Zagazig J. Agric. Res., Vol. 44 No. (3) 2017 1061-1071

Food and Dairy Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

# ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SOME PLANT EXTRACTS

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**Received: 27/02/2017 ; Accepted: 26/03/2017**

**ABSTRACT:** In this study, three plants; rosemary, ginger, and peppermint, were extracted using three solvents; ethanol, methanol and water. A comparison was held between different extracts concerning: chemical composition, efficiency of the extraction method, yield, antimicrobial and antioxidant potentials. Phenolic compounds profile were studied *via* High Performance Liquid Chromatography (HPLC).Antimicrobial activity of the extracts was examined against: *Escherichia coli, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus pyogenes, Candida albicans, Klebseilla pneumonia, Bacillus subtilis* and *Streptococcus*. spp using agar well diffusion method. The inhibition zones diameter (IZD) were ranged between 11- 37 mm. The results showed that ethanol extraction had the highest yield of rosemary and peppermint (19.17 and 17.19%, respectively). While; the lowest was obtained from ginger methanol extracts (12.78%). Rosemary water extract had the highest total phenolic contents (271.66 ± 12.2 µg/mg, while ethanol extract of ginger and peppermint gave 201.31 ± 8.99 and 165 ± 4.74 mg gallic acid equivalent (GAE/g) respectively. Concerning the total flavonoid contents; rosemary methanol extract gained the highest content (123.9 ± 2.99 µg/mg), while in ethanol extracts of ginger and peppermint showed the best results (44.06 ± 0.55, 89.54 ± 2.63 µg/mg, respectively). Antioxidant activity was used as a parameter to evaluate the protective antioxidant ability of examined herbs represented in IC50 (inhibition concentration). Results showed that in rosemary water extract 24.5µg/ml, while in ginger and peppermint ethanol extracts was 38.98 and 80 µg/ml, respectively. Depending on results stated above, it can be recommend using water for rosemary extraction and ethanol 70% for ginger and peppermint extractions for the best antioxidant and antimicrobial impact.

**Key words:** Natural antioxidants, plant extracts, antioxidant activity, phenolic compounds, flavonoids, antimicrobial activity.

## INTRODUCTION

There is a growing interest in natural antioxidants found in plants because of the worldwide trend toward the use of natural additives in foods, beverages and cosmetics. Herbs and spices are one of the most important targets to search for natural antioxidants from the point of view of safety (Yanishlieva *et al*., 2006).

Herbs and spices, which are important part of the human diet, have been used for thousands of years in traditional medicine and to enhance the flavour, colour and aroma of foods. In addition to boosting flavour, herbs and spices are also known for their preservative (Neilsen and Rios, 2000), antioxidative (Shobana and Naidu, 2000), and antimicrobial roles. Numerous studies have been published on the antioxidant capacity and the phenolic constituents of herbs (Konczak *et al*., 2010).

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The antioxidants can be of synthetic or natural origin. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallate (PG) have been widely used in meat and poultry products (Jayathilakan *et al*., 2007). The demand for natural antioxidants, especially of plant origin has increased in recent years due to the growing concern among consumers about these synthetic antioxidants because of their potential toxicological effects (Nunez de Gonzalez *et al*., 2008).

Unlike synthetic compounds, natural preservatives obtained from plants are rich in phenolic compounds and they can enhance the overall quality of food by decreasing lipid and protein oxidation and microbial growth. In Egypt rosemary, ginger and peppermint are important source of natural antioxidants. They are generally used as condiments to enhance the sensory quality and shelf-life of foods in Egypt, in addition to their health benefits, which have been widely studied (Shariatpanahi *et al*., 2010; Chandrashekar *et al*., 2011).

Natural antioxidants are important in food industry because of their healthy effects (Ibañez *et al*., 2003). Thus, their demand has increased for growing interest in foods obtained from natural sources (Aruoma *et al*., 1995; Kim *et al*., 1997). The extract quality is greatly influenced by the extraction methodology used and solvent extraction techniques. Several studies have shown that extraction method can alter the antioxidant activity and total phenol contents in the extracts (Chan *et al*., 2007; Sikora *et al*., 2008; Ding *et al.,* 2012).

Consequently, the aim of this study was to identify and determine the antioxidant activity, total phenolic and flavonoid contents and antimicrobial activity of ginger, rosemary and peppermint extracts.

# MATERIALS AND METHODS

## Materials

### Plant material and microbial strains

Dried leaves of rosemary (*Rosmarinus officinalis* L.), peppermint (*Mentha piperita* L.), and derived from the rhizome of ginger, (*Zingiber officinale* L) were obtained from local market in Alexandria, Egypt. Microbial strains used were (*Escherichia coli* BA 12296, *Staphylococcus epidermidis, Staphylococcus aureus* NCTC 10788, *Staphylococcus pyogenes, Candida albicans ATCCMYA-2876, Klebseillapneumonia* ATCC12296*, Bacillus subtilis* and *Streptococcus*. spp.) from Ain shams culture collection Cairo. Egypt

### Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu's reagent (FCR), sodium carbonate ($Na_2CO_3$), gallic acid, catechol, aluminum chloride ($AlCl_{3)}$, and butylated hydroxyl toluene (BHT) were purchased from Sigma-Aldrich Chemicals, Germany).

## Methods

### Chemical composition of three plants

After homogenization of the plant samples (to uniform size), proximate composition analysis (Moisture, protein, fat, ash, total fiber and carbohydrate) of three plants were carried out according to AOAC (2000). All analyses were conducted in Food Technology Lab, Arid Land Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

### Preparation of plant extracts

Plant extracts of tested plants were prepared according to Sung-Jin *et al*. (2013) with some modifications, dried plants were ground using mixer grinder, 50 grams of each plant powder were separately soaked in 1 L of ethanol 70%, methanol 70% and water (1: 20 *W/V*) and shacked for 24 hr., at room temperature using magnetic stirrer. The mixture was centrifuged at 3000 rpm for 15 min, filtered through a filter paper (What man No. 1). After filtering the obtained extract was concentrated under reduced pressure in a water bath set at 45ºC using a rotary evaporator (IKA RV 05 basic Type HB 4 B, Germany).The extra solvent was eliminated by a vacuum freeze-dryer (Model FDF 0350, Korea), The residual was weighed, and the extraction yield of each plant material was calculated. The dried powder of plant extract was then stored at -20ºC until analysis.

## Antioxidant Activity

### Determination of total phenol contents (TPC)

The total phenol compound contents were carried out using the Folin-Ciocalteu reagent, following the method of Singleton *et al*. (1999), Dewanto *et al*. (2002). 1mg extract was dissolved in 1ml methanol and 500 µl of dissolved sample was taken and added to 0.5 ml of distilled water and 0.125 ml of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before addition of 1.25 ml of 7% $Na_2CO_3$. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of Gallic acid (standard, from 0-1000 µg/ml). Total phenol contents (TPC) were expressed as Gallic acid equivalent (GAE)/mg of dry weight and calculated using the following liner equation based on the calibration curve:

$y = 0.001x - 0.141$, $R^2 = 0.998$ Where (y) is absorbance, (x) is the concentration (mg GAE/g extract), $R^2$ is correlation coefficient. All determinations were performed in triplicates.

### Determination of total flavonoid contents (TFC)

The total flavonoid contents of the plant extracts were determined by a modified colorimetric method described by Sakanaka *et al*. (2005), using catechol as a standard at concentrations of (20 – 200 µg/ ml). Extracts or standard solutions (250 µl) were mixed with distilled water (1.25 ml) and 75 µl of 5% sodium nitrite ($NaNO_2$) solution followed by the addition of 150 µl of 10% aluminum chloride ($AlCl_3$) solution after 5 min later. After 6 min, 0.5 ml of 1 M sodium hydroxide (NaOH) and 0.6 ml distilled water were added. The mixture was then mixed and absorbance was measured at 510 nm. Total flavonoids content was expressed as catechol equivalent (CE) and calculated using the following liner equation based on the calibration curve:

$y=0.004 x - 0.012$, $R^2 = 0.999$ where (y) is absorbance and (x) is the concentration (mg CE /g extract).

$R^2$ = correlation coefficient. All determinations were performed in triplicate.

### DPPH radical scavenging activity

The free radical scavenging activity of plant extracts was measured by the DPPH method as proposed by Brand-Williams *et al*. (1995), with some modifications. A solution of 0.2 mM DPPH in methanol (0.0078 g/100 ml) was prepared and 1 ml of this radical solution was added to 1 ml of sample or standard solution at different concentrations (1:1 *V/V*). The mixture was incubated for 30 min in the dark at room temperature and then the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid solutions as standards in the concentration range of (5 - 500 µg/ml) were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent (AAE)/g dried sample.

The percentage DPPH radical-scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (% inhibition)

$$= \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

For control, all reagents were added except plant extract and all determinations were performed in triplicate.

### HPLC analysis of phenolic compounds

The phenolic compounds of the plant samples; (rosemary, ginger and peppermint) the different solvents; (ethanol, methanol and water) were analyzed using high performance liquid chromatography (HPLC) according to Croci *et al.* (2009). Agilent 1260 infinity HPLC series (Agilent, USA), equipped with quaternary pump, a Zorbax Eclipse plus C18 column 100 mm x 4.6 mm i.d., (Agilent technologies, USA) operated at 25°C, was used for phenolic compound analysis. The injected volume was 20µ: VWD detector set at 284 nm. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade 0.2% $H_3PO_4$ (*V/V*), (B) methanol and (c) acetonitrile. The quantification of the phenolic compounds is based on the standards of phenolic acids; gallic acid, catechol, p-hydroxy benzoic acid, caffeine,

valnillic acid, caffiec acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, rutin, ellagic acid, benzoic acid, α-coumaric acid.

### Antimicrobial activity of plant extracts

The antimicrobial activity was performed by agar well diffusion essay (Perez *et al*., 1995) for all samples extract. Eight species known to be pathogenic to human such as microbial strains including *Escherichia coli* BA 12296, *Staphylococcus epidermidis, Staphylococcus aureus* NCTC 10788, *Staphylococcus pyogenes, Candida albicans* ATCCMYA-2876, *Klebseilla pneumonia* ATCC12296*, Bacillus subtilis* and *Streptococcus*. spp., were used. Hundred µl of the inoculums ($1\times10^8$ cfu/ml) were mixed with agar media and poured into the Petri plate. A well was prepared in the plates with the help of acork-borer (0.85 cm). and100 µl of the tested compound were introduced into the well. All the tested strains were incubated at 37°C for 24 hr., and microbial growth was determined by measuring the diameter of inhibition zone (mm). For each bacterial strain, controls were maintained as pure solvents instead of the extract. The experiment was done three times and the mean values were presented.

## Statistical Analysis

The results were reported as mean ± standard deviation (SD) (n = 3). The average contents of total phenolic content, total flavonoids and $IC_{50}$ of the extracts prepared by the different extraction methods were statistically investigated using one-way analysis of variance (ANOVA) with Duncan by SPSS for Windows 16.0. A statistical probability (p value) less than 0.05 indicated a statistically significant difference between groups (Steel and Torrie, 1980).

# RESULTS AND DISCUSSION

## Analysis of Chemical Composition

Chemical analysis of the three plants; (rosemary, ginger and peppermint) is represented in Table 1. The results of rosemary showed relatively high content of carbohydrate, fat and fiber 52.88, 14.7 and 10.02, respectively. While in ginger moisture content, fat and carbohydrate were 10.26, 11.32 and 57.62, respectively. In peppermint, the results showed relatively high content of ash and protein (16.11 and 12.07, respectively). From the obtained results, it could be seen that ginger has the highest moisture and carbohydrate content, but fat and total fibers content were the highest in rosemary, while ash and protein were higher in peppermint. These results agree with most data reported by USDA National Nutrient Database, Differences could be referred to different spices, seasons, or districts.

### Extraction yield

The yield of extracts obtained from the three spices; rosemary, ginger and peppermint for each solvent are shown in Table 2. Rosemary ethanol (70%) extract showed the highest yield, followed by methanol (70%) extract then water extract (19.17± 0.27, 17.61±0.44, and 16. 35± 0.61%, respectively).

The extraction yield of rosemary and peppermint with ethanol (19.17, 17.19%, respectively) was slightly higher than the other solvents but in ginger the highest was with water (15.85%). This may due to connected to polarity gained by water-solvent mix. Similar results were reported by Zhang *et al.* (2010), but disagree with Rodriguez-Rojo *et al.* (2012). also the Extraction yield obtained in the present study disagreed with the values described by Kejing *et al.* (2016) who reported the yield as (*V/W*%) 2.69 ± 0.32 from ginger, however the results were in harmony with Yeh *et al.* (2014) reported that yields of aqueous and ethanolic extracts from ginger were 11.95 ± 0.05 and 8.96 ± 0.08 (g/100 g).

### Contents of Total Phenolic

Results in Table 3 exhibit total phenolic contents of plant extracts (µg Gallic acid/mg extract).

Extraction of rosemary with distilled water gave the highest amount of phenolic contents (271.66 ± 12.2 mg GAE/g extract). Higher phenolic content in rosemary was reported by Wojdyło *et al.* (2007). While, TPC of ethanol extracts from ginger and peppermint showed significantly the highest between the examined extracts (201.31±8.99 and 165 ±4.74 mg GAE/ g respectively). These results suggest that the nature of these polyphenols is polar. The total phenolic contents obtained in the present study for ginger were higher than the values described by Sattar *et al.* (2013), Özlem *et al.* (2015) and

**Table 1. Chemical composition of rosemary, ginger and peppermint**

| Plant | Moisture | Ash | Protein | Fat | Total fiber | Carbohydrate |
|---|---|---|---|---|---|---|
| **Rosemary** | 7.78±0.21[b] | 7.88±0.19[b] | 6.73±1.63[b] | 14.7±0.81[a] | 10.02±0.99[a] | 52.88±3.03[a] |
| **Ginger** | 10.27±0.11[a] | 4.6±0.27[c] | 10.38±0.75[a] | 11.32±0.19[b] | 5.82±0.47[b] | 57.62±1.46[a] |
| **Peppermint** | 7.42±0.35[b] | 16.11±0.61[a] | 12.07±0.23[a] | 8.30±0.32[c] | 1.27±0.12[c] | 54.82±1.42[a] |

- Results are in g/100g sample
- Each reported value is the mean ± SD of three replicates. Means in the same row followed by different letters are significantly different ($p<0.05$).

**Table 2. Extraction yields of rosemary, ginger and peppermint with three different solvents**

| Solvent | Rosemary (%) | Ginger (%) | Peppermint (%) |
|---|---|---|---|
| **Water** | 16. 35± 0.61[c] | 15.85± 0.28[a] | 16.51±0.63[a] |
| **Ethanol** | 19.17± 0.27[a] | 14.48±0.1.3[a] | 17.19±0.24[a] |
| **Methanol** | 17.61±0.44[b] | 12.78±0.30[b] | 15.10±0.49[b] |

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$).

**Table 3. Total phenol contents in different solvent extracts (mg Gallic acid / g extract)**

| Solvent | Rosemary | Ginger | Peppermint |
|---|---|---|---|
| **Water** | 271.66± 12.2[a] | 94.82 ± 2.90[c] | 124.63±1.2[c] |
| **Ethanol** | 210.61± 8.44[b] | 201.31±8.99[a] | 165.00±4.73[a] |
| **Methanol** | 255.17±8.22[a] | 154.82±13.73[b] | 152.36±5.93[b] |

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$).

Kejing *et al.* (2016). But agreed with Jelled *et al.* (2015). Concerning TPC of peppermint, results agree with Dorman *et al.* (2003), Kosar *et al.* (2005), while Kanatt *et al.* (2007 and 2008) reported lower levels.

**Content of Total Flavonoids**

Total flavonoid contents of the plant extracts are shown in Table 4. Flavonoids are one of the most diverse and widespread groups of natural compounds. The flavones, isoflavones, flavonoids, anthocyanins, and catechins are considered to be the most important natural phenols Sim and Han (2008).

Rosemary methanol extract showed the highest flavonoids content (123.9 ± 2.99), followed by water extract then ethanol extract (112.71 ± 1.09, 77.63 ± 0.60), respectively.

In ginger TFCshowed the highest content in ethanol extract (44.06 ± 0.55). These results are in agreement with Jelled *et al*. (2015) and disagreed with Kejing *et al*. (2016).

In peppermint; ethanol extract gave the highest value (89.54 ± 2.63) and highest content by Santos *et al.* (2014).

**DPPH radical scavenging activity**

Fig. 1 shows the $IC_{50}$ values of the extracts hence the $IC_{50}$ value represents the lower concentration of plant extract required to scavenge DPPH radical to 50%. The lower the $IC_{50}$ value represents, the higher the antioxidant activity. From the obtained results, all plant extracts (with different solvents) showed high antioxidant activity potentials with no significant differences. $IC_{50}$of L-ascorbic acid as

**Table 4. Total flavonoids content in different solvent extracts (mg catechol/ g extract)**

| Solvent | Rosemary | Ginger | Peppermint |
|---|---|---|---|
| **Water** | 112.71±1.09[b] | 7.15±0.60[c] | 41.43±1.42[c] |
| **Ethanol** | 77.63±0.60[c] | 44.06±0.55[a] | 89.54±2.63[a] |
| **Methanol** | 123.9±2.99[a] | 26.52±1.09[b] | 73.82±4.42[b] |

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different upper case letters are significantly different ($p<0.05$).

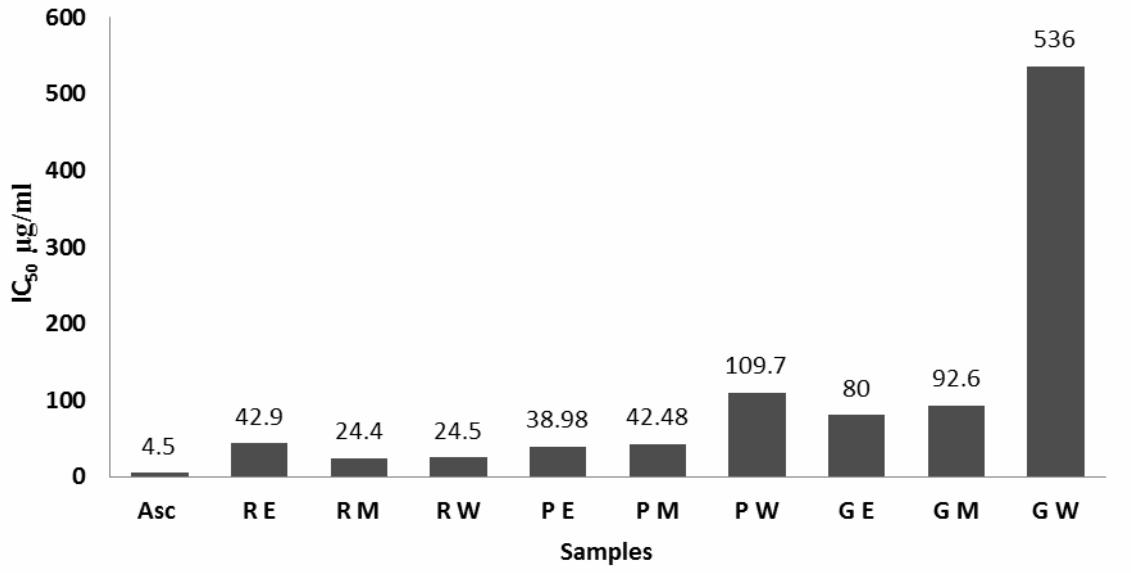


**Fig. 1. The inhibition concentration ($IC_{50}$) values of the extracts from three plants by different solvents**

- RE; Rosemary ethanol extract, RM; Rosemary Methanol, RW (Rosemary Water), GE (ginger Ethanol), GM (Ginger Methanol), GW (Ginger Water), PE (Peppermint Ethanol), PM (Peppermint Methanol), PW (Peppermint Water)

positive control was 4.5 µg/ml. Rosemary methanol extract (RM) and rosemary water extract (RW) showed the best $IC_{50}$ results among rosemary extract (24.4 and 24.5 µg/ml, respectively). Different results were reported by Wojdyło *et al.* (2007). hence $IC_{50}$ of ginger ethanol extract (GE) was the lowest comparing with other ginger extracts (80 µg/ml), the obtained results are in agreement with Jelled *et al.*, 2015), but higher $IC_{50}$ values were reported by Yeh *et al.* (2014) and Kejing *et al.* (2016). The lowest value of peppermint extracts was obtained in ethanol extract that reflect the highest antioxidant activity (38.98 µg/ml). This results agreed with that of Kanatt *et al.* (2007 and 2008) and Uribe *et al.* (2016). The obtained results of antioxidant activity were related with TPC and total flavonoid contents (Tables 3 and 4).

## HPLC Analysis of Phenolic Compounds

Results in Table 5 shows the phenolic compounds in rosemary, ginger and peppermint with different solvents; (ethanol, methanol and water) after analysis by HPLC. In ethanol extract of rosemary; p-hydroxy benzoic acid, syringic acid, and benzoic acid were higher (1122.7, 105.59 and 395.66 mg/100g, respectively) than in methanol or water. In contrary, the concentration of ellagic acid and

**Table 5. HPLC analysis of phenolic compounds in rosemary, ginger and peppermint extracted by different solvents**

| Conc mg/100g | Rosemary | | | Ginger | | | Peppermint | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ethanol | Methanol | Water | Ethanol | Methanol | Water | Ethanol | Methanol | Water |
| **Gallicacid** | 0.2 | ND | ND | ND | ND | ND | ND | ND | ND |
| **P-hydroxy benzoic** | 1122.7 | 742.3 | 627.1 | 12.6 | 8.0 | 0.1 | ND | ND | ND |
| **Valnillic** | 99.6 | 98.68 | 127.37 | 29.73 | 19.18 | 3.18 | 10.66 | 2.05 | 18.22 |
| **Caffiec** | 215.41 | 115.07 | 257.14 | ND | ND | ND | 166.21 | 8.78 | 96.76 |
| **Syringic** | 105.59 | 36.55 | 60.65 | 42.09 | 35.11 | 29.99 | 24.61 | 0.1 | ND |
| **Vanillin** | 0.1 | ND | ND | 36.69 | 23.31 | 15.95 | ND | ND | 0.1 |
| **P-coumaric** | 40.62 | 32.84 | 31.14 | 30.41 | 21.3 | 0.2 | 4.28 | 0.2 | ND |
| **Ferulic** | 77.19 | ND | 303.24 | 11.75 | 11.02 | 0.2 | 0.2 | 0.2 | ND |
| **Rutin** | 127.2 | 41.36 | 1691.75 | ND | ND | ND | ND | ND | ND |
| **Ellagic** | 3107.1 | 3827.6 | 2528.2 | 342.4 | 367.7 | 228.4 | 4688.9 | 1311 | 4342.8 |
| **Benzoic** | 395.66 | 105.51 | ND | 105.7 | ND | 0.2 | 283.58 | 93.41 | 254.57 |
| **O-coumaric** | ND | 1.75 | 13.68 | ND | ND | 0.2 | ND | 0.2 | 20.03 |
| **Salicylic** | ND | 9100.9 | ND | 56.13 | 97.58 | ND | ND | 0.1 | 53.72 |
| **Cinnamic** | 8.34 | 10.13 | 7.85 | ND | 0.2 | ND | ND | ND | ND |
| **Total** | 5299.7 | 14112.7 | 5648.1 | 667.5 | 583.4 | 278.4 | 5178.4 | 1416.1 | 4786.2 |

ND = Not detected

salicylic acid (3827.59 and 9100.9 mg/100g, respectively) were the highest in methanolic extract, and; valnillic acid, caffiec acid, rutin, and o-coumaric acid (127.37, 257.14 and 1691.75 mg/100g, respectively) were the highest in water extract. In ginger ethanol extract; p-hydroxy benzoic acid, valnillic acid, syringic acid, vanillin, p-coumaric acid, and benzoic acid were higher (12.58, 29.73, 42.09, 36.69, 30.41 and 105.7 mg/100g, respectively) than that in methanol or water. But in methanol; ellagic acid and salicylic acid (367.7 and 97.58 mg/100g, respectively) was the highest.

In ethanolic extract of peppermint the concentrations of (caffiec acid, syringic acid, p-coumaric acid, ellagic acid and benzoic acid were 166.21, 24.6, 4.28, 4688.9 and 283.58 mg/100 g, respectively) were higher than that in methanol or water. While; valnillic acid, o-coumaric acid and salicylic acid (18.22, 20.03 and 53.72 mg/100 g, respectively) were the highest in water peppermint extract.

These variabilities in the concentration of phenolic compounds may cause the differences in antioxidant activities between the three plants, the results of phenolic compounds content and concentrations obtained *via* HPLC were correlated with TPC, total flavonoids content as well as with DPPH results (Tables 3, 4 and Fig. 1).

**Antimicrobial activity**

The antimicrobial activity of the extracts was measured in terms of diameter of the inhibitory zones in agar. From the obtained results in Table 6, the three plants showed a reasonable antimicrobial activity against tested strains (*Escherichia coli* BA 12296, *Staphylococcus epidermidis, Staphylococcus aureus* NCTC 10788, *Staphylococcus pyogenes, Candida albicans* ATCCMYA-2876, *Klebseilla pneumonia* ATCC12296*, Bacillus subtilis* and *Streptococcus.* spp.) at a concentration of 100 mg/ml.

**Table 6. Antimicrobial activity of three plant extracts (with different solvents) against some microbial strains measured in terms of inhibition zone diameter (IDZ)**

| Sample | Inhibition zone (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Staph. epidermis*** | ***Bacillus subtilis*** | ***St. pyogenes*** | ***E. coli*** | ***Klebseilla* spp** | ***Steptococcus* spp** | ***Staph. aureus*** | ***Candida albicans*** |
| **RE** | ND | ND | ND | 21 | ND | 37 | 17 | 14 |
| **RM** | ND | 14 | 35.5 | 25 | ND | 29 | 20 | ND |
| **RW** | 11 | 17 | 21 | 13 | ND | 19 | 12 | ND |
| **GE** | ND | ND | 17 | 26 | 20 | 20 | ND | 19 |
| **GM** | 23.5 | 14 | 19 | 25 | ND | ND | 20.5 | ND |
| **GW** | ND | ND | ND | 21.5 | ND | ND | 19 | ND |
| **PE** | 30 | ND | 24 | 20 | ND | 15 | 18 | ND |
| **PM** | 25 | ND | ND | 24 | ND | 18 | 16 | 24.5 |
| **PW** | ND | ND | 13 | ND | ND | ND | ND | 30 |

RE (Rosemary Ethanol), RM (Rosemary Methanol), RW (Rosemary Water), GE (ginger Ethanol), GM (Ginger Methanol), GW (Ginger Water), PE (Peppermint Ethanol), PM (Peppermint Methanol), PW (Peppermint Water), ND Not detected

All the tested plant extracts showed antimicrobial activity against all tested microbial strains but variable values. The antimicrobial activity showed that the ethanolic extract of rosemary exhibited the maximum inhibitory zone diameter (IZD=37 mm) against *Streptococcus* Spp., and methanolic extract of rosemary (IZD=35.5 mm) against *Streptococcus pyogenes* while the water extract gave (IZD =21 mm). In ethanolic extract of ginger (against *E. coli)* showed a highest inhibition zone (26 mm) and IZD= 25 mm with methanol extract, followed by water extract (21.5 mm). In peppermint ethanol extract showed a highest inhibition zone (30 mm) against *Staphylococcus epidermidis* and the IZD was 25, 24.5 and 24mm against *Staphylococcus epidermidis*, *Candida albicans and E. coli,* respectively. while against *Candida albicans* the water extract showed the best results (IZD= 30 mm).The differences in the level of the effectiveness of plant extract as antimicrobial agent may refer to the action of phenolic compounds. The anti-bacterial activity of plant extract might be due the ability of phenolic compounds to bind with bacterial cell walls and prevent cell division and growth (Cowan, 1999; El Sohaimy, 2014). These results encourage the using of water for rosemary extraction and ethanol (70%) for ginger and peppermint which gave the best antimicrobial activity.

## Conclusion

The best antioxidant and antimicrobial results were achieved in water extract of rosemary, and ethanol for ginger and peppermint to obtain the highest content of phenolic and flavonoid compounds. Thus, these results recommend the use of water extraction method for rosemary and ethanol 70% for ginger and peppermint extraction for best antioxidant and antimicrobial impact.

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# النشاط المضاد للأكسدة والمضاد للميكروبات لمستخلصات بعض النباتات

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فى هذه الدراسة، تم عمل مستخلصات لثلاثة أنواع من النباتات هي الروزمارى، الزنجبيل والنعناع باستخدام ثلاثة مذيبات هى الايثانول ٧٠%، الميثانول ٧٠% والماء، تم إجراء التحليل الكيميائي لهذه النباتات وتم عمل مقارنة بين المستخلصات المختلفة من حيث: كفاءة وناتج الاستخلاص، النشاط المضاد للأكسدة والنشاط المضاد للميكروبات، تم دراسة المركبات الفينولية باستخدام جهاز HPLC وكذلك تقييم النشاط المضاد للميكروبات لهذه المستخلصات وتأثيرها المثبط للسلالات الممرضة التالية مثل *Escherichia coli* BA 12296, *Staphylococcus epidermidis,* *Staphylococcus aureus* NCTC 10788, *Staphylococcus pyogenes, Candida albicans* ATCCMYA-2876, *Klebseilla pneumonia* ATCC12296, *Bacillus subtilis* وSpp. *Streptococcus*. تراوح قطر منطقة التثبيط من ١١ إلى ٣٧ مم، وأظهرت النتائج أن أعلى نسبة استخلاص كانت بالايثانول للروزمارى والنعناع (١٩,١٧، ١٧,١٩% على التوالى) بينما اقل نسبة استخلاص تم الحصول عليها من الاستخلاص بالميثانول ٧٠% للزنجبيل ١٢,٧٨%، أعلى محتوى الفينولات الكلية تم الحصول عليه فى المستخلص المائى للروزمارى ٢٧١,٦٦ ملليجرام/جرام بينما كان الاستخلاص بالايثانول أفضل مع الزنجبيل والنعناع (٢٠١,٣١، ١٦٥ ملليجرام/جرام)، فيما يخص نتائج الفلافونيدات الكلية: أعلى محتوى تم الحصول عليه فى المستخلص الميثانولى للروزمارى ١٢٣,٩ ملليجرام/جرام، بينما أعطى الاستخلاص بالإيثانول للزنجبيل والنعناع (٤٤,٠٦، ٨٩,٥٤ ملليجرام/جرام على التوالى) أفضل النتائج، تم تقييم النشاط المضاد للأكسدة للنباتات بناءاً على قيم $IC_{50}$ والتى كانت فى المستخلص المائى للروزمارى ٢٤,٥ ميكرو جرام/ملليجرام بينما كانت في المستخلص الإيثانولى للزنجبيل والنعناع ٣٨,٩٨،٨٠ ميكرو جرام/مل على التوالى، وبناء على النتائج المذكورة أعلاه يوصى باستخدام الماء في الاستخلاص مع الروزمارى والايثانول ٧٠% مع الزنجبيل والنعناع للحصول على أعلى نشاط مضاد للأكسدة ونشاط مضاد للميكروبات.

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