ATR-FTIR Spectra Fingerprinting of Medicinal Herbs Extracts Prepared Using Microwave Extraction

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The present study aims to fingerprint 4 extracts of medicinal herbs: horsetail. (*Equisetum arvense L.),* milfoil (*Achillea millefolium L.*), comfrey (*Symphytum officinale L.*) and purple loosestrife (*Lythrum salicaria L.*) by ATR-FTIR spectroscopy. Extracts of medicinal plants were performed by Microwave-assisted extraction (MAE). In order to determine the fingerprint of each extract plants sample the FTIR was used and permit to characterize the presence of functional groups and to identify the specific fingerprint region. A good correlation was found between the concentration of total phenols calculated by UV-Vis spectrometry and FTIR method after calibration with gallic acid. Purple loosestrife has the highest phenol content.

Keywords: ATR-FTIR, MAE, microwave-assisted extraction, *Equisetum arvense*, *Achillea millefolium*, *Symphytum officinale*, *Lythrum salicaria*

Introduction:

Fourier Transform Infrared (FTIR) is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures (Topală and Tătaru, 2016), and has been used as a requisite method to identify medicines in pharmacopoeia of many countries. Owing to the fingerprint characters and extensive applicability to the samples, FTIR has played a significant role in pharmaceutical analysis in recent years (Cozzolino, 2015, Baseri and Baker, 2011, Movasaghi et *al*., 2008). These IR vibrational spectroscopic technique provides molecular-level information allowing investigation of functional groups, molecular conformations, quantitative analysis and permit to study structure with informing on the intra and intermolecular bonding types. Transmission mode, attenuated total reflectance (ATR), diffusive reflectance and microspectroscopy are the most common among infrared spectroscopy methods.

The advantages of Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique include a small amount of material required to experiment, and can be repeated many times very quickly to verify results, a short time to obtain spectra, high sensitivity, and in the case of tissue it

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is not necessary to do any preparatory operation of samples (Topală, 2013; Topală and Ducu, 2014). The use of attenuated total reflectance (ATR) device evolved rapid FTIR measurements of liquids such as oils and plant extracts, allowing the identification and quantification of valuable plant biomarkers (Schultz and Baranska, 2007).

The middle region of the infrared (MIR) radiation (wavenumbers from 4000 cm^{-1} to 400 cm^{-1}) is most widely used to study the biological samples. The infrared absorption spectrum of each compound has its own unique pattern fingerprint ranging from 1400 to 400 cm^{-1} (Alonso-Simon et *al*., 2004).

Additionally, the region from 1800 cm^{-1} to 1400 cm^{-1} could deliver information about functional groups occurring in investigated molecules. Infrared spectroscopy, as a very fast technique, has found an application in carbohydrates 'investigation, especially for cell wall polysaccharides with the large diversity of pectin, hemicelluloses and cellulose.

Numerous phytochemical studies into milfoil (*Achillea millefolium L.*) have shown that it is rich in pharmacologically active compounds with antimicrobial and antiviral activities. According to the literature the pharmacological effects are mainly due to the essential oil, proazulenes and other sesquiterpene lactones, dicaffeoylquinic acids, flavonoids, coumarins, pyrrolidine alkaloids (such as stachydrine and betonicine), tannins (Szymański et *al*., 2014). The diversity and complexity of the effective compounds of yarrow species explains the broad spectrum of their activity.

Horsetail (*Equisetum arvense L.*) showed an antiviral effect and antioxidant activity for which the polyphenols constituents appear to be responsible. Field horsetail contains more than 10% inorganic substances (two thirds of which are ***silicic acid*** and potassium salts) (Mimica-Dukic et *al*., 2008). The plant signal molecule salicylic acid (SA) can induce resistance to a wide range of pathogen types. In the case of viruses, SA can stimulate the inhibition of all three main stages in virus infection: replication, cell-to-cell movement and long-distance movement. SA may stimulate a separate downstream pathway, leading to the induction of an additional mechanism of resistance based on RNA interference (Carr, 2004).

Comfrey (*Symphytum officinale L.*) known to be rich in silicon and presents also antiviral and antibacterial activities. Previous studies showed that these beneficial properties of comfrey are due to the presence of numerous bioactive compounds (Savić et *al*., 2015). Andres et *al*., 1989 have reported that comfrey contains allantoin, 18 amino acids, A, B and C vitamins, ellagic acid, auxin, triterpenoids, tannins, rosmarinic acid, steroidal saponins, inulin, pyrrolizidine, alkaloids, calcium, potassium, iron, sulfur, copper and selenium. Although comfrey consists of numerous different compounds, allantoin, ellagic acid and rosmarinic acid are probably the most important for its beneficial activities.

Rosmarinic acid is an ester of caffeic acid and possesses antioxidant activities, antiviral, anti-inflammatory effects and shows a very low toxicity (Lamien-Meda et *al*., 2010). The rosemary

essential oil has long been known for its antimicrobial and antiviral activities (Ojeda-Sana et al., 2013).

The pharmacological activity of purple loosestrife (*Lythrum salicaria L.*) is mostly due to its phenolic compounds, mainly tannins. Antioxidant, antimicrobial, and hypoglycemic effects of loosestrife have been reported (Kahkonen et *al*., 1999).

The fundamental of the microwave-assisted extraction (MAE) process is different from those of conventional methods (solid-liquid or simply extraction) because the extraction occurs as the result of changes in the cell structure caused by electromagnetic wave.

In MAE, the process acceleration and high extraction yield may the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction. On the other hand, in conventional extractions the mass transfer occurs from inside to the outside, although the heat transfer occurs from the outside to the inside of the substrate. In addition, although in conventional extraction the heat is transferred from the heating medium to the interior of the sample, in MAE the heat is dissipated volumetrically inside the irradiated medium.

Microwave-assisted extraction (MAE), based on the rapid heating of solvent and sample due to direct effect of microwaves on molecules by ionic conduction and dipole rotation, is widely employed in the analysis and the extraction of natural compounds (Chan et *al*., 2011, Routray and Orsat, 2012). Microwave-assisted extraction gives several advantages with respect to classical extractive processes such as Soxhlet: MAE allows a gain of time, higher quality and yields (Capitani et *al*., 2014). It is also cheaper than supercritical fluid extraction (SFE) and faster than ultrasonic-assisted extraction (UAE) (De Monte et *al*., 2014). On the other hand, MAE shows some drawbacks: it is more expensive than UAE, less eco-friendly than SFE due to the use of organic solvents, and not efficient when the target molecules and/or the solvent of extraction are non-polar because they do not absorb energy from the source (Zhang et *al*., 2011).

The present study aimed to compare by ATR-FTIR spectroscopy the fingerprint regions of 4 ethanol plant extracts by microwave of the mentioned medicinal herbs, collected from wild flora of Romania. Medicinal plants used in this study: Horsetail, Milfoil, Comfrey and Purple loosestrife are recognized for their antiviral, antimicrobial activity.

These plant extracts will be used for making healing gels with anti-inflammatory properties due to antiseptic, healing, antimicrobial and anti-inflammatory effects.

Materials and methods:

Medicinal plants

Four types of dried medicinal plants, from wild flora of different areas of Muntenia, Romania were investigated. The plants were numbered as follows: 1- Milfoil (*Achillea millefolium L.*), 2- Horsetail

(*Equisetum arvense L.*), 3- Comfrey (*Symphytum officinale L.*) and 4- Purple loosestrife (*Lythrum salicaria L.*).

Preparation of extracts

The microwave-assisted extraction experiments for the extraction of bioactive compounds of 4 medicinal plants (*Lithrum salicaria*, *Achillea millefolium*, *Equisetum arvense* and *Symphytum officinale*) were carried out using a microwave laboratory system (230V-50Hz, NEOS-GR, Milestone, Italy). It is a multimode microwave reactor 2.45 GHz with a maximum delivered power of 900 W variable in 10 W increments. An open vessel 1.5 L Pyrex was used as the extraction vessel. Temperature was monitored by an external infrared sensor.

25 g of dry plant material were put into extraction vessel with 250 mL of ethyl alcohol as solvent. Then the samples were subjected to microwave irradiations in oven cavity, initially at ambient temperature, during a fixed processing time (30 min at 400 W). The microwave heating of the solvent contained inside the raw material allows releasing molecules constituting isolated oil. This oil was then driven by the generated vapor. A cooling system outside the microwave cavity permitted to condensate the distillate continuously. Condensed alcohol was refluxed to the extraction vessel in order to provide uniform conditions of temperature and humidity. Isolated extracts were dried with rotary evaporator and stored at 4°C in the dark until used.

ATR-FTIR measurements.

The Fourier Transform Infrared spectrum (FTIR) of each extract was recorded in the middle infrared (MIR) region, using a FTIR Jasco 6300 spectrometer. An ATR accessory equipped with a diamond crystal (Pike Technologies) was used for sampling. The spectral data were processed with JASCO SpectraManager II software. Samples were carried out at 100 scans with resolution of 4 cm^{-1} using Cosine apodization in the frequency regions of 4000-400 cm^{-1}. Total phenols were determined also by FTIR method, either using the intensity of the peak at 1710 cm^{-1} or from the area of the region 950-1800 cm^{-1}, considering the calibration curve with pure gallic acid (range of concentrations 5 to 30 mg/ml alcohol).

Results and discussions

The FT-MIR spectra (4000-400 cm^{-1}) of 4 ethanolic plant extracts (*Achillea millefolium*, *Equisetum arvense*, *Symphytum officinale, Lythrum salicaria*) were registered and the specific wavenumbers and intensities were considered. Fig. 1 presents the FTIR-ATR spectra of alcoholic extracts. Tab. 1 presents the different FT-IR absorption bands for 4 plant extracts. The assignments of both stretching and bending vibrations were compared with literature data (Szymanska-Chargot and Zdunek, 2013). Refering to the literature (Zavoi et *al*., 2011), the different eight areas were identified in the MIR domain and the fingerprint region was localized between 900 and 1760 cm^{-1}.

Area 1 (< 1000 cm^{-1}) corresponds out of plane to C-H bending vibrations, **area 2** (997-1140 cm^{-1}) corresponds to the absorptions of stretching vibrations of C-O (mono-, oligo- and carbohydrates) at 1033, 1042, 1072, 1104, and 1133, 1136 cm^{-1}, while **area 3** (1150-1270 cm^{-1}) corresponds to stretching vibrations of carbonyl C-O. **Area 4** (1300-1450 cm^{-1}) correspond to stretching vibrations C-O (amide) and C-C stretchings from phenyl groups, while **area 5** (1500-1600 cm^{-1}) to aromatic domain and N-H bending vibrations. **Area 6** is a complex one (1600-1760 cm^{-1}), corresponding to bending vibrations N-H (amino acids), C=O of associated fatty acids (1710 cm^{-1}) and C=O of ester such as glycerides (1740 cm^{-1}). **Area 7** (2800-2900 cm^{-1}), corresponds to C-H stretching vibrations specific to CH_3 and CH_2 from lipids, metoxy derivatives. **Area 8** (3350-3600 cm^{-1}) corresponds to stretching vibrations of OH groups (from water, alcohols, phenols, carbohydrates, peroxides).

Looking to region 1 (specific to terpenoids) it has been noticed that plants 1, 2 and 3 had higher peak areas in ethanol. In the other IR regions (5 and 6) there are significant differences between the four plant extracts; thus for the plants 1 (*Achillea millefolium*) and 4 (*Purple loosestrife*) the intense peaks were noticed. In regions 2 (corresponding to glucosides, Xyloglucan, cellulose) only for 3 (Comfrey) the peak area is small. For area 7 (lipids), in all plant extracts, the intensity peaks no obvious changes suffer. Tab. 2 includes the corresponding absorption peak areas for specific regions (1-7). In Tab. 2, is included the total phenols concentration in methanol extracts determined by VIS spectrometry (Neacsu et *al*., 2016).

Finally, it has been compared to the phenols concentrations, determined by FTIR method, based on the peak area in the region 950-1800 cm^{-1} and total phenols calculated from VIS spectrometry (Neacşu et *al*., 2016). The results lead to the conclusion that Purple loosestrife (4) has the highest phenol content.

Conclusions:

FTIR spectroscopy are recommended as rapid and reliable tool to investigate the fingerprint and to predict the composition of medicinal plants or to evaluate their quality and authenticity.

Based on the FT-MIR peaks from 8 regions, for each plant extract, has been determined the fingerprint region and identified the specific functional groups. The data presented in this study showed that FT-MIR spectroscopy is an adequate method to fingerprint of medicinal herbs with antiviral potential.

Table 1. Comparison of FT-IR absorption bands and the vibration assignments for plant extracts **1-4** obtained in present experiment

Region	Frequency of measured peaks	Assignment
Area 7	2916, 2849 (1) 2922, 2852 (2) 2924, 2854 (3) 2919, 2850 (4)	C-H stretching vibrations specific to CH_3 and CH_2
Area 6	1607 (1) 1709, 1645, 1633 (2) 1716, 1651, 1606 (3) 1713, 1687, 1650, 1614 (4)	C=O stretching vibration, Amide I C-N stretching, COO- antisymmetric stretching
Area 5	1513 (1) 1509 (2) 1526 (3) 1507 (4)	Amide II N-H deformation and aromatic domain
Area 4	1447, 1398 (1) 1448, 1409, 1376, 1304 (2) 1454, 1409, 1375, 1325 (3) 1448, 1416, 1317 (4)	C-O stretching vibrations (amide) and C-C stretching from phenyl groups, COO^- symmetric stretching, CH_2 bending, CH_2 symmetric bending
Area 3	1262 (1) 1243 (2) 1255 (3) 1269 (4)	C–O stretching
Area 2	1042 (1) 1133, 1042 (2) 1136, 1044 (3) 1136, 1072, 1039 (4)	C–O stretching, C–C stretching of carbohydrates
Area 1	922, 876, 816 (1) 919, 876, 816 (2) 991, 925, 874 (3) 918, 872 (4)	C-H out-of-plane bending vibrations

Table 2. Absorption peak areas of different regions (1-8) of FTMIR spectra recorded for the Microwave extracts (MAE) of the studied medicinal plants

Wavenumber (cm^{-1})	**1**	**2**	**3**	**4**
3300-3350 (8)	0.06	0.05	0.03	0.04
2800-3000 (7)	7.26	10.29	6.76	8.58
1600-1760 (6)	**5.38**	**4.35**	**4.80**	**6.21**
1500-1530 (5)	0.18	0.02	0.04	0.12
1300-1450 (4)	2.20	0.78	0.94	0.66
1150-1270 (3)	0.06	0.01	0.04	3.27
1000-1140 (2)	8.96	9.29	3.14	8.53
<1000 (1)	0.32	0.46	0.52	0.07
Total phenols(by FTIR) mg/ GA eq./ml M	8.13	5.22	7.12	16.08
Total phenols (by Vis spectrometry)[a] mg/ GA eq./ml M	7.84±0.20	3.23±0.20	6.76±0.20	22.40±0.10

[a]Expressed as grams of gallic acid equivalents (GAE) per 100 g dry extract (Neacşu et *al*., 2016)

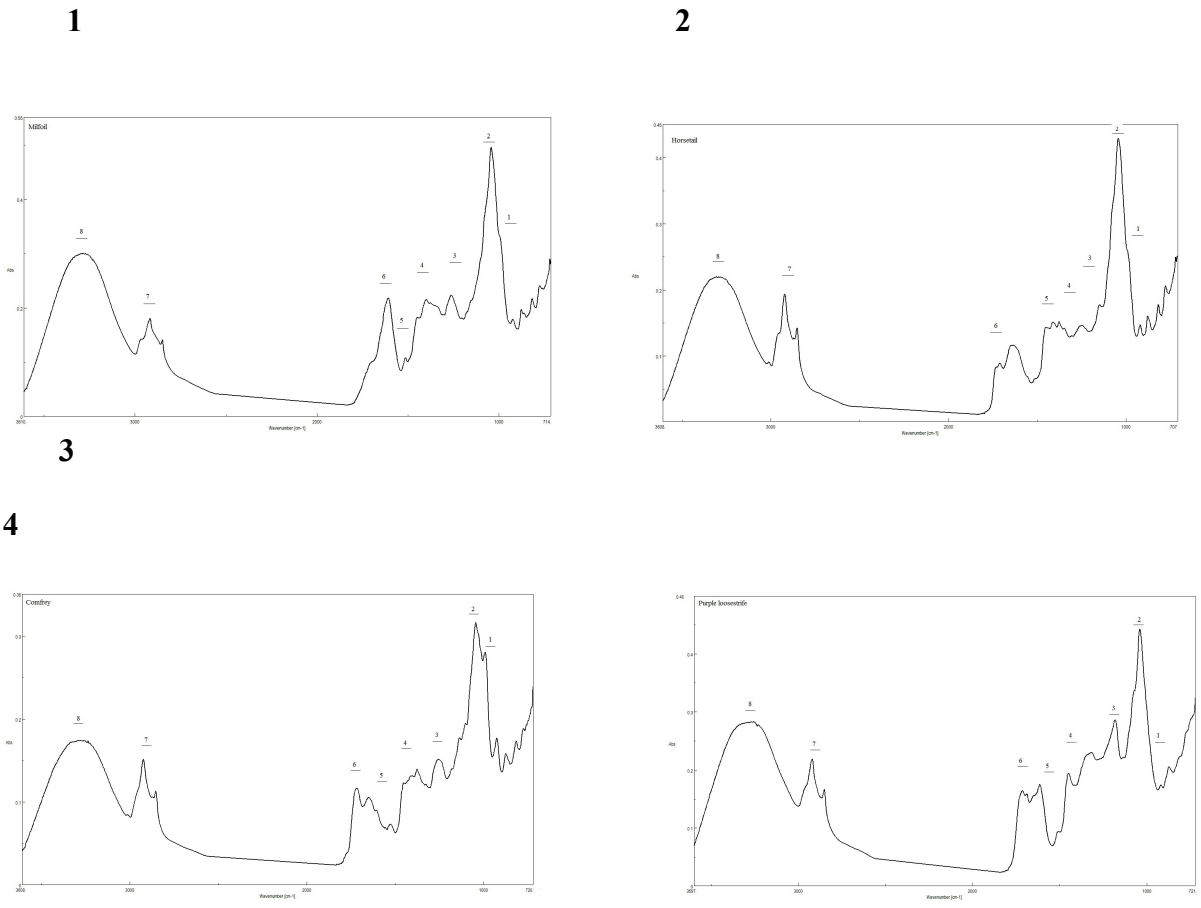

Figure 1. The FTIR fingerprint of the Microwave extracts (MWE) of the studied plants: *Achillea millefolium* (**1**); *Equisetum arvense* (**2**); *Symphytum officinale* (**3**); *Lythrum salicaria* (**4**). The specific regions are numbered 1 to 8.

Competing interests

The authors declare that they have no competing interests.

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References

Alonso-Simon A., Encina A. E., Garcia-Angulo P., Alvarez J. M., Acebes J. L. (2004). FTIR spectroscopy monitoring of cell wall modifications during the habituation of bean (*Phaseolus vulgaris L.*) callus cultures to dichlobenil, *Plant Sci*., 167, pp1273–1281.

Andres P., Brenneisen R., Clerc J.T. (1989). Relating antiphlogistic efficacy of dermatics containing extracts of *Symphytum officinale* to chemical profiles, *Planta Med*., 55, pp66–67.

Baseri M. K., Baker S., (2011). Identification of Cellular Components of Medicinal Plants Using FTIR, *Romanian J. Biophys*., 21 (4), pp277–284.

Capitani D, Sobolev A.P., Delfini M., Vista S., Antiochia R., Proietti N., Bubici N., Ferrante G., Carradori S., De Salvador F. R., Mannina L. (2014). NMR methodologies in the analysis of blueberries. *Electrophoresis*, 35(11), pp1615-1626.

Carr, J. P. (2004). Activation of multiple antiviral defence mechanisms by salicylic acid, *Mol. Plant. Patho*l., 5, pp57-63.

Chan C.H., Yusoff R., Ngoh G.C., Kung F.W.L. (2011). Microwave-assisted extraction of active ingredients from plants, *J. Chromatogr. A*, 1218(37), pp6213-6225.

Cozzolino D. (2015). Infrared Spectroscopy as a Versatile Analytical Tool for the Quantitative Determination of Antioxidants in Agricultural Products, Foods and Plants, *Antioxidants*, 4, pp482-497.

De Monte C., Carradori S., Granese A., Di Piero G. B., Leonardo C., De Nunzio C. (2014). Modern extraction techniques and their impact on the pharmacological profile of *Serenoa repens* extracts for the treatment of lower urinary tract symptoms, *BMC Urol*., 14(63), pp1-11.

Gierlinger N., Sapei L., Paris O. (2008). Insights into the chemical composition of *Equisetum hyemale* by high resolution Raman imaging, *Planta*, 227, pp969–980.

Kahkonen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Pihlaja K., Kujala T.S., Heinonen M. (1999). Antioxidant activity of plant extracts containing phenolic compounds; *J. Agric. Food Chem*., 47, pp3954–3962.

Lamien-Meda A., Nell M., Lohwasser U., Börner A., Franz C., Novak J. (2010). Investigation of Antioxidant and Rosmarinic Acid Variation in the Sage Collection of the Genebank in Gatersleben, *J. Agric. Food Chem*., 58, pp3813–3819.

Mimica-Dukic, N., Simin, J., Cvejic, N., Jovin E., Orcic D. and Bozin, B. (2008). Phenolic Compounds in Field Horsetail (*Equisetum arvense L*.) as Natural Antioxidants, *Molecules*, 13(7), pp1455-1464.

Movasaghi, Z., Rehman, S., Rehman, I.U., (2008). Fourier transform infrared spectroscopy of biological tissues, *Appl. Spectrosc. Rev.,* 4, pp134–179.

Neacsu, A. V., Ioniță G., Topală C., Oprea E., Tecuceanu V., Matei I. (2016). Poly(ethylene glycol)/β-cyclodextrin covalent gel networks: host matrices for studying radical processes in plant extract–riboflavin systems following UV irradiation, *Chem. Pap*., DOI 10.1007/s11696-016-0047-x

Nemeth E. and Bernath J. (2008). Biological Activities of Yarrow Species (Achillea spp.), *Curr. Pharm. Design*, 14(29), pp3151-3167.

Ojeda-Sana A.M., van Baren C.M., Elechosa M.A., Juárez M.A., Moreno S. (2013). New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components, *Food Control*, 31, pp189–195.

Routray W., Orsat V. (2012). Microwave-assisted extraction of flavonoids: a review. *Food Bioprocess Technol*, 5(2), pp409–424.

Savić V. Lj., Savić, S. R., Nikolić V. D., Nikolić L. B., Najman S. J., Lazarević J. S., Đorđević S. (2015). The identification and quantification of bioactive compounds from the aqueous extract of comfrey root by UHPLC–DAD–HESI–MS method and its microbial activity, *Hem. Ind*., 69, (1), pp1–8.

Schultz, H., Baranska M. (2007). Identification and quantification of valuable substances by IR and Raman spectroscopy, *Vib Spectrosc*., 43, pp13-25.

Szymanska-Chargot M. and Zdunek A. (2013). Use of FT-IR Spectra and PCA to the Bulk Characterization of Cell Wall Residues of Fruits and Vegetables Along a Fraction Process, *Food Biophys*., 8, pp29–42.

Szymański M., Witkowska-Banaszczak E., Klak N., Marciniak K., Wołowiec T., Szymański A., (2014). Effects of Trace Elements on Polyphenolic Compounds in Millefolii Herba, *Pol. J. Environ. Stud*., 23(2), pp459-466.

Topală C. M., Tătaru L. D. (2016). ATR-FTIR Study Of Thyme And Rosemary Oils Extracted By Supercritical Carbon Dioxide, *Rev. Chim*.(Bucharest), 67(5), pp842-846

Topală, C. M., Ducu, C. (2014). Spectroscopic Study of Sea Buckthorn Extracts, *Current Trends in Natural Sciences* 3(6), pp48-53.

Topală, C. (2013). Temperature Effects on the FTIR Spectra of Ribavirin, *Rev. Chim.* (Bucharest) 64(2), pp132-135.

Zavoi S., Fetea F., Ranga F., Pop R. M., Baciu A., Socaciu C. (2011). Comparative Fingerprint and Extraction Yield of Medicinal Herb Phenolics with Hepatoprotective Potential, as Determined by UV-Vis and FT-MIR Spectroscopy, *Not Bot Horti Agrobo*, 39(2), pp82-89.

Zhang H. F., Yang X. H., Wang Y. (2011). Microwave-assisted extraction of secondary metabolites from plants: current status and future directions. *Trends Food Sci Tech*. 22, pp672–688.

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