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Phytochemical Profile and Antioxidant Activities of *Zingiber officinale* (Ginger) and *Curcuma longa* L.(Turmeric) Rhizomes

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Abstract: The aim of our study was to evaluate and collate the chemical constituents and antioxidant properties of dry rhizomes of Ginger and dry rhizomes of Turmeric. The assay for quantification of the phenolic compounds in the samples was carried out using the reversed phase-high performance liquid chromatography (RP-HPLC). To determine mineral components in samples inductively coupled plasma optical out flow spectroscopy (ICP-OES) procedure was applied. The most abundant phenolic components in turmeric rhizomes (mg componenet 100 g^{-1} dried rhizome) are ferulic acid (93.59 mg), benzoic acid (40.09 mg), vanillin (26.69 mg) and p-coumaric acid (23.25mg) respectively. On the other hand, the most common phenolic components in ginger rhizomes are Benzoic acid (33.31mg), Ferulic acid (11.41 mg) and vanillin (11.83 mg). In addition, ethanolic extract ginger (EEG) and ethanolic extract turmeric (EET) had an effective DPPH• scavenging, hydrogen peroxide scavenging, ferric ions (Fe^{3+}) reducing power activities. According to ICP-OES analysis results of rhizomes and extracts, the potassium was, quantitatively, the most abundant mineral in samples. Subsequently, sodium, magnesium, phosphorus and calcium were identified, respectively.

Keywords: Antioxidant, mineral content, ginger, turmeric, phenolic compound.

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1. INTRODUCTION

Reactive oxygen species (ROS) is a common term that contains all reactive forms of oxygen, involving both radical and non-radical species that participate in the initiation and/or propagation of chain reaction (Kumar et al., 2011). ROS are superoxide radical ($O_2 \cdot -$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•), which are formed in small amounts during normal oxygen metabolism ROS are continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS can also damage important biomolecules such as nucleic acids, lipids, proteins, and carbohydrates, and cause DNA damage that can lead to mutations (Ak and Gulçin, 2008). This damage causes various diseases such as cancer, atherosclerosis, amyloidosis, age-related immune deficiency, senile dementia and hypertension and is known to play a role in the biological aging process (Kopáni et al., 2006). There are many defense mechanisms to prevent the formation of ROS and their damage. These mechanisms are known as "antioxidant defense systems" or simply "antioxidants." Antioxidant components can sweep free radicals and prolong shelf life by delaying the lipid peroxidation process, which causes food and pharmaceutical products to deteriorate (Halliwell, 1996). Recent research has shown that antioxidants with herbal-derived radical scavenging activity are crucial in curing free radical-borne diseases during the aging process (Mohan et al., 2015). An inquiry of normally happening antioxidant ingredients from plant sources may give prompts the advancement of novel medicines, which may diminish the danger of long-term infections brought about by free radicals (Abuja and Albertini, 2001).

Turmeric (*Curcuma longa* L.), a perennial plant that belongs to the Zingiberaceae family, is widely cultivated in Asian countries. The rhizomes of this plant are the most useful and are used for culinary and traditional medicinal purposes (Bagchi, 2012). Curcumin is the most important bioactive component of turmeric, which is also used as a spice. Turmeric powder, curcumin and its derivatives and

many other extracts from the rhizomes were found. Investigations of turmeric have uncovered various pharmacological properties (Wichitnitha et al., 2009). However, Ginger, whose Latin name is *Zingiber officinale*, is a plant of the Zingiberaceae family, which can grow up to one meter in length, with long leaves and yellow-red flowers. Ginger is a well-known herb to contain several bioactive compounds, anti-inflammatory, carminative, antiseptic properties and antioxidants that possesses health-promoting properties (Mushtaq et al., 2019).

The aim of our study was to evaluate and collate the chemical constituents and antioxidant properties dry rhizomes of Ginger and dry rhizomes of Turmeric. In the current study we have made an assay to determine the dietary advantages of these the two rhizomes.

2. MATERIAL AND METHODS

2.1. Chemicals

Gallic acid was purchased from Merck. BHA, BHT, L-Ascorbic Acid, DPPH•,the folin-ciocalteu reagent, Potassium ferricyanide, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Ferric chloride, Sodium Carbonate and trichloroacetic acid (TCA) were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals used were analytical grade and obtained from either Sigma–Aldrich or Merck.

2.2. Plant material

The turmeric and ginger were obtained from the Faculty of Agriculture- Isparta University of Applied Sciences. Plant specimens were also identified by Prof. Hasan Baydar and deposited at the herbarium of Faculty of Agriculture, Isparta University of Applied Sciences, with voucher specimen numbers TP32-2020 and GP32-2020. The preferred materials were cleaned to get rid from the dirt and other foreign particles, the cloves of turmeric ginger were peeled subsequently, for the preparation of ginger and turmeric extract and powders in the same way.

2.3. Preparation of ethanolic extract of turmeric and ginger powder

Ginger (EEG) and turmeric extracts (EET) were prepared using 96% ethanol. 100 grams dry powdered samples were soaked 500 mL in ethanol (96%) in a sealed 1 liter container for 24 hours at room tempurature, with intermittent shaking. Extracts were further washed with fresh ethanol (250 ml and 125 ml) and were filtered through Whatman No. 41 filter paper. Then the filtrates were concentrated under vacuum using a rotary evaporator under reduced pressure at 50 ^{o}C. From 100 grams of turmeric and ginger powder samples, 3.71 and 5.58 grams of extract were obtained, respectively (Table 1). EEG and EET were utilized for the discovery of their mineral profile and antioxidant capacity. To assess the anti- oxidative point of view, total phenols (TPC), DPPH radical scavenging potantial (1, 1-diphenyl-2-picrylhydrazyl), hydrogen peroxide scavenging and FRAP (Ferric reducing antioxidant power) assays were conducted and kept in in obscurity at +4 ^{o}C until use.

2.4. Elemental analysis

To determine components in samples inductively coupled plasma optical outflow spectroscopy (ICP-OES) procedure was applied. Sample preparation process was done by using wet burning method by adding 8 mL HNO_3 + 2 mL H_2O_2 to 0.2-0.3 gram sample using Milestone ETHOS ONE model microwave sample preparation unit according to EPA 3015 a method. The final volume was completed to 20 mL with distilled water. ICP OES measurements were made in accordance with EPA 6010 method by using Perkin Elmer OPTIMA 5300 DV device. The accompanying 16 substance components were determined: Al, Ca Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Se and Zn. The acquired outcomes are displayed in Table 3. Results were calculated as mg element g^{-1} dried rhizome using external calibration curves, constructed for each mineral standard. Investigation of each sample was done in triplicate. All the findings were indicated as the mean of triplicate measurements.

2.5. Determination of total phenolics content (TPC)

The quantity of total phenolic substance in the turmeric and ginger extracts was figured out with Folin-Ciocalteu reagent as indicated by the technique for Slinkard and Singleton (1977). Folin-Ciocalteu is a procedure used for the estimation of total phenolic compounds. Gallic acid was used as a standard phenolic compound. Briefly, 40 µl sample (1 mL of extract solution contains 1 mg extracts), a gallic acid calibration standard, or blank (deionized or distilled water) was put into a 15 ml falcon tube. Then, 3.16 ml water, followed by 200 µl FC reagent was added and mixed thoroughly by pipetting or inverting and incubate 1 to 8 min. After 5 min, 600 µL of Na_2CO_3 (20%) was added and afterward the mix was permitted to represent 2 h with discontinuous shaking. The absorbance was measured at 765 nm in a spectrophotometer (Perkin Elmer Lambda 20 UV VIS Spectrophotometer). A calibration curve was made by getting ready 40 µl aliquots of 31.25, 62.5, 125, 250, 500 and 1000 µg mL^{-1} arrangements of Gallic acid and the outcomes were stated as gallic acid equivalents in milligram per gram [mg GAE/g] of the sample. Absorbance=0:0011 x Total phenols [Gallic Acid Equivalent (mg)] +0.0174 (R^2: 0.9997). The assessment was performed in triplicate. The measure of phenolic content in extracts was determined by the accompanying equation:

$$T = C1 \times V/M$$

Where, T = Total phenolic content mg g^{-1} of extracts in GAE [Gallic acid equivalent]; C1 = The Concentration of Gallic acid established from the calibration curve mg mL^{-1}; V = The Volume of extract solution [mL] M = The Weight of the extract [g].

2.6. Analysis of Phenolic Components

The assay for quantification of the phenolic compounds has previously been described by Caponio et al., (1999). The reversed phase-high performance liquid chromatography (RP-HPLC) was used. The analytical HPLC system

employed consisted of a a SCL-10 Avp System controller, a SIL–10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array detector set at 278 nm. The separation was achieved on a Agilent Eclipse 5µm XDB 250 x 4,6 mm column at 30°C. The flow rate was 0.8 ml/min, injection volume was 20 µl. Gradient elution of two solvents was used: Solvent A consisted of acetic acid–water (3:97, v/v), solvent B: methanol and the gradient program used. The analytical data were evaluated using a Shimadzu Class-VP Chromatography Laboratory Automated Software System (Chiyoda-ku, Tokyo, Japan). The gradient used was similar to that used for the determination of phenolics in sage and rosemary (Baydar et al., 2007) with some modifications. The amount of phenolic compounds in the extract was calculated as mg 100 g^{-1} dried rhizome using external calibration curves, constructed for each phenolic standard.

2.7. 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), free radical scavenging activity

The free-radical-scavenging capacity of the extracts was evaluated, using the DPPH stable radical and following the methodology described by Gulçin (2006). Briefly, 0.1mM solution of DPPH• in ethanol was prepared and 1ml of this solution was added to 3 mL of EEG or EET solution in ethanol at different concentrations (10-20 µg/mL). After 30 min, the absorbance was measured at 517 nm against ethanol as a blank in a spectrophotometer (Perkin Elmer Lambda 20 UV VIS Spectrophotometer). The lower the measured absorbance value of the reaction mixture, the higher the free radical scavenging potential.

The ability to sweep the DPPH• radical was counted up using the following equation:

DPPH• scavenging effect (%) = [(AControl − ASample / AControl) × 100]

where AControl is the absorbance of the control reaction (ethanol solution containing 0.1 mM DPPH) and ASample is the absorbance in the presence of ginger and turmeric extracts and standarts (BHT and BHA) (Erdoğan and Gökçe, 2021).

2.8. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging test was carried out following the procedure of Ruch (1989). The fundamental of this assay that there is a reduce in absorbance of H_2O_2 upon oxidation of H_2O_2. 43 mM hydrogen peroxide solution was prepared in phosphate buffer (pH: 7.4). EEG or EET at different concentrations (10- 20 µg /mL) in 3.4mL phosphate buffer was added to 0.6mL of H_2O_2 solution (43mM) and absorbance of the reaction mixture was recorded at 230 nm. The blank solution contained phosphate buffer solution free hydrogen peroxide.

The percentage of H_2O_2 scavenging by EEG, EET and standard compounds was calculated using the following equation:

H_2O_2 scavenging effect (%) = [(AControl − ASample / AControl) × 100]

where Ac is the absorbance of the control and As is the absorbance in the presence of EEG, EET or other scavengers (BHA and BHT) (Benkeblia, 2005).

2.9. Ferric cyanide (Fe^{3+}) reducing antioxidant power assay

The reducing capacity (RP) of the extracts was assessed as described by Oyaizu (1986). The FRAP method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants (Benzie and Strain, 1996). 1 mL of EEG or EET solution in ethanol at different concentrations (250-1000 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K_3 Fe $(CN)_6$] solution. The reaction mixture was thoroughly mixed and the mixture was then left in an ultrasonic water bath for 20 min at 50 ºC. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 2000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Ascorbic acid was used as a reference standard. It was noteworthy that the iron reducing power of the samples was comparable to that of standard reference. Higher absorbance indicated greater reducing capacity.

2.10. Statistical analysis

The presented data (mean ±standard deviation) resulted from at least three independent experiments and analyzed by SPSS (version 17 for Windows 10 pro, SPSS Inc.). One-way analysis of variance (ANOVA) was performed by standard methodology and $p < 0.05$ was considered significant and $p < 0.01$ was very significant.

3. RESULTS AND DISCUSSION

3.1. Total phenolic content and ethanolic extraction yield

Extraction efficiency of turmeric and ginger powders and the mean values of total phenolics (TPC) in EET and EEG investigated in this study are presented in (Table 1). The total phenolic content of EET and EEG was found to be 82.47±2.70 and 48.56±1.64 respectively (mg GAE/g). As per the ongoing reports, a profoundly positive connection between total phenols and antioxidant capacity was found in many plant species (Velioglu et al., 1998). As it can be seen in Table 1, the extraction efficiency of GP (5.58 %) is higher than TP (3.71 %).

Table 1. Extraction efficiency of dry rhizomes of turmeric and ginger and total phenolic content (TPC) in EET and EEG.

	Extraction yield (% in 100 g)	Total phenolic contents (mg GAE/g)
TP	3.71	-
GP	5.58	-
EET	-	82.47 ± 2.70
EEG	-	48.56 ± 1.64

TP: Turmeric powder, GP: Ginger powder, EET: ethanolic extract turmeric, EEG: ethanolic extract ginger

3.3 HPLC analysis of phenolic compounds

The amounts of phenolic compounds detected in the samples are presented in Table 2. Results are expressed in mg 100 g^{-1} of dry sample. The most abundant phenolic components in turmeric rhizomes are ferulic acid (93.59 mg 100 g^{-1} dry sample), benzoic acid (40.09 mg 100 g^{-1} dry sample), vanillin (26.69 mg 100 g^{-1} dry sample dry sample) and p-coumaric acid (23.25mg 100 g^{-1} dry sample) respectively. Other phenolic components were identified as Caffeic acid (6.75 mg 100 g^{-1} dry sample) and Chlorogenic acid (4.37 mg 100 g^{-1} dry sample) respectively. On the other hand, the most common phenolic components in ginger rhizomes are Benzoic acid (33.31 mg 100 g^{-1} dry sample), Ferulic acid (11.41 mg 100 g^{-1} dry sample) and vanillin (11.83 mg 100 g^{-1} dry sample) respectively. Other phenolic components were identified as Caffeic acid (6.71 mg 100 g^{-1} dry sample), Chlorogenic acid (3.99 mg 100 g^{-1} dry sample), Cinnamic acid (3.46 mg 100 g^{-1} dry sample) and Syringic acid (2.12 mg 100 g^{-1} dry sample). From the results, it was observed that the phenolic component contents of turmeric and ginger rhizomes are partially similar.

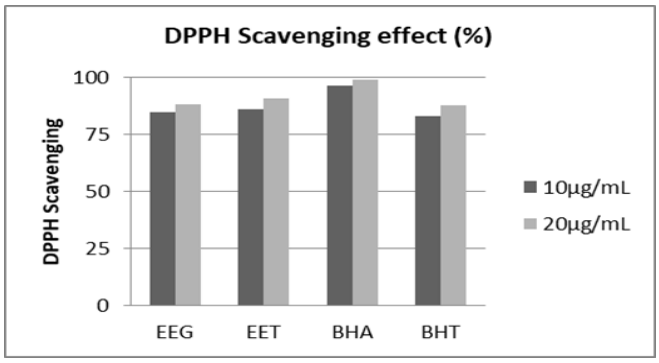
Table 2. Phenolic compounds of Ginger and Turmeric

Phenolic Compound	HPLC Retention Time (min)	Ginger (mg $100g^{-1}$of dry sample)	Turmeric (mg $100g^{-1}$of dry sample)
Chlorogenic acid	14.6	3.99	4.37
Caffeic acid	17.3	6.71	6.75
Syringic acid	19.9	2.12	-
Vanillin	21.0	11.83	26.69
p-Coumaric acid	24.5	-	23.25
Ferulic acid	28.1	11.41	93.59
Benzoic acid	34.8	33.31	40.09
Cinnamic acid	66.7	3.46	-

3.4. DPPH radical scavenging activity

In this study, free radical scavenging activities of EEG, EET and standards such as BHA and BHT were determined using a DPPH method. DPPH is often used to evaluate the free radical scavenging effects of different antioxidant substances (Ozcelik *et al.*, 2003). In the radical formation, this molecule had an absorbance at 517 nm which vanished after receipt of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthäus, 2002) Figure 1 displayed a significant decrease ($p < 0.01$) in the concentration of DPPH radical due to the scavenging ability of EET, EEG and standards. BHA and BHT were used as standarts for free radical scavengers. The scavenging effect of EET, EEG and standards on the DPPH radical decreased in the order of BHA > EET >EEG> BHT which were 98.8%, 90.9%, 88.3% and 87.8%, at the concentration of 20µg/mL, respectively. Free radical scavenging activity of these samples also increased with an increasing concentration.

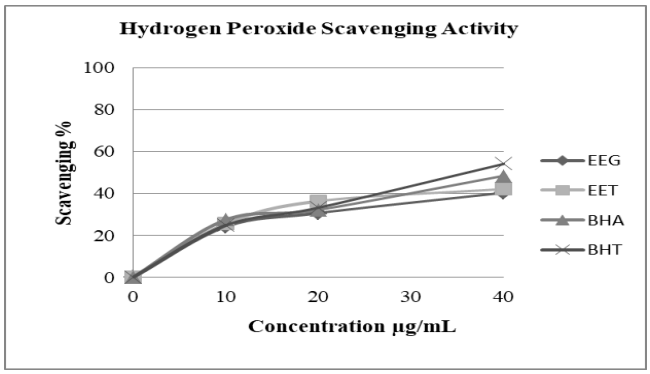
Figure 1. Scavenging effect of EET, EEG, BHA and BHT, on the stable DPPH• at different concentrations (10–20 µg/mL) (EET: ethanolic extract turmeric, EEG: ethanolic extract ginger, DPPH•: 1,1-diphenyl-2-picryl-hydrazyl free radicals, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, Data expressed as mean± S.D (n=3).



3.5. Hydrogen peroxide scavenging effects

The scavenging ability of EEG and EET on H_2O_2 is shown in Figure 2 and compared with BHA and BHT as standards. 40 µg/mL of EEG and EET exhibited 40.28 ± 0.73 and 42.04 ± 0.37 % scavenging activity ($p < 0.05$) on H_2O_2, respectively. However, BHA and BHT showed 48.32 ± 0.08 and 54.03 ± 1.39 % H_2O_2 scavenging activity at the same concentration. These findings demonstrated that EEG and EET had powerful hydrogen peroxide scavenging activity. The H_2O_2 scavenging effects 40 µg/mL concentration of EEG and EET and standards decreased in the order of BHT > BHA > EET > EEG. However, as the concentration of EEG and EET increases, radical scavenging activity in hydrogen peroxide increases.

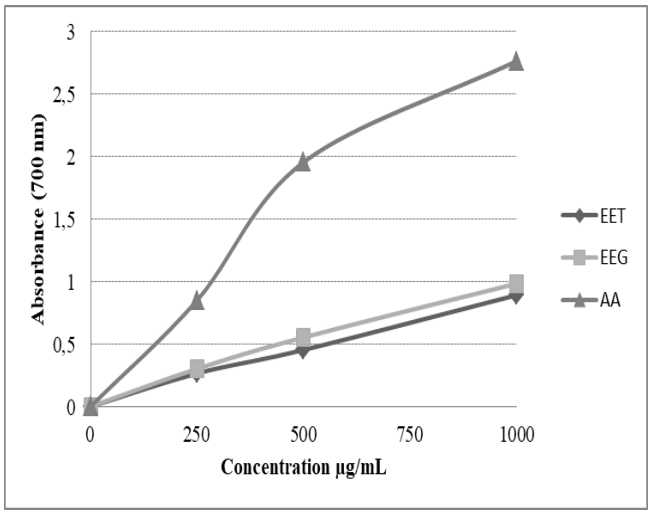
Figure 2. Scavenging effect of EET, EEG, BHA and BHT on H_2O_2, at different concentrations (10–40 µg/mL) (EET: ethanolic extract turmeric, EEG: ethanolic extract ginger, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, H_2O_2: Hydrogen Peroxide, The Scevenging (%) was the mean ± SD (n =3) $p < 0.05$).



3.6. Total reductive capability using the potassium ferricyanide reduction method

In this method, the reducing capacity of the extracts (EET and EEG) were accomplished using Fe^{3+} to Fe^{2+} reduction assay. In this analysis, yellow color turned pale green and blue color depending on the concentration of antioxidant capacity in the samples. The ability of a constituent to reduce iron may serve as a significant indicator of its potential antioxidant activity. As can be seen from Figure 3, EEG and EET had effective reducing power using the potassium ferricyanide reduction method when compared to the standard. At different concentrations (250–1000 µg/mL), EEG and EET demonstrated powerful reducing ability (r^2: 0.9973 and r^2: 0.9935, respectively) and these differences were statistically very significant ($p < 0.01$). The reducing power of EEG, EET and AA were increased with increase of sample concentrations. Reducing power ability of EEG, EET and standard compound exhibited the following order: AA >EET> EEG.

Figure 3. Total reductive potential of different concentrations (250–1000µg/mL) of EEG (r^2: 0.9973), EET (r^2: 0.9935), and reference antioxidant: ascorbic acid using spectrophotometric detection of the Fe^{3+}–Fe^{2+} transformations. In the presence of reductants, Fe^{3+}/ferricyanide complex reduces to the ferrous form (EET: ethanolic extract turmeric, EEG: ethanolic extract ginger, AA; ascorbic acid, Absorbance was the mean ± *SD* (n =3) $p < 0.01$).



3.7. Mineral content

Mineral content of the studied samples is given in Table 3. Elemental analysis of both ethanol extracts and powder of ginger and turmeric rhizomes were performed. The potassium was, quantitatively, the most abundant mineral in samples. Subsequently, sodium, magnesium phosphorus and calcium were identified, respectively. While the amount of these elements is higher in ginger and turmeric rhizomes, it decreased in EEG and EET.

Table 3. Mineral analysis of samples (Al, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Se and Zn) (mg element g^{-1})

Samples mg element g^{-1}				
Element	GP	TP	EEG	EET
Al	0.102 ± 0.0020	0.159 ± 0.0015	<0.012	<0.012
Ca	0.635 ± 0.0081	0.666 ± 0.0097	< 0.025	< 0.025
Cd	<0.005	<0.005	<0.005	<0.005
Cr	<0.004	<0.004	<0.004	<0.004
Cu	0.003 ± 0.0001	0.002 ± 0.0001	0.005 ± 0.0000	0.001 ± 0.0000
Fe	0.081 ± 0.0001	0.175 ± 0.0015	0.001 ± 0.0000	<0.010
Hg	<0.051	<0.051	<0.051	<0.051
K	13.84 ± 0.189	24.73 ± 0.173	1.388 ± 0.0129	3.782 ± 0.0240
Mg	1.686 ± 0.0115	1.582 ± 0.0181	0.002 ± 0.0001	0.018 ± 0.0000
Mn	0.244 ± 0.0015	0.019 ± 0.0002	0.001 ± 0.0000	<0.002
Mo	<0.003	<0.003	<0.003	<0.003
Na	3.069 ± 0.0336	0.406 ± 0.0091	0.889 ± 0.0103	0.315 ± 0.0038
P	0.996 ± 0.0074	1.584 ± 0.0133	0.304 ± 0.0019	0.090 ± 0.0003
Pb	< 0.030	< 0.030	< 0.030	< 0.030
Se	< 0.021	< 0.021	0.001 ± 0.0001	< 0.021
Zn	0.010 ± 0.0001	0.003 ± 0.0001	0.002 ± 0.0000	0.001 ± 0.0000

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4. CONLUSION

As indicated by the consequences of this investigation, it is obviously show that EEG and EET have high antioxidant capacity, high mineral profile and free radical scavenging potantial against different antioxidant systems in vitro. EEG and EET were discovered to be a powerful antioxidant in different in vitro assays including: RP, DPPH•, and hydrogen peroxide scavenging capacity when compared to standard antioxidant compounds such as BHA, BHT and ascorbic acid. Both the powders of turmeric and ginger rhizomes and their extracts have been found to be rich in bioactive components and mineral content. These essays have significant applications for the food and pharmaceutical industry. It should be encouraged to produce and novel products containing turmeric and ginger extracts, which have high antioxidant activities and are rich in bioactive substances.

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