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Antioxidant Capacity and Determination of Total Phenolic Compounds in Daisy (*Matricaria chamomilla, Fam. Asteraceae*)

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Abstract Daisy is a medicinal plant which is used for treating several diseases. This investigation describes the antioxidant capacity of different parts of daisy, collected from Tokat-Turkey, using various antioxidant assays. It was understood that all parts (flower, stem, and whole herb) of daisy have antioxidant activity. It was determined that there is extra activity of reduction power in the whole herb, extra activity of scavenging of superoxide anion radical in the stem of the plant, extra activity of total antioxidant activity in the whole herb, extra activity of metal chelating activity in the flower, but there is almost equal activity of scavenging free radical in the flower, in the stem and in the whole herb. In addition, total phenolic compounds were analyzed. The concentration of total phenolic compounds was 29.4 µg kg^{-1} dry weight in the flower, 22.3 µg kg^{-1} dry weight in the stem, and 32.1 µg kg^{-1} dry weight in the whole herb.

Keywords: *antioxidant capacity, phenolic compounds, free radical, matricaria chamomilla (Fam. Asteraceae), Daisy*

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1. Introduction

Matricaria chamomilla (MC) has a widespread use as a herbal medicine which posseses many medicinal properties [1]. Its aqueous infusion and tea preparations are used in folk medicine. There are two major varieties of chamomile namely: Roman chamomile (*Chamaemelum nobile L.*) and German chamomile (*Matricaria chamomilla*). The German variety is the commonly used for medicinal purposes. The flowers of chamomile can be used to cure, a range of disorders, specially inflammatory conditions [1]. The pharmacological properties of MC made it increasingly popular and is consumed in the form of tea [2].

Biologically active chemicals such as polyphenols (flavonoids) and essential oils had been isolated and identified from Matricaria chamomilla flowers [3]. Essential oil of Matricaria chamomilla consists of terpenoids and azulenes [4]. Terpenoids and bisabolol possess anti-inflammatory properties [1]. Chamazulene possesses antioxidant activity as it inhibits lipid peroxidation [5]. Several phenolic compounds of Matricaria chamomilla, including apigenin, quercetin, and luteolin had been analyzed [6,7]. Flavonoids play an important role in inflammatory processes and immune functions through inhibition by several enzymes [6]. One of the major reasons for deterioration of food and pharmaceutical products is due to lipid peroxidation which could be inhibited by the antioxidants which scavenge free radicals.

Therefore, antioxidants are widely used as food additives to retard the oxidative degradation of foods [8,9]. Currently, BHA, BHT, propyl gallate and *tert*–butylhydroquinone are the most commonly used antioxidants. However, BHA and BHT have been suspected to cause liver damage and carcinogenesis [10]. Therefore, it is more beneficial to use antioxidant compounds from natural sources since they will be safer than the synthetic ones [11,12,13,14].

The purpose of this work is to evaluate the antioxidant capacity of different parts of daisy collected from Tokat-Almus using various antioxidant assays e.g. antioxidant activity in linoleic acid emulsion, scavenging of superoxide anion radical activity, metal chelating activity, $DPPH^{\cdot}$ free radical scavenging activity and ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP). Furthermore, the determination of the total phenolic compounds were in the various daisy parts were carried out.

2. Materials and Methods

2.1. Chemicals

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), linoleic acid, α-tocopherol, polyoxyethylenesorbitan monolaurate (Tween-20) and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck (Darmstadt, Geramny). All other chemicals used were in analytical grade and obtained from either Sigma-Aldrich or Merck.

2.2. Plant Material and Extraction Procedures

Methanol extraction of dry flower, stem, and whole herb of *Matricaria chamomilla* samples was conducted. A 100 grams of samples were ground into a fine powder and was extracted with 500 mL methanol. The residue was re-extracted under the same condition until extraction solvents became colourless. The combined extracts were filtered over Whatman No.1 paper. The methanol was evaporated using a rotary evaporator at 50℃ to obtain dry extract. The extract was used for antioxidant assays and the total phenolic compounds determination.

2.3. Determination of Antioxidant Activity by Ferric Thiocyanate Method

The antioxidant activities were determined according to the method described by Mitsuda et al., [15]. 10 mg of methanol extract of *Matricaria chamomilla* parts (MEMCP) were dissolved in 10 mL methanol and used as a stock solution. Then, the solution containing different concentration of MEMCP from 25 to 75 µg mL^{-1} solution in 2.5 mL of sodium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion in sodium phosphate buffer (0.04 M, pH 7.0). Thus, 5 mL of the linoleic acid emulsion was prepared by mixing and homogenising 15.5 µL of linoleic acid, 17.5 mg of tween-20 as emulsifier, and 5 ml phosphate buffer (pH 7.0). On the other hand, 5 mL of negative control was composed of 2.5 mL of linoleic acid emulsion and 2.5 mL, 0.04 M sodium phosphate buffer (pH 7.0). The mixed solution (5 mL) was incubated at 37℃ in glass flask. The peroxide levels were determined by reading the absorbance at 500 nm in a spectrophotometer (Jasco V-530 UV/VIS Spectrophotometer), after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. Blank samples were prepared as described above but without adding the extract solution. Total antioxidant activity determined are the average of triplicate analyses. α-Tocopherol was used as positive control in this test. The inhibition of lipid peroxidation in % was calculated by the following equation:

$$\%\ Inhibition = 100 - [(A_1 / A_o)'100]$$

where A_o was the absorbance of the negative control reaction and A_1 was the absorbance in the presence of the sample or positive controls.

2.4. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power Assay (FRAP)

The reducing power of MEMCP was determined using the method reported by Oyaizu [16] but with slight modification [10,12]. Different concentrations of MEMCP (10-150 µg mL^{-1}) in 1 mL of distilled water were mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution. The mixture was incubated at 50℃ for 20 min. Aliquots (2.5 mL) of 10% trichloroacetic acid were added to the mixture. The 2.5 mL of this solution was mixed with 2.5 mL distilled water and 0.5 mL of 01% FeCl3, and the absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture indicates an increase of reduction capability.

2.5. Ferrous Ions (Fe^{2+}) Chelating Activity

The chelating of ferrous ions of MEMCP was estimated by the method of Dinis [17], where the chelating ability of MEMCP to the Fe^{2+} ions was monitored by the absorbance of the ferrous iron–ferrozine complex at 562 nm. Different concentration of samples (from 10 - 40 µg mL^{-1}) in 0.4 mL was added to a solution of 2 mM $FeCl_2$ (0.2 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.4 mL) and total volume was adjusted to 4 mL of ethanol. The mixture was shaken vigorously and left at room temperature for ten minutes. Absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used as negative control. The inhibition of ferrouse ion chelating activity in % was calculated by the following equation:

$$\%\ Inhibition = 100 - [(A_1 / A_o)$$

where A_o was the absorbance of the negative control (EDTA) and A_1 was the absorbance in the presence of the sample.

2.6. DPPH Free Radical Scavenging Activity

The free radical scavenging activities of MEMCP was measured by 1, 1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$) using the method of Blois [18], wherein the bleaching rate of a stable free radical, $DPPH^{\bullet}$, is monitored at a characteristic wavelength in the presence of the sample. $DPPH^{\bullet}$ absorbs at 517 nm in its free radical form, but upon reduction by an antioxidant or a radical species its absorption decreases. When a hydrogen atom or electron was transferred to the odd electron in $DPPH^{\bullet}$, the absorbance at 517 nm decreases proportionally to the increase of non-radical forms of $DPPH^{\bullet}$. 0.1 mM solution of $DPPH^{\bullet}$ in methanol was prepared and 1 ml of this solution was added 3 mL of methanolic extract at different concentrations (10-125 µg mL^{-1}). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 517 nm. Half maximal inhibitory concentration (IC_{50}) was calculated after percentage of scavenging acyivity calculation.

2.7. Superoxide Radical Scavenging Activity

Superoxide anion radicals were generated using the method described by Zhishen et al., [19]. Superoxide

radicals were generated in riboflavin, methionine, then illuminated and assayed by the reduction of NBT to form blue formazan. All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were performed using fluorescent lamps (20 W). The total volume of the reaction mixture was 3 mL, and the concentrations of the riboflavin, methionine and NBT were $1.33x10^{-5}$, $4.46x10^{-5}$ and $8.15x10^{-8}$ M, respectively. The reaction mixture was illuminated at 25°C for 40 min. The photochemically reduced riboflavin generated $O_2^{\bullet-}$, which reduced NBT to form blue formazan. The un-illuminated reaction mixture was used as blank. The illuminated reaction mixture without samples or other chemical was used as negative control. The absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. Half maximal inhibitory concentration (IC_{50}) was calculated after percentage of scavenging activity calculation.

2.8. Determination of Total Phenolic Compounds by Folin-Ciocalteu Reagent

The total phenolic contents in MEMCP was determined using Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [20]. Gallic acid was used as a standard phenolic compound. 1 mL of extract solution contains 1 mg extracts diluted with distilled water (46 mL). One millilitre of Folin-Ciocalteu reagent was added and the content was mixed thoroughly. After 3 min, 3 mL of 2% Na_2CO_3 was added and then the mixture was allowed to stand for 2 h with occasional shaking. The absorbance was measured at 760 nm. The amount of total phenolic compounds in MEMCP determined as microgram of gallic acid equivalent (GAE) using an equation obtained from the calibration curve of gallic acid graph.

2.9. Statistical Analysis

All the experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's.

3. Results

Antioxidant capacity is widely used as a parameter for medicinally bioactive and functional commpounds in food. The antioxidant activities of MEMCP were compared to BHA, BHT, trolox and α-tocopherol as positive control in this investigation. Table 1 shows the extraction yields and total phenolic contents of MEMCP. The antioxidant effect of plant phenols has been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [21,22]. Velioglu et al., [23] reported that in many plant species, there is a highly positive relationship between total phenols and antioxidant activity.

Table 1. Yield and total phenolic contents in percent of methanol extract of flower, stem, and whole herb of Matricaria chamomilla

Plant Part	Yield (%)	Total Phenolic Compounds (mg/kg DW*)
Stem	22.3	23.6
Flower	29.4	31.9
Whole Herb	32.1	37.1

* Dry weight

3.1. Determination of Antioxidant Activity in Linoleic Acid Emulsion

Methanol extract of MC exhibited effective antioxidant activity in this system as shown in Figure 1. The effect of 10 μg mL^{-1} concentration of MEMCP after 30 hours incubation were found to be 69, 60 and 63%, respectively and their activities are higher than the same concentration of α-tocopherol (57%). The differences are significant statistically ($p<0.05$).

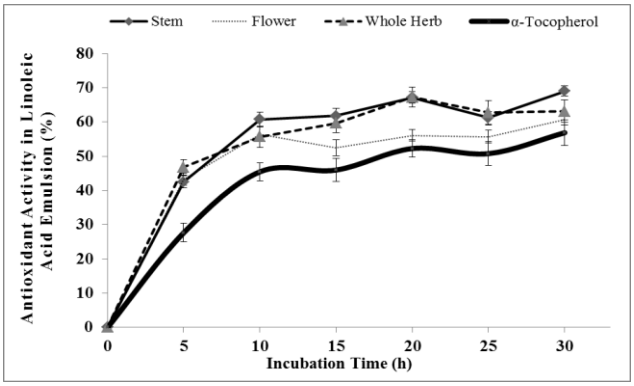

Figure 1. Aantioxidant activities in linoleic acid emulsion of methanol extract of flower, stem, and whole herb of *Matricaria chamomilla* and α-tocopherol at 10 μg mL^{-1} concentration

3.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power Assay (FRAP)

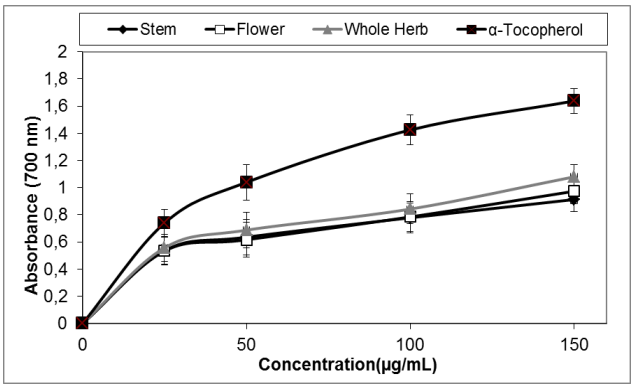

Figure 2. Total reductive potential of different concentrations (10-150 μg mL^{-1}) of methanol extract of flower, stem, and whole herb of *Matricaria chamomilla* and reference antioxidant; α-tocopherol

MEMCP had effective reducing power using the potassium ferricyanide reduction method when compared to α-tocopherol as shown in Figure 2. For the measurements of the reductive ability of MEMCP, the Fe^{3+}-Fe^{2+} transformation was investigated using the method of Oyaizu [16]. MEMCP demonstrated reducing ability at various concentrations (10-150 μg mL^{-1}). The reducing power of methanol extract of all parts of MC increased steadily with increasing concentration of samples. Reducing power of methanol extracts of all parts

of MC and standard compounds were in the following order: Whole Herb > Flower > Stem. The results on reducing power indicate the electron donor properties of methanol extract of MC, thus neutralizing free radicals by forming stable products.

3.3. Ferrous Ions (Fe^{2+}) Chelating Capacity

Ferrous ions (Fe^{2+}) chelating activities of methanol extract of all parts of MC are shown in Figure 3. The chelating effect of ferrous ions (Fe^{2+}) by MC extract was determined according to the method of Dinis [17]. As can be seen in Figure 3, methanol extract of all parts of MC exhibited marked chelation of ferrous ion at all used concentrations ($p<0.01$). The percentages of ferrous ions (Fe^{2+}) chelating capacity of the same concentration (40 µg mL^{-1}) of methanol extract of flower, stem, and whole herb of Matricaria chamomilla were found as 85, 67, and 73%, respectively. These results show that the chelating effect of methanol extract of flower, stem, and whole herb of Matricaria chamomilla are high to the ferrous ion (Fe^{2+}).

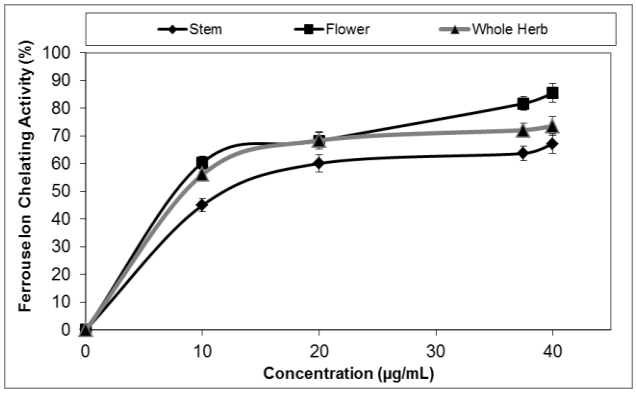

Figure 3. Metal chelating effect of different concentrations (10-40 µg mL^{-1}) of methanol extract of flower, stem, and whole herb of *Matricaria chamomilla*on ferrous ions (Fe^{2+})

3.4. Radical Scavenging Activity

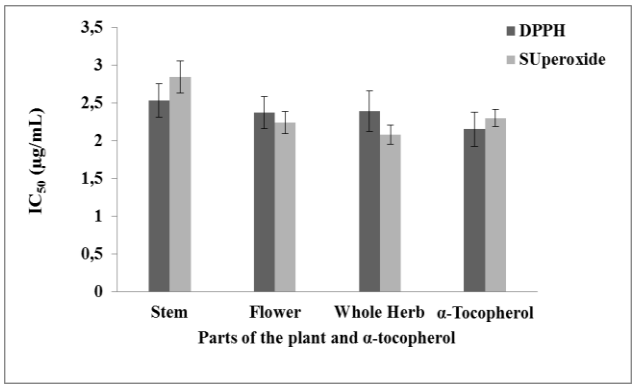

Figure 4. DPPH free radical and superoxide anion radical scavenging activities of methanol extract of flower, stem, and whole herb of *Matricaria chamomilla* and α-tocopherol at 40 µg/mL concentration. [DPPH•: 1,1-diphenyl-2-picryl-hydrazyl free radical]

The determination of potential radical scavenging activities of methanol extract of all parts of MC was accessed using DPPH• method which is based on the reduction of alcoholic DPPH• solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. This molecule has an absorbance at 517 nm in the radical form, which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. Figure 4 illustrates DPPH• scavenging activity as IC_{50}. DPPH• scavenging activities of flower and whole herb parts are almost same and are higher than stem (Figure 4). α-tocopherol scavenging activity is higher than MEMCP but the differences are statistically significant only in stem part ($p<0.05$).

3.5. Superoxide Radical Scavenging Activity

Figure 4 shows the scavenging activity of superoxide radical generation of MEMCP and α-tocopherol. The scavenging of superoxide radical is presented as IC_{50}. IC_{50} value of methanol extract of flower, stem, and whole herb of MC and α-tocopherol were found as 2.8, 2.2, 2.1, and 2.3 respectively (Figure 4). According to these results, methanol extract of flower, stem, and whole herb of MC had high superoxide anion radical scavenging activity. Except stem remind parts (flower and whole herb) of MC on superoxide radical scavenging activities are the almost same and statistically similar to α-tocopherol ($p>0.05$). Scavenging activity of stem is statistically most significant than α-tocopherol ($p<0.05$).

3.6. Determination of Total Phenolic Compounds by Folin-Ciocalteu Reagent

The amounts of total phenolic compounds in MEMCPs are given in Table 1. Phenolic contents of stem, flower, and whole part of *Matricaria chamomilla* were found as 23.6, 31.9, and 37.1 mg kg^{-1} dry weight (DW), respectively (Table 1).

4. Discussion

4.1. Determination of Antioxidant Activity in Linoleic Acid Emulsion

Lipid peroxidation is usually associated with several biological damage due the free radical- chain reactions involved [24]. The amount of peroxide produced during the initial stages of oxidation is measured by the ferric thiocyanate method, which is the primary product of lipid oxidation. Hydroperoxide which is produced by linoleic acid which was added to the reaction mixture, and was oxidized by air, was indirectly measured. All parts of *Matricaria chamomilla* showed antioxidant activity in linoleic acid emulsion which was higher than α-tocopherol. The highest activity was shown at stem part. Cemek et al., [25] reported that MCE diminished oxidative stress related to hyperglycemia in streptozotocin (STZ)-diabetic rats.

4.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power Assay (FRAP)

The reducing power of bioactive compounds is associated with their electron donating capacity and this is reflected with their antioxidant activity [26]. The presence of reductants in the antioxidant samples causes the

reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, F^{e2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [21,27]. There are a number of assays designed to measure overall antioxidant activity or reducing potential, as an indication of host total capacity to withstand free radical stress [28]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

4.3. Ferrous Ions (Fe^{2+}) Chelating Capacity

Ferrous iron (Fe^{2+}) can facilitate the production of reactive oxygen species (ROS) within animal and human systems. The ability of compounds to chelate iron can be a valuable indicator for their potential antioxidant capability [29]. Accordingly, ferrous ions (Fe^{2+}) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation. Iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity among transition metals. The effective ferrous ions (Fe^{2+}) chelators may also afford protection against oxidative damage by removing iron (Fe^{+2}) that may otherwise participate in $HO^{\bullet}$ generating Fenton type reactions.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^- \left(Fenton\ reaction\right)$$

The complex formation is disrupted in the presence of chelating agents, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity and therefore it is considered an important antioxidant property. The methanol extract of flower, stem, and whole herb of *Matricaria chamomilla* was assessed for its ability to compete with ferozzine for ferrous ions (Fe^{2+}) in the solution. In this assay, methanol extract of flower, stem, and whole herb of *Matricaria chamomilla* interfered with the formation of ferrous ions (Fe^{2+}) and ferrozine complex. This suggests that they possess chelating activity and are able to capture ferrous ion before ferrozine.

4.4. Radical Scavenging Activity

The use of $DPPH^{\bullet}$ spectrophotometric method for determination of the antioxidant capacity of food, beverages and vegetable extracts is a common assay to evaluate the antioxidant activity of substances [30]. This chromogen radical compound can directly react with antioxidants. Furthermore, $DPPH^{\bullet}$ scavenging method is simple, rapid, sensitive, and reproducible [31]. It is considered as a standard assay for evaluating the antioxidant activity of compounds [32].

4.5. Superoxide Radical Scavenging Activity

Superoxide is biologically toxic and is deployed by the immune system to kill invading microorganisms. It is an oxygen-centred radical with selective reactivity. It is produced by a number of enzyme systems in autooxidation reactions and by nonenzymatic electron transfers that univalently reduce molecular oxygen. The superoxide toxicity is due to its capacity to inactivate iron-sulfur cluster containing enzymes, which are critical in a wide variety of metabolic pathways, thereby liberating free iron in the cell, which can undergo Fenton-chemistry and generate the highly reactive hydroxyl radical. It can also reduce certain iron complexes such as cytochrome c enzyme system. Superoxide anions are a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage [29]. This can be rationalized by its transformation into more reactive species such as hydroxyl radical causing initiation of lipid peroxidation. Superoxide can also directly initiate lipid peroxidation [33]. In addition, it has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical [22]. Superoxide anion plays an important role in the formation of other reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA [34]. Superoxide radical is normally formed first, and its effects can be magnified by the production of other kinds of free radicals and oxidizing agents [35].

4.6 Correlations of Phenolic Compounds with Antioxidant Activities

The relationship between the antioxidant activities (radical scavenging activities, FRAP, and metal chelating activity) and phenolic contents of MEMEPs had a positive linear correlation. There was no correlation between antioxidant activities in linoleic acid emulsion of MEMEPs and phenolic contents. The correlations of total phenolic content with DPPH free radical scavenging, superoxide anion radical, FRAP, and metal chelating activities were high. The correlation coefficient of DPPH free radical scavenging, superoxide anion radical, FRAP, and metal chelating activities were analyzed as $r^2 = 0.7529$, 0.9589, 0.7206, 0.9999, respectively ($p<0.01$). Cos et al., [36] and Thaipong et al., [37] reported a highest correlation between total phenolic content and FRAP activity as compared to other antioxidant assays [36,37]. Plant phenolic compounds are considered to be the most important antioxidants as they act as the free radical terminators.

5. Conclusions

The results obtained in the present study indicate that the methanol extract of flower, stem, and whole herb of *Matricaria chamomilla* was effective antioxidant. *Matricaria chamomilla* can be used to minimize or prevent lipid oxidation in pharmaceuticals and food products, by retarding the formation of toxic oxidation products.

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Statement of Competing Interests

The authors have no competing interests.

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