IDENTIFICATION OF MATRICIN (CHAMAZULENE) IN THE ESSENTIAL OIL OF *MATRICARIA RECUTITA* L. WITH SPECTROSCOPIC TECHNIQUES

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Abstract

Medicinal and aromatic plants have a long history in the culture and traditional knowledge of Albania. About 30% of all known European plant species occur in Albania. Medicinal and aromatic plants of Albania are rich in essential oils. They are well-known for their curative properties with a large application in cosmetics as well. Therefore, it is of high interest essential oil extraction with different methods and their characterization with different techniques. Chamomile (*Matricaria Recutita* L.) is mostly known for its anti-inflammatory properties. The oil is effective in treatment of inflamed conditions such as eczema, ulcers, but also infections of the digestive tract, colitis and certain types of asthma. The essential oil of chamomile is rich in terpene and sesquiterpene such as bisabolol and matricin. Especially the amount of matricin or matricin as chamazulene is responsible for the quality of the oil. It is believed that the anti-inflammatory properties of the oil are due to chamazulene content only. In this work, essential oil and crude extract of chamomile are obtained by different extraction methods such as Clevenger and Soxhlet extraction. The essential oil and crude extract are analyzed with IR spectroscopy and UV-Vis spectrophotometry. Matricin or matricin as acid of chamazulene is identified in the range 1800-1600 cm^{-1} of the IR spectrum and at λ=245 nm of the UV-Vis spectrum in full agreement with reported IR or UV-Vis data in the literature. The blue color of the oil (oil obtained with hydrodistillation by Clevenger apparatus) is another indicator of chamazulene presence.

Keywords: Chamomile *(Matricaria recutita* L.*)*, essential oil, crude extract, spectroscopic techniques.

Përmbledhje

Vendi ynë është i pasur me bimë aromatike dhe mjekësore, madje 30% e bimësisë së Europës gjendet në Shqipëri. Këto bimë janë të pasura me esenca vajore të njohura për vetitë e tyre kuruese me përdorim të gjerë edhe në kozmetikë. Për këtë arsye është me mjaft interes përftimi i vajrave esenciale me metoda të ndryshme si dhe studimi i tyre me teknika të ndryshme karakterizuese. Kamomili (*Matricaria Recutita* L.) njihet për përdorimet e tij si një bimë mjekësore në rastet e ulçerës në stomak, irritimin e zorrëve si dhe kundër pagjumësinë. Ai përdoret si antiinflamator dhe antibakterial. Vaji esencial i kamomilit është i pasur me terpene dhe sekuiterpene siç janë bisabolol-i dhe matricina. Në veçanti sasia matricinës ose matricinës si kamazulen ose acid i kamazulenit përcakton cilësinë e vajit esencial të kamomilit. Është raportuar që sa më e madhe sasia e matricinës ose kamazulenit në vajin esencial të kamomilit aq më cilësor është ai. Në këtë punim vaji esencial i kamomilit dhe ekstrakti i tij është përftuar me metoda të ndyshme si esktraktimi me aparatin Klevenger (hidro-distilimi) dhe me aparatin Sokslet. Vaji esencial është analizuar me spektroskopinë IK dhe spektrofotometrinë UV-Vis. Matricina ose acidi i kamazulenit është identifikuar në intervalin 1800-1600 cm^{-1} të spektrit IK dhe në λ=245 nm të spektrit UV-Vis në përputhje të plotë me raportimet në literaturë. Prezenca e kamazulenit ose acidit të kamazulenit vërtetohet edhe nga ngjyra blu e vajit esencial të kamomilit.

Fjalëkyçe: Kamomil *(Matricaria recutita* L.*)*, vaj esencial, teknika spektroskopike.

Introduction

Nowadays, Southeast Europe is so far the most important European source region of medicinal plants (Kathe 2006). Albania and Bulgaria in particular, but also Romania, FYROM and other countries provide the European market with considerable amounts of raw material (Kathe, 2006). Two countries alone, Bulgaria (about10,000 tonnes annually) and Albania (about 7,600 tonnes annually), provide more than 50 % of the medicinal plant material (Lange, 2003). In this respect, between 1995 and 2000, Albania ranked in 15^{th} position among the most important countries for MAP (Medicinal and Aromatic Plant) export, with an annual average of 7,650 tonnes of dried material (Kathe *et al.* 2013).

Traditionally, Albania is Europe's leading sage (*Salvia officinalis* and *Salvia fruticosa*) producer (Schmiderer *et al*. 2013), exporting over 1,000 tonnes annually with a market value of about US$ 2.5 million (Kathe *et al.* 2013). Between 1996 and 1998, Albania became one of the world's leading low-cost suppliers of St John's-wort (*Hypericum perforatum*) exporting raw material with a value of over US$ 5 million annually (Kathe *et al.* 2013).

Additionally, it is reported by United States Agency for International Development that Aromatic and Medicinal Plants (in Albania) generates more than 16 million Euros per year. The value chain is mainly export-oriented, about 60% of MAPs are shipped to Germany (thyme) and USA (salvia species). Table 1 provides a list of the MAPs most commonly collected and traded in Albania, showing the Latin name, the English and the Albanian one (United States Agency for International Development). It is evident form Table 1 that chamomile is also part of this market.

Currently, Albania produces annually between 35 and 40 tons of essential oils, which are produced from an estimated 15 small, medium and large processing companies (United States Agency for International Development). Each of these companies has a distillatory operating with steam technology. The main essential oils produced include sage, juniper, oregano, thyme and winter savory essential oils.

Table 1. Main herbs and spices exported or sold in domestic market.

Latin Name	Name in Albanian	Name in English
Capsella bursa-pastoris	Shtraper	Shepherd's-purse
Capsicum	Spec djeges	Chili pepper
Centaurea Cyanus	Cian	Cornflower
Chamaimēlon	Kamomil	Chamomile
Cinnamomum verum	Kanelle	Cinnamon
Cirsium	Fare Gjembaci	Thistle Seed
Coriandrum sativum	Koriander	Coriander
Crataegus Oxycantha	Lule Murrizi	Hawthorn
Crocus sativus	Krokull	Saffron
Curcuma longa	Kurkume	Turmeric
Cynarae Folium	Argjinare	Artichoke
Foeniculum vulgare	Finok	Fennel
Gentiana	Sanzi	Gentian
Hypericum perforatum	Lule balsami	St John's wort
Juniperus Communis	Dellinja e Zeze	Repanda juniper
Laurus nobilis	Gjethe dafine	Bay Laurel/Bay leaves

Lavandula	Lavendul	Lavender
Malus Sylvestris	Molla e Eger	Wild Apple
Melissa officinalis	Bar Blete	Lemon balm
Mentha piperita	Meander i bute	Peppermint
Myristica	Arremyshk	Nutmeg
Ocimum basilicum	Borzilok	Basil
Orchis Mascula	Salep	Salep
Origonum Vulgare	Rigoni i Zakonshem	Oregano
Petroselinum crispum	Majdanoz	Parsley
Pimpinella anisum	Anasoni	Anise
Primula Veris	Agulice	Cowslip Genus
Rosa Canina	Trendafil i Eger	Dog Rose
Rosmarinus officinalis	Rozmarine	Rosemary
Rubus fruticosus	Manaferra	Blackberry
Salvia Officinalis	Sherebele	Sage
Sambucus Nigra	Shtogu	Elderberry
Satureja Montana	Trumez	Winter savoury
Sideritis Syriaca	Caj Mali	Mountain Tea
Syzygium aromaticum	Karafil	Clove
Taraxacum Officinale	Flete Qumeshtore	Dandelion
Thymus serpyllus	Zhumrica	Thyme
Tilia Cordata	Lule Bliri	Small-leaved Linden
Tussilago farfara	Thunderz	Coltsfoot
Urtica Dioica	Flete Hithre	Stinging nettle
Vaccinium Myrtillus	Boronice Frut	Blueberry
Vanilla	Vanilje	Vanilla
Viscum album	Veshtull i bardhe	Viscum album
Zingiber officinale	Xhinxhefil	Ginger

In this respect, chamomile is the most favored and most used medicinal plant over the world (Salomon *et al.* 2010). Curative effects of chamomile are determined by the essential oil content and composition (Salomon *et al.* 2010). Essential oil of chamomile is collected from flower heads, either by steam distillation or solvent extraction. Among the essential oil constituents the most active are α-bisabolol and chamazulene or matricine as chamazulene (Salomon *et al.* 2010). Chamazulene and α-bisabolol promotes wound healing and exhibit anti-inflammatory activity (Morgan, 1996).

Following our previous studies on the essential oils (crude oil) extraction from Albanian herbs (Taraj *et al.* 2013, Andoni *et al.* 2014, Dama *et al.* 2015, Taraj *et al.* 2017), we advanced this work by utilizing steam-distillation method (Clevenger apparatus) and solvent extraction (Soxhlet apparatus) to obtain essential oil or crude extract from Chamomile (*Matricaria Recutita* L.). The essential oil and crude extract are analyzed with IR spectroscopy and UV-Vis spectrophotometry. Matricin or matricin as acid of chamazulene is identified in the range 1800-1600 cm^{-1} of the IR spectrum and at λ=245 nm in the UV-Vis spectrum in full agreement with reported IR or UV-Vis data in the literature. The blue color of the oil (oil obtained with hydrodistillation by Clevenger apparatus) is another indicator of chamazulene presence. 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).

Materials and methods

The origin of the *Matricaria recutita* L. (flower heads) used in this work is from local Albanian herb. The herb (20 g) is dried at 40°C until constant weight and subjected to a grinding process before came into contact with the steam. The steam distillation extraction was carried out in a Clevenger apparatus using a ratio of 5:1 water/dried herbs. A Clevenger apparatus and a condenser were attached to the round flask placed on an electric mantle (heating bowl). The water-plant mixture was then subjected to distillation for an optimum number of hours which was determined to be 4 hours.

In the Soxhlet extraction, the plant was 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 (Ciko *et al.* 2016). The amount of the herb used for Soxhlet extraction was 20 g, whereas the amount of the solvent (hexane) used was 300 mL. In the current work the extraction process was allowed to run approximately 4 hours.

The essential oil (dissolved in hexane or dichloromethane) was then separated in a separating funnel and further analyzed by FTIR spectroscopy and spectrophotometer UV-Vis. FTIR spectra were obtained by Nicolet 6700 spectrometer, manufactured by Thermo Electron. The measurements were carried out in the transmission system in the mid-IR range (4000 – 400 cm^{-1}). The spectra were analyzed using OMNIC program. UV-Vis spectra measurements were carried out by 2400 PC Shimadzu spectrophotometer.

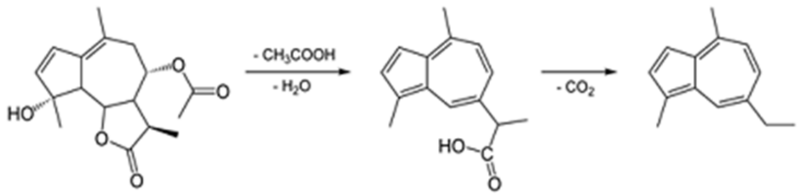
Results and discussion

Table 2 displays overall results of the yields of the oils obtained with different methods. It is evident from Table 2 that the Soxhlet extraction gives rise to higher yield when compared to the yield obtained with the Clevenger apparatus. This result is in good agreement with reported data for the same extraction methods (Taraj *et al*. 2017, Ciko *et al.* 2016).

Table 2. Overall results for the extraction of essential oil of M. recutita.

Extraction apparatus	Amount of *M. recutita*	Extraction solvent	Extraction time	Extraction temperature	Yield of oil and extract
Clevenger	20 g	Water	4 h	120^0C	0.19%
Soxhlet	20 g	Hexane	4 h	80^0C	5.54%

The essential oil of chamomile obtained by Clevenger apparatus had a blue light color due to chamazulene formation (Mwazighe, 2013), whereas crude extract of chamomile obtained by Soxhlet apparatus had a yellow color due to matricin presence. All extracts had the characteristic smell of chamomile essential oil. Fig.1 represents reaction scheme of matricin formation during water distillation extraction.



Matricin **Carboxylic acid of matricin** **Chamazulene**

Figure 1. Reaction scheme of matricin formation during hydro-distillation.

Fig. 2 exhibits FTIR spectra of chamomile essential oil and crude extract obtained by Clevenger and Soxhlet apparatuses respectively (left and middle spectrum). Additionally, for comparison reason, Fig. 2 introduces several IR spectra of chamomile extracts reported by Schulz *et al*. 2004 and 2005 (right spectra). The bands positioned at ~1716 cm^{-1} and ~1742 cm^{-1} are attributed to the stretching vibration of C=O of matricin (Smith 1999, Mwazighe 2013). This is in good agreement with the findings of Schulz *et al*. 2004 and 2005 (right spectra, band position at ~ 1713 cm^{-1}).

It is evident from the IR spectra that the intensity of the peak at ~1742 cm^{-1} is about three times in magnitude compared to the peak intensity at ~ 1713 cm^{-1}. The latter suggest presence of flavonoids in the crude extract of chamomile, most likely apigenin and related flavonoid glycosides (Mwazighe 2013). The lower intensity of the band at ~1716 cm^{-1} is indicator of partial degradation of matricin to chamazulene. It is also possible that matricin has partially converted to carboxylic acid of matricin and partially to chamazulene.

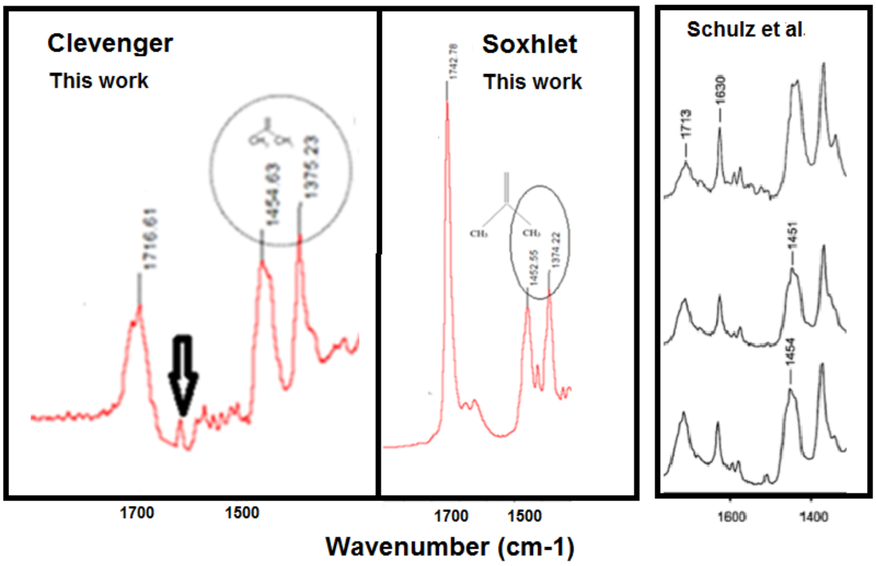


Figure 2. FTIR spectra of essential oil and crude extract of chamomile obtained by Clevenger and Soxhlet apparatuses (left and middle spectrum). FTIR spectra the of chamomile extracts reported by Schulz *et al*. (2004, 2005).

Additionally, the diagnostic IR band of chamazulene (-C=C-, alkene) appears at ~1640 cm^{-1} (indicated by arrow in the left spectrum) (Smith 1999). This is in good agreement with IR spectrum (signal) reported by Schulz *et al*. 2004 and 2005 (right spectra). The FTIR spectra of the essential oil and crude extract appear two sharp peaks (indicated by circles) positioned at ~1450 cm^{-1} and at ~1370 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. Additionally, the band at ~ 1450 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}. These peaks, also present in the IR spectra reported by Schulz *et al*. 2004 and 2005, and are attributed to α-bisabolol and its oxides (isopropyl or isobutyl groups). Lastly, Fig. 3 displays UV-Vis. spectra of chamomile extract. Mwazighe (2013) reported that UV-Vis. analysis of the chamomile extracts were characterized by strong absorption in the range 270-400 nm. This is in excellent agreement with the findings of this work. Fig. 3 reveals a strong absorption band in the range ~270-400 nm. To this end, Kaiser (2003) reported that matricin absorbs at 244 nm. The UV-Vis. spectrum reveals a band at 245 nm in excellent agreement with the findings of Kaiser (2003).

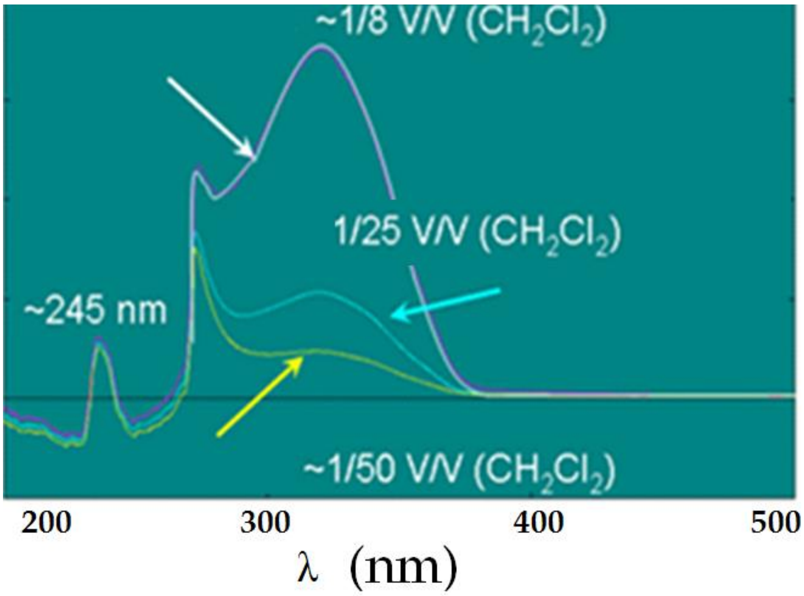


Figure 3. UV-Vis. spectra of chamomile extract.

Conclusions

The extraction of essential oil and crude extract from M. recutita L. flowers was performed using water distillation and Soxhlet extractions. The acquired oil and crude extract were characterized by FTIR and UV-Vis. spectroscopy. IR analysis indicated presence of matricin in the range and ~1742-1716 cm^{-1}, wheras chamazulene presence was identified by the IR signal at ~ 1630 cm^{-1}. UV-Vis. spectra supported IR findings. Matricin presence was revealed by the band at 244 nm (UV-Vis. spectra). The above mentioned findings are in excellent agreement with the reported data by Schulz *et al*. (2004, 2005), Mwazighe (2013) (IR data) and Kaiser (2003) and Mwazighe (2013) (UV-Vis. data).

References

Andoni A., (2009): A flat model approach to Ziegler-Natta olefin polymerization catalysts Eindhoven, PhD Thesis. Technische Universiteit Eindhoven (Eindhoven University of Technology). The Netherlands. Ch. 7

https://DOI:10.6100/IR638773

Andoni A., (2014): High resolution electron energy loss spectroscopy for studying planar model catalyst: A test of NO on Rh(100). Revue Roumaine de Chimie, Vol.59 (3-4): 245-249

Andoni A., Chadwick J.C., Niemantsverdriet J.W., Thüne P.C., (2009): Investigation of Planar Ziegler-Natta Model Catalysts Using Attenuated Total Reflection Infrared Spectroscopy. Catalysis Letters, Vol.130:278-285

Andoni A., Xhaxhiu K., Taraj K., Çomo A., (2014): An adsorption method for characterization of surface area and pore size of solid surfaces. Asian Journal of Chemistry, Vol.26:6833-6838

Ciko L., Andoni A., Ylli F., Plaku E., Taraj K., Çomo A., (2016): Extraction of essential oil from Albanian Salvia Officinalis (L.) and its characterization by FTIR spectroscopy: A Soxhlet method extraction, Asian Journal of Chemistry, Vol.28:1401-1402

Dama A., Taraj K., Ciko L., Andoni A., (2015): Extraction of essential oils from Salvia Officinalis L. Leaves with different extracting methods. International Journal of Ecosystems and Ecology Science (IJEES), Vol.5/3: 421-424

Kaiser C.S., (2003): Herstellung, In-line-einshcluss Und Charakterisierung Lipophiler und Hydrophylier Kamillenextrakte Unter Einsatz von Verdichtetem Kohlendioxid, Dizertacion der Fakultät für Chemie und Pharmazie der Eberhard-Karls-Universität Tübingen zur Erlangung des Grades eines Doktors der Naturwissenschaften, Germany

Kathe W., (2006): Conservation of Eastern-European medicinal plants, Ch. 14, Bogers R.J., Craker L.E., and Lange D. (eds.), Medicinal and Aromatic Plants, 203-211. © 2006 Springer. Printed in the Netherlands.

Kathe W., Honnef S. and Heym A. (eds.), 2003: Medicinal and aromatic plants in Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania: a study of the collection of and trade in medicinal andaromatic plants (MAPs), relevant legislation and the potential of MAP use for financing nature conservation and protected areas. Federal Agency for Nature Conservation, Bonn. BfN-Skripten no. 91.

[http://www.bfn.de/fileadmin/MDB/documents/skript91.pdf]

Lange D., (2003): The role of East and Southeast Europe in the medicinal and aromatic plant trade: with special focus on Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania. *In:* Kathe, W., Honnef, S. and Heym, A. eds. Medicinal and aromatic plants in Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania: a study of the collection of and trade in medicinal and aromatic plants (MAPs), relevant legislation and the potential of MAP use for financing nature conservation and protected areas. Federal Agency for Nature Conservation, Bonn, 64-77. BfN-Skripten no. 91.

[http://www.bfn.de/fileadmin/MDB/documents/skript91.pdf]

Morgan M., (1996): Chamomile from a clinical perspective. Modern Phytotherapist. Vol.3:17-19

Mwazighe F.M., (2013)**:** Extraction, physico-chemical characterization and stability monitoring of essential oil from Matricaria recutita L grown in selected areas in Kenia. A thesis submitted in partial fulfillment of the degree of Master of Science in environmental chemistry at the University of Nairobi.

http://erepository.uonbi.ac.ke/handle/11295/43499

Salamon I., Ghanavati M. and Khazaei H., (2010): Chamomile biodiversity and essential oil qualitative-quantitative characteristics in Egyptian production and Iranian landraces. Emir. J. Food Agric. Vol. 22 (1): 59-64

Schmiderer C., Londoño T. P., Novak J. (2013): Proof of geographical origin of Albanian sage by essential oil analysis, Biochemical Systematics and Ecology, Vol.51: 70-77

Schulz H., Baranska M., Belz H-H., Rösch P., Strehle M.A., Popp J., (2004): Chemotaxonomic characterisation of essential oil plants by vibrational spectroscopy measurements, Vibrational Spectroscopy, Vol. 35: 81-86

Schulz H., Özkan G., Baranska M., Krüger H., Özcan M., (2005): Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy, Vibrational Spectroscopy, Vol.39: 249-256

Smith B., (1999): Infrared spectra interpretation. A systematic approach, CRC Press, Boca Raton, London New York, Washington, D.C., 25-29

Taraj K, Malollari I., Andoni A., Ciko L, Lazo P., Ylli F., Osmëni A., Çomo A., (2017): Eco-extraction of Albanian chamomile essential oils by liquid CO_2 at different temperatures and characterisation by FTIR spectroscopy, Journal of Environmental Protection and Ecology, Vol.18(1):117-124

Taraj K., Delibashi A., Andoni A., Lazo P., Kokalari (Teli) E., Lame A., Xhaxhiu K., Çomo A., (2013): Extraction of chamomile essential oil by subcritical CO_2 and its analysis by UV-VIS spectrophotometer. Asian Journal of Chemistry, Vol.25(13): 7361-7364

The medicinal and aromatic plants value chain in Albania. (2009): USAID-Albania Agriculture Competitiveness (AAC) program. This publication was produced for review by the United States Agency for International Development. It was prepared by DAI.

[http://pdf.usaid.gov/pdf_docs/PA00JN4F.pdf]