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Extraction and Characterization of Peppermint (*Mentha piperita)* Essential Oil and its Assessment as Antioxidant and Antibacterial

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ABSTRACT

The aim of this study is to extract and characterize peppermint essential oil and assess it as antioxidant and antibacterial activity. The extraction processes, chemical composition, total phenolic contents (TPC), total flavonoid contents (TFC), antioxidant and antibacterial activity were studied. The oil was extracted by three methods: steam distillation, solvent and soxhelet. The results indicated that the highest oil extraction was obtained by soxhelet method 1.5±0.12 and 1.2±0.12 ml/100 gm and the lowest result recorded by steam distillation 1.1±0.09 and 0.9±0.14 ml/100gm from fresh and dry samples, respectively. The chemical composition of fresh and dry sample were determined by using gas chromatography and the results showed that there were 26 components of volatile compounds were identified in the essential oil isolated from peppermint. The antibacterial potential of mint essential oils was evaluated by Muller Hinton agar well diffusion method against selected bacteria. The essential oils showed higher activity against *Staphylococcus aureus* and *Salmonella* 19±1.41 and 16.5±2.12 mm, respectively, for fresh sample and showed lower activity against *Escherichia coli* and *Klebsilla pneumonia* 8.5±0.70 mm and nil, respectively, for dry sample. The TPC of fresh and dry samples were 14.00±0.12 and 8.80±0.09 mg /kg, respectively. TFC of the mint essential oil was determined in comparison with rutin were 8.1±0.09 and 5.0±0.07mg/kg, for fresh and dry sample, respectively. The results showed decrease in peroxide value at 30, 60, 90, 120 and 150 min compared with blank sample without essential oil for wet and dry mint. DPPH inhibition percentages were recorded and inhibition concentrations at 50% activity (IC_{50}) were 0.651±0.09, 0.683±0.6 and 0.161±0.07 mg/mL for wet, dry samples and standard respectively. It can be recommended that further study on sensory analysis of food products containing mint essential oil to evaluate its acceptability and shelf life.

Keywords: Peppermint essential oil, antioxidant, antibacterial, ethanolic extract, GC.

INTRODUCTION

Peppermint (*Mentha* × *piperita*, also known as *M. balsamea* Willd is a hybrid mint, a cross between water mint and spearmint. The plant, indigenous to Europe and the Middle East, is now widespread in cultivation in many regions of the world ('Wang *et al*., 2008). It is herbaceous rhizomatous perennial plant growing to 30–90 cm tall, with smooth stems, square in cross section. The rhizomes are wide-spreading, fleshy, and bare fibrous roots. The leaves are from 4–9 cm long and 1.5 – 4 cm broad, dark green with reddish veins, and with an acute apex and coarsely toothed margins. The leaves and stems are usually slightly fuzzy. The flowers are purple, 6–8 mm long, with a four-lobed corolla about 5 mm diameter; Peppermint is a fast-growing plant; once it sprouts, and it spreads very quickly ('Zaidy and Dahiya, 2015). Humankind has used plants for healing for many thousands of years, and it's from this tradition of that the use of aromatic plant compounds in medicine begun. Oils were used in the embalming process, in medicine and in purification rituals. Essential oils are highly concentrated substances extracted from flowers, leaves, stems, roots, seeds, barks, resins, or fruit rinds ('Yen *et al*., 2008). These oils are often used for their flavor

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and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Extraction of essential oils is one of the most time- and effort consuming processes. The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad spectrum activities. Since ancient times, plants and their derivatives, such as essential oils, have been used in folk medicine **(**'Virendra, 2007). Mint oil has a number of uses it can be used to add a minty flavor to drinks, add mint flavor to food such as chocolates and icing and used in a number of natural applications from deterring ants to clearing up chest congestion. Making your own takes a few weeks, but is cheap and easy to do ('Hayyan and Al-Taweil, 2014). Generally, Gram-negative bacteria are more resistant to essential oil than Gram-positive bacteria. Before examining the effects of essential oil on bacteria, should briefly consider the differing structures of the cell walls of Gram-positive and Gram-negative bacteria. Approximately 90%–95% of the cell wall of Gram-positive bacteria consists of peptidoglycan, to which other molecules, such asteicoic acid and proteins, are linked ('Felomina *et al*., 2013). Antioxidants are compounds found in virtually all plant foods. The primary job of antioxidants is to protect cells against the oxidative stress caused by free radicals, considered the primary cause of the aging process. Peppermint is an excellent source of antioxidants like vitamin C and beta-carotene, which is converted to vitamin A in the body. Peppermint is also a very good source of fiber, iron, calcium, magnesium, potassium, copper, vitamin B2 and omega 3 fats. The objective of this study is to extract and characterize peppermint essential oil and assess it as antioxidant and antibacterial.

MATERIAL AND METHODS

Fresh peppermint was collected from a local market in Wad Medani City, Gezira State, Sudan. Then, raw materials and bacterial strains: *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* were transported to the Laboratory of food Analysis, University of Gezira, Sudan. All other chemicals and reagents used of the highest grade commercially available.

Steam distillation:

Steam distillation is one of the most popular ways will be used to extract essential oils from plants, leaves and flowers. During steam distillation process, 100g of the plant raw material was being placed in the chamber of the essential oil distillation still, and steam passed through the plant matter. When the steam passed through the plant matter it picked up the oils and moved into another chamber where it is cooled and condensed. Then, essential oil was separated from the water and bottled for used (Hanbali *et al.,* 2005)**.**

Solvent extraction:

Sixty g of peppermint fresh and dry were mixed with 700 mL of ethyl alcohol using a shaker for 6 hour (70°C). The oil was then recovered by evaporating off the solvent using a rotary evaporator and the solvent was removed under a laboratory fume hood for 30 min at 37°C stream and was then stored in a refrigerator in dark bottle.

Soxhelet extraction:

100g of fresh and dry peppermint were weighted and put in soxhelet apparatus 300 mL of hexane solvent were added. The solvent was heated to reflux then vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor

cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly was filled with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber was almost full, the chamber was emptied by the siphon. The solvent was returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle was allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound was concentrated in the distillation flask. The advantage of this system was that instead of many portions of warm solvent being passed through the sample, just one batch of solvent was recycled. After extraction the solvent was removed, typically by means of a rotary evaporator, yielding the extracted oil. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Chemical composition of essential oil:

The volatile compounds were sampled with a SPAM–fiber and separated with a GC/MS. Volatile compounds were separated on a CP-Sil-8CB (Varian, Walnut Creek, CA, USA), fused silica capillary column (30 m length, 0.25 mm, id, and 0.25 μm film thicknesses) in a Varian model 3800 gas chromatograph. Volatile compounds identification were carried out by matching the compounds with the mass spectra of standard compounds found in the wily 130 K and national institute of standards and technology (NIST) 98 library of MS spectra based on their retention indices.

Determination of total phenolic (TPC) and flavonoid contents (TFC)

TPC was determined with the Folin-Ciocalteu method. Where, 100 mg oil equivalents·mL–1 were mixed with 0.5 ml of Folin-Ciocalteu reagent and 2 mL of methanol. The mixture was shaken for 1 min, and then 1.5 ml of 15% Na_2CO_3 was added and the mixture was shaken again. Finally, the solution was completed to 10 mL by adding distilled water. The mixture was incubated at 50°C for 20 min and centrifuged at 3,000 rpm for 10 min. The absorbance was recorded at 750 nm. TPC of the oil was calculated using Gallic acid as a standard (Liuand, Yao2007). The TFC of peppermint oil was determined according to the method reported by (Bayat and Borice, 2014) with slight modifications. Briefly, peppermint oil (2.5ml) was diluted with distilled water (10 ml), followed by the addition of 0.75 ml of 5% $NaNO_2$ solution. Then 10% $AlCl_3$ solution (0.75 mL) was added and the reaction mixture was allowed to stand at ambient temperature for 5 min. After incubation, 5 ml of 1 M NaOH were added and the volume of the mixture was made to 25 mL with distilled water. The mixture was shaken vigorously and the absorbance of the pink color developed was measured at 510 nm using spectrophotometer.

Peroxide value:

200 mL of cotton seed oil were placed in flask and put in water path 60^{o}C and added air. 0.50 ml and 1 mL peppermint oil added as antioxidant take recorded after 30 min, 1hour, 90 min, 2 hours and 150 min. About 1 mL of oil weighed into a clean dry boiling tube and 1g powder potassium iodide was added while still liquid. 20 mL of acetic acid–chloroform solution (at ratio2:1) were added. The tube was placed in the boiling water so that the liquid boils within 30

seconds. The content was poured into flask containing 20 mL of potassium iodide solution (5%). 25ml of distilled water was immediately added by graduated cylinder, and was titrated with 0.002 M sodium thiosulfate solution using starch indicator. The starting deep red orange color of the solution were the sign for titration with mixing slowly until the color lightness. When the blue gray color disappears (upper layer) the titration was then stopped the mL of titrant used two decimal places were accurately recorded.

DPPH radical scavenging activity:

The DPPH radical scavenging assay of peppermint oil was conducted using a method described by Bradly, (1992), with a slight modification. Extract concentrations of 0.25, 0.50, 0.75 and 1.00 mg oil equivalents/ mL were mixed with 3.5 mL of DPPH solution with absorbance at 520 nm. The mixtures were incubated for 30 min at 25°C. Then, the absorbance was recorded at 520 nm. The DPPH radical scavenging activity was calculated by the formula: DPPH scavenging activity % = (AC–AS /AC) ×100. Where: AS: absorbance of oil sample; AC: absorbance of control. The percentage of scavenging activity was plotted against sample concentration to obtain the IC_{50}, defined as the concentration of the sample required to cause 50% inhibition. Ascorbic acid was use as reference compounds.

Antibacterial activity:

The agar diffusion assay described by Smânia *et al*., (1999) was adopted. Briefly, bacterial strains were grown in Mueller-Hinton agar and broth. The strains were incubated at 36ºC for 18 h, and were diluted to a final concentration of approximately 106 CFU/ml. Each bacterial suspension was spread over the surface of Mueller-Hinton agar containing three wells of 7 mm diameter. The wells were filled with 0.25, 0.50, 0.75 and 1 ml of extract dissolved in the medium. The plates were incubated at 36ºC for 20 h. An antibiotic was used as a control.

Statistical analysis:

All experiments were conducted at least in triplicate and statistical analyses were performed using SPSS version 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to determine significant differences between means and Tukey's test were used to perform multiple comparisons between means. The significance level was defined as $p<0.05$.

RESULT AND DISCUSSION

Extraction process:

Peppermint leaves contains about 0.5 to 4 % volatile oil that is composed of 50-78% free menthol, monoterpene, menthofurane and traces of jasmine (Dew and Evans, 1984). Extraction of peppermint by different methods gave change in volume of oil and was noticed that the oil extracted from fresh peppermint more than from dry mint as shown in Table 1. Essential oil (EO)

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yield per plant on a dry weight basis was lower than that on a fresh weight basis for peppermint herb. Rabak, (1917) suggested that a reduction in EO yield may occur if plant was dried before extraction due to change that favor the formation of esters and the production of free acid in *M. piperita*.

Table 1: Quantity of essential oil (mL/100g) of peppermint extracted by different methods**.**

Extraction method	Fresh mint oil (mL/100g)	Dry mint oil (mL/100g)
Soxhlet	1.5 ± 0.12^{a}	1.2 ± 0.12^{a}
Solvent	1.2 ± 0.13^{a}	$1.0\pm0.16_{a}$
Steam distillation	1.1 ± 0.09^{a}	0.9 ± 0.14^{a}

Means ± standard deviation values having different superscript letter(s) in each row differ significantly ($p<0.005$).

Chemical composition of peppermint essential oil:

The chemical composition of fresh and dry sample was determined by using gas chromatography and the results were reported. As shown in Table 2 and 3, Twenty-six components of volatile compounds were identified in the essential oil isolated from peppermint fresh and dry sample. The results showed that α-Phellandrene acid, β-Bourbonene were the highest content (8.176 and 6.14 respectively), followed by Trans-Carveol, Methyl caproate (C6) 5.884 and 4.541, respectively in fresh sample. In Table 3, dry sample α-Humulene, Bornylacetate were the highest content (149, 5.887) followed by Cis-Limonene oxide, 4, Methyloleate (C 18:1) (5.143, 8.184), respectively.

Table 2: The chemical composition of fresh peppermint by GC.

No	Constituent	Retention time	Area
1	1,Hexane	4.281	107561924
2	2, Methylecaproate	4.404	11719
3	2, Methylecaproate (C6)	4.541	66683
4	α-Terpinene	4.681	5051
5	Trans-Sabinene hydrate	4.801	1255
6	Cis-Limonene oxide	4.983	2180
7	Trans-Limonene oxide	5.080	1097
8	Cis-p-Mentha-2.8-dien-1-ol	5.376	3977
9	Trans-p-Mentha-2.8-dien-1-ol	5.544	9397
10	Cis-Carveo	5.812	6717
11	Trans-Carveol	5.884	34050
12	Bornyl acetate	6.033	2884
13	β-Bourbonene	6.140	72946
14	α-Humulene	6.520	1256
15	Santene	6.670	1426

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16	Camphene	7.014	1898
17	Myrcene	7.087	3443
18	cis-3-Hexenyl acetate	7.730	9623
19	α-Phellandrene	8.176	259459
20	Linalool	8.613	4790
21	Nonanal	9.002	1410
22	trans-β-Caryophyllene	9.482	1114
23	Germacrene-D	10.582	2313
24	3, Methylepalmitoleate	10.898	6129
25	3, Methylepalmitoleate	14.568	1883
26	4, Methyleoleate (C 18:1)	15.721	10584

Table 3: The chemical composition of dry peppermint by gas chromatography

No.	Constituent	Retention time	Area
1	1,Hexane	4.276	109643237
2	2, Methylecaproate	4.431	47305
3	2, Methylecaproate (C6)	4.489	12282
4	3, Methylecaproate	4.538	19435
5	Trans-Sabinene hydrate	4.973	246820
6	Cis-Limonene oxide	5.143	390574
7	Trans-Limonene oxide	5.330	322749
8	Cis-p-Mentha-2.8-dien-1-ol	5.414	96728
9	Trans-p-Mentha-2.8-dien-1-ol	5.545	403518
10	Cis-Carveo	5.683	201447
11	Trans-Carveol	5.824	144494
12	Bornyl acetate	5.887	280072
13	β-Bourbonene	6.050	143714
14	α-Humulene	6.149	358481
15	Santene	6.296	66140
16	Camphene	6.380	154210
17	Myrcene	6.548	83188
18	Cis-3-Hexenyl acetate	6.684	141124
19	α-Phellandrene	7.015	10690
20	Linalool	7.293	4317
21	Nonanal	7.422	5616
22	Trans-β-Caryophyllene	7.623	7849
23	Germacrene-D	7.763	1982
24	3, Methylepalmitoleate	7.926	1519

25	3, Methylepalmitoleate	8.093	23611
26	4, Methyleoleate (C 18:1)	8.184	64552

Total phenolic compounds and total flavonoid content:

As shown in Table 4, the TPC value of fresh sample was 14.00 ± 0.12 mg/kg while the TPC values with dry samples 8.80 ± 0.09 mg/kg and all these values were significantly different (P< 0.05). The previous study of Zaidy and Dahiya, (2015) indicated that TPC of other kind of *Menthapiperita* had the highest contents of total phenolic (12.63± 0.878). In this study, the total phenolic content was determined in comparison with standard Gallic acid and the results expressed in terms of mg GAE/g dry extract. Phenolic compounds are responsible for the antioxidant activity of plant materials and they are highly effective radical scavengers (Pan *et al*., 2008). The antioxidant activities of phenolics are due to their redox properties. The phenol moiety helps them to work as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chua *et al*., 2008). Considering the heterogeneity of natural phenols and the possibility of interference from other readily oxidized substances, several methods including Folin-Ciocalteu, permanganate titration, colorimetric with iron salts, and ultraviolet absorbance have been used for total phenol determination. However, in most direct comparisons, Folin-Ciocalteu method has been found preferable and is being used by many researchers. In this method, phenols form a blue colored phosphomolybdic-phosphotungstic-phenol complex in alkaline solution (Singleton *et al*., 1999).

Table 4: TPC and TFC of peppermint extract (mg/kg).

Samples	TPC (mg/kg)	TFC (mg/kg)
Fresh mint sample	14.00± 0.12^{a}	8.10±0.09^{a}
Dry mint sample	8.80± 0.09^{b}	5.00±0.07^{b}

Mean values ± standard deviation having different superscript letter(s) in each column differ significantly (p<0.005).

TFC of the peppermint were determined in comparison with rutin as a standard and the results are shown as mg /kg dry extract (Table 4). The TFC value of sample extracted by steam distillation method was 8.1±0.09 mg/kg, 5.0±0.07 mg/kg for fresh and dry samples respectively. All these values were significantly different (P<0.05) from traditional treatment value.

Peroxide value:

As illustrated in Table 5, the peroxide values of control were increased and the measure was. for fresh and dry samples respectively. This is due to the formation of hydroperoxides of unsaturated fatty acids that were obtained as a result of lipid oxidation (Choe and Min, 2006). Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. Autoxidation is a free radical reaction involving oxygen that leads to deterioration of fats and oil which form off-flavor and off odors. Peroxide value concentration of peroxide in an oil and fat, is useful for assessing the extent to which spoilage has advanced (Cheuibini *et al* .,1999). Auto-oxidation, where peroxide is the main product that gives rise to objectionable flavor in food products, proceeds through the free radical chain reaction, where it attacks on the double bond at room temperatures. Photo-oxidation is a much faster reaction that involves attack at double bond (Lawson, 1997). Rancidity of food items can be the result of auto and photo-oxidation, which are

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natural oxidation and chemical degradation processes of oils, where fatty acid esters of oils are converted into free fatty acids giving a smell observed in many oils (Anwar *et al*., 2003). Oils that are more unsaturated are oxidized more quickly than less unsaturated oils (Parker *et al*., 2003).

Table 5: Peroxide value of peppermint oil (meq O_2·kg–1oil)

Dry peppermint oil sample (meq O_2·kg–1oil).			
Time (min)	**Blank**	**0.5 mL**	**1.0 Ml**
30	3.00	5.60	4.46
60	6.96	6.67	5.83
90	10.13	8.30	7.23
120	11.06	9.10	8.94
150	16.43	10.90	9.52
Fresh peppermint oil sample (meq O_2·kg–1oil).			
30	5.00	4.90	4.12
60	6.96	6.24	5.33
90	10.13	9.89	8.65
120	9.93	8.93	8.34
150	16.43	12.67	11.99

DPPH assay:

In the determination of antioxidant activity, the free- radical scavenging activity of *Menthapiperita* essential oils was evaluated using the DPPH method is presented in Figure 1A and 1B for dry and fresh peppermint oil. The absorbance decreases because of a color change from purple to yellow .DPPH assay is based on the reduction of DPPH radical in the presence of a hydrogen donating antioxidant, and it has been extensively used for screening antioxidant activity of natural compounds. It is sensitive enough to detect active ingredients at low concentrations and can accommodate many samples in a short period of time (Chua *et al*., 2008). DPPH is a stable free radical, which dissolves in ethanol or methanol.

The results indicated that the essential oils of *Menthapiperita* tested showed good antioxidant capacities compared with vitamin C as standard antioxidant compound. The results of fresh peppermint oil indicate that the radical scavenging activity inhibition of the essential oil was the highest (62.41±0.12%) at the concentration of 1mL and (60.7±0.08%) as highest for dry sample at same concentration 1 mL. It was noticed that the scavenging activities of the essential oils were increased with the increase of the essential oils concentrations. All the tested samples showed lower DPPH radical scavenging activity when compared with the standard. It is clear from the data that the concentration of 1ml of *Menthapiperita* essential oil gave a percentage inhibition of DPPH (62.41 and 60.7 %) for fresh and dry oil respectively while same concentration of 1 mL for vitamin C was 77.3±1.09% inhibition. According to the results recorded, the DPPH radical scavenging activity of fresh and dry peppermint oil of the IC_{50} were 0.651 and 0.681mg/mL respectively which were higher than the control with an IC_{50} of 0.161 mg/mL. A previous study by (Hussain *et al*., (2011) indicated that the radical scavenging activity of the essential oil from *Menthapiperita* was the highest (81.09±1.21%) at concentration of 150µg/mL. The result of the recent investigation was comparable with previous study made by Tenore *et al*., (2011). De Oliveira *et al*., (2012) reported that the DPPH radical scavenging activity of thymol rich essential oil from *Saturejamontana*. The radical scavenging activity of its edible oil could be credited to the presence

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of its main total phenolic contents, especially thymol and their recognized impact on oil lipid oxidation (Guimaraes *et al*., 2010).

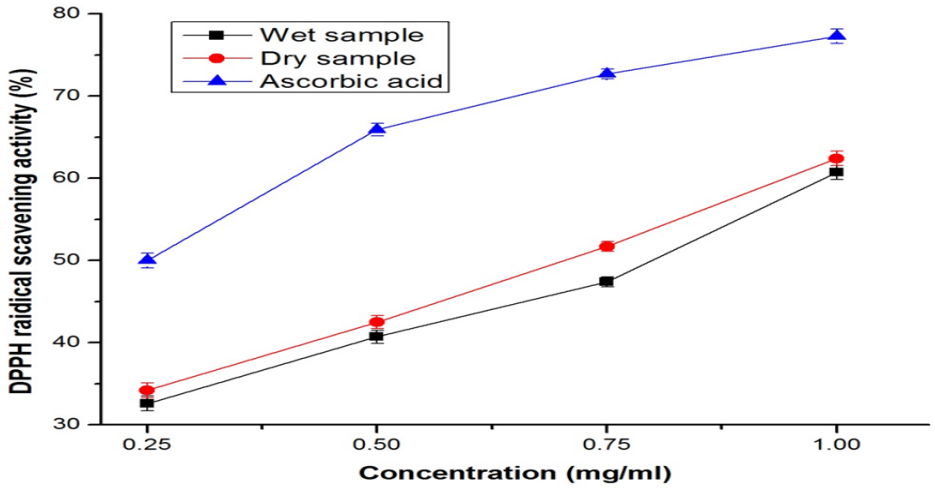

Figure 1: DPPH radical scavenging activity (means ± standard deviation) of A: fresh sample and B: dry sample with a reference (Ascorbic acid).

When an antioxidant scavenges the free radical by hydrogen donation, the purple color of DPPH in assay solution turns to yellow, which can be monitored spectrophotometrically at 517 nm (Yen *et al.*, 2008). Antioxidants effect on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them.

Antibacterial activity:

Peppermint extracts are bacteriostatic against *Streptococcus pyrogens ,Streptococcus aureus, Streptococcus pyrogens, Serratia marcescens, E. coli* and *Mycobacterium avium* (Gotshall, *et al*.,1949). For steam extraction of wet the results illustrated in Table 6, showed that the essential oil had the highest antibacterial activity against *Salmonella, Staphylococcus aureus* with a mean zone of inhibition of 18.5 and 17.5 mm respectively. The lowest mean zone of inhibition was as 16.5 and 11.5 mm obtained by *E. coli, Klepsilla pneumonia,* respectively, using a volume of 100µL, while 75µL gave 16.5 for *Staph* and *Salmonella* as highest zone and 11.5 and 10for *E. coli* and *Klepsilla pneumonia,* respectively.

Table 6: Antibacterial activity of essential oil extracted by steam and ethanolic extraction .

Volume	Fresh steam		Dry steam	
	100µL/disk	75 µL/disk	100µL/disk	75 µL/disk
Organism	Diameter zone (mm)			

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Staphylococcus aureus	17.5±0.70[b]	16.5±2.12[a]	11.5±0.70[b]	11±0.01[b]
Salmonella	18.5±0.70[a]	16.5±0.70[a]	13±1.41[a]	12±1.41[a]
Klebsilla pneumonia	12.0±0.01[d]	10±0.70[c]	0.0	0.0
E. coli	16.5±2.12[c]	11.5±070[b]	11±0.02[c]	8.5±0.70[c]
Antibacterial activity of essential oil extracted by ethanol extraction				
Staphylococcus aureus	19±1.41[a]	17±1.41[a]	15±1.41[a]	12.0±1.41[a]
Salmonella	16.5±2.12[b]	16 ±1.41[ab]	13.5±2.10[b]	12.5±0.70[a]
Klepsilla pneumonia	12.5±0.70[c]	10±0.70[c]	9.5±0.70[c]	8.5±0.70[b]
E.coli	16±1.41[b]	13.5±1.4[b]	15±1.41[a]	11±1.414[ab]

Mean values ± standard deviation having different superscript letter(s) in each column differ significantly (p<0.005).

The results showed that the essential oil of peppermint oil had antibacterial activity against all the bacterial strains tested .Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram-negative bacteria Diaz *et al*., (1989). The dry steam extract gave highest values of inhibition zone for *Salmonella* and *Staph*. as 13.5 and 11mm, respectively at volume 100 µL, lowest inhibitionzone of this extract shown by *E. coli* and *Klepsilla* as11 and 0.0 mm respectively while 75 µL showed highest values as 12 and11 mm for *salmonella* and *Staph* and in lowest 8.5 and 0 mm for *E.coli* and *Klepsilla*, respectively. For methanolic extraction of fresh the result showed in Table 6, the peppermint oil had highest antibacterial activity against *Staphylococcus aureus* and *Salmonella* with mean zone of inhibition19and 16.5mm respectively while the lowest mean zone of inhibition was as 16.0 and 12.5 mm obtained by *E.coli*, *Klepsilla pneumonia*, respectively using a volume of 100µL.while 75µL gave 17and 16 for *Staph* and *Salmonella* as highest zone and 13.5 and 10for *E.coli* and *Klepsilla pneumonia*, respectively. The dry methanol extract gave highest values of inhibition zone for *Staph* and *Salmonella* as 15 and 13.5 mm, respectively, at volume 100 µL, lowest inhibition zone of this extract shown by *E. coli* and *Klepsilla* as 15 and 9.5mm while 75 µL showed highest values as 12.5 and 12 mm for Salmonella and Staph and in lowest 11 and 8.5 for *E. coli* and *Klepsilla*, respectively. Zaidi and Dahia, (2015),the results showed in Table 6, of methanolic extraction dry and wet samples of Sudanese peppermint as15,16mm of dry and wet respectively higher as bacterial activity for *E. coli* and *Salmonella spp* as 13.5 and 16.5, while, lowest value no inhibition zone for *E. coli* and *Salmonella*. According to previous study also revealed the antibacterial activity of peppermint against *S.aureus*, *E.coli* and *Klebsilla spp* (Jeyakumar *et al*., 2011; Sujana et *al*., 2013), however, Sartoratto *et al*., (2004) reported that peppermint oil was found to be strongly effective against *Salmonella* species. The results are in fair correlation with the studies in which peppermint oil of antibacterial activities against Gram–ve and Gram+ve bacteria (Singh *et al*, 2011). Activity of inhibition was expressed in terms of the diameter of the inhibition zone>15 mm: sensitive, 14-15 Intermediate, <14 mm: resistance.

CONCLUSION

The Results revealed that the pepper mint extracts can be described as primary antioxidants, which suggests they can play beneficial role of preventing the initiation and propagation of free

radical-mediated chain reactions and consequently, prevent the oxidative damage on skin and avoid premature skin aging. The results also indicated that the mint essential oil can be used as a potential source of natural antimicrobial compound and the presence of phenolic and alkaloids possessing strong antioxidant potential. Hence it is essential to explore further by the identification of biologically active compounds, characterization and purification of the crude extracts. Based on the result obtained from the evaluation of the phenolic and flavonoid content and the DPPH values of peppermint as medicinal herbs we conclude that it is important to educate consumer on the benefit consumption, choosing those that have the highest antioxidant capacity in order to promote healthy diet.

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EDITORIAL

استخلاص الزيت الطيار من نبات النعناع (*Menthapiperita*) ودراسة خصائصه وتقييمه كمضاد أكسدة ومضاد بكتريا

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الملخص

الهدف الأساسي من هذا البحث استخلاص الزيت الطيار من النعناع ودراسة خواصه كمضاد اكسده ومضاد بكتريا .تمت دراسة عملية الاستخلاص، التركيب الكيميائي، محتوى الفينولية الكلية، محتوي الفلافونويد الكلي، مضادات الأكسدة والنشاط المضاد للبكتيريا. تم استخلاص الزيت الطيار بثلاثة طرق هي تقطير البخار و المذيب والسوكسليت. أشارت النتائج إلى أن الزيت الأعلى هو المستخلص بواسطة السوكسليت0.12 ± 1.5 و1.2 ±0.12 مل/100جم والنتيجة الأقل سجلت عن طريق التقطير0.09±1.1و0.14±0.9 مل/100جم من العينة الطازجة والجافة، على التوالي. تم تحديد التركيب الكيميائي للعينة الطازجة والجافة باستخدام كروماتوغرافيا الغاز، حيث أظهرت النتائج وجود 26 مكوناً من المركبات المتطايرة في الزيت الأساسي المعزول من النعناع للعينة الرطبة والجافة. وقد تم تقييم نشاط مضادات البكتيريا لزيوت النعناع الطيارة بواسطة طريقة Muller Hinton agar بشكل جيد ضد أنواع البكتيريا المختارة حيث أظهرت زيوت النعناع الطيارة نشاطاً أعلى ضد كلاً من *Staphylococcus aureus* و*Salmonella* 1.41±19 و2.12± 16.5ملم على التوالي، للعينة الرطبة والنشاط الأقل ضد *Escherichia coli* و *Klebsilla* 0.70 ±8.5 *pneumonia*ملم و zero، على التوالي، للعينة الجافة. تم تقييم المكونات الفينولية باستخدام طريقة Foline-Ciocaltue . كانت المحتويات الكلية الفينولية للعينة الطازجة0.12±14.00 مج/كج، بينما كانت للعينة الجافة 0.09±8.8. مج/ كج. تم تحديد المركبات الفلافونوديه الكلية من النعناع بالمقارنة مع rutin فكانت 0.09±8.1 و0.07±5.0 ملجم/كجم، للعينة الطازجة والجافة، على التوالي. أظهرت النتائج انخفاض في قيمة البيروكسيد لدى الازمان 30 ,60 ,120,90و150دقيقة مقارنة مع العينة بدون الزيت الطيار للنعناع الرطب والجاف. تم تحديد نشاط الـ DPPH من خلال المعالجة مع تركيزات مختلفة من الزيوت الأساسية وفيتامين ج كمركب قياسي مضاد للأكسدة. تم تسجيل نسب تثبيط الـ DPPHوكان تركيز التثبيط عند50% نشاط 0.09 IC_{50}±0.651 مج/مل، للعينة الرطبة،0.06±0.683 مج/مل للعينة الجافة 0.07±0.0161مج/مل للمركب القياسي. توصي هذه الدراسة بأجراء التحليل الحسي للمنتجات الغذائية المحتوية علي زيوت النعناع الطيارة لتقييم مدى القبول وتأثير النعناع علي العمر الافتراضي لها .