RESEARCH ARTICLE

QUALITY CONTROL TESTING OF VARIOUS SAMPLES OF PEPPERMINT OIL COLLECTED FROM LOCAL MARKET OF KARACHI, PAKISTAN

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ABSTRACT

Menthol is the most commonly used substance in many cosmetics and pharmaceutical products either as an active ingredient or in the form of excipient. In the present study, different samples of commercially available peppermint oil were subjected to standardization by determination of physicochemical characteristics, acid value, and resinified oil content. Thin layer chromatography (TLC) has been used to confirm the presence of menthol. The result showed that the quality control test performed for the evaluation of the physicochemical parameters of peppermint oil can be considered useful in its standardization. The results of acid value and the resinified oil tests, carried out on the raw material, have found to be within the standard limits. The results indicated specified number of free fatty acids and absence of greasy impurities. The data obtained from the study would be useful in the authentication of the commercial peppermint oil samples. In TLC studies, the Rf value of the active constituent has been determined by comparison with its standard spot. This technique may be used as a tool for the correct identification of the active constituent which could help in the standardization of the peppermint oil samples.

Keywords: Mentha piperita Linn., peppermint oil, physicochemical tests, TLC fingerprint, acid value.

1. INTRODUCTION

Peppermint oil is obtained from a botanical source Mentha piperita Linn. It belongs to the family of Labiatae¹. It has a mixed distribution across Europe, Africa, Asia, Australia and North America². Peppermint oil is used as a treatment for postoperative nausea³. Additionally, essential oils of Mentha piperita L. are also used as flavors and fragrances in pharmaceuticals. They have further been investigated for their antimicrobial properties against 21 human and plant pathogenic microorganisms and a pronounced activity has been found to be present in menthol⁴,⁵. Menthol is also responsible for the antifungal properties of peppermint essential oils while menthone alone does not show any effect⁶. Peppermint oil is a widely used essential oil that has also demonstrated larvicidal activity against different mosquito species such as Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus. The oil is known to possess strong repellent action against adult mosquitoes when applied on human skin⁷. The quality of peppermint oil also depends on the harvesting time. The best yield of high quality oil is obtained in late January to early February. At this time the composition of extracted oil is generally as follows: menthol 43.1%, menthone 28%, menthyl acetate 6.6%, cineole 4.1% and menthofuran 3.8%. After this period, oil yields become steadily declined because of leaf senescence, while the percentage of menthol, menthyl acetate and menthofuran increases with

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a decrease in menthone content⁸.

Various techniques have been employed by different workers for the analysis of peppermint oil. The chemical analysis of peppermint oil for the determination of its constituents is carried out by solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME-GC/MS). The content of peppermint-characteristic compounds such as menthol, menthyl acetate and neomenthol increases in a basipetal direction (older plant parts) whereas menthone and isomenthone shows higher levels in the acropetal direction (younger plant parts). The peppermint flowers show higher levels of menthofuran in contrast to those in the leaves. The SPME can detect relatively higher amounts of high-volatile monoterpenes and lower detection of less volatile compounds such as menthol and menthone compared to the solvent based material from essential oil distillation⁹. A total of 81 constituents have been identified by spectral data from the oil of Mentha piperita L. including a new ketoalcohol, (−)-mintlactone and (+)-isomentlactone¹⁰. The potential of near-infrared spectrometry (NIRS) for the determination of secondary metabolites in the leaves of different Mentha species have been investigated. The study demonstrated that NIRS can be successfully applied as a rapid method not only in breeding and during growth in the fields but also to determine the quality of the individual oil after distillation, blending and rectification processes¹¹. Bicchi and Frattini¹² have performed the quantitative determination of a minor component, pulegone, by gas liquid chromatography (GLC) that has a very close retention time to menthol and isomenthol. The overlapping of compound separation has been prevented by the silylation of the hydroxyl compound (menthol) to change its retention time. Capillary gas chromatography coupled on-line with isotope ratio mass spectrometry (GC/IRMS) has been used to determine the δ13CPDB-values of some typical peppermint oil constituents. By using the method of the internal isotopic standard (i-IST), a characteristic isotopic fingerprint of authentic peppermint oil has been established and used for the authenticity control of commercially available peppermint oils¹².

For the betterment of the efficacy, safety and quality of commercially available herbal drugs more efficient methods are needed for the standardization and quantification of the biological active constituents. Since mint oil is the most common ingredient of pharmaceutical and cosmeceutical formulations, it is necessary to ensure its quality for the formulations. The present study involves the performance of official quality control tests¹³ to authenticate the commercial samples of mint oil, physicochemical tests and TLC fingerprint profiles to identify the active constituents.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Samples
A total of 3 samples of peppermint oil were collected from different essential oil vendors of Karachi market at the same time. The samples were coded as P-1, P-2 and P-3 for test procedure. The authentic reference standard of peppermint oil was obtained from Sigma-Aldrich Company Ltd. (St. Louis, MO, USA).

2.2. Determination of Physicochemical Characteristics
The various physicochemical characteristics of the samples of peppermint oil like relative density, refractive index, optical rotation were determined by the methods given in British Pharmacopoeia¹³ and the values were compared with the official specified ranges.

2.3. Identification of Menthol in Different Samples of Peppermint Oil

Test solution: 0.1 g of the substance to be examined was dissolved in toluene and diluted to 10 ml with the same solvent.

Reference solution: 50 mg of menthol was dissolved in toluene and diluted to 10 ml with the same solvent.

Plate: TLC silica gel F₂₅₄ plate (5-40 μ) Mobile phase: Ethyl acetate and toluene (5:95, v/v).
Application: 10 µl of the reference solution and 20 µl of the test solution were applied as bands of 10 mm on TLC plate.

Development: Over a path of 15 cm.

Drying: Dried in air.

Detection: Examined in ultraviolet light at 254 nm (Camag, Muttenz, Switzerland).

Results: The zones of menthol present in the chromatograms were compared with that of with the reference standard.

2.3.1. Test protocol
A 10 ml quantity of each sample and the standard solution were applied with the help of Linomat 5 (Camag, Muttenz, Switzerland) as bands on the TLC plate and developed with the solvent system up to 15 cm. The developed chromatoplate was dried out in air. The spots were observed under UV light at 254 nm. The chromatogram was photodocumented with the help of photodocumentation system (Camag, Muttenz, Switzerland) and the Rf value of menthol in each sample was calculated.

2.4. Determination of Acid Value
The acid value was expressed as the number of the milligrams of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. A quantity of 5.0 g of the substance to be tested was placed in a conical flask and then 50 ml of a mixture of equal volumes of ethanol (96%) and light petroleum were added. It was then standardized with 0.1 M potassium hydroxide using 0.5 ml of phenolphthalein solution as an indicator. The substance was dissolved directly or by heating it to about 90°C and then titrated with 0.1 M sodium hydroxide until the pink color persisted for at least 15 sec. The acid value should not exceed from 1.4, determined on 5.0 g diluted in 50 ml of the prescribed mixture of solvents.

2.5. Determination of Fatty Oils and Resinified Essential Oils
One drop of the essential oil was placed on the filter paper. The drop was evaporated completely within 24 h without leaving any translucent or greasy spot. It was found to comply with the test for fatty oils and resinified essential oils.

3. RESULTS AND DISCUSSION
Various quality control tests have been performed to examine the authenticity of different samples of peppermint oil i.e. P-1, P-2 and P-3, according to British Pharmacopoeial specifications. The results obtained are discussed as follows:

3.1. Physicochemical Tests
3.1.1. Relative density
The range of RD for peppermint oil in British Pharmacopoeia is 0.900 to 0.916. The RD of P-1 and P-2 are found to be above the specified range while that of P-3 is below the range (Table 1). Thus, all the samples do not comply with the compendia specifications that may be due to some contamination in the oil samples.

3.1.2. Optical rotation
The optical rotation of samples of peppermint oil is given in Table 1. The pharmacopoeial range of optical rotation is between -30° to -10°. Thus, all the samples of peppermint oil (-15.8, -14.2 and -15.2) are optically active and the values comply with the official specifications.

3.1.3. Refractive index
The range of refractive index given in British

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Relative Density</th>
<th>Optical Rotation</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>P1</td>
<td>0.958</td>
<td>-15.8</td>
<td>1.460</td>
</tr>
<tr>
<td>2.</td>
<td>P2</td>
<td>1.065</td>
<td>-14.2</td>
<td>1.467</td>
</tr>
<tr>
<td>3.</td>
<td>P3</td>
<td>0.884</td>
<td>-15.2</td>
<td>1.533</td>
</tr>
</tbody>
</table>
Pharmacopoeia is 1.457 to 1.467\textsuperscript{13}. Refractive index of the peppermint oil samples have been found to be in the range of 1.460-1.533 (Table 1). Thus, the sample P-3 does not comply with the official range indicating the presence of contamination while P-1 and P-2 comply with the official range.

3.2. Identification of Menthol in Different Samples of Peppermint Oil by TLC

TLC of the samples of peppermint oil showed the presence of menthol (Fig. 1). The \(R_f\) values of menthol in peppermint oil samples are given in Table 2 along with the \(R_f\) values of menthyl acetate. The \(R_f\) values of all the samples corresponded to that of the standard spots of menthol \((R_f = 0.91)\) and menthyl acetate \((R_f = 0.82)\). The results indicated the presence of menthol and menthyl acetate in all the samples as the \(R_f\) values were found to be very close to that of the reference standard thus confirming their presence in the samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>(R_f) value of Menthol</th>
<th>(R_f) value of Menthyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Standard</td>
<td>0.91</td>
<td>0.82</td>
</tr>
<tr>
<td>2.</td>
<td>P1</td>
<td>0.91</td>
<td>0.82</td>
</tr>
<tr>
<td>3.</td>
<td>P2</td>
<td>0.90</td>
<td>0.80</td>
</tr>
<tr>
<td>4.</td>
<td>P3</td>
<td>0.91</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Fig. 1. TLC plate of Mentha piperita oil standard and samples.
3.3. Determination of Acid Value
The acid values of the peppermint oil samples are given in Table 3. The acid value as mentioned in British Pharmacopoeia\textsuperscript{13} should not be more than 1.4. All the samples of peppermint oil were found to have an acid value in the range of 1.299-1.396, thus complied with the pharmacopoeial specification.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Acid Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>P-1</td>
<td>1.396</td>
</tr>
<tr>
<td>2.</td>
<td>P-2</td>
<td>1.342</td>
</tr>
<tr>
<td>3.</td>
<td>P-3</td>
<td>1.299</td>
</tr>
</tbody>
</table>

3.4. Test for Fatty Oils and Resinified Essential Oil Impurities
The samples of peppermint oil were tested for fatty and resinified impurities by British Pharmacopoeia method\textsuperscript{13}. The test was carried out to check any fatty or greasy impurity present in peppermint oil samples. A single spot of each sample was applied to the filter paper and kept in observation for 24 h. After this period no spot was found that indicated the purity of the samples. Thus all the samples were found to be free from fatty and resinified oil impurities and complied with the official specifications.

4. CONCLUSION
Presently, the need for the standardization of peppermint oil samples have been emphasized for their therapeutic potential and efficacy. The official tests available for the authentication of peppermint oil and the identification of its active constituents in commercial samples are reliable, accurate and inexpensive. Thus, the values of relative density, refractive index, optical rotation, R\textsubscript{r} value, acid value and resinified oils in the samples have been reported. In recent years, there has been a great demand for the plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals as well as cosmeceuticals and are being supplied by many herbal manufacturers in Pakistan. In order to ensure the quality of raw materials used in the manufacturing of the finished products, it has become essential to conduct various quality control tests for confirming the authenticity of the commercial material. This can be achieved by the use of various quality control tests as specified in the British Pharmacopoeia and other pharmacopeias. The application of these tests to peppermint oil samples in the present study has shown the level of the quality of the tested material. The various tests can be used as a tool for the correct identification and standardization of the plant material and also to test the presence of adulterants, if present.

5. ACKNOWLEDGMENT
The authors are thankful to the Herbion Pakistan (Pvt.) Ltd. for providing its laboratory services for the quality control testing of given samples.

REFERENCES


