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Phytochemical Constituents, Antioxidant and Anticancer Activity of *Mentha citrata* and *Mentha longifolia.*

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ABSTRACT

The aim of the present work was to study the anti-cancer and antioxidant activity of two nutraceuticals (mixtures of different extracts) prepared from *Mentha citrata* and *Mentha longifolia* aerial parts separately. The anti-cancer activity was evaluated in three cancer cell lines. Antioxidant Activity was carried out adopting thiocyanate and β-carotene bleaching method. Total phenolic contents were determined in the methanol extracts of both plants. Fatty acids and phytosterols were assessed in petroleum ether extracts by GC. Bioactive constituents of the essential oil were determined by GC-MS. Results showed inhibition of the three tested cancer cells (liver, cervix and colon carcinoma) by the tested nutraceuticals with variable degrees. Both nutraceuticals showed comparable antioxidant activity. Total phenolic content of the ethanol extract of *Mentha longifolia* was less than that of *Mentha citrata*. *Mentha citrata* petroleum ether extract contained higher percentage of unsaturated fatty acids and total phytosterol than that of *Mentha longifolia.* The major volatile components were Linalyl acetate and linalool in *Mentha citrata* and Piperitone oxide in *Mentha longifolia.* Both nutraceuticals of *Mentha citrata* and *Mentha longifolia* possess antioxidant and anticancer effect that could be attributed to the presence of phytosterosl, phenolic compounds, unsaturated fatty acids and specific volatile constituents.

Keywords: *Mentha longifolia,* Mentha citrate, Nutraceuticals, Anticancer, Antioxidant, Phytochemical analysis.

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INTRODUCTION

Lamiaceae (Labiatae) is an important plant family that has been investigated for its medicinal properties due to its large amounts of phenolic acids, flavonoids and essential oils. Mentha is a member of family Lamiaceae and is a popular plant in Egypt and Arabic countries and is frequently consumed in form of hot beverage. It is used in Folk medicine for relief of colic and flatulence in almost all ages from infancy onward to Geriatric. Mentha is also used as spices in some food recipes. So, Mentha is widely used in food, flavor, cosmetic and pharmaceutical industries. The different herbal and food products from *Mentha* species have been in use since ancient times for the treatment of heart burns, indigestion, coughs and flu, nausea, gall-bladder and bile ducts, herpes, and certain skin infections including acne and pigmentation [1-3].

The genus *Mentha* which belongs to the family Lamiaceae consists of about 25-30 species, most of which are found in temperate regions of Eurasia, Australia and South Africa [4]. Various biological activities have been reported for some species of *Mentha*, such as antibacterial [5, 6], antifungal [7] and treatment of irritable bowel syndrome [8]. *Mentha longifolia* (L.), commonly known as wild mint or Horse mint, is a perennial herb that can grow 1-2 m high and is a member of the Lamiacea family [9]. The oils of *M. longifolia* are known to contain numerous monoterpenoids with piperitone oxide, piperitone, piperitenone, pulegone, d-limonene, carvone, menthone, β-caryophyllene, 1,8-Cineole, and menthol as dominating compounds; however, there have been some variations in the constituents of this oil from different countries, and a chemogeographical variation has been observed in essential oil composition of this species [5]. *Mentha longifolia* grows widely throughout the temperate regions of the world. Syrup made from *Mentha longifolia* L. was reported as a safe, well-tolerated, and effective choice in inducing bleeding and maintaining regular bleeding in women with secondary amenorrhea and oligomenorrhea [10].

Mentha citrata, called Bergamot mint and Eau-de-cologne Mint (plants for future) is also a member of Lamiacea family. A tea made from the fresh or dried leaves of *Mentha citrata* has traditionally been used for stomach aches, nausea, parasites and other digestive disorders and for fevers and headaches. The leaves and flowering plant have analgesic, antiseptic, antispasmodic, carminative, cholagogic, diaphoretic, and vasodilator properties [11].

The aim of the present work was to study the anti-cancer and antioxidant activity of two mixtures of polar and non polar extracts of *Mentha citrata* and *Mentha longifolia* aerial parts. The aim includes studying the bioactive constituents of both *Mentha* species including phenolic compounds, fatty acids, phytosterols and essential oils.

MATERIALS AND METHODS

Materials

Plant materials

Mentha longifolia, family Lamiaceae aerial parts were obtained from Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza. *Mentha citrate,* family Lamiaceae aerial parts were obtained from a farm in Ismalia, Egypt. The plants were authenticated by Dr. Tereez Labib, Consultant of Plant Taxonomy, Ministry of Agriculture, Giza, Egypt. A voucher specimen was deposited in the National Research Centre Herbarium, Cairo, Egypt.

Cancer cells

Three human tumor derived cell lines were supplied from National Cancer Institute, Cairo University, Egypt. HEPG2 (liver carcinoma cell), HELA (cervix cancer cell) and HTC-4 (colon carcinoma cell) were the studied cell lines.

Methods

Preparation of plant materials

Mentha citrata and *Mentha longifolia* aerial parts were dried separately in an air-circulated oven at 40 °C till complete dryness, and then they were reduced to No. 36 powder and kept in tightly closed containers. For extraction of volatile oil of both plants fresh samples were used.

Preparation of plant extracts

Petroleum ether and alcohol extracts

The dried powder of *Mentha citrata* and *Mentha longifolia* aerial parts were separately placed in a continuous extraction apparatus (Soxhelt) and subjected to successive extraction using petroleum ether (40-60°C) then ethanol. The solvent of each extract was removed by evaporation under reduced pressure. All extracts were kept in vacuum desiccators over anhydrous calcium chloride. The yield of different extracts was weighed and the mean ±SD was calculated from triplicate extractions.

Preparation of the essential oil

The essential oils were prepared from the aerial parts of *Mentha citrata* L. and *Mentha longifolia* L., separately, by hydro-distillation with Clevenger apparatus [12]. The obtained oils were dried over anhydrous sodium sulfate. The yield was weighed and the mean ±SD was calculated from triplicate extractions. The collected essential oils were immediately analyzed using GC-MS analysis.

Preparation of Nutraceuticals

Petroleum ether extract, alcohol extract and volatile oils of *Mentha citrata* and *Mentha longifolia* were mixed separately in the same ratio of their occurrence in the original aerial parts of each plant to have two nutraceuticals.

Determination of total phenolic contents in alcohol extracts

Total phenolic was determined in the ethanol extracts of *Mentha citrata* and *Mentha longifolia* aerial parts using Folin-Ciocalteu reagent [13] in triplicate. Absorbance was measured at 765 nm using UVPC spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in grams per 100 gram. The mean value of three determinations was calculated as the mean ±SD.

Assessment of fatty acids, hydrocarbons and phytosterols contents in the petroleum ether extracts (oils)

The unsaponifiable fraction and fatty acid methyl esters of the studied extracts were prepared according to A.O.A.C [14] and were subjected to GLC analysis of fatty acids, hydrocarbons and phytosterols.

The unsaponifiable fraction was analyzed by GLC adopting the following conditions: Column: 10% OV-101 packed column; Stationary phase: Chromosorb W-HP; Detector temperature: 290°C; Injector temperature, 28°C; Carrier gas N_2; flow-rate 30 ml/min; air flow-rate: 300ml/min; H_2 Flow-rate 30ml/min; Detector FID; Chart speed: 0.5 cm/min; Oven program: Initial temperature, 70°C; Final temperature, 270°C; programmed 4°C/min. For 35min at 270°C, total time, 85 min. Identification of hydrocarbons and sterols contents of the unsaponifiable matter was carried out by comparison of their retention times with co-injected authentic reference compounds. Quantization was based on peak area integration.

Analysis of the methyl ester by GLC was carried out according to the following conditions

Stationary phase: 10% diethylene glycosuccinate (DEGS) packed column; oven temperature, 170°C; detector temperature, 300°C; injector temperature, 250°C; Carrier gas, N_2; flow-rate, 30ml/min; air flow-rate, 350ml/min; H_2 flow-rate, 350ml/min; detector, FID; Chart speed, 2cm/min. Identification of the fatty acid methyl ester was carried out by direct comparison of retention times of each of the separated compounds with authentic samples of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

Gas chromatography - Mass spectrometry (GC-MS) analysis of essential oils

A gas chromatography (Hewlett-Packard model 5890) coupled to a mass spectrometer (Hewlett-Packard-MS (5970) was used for analysis. Volatiles were separated using a fused silica capillary column DB5 (60m x 0.32mm i.d. x 0.25 µm film thickness). The oven temperature was maintained initially at 50°C for 5 min, and then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The sample size was 2 µl, split ration 1:10, the injector temperature was 220 °C. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 400 amu . The retention indices (Kovats index) of the separated volatile components were calculated with reference to the retention time of a series of alkanes (C_{6-} C_{20}) as external standard run at the same conditions. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology, NIST) and comparison with those of authentic compounds and published data [15]. The quantitative determination was carried out based on peak area integration.

Anticancer Activity

The anticancer activity of the nutraceuticals was evaluated using the cell line technique according to Cordero *et al*. [16]. Cells of liver carcinoma cell (HEPG2), Colon carcinoma (HTC-4) and Cervix carcinoma (HELA) were plated in 96-multiwell plate (10^4cells/well) for 24h before treatment to be attached to the wall of plate. All nutraceuticals were dissolved in dimethyl sulfoxide (DMSO) at 10 mM as a stock solution. Dilutions with culture media were prepared just prior to addition to test plates. Different concentration of the nutraceuticals (0, 1, 2.5, 5 & 10 µg/ml) were added to the cell monolayer, triplicate wells were prepared for each individual dose of each nutraceutical. Plates were incubated for 48h at 37 °C and in atmosphere of 5% CO_2. After 48h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain, then excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction of cancer cell and nutraceutical dose was plotted. IC_{50} (concentration which reduces survival of the exposed cancer cells to 50%) was obtained from the curves. Mean±SD of IC_{50} was calculated from three determinations.

Antioxidant Activity of different nutraceuticals was carried out adopting thiocyanate method [17, 18] to measure percentage antioxidant activity and β-carotene bleaching method [19] to determine antioxidant protection factor (APF). D, L-α-Tocopherol was used as standard. Each sample was determined in triplicate and the mean ±SD was calculated.

RESULTS

Yield of different extracts was shown in table 1, it could be noticed that petroleum ether extract, alcohol extract and essential oil yields of *Mentha citrata* were more than that of *Mentha longifolia*. The moisture content of *Mentha longifolia* was 87.82% while that of *Mentha citrata* was 85.5%.

The anticancer effect of the nutraceuticals prepared from *Mentha longifolia* and *Mentha citrate* are present in table 2 and fig 1-3 . Results showed inhibition of the three tested cancer cell by the two nutraceuticals. Nutraceutical of *Mentha citrata* was more efficient than that of *Mentha longifolia* in inhibiting the survival of both

liver carcinoma (HEPG2) and cervix carcinoma (HELA**)** reflected in IC50. *Mentha longifolia* nutraceutical was superior in reducing colon carcinoma (HTC-4) where IC_{50} dose was 3.02±0.104 and 3.42±0.75 μg for *Mentha longifolia* and *Mentha citrata,* respectively.

Table 3 shows the antioxidant effect of both nutraceuticals. Nutraceuticals prepared from *Mentha longifolia* and *Mentha citratea* possess antioxidant effect. The percentage antioxidant activity of *Mentha longifolia* nutraceutical (60±2 %) was slightly higher than that of *Mentha citrata* (56±2.272 %). APF of both nutraceuticals was similar (2.3±0.126 for *Mentha longifolia* and 2.5±0.11for *Mentha citrata*).

Total phenolic content of the ethanol extracts of *Mentha longifolia* and *Mentha citrata* extracts were 30.327±1.4 and 31.452±1.25 g GAE/100g, respectively. Fatty acids' content of the petroleum ether extracts of *Mentha longifolia* and *Mentha citrata* oils as percentage of total fatty acids are shown in table 4. *Mentha citrata* petroleum ether extract contained higher percentage of unsaturated fatty acids (34.692) than that of *Mentha longifolia* (6.974). The unsaturated fatty acids were oleic (25.706 %) and linoleic acid (8.986%) in *Mentha citrata* while only linoleic (6.974%) was present in *Mentha longifolia.* Palmitic, stearic and behenic acid are the saturated fatty acids that present in both *Mentha* species.

Table 5 showed GLC analysis of unsaponifiable matter of *Mentha longifolia* and *Mentha citrata* oils as percentage of total unsaponifiable matter. *Mentha citrata* oil contained higher percentage of total phytosterol (14.657) than that of *Mentha longifolia* (9.901). Campesterol was present as 3.370 and 4.284% while stigmasterol was present as 1.877 and 0.341% and β-sitosterol as 4.654 and 5.748 % in *Mentha longifolia* and *Mentha citrata* oil, respectively. The major hydrocarbon in *Mentha longifolia* oil was C19 followed by C22 as 15.940 and 10.548 %, respectively. In *Mentha citrata* oil, the major hydrocarbon was C23 followed by C28 as 24.715 and 6.792%, respectively.

Qualitative and quanitative analysis of the *M. Longifolia* oil used in the present study is listed in Table 6. A total of 23 components were identified which formed 97.59% of the total oil components. The oxygenated monoterpenes were found to be the major chemical class of substances (86.24%) followed by the oxygenated sesquiterpene hydrocarbons (4.35%), sesquiterpene hydrocarbon (4.15%) and monoterpenes(2.85%). The oxygenated monoterpenes, piperitone oxide (59.05%) was the dominant identified component followed by 1,8-cineole (16.07%). β-Caryophyllene and Caryophyllene oxide were the major components identified in non oxygenated and oxygenated sesquiterpenes and oxygenated sesquiterpene hydrocarbons, respectively.

In the oil of *M. citrata*, 27 compounds were identified (table 7), representing about 96.72% of the whole oil. The oxygenated monoterpenes was the dominant class, accounting for 74.02% of the total oil followed by the monoterpenes (14.57%), sesquiterpene hydrocarbons (5.58%) and caryophylene oxide (2.55%) which was the only identified oxygenated sesquiterepene hydrocarbon. The major components identified in the oil were, linalyl acetate (35.01%), linalool (20.99%) and limonene (5.57%).

Table 1: Yield of different extracts (g/100g sample) (Mean±SD).

Sample name / Type of extract	g/100g fresh sample		g/100g dry sample	
	Mentha longifolia	***Mentha citrata***	***Mentha longifolia***	***Mentha citrata***
Petroleum ether extract	**0.491±0.008**	**0.763±0.015**	**4.02±0.075**	**5.24±0.07**
Alcohol extract	**2.30±0.15**	**3.7±0.132**	**18.9±0.757**	**25.52±0.5**
Essential oil	**0.12±0.01**	**0.17±0.007**	**0.99±0.085**	**1.17±0.125**

Table 2: IC_{50} dose of the different nutraceuticals (μg) (Mean±SD).

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Extract	Cell line		
	Liver carcinoma (HEPG2)	Colon carcinoma (HTC-4)	Cervix carcinoma (HELA)
***Mentha longifolia* mixture**	**2.15±0.05**	**3.02±0.104**	**3.62±0.101**
***Mentha citrata* mixture**	**1.74±0.065**	**3.42±0.75**	**3.02±0.085**

Table 3: Antioxidant activity and antioxidant protection factor (APF) of *Mentha longifolia* and *Mentha citrata* extracts (Mean±SD).

Extract	Antioxidant activity %	APF
***Mentha longifolia* mixture**	**60.0±2**	**2.3±0.126**
***Mentha citrata* mixture**	**56.0±2.272**	**2.5±0.111**
D, L. α-Tocopherol	**90.8±0.764**	**2.72±0.076**

Table 4: Fatty acids' content of the petroleum ether extracts of *Mentha longifolia* and *Mentha citrata* oils (as percentage of total fatty acids).

Fatty acids	***Mentha longifolia* oil**	***Mentha citrata* oil**
Palmitic, C16:0	1.630	11.645
Stearic, C18:0	4.200	8.437
Oleic, C18:1	-	25.706
Linoleic, C18:2	6.974	8.986
Behenic, C22:0	1.658	3.777
Total saturated fatty acids	**7.488**	**23.859**
Total unsaturated fatty acids	**6.974**	**34.692**

Table(5: GLC analysis of unsaponifiable matter of *Mentha longifolia* and *Mentha citrata* oils (as percentage of total unsaponifiable matter).

Hydrocarbon & sterols	***Mentha longifolia* oil**	***Mentha citrata* oil**
Hydrocarbon (Higher alkanes):		
Pentadecane (C15)	0.240	-
Hexadecane (C16)	0.038	0.005
Heptadecane (C17)	0.160	0.231
Octadecane (C18)	1.516	0.256
Nonadecane (C19)	15.940	0.527
Icosane (C20)	-	0.663
Heneicosane (C21)	3.624	0.142
Docosane (C22)	10.548	0.423
Tricosane (C23)	-	24.715
Tetracosane (C24)	1.737	1.816
Pentacosane (C25)	-	0.266
Hexacosane (C26)	0.576	4.217
Octacosane (C28)	4.098	6.792
Total hydrocarbon	**38.477**	**40.053**
Phytosterols:		
Campesterol	3.370	4.284
Stigmasterol	1.877	0.341
β-Sitosterol	4.654	5.748
Total phytosterols	**9.901**	**14.657**

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Table 6: Chemical composition (%) of hydrodistilled *Mentha longifolia* essential oil.

Peak No	RI[a]	Components	Relative area%
1	939	α-Pinene	0.24
2	976	Sabinene	0.14
3	980	β-Pinene	0.80
4	991	β-Myrcene	0.37
5	1035	Limonene	1.30
6	1045	1,8-Cineole	16.07
7	1088	Linalool oxide	0.20
8	1098	Linalool	0.51
9	1153	Menthone	1.51
10	1167	Borneol	1.21
11	1170	Piperitone oxide	59.05
12	1177	Terpinene-4-ol	0.14
13	1188	α-Terpineol	0.62
14	1220	trans-Carveol	0.31
15	1291	Thymole	0.82
16	1343	Piperitenone	1.80
17	1369	Piperitenone oxide	4.00
18	1418	β-Caryophyllene	3.20
19	1452	Humulene	0.35
20	1502	D-Germacrene	0.60
21	1582	Caryophyllene oxide	3.24
22	1595	Cedrol	0.61
23	1650	α-Cadinol	0.50
Monoterpenes			2.85
Oxygenated monoterpenes			86.24
Sesquiterpene hydrocarbons			4.15
Oxygenated sesquiterpene hydrocarbons			4.35
Total			97.59

Compounds listed according to their elution on DB5 column.
a: kovat index.

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Table 7: Chemical composition (%) of hydrodistilled *Mentha citrata* essential oil.

Peak No	RI[a]	Components	Relative area%
1	939	α-Pinene	2.20
2	976	Sabinene	0.21
3	980	β-Pinene	1.89
4	991	β-Myrcene	2.09
5	1018	α-Terpinene	0.25
6	1026	P-Cymene	0.82
7	1035	Limonene	5.57
8	1045	1,8-Cineole	2.82
9	1048	β-Ocimene	1.01
10	1088	α-Terpinolene	0.53
11	1098	Linalool	20.99
12	1156	iso-Pulegol	0.17
13	1173	Menthol	0.25
14	1177	Phenyl ethylalcohol	1.32
15	1189	α-Terpineol	2.89
16	1230	Nerol	2.21
17	1245	Carvone	1.00
18	1245	Geraniol	5.01
19	1257	Linalyl acetate	35.01
20	1270	Geranial	0.31
21	1294	Menthyl acetate	1.64
22	1380	Geranial acetate	0.40
23	1418	β-Caryophyllene	4.05
24	1450	β-Franesene	0.52
25	1454	α-Humulene	0.20
26	1506	Germacrene D	0.81
27	1582	Caryophyllene oxide	2.55
Monoterpenes			14.57
Oxygenated monoterpenes			74.02
Sesquiterpene hydrocarbons			5.58
Oxygenated sesquiterpene hydrocarbons			2.55
Total			96.72

Compounds listed according to their elution on DB5 column. a: kovat index.

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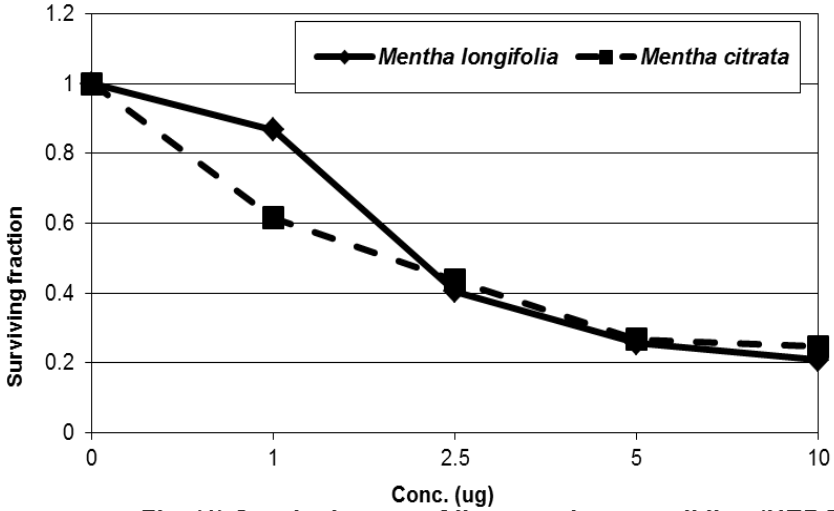

Fig. (1):Survival curve of liver carcinoma cell line (HEPG2)

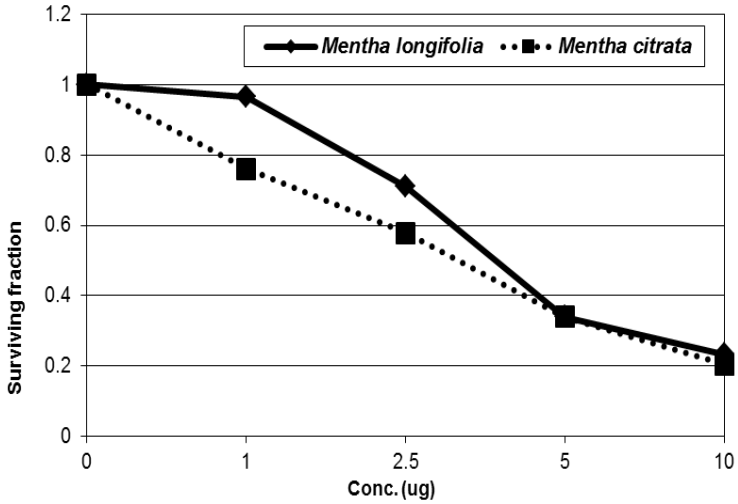

Fig. (2): Survival curve of cervix carcinoma cell line (HELA)

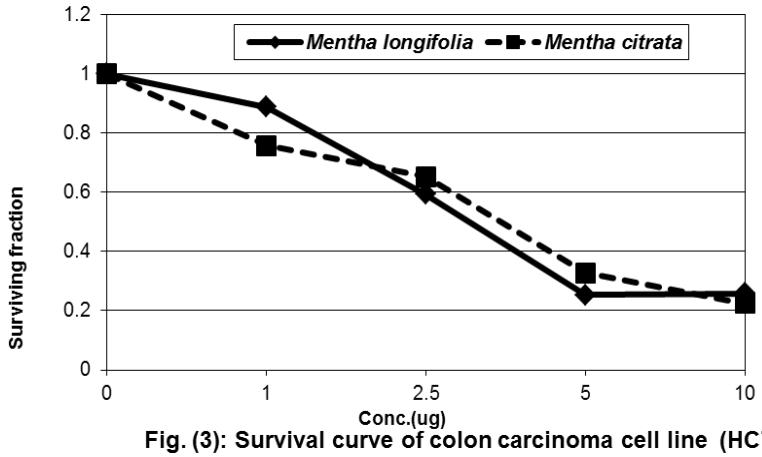

Fig. (3): Survival curve of colon carcinoma cell line (HCT-4)

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DISCUSSION

In the present study the dominant identified component in *Mentha longifolia* was piperitone oxide (59.05%) followed by 1,8-cineole (16.07%). β-Caryophyllene (3.2%) and Caryophyllene oxide (3.24%) were the major components identified in non oxygenated and oxygenated sesquiterpenes hydrocarbons, respectively. The results of the present study are in accordance with the previous published data [20] concerning the essential oil of *M. Longifolia* growing wild in Bosnia and Herzegovina. The most abundant constituents in the essential oil of *M. Longifolia* from Pakistani flora [21] were found to be piperitenone oxide (28.3%), piperitenone (24.9%), germacrene D (8.16%), borneol (5.96%) and β-caryophyllene (5.94%). Analyzed essential oil mainly consisted of oxygenated monoterpenes (67.24%) followed by sesquiterpene hydrocarbons (17.19%), monoterpene hydrocarbons (7.31%), and oxygenated sesquiterpenes (5.05%) [21]. Piperitone oxide and piperitenone oxide were the major components in the essential oil of *M. Longifolia* from the middle Blak Sea region of Turkey [22]. Carvone (7.9%), limonene (5.8%), 1,8-cineole (5.4%) and piperitone (4.8%) were the major components in the polish *M. Longifolia* oil [23]. In *Mentha longifolia,* the major volatile compounds were piperitenone (43.9%), tripal (14.3%), oxathiane (9.3%), piperitone oxide (5.9%), and d-limonen (4.3%) according to Khani and Asghari [24]. Essential oil of *Mentha longifolia* showed some major component as; 1, 8 cineole (11.58%), pulegone (21.90%), piperitone oxide (42.51%) and caryophyllene oxide (3.64%) [10]. The major volatile compounds of the Iranian *Mentha longifolia* oil were piperitone (43.9%), limonene (13.5%), and transpiperitol (12.9%) [25]. However, identification of piperitone as the major compound in the *Mentha longifolia* oil is in sharp contrast to other reports where the oil had carvone [26] or ciscarveol [27] as the major component. The variation in the essential oil composition of *M. longifolia* is reported in the literature from different part of the world [2, 28,29]. Concerning *Mentha citrara,* (3R)-(-)-linalool characterizes essential oils of Bergamot mint which is present as72 to 75% for *Mentha citrata* [30]. Indian *Mentha citrata* Ehrh. produced an essential oil containing only the acyclic monoterpenol (-)-3R-linalool and its acetate ester [31].

In the present study, linalyl acetate (35.01%) was the major component followed by linalool (20.99%), limonene(5.57%) geraniol (5.01%), β-Caryophyllene (4.05%), α-terpineol (2.89%), 1, 8 cineole (2.82%) in *Mentha citrata.* The results of the present study are in accordance with the previous study of Kumar *et al*. [32] and Mastelic *et al*. [33]. Linalool was the major constitutes (59.76%) in the oil of *M. citrata* grown in India [34] followed by linalyl acetate (18.4%), nerol (2.0%), trans-p-menth-1-en-2-ol (1.8%), α-terpineol (1.5%) and limonene (1.1%).

The essential oil of *M. citrata* might be a source of natural linalyl acetate and linalool [33]. However, the content of these components may be reduced during hydrodistillation of the oil. α-Terpineol, terpinene-4-ol and 1,8-cineol can be formed, as artifacts , from linalyl acetate and linalool. On the other hand the contents of β-myrcene, β-ocimene, neryl acetate and 1, 8-cineol were increased during hydrodistillation of *M. citrata* oil. At the same time the contents of linalyl acetate and linalool were decreased [33]. Variations in the chemical compositions of essential oil from different countries might be attributed to the varied climatic, seasonal, and geographical conditions, adaptive metabolism within plants and applied isolation methods.

In the present study the yield of methanol extract was higher than that of petroleum ether followed by essential oil for both *Mentha longifolia* and *citrata*. Concerning percentage yield of essential oil and different extracts yield (g/100 g of dry plant material) of *Mentha longifolia* native to dry region of Pakistan, maximum yield was obtained with methanol (12.60 g/100 g). The minimum yield was obtained with dichloromethane (3.50 g/100 g). The essential oil yield from the aerial parts of *Mentha longifolia* was found to be 1.07 g/100g. Nonpolar extract yield (*n*-hexane) was found to be 7.30 g/100 g [21]. The essential oil yield of the present study (0.99%) is comparable with that of Iqbal *et al.* [21] while the methanol yield (18.9%) was higher than that of Iqbal *et al.* [21]. In the present study, the non polar extract of *Mentha longifolia* represented by petroleum ether (4.02%) was lower than that of Iqbal *et al.* (2013) which represented by *n*-hexane.

Mint extracts are commonly used as food flavouring additive and are generally considered safe to use as they provide health benefits and good defense against oxidative damage [35]. Iqbal *et al*. [21] showed dichloromethane and methanol extracts of *Mentha longifolia* to exhibit excellent antioxidant activity. The essential oil and hexane extract showed comparatively weaker antioxidant and free radical scavenging activities [21]. Also,

Vladimir-Knežević *et al*. [36] reported antioxidant activity of *Mentha longifolia* methanol extract which was ascribed to the presence of rosmarinic acid. Rosmarinic acid content in different species of Labiatae was reported to be 0.0-58.5 mg/ g of dried plants. The highest amount of rosmarinic acid was found in *Mentha* species [37].

It was shown previously that Saudi Arabia M. *longifolia* methanol extract has powerful antioxidant activity [38]. The chemical composition and antioxidant activity of *M. Longifolia* were investigated [20]. The results revealed high free radical scavenging capacity of the oil, which was found to be in correlation to the content of mainly monoterpein ketones and aldehydes. The authors stated that *M. Longifolia* oil could serve as safe antioxidant and antiseptic supplements in pharmaceuticals.

Epidemiological studies have suggested a positive association between the consumption of phenolic rich foods or beverages and the prevention of disease due to the presence of antioxidant components sush as phenolics [39]. The phenolic hydroxyl groups present in aromatic plant have redox properties [40] allowing them to act as reducing agent and a hydrogen donator. However, the antioxidant activity of essential oils cannot be attributed only to the presence of phenolic constituents; monoterpene alcohols, ketones, aldehydes, hydrocarbons and ether also contribute to the free radical scavenging activity of some essential oils. Ruberto and Baratta [41] tested 100 pure constituents of essential oils and confirmed that the monoterpene hydrocarbons δ-terpinene, α-terpinene, P-cymene showed very high antioxidant activity. 1,8-Cineole, which was one of the major compounds present in *M. longifolia* oil under investigation, highly inhibited hexanal oxidation [42].

Similarly, previous studies attributed the high antioxidant activity of the essential oils of some aromatic plants to the presence of oxygenated and non oxygenated terpenoids such as α-pinene in Juniper berry and germacrene D in ylang-ylang [43], carvone, carveol, 1,8-cineol and limonene in essential oil of spearmint, limonene in celery seed and 4-terineol and carvon in *Mentha spicata* [42-44]. So, the antioxidant and APF of *Mentha* nutraceuticals in the present study might be attributed to the methanol extract and some essential oil components. Free radical scavenging potential of the methanol extract of *Mentha longifolia* was reported to be 79% while that of *Mentha citrata* was 64% [45]. The antioxidant activity of *Mentha longifolia* nutraceutical in the present study is also higher than that of M*entha. Citrata,* while their APF are equal. The antioxidant and APF effect of both *Mentha* nutraceuticals could be ascribed to the total phenolic contents that have been determined to be 30.327 and 31.452 g GAE/100g, in *Mentha longifolia* and *Mentha citrata* extracts methanol extract, respectively along with the essential oil.

Luteolin 7-O-glucoside, luteolin 7-O-rutinoside, luteolin 7-O-glucuronide and Apigenin 7-O-rutinoside isolated from *Mentha longifolia* (L.) Huds. subsp. longifolia showed antimutagenic effect reflecting great pharmacological importance that might be beneficial for reducing the risk of reactive oxygen species-related diseases [46-48] including cancer. *Mentha longifolia* possibly reduces NO secretion in macrophages by scavenging NO and inhibiting inducible NO synthase mRNA expression, and also decreases TNFα pro-inflammatory cytokine expression, thus showing its usefulness in the inflammatory disease process [49]. A relationship between exposure to inflammation and etiology of cancer was reported [50, 51]; therefore natural agents that reduce inflammation could have an impact in reducing cancer. This could explain the anticancer effect of *Mentha longifolia* in the present study. M. *longifolia* methanol extract was shown to protect against DNA damage and have cytotoxic activity against MCF-7 cell line [38]. Also, the phenolic content of *Mentha citrata* which is higher than that of *Mentha longifolia* could be responsible for the anticancer effect of *Mentha citrata.*

Among the important bioactive constituents that present in the present studied *Mentha* species are phytosterols which play an important role in human health including antioxidant and anti-inflammatory effects [52, 53]. Total phytosterol consumption was associated with a strong inverse relationship to cancer [54], through inhibiting proliferation, inducing apoptosis and increasing the sensitivity of patients to chemotherapeutic agents [55]. Unsaturated fatty acids that present in *Mentha* in the present study may participate in the anti cancer effect of the nutraceuticals. Unsaturated fatty acids have been reported to work as regulators of the immune response [56]. Phenolic compounds intake are associated with reduced risk of cancers [57]. These finding could explain the anticancer effect of the studied nutraceuticals in the present study.

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CONCLUSION

Both extracts' mixtures (nutraceuticals) prepared from *Mentha citrata* and *Mentha longifolia* possess antioxidant and anticancer effect that could be attributed to the synergestic effect of phytosterols, phenolic compounds, unsaturated fatty acids and specific volatile constituents.

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