



Phytochemical Analysis of *Minuartia hirsuta* subsp. *asiatica* and Evaluation of their Antioxidant and Antimicrobial Potentials

Rabia Nur Un¹

Received: 24 December 2025 / Accepted: 8 June 2026
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Abstract

Background Medicinal plants have been used for centuries to treat various diseases. They are rich sources of bioactive compounds with notable antioxidant and antimicrobial properties, making their chemical and biological evaluation highly important.

Objective This study aimed to investigate the phytochemical contents, as well as antioxidant and antimicrobial activities of extracts obtained from *Minuartia hirsuta* subsp. *asiatica*.

Methods Compounds were isolated with various chromatographic techniques. Phenolic profiles and fatty acid compositions were determined by LC-MS/MS and GC-FID, respectively. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified using the Folin-Ciocalteu reagent and the aluminum chloride colorimetric method. Antioxidant and antimicrobial activities were assessed using the DPPH and ABTS radical scavenging assay and the disc diffusion method.

Results Seven compounds were elucidated using NMR techniques. LC-MS/MS analysis identified 28 phenolic compounds in dichloromethane (DCM) and methanol (MeOH) extracts, while GC-FID analysis revealed the fatty acid compositions of the hexane extract. The MeOH and DCM extracts exhibited TPC values of 73.32 and 67.20 mg GAE/g and TFC values of 66.91 and 44.69 mg QE/g, respectively. Their radical scavenging activities were 72.83% and 68.72% relative to ascorbic acid, 75.46% and 63.22% relative to trolox. However, none of the extracts demonstrated significant antibacterial activity.

Conclusion This study provides valuable insights into the phytochemical diversity and biological properties of *M. hirsuta* subsp. *asiatica*. The extracts exhibited strong antioxidant activity and may serve as promising sources of natural antioxidants. These findings contribute to the identification of potential therapeutic agents for human health and may guide future studies toward the sustainable utilization of plant resources.

Keywords *Minuartia hirsuta* subsp. *asiatica* · Phytochemicals · Antioxidant activity · Antibacterial

Introduction

The therapeutic properties of medicinal plants have been recognized for centuries and they have been widely used in the treatment of various diseases since ancient times [1]. In recent years, advances in analytical techniques have enabled significant progress in the identification of secondary metabolites responsible for these therapeutic effects [2]. Natural compounds derived from plants have been and will continue to be an important source of pharmaceutical

agents [3, 4]. Currently, there is a growing demand for natural alternatives, such as plant-based products, rather than synthetic compounds. This demand arises from the fact that plants contain a wide range of phytochemicals with significant biological activities, including phenols, flavonoids, alkaloids, glycosides, lignins and tannins [5, 6]. Among these secondary metabolites, phenolic compounds are the most abundant, accounting for approximately 45% of plant secondary metabolites. They are followed by terpenoids and steroids (27%), alkaloids (18%), and other compounds (10%) [7].

Species belonging to the Caryophyllaceae family are widely used in traditional medicine and various industrial applications [8]. Their biological activities include anti-inflammatory, anticancer, antiviral, antifungal, antioxidant, and antibacterial effects, which underpin their traditional

✉ Rabia Nur Un
rabia.nur.un@ege.edu.tr

¹ Department of Chemistry, Faculty of Science, Ege University, Izmir 35040, Türkiye

medicinal use. Ethnopharmacological studies have demonstrated that Caryophyllaceae species possess a broad spectrum of bioactivities [9, 10]. Although many Caryophyllaceae species are well known for their established roles in traditional medicine, documented traditional uses of *Minuartia* genus appear to be scarce [11, 12]. To the best of current knowledge, no traditional medicinal applications have been reported for *M. hirsuta* subsp. *asiatica* in ethnobotanical references. The currently limited ethnopharmacological data available for this species suggest a gap in the existing literature and highlight the need for comprehensive phytochemical and biological investigations to better understand its potential medicinal properties.

The Caryophyllaceae is one of the largest families, comprising approximately 90 genera and 2,400 species, predominantly distributed in the Northern Hemisphere. In Türkiye, approximately 550 species have been recorded, of which nearly 250 are endemic, representing about 6% of the national flora [13, 14]. *Minuartia* is an important genus within the Caryophyllaceae family and, in terms of species richness, it is the third-largest genus in Türkiye, after *Silene* L. and *Dianthus* L. [15]. The genus comprises approximately 54 species, most of which are distributed in Mediterranean Europe and extend eastward into south-central Asia. In the flora of Türkiye, *Minuartia* is represented by 39 species [16]. *M. hirsuta* subsp. *asiatica* is one of these species. It is a perennial, densely hirsute herbaceous taxon of the Caryophyllaceae, typically occurring in rocky and mountainous habitats and characterized by narrow linear leaves and small, white, pentamerous flowers [17, 18]. Phytochemical screening studies have revealed that *Minuartia* species are rich in phenolic and flavonoid compounds with potential biological activities [19, 20]. The diverse biological activities observed in plant extracts are closely associated with their rich phytochemical composition [21]. Therefore, establishing the relationship between phytochemical profiles and biological activity is essential for understanding the therapeutic potential of plant species [22].

This study aims to elucidate the phytochemical composition and biological activities of hexane (Hex), dichloromethane (DCM), and methanol (MeOH) extracts of *M. hirsuta* subsp. *asiatica*. Although members of the Caryophyllaceae family are recognized as important sources of bioactive secondary metabolites, this species remains chemically and pharmacologically unexplored. To the best of our knowledge, no previous study has investigated its secondary metabolite composition or evaluated its antioxidant and antibacterial activities. This absence of data underscores a significant knowledge gap for the genus. Therefore, this study represents the first comprehensive phytochemical and biological investigation of *M. hirsuta* subsp. *asiatica*. A systematic strategy was employed using solvents of

increasing polarity (Hex, DCM, and MeOH) in combination with advanced analytical techniques to achieve broad metabolite characterization. Compounds were isolated and structurally elucidated from the MeOH extract, providing the first chemical data for this species. Furthermore, phenolic profiling of the DCM and MeOH extracts was performed using LC-MS/MS, while the fatty acid composition of the Hex extract was determined by GC-FID, enabling detailed characterization of both polar and non-polar constituents. By combining compound isolation, chemical profiling, and biological activity evaluation, this study goes beyond preliminary screening and establishes a structured chemical-biological framework for *M. hirsuta* subsp. *asiatica*.

Materials and Methods

General Experimental Procedure

Phytochemical analyses were performed using an Agilent 1260 Infinity liquid chromatography system coupled with a 6420 Triple Quadrupole mass spectrometer (LC-MS/MS). Fatty acid compositions were determined using an Agilent 6890 Gas Chromatography-Flame Ionization Detector (GC-FID). Spectroscopic measurements were carried out using a Thermo Scientific Evolution 201 UV-Visible and an Agilent Cary 60 Spectrophotometer. Nuclear Magnetic Resonance (NMR) spectra were recorded on an Agilent 600 MHz Premium Compact NMR spectrometer.

All chemicals, solvents and materials required for LC-MS/MS, GC-FID, antioxidant and antimicrobial analyses were purchased from Sigma-Aldrich and Merck. Sephadex LH-20 (25–100 μ m, Sigma-Aldrich), Lichroprep RP-18 (25–40 μ m, Merck) and Silica gel (60 Å, 0.063–0.200 mm, Merck) were used as packing materials for column chromatography.

Plant Material

Minuartia hirsuta subsp. *asiatica* (whole plant) was collected from Bozdag/Odemis/Izmir, Türkiye (GPS coordinates 38°33'26.38"N; 28°11'03.85"E). A voucher specimen has been deposited in the Herbarium of Ege University (EGE-HERB 43177).

Extraction and Isolation

The powdered and air-dried *M. hirsuta* subsp. *asiatica* plant material (410.0 g) was extracted with Hex (1 $\times$ 2.5 L), DCM (2 $\times$ 2.5 L) and MeOH (3 $\times$ 2.5 L). After filtration and evaporation, crude extracts of Hex (1.7 g), DCM (2.5 g), and MeOH (27.0 g) were obtained. The MeOH extract (27.0 g)

was dissolved in water (H₂O-100.0 mL) and partitioned with *n*-butanol (*n*-BuOH). The *n*-BuOH phase (12.7 g) was fractionated by vacuum liquid chromatography (VLC), on a RP-18 (135.0 g) eluting with H₂O: MeOH (10:0–0:10) to afford five fractions (Fr. 1–5).

Fraction 2 (575.0 mg) was applied to a silica gel column (65.0 g), eluting with DCM: MeOH: H₂O (80:20:2; 61:32:7) to yield seven subfractions (Fr. 2.1–2.7). Subfractions 2.2 (127.0 mg), 2.5 (158.0 mg) and 2.6 (97.0 mg) were further purified on a RP-18 column (7 g) using H₂O: MeOH (4:6, 2:8 and 6:4, respectively) to give compounds 29 (3.4 mg), 30 (2.6 mg), and 31 (7.4 mg). Fraction 3 (65.0 mg) yielded three subfractions (Fr. 3.1–3.3) by a silica gel column (25.0 g), with DCM: MeOH: H₂O (90:10:1; 80:20:2). After purification of subfraction 3.2 by RP-18 (H₂O–MeOH, 40:60) to afford compound 32 (8.6 mg). Fraction 4 (372.0 mg) was fractionated on a silica gel column (125 g), with DCM: MeOH: H₂O (90:10:1; 70:30:3), which provided five subfractions (Fr. 4.1–4.5); further separation of subfraction 4.4 obtained compounds 33 (3.7 mg) and 34 (5.2 mg). Fraction 5 (180.0 mg) was subjected to silica gel column chromatography (40.0 g) with DCM–MeOH–H₂O (90:10:1 then 70:30:3) to yield three subfractions (Fr. 5.1–5.3), and subsequent RP-18 chromatography of subfraction 5.3 with H₂O–MeOH (6:4) afforded compound 35 (8.1 mg).

Fatty Acid Profiling by GC – FID Analysis

Fatty acid analysis was performed using an Agilent 6890 gas chromatography system equipped with a flame ionization detector (GC-FID) and a Supelco 2560 capillary column (100 m × 0.25 mm i.d., 0.20 µm film thickness). The injector and detector temperatures were set at 260 °C and 250 °C, respectively. The oven temperature program was as follows: initially held at 140 °C for 1 min, then increased to 240 °C and maintained for 5 min. Fatty acids were identified by comparing their retention times with those of a 37-component fatty acid methyl ester (FAME) standard mixture [23].

Phenolic Compounds Analysis via LC-MS/MS

Quantitative analysis of phenolic compounds was carried out using an Agilent Technologies 1260 Infinity liquid chromatography system coupled with a 6420 Triple Quadrupole mass spectrometer (LC-MS/MS) [24]. Electrospray ionization (ESI) was operated in both positive and negative ion modes under multiple reaction monitoring (MRM) conditions. The mobile phase consisted of solvent A (0.1% v/v formic acid in water) and solvent B (methanol). Chromatographic separation and gradient elution were performed according to a previously reported method [25].

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Determination

The TPC was determined using the Folin-Ciocalteu method [26]. A standard calibration curve was prepared using Gallic acid (GA) at concentrations of 1, 20, 40, 60, and 80 ppm in MeOH from a GA stock solution. GA solution (1 mL) and plant extracts (MeOH, DCM, and Hex; 1 mL each) were mixed with the Folin-Ciocalteu reagent. After 6 min., 4 mL of 10% sodium carbonate (Na₂CO₃) solution was added. Following incubation for 30 min at 25 °C, the absorbance was measured at 765 nm. The TPC values of the Hex, DCM, and MeOH extracts were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) [27]. All analyses were performed in triplicate. The TFC was determined using the aluminum chloride (AlCl₃) colorimetric assay [28]. *M. hirsuta* subsp. *asiatica* extracts (1 mL) and Quercetin standard solutions (QE; 1, 20, 40, 60, and 80 ppm) were transferred into test tubes. 4 mL H₂O and 0.3 mL of 5% sodium nitrite (NaNO₂) solution were added to each tube and mixed thoroughly. After 5 min, 0.3 mL of 10% AlCl₃ and 2 mL of 1 M sodium hydroxide (NaOH) were added. The final volume was adjusted to 10 mL with H₂O. The absorbance was measured at 510 nm. The TFC values of the Hex, DCM, and MeOH extracts were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g) [29]. All analyses were performed in triplicate.

Determination of Antioxidant Activity

DPPH and ABTS free radicals have been widely used to evaluate the radical scavenging ability of antioxidants. Therefore, the radical scavenging potential of the extracts was examined using two different test systems, namely DPPH and ABTS [30]. The radical-scavenging activity of the *M. hirsuta* subsp. *asiatica* extracts were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [31] and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (ABTS) [32]. To measure the scavenging ability of DPPH in the samples, DPPH solution and the extracts were mixed, shaken, and allowed to stand for 30 min. The absorbance of the mixture was measured at 517 nm using a spectrophotometer. Ascorbic acid (AA) was used as a positive control. To measure the scavenging ability of ABTS in the samples, ABTS solution and the extracts were mixed and allowed to react in the dark for 30 min. The ABTS⁺ scavenging activity was determined by measuring the decrease in absorbance at 734 nm. A standard curve was prepared using Trolox. All analyses were performed in triplicate.

Determination of Antimicrobial Activity

The antibacterial activity of the *M. hirsuta* subsp. *asiatica* extracts were evaluated against the gram-negative *Escherichia coli* ATCC 25,922 and the gram-positive *Staphylococcus aureus* ATCC 25,923 using the disc diffusion method. Bacterial strains were cultured in Muller-Hinton Broth (MHB) at 37 °C for 16–24 h until a turbidity equivalent to 0.5 McFarland standard was achieved. The plant extracts were diluted to 50% with MHB, and 20 µL of a microorganism culture solution was added to the microplate. After application of the extract onto the agar plates, the plates were allowed to stand for 15 min to facilitate diffusion and were then incubated at 37 °C for 24 h. Ofloxacin (OFX, 5.0 µg/disc) was used as the positive control. All analyses were performed in triplicate [33–35].

Statistical Analysis

All experiments were performed in triplicate ($n=3$), and the results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) to evaluate differences among the extracts. When significant differences were observed, Tukey's honestly significant difference (HSD) post hoc test was applied for multiple comparisons. Differences were considered statistically significant at $p<0.05$.

Results and Discussion

Fatty Acid Content by GC – FID

A total of 24 fatty acids were identified in the hexane extract by GC-FID analysis (Table 1, Fig. S1). The fatty acid profile indicated that *M. hirsuta* subsp. *asiatica* possesses a diverse and abundant fatty acid composition. The total saturated fatty acid content was 62.29%, whereas unsaturated fatty acids accounted for 36.14% of the total fatty acid content. The main saturated and unsaturated fatty acids in the hexane extract were heneicosanoic acid (24.17%) and cis-11,14-eicosadienoic acid (10.69%), respectively. These compounds are lipid biomolecules widely present in living organisms and are commonly utilized in food and cosmetic products [36]. Previous studies on species belonging to the Caryophyllaceae family have generally reported linoleic, linolenic, palmitic, and (Z, Z)-9,12-octadecadienoic acids as the predominant fatty acids [10, 37, 38]. In contrast, in this study, heneicosanoic acid was identified as the major saturated fatty acid, while cis-11,14-eicosadienoic acid was the major unsaturated fatty acid. This differs from the commonly reported profiles in related species. Furthermore,

Table 1 Fatty acid composition of *M. hirsuta* subsp. *asiatica* hexane extract

	Retention time	Compound name	Abundance
1	5.908	capric a. (c10:0)	0,18
2	7.707	lauric a. (c12:0)	0,64
3	9.435	myristic a. (c14:0)	1,13
4	10.294	myristoleic a. (c14:1)	0,58
5	10.751	pentadecanoic a. (c15:0)	0,59
6	11.766	palmitic a. (c16:0)	9,47
7	12.987	palmitoleic a. (c16:1)	1,13
8	13.810	heptadecanoic a. (c17:0)	0,70
9	15.337	stearic a. (c18:0)	2,43
10	16.708	oleic a. (c18:1 cis)	5,97
11	18.605	linoleic a. (c18:2 cis)	6,22
12	20.059	arachidic a. (c20:0)	2,54
13	20.707	linolenic a. (c18:3n3)	0,91
14	21.983	cis-11-eicosenoic a. (c20:1)	2,81
15	22.938	heneicosanoic a. (c21:0)	24,17
16	22.967	cis-11,14- eicosadienoic (c20:2)	10,69
17	23.894	behenic a. (c22:0)	3,12
18	24.356	cis-8,11,14-eicosatrienoic a. (c20:3)	1,65
19	25.553	erucic a. (c22:1)	1,92
20	26.492	tricosanoic a. (c23:0)	17,32
21	27.509	lignoceric a. (c24:0)	1,57
22	28.288	cis-5,8,11,14,17-eicosapentaenoic a. (c20:5)	0,31
23	30.411	nervonic a. (c24:1)	1,26
24	31.195	cis-4,7,10,13,16,19-docosaheptaenoic a. (c22:6)	2,69

linolenic acid, which has been reported at relatively high levels (e.g., 15.6%) in *M. laricifolia* [39], was not a major component in the present study. These differences indicate a distinct fatty acid composition for *M. hirsuta* subsp. *asiatica*, suggesting significant interspecific variation within the Caryophyllaceae family. Such variation may be attributed to genetic factors, environmental conditions, or geographical origin, all of which are known to influence lipid composition in plants [40, 41].

Phenolic Compounds by LC-MS/MS

The phenolic profiling of the DCM and MeOH extracts were performed by LC-MS/MS using 31 reference standards. As a result, 28 phenolic compounds were detected in both extracts based on the chromatograms (Fig. 1), and their concentrations were quantified in mg/kg of extract, as shown in Table 2; Fig. 2.

Based on the results, although the DCM and MeOH extracts exhibit broadly similar overall profiles, they differ markedly in the specific compounds and their quantities. The analyses confirmed that both extracts contained twelve phenolic acids, seven flavonoid aglycones, four flavonoid glycosides, one flavanone glycoside, three organic

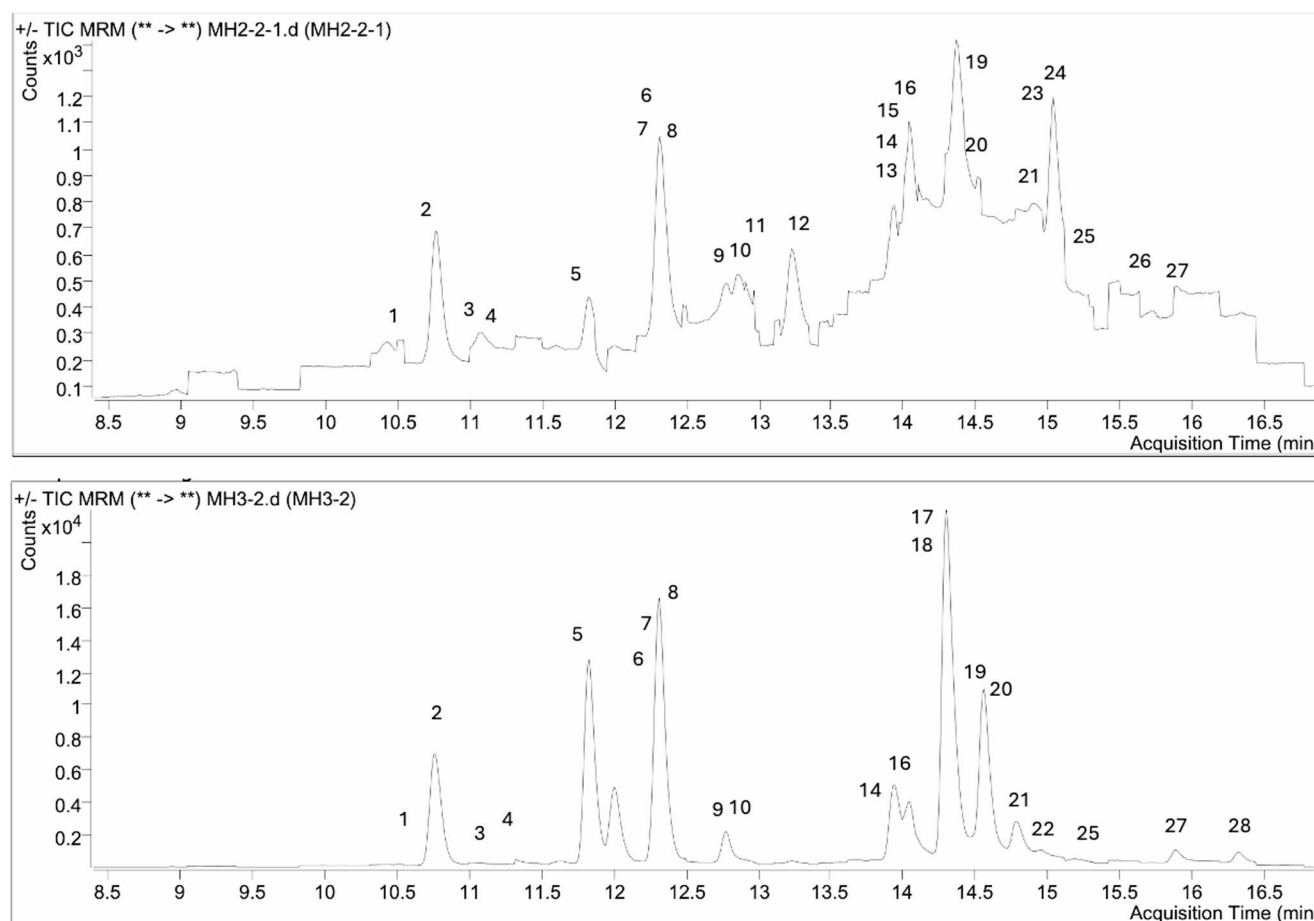


Fig. 1 LC-MS/MS profile of dichloromethane (a) and methanol (b) extracts from *M. hirsuta* subsp. *asiatica*

acids, two caffeic acid esters and one phenolic aldehyde. The DCM extract exhibited the highest contents of protocatechuic acid (2) (49.634 mg/kg) and syringic acid (11) (21.736 mg/kg). In contrast, the MeOH extract showed the highest concentrations of 3-hydroxybenzoic acid (6) (93.757 mg/kg), 4-hydroxybenzoic acid (7) (93.370 mg/kg), and protocatechuic acid (2) (67.686 mg/kg), indicating notable differences in the phenolic profiles of the two extracts. Apigenin (28) and Luteolin 7-glucoside (17) were detected exclusively in the MeOH extract. The LC-MS/MS results are consistent with previous studies reporting the analysis of phenolic compounds in plant extracts using similar extraction methodologies [42]. Overall, phenolic compounds were present in higher concentrations in the MeOH extract compared to the DCM extract.

Comparison with the literature revealed that 43 phenolic compounds were previously identified in the MeOH extract of *M. geniculata* using LC-MS/MS analysis. Several phenolic compounds, including coumarin, salicylic acid, ferulic acid, luteolin, rutin, hesperidin, and rosmarinic acid were also detected in the present study and have been reported in *M. geniculata* extracts. Among them, coumarin and

luteolin-6-arabinose-8-glucose were identified as the major phenolic constituents, whereas protocatechuic acid and quercetin were present in the lowest amounts [19]. According to the LC-MS/MS results, the DCM and MeOH extracts of *M. hirsuta* subsp. *asiatica*, particularly the MeOH extract, are rich in valuable phenolic secondary metabolites, mainly phenolic acid derivatives, flavonoid glycosides, and flavones. In addition, these compounds, which have not previously been reported in the literature for this species, were detected in this study. This is the first comprehensive study on *M. hirsuta* subsp. *asiatica*.

Isolation and Identification of Secondary Metabolites

This study represents the first investigation of the secondary metabolites of *M. hirsuta* subsp. *asiatica*, leading to the identification of seven previously reported compounds, vitexin 2''-O- α -L-rhamnopyranoside (29), Isovitrin 7-O- β -D-glucoside (30), isorhamnetin-7-O- β -D-neohesperidoside (31), Biochanin A-7-O- β -D-glucoside (32), 22-dihydrospinasterol-3 β -D-O-glucoside (33),

Table 2 Phenolics compounds of *M. hirsuta* subsp. *asiatica* extracts by LC-MS/MS

Number	Compound name	Retention time	Mass-to-charge (m/z)	Molecular formula	DCM extract (mg/kg)	MeOH extract (mg/kg)
1	3-hydroxytyrosol	10.4	153.0	C ₈ H ₁₀ O ₃	0.763	0.488
2	Protocatechuic acid	10.8	152.9	C ₇ H ₆ O ₄	49.634	67.686
3	3,4-Dihydroxyphenylacetic acid	11.1	167.0	C ₈ H ₈ O ₄	1.454	0.542
4	(+)-Catechin	11.4	289.0	C ₁₅ H ₁₄ O ₆	1.043	0.454
5	Chlorogenic acid	11.8	355.0	C ₁₆ H ₁₈ O ₉	1.964	34.510
6	3-Hydroxybenzoic acid	12.3	137.0	C ₇ H ₆ O ₃	4.568	93.757
7	4-Hydroxybenzoic acid	12.3	136.9	C ₇ H ₆ O ₃	4.511	93.370
8	(-)-Epicatechin	12.5	291.0	C ₁₅ H ₁₄ O ₆	1.467	1.633
9	Homovanillic acid	12.7	181.0	C ₉ H ₁₀ O ₄	6.324	3.348
10	Caffeic acid	12.8	179.0	C ₉ H ₈ O ₄	0.651	8.739
11	Syringic acid	12.9	196.9	C ₉ H ₁₀ O ₅	21.736	14.176
12	Vanillin	13.2	151.0	C ₈ H ₈ O ₃	13.622	5.711
13	Sinapic acid	14.0	222.9	C ₁₁ H ₁₂ O ₅	0.359	0.576
14	p-Coumaric acid	13.9	162.9	C ₉ H ₈ O ₃	0.895	23.168
15	Taxifolin	13.9	303.0	C ₁₅ H ₁₂ O ₇	0.146	0.145
16	Ferulic acid	14.0	193.0	C ₁₀ H ₁₀ O ₄	6.414	30.706
17	Luteolin 7-glucoside	14.3	447.1	C ₂₁ H ₂₀ O ₁₁	not detected	33.153
18	Hesperidin	14.4	611.1	C ₁₆ H ₁₄ O ₆	not detected	0.009
19	Rosmarinic acid	14.5	359.0	C ₁₈ H ₁₆ O ₈	0.416	47.866
20	Hyperoside	14.6	465.1	C ₂₁ H ₂₀ O ₁₂	0.007	0.874
21	Oleuropein	14.9	539.2	C ₂₅ H ₃₂ O ₁₃	0.679	0.674
22	Apigenin 7-glucoside	14.8	433.1	C ₂₁ H ₂₀ O ₁₀	0.000	3.015
23	2-Hydroxycinnamic acid	14.9	162.9	C ₉ H ₈ O	0.445	0.363
24	Resveratrol	14.7	226.9	C ₁₄ H ₁₂ O ₃	0.098	0.110
25	Eriodictyol	15.2	287.0	C ₁₅ H ₁₂ O ₆	0.451	0.448
26	Quercetin	15.7	301.0	C ₁₅ H ₁₀ O ₇	0.129	0.090
27	Luteolin	15.9	287.0	C ₁₅ H ₁₀ O ₆	0.402	2.900
28	Apigenin	16.3	271.0	C ₁₅ H ₁₀ O ₅	not detected	1.781

spinasteryl- β -D-O-glucoside (34), gysogenin (35) (Fig. 3, Fig. S2-S15, Tables S1-S4).

Among these metabolites, compounds 29 and 30 are apigenin glycosides. Apigenin is the major flavone in the Caryophyllaceae family and was reported in 28 species. Although vitexin was isolated from various Caryophyllaceae species, compound 29 was reported from *Vaccaria pyramidata* [43]. Isovitexin, a C-glycosylated apigenin derivative, was isolated from *M. elegans* and more than 70 plant species. Compound 30 was reported in several Caryophyllaceae species, including *Gypsophila* spp., *Dianthus* spp., *Silene* spp. and *Vaccaria* spp. Another aglycone commonly found in the Caryophyllaceae family is quercetin, one of the most important flavonoids, which is widely distributed in plants and was isolated from more than 20 species [44]. Compound 31 is also a natural flavonol quercetin glycoside and was isolated from Caryophyllaceae species [45]. In contrast, compound 32 was reported for the first time from *Stellaria media* in Caryophyllaceae family [46]. Saponins such as gypsogenin, gypsogenic acid, quillaic acid (oleanane type), and 22-dihydrospinasterol (sterol type) were isolated previously from various Caryophyllaceae species [8, 47]. However, the

isolation of these compounds from *M. hirsuta* subsp. *asiatica* is reported here for the first time.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC of the *M. hirsuta* subsp. *asiatica* extracts were determined using the Folin–Ciocalteu method, and expressed as GA equivalents, as shown in Fig. 4. The TPC values of Hex, DCM, and MeOH extracts were calculated from the GA calibration curve with the equation $y=0.0049x+0.1637$, $R^2=0.9953$. The TFC of the *M. hirsuta* subsp. *asiatica* extracts were determined using the aluminum chloride spectrophotometric method and expressed as QE equivalents, as shown in Fig. 4. The standard calibration curve equation used for the calculation was $y=0.0033x+0.1992$, $R^2=0.9961$. The results are presented in Table 3.

According to the literature, the MeOH extract of the *M. geniculata* has been reported to exhibit total phenolic and total flavonoid contents of 52.80 and 34.50, respectively [19]. In comparison with previously reported data,

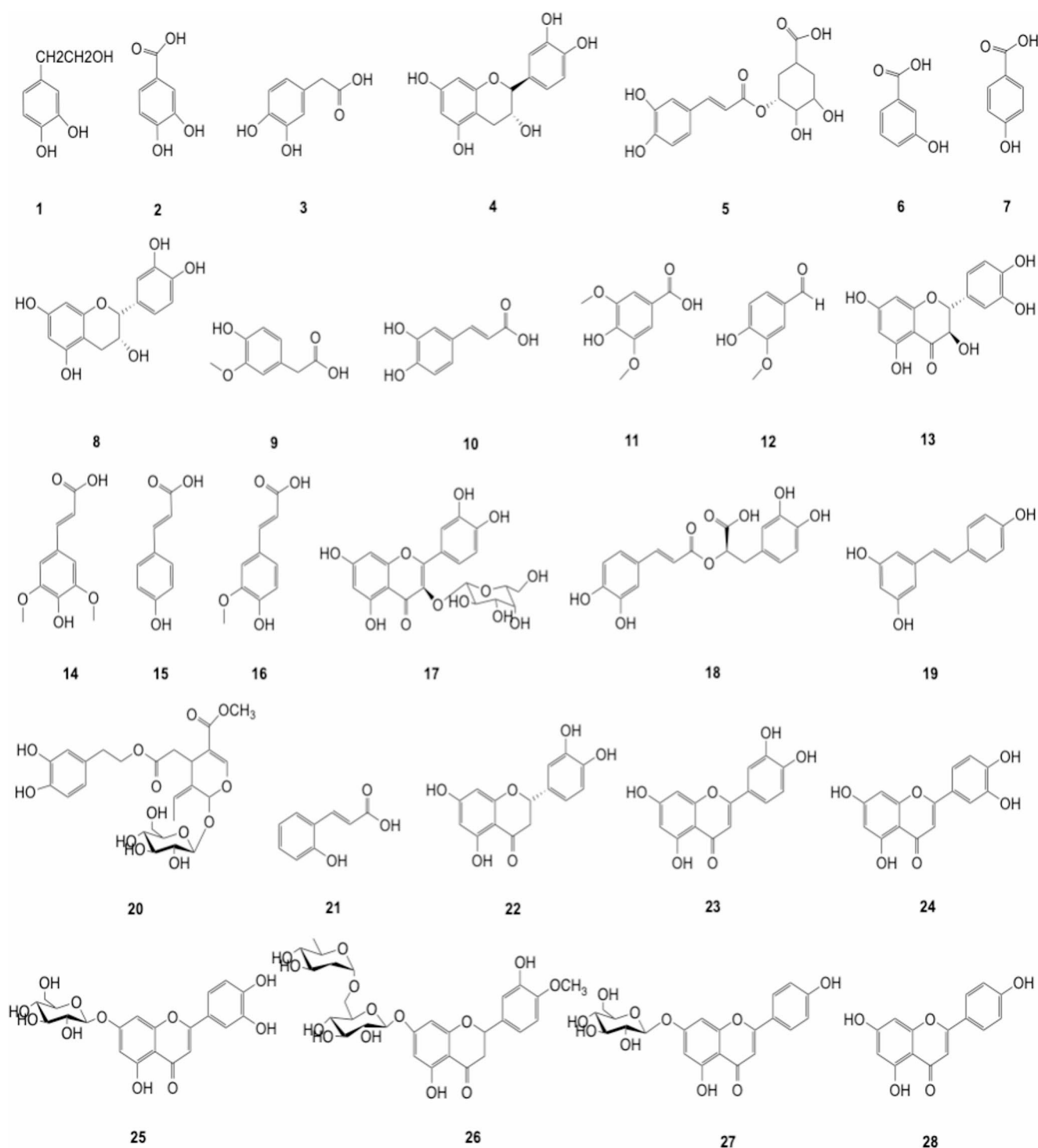


Fig. 2 Compounds occurring in dichloromethane and methanol extracts of *M. hirsuta* subsp. *asiatica*

the phenolic content of *Minuartia* species in present study appears to be at an average level among closely related species. Moreover, both TPC and TFC values of *M. hirsuta* subsp. *asiatica* are consistent with those reported for other medicinally important plant species [48–52]. The

results demonstrated that the MeOH extract exhibited the highest TPC, 73.32 mg GAE/g extract, and TFC, 66.91 mg QE/g extract, followed by the DCM extract, which showed TPC and TFC values of 67.20 mg GAE/g extract and 44.69 mg QE/g extract, respectively.

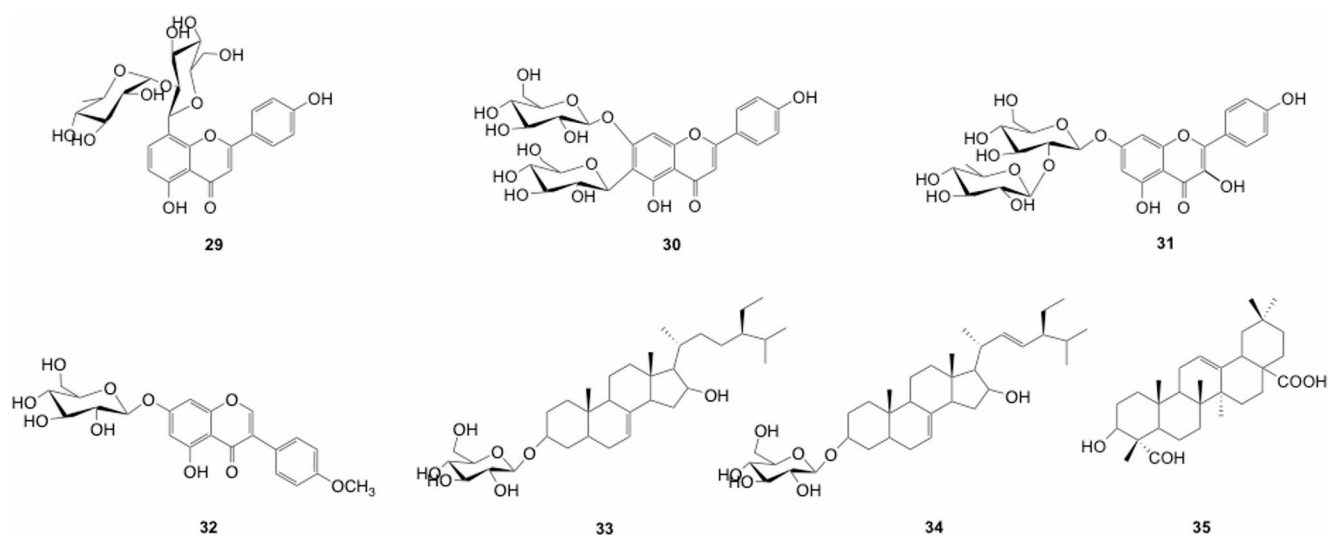


Fig. 3 Isolated compounds from the methanol extract of *M. hirsuta* subsp. *asiatica*

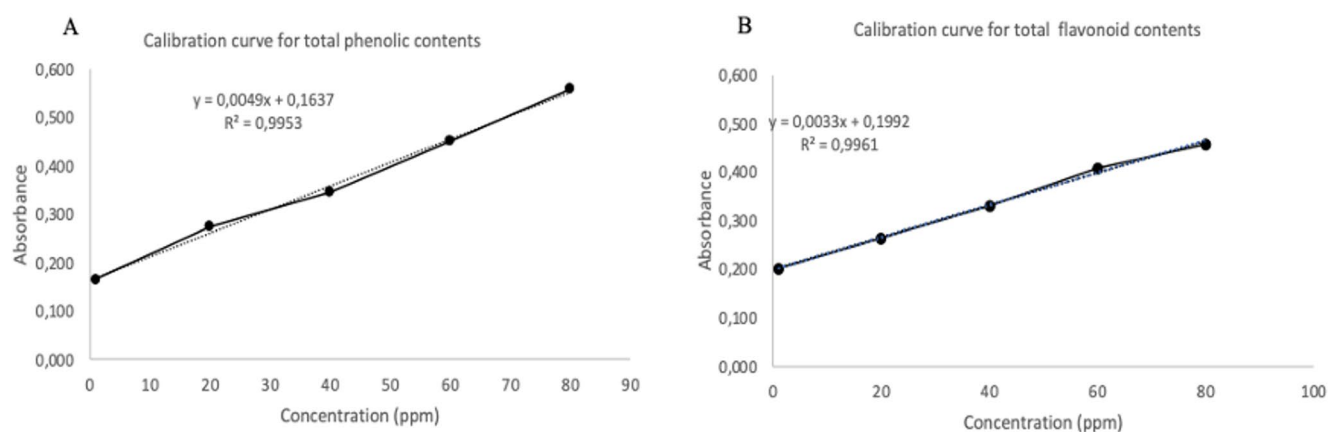


Fig. 4 Calibration curve for standards gallic acid (A) and quercetin (B)

Table 3 Total Phenolic and Flavonoid Content and Antioxidant Activity Capacity of *M. hirsuta* subsp. *asiatica* Extracts

Extracts	Total phenolic content (mg GA/g)	Total flavonoid content (mg QE/g)	% of inhibition (DPPH)	mM AAE/mL	% of inhibition (ABTS ⁺)	mM TE/mL
Hex	3.64 ± 0.26 ^a	1.25 ± 0.14 ^a	...	...	...	...
DCM	67.20 ± 0.54 ^b	44.69 ± 0.38 ^b	68.72 ± 0.41 ^b	14.59 ± 0.16 ^b	63.22 ± 0.33 ^b	14.66 ± 0.15 ^b
MeOH	73.32 ± 0.41 ^c	66.91 ± 0.25 ^c	72.83 ± 0.39 ^c	39.90 ± 0.29 ^c	75.46 ± 0.32 ^c	37.57 ± 0.29 ^c

...: Not determined

Antioxidant Activity of *Minuartia hirsuta* subsp. *asiatica*

The radical scavenging activities of the *M. hirsuta* subsp. *asiatica* extracts were evaluated using the DPPH assay, with ascorbic acid (AA) and ABTS⁺ assay with trolox as the reference standards (Fig. 5). The antioxidant activities of the extracts are presented in Table 3. The radical scavenging capacity was calculated using the equation derived from the AA calibration curve $y = -0.0032x + 0.3687$, $R^2 = 0.994$ and TE calibration curve $y = -0.0034x + 0.4416$, $R^2 = 0.9871$.

Values are expressed as mean ± standard deviation ($n = 3$). Different superscript letters (a, b, c) within the same row indicate significant differences between extracts ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD test.

At a concentration of 500 µg/mL, the MeOH and DCM extracts exhibited antioxidant activities with DPPH scavenging activities of 72.83% and 68.72%, and ABTS radical scavenging rates of 75.46% and 63.22%, respectively. All *M. hirsuta* subsp. *asiatica* extracts, except for Hex extract, showed high inhibition percentages in the DPPH and ABTS

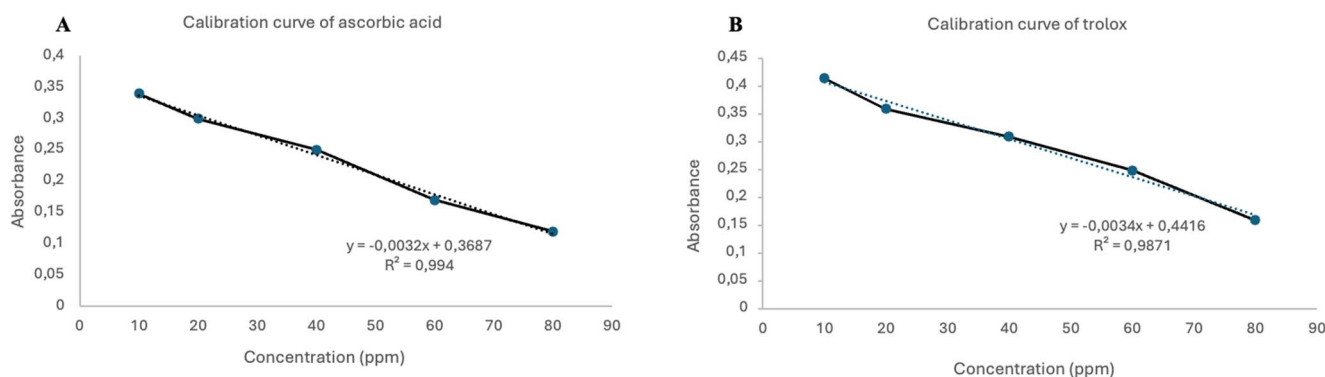


Fig. 5 Calibration curves of ascorbic acid and trolox by DPPH and ABTS⁺ assay. **(A)** DPPH radical scavenging activity; **(B)** ABTS⁺ radical scavenging activity

assay. Comparable results have been reported in the literature for *M. geniculata*, where the MeOH extract demonstrated a DPPH scavenging activity of 72.24% [19]. In a study evaluating the DPPH scavenging abilities of 34 different plant extracts, inhibition values ranged from 6.32% to 87.98%, with Caryophyllaceae flowers exhibiting an average scavenging rate of 37.82% [53]. Similar results have been observed for ABTS radical scavenging activity. In one study, ABTS inhibition values of 32 plant species ranged from 0.45% to 140%, with *Herniaria glabra* (Caryophyllaceae) exhibiting 48.1% inhibition [54]. Differences in total phenolic and flavonoid content among the studied species explain the difference in ABTS values. Furthermore, the wide range of ABTS activities among Caryophyllaceae species reflects both phytochemical diversity and differences in extraction methods and solvent polarity [55, 56]. Compared to the literature data, the present DPPH and ABTS results (0–72.83% and 0–75.46%, respectively) indicate that *M. hirsuta* subsp. *asiatica* extracts possess a relatively high antioxidant capacity. Accordingly, *M. hirsuta* subsp. *asiatica* can be considered a promising source of natural antioxidants, highlighting the potential of *Minuartia* species as valuable antioxidant resources.

The slightly higher inhibition observed for the MeOH extract may be attributed to its higher phenolic and flavonoid contents compared to the DCM and Hex extracts, which enhance hydrogen atom transfer mechanisms [57]. These findings are consistent with reports indicating that samples exhibiting strong antioxidant activity generally contain higher relative abundances of bioactive metabolites [58].

Flavonoids, which constitute an important class of polyphenolic compounds identified in *M. hirsuta* subsp. *asiatica*, are known to exhibit antioxidant properties due to the presence of specific functional groups and intra-ring molecular structures that can interact with and influence the oxidation state of other compounds. In particular, the number and position of hydroxyl groups play a crucial role

in determining antioxidant capacity. It has been reported that an increased number of hydroxyl groups enhances antioxidant activity, whereas structural modifications such as glycosylation or methylation may significantly reduce this activity [59–61]. Among the identified compounds, vitexin, an apigenin flavone glycoside, has been widely reported to exhibit significant antioxidant activity, including protective effects against reactive oxygen species, lipid peroxidation, and oxidative damage in various pathological conditions [62]. Similarly, isovitexin derivatives are known to regulate signaling pathways associated with antioxidant and anti-inflammatory responses. Isovitexin glycosides have been extensively studied for their potent antioxidant activity, particularly due to their ability to protect essential biomolecules such as DNA, lipids, and proteins from oxidative damage [63]. Moreover, isorhamnetin glycosides are biologically important flavonols with well-documented antioxidant and anti-inflammatory properties, along with other pharmacological activities [64]. Likewise, biochanin glycosides have been reported as a potential compound in combating diseases associated with oxidative stress [65].

Furthermore, phenolic acids identified in the study, including hydroxybenzoic acids (such as 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, protocatechuic acid, and vanillic acid) and hydroxycinnamic acids (such as caffeic acid, ferulic acid, sinapic acid, and p-coumaric acid), are widely recognized for their antioxidant, anti-inflammatory, and metabolic properties. These compounds contribute to antioxidant activity through mechanisms such as radical scavenging and protection against oxidative damage [66]. Protocatechuic acid, in addition to its remarkable antioxidant activity, has been extensively studied and exhibits biological properties such as antibacterial, anti-inflammatory, antimutagenic, antidiabetic, anti-aging, and analgesic effects [67]. Syringic acid is also an antioxidant and exhibits antibacterial and hepatoprotective effects [68]. In addition, hydroxybenzoic acids have been shown to exhibit various biological activities, including antibacterial, estrogenic, antisickling,

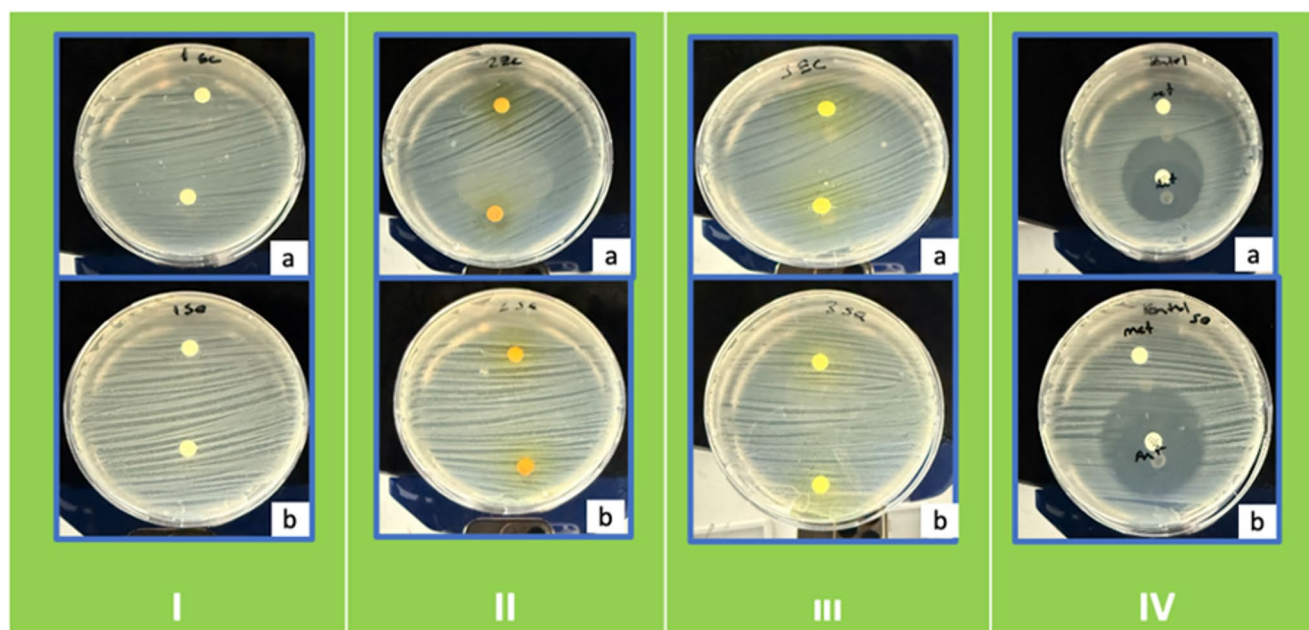


Fig. 6 Antibacterial activity of *Minuartia* extracts in (I) hexane, (II) dichloromethane, and (III) methanol against bacterial pathogens (a) *Escherichia coli* (b) *Staphylococcus aureus*

antifungal, and antimutagenic effects [69]. Many of these compounds are well-known for their antioxidant properties, which likely contribute to the radical scavenging activity observed in both DPPH and ABTS assays, providing a link between the phytochemical composition and the measured antioxidant potential of the extracts.

Overall, the observed antioxidant activity in this study can be attributed to the presence of these phenolic constituents and their structural characteristics, which enable them to interact with reactive species and reduce oxidative stress. Furthermore, recent studies have demonstrated that extraction solvents and processing conditions significantly influence phenolic composition and antioxidant potential in plant materials, supporting the differences observed among the solvent extracts of *M. hirsuta* subsp. *asiatica* [70].

Antimicrobial Activity of *Minuartia hirsuta* subsp. *asiatica*

The antibacterial activity of the *M. hirsuta* subsp. *asiatica* extracts were evaluated using the disc diffusion method against *ololi* and *S. aureus*. None of the *M. hirsuta* subsp. *asiatica* extracts exhibited detectable antibacterial activity against the tested bacterial strains, as shown in Fig. 6.

Plants are recognized as potential sources of antimicrobial agents due to their ability to produce a wide range of bioactive secondary metabolites. The identification of plant-derived compounds with antimicrobial properties is therefore of considerable interest for the development of novel therapeutic agents [71]. Previous studies have reported

antibacterial activity in certain *Minuartia* species; for instance, the ethyl acetate fraction of *M. genculata* exhibited moderate antibacterial activity against *S. aureus* and *E. coli*, whereas the essential oil of *M. meyeri* showed antibacterial activity against *S. aureus* but not against *E. coli* [19, 20]. In the present study, *M. hirsuta* subsp. *asiatica* extracts did not exhibit antibacterial activity against the selected bacterial strains under the applied experimental conditions.

The antibacterial efficacy of plant extracts largely depends on their phytochemical composition, particularly the presence of bioactive secondary metabolites such as phenolics, terpenoids, and alkaloids [72, 73]. Therefore, the absence or low concentration of these compounds may account for the observed inactivity. In addition, extraction methods and solvent polarity play a critical role in determining the chemical profile and biological activity of plant extracts, as different solvents selectively extract different classes of compounds. Accordingly, extracts obtained from the same plant using different solvents may exhibit markedly different biological activities [74–76]. Overall, the absence of antibacterial activity can be explained by both the phytochemical profile of the species and the experimental conditions employed.

Conclusion

In this study, the phytochemical properties of *M. hirsuta* subsp. *asiatica* were investigated for the first time through isolating and identifying seven previously reported

compounds (29–35) from the methanol extract. These isolated compounds exhibit diverse structural classes, including two flavones (29 and 30), one flavonol (31), one isoflavone (32), two sterols (33 and 34), and one triterpene (35). Furthermore, LC-MS/MS analysis revealed the presence of 28 phenolic compounds in the DCM and MeOH extracts, while GC-FID analysis confirmed the presence of fatty acids in the hexane extract.

Among the extracts, the DCM and MeOH extracts of *M. hirsuta* subsp. *asiatica*, which exhibited significant total phenolic and total flavonoid contents, demonstrated moderate to strong antioxidant activity. In contrast, none of the extracts showed antibacterial activity against the tested microorganisms. The higher antioxidant activity observed in the MeOH and DCM extracts, compared to the hexane extract, can be attributed to the greater abundance and diversity of phenolic compounds in medium-polar and polar extracts, which are known to play a crucial role in antioxidant capacity.

Overall results obtained from TPC and TFC assays, DPPH and ABTS radical scavenging activities and LC-MS/MS analysis provide a comprehensive evaluation of the relationship between the phytochemical composition of *M. hirsuta* subsp. *asiatica* and its antioxidant potential. These findings indicate that *M. hirsuta* subsp. *asiatica* possesses a significant antioxidant property and may serve as a valuable source of natural antioxidants. Moreover, this study may contribute to the discovery of novel therapeutic agents for human health and will support future research aimed at improving the understanding of plant biochemistry and the sustainable utilization of plant resources.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12247-026-10854-x>.

Acknowledgements The author would like to acknowledge Prof. Dr. Ozgen Alankus and Prof. Dr. Tamer Karayildirim for their support and the access to Organic Chemistry Laboratory facilities at the Department of Chemistry, Ege University and MATAI (Ege University) for bioactivity results.

Author Contributions R.N.U. conducted the research project and wrote the manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This work was supported by Ege University Scientific Research Projects [BAP-32306].

Data Availability All experimental data and analyses of the study are included in this manuscript.

Declarations

Competing interests The authors declare no competing interests.

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