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Original Article

COMPARATIVE ANALYSIS OF PHYTOCHEMICAL PROPERTIES OF EXTRACTS OBTAINED FROM FLOWERS AND LEAVES OF *ASTRAGALUS ANGUSTIFOLIUS* COLLECTED FROM DIFFERENT LOCATIONS

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ABSTRACT

Plants containing phenolic secondary metabolites such as tannins and flavonoids are of great interest due to their antioxidant and antimicrobial effects on health. Many species belonging to the *Astragalus* genus possess promising bioactive phyto-compounds and are used in folk medicine worldwide. In this study, extracts were obtained using two different solvents (ethanol and methanol) from the flowers and leaves of *Astragalus angustifolius* collected from two different locations in Kahramanmaraş. The antioxidant and antimicrobial activities of these extracts were compared, along with their total phenolic and flavonoid contents. Additionally, the fixed oil content of the extracts was examined using GC-MS analysis, revealing 8 different fatty acid components. The main fatty acid components of the plant extracts were found to be linoleic, oleic, and gamma-linolenic acids in high abundance at both locations. In the methanol extracts of *A. angustifolius*, total phenolic content ranged from 14.82 to 15.47 mg GAE/g dw in leaves and from 4.38 to 5.30 mg GAE/g dw in flowers. Similarly, flavonoid content ranged from 8.14 to 9.26 mg QE/g dw in leaves and from 5.08 to 5.20 mg QE/g dw in flowers. Leaves exhibited higher phenol and flavonoid content and antioxidant activity compared to flowers. Moreover, methanol was observed to be more successful than ethanol in retrieving the bioactive content of the plant material. This conclusion was confirmed by both antioxidant capacity tests such as FRAP and IC50 values, and the difference in phenol and flavonoid levels between leaves and flowers. Antimicrobial activity determination using the well-diffusion method showed that *A. angustifolius* leaf extracts had a broad spectrum of effects on test microorganisms.

KEYWORDS: *Astragalus angustifolius*, antioxidant activity, antimicrobial activity, fatty acids.

1. Introduction

Plants are used for many purposes by different communities around the world. People often prefer herbal remedies over synthetic drugs for ailments such as the common cold, skin diseases, and cancer, believing that these plants strengthen the immune system in combating diseases. Although the recovery of patients treated with medicinal plants may seem like a slow process, they have become more popular due to their limited side effects compared to modern drugs [1]. In our study, some biological activities and fatty acid profiles of *Astragalus angustifolius* samples collected from different regions were determined.

The genus *Astragalus* derives its name from the Greek words "Astron", meaning star, and "Gala", meaning milk. This nomenclature originates from the belief that animals fed with this plant experienced increased milk production [2]. Literature surveys reveal that *Astragalus* species are predominantly utilized in medicine [2]. These plants hold significant positions in traditional medicine due to their potent healing properties, including cancer prevention, anti-ageing effects, autoimmune system strengthening, liver protection, anti-inflammatory, analgesic, diuretic, hypotensive, sedative, and antiviral properties [3,4]. Moreover, *Astragalus* species are known to regulate the

functioning mechanism of the cardiac ventricle and the heart's blood-pumping volume. Especially in Chinese medicine, *Astragalus* species have been widely used for centuries to strengthen the heart [5]. The discovery that the smoke from burning *Astragalus* plants, used as fuel by shepherds during cold winter months, alleviated cold symptoms led to various scientific studies supporting the notion that certain *Astragalus* species enhance antibody production, aiding in the easier and faster recovery from various ailments [2,6]. Additionally, due to the plant's neurotransmitter effect, it has been reported to be used in autism treatment [7]. In this context, determining the biological activity of plants is very important in terms of their usage potential.

This study aims to investigate the bioactive components, antioxidant activity, and antimicrobial activity of extracts obtained from *Astragalus angustifolius* collected from two different sites of natural occurrence in Kahramanmaraş. Additionally, the fatty acid profile of the plant flowers and leaves was analyzed using GC-MS. This study represents the first comprehensive investigation conducted on *Astragalus angustifolius*.

2. Matherials and Methods

2.1. Plant Material

Samples of *Astragalus angustifolius* were collected from two different locations in Kahramanmaraş, Turkey, namely Gündere and Yoğunsöğüt, in June 2020. This perennial shrub, a steppe plant, is distributed in both North and South Anatolia in Turkey and is characteristic of terrestrial Anatolia. The plants were identified according to the Flora of Turkey Volume 3 and specimens with assigned herbarium numbers were deposited in the KSU Herbarium for preservation [8].

2.2. Sample Preparation

Plant samples were separated into flowers and leaves and dried in a shaded, moisture-free environment at room temperature for approximately one week. The dried plants were then ground separately into powder using a Waring blender and stored protected from light and moisture for use in the experiment.

2.3. Extraction Method

Preparation of extracts was carried out by modifying the method of Çolak et al. [9]. Two different solvents, methanol (Sigma-Aldrich, 99.9%), and ethanol (Sigma-Aldrich, 99.9%), were used. After extraction in an ultrasonic water bath, the samples underwent centrifugation. The upper liquid portion was transferred to another container. The solvent was then removed using a vacuum rotary evaporator to obtain dry extracts. The obtained dry extracts were stored at -20 °C.

2.4. Determination of Total Phenolic Content

The total phenolic content of the samples was determined using the Folin-Ciocalteu Reagent (FCR) method modified from the procedure of Blainski et al. [10]. Gallic acid (Sigma) was used as a standard. The absorbance values were read at 750 nm using a spectrophotometer (Perkin-Elmer Lambda EZ 150, USA). The values obtained for prepared solutions were expressed as mg gallic acid equivalent (GAE)/g of dry sample weight using a calibration curve prepared with gallic acid solutions.

2.5. Determination of Total Flavonoid Content

The total flavonoid content in plant extracts was determined spectrophotometrically according to Chang et al. [11]. Quercetin (Sigma) was used for preparing standard solutions at different concentrations (25-200 µg/mL) following the above procedure. Absorbance was measured at 415 nm using a spectrophotometer, and the absorbance values were converted to µg quercetin equivalents/g of dry sample weight.

2.6. Determination of Antioxidant Capacity

DPPH Method

The antioxidant capacity (capacity of reducing free radicals) was determined by modifying the DPPH method described by Brand-Williams et al. [12]. Solutions were prepared from each plant extract at five different concentrations by dilution. The results were presented as the concentration value (IC50) required to reduce 50% of DPPH free radicals. All experiments were performed in triplicate, and ascorbic acid was used as a positive control.

FRAP Method

The FRAP method was performed according to Benzie and Strain [13]. Plant extracts (50 µl) were transferred to 2 ml Eppendorf tubes, and 600 µl of FRAP reagent (Sigma) was added. Absorbance was measured at 593 nm, and the results were expressed as µmol ascorbic acid equivalents/g of dry plant weight using a calibration curve with ascorbic acid (100-1000 µmol/L).

2.7. Determination of Oil Content and Fatty Acid Composition

The analysis of fatty acids in the fixed oil obtained by the Soxhlet method was conducted according to Comlekcioglu [14] using GC-MS. GC-MS analyses were performed using a Schimadzu GC 2025 system with a TRCN-100 (60m x 0.25 mm x 0.20 µm film thickness) SE-54 fused silica capillary column. The electron energy was set at 70 eV, and the injection volume was 1 µl. Injections were performed in split mode (1:50) at a temperature of 240 °C, and the detector temperature was set at 250 °C. Helium was used as the carrier gas with a flow rate of 30 ml/min. The gas flows used were H2=40 ml/min and dry air=400 ml/min.

2.8. Determination of Antimicrobial Activity

The potential antimicrobial activity of *A. angustifolius* plant extracts was determined using the well-diffusion method with eight microorganisms, including six bacteria and two yeast strains, obtained from the culture collection of the KSU Faculty of Science Biotechnology Laboratory. Standard strains of *Escherichia coli* NTCC 13846, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341NA, as well as clinical isolates of *Serratia marcescens*, *Staphylococcus aureus*, *Candida albicans*, and *Saccharomyces cerevisiae* (baker's yeast), were selected as test microorganisms.

Isolates were revived in Luria Bertani (LB) and Sabouraud Dextrose Broth nutrient broths one day before

the experiment, and the turbidity was adjusted to 0.5 McFarland standard ($1.5x10^8$ bacteria and 0.5-3 x 10^4 yeast/mL) with sterile saline solution. From each isolate, 0.1 mL was inoculated onto previously prepared sterile Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates. Plant extracts were dissolved in DMSO (dimethyl sulfoxide) at a concentration of 16 mg/mL. Different concentrations of plant extracts were added to sterile wells prepared aseptically using a 6 mm diameter cork borer and a sterile micropipette. The plates were incubated at 37 °C for bacteria and 25 °C for yeast for one night and two days, respectively. After incubation, the inhibition zones around the wells were measured. DMSO (50 µl) was used as a solvent control. Minimum Inhibitory Concentrations (MIC) for extracts showing antimicrobial activity were determined in microplates containing decreasing concentrations of plant extracts in Mueller Hinton broth and Sabouraud Dextrose broth [15]. The MIC values were noted as the lowest extract concentration that prevented observable growth/turbidity. All antimicrobial assays were performed in triplicate under aseptic conditions, and the mean results were used.

2.9. Statistical Analysis

All statistical analyses were conducted using Python (version 3.11.4). The data was first examined for normality and homogeneity of variances using standard diagnostic procedures. One-way ANOVA was performed using the ordinary least squares (OLS) method. The analysis was conducted using the `statsmodels` library. Additionally, post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to determine which groups differed from each other.

3. Results and Discussions

3.1. Total Phenol and Phenolic Content

Solvent extraction is one of the most commonly used techniques for isolating plant antioxidant compounds. However, the selection of the correct solvent is an important factor for the extraction of bioactive components in plants [15]. In this study, methanol and ethanol were used as extraction solvents. The results indicated that the solvent effect is significant in the extraction of phenolics, with methanol extracts showing higher phenolic content in both leaves and flowers compared to ethanol extracts ($p<0.001$). However, no significant difference was observed in the phenolic content among locations. It was found that the phenolic content of the leaves of *A. angustifolius* is higher than that of its flowers ($p<0.001$). The methanolic extracts of *A. angustifolius* leaves and flowers had average phenolic contents of 15.15 and 4.84 mg GAE/g dw, respectively. Similarly, methanol extraction resulted in higher levels of flavonoid content compared to ethanol extraction ($p<0.001$). There was no significant effect of different locations on the flavonoid content of *A. angustifolius*. However, it was observed that the leaves of *A. angustifolius* have higher flavonoid content compared to its flowers ($p<0.001$). The methanolic extracts of both leaves and flowers of *A. angustifolius* had the highest flavonoid content (average 8.70 and 5.02 µg QE/g dw, respectively). The interaction between plant parts and extraction solvent was found to be significant in terms of phenolic and flavonoid yield results ($p<0.05$). The Pearson correlation coefficient indicated a high positive correlation between phenolics and flavonoid content ($r^2 = 0.93$). The phenol and flavonoid contents of the ethanol and methanol extracts of *A. angustifolius* leaves and flowers are presented in Figure 1.

3.2. Antioxidant Activity

In this study, DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays were used to measure the antioxidant activity of methanol and ethanol extracts obtained from leaves and flowers of *A. angustifolius* collected from two different locations. The results indicate that all tested extracts exhibited high activity at DPPH scavenging and FRAP activity. The difference in DPPH values between flower extracts from different locations was found to be statistically significant ($P<0.01$). However, the difference in DPPH values between leaf extracts from different locations was found to be insignificant. Similarly, the difference in FRAP values between locations was insignificant for both flower and leaf extracts. When examining the effect of different solvents, no significant

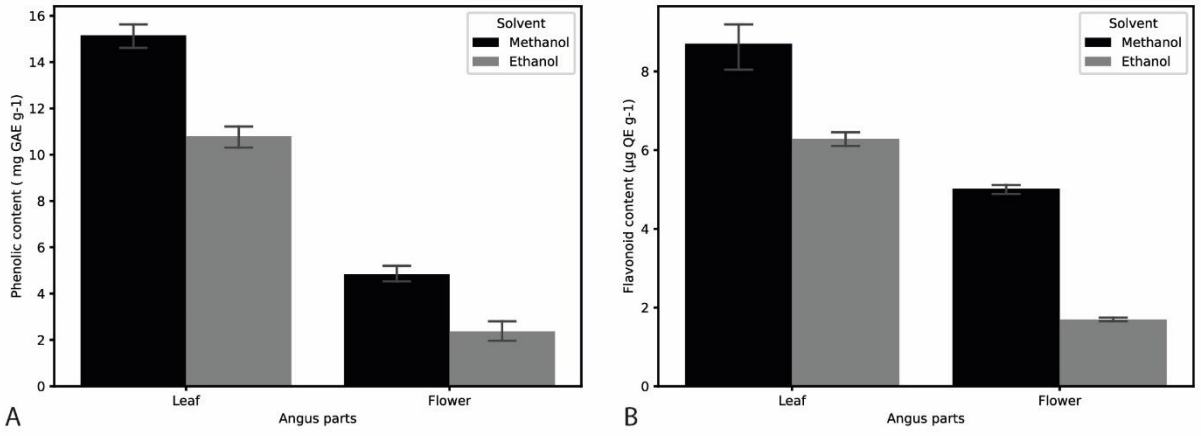

Figure1. The total phenolic (A) and flavonoid (B) content of methanol and ethanol extracts *of A. angustifolius*. Error bars represent the standard error (n = 3).

Table 1. Antioxidant activity values of *A. angustifolius* flower and leaf extracts

		Gündere		Yoğunsöğüt	
		Flower	Leaves	Flower	Leaves
IC50 (DPPH) (µg AAE/mL)	Methanol	7.01± 0.09	3.02± 0.41	4.5± 0.33	2.86± 0.32
	Ethanol	7.33± 0.21	3.16± 0.23	4.85± 0.12	2.97± 0.18
FRAP (mg AAE/ g dw)	Methanol	3.90± 0.09	18.44± 0.58	3.61± 0.03	18.68± 0.15
	Ethanol	2.36± 0.02	12.14± 0.63	2.43± 0.02	13.06± 0.17

difference was found between methanol and ethanol extracts for DPPH. However, methanol extracts showed higher FRAP values compared to ethanol extracts ($P<0.001$). Additionally, the antioxidant activity of leaf extracts was found to be higher than that of flower extracts ($P<0.001$). The IC50 values in methanol extracts of leaves and flowers were found to be 2.86-3.02 µg AAE/ ml and 4.5-7.01 µg AAE /ml, respectively. The FRAP values in methanol extracts of leaves and flowers were found to be 18.44-18.68 µg AAE/ ml and 3.61-3.90 µg AAE /ml, respectively. Positive correlations were found between DPPH and flavonoids ($r2 = 0.81$) and between FRAP and phenolics ($r2 = 0.89$). The DPPH and FRAP values of ethanol and methanol extracts of *A. angustifolius* leaves and flowers are presented in Table 1.

In general, it has been observed that methanolic extracts are more efficient compared to ethanolic extracts. In a previous study [1], methanol-extracted *A. angustifolius* leaf extract exhibited higher phenolic (60.14 mg GAE/g dw) and flavonoid (41.97 mg GAE/g dw) contents compared to those obtained in the present study. Another study [17] reported high flavonoid (26.15 mg QE g^{-}) but low phenolic (11.78 mg GAE/g dw) content in methanolic extracts of *A. angustifolius* leaves. The phenolic (4.59 and 10.39 mg GAE/g dw) and flavonoid (1.63 and 5.88 mg QE /g dw) contents of underground and aboveground parts of a different species, *A. argaeus*, vary depending on the examined organ [18]. This inconsistency indicates that different parts of the plant tend to accumulate varying amounts of phenolic compounds, emphasizing the variability in the distribution of bioactive compounds within the plant.

Regarding antioxidant capacity, our data showed that leaves have higher antioxidant activity compared to flowers. This result was corroborated by both antioxidant capacity tests, FRAP and IC50 values, as well as the difference in phenol and flavonoid quantities between leaves and flowers. Plant-derived phenolic compounds are effective in scavenging free radicals and active oxygen intermediates due to their antioxidant properties. These compounds contribute to the modification of hydroxyl groups through their ability to donate hydrogen by interacting with free radicals. Natural compounds like flavonoids constitute a significant portion of total phenolics in plants and serve as important antioxidant sources due to their properties [1]. Antioxidants play a vital role in protecting cellular structures and biomolecules from oxidative damage caused by reactive oxygen species. Their key role in reducing oxidative stress significantly contributes to the prevention of various diseases and health disorders [17]. Previous studies have reported significantly high antiradical activity levels in methanol extracts obtained from *A. angustifolius* in both DPPH assays and other antioxidant capacity tests [1,17].

3.3. Fatty Acid Composition

In this study, the oil content in the flowers and leaves of the examined species *A. angustifolius* was determined to be 1.62% and 2.35% at the Gündere location, and 1.22% and 1.90% at the Yoğunsöğüt location, respectively. The results of the analysis of the fatty acid composition of the plant's flowers and leaves are presented in Table 2. GC-MS analysis revealed that the plant contains a total of 8 different fatty acids in varying proportions. The main fatty acid component of the flower and leaf extracts collected from the Gündere location was linoleic acid (56.18% and 61.15%, respectively), while oleic and gamma-linolenic acids were the other high-abundance fatty acids. It was determined that the main fatty acid components of the flower and leaf extracts collected from the Yoğunsöğüt location were linoleic and oleic acids. In both locations, the amounts of palmitic acid and oleic acid are high, but these amounts are higher in the Yoğunsöğüt location than in the Gündere location. While the ratios of gamma-linolenic acid are similar, the amount of linoleic acid is significantly higher in the Gündere location compared to the Yoğunsöğüt location. These three fatty acids (oleic, linoleic, and gamma-linolenic acids) are known to be beneficial for health, and their presence as the main fatty acids in the examined organs indicates a positive aspect of the plant's potential. No study has been found in the literature regarding the fatty acid composition of *A. angustifolius* extracts, hence no comparison could be made. Differences in fatty acid ratios observed in various studies and different locations can be attributed to the responses of plants to a combination of geographical or local ecological conditions. Environmental factors such as temperature, soil composition, exposure to sunlight, and altitude can influence the fatty acid composition in plants. These differences highlight the ability of plants to adapt to specific environments and emphasize the complex interaction between genetic and environmental factors, shaping the biochemical composition of plants.

In both locations, polyunsaturated fatty acids (PUFAs) were found to be the highest, while monounsaturated fatty acids (MUFAs) were lower, and saturated fatty acids (SFAs) were the lowest. Linoleic acid and gamma-linolenic acid are polyunsaturated omega-6 fatty acids, essential fatty acids that need to be obtained from the diet [19]. Oleic acid, on the other hand, is an omega-9 fatty acid that can be obtained from the diet or synthesized endogenously in the body. Saturated fats are required for energy, hormone production, cellular membranes, and organs. Palmitic acid, one of the saturated fatty acids found in the diet and synthesized endogenously, plays a significant role in these processes [20].

Table 2. Fatty acid compositions (%) of *A. angustifolius* flowers and leaves

			Gündere		Yoğunsöğüt	
			Flower	**Leaves**	**Flower**	**Leaves**
1	C16:0	Palmitic Acid	9.82±1.15	7.31±0.96	21.5±1.23	22.02±1.43
2	C18:0	Stearic Acid	2.68±0.89	-	2.1±0.78	-
3	C21:0	Behenic Acid	0.90±0.25	0.14±0.12	0.96±0.52	-
4	C16:1	Palmitoleic Acid	-	0.13±0.09	-	-
5	C17:1	Cis-10-Heptadecanoic	0.37±0.23	2.79±0.89	-	-
6	C18:1	Oleic Acid **Ω9**	16.29±2.34	17.88±1.64	26.87±3.63	32.01±2.03
7	C18:2	Linoleic Acid **Ω6**	56.18±2.21	61.15±2.02	35.37±2.16	32.5±2.55
8	C18:3	Gamma-Linolenic Acid **Ω6**	13.74±1.34	10.58±1.16	13.17±0.69	13.46±0.58

Palmitic acid, thought to have negative effects on chronic diseases in adults, is a major component of membrane, secretory, and transport lipids that play important roles in modifying proteins and signaling molecules [21]. Oleic acid is the major MUFA in the human circulatory system. It is a significant component of brain membrane phospholipids and is highly abundant in myelin. Significant reductions in oleic acid have been observed in the brains of patients with major depressive disorders and Alzheimer's disease. Oleic acid is also defined as an antioxidant and anticancer molecule [22]. Polyunsaturated fatty acids (PUFAs) are essential components in the structure and function of cellular membranes and play a key role in various biological processes. PUFAs act as endogenous mediators for cellular signaling and regulate gene expression [23]. Furthermore, the benefits of PUFAs in preventing cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorders, and cancer, as well as reducing diabetes, body fat, and obesity, are increasingly attracting attention [20]. Therefore, a diet rich in foods containing omega-3, omega-6, and omega-9 fatty acids is highly important for health. The fatty acid composition obtained from *A. angustifolius* plant extracts, which have higher levels of mono and polyunsaturated fatty acids than saturated fatty acids, is highly suitable for human nutrition.

3.4. Antimicrobial Activity

In the literature, it has been reported that the chloroform extracts of *Astragalus densifolius* subsp. *amasiensis* and *A. angustifolius* subsp. *angustifolius var. angustifolius* (current name: *Astragalus angustifolius* Lam.), collected from Turkey, are effective against *Escherichia coli, Klebsiella pneumoniae, Yersinia enterecolitica, Salmonella enteritidis, Proteus vulgaris, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus,* and *B. subtilis* [24]. It has also been reported that the aqueous and methanol extracts of *Astragalus pelecinus*, collected from Palestine, are effective against *Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* [1]. Additionally, it has been reported that *A. argaeus,* collected from Turkey, is effective against *Pseudomonas aeruginosa* [18]. In this study, ethanol and methanol extracts obtained from *A. angustifolius* were tested against both clinical and standard strains. All extracts exhibited varying degrees of inhibition on the test microorganisms, and the results are summarized in Table 3. Tests conducted with other *Astragalus* species found in Turkey, including *A. angustifolius*, reported similar antimicrobial effects against Gram-negative and Gram-positive bacteria [24]. The effects of *A. angustifolius* collected from different countries on pathogenic and standard strains also showed similarities [1].

In this study, extracts from flowers and leaves of *A. angustifolius* collected from the Gündere location were able to generate inhibition on the test microorganisms. However, extracts obtained from the Yoğunsöğüt location showed inhibition only against the *E. coli* NTCC 13846, excluding other microorganisms. It is known that many *Astragalus* species are less effective against Gram-positive bacteria compared to Gram-negative bacteria, as mentioned in the literature [1]. The widest inhibition zone was observed for the ethanol extract of flowers from the Yoğunsöğüt location (16±2.78 mm). However, according to the results in Table 3, the antimicrobial activity of the plant collected from the Gündere location had a broader spectrum among the test microorganisms. As a generalization for the extraction solvent of antimicrobial bioactive substances, methanol was observed to be a more effective solvent compared to ethanol. Similarly, Jaradat et al. [1] reported that methanol extractions were more effective than aqueous extractions. Nonetheless, flower extracts of both regions created wider inhibition zones on the test microorganisms compared to leaf extracts. Particularly, while leaf extracts did not exhibit inhibition against the clinical isolate *Candida albicans*, flower extracts could produce some level of inhibition in all solvents. Jaradat et al. [1], unlike the findings of this study, were unable to detect antifungal activity with methanol extracts but could determine some antifungal activity with aqueous *A. angustifolius* extracts. In terms of minimum inhibitory concentration (MIC) values, the lowest value, generally 16 mg/mL, was obtained mostly in leaf extracts against *S. marcescens* and *S. aureus* ATCC 25923 strains.

Overall, the inhibition effects of plant extracts are attributed to compounds such as phenolics, flavonoids, terpenoids, alkaloids, saponins, and glycosides. In this study, the higher amounts of phenolic compounds and flavonoids in the leaves of plants collected from both regions were associated with the broad spectrum of antimicrobial activity observed in leaf-derived extracts, with the exception of ethanol extracts of Gundere plant.

Table 3. Inhibition zones (mm) and Minimum Inhibitory Concentration (MIC) values of methanol and ethanol extracts obtained from *A. angustifolius*.

	Gündere				**Yoğunsöğüt**				**Antibiotic**	
	MeOH/**MIC**		EtOH/**MIC**		MeOH/**MIC**		EtOH/**MIC**		Control	
	Inhibition Zone (mm)/ MIC (mg/mL)		Inhibition Zone (mm)/ MIC (mg/mL)		Inhibition Zone (mm)/ MIC (mg/mL)		Inhibition Zone (mm)/ MIC (mg/mL)			
Organism	Flower	Leaves	Flower	Leaves	Flower	Leaves	Flower	Leaves	AMP	NYS
E. coli NTCC 13846	-	10±0,91/**32**	-	-	-	-	-	-	-	NT
B. subtilis ATCC 6633	-	11±0,12/**32**	11±1,22/**32**	-	-	10±0,78/**32**	-	9±0,69/**32**	10	NT
*S. marcescens**	-	9±0,49/**32**	11±0,63/**32**	11±0,48/**32**	-	8±0,87/**32**	-	8±0,74/**16**	13	NT
*S. aureus**	10±0,16/**32**	10±0,25/**32**	14±0,96/**32**	11±2,02/**32**	11±2,23/**32**	10±1,87/**32**	12±2,42/**32**	9±2,63/**32**	8	NT
S. aureus ATCC 25923	-	8±0,86/**16**	9±0,28/**32**	8±1,11/**16**	10±1,16/**16**	8±0,52/**16**	8±0,98/**32**	8±0,90/**16**	15	NT
S. lutea ATCC 9341NA	14±2,86/**32**	10±2,21/**32**	14±1,77/**32**	10±2,34/**32**	15±2,94/**32**	9±0,58/**32**	16±2,78/**32**	7±1,52/**32**	33	NT
*C. albicans**	8±0,92/**32**	-	7±0,76/**32**	-	8±0,34/**32**	-	7±0,82/**32**	-	NT	18
S. cerevisiae	-	7±0,52/**32**	9±1,66/**32**	7±0,36/**16**	10±1,69/**32**	7±0,88/**32**	9±0,40/**32**	7±0,59/**16**	NT	18

MeOH: Methanol, **EtOH**: Ethanol, **MIK**: Minimum Inhibition Concentration, **AMP:** Ampicillin (10 µg), **Nys:** Nystatin (100U), **NT:** Not Tested, ***:** Clinical isolate; **(-):** No result detected.

4. Conclusions

This study highlights the chemical composition, antioxidant activity, and antimicrobial activity of the aerial parts (flowers and leaves) of naturally occurring *A. angustifolius* in our region. The results indicate that the plant is rich in phenolic and flavonoid compounds and possesses antioxidant and antimicrobial properties. It has been shown that the contents vary somewhat depending on the location and that methanol is more successful than ethanol in obtaining the chemical content of the extracts. With its high omega-6 and omega-9 content, *A. angustifolius* oils can be evaluated as a valuable raw material in various fields such as pharmaceuticals, cosmetics, perfumes, food, and the pharmaceutical industry. Further studies on the isolation and characterization of bioactive compounds are believed to be beneficial for better and particularly directed applications. Some of the bacteria used in this study are opportunistic pathogens that can cause serious diseases in the community. *Candida albicans*, in particular, is a pathogenic fungus that can be deadly in patients with immunodeficiency. The findings obtained in this study have shown that *A. angustifolius* is a potential source of antimicrobial agents, and plant bioactive compounds can be used in various fields for the control of microorganisms. Further studies should be conducted on the isolation and characterization of compounds from the extracts, and the mechanisms of action of these compounds should be clarified. Toxicological studies should be conducted to assess the safety of extracts and isolated compounds. Animal experiments and clinical trials are recommended to confirm their potential uses in various pharmaceutical applications.

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References

1. Jaradat, N.A.; Zaid, A.N.; Abuzant, A.; Khalaf, S.; Abu-Hassan, N. Phytochemical and biological properties of four Astragalus species commonly used in traditional Palestinian medicine. *Eur. J. Integr. Med.* **2017**, *9*, 1-8. https://doi.org/10.1016/j.eujim.2017.01.008
2. Colak, S. *Determination of Photochemical Properties of Some Naturally Growing Astragalus Species in Kahramanmaraş*. Master's thesis. Kahramanmaras Sutcu Imam University, 2022.
3. Denizli, N. Isolation and Characterization of Secondary Metabolites from *Astragalus plumosus* var. Krugıanus. Master's thesis. Ege University, 2011.
4. Turker, A.U.; Yıldırım, A.B. Evaluation of antibacterial and antitumor activities of some Turkish endemic plants. *Trop. J. Pharm. Res.* **2013,** *12(6)*, 1003-1010. DOI: 10.4314/tjpr.v12i6.20
5. Liu, P., Zhao, H. & Luo, Y. Anti-aging implications of

Astragalus membranaceus (Huangqi): a well-known Chinese tonic. *Aging Dis.* **2017**, *8(6)*, 868. doi: 10.14336/AD.2017.0816

6. Rios, J.L.; Waterman, P.G. A review of the pharmacology and toxicology of Astragalus. *Phytother. Res.* **1997**, *11(6)*, 411-418. https://doi.org/10.1002/(SICI)1099-1573(199709)11:6<411::AID-PTR132>3.0.CO;2-6

7. Sevindik, M; Mohammed, F. S.; Uysal, I. Autism: plants with neuro-psychopharmacotherapeutic potential. *Prospects Pharm. Sci.* **2023**, *21(3)*, 38-48. https://doi.org/10.56782/pps.143

8. Davis, P.H. (ed*). Flora of Turkey and the East Aegean Islands*, Edinburgh Univ. Press, England, 1970; Volume 3, pp. 247.

9. Çolak, S.; Dağlı, F.; Çömlekcioğlu, N.; Kocabaş, Y. Z.; Aygan, A. Antimicrobial activity and some phytochemical properties of extracts from *Achillea aleppica* subsp. aleppica. *J. Food* **2020**, *45(5)*, 929-941. http://dx.doi.org/10.15237/gida.GD20048

10. Blainski, A., Lopes, G.C. & De Mello, J.C.P. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules* **2013**, *18*, 6852-6865. https://doi.org/10.3390/molecules18066852

11. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*. **2002**, *10*, 178-182. https://doi.org/10.38212/2224-6614.2748

12. Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT* **1995**, *28(1)*, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

13. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239(1)*, 70-76. https://doi.org/10.1006/abio.1996.0292

14. Comlekcioglu, N. Bioactive Compounds and Antioxidant Activity in Leaves of Endemic and Native Isatis spp in Turkey. *Braz. Arch. Biol. Techn.* **2019**, *62*, 1-13. https://doi.org/10.1590/1678-4324-2019180330

15. Collins, C.H.; Grange, J.M. *Collins and Lyne's Microbiological Methods*. Sixth Edition, Butterworths Co. Ltd. London, 1989.

16. Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K.M.; Latha, L.Y. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr. J. Tradit. Complement. Altern. Med.* **2011**, *8(1)*, 1-10. doi: 10.4314/ajtcam.v8i1.60483

17. Zengin, G., Yagi, S., Sinan, K. I., Yildiztugay, E., Jekő, J., Cziák, Z., Aktumsek, A. Screening for Chemical Components, Antioxidant, and Anti-enzymatic Activities of Three Astragalus Species from Turkey. *J. Biol. Regul. Homeost. Agents* **2024**, ***38(2)***, 973–987. DOI: 10.23812/j.biol.regul.homeost.agents.20243802.78

18. Albayrak, S.; Kaya, O. Antioxidant, Antimicrobial and Cytotoxic Activities of Endemic *Astragalus argaeus* Boiss. from Turkey. *Hacettepe J. Biol. Chem.* **2019**, *47(1)*, 87-97. DOI: 10.15671/HJBC.2019.278

19. Whitney, E.; Rolfes, S.R. *Understanding Nutrition* (11th ed.). Wadsworth Publishing, California, USA. 2007, p. 154.

20. Comlekcioglu, N.; Kutlu, M. Fatty acids, bioactive substances, antioxidant and antimicrobial activity of Ankyropetalum spp., a novel source of nervonic acid. *Grasas Aceites* **2021**, *72(1)*, e399-e399. DOI: https://doi.org/10.3989/gya.0105201.

21. Innis, S.M. Palmitic acid in early human development. *Crit. Rev. Food Sci. Nutr.* **2016**, *56(12)*, 1952-1959. https://doi.org/10.1080/10408398.2015.1018045

22. Santa-María, C.; López-Enríquez, S.; Montserrat-de la Paz, S.; Geniz, I.; Reyes-Quiroz, M.E.; Moreno, M.; Palomares, F.; Sobrino, F.; Alba, G. Update on anti-inflammatory molecular mechanisms induced by oleic acid. *Nutrients* **2023**, *15(1)*, Art. No.:224. https://doi.org/10.3390/nu15010224

23. Mercola, J.; D'Adamo, C.R. Linoleic acid: a narrative review of the effects of increased intake in the standard American diet and associations with chronic disease. *Nutrients*, **2023**, *15(14)*, Art. No.: 3129. https://doi.org/10.3390/nu15143129

24. Dogan N.M.; Cansaran A.; Acar G.; Öztekin M. Antimicrobial activity of extracts of some plants from Amasya (Turkey). *Adv. Biores.*, **2010**, *1(1)*, 87-91.