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CHARACTERIZATION AND IDENTITY CONFIRMATION OF ESSENTIAL OILS BY MID INFRARED ABSORPTION SPECTROPHOTOMETRY♣

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Characterization and confirmation of identity as part of the structural interaction perspective of essential oils biostructure from lavender (*Lavandula officinalis*), peppermint (*Mentha piperita*), green Douglas (*Pseudotsuga menziesii*), fir (*Abies alba*) and chicory (*Cichorium intybus*) has been addressed by qualitative detection and quantification of specific natural clusters, by infrared absorption spectrophotometry. The method allows the identification of structural components that are characteristic to essential oils, due to steric and electronic effects of vibrational coupling (neighbouring bonds acting on force constant of the bond, changing the position of the absorption band). Through this research the essential oil identity was confirmed by quantifying the structural components and by assigning characteristic absorption bands in the spectrum measured. Comparisons of spectra by calculating the correlation coefficient is a convenient, fast and secure method for objective confirmation of essential oils identity.

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1. Introduction

Essential oils are used in order to exploit physiological and psychological properties of individual response to volatile biostructures, with the aim to reduce stress and to speed up the healing processes [1], including prevention and also treatment after the onset of disease [2,3]. The terms "essential oil", "volatile oil", "etheric oil" are used for denominating a liquid substance contained in specialized cells of plants, and which is obtained using certain methods of extraction [4]. Most essential oils are liquid, but there are also are solid or semi-solid oils depending on the temperature of the environment in which they are stored. They are soluble in organic solvents such as alcohol, fats, alcohol and insoluble in water. Their smell comes from the volatile substances contained, that give the characteristic scent of plants, flowers, fruits, seeds, bark of trees. From chemical point of view these are complex mixtures of aliphatic and aromatic hydrocarbons, aldehydes, alcohols, esters, and other constituents, but the predominant compounds class is represented by terpenoids [5]. Volatile oils extraction methods depend on the vegetal material used. For example cold pressing is used for oranges, whose bark contains essential oils, solvent extraction for fragile flowers and steam distillation for other plants [6]. Extraction of essential oils is an expensive process, due to the large amount of raw material required; it takes approximately

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35 kg of vegetal material to obtain 1 L of essential oil, and even more in the case of roses (to obtain 1 L of rose essential oil requires about 2000 kg of petals). However, in the perfume industry are used synthetic oils, which are not suitable for aromatherapy [7]. Essential oils contain hundreds of biostructures formed from different molecules, each having specific properties (antiseptic, antibacterial, immunostimulatory, decongestants, etc.). For example, the sage contains 250 different molecules, of which 75% came from the family of esters and 15% monoterpenes [8]. Green Douglas needles exudes a distinct odour when crushed (lemon flavour) hence the name of the tree, lemon flavour fir. The biostructures act synergistically, and this explains the versatility and broad spectrum of essential oils [9]. If the biostructure family properties and their concentration in essential oils are known, then their effects (beneficial or harmful) may be deduced. The essential oils isolated from different plants are complex biostructures comprising different compounds belonging to various chemical classes (terpenoids, ketones, aldehydes, esters) saturated or with some degree of unsaturation [10]. The compounds have common structural fragments as isopropyl group, isopropenyl, normal aliphatic chains, oxygen heterocycles, etc. [11]. The variability of the essential oils from the same species, is determined by the increased chemical complexity, geographical and climatic factors, and the particularity of technology of production and / or purification. For these reasons the confirmation of the identity of the essential oil biostructure seems to be a complex issue [12]. The studied species varieties have different flavours because plants (woody or herbaceous) contain volatile biostructures combined in various proportions for each species. The clove aroma of studied species is given by eugenol. Lemon flavour is provided by a high content of citral and limonene. Camphor aroma is due to camphor and camphene. The vegetal material may contain anethole, or other chemicals involved in the production of distinct flavours, depending on the proportion that are specific to each species such as: cinnamate, citronellol, geraniol, linalool, methyl-cavicol, myrcene, pinene, ocimene, terpineol, etc. A rational approach to the issue of identifying essential oil may lie in separating chemical constituents and their quantitative determination by different methods of chromatography (gas chromatography, HPLC) coupled with mass spectrometric detection. However, these techniques are laborious and often involve extensive investigations on finding optimal separation conditions for each type of essential oil [13]. Another impediment is costly laboratory infrastructure, which few laboratories own. For the reasons listed above is interesting to develop a technique for comparing an essential oil with a standard, in order to confirm its identity [14]. It is required that the technique of identity confirmation to be robust in the sense that variations related to geographical, climatic and processing peculiarities have minor influence on the results, however the identity of the species to have a significant influence.

Due to the fact that the mid infrared absorption spectrophotometry has a particular specificity, the use of this technique seems to be promising. Essential oils are immiscible with water. For this reason, the problems related to the presence of water in the analysed samples, which often compromise the samples in the infrared spectrophotometric analysis, do not occur. Although some components of the oils contain components with hydroxyl groups, capable of intermolecular hydrogen bonds, this detail does not compromise the analysis. This study aimed to measure IR spectra of several essential oils obtained from lavender, peppermint, green Douglas, fir and chicory.

2. Experimental

2.1 Essential oil extraction

The samples were collected from two locations in the western part of Romania: Trei Ape–Gărâna (22.10° East longitude and 45.22° North latitude) and Bazoş Arboretum Park (90 m altitude, 21.49° East longitude and 45.73° North latitude). Solid-liquid extraction was run with a continue Soxhlet extractor.

The extracted component is contained by a solid material (lavender, peppermint, green Douglas, fir and chicory). After repeated contact with the chosen solvent to dissolve essential oil, the solvent enriches in essential oils extracted. Soxhlet apparatus consists of a round-bottomed

flask (or bottom), an extractor and a reflux cooler (with bubble). The flask was placed in a water bath or sand, and the solvent (petroleum ether) was introduced, selectively chosen to extract the component of interest from the solid material. The extractor was attached above the flask sealed with a rubber stopper. The cartridge with vegetal material (lavender, mint, green Douglas, fir and chicory) was weighted and fixed into the extractor. The cartridge is made up of filter paper, and the vegetal material was fine grounded. The cartridge must be positioned so as to not touch the siphon. Above the extractor was mounted the refrigerant and water connections were linked to the source. The solvent was heated and the vapours reached the refrigerant where they were cooled and condensed, the condensate flowing in the extractor over the cartridge. At this moment, the condensation level increases, while the essential oil is extracted from the solid. When the liquid from the extractor reaches the top of the siphon, essential oil extracted will flow into the flask. Continuing the heating, further amounts of pure solvent vapours reached the refrigerant, were cooled and condensed, reaching over the solids in the extractor, where further amounts of essential oil was taken up by solvent. Again trap level was reached and the liquid in the flask reached the extractor. With each siphoning the solvent enriches with essential oil extracted. After 10-15 cycles, if there is no component to be extracted, the heating was stopped, the system was allowed to cool down (with the water circuit running during the cooling operation), and the cartridge was removed, dried and weighed. The difference between the initial mass of the cartridge and the final mass is the amount of essential oil (substance / component) extracted from the solid. Knowing the amount of solids introduced into the cartridge may be calculated the % of essential oil that was extracted from the solid material (assuming that the extraction was totally) [15].

2.2 FTIR spectra of essential oils

Absorption spectra were measured in the 1000-4000 cm^{-1} domain using a Fourier transform infrared spectrophotometer (FTIR technique), model FTIR 640, product of JASCO. This measuring technique has the advantage of a signal / noise ratio particularly advantageous. For this reason a full spectrum measuring time is reduced.

The high measuring speed (under a second) enables repeated measurements (the device was set for 64 consecutive measurements), following to calculate the average for each value of the wave number (operation "acquisition of spectra"). Due to the measurements of 64 spectra, signal / noise ratio improved 8 times. Samples of essential oil and essential oil used as a reference were compressed in the form of thin film (about 20 μm) between two slides of calcium fluoride crystal (transparent between 1000-4000 cm^{-1}) and interposed in the optical path of the radiation source with infrared emission [16]. Fourier transform spectrophotometer operation is based on light interference and a mathematical transformation (Fourier transform) of the detector signal, operation after which is obtained the absorption spectrum of the sample and reference material [17].

3. Results and discussion

Figures 1-5 present the absorption spectra of samples of essential oil obtained from lavender, peppermint, green Douglas, fir and chicory measured in the wavelength range 4000-1000 cm^{-1}, with a resolution of 4 cm^{-1}. Each spectrum has been obtained as a result of accumulation of 64 individual spectra. Vegetal samples were collected from the area Trei Ape–Gărâna and Arboretum Park Bazos. The spectrum of the reference oil is presented in Figure 1. The region 1400- 400 cm^{-1} (fingerprint) of the IR spectrum contains absorption bands that characterize the entire molecular structure by vibrations of the spectrum: deformation, combining, harmonic bands that cannot generally be attributed to normal vibrations.

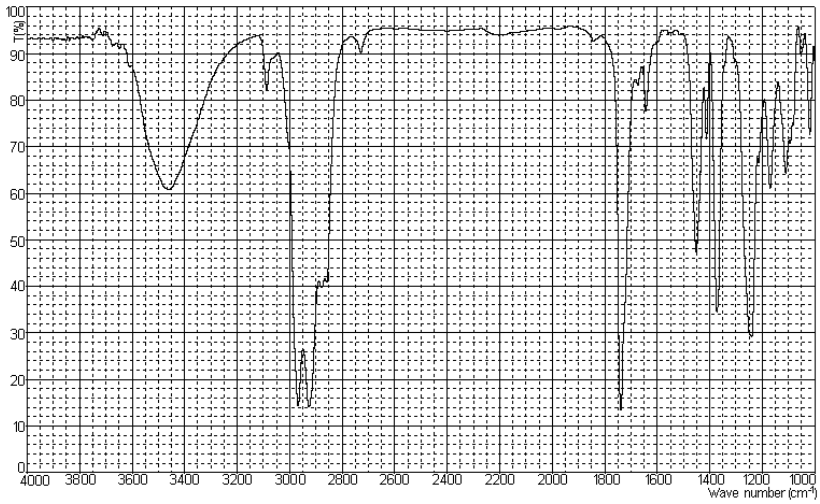

Fig. 1. Absorption spectrum of lavender (Lavandula officinalis) essential oil

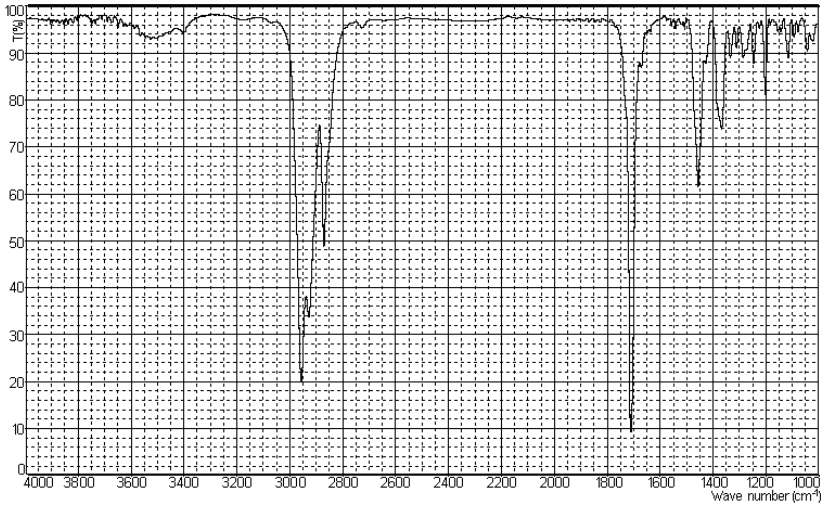

Fig. 2. Absorption spectrum of peppermint (Mentha piperita) essential oil

Fingerprint region can be used to identify a biostructure by comparing the IR spectrum of a standard compound. If the two compared spectra (the sample and the standard compound) has the same absorption in the fingerprint, it is strongly confirmed, more than with other methods (e.g. comparing the melting points or thin-layer chromatograms), that the structure of the compound to be analysed is the same as that of the standard, but the IR spectra must be measured in the same conditions. In the charged region of the absorption bands can be identified intense absorptions due to the deformation vibrations of the C-H bond and the valence vibrations of C-O single bonds of alcohols, ethers, esters and the C -halogen bonds [18].

The occurrence of characteristic absorption bands for functional groups can be explained by structural assignments, but should not be based solely on absorptions located in this area, because they are most often used only for confirming the proposed structure taking into account the group characteristic absorptions in other areas of the spectrum. The hydrogen bond modify the vibration frequencies of the essential oils that contain O-H and N-H bonds. The position of the absorption band due to valence vibration of O-H bonds is used to quantify the strength of association through hydrogen bonding. When the association through hydrogen bonding is stronger, the O-H bond length increases and bond force constant decreases, so the valence vibration is identified at lower frequency values compared to the values determined in the absence of association with hydrogen bonds. The vibration absorption band given by the free O-H bond

valence (monomeric ν_{OH}) occurs in the region of 3590-3650 cm^{-1}, and the association through polymer hydrogen bonds leads to wide bands in the region 3200-3600 cm^{-1} (carboxylic acid broadband absorption from low frequencies, in 2500-3000 cm^{-1} domain, due to strong dimers associations).

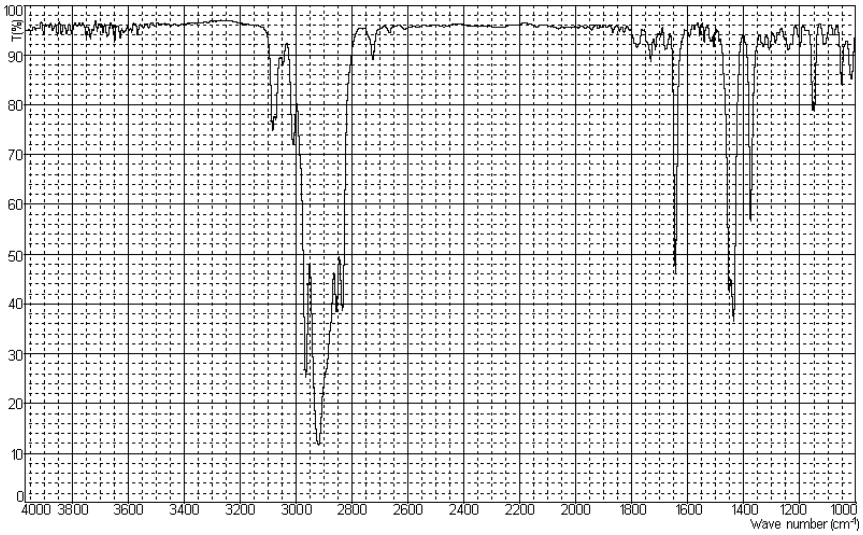

Fig. 3. Absorption spectrum of green Douglas (Pseudotsuga menziesii) essential oil

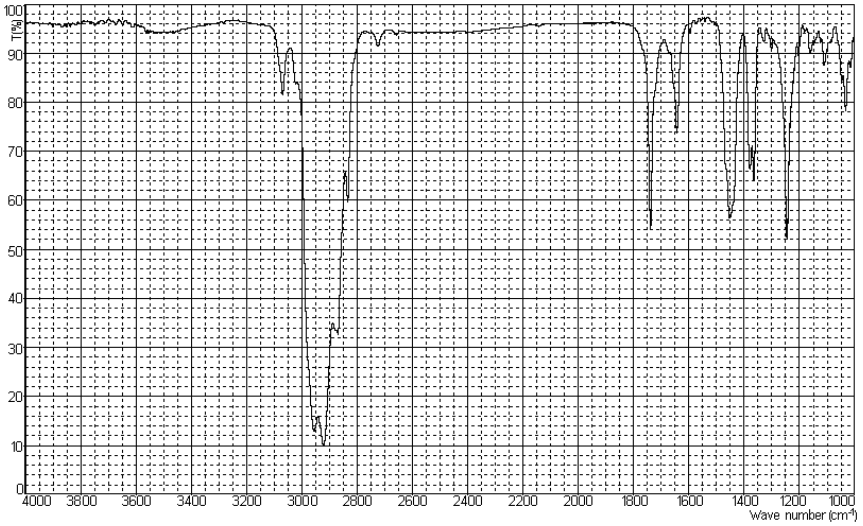

Fig. 4. Absorption spectrum of fir (Abies alba) essential oil

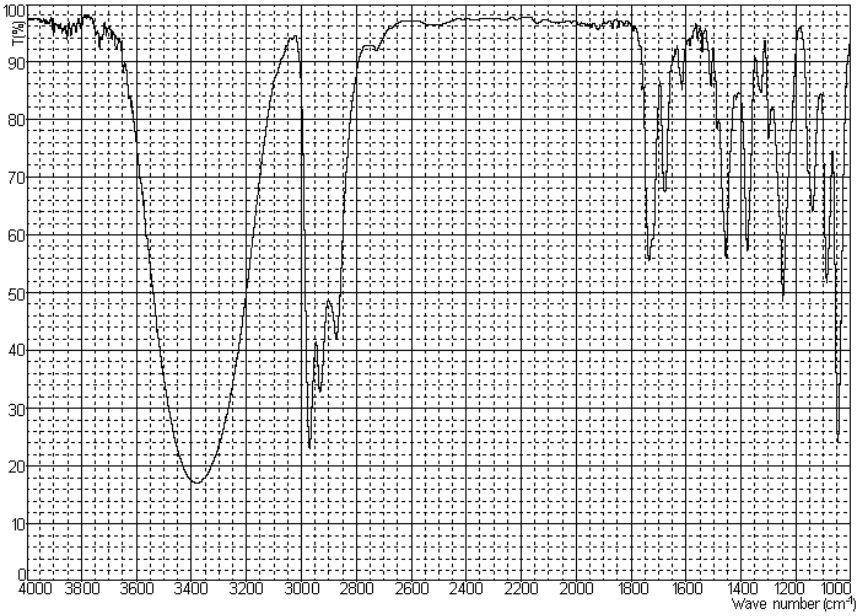

Fig. 5. Absorption spectrum of chicory (Cichorium intybus) essential oil

Fig. 6 and 7 present the infrared spectrum of the reference essential oil (from Fig. 1), compared with the spectrum of sample lavender oil.

The destruction of the intermolecular hydrogen bonds by diluting with a solvent not participating in hydrogen bonding (non-polar organic solvent) leads to the reduction of the polymer band intensity and the occurrence of a narrow band range located at higher frequencies, characteristic to the valence vibration of the O-H free bond (unassociated), so it appears that the absorption spectra of essential oils from different species differ significantly, while the spectrum of lavender oil, used as sample, and lavender essential oil, used as a reference, shows obvious similarities [19]. To express the similarity between the spectra of the two samples, the spectra in question are digitized, represented as a set of pairs of values (wavelength number vs percentage transmission).

Each spectrum consists of 3113 such pairs of values. Comparison of the two spectra, from biosystems which are to be expressed quantitatively for similarity is a comparison of the absorbance values for each pair of values of the 3113 wave number vs. percentage transmission. In the case of theoretical identity of the two spectra, for each value of the wavenumber, the transmission associated with the two spectra should be equal [20].

In the Figures 6 and 7 are presented the superimposed spectra of lavender oil (considered sample) and lavender essential oil (considered standard reference). The similarity between the two samples is visible, for the entire spectrum (4000-1000 cm^{-1}) and the extended representation of the subdomain from 2000 to 1000 cm^{-1}.

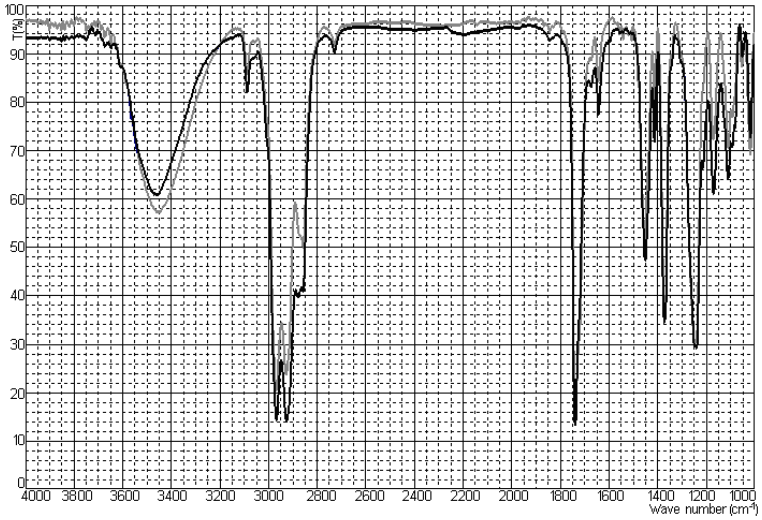

Fig. 6. Comparative absorption spectra of lavender (Lavandula officinalis)

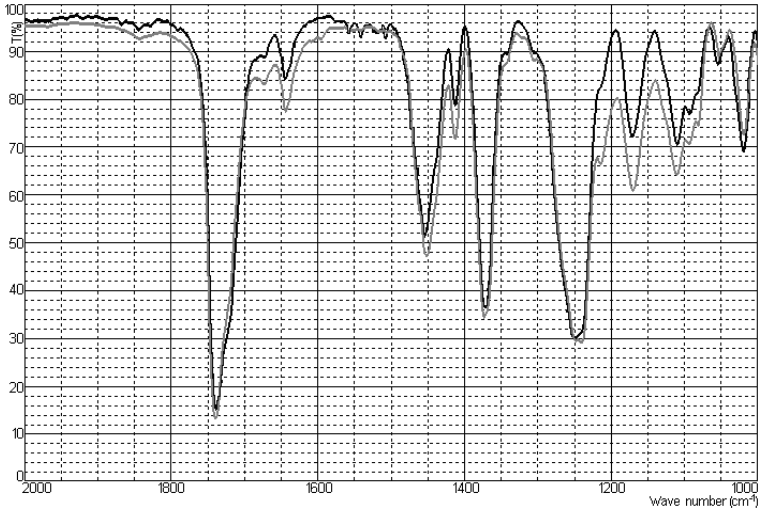

Fig. 7. Comparative absorption spectra of lavender (Lavandula officinalis)

For quantitative expression of the similarity of the two spectra, linear correlation coefficient calculated for the corresponding percentage transmission values ($\mathbf{T_a}$ and $\mathbf{T_b}$) of the two spectra (the spectrum **a** and **b**), appears to be a suitable statistical test. Linear correlation coefficient **(r**) for spectra **a** and **b** considered, calculated for the spectral range of digitalised values with serial number **m** and **n**, is expressed by equation (1).

$$r_{a,b}(m,n) = \frac{\sum_{i=m}^{n}(T_{a,i} - \overline{T}_a)\cdot(T_{b,i} - \overline{T}_b)}{\sqrt{\sum_{i-m}^{n}(T_{a,i} - \overline{T}_a)^2 \cdot \sum_{i=m}^{n}(T_{b,i} - \overline{T}_b)^2}} \tag{1}$$

In the above relation $\mathbf{T_{a,\,i}}$ is the value of optical transmission associated with point no. "**i**" of the spectrum "**a**" digitized, and $T_{\mathbf{b,i}}$, and is the same value for the spectrum "**b**". The symbols $\overline{T}_{\mathbf{a}}$ and $\overline{T}_{\mathbf{b}}$ are respectively the average value of transmission from spectra "**a**" and "**b**" above summation parameter "**i**". If the values $\mathbf{T_{a,i}}$ and $T_{\mathbf{b,i}}$ are equal for all values of "**i**" (included in the

m-n domain), the linear correlation coefficient $\mathbf{r_{a,b}(m.n)}$ is 1. For a real similarity, but non-ideal, the linear correlation coefficient is between **0** and **1**. The closer the coefficient of correlation of the theoretical value "**1**", the more advanced the similarity between the two spectra will be, so the more reliable the chemical identity of the sample and reference.

Applying the algorithm described were compared spectra of lavender oil (sample investigated) and lavender oil (reference) for the entire spectral range measured (4000-1000 cm^{-1}) and for spectral subdomains ranged 2000-1000 cm^{-1} and between 4000-2000 cm^{-1} [21]. Thus, it is presented the correspondence between the values of wave numbers marking the spectral range and the corresponding "**m**" and "**n**" in equation (1). (Table 1).

Table 1. The correspondence between the values of wave numbers marking the spectral range and the corresponding "m" and "n" in equation (1)

Spectral domain (cm^{-1})	**m**	**n**	**r(m,n)**
4000–1000	1	3113	0.97842
2000–1000	1	1039	0.97579
4000–2000	1039	3113	0.98143

It is found that the correlation coefficient calculated for subdomain 2000-1000 cm^{-1} is about as "permissive" as that calculated for the entire spectral scan (4000-1000 cm^{-1}), whereas the correlation coefficient calculated for subdomain 4000-2000 cm^{-1} is more permissive. This phenomenon is related to the fact that subdomain 4000-2000 cm^{-1} offer less spectral information than the subdomain 2000-1000 cm^{-1} [22].

Using linear correlation coefficient or comparing spectra is justified only in the case when measuring the two spectra, a baseline shift appears, and this does not affect the value of the correlation coefficient.

Furthermore were calculated the values for linear correlation coefficient between lavender essential oil (considered the reference) and samples of essential oils from the five samples of vegetal material, namely, lavender, peppermint, green Douglas, fir and chicory, calculated over the entire spectrum (4000-1000 cm^{-1}) (Table 2).

Table 2. The values of the linear correlation coefficient between lavender reference oil and essential oil samples studied.

Essential oil	Linear correlation coefficient with lavender reference oil spectra
Lavender oil	0.978425
Peppermint oil	0.685982
Green Douglas oil	0.586275
Fir oil	0.745359
Chicory oil	0.699587

From the data presented in Table 2 it can be observed that the correlation coefficient values for the oils derived from mint, green Douglas, fir and chicory were significantly lower than 0.97, the value calculated for sample lavender, collected from the Trei Ape-Gărâna vs the essential oil of lavender collected from the Bazoş Arboretum Park.

4. Conclusion

The method described presents several advantages such as: shorter period of time necessary for spectra recording (being overlapped the approximately 10 minutes required for frequency sweep) and a much more accurate reading of wave lengths characteristic for absorption bands peaks. IR spectroscopy provides a convenient opportunity to highlight how the association through hydrogen bonds, hard or impossible to study by other methods. Digitized spectra comparison by

calculating the correlation coefficient is a convenient way, fast and safe for objective confirmation of the identity of essential oils. The method is also suitable for detecting counterfeits.

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