Hungarian Association of Agricultural Informatics
European Federation for Information Technology in Agriculture, Food and the Environment

Journal of Agricultural Informatics. Vol. 9, No. 1
journal.magisz.org

Spectroscopic study on chemical composition of essential oil and crude extract from Albanian *Pinus halepensis* Mill.

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INFO
Received 28 Feb 2018
Accepted 17 Mar 2018
Available on-line 23 Mar 2018
Responsible Editor: M. Herdon

Keywords:
Pinus halepensis Mill., Soxhlet and Clevenger extraction, FTIR and UV-Vis. spectroscopy

ABSTRACT

Balkan countries are home to outstanding natural bioresources such as large number of herbs, plants and forestry species. They are also, among the most important export regions for medicinal and aromatic plants in Europe. Balkan has various flora with different tree species as well. *Pinus halepensis*, also known as the Aleppo pine, is native to the Mediterranean region. However, its range extends to Balkan countries as well, such as Albania, Greece, Croatia and Montenegro. It is one of the many trees that are well known for their medicinal properties, as well as for their economical significance. In this work essential oils/extracts of *Pinus halepensis* needles were acquired by hydro-distillation (Clevenger type) and Soxhlet extraction. The pine oils and crude extracts were analysed by FTIR spectroscopy and UV-Vis spectrophotometry aiming the identification of the main chemical constituents of the oil extracts of Albanian pine needles. FTIR analyses indicated presence of caryophyllene and pinene as the main chemical constituents in the essential oil and extract of pine needles.

1. Introduction

Balkan countries possess rich ecosystems with enormous natural and biological value (Kathe et al. 2003, Metaj 2007, Pieroni & Quave 2014, Schmiderer et al. 2013*)*. Balkan region has very various flora with a great deal of different tree species as well, including pine species. *Pinus halepensis* (Pinaceae), also known as the Aleppo pine, is native to the Mediterranean region (Abi-Ayad et al. 2011). It is one of the many trees that are well known for their medicinal properties, as well as for their economical significance (Cheikh-Rouhou et al. 2006). Its range expands from Morocco, Algeria, Spain to France, Italy, Croatia, Albania (Toromani et al. 2015) and Greece (Roussis et al. 1995). In Albania, conifer forests cover an area of 104780 ha with a standing volume of 10.7 million cubic

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doi: 10.17700/jai.2018.9.1.440

meters, while the Mediterranean pine species occupy 18440 ha. Pinus species (*P. halepensis* and *P. pinea*) cover approximately 85% of that area (Toromani et al. 2015).

Essential oils and crude extracts from *Pinus* species are reported to have different therapeutic properties. They are used as fragrances, flavoring additives for food and beverages, and intermediates in synthesis of perfumes (Fekih et al. 2014). In this respect, many authors have reported, the major chemical components of *Pinus halepensis* extract oils are β-caryophyllene and α-pinene (Roussis et al. 1995, Dob et al. 2005, Cheikh-Rouhou et al. 2006, Abi-Ayad et al. 2011, Fekih et al. 2014).

Usually the oil extraction from plants are carried out by water distillation and organic solvent extraction using a Soxhlet technique (Dob et al. 2005, Taraj et al. 2013, Andoni et al. 2014, Ciko et al. 2016, Taraj et al. 2017). Following our previous studies on the essential oils extraction from Albanian plants (Taraj et al. 2013, Andoni et al. 2014, Ciko et al. 2016, Taraj et al. 2017), we further extended this work by utilizing a Clevenger type apparatus (hydrodistillation extraction) and a Soxhlet extractor to obtain essential oil from *P. halepensis* needles. Chemical composition analyses were carried out by spectroscopy techniques. Spectroscopy methods are effective in assessing the qualitative difference between samples (Andoni 2009, Andoni et al. 2009, Andoni 2014, Schulz et al. 2004, Schulz et al. 2005).

The first contribution of this paper is to obtain essential oils or extracts from Albanian *P. halepensis* by different traditional methods. The second contribution is to determine the chemical composition (major bioactive components) by spectroscopic techniques and subsequently compare the obtained findings with those reported in the literature.

The paper is structured as follows. Section 2 presents the methodology used for all the oil extractions. Section 3 presents the results on extractions by different methods and the relevant spectra by different spectroscopic techniques. Section 4 presents a brief discussion on the results. At last some conclusions are drawn in Section 5.

2. Methodology

The origin of *P. halepensis* used in this work is from Western Albania. The pine needles were dried in shadow at room temperature and cut off in small pieces. The water distillation extraction was carried out by means of Clevenger apparatus in a round bottom flask using a ratio of 10:1 water/dried needles. The water-flower mixture was then subjected to distillation for an optimum number of hours, which was determined to be 3 hours. In the first 30 minutes once the oil had started collecting in the collecting column of the Clevenger apparatus, about 1 ml of hexane (oil phase) was put through the condenser to wash down any oil which had stuck to the walls of the condenser. The essential oil (dissolved in hexane) was then separated in a separating funnel (Ciko et al. 2016).

In the Soxhlet extraction, pine needles were placed inside a container made of thick filter. The container is located into the main chamber of the Soxhlet extractor. The Soxhlet can be slotted onto a flask which contains hexane (in this work), as extraction solvent. The Soxhlet is afterward equipped with a condenser, whereas the hexane is heated and allowed to reflux. The amount of the herb used for Soxhlet extraction was ~10 g, whereas the amount of the solvent (hexane) used was 300 mL. In the current work the extraction process was allowed to run approximately 3 hours. Hexane is removed, by means of a rotary evaporator, yielding though the extracted compounds (Ciko et al. 2016).

FTIR spectra were obtained by Nicolet 6700 spectrometer, manufactured by Thermo Electron. In this study, measurements were carried out in the range mid Infra-Red (4000 – 400 cm^{-1}). The spectra were analyzed using OMNIC software. UV-Vis spectra measurements were carried out by 6800 UV-VIS Jenway spectrophotometer.

3. Results

Table 1 displays overall results of the yields for the oils obtained with different methods. It is evident from table 1 that the Soxhlet extraction gives rise to higher yield when compared to the yield obtained from the extraction with the Clevenger apparatus. In this respect Dob et al. 2005, reported yield of 0.52% for the essential oil of *P. halepensis* extracted by hydro-distillation.

Table 1. Overall results for the extraction of essential oil and crude extract of *P. halepensis*.

Extraction method	Amount of *P. halepensis*	Extraction solvent	Extraction time	Extraction Temperature	Yield of extract
Clevenger (distillation)	10 g	Water	3 h	120^0C	0.30%
Soxhlet (distillation)	10 g	Hexane	3 h	80^0C	3.54%

Figure 1 exhibit FTIR spectrum of the *P. halepensis* oil extract obtained with the hydro-distillation method (in the insert) and FTIR spectrum of the *P. halepensis* crude extract obtained with Soxhlet extractor. The spectra of all extracts revealed very similar features. The acquired essential oil obtained by hydro-distillation had a pale yellow color whereas the crude extract obtained with the Soxhlet apparatus had yellow color.

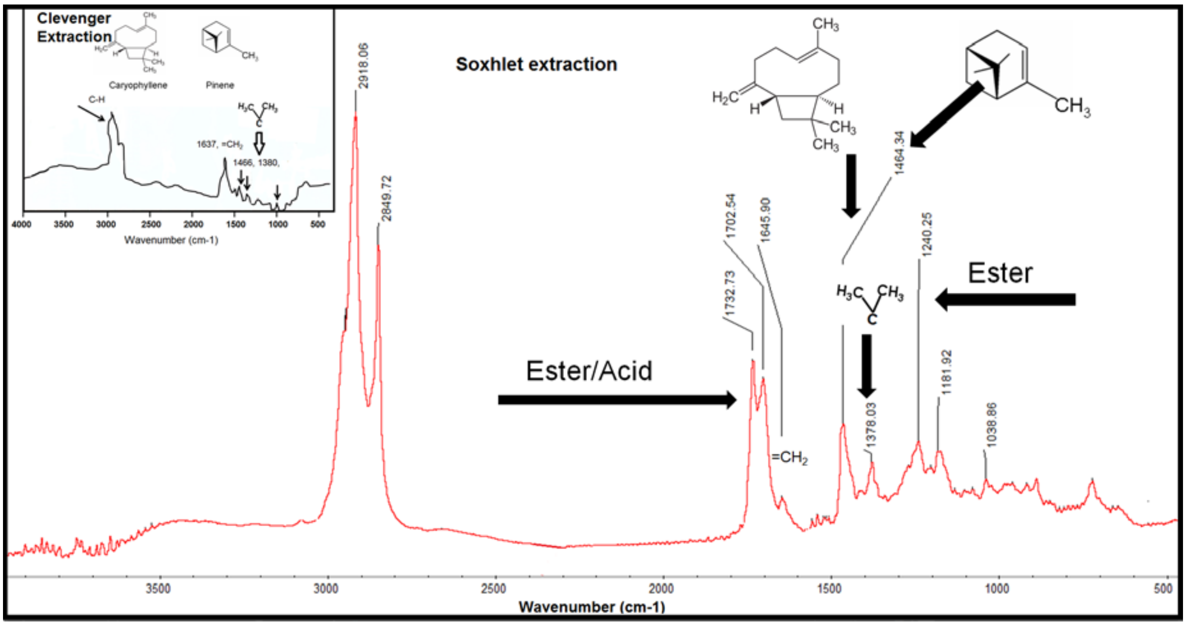

Figure 1. FTIR spectra of *P. halepensis* essential oil obtained by a Clevenger type hydro-distillation (in the insert) and Soxhlet type distillation. The main components identified in the FTIR spectrum are indicated by arrows.

FTIR spectrum of the oil extract obtained by Clevenger apparatus (inserted spectrum) indicates peaks positioned at ~1637 cm^{-1}, ~1466 cm^{-1} and ~1380 cm^{-1}. Other IR signals appear in the region ~3000-2800 cm^{-1} along with minor peaks evident at ~ 1000 cm^{-1} and at ~700 cm^{-1}. Additionally, FTIR spectrum of the crude extract (Soxhlet extraction) reveals similar features. IR spectrum indicates peaks positioned at ~1645 cm^{-1}, ~1464 cm^{-1} and ~1378 cm^{-1}. Other IR signals appear in the region ~3000-2800 cm^{-1} along with minor peaks evident at ~ 1038 cm^{-1} and at ~700 cm^{-1}. Furthermore, there are three distinctive bands in the IR spectrum of the crude extract. An intense band appears in the region 1730-1700 cm^{-1}, whereas two other bands appear at ~1240 cm^{-1} and at ~1181 cm^{-1}. Additionally, UV-VIS spectrum (Figure 2) of *P. halepensis* oil extract (Clevenger extraction) indicates peaks in the regions, 200-210 nm, 230-235 nm and a minor peak at 270-290 nm.

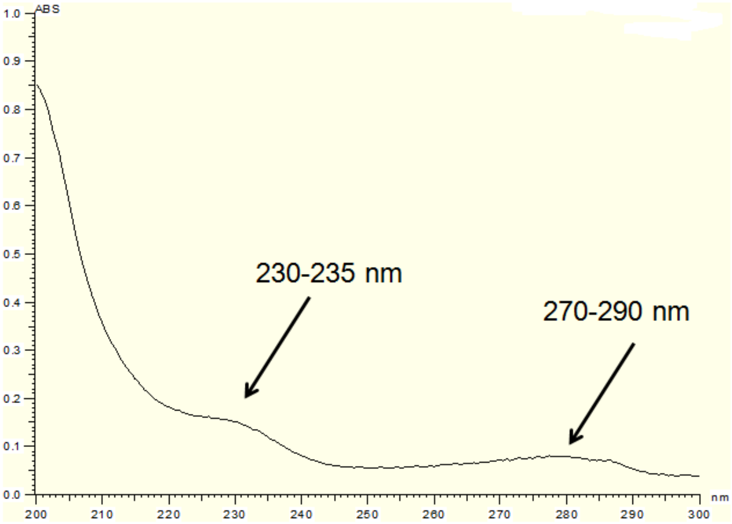

Figure 2. UV-Vis spectrum of *P. halepensis* essential oil obtained by hydro-distillation.

4. Discussion

In the FTIR spectrum of the *P. halepensis* essential oil appears two peaks positioned at ~1466 cm^{-1} and at ~1380 cm^{-1}. It is known that isopropyl and *gem*-dimethyl groups give rise to a split umbrella mode with two peaks in the IR spectrum positioned at ~1385 to 1365 cm^{-1} (Smith 1999). The splitting is caused by vibrational interaction between the umbrella modes of the two methyl groups. The split of the umbrella modes is of about equal intensity. Meanwhile, t-butyl and isopropyl groups also give rise to a split umbrella mode with two peaks positioned between ~ 1393 to 1366 cm^{-1} (Smith 1999). However, the approximate intensity ratio in this case is 1:2 (Smith 1999). Additionally, the band at ~ 1466 cm^{-1} can also indicate the presence of a CH_3, a CH_2 or both groups; whereas CH_3 symmetric bend (umbrella mode) shows up at 1375±10 cm^{-1} (Smith 1999). The chemical structures of caryophyllene consists of isopropyl or isobutyl groups, therefore we assign the peaks at at ~1466 cm^{-1} and at ~1380 cm^{-1} to caryophyllene. These findings are in good agreement with IR data reported for oil extracts of different herbs (peak position of the same functional groups) (Schulz et al. 2004, Schulz et al. 2005). In this respect, the same authors (Schulz et al. 2004, Schulz et al. 2005) reported IR spectrum of caryophyllene and indicated presence of three peaks in the region 1500-1300 cm^{-1}. The latter is in good agreement with our findings. The C=C stretches appear at ~1660-1630 cm^{-1} (Smith 1999), therefore the IR at ~1630 cm^{-1} belong to pinene and caryophyllene (vinyl group, =CH_2) (Schulz et al. 2004, Schulz et al. 2005).

The FTIR spectrum of the crude extract of *P. halepensis* features similiar signals as the IR spectrum of essential oil of pinus needles. The FTIR peaks in the region 1464-1378 cm^{-1} and 1645 cm^{-1} suggests presence of caryophyllene and pinene respectively. In comparison to the IR spectrum of the essential oil, the IR spectrum of the crude extract is characterised by three additional signals in the region 1730-1700 cm^{-1}, ~1240 cm^{-1} and ~1181 cm^{-1}. It has been reported by Mohareb et al. 2017, that crude extracts of pinus species contain fatty acids, resin acids and phthalates as well. In this respect, the additional signals in the IR spectrum are characteristic for the ester or acid functional group (Smith 1999). In addition, UV-VIS spectrum of pine essential oil gives rise to features in 200-290 nm region. These features originate from the double bonds (or conjugated double bonds) of caryophyllene and pinene (Taraj et al. 2013).

5. Perspectives and conclusion

The extracts from *P. halepensis* were obtained by means of Clevenger apparatus (hydro-distillation) and Soxhlet extractor using hexane as solvent. The extraction with Clevenger apparatus gave rise to lower yield compared to the yield obtained with Soxhlet method extraction. IR spectra of *P. halepensis* essential oil and crude extract indicated presence of caryophyllene and pinene as two major chemical constituents in the extract. IR signals for the isopropyl groups (1466-1380 cm^{-1}) and vinyl group (1645-1637 cm^{-1}) were in good agreement with IR data reported by Schulz and co-authors. UV-Vis signals supported IR findings for the caryophyllene and pinene presence. To this end, the chemical composition of Albanian *P. halepensis* oil is similar to the chemical composition reported in the literature for the pine needles (*P. halepensis*). Extractions by Clevenger and Soxhlet apparatuses could also be combined with the extractions with liquid CO_2 at different temperatures (sub-critical conditions). Compressed CO_2 is frequently used as it represents an environmentally friendly solvent (Taraj et al. 2013). The extraction by liquid CO_2 is a very selective extraction. The lower temperature of the extraction allows better separation of thermally labile compounds (Taraj et al. 2013).

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