Global Advanced Research Journal of Medicine and Medical Sciences (ISSN: 2315-5159) Vol. 6(9) pp. 224-233, September, 2017
Available online http://garj.org/garjmms

Full Length Research Paper

The Antibacterial, Antifungal, and Antioxidant Activities of Essential Oil from Different Aromatic Plants

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Accepted 24 September, 2017

Plant's natural extracts such as Essential Oil, are being increasingly used as preservatives as well as in many human used products. The antimicrobial and antioxidant properties are essential for the use of such products, and therefore, their comparison with synthesized preservatives is the basis to replace the latter and for better biological activities with safer sources. The present study determined the antioxidant and antimicrobial activities of hydrodistilled EO from sixteen aromatic plants grown in Yemen. Antioxidant activity was examined by two methods: reducing power assay (RPA) and determination of antioxidant activity with thiobarbituric acid reactive substances (TBARS). Thus, on the bases of the lowest concentrations used in both methods, the antioxidant activities of the EOs according to TBARS can be sorted in the following descending order: *Thymus laevigatus* > Clove *Eugenia caryophyllus* > *Cinnamomum zylanicum* > *Chenopodium Ambrosioides* = *Eucalyptus camaldulensis* = *Marjoram majorana hortensis* = *Schinus molle* > *Pulicaria jaubertii* = *Ocimum basillicum* > *Artemisia abrotanum* > *Conyza incana* (vahl) willd > *Coriandrum sativum* > *Tagetes minuta* > *Rosmarinus officinalis* > *Lantana camara* > *Peppermint mentha piperita*. On the other hand these activities as obtained by RPA can be arranged as follows: Clove *eugenia caryophyllus* > *Ocimum basillicum* > *Thymus laevigatus*> *Artemisia abrotanum* > *Tagetes minuta* > *Eucalyptus camaldulensis* = *Marjoram majorana hortensis* = *Cinnamomum zylanicum* = *Schinus molle* > *Coriandrum sativum* = *Conyza incana* (vahl) willd = *Lantana camara* > *Chenopodium Ambrosioides* = *Pulicaria jaubertii* > *Peppermint mentha piperita* = *Rosmarinus officinalis* .The obtained EOs were screened against both gram-negative bacteria, gram-positive bacteria, and against three fungal species (*Aspergillus flavus*, *Fusarium oxyporium*, and *Candida albicans*). Most of these EOs have shown important antibacterial and antifungal effects against the tested strains. Some of the obtained EOs are promising as sources of natural antioxidants and antimicrobial agents in cosmetics and pharmaceutical applications.

Keyword: Essential oil (EO); Antimicrobial; Antioxidant; Antifungal; TBARS; RPA.

INTRODUCTION

Essential oils (EOs) are valuable commodities gain their importance due to increase of their application in perfumery, aromatherapy, cosmetics, incense, medicine, pharmaceutical and food industries. They have been reported to possess great biological activities, such as, antimicrobial and antioxidant (Aligiannis et al., 2001; Salehi et al., 2005; Magwa et al., 2006; de Sousa Barros et al., 2015) activities. As a result, there are much attention is being paid in attempts to replace synthetic

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antimicrobial and antioxidant compounds by natural secondary metabolites, such as, EO. EOs are volatile and aromatic oily liquids from various plant materials (flowers, buds, seeds, twigs, bark, herbs, wood, fruits, and roots). They are very complex mixtures of organic compounds include terpenes, terpenoids, aromatic, and aliphatic constituents (Bassole and Juliani, 2012). Chemically they are derived from terpenes, and their oxygenated derivative compounds, such as, monoterpenes and sesquiterpines , which are hydrocarbons with the general formula $(C_5H_8)_n$. The group of oxygenated compounds derived from these hydrocarbons includes alcohols, aldehydes, ketones, acids, esters, ethers, phenols, and oxides.

EOs have shown to possess antibacterial, antifungal, antiviral, and antioxidant activities (Burt, 2004; Kordali et al., 2005; Costa et al., 2015). In addition, some EOs have been used in cancer treatment (Sylvester et al., 2006), aromatherapy, food preservation (Faid et al., 1995), and fragrance industries. At present time attention of various industries is focused on alternative sources of more natural and environmentally friendly antimicrobials, antibiotics, antioxidants and crop protection agents, instead of the synthetic compounds, such as, butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), which could be promoters of carcinogensis (Barlow, 1990). In other words, the concerns of pharmaceutical industries are mostly in the development and preparation of synthetic analogues from the active chemical structures of the natural products. These products are more controllable from point of reproducibility, patentability, safety, and are more economically viable. Since aromatic plants are widely distributed and commonly grown in Yemen for different purposes, such as, appetizers, flavors and also used in folk medicine and perfumery, so the present work, aims to screen essential oils from these plants for their biological activities, which may be more effective, cheaper and safer than the synthetic organic compounds.

MATERIALS AND METHODS

Plant materials

Plant materials were collected from three different regions in Yemen. They were identified by Dr. Abdulwali Ahmed Al-Khulaidi, the botanists at the Department of Biology, Faculty of Sciences, Taiz University. The plants, which screened for their biological activities are shown table 1.

Extraction of EO

Two hundreds grams (200 g) of the plants samples were collected during summer 2008 and were subjected to hydrodistillation using a Clevenger-type apparatus for approximately three hours. This method of extraction is the most simple and old traditional method used for extraction of EO (Meyer-Warnod, 1984). After the process of hydrodistillation, the oil layer was collected and in some cases, the remaining distillate aqueous layers were washed with ether to extract any dissolved oils in water. Then, the ether was separated by separatory funnel and evaporated on a water bath at 40°C and the residue essential oil was added to the first collected portion. The EOs were dried over anhydrous sodium sulfate and the yield was calculated.

Antibacterial activity test

Tested bacteria

The tested bacteria were selected on the basis that they are pathogenic to humans. Both Gram-positive and Gram-negative pathogenic bacterial species were obtained from the Central Public Health Laboratory (*Staphylococcus aureus* and *Pseudomonaaeruginosa*) and Kuwait Hospital (*Escherichia coli* and *Proteus vulgaris*) and used as tested bacteria.

Inoculation method

The isosensitest broth was inoculated aseptically with the corresponding bacterial strain 24 hour before testing to ensure the adaptation of bacteria to the broth. The inoculated bacterial strains were incubated at 37°C for 24 hour to obtain the bacterial suspension, which is going to be used in the determination of antibacterial activity of EO in the next method. This procedure is carried out for each tested bacterial species.

Determination of antibacterial activity of EO

Agar well diffusion method is used in this study which is widely used method to evaluate the antimicrobial activity of plants or microbial extracts (Bassolé and Juliani, 2012 and Magaldi et al., 2004). EOs were diluted with Tween 40 to produce the following concentrations: 0, 25, 50, 75 and 100 % (v/v). Agar was melted in a steam bath set at 30°C to prevent solidification. Three Petri dishes were pre-inoculated with the corresponding bacteria as follows: 100 µl of the bacterial suspension (10^8cfu / mL) pipetted into the corresponding Petri dish. Then, 25 mL of the molten agar was added to the Petri dish and mixed thoroughly to obtain a homogeneous suspension, and allowed to set for 1 hour. Three wide holes (diameter of 6 mm), were then made in the agar using a cork borer. To each hole, 25 µL of a specific concentration of essential oil was introduced using a sterile micropipette. Gentamicin (10 µg / mL) was used as a positive control and Tween 40 (solvent) as a negative control. Then, all Petri dishes were incubated at 37°C for 24 hours. After that, zones of inhibition were measured and recorded. The zone of inhibition was taken to be the diameter of the

zone visibly showing the absence of growth including the 6 mm hole. In case of the absence of inhibition, the value 0.0 mm was assigned to the test sample.

Antifungal activity test

Tested fungi

The fungal species were chosen on the basis that they cause serious systemic and skin infections in humans. Three fungal species were tested in this work, namely: *Candida albicans, Aspergillus flavus*, and *Fusarium oxyporium*. They were obtained from the Modern National Laboratory (*Aspergillus flavus* and *Candida albicans*) and the Faculty of Agriculture (*Fusariumoxysporium*) and subjected for the antifungal activity of EO.

Determination of antifungal activity of EO

The antifungal activity of the EO was evaluated using the hole plate (agar well) diffusion method (Bassolé and Juliani, 2012). Suspensions were standardized to 1-5 x 10^6cfu / mL (in sterile normal saline) matching the turbidity of 0.5 McFarland standard. EOs were diluted with Tween 40 to produce 0.0, 25, 50, 75, and 100 % of its concentration. Three Petri dishes were pre-incubated with the appropriate fungal species as follows: 0.1 ml of the fungal suspension (1-5 x 10^6cfu / mL) was spread on all surface of the Sabouraud Dextrose Agar using sterile cotton swabs. Three wide holes (6 mm) were then made in the agar using cork borer. Then, 20 µL of specific concentration was introduced into each of the holes in appropriately labeled Petri dish using a sterile micropipette. Three plates free from EO (only media, fungi, and Tween 40) were used as negative control and three plates were used for positive control (trimoxazole instead of EO). All plates were incubated at 25°C for 7-10 days. At the end of incubation period, the zone of inhibition was taken to be the diameter of the zone visibly showing the absence of growth including the 6 mm hole. In case of the absence of inhibition, the value of 0.0 mm was assigned to the test sample. The mean value of diameter of the inhibition zone was recorded.

Antioxidant activity test of EO

The antioxidant activity of plant EO was carried out by two *in vitro* methods, namely: the reducing power method and the TBARS method. The EOs were dissolved in methanol at the concentration of 100 µl / mL. All assays were carried out in triplicate and the average value was obtained. All methods were made using UV-VIS spectrophotometer (1061-Shimadzu, Japan).

The reducing power assay (RPA) of EO

The reducing power was determined according to the method described by Oyaizu (1986). A stock solution of each essential oil in methanol (10 µl / mL) was prepared and different levels (25 µL, 50 µL, and µL 100) from each stock solution were transferred to different test tubes, were adjusted to 1 mL with the solvent (methanol). Then, 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6), and 2.5 mL of 1 % potassium ferricyanide were added to each test tube. The mixture was incubated at 50°C for 20 min and 2.5 ml of 10 % trichloroacetic acid was added. The upper layer (2.5 mL) of the centrifuged mixture was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1 % ferric chloride. The absorbance was measured at 700 nm against a blank. The reducing power increases with the increase of absorbance. Butylatedhydroxyanisole (BHA) was used as a positive control. All determinations were carried out in triplicates. The reducing power of each essential oil was expressed as µg of BHA equivalent to 1 µl of essential oil.

Total antioxidant activity of EO using TBARS method

The thiobarbituric acid reactive substances (TBARS) method was used as described by Rubertov *et al*. (2000), which measure the total antioxidant activity with slight modifications. Briefly, this method was carried out using egg yolk homogenate (10 %) in phosphate buffer (pH 7.4) as lipid rich media. A stock solution of (10 µL / mL in methanol) was prepared from each essential oil. Then, different levels (100, 250, 500, and 1000 µL) from each stock solution were transferred to different test tubes and were adjusted to 1 ml with the solvent (methanol). 3.0 mL of 10 % yolk homogenate was added to each test tube and incubated for 30 min. Then, 0.1 mL of ferrous sulfate solution (50 µmol/L FeSO4; 1 mmol/L KH2PO4; 0.2 mmol/L ascorbic acid in 0.15 M Tris-HCl buffer, pH 7.4) was added and incubated for 30 min at 37°C. Next, 3.0 ml of 10 % trichloroacetic acid was added and centrifuged at 2000 g for 5 min. After that, 1.0 mL of the supernatant was mixed with 3.0 ml of 0.67 % TBA solution in 50 % glacial acetic acid. Then the mixture was heated in a boiling water bath for about 30 min. In case of turbidity appearance, the mixture is centrifuged at 2000 g for 5 min. Finally, the absorbance was measured in spectrophotometer at 535 nm. The decrease in absorbance indicates increase of antioxidant activity. Butylatedhydroxyanisole was used as a positive control. All determinations were carried out in triplicates. The values of antioxidant activity were expressed as the percentage inhibition of egg yolk lipid peroxidation and compared with the value obtained from the untreated egg

Table 1. Botanical names of aromatic plants collected from different regions, site of their collection, and parts used for EO hydrodistillation.

Botanical name	Site of collection	Plant's Part used
Artemisia abrotanum	Thamar	Aerial parts (leaves and stem)
Chenopodium Ambrosioides	Sana'a	Fresh whole plant
Cinnamomum zylanicum	Imported (India)	Bark
Clove *eugenia caryophyllus*	Imported (India)	Fruits
Conyza incana (Vah) willd	Taiz-Hojariah	Seeds
Coriandrum sativum	Sana'a	Seeds
Eucalyptus camaldulensis	Sana'a	Dried leaves
Lantana camara	Sana'a	Fresh leaves
Peppermint mentha piperita	Amran	Fresh leaves
Ocimum basillicum	Sana'a	Fresh leaves
Marjoram majorana hortensis	Sana'a	Fresh aerial parts(leaves and stem)
Rosmarinus officinalis	Sana'a	Fresh leaves
Pulicaria jaubertii	Sana'a	Fresh aerial parts(leaves and stem)
Schinus molle	Sana'a	Fruits
Tagetes minuta	Sana'a	Fresh aerial parts(leaves and stem)
Thymus laevigatus	Sana'a-Alhimah	Dried aerial parts(leaves and stem)

yolk lipid peroxidation (i.e. it must give the highest absorbance). Thus, The antioxidant activity (% Inhibition of peroxidation) = $[(A_b - A_s) / A_b] \times 100$

Where A_b- is the absorbance of blank, A_s- is the absorbance of positive control or sample.

RESULTS AND DISCUSSION

The EO extracted from *Cinnamomum zylanicum*and *Clove eugenia caryophyllus* have clear antibacterial activity, which was increased with the increase of essential oil concentration as noticed from the inhibition zones of bacterial growth (Table 2). These results are in agreement with those obtained by other researchers (Unlu et at., 2010). However, it was noticed that, all tested bacterial strains were not susceptible to any level of the EO obtained from *Chenopodiumabrosioides*, *Schinus molle*, *Pulicaria jaubertii* and *Coriandrum sativum* (Table 2). It seems that the antibacterial activity of these EOs could be clarified if the activities of these oils on each tested bacterial species were discussed separately. Therefore, it was observed that *E. coli* was sensitive to the EO extracted from *Cinnamomum zylanicum, Clove eugenia caryophyllus, Marjoram majorana hortensis, Artemisia abrotanum, Thymus laevigatus,* and *Ocimumbasilicum*. Their antibacterial activities were increased with the increase of their concentrations and the highest levels of these EOs showed greater effect than the standard antibiotic (gentamicin, 10 µg).

On the other hand, the growth inhibition of Pseudomonas aeruginosa was observed with application of EO obtained from *Cinnamomum zylanicum*, Clove *eugenia caryophyllus*, *Marjoram majorana hortensis*, *Artemisia abrotanum*, *Thymus laevigatus*, *Eucalyptus camaldulensis*, *Lantana camara*, *Peppermint mentha piperita* and *Ocimum basillicum* respectively. The inhibition zone of bacterial growth was increased with the increase of their concentrations (Table 2). The most virulent Staphylococcus species is Staphylococcus aureus, is isolated from hospitalized patients and also prevalent pathogen in outpatient. Generally, bacteria have the genetic ability to transmit and acquire resistance to some drugs utilized as therapeutic agents. The present study showed that these bacterial species were sensitive to EOs extracted from Clove *eugenia caryophyllus*, *Marjoram majorana hortensis*, *Artemisia abrotanum*, *Eucalyptus camaldulensis*, *Thymus laevigatus*, *Lantana*

Table 2. The antibacterial effect (the inhibition zone in mm) of 25 µL containing different levels of EO (0%, 25%, 50%, 75%, and 100%)

Plant name	*Escherichia coli*					*Pseudomonas aeruginosa*					*Staphylococcus aureus*					*Proteus vulgaris*				
	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Chenopodium Ambrosioides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cinnamomum zylanicum	0.0	10.00 ±0.58	11.33 ±0.58	13.00 ±0.0	17.67 ±1.53	0.0	13.00 ±0.0	16.00 ±1.0	18.00 ±2.65	21.78 ±2.17	0.0	0.0	0.0	0.0	0.0	0.0	13.67 ±0.50	15.78 ±0.44	18.00 ±2.60	21.89 ±2.37
Schinus molle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pulicaria jaubertii	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tagetes minuta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.22 ±0.44	12.93 ±0.74	16.56 ±0.53	17.78 ±0.44
Artemisia abrotanum	0.0	0.0	0.0	11.33± 1.16	19.67 ±2.08	0.0	8.00 ±0.0	9.10 ±0.60	10.22 ±0.44	10.33 ±0.58	0.0	8.00 ±0.0	9.00 ±0.0	10.22 ±0.44	16.89 ±1.17	0.0	0.0	15.22 ±0.44	16.00 ±0.0	18.89 ±0.0
Marjoram majorana hortensis	0.0	0.0	0.0	18.00 ±2.65	22.33 ±2.08	0.0	10.33 ±0.58	11.67 ±1.16	14.67 ±1.16	16.11 ±0.60	0.0	11.67 ±0.58	12.87 ±1.16	14.56 ±0.73	16.22 ±1.05	0.0	12.78 ±0.44	13.44 ±1.01	15.56 ±0.53	19.33 ±1.50
Eucalyptus camaldulensis	0.0	0.0	11.67 ±1.16	15.00 ±1.00	18.00 ±2.65	0.0	0.0	0.0	15.63 ±0.74	18.00 ±2.55	0.0	0.0	11.00 ±0.50	15.63 ±0.74	18.00 ±2.55	0.0	0.0	11.50 ±1.07	19.33 ±1.66	21.89 ±0.33
Clove eugenia caryophyllus	0.0	8.33 ±0.58	10.33 ±0.58	19.00 ±1.0	20.00 ±1.00	0.0	11.33 ±0.58	13.33 ±0.58	16.33 ±0.50	18.00 ±2.29	0.0	11.33 ±0.58	13.33 ±0.58	16.33 ±0.50	18.00 ±2.29	0.0	13.56 ±0.58	14.33 ±0.50	18.67 ±1.32	16.56 ±1.24
Thymus laevigatus	0.0	0.0	0.0	11.33 ±1.53	15.67 ±0.58	0.0	0.0	10.67 ±0.58	12.00 ±1.00	15.11 ±0.58	0.0	0.0	11.00 ±1.0	12.44 ±1.24	15.22 ±0.67	0.0	0.0	0.0	11.75 ±0.71	14.89 ±0.33
Lantana camara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.33 ±1.16	14.33 ±0.50	0.0	0.0	0.0	11.33 ±1.03	14.56 ±0.88	0.0	0.0	0.0	0.0	0.0
Rosmarinus officinalis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.00 ±0.0	21.20 ±2.05	21.78 ±2.17
Conyza incana (vahl) willd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.44 ±1.01	15.89 ±0.33
Peppermint mentha piperita	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.11 ±0.93	0.0	0.0	0.0	0.0	16.11 ±1.05	0.0	0.0	0.0	0.0	0.0

Table 2 continue

Coriandrum sativum	0.0	0.00.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ocimum basillicum	0.0	0.0	0.0	0.0	12.00 ±0.0	0.0	0.0	0.0	0.0	13.38 ±0.52	0.0	0.0	0.0	0.0	14.11 ±1.17	0.0	0.0	0.0	0.0	11.89 ±0.33
Gentamicin	11±0.52					25±0.73					25±1.05					23±0.8				

Table 3. The antifungal effect (the inhibition zone in mm) of 25 µL containing different levels of EO (0%, 25%, 50%, 75%, and 100%)

Plant name	***Candida albicans***					***Aspergillus flavus***					***Fusariumoxysporium***				
	0%	**25%**	**50%**	**75%**	**100%**	**0%**	**25%**	**50%**	**75%**	**100%**	**0%**	**25%**	**50%**	**75%**	**100%**
Chenopodium Ambrosioides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cinnamomum zylanicum	-	14.89 ±0.33	18.56 ±1.13	31.67 ±2.06	32.22 ±1.99	-	0.0	0.0	0.0	14.89 ±0.33	-	11.89 ±0.33	13.89 ±0.33	28.22 ±2.64	31.89 ±2.37
Schinus molle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pulicaria jaubertii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tagetes minuta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Artemisia abrotanum	-	-	-	-	-	-	-	-	11.89 ±0.33	-	-	-	-	-	-
Marjoram majorana hortensis	-	-	-	-	12.44 ±1.01	-	-	-	11.89 ±0.33	-	-	-	-	-	-
Eucalyptus camaldulensis	-	-	11.33 ±0.50	16.56 ±1.81	18.00 ±2.87	-	-	11.33 ±0.50	16.56 ±1.81	17.89 ±0.33	-	-	-	-	7.89 ±0.33
Clove eugenia caryophyllus	-	11.75 ±0.71	22.89 ±1.36	27.11 ±1.27	30.11 ±0.33	-	20.11 ±0.33	24.21 ±0.63	28.14 ±2.22	28.22 ±2.64	-	16.89 ±0.33	20.00 ±0.50	28.89 ±0.33	32.11 ±2.71
Thymus laevigatus	-	12.67 ±0.50	13.44 ±0.88	22.67 ±3.54	29.89 ±0.33	-	-	-	-	-	-	10.89 ±0.33	14.00 2.67	22.03 ±3.04	24.89 ±0.33
Lantana camara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rosmarinus officinalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3 continue

Conyza incana (vahl) willd	-	-	-	-	-	-	-	-	-	-	-	9.89 ±0.33	11.78 ±1.20	20.22 ±1.48	22.89 ±1.36
Peppermint mentha piperita	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coriandrum sativum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ocimum basillicum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.89 ±0.33

Table 4. The Antioxidant Activity of Different Levels of EOs

Plant name	TBARS-Method (% inhibition)				Reducing Power Activity (as µg BHA equivalent to 1 µl of EO)		
	100 µL	250 µL	500 µL	1000µL	25 µL	50 µL	l00 µL
Chenopodium Ambrosioides	68.51 ±4.20	78.84 ±3.96	85.04 ±1.35	89.00 ±1.41	23.80 ±3.02	43.12 ±5.70	95.97 ±1.67
Cinnamomum zylanicum	79.56 ±2.75	89.65 ±1.82	90.45 ±0.63	93.00 ±0.16	30.43 ±3.69	54.85 ±2.78	107.38 ±7.07
Schinus molle	65.23 ±3.66	70.30 ±2.43	72.17 ±2.84	76.87 ±1.99	28.53 ±2.88	52.89 ±4.16	94.08 ±1.94
Pulicaria jaubertii	60.32 ±3.75	80.12 ±4.13	88.21 ±0.75	90.27 ±1.85	22.61 ±0.95	27.90 ±0.91	40.52 ±2.03
Tagetes minuta	20.67 ±1.89	69.13 ±3.67	80.06 ±1.44	90.12 ±2.04	37.78 ±1.08	63.78 ±2.46	101.70 ±3.49
Artemisia abrotanum	58.35 ±2.46	72.29 ±4.77	80.11 ±0.16	82.34 ±0.47	50.42 ±3.41	80.98 ±0.31	135.97 ±12.10
Marjoram majorenahortensis	66.53 0.47	79.70 ±1.21	85.26 ±3.75	89.00 ±2.43	31.01 ±2.00	56.71 ±2.31	104.22 ±1.63
Eucalyptus camaldulensis	66.19 ±0.85	66.78 ±1.19	57.96 ±1.66	57.54 ±0.42	31.32 ±3.55	51.20 ±0.58	88.05 ±3.41

Table 4 continue

Clove eugenia caryophyllus	87.58	91.53	94.17	98.22	131.36	156.58	249.12
	±3.82	±5.55	±6.14	±10.22	±4.34	±4.94	±3.11
Thymus laevigatus	92.90	95.28	97.63	98.67	78.36	116.01	144.85
	±9.90	±8.46	±8.94	±7.26	±1.93	±5.85	±11.20
Lantana camara	11.72	16.48	23.58	32.55	25.48	34.41	68.00
	±2.13	±1.74	±2.31	±0.88	±2.96	±0.62	±0.57
Rosmarinus officinalis	15.09	40.40	69.72	78.65	17.56	23.03	36.24
	±1.06	±2.12	±2.98	±1.16	±2.46	±1.14	±2.72
Conyza incana (vahl) willd	55.24	69.83	72.70	74.33	26.19	41.56	75.64
	±3.55	±4.59	±0.88	±1.02	±2.07	±4.70	±6.96
Peppermint mentha piperita	10.10	31.43	43.89	66.48	18.02	22.28	34.47
	±1.94	±1.76	±0.71	±1.96	±1.60	±0.32	±1.68
Coriandrum sativum	35.54	42.92	46.87	57.49	26.55	35.80	47.13
	±3.00	±2.82	±1.78	±0.88	±2.60	±2.75	±1.90
Ocimum basillicum	60.23	63.95	69.95	75.19	91.68	144.85	209.05
	±4.05	±2.21	±3.71	±0.88	±2.68	±9.20	±7.98
BHA	81.67	84.17	84.04	85.17			
	±0.88	±0.18	±0.35	±0.18			

camara, *Peppermint mentha piperita* and *Ocimum basillicum*. Their antibacterial activities were increased with the increase of their concentrations, but all of them revealed lower antibacterial potency than the gentamicin even at their highest levels.

The new data showed that the growth of Proteus vulgaris was affected by most of the hydrodistilled EOs, namely: *Cinnamomum zylanicum*, Clove *eugenia caryophyllus*, *Marjoram majorana hortensis*, *Tagetes minuta*, *Artemisia abrotanum*, *Rosmarinus officinalis*, *Eucalyptus camaldulensis*, *Conyza incana* (Vahl) wild, *Thymus laevigatus*, and *Ocimum basillicum*. The inhibition zones were increased as the added amounts of EO increased, but all of them revealed lower antibacterial activity than the reference standard even at their highest levels (Table 2). Generally, it is that, as the concentration of EO increases as the inhibition zone increases, since it is used as a measure of the antibacterial activity of EO. In addition, the increase of concentration of EO, leads to broader spectrum, which means more bacterial strains are affected. These phenomena can be observed from the effects of *Artemisia abrotanum*, *Marjoram majorana hortensis*, *Eucalyptus camaldulensis*, *Thymus laevigatus*, *Lantana camara* and *Peppermint mentha piperita*. The present study has shown that, some of the tested EOs could be promising natural antibiotics. This statement is justified on the basis of their broad spectrum and their effectiveness as antibacterial agents that could be arranged in the following order: the *Cinnamomum zylanicum*, Clove *eugenia caryophyllus* , *Artemisia abrotanum*, *Marjoram majorana hortensis*,*Eucalyptus camaldulensis*, *Thymus laevigatus*, and *Lantana camara*.

The chemical components in EO may exert their toxic effects against microorganisms through the disruption of bacterial or fungal membrane integrity (Andrews et al., 1980; Uribe et al., 1985; Knoblock et al., 1988; Helander et al., 1998; Mann, 2000). However, the EOs tested were shown to have smaller inhibition zone against Gram-negative bacteria (E. coli and P.aeruginosa) than Gram-positive bacteria (Proteus vulgaris and Staphylococcus aureus) as shown in the results of *Tagetes minuta* , *Rosmarinus officinalis* and *Conyza incana* (Vahl) wild. This might be due to the protection by a hydrophilic outer membrane of the Gram-negative bacterial membrane, which suppressed the passage of lipophilic essential oil (Nazzaro et al., 2013). Consequently, this can also be explained by the lipophilic nature of some components of the EO, that allow to cross the phospholipidic cell membrane; which can alkylate proteins and thus disturb their conformation (Mulyaningsih et al., 2010). Bacterial resistance varied among bacterial strains as for example in P. aeruginosa could be due to the absence of the porin gaps which are required for the input of the antibiotics (Carle, 2010). This variation in the

antibacterial sensitivity of different antimicrobial agents could be explained by the structural differences among bacteria (Rather et al., 2012).

The present study has shown that, most of the tested EOs (*Chenopodium Ambrosioides* , *Schinus molle* , *Pulicaria jaubertii* , *Tagetes minuta* , *Lantana camara*, *Rosmarinus officinalis*, *Peppermint mentha piperita*, *Coriandrum sativum* and *Ocimum basillicum*) did not show any notable antifungal activity with all levels used against the three tested fungal species (Table 3). On the other hand, EOs from Clove *eugenia caryophyllus* and *Cinnamomum zylanicum*, *Thymus laevigatus*, *Conyza incana* (Vahl) wild, *Eucalyptus camaldulensis*, *Marjoram majorana hortensis*, and *Artemisia abrotanum* have shown to have variable antifungal activities, which were appeared to be dependent on the type of fungal species, the type and concentration of the tested essential oil (Table 3). The high antifungal activities of EO obtained from Clove *eugenia caryophyllus* and *Cinnamomum zylanicum*are well documented and the present results are in agreement with those obtained by other researchers (Unlu et al., 2010; Trajano et al., 2012; El-Ahmady et al., 2013). *Conyza incana* (Vahl) wild essential oil has a notable antifungal activity only against the Fusariumoxysporium, while *Artemisia abrotanum* has shown antifungal activity at the second (75%) highest level only against *Aspergillus flavus*. Clove *eugenia caryophyllus* followed by *Thymus laevigatus*, *Cinnamomum zylanicum*and *Eucalyptus camaldulensis* have shown a broader antifungal activity and the increase of their concentrations leads to the increase of the inhibition zones of fungi growth (Table 3).

The results of antioxidant activities of EOs obtained by hydrodistillation from different aromatic plants grown in Yemen are shown in Table 4. In addition, two sources of EO, which are well known as antioxidants, and antimicrobial agents, namely, *Cinnamomum zylanicum*and *Clove eugenicacaryophyll*, were subjected to the process of hydrodistillation and were studied in parallel for their biological activities. The antioxidant activities of different levels of EOs were obtained using two methods of measurements: RPA and TBARS. As EOs are complex mixtures of different compounds, therefore, the results of antioxidant activities of the studied EOs could be attributed to some active compounds exist in the mixture of essential oil, which may be present in high percentage, or due to the presence of other active constituents in small quantities, or to synergy among them (Abdalla, and Roozen, 1999; Manjamalai, 2012). The present results (Table 4) show that, most of the EOs have antioxidant activity and this activity increases with the increase of essential oil concentration. However, this relationship does not reflect linear relationship between the concentration of essential oil and the antioxidant activity. This phenomenon can be explained on the basis that, antioxidant activities of EO from aromatic plants are mainly attributed to their phenolic and flavonoids contents (Calo et al., 2015) as well as the active compounds present in their mixtures, which can be due to the high percentage of main constituents, and to the presence of other constituents in small quantities, or to synergy among them (Abdalla, and Roozen, 1999; Jukic and Milos, 2006). Studies by other researchers have shown that, monoterpenes contents of EO may act as free radical scavenging agents, but it seems to be that the EO, which contain oxygenated monoterpenes, monoterpene hydrocarbons and/or sesquiterpenes have greater antioxidative power (Helander et al., 1998; Brits et al., 2011; Mau et al., 2003; Tepe et al., 2004).

CONCLUSION

The present study has shown that, the EOs from four aromatic plants, namely: *Ocimum basillicum* > *Conyzaincane* > *Thymus laevigatus* > *Tagetes minuta,* could be used as a potential source of natural antioxidants with possible applications of *Ocimum basillicum* and *Thymus laevigatus* in food systems, while the *Conyza incana* and *Tagetes minuta* can be used as a source of natural antioxidants for pharmaceutical and cosmetics industry. Generally, the present aromatic plants, which can be potential sources of natural antioxidants as observed from the antioxidant activities of their EOs according to TBARS method can be arranged according to the following order: *Thymus laevigalus* > *Clove eugenia caryophyllus* > *Cinnamomum zylanicum* > *Chenopodium Ambrosioides = Eucalyptus camaldulensis = Marjoram majorana hortensis = Schinus molle* >*Pulicaria jaubertii = Ocimum basillicum* > *Artemisia abrotanum* > *Conyza incana (vahl) willd*>*Coriandrum sativum* >*Tagetes minuta* > *Rosmarinus officinalis* > *Lantana camara* > *Peppermint mentha piperita*. On the other hand these activities as obtained by RPA can be arranged as follows: *Clove eugenia caryophyllus* > *Ocimum basillicum* > *Thymus laevigalus*> *Artemisia abrotanum* > *Tagetes minuta* > *Eucalyptus camaldulensis = Marjoram majorenahortensis = CinnamonumZylanicum = Schinus molle* > *Coriandrum sativum = Conyza incana (vahl) willd = Lantana camara* > *Chenopodium Ambrosioides = Pulicariajubertii* > *Peppermint mentha piperita = Rosmarinus officinalis.* Meanwhile, some of the tested EOs could be promising natural antibiotics. Due to their broad spectrum and their effectiveness as antibacterial agents they could be arranged in the following order: the *Cinnamomum zylanicum*, *Clove eugenia caryophyllus*, *Artemisia abrotanum*, *Marjoram majorana hortensis*, *Eucalyptus camaldulensis*, *Thymus laevigatus*, and *Lantana camara*. While most of the tested EOs (*Chenopodium Ambrosioides*, *Schinus molle* , *Pulicaria jaubertii*, *Tagetes minuta*, *Lantana camara*, *Rosmarinus officinalis*, *Peppermint mentha piperita*, *Coriandrum sativum* and *Ocimum basillicum*) did not show any notable antifungal activity with all levels used against the three tested fungal species, EOs from *Clove eugenia caryophyllus* and

Cinnamomum zylanicum, *Thymus laevigatus*, *Conyza incana* (Vahl) wild, *Eucalyptus camaldulensis*, *Marjoram majorana hortensis*, and *Artemisia abrotanum* have shown to have variable antifungal activities. However, future work on these plants must be oriented to identify the active components in each essential oil and to explore the mechanism of actions and their toxicity levels on the bases of their applications.

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