European Journal of Medicinal Plants

26(1): 1-12, 2018; Article no.EJMP.45751
ISSN: 2231-0894, NLM ID: 101583475

GC-MS Analysis, Antioxidant and Cytotoxic Activities of *Mentha spicata*

Heba Abdel-Hady[1*], Eman Ahmed El-Wakil[1] and Mahfouz Abdel-Gawad[1]

[1]Department Medicinal Chemistry, Theodor Bilharz Research Institute, Warrak El-Haddar, Imbaba, 30 P.O. 12411, Giza, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/45751
Editor(s):
(1) Dr. Patrizia Diana, Professor, Department of Molecular and Biomolecular Sciences and Technologies, University of Palermo, Palermo, Italy.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Kevison Romulo da Silva França, Federal University of Campina Grande, Brazil.
(2) Veerareddy Arava, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/27779

Original Research Article

Received 19 September 2018
Accepted 01 December 2018
Published 16 December 2018

ABSTRACT

Aims: *Mentha spicata* medicinal properties are well known. In this study, Total phenolic and flavonoid contents and the antioxidant activity of the methanol extract of *Mentha spicata* were determined as well as evaluation of the cytotoxic activity of it. Also, the identification of some bioactive compounds in the plant was analysed.
Place and Duration of Study: Extraction and antioxidant at Medicinal Chemistry Department, Theodor Bilharz Research Institute, cytotoxic evaluation and GC-Ms analysis at Al-Azhar University, Egypt.
Methodology: The antioxidant activity was determined by two methods, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2-2`azinobis (3-ethylbenzthiazoline-6-sulphonic acid) while, the cytotoxic assay was determined by MTT assay on HepG-2 (Human hepatocellular carcinoma) and MTC-116 (Human colon carcinoma). Regarding to the chemical identification of methanol extract was carried out by GC-MS analysis.
Results: The study proved that *Mentha spicata* has high Total phenolic and flavonoid contents (388.20±2.38mg GAE/gm of extract &204.01±17.93mg RE/gm of extract) respectively. Also, exhibited promising antioxidant activity by DPPH & ABTS (IC_{50}=65.13±1.29 µg/ml & 52.31±0.81

**Corresponding author: E-mail: h_hady10@yahoo.com;*

µg/ml) respectively. The methanol extract of the plant showed a good cytotoxic effect on HepG2 and HTC-116 (IC_{50}=25.2±3.6µg/ml & 62.1±4.9µg/ml) respectively. GC-MS analysis of the methanol extract of *Mentha spicata* showed 43 oxygenated hydrocarbon compounds. The major ones are Hexadecanoic acid, methylEster (palmitic acid ester) (31.51%) followed by 9,12,15-Octadecatrienoic acid,methyl ester (CAS)(methyl linolenate) (22.10%), 2-Pentadecanone,6,10,14-trimethyl- (CAS)(6.82%), Phytol (6.20%), 9,12-Octadecadienoic acid(Z,Z)-, methyl ester (6.18%), Hexadecanoic acid (palmitic acid) (5.95%) and Methyl stearate (4.49%).
Conclusion: *Mentha spicata* is a potential antioxidant and anticancer agents.

Keywords: ABTS; DPPH; cell viability; HepG2; HTC-116; inhibition percentage.

1. INTRODUCTION

Medicinal plants are used as therapies for diseases. According to WHO reported that more than 80% of world populations depend on traditional medicine for their health needs [1]. Many species of fruits, vegetables, herbs, spices and seeds have been demonstrated their antioxidant activity to protect from cell damage, diseases and cancers that induced by oxidative stress [2].

Natural antioxidants as medicinal plants are recommended to use due to their safety while, the commercially ones that have been used as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetic and have negative health problem [3].

Also, cytotoxic activity of many medicinal plants and herbs has been reported as well as the antioxidant properties of them [4]. Herbs contain a variety of phytosterols, phenolic acids, triterpenes, flavonoids, anthocyanins, saponins and carotenoids have been shown to apply cancer chemo-preventive and antioxidant activities [5].

Mentha is a member of family Lamiaceae (Labiatae) with about 220 genera and nearly about 4000 species worldwide. The two major forms of Lamiaceae named: *Mentha piperita* L. and *Mentha spicata* L. The English name is spearmint. *Mentha* species are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor [6].

Mentha spicata has a smooth grey haired leaves with long and narrow spike carries on its top a pale blue flowers [7]. Moreover, it was used in traditional medicine as it is potent in common cold, sinusitis, cough, bronchitis, nausea, vomiting, indigestion, intestinal Colic and appetite loss [8].

It has been documented that aqueous extract of *Mentha spicata* and its essential oil were effective in gastro-intestinal disorders as well as anti-microbial, anti-inflammatory and anti-tumoral activity on some cancer cell lines [9]. Because of, much less studies have been reported on methanol extract of *Mentha spicata*. Therefore, the objective of this study is (1), to evaluate the antioxidant and anticancer activities of *Mentha spicata* methanol extract on human hepatocellular carcinoma (HepG-2) and human colon carcinoma (HCT-116) cell lines. (2), to identify the chemical compounds responsible for Activity of *Mentha spicata* methanol extract collected from Egypt by GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Chemicals

Dimethyl sulfoxide (DMSO), MTT and trypan blue were purchased from Sigma (St. Louis, Mo., USA). DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, ABTS [2-2`azinobis (3-ethylbenzthiazoline-6-sulphonic acid)], Gallic acid, Rutin, Ascorbic acid and Folin-Ciocalteau reagent were obtained from Sigma-USA. Sodium hydrogen phosphate and Potassium persulphate, Sodium nitrite, Sodium hydroxide, Sodium bicarbonate and Aluminum chloride were purchased from Merck (Germany). Fetal Bovin serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine gentamycin and 0.25% trypsine-EDTA were purchased from Lonza.

2.2 Biological Materials

2.2.1 Cell lines

Human hepatocellular carcinoma (HepG2) and human colon carcinoma (HCT-116) cells were obtained from The American Type Culture Collection (ATCC, Rockville, MD).

2.2.2 Plant material

The whole plant was obtained from the Local market in Giza, Egypt. It was identified by Professor El-Sayed Hassan Hussien Shaban, Head of Aromatic and Medicinal Plant Research Department, Agriculture Research Centre. The plant dried and grounded into powder then stored for extraction.

2.3 Plant extraction

500 gm of dried *Mentha spicata* were soaked in 85% methanol for one week then, the extract was filtered through Whatman No.1 filter paper for several times. After that, the filtrate was concentrated by Buchi Rotatory evaporator at 60^0C to remove methanol completely. Finally, the crude dried methanol extract was kept in Medicinal Chemistry department, Theodor Bilharz Research Institute for more studies.

2.3.1 Total phenolic content

The phenolic content of the methanolic extract of *Mentha spicata* was determined using a spectrophotometric method described by Abdel-Hady et al. [10]. 0.5ml of the extract (250 µg/mL); 2.5ml of Folin- Ciocalteus reagent (10 %) dissolved in water and 2.5ml NaHCO3 (7.5%). Blank sample contains 0.5ml MeOH, 2.5ml of Folin-Ciocalteus reagent (10 %) dissolved in water and 2.5ml NaHCO3 (7.5%). Gallic acid was used as the standard contains 2.5ml gallic acid (200 µg/ml), 2.5ml of Folin-Ciocalteus reagent (10 %) and 2.5ml of NaHCO3 (7.5%). All mixtures were shaken and incubated at 45°C for 45 min. the absorbance was recorded at 765 nm against a blank sample. The experiment was carried out in triplicate. The total phenolic content was expressed in mg gallic acid equivalent (GAE) per gram dry weight of the extract (mg GAE/gm dry extract).

2.3.2 Total flavonoid content

The content of flavonoids of methanolic extract of *Mentha spicata* was determined using a colorimetric assay reported by Rohman et al. [11]. 0.5 ml of the extract was mixed with 2ml distilled water and 150µl of NaNO2 (5%) for 6 min, then 150µl of AlCl3 (10%) was added and allow to stand for 5 min then added of 2ml of NaOH (4%) and adjusted to 5ml with 200µl distilled water. Blank sample contains 0.5ml MeOH instead of extract and rutin was used as standard. The mixture was incubated at room temperature for 15 min. the absorbance was measured at 510 nm against a blank sample. The experiment was carried out in triplicate. The total flavonoid content was estimated as mg rutin equivalents (RE) per gram extract (mg RE/g of extract).

2.4 Antioxidant Assays

2.4.1 DPPH radical scavenging assay

The antioxidant activity of *Mentha spicata* methanol extract was evaluated using DPPH free radical scavenging method according to Akroum et al. [12]. Various concentrations of the extract from 5-500 µg/ml were prepared. 2 ml of each extract concentration was mixed with 2ml of DPPH in MeOH (0.1 mM/l). The control contained solvent and DPPH without extract. The mixtures were shaken well and kept in dark for 30 min at 37˚C. The absorbance was measured at 517nm. Ascorbic acid was used as standard. The experiment was carried out in triplicate. The DPPH scavenging % of the plant extract was calculated from this equation.

$$\text{Scavenging activity \%} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample. Data were expressed as IC_{50}. The lower IC_{50} value is an indication of more powerful antioxidant activity.

2.4.2 ABTS radical scavenging assay

The plant extracts can able to quench $ABTS^{\cdot}$+ (2-2`azinobis (3-ethylbenzthiazoline-6-sulphonic acid) in comparison to Trolox® [13]. ABTS assay was evaluated according to Re et al. [14] with some modifications. The concentrated reagent solution was prepared by dissolving 9.6 mg ABTS in 2.5ml water(final concentration 7mM) and then adding 110µl of a solution made by dissolving 37.5 mg of potassium persulphate (K2S2O8) in 1ml of water (final concentration 2.45mM) to produce $ABTS^{\cdot+}$ radical cation. The stock solution was kept in the dark room for 12-16 hours before use; for study the $ABTS^{\cdot}$+solution was diluted to an absorbance value 0.700±0.020 at wavelength 734 nm. Subsequently, 100µl of aqueous or alcoholic plant extract (according to solubility) with various concentrations from 5-500 µg/ml was added to 1ml of work solution, and it was measured exactly after 2.5 min. Also, an appropriate solvent blank was measured. $ABTS^{\cdot+}$ methanol are used as control. The experiment was carried

out in triplicates. Results were expressed in terms of mm Trolox® equivalent per 100 g dry weight of plant extract. The ABTS scavenging % was calculated from the following equation.

$$\text{Scavenging activity \%} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample. Data were expressed as IC_{50}. The lower IC_{50} value is an indication of more powerful antioxidant activity.

2.5 Anticancer Assay

Antitumor assay of *Mentha spicata* methanol extract was carried out according to Mosmann [15,16] on HepG-2 cells (Human hepatocellular carcinoma) and HCT-116 cells (human colon carcinoma). The cells were grown on RPMI-1640 medium with 10% inactivated fetal calf serum and 50µg/ml gentamicin then maintained in humidified atmosphere at 37ºC with 5%CO_2. The cells were subcultured two or three times a week. After that tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in 96-well tissue culture plates. After incubation for 24 hr. the concentration of tested extract was added in into96-well plats (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/ml) (three replicates). 0.5% DMSO was run as negative control. After incubating for 24hr., the number of viable cells was determined by MTT test. As, briefly the media was removed from the 96 well plat and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of 12mM MTT stock solution (5 mg/ml PBS) was added to each well. The 96 well plate were incubated at 37ºC and 5% CO_2 for 4 hr. and 85 µl aliquot of the media was removed from wells and 50 µl DMSO was added then incubated at 37ºC for 10 min. the optical density was measured at 590nm with the microplate reader to determine the viable cells number and the percentage of viability was calculated as:

$$[1-(ODt/ODc)] \times 100\%$$

Where, ODt is the mean optical density of wells treated with the tested sample while, ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC_{50}), the concentration that is required to cause toxic effect to 50% of cells was estimated.

2.6 GC-MS Analysis

The methanol extract of *Mentha spicata* was subjected to GC-MS analysis using Thermo Scientific TRACE 1310 Series Gas Chromatograph on Helium as a carrier gas in TG-SQC column. Analysis of extract was held on the following temperature program: initial temperature 50ºC for 1min then increased to 250ºC for 5 min, finally increased to 290ºC for 2min. The extract was injected in split mode constant flow 1.5ml/min. Mass spectral range was set as 40-1000Hz also, mass transfer line temperature was 300ºC and ion source temperature was 300ºC then identification of components was carried out by using their MS data compared to the NIST mass spectral library.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic and Flavonoid Contents

Phenolic and flavonoid compounds are the main secondary metabolites act as natural antioxidants in plants [17]. They are mainly responsible for antioxidant activity due to their hydroxyl group and their ability to act as hydrogen donors [18,19,20]. The results revealed that methanolic extract of *Mentha spicata* showed high phenolic content (388.20±2.38mg GAE/gm of extract) as well as flavonoid content (204.01±17.93mg RE/gm of extract). The Phenolic and flavonoid contents of *Mentha* species are frequently studied through literature and comparing between them is so difficult due to the difference in methodology. However, Mata et al. [21] studied the phenolic content of *Mentha* species and proved that *M. spicata* contains the most total phenolic content while, Nickavar *et al*. [3] also studied the phenolic content for five *Mentha* species from Iran and his results revealed that *M. spicata* was the lowest one. Also, Dorman et al. [22] proved that total phenolic content of different *Mentha* species ranged from 128-230 mg GAE/gm of extract.

3.2 Antioxidant Assays

There are numerous assays to investigate the antioxidant activity of the plants. Among these assays, DPPH and ABTS which are common and promising methods to investigate antioxidant activity and free radical scavenging ability of the extracts [3]. The two assays are perfect ones to determine the scavenging activity of hydrogen donating and chain breaking antioxidants [23]. In this study, the IC_{50} of methanol extract obtained from *Mentha spicata* as well as the inhibition percentage of the extract for concentrations

ranged from 5-500µg/ml were measured by the two assays. According to DPPH, *Mentha spicata* methanol extract revealed obvious antiradical activity, the plant inhibition concentration of 50% of DPPH concentration (IC_{50}) was (65.13±1.29 µg/ml) as shown in Table 1. Also, the plant showed inhibition percentage ranged from 9%-95% at concentrations from 5-500µg/ml as shown in Fig 2. On the other hand, the plant demonstrated that ABTS scavenging activity is weaker than DPPH. IC_{50} which represents the required concentration to inhibit 50% of ABTS was (52.31±0.81 µg/ml) as shown in Table 1. Also, the plant showed inhibition percentage ranged from 7%-80% at concentrations from 5-500µg/ml as shown in Fig 2. The lower IC_{50} observed for ABTS than DPPH may be due to the variation in redox potentials or in other words, the mechanism of the reaction between each radical; ABTS and DPPH; and the antioxidant compounds in the extract is different than each other [3,24]. However, the antioxidant activity of aqueous and methanol extracts of *Mentha spicata* was proved previously by [17,18]. In fact, the methanol extract of *Mentha spicata* had high phenolic and flavonoid content. In earlier study, it was reported that the antioxidant activity was due to the presence of polyphenolic compounds in the plant [25,26,10] but it must be taken in consideration that the antioxidant activity might be attributed not only to phenolic and flavonoid compounds but also to the other chemical compounds in the extract as shown in Table 2 [27,19].

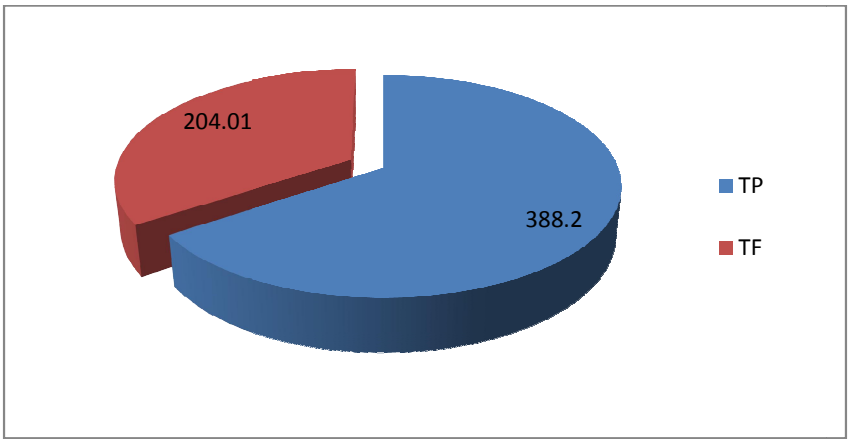

Fig. 1. Total phenolic and flavonoid contents of methanol extract of *Mentha spicata*

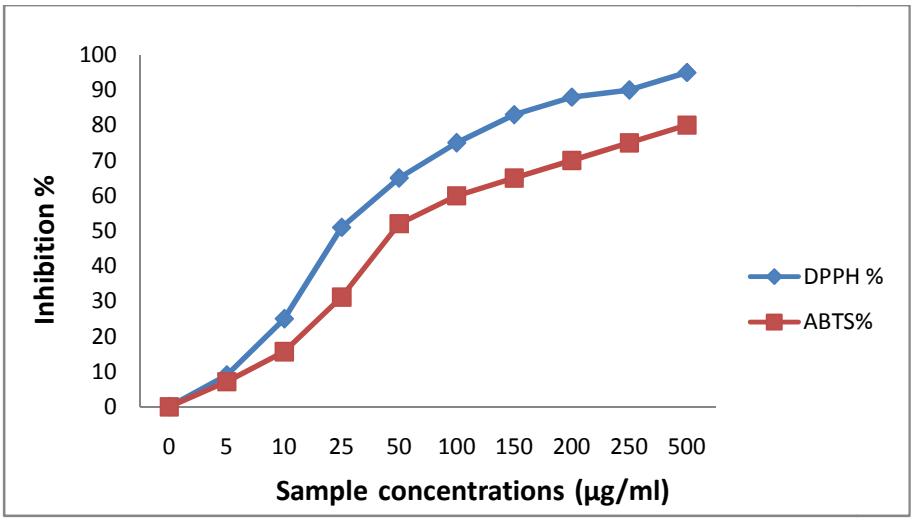

Fig. 2. Inhibition percentage of *Mentha spicata* methanol extract by DPPH and ABTS

Table 1. IC_{50} values of DPPH and ABTS scavenging activities of *Mentha spicata*

The plant	IC_{50} of DPPH (µg/ml)	IC_{50} of ABTS (µg/ml)
Mentha spicata	65.13±1.29	52.31±0.81

3.3 Anticancer Assay

Cancer is the principle causes that threating life as it is malignant expansion of cells ends with death [28]. Anticancer activity of some medicinal plants has been well known [29, 30, 31]. In our survey the anticancer activity of *Mentha spicata* methanol extract was investigated on HepG-2 and HCT-116 cell lines by MTT assay. The results proved that the methanol extract of the plant has promising inhibitory activity on HepG-2 cell lines (IC50= 25.2±3.6µg/ml) while it has inhibitory activity on HCT-116 cell lines (IC50=62.1±4.9µg/ml). Also, the plant showed cell viability in concentration dependent – manner. The cell viability determined by treating HepG-2 was < 6% at 500µg/ml while, by treating HCT-116 was <10% at 500µg/ml as shown in Fig 3. The cytotoxicity of *Mentha* species has been reported by Hoffman [32]. Hajighasemi et al. [9] proved the cytotoxic activity of aqoueous extract of *Mentha spicata* leaves on both human and animals. Previous studies on *Mentha* sp. Proved that it has been used in treatment human cervical carcinoma [33]. Also, Shirazi et al. [34] proved the cytotoxic activity of methanol extract of *Mentha pulegium* on ovarian adeno-carcinoma (SK-OV-3), human lung carcinoma (A549) and human malignant cervix carcinoma (Hela).

3.4 GC-MS Analysis

Gas chromatography mass spectroscopy analysis was carried out on the methanol extract of *Mentha spicata*. GC-MS chromatogram of the plant showed 43 peaks as shown in Table 2 & Fig. 4. GC-MS analysis of the methanolic extract of *Mentha spicata* was identified after comparison of mass spectra with libraries and the compounds were enumerated along with their molecular formula, retention time and peak area (Figs. 5-14). The major compounds were identified as: Hexadecanoic acid,methyl Ester (31.51%), 9,12,15-Octadecatrienoic acid,methyl ester (Synonym: methyl linolenate) (22.10%), 2-Pentadecanone,6,10,14-trimethyl (6.82%), Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol) (6.20%), 9,12-Octadecadienoic acid (Z,Z), methyl ester (6.18%), Hexadecanoic acid (Synonym: Palmitic acid) (5.96%) and Methyl stearate (4.49%). Our results are in full agreement with other previous studies that have been proved the presence of these compounds on methanol extract of *Mentha spicata* and the essential oil of different *Mentha* species [5,35]. Also, these results agreed with Boukhebti et al. [36] who proved the presence of some minor compounds in the methanol extract as major in the essential oil of *Mentha spicata*.

However, the biological properties of these compounds were exhibited previously. As Hexadecaionic acid, methyl ester is reported as antioxidant, anti-inflamatory and hypochlosterolemic agent [37]. Also, 9,12,15-Octadecatrienoic acid, methyl ester (CAS)(methyl linolenate) is said to be anti-inflamatory, cancer preventive,

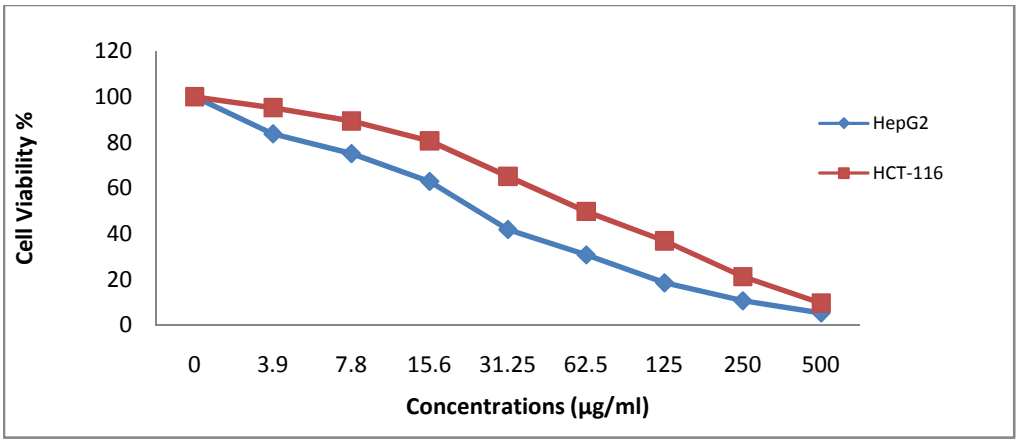

Fig. 3. Cytotoxicity activity of *Mentha spicata* methanol extract on HepG-2 and HCT-116

Table 2. Chemical constituents present in the methanolic extract of *Mentha spicata*

No	RT	Area %	M.WT	M. Formula	Name
1	7.59	0.08	154	C10H18O	Dihydro-carveol
2	8.05	0.11	152	C10H16O	Limonene oxide
3	8.45	0.30	150	C10H14O	D-Carvone
4	12.25	0.19	204	C15H24	á-copaene
5	13.07	0.14	212	C12H20O3	Cyclohexan-1-ol-2-carboxylic acid, 2-allyl-3-methyl-, methyl ester (1R,2S)-
6	13.29	0.30	202	C15H22	trans-calamenene
7	13.37	0.09	206	C14H22O	Phenol, 2,4-bis(1,1-dimethylethyl)-
8	14.26	1.29	220	C15H24O	(+) spathulenol
9	14.35	0.19	220	C15H24O	Caryophyllene oxide
10	14.84	0.45	222	C15H26O	Cubenol
11	15.50	0.27	222	C15H26O	1,1,4,7-Tetramethyldecahydro-1H-Cycloprop A [E] Azulen-4-Ol
12	16.08	0.15	220	C15H24O	trans-Z-à-Bisabolene epoxide
13	16.82	0.66	242	C15H30O2	Methyl tetradecanoate
14	18.20	0.25	280	C15H20O5	Tetraneurin- A-Diol
15	18.77	0.30	252	C16H28O2	14-Pentadecynoic acid, methyl ester (CAS)
16	19.16	6.82	268	C18H36O	2-Pentadecanone,6,10,14-trimethyl- (CAS)
17	19,99	0.46	270	C17H34O2	Pentadecanoic acid, 14-methyl-, methyl ester (CAS
18	20.27	0.21	254	C16H30O2	9-Hexadecenoic acid (CAS)
19	20,85	31.51	270	C17H34O2	Hexadecanoic acid, methylEster
20	22.03	5.96	256	C16H32O2	Hexadecanoic acid (Palmitic)
21	22.56	0.46	284	C18H36O2	Heptadecanoic acid, methylEster
22	22,84	0.10	282	C18H34O2	9-Octadecenoic acid (Z)- (CAS
23	23.40	0.20	282	C18H34O2	Oleic acid
24	23.79	6.18	294	C19H34O2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
25	23.98	22.10	292	C19H32O2	9,12,15-Octadecatrienoic acid, methyl ester (CAS) (methyl linolenate)
26	24.16	6.20	296	C20H40O	Phytol
27	24.40	4.49	298	C19H38O2	Methyl stearate
28	24.78	0.21	268	C18H34D2O	2,2-Dideutero,octadecanal
29	25.00	1.23	306	C20H34O2	8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (CAS)
30	25.35	0.43	341	C21H29N2O2	10-Methylethoxy-NB-Alphamethylcorynantheol
31	25.93	0.55	296	C20H40O	3,7,11,15-Tetramethyl-2-hexade cen-1-ol
32	26.71	2.85	258	C16H34S	tert-Hexadecanethiol
33	27.18	0.85	431	C24H33NO6	Benzyl 3-oxo-5-(nitro-2-oxocyclodode cyl)pentanoate
34	27.28	0.51	306	C20H34O2	8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (CAS)
35	27.75	1.03	326	C21H42O2	Eicosanoic acid, methyl ester
36	27.85	0.83	318	C21H34O2	Methyl arachidonate
37	28.70	0.71	296	C20H40O	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
38	29.18	0.10	344	C18H16O7	Quercetin 7,3',4'-Trimethoxy
39	30.87	0.28	354	C23H46O2	Docosanoic acid, Methyl ester
40	31.13	0.46	390	C24H38O4	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS)
41	31.56	0.35	312	C20H40O2	Ethanol, 2-(9-octadecenyloxy)-,(Z)- (CAS)
42	34.27	0.12	280	C15H20O5	Tetraneurin – A-DIOL
43	42.98	0.32	414	C29H50O	Stigmast -5-EN-3-OL,(3á,24S)-

hypochlosterolemic and hepatoprotective [38]. Regarding to Phytol, 2Pentadecanone,6,10,14-trimethyl, Hexadecanoic acid and Methyl stearate are reported as antioxidants, cancer preventives, antidiarrheal, anti-inflamatory, pesticides, nematicides and antimicrobials [39,40,41,42,10].

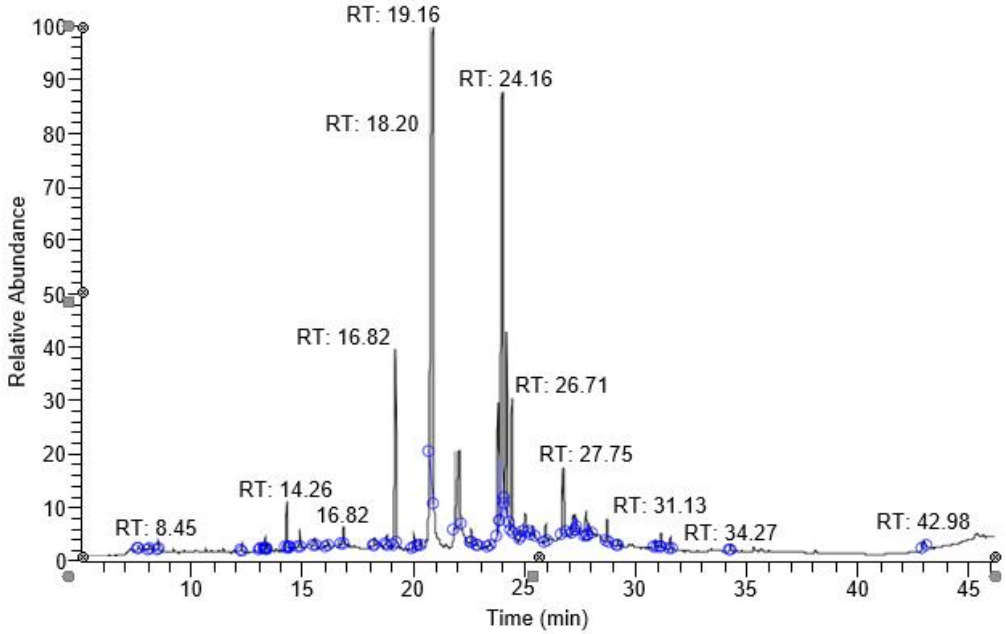

Fig. 4. Total ion chromatogram of methanol extract of *Mentha spicata*

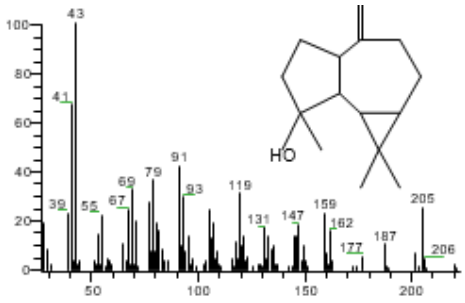

Fig. 5. spathulenol

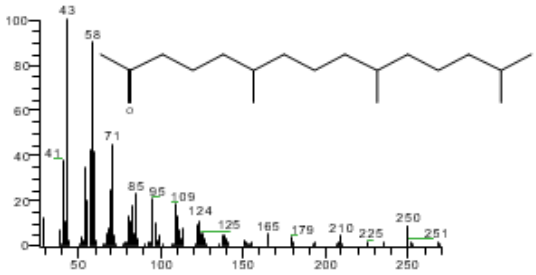

Fig. 6. 2-Pentadecanone,6,10,14-trimethyl

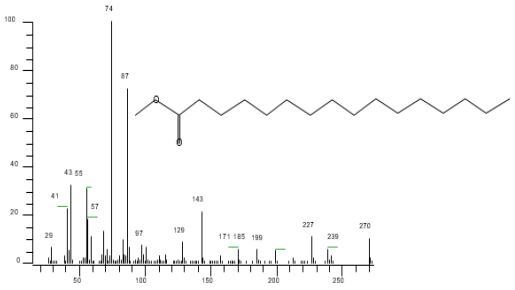

Fig. 7. Hexadecanoic acid, methy Ester

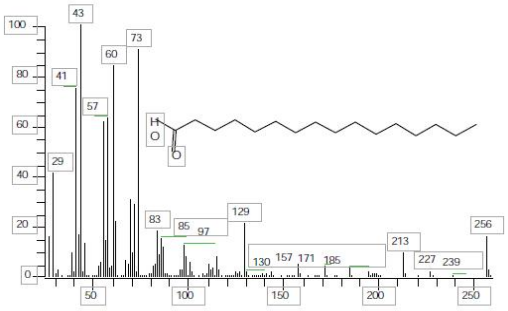

Fig. 8. Hexadecanoic acid

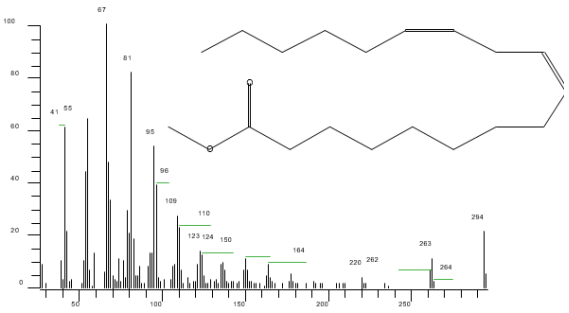

Fig. 9. 9,12-Octadecadienoic acid

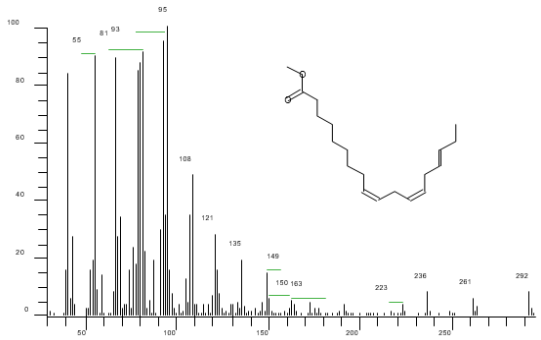

Fig. 10. Methyl linolenate

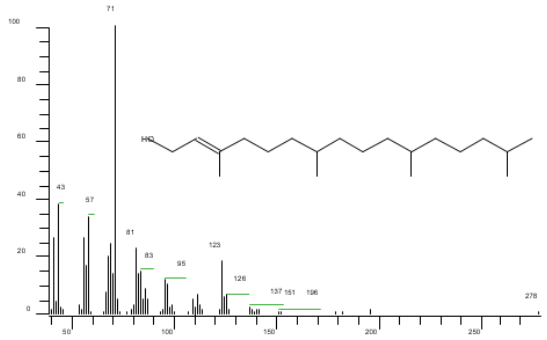

Fig. 11. Phytol

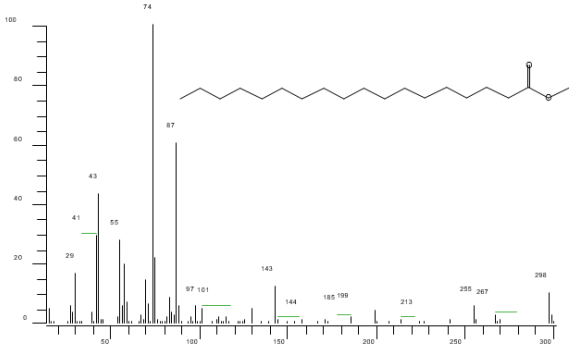

Fig. 12. Methyl stearate

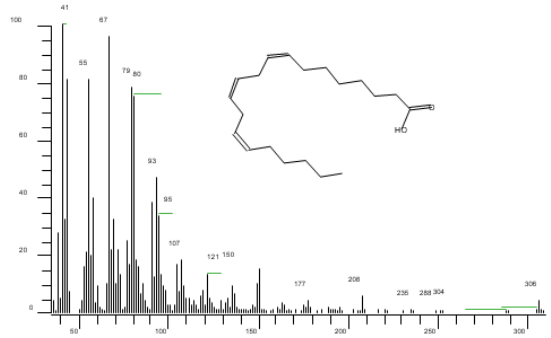

Fig. 13. 8,11,14-Eicosatrienoic acid

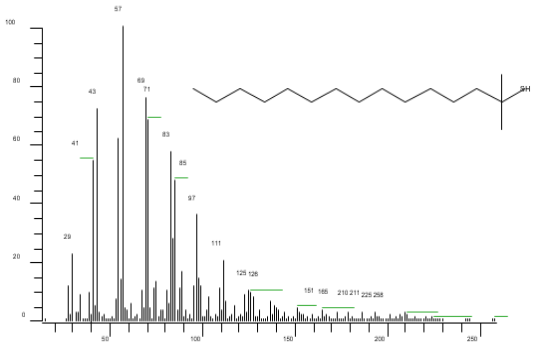

Fig. 14. Tert-Hexadecanethiol

Figs. 5-14. Mass spectrum of the ten major compounds

4. CONCLUSION

The presence of Hexadecanoic acid, methyl Ester, methyl linolenate, Phytol and Palmitic acid may be responsible for the antioxidant and the anticancer activities. Working in synergy with the extract to exert the biological activities of all other phytochemicals of *Mentha spicata* by chromatographic isolation will be our future plan.

DISCLAIMER

This manuscript was presented in the conference.

Conference name: - 18th Annual Pharmaceutical and Chemical Analysis Congress

Available link: - https://analysis.pharmaceuticalconferences.com/abstract/2018/gc-ms-analysis-antioxidant-and-cytotoxic-activities-of-mentha-spicata

November 05-06, 2018 Madrid, Spain

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that there is no competing interests exist.

REFERENCES

1. Keawsaard S, Kongtaweelert S. Antioxidant, antibacterial, anticancer activities and chemical constituents of the essential oil from *Mesua ferrea* leaves. Chiang Mai J. Sci. 2012;39(3):455-463.
2. Elmastaş M, Dermirtas I, Isildak O, Aboul-Enein HY. Antioxidant activity of s-carvone isolated from spearmint (*Mentha spicata* L. Fam Lamiaceae). J Liquid Chrom Related Technol. 2006; 29(10):1465-1475.
3. Nickavar B, Alinaghi A, Kamalinejad M. Evaluation of the antioxidant properties of five *Mentha* species. Iran J Pharm Res. 2008;7(3):203–209.
4. Bisi-Johnson MA, Obi CL, Hattori T, Oshima Y, Li S, Kambizi L, Eloff JN, Vasaikar SD. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. BMC Complement Altern Med. 2011;11:14.
5. Naidu JR, Ismail RB, Yeng C, Sasidharan S, Kumar P. Chemical composition and antioxidant activity of the crude methanolic extracts of *Mentha spicata*. Journal of Phytology. 2012;*4*(1):13-18.
6. Barchan A, Bakkali M, Arakrak A, Pagán R, Laglaoui A. The effects of solvents polarity on the phenolic contents and antioxidant activity of three *Mentha* species extracts. Int J Curr Microbiol App Sci. 2014; 3(11):399-412.
7. Yousuf PMH, Noba NY, Shohel M, Bhattacherjee R, Das BK. Analgesic, Anti-Inflammatory and Antipyretic Effect of *Mentha spicata* (Spearmint). British J Pharm Res. 2013;3(4):854-864.
8. Starburck J. Herbs for sleep and relaxation. Men's Health. 2001;16:24–26.
9. Hajighasemi F, Hashemi V, Khoshzaban F. Cytotoxic effect of *Mentha spicata* aqueous extract on cancerous cell lines in vitro. J Med Plants Res. 2011;5(20):5142-5147
10. Abdel-Hady H, Abdel-Gawad MM, El-Wakil EA. Characterization and evaluation of antioxidant activity of *Ocimum canum* leaves and its efficiency on *Schistosoma mansoni* larval stage. Indo Amer J Pharma Res. 2017;7(11):978-994.
11. Rohman A, Riyanto S, Yuniarti N, Saputra WR, Utami R, Mulatsih W. Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). International Food Research Journal. 2010;17(1):97-106.
12. Akroum S, Bendjeddou D, Satta D, Lalaoui K. Antibacterial, antioxidant and acute toxicity tests on flavonoids extracted from some medicinal plants. Int J Green Pharmacy. 2010;4:165-169.
13. Andarwulan N, Batari R, Sandrasari DA, Bolling B, Wijaya H. Flavonoid content and antioxidant activity of vegetables from Indonesia. Food chemistry. 2010;121(4): 1231-1235.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26:1231–7.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983; 65(1-2):55-63.
16. Gomha SM, Riyadh SM, Mahmmoud EA. Synthesis and anticancer activities of thiazoles, 1, 3-thiazines, and thiazolidine using chitosan-grafted-poly (vinylpyridine) as basic catalyst. Heterocycles: an international journal for reviews and communications in heterocyclic chemistry. 2015;91(6):1227-1243.
17. Kanatt SR, Chander R, Sharma A. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chemistry. 2007;100(2):451-458.
18. Hosseinimehr SJ, Pourmorad F, Shahabimajd N, Shahrbandy K, Hosseinzadeh R. In vitro antioxidant activity of *Polygonium hyrcanicum, Centaurea depressa, Sambucus ebulus, Mentha spicata* and *Phytolacca americana.* Pakistan J Biological Sci. 2007;10(4):637-640.
19. Benabdallah A, Rahmoune C, Boumendjel M, Aissi O, Messaoud C. Total phenolic

content and antioxidant activity of six wild *Mentha* species (Lamiaceae) from northeast of Algeria. Asian Pacific J Tropical Biomedicine. 2016;6(9):760-766.

20. Abdel-Hady AA, El-Nahas HA, El-Nabarawy SK, Abdel Raouf HA. Evaluation of the Antioxidant Activity and the Acute Oral Toxicity of Three Plant Extracts on Albino Mice. Mid East J Appl Sci. 2014; 4(2):207-216.
21. Mata AT, Proença C, Ferreira AR, Serralheiro MLM, Nogueira JMF, Araújo MEM. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. Food Chem. 2007;103(3):778-786.
22. Dorman HJD, Kosar M, Kahlos K, Holm Y, Hiltunen R. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. J Agric Food Chemistry. 2003; 51:4563–4569.
23. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Anal. 2006;19:669-675.
24. Fatiha B, Didier H, Naima G, Khodir M, Martin K, Léocadie K, Caroline S, Mohamed C, Pierre D . Phenolic composition, in vitro antioxidant effects and tyrosinase inhibitory activity of three Algerian *Mentha* species: *M. spicata* (L.), *M. pulegium* (L.) and *M. rotundifolia* (L.) Huds (Lamiaceae). Industrial Crops and Products, Elsevier. 2015;74:722-730.
25. Rice-Evans CA, Miller NJ. Total antioxidant status in plasma and body fluids. Meth. Enzymol. 1994;234:279–293.
26. Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M, Kazaz C. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* (L.) by activity-guided fractionation. J Ethnopharmacol. 2003;87:73–83.
27. Al-Owaisi M, Al-Hadiwi N, Khan SA. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrine (Forssk.) Fiori leaves. Asian Pac J Trop Biomed. 2014;4(12):964-70.
28. Abdel-Hady H, Abdel-Wareth MTA, El-Wakil EA, Helmy EA. Identification and evaluation of antimicrobial and cytotoxic activities of *Penicillium islandicum* and *Aspergillus tamarii* ethyl acetate extracts. World J Pharm Pharm Sci. 2016;5(9): 2021-2039.
29. Kaefer CM, Milner JA. The role of herbs and spices in cancer prevention Review. J Nutr Biochem. 2008;19(6):347-361.
30. Afifi-Yazar FU, Kasabri V, Abu-Dahab R. Medicinal plants from Jordan in the treatment of cancer: Traditional uses vs. in vitro and in vivo evaluations–Part 1. Planta medica. 2011;77(11):1203-1209.
31. Sertel S, Eichhorn T, Plinkert PK, Efferth T. Chemical Composition and antiproliferative activity of essential oil from the leaves of a medicinal herb, *Levisticum officinale*, against UMSCC1 head and neck squamous carcinoma cells. Anticancer. Res. 2011;31(1):185-191.
32. Hoffman EJ. Plant biochemistry and cancer. In: Hoffman EJ. Cancer and the search for selective biochemical inhibitors. Boca Raton: CRC Press, Florida, U.S.A., 1999;76.
33. Duke JA. *Mentha pulegium* L. (Lamiaceae) – Pennyroyal. In: Duke JA. Handbook of medicinal herbs. Boca Raton: CRC Press, Florida, U.S.A. 2001;307-308.
34. Shirazi FH, Ahmadi N, Kamalinejad M. Evaluation of northern Iran *Mentha pulegium* L. cytotoxicity. Daru. 2004;2(3): 106-110.
35. Hameed IH, Hussein HJ, Kareem MA, Hamad, NS. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). J Pharm Phytother. 2015;7(7):107-125.
36. Boukhebti H, Chaker AN, Belhadj H, Sahli F, Ramdhani M, Laouer H, Harzallah D. Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils. Der Pharmacia Lettre. 2011;3(4):267-275.
37. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from *Thesium humile* Vahl. J Mater Environ Sci. 2015;6(10):2778-2783.
38. Devi J, Muthu AK. Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of

Saccharum spontaneum Linn. Int J Pharm Pharm Sci. 2014;6(2):755-759.

39. Kessler M, Ubeaud GC, Jung L. Anti- and pro-oxidant activity of rutin and querectin derivatives. J Pharm Pharmacol. 2003;55: 131-142.
40. Rajeswari G, Murugan M, Mohan VR. GC-MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae). Research J Pharm Biol Chem Sci. 2012;3(4):301-308.
41. Ponnamma SU, Manjunath K. GC-MS Analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (nees) T. anders. Int J Pharm Bio Sci. 2012;3(3):570-576.
42. Idan SA, Al-Marzoqi AH, Hameed IH. Spectral analysis and anti-bacterial activity of methanolic fruit extract of *Citrullus colocynthis* using gas chromatography-mass spectrometry. African J Biotechnology. 2015;14(46):3131-3158.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27779