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QUALITY CONTROL, CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOME MARKETED PEPPERMINT OIL SAMPLES

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ABSTRACT: This work aimed to evaluate the quality of some commercial peppermint oil products available in the Egyptian market. Also, analysis of peppermint oil extracted from *Mentha piperita* L. leaves marketed in Egypt. Thus, quality control of both herb extracted and purchased oil products. Essential oils were analyzed using GC/MS, and the main ingredients of each peppermint oil sample were quantified. The antioxidant potential for each of the tested six oil samples was performed using the DPPH radical scavenging assay using ascorbic acid as a reference standard. Also, their $EC_{50\%}$ values were calculated. The quality of the samples had been evaluated according to the European Pharmacopeia limits for each of the major peppermint oil components. Thus, menthol, menthone, menthyl acetate, carvone, and pulegone percent composition in each sample was used to evaluate its quality. All the tested samples were found to possess strong to moderate antioxidant activities with $EC_{50\%}$ values ranged from 20.44 and 66.10 depending on the oil chemical composition variation. Percent composition of the major peppermint oil components varied within the tested samples.

INTRODUCTION: Phytotherapy is a form of alternative and complementary medicine using plants and their extracts for healthcare. Essential oils are well-known in traditional medicine due to their different beneficial uses. Some of the essential oils are used in food industry as well as in cosmetics and pharmaceutical preparations. Essential oils can be obtained from pharmacies, drug stores, and markets. Due to the increased number of oil suppliers, demand for efficient analysis and quality control is increased.

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Peppermint oil is commonly used in cases of common cold and flu, and to aid digestion. It may be used as an analgesic in menstrual cramps [1]. Additionally, peppermint oil may be an effective remedy for Irritable Bowel Syndrome (IBS) and in constipation [2, 3]. It has been used to soothe the gastrointestinal tract for hundreds of years.

A study showed that it relaxes intestinal smooth muscle by decreasing calcium influx into the smooth muscle cells [4]. It is also used as a natural remedy for toothache [5]. Moreover, peppermint oil is reported to have antiviral in the treatment of herpes [6]. Oil lipophilic nature provides penetration into the skin, and it may, therefore, have topical therapeutic use as a virucidal agent against recurrent herpes infections. Antimicrobial activity of peppermint oil was tested and proved against gram positive and gram-negative bacteria [7, 8].

Recently, an antifibrogenic activity of the Egyptian *Mentha piperita* L. essential oil against CCl_4-induced liver fibrosis in rats was tested and proved [9]. Topical application of peppermint oil was equally effective as acetaminophen in relieving headache in a double-blind, placebo-controlled patients [10]. Furthermore, peppermint oil is commonly used as insecticide [11]. Also, the oil can be used as a physical performance enhancer [12]. Although peppermint oil is a low-risk skin allergen, it was reported to be neurotoxic in high doses [13]. Peppermint oil can be used in different ways. It may be inhaled, rubbed on reflexology points on the bottom of the feet, diffused into the air, or as a therapeutic bath. It also may be administered as an enteric coated capsule. It is highly recommended that extreme caution should be used when administering to children less than five years as the menthol can cause a choking reaction in children [14].

According to European Pharmacopoeia, official peppermint grade must undergo quantitative analysis of composition by gas chromatography. It must contain 30.0-55.0% menthol, 1.0-5.0% limonene, 3.5-14.0% cineole, 14.0-32.0% menthone, 1.0-9.0% menthofuran, 1.5-10.0% isomenthone, 2.8-10.0% menthyl acetate. Pharmacopoeial maximum limits of pulegone and carvone are 4% and 1% respectively (Ph.Eur.3, 1997). Somewhat different values are described for French pharmacopoeial grade peppermint oil. It must contain not less than 44% menthol, from 4.5-10% esters calculated as methyl acetate, and from 15-32% carbonyl compounds calculated as menthone. TLC is used for identification, quantification of compounds, and verification of the absence of visible bands corresponding to carvone, pulegone, and isomenthone.

In the Western world, it was reported by the Joint Food Safety and Standards Group in the United Kingdom that commercial peppermint oils must contain 0.2-2.9% pulegone [15]. The highest known recommended daily dose as stated in European herbal monograph is 1.2 ml peppermint oil. This means that 1099 mg peppermint oil (based on relative density 0.916 -according to Ph. Eur. 8.1 (2014). This dose contains a maximum of 32.97 mg pulegone and 87.92 mg menthofuran (according to Ph. Eur. 8.1 limits for pulegone and menthofuran in peppermint oil).

If being administered as enteric-coated peppermint capsules, the adults: 0.2 to 0.4 mL of oil three times daily while for children older than eight years, the recommended dose is 0.1 to 0.2 mL three times daily [16].

The chemical composition of peppermint essential oil shows significant variations following different species and chemotypes. Other variation factors are geographical origins, cultivation conditions, plant maturity and age [15].

Even within *Mentha pepperita* L. varieties cultivated in Egypt, there is significant variation in chemical composition following cultivation areas [17, 18]. Menthol content of peppermint oil determines its quality. Menthyl acetate is responsible for peppermint's minty aroma and flavor. Quality control of peppermint oil by gas chromatography with a flame ionization detector (FID) was done in a previous study [18]. It resulted in the isolation of 10 primary components ranging from menthol (30-50%) to isopulegol (< 0.2%).

The present study aimed to evaluate the quality of 6 peppermint oil samples from different suppliers either by extraction from a plant cultivated in Egypt or oil samples already extracted and marketed in Egypt by determination of their chemical composition. Also to determine, compare oils antioxidant potential and correlate them with oil composition. Both oil composition and antioxidant activity were used to compare and evaluate samples quality.

MATERIALS AND METHODS:

General Experimental Procedure:

Plant Material: The whole herb of *Mentha pippereta* L. belonging to the Family Lamiaceae cultivated in an Egyptian farm was collected in April 2018. A voucher specimen of the studied plant material was given accession number MP117. It was deposited at Pharmacognosy Department, Faculty of Pharmacy, Damanhour University. Botanical identification has been confirmed by macroscopic and microscopic examinations.

Extraction of the Essential Oil from Leaves: About 100 gm of air-dried freshly cut leaves just before use was subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous Na_2SO_4. The

oil was measured, coded as Supplier 1and stored at –3 °C in a sealed vial until use.

Commercial oil Samples: Five commercial peppermint oil was purchased from the Egyptian market. The oil samples were coded Supplier 2, Supplier 3, Supplier 4, Supplier 5 and Supplier 6. The sample coded Supplier 3 was prepared by cold press extraction method.

General Experimental Procedures: TLC of the purchased essential oil samples together with that was extracted from the plant was done on precoated silica gel 60 F254 plates (Germany).

UV detection of the TLC plates was done using Camag, Switzerland UV Lamp.).

GC/MS analysis was performed on Perkin Elmer model: Clarus 580/560 S).

Clevenger type apparatus was used for essential oil extraction.

Spectrophotometer (Optima SP-300, Japan).

All solvents and Chemicals were purchased from Sigma Chemical Co. (St., Louis, USA

Menthol was obtained from Sigma-Aldrich.

DPPH was purchased from Sigma Chemical Co. (St. Louis, USA).

TLC of Peppermint Oil: All oil samples were spotted on gel F percolated plates and developed using 10% ethyl acetate in hexane as mobile phase. Plates were visualized under UV and sprayed with anisaldehyde/H_2So_4.

Peppermint Oil Samples were Co-Chromatographed with Authentic Menthol: All essential oil samples (Supplier 1, 2, 3, 4, 5 and 6) showed menthol recognized by the same authentic R_f = 0.35. Menthol spot was not visualized under UV but appear as blue spot after spraying with anisaldehyde/H_2So_4 and heat at 120 ºC for 10 min. The intensity of menthol spot varied as being faint in samples 1 and 6. Essential oil samples showed major 3 spots with R_f values 0.35, 0.6 and 0.95. The 3 spots appeared after spraying with anisaldehyde/H_2So_4 as blue, blue and green respectively. Some samples should major UV active spot with higher R_f than menthol. This spot varies in intensity and being more intense in samples coded Supplier 1 and 6.

Gas Chromatography/ Mass Spectroscopy of the Peppermint Oil Samples: GC /MS analysis was performed on Perkin Elmer model: Clarus 580/560 S).

Oven: Initial temp 60 °C for 5 min, ramp 4 °C/min to 200 °C, hold 2 min, Injection temperature was 220 °C, Volume = 0 HL, Split = 20:1, Carrier Gas was Helium,

Solvent Delay = 3.00 min, Transfer Temp = 280°C, Source Temp = 200 °C, Scan: 50 to 500Da, column (Rxi-5Sil MS column 30 m, 0.25 mm ID, 0.25 df). Samples were diluted with ethanol before injection.

Antioxidant Activity Assay Using DPPH Radical Scavenging Method: Assessment of the antioxidant activity of both the isolated and commercial peppermint oil samples was done by the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical using ascorbic acid as a standard. The antioxidant radical scavenging effects on DPPH are due to their hydrogen donating ability which causes an absorbance drop at 517 nm. Serial dilutions (25-100 µg/ml) of the tested oil samples were measured by the same assay to obtain EC_{50} (effective concentration at which the DPPH scavenging activity being half its maximal activity).

In the DPPH radical scavenging, antioxidants react with the DPPH radical, which is a stable free radical naturally has deep violet color, to turn yellow-colored compound (diphenyl picryl hydrazine). The degree of discoloration indicates the radical scavenging potential of the antioxidant. Oil samples were prepared and tested as previously described [19].

By $EC_{50\%}$ values (mg /ml), we mean the effective concentration at which the DPPH scavenging effect being 50%. It was obtained by interpolating from the linear regression analysis.

Statistical Analysis: Antioxidant assays were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA.

RESULTS: The yield of the plant isolated essential oil was about 0.66% based on the dry weight of plant material. As described in the European Pharmacopoeia, the essential oil is the major constituent of the leaves (0.5-4%).

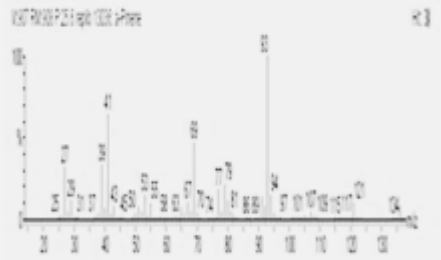
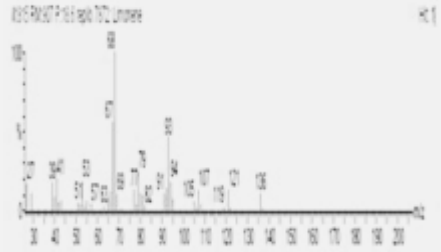
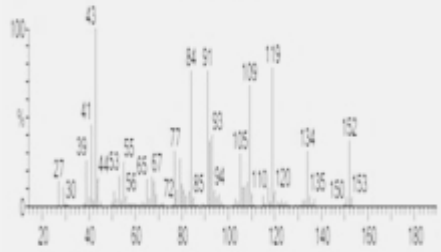
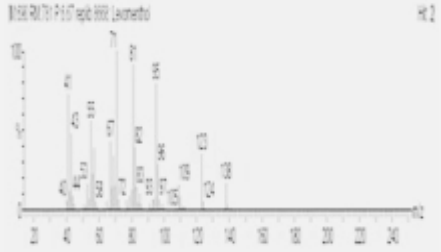
All the oil samples (isolated and purchased) were colorless to pale yellow except that coded Supplier 6 that was yellow and had a somewhat different odor. All were liquid having characteristic odor and taste followed by a sensation of cold. Samples are freely soluble in 70% ethanol.

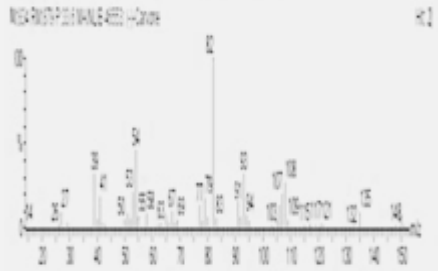
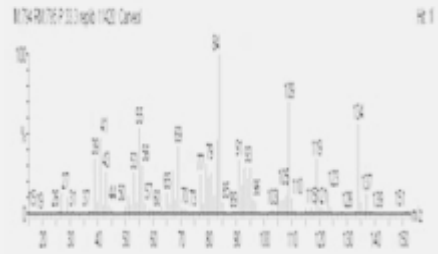
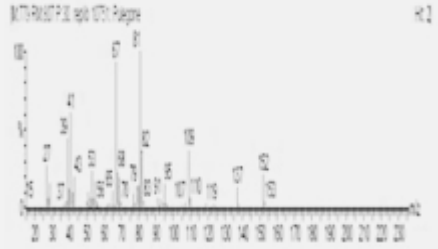
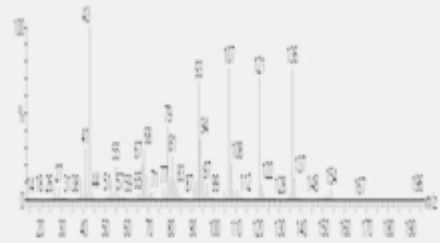
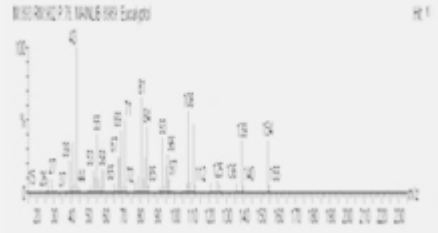
TLC of all tested samples showed menthol spot in all samples with variable density.

Visible bands under UV were identified in TLC analysis of samples; Supplier 1 and 6.

Chemical Composition of Peppermint Oil Samples: All the tested six samples tested showed thirty peaks in the GC/MS diagram. GC/MS diagrams are shown in **Fig. 1**. The major compounds in peppermint oil samples either extracted or purchased are represented in **Table 1**.

TABLE 1: PERCENTAGE OF MAJOR COMPOUNDS IN GC/MS OF THE SIX TESTED PEPPERMINT OIL SAMPLES EXTRACTED AND PURCHASED

Compound / Mass spectrum	% composition					
	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5	Supplier 6
α-pinene	1.223	3.318	1.524	4.526	3.687	0.305
						
Limonene	6.466	3.008	1.369	5.646	5.229	3.788
						
Menthone	0.667	11.034	6.561	24.213	25.096	0.154
						
Menthol	1.674	23.112	43.092	44.364	43.897	1.56
						

Carvone	50.290	----	0.402	0.358	----	80.600
						
Carveol	19.608	------	-------	----		0.290
						
Pulegone	0.192	--------	------	1.257	1.299	4.526
						
Menthylacetate	0.532	3.199	0.874	0.183	2.815	0.269
						
Eucalyptol	2.551	7.891	----	0.274	0.280	1.857
						

Major compounds in peppermint oil samples were menthol, menthone, methyl acetate, limonene, carvone, carveol, pulegone, eucalyptol, and α-pinene. Quality control of the oil samples will be assessed depending on the percent concentration of these major compounds compared with those described in the European Pharmacopeia.

Regarding menthol concentration, samples 3, 4 and 5 agreed on the Pharmacopoeia limits (30-55%).

Regarding carvone content, samples are official (<1%) except the oil distilled from the tested plant sample (supplier 1) and the sample coded (supplier 6). All samples tested showed the absence of menthofurane which is a metabolite of pulegone reported to be dangerous due to proximate hepatotoxicity in humans [20].

All samples were found to contain pulegone within its described Pharmacopoeia limits (4%). Also,

limonene content of all samples full within the official range in all samples except sample coded supplier 1 which showed slightly higher content (6.466%).

Sample coded supplier 2 contained menthol and menthone in lesser than their official limits. The oil thought to be extracted from exhausted *Mentha piperita* leaves.

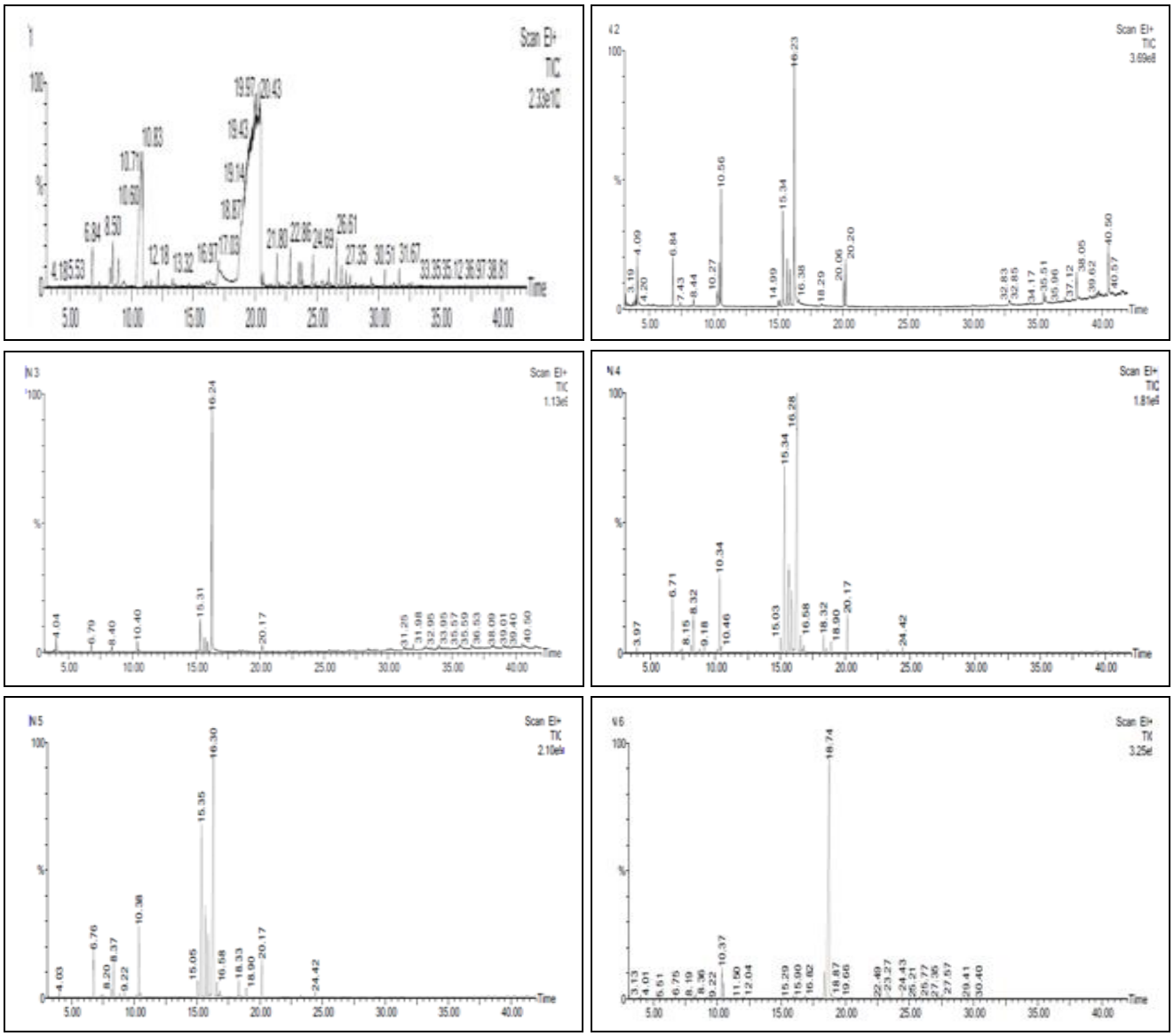


FIG. 1: GC/MS DIAGRAMS OF THE SIX PEPPERMINT OIL SAMPLES ANALYZED FOR QUALITY CONTROL STARTING FROM SUPPLIER I TO SUPPLIER VI

The Antioxidant Potential of the Tested Peppermint Oil Samples: All oil samples showed antioxidant activity varied to different extents. Results are tabulated in **Table 2**. The percentage of scavenging ability of all the tested six peppermint oil samples using DPPH method were shown in **Fig. 2**.

Sample coded Supplier 1 showed the highest antioxidant potential (70.56 ± 1. 34%) followed by sample Supplier 5 (66.28%). $EC_{50\%}$ values 20.44 and 21.74 respectively.

TABLE 2: DECREASE OF DPPH ABSORBANCE (%) AND EC_{50} VALUES FOR THE SIX TESTED PEPPERMINT OIL SAMPLES

Supplier	The decrease of DPPH absorbance % mean ± SD (*n* = 3)	EC_{50} (µg/ml)
1	70.56 ± 1.34	20.44
2	39.56 ± 0.76	59.30
3	47.87 ± 1.08	43.95
4	36.94 ± 0.37	66.10
5	66.28 ± 0.89	21.74
6	58.44 ± 1.17	27.85
Ascorbic acid (standard)	81.60 ± 0.82	8.59

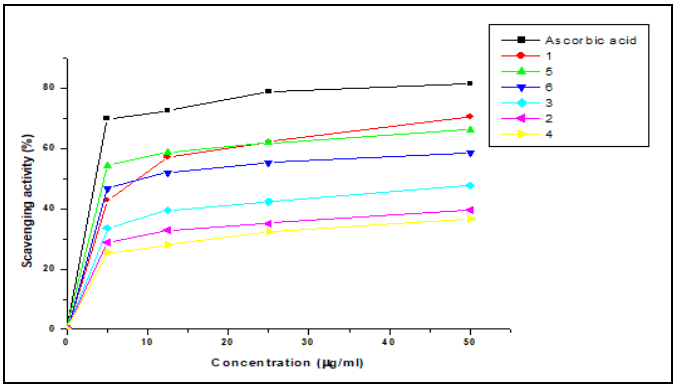


FIG. 2: COMPARISON BETWEEN THE PERCENTAGES OF SCAVENGING ABILITY OF ALL THE TESTED SIX PEPPERMINT OIL SAMPLES USING DPPH METHOD WITH ASCORBIC ACID AS REFERENCE

DISCUSSION AND CONCLUSION: The existence of different chemotypes is a common feature in most *Mentha species* and hybrids. *Mentha piperita* L. is a hybrid of spearmint (*Mentha spicata*) and water mint (*Mentha aquatica*) [21]. This results in variations in the chemical composition of the commercially used peppermint oil. The plant yield of volatile oil sample (coded as Supplier I) agreed with the European pharmacopeia standardization (0.5-4%). While as regards its chemical composition, it cannot be considered official.

According to European Pharmacopoeia grade, peppermint oil must contain 30–55% menthol and maximum 1.0% carvone and 4.0% pulegone to be considered official. Not all samples showed excellent quality and high purity. One of the tested samples (Supplier 2) was found to be hydrodistilled from the exhausted plant as containing a lower percentage of menthol. Pulegone content of peppermint oil depends on the type of soil in which the plant is grown and the time of picking, as well as on other, more elusive factors [15, 21].

Samples coded Supplier 3, 4 and 5 were found to be official. All contained menthol within official Pharmacopoeia range (43%) and accepted limits of the other major oil constituents. The three samples differed in their antioxidant potential. EC_{50} of samples Supplier 3, 4 and 5 were 43.95, 66.1 and 21.74 respectively. The increased antioxidant potential of sample Supplier 5 than Supplier 3 may be due to its higher content of limonene (5.22 and 1.369 respectively). It was reported that limonene showed higher antioxidant activity (98.74%) while pulegone exhibited low or almost null antioxidant activity determined by DPPH and ABTS methods [22]. Thus, sample coded Supplier 4 was superior to other official samples regarding both chemical composition and antioxidant activity

According to the European Commission, a review of human intoxication cases following ingestion of 10 mL oil contained high pulegone (62-97%) were reported [23]. Sánchez-Borzone *et al*., 2017 suggested that the ketone components of Mentha can exhibit convulsions in mammalian organisms. They related this effect to the contents of ketone monoterpenes that are most common in different Mentha species. Among them pulegone was observed as the most active, followed by carvone and finally menthone. That is why limitation of their content in the official peppermint oil is necessary. Indeed all the tested six samples are safe and accepted in concern with pulegone content.

Adulteration with spearmint oil (*Mentha spicata*) gives a different characteristic odor that is known as carvone-scented mint plants as Samples 1 and 6. Carvone; the natural terpenoid ketone was found to be responsible for the powerful antioxidant activity in the Mediterranean Mentha species [18]. This explained the increased antioxidant potential of samples (Supplier 1 and 6) due to their high contents of carvone (50.29 and 80.6% respectively. Despite this high antioxidant potential, these samples are to be rejected or applied only externally.

RECOMMENDATION: Quality control of the chemical composition of the essential oil in the market is very important especially if used internally. The maximum daily dose is to be written in peppermint oil bottle. Also warning from used in high doses in children as some of the commercial products continue to be marketed without fulfilling the quality criteria. Gas chromatography despite being expensive is an accurate and efficient method in quality control of essential oils.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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