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Essential Oil Quality and Purity Evaluation-Spectroscopy

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Abstract: Essential oils are fragrant concentrated extracts from plants that are very volatile and have a wide range of applications. In this study, chemometric approaches were integrated with quick, easy-to-use attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) to validate essential oils' taxonomy and purity. Based on plant botanical family and concentration, principal component analysis (PCA) divided 30 essential oil samples into three categories. The first group had highly concentrated Asteraceae oils, the second group contained highly concentrated Lamiaceae oils, and the third group contained three highly concentrated essential oils from various botanical families as well as commercial-grade essential oils. Despite their comparable spectral patterns or botanical family, commercial-grade oil samples did not cluster with the matching concentrated oil samples. The most important spectral bands that can be utilized as marker bands for discriminating between distinct botanical plant family groupings are infrared (IR) bands that correspond to carbonyl, vinyl, methyl, and methylene group vibrations, according to a loading plot. The results of PCA were confirmed by hierarchical cluster analysis (HCA). ATR-FTIR spectroscopy paired with chemometric algorithms provides a non-destructive and direct method for chemotaxonomic classification and purity assessment of medicinal and aromatic essential oils.

I. INTRODUCTION:

Almost every major end-use industry, including food and beverage, personal care, cosmetics, and aromatherapy, has seen continuous and strong growth in the worldwide essential oils market. The demand for essential oils in pharmaceutical and medical applications is predicted to grow as a result of the health benefits connected with their use. Essential oils, unlike most conventional medications and drugs, have little negative effects. Essential oils' properties are predicted to be a major influence in market growth. Furthermore, the rising prevalence of age- and stress-related health conditions like cardiovascular disease, Alzheimer's disease, diabetes, and anxiety is increasing demand for therapeutic essential oils in aromatherapy applications. Unfortunately, essential oils' great medicinal capabilities and market value [1–3] make them prime candidates for counterfeiting or adulteration with low-quality, low-cost substitutes [4,5].

Chemical composition, which is influenced by geographical and climatic conditions, as well as the process of extraction and purification, determine the variety of essential oils from the same botanical species. As a result, confirming the essential oil's identity is a difficult task. Separating chemical ingredients and then quantifying them using various chromatographic methods (gas chromatography, HPLC) along with mass spectrometric detection is the sensible approach. Analytical methods such as gas chromatography (GC) [6,7], high-performance liquid chromatography (HPLC) [8,9], nuclear magnetic resonance (NMR) spectroscopy [10], and electroanalytical techniques [11] are widely used for the accurate and precise analysis and evaluation of the authenticity of consumable products.They frequently include considerable chromatographic separation conditions optimization for each essential oil type. Reading a GC/MS chromatogram, on the other hand, necessitates talent and experience. Standards, as well as predicted ranges for each constituent, are required for the quantification of constituents in order to establish whether their concentration is within these ranges. For identification and quality assurance of food products, non-destructive, easy-to-use analytical spectroscopic approaches such as infrared spectroscopy as a green tool paired with chemometric analysis have been used [12,13]. Because it provides for fast, green, non-destructive, and cost-effective testing of essential oil quality, non-targeted fingerprinting by Fourier-transform infrared (FTIR) spectroscopy has gained favour as an alternative to traditional GC-based approaches [14]. The main benefit of IR spectroscopy is that it requires little or no sample preparation and allows for simple and quick analysis. Most IR spectrometers are simple to operate, portable, and affordable, allowing for online sample examination.

Mid-infrared spectroscopy was previously primarily used as a qualitative method to identify unknown pure substances by providing structural characterization based on functional group vibration and a fingerprint spectrum (identification), or to verify quality markers in plant extracts or distillates [15]. Because plant extracts are multicomponent mixtures, infrared spectra obtained from them are typically quite complicated. Because each functional group in a single molecule contributes to the spectral pattern, band assignments can be challenging due to the complexity of the final spectrum caused by overlapping peaks and vibrational mixing.

For the classification and quality evaluation of food products, the use of chemometric methods is a particularly active research topic. Chemometric methods have been used to classify plant foods in a number of research. Principal component analysis (PCA) was used, for example, to outline the similarities and differences among 16 algal species and to identify 40 wine samples based on their HPTLC fingerprints. Each plant species contains a unique, complex blend of bioactive natural compounds, each of which contributes to the overall bioactivity of the plant.Similar types of biologically active secondary metabolites are commonly found in

related botanical families, and knowing the systematic position of a medicinal plant species allows some assumptions to be made about the substances that are present. Plants from the Asteraceae and Lamiaceae families have long been used to treat a variety of ailments. This is due to the fact that they produce a variety of secondary metabolites that have antibacterial, antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer properties. Plant extracts from the Lamiaceae family contain more known medicinal species and contain higher levels of phenolics (and flavonoids) than extracts from the Asteraceae family.The current study's major goal was to look at the impacts of plant taxonomy or botanical origin on essential oil purity and purity based on their spectral fingerprint in the mid-infrared range. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy was used in conjunction with pattern recognition techniques like PCA and cluster analysis to achieve this goal.

II. MATERIALS AND METHOD OF EXPERIMENT:

Samples:

Thirty essential oil samples were analyzed. Sixteen samples of concentrated essential oils (Table 1)that were obtained by supercritical fluid extraction with natural carbon dioxide (se-CO_2) were kindly donated by FLAVEX® Naturextrakte GmbH, and 14 remaining samples were commercial-grade essential oils purchased from the local market (lemongrass, two geranium oil samples, lavender, orange,two peppermint oil samples, forgive oil, breathe blend, frankincense, juniper, two rosemary samples,and bergamot essential oil).

Sample No	Extract	Species	Family
1	Frankincense carteri resin extract	*Boswellia carteri*	Burseraceae
2	Evening primrose seed extract	*Oenothera biennis*	Primulaceae
3	Arnica flower extract	*Arnica montana*	Asteraceae
4	Echinacea root extract	*Echinacea purpurea*	Asteraceae
5	Marigold flower extract	*Calendula officinalis*	Asteraceae
6	Tagetes flower extract	*Tagetes erecta/Tagetes patula*	Asteraceae
7	Chamomile flowers and flowering shoots extract	*Chamomilla recutita*	Asteraceae
8	Oregano phenol type leaf extract	*Origanum vulgare*	Lamiaceae
9	Oregano terpineol type leaf extract	*Origanum vulgare*	Lamiaceae
10	Sage leaf extract	*Salvia officinalis*	Lamiaceae
11	Lemon myrtle leaf extract	*Backhousia citriodora*	Lamiaceae
12	Thyme leaf extract	*Thymus vulgaris*	Lamiaceae
13	Rosemary extract, cineole type, and leaf extract	*Rosmarinus officinalis*	Lamiaceae
14	Rosemary plus, leaf extract	*Rosmarinus officinalis*	Lamiaceae
15	Peppermint leaf extract	*Mentha piperita*	Lamiaceae
16	Lavender flower extract	*Lavandula angustifolia*	Lamiaceae

Table 1. Concentrated essential oils obtained by supercritical fluid extraction (FLAVEX®).

ATR-FTIR-Spectroscopy:

The FTIR spectra were acquired using a Cary 630 FTIR (Agilent Technologies Pty Ltd., Mulgrave, Australia) interfaced with an ATR (attenuated total reflectance) sampling accessory with a single bounce diamond crystal. Spectra, in the absorbance mode, were measured from 4000 cm^{-1} to 600 cm^{-1},by accumulation of 64 scans at a spectral resolution of 4 cm^{-1}. A reference (background spectrum of air)was scanned under the same instrumental conditions before each sample measurement. Spectra were processed with Resolution Pro FTIR spectroscopy software (version 5.2.0, Agilent Technologies Pty Ltd., Mulgrave, Australia). A small drop of essential oil sample is simply placed on the surface of the diamond ATR crystal and the sample spectrum collected.

For the PCA analysis, the original 1858 spectral intensities were reduced into 254 averaged spectralvalues, each from five consecutive wavenumbers (dB = 5).

Principal Component Analysis:

PCA analysis was performed with the Principal Component Analysis for Spectroscopy App for OriginPro®. 2019 version 9.6.0.172 (OriginLab Corporation, Northampton, MA, USA). Hierarchical clusteranalyses (HCA) were performed using the PLS Toolbox software package (Eigenvector Research, Inc., Manson, WA, USA) for MATLAB (Version 7.12.0 R2011a). HCA was performed using the Ward methodto calculate Euclidean distance as a measure of distance between samples.

III. RESULT AND DISCUSSION:

ATR-FTIR Spectrometry:

Essential oils are concentrated solutions of volatile molecules made up of complicated homogeneous combinations of different substances, with each species containing over 100 ingredients (taxon).

Due to the overlapping spectra of distinct components and the mixing of numerous vibrational modes, their FTIR spectra are complex. Although main components seldom account for more than 25% of the total content, chemicals in essential oils that appear in low amounts (less than 1%) have no effect on the ATR-IR spectrum. As a result, ATR-FTIR spectra obtained from essential oil samples exhibit distinctive spectral fingerprints that can be utilized to distinguish between plant species and chemotypes.

ATR-FTIR absorption spectra of the concentrated essential oils show the expected characteristic C-H stretch (~2900 cm^{-1}), C=O stretch (~1700 cm^{-1}), broad O-H stretch (~3400 cm^{-1}) and C-O stretch(~1100 cm^{-1}) of terpenoid components present in the essential oils (Figure 1 a,b). As expected, the FTIR spectra of these oils are dominated by vibrational modes from monoterpenes which are observed at886, 1436 and 1644 cm^{-1}. Another useful band in terms of differential identification of the oils is theband for C=O stretching, which appears at 1740 cm^{-1} in lavender, rosemary and sage oil, and is shifted to lower wavenumbers in lemon myrtle, oregano (terpineol type) and peppermint oil.

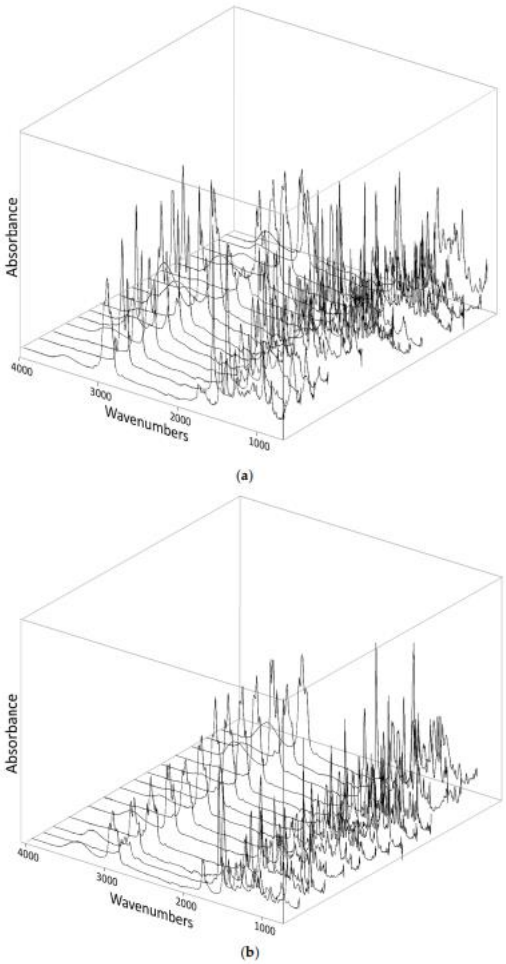

Figure 1. Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) superimposedspectra of concentrated essential oil samples (**a**) and commercial-grade essential oils (**b**).

Principal Components Analysis and Cluster Analysis:

Principle components analysis is a technique for condensing a large dataset into a smaller number of latent variables called principal components (PCs). With a high number of spectral bands (input variables), it is important to minimise the number of variables to a few linear combinations of the spectral bands that can be simply explained in order to interpret the data in a meaningful fashion. The steep line that bends fast and then flattens out indicates that the first two PCs captured the majority of the information and were sufficient to express the essence of the data in the PCA correlation matrix.Figure 2a,b displays clustered samples with similar input spectral data values in a score plot (i.e., principal components). PC1 accounts for 38.2% of total variability, PC2 for 23.7 percent, and PC3 for 7.9%.

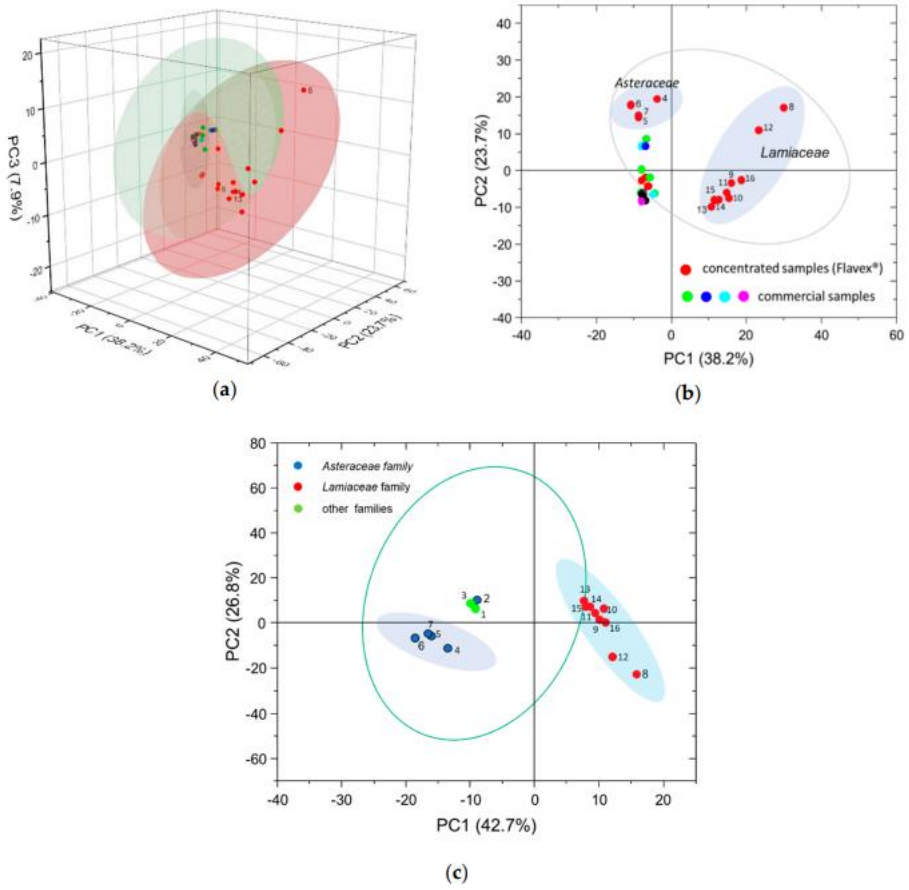

Figure 2. (**a**) 3D and (**b**) 2D PCA score plots for all essential oil samples, and (**c**) 2D PCA score plots forconcentrated essential oil samples obtained from FLAVEX®.

Plant materials from the Asteraceae and Lamiaceae families formed different clusters along PC1 according to principal component analysis. Asteraceae samples (samples 4–7) were also separated from commercial samples as well as the Lamiaceae (samples 9–16) using PC2.

Essential oil samples isolated from plants belonging to distinct botanical families differ greatly in their contents, resulting in unique spectral fingerprints, according to a cluster analysis. The principal component analysis of 16 samples of concentrated essential oils from FLAVEX® grouped samples yielded a two-component model that explained 69.50 percent of the total variance after filtering and normalisation (Standard Normal Variate, SNV). PC1 accounted for 42.7 percent of the overall variance, whereas PC2 accounted for 26.8%. (Figure 2c). All samples were divided into three groups in the same manner as before.According to PC1, Lamiaceae samples are clearly distinguished from other concentrated essential oils, which are divided into two groups in PC2: one containing four Asteraceae family samples (samples 4–7), and the other containing frankincense (Burseraceae family), arnica (Asteraceae family), and primrose (Asteraceae family) (Primulaceae family).

Four samples from the Asteraceae plant family were included in the first group; nine concentrated oils from the Lamiaceae were included in the second group; and frankincense, arnica, and primrose essential oils from various plant families were included in the third group.

The PC loadings plot was used to identify IR bands that are characteristic for the sample groups and have the strongest influence on each group of samples and thus contribute to the unique FTIR profile in the group (Table 2). The bands with the strongest influence on the principal components

in the Lamiaceae family group essential oils are bands at ~1375 and 1450 cm^{-1}, a broad band at 3400–3500 cm^{-1}, in addition to a band at 842 cm^{-1} that was shifted to 862 cm^{-1} in oregano essential oil(Figure 3a). The vibrational frequencies for linalool and linalool acetate fit correctly in the vinyl group vibration RHC=CH_2 at 1635–1650 cm^{-1}, but the intensities of these peaks are very low. Linalool is foundin the essential oils of over 200 plant species, belonging to different families. Linalool and its ester linalyl acetate are the main constituents in lavender oil. The C=CH_2 in-plane deformation vibration occurs near 1420 cm^{-1}. Only linalool, linalyl acetate and myrcene show this band, while limonene and β-pinene show no trace of it. However, this band may be hidden under the CH_2 and CH_3 deformation bands near 1450 cm^{-1}. The =CH_2 in plane deformation vibration was not found as a separate band near 1410 cm^{-1}, and was most probably hidden under the $-CH_3$ and =CH_2 absorption bands. The presence of a =CH_2 group may also explain why the 1330–1410 cm^{-1} intensity is higher for some terpenes.

Family	Species	ATR-IR Peaks	Compound Assignment
Lamiaceae family cluster	Oregano (terpineol type), sage, lemon myrtle, rosemary (cineol type), rosemary plus, peppermint and lavender	~1375 and 1450 cm^{-1}	$=CH_2$ in plane deformation at 1420 cm^{-1} (presence of $=CH_2$ group will increase intensity of the peaks from 1330–1410 cm^{-1} for some terpenes) Peak at ~1450 cm^{-1} is a result of overlap of CH_2 deformation and asymmetrical CH_3 deformation (intensity of this peak is proportional to the number of CH_2 and CH_3 groups present)
		3400–3500 cm^{-1} (broad band)	Lamiaceae family plant extracts have a higher content of phenolics (and flavonoids).
		842 cm^{-1} (shifted to 862 cm^{-1} in oregano)	Weak skeletal vibration for isopropyl ($R_1R_2C=CHR_3$ out-of-plane deformation of non-strained, weakly strained (cyclohexene derivatives) and strongly strained systems); key characteristic peak for carvacrol occurs at 862 cm^{-1}
		1635–1650 cm^{-1} (low intensity peaks)	Vibration for $RHC=CH_2$ (linalool and linalool acetate).
		1745 cm^{-1}	Carbonyl stretching from α-thujone and camphor (sage essential oil)
	Oregano (phenol type), thyme	1458 and 1380 cm^{-1}	Double bands at 1370 and 1380 cm^{-1} from isopropyl groups (tetrahydrolinalool, tetrahydrogeraniol and their acetates), gem dimethyl ($>C(CH_3)_2$) in α- and β-pinene band at 1385 cm^{-1} could be related to bending symmetric $CH_3(CO)$ vibration of 1,8-cineole
		~810 cm^{-1}	C-H out-of-plane bending for carvacrol (oregano); ring vibration of thymol is seen at 807 cm^{-1}, while for carvacrol this corresponding signal appears at 811 cm^{-1}
Asteraceae family cluster	Echinacea, marigold, tagetes and chamomile	1733 cm^{-1}	Carbonyl stretching (chamomile has a double C=O band at 1711 and 1735 cm^{-1}; this band is smaller in intensity for echinacea).
		850-920 cm^{-1}	Methylene $=CH_2$ out-of-plane deformation (β-pinene absorbs at 875 cm^{-1} due to the strained ring structure with an exocyclic $=CH_2$ group). This band indicates the presence of myrcene in chamomile (high intensity due to the conjugation with the vinyl group)
Mixed cluster	Arnica, primrose and frankincense	1159 cm^{-1}	Stretching of C-O and bending of C-OH due to the presence of lipids and alcohol groups in unsaturated fatty acids (primrose), diterpene alcohols (frankincense) and triterpenediol esters (arnidiol/faradiol) in arnica
		1743 cm^{-1}	Carbonyl stretching

Table 2. The most significant infrared (IR) bands with the strongest influence on different cluster groups.

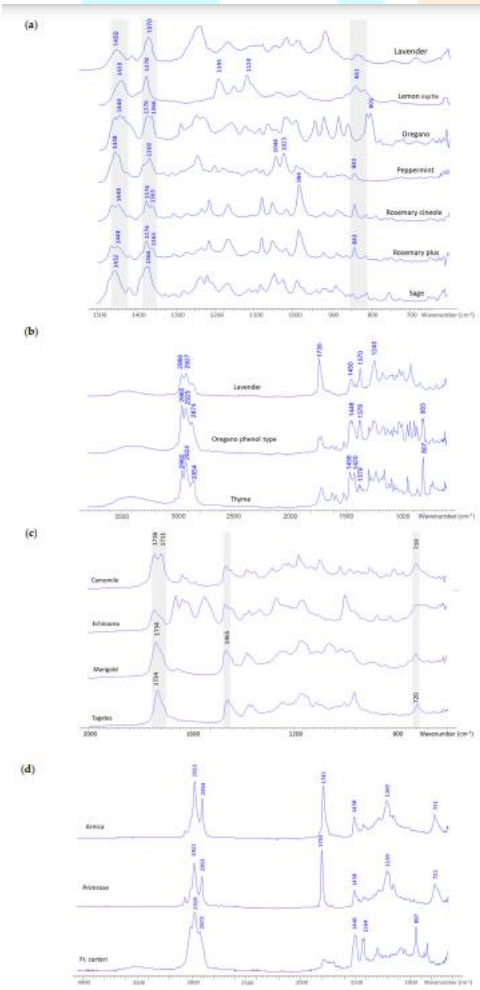

Figure 3. ATR-FTIR spectra for (**a**) Lamiaceae cluster with positive correlation (lavender, lemon myrtle,oregano terpineol type, peppermint, rosemary cineole type, rosemary plus and sage essential oils);
(b) lavender against Lamiaceae cluster with negative correlation (oregano phenol type, and thyme);
(c) Asteraceae cluster (chamomile, echinacea, marigold and tagetes) and (**d**) mixed oil samples cluster(arnica, primrose and frankincense).

The CH_2 deformation and asymmetrical CH_3 deformation appear at 1450 cm^{-1}, while the symmetrical CH_3 deformation is near 1380 cm^{-1}. Although the two 1450 cm^{-1} vibrations actually occur at two different frequencies (1460 and 1440 cm^{-1} as average values), the two peaks usually overlap andonly one peak is observed. The integrated intensity of this band can supply information about the number of CH_2 and CH_3 groups present. The strong band at 1745 cm^{-1} for rosemary essential oil, especially in the rosemary cineole type, suggests the presence of 1,8-cineole [12]. For sage essential oil, the carbonyl stretching band at 1745 cm^{-1} indicates the presence of α-thujone and camphor.

Oregano (phenol type) and thyme essential oils, although in the same group, were separatedfrom the other Lamiaceae plant essential oils. The loading plot for oregano and thyme suggested the importance of the FTIR area around 1380 cm^{-1}. They also exhibit the same characteristic bands at 1458 and 1380 cm^{-1}, but the band at 1740 cm^{-1} was reduced in intensity. This is clearly seen when compared to the spectrum of lavender (Figure 3 b). For oregano essential oil, the signature band for carvacrol is located at ~810 cm^{-1}, indicating C-H out-of-plane bending. Oregano (phenol type) essential oil contains 60–80% phenolic carvacrol according to the manufacturer's claim. The band near 1380 cm^{-1} has been assigned to the symmetrical CH_3. Isopropyl groups produce double bends near 1370 and 1380 cm^{-1} (e.g., tetrahydrolinalool, tetrahydrogeraniol and their acetates). The gem dimethyl ($R_1R_2C(CH_3)_2$) group present in the in α- and β-pinene also produces this doublet. α- and β-pinene are two structural isomers, bicyclic monoterpenes, present in many essential oils.

The most important bands for the Asteraceae family cluster were at 726, 1465 and 1733 cm^{-1},together with the area between 850 and 920 cm^{-1}. Chamomile, marigold and tagetes were very close together, while echinacea was apart from the group (Figure 2b), perhaps because the peak at 1700 $cm^{-1}$is smaller in intensity (Figure 3c). Chamomile has a double C = O band at 1711 and 1735 cm^{-1}. Methylene $=CH_2$ out-of-plane deformation is seen in the region around 890 cm^{-1}. β-pinene, however, absorbs at 875 cm^{-1} with an increased intensity that is typical for strained ring structures with an exocyclic $=CH_2$ group. The intensity of this band in myrcene is high, due to the conjugation with the vinyl group, which shows enhanced intensities for the out-of-plane deformation frequencies. Myrcene is a typical example of an unsaturated acyclic hydrocarbon, and is detected in chamomile flower essential oil.

A loading plot identified significant bands at 1159 and 1743 cm^{-1} for the last cluster containing pure essential oils of arnica, primrose and frankincense (Figure 3d). The strong absorption at 1160 cm^{-1} occurs due to the presence of lipids and alcohol groups (stretching of C-O and bending of C-OH). Frankincense essential oil contains diterpene alcohols, primrose oil contains high content of polyunsaturated fatty acids, while arnica contains sesquiterpene lactones and triterpenediol esters (arnidiol/faradiol). Although alcohols show two strong bands, the band in the 1300–1450 cm^{-1} region is usually overlapped by CH_2 and CH_3 absorptions, while the second band, assigned to the C-O stretching vibration, is in the 1000–1150 cm^{-1} region. Primary alcohols like nerol and geraniol, due to the presence of a double bond and secondary alcohols, absorb about 50 cm^{-1} lower.

Essential oil samples were classified into three categories based on their resemblance using principal component analysis. Commercial essential oil blends were classified together regardless of botanical family. Highly concentrated extracts were grouped together according to botanical family. Four samples of concentrated essential oils from the Asteraceae plant family were grouped together in the first group; nine samples of concentrated extracts from the Lamiaceae plant family were grouped together in the second group; and seventeen samples (three remaining concentrated extracts and commercial essential oils) from various sources, regardless of purity or plant source, were grouped together in the third group.Thus, all samples from the Lamiaceae (Mint) family and all samples from the Asteraceae (Sunflower) family were clustered together except for arnica essential oil (sample 2), that belongs to theAsteraceae family but was not clustered together with the rest of the oils from this family. Instead, arnica was clustered in the same group with concentrated frankincense and primrose oils. Arnica oil exhibits a similar ATR-FTIR spectrum to the primrose oil with a strong sharp C=O band at 1743 cm^{-1} and a medium wide band at 1159 cm^{-1} that are not present in frankincense essential oil.

Despite having identical spectral patterns, commercial lavender, rosemary, frankincense, and peppermint essential oil samples did not cluster with their respective concentrated extracts. PCA of commercial blends could not be explained by 2D or 3D PC plots, showing that there is no relationship between two main qualities. To achieve the defined oil profile standards, commercial essential oils are normally made with roughly 20% of the identified species of plant plus natural extractions from other essential oils. The highly concentrated FLAVEX® essential oils employed in this study, on the other hand, were 70–90 percent pure.

Although same oil samples produce very similar spectral fingerprints, there is a significant difference in in the ATR-FTIR spectra from concentrated essential oils and commercial-grade essential oils. Dilution of an essential oil with a non-polar organic solvent not participating in hydrogen bonding leads to significant differences in terms of the shape and intensity of the respective bands, particularly in the region from 1000–1300 cm^{-1}. The peaks in the FTIR spectra of commercial oils samples are better separated with less overlapping IR bands. The area between 1100 and 1300 cm^{-1} corresponds to stretching vibrations of the C-O group, while the O-C-O band originating from primary alcohols appears in the region from 1100 to 1020 cm^{-1}.

For example, concentrated lavender oil sample shows a clearly defined shoulder peak for the peak at 1740 cm^{-1} (corresponding to the vibrations of the C=O group) on the lower wavenumber side, witha clear maximum at 1685 cm^{-1} , which can be associated with the formation of a hydrogen bond between C=O and –OH groups (Figure 4).

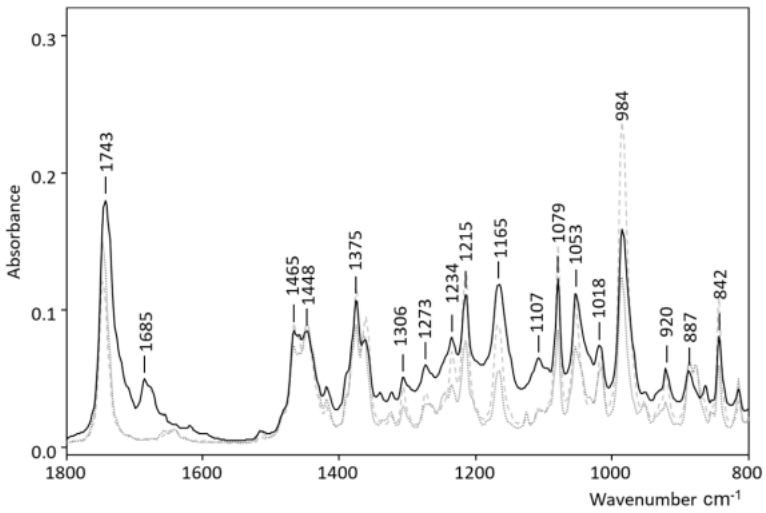

Figure 4. Superimposed ATR-FTIR spectra of concentrated lavender essential oil sample (solid black line)and commercial-grade lavender oil samples.

As a result, spectra from the same botanical family are more comparable than spectra from concentrated and diluted oil samples from the same origin.

A hierarchical cluster analysis (HCA) of spectra was done to confirm the results produced by the PCA (Figure 5). Unlike PCA, HCA takes into account all data variability and indicates how similar or different each pair of samples are. The clustering algorithm's purpose is to divide the items into homogeneous groups, with within-group similarities being greater than between-group similarities.According to the hierarchical clustering result, concentrated essential oils samples extracted from the Lamiaceae family (samples 8–16) and the Asteraceae family (samples 3–7) were near to each other, confirming the results of PCA.

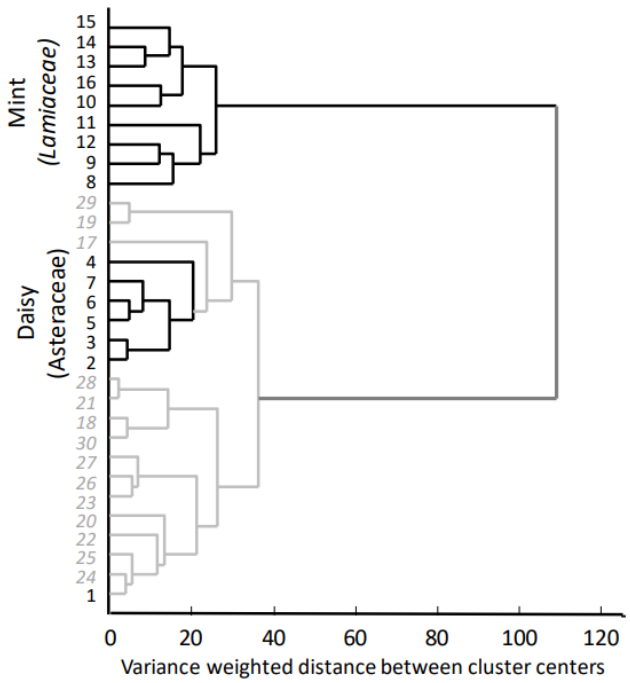

Figure 5. Hierarchical cluster analysis (HCA) of ATR-FTIR essential oil spectra.

IV. CONCLUSION:

The objective ATR-FTIR approach with chemometric evaluation was successfully used to the qualitative discrimination of essential oils from various plant species. PCA was used to characteristic key spectral bands that can be utilized as marker bands to discriminate distinct plant botanical families, using ATR-FTIR spectra acquired from essential oils as spectral fingerprints.

Using ATR-FTIR spectra with PCA and cluster analysis, it was proved that essential oils could be categorized based on their purity and taxonomy (botanical family).

For the quality control of essential oils, ATR-FTIR spectroscopy is a green, direct, and cost-effective alternative analytical approach. The use of vibrational spectroscopy in conjunction with chemometric algorithms allows for the efficient and non-destructive categorization of medicinal and aromatic essential oils. Furthermore, PCA analysis of IR spectra identifies distinctive key bands of a specific essential oil, allowing for the differentiation of different essential oil profiles of individual oil plants within the same species (chemotypes).

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