https://doi.org/10.31925/farmacia.2025.4.23 **ORIGINAL ARTICLE**

PHYTOCHEMICAL PROFILING AND ANTIOXIDANT PROPERTIES OF *AJANIA FASTIGIATA* (C. WINKL.) POLJAKOV (*ASTERACEAE*)

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Abstract

This study aims to evaluate the phytochemical composition and antioxidant properties of *Ajania fastigiata* (C. Winkl.) Poljakov, a medicinal plant native to Kazakhstan. For the first time, a comprehensive GC-MS-based phytochemical profiling of this species was conducted, revealing a unique chemical composition. GC-MS analysis identified 26 bioactive compounds in the ethanol extract, with bicyclo[3,1,1]hept-3-en-2-ol, 4,6,6-trimethyl- (30.6%), hexadecanoic acid (11.0%) and 9,12,15-octadecatrienoic acid (10.7%) as the major constituents. The ethanol extract showed the highest total phenolic content (65.7 mg GAE/g) and flavonoid content (7.18 mg QE/g). Antioxidant activity, evaluated through DPPH and FRAP assays, demonstrated significant radical scavenging capacity (79.21% at 1 mg/mL) and reducing power (1.3727 OD at 1 mg/mL), comparable to gallic acid. The essential oil contained camphor, α-pinene and carvacrol. These findings highlight *Ajania fastigiata* as a promising natural source of antioxidants and support its potential for pharmaceutical and therapeutic applications.

Rezumat

Studiul a evaluat compoziția fitochimică și proprietățile antioxidante ale speciei *Ajania fastigiata* (C. Winkl.) Poljakov, o plantă medicinală originară din Kazahstan. A fost realizat un profil fitochimic al acestei specii, utilizând GC-MS. S-au identificat 26 de compuși bioactivi în extractul etanolic, dintre care principali au fost: biciclo[3,1,1]hept-3-en-2-ol, 4,6,6-trimetil (30,6%), acidul hexadecanoic (11,0%) și acidul 9,12,15-octadecatrienoic (10,7%). Extractul etanolic a prezentat cel mai mare conținut total de compuși fenolici (65,7 mg GAE/g) și flavonoide (7,18 mg QE/g). Activitatea antioxidantă, evaluată prin testele DPPH și FRAP, a demonstrat o capacitate semnificativă de neutralizare a radicalilor liberi (79,21% la 1 mg/mL) și activitate reducătoare (1,3727 OD la 1 mg/mL), comparabilă cu cea a acidului galic. În uleiul volatil au fost identificați camfor, α-pinen și carvacrol.

Keywords: *Ajania fastigiata*, phytochemical profiling, antioxidant activity, GC-MS

Introduction

Medicinal plants have long played a crucial role in pharmaceutical research, offering a rich source of bioactive compounds with diverse therapeutic properties [25]. The rising interest in plant-based medicines stems from their natural origin, biocompatibility and broad spectrum of biological activities, including antioxidant, antimicrobial and cytotoxic effects [8]. Among these, plants producing essential oils have garnered special attention due to their potential applications in treating infections, inflammation and oxidative stress-related disorders [1]. Several species of the *Asteraceae* genus have demonstrated antimicrobial, anti-inflammatory and antioxidant properties, making them valuable candidates for pharmaceutical applications [2]. Despite this, the *Ajania* genus remains underexplored compared to other medicinal plants, with only a limited number of studies detailing its chemical composition and biological potential [41].

One of the most promising yet understudied species within this genus is *Ajania fastigiata* (C. Winkl.) Poljakov is a perennial plant native to Kazakhstan. The flora of Kazakhstan is remarkably diverse, comprising over 6,000 plant species, many of which remain untapped for their medicinal potential [15]. *A. fastigiata* is predominantly found in the mountainous regions of southern and southeastern Kazakhstan, where it has been traditionally used for its wound-healing and antimicrobial properties [14]. While several studies have explored the essential oil composition of *Ajania* species, limited data are available on the detailed phytochemical profile and antioxidant activity of *A. fastigiata* [32].

One of the most significant bioactive properties of *A. fastigiata* is its antioxidant potential [10]. Oxidative stress is a major contributor to various chronic diseases, including cardiovascular disorders, neurodegenerative diseases and cancer [35]. Antioxidants neutralise reactive oxygen species, preventing cellular damage and disease progression [3]. This study aims to conduct a detailed phytochemical analysis of *A. fastigiata* extracts and evaluate their antioxidant activity. The plant material was extracted using the sequential solvent extraction (hexane, chloroform, ethyl acetate, ethanol) and hydro-distillation for essential oil extraction. The chemical composition was analysed using gas GC-MS and spectrophotometric assays. Antioxidant activity was assessed using DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays [7]. The results of this study will contribute to a better understanding of *A. fastigiata* as a promising source of natural bioactive compounds, supporting its potential pharmaceutical applications.

Materials and Methods

Plant material

The plant material used in this study, *A. fastigiata* (C. Winkl.) Poljakov was collected early in the morning during its flowering period in June 2023 in Kegen District, Almaty Region, Kazakhstan; GPS (Garmin eTrex22x) coordinates: N 43.0609796° E 78.30461082°, altitude: 1290 m. The species was identified and authenticated by researchers from the Institute of Botany and Phytointroduction in Almaty, Kazakhstan, where the control sample is deposited under reference number 5/237.

The collection of plant material was carried out in accordance with international and national ethical standards for biodiversity research. Specifically, the procedures complied with the Convention on Biological Diversity (CBD) principles and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Prior informed consent was obtained from relevant local authorities, and no protected or endangered species were involved. Collection activities ensured minimal disturbance to native habitats and followed Kazakhstan's legal frameworks for the sustainable use of plant genetic resources.

After collection, the plant material was thoroughly cleaned and washed with distilled water to remove impurities. It was then air-dried under controlled conditions in the dark at room temperature for at least ten days. After drying, the plant material was ground into a fine powder using an Electric Micro Plant Grinding Machine (110 V, 1400 rpm/min).

Extraction procedure

To maximise the yield of phytochemicals, 100 g of dried plant powder was subjected to Soxhlet extraction using solvents of varying polarity [20]. Solvents with different polarities were chosen for the extraction of biologically active compounds of *A. fastigiata*: hexane (non-polar) for the extraction of lipids and terpenes, chloroform (weakly polar) for the isolation of alkaloids and phenols, ethyl acetate (medium polar) for flavonoids and phenolic acids and ethanol (highly polar) for hydrophilic compounds, including polyphenols [11] each extraction was carried out for 5 hours with 500 mL of solvent. After extraction, the solvents were removed under reduced pressure at 40°C using a rotary evaporator (IKA RV10 auto V-C, Staufen, Germany). The resulting extracts were filtered through Whatman filter paper 1 and concentrated under reduced pressure. The semi-solid mass underwent transformation into a solidified mass through exposure to ambient air. The percentage yield of each extract was calculated using the following formula: The quantification of extract contents (W, %) was determined utilising the following Equation (1), representing the percentage ratio of the final dry mass of extracts (m_1) to the initial dry mass of the plant material (m_0) subjected to extraction:

$$W\ (\%) = (m_1/m_0) \times 100, \quad (1).$$

Essential oil extraction

The essential oil from the aerial parts of *A. fastigiata* was obtained using a hydrodistillation method with a Klevenger apparatus [13]. A 100 g sample of finely ground plant material was placed in a 2000 mL round-bottom flask and mixed with 1 L of distilled water. The flask was connected to the Klevenger apparatus, and 1 mL of hexane was added through the side receiver. The mixture was subjected to hydrodistillation for 2 - 3 hours, beginning when the first distillate drops appeared. The distillation rate was maintained at 45 - 50 drops *per* minute. After distillation, the volume of the oil was measured at room temperature, and the oil was then passed through the apparatus with steam for 30 minutes to ensure complete extraction.

Phytochemical analysis

Determination of total phenolic and flavonoid content (TPC and TFC). The total flavonoid content (TFC) and total phenolic content (TPC) were determined using spectrophotometric analysis. TFC was determined using aluminium chloride ($AlCl_3$) and quantified by comparing the absorption of the sample with a standard quercetin calibration curve. The standard solutions of quercetin were prepared at various concentrations (6.25, 12.5, 25, 37.5, 50, 75, 100 µg/mL). A 1 mL sample of the extract was mixed with 1 mL of $AlCl_3$ solution and incubated for 30 minutes in the dark. Absorbance was measured at 430 nm. TFC was calculated as quercetin equivalents (QE) *per* unit weight or volume of the sample [24].

For the determination of TPC, the Folin-Ciocâlteu method was employed. A series of GA (gallic acid) standard solutions (6.25, 12.5, 25, 37.5, 50, 75, 100 µg/mL) was prepared for calibration. A 0.25 mL sample of extract was mixed with 1 mL of Folin-Ciocâlteu reagent, and after 3 minutes, 1% sodium carbonate

solution was added. The mixture was kept in the dark at room temperature for 2 hours. Absorbance was measured at 760 nm, and TPC was calculated as gallic acid equivalents (GAE) using the standard curve. The phenolic content was reported as GAE *per* gram of sample, and the flavonoid content was expressed as QE *per* gram of sample [8].

Calibration curves for TPC and TFC were established using GA and quercetin, respectively. The method's linearity was confirmed within the concentration range of 6.25 - 100 µg/mL, with correlation coefficients (R^2) exceeding 0.99, indicating high quantification accuracy.

GC-MS analysis. The composition of the extracts was performed using gas chromatography-mass spectrometry (GC-MS) with an Agilent 7890A/5975C system using electron impact (EI) ionisation at 70 eV [26]. A sample volume of 0.5 µL was injected at 280°C, with a split ratio of 10:1 and a solvent delay of 10 minutes. Compound separation was performed using a DB-WAXetr capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) under a constant helium carrier gas flow of 1 mL/min. The chromatographic temperature program was set as follows: an initial temperature of 40°C (held for 5 minutes), followed by a ramp of 5°C/min up to 260°C, with a final hold time of 5 minutes. The total analysis time was 54 minutes. Detection was performed in SCAN mode (m/z 34 - 850). The Agilent MSD ChemStation (version 1701EA) software was used for system control, data acquisition and processing. Data analysis included determining retention times, peak areas and spectral information obtained from the mass spectrometric detector. Compound identification was conducted by comparing the acquired mass spectra with reference libraries, Wiley 7th edition and NIST'02, which contain over 3 million spectra.

DPPH radical scavenging assay. The antiradical activity (ARA) of the plant extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [3]. A 0.1 mL aliquot of the sample solution was mixed with 3 mL of a 6×10^{-5} M DPPH solution at concentrations of 0.25, 0.5, 0.75 and 1.0 mg/mL. The mixtures were kept in the dark for 30 minutes after thorough mixing. The absorbance was measured at 520 nm using a spectrophotometer. The antiradical activity of the samples was calculated using the following formula:

$$APA\ (\%) = (A_0 - A_t) / A_0 * 100, \quad (2)$$

where, A_0 is the absorbance of the control sample and A_t is the absorbance of the test sample. The values of ARA were compared with the GA as a reference, and the results were tabulated for each concentration.

FRAP antioxidant assay. The antioxidant activity (AOA) was determined using the FRAP (Ferric Reducing Antioxidant Power) method [3]. A 0.1 mL aliquot of the sample solution was mixed with 0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of a 1% potassium hexacyanoferrate (III) solution. The reaction mixture was incubated for 20 minutes at 50°C. After incubation, 0.25 mL of 10% trichloroacetic acid was added to stop the reaction, and the mixture was centrifuged at 3000 rpm for 10 minutes. The upper layer (0.5 mL) was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% $FeCl_3$ solution. The absorbance was measured at 700 nm. The antioxidant activity was calculated by comparing the absorbance of the samples to that of the gallic acid standard. The concentrations tested were 0.25, 0.5, 0.75 and 1.0 mg/mL. Results were reported as the reduction of Fe^{3+} to Fe^{2+}, with the formula for the calculation of AOA:

$$AOA = A_{sample} / A_{control} \times 100, \quad (3)$$

where, A_{sample} is the absorbance of the sample solution, $A_{control}$ is the absorbance of the GA standard solution. This method provides insight into the antioxidant potential of the extracts based on their ability to reduce Fe^{3+} ions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to determine statistically significant differences among multiple extract groups with varying solvent polarities. ANOVA was used to compare more than two groups when the data were approximately normally distributed, and the variances were homogeneous. Tukey's post hoc test was applied for multiple comparisons to identify specific group differences while controlling the type I error rate. Data are presented as mean ± standard deviation (SD) based on triplicate measurements.

Results and Discussion

Chemical composition of A. fastigiata essential oil

The essential oil extracted from the aerial parts of *A. fastigiata* was obtained through hydrodistillation using a Clevenger apparatus. The process involved four separate extractions, yielding a total of 2.8116 g of essential oil (1. m_1 (A.Fas.0) = 0.3042 g; 2. m_2 (A.Fas.0) = 0.7836 g; 3. m_3 (A.Fas.0) = 1.1054 g; 4. m_4 (A.Fas.0) = 0.6184 g). These results indicate a consistent extraction process, with the third extraction yielding the highest amount of oil. The essential oil of *A. fastigiata* is primarily composed of camphor, p-cymene, butyric acid, camphene, (+)-isoborneol, carvacrol and α-pinene [36]. In contrast, the essential oil of *Ajania fruticulosa* (*Asteraceae*) is dominated by β-thujone (19.18%) and thymol (18.04%), highlighting significant variations in the phytochemical profile [37]. Similarly, species within the *Artemisia* genus, such as *Artemisia absinthium*, are characterised by the presence of α-thujone, β-thujone, bornyl acetate and 4-terpineol, which contribute to their well-documented antimicrobial and pharmacological properties [38]. These compositional

differences suggest a diverse range of biological activities and potential therapeutic applications unique to each species.

TPC and TFC in A. fastigiata

The results of the analysis of TPC and TFC in *A. fastigiata* extracts, including the ethanol extract, are presented in Table I. The highest TPC (65.7 mg GAE/g) and TFC (7.18 mg QE/g) (Figure 1) values were observed in the ethanol extract (A.Fas.4), which is attributed to the high polarity of ethanol facilitating the efficient extraction of polyphenols [34]. Compared to other species within the *Artemisia* genus, *A. fastigiata* exhibits notably higher phenolic and flavonoid contents. For instance, in *Artemisia absinthium*, the TFC reached 3.80 ± 0.13%, with main components including astragalin, cynaroside, ononin and rutin [17]. Similarly, studies on *Artemisia abrotanum* reported total phenolic acid contents ranging from 0.35 to 217.39 mg/g during different vegetation phases [31]. The elevated levels of phenolic and flavonoid compounds in *A. fastigiata* are indicative of its strong antioxidant potential. Phenolic compounds are known for their ability to scavenge free radicals, thereby mitigating oxidative stress and reducing the risk of chronic diseases [4]. On the other hand, Flavonoids contribute to various biological activities, including anti-inflammatory, antimicrobial and anticancer effects [33].

Table I

TFC and TPC in *A. fastigiata* extracts

№	Extract	Abs (TFC)	Abs (TPC)	TPC (mg GAE/g)	Solvent
1	*A.Fas.1*	0.365	0.071	13.29	Hexane
2	*A.Fas.2*	0.36	0.12	19.53	Chloroform
3	*A.Fas.3*	0.414	0.192	29.4	Ethyl acetate
4	*A.Fas.4*	0.456	0.457	65.7	Ethanol

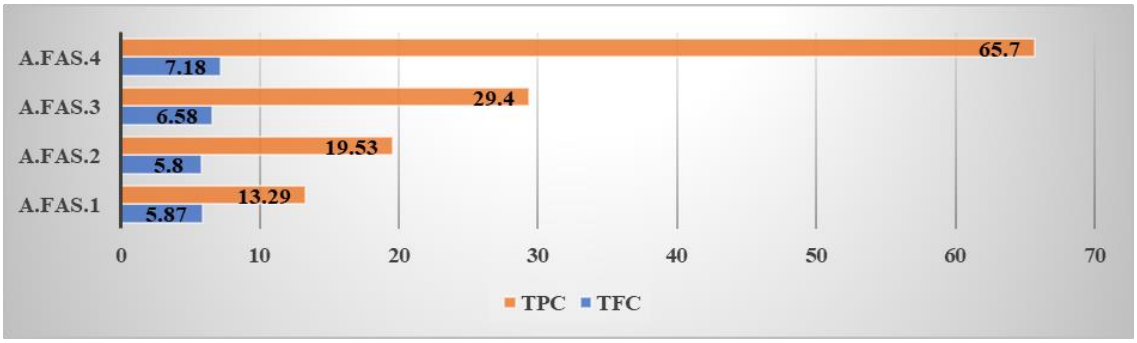


Figure 1.

Total flavonoid content (TFC) and total phenolic content (TPC) of *A. fastigiata* extracts obtained using different solvents (hexane, chloroform, ethyl acetate and ethanol)

Chemical composition of A. fastigiata ethanol extract (A.Fas 4.1) via GC-MS analysis.

The chemical composition of the ethanol extract (A.Fas 4.1) of *A. fastigiata* was determined using gas chromatography-mass spectrometry (GC-MS). The analysis identified 26 compounds, with their retention times, probability of identification and relative content presented in Table II.

Table II

Results of chromatographic analysis of (A.Fas 4.1) ethanol extract

No.	Retention Time, min	Compound	Identification Probability, %	Content, %
1	12.21	Thujone	88	1.7
2	12.85	Propanoic acid, 2-oxo-, methyl ester	73	1.6
3	14.24	(+)-2-Bornanone	97	12.6
4	15.60	p-Menth-8-en-1-ol, stereoisomer	74	0.9
5	16.04	Bicyclo[3,1,1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	87	2.8
6	17.90	Bicyclo[3,1,0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1α,3α,5α)-	69	1.7
7	18.37	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	81	0.9
8	18.72	Bicyclo[2,2,1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	85	3.2
9	19.03	Bicyclo[2,2,1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	86	0.9
10	19.64	Bicyclo[3,1,1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1α,2β,5α)]-	87	30.6
11	20.28	Bicyclo[2,2,1]heptane-3-methylene-2,2-dimethyl-5-ol acetate	77	0.7
12	24.66	Phenol	88	0.5
13	25.36	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	78	0.8
14	27.51	(-)-Spathulenol	77	0.8

No.	Retention Time, min	Compound	Identification Probability, %	Content, %
15	28.98	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	78	1.0
16	29.22	α-Bisabolol	83	0.8
17	31.63	Benzofuran, 2,3-dihydro-	83	1.0
18	33.94	Benzeneacetic acid	72	0.4
19	36.01	Phytol	88	2.4
20	39.74	Hexadecanoic acid	92	11.0
21	40.80	Hydroquinone	66	1.1
22	43.49	9,12-Octadecadienoic acid (Z,Z)-	85	5.4
23	43.86	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	86	1.5
24	44.31	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	88	10.7
25	44.45	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1,fwdarw,3)-β-D-fructofuranosyl	70	3.2
26	48.42	Achillicin	87	1.9

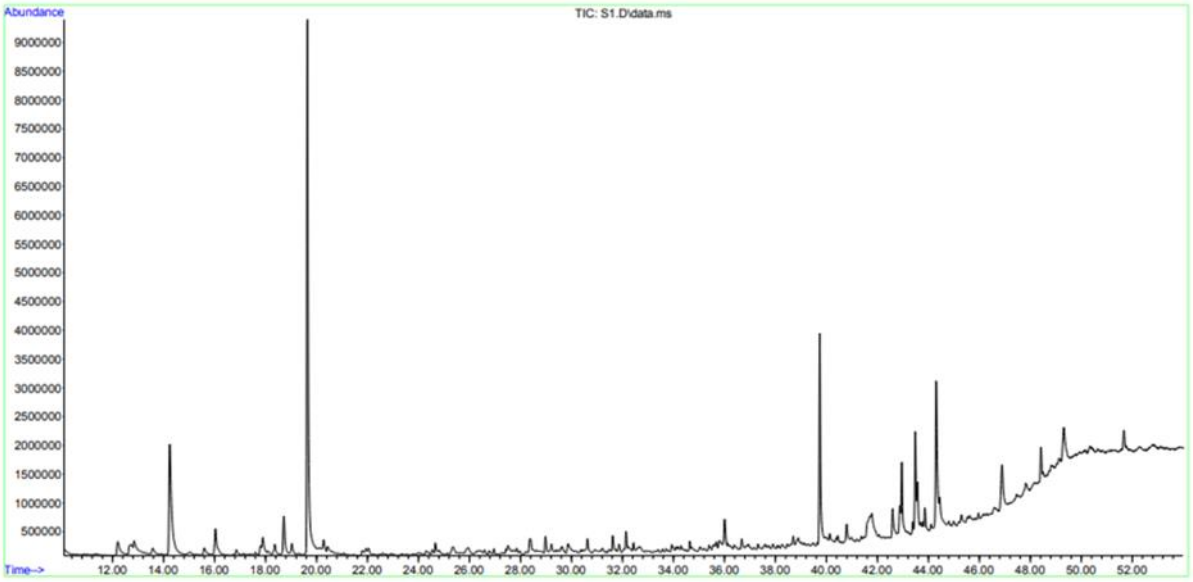


Figure 2.
GC-MS chromatogram of the ethanol extract of *A. fastigiata* (A.Fas 4.1)

Among the identified constituents, terpenoids [7] such as thujone (1.7%), spathulenol (0.8%) and α-bisabolol (0.8%) were present, indicating possible antimicrobial and anti-inflammatory properties [22]. Notably, phytol (2.4%) [18] and hydroquinone (1.1%) [12], which are known for their antioxidant and cytoprotective effects, were also detected. Phenol (0.5%) and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (1.0%) [9], compounds with documented antioxidant properties, were also present.

In *Ajania tibetica* (*Asteraceae*), essential oils rich in terpenoids and flavonoids exhibited strong antimicrobial and moderate antioxidant activity [16]. Similar to our findings, bornanone derivatives and bicyclic monoterpenes were predominant, indicating their role in biological activity.

The comparative analysis of the ethanol extract of *A. fastigiata* (A.Fas.4.1) and *Ajania fruticulosa* highlights key phytochemical similarities and differences that underscore the potent bioactivity of the *Ajania* genus. Both species contain bioactive terpenoids, including thujone, spathulenol and α-bisabolol, known for their antimicrobial and anti-inflammatory properties. However, *A. fastigiata* is distinguished by its high content of bicyclo[3,1,1]hept-3-en-2-ol, 4,6,6-trimethyl- (30.6%), which is absent in *A. fruticulosa*, suggesting a unique antioxidant potential [21].

These comparisons indicate that *A. fastigiata* shares key bioactive compounds with other *Ajania* species but is unique in its relatively high content of fatty acids and bicyclic terpenoids, which may offer distinct pharmacological applications. The high content of 9,12,15-Octadecatrienoic acid is particularly noteworthy, as this compound has been associated with antitumor and cardioprotective properties in pharmacological studies [27].

Antiradical activity of A. fastigiata extracts

The ethanol extract (A.Fas.4) exhibited the highest activity (up to 79.21% at 1.0 mg/mL) (Table III) because ethanol effectively extracts polyphenols and flavonoids, which have potent antioxidant properties [28]. These findings are consistent with previous studies on the genus *Ajania*, which have reported that ethanol and methanol extracts tend to exhibit the highest anti-

oxidant activities due to their ability to extract polar compounds [39].

The ethyl acetate extract (A.Fas.3) showed moderate activity, as it can extract phenolic compounds, but to a lesser extent than ethanol [19]. The hexane (A.Fas.1) and chloroform (A.Fas.2) extracts demonstrated low activity since nonpolar solvents primarily extract terpenes and sterols, which have weak antioxidant effects [30]. The essential oil (A.Fas.0) had the lowest activity, explained by its composition, which mainly includes volatile compounds with minimal antiradical properties. Compared to gallic acid (93.40% at 1.0 mg/mL) (Figure 3), only the ethanol extract approached its effectiveness, confirming its high content of active antioxidant compounds.

Table III

Antiradical activity (%) of *A. fastigiata* extracts at different concentrations

№	Sample	Different Concentrations (%)			
		0.25	0.5	0.75	1.0
1	Gallic Acid (GA)	83.94	91.32	92.50	93.40
2	Hexane Extract (A.Fas.1)	8.72	9.41	9.63	12.80
3	Chloroform Extract (A.Fas.2)	10.76	11.81	14.03	16.90
4	Ethyl Acetate Extract (A.Fas.3)	17.78	17.92	24.68	27.01
5	Ethanol Extract (A.Fas.4)	36.10	45.15	58.67	79.21
6	Essential Oil (A.Fas.0)	8.98	9.81	10.58	10.98

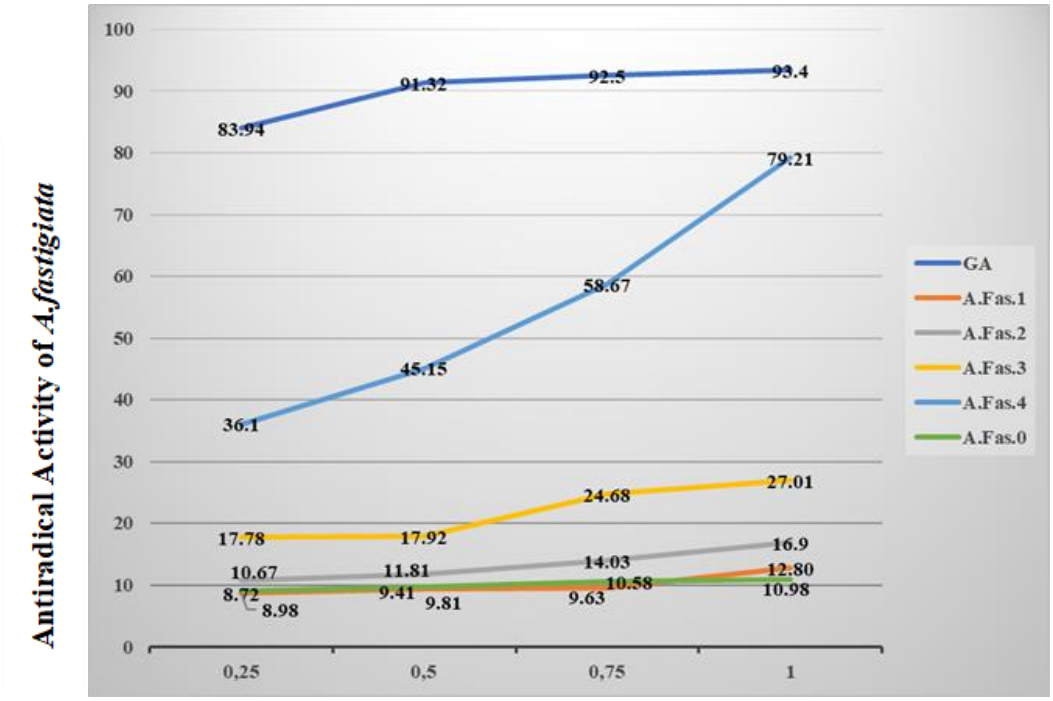


Figure 3.

Antiradical activity (%) of *A. fastigiata* extracts at varying concentrations (0.25 - 1.0 mg/mL), assessed using the DPPH assay

Antioxidant activity of A. fastigiata extracts (FRAP assay).

The antioxidant properties of different extracts of *A. fastigiata* (C. Winkl.) Poljakov were evaluated using the FRAP assay (Table IV).

The ethanol extract (A.Fas.4) demonstrated the highest antioxidant activity among the tested extracts, with an OD of 1.3727 at 1.0 mg/mL, the closest value to GA (1.8705 OD at 1.0 mg/mL). At lower concentrations, its OD values were 0.9099 (0.25 mg/mL), 1.1563 (0.5 mg/mL) and 1.3055 (0.75 mg/mL), confirming its strong reducing power. The chloroform extract (A.Fas.2) exhibited moderate antioxidant activity, with OD values increasing from 0.7144 (0.25 mg/mL) to 1.0677 (1.0 mg/mL) (Figure 4). This suggests that mid-polarity compounds may contribute to its antioxidant potential. The ethyl acetate extract (A.Fas.3) showed relatively low activity, with an OD of 0.8288 at 1.0 mg/mL, indicating that it lacks a significant amount of reducing agents compared to ethanol and chloroform extracts. The hexane extract (A.Fas.1) demonstrated the lowest antioxidant activity, with OD values ranging from 0.5900 (0.25 mg/mL) to 0.7700 (1.0 mg/mL), suggesting that non-polar compounds in *A. fastigiata* do not contribute significantly to antioxidant activity.

Table IV

Absorbance values indicating antioxidant activity (FRAP assay)

№	Sample	Optical Density value at concentration (ng/mL)			
		0.25	0.5	0.75	1.0
1	Gallic Acid (GA)	1.3480	1.5237	1.7191	1.8705
2	Hexane Extract (A.Fas.1)	0.5900	0.6127	0.6333	0.7700
3	Chloroform Extract (A.Fas.2)	0.7144	0.8425	0.9971	1.0677
4	Ethyl Acetate Extract (A.Fas.3)	0.6533	0.7721	0.7810	0.8288
5	Ethanol Extract (A.Fas.4)	0.9099	1.1563	1.3055	1.3727

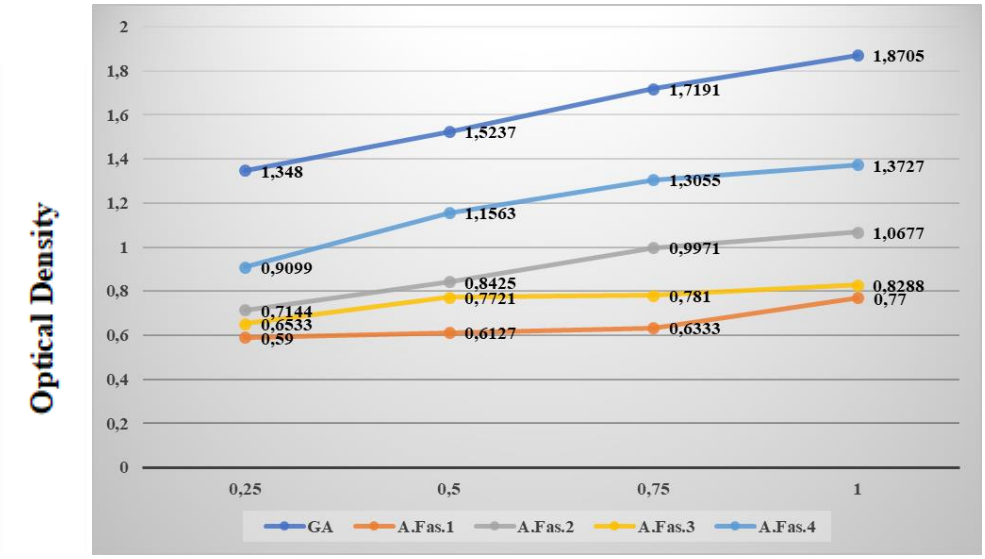


Figure 4.

The antioxidant activity of *A. fastigiata* extracts was measured using a FRAP assay. Optical density (OD) values are shown for different concentrations (0.25 - 1.0 mg/mL), comparing extracts with gallic acid as a standard

Similarly, *Ajania semnanensis (Asteraceae)* extracts demonstrated moderate FRAP activity, supporting the hypothesis that mid-polarity solvents (chloroform and ethyl acetate) extract antioxidant compounds to a lesser extent than ethanol [32].

In another study, essential oils from *Ajania* species showed only minimal antioxidant activity, aligning with the findings for *A. fastigiata* essential oil (A.Fas.0), which exhibited the lowest antiradical activity in previous experiments [16]. Given that oxidative stress plays a key role in the development of diseases such as cardiovascular disorders, neurodegenerative diseases and cancer, plant-based antioxidants are gaining attention for their therapeutic and preventive potential [35].

Conclusions

This study confirmed the antioxidant potential of *Ajania fastigiata* (C. Winkl.) Poljakov, through phytochemical profiling and GC-MS analysis, identified several bio-active compounds with therapeutic relevance. The results suggest potential applications in the development of antioxidant agents for pharmaceutical and nutraceutical products. Further investigations should focus on *in vivo* models, safety profiling and standardisation of extract composition. Overall, these findings provide a scientific basis for the future development and conservation of *Ajania fastigiate*.

Conflict of interest

The authors declare no conflict of interest.

References

1. Aidarbayeva D, Sholpankulova G, Jarkylkapova S, Schokanova A, Natural resources of some medical plants of Kazakhstan. In: *18th International Multi-disciplinary Scientific Geo Conference SGEM*, Sofia, Bulgaria, June 30 – July 9, 2018; 385-393.
2. Amertayeva GA, Mamurova AT, Osmonali BB, Morphological characteristics and anatomical features of the perennial herb *Ajania fastigiata* (C. Winkl.) Poljakov (*Asteraceae*). *Bull Karaganda Univ Biol Med Geogr Ser.*, 2023; 1(109): 28-36.
3. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang C-M, Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, 2022; 27(4): 1326.
4. Bordean ME, Ungur RA, Toc DA, Borda IM, Marțiș GS, Pop CR, Filip M, Vlassa M, Nasui BA, Pop A, Cinteză D, Popa FL, Marian S, Szanto LG, Muste S, Antibacterial and phytochemical screening of *Artemisia* species. *Antioxidants*, 2023; 12(3): 596.

5. Baba SA, Malik SA, Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J Taibah Univ Sci.*, 2015; 9(4): 449-454.
6. Bursal E, Köksal E, Evaluation of reducing power and radical scavenging activities of water and ethanol extracts from sumac (*Rhus coriaria* L.). *Food Res Int.*, 2011; 44(7): 2217-2221.
7. Bergman ME, Davis B, Phillips MA, Medically useful plant terpenoids: Biosynthesis, occurrence, and mechanism of action. *Molecules*, 2019; 24(21): 3961.
8. Chaudhary A, Sharma S, Mittal A, Gupta S, Sharma BK, Singh B, Phytochemical and antioxidant profiling of *Ocimum sanctum*. *J Food Sci Tech.*, 2020; 57(10): 3852–3863.
9. Chen Z, Liu Q, Zhao Z, Bai B, Sun Z, Cai L, Fu Y, Ma Y, Wang Q, Effect of hydroxyl on antioxidant properties of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one to scavenge free radicals. *RSC Adv.*, 2021; 11(55): 34456-34461.
10. Chumbalov TK, Mukhamed'yarova MM, C-glycosides of *Ajania fastigiata*. *Chem Nat Compd.*, 1976; 12(5): 594-595.
11. El Kamari F, El Omari H, El-Mouhdi K, Chlouchi A, Harmouzi A, Lhilali I, El Amrani J, Zahouani C, Hajji Z, Ousaaid D, Effects of different solvents on the total phenol content, total flavonoid content, antioxidant, and antifungal activities of *Micromeria graeca* L. *Biochem Res Int.*, 2024; 2024: 9027997.
12. Enguita FJ, Leitão AL, Hydroquinone: environmental pollution, toxicity, and microbial answers. *Biomed Res Int.*, 2013; 2013: 542168.
13. Fagbemi KO, Aina DA, Olajuyigbe OO, Soxhlet extraction *versus* hydrodistillation using the Clevenger apparatus: A comparative study on the extraction of a volatile compound from *Tamarindus indica* seeds. *Sci World J.*, 2021; 2021: 5961586.
14. Gemejiyeva NG, Sitpayeva GT, Karzhaubekova ZhZh, Choi SH, Paik JH, Medicinal Plants of Kazakhstan, Vol. 1. Korea Research Institute of Bioscience and Biotechnology: Daejeon, Korea, 2022.
15. Grudzinskaya LM, Gemejiyeva NG, Nelina NV, Karzhaubekova ZhZh, Annotated List of Medicinal Plants of Kazakhstan. Almaty, Kazakhstan, 2014; 20(1): 26, 86.
16. Han C, Zhou S, Mei Y, Cao Q, Shi K, Shao H, Phytotoxic, insecticidal, and antimicrobial activities of *Ajania tibetica* essential oil. *Front Plant Sci.*, 2022; 13: 971621.
17. He M, Yasin K, Yu S, Li J, Xia L, Total flavonoids in *Artemisia absinthium* L. and evaluation of its anticancer activity. *Int J Mol Sci.*, 2023; 24(22): 16348.
18. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Chandra Shill M, Karmakar UK, Yarla NS, Khan IN, Billah MM, Pieczynska MD, Zengin G, Malainer C, Nicoletti F, Gulei D, Berindan-Neagoe I, Apostolov A, Banach M, Yeung AWK, El-Demerdash A, Xiao J, Dey P, Yele S, Jóźwik A, Strzałkowska N, Marchewka J, Rengasamy KRR, Horbańczuk J, Kamal MA, Mubarak MS, Mishra SK, Shilpi JA, Atanasov AG. Phytol: A review of biomedical activities. *Food Chem Toxicol.*, 2018; 121: 82-94.
19. Kaczorová D, Karalija E, Dahija S, Bešta-Gajević R, Parić A, Ćavar Zeljković S, Influence of extraction solvent on the phenolic profile and bioactivity of two *Achillea* species. *Molecules*, 2021; 26(6): 1601.
20. Kozhantayeva A, Tursynova N, Kolpek A, Aibuldinov Y, Tursynova A, Mashan T, Mukazhanova Z, Ibrayeva M, Zeinuldina A, Nurlybayeva A, Iskakova Z, Tashenov Y, Phytochemical profiling, antioxidant and antimicrobial potentials of ethanol and ethyl acetate extracts of *Chamaenerion latifolium* L. *Pharmaceuticals*, 2024; 17: 996.
21. Khan AM, Shaikh A, Rahman A, Choudhary MI, Atta-ur-Rahman. Biological and phytochemical investigations on *Ajania fruticulosa* (Ledeb) Poljakov. *Chem Nat Compd.*, 2011; 47(5): 807-809.
22. Lee JY, Park H, Lim W, Song G, Therapeutic potential of α,β-thujone through metabolic reprogramming and caspase-dependent apoptosis in ovarian cancer cells. *J Cell Physiol.*, 2021; 236(2): 1545-1558.
23. Li X, Wu X, Huang L, Correlation between antioxidant activities and phenolic contents of *Radix Angelicae sinensis* (*Danggui*). *Molecules*, 2013; 18(7): 5349-5361.
24. Marboh V, Mahanta CL, Characterisation and antioxidant activity of sohphlang (*Flemingia vestita*), a tuberous crop. *J. Food Sci Technol.*, 2020; 57: 3533-3544.
25. Mostafa AA, Al-Askar AA, Almaary KS, Aly AA, Dawoud TM, El Hamid A, Moussa SH, Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J Biol Sci.*, 2018; 25: 361–366.
26. Mukanova A, Datkhayev U, Abdullabekova R, Alimzhanova M, Khrustalev D, Iskakova Z, Zhurinov M, Rakhimzhanova M, Composition of *Scabiosa ochroleuca* L. extracts prepared by ultrasonic and microwave methods and their antiradical and antioxidant activity assessment. *Farmacia*, 2024; 72(4): 867-874.
27. Nava Lauson CB, Tiberti S, Corsetto PA, Conte F, Tyagi P, Machwirth M, Ma X, Maiorino L, Lagomarsino MC, Taramelli R, Linoleic acid potentiates CD8+ T cell metabolic fitness and antitumor immunity. *Cell Metab.*, 2023; 35(4): 633-650.e9.
28. Palaiogiannis D, Chatzimitakos T, Athanasiadis V, Bozinou E, Makris DP, Lalas SI, Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. *Oxygen*, 2023; 3(3): 274-286.
29. Qun L, Zhang Z, Li Y, Wang H, Li W, Sun C, Zhao L, Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. *J Food Sci Technol.*, 2018; 55(2): 586-593.
30. Saklani S, Mishra AP, Chandra H, Atanassova MS, Stankovic M, Sati B, Shariati MA, Nigam M, Khan MU, Plygun S, Elmsellem H, Suleria HAR, Comparative evaluation of polyphenol contents and antioxidant activities between ethanol extracts of *Vitex negundo* and *Vitex trifolia* L. leaves by different methods. *Plants*, 2017; 6(4): 45.
31. Saunoriūtė S, Ragažinskienė O, Ivanauskas L, Marksa M, Laužikė K, Raudonė L, Phenolic diversity and antioxidant activity of *Artemisia abrotanum* L. and *Artemisia absinthium* L. during vegetation stages. *Separations*, 2023; 10(10): 545.
32. Salehi P, Abedini N, Sonboli A, Aliahmadi A, Ayyari M, Chemical composition and antimicrobial activity of *Ajania semnanensis* essential oil in two growing stages. *J Essent Oil Res.*, 2015; 27(2): 128-134.

33. Seyoum A, Asres K, El-Fiky FK, Structure-radical scavenging activity relationships of flavonoids. *Phytochemistry*, 2006; 67(18): 2058-2070.
34. Sant'Ana da Silva A, Martins GR, Monteiro AF, Amaral FRL, A validated Folin-Ciocalteu method for total phenolics quantification of condensed tannin-rich açaí (*Euterpe oleracea* Mart.) seeds extract. *Food Sci Technol.*, 2021; 58(12): 4693-4702
35. Senoner T, Dichtl W, Oxidative stress in cardiovascular diseases: still a therapeutic target?. *Nutrients*, 2019; 11(9): 2090.
36. Sharipova FS, Chumbalov TK, El'chibekova LA, Zhakipbekova AK, Study of the essential oil of *Ajania fastigiata*. *Chem Nat Compd.*, 1974; 10: 104-105.
37. Shatar S, Staneva J, Altantsetseg S, Todorova M, Essential oil composition of three *Ajania* species from Mongolia. *J Essent Oil Bear Plants.*, 2010; 13(4): 460-464.
38. Sharifi-Rad J, Herrera-Bravo J, Semwal P, Painuli S, Badoni H, Ezzat SM, Shariati MA, Sytar O, Simental-Mendía LE, Scalbert A, *Artemisia* spp.: An update on its chemical composition, pharmacological and toxicological profiles. *Oxid Med Cell Longev.*, 2022; 2022: 5628601.
39. Taldybay A, Aidarbayeva D, Aksoy A, Sitpayeva G, Baiseitova A, Jenis J, *Ajania fastigiata* in Zhetysu Alatau: Distribution, morphological characterization, phytochemical profiles, and optimization of extraction of bioactive constituents. *J Appl Res Med Aromat Plants.*, 2024; 40: 100540.
40. Wiley. *Wiley Registry of Mass Spectral Data & NIST Mass Spectral Library.* https://sciencesolutions.wiley.com/solutions/technique/gc-ms/wiley-registry-nist-mass-spectral-library/.
41. Yao M, Di XJ, Jing ZX, Zhang ZW, Zhang Y, Zhang JL, Jiang W, Yang Y, Research progress on chemical constituents and pharmacological effects of *Ajania* plants. *Zhongguo Zhong Yao Za Zhi*, 2023; 48(11): 2904-2918.