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Determination of the antibacterial properties of chaste tree leaf and seed essential oils

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The essential oils of the leaves and seeds of *Vitex agnus-castus* L. (Chaste Tree), a plant of medical importance, were analyzed for antimicrobial effects. Total phenolic content was determined, and antimicrobial effects against Gr (-) *Escherichia coli* ATCC 25922, Gr (-) *Pseudomonas aeruginosa* ATCC 27853, Gr (+) *Staphylococcus aureus* ATCC 25923, and Gr (+) *Micrococcus luteus* NCIMB 13267 bacteria was assessed using the standard disk diffusion method. Analysis of the compounds in chasteberry seed essential oil reveals that the most abundant compound is 1,8-cineol, followed by a high proportion of α-pinene. Overall, the data have once again confirmed that the antibacterial effect of chasteberry seeds is greater on Gram-negative bacteria. 1,8-cineole is also known as eucalyptol. Thanks to the rich components of the chaste tree plant, our antibacterial study observed that the leaf and seed oils of *Vitex agnus-castus* L. are effective against Gram (+) and Gram (-) bacteria, and it was determined that the inhibition zones (cm) are larger on Gram (-) bacteria. The antibacterial effect of the leaf oil was found to be greater than that of the seed oil. The inhibition zone formed by the seed oil on *Pseudomonas aeruginosa* bacteria was found to be greater than that formed on *Escherichia coli* bacteria.

Keywords: Antibacterial, Antimicrobial, chasteberry (*Vitex agnus-cactus),* essential oil.

INTRODUCTION

Vitex agnus-castus L. (Chasteberry) is known by various names, including ‘Chasteberry’, ‘Hemp tree’, ‘Bitter Hess’, ‘Five-finger Herb’, ‘Monk’s pepper’ and ‘Chaste tree’ (Gülsoy, 2011). In the Middle Ages, it was used as a spice in monastic kitchens, and was believed to be beneficial in reducing the sexual desires of monks, which led to it named ‘Monk’s pepper’ (Odenthal, 1998). The species, *Vitex agnus-castus* L., has been used in traditional medicine for over 2000 years (Meier et al., 1994; Asdadi et al., 2015). It was first mentioned in the writings of Hippocrates in the 4th century BC. Its fruits have been used as an infusion (2-5%) as a diuretic, carminative, and sedative (Fakir et al., 2014). In addition, *Vitex agnus-castus* L. extracts have been shown to have moderate cytotoxic and pro-apoptotic effects on human cancer cells (Sezik et al., 2013).

Essential oils, also known as volatile oils, are complex mixtures obtained from plants or plant parts by distillation or pressing (Evren and Tekgüler, 2011). These oils can easily penetrate cell membranes and easily absorbed through organs such as the skin and lungs. Volatile oils used as medicines or food additives have a very high pharmaceutical value, and their biological activation is still a matter of curiosity (Erdoğan, 2012). They are used in various fields such as cosmetics, perfumery, pharmaceuticals, and the food industry, with their extraction and usage dating back to the Roman period.

Since the 1980s, they have been investigated in numerous studies in the terms of their antimicrobial effects. The properties of extracts, essential oils and their individual components have generally been used *in vitro* as antibacterial and antifungal agents (Arslan and Karabulut, 2005). The chemical composition and biological activity of these essential oils are affected by factors such as geographical origin, climatic conditions, and environmental stress (Yazlık and Üremiş, 2015).

In the literature review, we have come across a wide range of biological activities, including antifungal, antibacterial, antitumor, hormonal, and anti–prostate cancer effects related to the species *Vitex agnus-castus* L. The richness in secondary components has been well documented in many studies, and its areas of application have been indicated. In this study, antibacterial tests were conducted using the disk diffusion method to evaluate the antimicrobial effects of leaf and seed essential oils and to correlate these effects with their chemical composition determined by total phenolic content and GC-MS analysis. The antimicrobial activity of leaf and seed essential oils of V. agnus-castus against both Gram-positive and Gram-negative bacteria was investigated.

MATERIALS AND METHODS

Plant collection

Extraction procedures were carried out in the Plant Physiology Laboratory of the Department of Biology, Faculty of Science, Pamukkale University. Plant materials were dried under suitable environment, protected from direct sunlight, preserving their morphological characteristics (Baydar et al., 2008; Yaşar, 2005). Fieldwork was carried out at different times to collect leaf and seed samples (Fig. 1). The collection period was from June to November. To minimize the loss of essential oil yield, leaf, seed, and flower parts were collected during the early morning hours, and a total of 2–3 kg of fresh plant material were taken. The collection site was Karahayıt, Denizli, at an altitude of 253 m. The identification of the plants was performed by Prof. Dr. Mehmet Çiçek (Department of Biology, Pamukkale University).

Method

Fats are fundamental components that occupy an important place in diets. The fatty acid composition of seeds determines their physical and chemical properties, enabling plants to be used in different industrial fields. Fatty acids found in plants are divided into two groups: saturated and unsaturated fatty acids. Myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) are the most important saturated fatty acids found in plants. Palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) are among the most important unsaturated fatty acids present in plants. The importance of plant fatty acids, especially omega fatty acids, for human health is well known.
This study aimed to determine the oil content of chia seeds grown under different environmental conditions.

Instrument used for oil analysis: Agilent gas chromatography/mass spectrometry (AGILENT 5975 °C, AGILENT 7890A GC)
Software: MSDCHEM
Column: DB-WAX (50 × 0.20 mm, 0.20 µm)
Operating conditions:
The initial oven temperature was 80 °C. After the chia sample was held at 60 °C for 4 minutes, the temperature was increased to 175 °C at a rate of 13 °C per minute and held at this temperature for 27 minutes. Then, the temperature was increased to 215 °C at a rate of 4 °C per minute and held for 5 minutes. Subsequently, the temperature was increased to 240 °C at a rate of 4 °C per minute and held for 15 minutes. The detector and injector temperatures were set at 240 °C. The injection volume was 1 µL.

Methods

Oil was extracted from 10 g of chia seed sample using chloroform–methanol (2:1, v/v). The extracted oil was derivatized using 1.5 M methanolic HCl and injected into the GC–MS system (Bardakcı, 2006). Essential oils were obtained from dried leaves and seeds of the plant using a Clevenger apparatus. The oils obtained by boiling the powdered seeds for 3 hours were transferred to dark glass bottles and stored at +4 °C until use (Umay, 2007; Batish et al., 2012).

GC MS analysis method

GC-MS analysis was performed using an Agilent gas chromatography-mass spectrometry system (Agilent 5975C MSD, Agilent 7890A GC). MSDCHEM software was used for data gathering. Separation was done on a CPWAX 52CB capillary column (50 m × 0.32 mm × 0.20 µm). The initial oven temperature was set to 60 °C and held at this temperature for 2 minutes, then it was increased to 220 °C at a rate of 2 °C/min and kept at this temperature for 20 minutes. The detector and injector temperature were 250°C (Ulusoy et al., 2009).

HPLC analysis method

For HPLC analysis, 5 g of the sample was extracted with 300 mL of hexane with a hot extraction system. After the hexane was evaporated, the residue was dissolved in the mobile phase and injected into the system. The system used was a Shimadzu Prominence brand HPLC (Tokyo, Japan) (Ulusoy et al., 2009). It consisted of a CBM-20A controller, an RF-10AXL fluorescence detector (excitation at 295 nm, emission at 330 nm), a CTO-10ASVp column oven, an LC-20AT pump, and a SIL-20ACHT autosampler. The data gathering was done using LC Solution software. Separation was done on a Luna Silica column (250 × 4.6 mm, 5 µm) using n-heptane/THF (95:5, v/v) as the mobile phase at a flow rate of 1.2 mL/min. The injection volume was 20 µL.

Standard disk diffusion method

Antimicrobial activities of seed and leaf extracts were determined using the standard disk diffusion method (Collins et al., 1995, Murray et al., 1995). The densities of microorganisms were prepared using 0.5 ml McFarland standard (108 CFU/mL). 250 µL of bacterial suspension was taken and inoculated onto Müeller-Hinton Agar (Difco). Standard sterile disks (Schleicher & Schuell) with a diameter of 6 mm, each impregnated with 10 µl of extract, were placed on the culture medium. Sterile ampicillin (AM, 10 µg, Bioanalyse) and penicillin (P, 10 U, Bioanalyse) antibiotics were placed on the culture media for comparison. Culture media were incubated at 37 ± 0.1°C for 24 hours. After incubation, the diameters of the inhibition zones formed around the discs were measured in millimeters. All culture media were sterilized at 121°C for 15 minutes. Nutrient Broth was used for bacterial activation, and Müeller-Hinton Agar medium was used to determine antimicrobial activity.

Figure1. Dried leaves and seeds of the Chaste tree *(Vitex agnus-cactus)* plant.

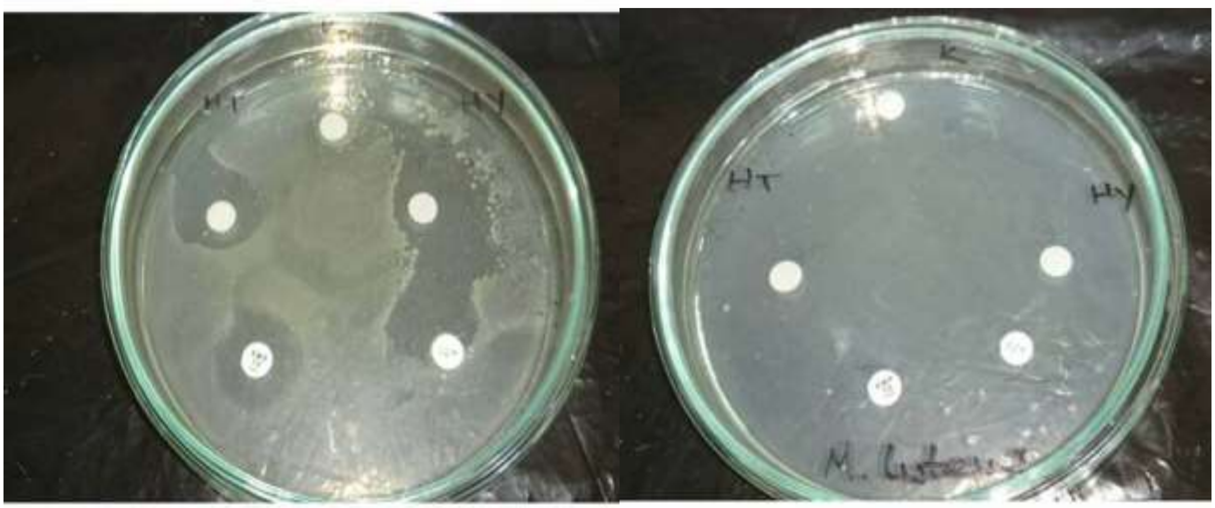

Figure 2. The effect of chaste tree seed and leaf oil on Gr (+) *Staphylococcus aureus* and *Micrococcus luteus.*

RESULTS AND DISCUSSION

Analysis of the chasteberry seed oil revealed that 1,8-cineole was the predominant compound. In addition, α-pinene was present in large amounts. In our study on antibacterial effects, the results of seed and leaf essential oils against Gram-negative *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, as well as Gram-positive *Staphylococcus aureus* ATCC 25923 and *Micrococcus luteus* NCIMB 13267, are presented in Fig. 2.

In another study, the antioxidant activity of the essential oil of *Vitex agnus-castus* L. plant was investigated. In GC and GC-MS analysis, the presence of 27 compounds representing 94.5% of the total oil was reported. The percentages of essential oils were determined as 1,8-cineole (24.98%), sabinene (13.45%), α-pinene (10.60%), α-terpinyl acetate (6.66%) and (Z)-β-farnesan (5.40%) (Sarıkürkçü et al., 2009). The contents of leaf, flower and seed oils of *Vitex agnus-castus* L. collected from the Amazon region were compared. As a result of the study, the most abundant compounds were found to be, in order, 1,8-cineole, β-farnesan, sabinene, α-pinene, α-terpinyl acetate, β-caryophyllene and bicyclogermacrene (Zoghbi et al.1999). The antimicrobial effect of essential oils acquired from the fruits and leaves of *Vitex agnus-castus* L. was investigated and their chemical composition was examined. In the chemical composition of the oil obtained from the unripe fruits of the plant, sabinene and 1,8-cineol compounds were found to be the most abundant, respectively. In the ripe fruits, this order was observed to be 1,8-cineol and sabinene (Yamaner 2024). In the oil obtained from the leaves, the main compound was found to be 1,8-cineol. It has been proven to have a positive effect against bacteria and fungi, and the 1,8-cineol compound has been found to be effective against the *Aspergillus niger* pathogen, which causes problems during the storage of apples (Stojkovic et al., 2011). In commercial products, it is often unclear which extraction method, solvent, or plant part is utilized. Consequently, the limited antibacterial activity observed in commercial preparations of Sambucus nigra may be linked to the product's formulation, the presence of additional ingredients, or variations in the concentration of the active compounds.The findings revealed that only one of the 11 tested products demonstrated activity against Acinetobacter baumannii, Klebsiella quasipneumoniae, *Proteus vulgaris, Bacillus subtilis, Enterococcus faecalis, and Staphylococcus aureus.* Using the microdilution method, it was established that the effective product, SN7, had MIC values of 200 and 250 µL/mL and exhibited a bactericidal effect (Ateş 2025). Our research has shown that the antibacterial activities of leaf and seed essential oils of *V. agnus-castus* L. were evaluated against both Gram-positive and Gram-negative bacteria. However, the inhibition zones (cm) on Gram-negative bacteria were found to be larger. Overall, the leaf oil was found to have a greater antibacterial effect than the seed oil. Notably, the inhibition zone created by the seed oil on *Pseudomonas aeruginosa* bacteria was found to be larger than that created by *Escherichia coli* bacteria. Based on this result, we can say that the antibacterial effect of chaste tree seeds is greater on Gram-negative bacteria.

CONCLUSIONS

The other name of the 1,8 cineol compound is eucalyptol. Thanks to the rich components of the chaste tree plant, in our antibacterial study, it was observed that the leaf and seed oils of *Vitex agnus-castus* L. were effective against Gr (+) and Gr (-) bacteria, and it was determined that the inhibition zones (cm) on Gr (-) bacteria were greater. The antibacterial effect of the leaf oil was found to be greater than that of the seed oil. The inhibition zone created by the seed oil on *Pseudomonas aeruginosa* bacteria was found to be greater than that created by *Escherichia coli* bacteria.

Supplementary materials

The supplementary material / supporting for this article Not applicable.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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