

Original Article

Effect of essential oil of traditional two Saudi mint types and its possible role in cardiovascular and throat health

Ahmed M Ahmed, Hani A Ozbak, Hassan A Hemeg

Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, Taibah University, Al-Madinah Al-Munawarah, Kingdom of Saudi Arabia

Received March 4, 2015; Accepted April 30, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Essential Oil (EO) of two *Mentha* species (popularly used in Saudi Arabia), *Mentha Longifolia* (ML), and *Mentha pulegium* (MP) was isolated and subjected to inhibit LDL oxidation in 22 hypercholesterolemic samples collected from 22 subjects, and inhibited of 5 bacterial pathogen *in vitro*. LDL was isolated by ultracentrifugation and enhanced to oxidation with CuSO_4 and EO was added to prevent this oxidation, free radical scavenging activity was measured (decrease 50% DPPH radicals). EO content of phenolic and flavonoid was estimated. Five bacterial strains infect human throat was tested against EO of ML and MP in diffusion agar method. EO of the two herbals possess highly significant results, success to inhibit LDL oxidation ($P < 0.001$ for both herbal than non addition against lipid peroxidase, $P < 0.001$ ML better than MP $P < 0.001$), decreased DPPH free radicals ($P < 0.001$ for both than control, $P < 0.001$ for ML than MP), and possess strong antioxidant activity for ML than MP (polyphenol and flavonoids contents was higher in EO of ML than MP, $P < 0.001$). EO of ML possess strong antioxidant and antibacterial activity than MP, these attributed to its high flavonoid contents enable it to be a good for cardiovascular and throat health. The study supported the traditional uses of ML.

Keywords: *Mentha logifolia*, *mentha pulegium*, LDL oxidation, essential oil, antioxidant activity, antibacterial activity

Introduction

Oxidative modification of Low Density Lipoprotein (OxLDL) plays strongest role in the atherosclerosis pathogenesis, Oxidized LDL can engage monocytes to transform into macrophages in the arterial wall, the precursors of foam cells, leading to the early atherosclerotic lesions formations [1]. Highly efficient defense mechanisms induced by plasma antioxidants is well protected circulated LDL against active oxidation, but if this oxidative stress more than antioxidant defense capacity, then LDL may be oxidized [2]. Inhibition of LDL oxidation is a target for early reduction the incidence of atherosclerosis. Antioxidants, which can actively inhibit this oxidation and may protect against atherosclerosis due to the early prevention of atherogenesis [3].

The wild *Mentha* belong to Lamiaceae family which includes about 25-30 species that grow extensively worldwide [4]. *Mentha longifolia*

(ML), Lamiaceae family grows widely in Mediterranean regions, Europe, Australia, and North Africa [5]. The most common species grown in Saudi Arabia is ML which is locally known as Al-Madinah mint, (also local name called Hassawi and Habaq). Another species is MP, which locally known as (Mugrabi), both species used in herbal teas, alone or as spice mixtures for many foods to offer aroma and flavor. In addition, *Mentha* species had been used for treatment of many diseases such as throat infection, bronchitis, nausea, and ulcerative colitis [6]. Furthermore, it is well-documented the essential oils of *Mentha* species have strong antibacterial properties against much bacterial growth [7]. Very few studies done about antioxidant and antimicrobial activity of *Mentha* species in Saudi Arabia, but no published data concern about inhibition of LDL oxidation by *Mentha* types so, this work undertaken to evaluate the ability of essential oil of ML and MP for inhibition of LDL oxidation and inhibit bacterial growth *in vitro*.

Subjects and methods

Clinical samples

Twenty two Saudi hyperlipidemic subjects randomly selected from different hospitals in (Al-Madinah Al-Munawarah, Saudi Arabia); baseline serum total cholesterol and LDL-cholesterol concentrations were (mean \pm SD) (5.60 ± 0.15 and 4.00 ± 0.13 mmol/L respectively). All subjects were look healthy and not take any medications at least for last month before sample collection. Subjects with acute or chronic illness had been excluded. Blood samples were collected from all subjects after overnight fasting (9-12 hour). Ethical considerations: All participants were signed an informed consent before sampling.

Methods

Plant materials

ML and MP were purchased from the famous local market in Al-Madinah (mint market), and then it dried in direct sun light and open environment for 5 days.

Essential oil (EO) isolation procedure

50 g of ML and MP powder were put in hydro distillation (3 hour) in 250 ml of DW by Clevenger-type apparatus (European Pharmacopoeia procedure) [8]. Then EO were isolated and stored at -20°C until analysis in brown container.

LDL isolation

EDTA blood centrifuged for 10 min, 200 RBM at 4°C , and then LDL was separated with ultra-centrifugation 400,000 g for at least 40 min and 4°C with Beckman Instruments, Glenrothes, UK ultra-centrifuge. Concentrated LDL protein was determined with kits of Micro BCA Protein (Pierce Laboratories, Rockford, IL). The samples of LDL were diluted using PBS to give a final concentration of $70 \mu\text{g}/\text{mL}$ LDL protein prior to oxidation experiments.

Kinetic oxidation of LDL

LDL oxidation was measuring according to the method described by Esterbauer, [9] $100 \mu\text{g}/\text{ml}$ of LDL were placed and mixed with or without $20 \mu\text{l}$ of EO of ML and MP separately at

37°C for 15 min. $10 \mu\text{l}$ of 0.167 mM copper sulphate (CuSO_4) freshly prepared was used to enhancement the oxidation process, the kinetics of oxidation were calculating the absorbance changing at 10 min intervals for a total period of 200 min at 234 nm with Hitachi U2000 spectrophotometer. The Lag time was obtained from the intercepts of the twice tangents pulled to this phase (lag) and another phase (propagation phase). The oxidation rate was measured from propagation phase slope. Conjugated diene (CD) concentration measured with a molar extinction coefficient of $2.95 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Maximum CD concentration was calculated as difference between CD (zero time) and CD (at maximum diene). The tests in all tubes were done in triplicate then mean and SD was calculated.

Free radical-scavenging activity

Estimated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (SIGMA-ALDRICH, Chemistry and Materials Company, USA). Aliquot amounts of each herbal EO were added to 2 ml of DPPH solution (0.1 mM DPPH dissolved in ethanol), then wait for 30 minutes in 37°C , and then determined in 516 nm wavelength using Hitachi U2000 spectrophotometer. Each volume of herbals required to lead to decrease 50% DPPH radicals in the absorbance at 516 nm proportional to the control was then recorded. Free radicals-scavenging activity recorded as inhibition according to (%) scavenging activity of DPPH radical: $1\% = \frac{[A_0 - A_s]}{A_0} \times 100$. A_0 : blank absorbance. A_s : sample absorbance. The samples were analyzed in triplicate then mean and SD was calculated.

Determination of total phenolic content

The method described by Singleton et al, [10]. With Folin-Ciocalteu reagent (FCR) using spectrophotometer for quantification. Briefly, $125 \mu\text{l}$ of FCR (10%) and $125 \mu\text{l}$ of sample were mixed with each other and incubated for 6 min, and then $125 \mu\text{l}$ of 7% Na_2CO_3 for 30 min. then the phenolic compounds and phosphomolybdic acid were reacted and produce a blue-colored complex its absorbance recorded spectrophotometrically with wavelength 760 nm. With standard calibration curve using gallic acid as standard, total phenolic contents was explained as Gallic Acid Equivalents (GAE) mg/g dry weight (DW).

Essential oil of mint and human health

Estimation of flavonoid content

the method described by Eberhardt et al. [11]. 250 μ l of EO of herbalmixed with 780 μ l of distilled water, followed by addition of 60 μ l of 5% NaNO_2 solution and incubated for 6 min, so 60 μ l of 10% AlCl_3 solution were mixed, again incubated for 6 min at room temperature, then 400 μ l of 1 M NaOH was mixed well. Then immediately the absorbance was recorded spectrophotometrically at 510 nm. With standard calibration curve using Runtin as a standard, flavonoid contents was expressed as Runtin Equivalents (RE) mg/g DW.

Estimation of end product of LDL oxidation

[estimation of: Malonaldehyde (MDA), Lipid hydroperoxide (LHP), and Protein carbonyl (PC)]. The oxidized LDL enhanced by CuSO_4 was tested with EO of ML and MP till 24 h and without addition of EO (control). After incubation time, 10 mM of EDTA was mixed to stop LDL oxidation, and all samples was further incubated to determination of MDA, LHP, and PC.

MDA determination

100 μ l of aliquot serum was added to 900 μ l of thiobarbituric acid reactive substances (TBARS) (0.37% TBA, 15% TCA in 0.25 N HCl) placed in water bath with 100°C, for 30 min. And after 40 min incubation at room temperature the mixture was centrifuged at 3000 RPM-10 min at 532 nm with Hitachi U2000 spectrophotometer the absorbance was recorded. With a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$, MDA was calculated [12].

LHP determination

100 μ l of aliquot serum was added to 1000 μ l of Fox reagent (0.25 mM Ammonium sulfate, 0.1 mM xylenol orange, 25 mM H_2SO_4 , and 4 mM BHT in 90% (v/v) HPLC-grade methanol). After incubation in water bath at 37°C -30 min, with 560 nm spectrophotometer the absorbance was measured. With a molar extinction coefficient of $4.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, LHP was calculated (13).

For PC determination

100 μ l of aliquot serum was added to 200 μ l of DNPH solution (with 2 M HCl). After incubation in room temperature for 70 min, 600 μ l of

denaturing buffer (150 mM sodium phosphate buffer containing 3% SDS) was mixed thoroughly. 1800 μ l of each of Ethanol and heptanes were added later; the all contents then were centrifuged for precipitation of protein after well mixed. With 1500 μ l with each of ethyl acetate/ethanol (1:1, v/v) the protein was washed 3 times and dissolved in 1000 μ l with each of denaturing buffer. At 360 nm spectrophotometer the absorption was recorded and with a molar extinction coefficient of $22.000 \text{ M}^{-1} \text{ cm}^{-1}$, PC was calculated [14].

Microbiological assay

Five bacterial strains generally infect the human throat were tested. These strains are: Gram (+ve) species: Streptococcus pyogenes (Group A streptococci). (ATCC 19615), Streptococcus pneumonia (ATCC 6303), and Corynebacterium diphtheria (ATCC 296). Gram (-ve) species: Haemophilus influenza (ATCC 33533), and Neisseria gonorrhoeae (ATCC 19424). The strains of bacteria were challenged and tested against EO of ML and MP. The diffusion on agar method in Muller Hinton medium was used. 0.2 ml of each type of bacteria was taken and put into 20 ml of sterile nutritive broth and incubated at 37°C for 3-5 hours to standardize the cultures. Then each type of bacteria was cultured in the medium followed by 0.2 ml of ML and MP EO then allowed for 1 day at 37°C. The test was repeated three times, mean and SD was calculated. A reference antibacterial antibiotic (Amoxicillin) was used.

Minimum inhibitory concentration (MIC)

The method recommended by the Standard Institute of Clinical Laboratory [15]. 15 μ g/ml of EO of ML and MP was dissolved with 20% of dimethyl sulfoxide (DMSO; aqueous solution. In 96 well plates the mixture was added (100 μ l in every well), and then 100 μ l of Mueller Hinton broth (MHB) was mixed in all well. 5 μ l (1.5×10^8) of colony forming unit/ml of microorganisms was mixed in all well. For positive control Amoxicillin antibiotic was applied in one well. Then incubated for 24 h aerobically at 36°C. To detect active metabolism of bacteria; 10 μ l of 2, 3, 5-triphenyl-tetrazolium chloride (methanol solution 5 mg/ml) was added. MIC was known as lowest EO concentration that appears to inhibit bacterial growth spot detected with TTC

Table 1. Antioxidant activity of ML and MP against DPPH, lag time, oxidation rate, maximum CD, total phenol and flavonoid contents

	DPPH test µg/ml	Lag time (min)	Oxidation ratio (nmol/min/mg LDL protein)	Maximum CD (nmol/mg LDL protein)	Total phenol (mg GAE/g DW)	Flavonoid content (mg RE/g DW)
	Mean ± SD	Mean ± SD (Fold)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	-	25.3±2.9 (1.0)	10.1±1.4	397.1±20.3	-	-
ML	7.7±1.1#	208.4±27.2 (8.2)*,#	4.6±1.1*,#	287.3±16.7*,#	182.2±18.6#	26.3±2.1#
MP	10.2±1.7	138.4±11.8 (5.5)*	6.6±1.3*	316.2±19.1*	153.7±13.1	17.1±1.9

Abbreviations: DPPH: 1, 1-diphenyl-2-picrylhydrazyl. GAE: Gallic Acid Equivalent. RE: Runtin Equivalent. DW: Dry Weight. SD: Standard Deviation. µl: micro liter. *: Significant compared to control level ($P<0.001$). #: Significant compared to MP level ($P<0.001$).

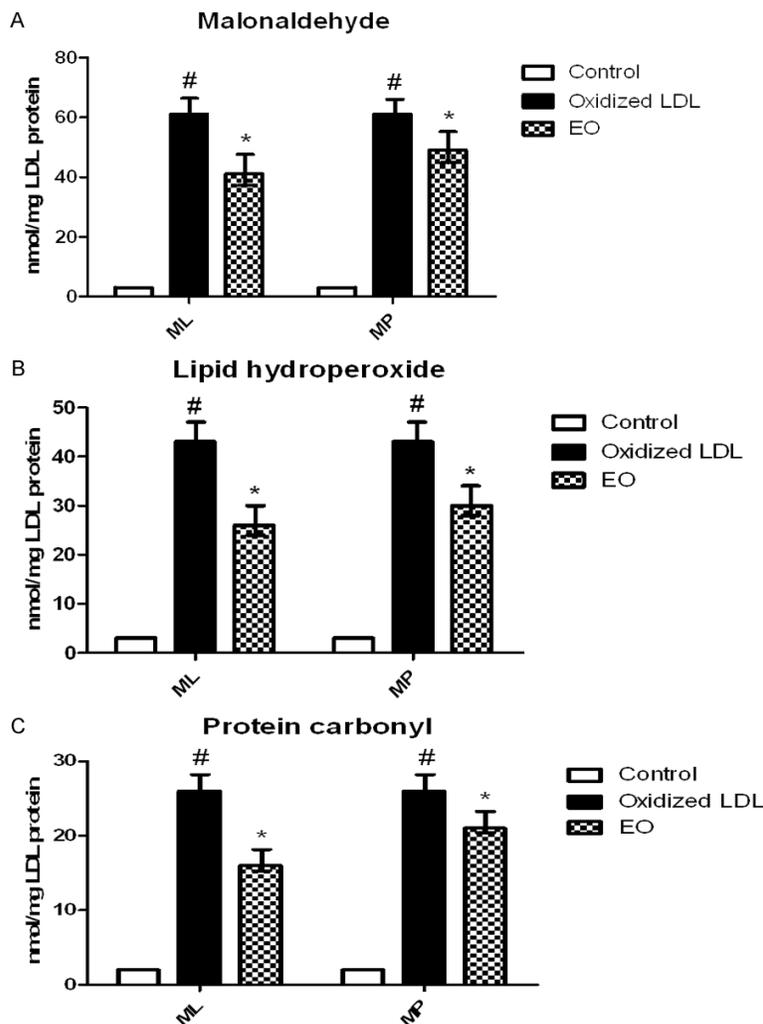


Figure 1. Effect of ML and MP EO on MDA, LHP and PC constituents, in CuSO_4 enhancement LDL oxidation. A-C: #: significant compared to control ($P<0.0001$). *: Significant compared to control ($P<0.001$). A: MDA: $P<0.001$ (ML vs. MP). B: LHP: $P<0.001$ (ML vs. MP). C: PC: $P<0.001$ (ML vs. MP).

solution. This test repeated three times and mean and SD was calculated.

Minimum bactericidal concentration (MBC)

10 µl of the broth (aliquot broth) were collected from each well and placed in MHB for 24 h at 37°C. MBC appear as necessary concentration to kill 99% or more (complete) of the inoculums. Also the test repeated three times and mean and SD was calculated.

Statistical analysis

Done by using SPSS version 19 (SPSS, Chicago, IL), all experiment were calculated in triplicate and the mean and standard deviation (SD) were applied using unpaired t-test for comparison of two variables and ANOVA for three, with 95% confidence interval, (P value of ≤ 0.05) consider significant. GraphPad prism software version 5 was used to design the figure.

Results

Antioxidant activity of ML and MP concerning DPPH, oxidation of LDL, lag time, oxidation rate, maximum CD, total phenol and flavonoid contents in this study was delivered in **Table 1**. For scavenging of 50% of the radicals of DPPH

(Inhibitory Concentration IC_{50}), 7.7±1.1 and 10.2±1.7 µg/ml of EO of ML and MP respec-

Table 2. Antibacterial activity of EO of ML and MP showing inhibition zone diameter in mm

Bacterial species	Inhibition zone diameter in mm of (Mean and SD) surround disc					
	ML EO (10 µl)		MP EO (10 µl)		Amoxicillin (10 µg/disc)	
	Mean	SD	Mean	SD	Mean	SD
<i>Streptococcus pyogenes</i> (Group A)	13.1	0.32	11.2	0.31	27.8	1.9
<i>Streptococcus pneumoniae</i>	14.9	0.38	11.6	0.41	28.7	2.7
<i>Corynebacterium diphtheria</i>	15.2	0.29	11.8	0.28	29.9	2.4
<i>Haemophilus influenzae</i>	9.9	0.4	7.9	0.16	26.1	1.8
<i>Neisseria gonorrhoeae</i>	10.5	0.83	9.2	0.22	26.4	1.7

Abbreviations: mm: millimeter. SD: Standard Deviation. ML EO: Mentha longifolia Essential Oil. MP: Mentha pulegium Essential Oil.

tively were required. Inhibitory effect of ML and MP against LDL oxidation mediated by CuSO_4 compared with control as follow; ML was highly effective against oxidation rate (4.6 nmol/min/mg LDL proteins respectively) and increased lag time 8.2 times than control (no addition of EO). MP inhibitory effect against rate of oxidation was less than the effect of ML (6.6 nmol/min/mg LDL protein) and lag time increased 5.5 times than control. Maximum CD formation was significantly reduced in the presence of EO of ML and MP (from 397.1 ± 20.3 nmol/mg LDL protein) in control without herbals addition to 287.3 ± 16.7 in presence of ML EO, and 316.2 ± 19.1 of MP. Total polyphenol of ML expressed as (mean \pm SD) (182.2 ± 18.6 gm GAE/g DW), higher than that of MP (153.7 ± 13.1 gm GAE/g DW). Also the flavonoid content of ML expressed as (mean \pm SD) (26.3 ± 2.1 gm RE/g DW), more than MP levels (highly significant $P < 0.001$), (17.1 ± 1.9 gm RE/g DW).

Mediation for LDL oxidation induced by CuSO_4 was recorded by determination the concentration of MDA, LHP and PC. The concentration was increased in the presence of oxidize LDL mediated by CuSO_4 without addition of EOs of the two herbal types, and decreased in control (LDL without addition of CuSO_4). When EO of ML and MP was mixed, it was significantly minimizing the concentration of MDA, LHP and PC; this was showed in **Figure 1**.

Antibacterial activity of EO of ML and MP was compared with Amoxicillin antibiotic (standard antibiotic) as inhibition zones in mm in the media surround disc. The EO of ML has essential sensitivity as antibacterial against the 5 species of bacteria rather than MP which pos-

sess weak activity, presented in **Table 2**.

MIC and MBC values compared with Amoxicillin, present as diameter of inhibition zones, the results was expressed in **Table 3**, showed that EO of ML was active against the 5 bacterial strains than MP.

Discussion

It's well documented that oxidized LDL play a major role in the modification of

atherosclerosis; the inhibition should be reducing the progression [16]. There were many studies show that LDL oxidation can be inhibited by an appropriate antioxidants; many natural antioxidants and herbal medicine was used to inhibit this oxidation [17]; for example Ohmori [18] *et al*, use green tea, Thounaojam [19] *et al*, use Sida rhomboidea and Gajaria [20] *et al*, use Murraya Koenigii. In this study the EO of ML and MP was subjected for inhibition of LDL oxidation in vitro. According to our results the antioxidant activity of ML and MP Eos for scavenging of 50% (IC_{50}) of the radicals of DPPH, 7.7 µg/ml of ML and 10.2 µg/ml of MP were required. The antioxidant activity of EO of ML and MP was determined with the ability to remove free radical DPPH and with the ability to remove the reactive oxygen species (superoxide anion O_2^-), the EO of both herbals possess high ability to inhibit the free radical of DPPH, agreed with Hajlaoui [21] *et al*. ML was better than MP for inhibit free radical of DPPH ($P < 0.05$). Inhibitory effect of ML and MP against LDL oxidation mediated by CuSO_4 compared with control explained that ML was higher effective than MP against oxidation rate (4.6 and 6.6 nmol/min/mg LDL protein respectively, significant $P < 0.05$) and increased the lag time (8.2, 5.5 times than control for ML and MP respectively, significant $P < 0.05$). Maximum CD formation was significantly reduced in the presence of EO of ML and MP, this finding agreed with Thounaojam [19] *et al*, and Gajaria [20] *et al*, in which they found other herbal types (Sida rhomboidea and Murraya Koenigii respectively), decreases maximum CD formation. EO of ML was the most effective in lag time prolongation and the rate of oxidation than MP and con-

Essential oil of mint and human health

Table 3. MIC and MBC showed antibacterial activity of EO of ML and MP and Amoxicillin ($\mu\text{g/ml}$)

<i>Bacterial species</i>	ML EO		MP EO		Amoxicillin	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Streptococcus pyogenes</i> (Group A)	3.4 \pm 0.3	3.7 \pm 0.4	1.4 \pm 0.1	2.4 \pm 0.3	28.3 \pm 6.2	29.5 \pm 6.4
<i>Streptococcus pneumoniae</i>	3.9 \pm 0.3	3.4 \pm 0.2	1.6 \pm 0.2	2.4 \pm 0.5	29.8 \pm 8.4	30.3 \pm 5.2
<i>Corynebacterium diphtheria</i>	4.0 \pm 0.3	4.5 \pm 0.4	1.7 \pm 0.2	2.5 \pm 0.3	31.1 \pm 12.4	32.3 \pm 8.5
<i>Haemophilus influenzae</i>	2.2 \pm 0.4	2.9 \pm 0.3	0.9 \pm 0.1	1.4 \pm 0.2	23.4 \pm 7.9	24.8 \pm 4.5
<i>Neisseria gonorrhoeae</i>	2.4 \pm 0.2	3.0 \pm 0.2	1.0 \pm 0.1	1.7 \pm 0.2	25.2 \pm 9.2	26.7 \pm 7.8

Abbreviations: MIC: minimum inhibitory concentration. MBC: minimum bactericidal concentration. Data represent as mean \pm SD.

trol (significant $P < 0.05$), however Ohmori *et al* [18], found the green tea prolongation of lag time (9.9 folds) and oxidation rate was (3.7 nmol conjugated diene/min/mg LDL protein) was comparable with that of ML. The prolongation of the lag phase in the presence of ML and MP (especially ML) indicating their antioxidant effect to reduce the role of CuSO_4 to enhance LDL oxidation. This is not surprise because many studies showed that ML has good antioxidant activity. Flavonoid content and total polyphenol of the two herbals [21], justify their antioxidant activity, however ML EO was higher in both phenolic and flavonoid contents than MP (significant $P < 0.05$). Antioxidant activity of herbal materials attributed to its total phenolic and flavonoid content [22]. There were two previous studies done with methanolic extract of ML and MP which cultivated in our region (Al-Madinah); the first was done by Al-Ali [23] *et al*, who tested ML and showed that ML it has a powerful antioxidant activity attributed to the flavonoid compound content and Quercetin 3-O-glycoside which support antioxidant activity (it's a part of flavonoids), our results agreed with this findings. Where the second done by Osman [24] who tested MP and concluded to MP possess strong antioxidant activity due to its content of total phenolic and flavonoid which is very rich compared to other species cultivated in different world region (more than Tunisian MP [25], also our result was agreed with this findings. In the same way, phenolic and flavonoid contents of ML and MP was higher significant than those Tunisian one determined by Hajlaoui [21] *et al*.

Mediation for LDL oxidation induced by CuSO_4 was recorded by determination the concentration of MDA, LHP and PC. The concentration was increased in presence of oxidize LDL mediated by CuSO_4 and decreased in control (LDL

without addition of CuSO_4). In presence of EO of both ML and MP minimize the concentration of MDA, LHP and PC gradually; this suggests the protective impact of ML and MP against the oxidation modification of polyunsaturated fatty acids and split of protein and lipid molecules. The decrease in TBARS level expressed as MDA paralleled. Said [6] *et al*, determine the antioxidant effect of herbal called Weighlevel (a mixture of 4 herbal types) against lipid peroxidation, he found in addition of Weighlevel lead to further reduction in MDA level, one of the 4 mixture extract is ML, this is agreement to our results. Berselli *et al*. [27] shown the efficacy of high (but nontoxic dose) of ML was significantly reduced lipid peroxidation, in agreement with our findings.

Antibacterial activity of EO of ML and MP in comparison with Amoxicillin antibiotic was expressed as inhibition zones in mm in the media surround disc. The antibacterial activity of ML attributed to its highest flavonoid content as the report of Akroum [28] *et al*, in contrast MP also possess high content of flavonoid but less than ML and possess weak antibacterial activity than ML. Antibacterial activity of ML was examined in previous studies [29, 30]; the present study was agreed with both studies although the bacterial species examined was different. Hajlaoui [31] *et al*, in Tunisia concluded to that EO of ML is more active than MP due to presence of active compounds such as 1, 8 cineole and α -pinene. Oxygenated monoterpenes of the EO of these herbals suggested to have ability to distraction bacterial membrane with lipophilic constituents although the mechanism of its action was not fully understood [32]. However Rodrigues [33] *et al*, attributed the antibacterial activity in the EO of *Mentha cervina* for three oxygen-containing monoter-

pene compounds, mainly pulegone, isomenthone and menthone; and also describe the ability of gram -ve bacterial species to resist EO than gram +ve one because it have complex membrane structure. The present study showed significant activity against gram +ve bacterial species of EO of ML and weak inhibition zone against gram -ve species compare to the former. According to MIC and MBC, EO of ML showed high sensitivity against gram +ve strains and less against gram -ve bacteria in agree with other authors [21, 34-37]. Regarding to antibacterial activity, MIC and MBC of ML and MP in our results it's possible to say that EO of ML possess strongest antibacterial activity than MP and comparable to other antibacterial activity of ML in different region in the world with minimal quantification differences. The different between antioxidant and antibacterial activities between (ML and MP) in our study and other studies in different world regions are suggested due to different constituents in EO, the frequency of these constituents, the soil in which the plant grow, environment, water, use of fertilizer, pesticides and insecticides.

To our knowledge, in KSA there are many reports describe the prevalence of hyperlipidemia which reach a serious rate [38-41], this help us to justify and recommended more studies for more awareness of the risk of oxidized bad lipid behaviors which play the key role -as mentioned - in cardiovascular disease. The present study considers the first report about antioxidant ability of ML and MP to inhibit LDL oxidation induced by CuSO_4 in KSA, and very few published report. But we did not elucidate the constituents of the EO and describe its antioxidant and antimicrobial. Further studies should be done *in vivo* to confirm antioxidant and antimicrobial of the EO; and for more expression and identification of EO components and physiologically and pharmacologically impact.

Conclusion

EO of ML possess strong antioxidant and antibacterial activity than MP, these activities of ML attributed to its high flavonoid contents enable it to be a good for cardiovascular and throat health. The study supported the traditional uses of ML.

Acknowledgements

This study was financially funded by the Deanship of Scientific Research, Taibah University, Al-Madinah, Saudi Arabia. By grant No. (435/6034).

Disclosure of conflict of interest

None.

Address correspondence to: Ahmed M Ahmed, Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, Taibah University, Al-Madinah Al-Munawarah, Kingdom of Saudi Arabia. E-mail: ahmedlab1@hotmail.com

References

- [1] Rosenfeld, ME, Khoo JC, Miller E. Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, prompt oxidation of low-density lipoproteins, and contain oxidation specific lipid-protein adducts. *The Journal of Clinical Investigation* 1991; 87: 90-99.
- [2] Regnstrom J, Nilsson J, Tornvall P, Landou C, Hamsten A. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* 1992; 339: 1183-1186.
- [3] Stefen Y, Jung T, Klotz LO, Schewe T, Grune T, Sies H. Protein modification elicited by oxidized low-density lipoprotein (LDL) in endothelial cell. Protection by (-)-epicatechin. *Free Radic Biol Med* 2007; 42: 955-970.
- [4] Moreno L, Bello R, Primo-Yufera E. Pharmacological properties of the methanol extract from *Mentha suaveolens* Ehrh. *Phytother Res* 2002; 16: 10-13.
- [5] Harley RM, Brighton CA. Chromosome number in the genus *Mentha* L. *Bot J Linn Soc* 1977; 74: 71-96.
- [6] Iscan G, Kirimer N, Kurkcuglu M, Baser KH, Demirci F. Antimicrobial screening of *Menthapiperita* essential oils. *J Agric Food Chem* 2002; 50: 3943-3946.
- [7] Daferera DJ, Ziogas BN, Polissiou MG. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* sub sp. *michiganensis*. *Crop Protection* 2003; 22: 39-44.
- [8] Council of Europe (COE). European Directorate for the Quality of Medicines. 6th Edition. Strasbourg: European Pharmacopoeia; 2007.
- [9] Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radic Res Commun* 1989; 6: 67-75.

Essential oil of mint and human health

- [10] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 1999; 299: 152-178.
- [11] Eberhardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apples. *Nature* 2000; 405: 903-904
- [12] Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978; 52: 302-310.
- [13] Nourooz-Zadeh J, Tajaddini-Sarmadi J, Ling KL, Wolff SP. Low-density lipoprotein is the major carrier of lipid hydroperoxides in plasma. Relevance to determination of total plasma lipid hydroperoxide concentrations. *Biochem J* 1996; 1: 781-786.
- [14] Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol* 1994; 233: 357-363.
- [15] Clinical and Laboratory Standards Institute (CLSI), performance standards for antimicrobial susceptibility testing: eighteenth informational supplement M100-S18. CLSI, Wayne 2008.
- [16] Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997; 272: 20963-20966.
- [17] Sánchez-Moreno C. Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Sci Technol Int* 2002; 8: 121-137.
- [18] Ohmori R, Iwamoto T, Tago M, Takeo T, Unno T, Itakura H, Kondo K. Antioxidant Activity of Various Teas against Free Radicals and LDL Oxidation. *Lipids* 2005; 40: 849-53.
- [19] Thounaojam MC, Jadeja RN, Devkar RV, Ramachandran AV. In vitro evidence for the protective role of *Sidarhomoidea*. *Roxb extract against LDL oxidation and oxidized LDL-induced apoptosis in human monocyte-derived macrophages*. *Cardiovasc Toxicol* 2011; 11: 168-79.
- [20] Gajaria TK, Patel DK, Devkar RV, Ramachandran AV. Flavonoid rich extract of *Murraya Koenigii* alleviates in-vitro LDL oxidation and oxidized LDL induced apoptosis in raw 264.7 Murine macrophage cells. *J Food Sci Technol* 2015; 52: 3367-75.
- [21] Hajlaoui H, Trabelsi N, Noumi E, Snoussi M, Fallah H, Ksouri R, Bakhrouf A. Biological activities of the essential oils and methanol extract of two cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. *World J Microbiol Biotechnol* 2009; 25: 2227-2238.
- [22] Rice-Evans CA, Miller JM, Paganga G. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic Biol Med* 1996; 20: 933-956.
- [23] Al-Ali KH, El-Beshbishy HA, Alghaithy AA, Abdallah H, El-Badry AA, Abdel-Sattar E. *In vitro* antioxidant potential and antiprotozoal activity of methanolic extract of *Mentha longifolia* and *Origanum syriacum*. *J Biol Sci* 2013; 13: 207-216.
- [24] Osman IH. *In Vitro* Antioxidant activity of *Mentha pulegium* from Saudi Arabia. *Bioscience Research* 2013; 10: 33-37.
- [25] Karray-Bouraoui N, Ksouri R, Falleh H, Rabhi M, Abdul-Jaleel C, Grignon C, Lachaâl M. Effects of environment and development stage on phenolic content and antioxidant activities of Tunisian *Mentha pulegium* L. *J Food Biochemistry* 2010; 34: 79-89.
- [26] Said O, Saad B, Fulder S, Khalil K, Kassis E. Weight loss in animals and humans treated with "weighlevel", a combination of four medicinal plants used in traditional arabic and islamic medicine. *Evid Based Complement Alternat Med* 2011; 2011: 874538.
- [27] Berselli PV, Zava S, Montorfano G, Corsetto PA, Krzyzanowska J, Oleszek W, Berra B, Rizzo AM. A mint purified extract protects human keratinocytes from short-term, chemically induced oxidative stress. *J Agric Food Chem* 2010; 58: 11428-34.
- [28] Akroum S, Bendjedou D, Satta D, Lalaoui K. Antibacterial Activity And Acute Toxicity Effect of Flavonoids Extracted From *Mentha longifolia*. *AM. Eurasia J. Sci Res* 2009; 4:93-96.
- [29] Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. *Food Chem* 2007; 103: 1449-1456.
- [30] Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavoulj M. Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med* 2003; 69: 413-419.
- [31] Hajlaoui H, Snoussi M, Ben Jannet H, Mighri Z, Bakhrouf A. Comparison of chemical composition and antimicrobial activities of *Mentha longifolia* L. ssp. *longifolia* essential oil from two Tunisian localities (Gabes and Sidi Bouzid). *Ann Microbiol* 2008; 58: 103-110.
- [32] Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; 12: 564-582.
- [33] Rodrigues L, Duarte A, Figueiredo AC, Brito L, Teixeira G, Moldão M, Monteiro A. Chemical composition and antibacterial activity of the essential oils from the medicinal plant *Mentha cervina* L. grown in Portugal. *Med Chem Res* 2012; 21: 3485-3490.
- [34] Stanislavljević D, Đorđević S, Milenković M, Lazić M, Veličković D, Randelović N, Zlatković B. Antimicrobial and Antioxidant Activity of the Essential Oils Obtained from *Mentha longifolia* L.

Essential oil of mint and human health

- Hudson, Dried by Three Different Techniques. *Rec. Nat. Prod* 2014; 61-65.
- [35] Nikšić H, Kovač-Bešović E, Makarević E, Durić K. Chemical composition, antimicrobial and antioxidant properties of *Mentha longifolia* essential oil. *J Health Sci* 2012; 2: 192-200.
- [36] Sarac N, Ugur A. The in vitro antimicrobial activities of the essential oils of some lamiales species from Turkey. *J Med Food* 2009; 12: 902-907.
- [37] Mahboubi M, Haghi G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *J Ethnopharmacol* 2008; 119: 325-327.
- [38] Al-Nuaim AR, Al-Rubeaan K, Al-Mazrou Y, Al-Attas O, Al-Daghari N. Prevalence of hypercholesterolemia in Saudi Arabia, epidemiological study. *Int J Cardiol* 1996; 54: 41-49.
- [39] Al-Nuaim AR. Serum total and fractionated cholesterol distribution and prevalence of hypercholesterolemia in urban and rural communities in Saudi Arabia. *Int J Cardiol* 1997; 58:141-9.
- [40] Al-Nozha MM, Arafah MR, Al-Maatouq MA, Khalil MZ, Khan NB, Al-Marzouki K, Al-Mazrou YY, Abdullah M, Al-Khadra A, Al-Harhi SS, Al-Shahid MS, Al-Mobeireek A, Nouh MS. Hyperlipidemia in Saudi Arabia. *Saudi Med J* 2008; 29: 282-7.
- [41] Al-Kaabba AF, Al-Hamdan NA, El Tahir A, Abdalla AM, Saeed AA, Hamza MA. Prevalence and Correlates of Dyslipidemia among Adults in Saudi Arabia: Results from a National Survey. *Open Journal of Endocrine and Metabolic Diseases* 2012; 2: 89-97.