



DIFFERENT PERSPECTIVES IN MEDICINAL AND AROMATIC PLANTS

EDITORS

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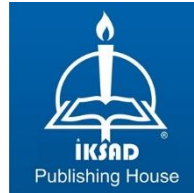
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PREFACE

Türkiye is at the intersection of three floristic regions, namely Europe-Siberia, Iran-Turanian and Mediterranean, and is located in the Fertile Crescent region. Due to the geographical location of Turkey, the diversity of plant species in its natural flora is quite high, and about one-third of them are endemic. About 500 of the 10,000 plant species spread throughout Türkiye are used for medicinal and aromatic purposes.

Today, medicinal and aromatic plants are used in many fields such as medicine and pharmacy, cosmetics and perfumery, spices, food additives, plant protection, animal health and feed additives, natural dyestuffs and landscape applications. Phenolic compounds, terpenoids and alkaloids possessed by aromatic plants give plants their medicinal and aromatic properties. These compounds constitute the raw material of many drugs in the pharmaceutical industry today, either by isolating or synthesizing. According to the WHO (World Health Organization), 25% of prescription drugs are medicinal and aromatic plant-based and when the FAO (Food and Agriculture Organization) data are examined, 30% of the drugs sold worldwide consist of compounds derived from medicinal plant. Some compounds found in plants can be produced by synthesis, while others cannot. Although drugs produced from synthetic compounds have an important place today, they can be seen in cases where synthetic drugs have side effects or fail to respond to treatment over time. At the same time, some bacteria may become resistant to antibiotic-style drugs. In such cases, there is a rapid return to natural products and nature.

This book consists of twenty chapters from different disciplines related to medicinal and aromatic plants. We would like to express my special thanks to our valuable authors who shared their research with us in this book.

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CHAPTER 10

CHARACTERIZATION TECHNIQUES OF HERBAL ESSENTIAL OILS

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INTRODUCTION

Essential oils, which can be obtained from the leaves, fruit, bark or root parts of plants, are natural products that are liquid at room temperature, usually colorless or light yellow. Although essential oils are defined as oils because they do not mix with water, they are different from fixed oils and are largely composed of terpenes in their chemical structure. (Ceylan, 1983). It is also known that essential oils can be produced by many aromatic plants and consist of a mixture of various components such as terpenes, terpenoids, phenylpropenes (Mejri et. al, 2018). Essential oils are generally known for their fragrant, flavor-enhancing and therapeutic properties, as oxygenated derivatives exhibit odor, taste and therapeutic properties by oxidation of terpenes (Linskens and Jackson, 1997a). In recent years, the use of essential oil has increased, especially due to the interest in alternative medicine. In addition, herbal essential oils are widely used in production, cosmetics, food and cleaning industries, apart from alternative medicine. From this perspective, the production of essential oils from plants and their characterization become important. Distillation, extraction and pressing processes are generally used methods from past to present in the production of essential oils. Today, it is preferred in different modern methods, especially in order to increase the yield and quality of the product. These methods are microwave-assisted extraction, supercritical fluid extraction and solid-phase microextraction etc. can be classified as techniques.

Water distillation, steam distillation and vacuum distillation types are generally preferred among the distillation processes and carried out by utilizing the differences in boiling points of liquids. Water distillation is widely used and is carried out in small-scale and large-scale production in clevenger and distillation boilers, respectively. The basis of this method is based on the transportation of volatile oil molecules with the water vapor formed as a result of boiling the plant with water and then separating it from the water by condensation (Linskens and Jackson, 1997b). In the steam distillation method, the water vapor applied to the plants with the help of pressure ensures that the essential oils are entrained with steam and the essential oil is separated from the water as a result of condensation (Linskens and Jackson, 1997b). In vacuum distillation, it is generally applied to components with high boiling points. By reducing the pressure below the vapor pressure of the compound, boiling and distillation processes are applied.

In the extraction method, which is another separation technique, solvent extraction is generally used. In this method, plants can be kept directly in organic solvents at room temperature or boiled in soxhlet. After extraction, the organic solvent is removed by distillation and volatile compounds can be obtained in the remaining part.

Cold hydraulic pressing of the raw material can be applied in the production of essential oils that can deteriorate when distillation and extraction processes are applied.

Microwave-assisted extraction method, which is among the preferred modern methods today, is closed system extraction with temperature and pressure control. The basis of this method is based on heating the plant sample with the solvent by applying microwave. In this method, while product losses are reduced compared to other methods, the extraction time and the amount of solvent used are also significantly reduced (Kaufmann and Christen 2002; Kaufmann et al., 2007; Beejmohun et al., 2007).

Supercritical fluid extraction, which is among the modern separation techniques, attracts great attention with its feature of separating components dissolved at high temperature under normal conditions (Yamani et al., 2008). In this method, substances with supercritical fluid properties are used as solvents instead of organic solvents. Carbon dioxide is generally preferred as this solvent because of its low cost, high purity, ease of use and minimal environmental impact (Linskens and Jackson, 1997b).

Another method, solid-phase microextraction, combined sample preparation, extraction and concentration in a single solvent-free step. This method, which provides significant savings in processing time and costs, is especially used in the extraction of volatile and semi-volatile organic compounds in the samples (Vas and Vekey, 2004).

After the separation method is selected and applied depending on the properties of the essential oil components, their characterization is the second important step. In this study, we briefly summarized the

separation techniques. The next section contains information about the characterization methods of the essential oils obtained.

In the characterization of essential oils, techniques such as Fourier-Transform Infrared Spectroscopy, Raman Spectroscopy, Gas Chromatography-Mass Spectrometry, Differential Scanning Calorimetry, Nuclear Magnetic Resonance Spectrometer are generally used. In this part of the chapter, information about these techniques is separately given.

Fourier-Transform Infrared Spectroscopy (FT-IR)

FT-IR is used to characterize organic and/or inorganic compounds. In FT-IR, measurements of the samples can be taken directly with the Attenuated Total Reflectance (ATR) technique or by preparing pellets with potassium bromide (KBr). The spectra obtained by this method contain vibrations (wave number) of molecular bonds in the infrared region (4000 to 400 cm^{-1}), and absorption peaks at the frequencies formed by these vibrations give the characteristic peaks and fingerprint region of the samples. Samples with different molecular bonds can be detected with different vibration frequencies, as each component has characteristic peaks and fingerprint region depending on the chemical structure (Çağlar, Demirci and Çağlar, 2019; Büyüksırt and Kuleasan, 2014).

The spectrum bands in the FT-IR correlate with the chemical bond structure of the functional groups in the molecules in the sample. The

approximate values of the wave number range of bond types according to chemical groups are given in Table 1.

Table 1: Approximate wavenumber ranges of bond structures of functional groups (Settle, 1998)

Bonds	Wave Number Range
C–H, N–H, O–H or S–H	4000 - 2500 cm^{-1}
O–H and N–H	3700 - 2500 cm^{-1}
C–H	3300 - 2800 cm^{-1}
C–H aldehyde	2900 - 2700 cm^{-1}
$\text{C}\equiv\text{C}$, $\text{C}\equiv\text{N}$ and $\text{C}=\text{C}=\text{C}$	2700 - 1850 cm^{-1}
$\text{C}=\text{C}$, $\text{C}=\text{N}$ and $\text{C}=\text{O}$	1950 - 1450 cm^{-1}

Herbal essential oils can be characterized by determining the functional groups of the molecules in their chemical composition by FT-IR. Classification can also be made by taking into account the differences in the spectrum depending on the type of plant containing the essential oil, the production conditions, the geography where it is produced, etc. Depending on the composition of analyzed sample, FT-IR may be an adequate analysis technique for characterization. However, it is not an adequate characterization technique for the characterization of multicomponent samples. Because molecular bands in multicomponent samples may overlap and/or some bands may be masked (Filoda et al., 2018; Yadav, 2018; Yang et al., 2005). In this case, the spectral results obtained do not give accurate results about the chemical structure of the components.

In addition, the FT-IR technique is a very suitable alternative technique in terms of time and savings, without using solvents for purity evaluation with its high reproducibility.

FT-IR spectroscopy was also used for qualitative and quantitative analysis of different oil components mixed in certain portions in the literature. For example, FT-IR was used to detect blended soybean oil and sunflower oil to reduce the cost of avocado oil, which is mostly used in the cosmetics industry. For this purpose, it was analyzed by FT-IR by Jimenes-Sotelo et al and the results showed that it can be an accurate and reliable technique for the analysis of avocado oil (Jiménez-sotelo et al., 2016).

In another study, 36 samples prepared by mixing wheat oil obtained by cold pressing with pure sunflower and soybean oil in certain proportions were analyzed with FT-IR spectrometer. In the study conducted by Arslan et al., it was reported that oil mixtures below 1% could be determined by FT-IR (Arslan and Ça, 2018).

Many similar examples are given in the literature. Considering the advantages of the FT-IR method, such as its practicality, short analysis time, low cost, and no need for sample preparation, we can say that FT-IR is an effective characterization method that can be used to determine the chemical components and purity of oils.

Raman Spectroscopy

Raman spectroscopy is a branch of vibrational spectroscopy in which the spectrum of Raman-active vibrational modes generated by excited molecules as a result of exposure to an intense light beam is obtained (LiChan, 1996). As a result of the scattering of light by interaction with molecules, it has been observed that most of the wavelength is the same as the wavelength of the incoming light, and a very small part of it has been found to shift to different wavelengths. The shift of light to different wavelengths is Raman scattering, also known as inelastic scattering. During Raman scattering, the total energy is conserved, and the energy gained or lost by the photon must be equal to the energy between the vibrational energy levels of the molecule. Therefore, vibrational spectroscopy, in which information about the vibrational energy levels of molecules can be obtained thanks to the amount of energy gained or lost by the photon, is called Raman Spectroscopy (Ersöz, 2010).

Raman scattering is a spectroscopic analysis method that depends on the polarizability of functional groups as atoms vibrate, and functional groups such as C=O, N-H and O-H have strong infrared stretching vibrations and groups such as C=C, C-C and S-S form intense Raman lines. In this sense, FT-IR absorption and Raman scattering can also be seen as complementary to each other (LiChan, 1996).

For example, Raman spectroscopy analysis of 15 different essential oils preferred in the cosmetic industry was performed by Jentzsch et al. (Jentzsch et al., 2015) and principal component analysis of terpenoids in these essential oils with Raman spectra were successfully performed.

Gas Chromatography-Mass Spectrometry (GC-MS)

Chromatography is defined as the method used to separate substances with similar properties in complex mixtures. In the gas chromatography technique, the mobile phase is an inert gas and the sample is evaporated and injected into the column used as the stationary phase. Elution is carried out by the flow of the mobile phase, which is an inert gas. Elution is carried out by the flow of the mobile phase. The mobile phase in gas chromatography does not interact with the analyte molecules, but carries that analyte into the column. The GC-MS system is formed by connecting the gas chromatography device directly to various mass spectrometers. GC-MS is an analytical method that combines features of gas chromatography and mass spectrometry. The contents of the samples, which can be found in the gas phase or gasified by GC, can be mass separated and illuminated by advanced molecular determination. GC-MS system is used to recognize hundreds of substances in natural systems simultaneously (Skoog et al. 1998). GC-MS is a method mostly used for the determination of herbal essential oil components.

In a literature study, GC-MS was used for the characterization of essential oils and extracts obtained from the flowers, leaves and stems of *Lippia origanoides* plants growing wild in different regions of

Colombia. By principal component analysis of 139 substances detected in these essential oils and extracts, it was possible to classify *L. origanoides* into three chemotypes characterized by essential oil main components (Stashenko et al., 2010).

In another study in the literature, volatile compounds of cinnamon bark from three species and seven habitats were detected and identified for the first time by GC-MS and FTIR analysis. GC-MS analysis showed that the main compound in the essential oils of nine samples was trans-cinnamaldehyde (66.28–81.97%). In conclusion, this study has proven to be an effective strategy for species identification and geographic distribution, especially for assessing the quality of cinnamon for use in crude herbal medicines (Li et al., 2013).

Differential Scanning Calorimetry (DSC)

DSC provides important information about oils obtained from plants. DSC is generally known as a technique used to monitor the thermo-oxidative process against temperature. The transition between phases (liquid-solid, solid-liquid) is affected by the composition of the molecule. In this way, it can provide information about the purity of oil components. At the same time, melting and crystallization processes can provide information about oil quality, structure and origin. In the literature, the effect of heating and cooling rate on melting and crystallization thermograms is investigated, and in this way, researches are carried out on the use of calorimetric methods in quality control

(Anguili et al., 2006; Ferrari et al., 2007). Using DSC analysis, a relationship can be established between the quality and stability of oils.

In the literature, in a study examining the thermal properties of oils obtained from various plants, it was observed that the number of exothermic peaks occurring during the crystallization process and the number of endothermic peaks occurring during the melting process is different from oil to oil. The starting and ending temperatures of the peaks were calculated and it was examined whether there was a difference between them. As a result, it was determined that the difference between the crystallization initiation and melting end temperatures was statistically significant. It has been determined that DSC does not give direct information about the chemical composition of the herbal essential oils, but gives useful information about the thermodynamic changes that occur between phase transitions. Since these thermodynamic properties are sensitive to chemical composition, it has been concluded that they can be used in qualitative and quantitative evaluations. It has been suggested that the crystallization start temperature and the melting point temperature can be used to define the oil type (Tan and Man, 2000).

In another study in the literature, the relationship between the thermal properties and chemical composition of Biancolilla, Cerasuola and Nocellara type Sicilian olive oils were investigated. It was determined that in the Cerasuolo type olive oil, which has a high unsaturation rate, the crystallization starts at a lower temperature and the melting starts at a higher temperature. Analysis results show that thermal properties are

affected by olive oil composition, especially the unsaturation rate (Chiavaro et al., 2007; Chiavaro et al., 2008).

Nuclear Magnetic Resonance Spectrometer (NMR)

NMR is a spectroscopic technique that provides information about the structures, binding properties and chemical properties of organic molecules. Magnetic resonance occurs as a result of the interaction between an external magnetic field and the magnetic moment of atomic nuclei. NMR spectroscopy is a technique in which this magnetic resonance is monitored by radio waves. By measuring the magnetic moment of an atomic nucleus, which changes depending on its chemical environment, with NMR spectrometry, structure elucidation of organic molecules can be performed (Keeler, 2010; Günther, 2013).

The use of NMR, especially in the characterization of essential oils, has attracted attention in recent years. A major advantage of NMR is that essentially any organic compound can be easily detected with equal sensitivity without being subject to the detection limitations encountered in GC, such as volatility or lack of thermal stability (Schripsema et. al, 2022). In recent studies, it is seen that especially ^1H -NMR is used to determine the composition of herbal essential oils. If the results cannot be decided, the results can be supported with ^{13}C -NMR (Schripsema et. al, 2022).

In the literature, the components of the essential oils of *Helichrysum* species from South African medicinal plants by Adewinogo et al. were comparatively analyzed by GC-MS and NMR spectroscopy

(Adewinogo et. al, 2022). In recent years, it is observed that the number of similar studies has increased in the literature.

CONCLUCTIONS

When all the methods used in the production of essential oil are evaluated, the characteristics of the essential oil component and the purpose of producing the product are important when choosing the method. In particular, modern methods have attracted more attention in recent years due to their advantages such as giving results in a shorter time, creating less toxic waste, and high product purity. After the essential oils are obtained by appropriate separation techniques, the second important step is their characterization. As with the characterization of many components, many analysis techniques are used in the characterization of essential oil components. When the literature is investigated in detail, it is seen that methods such as FT-IR, GC-MS, Raman, DSC are widely used in the characterization of essential oils. In addition to these techniques, the use of NMR technique in the characterization of essential oils is also very important and its importance has been especially noticed in recent years. In summary, while a single analysis method may be sufficient to determine the content of some essential oils, comparisons need to be made using several techniques to determine the content of some components. This situation is closely related to the method used to obtain essential oils. If essential oils can be obtained pure, it will be easier to identify the components.

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