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Research Article

Modern Chromatographic Techniques for the Analysis of Thymol and Carvacrole from the Thyme

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Abstract

Researchers are interested in the natural antibacterial and antifungal properties of thymol and carvacrol found in the leaves of the *Origanum syriacum* "Za'atar" plant. However, it is still challenging to employ them as natural preservatives in the modern pharmaceutical and cosmetics industries. The best vegetable oil for extraction was determined by analysing the thymol and carvacrol concentration data using a recognised analytical method. According to the results, sesame oil had the A consistent extraction result raises the possibility of usage in the pharmaceutical and cosmetics industries. Thus, the key extraction parameters that influence the final concentration of thymol and carvacrol were evaluated. Sesame oil quality and amount had the biggest impact. In cold-pressed sesame oil, thymol increased by 23% while carvacrol decreased. Sesame The second aspect was the growing circumstances of the Za'atar; the content of thymol and carvacrol in the Za'atar from Hebron differed by less than 10%. Last but not least, the provenance of the sesame seeds used to make sesame oil had the least impact.

Keywords:Chromatographic Techniques, Thymol, Carvacrole, Thyme

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Introduction

It is considered the most stable and useful plant in Arabic breakfasts. Bread and olive oil are served with a mixture of sesame seeds, the fruits of the *Rhus coriaria* tree, salt, and crushed dry leaves [1,2]. Because of its high content of volatile oils, the herb's leaves have a strong, warm aroma that is a little harsh or unpleasant. Because of the volatile oils in the belongs to either the Thyme or

Oregano genera. *Origanum syriacum* var. *syriacum* is a subspecies of *Origanum syriacum* that grows to about 40 cm in height. Its simple, ovate-shaped green leaves are covered in long and short white hairs, and its young leaves are tetragonal and rounded [2]. This subspecies may be found in Jordan, Lebanon, the Palestinian territories, and Antibacterial and antioxidant qualities are conferred by this herb's high concentration of phenolic

components [3], Thymol is thought to be the primary monoterpene with antibacterial activity [6] due to the hydroxyl group at the meta position [5]. A synergistic antibacterial effect is produced when thymol and its isomer, carvacrol, are combined [7]. Other terpenes include o-cymene [3] and γ -terpinene [6]. These four terpenes are the most common ones found in Za'atar

Materials and Methods

Fresh za'atar leaves were collected in two different locations in Hebron. The leaves were separated from the stem, sieved, and let to dry for 14 days before being stored in a dark plastic bag.

Plant material was ground in a coffee mill. One gramme of powdered material was separately combined with one of the solvents (sunflower oil, olive oil, or sesame oil) in a 100 ml volumetric flask. The mixture was sonicated for 25 minutes at was given time to cool before being shaken vigorously and loaded with solvent. Following its passage through a coffee filter, the mixture was transferred to a beaker and well mixed before 1:1.5 (mixture: absolute ethanol) was added. The mixture was transferred to a separating funnel, vigorously agitated, and allowed to stand for one hour. The supernatant The sesame seeds were pressed using an oil pressing machine, and the temperature was maintained below 45°C throughout the process. Low temperatures protect the oil's constituents from degradation [19]. Two different kinds of sesame seeds, from India and Turkey, were compacted with a single screwdriver. The oil is collected from underneath, and the sesame peels are discharged out one side of the screwdriver. The sesame oil was filtered through a coffee filter and then left for a whole day. The upper yellow component was collected, and the white portion was put in a small jar to get additional precipitation. There was a brown jug with sesame oil. 30 grammes of dry plant material were ground in a coffee mill. The milled material was weighed and transferred to a 2000 ml beaker, where 350 ml of sesame oil was added. The beaker was sonicated at 50°C for 25 minutes, with agitation taking place every 7 minutes. The mixture was transferred to a beaker and filled with absolute ethanol at a ratio of 1:1.5 (mixture: absolute ethanol). The remaining oil was placed in a separating Buchner funnel to remove any remaining alcohol after the supernatant was collected, and it was allowed to stand for an hour. The supernatant was combined with the collected alcohol and allowed to evaporate at 40°C.

Sample Preparation for HPLC Analysis

One gramme of the extracted sample was weighed into a 50 ml VF, topped to volume with diluent (ACN 80: PW 20), and then sonicated for five minutes at room temperature. Five (5.0) millilitres of the prepared sample were diluted to a 25 millilitre VF using the same diluent.

All samples were injected into HPLC according to the authorised protocol.

Extraction Optimization

Using the scale-up extraction method, Hebron Za'atar B was extracted from sesame oil made by cold pressing Indian sesame seeds. The Za'atar powder was moistened with the solvent after the volume of sesame oil was decreased from 350 ml to 250 ml.

Results and Discussion

The dependence of the mass of carvacrol (K : mg), i.e., of the mass of thymol (m : mg), in the investigated sample, on the integrated peak area (P), can be expressed by the following equations (07): $mK = (P + 3178115)/674321$ $r = 119988$ and $mK = (P + 3119115)/7685471$ $r = 119955$ $mT = (P - 681149)/7876541$ $r = 119985$ where r is the coefficient of regression. The yield of thyme essential oil (Table I) in plant material obtained by steam distillation (00°43) is a little lower than that prescribed by Yugoslav pharmacopoeia. The 15 qualitative composition of extracts obtained by investigated extraction procedures, determined by TLC, shows that they are very similar (R_f values of detected thyme compounds are given).

HPLC conditions: Column: NovaPak d07 (Waters; 345 mm LD. x 04 mm; μ urn) Precolumn: Waters Guard-Pak (01 urn) Mobile phase: Acetonitrile - water (51351; V/V) Flow rate: 232 mL/min Pressure: 001 bar Detection: UV at 044 nm / 0.1 AUFS

Various vegetable oils have been used as cosolvents for extraction. The advantages of employing vegetable oils as cosolvents to boost production have been shown to vary depending on the physicochemical properties of the used oil [20–23]. The oils used for this study's comparison are widely available in the Palestinian market. In contrast to olive oil, which produced very little thymol and carvacrol, sesame oil-absolute ethanol was 42% more effective than sunflower oil-absolute ethanol for both thymol and carvacrol. Carvacrol and This disparity may be explained by using a magnetic stirrer to extend the mixing time between sesame oil and 100% ethanol and by gradually increasing extraction expertise.

For every 10 grammes of plant leaves, Mnayer extraction yielded 5.93 grammes of oil, with a total phenolic content of 171.22 mg/g composed of 5.7% carvacrol and 80.5% thymol. 817.34 mg of thymol and 57.87 mg of carvacrol were obtained using the method of Mnayer. However, for every 100 grammes of plant removed, 66.66 grammes of oil were generated using the scaled-up sesame oil-absolute ethanol combination. This oil yielded 1559.84 mg of thymol and 88.66 mg of carvacrol since it contained 1.33 mg/g of carvacrol and 23.4 mg/g of thymol. The cold-pressed sesame oil-absolute ethanol Bermejo compared the green solvents ethanol, limonene, and ethyl lactate in thymol extraction from *Thymus vulgaris* using pressurised liquid extraction (PLE) and

supercritical fluid extraction (SFE). The ultrasonic method using sesame oil-absolute ethanol yielded 20–25 mg of thymol in 1 gramme of dried leaves, while the SFE method yielded the highest thymol content in this study, ranging from 7 to 11 mg for 1 gramme. The pharmaceutical and cosmetic sectors can utilise the extracted Za'atar oil as a natural preservative [25]. For regular batches with little fluctuations, extraction techniques must be thoroughly understood. The majority of

The place of origin of plant leaves and the conditions of cultivation are additional elements that affect the uniformity of extraction. Hebron Za'atar's origin was selected since it has the most market share in Palestine. The acrid-tasting leaves of the Za'atar plant are a sign that thymol is present. This criterion is widely accepted since only bitter Za'atar leaves from the Hebron region were selected, and the thymol content of the provenance of the sesame seeds used for pressing. The Turkish and Indian sesame seeds, which are the two main sources of sesame seeds that enter Palestinian territory, were found to have somewhat greater levels of thymol and carvacrol in the Indian seeds.

Conclusion

A straightforward, reasonably priced, and efficient method for extracting thymol and carvacrol involves combining ultrasonic technology with a sesame oil-absolute ethanol solvent. Mnayer's method, which combined ultrasonic and sunflower oil-absolute ethanol, produced results that were almost exactly the same as those of a conventional extraction procedure using hexane. Using sesame oil instead of sunflower oil increases the extraction efficiency by 50.2%.

References

- Chevallier, A. in: The Encyclopedia of Medicinal Plants, Dorling Kindersley Limited, London (1993). p. 0°0.
- Kisgeci, O., Adamovic D. i M. Soldatovic: Sistematskiprikazprivrednoznacajnihlekovitih, aronaticnihizacinskihbiljakainjihovizbor za gajcnjc u Vojvodini, Bilten za hmelj, sirakiIckovitobilje, Vol. 08,°9-51 (1975),°°-80.
- Pekic, B. in: Hemijaitehnologijafarmaceutskihproizvoda (alkaloidi i etarskaulja), TehnoloskifakultetUniverziteta u Novom Sadu (1976), p. 006. °. The Merck Index, Tenth Edition, Merck & Co., Inc., Rahway,N. J., U.S.A. (1976). p. 987.
5. Wagner, H., Bladt S. and E.M. Zgainski, in: Plant Drug Analysis (Translated by A. Scott). Springer-Verlag, Berlin HeidelbergNewYorkTokyo(197°), p. 06.19
- Pharmacopoeia.Iugoslavica, Editioquarta (Ph. Jug. IV), Saveznizavod za zdravstvenuzastitu, Beograd (197°).
- Pekic, B., Lepojevic Z. i Z. Zekovic, L. Pekic: Ispitivanjcsadrzajetaraskoguljaifenolnihjedinjenja u nekimbiljnimvrstamaroda Thymus iSatureja, Proceedings of Faculty of Technology (Novi Sad) 00 (1990), 78-90.
- Fehr, D. und G. Stenzhorn: UntersuchungenzurLagerstabilitat von Pfefferminzblattern, Rosmarinblattern und Thymian, Pharmazeutische Zeitung 00°,°3 (1989),06°0-06°9.
- Lukic, P. in: Farnakognozija, FarmaceutskifakultetUniverziteta u Beogradu (1979).
- Deighton,N., Glidewell S.M., S.G. Deans, B.A.Goodman: Identification by EPR Spectroscopy of Carvacrol and Thymol as the Major Sources of Free-Radicals in the Oxidation of Plant Essential Oils, Journal of the Science of Food and Agriculture 36, 0 (1996),000-005.
- Aleksovski, S., Sovova H., Poposka F. and B. Curapova: Supercritical fluid extraction of essential oils from peppermint and wild thyme and its comparison with hydrodistillation, Xlii CHISA '97, Summaries ° (1997), 007.
- 10.VanDen Broucke, e.1. and lA.Lemli:ChemicalInvestigationof the Essential Oil of Origanum compactum, Planta medica 67 (1971), 03°-033.
- Solinas, v., Oessa e. and L. Falchi Delitala: High-performance Liquid Chromatographic Analysis of Carvacrol and Thymol in the Essential Oil of Thymus capitatus,.I. Chromatogr. 009 (1970),660-668. 0°.
- VanDenBroucke, C.O. and lA. Lemli: Pharmacologicaland Chemical Investigation of Thyme Liquid Extracts, Plantamedica°0 (1970),009- 065.
- Pekic, P., Lepojevic Z. i Z. Zekovic: Odredivanjetimolaikarvakrola u etarskomuljutimijana (Thymus vulgaris L.) primenom HPLC, Proceeding for Natural Sciences (Novi Sad) 71 (1990),08-00. 300
- Jackson, S.A.L. and R.K.M. Hay: Characteristics of Varieties of Thyme (Thymus vulgaris 0,) for Use in the UK: Oil Content, Composition and Related Characters, Journal of Horticultural Science39, 0 (199°),085- 070.
- Pekic, B., Zekovic Z., Petrovic L. and A. Tolic: Behavior of (-)-a- Bisabolol and (-)-a Bisabololoxides A and B in Camomile Flower Extraction with Supercritical Carbon Dioxide, Separation Science andTechnology 61, 07(1995),6538-6583.
- Zekovic, Z., Lepojevic Z. and Dj. Vujic: Supercritical Extraction of Thyme (Thymus vulgaris L.), Chromatographia 50,61° (0111), 085-089.