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Türkiye-Erzurum Orijinli *Micromeria fruticosa* Yaprak Etil Asetat Ekstresinin Fitokimyasal Profili

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ÖZET

Bitkiler, eski zamanlardan beri beslenmenin yanı sıra tıbbi ve geleneksel amaçlarla kullanılmaktadır. Bitkilerin yapısında yer alan çeşitli biyoaktif bileşikler ve ikincil metabolitlerden, ilaç ve biyoteknoloji endüstrilerinde çeşitli amaçlarla etkin bir şekilde yararlanılmaktadır. Türkiye bitki çeşitliliği bakımından oldukça zengin bir coğrafyaya sahiptir ve bu nedenle ilaç etken maddesi olarak kullanılabilecek çok sayıda bitki türü mevcuttur. Bu bitkilerden biri olan *Micromeria fruticosa*, *Lamiaceae* ailesine ait çok yıllık aromatik bir çalıdır. Bitki, geleneksel tıpta birçok rahatsızlığın tedavisinde semptomları hafifletmek için yaygın olarak mevcılmaktadır. Biyotik ve abiyotik koşullara göre bitkinin içerdiği bileşenler ve oranları büyük ölçüde değişebilmektedir. Bu nedenle, bu çalışma Türkiye'nin Erzurum ili Tortum ilçesinde yetiştiği bilinen *M. fruticosa* bitkisinin yapraklarından elde edilen etil asetat ekstraktındaki biyoaktif bileşiklerin belirlenmesi amacıyla gerçekleştirilmiştir**.** Analizin gerçekleştirilmesi için ilk adım olarak, *M. fruticosa* yaprak etil asetat (EA-MFL) ekstraksiyonu maserasyon yöntemi kullanılarak elde edildi. *M. fruticosa* bitkisinin kimyasal bileşenlerinin belirlenmesi için Gaz Kromatografisi Kütle Spektroskopisi (GC-MS) yöntemi kullanılmış ve yaprakta 7 bileşik tanımlanmıştır. Major bileşenler heptacosane (%73,939) ve piperitenone (%12,658) olup; tespit edilen diğer bileşenler sırasıyla ethyl iso-allocolate (%5,722), 3-ethyl-5- (2-ethylbutyl) octadecane (%3,861), pulegone (%2,590), toluene (%0,898) ve o-acetylserine (0,332) olarak belirlenmiştir.

Phytochemical Profile of *Micromeria fruticosa* Leaf Ethyl Acetate Extract from Erzurum, Türkiye

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ABSTRACT

The utilization of plants for nutritional, medicinal, and traditional purposes dates back to ancient times. Various bioactive compounds and secondary metabolites found in plants are effectively utilized for various purposes in the pharmaceutical and biotechnology industries. Türkiye's extensive plant biodiversity has led to the identification of numerous plant species as potential active pharmaceutical ingredients. One of these plants, *Micromeria fruticosa*, is a perennial aromatic shrub belonging to the Lamiaceae family. The plant is widely used in traditional medicine to alleviate symptoms associated with the treatment of numerous ailments. The components and proportions contained in the plant can vary greatly depending on biotic and abiotic conditions. Therefore, this study was conducted to determine the bioactive compounds present in the ethyl acetate extract obtained from the leaves of *M. fruticosa*, a plant species known to be native to the Tortum district of Erzurum province, Türkiye. The initial phase of analysis involved the extraction of *M. fruticosa* leaf ethyl acetate (EA-MFL) through the maceration method. The chemical components of *M. fruticosa* were determined by Gas Chromatography Mass Spectrometry (GC-MS). The analysis revealed the presence of seven compounds in the leaf. The major components were identified as heptacosane (73.939%) and piperitenone (12.658%). Other detected components included ethyl iso-allocolate (5.722%), 3-ethyl-5-(2-ethylbutyl) octadecane (3.861%), pulegone (2.590%), toluene (0.898%), and o-acetylserine (0.332%).

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INTRODUCTION

The most important sources of bioactive compounds are plant-based products [1]. In recent years, there has been a growing interest in natural products rich in these compounds especially fenolics and flavonoids due to their antiviral, antioxidant, antibacterial, anti-inflammatory and antitumor potential [2,3]. In this regard, the focus of research has shifted to medicinal and aromatic plants that are notable for their high levels of phenolic and flavonoid compounds [4, 5].

The *Lamiaceae* family, historically of significant interest due to its pleasant aromatic odor, medicinal properties, and consumption as tea, consists of 236 genera and over 7000 species [6]. Like many other *Lamiaceae* species, plants belonging to the *Micromeria* genus are a component of the typical Mediterranean flora [7]. The genus is represented in the Turkish flora by 14 species and 22 taxa, 12 of which are endemic [8].

Micromeria species are widely used as herbal tea and as a substitute for mint in folk medicine. Studies have also reported sedative, analgesic, anesthetic, eye infections, wound healing, antiseptic, abortifacient, antirheumatic, antioxidant, antimicrobial activity, insecticidal effect, myeloperoxidase inhibition, CNS stimulant, antimutagenic, hypertension, anti-biofilm formation, fatigue, acaricidal effect and cold, insecticidal effect [9-18].

M. fruticosa is a widely distributed perennial plant found in rocky areas in the Eastern Anatolia Region of Türkiye [9, 19]. It grows up to 0.2-0.8 m in height and gives off an aromatic mint odor when pressure is applied to it [20]. Its dried leaves are used as flavoring in beverages, foods and especially soups in villages and districts of Erzurum Province, Türkiye [9, 21]. Previous studies on *Micromeria* species and other medicinal plants have shown that the composition of extracts prepared using different solvents may vary due to differences in cultivation, origin, vegetative stage and growing season of the plants [22]. Several attempts have been made to investigate the chemical composition of some *Micromeria* species growing in various regions of Türkiye [23-26]. However, our literature review revealed that no study has yet investigated the chemical content of ethyl acetate extracts of *Micromeria fruticosa* (L.). Druce ssp. *serpyllifolia* (Bieb.) PH Davis. leaves growing in Tortum District of Erzurum Province, Türkiye. The present study aims to investigate the chemical composition of *M. fruticosa* ssp. collected in Tortum district of Erzurum province, Türkiye. The aim of the study was to analyze the chemical composition of leaf ethyl acetate extract of *M. fruticosa* plants.

MATERIALS AND METHODS

Materials

Micromeria fruticosa (L.). Druce ssp. *serpyllifolia* (Bieb.) PH Davis. The plant leaves selected for analysis were obtained from the Tortum district of Erzurum (Türkiye) during the flowering period (2023).

Methods

Preparation of Leaf Ethyl Acetate Extract of M. fruticosa for Analysis

The leaves of *Micromeria fruticosa* were separated and then washed with distilled water. The drying process involved the application of a thin layer of blotting paper, which was used to absorb moisture from the surface of the samples. This procedure was carried out at room temperature, protected from direct sunlight to minimize possible damage to the integrity of the samples [27, 28]. Most of the work was conducted in the Molecular Biology and Genetics and Organic Chemistry Laboratories of the Faculty of Science, Atatürk University.

Ethyl Acetate Extract of Aerial Part of M. fruticosa Plant

The leaves of the *M. fruticosa* plant were subjected to desiccation at ambient temperature, subsequently resulting in their transformation into a pulverised state. A quantity of 10 g of the powdered plant material was taken, 50 ml of ethyl acetate ($C_4H_8O_2$) was added and the mixture left to stir for 72 hours at room temperature (25±3 °C) with a standard heated magnetic stirrer. Following the maceration process, the pulp part (i.e. the plant waste) in the glass bottle was filtered with Whatman No. 1 filter paper [9]. The residual liquid extract was then removed by means of an evaporator device at the boiling point of ethyl acetate, with a rotational speed of 155 rpm. This process was repeated on four occasions for the pulp part [29, 30].

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The chemical constituents of *M. fruticosa* leaf extracts in ethyl acetate were ascertained by means of GC-MS analysis.

GC-MS System and Chromatographic Conditions

GC-MS analysis was performed using an Agilent 7820A with Chemstation software and a 7673 autosampler. The separation process used an HP-5 MS column (0.25 µm) and the temperatures of the inlet and transfer line were set at 250°C and 300°C. The injection parameters were as follows: helium (1 mL/min), 1 µL splitless, ionisation energy of 70 eV [31, 32].

During the procedure, a programmed temperature gradient was applied: initially increased by 50°C for one minute, followed by a ramp of 20°C per minute up to 100°C, then 10°C up to 180°C and finally 5°C per minute for an additional minute. The chromatographic peaks and mass spectra of the extract were identified through comparison with reference standard substances.

Identification of Components

The 2005 version of the National Institute of Standards and Technology (NIST) Library, specifically the Turbomass 5.2 software, analysed the obscure segment's range in comparison to the reference section. The direct Kovats retention index, evaluated with mass spectra data from the MS library, differentiated components. The NIST database, which contains 62,000 records, facilitated insights into the GC-MS mass range. The relative concentration of each component was determined by assessing its peak area compared to the overall detected areas. This analysis identified the test materials, revealing their respective names, molecular weights, and structural compositions.

RESULTS

During the extraction process, the chemical composition of the materials obtained from different parts of the plant (tissues and organs) changes [33]. This discrepancy is attributable to physiological processes such as synthesis, storage and transportation of primary and secondary metabolites in plants [34]. The variation in chemical content is not solely attributable to the plant material employed; the preferred solvent and the method applied during the extraction process can also influence this change. As posited by Alawode *et al.* [35], it is evident that plants contain a variety of bioactive components, which have been demonstrated to influence their biological activities.

The chemical composition of Micromeria fruticosa leaf ethyl acetate (EA-MFL) extraction was determined by gas chromatography-mass spectrometry (GC-MS) analysis. Following GC-MS analysis, 7 compounds were identified in EA-MFL. These compounds are shown in Table 1, Figure 1 and Figure

2.

Table 1
Chemical Composition of Compounds Identified in EA-MFL Extract.

Peak	t_R(min)[a]	% of total	Compound	Molecular Formula
1	3.196	0.332	o-acetylserine	$C_5H_9NO_4$
2	3.587	0.898	Toluene	$C_6H_5CH_3$
3	9.765	2.590	Pulegone	$C_{10}H_{16}O$
4	11.332	12.658	Piperitenone	$C_{10}H_{14}O$
5	32.493	3.861	3-ethyl-5- (2-ethylbutyl) octadecane	$C_{26}H_{54}$
6	33.620	73.939	Heptacosane	$C_{27}H_{56}$
7	35.340	5.722	Ethyl iso-allocolate	$C_{26}H_{44}O_5$

[a]Retention time

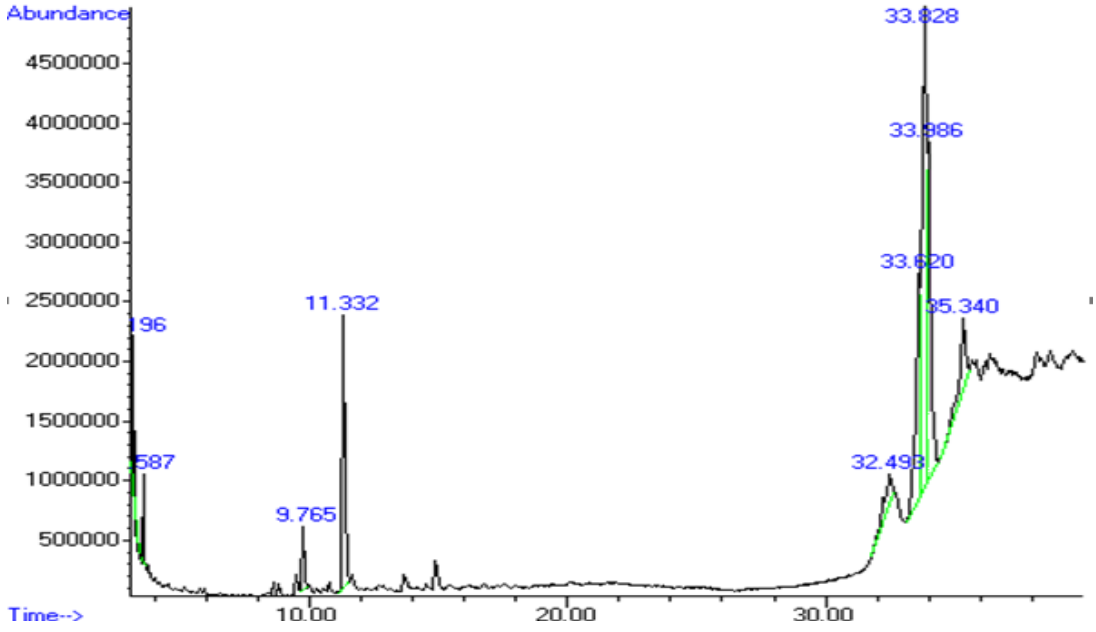

Figure 1
GC-MS Chromatogram of EA-MFL.

During the 40-minute gas chromatography-mass spectrometry (GC-MS) analysis period, the compounds began to appear between 3 and 36 minutes. During this period, heptacosane (73.939%) and piperitenone (12.658%) were the major components; other components were ethyl iso-allocolate (5.722%), 3-ethyl-5-(2-ethylbutyl) octadecane (3.861%), pulegone (2.590%), toluene (0.898%) and o-acetylserine (0.332), respectively. As illustrated in Table 1, a comprehensive account of the bioactive compounds is provided, as determined by GC-MS analysis of *M. fruticosa* leaf ethylacetate extract. Furthermore, the gas chromatography-mass spectrometry (GC-MS) chromatogram of these substances is presented in Figure 1, and their three-dimensional (3D) representation is shown in Figure 2.

DISCUSSION

A review of the extant literature on medicinal plants reveals that the most salient aspect of these plants is the presence of effective, natural, and readily accessible therapeutic agents with minimal or

nonexistent side effects from the compounds in their structure [36].

The findings obtained in this study revealed that the extract obtained from *M. fruticosa* leaves with ethyl acetate solvent had a phytochemical-rich content. The most prevalent components, as determined by GC-MS analysis, were identified as heptacosane (73.939%) and piperitenone (12.658%).

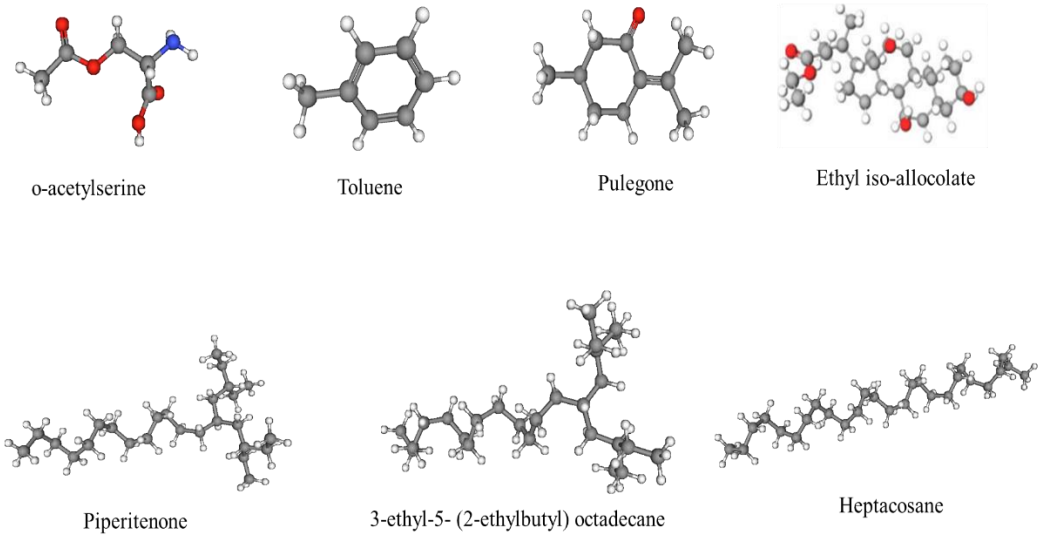

Figure 2
3D Structure Visualization of Bioactive Components of EA-MFL (MolView).

In the literature, some studies on *M. fruticosa* have investigated the composition of essential oils and different results have been obtained. For example, Güllüce *et al.* [9] identified 29 components in the essential oil obtained from *M. fruticosa,* accounting for 93.9% of the total content. The most significant components were piperitone (50.61%) and pulegone (29.19%). However, when compared with the composition of the ethyl acetate extraction obtained in our study, significant differences were found in terms of both the number of chemical components and the ratios of common chemical components. In our study, the main components were heptacosane (73.939%) and piperitenone (12.658%). The amounts of piperitenone (12.658%) and pulegone (2.590%) differed between the two studies. The results differed from those in the literature in many ways. The content and composition of active ingredients in extracts and essential oils may vary depending on the genetic structure of the plants, their developmental periods, the region where they are grown (ontogenetic variability), biotic and abiotic factors, diurnal variability, the method and solvent used [22]. Consequently, the observation that the active ingredients obtained in this study differ from those reported in the extant literature is a common phenomenon in the study of medicinal and aromatic plants. In this context, it is understood that extractions with solvents of medium polarity such as ethyl acetate can yield different bioactive profiles compared to essential oil distillation.

Heptacosane, one of the primary compounds identified in our study, is a straight-chain alkane with 27 carbon atoms and it acts as an oil component and plant metabolite. While it is a component of petroleum products, it is also naturally present in a variety of plants [37]. A multitude of research studies have demonstrated that heptacosane possesses a variety of biological activities, including antioxidant, anti-inflammatory [38], antimicrobial [39, 40], and anticancer properties [41]. In this regard, heptacosane is considered a potential contributor to the therapeutic efficacy of the *M. fruticosa*.

Another important component identified in the *M. fruticosa* leaf ethyl acetate extract in our study is piperitenone, a monoterpene commonly found in various Mentha species and plants belonging to the Lamiaceae family. This chemical has been shown to have biological effects such as antimicrobial [42], antioxidant [43], anti-inflammatory [44], and acetylcholinesterase inhibitor properties [45]. These

properties align with the utilization of *M. fruticosa* in traditional medicine.

Furthermore, the presence of other compounds, though at low concentrations, such as ethyl iso-allocolate, pulegone, and o-acetylserine, detected in the extract, may enhance its biological activity through synergistic interactions. This finding supports the concept of phytocomplex, which posits that complex plant extracts are more effective when used in conjunction with multiple components rather than a single active ingredient.

CONCLUSION

In recent years, a multitude of chronic and infectious diseases have emerged as significant global health concerns, including cancer, diabetes, immune system disorders, and bacterial and fungal infections. The identification of effective, safe, and innovative drug compounds for the treatment of these diseases is a primary objective of contemporary medicine. Extensive scientific research conducted in recent years has revealed the therapeutic potential of compounds obtained from natural sources, particularly. Herbal products have been utilized in folk medicine since ancient times and continue to serve as a valuable source of inspiration in novel drug development processes to the present day. In this context, the ethyl acetate extract of *M. fruticosa* leaves was subjected to GC-MS analysis, which revealed the presence of compounds with pharmacological potential, including heptacosane and piperitenone, as reported in our study. It is hypothesized that the detailed biological activities of these compounds, to be investigated in future studies, will contribute to the development of new generation therapeutic agents.

Ethical Statement
The present study is an original research article designed and produced by the authors.

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Author Contributions
Research Design (CRediT 1) H.U.B. (%100)
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Research- Data Analysis- Validation (CRediT 3-4-6-11) H.U.B (%70) – Ş.B. (%30)
Writing the Article (CRediT 12-13) H.U.B. (%60) – Ş.B. (%30) – A.K.K. (%10)
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Conflict of Interest
The authors declare no conflict of interest for the present study.

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