Comparison of Antioxidant and Antimicrobial Properties for Ginkgo biloba and Rosemary (Rosmarinus officinalis L.) from Egypt

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Abstract

The widespread use of medicinal plants for health purposes has increased dramatically due to their great importance to the public health. In this study levels of phenolic, flavonoid contents of Ginkgo biloba and Rosmarinus officinalis from Egypt were determined. HPLC was used to identify and quantify the phenolic compounds in selected plants. The plant extracts were evaluated for their antioxidant activities using various antioxidant methodologies, (i) scavenging of free radicals using 2, 2-diphenyl-1-picrylhydrazyl, (ii) metal ion chelating capacity, and (iii) scavenging of superoxide anion radical. The antimicrobial activity of both plant’s extracts were evaluated against a panel of microorganisms by using agar disc diffusion method. The total phenolic content (75.30 and 98.31 mg/g dry weight in G. biloba and R. officinalis, respectively) was significantly (p<0.05) different. Among the identified phenolic compounds, quercetin, kaempferol and caffeic acid were the predominant phenolic compounds in Ginkgo biloba, whereas carnosic acid, rosmarinic acid, naringen and hispidulin were the predominant phenolic compound in Rosmarinus officinalis leaves. The antioxidant activity increased with the concentration increase. The R. officinalis was more active than G. biloba extract against Gram-negative bacteria. This study reveals that the consumption of these plants would exert several beneficial effects by virtue of their antioxidant and antimicrobial activities.

Keywords: antimicrobial activity, antioxidant activity, Ginkgo biloba, phenolic compounds, Rosmarinus officinalis

Introduction

Nature has been a source of medicinal agents since time immemorial. Herbal medicine is still the mainstay of about 65-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines (Afify et al., 2011a, 2011b, 2012a; Ali et al., 2011). The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body (Afify and El-Beltagi, 2011). The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds (Shariff, 2001). Phytochemicals are extensively found at different levels in various medicinal plants and used in herbal medicine to treat diverse ailments such as cough, malaria, wounds, toothache and rheumatism diseases (Exarchou et al., 2002). The majority of disease/disorders are mainly linked to oxidative stress due to presence of reactive oxygen species (ROS). The most common ROS are superoxide anion (O2·−), hydroxyl radicals (OH·) and hydrogen peroxide (H2O2) which has been implicated in the etiology and pathophysiology of human diseases such as inflammation, viral infections, autoimmune pathologies and ulcer (Surh and Ferguson, 2003). ROS can readily react with and oxidize most bio-molecules including carbohydrates, proteins, lipids and DNA. In addition, oxidative damage caused by ROS is one of the major factors for the deterioration of food products during processing and storage (Aly and El-Beltagi, 2010; El-Beltagi et al., 2008, 2010, 2011a, Ibrahim et al., 2012; Kesba and El-Beltagi, 2012; Kobeasy et al., 2011; Mohamed et al., 2009; O’Kane et al., 1996), particularly when plants are exposed to stress conditions such as chilling stress, salt stress, Fe deficiency, cadmium stress, Lead toxicity and ionizing radiation, nematode infection, organisms and micro-orgaisms. Effective synthetic antioxidants such as butylated hydroxytoluene (BHT) have been used for industrial processing but these synthetics are suspected of being responsible for liver damage and carcinogenesis (Barlow, 1990). Recently, there is an increasing interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant compounds which widely distributed in plants are capable to terminate free radical-mediated oxidative reaction and would have beneficial activities in protecting the human body from such diseases (Havsteen et al., 2002). The ability of phenolic compounds to serve as antioxidants has been recognized by donating a hydrogen atom (Abdel-Rahim
et al., 2013; Abdel-Rahim and El-Beltagi, 2010; Shallan et al., 2010a, 2010b; Soong and Barlow, 2004). Furthermore, flavonoid are a large group of naturally-occurring plant phenolic compounds that inhibit lipid oxidation by scavenging radicals or by other mechanisms such as singlet oxygen quenching, metal chelation, and lipoxygenase inhibition (Abdel-Rahim and El-Beltagi, 2011; El-Beltagi et al., 2011b; Mohamed et al., 2010; Yanishlieva-Maslarova, 2001). Within the recent years, infections have increased to a great extent and resistance against antibiotics becomes an ever-increasing therapeutic problem (Austin et al., 1999). Antimicrobials of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Parekh et al., 2005). The mechanism of polyphenols toxicity against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions to inactive microbial adhesins, cell envelope transport proteins, non specific interactions with carbohydrates (Cowan, 1999). However, Maidenhair Tree (Ginkgo biloba) (Family- Ginkgoaceae) and Rosemary (Rosmarinus officinalis L.) (Family- Lamiaceae) are widely used as medicinal plants either by themselves or in combination with other herbs.

Ginkgo biloba leaves used as traditional Chinese herbal medicine to treat asthma and chilblains and prevent to drunkenness for thousand years (Nakanishi, 2005). In recent years, Ginkgo biloba extract has been extensively studied due to its various medicinal properties in the world. It is known that, it is effect as memory enhancing supplement for elderly people. However, it is used in treating cardiac and cerebral diseases. The main reason of medicinal effect of Ginkgo biloba is to contain phytochemicals which have been reported to have protective effect cardiovascular diseases, diabetes, aging and several cancer types (Chan et al., 2007; Saw et al., 2006). This protective effect is attributed to antioxidant activity of Ginkgo biloba leaves (Malta et al., 2011). The leaves extract of Ginkgo biloba is a standardized extract to contain 24% flavonoids, 7% proanthocyanidins and 6% terpenoids (Goh and Barlow, 2002). The flavonoids are primarily flavonol-glycosides of kaempferol, quercetin and isohamnetin with glucose or rhamnose. The terpenoid fraction consists of a unique group of diterpenes (ginkgolides A, B, C and J) and the sesquiterpene, bilobalide. The Ginkgo biloba extract also contains a number of organic acids including kinureninc, hydroxykinureninc and vanillic acid. Rosemary (Rosmarinus officinalis L.) is a common household plant grown in many parts of the world. It is used for flavoring food, as a beverage, and in cosmetics as well as in folk medicine for its choleretic, hepatoprotective, antiarthrombic, antiulcerogenic, diuretic, antidiabetic, antinociceptive, anti-inflammatory, and antitumorigenic activity (Borrás Linares et al., 2011). Rosemary is known to contain appreciable amount of tannins (Perez et al., 2007; Varyar et al., 1998) which on irradiation might have a spike in the content of polyphenols. Rosemary and its constituents (carnosol, carnosic acid, ursolic acid, rosmarinic acid, caffeic acid) have been intensively studied during the last 10 years. Different effects of this spice important from the point of view of cancer prevention were observed (Slamenova et al., 2002). Therefore, the objectives of the present study are: (i) to determine the chemical composition of methanolic extracts of Ginkgo biloba and Rosmarinus officinalis L. leaves; (ii) to determine the content of total phenolic and flavonoids of both leave extracts, and (iii) to evaluate their in vitro antioxidant and antibacterial properties.

Materials and methods

Chemical reagents
Folin-Ciocalteu reagent, 2, 2-diphenyl-1- picrylhydrazyl (DPPH), sodium carbonate and aluminum chloride were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were obtained from Merck (Darmstadt, Germany). Ferrozone or 3-(2-pyridyl)-5, 6-bis (4-phenylsulfonylacid)-1,2,4- triazine monosodium salt were purchased from Sigma-Aldrich. All other reagents were of analytical grade.

Collection of plant materials
Plant materials of Ginkgo biloba and Rosemary (Rosmarinus officinalis L.) were purchased from the Egyptian local market. Plant material consists of mature leaves.

Microbial strain
Microorganisms used in this study (Tab. 1) were obtained from the American Type Culture Collection (ATCC).

Extraction of plant materials
Ginkgo biloba and Rosmarinus officinalis (leaves) was oven dried at 38ºC for 48 h until the powder did not form. Extraction of plant materials

<table>
<thead>
<tr>
<th>Microbial group</th>
<th>Indicator strain</th>
<th>Cultivation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive bacteria</td>
<td><strong>Staphylococcus aureus</strong> (ATCC 25923)</td>
<td>TSA + YE, 37ºC</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td><strong>Escherichia coli</strong> (ATCC 19404)</td>
<td>TSA + YE, 37ºC</td>
</tr>
<tr>
<td>Fungus</td>
<td><strong>Aspergillus niger</strong> (ATCC 16404)</td>
<td>PDA, 25 ºC</td>
</tr>
</tbody>
</table>

*Obtained from Department of Microbiology, Agriculture Faculty Cairo University, G*. Gram positive bacteria. G. Gram negative bacteria, TSA+YE, Trypticase Soy Agar + 0.6% Yeast Extract, PDA, Potato Dextrose Agar
Determination of total phenolic contents

Phenolic contents were determined based on a method described by Singleton et al. (1999). Briefly, 1 ml of methanolic extract was mixed with 1 ml of Folin Ciocalteu reagent. After 3 min, 1 ml of saturated sodium carbonate solution (20%) was added to the mixture and adjusted to 10 ml with distilled H2O. The reaction mixture was kept in the dark for 1 h with intermittent shaking. The absorbance was measured at 725 nm using a spectrophotometer (UNICAM UV300). Phenolic contents were calculated on the basis of the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per g of dry extract.

Determination of total flavonoid contents

The methanolic extract (250 μl) was mixed with 1.25 ml of distilled H2O and 75 μl of a 5% NaNO2 solution. After 5 min, 150 μl of a 10% AlCl3·H2O solution was added and filtered for 6 min. About 500 μl of 1 M NaOH and 275 μl of distilled H2O were added to the mixture, mixed well and the intensity of pink color was measured at 510 nm. The level of total flavonoid concentration was calculated using quercetin (QU) as a standard (Jia et al., 1999). The results were expressed as mg of quercetin equivalents per g of dry extract.

HPLC analysis of phenolic compounds

One gm of fresh leaves of each sample were homogenized with methanol 40% and stirred on a shaker. The extract was filtered through a whatman filter paper No. 1 and the solvent was evaporated in vacuum. The dried residues containing phenol compounds were dissolved in a solution consists of methanol, water, acetic acid (40:59.3:0.7, v:v:v) and stored in vials. The method suggested by Christian (1990) was used as follows, HPLC analysis was used to detect and determine the phenolic compounds from the plant tissues. The phenolic compound extracts were passed through micro-filter 0.45 μm. The analysis of phenolic compounds was performed on HPLC model (HP1050). HPLC equipped with UV detector. The separation and determination were performed on C18 column (150x4.6 mm). The mobile phase yielded results of methanol, water, acetic acid, (40:59.3:0.7, v:v:v). The wave length of UV detector was 254 nm and the total run time for the separation was approximately 25 min at a flow rate of one ml/min. Identification of phenolic compounds was carried out by comparing retention times and spectral data with those of the standard mixture chromatogram. Quantification was done by an external standard method, in triplicate.

Determination of antioxidant properties

Radical scavenging ability using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical

The antioxidant activity of plant methanol extracts was determined based on the radical scavenging ability in reacting with a stable DPPH free radical according to Blois (2002). Briefly, 0.1 mM of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of methanolic plant extract (50-150 μg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm. The radical scavenging activities of BHT and BHA were also determined as positive controls. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Purple colored stable free radicals were reduced to the yellow colored diphenylpicrylhydrazine when antioxidant was added. The corresponding blank readings were taken and the capability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH}^\cdot \text{scavenging effect} \% = \left( \frac{A_0 - A}{A_1} \right) \times 100
\]

where, \(A_0\) = The absorbance of the control reaction (containing all reagents except the test compounds) and \(A_1\) = The absorbance in the presence of the tested extracts.

Determination of iron chelating agent using ferrozine

The iron-chelating capacity was determined according to the method of Dinis et al. (1994). Sample solutions at various concentrations (150 to 300 μg/ml) were prepared from methanolic plant extract. One ml aliquot was mixed with 100 μl of 1 mM FeCl2 and 3.7 ml of distilled H2O. The reaction was initiated by adding 200 μl of 5 mM ferrozine. After 20 min incubation at room temperature, the absorbance at 562 nm was recorded. NaEDTA was used as positive control. Percent activity was calculated using the following formula:

\[
\text{Metal chelating effect} \% = \left( \frac{A_0 - A}{A_1} \right) \times 100
\]

where, \(A_0\) = The absorbance of the control reaction and \(A_1\) = The absorbance in the presence of the samples.

Determination of superoxide anion (O2^-) scavenging activity

A measurement of superoxide anion scavenging activity was done based on the method described by Nishimiki...
et al. (1972). Sample solutions at various concentrations (100 to 400 μg/ml) were prepared from methanolic extract. About 1 ml of nitroblue tetrazolium (NBT) solution (156 M NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution were mixed. The reaction started by adding 100 μl of phenazine methosulfate solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated for 5 min at 25°C and the absorbance was measured at 560 nm. Quercetin was used as a positive control. The superoxide anion scavenging activity was calculated according to the following equation:

% Scavenging = \[1 - \frac{A_1 - A_2}{A_0}\] \times 100

where \(A_0\) was the absorbance of the control (blank, without extract) and \(A_1\) was the absorbance in the presence of the extract. \(A_2\) was the absorbance without PMS.

**Antimicrobial activities**

The antimicrobial activities of the tested plants were measured by disk assay procedure (Bauer et al., 1966) against indicator microorganisms such as food spoilage bacteria (Bacillus subtilis ATCC 6633), pathogenic bacteria (Escherichia coli ATCC 19404), (Salmonella typhi ATCC 14028), (Staphylococcus aureus ATCC 25923) and onion post-harvest spoilage fungus (Aspergillus niger ATCC 16404). Discs were used in assay agar plates. Soft agar medium culture seeded or inoculated with the tested microorganisms was layered over 10 ml of hard agar (2%). Plates were incubated at various temperatures for required incubation periods according to strain type (Tab. 1). A specific volume containing 40 μg/ml of each extract and specific volume containing 40 μg/ml of Tetracycline which used as positive control was impregnated into sterilized paper discs (Whatman No. 1) of 6 mm in diameter. Filter paper discs soaked in solvent were used for negative controls. After drying, the paper discs were plated on the assay plates in triplicate and left at 4°C for 24 h to allow maximum diffusion of the test sample. After incubation time, the distinct zone of inhibition surrounding the disc was measured. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm) as follows, - (negative) = 0 mm; + (weak) = 1-4 mm; ++ (moderate) = 5-10 mm; +++ (strong) =10-15 mm and ++++ (very strong) ≥ 16 mm. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

**Statistical analysis**

All experimental results were expressed as means ± S.D. Analysis of variance was performed by ANOVA procedures. The results with \(p<0.05\) were regarded to be statistically significant. Data were statistically analyzed using Costate Statistical Package (Anonymous, 1989).

**Results and discussion**

**Total phenolic, flavonoid contents and HPLC analysis of phenolic compounds**

It is well-known that plant phenolic contents are highly effective free radical scavengers and antioxidants. In this study, the total phenolic contents of methanolic extracts were determined using Folin-Ciocalteu reagent and expressed as mg gallic acid (GAL) equivalent/g dry weight. Significant differences \((p<0.05)\) were observed between both plants (Tab. 2). *R. officinalis* contained phenolic compounds at 98.31 mg/g d.w., whereas, *G. biloba* contains 75.31 mg/g d.w. The leaves of *R. officinalis* had higher content of phenolic compounds than that in *G. biloba* (Zheng and Wang, 2001). The content of flavonoid (mg/g), in quercetin equivalent varied from 84.59 to 113.55 mg/g d.w. in both plants. Total flavonoid contents had higher than total phenolic compounds in both plants.

Selected phenolics in extracted plants, separated and identified by using reversed-phase high performance liquid chromatography (HPLC), are presented in Tab. 3. Considerable variation was found in phenolic compounds of *G. biloba* and *R. officinalis*. *Ginkgo biloba* leaf extract, is a complex product containing different active compounds, is used as a phytomedicine to increase peripheral and cerebral blood flow (Hasler et al., 1992). The results of HPLC analysis of *G. biloba* leaf extract showed that, the major components were quercetin, kaempferol, caffeic acid and rutin which recorded (173.2, 157.41, 44.90, 35.98 mg/100 g of fresh weight) respectively (Tab. 3), whereas, the minor components were *p*-coumaric acid, vanillic acid, ferulic acid and naringen which recorded (14.25, 12.65, 9.68, 8.45) respectively. The extract of rosemary was the first marketed natural antioxidants. Several phenolic compounds of rosemary determined in this study were similar in content and concentration to those in previous reports (Cuvelier et al., 1996) i.e., rosmarinic acid (75.27 mg/100 g of fresh weight), naringin (96.29 mg/100 g of fresh weight), hispidulin (48.58 mg/100 g of fresh weight), carnosic acid (227.49 mg/100 g of fresh weight), and caffeic acid (13.41 mg/100 g of fresh weight). These phenolic compounds of *G. biloba* and *R. officinalis* are presented in Tab. 3.

**Tab. 2. Total phenolic, flavonoid contents of the methanolic extracts obtained from Ginkgo biloba and Rosmarinus officinalis plants**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Phenolic*</th>
<th>Flavonoids**</th>
<th>Flavonoids/ phenolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginkgo biloba</td>
<td>75.30 ± 0.69*</td>
<td>84.59 ± 1.43*</td>
<td>1.12 ± 0.009*</td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>98.31 ± 0.97*</td>
<td>113.55 ± 1.60*</td>
<td>1.155 ± 0.007*</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>1.92</td>
<td>3.43</td>
<td>0.0179</td>
</tr>
</tbody>
</table>

Data with different superscript letters in the same column were differed significantly \((p<0.05)\). * Mean of triplicate determinations ± SD expressed as mg GAL acid equivalent /g dry weight. ** Mean of triplicate determinations ± SD expressed as mg QU equivalent /g dry weight.
compounds in rosemary extracts are very potent antioxidants and are utilized in many food products (Zheng and Wang, 2001).

It has been found that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and in providing health beneficial effects. In addition, they serve in plant defense mechanisms to counteract ROS in order to survive and prevent molecular damage (Vaya et al., 1997). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits, vegetables and other plants (Tanaka et al., 1998).

Phenolic acids such as, caffeic acid, ferulic acid and vanillic acid are widely distributed in the plant kingdom. Caffeic acid has been found to have high activity comparable to that of the flavonoid, quercetin. Ferulic acid was shown to inhibit the photoperoxidization of linoleic acid at the somewhat high concentration of 10^3 M (Larson, 1988). The most widespread and diverse phenolics are the flavonoids which have the same C15 (C6-C3-C6) skeleton and possess antioxidant capacity toward a variety of easily oxidizable compounds (Robards et al., 1999). In many plants, the main flavonoid constituents are flavonol aglycones such as quercetin, myricetin, kaempferol, and their glycosides (Kähkönen et al., 1999). It has been recognized that flavonoid show antioxidant activity and their effects on human nutrition and health are considerable. The action mechanisms of flavonoid are through scavenging or chelating process (Kessler et al., 2003). The compounds such as flavonoid, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants (Das and Pereira, 1990). However, the flavonoid glycosides (including rutin, naringin, and hesperidin) usually have low antioxidant values (Robards et al., 1999).

### Antioxidant activity

**Free radical scavenging activity by DPPH method**

The proton radical scavenging action is known as an important mechanism of antioxidants. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability (Shimada et al., 1992). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of natural antioxidants. Scavenging effects of methanolic extracts from our two plants on DPPH radicals increased with concentration (Fig. 1). The decrease in the concentration of DPPH radical due to the scavenging ability of methanolic extracts from both plants and antioxidant standards such as BHA and BHT was significant (p<0.05). Methanolic extract of the *R. officinalis* and *G. biloba* has shown strong DPPH scavenging activity. We used BHA and BHT as standards. The scavenging effects of methanolic extracts from both plants and standards on the DPPH radical decreased in the order of BHA > BHT > *R. officinalis* > *G. biloba* which were 89.33, 85.26, 80.2 and 75.86% at the concentration of 150 µg/ml, respectively. These results indicated that methanolic extracts of *R. officinalis* and *G. biloba* have a noticeable effect on scavenging free radical. The strong antioxidant activity of rosemary extracts is primarily re-

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ginkgo biloba (mg GAL / 100 g of fresh weight)</th>
<th>Rosmarinus officinalis (mg GAL / 100 g of fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillic acid</td>
<td>12.65±0.09</td>
<td>25.13±1.11</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>44.90±1.17</td>
<td>13.41±0.82</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>-</td>
<td>75.27±2.67</td>
</tr>
<tr>
<td>hispidulmin</td>
<td>-</td>
<td>48.58±1.48</td>
</tr>
<tr>
<td>Carnosic acid</td>
<td>-</td>
<td>227.49±2.33</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>14.25±0.78</td>
<td>-</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>9.68±0.59</td>
<td>-</td>
</tr>
<tr>
<td>Luteolin</td>
<td>-</td>
<td>8.09±0.47</td>
</tr>
<tr>
<td>Rutin</td>
<td>35.98±1.08</td>
<td>-</td>
</tr>
<tr>
<td>Apigenin</td>
<td>-</td>
<td>5.34±0.39</td>
</tr>
<tr>
<td>Naringen</td>
<td>8.45±0.76</td>
<td>96.29±2.67</td>
</tr>
<tr>
<td>Quercetin</td>
<td>173.2±2.23</td>
<td>-</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>157.41±2.64</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>461.87</td>
<td>499.60</td>
</tr>
</tbody>
</table>

Tab. 3. HPLC analysis of phenolic compounds in methanolic extract of the *Ginkgo biloba* and *Rosmarinus officinalis* (mg GAL / 100 g of fresh weight)
Many plant phenolic compounds have been described as antioxidants due to their chelating ability to iron ion. As shown in Fig. 2, the plant extracts displayed the Fe^{2+} chelating effect in a concentration dependent manner.

The percentages of metal scavenging capacity at 200 µg/ml of tested methanol extracts of G. biloba, R. officinalis and EDTA was found to be 32.2, 38.31 and 51.21% respectively. As can be seen, EDTA hardly carried the ferrous ion chelating ability due to their chemical structure properties. Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh et al., 1999). Several antioxidants possess metal chelating ability to reduce the redox potential and stabilize the oxidized form of the metal ions, which related to the obstruction on the peroxidative process and oxidative damage. Iron and copper are essential transition metal elements in the human body for the activity of a large range of enzymes and for some proteins involved in cellular respiration, O₂ transport and redox reactions. But, because they are transition metals, they contain one or more unpaired electrons that enable them to participate in redox reactions, such as participation in the conversion of H₂O₂ to OH⁻ to the highly reactive alkoxy and hydroxyl radicals (Lloyd et al., 1997).

Due to this property, transition metal chelation to form low redox potential complexes is an important antioxidant property (Halliwell et al., 1995) and measuring chelation of iron (II) is one method for assessing this property.

**Superoxide anion scavenging activity**

The superoxide anion radical scavenging activity of G. biloba and R. officinalis were assayed by the PMS-NADH system. The inhibition percentage of superoxide radical generation by the plant extracts and comparison with quercetin as standard is shown in Fig. 3. The percentage inhibition of superoxide generation at 300 µg/ml concentration of G. biloba was found as 62.31%, whereas for R. officinalis the value was 69.12%, the differences were found statistically significant (p<0.05). On the other hand, quercetin at 300 µg/ml concentrations showed 75.31%, inhibition of superoxide radical. A decrease in the absorbance at 560 nm in the presence of antioxidants is indicative of the consumption of superoxide anions in the reaction mixture. Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985).
The superoxide radical is known to be produced in vivo and can result in the formation of H₂O₂ via dismutation reaction. Moreover, the conversion of H₂O₂ into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavorable effects caused by superoxide radicals (Halliwell, 1991). The extracts are found to be an efficient scavenger of superoxide radical generated in PMS-NADH system in vitro and their activity are comparable to that of quercetin. This result clearly indicates that the tested extracts have a noticeable effect on scavenging superoxide radical. In general, the methanol extracts of R. officinalis showed strong antioxidant activity, DPPH radical, metal chelating and superoxide anion scavenging activities. The antioxidative effect of R. officinalis extract may be due to the phenolic components. Thus, the DPPH radical scavenging activity of R. officinalis extracts may be mostly related to their phenolic hydroxyl group. These results are in agreement with many previous studies which confirmed that, the strong antioxidant efficiency of R. officinalis extracts due to the highly predominant content of carnosic acid (Cavero et al., 2005; Nogala-Kalucka et al., 2005). This study has examined various reactions that might contribute to antioxidative activity present in R. officinalis which could play an important nutritional role in the diet of adults and children alike in some of the poorest regions of the world (Egypt, and sub-Saharan Africa). The results proved that R. officinalis exhibited higher antioxidant activity than G. biloba.

**Antimicrobial activity**

The result of the antimicrobial activity is presented in Tab. 4. As it is shown, the extracts of both plants presented variable inhibition effects against pathogenic bacteria and fungus. In general, both methanolic extracts of R. officinalis and G. biloba showed stronger inhibition effects against pathogenic bacteria. Also, the both extracts exhibit moderate antifungal activities. In contrast, methanolic extract of G. biloba did not exhibit any antibacterial effects against the gram negative bacteria E. coli. On the other hand, methanolic extracts of R. officinalis exhibited similar strong inhibition effects against gram positive and gram negative bacteria. The antibacterial activities against both gram positive and gram negative bacteria may indicate the presence of broad spectra antibiotic compounds or simply metabolic toxins (Moniharapon and Hashi-naga, 2004). Such results was supported by Abramovic et al. (2012), who showed that, Rosemary leaves extracts showed a stronger antimicrobial activity for gram positive and gram-negative bacteria, which could be explained by the presence of carnosic acid as the main bioactive antimicrobial compound in rosemary extracts (Moreno et al., 2006). As was previously reported, carnosic acid is more efficient against gram-positive bacteria than rosmanic acid (Klancnik et al., 2009). From these results, it is obvious that the rosemary leaves have different modes of action and exhibited stronger biological activity against gram-positive and gram-negative bacteria.

Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nuclide acids (Oyaizu et al., 2003). Taking into consideration the properties of the organic solvent used for the extraction, the extract seems to contain diverse substances, ranging from non-polar to polar compounds. Further research is necessary to determine the identity of the antibacterial compounds from these plants and also to determine their full spectrum of efficacy. However, the present study is a primary platform for comparison of further phytochemical and pharmacological studies on G. biloba and R. officinalis.

In conclusion, the findings of this study support the view that some medicinal plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. It can be also used in stabilizing food against oxidative deterioration.

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