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Investigation of *in vitro* antioxidant activity of *Glycrrhiza glabra* and *Syzygium aromaticum* extracts

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Abstract: In this study, total antioxidant activity (TAC), phenolic compound amounts (TPC) and reduction power (RP) of *Syzygium aromaticum* and *Glycyrrhiza glabra* species, widely used worldwide and our country, were investigated. Ethanol-water and methanol extracts of each type of spice samples were obtained by using the literature methods. In the ethanol-water extracts of S. aromaticum and G. glabra the TAC levels were determined to be at the highest level. When the TPC and RP between extracts listed, it was detected as methanol < ethanol-water. It was concluded that types of spices used as experimental materials thanks to TAC, TPC and RP could be priority in several in vivo and in vitro biological activity studies.

Keywords: *Glycrrhiza glabra*, *Syzygium aromaticum*, antioxidant activity

1. Introduction

Free radicals containing one or more unpaired electrons in their atomic or molecular structure are high-energy, unstable, short-lived, low molecular weight compounds. Unpaired electrons in their structure giving reactivity free radicals damage the cell membranes, lipids, proteins, nucleic acids and DNA in the cells. Thus, diabetes, cancer, cardiovascular diseases, nervous system degenerative diseases are caused. Antioxidant system components of cells and tissues that inhibit free radicals caused by exogenous and endogenous sources prevent the progression of autoxidation / peroxidation (Odabasoglu et al., 2004, 2005; Odabasoglu, 2006a).

Functions of the antioxidants are repairing the damaged lipids, proteins and DNA molecules in the cell structure, neutralizing free radicals, suspension or suppression of free radical generating reactions and increasing the enzymatic and non-enzymatic antioxidant synthesis. So the high level of antioxidants in the organism is more advantageous. To sustain this advantage organisms can choose to increase their own antioxidants or to provide the outsourcing needs of antioxidants as well (Odabasoglu et al.,2004;2005; Yucel et al., 2007).

The most remarkable parameters are reducing power and amounts of phenolic compounds in the effectivelly determining of antioxidant potential The amount of phenolic substances and reducing power shows compatibility with antioxidant potential, depending on species and varieties. So, it is widely accepted that antioxidant activity in many plants is due to phenolic compound in the extracts. (Lee et al., 2000; Odabasoglu et al.,2004, 2005; 2006b; Yucel et al., 2007).

Today, although studies of antioxidants in higher plants have been conducted, searches have been limited in spices that has interesting features. Spice is obtained by grinding, drying or disintegration of seeds, fruit, flowers, bark, roots, leaves of various plants. It is defined as natural compounds or mixtures that are colouring and flavour agents (Odabasoglu, 2006a; Benavente-Garcia et al., 2000). Spices today alongside of flavor to food, antimicrobial, anti-oxidative, anti-hypertensive, anti-spasmolytic, anti-inflammatory, antiallergic, antiulcerogenic, antipyretics, sedatives, neuroprotective, anesthetics, anti-tumor, antikolesterolemik and antiseptic effects of spices have been reported (Shan et al., 2005; Gruenwald et al., 2010; Allahghadri et al., 2010, Rohan et al., 2012; Rui et al., 2014, Shashank et. al., 2018, Vagih et. al., 2019; Cevik et al., 2019). In our country, clove and licorice are among the most

commonly consumed spices and there are some literature records about them. Antidiabetic, antiseptic, antifungal, antiviral, local anesthetic, antioxidant, neuroprotective, antithrombotic, anti-inflammatory, anticarcinogenic properties of clove have been explained (Gruenwald et al., 2010, Shashank et. al., 2018, Cevik et al., 2019). On the other hand there are some resources for licorice such as including inhibition histamine-induced ulceration, antioxidative, antimicrobial, detoxification, anti-platelet, laxative, antipyretic, atherosclerotic, hyperlipidemia, hypoglycemic, hypocholesterolemic, antitumoral, antiatherogenic, hepatopropektif and memory booster effects (Lee and Shibamoto T 2001; Rohan et al., 2012; Rui et al., 2014, Abo El-Maati et al., 2016; Vagih et. al., 2019; Radünz et. al., 2019; Cevik et al., 2019).

In the present study, we aimed to offer an insight into consumption of some spices by measuring antioxidant potentials, the amounts of phenolic compounds and reducing power. In our research, antioxidant activity and reducing powers of ethanol-water, methanol extracts derived from clove and licorice spices consumed in our country was determined and we tried to show relationship between antioxidant potential and total phenolic compounds of extracts.

2. Materials and Method

Plant Materials: The species were provided by "Baghdad Spice"- (Turkey). After the materials were taken, they were stored in a dry and cool cabinet.

Extraction of plant materials: 100 g of spices samples were extracted separately with methanol (50 ºC, 250 ml × 4) and ethanol-water (50 ºC, 250 ml × 4, 50:50) for 2 days in a water bath with a shaking attachment. Then, the methanol and ethanol-water extracts were concentrated under reduced temperature and pressure using a rotary evaporator.

Antioxidant activity assays: Antioxidant activities of extracts were measured using the thiocyanate method of the protocol described previously by Mitsuda et al. (1996). For stock solutions, 1 mg sample was dissolved in 1 ml distillate water and added into 4 ml of 0.2 M phosphate buffer (pH 7.0) and 5 ml linoleic acid mixture. The same mixture without the sample was used as the negative control. The mixed solution in tube was incubated at 40ºC. At 10-h intervals, aliquots of the reaction mixtures were taken for oxidation activity measured by ferric thiocyanate (FTC) assay. An aliquot 0.1 ml of the incubation mixture was mixed with 4.7 ml 75% ethanol followed by the addition of 0.1 ml 30% ammonium thiocyanate and 0.1 ml 20 mM ferrous chloride solution in 3.5% HCl. After 3 min, samples was measured at 500 nm (Mitsuda et al., 1996).

Reducing power assay: 0.5 mg sample was dissolved in 0.5 ml distillate water and added into 2.5 mL of $K_3Fe(CN)_6$ 1% w/v and 2.5 mL of 0.2 M phosphate buffer (pH 6.6). The resulting mixture is incubated at 50 °C for 30 min, followed by the addition of 2.5 ml of trichloro acetic acid (10% w/v). This incubation mixture is centrifuged at 3000 rpm for 10 min to 2.5 ml supernatant, mixed with 2.5 ml distilled water and 0.5 ml of $FeCl_3$ (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample. This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity (Yen and Chen, 1997).

Determination of total phenolic contents: Spice extracts in total amount of phenolic compounds in accordance with the procedure described by Slinkard and Singleton (1997) and was determined using the Folin-Ciocalteu solution. The samples (0.5 mg in 0.5 ml solvent) were added into 2.5 ml of Folin–Ciocalteu oxidising reagent and 2 ml of Na_2CO_3 (7.5%). The resulting mixture is incubated at 30 °C for 90 min. After 90 min, absorbance of all samples was measured spectrophotometrically at 765 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of lyophylisates.

Statistical analyses:

Statistical calculations were done by using SPSS 20.0 software. To determine the statistical significance of TAC, TPC and RP, one-way variance analyses (ANOVA) was applied showing that there was a statistically significant difference ($P < 0.05$).

3. Results and Discussion

Phenolic compounds one of the most important parameters in determination of antioxidant potential. Although antioxidant capacity can change with respect to the feature of phenolic compond, total phenolic content of the extract generaly shows a good correlation with the antioxidant activity of the sample. Therefore, it is commonly accepted that antioxidant activity of many plant extract is explained by their phenolic content (Mitsuda et al., 1996; Yen and Chen,1997; Odabasoglu et al., 2004; 2005).Today, due to the doubts on synthetic antioxidants, people prefer natural atioxidants (Schwarz et al., 2001; Odabasoglu et al., 2006a). So many studies are reported about investigation of antioxidant effects of plants and spices (Mathew and Abraham, 2006; Allahghadri et al., 2010; Gruenwald et al., 2010; Bettaieb et al., 2010; Rohan et al., 2012; Rui et al., 2014, Shashank et. al., 2018, Vagih et. al., 2019; Cevik et al., 2019). Although antioxidant capacity can change with respect to the feature of phenolic compound, total phenolic content of the extract generaly shows a good correlation with the antioxidant activity of the sample. Reduction power is described as electron donor or ability to give electron to the free radicals and accepted to be one of the important parameters for a molecule which has antioxidant effect (Odabasoglu et al., 2004; 2005; Gulcin et al., 2006a; Koksal and Gulcin, 2008).

Dose dependent total antioxidant activity, reduction power and total phenolic content values of *S.aromaticum* and *Glycyrrhiza glabra*- ethanol-water extracts were monitored in Table 1. The ethanol-water extracts of of *S.aromaticum* and *Glycyrrhiza glabra* exhibited potent antioxidant activities 92.5% inhibition of linoleic acid peroxidation. The highest inhibition, reduction power and total phenolic content values were obtained in 10 mg/ml (Table 2). The highest TAC was shown by the ethanol- water extracts of *S.aromaticum* and *Glycyrrhiza glabra*. In the present study,

there was no linear correlation between the TAC and TPC values of the all extracts. For example, although the ethanol-water extract of *S.aromaticum* and *Glycyrrhiza glabra* had highest TAC value, its exhibited a prooxidant activity in comparison with the control. On the contrary, the ethanol-water extract of the *S.aromaticum* had the highest value of TPC (Table 1) They also develop synergistic or antagonistic interactions with other phenolics or other types of components such as carbohydrates and proteins (Rice-Evans et al. 1997). In addition, nonphenolic compounds may play a majör role in the antioxidant activity of plant material (Velioglu et al. 1998). Methanol is known to be one of the best solvents for extracting compounds such as phenolics and other polar materials in plants (Velioglu et al. 1998).The highest amount of TPC was shown by the methanol extract of S.aromaticum (Table 2). There are strong relationships between the TPC and TAC values of methanol extracts of S.aromaticum and Glycyrrhiza glabra. It has been found that spices have higher antioxidant activity as compared to fruits, cereals and nuts. Present results suggest that the antioxidant activity of some tested extracts might be attributed to the presence of phenolic and non-phenolic compounds. The active components in spices phthalides, polyacetylones, phenolic acids, flavonoids, coumarins and terpenes are reported as powerful antioxidants. The different phytochemicals present greatly influence the biological activities possessed by plants/spices (Odabasoglu et al. 2006b; Gupta et al. 2017). Nevertheless, it should be taken into consideration that individual phenolic and non-phenolic may have distinct antioxidant activities; there may be antagonisstic or synergistic interactions between phenolic, non-phenolic and other compounds like carbohydrates, proteins, etc.

Table 1. Antioxidant activity, total phenolic content and reducing power of *Syzygium aromaticum* and *Glycyrrhiza glabra*' ethanol-water extracts

Samples	Doses (mg/ml)	TAC		RP	TPC
		Mean Absorbance (50. hour, 500 nm)	% Inhibition	Mean Absorbance (700 nm)	(mg GAE/g lyophilisate)
SAEWE	1	0.173±0.002^{c}	87.7	2.057±0.002^{a}	3.705±0.001^{a}
	5	0.153±0.005^{b}	89.1	3.699±0.001^{b}	3.798±0.002^{b}
	10	0.105±0.002^{a}	92.5	3.809±0.003^{c}	3.896±0.002^{c}
GGEWE	1	0.227±0.003^{c}	83.9	0.329±0.001^{a}	0.430±0.002^{a}
	5	0.149±0.020^{b}	89.4	0.933±0.001^{b}	1.463±0.001^{b}
	10	0.105±0.001^{a}	92.5	1.762±0.002^{c}	2.472±0.001^{c}
Ascorbic acid	1	0.152±0.001^{b}	89.2	-	-
Trolox	1	0.142±0.001^{b}	90.0	-	-
Control (water)	-	1.407±0.002^{d}	-	-	-

The values are presented as mean ± SD. Significant at $p < 0.05$. Values with the same letter are not different according to Duncan test for statistical purposes. SAEWE: Ethanol-water extract of *Syzygium aromaticum* and GGEWE: Ethanol-water extract of *Glycyrrhiza glabra*

Table 2. Antioxidant activity, total phenolic content and reducing power of *Syzygium aromaticum* and *Glycyrrhiza glabra*' methanol extracts.

Samples	Doses (mg/ml)	TAC		RP	TPC
		Mean Absorbance (50. hour, 500 nm)	% Inhibition	Mean Absorbance (700 nm)	(mg GAE/g lyophilisate)
SAME	1	0.252±0.001^{e}	76.8	1.366±0.002^{a}	3.507±0.002^{a}
	5	0.234±0.001^{d}	78.5	3.551±0.002^{b}	3.873±0.002^{b}
	10	0.225±0.002^{c}	79.3	3.631±0.052^{b}	3.975±0.001^{c}
GGME	1	0.355±0.003^{e}	64.7	0.264±0.002^{a}	0.421±0.001^{a}
	5	0.203±0.001^{d}	81.3	0.864±0.003^{b}	3.507±0.001^{b}
	10	0.193±0.001^{c}	82.2	1.112±0.008^{c}	3.871±0.001^{c}
Ascorbic acid	1	0.134±0.001^{a}	87.7	-	-
Trolox	1	0.171±0.002^{b}	84.3	-	-
Control (water)	-	1.088±0.001^{f}	-	-	-

The values are presented as mean ± SD. Significant at $p < 0.05$. Values with the same letter are not different according to Duncan test for statistical purposes. SAEWE: Methanol extract of *Syzygium aromaticum* and GGEWE: Methanol extract of *Glycyrrhiza glabra*

5. Conclusion

It is concluded that spices used in this study as experimental material may be evaluated in *in vivo* and *in vitro* biological activity studies due to their antioxidant activity, reduction power and total phenolic content characteristics.

Conflict of interest disclosure:

No conflict to interest.

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