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Chemical Composition and Antioxidant Potential of *Aristolochia maurorum* Above-groud Extract: Phenolic and Phytochemical Analysis

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Abstract
The aim of this study is to evaluate the antioxidant activity, chemical composition, phenolic components, and fatty acid content of extracts obtained from the aerial parts of *Aristolochia maurorum*. The above-groud of *A. maurorum* were collected from remote areas in Havza and Kavak districts of Samsun province, Turkey. The plants were dried, powdered, and extracted with methanol. The total phenolic contents, total flavonoid contents, total tannin contents, and total proanthocyanidin contents of the extracts were determined using spectrophotometric methods. Additionally, DPPH free radical scavenging activity was measured. The fatty acid contents were analyzed using gas chromatography-mass spectrometry (GC-MS). The methanol extract of *A. maurorum* above-groud contains high levels of phenolic compounds and exhibits strong antioxidant activity. The total phenolic content of the extract was found to be 12.24 ± 0.91 mg GAE/g, total flavonoid content 27.45 ± 0.31 mg QE/g, total tannin content 28.41 ± 0.31 mg GAE/g, and total proanthocyanidin content 155.24 ± 32.20 mg CAE/g. The DPPH free radical scavenging activity showed an IC_{50} value of 506.29 ± 38.35 mg/mL. GC-MS analyses demonstrated that the extract contains various bioactive compounds, notably eicosane, which is reported to have anti-inflammatory, analgesic, and antipyretic properties. In conclusion, extracts derived from the above-groud of *A. maurorum* are rich in phenolic compounds and exhibit high antioxidant activity. These findings may contribute to a better understanding of the potential therapeutic uses of *A. maurorum* and could aid in the development of future plant-based medicines.

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1. Introduction

Aristolochiaceae belongs to the order Piperales and comprises seven different genera including *Asarum, Saruma, Lactoris, Hydnora, Prosopanche, Thottea,* and *Aristolochi*a (Wagner et al., 2014). In this family, there are approximately 550 species, which are commonly found in tropical and temperate regions (Zhou et al., 2019). *Aristolochia* is the best-known and most widespread genus within this family (Zhou et al., 2017). *Aristolochia* species are typically perennial, have tuberous roots, and exhibit herbaceous or vine-like structures, known for their striking flowers (Kuo et al., 2012).

Due to its diverse climate, Turkey hosts 29 different species of *Aristolochia*, particularly in regions where the influence of the Mediterranean climate is prevalent. *Aristolochia maurorum* L. is a native species to Türkiye, Lebanon, Syria, and Sinai. Because of its limited distribution range, *Aristolochia* is considered an important species. It is commonly found in the western and central parts of the Black Sea Region, as well as in the western regions of Central Anatolia, extending to the Upper Euphrates basin and the Mediterranean Region.

The various metabolites found in *Aristolochia* plants are chemical compounds that determine the biological effects of the plant and play roles in various biological functions. Among these metabolites are compounds from different chemical groups such as aristolochic acids and derivatives, monoterpenes, diterpenes, triterpenes, alkaloids, and lignans (Al-Barham et al., 2017; Ali et al., 2022; Karaşin et al., 2019; Pacheco et al., 2009).

In the past, *Aristolochia*, which was widely used in traditional Chinese medicine, has also been frequently preferred in other countries for preparing traditional medicines used for human and animal health (Wanke et al., 2006).

Aristolochia species exhibit a wide range of activities including anti-fungal, antioxidant, anti-inflammatory, anti-fibrotic, anti-neuroprotective, anti-ulcer, antiallergic, antidiabetic, anti-dyslipidaemia, antihistaminic, and anticancer activities. They are used in traditional medicine for various purposes including treating bronchitis, constipation, rheumatism, bladder diseases, as well as for the treatment of various animal and insect bites (Pacheco et al., 2009).

Plants harbor a variety of phytochemicals, notably polyphenols, which boast potent antioxidant properties pivotal in neutralizing free radicals, quenching singlet and triplet oxygen, and degrading peroxides (Anderson et al., 2001). The surge in research endeavors in recent years has focused on investigating the biological activities and compositions of plants (Dicson et al., 2015) crucial for assessing antioxidant potential and discerning associated health advantages.

Lipids are ubiquitous across plant tissues, serving as nutritional reservoirs and contributing to the management of diverse cardiovascular ailments (Chen and Chuang, 2002; López-Miranda et al., 2006). The lipogenic attributes of polyunsaturated fatty acids can be leveraged to augment the therapeutic efficacy of anticancer medications (Wang et al., 2012). Noteworthy fatty acids like palmitic and stearic acid find widespread applications in pharmaceutical and cosmetic sectors (Kalustian, 1985). The extraction and preservation of natural antioxidants, with the aim of supplanting synthetic oxidants, emerge as pivotal objectives for the future landscape of the health, pharmaceutical, and cosmetic industries (Binic et al., 2013).

Although numerous phytochemical and biochemical studies have been conducted on *Aristolochia*, there is no study available specifically on *A. maurorum*. We know that the biological activities of *A. maurorum* have not been fully explored, and their therapeutic potentials have yet to be fully discovered. This study aims to evaluate extracts obtained from the above-groud parts of *A. maurorum*. This evaluation includes investigating antioxidant activity, chemical composition, phenolic compounds, and fatty acid content.

2. Materials and Methods

2.1. Collection of plant material

A. maurorum samples were collected from grassy and rocky areas along the old road between Havza and Kavak, specifically at the 3rd kilometer opposite Boğaziçi Petrol. The collected plants were identified by Dr. Alper Durmaz. The collected specimens were recorded under the herbarium number OMUB-9032. When sampling from *A. maurorum* populations, only the aboveground parts were pruned to ensure the plant's undamaged continuity and population sustainability. The collected plant materials were dried in a plant and soil laboratory and prepared for analysis. These materials were stored in containers without air until the analysis process.

2.2. Plant extraction

The above-groud parts of *A. maurorum* were initially dried in an oven at 40°C and then ground into powder using a blender. The maceration method suggested by Aytar (2024) was employed for extraction. The aboveground parts, dried at 40°C, were extracted with methanol at room temperature for two days in a dark environment. The obtained methanolic extracts were filtered with filter paper. After filtration, the solvent was evaporated under reduced pressure at 40°C using a rotary evaporator, and solid extracts were stored at +4°C (Aytar et al., 2024). The morphological structure of the *A. maurorum* plant is shown in Figure 1.

Figure 1. *A. maurorum plant morphological structure*

2.3. Spectroscopic analysis of secondary metabolites

2.3.1. Total phenolic content

In this study, equal volumes of the sample and diluted Folin-Ciocalteu reagent were mixed. After incubating at room temperature for 3 minutes, 1 mL of 2 % sodium carbonate solution was added. The mixture was then left to incubate in the dark at room temperature for 1 hour, followed by measuring the absorbance at 760 nm using a UV spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g extract). All measurements were performed in triplicate (Singleton and Rossi, 1965).

2.3.2. Total flavonoid content

This method was assessed using the $AlCl_3$ method with minor adjustments, following the procedure by Osuna-Ruiz et al., (2016). Extracts were mixed with distilled water, followed by NaNO2 (5 %) addition and a standing period. Subsequently, $AlCl_3$ (10 %) was added, and the solution underwent

incubation. NaOH (1M) was then introduced, and the solution was left at room temperature. Absorbance was measured using a UV spectrophotometer, and total flavonoid content was quantified as quercetin equivalent (QE) per gram of dried extract (mg QE/g). All measurements were performed in triplicate (Osuna-Ruiz et al., 2016).

2.3.3. Total flavanols content

The total flavonoid content was determined using the aluminium chloride method. Briefly, 1 mL of the extracts was mixed with of $AlCl_3$. Subsequently, 3 mL of sodium acetate solution was added. The mixture was then kept at room temperature in a dark environment for 30 minutes. After the incubation period, the absorbance of the sample was measured at 415 nm using quercetin as the standard. The total flavanols was expressed as quercetin equivalents (mg QE/g) (Mahmoudi et al., 2023).

2.3.4. Total tannin content

Modified according to the method specified by Lou et al. (2014). A calibration curve was prepared using different concentrations of gallic acid in methanol. Samples were mixed with diluted Folin–Ciocalteu reagent in water and aqueous sodium carbonate solution. After incubation in darkness at room temperature, absorbance was measured at 760 nm. Total tannin content was quantified as gallic acid equivalent (GAE) per gram of dried extract (mg GAE/g). All measurements were performed in triplicate.

2.3.5. Total proanthocyanin content

Modified according to the method specified by Porter et al. (1985). Diluted phenolic extract was mixed with n-butanol/HCl reagent, followed by the addition of ferric ammonium sulfate in HCl. After boiling and cooling, the absorbance of the solutions was measured at 550 nm. Total proanthocyanidins content was expressed as catechin equivalent (CAE) per gram of dried extract. All measurements were performed in triplicate.

2.4. Determination of antioxidant capacity

2.4.1. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay

Modifications were made based on the procedure of Braca et al. (2001). The free radical scavenging potential of the extract was determined using the DPPH assay. For this test, various concentrations of 1 mL extract were mixed with a methanol solution of DPPH radical (0.1 mM) in a tube. The mixture was left to incubate in the dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm using a UV spectrophotometer against a blank. Ascorbic acid was used as the standard (Braca et al., 2001).

The percentage inhibition of DPPH radical scavenging activity of the extract was calculated using the following equation:
DPPH scavenging activity (% inhibition) =

$$[(A_{\_control} - A_{\_sample}) / A_{\_control}] \times 100.$$

To determine the extract concentration that would cause a 50% reduction in the initial DPPH concentration, a concentration curve against the percentage inhibition of the extract was plotted, and the IC_{50} value was obtained by linear regression analysis. A lower IC_{50} value indicates a stronger antioxidant capacity of the extract.

2.5. Extraction and analysis of fatty acids

Fatty acid analysis of *A. maurorum* samples was conducted according to the procedure outlined by Aytar et al. (2023). The above-groud parts of the plant at the flowering stage were collected and dried at 40°C before being ground into powder. Oil extraction was performed using a Soxhlet apparatus. For gas chromatography analysis (Thermo Scientific Trace 1310), fatty acids were converted into methyl esters. Samples were analyzed using a Thermo Scientific ISQ LT GC/MS system with a Trace Gold TG-WaxMS capillary column. Fatty acids were identified by comparing their retention times with a standard FAME mixture containing 37 components.

2.6. Gas Chromatography-mass spectroscopic analysis of *A. maurorum*

For gas chromatography-mass spectrometry (GC-MS) analysis following the methodology outlined by (Aytar et al. 2023). Following this, the samples underwent centrifugation at 3500 revolutions per minute for 10 minutes, and the resulting supernatant was utilized for GC-MS analysis. The GC-MS analysis was conducted in accordance with the protocol, utilizing the NIST Standard Reference Database.

2.7. Statistical analysis

To determine the relationship between two variables, correlation coefficients (R) were calculated using the CORREL statistical function in MS Excel software. Data are expressed as mean ± SD obtained from three separate observations. The analysis of the data was conducted using SPSS 21.

3. Results and Discussion

The total phenolic content of the extract obtained from the above-ground parts was determined to be 12.24 ± 0.91 mg GAE/g crude extract, the total flavonoid content was 27.45 ± 0.31 mg QE/g crude extract, the total flavonol content was 98.92 ± 9.08 mg QE/g crude extract, the total tannin content was 28.41 ± 0.31 mg GAE/g crude extract, and the total proanthocyanidin content was 155.24 ± 32.20 mg CAE/g crude extract. The DPPH content (IC_{50} mg/mL) of the extract obtained from the above-ground parts was found to be 506.29 ± 38.35 mg QE/g crude extract. These results indicate that the above-ground parts are rich in phenolic compounds, and the contents are shown in the Table 1. Additionally, Table 1 demonstrates the DPPH radical scavenging activity of the above-groud parts of *A. maurorum*, which is associated with high levels of radical scavenging activity, indicating high antioxidant activity.

Table 1. DPPH radical scavenging activities of *A. maurorum* extracts (IC_{50} (μg mL^{-1}) ± SD) and total flavonol, flavonoid, phenolic, proanthocyanidin, and tannin contents ± SD* Values

Plant Name	DPPH (IC_{50} μg mL^{-1})	Total Flavonol Compound (mg QE/g extract)	Total Flavonoid Compound (mg QE/g extract)	Total Phenolic Compound (mg GAE/g extract)	Total Proanthocyanidin content (mg CAE/g extract)	Total Tanen Content (mg GAE/g extract)
Aristolochia maurorum	506.29±38.35	98.92 ± 9.08	27.45 ± 0.31	12.24± 0.91	155.24 ± 32.20	28.41 ±0.31

In the study conducted by El et al. in 2020, the extract was obtained from the roots of *Aristolochia longa*. Additionally, the methanolic extract was reported to have a flavonoid content of 34.02 ± 1.87 mg QE/g and a total polyphenol content of 89.41 ± 4.96 mg GAE/g. When evaluated using the DPPH method, the IC_{50} value of the methanolic root extract of *A. longa* was determined to be 1.32 mg/mL (El et al., 2020). In the study conducted by Bourhia et al. in 2019, the total phenolic and flavonoid contents of methanolic extracts of *A.baetica* and *A. paucinervis* were determined to be 360 ± 20 mg GAE/g and 280 ± 27 mg GAE/g, respectively. Additionally, the total flavonoid contents of *A. baetica* and *A. paucinervis* methanolic extracts were found to be 35 ± 8 mg QE/g and 235 ± 7 mg QE/g, respectively. For DPPH radical scavenging activity, the IC50 values of *A. baetica* and *A. paucinervis* methanolic extracts were approximately determined to be 150 ± 8 μg ml^{-1} and 160 ± 10 μg/ml, respectively (Bourhia et al., 2019).

The methanol extract of the combination of *A. indica* and *Piper nigrum* was reported to have a total phenolic content of 150.65 ± 0.08 mg GAE/g extract and a flavonoid content of 158.97 ± 0.93 mg RE/g extract. For DPPH radical scavenging activity, the IC_{50} values of

the methanol extract of the combination of *A. indica* and *P. nigrum* were approximately determined to be 3.05 µg/mL. In the same study, it was reported that the phenolic content of *A. indica* root extract was determined to be 54.27 ± 0.77 mg GAE/g extract (Sivaraj et al., 2018).

It can be said that in this study, we determined the DPPH radical scavenging activity of the methanol extract of *A. maurorum*, demonstrating its ability to effectively neutralize free radicals. These findings highlight the high antioxidant properties of *A. maurorum* and the significant role of phenolic compounds in this activity.

We analyzed the methanol extract of the above-ground parts of *A. maurorum* to determine various bioactive contents. The retention time (RT), concentration (% area), and chemical structure of 15 bioactive phytochemical compounds in the extract are presented in Tables 2. The main components identified in the above-ground part of *A. maurorum* were eicosane (%31.57), 2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxane (% 17.26), heneicosane (% 6.28), pentadecane (% 6.13), myristate <methyl-> (% 3.82), 12-methoxy-3-methylcholan (% 3.64), and 9,12-octadecadienoic acid (Z, Z) (% 3.63) (Table 2).

Table 2. GC-MS analysis results of *A. maurorum*

No	Retention time(min)	Name of the compound	Area %
1	3.400	Glycerin	2.11
2	3.880	2,2-dimethoxybutane	1.55
3	18.564	o-Tolualdehyde	2.12
4	22.265	Benzenemethanol, 3-hydroxy-	2.52
5	25.821	Levoglucosan	2.02
6	29.001	Pentadecane	6.13
7	33.646	Eicosane	31.57
8	36.350	Myristate	3.82
9	37.723	1-docosanol	1.48
10	39.763	9,12-Octadecadienoic acid (Z, Z)-	3.63
11	39.885	9,12,15-Octadecatrienoic acid, methyl ester, (9Z,12Z,15Z)-	1.49
12	41.684	Heneicosane	6.28
13	43.334	3-Ethoxy-3-methylhexane	3.64
14	43.466	1-Pentacontanol	2.34
15	43.772	1-(3-Hydroxy-4-methoxyphenyl) ethanone	17.26

Furthermore, we conducted an analysis to determine various bioactive components of the methanol extract of *A. maurorum* above-groud parts. Among the major components identified in the above-groud parts, linoleic acid (% 25.96), alpha-linolenic acid (% 35.94), palmitic acid (% 20.55), stearic acid (% 3.20), lignoceric acid (% 1.55), palmitoleic acid (% 1.21), and oleic acid (% 6.19) were found (Table 3 and Figure 2).

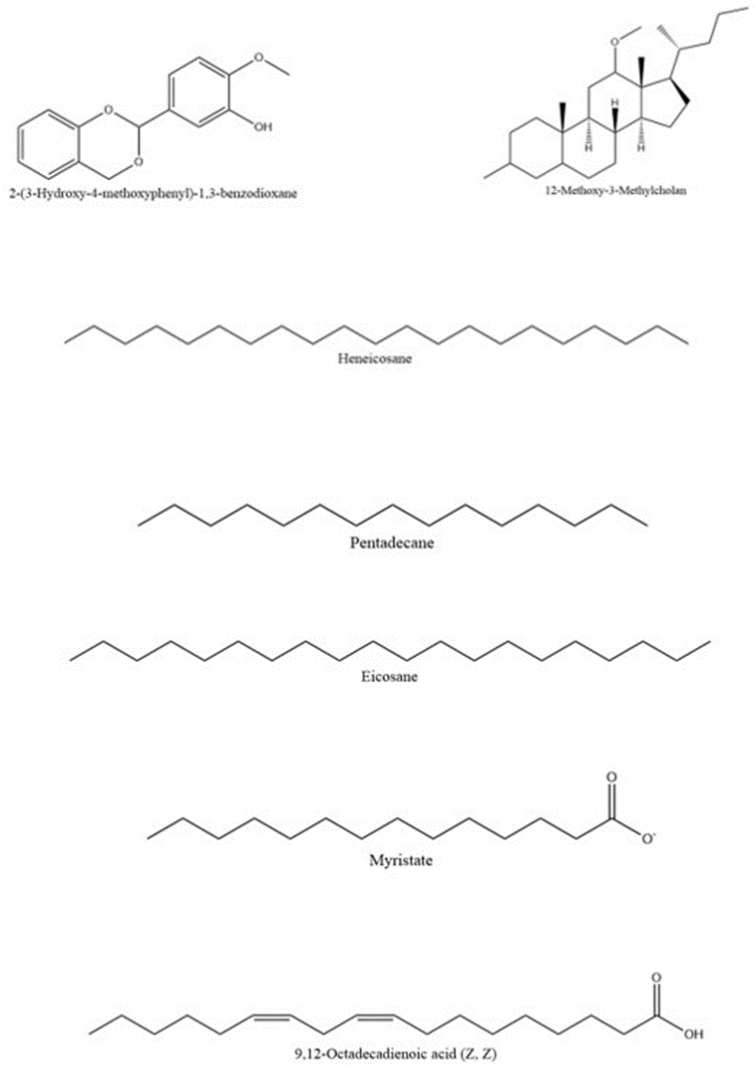

Figure 2. *A. maurorum* major component structure

In the study by Okechukwu et al. (2020), among the identified major compounds of *A. maurorum*, Eicosane has been reported to possess anti-inflammatory, analgesic, and antipyretic properties (Okechukwu, 2020). Additionally, linoleic acid has been reported to possess anti-inflammatory effects. (Kolar et al., 2019). The study has extracted compounds with diverse pharmacological properties from all parts of *A. maurorum*.

Table 3. Results of fatty acid composition in *A. maurorum*

No	Retention time(min)	Name of the fatty acid	Area %
1	24.40	Palmitic Acid	20.55
2	25.07	Palmitoleic Acid	1.21
3	27.94	Stearic Acid	3.60
4	28.57	Oleic Acid	6.19
5	29.67	Linoleic Acid	25.96
6	30.95	Alpha Linolenic Acid	35.94
7	36.94	Lignoceric Acid	1.55

In the study by Bourhia et al. (2019), the phytochemical composition of *A. paucinervis* methanol extracts were analyzed using GC-MS, uncovering the presence of dihydro-4,4,5,5-tetramethyl 2(3H)-furanone, mesitylene, dodecane, maaliol, 2-palmitoylglycerol, dioctyl terephthalate, phthalic acid, and di(2-propylpentyl) ester. Furthermore, the phytochemical composition of A. baetica methanolic extracts was determined via GC-MS analysis, revealing the presence of pseudocumen, tetrasiklodekan-10-one, p-vinylguaiacol, 2-epi-trans-p-caryophyllene, guaia-6,9-diene, pacifigorgiol, maaliol, methylglucoside, isoaromadendrene epoxide, and trans-sinapyl alcohol (Bourhia et al., 2019). In the study by Das et al. (2016), the phytochemical composition of A. bracteolata methanol extracts were determined using GC-MS analysis, revealing the presence of dodecane (7.50 %), tetradecane (7.31 %), tridecane (6.59 %), undecane (6.29%), pentadecane (3.99 %), hexadecane (2.69 %), and decane (1.49 %) (Das et al., 2016).

The diverse phytochemical compositions observed across *Aristolochia* species indicate variations in their potential medicinal uses. Phytochemical analyses play a crucial role in understanding the pharmacological effects of plants and help in identifying potential therapeutic compounds. Such studies provide valuable insights for the development of plant-based medicines and the advancement of herbal medicine.

This research represents one of the first significant investigations on *A. maurorum*, providing detailed insights into its antioxidant activity and phenolic components. Previous studies have indicated similar antioxidant properties in *Aristolochia* species. Given the absence of prior research on *A. maurorum* in the literature, this study stands as one of the pioneering inquiries into this plant.

4. Conclusions

In conclusion, this study demonstrates that the methanol extract obtained from the above-groud parts of *A. maurorum* possesses a wide range of phenolic compounds and exhibits potent antioxidant activity. The high content of total phenolics, flavonoids, flavonols, tannins, and proanthocyanidins in the extract suggests that it may serve as a source of biological activities in the plant. Additionally, significant levels of fatty acids such as linoleic acid and alpha-linolenic acid were identified in the extract, enhancing its nutritional value. These findings suggest that *A. maurorum* harbours significant pharmacological and nutritional potential. Further research is warranted to elucidate the biological activities of this plant in more detail and to better understand its health benefits. The outcomes of this study could contribute to the development of new products in the pharmaceutical and food industries by providing insights into the usability and potential therapeutic applications of the plant.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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