QUALITATIVE ASSAY OF ESSENTIAL OILS OF LAVENDER AND PEPPERMINT IN COMMERCIAL PRODUCTS THROUGH SPECTRAL AND CHROMATOGRAPHIC METHODS

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Abstract

Essential oils are largely employed for their therapeutic properties, being marketed extensively in pharmaceutical and cosmetic industry. The aim of our study was to assess the purity and quality of peppermint and lavender volatile oils, available on the market, from various commercial producers. The methods used for testing the quality of these oils were refractometry, polarimetry, thin layer and gas chromatography, which are official techniques in Pharmacopoeia monographies of essential oils, and IR spectrometry, a non-official one. Following the analytical results, not all samples proved to be of excellent quality and high purity, some of them being contaminated with other substances or even diluted. Gas chromatography coupled with mass spectrometry and IR spectrometry methods had proved effective in assessing without doubt the qualitative difference between samples, the other techniques remaining a fast alternative, although there are limitations in terms of specificity and sensitivity.

Rezumat

Uleiurile esențiale sunt folosite în mare măsură pentru proprietățile lor terapeutice, fiind comercializate intens în industria farmaceutică și cosmetică. Scopul studiului nostru a fost de a evalua purețatea și calitatea uleiurilor volatile de mentă și lavandă existente pe piață, de la diferiți producători comerciali. Tehnicile de laborator aplicate pentru controlul calității uleiurilor volatile au fost cele prevăzute de farmacopee, refractometrie, polarimetrie, cromatografie în strat subțire, cromatografie de gaze, și o metodă neoficială în monografiile uleiurilor volatile, spectrometrie IR. Nu toate probele s-au dovedit a prezenta o calitate excelentă, unele dintre ele fiind contaminate cu alte substanțe sau chiar diluate. Tehnicile de cromatografie de gaze cuplată cu spectrometrie de masă și spectrometrie IR au permis diferențierea calitativă certă dintre probe, celelalte tehnici rămânând o alternativă rapidă, cu rezerva limitării acestora în ceea ce privește specificitatea și sensibilitatea.

Keywords: volatile oils, peppermint, lavender, qualitative analysis

Introduction

Nowadays, the use of essential oils as alternative therapies has gained the worldwide concern, owing to their various biological activities. Considerable attention has been devoted to peppermint oil, which is widely used for its important properties including antimicrobial, anti-inflammatory, antispasmodic, cytoprotective, hepatoprotective, with strong antioxidant actions. It is also used for its cooling effect, to enhance the dermal penetration of pharmaceuticals and is one of the most important flavouring additives in the world [5, 14].

The major components of peppermint oil are menthol (30 - 55%) and menthone (14 - 32%). Monoterpenes including limonene (1 - 5%), cineole (3.5 - 14%), menthofuran (1 - 9%), isomenthone (1.5 - 10%), menthyl acetate (2.8 - 10%), pulegone (up to 4%) and carvone (up to 1%) are present in smaller proportions [16].

Lavender oil, also, is widely used for its medicinal actions including antispastic, anti-inflammatory, sedative, antimicrobial and general tonic action. Externally, it can be used for wounds and superficial burns. It contains as major compounds linalool (20 - 45%), linalyl acetate (25 - 46%), monoterpenes, alcohols and esters [1, 10, 13].

Even if essential oils are marketed extensively in pharmaceutical and cosmetic industry, not all products available to use are properly controlled in terms of quality of their composition. The analytical methods applied for the analysis of essential oils are numerous and include methods exploiting volatility (gas chromatographic methods) or their optical activity (polarimetric method), along with
the IR spectral methods, refractometry and thin layer chromatography [6, 7, 17].

Gas chromatography coupled with mass spectrometry (GC-MS) is the elected technique for analysing the main constituents of essential oils. The scientific literature regarding GC-MS analysis of essential oils reported a large number of components quantitatively and qualitatively analysed [2-4, 8-9, 11, 15].

Other techniques, inexpensive, remain a fast alternative, although there are limitations in terms of specificity and sensitivity.

This study was designed to assess the purity and quality of peppermint and lavender volatile oils available in herbal stores, through standardized or modified methods provided by pharmacopoeia, and to test the ability of IR spectrometry, a powerful identification method, to compare the same type of volatile oils from different producers.

Materials and Methods

Five peppermint oil samples, M1-M5, and four lavender oil samples, L1-L4, from different producers were analysed through standardized or modified methods provided by pharmacopoeia: refractive index, optical rotation, thin layer chromatography, GC-MS and through a non-official method, ATR-FTIR spectrometry. The gas chromatography coupled with mass spectrometry was used as reference method.

Thin-layer chromatographic analysis (TLC)

Reference substance: linalool, linalyl acetate, menthol, menthyl acetate. Reagents: ethyl acetate (Lachner), toluene (Lachner), ethanol (Chimopar), anisaldehyde (Sigma-Aldrich). Sample preparation: a volume of 20 µL commercial essential oil was diluted with 1 mL of toluene.

Chromatographic method: volumes of 5 µL samples were applied and eluted with toluene: ethyl acetate 95:5 as mobile phase on 20 x 10 cm chromatographic plate of Silicagel 60, 0.25 mm (Macherey Nagel, Duren, Germany); the plates were then sprayed with anisaldehyde solution and kept at 100 - 105°C in oven, for 5 - 10 minutes.

Physico-chemical constants determination

Polarimetric measurements were carried out on a Polamat A (Karl Zeiss Jena) polarimeter with 1 dm tube. Refractometry measurements were carried out on Abbé refractometer and the temperature was maintained at 20°C.

IR spectrometry analysis

IR spectrometry analysis were made with FTIR Nicolet 380 spectrometer (Thermo Electron Corp.), equipped with ATR Smart (Multi - bounce HATR) with Zn Se 45° plate. Omnic software version 8.3 (Thermo Scientific) was used for instrumental spectral recording and data acquisition. All FT-IR absorbance spectra were recorded in 4000 - 700 cm⁻¹ spectral region.

GC-MS analysis

Equipment: Agilent 7890A GC System with Agilent 5975C Inert XL E1/E1 MS MSD. Chromatographic conditions: injector temperature 250°C, column temperature 60°C, isotherm for 2 min, 3°C/min, to 240°C, isotherm for 5 min. Column: HP - 5 ms 30 m x 0.25 mm x 0.25 µm. Injection 1 µL/split less. Detector parameters: temperature 230°C, scan m/z = 50 - 800, solvent delay 4 min, (automatic) reference voltage, gas flow He 1 mL/min. The samples were diluted 1:1000 in hexane before the analysis. The identification was made by mass spectra comparison.

Results and Discussion

TLC analysis

Peppermint essential oil. Identification of menthol: by comparing the retention factor (Rf = 0.25) of standard solution spot with the retention factors of samples, it was observed that all samples contained menthol, M1 and M4 containing the highest amount. Identification of menthyl acetate: all samples contained this compound, M4 and M5 having the highest amount. The comparison of the chromatographic profiles for the five samples (Figure 1) showed that M1 and M2 were virtually identical in terms of number of spots, colour and size. M3 and M4 samples presented a spot just below the Rf 0.79 which proves that this sample contains a compound that is not found in other studied oils.

Lavender essential oil. Identification of linalool: all samples contain linalool (Rf = 0.59), L3 with lower concentrations than other L samples. Figure 2 shows clearly that sample L3 differs qualitatively from the other samples: the common identified spots have low intensity, which means diluted product, and the compound with Rf = 0.87 is missing.
Physico-chemical parameters

The results were analysed in terms of Pharmacopoeia provisions for peppermint and lavender oil monography, respectively.

Lavender samples. According to Pharmacopoeia provisions [18-19], L1, L2 and L4 samples were within the limits for refractive index and optical rotation.

Table I shows that L3 lavender oil has a very high refractive index and an angle of rotation nearly 0, which proves again dilution or contamination.

Peppermint oil samples. Regarding the refractive index and the optical rotation, all peppermint oil samples fall within Pharmacopoeia limits (Table II).

Table I

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Eu. Ph. limits</th>
<th>L₁</th>
<th>L₂</th>
<th>L₃</th>
<th>L₄</th>
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<tbody>
<tr>
<td>$n_0^{20}$</td>
<td>1.455 - 1.466</td>
<td>1.4660</td>
<td>1.4610</td>
<td>1.4750</td>
<td>1.4600</td>
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<tr>
<td>$\alpha_0^{20}$</td>
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<td>-11°</td>
<td>-11°</td>
<td>-0.2°</td>
<td>-6.8°</td>
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</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Eu. Ph. limits</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
<th>M₄</th>
<th>M₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_0^{20}$</td>
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<td>1.4615</td>
<td>1.4615</td>
<td>1.4620</td>
<td>1.4615</td>
<td>1.4595</td>
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<tr>
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<td>-24.8°</td>
<td>-16.8°</td>
<td>-23°</td>
<td>-22.2°</td>
</tr>
</tbody>
</table>

IR spectrometry analysis

Peppermint oil samples. The spectrum of each commercial product was obtained and the spectral fingerprint was found virtually identical in all cases (Figure 3). Making the comparison with the reference spectra from the spectrometer library, menthol and sample M1, for example, has a similarity score of 81.70 and menthone, 66.10.

Lavender oil samples. The samples spectra were identical for all samples, except L3 sample, which presented a supplementary band around 1600 cm⁻¹, in contrast to the other samples with a single band.
in this spectral region (Figure 4). For L3 sample the fingerprint region showed significant differences. Making the comparison of samples spectra with the reference spectra, standardized Lavender oil and linalyl acetate spectra, the similarity score was consistent for L1, L2 and L4 samples.

**Figure 4.**
IR spectra of lavender essential oil samples (from top to bottom L4, L1, L2, L3 samples)

**GC-MS analysis**
The GC-MS confirmed the preliminary tests results. The M samples had identical chromatographic patterns. The L3 sample chromatogram (Figure 5) and mass spectrometry profile were different in comparison with L1, L2 and L4: 19 identified compounds were common, including linalool and linalyl acetate with low concentrations, and 14 compounds were different.

**Figure 5.**
GC-MS chromatograms of lavender essential oils
The applied tests confirmed the fact that lavender oils are often falsified. Prusinowska et al. [12] showed that Lavandula latifolia essential oil, hybrid Lavandula oils or synthetic chemicals are often used as substitutes in order to reduce the costs of the final product.

According to our results, this kind of adulteration could be rapidly identified by simple determinations of refractive index or optical rotation. GC-MS is the confirmatory method, but the high cost of analysis is not affordable for the herbal products companies to test the quality of their raw materials by this method. However, a set of three simple assays, TLC, refractive index and optical rotation determinations, together with a powerful qualitative analysis technique, namely IR spectrometry, cheaper and easy to use than GC-MS, could be apply as a relatively fast methodology for quality control of essential oils.

Conclusions

Focusing on the qualitative assessment, all samples of peppermint oil contained menthol and menthyl acetate and all samples of lavender oil contained linalool and linalyl acetate, respectively. But, regarding the overall analytical results, it can be observed that not all samples were of excellent quality and high purity, some of them being contaminated with other substances or even diluted. The greatest difference was observed in the case of one lavender oil sample, which, probably, has a synthetic origin. We may conclude that commercial products continue to be marketed without fulfilling the quality criteria. Regarding the methods used for quality control, gas chromatography is a complex method, allowing the identification and quantification of constituents of essential oils, accurately and precisely. On the other hand, IR spectrometry method has proved effective in assessing the similarity in the composition of essential oils, being able to distinguish the difference between samples, in terms of compounds. In conclusion, fingerprinting IR spectrometry it’s a safe alternative for fast pre-testing of the quality of essential oils.

References

