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Studying Structural Differentiation of Plant Parts of *Sideritis pisidica* Boiss. & Heldr. Using FTIR Spectroscopy

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Abstract

Fourier Transform Infrared Spectroscopy (FTIR) is a novel tool for exploring compositional structures of diverse organic or inorganic compounds employed by many researches from physicists to horticulturists due to its time-saving and easy-to-use application. FTIR was performed to differentiate structural differentiation of plant parts of wild-grown *Sideritis pisidica* Boiss. & Heldr. from Turkey. Some minerals Na, Cr, Zn, Fe, Mg, Mn, Co, Cu and Ni; protein, lipid, soluble sugar and moisture contents in the stalk, leaf and blossom of the plant were analyzed whether the FTIR spectra may predict major structural compositions. As expected most of the minerals were presented higher amount in the leaf followed by the blossom and stalk. The blossom was however found be richer in Ca, Cr, Ni and Zn than leaf and stalk. Total protein contents of the leaf were higher than those of the stalk or the leaf. Crude lipid amounts were however more prominent in the stalk followed by the blossom and leaf respectively, unlike the total protein contents. The blossom was found be richer in moisture content than the leaf and stalk followed, respectively. The FTIR spectra of the plant parts clearly revealed that protein and total soluble sugar contents are varied in stalk, leaf or blossom of *Sideritis pisidica* Boiss. & Heldr. interpreted from the peak intensities. The spectra however did not reveal the crude lipid or moisture contents of the plant parts.

Keywords: Herbal tea, Mountain tea, FTIR, *Sideritis spp*, Dedegöl Mountain

FTIR Spektroskopisi Kullanarak *Sideritis pisidica* Boiss. & Heldr. Bitki Aksamlarındaki Yapısal Farklılığın Araştırılması

Özet

Fourier dönüşümlü kızılötesi spektroskopisi (FTIR) kısa sürede ve kolay uygulanabilmesi nedeni ile fizik bilim insanlarından bahçe bitkileri bilim insanlarına kadar birçok araştırmacının başvurduğu değişik organik ve inorganik maddelerin yapılarını araştırmak amacıyla kullanılan yenilikçi bir yöntemdir. Bu çalışmada, FTIR, Türkiye'de doğal olarak yetişen yabani *Sideritis pisidica* Boiss. & Heldr. bitki aksamlarındaki farklılığı ortaya koymak için kullanılmıştır. Bitkinin gövdesi, yaprakları ve çiçeklerinde bulunan Na, Cr, Zn, Fe, Mg, Mn, Co, Cu ve Ni gibi bazı mineraller ile protein, yağ, çözünür şeker ve nem içeriği, FTIR spektrumlarının ana yapısal içerikleri tahmin edip edemeyeceği amacıyla analiz edilmiştir. Tahmin edildiği gibi, çoğu minerallerin miktarı yaprakta yüksek miktarda bulunmuş ve yaprağı sırası ile çiçek ve gövde takip etmiştir. Bununla birlikte çiçek, yaprak ve gövdeye göre Ca, Cr ve Zn mineralleri bakımından daha zengin bir içeriğe sahip olmuştur. Yaprak, gövde ve çiçeğe göre daha fazla protein içermiştir. Toplam proteinin aksine, ham yağ içeriği gövdede en yüksek bulunmuş ve gövdeyi sırası ile çiçek ve yaprak takip etmiştir. Pik içeriği dikkate alındığında, FTIR spektrumları *Sideritis pisidica* Boiss. & Heldr. bitkisinin gövde, yaprak ve çiçekte bulunan protein veya toplam çözünür şeker içeriklerindeki farklığı açıkça ortaya koymuştur. Fakat spektrumlar bitki aksamlarındaki ham yağ veya nem içeriğini tahmin edememiştir.

Anahtar Kelimeler: Bitkisel çay, dağ çayı, FTIR, *Sideritis spp*, Dedegöl Dağı

Introduction

Sideritis genus covers a very wide range of species (150) spreading from the Bahamas to China and from Germany to Morocco but the genus is primarily located in Mediterranean basin (Güvenç and Duman, 2010). *Sideritis pisidica* Boiss. and Heldr. is one the member of this genus and widely consumed as an herbal tea in Turkey. The plant is locally called "Dağ Çayı" (Mountain Tea), "Yayla Çayı" (Flatland Tea) or "Ada Çayı" (Sage Tea). Herbal tea of the plant is not only consumed for its pleasure originated from mostly rich volatile compounds but also for the treatments of several ailments such as cough and stomachache by locals in folk medicine. The plant is very rich in volatile composites, more than 35 volatiles were identified so far (Ergun et al., 2016). α-Pinene, β-pinene, sabinene, sabinene hydrate, β-caryophyllene have been reported to be found in greater amounts in the plant (Ergun et al., 2016). Mineral content of *Sideritis pisidica* Boiss. & Heldr. has yet be reported. In fact, most of the species of the genus have been extensively studied for volatiles, antimicrobial or pharmacological activities, however, there are few studies on mineral content. *Sideritis scardica* and *Siderritis raeseri* are two examples in which mineral contents were detailed (Karapandzova et al., 2013).

Fourier Transform Infrared Spectroscopy (FTIR) is a rapid, time and money saving method which has recently attracted plenty of scientists' attention. This method is very simple and requires only a small amount of matters (micrograms to nanograms). FTIR functions on the basis functional groups using a vibrational technique with giving details in the form of peaks (Amir et al., 2013; Basnet al., 2016). Thus FTIR can be used to acquire detailed information of plant structure and cell constituents like protein, carbohydrates or lipids which are rich in functional groups (Amir et al., 2013). Plant scientists have started to using FTIR in their respected fields for a variety of porpoises such as exploring chemical constituents some medicinal plants (Prasad et al., 2011), identification of wheat varieties (Amir et al., 2013), characterizing extracellular matrix components in Arabidopsis (Mazurek et al., 2013), comparison of essential oils from 15 different herbs (Jentzch et al., 2015), identification potential metal-ligand binding sites in rice seeds (Basnet et al., 2016).

In the present study, we primarily aimed to predict plant part compositions and mineral quantities that specific to wild-grown *Sideritis pisidica* Boiss. & Heldr. plant from Turkey by applying FTIR spectroscopy. Complex plant molecules, such as enzymes or cell wall constituents, are formed only in the presence of minerals. Thus, micro or trace minerals contribute to proteins, carbohydrates, lipids or related structures which bearing an array of structural groups. With having on our mind that FTIR has been proved detect a range of functional groups in plants, we tried to find a link between minerals and FTIR spectra if any, which represented our secondary aim. Finally, with this research, we intended to exemplify the use and success of FTIR spectroscopy in especially herbaceous plants to characterize their plant structure with a time and money-saving manner for not only scientific researches but also food technology as well.

Materials and Methods

Plant Material

Plant materials from 5 plant tufts (*Sideritis pisidica* Boiss. & Heldr.) were collected from Dedegöl Mountains in the providence of Konya, Turkey located at latitude of 37° 34' 42.49" N and longitude of 31° 23' 48.12" E longitude with an elevation of 1617.55 m (near Yeşildağ village). When a resin-like substance appeared on all aerial plant parts, an evidence of the best harvesting time for particularly the use of herbal tea, the plant samples were chosen to collect. The harvested plants were air-dried at circa 23 °C under in the shade for 1 day. The plants were afterwards bunched and stored at the room temperatures with protecting from light and moist. The plant material was identified by Prof. Dr. Lütfi Behcet (Faculty of Science and Literature of Bingol University). A voucher specimen (No. BIN 3321; Collector No. ME01) was deposited at the Herbarium of Faculty of Science and Literature of Bingöl University, Turkey.

Microelement analyses

Dried plant samples (1 g) were placed in a porcelain crucible followed by incubating at 500 °C in a muffle furnace overnight. After cooling, the ash was dissolve in 5 mL HCL (5 %). The mixed solution was then filtered by using an acid washed filter paper into a volumetric flask (50 mL). After washing the filter paper, the solution diluted to volume (50 mL) with deionized water and mixed thoroughly. Analyses for K, Na, Cr, Zn, Fe, Mg, Mn, Co, Cu and Ni were carried out with a Perkin-Elmer model Analyst 800 atomic absorption spectrometer equipped Perkin-Elmer model AS-800 autosampler and with a flame atomizer. Running conditions were set as in described in the operation manual of the instrument. Operation was assisted a WinLab32 software. After adjusting the spectrophotometer, nitric acid, standards, the

HCl and finally samples were inserted from lowest to highest or highest to lowest depending on the elements with tree times then absorbance were read. The standards were used to obtain straight lines for calibration. The WinLab32 software was used for calculation. Ca and Mg elements were detected by complexometric titration method.

Protein, lipid and moisture constituents

Protein constituents were determined by using Dumatherm (Gerhardt, Dumas analytical system, DTM) nitrogen/protein analyzer with the appropriated analyzing method using its software from the samples after quantifying moisture portion. Total nitrogen content obtained by combustion was used to calculate the amount of protein. Crude lipid was determined by hexane extraction using a Soxhlet extractor (Velp SER 148; Usmate, Italy). Moisture content was determined by oven-drying at 130 °C for 25 h.

FTIR measurement

The plant samples were separated into stalks, leaves and blossom and then ground into very fine particles by Ultra Centrifugal Mill (Retsch ZM 200; Haan, Germany) for FTIR analysis. An FTIR spectrum (Perkin-Elmer 100, Perkin-Elmer Inc., Norwalk, CT, USA) equipped with Attenuated Total Reflectance accessory (ATR; Perkin-Elmer) was used to acquire spectra from the stalk, leaf or blossom of the plant. The powdered plant samples were put on Diamond/ZnSe crystal cell and scanned with 4 cm^{-1} resolution for 5 scans in the wave number of 4000 – 650 cm^{-1}. Each sample was run tree times, giving identical spectra. Therefore, average spectra within sample were used for processing. Spectra were processed using Spectrum 100 (version 6.3.5, 1999) and Spekwin32 (version 1.71.6.1, 2012) software.

The data representing microelements, protein, lipid and moisture constituents in the plant parts were statistically analyzed, and treatments were compared using Duncan multiple range test.

Results and Discussion

Mineral contents of the stalk, leaf and blossom are displayed in Table 1. K was the most abundant mineral flowed by Na and Ca, Mg, Fe, Mn, Co, Cr, N, and Cu irrespective of plant parts. K presented almost same values in all three plant parts while others did in various quantities. Leaves contained higher amount of Na concertation than stalks or blossoms. Ca was very abundant in blossoms over in stalks or leaves. Mg and Fe levels were higher in leaves than stalks or blossoms. Leaves and blossoms had a very similar level of Co while stalks had a much lower level of Co. Blossoms contained more abundant values of Cr, Ni or Cu over stalks or leaves while stalks and leaves contained more Zn concentration than blossom did.

We are unaware of any research containing mineral composition of *Sideritis pisidica* Boiss. & Heldr. In fact, there are only few literatures of about whole Siderites species. One of study was done on trace metal concentrations of *Sideritis congesta* from Gözce County in Anamur, Mersin, Turkey which is about 350 km from where the *Sideritis pisidica* Boiss. & Heldr. collected (Koc and Sari, 2009). The researcher analyzed the plant for Mn, Zn, Cu, Ni, Pb, Cd, Fe, Cr and Co and found that their levels of 99, 74.2, 187.3, 214, 6.4, 0.9, 314, 0.1 and 0.1 mg kg-1, respectively. Only Fe and Mn level in *Sideritis congesta* is comparable to the level of *Sideritis pisidica* Boiss. & Heldr.

Aerial parts of cultivated *Sideritis raeseri* subsp. raeseri in four different stages of the flower development were analyzed for Na, K, Mg, Ca, Cu, Fe Mn and Zn concentrations (Pljevljakusic et al., 2011). K, Ca and Mg levels in *Sideritis raeseri* subsp. Raeseri were found comparable to ours while Na, Cu, Fe, Mn and Zn levels in the *Sideritis raeseri* subsp. Raeseri was lower than those in *Sideritis pisidica* Boiss. & Heldr.

The mineral contents of *Sideritis scardica* and *Sideritis raeseri* were thoroughly investigated in Macedonia and Albania using eight or more specimens (Karapandzova et al., 2013). K and Na levels of *Sideritis scardica* and *Sideritis raeseri* were lower than those of *Sideritis pisidica* Boiss. & Heldr. employed in the present study. Ca and Fe concentrations of some specimens from *Sideritis scardica* or *Sideritis raeseri* were comparable to ours. On the other hand, Mg levels of the both *Sideritis* species were very high while Cr, Zn, Mn, Co and Ni levels very low compared those of *Sideritis pisidica* Boiss. & Heldr.

Sideritis pisidica Boiss. & Heldr. employed in this study contained high quantities of Cr, Zn, Mn or Co when compared to other *Sideritis* species mentioned above. This could be due to the soil composition of the field where *Sideritis pisidica* Boiss. & Heldr. plants were harvested. There is an active chrome mine about 6-7 km from where the plant specimens were collected. The chemical composition of the chromite ore mined from the mine is made of Cr_2O_3 (47.68%), MgO (17.45%), SiO_2 (6.78%), Al_2O_3 (9.21%), Fe_2O_3 (15.25%) and CaO (0.78%) (Ağaçkayak et al., 2006).

Total protein, soluble sugar and crude lipid ratios of the plant parts are given in Table 2. Leaves represented the highest protein content value fallowed by blossoms and stalks. The protein

content of the leaf was almost a twice as that of the stalk. Soluble sugar content was very high in the leaf (0.61 %) compared to the blossom (0.35 %) or stalk (0.34 %), almost doubling the amounts of the blossom or stalk (Table 2). The stalk contained the highest amount of crude lipid (2.88 %) followed by blossom (2.73 %) and leaf (2.66 %) as expected because the plant secretes a resin-like and perfumy substance from the stalk when flowers (Table 2). As expected, blossom held more moisture (7.20 %) over leaf (7.12 %) and (6.87 %).

Table 1. Mineral contents in dried aerial parts of *Sideritis pisidica* Boiss. & Heldr. from Turkey (mg kg^{-1})

Minerals	Stalk	Leaf	Blossom
K	53150 a	53040 a	52920 a
Na	5064 b	6024 a	4770 c
Ca	944 c	1053 b	1636 a
Mg	243 b	429 a	140 c
Fe	267 b	373 a	169 c
Mn	96 c	292 a	168 b
Co	69 b	124 a	128 a
Cr	84 c	94 b	124 a
Ni	55 b	58 b	76 a
Cu	60 b	54 b	100 a
Zn	43 a	38 a	12 c

[a, b, c]Data are presented as mean ± SD. Numbers with no common superscript in the same row are statistically different at a level of significance of 0.05

Table 2. Protein, soluble sugar, lipid and moisture contents aerial parts of *Sideritis pisidica* Boiss. & Heldr. from Turkey.

	Stalk	Leaf	Blossom
Total proteins (%)	8.43 c	17.30 a	14.03 b
Total soluble sugars (%)	0.34 b	0.61 a	0.35 b
Total lipids (%)	2.88 a	2.66 c	2.73 b
Total moisture (%)	6.87 c	7.12 b	7.40 a

[a, b, c]Data are presented as mean ± SD. Numbers with no common superscript in the same row are statistically different at a level of significance of 0.05

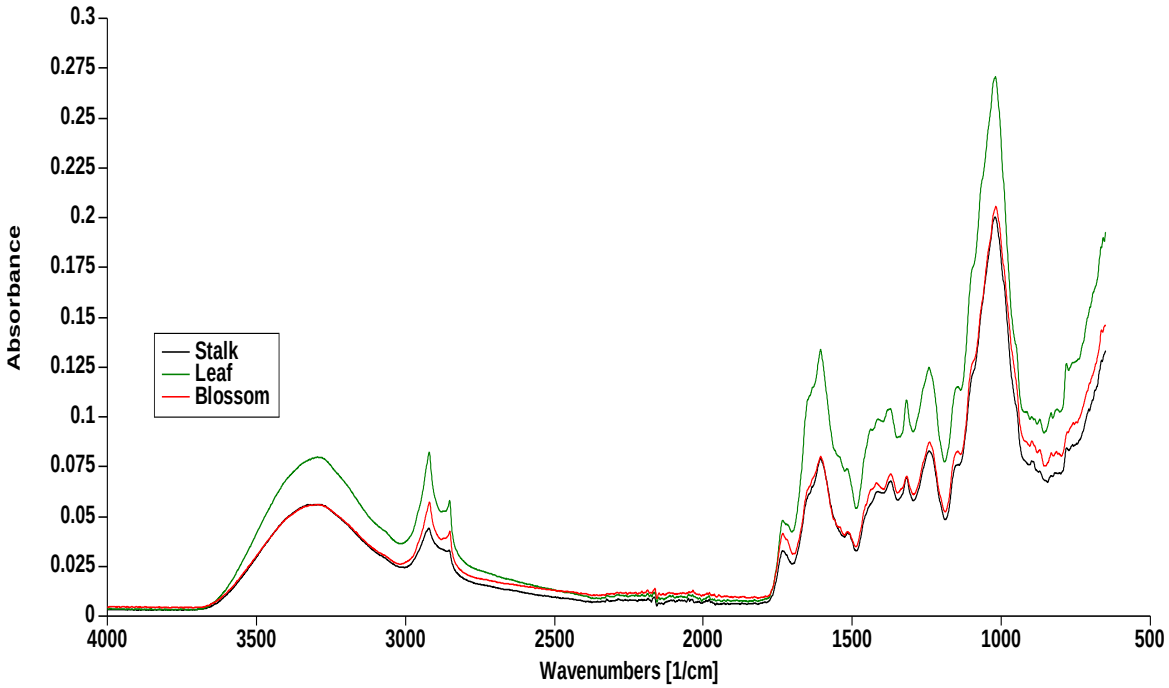

Figure 1. FTIR sepctra of stalk, leaf and blossom of *Sideritis pisidica* Boiss. & Heldr. plant

Similar to our findings, Yücel et al. (2011) reported that aerial parts of *Sideritis germanicopolitana* subsp. germanicopolitan plant contain approximately 2.30 % lipid.

No literature covering protein or soluble sugar content of any *Sideritis pisidica* Boiss. & Heldr. has yet be reachable. However, protein content of one of *Sideritis germanicopolitana* subsp. germanicopolitana was studied and reported that the content in its root was 1.07, in its stalk 0.63 and its leaf 0.10 g 100 g^{-1} (Yücel et al., 2011).

The FTIR spectra of the stalk, leaf and blossom demonstrated the same qualitative absorption bands while displaying some quantitative variations as seen in Figure 1. From 3600 to 2600 cm^{-1}, the leaf spectra had higher absorbance values than the stalk or blossom. From 3000 through 2600 cm^{-1}, the blossom spectra markedly rose over the stalk spectra and gave 3 the intense peaks at around 3300, 2920 and 2846 cm^{-1}. Infrared region from around 2400 to 1800 cm^{-1}, the leaf spectra very slightly fell under the blossom spectra. However, starting from around 1750 through 650 cm^{-1} the leaf spectra rose over again the blossom spectra. From 1750 to 650 cm^{-1} spectrum section, 9 intense peaks were observed nearby at 1735, 1607, 1516, 1413, 1371, 1328, 1243, 1020, and 652 cm^{-1}.

The absorption bands in the vicinity 3300 cm^{-1} correlated to C-H (Gallardo-Velázquez et al., 2009), O-H (Movasaghi et al., 2008) and N-H (NH_3) (Gallardo-Velázquez et al., 2009) stretching vibrations in carbohydrates and proteins. From 3600 to 2600 cm^{-1}, the leaf spectra displayed higher absorbance values than the stalk or blossom, which may indicate protein and carbohydrate contents of the leaf are richer than the stalk and blossom. From 3000 through 2600 cm^{-1}, the blossom spectra rose over the stalk spectra and gave 3 the intense peaks at around 3300, 2920 and 2846 cm^{-1}, which may be attributed polysaccharides, lipophilic components and proteins (Prasad et al., 2011). Indeed, the spectrum region from 3000 to 2800 cm^{-1} has been correlated to NH_3 stretching (free amino acids; Gallardo-Velázquez et al. 2009; Sivakesava and Irudayaraj 2001), O–H stretching (carboxylic acids; Movasaghi et al., 2008) and C–H stretching (carbohydrates; Gallardo-Velázquez et al., 2009). The bands around 2920 cm^{-1} likely indicatives of chlorophylls (Bellamy, 1975).

From 2400 to 1800 cm^{-1} spectrum section, the absorbance values of the blossom very slightly rose over the leaf, which could be due to from a compound(s) quantities rich in Ca (Varetti and Volponi, 1995).

Infrared region starting 1750 through 650 cm^{-1} displayed a clear rise of the leaf spectra over others, which mainly corresponded to carbohydrates, proteins, lipids and water. The absorption band from 1700 to 1600 cm^{-1} is correlated to N–H bending of amide I (mainly proteins; Philip 2009), C=O stretching (mainly from carbohydrates; Gallardo-Velázquez et al., 2009) and O–H stretching/bending (water; Cai and Singh, 2004; Stuart, 1997); from 1175 to 1145 cm^{-1} is correlated to C-O stretching of ketones (Tewari and Irudayaraj, 2004), C–H stretching (carbohydrates; Tewari and Irudayaraj, 2005), C–O stretching (carbohydrates; Tewari and Irudayaraj, 2004) and O–H stretching/bending (Gallardo-Velázquez et al., 2009; Tewari and Irudayaraj, 2004); from 1145 to 940 cm^{-1} is correlated to ring vibrations (mainly from carbohydrates; Gallardo-Velázquez et al. 2009; Tewari and Irudayaraj, 2004) and C–O and C–C stretching (carbohydrates; Subari et al. 2012; Tewari and Irudayaraj, 2005); and from 940 to 700 cm^{-1} is correlated to ring vibrations (mainly from carbohydrates; Tewari and Irudayaraj, 2004), C-H bending (mainly from carbohydrates; Tewari and Irudayaraj, 2004; Gallardo-Velázquez et al. 2009) and anomeric region of carbohydrates (Mathlouthi and Koenig, 1986; Subari et al., 2012). These 4 spectrum sections may imply that water, carbohydrate and protein contents of the leaf are significantly higher than those of the stalk and blossom both of which had a very similar absorbance values except for the fraction of 1000 to 650 cm^{-1} where carbohydrate contents of the blossom may be slightly higher compared to those of stalk.

From 1750 to 650 cm^{-1} spectrum section, 9 intense peaks were observed nearby at 1735, 1607, 1516, 1413, 1371, 1328, 1243, 1020, and 652 cm^{-1}. Wavenumbers of 1735 cm^{-1} may indicate carbonyl compounds (Geethu et al., 2014), of 1607 cm^{-1} does carboxylic groups or aromatics (Dick et al. 2006), of 1413 cm^{-1} does carboxylic acid (Ibrahim et al. 2005), of 1516 cm^{-1} does proteins especially tyrosine (Kaposi et al. 1999), of 1371 cm^{-1} does proteins mainly cytosine and guanine (Prasad et al. 2011), of 1328 cm^{-1} does carboxylate groups (Pradhan et al., 2007), of 1243 cm^{-1} does phosphate moieties (Parikh and Chorover, 2007), of 1020 cm^{-1} does carbohydrates (Li et al., 2004), and of 652 cm^{-1} does aromatic compounds (Jentzch et al., 2015).

Conclusion

FTIR in the current study revealed some of the quantitative differences of chemical components by the spectral profiles in the stalk, leaf and blossom of *Sideritis pisidica* Boiss. & Heldr.

plant. The leaf of the plant contained more protein and soluble sugar content which supported by the spectra. Moreover, the blossom held slightly more Ca content over leaf and stalk which can also be tracked in the spectra as well. Moisture and lipid content however were not clearly revealed by the spectra.

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