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Antioxidant and antimicrobial properties of kaff maryam *(Anastatica hierochuntica)* and doum palm *(Hyphaene thebaica)*

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RESUMEN

Propiedades antioxidantes y antimicrobianas de la rosa de Jerico *(Anastatica hierochuntica)* y duma *(Hyphaene thebaica)*

El extensivo uso de plantas medicinales con fines médicos ha aumentado dramáticamente debido en gran parte a la importancia que tienen en la salud pública. En este estudio, los niveles de compuestos fenólicos, flavonoides, β-caroteno y licopeno de *Anastatica hierochuntica* y *Hyphaene thebaica* fueron determinados. Los extractos de plantas fueron evaluados mediante su actividad antioxidante usando varias metodologías: (i) captación de radicales libres usando 2,2-difenil-1-picrilhidrazina, (ii) capacidad quelatante de iones metálicos, y (iii) captación de radicales superóxidos. La actividad antimicrobiana de ambos extractos de planta fue evaluada mediante un panel de microorganismos usando el método de difusión en disco de agar. El contenido total de fenoles (51.97 y 64.9 mg/g peso seco en *A. hierochuntica* y *H. thebaica*, respectivamente) fue significativamente diferente ($p < 0.05$). La actividad antioxidante incremento con un aumento de la concentración. Los extractos de planta fueron más activos contra bacterias Gram-positiva que contra bacterias Gram-negativas. También, la actividad antimicrobiana de los extractos metanólicos de *H. thebaica* fue mayor que la de los extractos metanólicos de *A. hierochuntica.* Este estudio revela que el consumo de estas plantas podría ejercer algunos efectos beneficiosos a causa de sus actividades antioxidantes y microbianas.

PALABRAS CLAVE: Actividad antimicrobial – Actividad antiradicalaria – Compuestos fenólicos – Ensayo DPPH – Licopeno.

SUMMARY

Antioxidant and antimicrobial properties of kaff maryam *(Anastatica hierochuntica)* and doum palm *(Hyphaene thebaica)*

The widespread use of medicinal plants for health purposes has increased dramatically due to their great importance to public health. In this study, the levels of phenolic, flavonoid, β-carotene and lycopene compounds of *Anastatica hierochuntica* and *Hyphaene thebaica* were determined. 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 extracts was evaluated against a panel of microorganisms using the agar disc diffusion method. The total phenolic content (51.97 and 64.9 mg/g dry weight in *A. hierochuntica* and *H. thebaica*, respectively) was significantly ($p < 0.05$) different. The antioxidant activity increased with an increase in concentration. The plant extracts were more active against Gram-positive bacteria than Gram-negative bacteria. Also, the antimicrobial activity of *H. thebaica* was higher than that of *A. hierochuntica* methanolic extracts. This study reveals that the consumption of these plants would exert several beneficial effects by virtue of their antioxidant and antimicrobial activities.

KEY-WORDS: Antimicrobial activity – DPPH assay- Lycopene – Phenolic compounds – Radical scavenger activity.

1. INTRODUCTION

Nature has been a source of medicinal agents since the beginning of time. Herbal medicine is still the most common source for primary health care of about 65-80% of the world's population, mainly in developing countries, 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. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. 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 the 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 have 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. 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 are widely distributed in plants are capable of terminating a 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 (Soong and Barlow, 2004). Furthermore, flavonoids 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 (Yanishlieva-Maslarova, 2001). Within recent years, infections have increased to a great extent and resistance against antibiotics becomes an ever-increasing therapeutic problem (Austin *et al*., 1999).

The 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 polyphenol toxicity against microbes may be related to the inhibition of hydrolytic enzymes (proteases) or other interactions to inactivate microbial adhesins, cell envelope transport proteins and non specific interactions with carbohydrates (Cowan, 1999). However, *Anastatica hierochuntica* (Family-Brassicaceae) and *Hyphaene thebaica* (Family- Arecaceae) are widely used as medicinal plants either by themselves or in combination with other herbs. The whole plant of *A. hierochuntica* is commonly called “Kaff maryam” or “Rose of Jericho”, which is a winter annual plant of the Sahara-Arabian deserts, and was prescribed in Egyptian folk medicine and used as a charm for child birth (Rizk and El-Ghazaly, 1995). The aerial part of the Rose of Jericho is still attractive for Egyptian people as a remedy for asthma and diseases of the respiratory system. The methanolic extract from the whole plant of *A. hierochuntica* was found to show potent hepatoprotective effect on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes (Yoshikawa *et al*., 2003).

H. thebaica, a desert palm native to Egypt, sub-Saharan Africa and West India; is commonly called “African doum palm” or ginger bread palm (Dosumu *et al*., 2006). Previous studies on Doum had focused on the fruit because, besides its nutritional value, the fruit drink brewed from a hot water infusion of the dried fruit pulp is widely consumed as a health tonic and has been valued in the region for its many anecdotal medicinal properties. Research on the fruit pulp of *H. thebaica* showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Kamis *et al*., 2003). The identification of compounds, by thin-layer chromatography, showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids.The fruit also lowers blood pressure in animal models (Sharaf *et al*., 1972). The aqueous extract of doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents (Hsu *et al.,* 2006). In this study, the crude extract of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*) were evaluated in terms of antioxidant and antimicrobial activities.

2. MATERIALS AND METHODS

2.1. 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.

2.2. Collection of plant materials

Plant materials (*A. hierochuntica* and *H. thebaica*) were purchased from the Egyptian local market. Voucher specimens were deposited in the Herbarium of Phytochemistry and Plant Systematic Dept, National Research Center of Egypt (CAIRC) for *A. hierochuntica* (No, 14562) and *H. thebaica* (No, 1357) respectively.

Microbial strain

Microorganisms used in this study (Table 1) were obtained from the American Type Culture Collection (ATCC) as well as the culture collection of the Microbiology Dept (CAICC), Faculty of Agriculture, Cairo University.

2.3. Extraction of plant materials

Methanolic extract

A. hierochuntica (whole plants) and *H. thebaica* (fruit) were oven dried at 38 °C for 48 h until the

Table 1
Microbial strains used to test the antimicrobial activities of *A. hierochuntica* and *H. thebaica* extracts.

Microbial group	Indicator strain	Cultivation conditions*
Gram positive bacteria	*Staphylococcus aureus* (ATCC 29213)	TSA + YE, 37ºC
	Bacillus subtilis (CAICC 11)	TSA + YE, 30ºC
	Listeria monocytogenes (NCIMB 50007)	TSA + YE, 37ºC
Gram negative bacteria	*Escherichia coli* (ATCC 25922)	TSA + YE, 37ºC
	Pseudomonas aeruginosa (CAICC 21)	TSA + YE, 37ºC
	Salmonella typhi (CAICC 31)	TSA + YE, 37ºC
Fungus	*Aspergillus niger* (CAICC 41)	PDA, 25 ºC
Yeast	*Candida albicans* (CAICC 51)	TSA + YE, 30ºC

*TSA = Trypticase Soy Agar; YE: = Yeast Extract; PDA = Potato Dextrose Agar.

powder did not form 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, 10g 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, β-carotene, lycopene, antioxidant and antimicrobial activities.

Aqueous extract

The aqueous extractions were carried out as described by Asuzu (1986). Ten grams of dried plant material were added to 100 ml. of sterile distilled water in a round bottom flask with a glass stopper. The mixture was then shaken well and allowed to stand for 1 h. Then a reflux condenser was attached to the flask and boiled gently for 1 h, cooled, shaken well and filtered through a dry Whatman filter paper No 1. The filtrate was then poured into a sterile beaker and evaporated to dryness in a water bath at 50 °C. It was cooled in a desiccator for 30 min and then re-dissolved in 1% Dimethyl sulfoxide.

2.4. Determination of bioactive compounds

Total phenolic compounds

Phenolic compounds 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.

Flavonoid determination

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% $AlCl_3.H_2O$ 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.

β-carotene and lycopene determination

β-carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of an acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance was measured at various wavelengths 453, 505, and 663 nm. The contents of β-carotene and lycopene were calculated according to the following equations:

$$\beta\text{-carotene (mg/100 ml)} = 0.216\ (A_{663}) - 0.304\ (A_{505}) + 0.452\ (A_{453})$$

$$\text{Lycopene (mg/100 ml)} = 0.0458\ (A_{663}) + 0.372\ (A_{505}) - 0.0806\ (A_{453})$$

where A = absorbance.

2.5. 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:

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

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 $FeCl_2$ 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:

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

where:

A_0: The absorbance of the control reaction

A_1: The absorbance in the presence of the samples

Determination of superoxide anion ($O2^{\cdot -}$) 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:

$$\text{\% Scavenging} = [1 - (A_1 - A_2/A_0)] \times 100$$

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.

2.6. 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* and *Pseudomonas aeruginosa*), pathogenic bacteria (*Escherichia coli* ATCC 25922), *Listeria monocytogenes* NCIMB 50007), *Salmonella typhi*, onion post-harvest spoilage fungus (*Aspergillus niger*) and pathogenic yeast (*Candida albicans*). 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 (Table 1). A specific volume containing 40 µg/ml of each extract was impregnated into sterilized paper discs (Whatman No. 1) of 6 mm in diameter. 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.

2.7. 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).

3. RESULTS AND DISCUSSION

3.1. Bioactive compounds

It is well-known that plant phenolic compounds are highly effective free radical scavengers and antioxidants. In this study, the total phenolic compounds of methanolic extracts were determined using the Folin-Ciocalteu reagent and expressed as mg gallic acid (GAL) equivalent/g dry weight. Significant differences

($p < 0.05$) were observed between plants (Table 2). *H. thebaica* contained phenolic compounds at 64.90 mg/g d.w., whereas, *A. hierochuntica* contained 51.97 mg/g d.w. The fruits of *H. thebaica* had a higher content of phenolic compounds than *A. hierochuntica*. The content of flavonoids (mg/g), in the quercetin equivalent varied from 42.53 to 46.28 mg/g d.w. in both plants. β-carotene and lycopene were found only in vestigial amounts (2.27 and 2.43 µg/g) in *A. hierochuntica* compared to (4.04 and 3.33 µg/g) in the *H. thebaica* extract, respectively.

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 beneficial health 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 is ingested from a diet rich in fruits and vegetables (Tanaka *et al*., 1998). However, the total phenolic content of the Doum fruit is low, but the extract exhibited potent antioxidant activity (Sharaf *et al*., 1972). It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The action mechanisms of flavonoids are through the scavenging or chelating process (Kessler *et al*., 2003). The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for the antioxidant effect in the plants (Das and Pereira, 1990). The high antioxidant activities can be attributed to their phenolic and β-carotene contents and a wide range of β-carotene (ranging from 1 to 190 µg/g d.w.) among various sweet potato breeding lines were observed (Simonne *et al*., 1993). Carotenoids may act as a singlet oxygen quencher and can transfer one electron to the radicals, giving rise to a stable carotenoid radical cation regenerating the original molecule (Mortensen and Skkibsted, 1997). The high or low β-carotene content given for both plants could be dependent on various factors: the level of expression of the genes governing carotenogenesis, physiological and morphological characteristics intrinsic to the plant and growth conditions. All these factors taken together may influence the performance of both plants with respect to phytochemical content.

3.2. 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 activity 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 the $DPPH^{\cdot}$ 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 the antioxidative activity of natural antioxidants. The scavenging effects of methanolic extracts from our two plants on $DPPH^{\cdot}$ 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 *H. thebaica* and A. hierochuntica 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 > *H. thebaica* > *A. hierochuntica* which were 70.45, 67.55, 64.55, and 55.87% at the concentration of 150 µg/ml, respectively. These results indicated that the methanolic extracts of *H. thebaica* and *A. hierochuntica* have a noticeable effect on scavenging free radicals. However, the scavenging effects of BHA and BHT are higher than our methanolic extracts of *H. thebaica* and *A. hierochuntica*. 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 decolourize $DPPH^{\cdot}$ by their hydrogen donating ability (Yokozawa *et al*.,

Table 2
Bioactive compounds of the methanolic extracts obtained from *A. hierochuntica* and *H. thebaica* plants

Lycopene (µg/g)	β-carotene (µg/g)	Flavonoids/ phenolic	Flavonoids**	Phenolic*	Plant species
2.43 ± 0.09^{b}	2.27 ± 0.06^{b}	0.82 ± 0.045^{a}	42.53 ± 1.06^{b}	51.97 ± 2.01^{b}	*A. hierochuntica*
3.33 ± 0.17^{a}	4.04 ± 0.14^{a}	0.71 ± 0.03^{b}	46.28 ± 0.94^{a}	64.9 ± 1.92^{a}	*H. thebaica*
0.308	0.244	0.086	2.62	4.44	LSD 0.05

Data with different superscript letters in the same column were significantly different ($P < 0.05$).
* 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.

1998). Phenolic compounds of the *H. thebaica* extracts are probably involved in their antiradical activity (Hsu *et al.*, 2006). Although the activity is relatively lower than that of BHT and BHA, the extracts may be viable source of bioactive compounds 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 ions. 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 *A. hierochuntica*, *H. thebaica* and EDTA were found to be 16.72, 24.3, and 50.41% 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 of 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 *A. hierochuntica* and *H. thebaica* was 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 *A. hierochuntica* was found as 52.61%, whereas for *H. thebaica* the value was 63.22%, the differences were found statistically significant ($P < 0.05$). On the other hand, quercetin at 300 µg/ml concentration showed 75.31%, inhibition of the 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 H_2O_2 via a 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 radicals generated in a PMS-NADH system *in vitro* and their activities are comparable to that of quercetin. This result clearly indicates that the tested extracts have a noticeable effect on scavenging the superoxide radical. In general, the methanol extracts of *H. thebaica* showed strong antioxidant activity, DPPH radical, metal chelating and superoxide anion scavenging activities. The antioxidative effect of *H. thebaica* extract may be due to the phenolic components. Thus, the DPPH radical scavenging activity of *H. thebaica* extracts may be mostly related to their phenolic hydroxyl group. This study has examined various reactions that might contribute to antioxidant activity present in Doum fruit which could play an important nutritional role in the diet of adults and children alike in some of the poorest

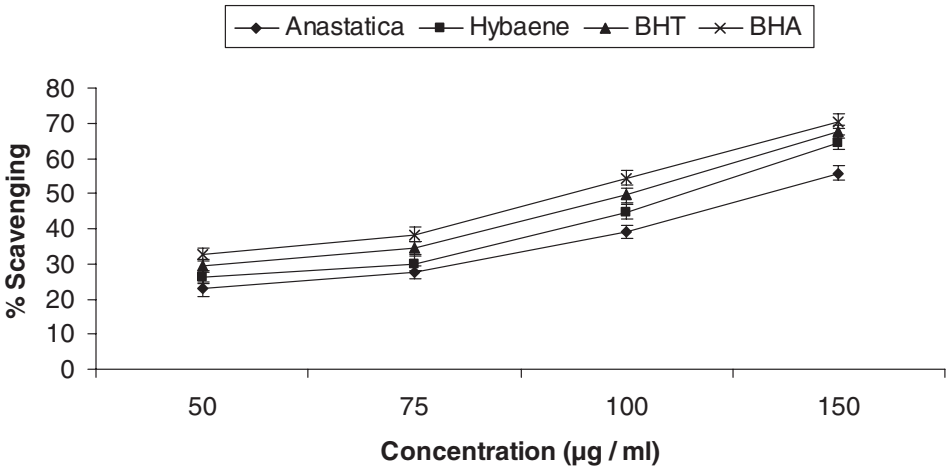

Figure 1
Free radical scavenging activity of different concentrations of methanolic extracts of *A. hierochuntica, H. thebaica*, BHT and BHA by DPPH radicals. Each value is expressed as mean ± standard deviation (n = 3).

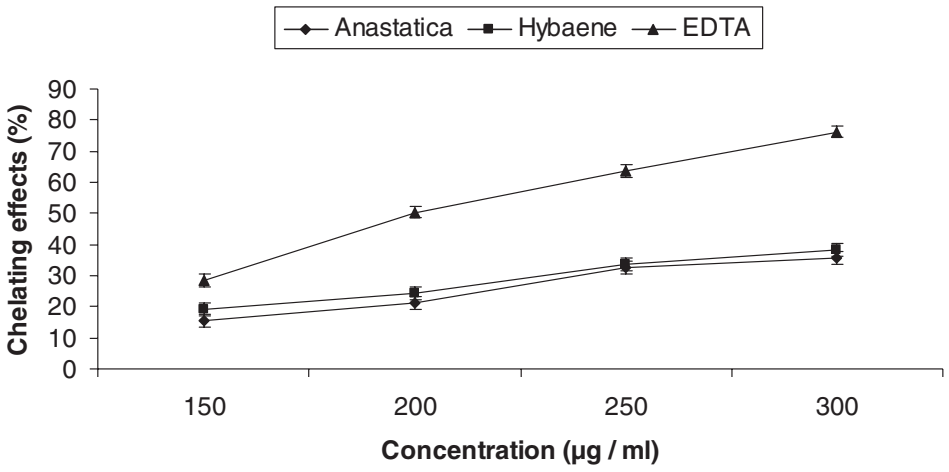

Figure 2
Metal chelating effects of different concentrations of methanolic extract of *A. hierochuntica, H. thebaica* on ferrous ions. Each value is expressed as mean ± standard deviation (n = 3). Na_2EDTA was used as positive control.

regions of the world (Egypt, and sub-Saharan Africa). The results proved that Doum fruit exhibited higher antioxidant activity than kaff maryam.

3.3. Antimicrobial activity

The result of the antimicrobial activity is presented in Table 3. As shown, the extracts of both plants presented variable inhibition effects against pathogenic bacteria, yeast and fungus ranging from negative (−) to very strong inhibition (++++). In general, methanolic and aqueous extracts of *H. thebaica* showed stronger inhibition effects than those of *A. hierochuntica* against pathogenic bacteria. However, the extract of *A. hierochuntica* did not exhibit any antifungal or anti-yeast activities. On the other hand, both methanolic and aqueous extracts of *H. thebaica* exhibited similar inhibition effects against gram positive and gram negative bacteria, except for *L. monocytogenes*, where only a slight inhibition was observed. The antibacterial activities against both gram positive and gram negative bacteria may indicate the presence of broad spectra antibiotic compounds or simply metabolic toxins in plant extracts (Moniharapon and Hashinaga, 2004).

We also observed that the methanolic extract of *H. thebaica* showed stronger antifungal and antiyeast activities than aqueous extracts. Such observation was supported by Irobi and Adedayo (1999), who found that polar solvent extract has high antifungal activity against a wide range of fungal isolates including *Aspergillus niger* and *Candida albicans*. 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.

Despite many published reports dealing with the bioactivity of compounds isolated from *A. hierochuntica* little was known about its antimicrobial activity prior to our investigation. Further research is

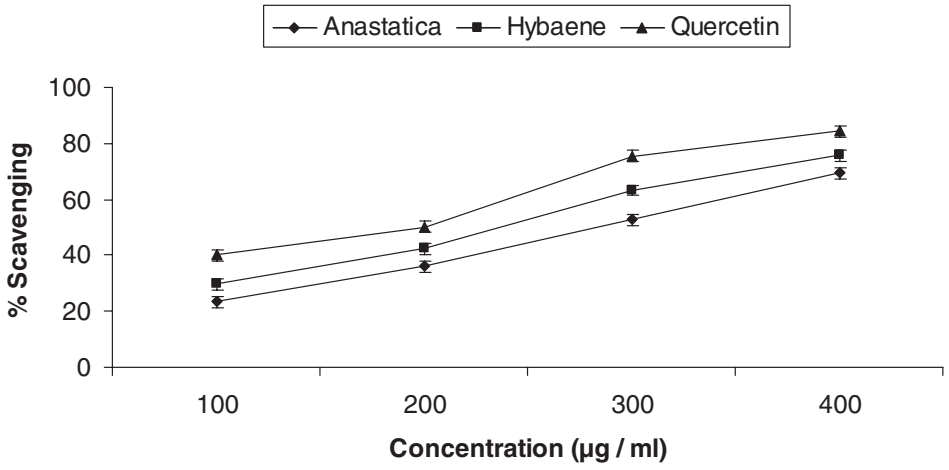

Figure 3
Comparison of superoxide anion radical scavenging activity of different concentrations of methanolic extract of *A. hierochuntica, H. thebaica* and quercetin standard.

Table 3
Antimicrobial activities of *Hyphaene thebaica* and *Anastatica hierochuntica* extracts

	Antimicrobial activities*							
	Gram positive bacteria**			Gram negative bacteria			Fungus & Yeast	
Plant extracts	*S. aureus*	*B. subtilis*	*L. monocytogenes*	*E. coli*	*P. aeruginosa*	*S. typhi*	*A. niger*	*C. albicans*
Methanolic *H. thebaica* extract	++++	+++	++	−	+++	+++	++++	+++
Aqueous *H. thebaica* extract	++++	++++	−	−	++++	+++	+++	+++
Methanolic *A. hierochuntica* extract	−	+++	−	−	−	−	−	−
Aqueous *A. hierochuntica* extract	−	+++	−	−	−	−	−	−

* 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 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).

necessary to determine the identity of the antibacterial compounds from these plants and also to determine their full spectrum of efficacy.

4. 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. However, the present study is a primary platform for further phytochemical and pharmacological studies on *A. hierochuntica* and *H. thebaica*.

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