Research Article

Honey Protects Human Low Density Lipoprotein (LDL) from Peroxidation (*In vitro* study)

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ABSTRACT

The oxidative modification hypothesis of atherosclerosis predicts that low-density lipoprotein (LDL) peroxidation is an early event in atherosclerosis. Therefore, inhibition of LDL peroxidation might be an important step in preventing atherogensis. It was hypothesized that honey extracts contain varying amounts of phenolic compounds and that they possess different *in vitro* antioxidant activity. Honey extracts were analyzed using HPLC and GC/MS to identify and compare phenolic compounds, whereas high-performance liquid chromatography was used for their quantification. The antioxidant activity of Thyme honey and another multifloral honey (MF, from Red Sea area) were evaluated with low density lipoprotein (LDL) peroxidation assay, in different concentrations (100 - 0.1 mg honey/1 ml). The very interesting result that, although the two samples have different chemical composition by GC/MS and HPLC analysis; they exhibited highly significant antioxidant activity within the concentration of 0.1mg honey/1ml for MF and 1mg honey/1ml for Thyme honey in LDL peroxidation assays than that of the very high concentration 100mg honey/1 ml. The IC_{50} (mg/ml) of the honey samples are 0.89 ± 0.02 and 0.52 ± 0.03 respectively. HPLC analysis revealed the presence of four aromatic acids in the two honey samples (total content; 1.26 and 62.02 μg / 100 g honey). Vanillic and coumaric acids were highly present in MF honey (15, 45 μg / 100 g honey). 14 Flavonoids were identified (total content; 204.66 and 35.42 μg / 100 g honey). Thyme honey has the highest content of kaempferol and luteolin. Pinobankasin and quercetin were present only in the multifloral honey. The GC/MS study showed the presence of 10 and 16 volatile compounds, respectively, while ether extract of the two honeys showed presence of 68 compounds (44 and 53 compounds, respectively). The samples were characterized by the presence of 22 aliphatic Acids (9.59 and 10.45%), 24 aromatic acids (20.37 and 12.12%) and 12 esters (4.12 and 11.04 %) respectively.

Keywords: GC / MS, HPLC, Honey, LDL peroxidation.

INTRODUCTION

Oxidation of lipids is assumed to be implicated in the pathophysiology of atherosclerosis. It has been suggested that scavenging of lipid peroxyl radicals contributes to the anti atherosclerotic effects of naturally occurring compounds such as polyphenol compounds. These compounds are capable of inhibiting lipoprotein oxidation *in vitro* and suppressing formation of plasma lipid oxidation products *in vivo*.[1] Therefore, inhibition of LDL oxidation might be an important step in preventing atherosclerosis. Humans protect themselves from reactive oxygen species, in part, by absorbing dietary antioxidants.

During the past decade, the use of honey as a therapeutic substance has been reevaluated in a more scientific setting. Studies have shown that honey has both antibacterial and anti-inflammatory properties,[2,3] useful in stimulation of burn and wound healing as well as honey had antioxidant activity.[4-6]

Honey is rich in phenolic compounds, which act as natural antioxidants and are becoming increasingly popular because of their potential role in contributing to human health. A wide range of phenolic constituents is present in honey like quercetin, caffeic acid phenethyl ester, acacetin, kaempferol, galangin which have promising effect in the treatment of cardiovascular diseases. Many epidemiological studies have shown that regular intake of phenolic compounds is associated with reduced risk of heart diseases. In coronary heart disease, the protective effects of phenolic compounds include mainly antithrombotic, anti-ischemic, anti-oxidant, and vaso-relaxant. It is suggested that flavonoids decrease the risk of coronary heart disease by three major actions: improving coronary vasodilatation, decreasing the ability of platelets in the blood to clot, and preventing low-density lipoproteins (LDLs) from oxidizing.[7]

The aim of this study was to evaluate the antioxidant activity of honey from two different floral sources to find out the highly effective antioxidant one which could protect the human low density lipoprotein (LDL) against copper-induced oxidation *in vitro*, in correlation with investigating their chemical composition by GC/ MS and HPLC analysis.

MATERIALS AND METHODS

Reagents and Honey

All reagents are of analytical purity grade. Distilled water was used for all dilution steps. Thyme honey was collected as market sample (From Authorized apiary farm) of Libyan origin, while the multifloral honey (from Red

Sea area) was provided (From Authorized apiary farm) of Egyptian origin (2011, flowering season).

Measurement of Copper-Induced Low Density Lipoprotein (LDL) oxidation in-vitro

Isolation of LDL

LDL was isolated according to the method of Gugliucci and Menini.[8] LDL (1.019-1.055 g/ml) was separated by sequential ultra-centrifugation using TL-100 Ultracentrifuge (Beckman, U.S.A.) from plasma LDL then extensively dialyzed against phosphate buffered saline (PBS), pH 7.2, containing 0.01% EDTA at 4°C. Samples were stored at 4°C in the dark and used within 24hr. Protein content was determined according to Lowry's method.[9]

Thiobarbituric Acid Reactive Substances (TBARS) assay

LDL was oxidized using 5 µM/ml CuSO4.[10] Oxidation of LDL was monitored in the presence or absence of honey samples by measuring the thiobarbituric acid reactive substances (TBARS). The absorbance was measured at 534 nm using UV Spectrophotometer [UNICAM UV300], malondialdehyde-bis-(dimethylacetal) which yields malondialdehyde (MDA) by acid treatment, was used as a standard.

Flavonoid extraction from honey for HPLC analysis

200 g of each honey sample was thoroughly mixed with 5 parts of water (pH 2 with HCl) until completely fluid and filtered through cotton to remove solid particles. The filtrate was then passed through a column (25 x 2 cm) of Amberlite XAD-2 (Supelco; pore size 9 nm, particle size 0.3-1.2 mm). The various phenolic compounds remained in the column while sugars and other polar compounds were eluted with the aqueous solvent.[11] The column was washed with acid water (100 ml) and subsequently with distilled water (300ml). The whole phenolic fraction was then eluted with methanol (300ml until no more color was eluted) and concentrated under reduced pressure (40°C). This phenolic fraction was re-dissolved in 5 ml distilled water. This water extract was partitioned with ethyl ether (5ml x 3). The ether extracts were combined and the ether removed under reduced pressure. The dry extract was dissolved in methanol and filtered through 0.45-µm filter before direct HPLC analysis.

HPLC analysis of honey flavonoids

The HPLC analysis was achieved with Agilent 1100 series liquid chromatograph with UV detector and an auto-sampler. The column used was a Lichrochart RP-18 (Merck, Darmstadt, Germany; 25 x 0.4 cm, 5-µm particle size). Elution was with water: formic acid (19:1 v:v; solvent A) and acetonitrile (solvent B), and the flow rate was 1ml/min. Gradient elution started with 20% B, reaches 25 % B at 25 min and 30% B at 35 min, and then the system became isocratic until 50 min, reaches 50 % B at 60 min and 70 % B at 67 min. The flavonoids were detected with UV detector and the chromatograms were recorded at 340 and 290 nm.

Flavonoid identification and quantification

The different flavonoids were identified by chromatographic comparisons with authentic flavonoids. The flavanones were detected at 290 nm, the flavones at 340 nm and phenolic acids at 290 nm.[12] The compounds identification was carried out by direct HPLC comparison of authentic samples and was based on co-chromatography in 290 and 340 nm. Response factors for the authentic flavonoids and the concentration of flavonoids in each honey sample were calculated according to Ogan and Katz and Annual Book of ASTM Standards.[13,14]

Extraction of honey volatiles for GC/MS

Honey samples were extracted according to Graddon *et al.*[15], where 50 g honey were thoroughly mixed with 25 ml n-hexane and then decanted. This was repeated with four further 25 ml portions. Aliquots of solvent and the combined extract were concentrated by evaporating the solvent under vacuum at 25°C. This led to an extract with a strong overall honey-like aroma similar to that of the original sample.

Extraction of honey for GC/MS

50 g of each honey sample was extracted with diethyl ether,[16] and concentrated by evaporation under vacuum at 40 °C. 5 mg of the ether extract was dissolved in 0.05ml pyridine + 0.1 ml BSTFA (N,O-bis(trimethylsilyl) trifluoro-acetamide(BSTFA), from Sigma) and heated for 30 min. at 60 °C and injected in the GC/MS.[17]

GC/MS analysis

A finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph. DB-5 column, 30m x 0.32 mm (internal diameter), was employed with helium as carrier gas and the temperature programmed from 40 to 260ºC at 5ºC / min. (3-min. initial hold, 10-min. final hold). The mass spectra were recorded in electron ionization (EI) mode at 70 eV, ion source temperature 150ºC. The scan repetition rate was 0.5 s.

Identification of compounds

Peaks were identified by computer search of user-generated reference libraries, incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity; mixed peaks were resolved by computer program aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another.

RESULTS

Susceptibility of LDL to Cu^{2+} - induced oxidation

The antioxidant activity of Thyme honey and another multifloral honey (MF, from Red Sea area) was evaluated with low density lipoprotein (LDL) peroxidation assay (in different concentrations 100 - 0.01 mg honey/1 ml). Pre-incubation of LDL with honey samples resulted in significant inhibition of TBARS accumulation (Fig.2, Table

1). A very interesting result showed that, although the two samples have different chemical composition by GC/MS and HPLC analysis; they exhibited highly significant antioxidant activity within the concentration of 0.1mg honey/1ml for MF and 1mg honey/1ml for Thyme honey in LDL peroxidation assays than that of the very high concentration 100mg honey/1 ml. the IC_{50} (mg/ml) of the honey samples are 0.89 ± 0.02 and 0.52 ± 0.03 respectively. It is clear that MF honey is the highly active in the concentration of 0.1mg honey/1ml, also has the lower IC_{50}.

Table 1: Antioxidant activity of honey samples on Copper-Induced human LDL peroxidation *in-Vitro*

Honey sample	100 mg/ml	10 mg/ml	1mg/ml	0.1mg/ml	0.01mg/ml	IC_{50} (mg/ml)
Control	0.0965± 0.001	0.0965± 0.001	0.0965± 0.001	0.0965± 0.001	0.0965± 0.001	
Control oxidized	0.2023± 0.0005	0.2023± 0.0005	0.2023± 0.0005	0.2023± 0.0005	0.2023± 0.0005	
Thyme Honey	0.1133±0.001	0.1012± 0.0006	0.0993± 0.002	0.1027± 0.001	0.1031± 0.0002	0.89 ± 0.02
MF honey	0.1033± 0.0007	0.1012± 0.0009	0.1003± 0.0003	0.0991± 0.001	0.1011± 0.001	0.52 ± 0.03

Oxidation of LDL was monitored in the presence or absence of honey sample by measuring the thiobarbituric acid reactive substances (TBARS) at 534 nm. (Results are expressed as mean ± SD, n=3).

Table 2: Polyphenolic compounds assessed by HPLC of Thyme and MF (Multifloral Honey) (Conc. µg / 100 g honey)

Name	Structure	Thyme	MF	Name	Structure	Thyme	MF
Hydrocinnamic acid	3-Phenylpropanoic acid	0.2	0.6	Pinobankasin	3,5,7-trihydroxyflavanone	-	3.38
Vanillic acid	4-Hydroxy-3-methoxybenzoic acid	0.05	15.4	8-Methoxykaempferol	3,5,7,4'-Tetrahydroxy-8-methoxyflavone	2.9	0.91
Coumaric acid	*trans*-4-Hydroxy-cinnamic acid	0.31	45.12	Kaempferol	3,5,7,4'-tetrahydroxyflavone	114.4	2.66
Ferulic acid	4-Hydroxy-3-methoxy cinnamic acid	0.7	0.9	Luteolin-3'-methylether	5,7,4'-trihydroxy-3'-methoxyflavone	-	0.7
Major unknown (Mu)	----------	-	(Mu)	Kaempferol-3-methylether	5,7,4'-trihydroxy-3-methoxyflavone	2.5	2.8
Major unknown (Mu)	----------	-	(Mu)	Quercetin-3,3'-dimethyl ether	5,7,4'-trihydroxy-3,3'-dimethoxyflavone	0.05	0.7
Eriodictyol	5,7,3',4'-tetrahydroxyflavanone	0.12	--	Formonontin	7-hydroxy-4'-methoxyflavanone		5.50
Liquiritegenin	7,4'-dihydroxyflavanone	0.47	1.94	Pinocembrin	5,7-Dihydroxy flavanon	4.84	4.52
Luteolin	5,7,3',4'-tetrahydroxyflavone	79.38	-	Formononetin	7-hydroxy-4'-methoxyflavone	-	0.82
Quercetin	3,5,7,3',4'-pentahydroxyflavone	-	7.77	Biochanin A	5,7-Dihydroxy-4'-methoxyflavone		3.72

Table 3: Volatile compounds from honey samples assessed by GC/MS

Compound	Thyme[a]	MF[a]	Compound	Thyme[a]	MF[a]
2-Ethoxypropane	1.70		Hexadecane	4.16	11.67
1,2-Diethoxyethane	0.18		2-Tetradecanone		3.30
2-Propanol-1-(1-methylethoxy)	0.49		3,4,5-Trimethoxy benzylmethylether		0.73
Undecanal	0.50		Butanoic acid-4-methoxy		0.63
2-Hydroxycineol		0.42	Octadecene		5.42
Tridecane		0.93	Eicosene	0.27	
Citronellol		0.51	Heneicosane	2.50	
Citronellyl formate		0.61	Tricosene		0.54
2-propanone-1-(4-methoxyphenyl)		1.3	Tricosane		5.37
2,6-octadienal,3,7-dimethyl-(E)		2.63	Pentacosane		0.55
Phenol, 4-ethyl-3-methoxy	1.00	7.70	1- Docosanol	0.28	
Phenol-5-methoxy-2,4,6-trimethyl	0.40		Heptacosane		2.39

[a]The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation; MF = multifloral honey.

Table 4: Chemical composition of honey samples ether extract assessed by GC/MS

Compound	Thyme[a]	MF[a]	Compound	Thyme[a]	MF[a]
Aliphatic Acids					
Hydroxyacetic acid	0.12	0.08	Pentanedioic acid	0.28	0.02
2-Hydroxypropanoic acid	1.35	0.40	2,4-Hexadienedioic acid		0.19
4-Hydroxybutanoic acid	0.08	0.05	Octanedioic acid		0.37
2-Hydroxy-3-methylbutanoic acid	0.07	0.12	7-Hydroxy Octanedioic acid	0.06	
2-Hydroxy-4-methylpentanoic acid	0.09	0.09	9-Hydroxydecanoic acid	0.4	0.13
Sebacic acid	0.40		Hexadecanoic acid	1.8	3.70
3-Hydroxy Sebacic acid	0.10	0.09	Oleic acid	1.93	0.56
5-Hydroxy-n-valeric acid	2.16	1.00	Oleic acid isomer		0.42
2-Methyl-2-hydroxybutanoic acid		0.83	Octadecanoic acid	0.10	2.20
2,2-Dimethylbutanedioic acid		0.07	Stearic acid	0.65	
2,3-Dihydroxypropanoic acid		0.05	Nonadecanoic acid		0.08
Aromatic acids					
Benzoic acid	15.75	6.67	4-Phenyl butyric acid		0.13
Benzeneacetic acid	1.0	0.24	Benzoic acid-4-(2-methyl-2-butenyl) [b]	0.08	
2-Phenyl-2-hydroxyacrylic acid		0.13	3,4-Dihydroxybenzoic acid		0.04
Benzeneacetic acid-α-hydroxy		0.45	2-Phenyl-2-hydroxy acrylic acid	0.68	
Dihydro Cinnamic acid	0.10		3,5-Dimethoxymandelic acid	0.10	
cis-Cinnamic acid	0.21	0.07	4-Phenyl butyric acid isomer		0.33
1,2-Benzenedicarboxylic acid	0.51	0.10	2,5-Dihydroxybenzoic acid		0.41
3-Hydroxybenzeneacetic acid		0.39	2,4,5-Trihydroxybenzoic acid	0.34	
3-Hydroxybenzoic acid	0.62	0.18	Dihydro Coumaric acid	0.75	
4-Hydroxybenzoic acid	0.15	0.89	cis-Coumaric acid	0.03	0.07
Vanillic acid	0.11	0.47	p- Coumaric acid	0.07	1.35
4-Hydroxybenzeneacetic acid	0.20	0.17	Ferulic acid	0.03	0.03
Esters					
4-Hydroxyphenyllactic acid ethyl ester		0.27	1,2-benzendiol,3,5-bis(1,1-dimethylether)	1.90	
Octadecanoic acid methyl ester	0.10	0.29	Mandelic acid ethyl ester	1.72	0.45
Methyl-4-t-butylbenzoate		0.21	m-Anisic acid-4-hydroxy-methylester		5.86
3-Butanyl-4-methoxy-methylbenzoate		1.40	3,4,5-Trimethoxybenzyl methyl ether		0.85
4-Isopentanyl-3-methoxy-methylbenzoate		0.51	Phenyl ethyl-p- coumarate-4- heptadecatriene [b]	0.30	
3-Methoxy-4-pentanyl-methylbenzoate		1.20	Tetradecyl ferulate	0.10	
Others					
2-furancarboxylic acid	0.23	0.15	4H-Pyran-4-one-2,5-dihydroxy		0.16
1,2-Cyclohexanedicarboxylic acid	0.5		Hippuric acid	0.20	
2'-Hydroxypropiophenone	0.27		4-Pyrimidinamine-5-methylamine-2-OH		17.94
5-Methyl-2,4-Di isopropylphenol		0.13	1,3,5-Trihydroxybenzene		0.17
2-Phenyl-1,2-dihydroxy propane	0.21		1,4-Dihydroxybenzene	0.05	0.31

[a] The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation. MF = multifloral honey. [b] for first time in honey.

Chemical composition of honey

Quantitative comparison of polyphenolic profile of two honey samples were studied by HPLC analysis. 20 compounds were detected, from which 18 were completely identified by comparison with authentic markers. The results of HPLC analysis of polyphenolic compounds in honey samples (μg / 100 g honey) were presented in (Figure 1A, Table 2).

Four aromatic acids were present in the two honey samples (total content; 1.26 and 62.02 μg / 100 g honey).

Vanillic and coumaric acids were highly present in the MF honey (15, 45 μg / 100 g honey). 14 Flavonoids were identified (total content; 204.66 and 35.42 μg / 100 g honey), the difference in the flavonoid composition between the two honey samples is clear (Figure 1A, Table 2). Thyme honey has the highest content of kaempferol and luteolin. Luteolin was detected only in thyme honey. Pinobankasin and quercetin were present only in the multifloral honey.

The GC/MS study of the volatile components revealed that, Thyme honey and the multifloral honey (MF, from Red Sea area) contained 10 and 16 volatile compounds, respectively (Fig. 1B, Table 3). Thyme honey was characterized by the presence of 2-ethoxypropane, 2-propanol-1-(1-methylethoxy), undecanal, phenol-5-methoxy-2,4,6-trimethyl. MF honey was characterized by the presence of 2-hydroxycineol, citronellol, citronellyl formate, 2-propanone-1-(4-methoxyphenyl), 2,6-octadienal,3,7-dimethyl-(E), 2-tetradecanone. Phenol-4-ethyl-3-methoxy presented in a large amount in MF honey (7.7%) rather than that in Thyme honey (1.0%) (Figure 1B, Table 3).

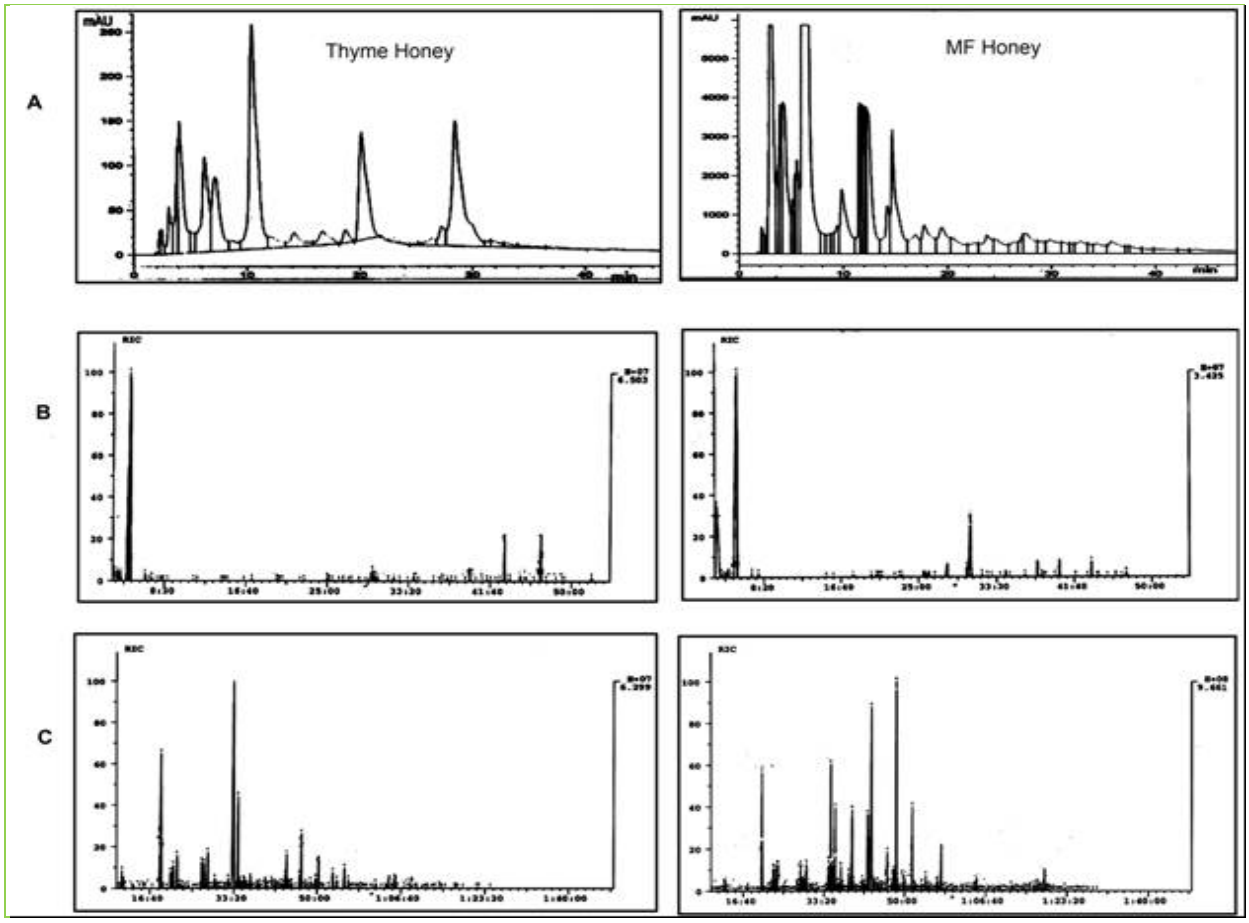

Figure 1: Comparative chromatographic studies for Thyme and Multifloral (MF) honeys; **A** for HPLC, **B** for GC/MS of volatiles and **C** for GC/MS of honeys ether extracts.

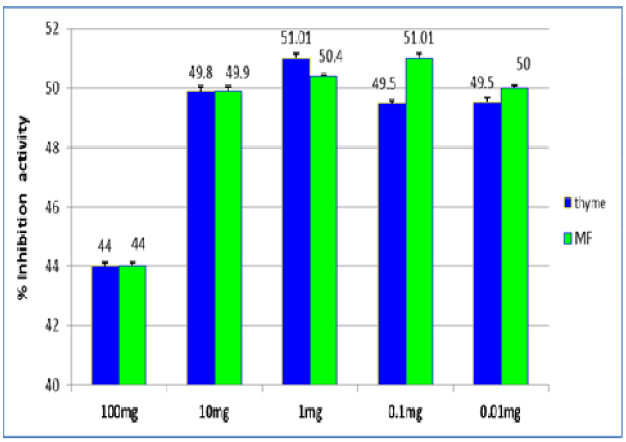

Figure 2: Inhibitory activity of honey samples on cupper-induced LDL peroxidation**.** Values are mean ±SD

The investigation of the ether extract of the two honey samples by GC/MS revealed the presence of 68 compounds. The samples were characterized by the presence of 22 aliphatic Acids (9.59 and 10.45%), 24 aromatic acids (20.37 and 12.12%) and 12 esters (4.12 and 11.04 %) for Thyme and MF honeys respectively (Figure 1C, Table 4). The aliphatic acids: 2-hydroxypropanoic acid, 5-hydroxy-n-valeric acid, pentanedioic acid, 7-hydroxy octanedioic acid, hexadecanoic acid and oleic acid were present with high percent in thyme honey, while 4,6-dioxohept-2-enoic acid, hexadecanoic acid and octadecanoic acid were present with high percent in MF honey. Benzoic acid existed in a very large concentration in thyme honey (15.75%). The new aromatic acid: benzoic acid-4-(2-

methyl-2-butenyl was present only in thyme honey. Dihydrocoumaric, cis-coumaric, p- coumaric and ferulic acids were present in the two samples. The new compounds: phenylethyl-p-coumarate-4-heptadecatriene, tetradecyl ferulate and hippuric acid were present only in thyme honey.

DISCUSSION

LDL peroxidation is considered to be essential in the pathogenesis of atherosclerosis.[1] Although data concerning the mechanisms by which lipid peroxidation occurs *in-vivo* are scarce, several lines of evidence suggest that some endogenous and exogenous compounds with antioxidant activity could have some beneficial effects in the prevention of the disease. Many plant phenols and flavonoids may be important dietary antioxidants.[18-20]

Honey has been found to contain several antioxidant enzymes including glucose oxidase, catalase and other antioxidant components like ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins.[21,22] Various polyphenols are reported in honey; some of honey's polyphenols are caffeic acid, caffeic acid phenyl ester, chrysin, galangin, quercetin, acacetin, kaempferol, pinocembrin, Pinobanksin and apigenin.[6]

The antioxidant activity of Thyme and the multifloral honey (MF) were evaluated with low density lipoprotein (LDL) peroxidation assay, in different concentrations (100 - 0.1 mg honey/1 ml). The very interesting result that they exhibited highly significant antioxidant activity within the concentration of 0.1mg honey/1ml for MF and 1mg honey/1ml for Thyme honey than that of the very high concentration 100mg honey/1 ml, although the two samples have different chemical composition by HPLC and GC/MS analysis.

The highest activity of the concentration 0.1mg honey/1ml could be attributed to the increasing activity of glucose oxidase enzyme by dilution (honey pH 3.9 - 6.1, the optimum pH for glucose oxidase activity is 6.5 - 8; increasing activity of glucose oxidase enzyme lead to the increase of the concentration of hydrogen peroxide.[23] More the hydrogen peroxide generation, more potent is the radical trapping.[24] The action of some but not all of honeys is linked to the production of hydrogen peroxide on dilution of the honey with wound exudates. All honeys tested had antioxidant potential, with manuka able to completely quench added radicals within 5 min of spiking.[5] Navab *et al.*[25] showed that 8 mM hydrogen peroxide added to 10 µg of PAPC **(**1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine) for 8 h dramatically decreased the relative abundance of PAPC and increased the formation of the biologically active phospholipids, while 2 and 4 mM hydrogen peroxide had no effect. Because the molecular weight of PAPC is 782 the molar ratio required for the enhanced oxidation of PAPC by hydrogen peroxide was approximately 62:1 (H_2O_2: PAPC). Intermolecular hydrogen bonds are broken on dilution and as a result there is a decrease in the bonded O - H absorption and an increase in or the appearance of free O - H absorption.[26]

From this point of view, our data are in agreement; where dilution of honey activates glucose oxidase to convert glucose to glucoronic acid and hydrogen peroxide. Further dilution decrease the molar concentration of the H_2O_2 produced, so its peroxidation effect on LDL (ox-LDL) decreased, besides; hydrogen bonding between glucose and flavonoids decreased with dilution, so increase their inhibitory activity for LDL peroxidation.

Tiwari demonstrated that the reactivity of flavonoids in protecting LDL against Cu^{2+} ion-induced oxidation are dependent in their structure properties in terms of the response of a particular flavonoid to Cu^{2+} ions. Whether chelation or oxidation their partitioning abilities between the aqueous compartments and lipophilic environment within LDL particles and their hydrogen donation antioxidant properties are important aspects.[24] Schramm *et al.*[4] found that phenolic antioxidants from processed honey are bioavailable, and that they increase antioxidant activity of plasma.

The HPLC and GC/MS data (Figure 1, Tables 2-4) revealed that the two honey samples were rich in phenolic acids and flavonoids. The enrichment of flavonoids such as luteolin, naringenin, and Kaempferol significantly increased the resistance of LDL to oxidation.[27] Dihydroxy benzoic acid, caffeic acid, ferulic acid and cinnamic acid were the major phenolic constituents found in the honey samples.[28] Some of the polyphenols like quercetin, acacetin, kaempferol, and galangin present in honey have been reported as promising pharmaceutical drugs in the treatment of cardiovascular diseases.[7] Acacia, Coriander, Sider and Palm honey samples were found to exhibit more or less the same highly significant antioxidant activity within the concentration of 1mg honey / 1 ml in LDL peroxidation assay.[6]

CONCLUSION

Our study provides primary evidence suggesting that these honeys in further *in vivo* studies could play an important role in inhibiting lipid peroxidation in biological systems through their antioxidant, metal chelating and free radical scavenging activities.

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