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Comparison of Phenolic Compounds and Antioxidant Activities of the Extracts of Grape Seed, Rosemary, Green Tea and Olive Leaf

Üzüm Çekirdeği, Biberiye, Yeşil Çay ve Zeytin Yaprağı Ekstraktlarının Fenolik Bileşenleri ve Antioksidan Aktivitelerinin Karşılaştırılması

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Key Words:

Plant extracts, antioxidant activity, phenolic compounds, DPPH, TEAC

Anahtar Sözcükler:

Bitkisel ekstraktlar, antioksidan aktivite,fenolik bileşenler, DPPH, TEAC

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ABSTRACT

Objective: Total antioxidant activities and phenolic compounds of ethanol extract of grape seed, rosemary, green tea and olive leaf were investigated in this study.

Material and Methods: The total antioxidant activities of plant extracts were analyzed by two different methods, including 2.2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging activity and trolox equivalent antioxidant capacity (TEAC). Carnosic acid, rosmarinic acid in rosemary extract (RE), total catechins in green tea extract (GTE) and oleuropein in olive leaf extract (OLE) were identified by high performance liquid chromatography (HPLC) method. Condensed tannins of grape seed extract (GSE) was analyzed by butanol-HCl method.

Results: The total phenolic contents of plant extracts determined by the Folin-Ciocalteu method ranged from 141.7 to 315.56 mg of gallic acid equivalents (GAE) g^{-1}. According to results obtained this study, RE exhibited the highest DPPH radical scavenging activity, and GTE exhibited highest TEAC activity. Additionally, obtained data revealed high linear correlation (r=0.82) between total phenolic contents and TEAC values and low linear correlation (r=0.47) between total phenolic contents and DPPH values.

Conclusion: The potent antioxidant activity of plant extracts provided from agro-industrial by-products, medicinal and aromatic plants makes it possible used them as natural sources of antioxidants in feed, food and pharmaceutical industries.

ÖZ

Amaç: Bu çalışmada, üzüm çekirdeği, biberiye, yeşil çay ve zeytin yaprağı etanol ekstraktlarının fenolik bileşen miktarları ile toplam antioksidan aktiviteleri araştırılmıştır.

Materyal ve Metot: Bitkisel ekstraktların toplam antioksidan aktiviteleri 2.2-difenil-1-pikrilhidrazi hidrat (DPPH) radikal süpürme aktivitesi ve troloks eşdeğeri antioksidan kapasitesi (TEAC) olmak üzere iki farklı yöntemle belirlenmiştir. Biberiye ekstraktı (BE) karnosik asit ve rosmarinik asit, yeşil çay ekstraktı (YÇE) toplam kateşinler ve zeytin yaprağı ekstraktı (ZYE) oleuropein içerikleri, yüksek performanslı sıvı kromatografisi (HPLC) yöntemi ile bulunmuştur. Üzüm çekirdeği ekstraktı (ÜÇE) kondanse tanenleri ise butanol-HCl yöntemi ile analiz edilmiştir.

Bulgular: Bitkisel ekstraktların Folin-Ciocaltaeu yöntemine göre saptanan toplam fenolik içerikleri 141.7 ile 315.56 mg gallik asit eşdeğeri (GAE) g^{-1} arasında değişim göstermiştir. Bu çalışmadan elde edilen bulgulara göre; BE en yüksek DPPH radikal süpürme aktivitesi gösterirken, YÇE en yüksek TEAC aktivitesi göstermiştir. Bununla birlikte, elde edilen verilerin toplam fenolik içerikleri ile TEAC değerleri arasında yüksek doğrusal korelasyon (r=0.82) ve toplam fenolik içerikleri ile DPPH değerleri arasında düşük doğrusal korelasyon (r=0.47) saptanmıştır.

Sonuç: Agro-endüstriyel yan ürünlerden, tıbbi ve aromatik bitkilerden elde edilen bitkisel ekstraktların güçlü antioksidan aktivitelerinin ortaya konulması, bunların gıda, yem ve ilaç endüstrilerinde doğal antioksidan kaynağı olarak kullanılmasını mümkün kılmaktadır.

INTRODUCTION

In recent years, there is growing interest in the evaluation of economic antioxidants from agro-industrial by-products, medicinal and aromatic plants as well as from other plant products. Polyphenolic compounds are commonly found in both edible and inedible plants (Kahkönen et al., 1999). Polyphenolic compounds (=phytochemicals) extracted from grape *(Vitis viniferae L.)* seeds, rosemary *(Rosmarinus officinalis L.)*, green tea *(Camellia sinensis L.)* and olive leaves *(Olea europea L.)* are widely used, mainly as nutritional supplements and have in vitro antioxidant and antibacterial activity (Meagher et al., 2005; Kahkönen et al., 1999; Shahidi and Marian, 2003; Rababah et al., 2004; Tsai et al., 2008; Erkan et al., 2008). Also, these phytochemicals have been rising interest as feed supplements in animal nutrition for decrease the oxidative stress factors and so protect the animal products (egg and meat) from lipid peroxidation and produce antioxidant rich functional products (Basmacıoğlu et al., 2004; Bou et al., 2009).

In animals, oxidative stress may occur due to feeding management (toxins, high levels polyunsaturated fatty acids and oxidized fat intake, etc.) environmental conditions (temperature, humidity, transport, etc.) and disease factors (bacterial and viral). In a normal live organism, there is a balance between the substances causing oxidation and a system that prevents this oxidation. Oxidative stress occurs when this balance is impaired. The antioxidant effects of plant extracts are due to the polyphenolic compounds. They can play an important role in preventing oxidative stress conditions in the cells to remove superoxide anions and hydroxyl radicals from activated neutrophils, to bind metal ions such as iron and copper and to prevent the formation of free radicals (Rice-Evans et al., 1996).

Rosemary is the unique medicinal and aromatic plant as a commercially available natural antioxidant in the food sector. The principal compounds responsible for the antioxidant activity of rosemary are reported to relate to the presence of three phenolic compounds, carnosic acid, carnosol and rosmarinic acid (Frankel et al., 1996). Grape seed, green tea and olive leaf, as agro-industrial by-products, are considered as natural antioxidant resources (Aktaş et al., 2013; Uydu et al., 2011). Proanthocyanidins are polymers of flavan-3-ol units found in green tea and grapes (Rababah et al., 2004) and also oleuropein found in olive leaf extract (Benavente-Garcia et al., 2000). These plants antioxidant properties are arose from to present catechol structure in their moieties.

The main purpose of this study was to evaluate grape seed, rosemary, green tea and olive leaf extracts as new sources of natural antioxidants by determining their phenolic compounds and their antioxidant activities by two common DPPH and TEAC analysis. Also, this study shows a correlation between antioxidant activity methods and total phenolic contents.

MATERIAL and METHODS

Plant Material

Grape seed was obtained from local juice-processing industry (Dimes A.Ş, Kemalpaşa-İzmir). Rosemary was collected from Mersin. Green tea leaves collected from the waste part after the tea processing leaves taken. This green tea waste leaves were obtained from local tea industry (Çay-kur A.Ş, Rize). Olive leaves were collected from Edremit-Balıkesir. The plant extracts were obtained from commercial firm (Edremit/Balıkesir, Turkey).

Main Phenolic Compounds of Plant Extracts

Grape Seed Extract

Butanol-HCl method was used for determination of condensed tannin of GSE (Makkar, 1995). 0.01 gram of grape seed extract was weighed into glass tubes and 6 ml butanol-HCl solution (95 ml butanol +5 ml HCl + 1 g Fe_2SO_4) was added. The tubes were waited in 100 °C water bath for 1 hour and then cooled to room temperature. The tubes were centrifuged at 3000 X g for 100 minutes. The absorbance measured at 550 nm by using a spectrophotometer (Amersdam, 2100 UV, UK).

Rosemary Extract

The rosmarinic and carnosic acid content of the rosemary extract was determined by HPLC. Operating conditions of the device; mobile phase: A (methanol) + B (10 mM 850 ml acetic acid and 150 ml acetonitrile mixture) elution condition: linear gradient, flow rate: 1.1 ml min^{-1}; column type: Zorbax, 5 µm. 15 cm x 4.6 mm, detector: Waters 2487 Dual absorbance UV 285 nm; injection volume: 20

Green Tea Extract

The total catechin content of GTE was determined by HPLC. For this purpose, 50 mg GTE was dissolved in 20 ml of methanol and stored in an ultrasonic bath for 15 min. The extracted extract was passed through a 0.45 µm filter and the obtained clear solution was applied an HPLC device. Operating conditions of the device; wavelength: 270 nm; flow rate: 1 ml min^{-1}; mobile phase: A (0.2% Formic acid + Water) + B (0.2% Formic acid + Acetonitrile); column: Varian C8 column (250 x 4.6 mm x 5 um).

Olive Leaf Extract

The oleuropein content of the OLE was determined by HPLC. For this purpose, 250 mg sample was weighed and extracted with 10 ml of 80% methanol for 2 times. It was then stirred for 30 s and the liquid fraction was removed after centrifugation 25 ml of 80% methanol added. The solution was filtered and then injected into the HPLC with a syringe. Operating conditions of the HPLC device; detector: UV Visible; wavelength: 280 nm; flow rate: 1 ml min^{-1}; column temperature: 35 °C; injection volume: 20 µl; column: C18 250x4.6x5 um; mobile phase: A (100% Acetonitrile) + B (0.02% trifluoroacetic acid + Water). Gradient system; 0: 5% A, 95% B; 0-10.dk: linear change to 10% A, 90% B; 10-24.dk: linear change to 30% A, 70% B; 24-35. min: linear change to 40% A, 60% B; 35-45. min: linear change to 80%, 20% B; 45-65.dk: isocratic change to 5%, 95% B.

Total Phenolic Contents and Antioxidant Activities of Plant Extracts

Total Phenolic Content Analysis

Folin-Ciocalteu method was used for determination of the total phenolic contents in the plant extracts (Vinson et al 1995). 7 mg of plant extract was dissolved with 2 ml of

methanol and 10 µl aliquot plant extract was added in a tube. Then a total volume of 10 ml with distilled water and 500 µl of Folin-Ciocalteu-reagent were added. The solution was mixed by vortex and waited for 5 minutes. Then 1.5 ml of saturated sodium carbonate solution were added, mixed again and kept at room temperature for an hour. The absorbance solution was read at 760 nm by using a spectrophotometer (Amersdam 2100 UV, UK). A standard curve was prepared by gallic acid and the results were expressed as GAE per gram of extract.

DPPH Radical Scavenging Activity Analysis

DPPH method was carried out as described by Amarowicz et al. (2004) with minor modifications. Plant extracts were dissolved in 4 ml of methanol, then 0.5 ml of 1mM DPPH methanolic solution was added. The contents were mixed for 15 seconds, it was kept at room temperature for 30 minutes. The absorbance of the solution was read against methanol at 517 nm by using a spectrophotometer (Amersdam 2100 UV, UK). The radical scavenging activity (RSA) was calculated according to the following formula;

$\% RSA = 100 \times (1 - AE\ AD^{-1})$

AE : the absorbance of the solution containing antioxidant extract

AD : the absorbance of the methanolic DPPH solution.

Trolox Equivalent Antioxidant Capacity (TEAC) Analysis

TEAC method was carried out as described by Re et al. (1999) with some modifications. ABTS· [2′, 2′- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium] is dissolved in water to a concentration of 7 mM and reacted with 2.45 mM potassium persulfate at a molar ratio of 2:1 to form the ABTS· radical, stayed in the dark room overnight for 16 hours. The ABTS· solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 AU at 734 nm was reached. After addition of 1.9 ml of diluted ABTS· solution to 10 µl plant extracts or trolox standards (final concentration 5-25 µM) in ethanol the absorbance reading was taken at 1 min after initial mixing and up to 6 min. The results were expressed as µM Trolox per 100 g of sample.

Statistical Analysis

Statistical analysis of the obtained data was determined by Student's t-test in the SPSS 13 (SPSS Inc., Chicago, USA, 2007) package program. The significance level of difference between groups was taken into account at $P \leq 0.05$. Data were given as the mean ± standard deviation (SD). Additionally, graphs for the correlation between antioxidant activity methods and total phenolic contents figure out in Excel program.

RESULTS and DISCUSSION

Figure 1, 2 and 3 show a typical HPLC chromatogram of RE, GTE and OLE, respectively. Main phenolic compound or compounds identified in plant extracts were shown in Table 1.

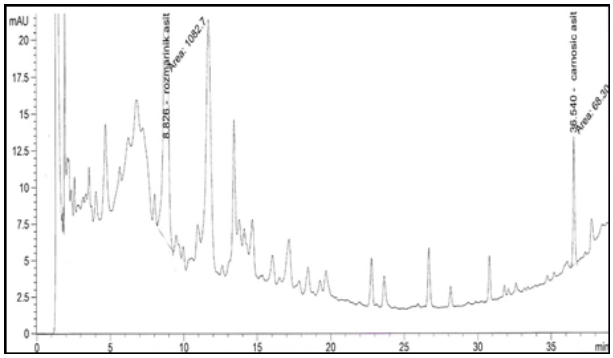

Figure 1- HPLC chromatogram of an ethanol extract of rosemary
Şekil 1- *Biberiye etanol ekstraktının HPLC kromatogramı*

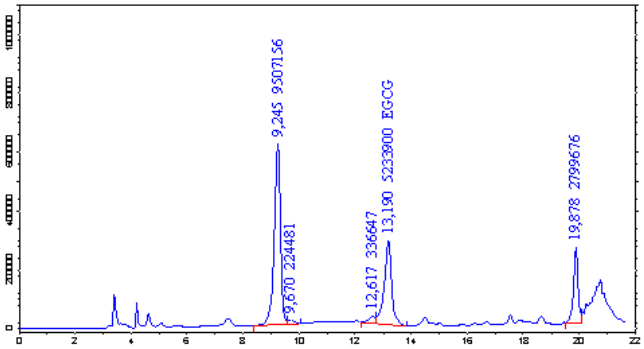

Figure 2- HPLC chromatogram of an ethanol extract of green tea
Şekil 2- *Yeşil çay etanol ekstraktının HPLC kromatogramı*

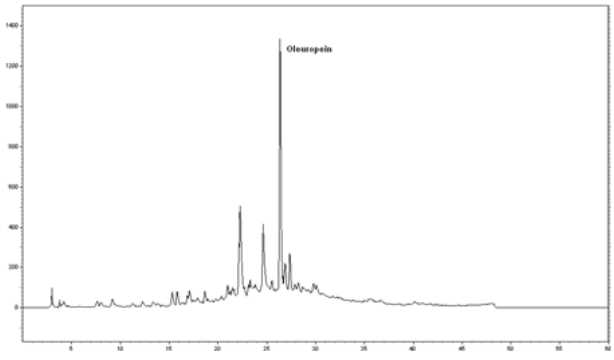

Figure 3- HPLC chromatogram of an ethanol extract of olive leaf
Şekil 3- *Zeytin yaprağı etanol ekstraktının HPLC kromatogramı*

Table 1. Main phenolic compound or compounds of plant extracts
Çizelge 1. *Bitkisel ekstraktların başlıca fenolik bileşen veya bileşenleri*

Grape seed	**Condensed tannin (g/100g)**	
	41.07	
Rosemary	**Carnosic acid (g/100g)**	**Rosmarinic acid (g/100g)**
	14.90	35.59
Green tea	**Total catechins (g/100g)**	
	85.31	
Olive leaf	**Oleuropein (g/100g)**	
	15.49	

The condensed tannin of GSE determined with butanol-HCl method was 41.07 g 100 g^{-1}. In our previous study (Aktaş et al., 2018) the condensed tannin of GSE (Antep Karası) was found 45.88 g 100 g^{-1}. In another study (Cross et al., 2004) where the same method was used, it was found to be 37.2 g 100 g^{-1} similar to the findings obtained from this study.

In this study, the carnosic acid and rosmarinic acid contents of RE were found to be 14.90 g 100 g^{-1} and 35.59 g 100 g^{-1}. Basmacıoğlu-Malayoğlu et al. (2008), found in their study that the carnosic acid and rosmarinic acid contents of RE were 105.72 mg g^{-1} and 72.12 mg g^{-1}, respectively. In a study (Yeşil-Çeliktas et al., 2007), with RE obtained by the supercritical carbon dioxide method, the carnosic acid content varied between 60.9-115.5 mg g^{-1}. In another study (Wellwood and Cole, 2004), comparing extraction of three different solvents (ethanol, petroleum ether, dichloromethane), 29.77 mg g^{-1} carnosic acid in RE obtained with ethanol extract was close to the content set in this study. On the other hand, the content of rosmarinic acid (2.19 mg g^{-1}) is much lower than that found in this study. As a matter of fact, it is suggested that the seasonal and regional differences of the active compounds of plant products are different according to the harvesting time, the plant part used, the phenolic structure and concentration, the extraction conditions (Bano et al., 2003).

The total catechin content of the GTE was found to be 85.31 g 100 g^{-1}. When the findings obtained this study were compared with the findings obtained from the literature, Tang et al. (2002) determined total catechin values as 86 % similar to our results. But Eid et al. (2003) and Babu et al. (2006) reported total catechin as 63.9 % and 79.36 %, respectively.

The oleuropein content of the OLE was found to be 15.49 g 100 g^{-1}. In similar studies, the content of oleuropein in OLE was found to be 19 % (Le Tutour and Guedon, 1992) and 24.54% (Benavente-Garcia et al., 2000) and 9.04-14.14 % (Savournin et al., 2001) consitent with the findings obtained this study.

The total phenolic contents of GSE, RE, GTE and OLE were determined as 157.20, 248.50, 315.56 and 141.74 mg GAE g^{-1}, respectively (Table 2). In this study, the total phenolic content of GTE was found ≈ 1.27, 2.00 and 2.24 fold higher than RE, GSE and OLE, respectively. Similar to the findings obtained from this study, Tsai et al. (2008) found total phenolic content in green tea higher than among the 12 herbs.

The total antioxidant activities of the plant ethanolic extracts for each method are shown in Table 2.

Table 2. Total phenolic contents and antioxidant activities of the plant extracts[1]
Çizelge 2. *Bitkisel ekstraktların toplam fenolik içeriği ve antioksidan aktiviteleri*[1]

Plant extracts	Total phenolic content[2]	DPPH Radical scavenging activity[3]	TEAC[4]
Grape seed	157.2 ± 1.00	61.7 ± 1.99	69.5 ± 1.77
Rosemary	248.5 ± 2.36	94.0 ± 1.10	58.8 ± 1.91
Green tea	315.6 ± 0.66	74.4 ± 0.83	285.4 ± 0.46
Olive leaf	141.7 ± 2.16	72.1 ± 1.06	43.7 ± 0.90

[1] Each value corresponds to the mean and standard deviation (n=3).
[2] Data of total phenolic contents are expressed as milligrams of GAE per gram plant extract.
[3] Data of DPPH Radical scavenging activity are expressed as %.
[4] TEAC are expressed as micromoles of Trolox equivalents per 100 gram plant extract.

Several researchers investigated that the evaluation of antioxidant activities of plant extracts in *in-vitro* with a single method is not accurate due to the multiple reaction properties and mechanisms of plants. For this purpose, the antioxidant activities of GSE, RE, GTE and OLE were determined by two different methods provided the values of 61.7, 94.0, 74.4 and 72.1 % for DPPH radical scavenging activity and 69.5, 58.8, 285.4 and 43.7 g µM/100g for TEAC activity, respectively. Despite the fact that both methods (DPPH and TEAC) used in this study related to the measurement of the electron transfer propensity of antioxidants, there was a difference in the comparison of the plant extracts according to the method. According to the TEAC method the highest antioxidant activity was found in GTE with the highest total phenolic content whereas according to DPPH method the highest antioxidant activity found in RE. According to Furiga et al. (2009) GSE showed stronger antioxidant activity than vitamins E and C in an *in vitro* study. In another *in vitro* study, comparing the antioxidant effects of different plant extracts showed the highest antioxidant activity of GSE and GTE (Rababah et al., 2004). Also Peschel et al. (2007) found that in their *in vitro* study; GSE and GTE have stronger antioxidant activity than other antioxidants (BHA, sesame, indigo herb, blackcurrant, pitrach) including RE. High antioxidant activity is attributed to phenolic compounds (catechin, epicatechin and caffeic acid) in the grape seed and the green tea structure.

The correlation between total phenolic contents of plant extracts and antioxidant activities were determined by two different methods. There was a low correlation (r=0.47) between total phenolic content and DPPH value (Figure 4) and a high correlation (r=0.82) between total phenolic content and TEAC value (Figure 5).

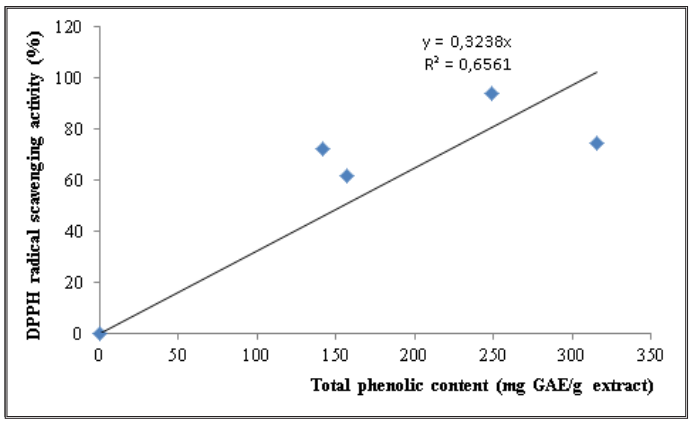

Figure 4- Linear correlation of DPPH radical scavenging activity (Y) versus the total phenolic content (X) of plant extracts.

Şekil 4- *Bitkisel ekstraktların toplam fenolik içeriğine (X) karşı DPPH radikal süpürme aktivitesinin (Y) lineer korelasyonu*

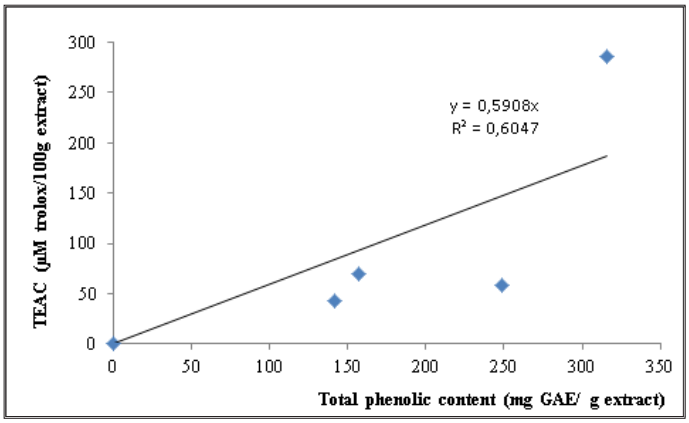

Figure 5- Linear correlation of Trolox equivalent antioxidant capacity (TEAC) (Y) versus the total phenolic content (X) of plant extracts.

Şekil 5- *Bitkisel ekstraktların toplam fenolik içeriğine (X) karşı Troloks eşdeğeri antioksidan kapasitesinin (TEAC) (Y) lineer korelasyonu*

Tsai et al (2008) suggested that the different observations using TEAC and DPPH methods due to the TEAC values were not strongly related to chemical structures or numbers of electrons that an antioxidant give away (Huang et al., 2005). Some researchers (Cai et al., 2004; Djeridane et al., 2006; Katalinic et al., 2006; Basmacıoğlu-Malayoğlu et al., 2011) have demonstrated a high correlation between the content of phenolic compounds and their antioxidant activities, while others (Czapecka et al., 2005; Wong et al., 2006) have demonstrated a low correlation. It has been suggested by researchers (Kahkönen et al., 1999; Shahidi and Marian, 2003) that differences of plant extracts antioxidant activities were related to the different structures from phenolic acids and flavonoids compounds and their derivates. On the other hand, differences between the methods may be due to the environmental changes (climate, temperature, location, fertility, diseases and pest exposure) in the plants, the plant part tested, seasonal differences (harvesting time), extraction conditions, method used in pre-extraction, product and oxidation conditions and analytical method (Shan et al., 2005).

CONCLUSION

The results confirm that plant extracts were rich in phenolic compounds and exhibited different antioxidant activities in relation to the method applied. DPPH radical scavenging activity of four plant extracts decreased in the following: RE>GTE> OLE > GSE. TEAC activity of four plant extracts decreased in the following: GTE> GSE> RE> OLE. A positive correlation existed between antioxidant activity and total phenolic contents, measured by TEAC analysis in plant extracts, revealing that phenolic compounds were the dominant antioxidant component. The results obtained by these methods provide simple datas for the classification of plant extracts according to their antioxidant activity. In conclusion, these plant extracts provided from agro-industrial by-products, medicinal and aromatic plants have potent antioxidant activity. They could be utilized as natural sources of antioxidants in feed, food and pharmaceutical industries.

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