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Chemical composition and antioxidant activity of the crude methanolic extracts of *Mentha spicata*

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Abstract
The chemical constituents and antioxidant activity of the crude extracts of *Mentha spicata* were investigated. Phytochemical analysis indicated the presence of sugar, flavonoids and alkaloids in the crude extracts of *Mentha spicata*. GC-TOFMS (Gas Chromatography Time-of-Flight Mass Spectrometry) analysis indicated the presence of fatty acid methyl esters (hexa decane, hepta decane, octa decane) terpenoids, terpenoid alcohol, caryophyllene and glycosides. Total phenolic components of the crude extracts was found to be 27.26±0.62 mg/g gallic acid equivalent which was determined by Folin-Ciocalteu method. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was found to increase with increasing concentrations and was found to be 54.84±0.57% with an IC50 value of 25.2µg/ml. The reported antioxidant activity may be due to the presence of flavonoids and fatty acid methyl esters which has the scavenging potential by reducing the free radicals.

Keywords: *Alocacia Mentha spicata,* GC-TOFMS, chemical profiling, antioxidant activity

INTRODUCTION

In recent years, the essential oils and herbal extracts have attracted a great scientific interest due to their potential as a source of natural antioxidants and biologically active compounds [1]. Diets rich in selected natural antioxidants such as polyphenols, flavonoids, vitamin C and vitamin E are related to reduced risk of incidence of cardiovascular, other chronic diseases and certain types of cancer and also has led to the revival of interest in plant-based foods [2, 3, 4]. Many herbs contains a variety of phytosterols, phenolic acids, triterpenes, flavonoids, anthocyanins, saponins and carotenoids, which have been shown to exert cancer chemo-preventive and antioxidant properties [5].

Mint species have been exploited by man for more than 2000 years. The genus *Mentha* (Lamiaceae) is composed of 19 geographically wide spread species and 13 hybrids [6, 7]. Spearmint belongs to the genus Mentha in the familty Labiateae. This family is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties [8, 9]. Three *Mentha* species, *M*. x piperita L. (Peppermint), *M. arvensis* L. (cornmint) and M. *spicata* (spearmint) are commonly cultivated in the world for essential oil production that is used extensively in the liquor and confectionary industries, flavoring, perfume production and medicinal purposes [10]. *Mentha* species are widely used in conventional medicine and as culinary herb and spice. *M. spicata* has been produced by cross breeding form *M. longifolia* and *M. rotundifolia*. *Mentha spicata* L. (spearmint) is a creeping rhizomatous, glabrous and perennial herb with a strong aromatic odor.

The oil of *Mentha spicata* is rich in carvone and presents a characteristic spearmint odor. The fresh and dried plants and their essential oil is used in food, cosmetic, confectionary, chewing gum, toothpaste and pharmaceutical industries [11, 12]. Leaves, flowers and the stem of *Mentha spp* are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor [10]. It is well documented that the essential oil from mentha species posses antimicrobial and antioxidant properties [13, 14]. This herb is considered as a stimulant, carminative, antispasmodic, stomachic and diuretic and is used in the treatment of gas pain, rheumatism, toothache, muscle pain. Mint possesses antioxidant properties due to the presence of active constituents like menthone, menthol, rosmarinic acid and carvone [15]. Pudina extracts (*Mentha spicata*) are used as flavoring in culinary preparation through out the plains of India and possessed DNA damage protecting activity and antioxidant potential [16].

Flavonoids which are widely distributed in the leaves, seeds, seeds, bark and flowers of plants are a broad class of low molecular weight compounds and highly effective antioxidant and less toxic than synthetic antioxidants such as BHA and BHT and have received greater attention and studied extensively [17]. By considering adverse effects of synthetic antioxidant on human health, alternative nature and safe sources of food antioxidant should be identified [18, 19]. The potential of the antioxidant constituent of plant materials for the maintenance of health disease and cancer is also raising interest among scientists. The present study determined the chemical constituents and antioxidant activity of the crude extracts of *Mentha spicata*. Qualitative phytochemical analysis and GC-TOFMS (Gas Chromatography Time-of-Flight Mass Spectrometry) analysis of the crude extracts of the plants were done to determine the chemical constituents. Antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method and total phenolic content by Folin-Ciocalteu method.

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MATERIALS AND METHOD

Extraction of plant material

Fresh plants were purchased from a local hyper market. A voucher specimen was submitted to the Herbarium unit, School of Biological sciences, USM, Penang, Malaysia. The procured plant samples were washed and dried at room temperature (30°C) for a week, grounded into a powdered using an electrical blender. 200 g of the finely powdered material were macerated and soaked in 80% methanol for 4 days. The extracts were clarified by filtration with Whatman No. 1 paper and concentrated *in vacuo* in a rotary evaporator. Finally the crude extracts were obtained.

Phytochemical analysis

Qualitative phytochemical tests of the crude extracts were done to identify the presence of sugar, flavonoids and alkaloids [20].

GC-TOFMS (Gas chromatography-time of flight mass spectrometer) analysis

For GC-TOF-MS analysis, 0.1 g of crude extract was mixed with 1 ml of distilled water and 4 ml of solvent mixture (ethyl acetate: hexane: methylene chloride). The mixture was agitated for 2 min at 3000 rpm. The supernatant was filtered and injected in to the GC column (B-5 20 x 0.18 mm, ID,0.18 µm).The chemical constituents of the crude extracts of the plants were analyzed by Pegasus®, a time-of-flight mass spectrometer (TOFMS) for GC/MS analysis. The m/z of each ion was determined by its time of flight and equation (t = Slope*(m/z)1/2 + Offset). Slope and offset values is determined using a mass calibration standard which is done automatically by the mass calibration routine of the Pegasus® ChromaTOF TM software [21].

Determination of antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method

The free radical scavenging capacity of the extracts was noted through the change of optical density of DPPH radicals at 517 nm after 20 min incubation at room temperature. The total free radical scavenging capacity of the methanolic crude extracts was determined spectrophotometrically by measuring the disappearance of DPPH radical at an absorbance of 517 nm. DPPH scavenging activity was calculated by using the equation:

DPPH scavenging effect (%) = $A_0 - A / A_0 \times 100$ (A_0 – absorbance of the negative control; A – absorbance of the test) [22].

Total phenolic content

The total phenolic content (TPC) of extracts was determined spectrophotometri cally by Foiln-Ciocalteu method. Gallic acid is used as a standard, results were expressed as gallic acid equivalents (GAE) per gram of fresh mass. Serial dilution of a standard solution (10 mg/ml) of gallic acid was made to obtain the calibration curve [23].

RESULTS

Phytochemical analysis

Results from the phytochemical analysis indicated the presence of reducing sugar, flavonoids and alkaloids (Table 1.0).

Table 1. Phytochemical analysis of the plant extracts. + indicates the presence and - indicates the absence of the organic compounds tested by qualitative color reaction.

Plant	Benedict's test	Frothing test	Borntrager's Test	Flavonoid test	Ferric chloride test	Alkaloid test
Mentha spicata	+	-	-	+	-	+

(Benedict's test - reducing sugar, frothing test - saponins, Borntrager's test - anthroquinones, flavonoid test - flavonoids, ferric chloride test - tannins, alkaloid test-alkaloids)

GC-TOFMS (Gas chromatography- time of flight mass spectrometer) analysis

GC profiling indicated the presence of fatty acid methyl esters (hexa decane, hepta decane, octa decane) terpenoids, terpenoid alcohol, caryophyllene and glycosides. *Mentha spicata* crude extracts indicated the presence of R-(-)-2-amino-1-propanol, 1,2-epoxy-5,9-cyclododecadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 9,12,15-octadecatreinoic cid, methyl ester, (Z,Z,Z)-9,12-octadecadienoic cid, methyl ester, 9,12,15-octadecatrein-1-ol (Z,Z,Z), benzophenone, caryophyllene oxide, 2-cyclohexene,1-ol, 2-methyl-5 (1-methylethenyl), 2-cyclohexene,1-one, 3,5,5 trimethyl-4 (3-oxo-1-butenyl)-, 2-pentadecanone, 6, 10, 14-trimethy-3-butyn-1-ol, 3-cyclohexane-1-carboxaldehyde, 3, 4-dimethyl-, 7-hexadecenoic acid, methy ester, 3-octyn-2-one, 4-O-methyl-12b, 13, 20-triacetoxy-2,9-dihydroxy-3a-carboxy-2,3-seco-tigia-1 (10), 6-diene-3,9-lactone, Bicyclo (2.2.1) heptan-2-one, ethyl isochollate, glucopyranuronamide, hexa decanoic cid, 15 methyl-methyl ester, N-carbobenzyloxy-cysteinylcysteine, octadrine, O-methylisourea hydrogen sulphate, phenylethyamine, p â-dimethyl-, tungsten, dicarbonyl- (u-4-pinocarvone) (1, 2bis (dimethylphosphino) ethane), Z,E-3,13-Octadecedien- 1-ol, Z,Z,Z-1,4,6,9-Nonadecatetraene, -2,5-Dimethoxy-4- (methoxy-sulfonyl) amphetamine, 4,7,7-trimethyl-semicarbazone, and 9-Dodecanoic cid, methyl ester (Table.2.0).

Table 2.Table of suggested chemical constituents of the crude methanolic extracts of the *Mentha spicata* analysed by GC-TOFMS at an acquisition rate of 10 spectra/sec

Sl. No	Name of the compound	Formula	% Area	R.T (s)
1.	(R)-(-)-2-Amino-1-propanol	C_3H_9NO	0.95	345.928
2.	1,2-Epoxy-5,9-cyclododecadiene	$C_{12}H_{18}O$	0.72	410.93
3.	2,5-Dimethoxy-4-(methoxysulfonyl)amphetamine	$C_{12}H_{19}NO_4S$	0.61	302.571
4.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.63	3118.02
5.	9,12,15-Octadecatreinoic cid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	24.88	1760.31
6.	9,12-Octadecadienoic cid, methyl ester	$C_{19}H_{34}O_2$	1.03	1748.79
7.	9, Dodecanoic cid, methyl ester	$C_{13}H_{24}O_2$	0.77	1554.78
8.	9,12,15-Octadecatrein-1-ol (Z,Z,Z)-	$C_{13}H_{24}O_2$	6.83	1757.58
9.	Benzophenone	$C_{13}H_{10}O$	0.43	1173.57
10.	Caryophyllene oxide	$C_{15}H_{24}O$	0.37	1223.65
11.	2-Cyclohexene,1-ol, 2-methyl-5 (1-methyletheny)-	$C_{10}H_{16}O$	0.45	2139.27
12.	2-Cyclohexene,1-one, 3,5,5 trimethyl-4 (3-oxo-1-butenyl)-	$C_{13}H_{18}O_2$	0.16	981.026
13.	2-Pentadecanone, 6, 10, 14-trimethy-	$C_{18}H_{36}O$	0.47	1461.54
14.	3-Butyn-1-ol	C_4H_6O	0.12	369.571
15.	3-Cyclohexane-1-carboxaldehyde, 3, 4-dimethyl-	$C_9H_{14}O$	1.07	903.037
16.	7-hexadecenoic acid, methy ester	$C_{17}H_{32}O_2$	0.85	1532.61
17.	3-Octyn-2-one	$C_8H_{12}O_2$	3.89	1284.60
18.	4 -O-Methyl-12b, 13, 20-triacetoxy-2,9-dihydroxy-3a-carboxy-2,3-seco-tigia-1 (10), 6-diene-3,9-lactone	$C_{27}H_{36}O_{10}$	0.35	1260.75
19.	Bicyclo (2.2.1)heptan-2-one, 4,7,7-trimethyl-semicarbazone	$C_{11}H_{19}N_3O$	0.95	1473.13
20.	Ethy isochollate	$C_{26}H_{44}O_5$	1.08	1312.63
21.	Glucopyranuronamide	$C_{16}H_{25}N_7O_8$	0.71	984.689
22.	Hexa decanoic cid, 15 methyl-methyl ester	$C_{15}H_{30}O_2$	16.37	1561.91
23.	N-carbobenzyloxy-cysteinylcysteine	$C_{14}H_{18}N_2O_5S_2$	0.28	231.842
24.	Octadrine	$C_8H_{19}N$	0.52	1779.49
25.	O-methylisourea hydrogen sulphate	$C_2H_6N_2O$	0.31	1300.57
26.	Phenylethyamine, p, â-dimethyl-	$C_{10}H_{15}N$	0.12	185.555
27.	Tungsten, dicarbonyl- (υ-4-pinocarvone)(1,2-bis(dimethylphosphino)ethane)	$C_{18}H_{30}O_3P_2W$	0.05	1673.4
28.	Z,E-3,13-Octadecedien-1-ol	$C_{18}H_{34}O$	30.64	1774.9
29.	Z,ZZ-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	0.01	873.067

Antioxidant activity

The DPPH radical scavenging method was used to evaluate the antioxidant property of *M. spicata* in comparison with those of known natural antioxidant, ascorbic acid. The concentrations of the plant extracts required to scavenge DPPH showed a dose-dependant response. Free radical scavenging capacity of the extracts were found to be increased with increasing concentrations. The disappearance of DPPH* is directly proportional to the amount of antioxidant present in the reaction mixture. The DPPH (2,2-diphenyl-1-1-picrylhydrazyl radical) radical scavenging activity was found to be 54.84±0.57% with an IC50 value of 25.2 µg/ml when compared to standard Vitamin C which was used as the positive control. The total phenolic content of the crude extracts was found to be 27.26±0.62 mg/g gallic acid equivalent (Table 3.0 & 4.0).

Table 3. % Total phenolic content of the crude methanolic extracts of the selected plants.

Sample	Total phenolic content (GAE)(mg/100g of fresh mass) (mean ± SD)
M. Spicata	27.26±0.62

Values are represented in mean, sd, n= 3 replicates. Control observed at 517 nm (1.725 OD).

Table 4.% Scavenging activity (DPPH) of vitamin C and *Mentha spicata*

Concentration (µg/ml)	Standard % inhibition	Mentha spicata % inhibition
10	23.26±0.86	10.71±0.80
20	37.13±0.32	22.10±0.94
30	46.50±0.30	34.00±0.34
40	59.28±0.45	42.50±0.41
50	68.00±0.09	54.84±0.57
IC_{50}	18	25.2

Discussion

Phytochemical analysis indicated the presence of reducing sugar, flavonoids and alkaloids. Mint extracts had good flavonoid content and total phenolic content [6]. GC profiling indicated the presence of fatty acid methyl esters (hexa decane, hepta decane, octa decane) terpenoids, terpenoid alcohol, caryophyllene and glycosides. A recent report on GC-MS analysis indicated the presence of menthone, isomenthone, and hexadecanoic acid in *Mentha* extracts [15]. Bioassay guided separation of the ethyl acetate soluble portion of *Mentha* extracts afforded 13 compounds including flavonoids, glycosides and lower alcohols and rosmaniric acid [24].

The results from the GC-TOFMS analysis of the present study indicated the presence of 45 compounds including 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 9,12,15-octadecatreinoic cid, methyl ester, (Z, Z, Z)-9, 12-Octadecadienoic acid, methyl ester, 9, 12, 15-Octadecatrein-1-ol (Z, Z, Z)-benzophenone, caryophyllene oxide, 2-cyclohexene,1-ol, 2-methyl-5 (1-methylethenyl), 2-cyclohexene,1-one, 3, 5, 5 trimethyl-4 (3-oxo-1-butenyl)-, 2-pentadecanone, 6, 10, 14-trimethy-3-butyn-1-ol, 3-cyclohexane-1-carboxaldehyde, 3, 4-dimethyl-, 7-hexadecenoic acid, methy ester, 3-octyn-2-one, tungsten, dicarbonyl- (u-4-pinocarvone, 9-dodecanoic cid, methyl ester. A recent study reported that 45 constituents including cis-piperitone epoxide, pulgeone and piperitenone were identified by GC-MS analysis of the essential oil from *mentha* oil [25].

Another study stated that the essential extracted form *M. spicta* contained mainly carvone (50-70%) and menthone [26, 27]. Monoterpenes are the major essential components of the mint including peppermint [28]. The chief components of the essential oil from *M. longifolia* from South Africa were found to be the monoterpene ketone, menthone. Some other chemotypes had carvone, piperitone, β-pinene, cineole, pulgone, limonene, germacrene and β-caryophyllene [29, 30, 31]. External lipophilic methylated flavonoids have been extracted from dried leaves of *Mentha aquatica, Mentha spicata* and *M. x piperita* have been identified by mean of spectrophotometric methods (UV, NMR) [32]. The main element identified in the volatile essential oil of *Mentha s* are menthol (33-60%) menthone (15-32%), isomenthone (2-8%), 1,8 cineol (eucalyptol), (5-13%), methyl acetate (2-11%), menthofuaran (1-10%), limonene (1-7%), B-myrcene, B-caryphyllene, pulegone, carvone [24].

The DPPH (2,2-diphenyl-1-1-picrylhydrazyl radical) radical scavenging activity was found to increase with increasing concentrations, it was found to be 54.84±0.57% with IC50 values of 25.2µg/ml. A latest report indicated that the extracts of spearmint obtained by supercritical fluid extraction had significantly higher antiradical activities varying form 35.62±0.34% to 72±2.17% [33]. The DPPH assay of the crude methanolic extracts of pudina extracts showed an IC50 value of 17.46 µg/ml. One-mg of pudina extracts was equivalent to 500 µg of gallic acid and 487 µg of quercetin. Another study reported 91.5% RSC (radical scavenging) with 16.6 µg/ml of PE by DPPH method [16]. The total polyphenol content of the pudina extracts were expressed as gallic acid equivalent [34]. Significantly high total flavonoid content in the pudina extract may be corroborated with the traditional use of the plant in India in daily life as well as reported use in several free-radical mediated diseases since time immemorial [16]. The essential oil from *Mentha longifera* showed an IC50 of 57.4 µg/ml [25]. Mint extract showed a strong correlation between its antioxidant activity and concentration of which 50% scavenging was obtained IC 50 was found to be 25.8 µg/ml when compared to the synthetic antioxidant BHT (10.1 µg/ml). A direct correlation was between the total phenolic content and DPPH radical scavenging activity was found [6]. The essential oil and different extracts of *M. Piperita* exhibited 70.3±6.1% inhibition of DPPH radical scavenging activity with an IC50 of 15.2 µg/ml when compared to BHT (6.1 ±0.3) [35].

The reported antioxidant activity may be due to the presence of flavonoids which has the scavenging potential by reducing the free radicals. Naringenin a flavanoid found in *Piper. s* was found to possess high super oxide scavenging activity [36]. Most studies on antioxidant compounds in the Lamiaceae family are directed to phenolic diterpenes, flavonoids and phenolic acids [37]. Methyl esters are proven to possess antimicrobial, antioxidant and anti-tumor activity. Various terpenoids have been reported from the petroleum ether extract of *C.officinalis* flowers which included fatty acid methyl esters with proven pharmacological activities including anticancer and antioxidant activity [38] . Antioxidant and antimicrobial properties of methanol extracts of pummelo fruits (*Citrus grandis*) as reported in a recent study revealed the presence of fatty acid methyl esters [39]. GC-MS analysis revealed the presence of more saturated fatty acids than unsaturated fatty acids of halophytic plants, which showed antibacterial and antifungal activities [40]. Over the last decades, an increasing body of evidence has been accumulated on the beneficial effect of polyunsaturated fatty acids both in primary and secondary prevention of cardiovascular diseases. Vast majority of the studies has been performed on long-chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Some important evidences have been raised on the association between alpha linolenic acid (ALA) and cardiovascular mortality [41]. From these reports it is evident that the presence of fatty acid methyl esters (hexa decane, hepta decane, octa decane) terpenoids, terpenoid alcohol and caryophyllene and glycosides and flavonoids may be the factor contributing to the antioxidant property of the extracts.

CONCLUSION

The results from the present study indicated that the crude methanolic extracts of *Mentha spicat*a has antioxidant potential and the presence of flavonoids and fatty acid methyl esters may be the contributing factor for the scavenging potential. Hence it is essential to explore further by characterization and purification of the crude extracts of *Mentha spicata* to understand the underlying mechanisms for the antioxidant potential.

REFERENCES

[1] Wannissorn, B., Jarikeserm, S., Siriwangchai, T and Thubthimthed, S. 2005. Antimicrobial properties of essential oils from Thai medicinal plants, *Fitoterapia,* 76: 233-236.

[2] Mata, A.T.C., Proenca, A. R., Ferreira, M. L .M ., Serraleiro, J. M. F., Nogueira, M. E. M, Araujao. 2007. Antioxidant and acetyl cholinesterase activities of five plants used as Portuguese food species. *Food. Chem*, 103: 778-786.

[3] Halliwell B. 2007. "Dietary polyphenolics: good, bad or indifferent for your health?" *Cadiovascular Research,* 73 (2): 341-347.

[4] Rois, A. D.O, L. M.G, Antunes, M. D.L. P, Bianchi. 2009. "Bixin and Lycopene modulation of free radical generation induced by cisplatuin-DNA interaction". *Food Chemistry,* vol 133:1113-1118

[5] Ighodaro, O. M., Agunbiade, S.O and O. A. Akintobi. 2010. Phytotoxic and antimicrobial activities of flavonoids in *Ocimum gratissimum, Eurpoean Journal of Applied sciences* 2 (1): 37-40.

[6] Sweetie, R., Kanatt, Ramesh Chander., Arun Sharma. 2007. Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. *Food Chemistry* 100: 451-458.

[7] Reverchon E, 2006. Review supercritical fluid extraction and fractionation of natural matter. *Journal of supercritical fluids* 38: 146-166.

[8] Choudury, P., Kumar, R and Garg. A. N. 2006. Analysis of Indian mint (*Mentha spicata*) for essential , trace and toxic elements and its antioxidant behaviour. *J. Pharm Biomed Anal,* 41: 825-832.

[9] Bimakr, Mandana., Russly, Abdul. Rahman., Farah, Saleena., Taip, Ali Ganjiloo, Liza Md, Salleh., Jinap, Selamat., Azizh, Hamid., I. S.M. Zaidul. 2011. Comparison of different extraction for the extractiom of major bioactive flavanoid compounds from spearmint (*Mentha spicata* L.) leaves. *Food and Bioproducts Engineering,* 89:67-72.

[10] Moreno, L., Bella, R., Primo-Yufera, E., and Esplugues, J. 2002. Phramacological properties of the methanol extract from *Mentha suaveolens* Ehrh. *Phytotherapy. Research,* 16:10-13.

[11] Lawrence B. M. 2006. Mint: The Genus Mentha, CRC Press, Boca Raton.

[12] Znini, M., M, Bouklah., L, Majidi., S, Kharchouf,, A, Anouniti., A, Bouyanzer., B, Hammouti. 2010. Chemical composition and inhibitory effect of Mentha spicata essential oil on the corrosion of steel in molar hydrochloric acid. *Int J. Electrochem. S Co,* 6: 691-704.

[13] Daferera, D. J., Ziogas, B. N and Polissiou, M. G. 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea, Fusarium. sp* and *Clavibacter michiganensis*. *Crop protection,* 22: 39-44.

[14] Kaur, C., and Kapoor, H .C. 2002. Antioxidant activity and total phenolic content of some Asian vegetables. International *Journal of Food Science and Technology*, 37 (2): 153-161.

[15] Padmini E, A. Valarmathi M, Usha Rani. Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camella sinensis. Asian .J. exp. Biol. Science,* 1 (4) 2010*:* 772-781.

[16] Akhilesh kumar, Sharmila Chaatopadhyay. 2007. DNA damage protecting activity and antioxidant potential of pudina extracts. *Food chemistry* 100:1377-1384.

[17] Naczk, M., 2004. Extraction and analysis of phenolics in fo. *J Chromatogr A,* 1054: 95-11.

[18] Sreenivasan S, Ibrahim D, Mohd Kassim M. J. N. 2007. "Free radical scavenging activity and total phenolic compounds of *Gracilaria changii". International Journal of Natural Engineering Sciences,* 1 (3): 115-117

[19] Goli A, Barzegar, M and Sahari M, 2005. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chemistry* 92:522-525.

[20] Harborne. J. B. 1993**.** Phytochemistry. Academic Press, London, pp 89-131.

[21] Carmen, Gherman., Monica, Culea., O, Cozar. 2000. Comparative analysis of some active principles of herb plants by GC/MS. *Talanta*, 53, 1:10-253-262.

[22] Ramesh B & Satakopan V N. (2010). *Invitro* antioxidant activities of *Ocimum* species: *Ocimum basilicum* and *Ocimum sanctum*. *Journal of Cell and Tissue Culture Research* , 10 (1): 2145-2150.

[23] Kumar, S, Kumar, D, Prakash, O. 2008. "Evaluation of antioxidant potential, phenolic and flavonoid contents of *Hibiscus. t* flowers". *Eletronic journal of Environmental, Agricultural and food chemistry,* 7 (4) 2863-2871.

[24] Satoshi, Yamamura., Koichiro, Ozawa., Kazuhiro, Ohtani,, Ryoji, Kasai and Kazuo, Yamasaki. 1998. Antihistaminic flavones and aliphatic glycosides from *Mentha spicata*. *Phytochemistry.* Vol 48 (1):131-136.

[25] Gulluce M, F. Sahin, M. Sokmen, H. Ozer, D.Daferara . A. Sokmen, M Poliisiou , A Adiguzel, H. Ozkan. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract form Mentha Longifolia L. ssp. Longifoila. *Food Chemistry* 103: 1449-1456.

[26] Aflatuni. A. 2005. The yield and essential oil content of mint Mentha spp in Northern Ostrobonthia. (http://herkules.oulu.fi). Oulu University Press, Finland, pp 52 .

[27] Hussian, A.I. 2009. Characterization and biological activities of essential of some species of Lamiaceae: Ph. D Thesis, Faisalbad, Pakistan, pp 257.

[28] Soheil, S., Mahmoud, Mathew, Williams., Rodney, Croteae. 2004. Co-suppression of limoene-3-hydroxylase in peppermint promoted accumulation of limonene in the essential oil. *Phytochemistry* 65: 547-554.

[29] Oyedeji , O. and Afloyan, A.J. (in press). Chemical composition and antibacterial activity of essential oil of the South African *Mentha longifolia*. *Journal of Ethnopharmocology.*

[30] Younis, Y. M. H., and Beshir, S. H. 2004. Carvone-rich essential oils from *Mentha longifolia* (L.) Huds. ssp. *Schimperi Briq* and *Mentha spicata* L., Grown in Sudan. *Journal of Essential oil research,* 16(6):539-541.

[31] Karousou, R. A., Lanaras, T. B and Kokkini, S. 1998. Piperitone oxide-rich essential oils from *Mentha longifera* subsp. Petiole and M. x. villoso-nervata grown wild on the Island of Crete (S.Greece). *Journal of essential oil research,* 10(4), 375-379.

[32] Bernard, Voirin., Chridtine, Bayet., Olivier, Faure., Frrederic, Jullien. 1999. Free flavonoid aglycones as markers of parentage in *Mentha aquatica*, M. citrate and M.x. piperita. *Phytochemistry* 50:1189-1193.

[33] Mandana B., Russly A. R., Ali, G and Farah, S. T. 2011. Antioxidant activity of spearmint (*Mentha spicata* L) leaves

extracts by supercritical carbon dioxide (SC-CO2) extraction. *International Food Research Journal* 18: 543-547.

[34] Capecka, E., Mareczek, A., and Leja 2005. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food chemistry,* 93:223-226.

[35] Rajender, singh., Muftah, A.M., Shushni., Asma, Belkheir. 2011. Antibacterial and antioxidant activities of *Mentha Piperita* L. Arabian Journal of Chemistry - article in press.

[36] Subramaniam V, Adenan M.I, Ahmad A.R, Sahdan R. 2003. "Natural antioxidants: Piper s. (Kadok) and Morinda. e (Mengkudu)". *Malaysian Journal of Nutrition,* 9 (1): 41-51.

[37] Kivilompo, M and Hyotylainen, T. 2007. Comprehensive two-dimensional liquid chromatography in anlysis of Lamiaceae herbs: characterization and quantification of antioxidant phenolic acids, *J. Chromatogr. A,* 1145:155-164.

[38] Muley, B. P., S. S Khadabadi., N. B. Banarase. 2009. "Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae):A Review". *Tropical Journal of Pharmaceutical Research*, 8 (5): 455-465.

[39] Mahtook S. M, Toshihiko S. 2006. "Antioxidant and antimicrobial activities of the methanol extracts from (*Citrus grandis Osbeck*) fruit albedo tissues". *Eur. Food. Res. Technol,* 224: 39-47.

[40] Manivachagam. C., Krishnan. K, Venugopalan V. 2008**.** "Antimicrobial activity of fatty acid methyl esters of some members of chenopodiaceae". *Z. Naturforsch.,* 63: 331-336.

[41] William S. Harris. 2005. "Alpha-Linolenic Acid: A Gift From the Land?" *Circulation,* 111:2872-2874.