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A Review on Peppermint Oil

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ABSTRACT:

The principal pharmacodynamic effect of peppermint oil relevant to the gastrointestinal tract is a dose-related antispasmodic effect on the smooth musculature due to the interference of menthol with the movement of calcium across the cell membrane. The choleretic and antifoaming effects of peppermint oil may play an additional role in medicinal use, In the present study, the protective effect of peppermint (Mentha piperita) and parsley (Petroselinum crispum) leaves oils against hepatotoxicity is induced by carbon tetrachloride (CCl4) in experimental rats. GC/MS results indicated that the main components in peppermint oil were menthol (35.9%) and menthone (25.6%), while in parsley oil were a-Pinene (26.6%) and Myristicin (20.3%). Hepatotoxicity by CCl4 resulted in significant elevation of serum triglycerides, total cholesterol, low density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C) and decreasing in serum high density lipoprotein (HDL-C). Moreover, kidney function tests for serum urea nitrogen, creatinine, and uric acid were found to be increased. 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 EC50% values were calculated. And Peppermint leaf oil was isolated by a supercritical fluid extraction (SFE) using COz in which the extraction was followed by a two-stage fractional separation

Key Words: Mentha piperita, peppermint, menthone, GC/MS, Quality control, Antioxidant

INTRODUCTION:

Peppermint (Mentha x piperita) is a recurrent flowering member of the mint family, which grows widely in Europe and North America. The medicinal use of peppermint and other mint plants possibly dates back to the herbal pharmacopoeia of early Greece, where peppermint leaf traditionally was used inter-Nally as a gastric aid and intended for management of gallbladder disease; it also was used in inhaled form for upper respiratory symptoms and cough. Peppermint oil, which is extracted from the stem, leaves, and flowers of the plant, has become popular as a treatment for a variability of conditions, including irritable bowel syndrome (IBS), headache, and non-ulcer dyspepsia.

Extracts of peppermint are widely used as flavoring (rather than for their medicinal properties) in many products, including toothpastes, mouthwashes, and over-the-counter gastro-intestinal (GI) products. Menthol, which is extracted from peppermint, is a common ingredient in over-the-counter topical products used for respiratory congestion, head-ache, and muscle pain. Peppermint (Mentha x piperita) is a perennial flowering member of the mint family, which grows widely in Europe and North America.

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The Irritable Bowel Syndrome Drop Evaluation and Safety Trial (IBSREST) was conducted to compare a novel formulation of triplecoated microspheres of solid state, highly purified PO (IBgard, IM Health Science, Boca Raton, FL, USA) with placebo in patients with moderate to severe IBS-M and IBS-D. This PO formulation was designed to provide quick, reliable, and sustained release in the small intestine. The aim of the IBSREST was to estimate the effectiveness, safety, and tolerability of this novel formulation of PO for the management of worldwide and individual gastrointestinal symptoms in patients with non-constipated IBS.

Gastritis commonly utilizedinpediatric sufferers for treating abdominal pain, irritable gut syndrome, nausea, and symptomatic comfort of coughs and colds, it is far one of the biosphere's most pro-therapeutic sages and is applied in respectively Eastern and Western conventions. Old Greek, Egyptian, and Roman societies utilized the mint in medication and cookery. Mint is as of now one of the principals financially essential fragrant and medicinal crops brought within the U.S. The sector technology of mint oil is about 8000 tons consistent with 12 months [9]

Phytotherapy is a form of alternative and complementary medicine using plants and their extracts for healthcare. Essential oils are well-known in outmoded 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 [10]

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symptoms and cough. Peppermint oil, which is extracted from the stem, leaves, and flowers of the plant, has become prevalent as a treatment for a variety of conditions, including irritable bowel syndrome (IBS), headache, and non-ulcer dyspepsia [11]

Supercritical fluid extraction (SFE) is an interesting method for the extraction of flavoring compounds from vegetable material. It can constitute an industrial alternative to solvent extraction and steam distillation processes. SFE allows a continuous modification of solvent power and selectivity by changing the solvent density. Nevertheless, the simple SFE process, consisting of supercritical Cot extraction [14]

DATA AND MATERIAL:

- **Peppermint biological picture and their scientific classification:**

Table 1. Scientific classification of peppermint [6,7]

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asteroids
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Mentha*
Species:	***M. × piperita***

- **Peppermint and its component**:

Key compounds in peppermint oil samples were menthol, menthone, methyl acetate, limonene, carvone, carveol, pulegone, eucalyptol, and αpinene. Quality control of the oil samples will be assessed depending on the out of a hundred concentration of these major compound compared with those described in the European Pharmacopeia [10]

➢ Extraction of peppermint oil:

- **Experimental Plant:**

Material Dried leaves of Mentha x piperita L. were purchased from Aboca (lot 13442, Arezzo, Italy) in vacuum package. The plants were harvested in fields near Arezzo (Italy). The leaves were air-dried and comminuted until an approximate size of 0.3 mm was attained. The moisture content was about 10% by weight on dry basis. The essential oil content, determined by the supplier using steam distillation, was about 2.0% by weight.[14]

- **Isolation of Essential Oil:**

The supercritical extraction apparatus contained of a 400-ml extractor and two separation vessels, operated in series, with a volume of 200 ml each.

C02 circulation was obtained by a Milton Roy high-pressure diaphragm pump (Milroyal B).

Additional details and a schematic representation of the apparatus have been given elsewhere.'.3 Around 110 g of comminuted peppermint leaves were submitted to extractionin each run.

A COz flow rate of 0.8 kg/h and an extraction period of 160 min were used.

First the SFE process was performed at various COz densities.

GC-MS and sensory analysis were used to determine the extraction conditions that diminish the co-extraction of unwanted compounds; subsequently the optimum fractional distillation conditions to be used in the two separators were studied. The yield of the various fractions was measured by weight with respect to the dried material charged in the extractor. The plant material was also subjected to hydro distillation (HD) for 2 h conferring to the standard procedure described in the European Pharmacopoeia.

Table 2. Key Points About Peppermint Oil:[11]

Effectiveness:	Irritable bowel syndrome symptoms: probably effective non-ulcer dyspepsia: probably effective Reducing spasm during gastrointestinal procedures: probably effective Tension, headache probably effective
Adverse effects Common:	allergic reactions, heartburn, perianal burning, blurred vision, nausea, and vomiting Rare: interstitial nephritis, acute renal failure
Interactions:	May inhibit the cytochrome P450 1A2 system
Contraindications:	Hiatal hernia, severe gastroesophageal reflux, gallbladder disorders; use with caution in pregnant and lactating women
Dosage Adults:	0.2 to 0.4 mL of oil three times daily in enteric-coated capsules Children older than eight years: 0.1 to 0.2 mL three times daily
Bottom line:	Safe at proper dosages and moderately effective in patients with functional gastrointestinal conditions

1. **Scope:**

This analysis method is applied to peppermint oils of subheadings 3301.24 and 3301.25 of Customs Tariff Law (Appendix Table – Customs Tariff Schedule), for distinguishing between those obtained from Mentha piperita and those from Marvensis.

2. Outline of Test Method:

Under Customs Tariff Law (Appendix Table – Customs Tariff Schedule), peppermint oils are divided into three group, i.e., those obtained from M. piperita, those obtained from M. arvensis and others. This analysis method enable to differentiate between peppermint oil obtained from M. piperita and that M. arvensis.

The peppermint oils from these two Mentha species are differentiate using gas chromatograph mass spectrometer, by detecting Menth furan, sabinene hydrate and viridiflorol, all of which are specifically contained in those obtained from M. piperita, and comparing the content percentage of cineole and limonene commonly contained in these two types of oil.

3. Apparatus:

1) Gas chromatograph-mass spectrometer (GC-MS)
2) Capillary column
3) Use a DB-WAX (30 m × 0.25 mm i.e., film thickness 0.5 µm) or equivalent one

- **GC-MS, operating under the following conditions:**

a. GC temperature program
b. Program the oven temperature so that each constituent of peppermint oils is divided sufficiently (e.g., initial temperature 50 °C for 3 min; increase at 15 °C per min; 110 °C; increase at 3 °C per min; 150 °C; increase at 15 °C per min; final temperature 200 °C for 5 min).
c. Injection port temperature - 250 °C
d. Other
e. Set other instrument parameter to the optimum condition for the analysis

4. Reagents

a) Standard peppermint oil
b) Use peppermint oil obtained from M. piperita and M. arvensis whose constituent being main peaks in their gas chromatogram have been identified.
c) JIS special reagent grade or equivalent
d) Diethyl ether

Note 1) Standard peppermint oil have to be stored in a cool and dark place.

5. Procedure:

5.1. Measurement

Prepare a test solution of approx. 1 % concentration by adding diethyl ether to the test sample. Inoculate 1 all of the test solution into GC-MS to obtain a total ion chromatogram and to measure mass spectra of object peaks.

- **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 plate (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) [10]

5.2. Identification of constituents

Identify object peaks in the total ion chromatogram of the test sample, based on their mass spectra and by comparing their retention times with those of the same compound present in the standard peppermint oil.

5.3. Identification of peppermint oils

Identify the kind of peppermint oil sample using the table below indicating difference between peppermint oil obtained from M. piperita and that obtained from M. arvensis.

5.4. Determination of Total Menthol

If the sample is identified to be peppermint oil obtained from M. arvensis according to the procedure in 5.3, its total content of menthol is analyze by the following quantitative method as test method based on Article 74 of the Cabinet Order for Enforcement of the Customs Tariff Law.

- **Methods**
- **Study Subjects:**

To be eligible for the trial, subjects had to meet Rome III criteria for IBS-M or IBS-D with an average daily IBS- related abdominal pain rating of C4 on a 0–10 scale and a Total IBS Symptom Score (TISS) of C2 on a 0–4 scale. Subjects had to be between 18 and 60 years of age, and had to confirm that they were not planning to change their usual diet and lifestyle during the study. Exclusion criteria included a diagnose of IBS-C or IBS- U as defined by the Rome III criteria or a history of inflammatory or immune-mediated gastrointestinal disor- ders, including celiac disease. Also excluded were subjects with a history of organic gastrointestinal disorders includeing intestinal obstruction, stricture, toxic megacolon, per- formation, fecal impaction, adhesions, ischemic colitis or impaired intestinal circulation, gastrointestinal surgery cholecystitis, or major including cholecystectomy. Additional exclusion criteria included a history of cardiovascular events, uncontrolled hypertension, unstable renal, hepatic, metabolic, or hematologic conditions, human immunodeficiency virus (HIV) infection, or a history of alcohol abuse or binge drinking. Subjects who refused to discontinue one or more prohibited medications for at least 7 days before start the baseline diary and throughout the remainder of the study were excepted. The protocol did not allow concomitant or rescue medications during the trial.

- **Experimental Design:**

The trial was directed at four geographically diverse study sites in the USA, in accordance with good clinical practice (GCP) and applicable regulatory requirement and ethical principles. The protocol was approved by the Chesapeake Institutional Review Board and the Palm Beach Clinical Research Organization (West Palm Beach, FL, USA) was responsible for conducting the study.

➢ **Therapeutic uses and health benefits:**

- IMMUNE MODULATION: Menthol has anti-inflammatory effects when applied Topically. In one study it was claimed that it could suppress Antigen induced allergy. Menthol also has a property of Inhibiting cutaneous anaphylaxis that's mediated by IgE. Antibody

Figure: health benefits of peppermint oil [8]

- DENTAL HEALTH: Pepper mint is used in making oral dentifrices as it can Run over all freshness in breath and also keep away bad Breath. Additional studies are being done as to whether or not it Directly contribute to preventing caries and plaque, however it is confirmed that it does create an un-favorable Environment for bacteria.

- GASTROINTESTINAL BENEFITES: Peppermint is used for treatment of non-obstructive Dyspepsia without any known side effect. It improves the Gastric emptying rate. There is a significant antiemetic Effect of peppermint in reducing postoperative nausea for patient with very sensitive gag reflex
- NEURO-PSYCHIATRIC EFFECTS: studies have suggested that peppermint is a central Nervous system stimulant. Studies have been directed on the effectiveness of aromas on cognitive performance, perceived physical workload, and pain responses were Conducted based on possible change in the brain activity

- **CHEMICAL CONSTITUENTS:**

Various constituent of peppermint oil as per monograph Of International Pharmacopoeia are limonene (1.0-5.0%), Cineole (3.5-14.0%), menthone (14.0-32.0%), Menthofuran (1.0 -9.0%), isomenthone (1.5-10.0%),

Menthyl acetate (2.8-10.0%), isopulegol (max. 0.2%), Menthol (30.0-55.0%), pulegone (max. 4.0%) and carvone (max. 1.0%). [4]

- **Pharmacological actions of peppermint oil:**

- In vitro research shows peppermint oil to be effective in relaxing GI smooth muscle, possibly through an antagonistic effect on calcium channels in the gut.
- Peppermint oil also has been shown to relax the lower esophageal sphincter, which can result in gastroesophageal reflux.
- This finding has led to the popularity of enteric-coated peppermint formulations, which bypass the upper GI tract unmetabolized, thereby facilitating its effect in the lower GI tract without effects in the upper tract

- **OTHER USES:**

Anti-bacterial property of peppermint in the current scenario, there are huge demand for medicinal plants as secondary metabolite because of their application in pharmaceutical unit in the form of antimicrobial agent (Mahboubi and Kazempour, 2014; Almajano et al., 2008) [82, 6]. Some scientists revealed that peppermint oil and its extracts show the strong barrier in contradiction of growth of various microbes such as: Escherichia coli, Salmonella pullorum, Comamon asterrigena, Streptococcus faecalis, Acinetobactersp, Streptococcus thermophiles, Lactobacillus bulgaricus, Staphylococcus pyogenes, Staphylococcus aureus, Streptococcus pyogenes, Serratiamarcescens, Mycobacterium avium, Salmonella typhi, Salmonella paratyphi A/B, Proteus vulgaris, Enterobacteraerogenes, Yersinia enterocolitic

Hot flushes in women A single-blind randomised control crossover study15 Was Performed to look at the effects of a peppermint and neroli spray on hot flushes in women being treated for Breast cancer. Only 18 of the 44 patients (41%) preferred. The hydrolat spray to a plain water spray, which was less Than the 80% required to offer this spray as a standard Suggestion for hot flush management. However, a small Number of those choosing it found it extremely helpful. Both sprays appeared to lessen hot flush annoyance.

Previous chemotherapy appeared to be a factor influencing the choice of spray. Irritable Bowel Syndrome Small intestine bacterial overgrowth and lactose intolerance. They are associated with increased gas production, which may Sometimes trigger abdominal discomfort and bloating Which are also considered also the cardinal symptoms in IBS.16-17 Furthermore, a high prevalence of celiac disease Has been observed in

patient with bloating and diarrhea and positive H2 -lactose breath test. In these patients the Symptom related to lactase deficiency seem to be the only

Manifestation of celiac disease18. Basing themselves on These data, some authors suggest that these diseases should Be previously excluded in clinic therapeutic trials with Investigational drug that affect IBS19.

Peppermint oil has Been tested in children 20 and adults21 with IBS, with Conflicting results. A recent meta-analysis on this topic Concluded that the role of peppermint oil has not yet been Established beyond a reasonable doubt.22 In this regard one Double blind study by L. Marzio et al.23 57 patient with irritable bowel syndrome were treated with peppermint Oil (two enteric-coated capsule twice per day or placebo) And 4 weeks treatment with peppermint oil progresses Abdominal symptoms in patients with irritable bowel syndrome.

General test:

1. **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/H2So4

   Thin-layer chromatography:

   (TLC) has been an important analytical tool for many years, Recently, TLC was described as "The 'Eyes' of the Organic Chemist". In this inquiry-based activity, the usefulnes of TLC to visualize the difference between spearmint and peppermint is discovered. The experiment, an adaptation of an earlier report, may be used in any class where TLC is discussed from high school to college level. We have used this activity with science major in an organic chemistry laboratory, with non-science major in a brewing science class, and in a general science class for elementary education major. The experiment can be completed in a two-hour period.

   After a general explanation of the TLC technique, the students are asked to determine whether this method could be useful for peppermint oil. Students are provided with dilute samples of carvone, menthol, spearmint oil, and peppermint oil for spotting on TLC plates. A developing chamber is also provided with an appropriate developing solvent (10% ethyl acetate in hexane). Two visualization methods are also provided, a UV light and anisaldehyde solution, Students are given instruction on the use of each method and cautioned to use the UV light for visualization prior to dipping the developed plates in anisaldehyde solution. Students determine that an effective experimental method is to spot a TLC plate with both known components and one of the oils.[12]

   Hazards:

   All of the chemicals and reagents used in the experiment are flammable. The TLC visualization stain is corrosive. Silica gel should not be inhaled. Students should wear safety goggles and gloves

Results:

Typical student results are shown in Table 3. Carvone is visible under UV light but menthol is not. This causes some concern with the students, and they must be reminded to develop their TLC plate with the anisaldehyde solution before they conclude the experiment

Table 3. Student Results from TLC

Sample)	Rf Value(s]	Number of Spots Visible	
		Light Under UV	**After Treatment with Anisaldehyde**
Carvone	0.5–0.6	1	1yellow
Spearmint extract	0.5–0.6	1	1yellow
Peppermint extract	0.35–0.4 0.6 0.95	2	2 blue 1 green
Menthol	0.35–0.4	None	1 blue

2. **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

Table 4 Gas Chromatography (GC/GC–MS) analysis of the peppermint.

Compound	**Content (%**
1,8-Cineol	4.6
Menthone	25.9
Menthol	35.6
Methylurethane	3.55
Caryophyllene	2.17

3. **Antioxidant Activity Assay Using DPPH Radical Scavenging Method**:

Assessment of the antioxidant activity of both the inaccessible 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 EC50 (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 EC50% 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.

4. **Statistical Analysis**: Antioxidant assay were conducted in triplicate. Data were voiced as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA.[10]

The Antioxidant Potential of the Tested Peppermint Oil Samples: All oil samples showed antioxidant action varied to different extents. Results are tabulated in Table 5. The percentage of scavenging ability of all the tested six peppermint oil samples using DPPH method were shown in Fig. 2.

TABLE 5: decrease of dpph absorbance (%) and ec50 values for the six tested peppermint oil samples [10]

Supplier	The decrease of DPPH absorbance % mean ± SD (n = 3)	EC50 (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

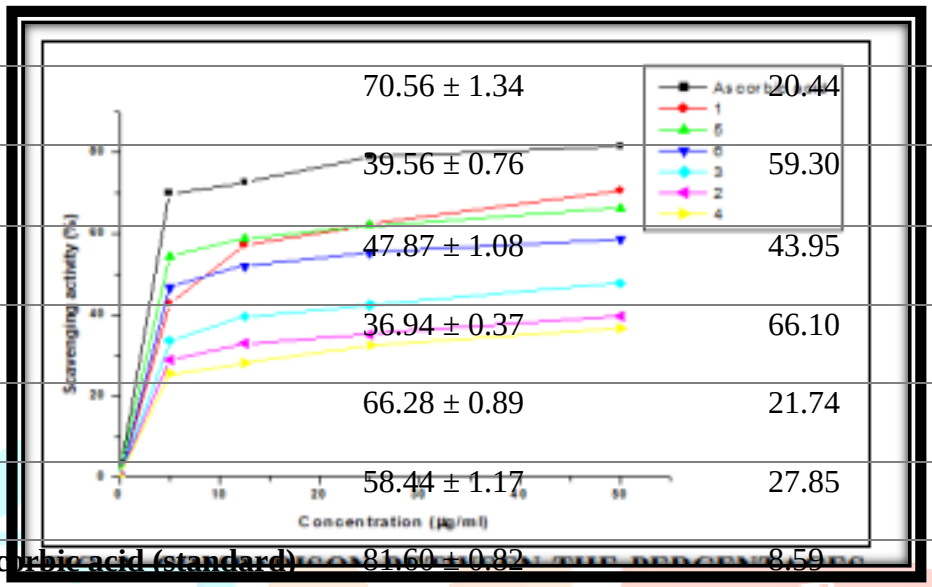

FIG. 2: COMPARISON BETWEEN THE PERCENTAGES OF SCAVENGING ABILITY OF ALL THE TESTED SIX PEPPERMINT OIL SAMPLES USING DPPH METHOD WITH ASCORBIC ACID AS REFERENCE [10]

DISCUSSION AND CONCLUSION:

The existence of different chemotype is a common feature in most Mentha species and hybrid. 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. In the present study pretreatment with peppermint, parsley and their mixture leaves oils showed increased activity of antioxidant enzyme compared to CCl4 treated animals indicating the potentiality of peppermint, parsley and their mixture leaves oils to act as an antioxidant by avoiding the peroxidative damage caused by CCl4.

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