *Sideritis* taxa are used as tonics, carminatives, antispasmodics, diuretics, digestives and in the treatment of colds (46). Also *S. montana* subsp. *montana* showed highest TAS activity (Table 2). Kilic et. al., (2016) reported that, pollen types of Fabaceae, Asteraceae and Lamiaceae plant taxa were the most abundant among the samples from the Hizan district of Bitlis province, eastern region of Turkey (47). In another study, 61 wild plant taxa that are used as medicine are documented in Keban (Elazığ); the most encountered medicinal plant families were Lamiaceae (10 taxa), Asteraceae (8 taxa), so Lamiaceae and Asteraceae taxa are widely used in ethnobotany from Turkey (29). In recent years, phytochemical investigations on different *Stachys* taxa have shown that extracts or isolated constituents of *Stachys* taxa exert various pharmacological effects, such as anti-inflammatory, antitoxic, hypoazotemic, antihepatitis, antibacterial and antioxidant (48). In Turkey, some *Stachys* taxa are used as tonic and stomachic and have been reported in folk medicine to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers (49). Besides, whole plants are used in phytotherapy, possessing sedative, antispasmodic, diuretic and emmenagogue activities as a tea preparations (50). Despite its economic significance, *Origanum* taxa are often referred to as an under-utilized, in the sense that its genetic resources and variability (51); Oregano essential oils have antibacterial, antioxidant, antifungal, carminative, diaphoretic, antispasmodic, antifungal, antimicrobial and analgesic effects (52). *Salvia* taxa have antioxidant, antifungal, analgesic and antiinflammatory effects (53). There are many studies about antioxidant properties of medicinal plants (54-58).

It was found to be higher ($p<0.38$) TAS levels of Asteraceae than Lamiaceae family that compared to the TAS levels of each family (71.61±37.05 mmol/L) and (36.97±9.6 mmol/L) respectively (Fig. 1). Its levels were observed to be no significant ($p=0.08$) difference that

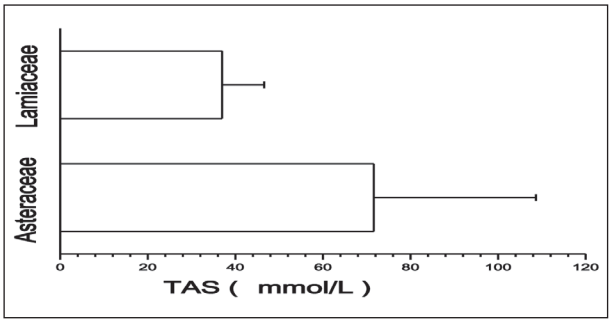

Figure 1. TAS levels in Asteraceae (n=13) and Lamiaceae (n=21) family (mean SEM, $p<0.05$)

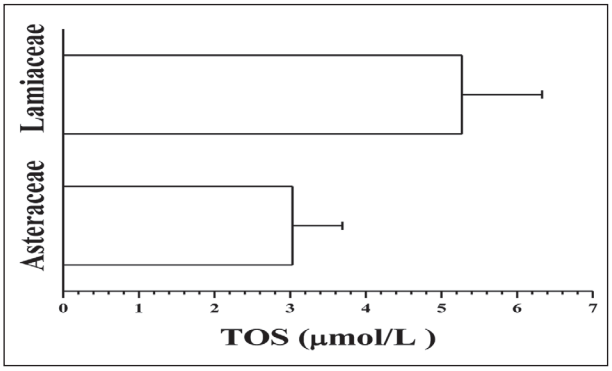

Figure 2. TOS levels in Asteraceae (n=13) and Lamiaceae (n=21) family (mean SEM, $p<0.05$)

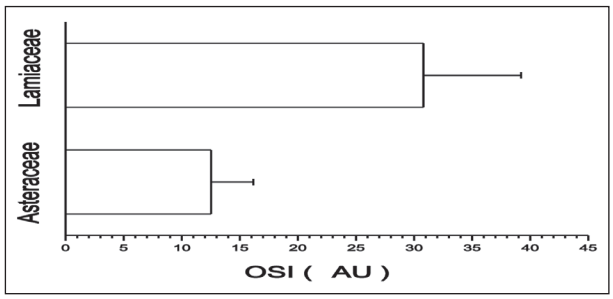

Figure 3. OSI levels in Asteraceae (n=13) and Lamiaceae (n=21) family (mean SEM, p<0.05)

compared to TOS levels between Asteraceae (3.02±0.66) and Lamiaceae families (5.26±1.06) (Fig. 2). In the similar way OSI levels in the Asteraceae (12.52±3.63 AU) Lamiaceae families (30.8±8.4 AU) were found to be no significant difference (p=0.05) (Fig. 3).

Conclusion

In conclusion, with this study, total antioxidant level, total oxidant level and oxidative stress index of 34 plant were determined. In addition with this research in vitro analyses average total antioxidant status, total oxidant status and oxidative stress index of selected economically important plants were done and productive results were achieved. Comparative analysis of total antioxidant, total oxidant and oxidative stres compound of plant extracts were detected. Studied plants can be used in pharmaceutical products as a source of natural antioxidants and that might be helpful in potential usefulness, biological activities and other phytochemical studies of these plants. In addition we think studies on how to ensure the existence of these taxa, which is in danger of extinction, should be emphasised.

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