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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, narinigen 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 ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) 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 inactivate 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 (Maltas *et al.,* 2011). The leaves extract of *Ginkgo biloba* is a standardized extract to contain 24% flavonoids, 7% proanthocyanidins and 6% terpeniods (Goh and Barlow, 2002). The flavonoids are primarily flavonol-glycosides of kaempherol, quercetin and isohamnetin with glucose or rhamnose. The terpenoid fraction consists of a uniqe 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 kinurenic, hydroxykinurenic 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, antithrombotic, 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; Variyar *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). Ferrozine or 3-(2-pyridyl)-5, 6-bis (4-phenylsulfonic acid)-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 38°C for 48 h until the powder did not form

Tab. 1. Microbial strains used to test the antimicrobial activities of *Ginkgo biloba* and *Rosmarinus officinalis* extracts

Microbial group	Indicator strain	Cultivation conditions
Gram positive bacteria	*Staphylococcus aureus* (ATCC 25923)	TSA + YE, 37°C
	*Bacillus subtilis** (ATCC 6633)	TSA + YE, 30°C
Gram negative bacteria	*Escherichia coli* (ATCC 19404)	TSA + YE, 37°C
	*Salmonella typhi** (ATCC 14028	TSA + YE, 37°C
Fungus	*Aspergillus niger** (ATCC 16404)	PDA, 25 °C

*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

lumps when touched and then ground with a coffee grinder into a fine powder that would pass through a 0.4 mm screen. The plant extracts were prepared using the modified method of Matkowski and Piotrowska (2006). Briefly, 10 g of the dried powder from the plant were soaked separately in 100 ml of methanol (98.8%). Then, each mixture was refluxed in a water bath in the dark at 45°C. The extracts were filtered through Whatman filter paper No. 42. The collected filtrates were dried under vacuum at 40°C using a rotary evaporator (Buchi, Switzerland); the extraction was repeated twice. The resulting residue was re-dissolved in methanol and used for the determination of phenolic, flavonoid, antioxidant and antimicrobial activities.

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 H_2O. 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 H_2O and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of a 10% $AlCl_3 \cdot H_2O$ solution was added and filtered for 6 min. About 500 µl of 1 M NaOH and 275 µl of distilled H_2O 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 (150×4.6 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:

$DPPH^{\cdot}$ scavenging effect (%) = $[(A_0 - A_1 / A_0)] \times 100$

where, A_0 = The absorbance of the control reaction (containing all reagents except the test compounds)

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 H_2O. 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. Na_2EDTA was used as positive control. Percent activity was calculated using the following formula:

Metal chelating effect (%) = $[(A_0 - A_1 / A_0)] \times 100$

where, A_0 = The absorbance of the control reaction

A_1 = The absorbance in the presence of the samples

Determination of superoxide anion ($O_2^{\cdot -}$) 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 methosulphate 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 = $[1 - (A_1 - A_2 / A_0)] \times 100$

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 to113.55 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 narinigen 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

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

Plant species	Phenolic*	Flavonoids**	Flavonoids/ phenolic
Ginkgo biloba	75.30 ± 0.69^b	84.59 ± 1.43^b	1.12 ± 0.009^b
Rosmarinus officinalis	98.31 ± 0.97^a	113.55 ±1.60^a	1.155 ± 0.007^a
LSD 0.05	1.92	3.43	0.0179

Data with different superscript letters in the same column were differed significantly ($p \leq 0.05$), * 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

Tab. 3. HPLC analysis of phenolic compounds in methanolic extract of the *Ginkgo biloba* and *Rosmarinus officinalis* (mg_{GAL} / 100 g of fresh weight

Compounds	*Ginkgo biloba*	*Rosmarinus officinalis*
Vanillic acid	12.65±0.09	25.13±1.11
Caffeic acid	44.90±1.17	13.41±0.82
Rosmarinic acid	-	75.27±2.67
hispidulin	-	48.58±1.48
Carnosic acid	-	227.49±2.33
p-Coumaric acid	14.25±0.78	-
Ferulic acid	9.68±0.59	-
Luteolin	-	8.09±0.47
Rutin	35.98±1.08	-
Apigenin	-	5.34±0.39
Narinigen	8.45±0.76	96.29±2.67
Quercetin	173.2±2.23	-
Kaempferol	157.41±2.64	-
Total	461.87	499.60

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 photoperoxidation 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 (Kahkonen *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 $DPPH^{\cdot}$ radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on $DPPH^{\cdot}$ radical scavenging was thought to result from their hydrogen donating ability (Shimada *et al.*, 1992). $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ 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, $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ scavenging activity. We used BHA and BHT as standards. The scavenging effects of methanolic extracts from both plants and standards on the $DPPH^{\cdot}$ 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-

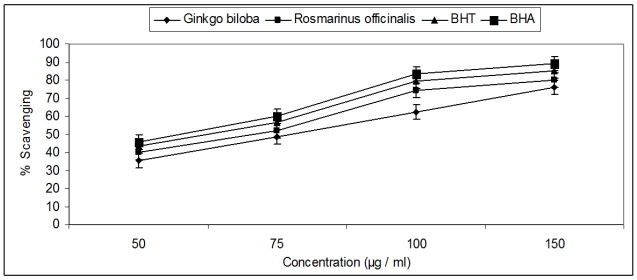

Fig. 1. Free radical scavenging activity of different concentrations of methanolic extracts of *Ginkgo biloba*, *Rosmarinus officinalis,* BHT and BHA by DPPH radicals. Each value is expressed as mean ± standard deviation (n = 3)

lated to the presence two phenolic diterpenes, carnosic acid and carnosol (Frankel *et al.,* 1996; Nogala-Kalucka *et al.,* 2005). Carnosic acid was the most correlated compounds to free radical scavenging activity (Cavero *et al.,* 2005). Rosmarinic acid, a caffeic acid ester, also appears as an important component in extracts of rosemary for having superior antioxidant activity than α-tocopherol and butylated hydroxyl toluene (BHT) (Tepe, 2008). However, the scavenging effect of BHA and BHT are higher than our methanolic extracts of *R. officinalis* and *G. biloba*. The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (Deighton *et al.,* 2000). It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoid, tannins, and aromatic amines (*p*-phenylene diamine, *p*-aminophenol, etc.), reduce and decolourise $DPPH^{\cdot}$ by their hydrogen donating ability (Yokozawa *et al.*, 1998). Phenolic compounds of the *R. officinalis* and *G. biloba* extracts are probably involved in their antiradical (Ramalakshmi *et al.,* 2009). Although the activity is relatively lower than that of BHT and BHA, the extracts maybe veritable source of bioactive compound with better activities after fractionation.

Ferrous ion chelating activity

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.

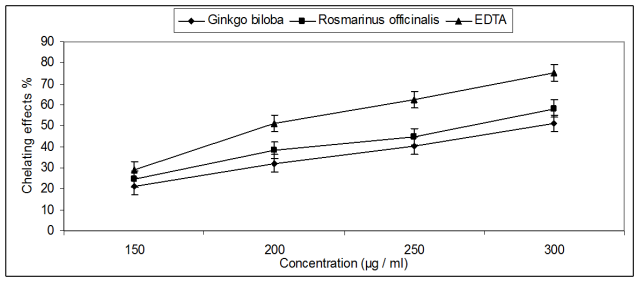

Fig. 2. Metal chelating effects of different concentrations of methanolic extract of *Ginkgo biloba, Rosmarinus officinalis,* and EDTA on ferrous ions. Each value is expressed as mean ± standard deviation (n = 3)

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 activity 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, O_2 transport and redox reactions. But, because they are transition metals, they contain one or more unpaired electrons that enable them to contribute one-electron transfer reactions. Hence, they are powerful catalysts of autoxidation reactions, such as participation in the conversion of H_2O_2 to $OH^{\cdot}$ to the highly reactive alkoxyl 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).

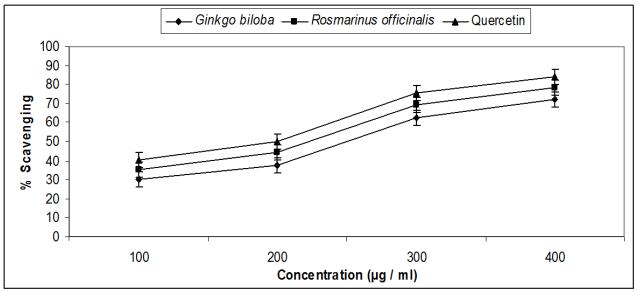

Fig. 3. Comparison of superoxide anion radical scavenging activity of different concentrations of methanolic extract of *Ginkgo biloba, Rosmarinus officinalis* and quercetin standard

Tab. 4. Antimicrobial activities of *Ginkgo biloba* and *Rosmarinus officinalis* extracts

Plant methanolic extracts	Antimicrobial activities*				
	Gram positive bacteria**		Gram negative bacteria		Fungus
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*	*A. niger*
Ginkgo biloba	++++	++++	-	++++	++
Rosmarinus officinalis	++++	++++	++++	++++	++
Tetracycline antibacterial agent	++++	++++	++++	++++	++++

* Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm), - (negative) = 0 mm; + (weak) = 1-4 mm; + + (moderate) = 5-10 mm; +++ (strong) = 10-15 mm and ++++ (very strong) ≥ 16 mm;

** Microorganisms used in this study were *S. aureus* (ATCC 29213), *B. subtilis* (CAICC 11), *L. monocytogenes* (NCIMB 50007), *E. coli* (ATCC 25922), *P. aeruginosa* (CAICC 21), *S. typhi* (CAICC 31), *A. niger* (CAICC 41), *C. albicans* (CAICC 51)

The superoxide radical is known to be produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of H_2O_2 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 antioxidant 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 Hashinaga, 2004). Such results was supported by Abramovic *et al.* (2012), who showed that, Rosemary leaf 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 rosmarinic acid (Klancnik *et al.,* 2009). From these results, it is obvious that the rosemary leaf extracts 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 seams 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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