MedCrave
Step into the World of Research
Pharmacy & Pharmacology International Journal

Research Article
Open Access
CrossMark

UPLC-PDA-ESi-MS based profiling of phytochemicals in wild *Achillea fragrantissima* from Palestine

Abstract

Drug development often begins with the discovery of bioactive compounds derived from medicinal plants. This process involves isolating, purifying, and identifying the active ingredients responsible for the plant's therapeutic effects. Phytochemicals from ethnopharmacologically recognized plants are valuable resources for drug discovery. In Palestine, *Achillea fragrantissima (A. fragrantissima)* is one of the most commonly used medicinal plants, known for its diverse therapeutic properties in treating various ailments. The aim of this study was to identify the primary phenolic compounds present in a wild water infusion of *A. fragrantissima*. Using a UPLC-PDA-ESi-MS approach, we successfully identified a range of phenolic compounds, including several flavonoid derivatives such as kaempferol 3-*O*-(6"-malonyl-glucoside), pelargonidin 3-*O*-arabinoside, phloretin, catechin, and rutin. These compounds are well-known for their antioxidant and anti-inflammatory effects. Additionally, we identified a variety of phenolic acids, including hydroxycaffeic acid, dihydrocaffeic acid, ferulic acid 4-*O*-glucoside, and gallic acid. Notably, resveratrol 3-*O*-glucoside and resveratrol 5-*O*-glucoside were also detected, compounds recognized for their potential anti-cancer, cardiovascular, and neuroprotective properties. This study highlights the rich phytochemical profile of *A. fragrantissima*, reinforcing its therapeutic potential and providing a foundation for future drug development from this plant species.

Keywords: *Achillea fragrantissima*, leaves, infusion, polyphenols, flavonoids, phenolic acids, UPLC-PDA-ESi-MS

Volume 12 Issue 6 - 2024

Sawsan Salameh, Saleh Abu-Lafi
Faculty of Pharmacy, Al-Quds University, Palestine

Correspondence: Saleh Abu-Lafi, Faculty of Pharmacy, Al-Quds University, P. O. Box 20002, Abu-Dies, Palestine, Tel + +972-2-2799360, Email sabulafi@staff.alquds.edu

Received: November 11, 2024 | **Published:** November 22, 2024

Abbreviations: LC-PDA-ESi-MS, UPLC coupled to photodiode array and quadrupole mass spectrometry in the electrospray ionization mode; UPLC, ultra-performance liquid chromatography; PDA, photodiode array; ESi, electrospray ionization; MS, mass spectrometry; QDa, quadrupole mass analyzer detector; GC-EI-MS, gas chromatography electron impact mass spectrometry; LC-MS/MS, liquid chromatography triple quadrupole mass spectrometry; ACN, acetonitrile; FA, formic acid; H_2O, water; UV-Vis, ultraviolet-visible spectroscopy; PTFE, polytetrafluoroethylene; min, minute; (w/v), weight by volume; RT, retention time; Mwt, molecular weight

Introduction

Palestine is home to a remarkably rich and diverse flora, with over 2,953 documented plant species, approximately 700 of which are recognized for their medicinal properties.[1] Ethnopharmacological practices have long been a significant part of Palestinian culture, playing an important role in both human and animal healthcare, particularly in treating a variety of ailments.[1] Traditional knowledge and the use of medicinal plants are integral to the daily lives of many Palestinians, reflecting a deep connection between people and their natural environment.

Among the diverse plant species, the genus *Achillea* (family Compositae/Asteraceae) stands out, with approximately 140 known species.[2] Of particular interest is *Achillea fragrantissima* (locally known as Qaisoom), a wild medicinal shrub native to the Arabian region. This species thrives in the dry, semi-desert and desert climates typical of the area.[3] Notably, *A. fragrantissima* is the only member of the *Achillea* genus commonly used in Palestinian folk medicine. The plant is primarily consumed as an infusion tea to treat a variety of health conditions. Its traditional uses include alleviating nerve spasms, treating allergies, and controlling bleeding. The infusion is believed to help with both internal and external bleeding, including conditions like stomach ulcers and bleeding wounds. Additionally, it is used to reduce stomach acidity. In certain areas, particularly in the eastern foothills of the Palestinian mountains, the flowers of *A. fragrantissima* are used in the form of an infusion or decoction to treat skin conditions such as acne and hemorrhoids. Bedouins living in these regions also use the plant to relieve toothaches by rubbing the flowering branches on inflamed gums. In pharmacological terms, *A. fragrantissima* is known for its potential to treat gastrointestinal spasms, hepatobiliary disorders, and skin inflammations, in addition to promoting wound healing and acting as an appetite stimulant.[3]

Extensive research has been conducted on the pharmacological activities and chemical constituents of *Achillea* species, including *A. fragrantissima*.[4–16] These studies have revealed a complex array of bioactive compounds, including polyphenols and flavonoids, whose profiles vary depending on the *Achillea* species, solvent used in extraction, and other experimental conditions. This variability highlights the importance of analyzing different species, parts, extracts to better understand the phytochemical profiles of the plant and their corresponding biological activities.

Building on this, we conducted a study on two *Achillea* species, *Achillea santolina* and *Achillea fragrantissima*, both of which grow wild in Jordan.[4]

The plants were extracted using ethyl acetate, and the resulting extracts were evaluated for their antibacterial activity. HPLC-PDA analysis of the extracts revealed the presence of significant amounts of flavonoids and phenolic compounds. The antibacterial activity observed was primarily attributed to the high concentration of phenol-based compounds, which are known for their antimicrobial properties. These results reinforce the therapeutic potential of *Achillea* species, particularly in traditional medicine for treating infections.[5] *Achillea*

fragrantissima, a desert plant native to Saudi Arabia, has been subjected to various analyses that highlight its impressive therapeutic properties. In a study using LC-MS in both negative and positive ion modes, 47 distinct phytochemicals were identified in the plant, demonstrating its potential for antibiofilm, antimicrobial, and wound-healing activities *in vivo*.

These findings underscore *A. fragrantissima* as a valuable medicinal plant, particularly in combating infections and aiding in tissue repair.[5] Further research on the pharmacological properties of *A. fragrantissima* has highlighted its anticonvulsant potential. In a study of the ethanolic extract from *A. fragrantissima* grown in Saudi Arabia, the plant demonstrated potent anticonvulsant effects in a PTZ induced seizure model. This activity may be due to its GABA agonistic properties and/or its antioxidant activity. While the precise mechanism behind this anticonvulsant effect remains unclear, these findings suggest that *A. fragrantissima* could hold promise as a natural alternative in seizure management.[6] Moreover, the antioxidant properties of *A. fragrantissima* were further explored through the *in vitro* analysis of a hydroalcoholic extract derived from the plant's aerial parts, collected from Egypt.

Using HPLC-MS, polyphenolic compounds were identified, with the primary constituents being flavones, benzoic acids, cinnamic acids, and flavonols. These compounds are known for their antioxidant effects, suggesting that *A. fragrantissima* could serve as a natural source of antioxidants, potentially beneficial in mitigating oxidative stress damage, particularly in cells of the central nervous system.[7] N-alkylamide profiling has been carried out on ethanolic extracts of two *Achillea* species, *Achillea ptarmica* and *Achillea millefolium*, using HPLC-ESi-MS and GC-EI-MS.[8] Using LC-MS/MS, feruloylquinic acid, luteolin, luteolin glucoside, isorhamnetin, isovitexin, methoxyflavonoid like chrysoeriol and its glycoside derivative were detected.[11] The volatile compounds in *Achillea falcata L.* were analyzed through GC-MS of water-distilled oil collected from Turkey. A total of 60 components were identified, accounting for approximately 95% of the overall oil composition. The primary constituents identified were 1,8-cineole, camphor, and α-pinene.[13]

Other *Achillea* genus plants have also been investigated for their wide range of bioactive compounds and pharmacological activities. These include antidiabetic, anti-tumor, antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties, further establishing the genus as a significant source of therapeutic agents.[3, 9–16]

To date, no comprehensive study has documented on the phenolic compounds present in wild populations of *A. fragrantissima* in Palestine. Our study aims to fill this gap by providing the first detailed analysis of the plant chemical constituents using UPLC-PDA-ESi-MS. This analysis offers a better understanding of the plant medicinal potential and paves the way for future pharmacological research into its health benefits.

Materials and methods

Collection and identification of plant material

The wild *Achillea fragrantissima* plant was randomly selected and collected from Bani Naim, near Hebron, Palestine, in June 2024. The plant samples were harvested, and the leaves and stems were separated. These parts were dried individually in the shade at room temperature to prevent any degradation of active compounds. The air-dried leaves were then mechanically ground into a fine, homogeneous powder and stored at 4°C until extraction.

Sample preparation

One gram of the dried *A. fragrantissima* plant powder was infused in 100 mL of 100% pure boiled water (*1% w/v*) for 30 minutes. The resulting extract was filtered through filter paper. It was then frozen and lyophilized to obtain the active raw water extract. The stock extract was stored in the refrigerator for further analysis. Before injection into the UPLC-PDA-ESI-MS system, the sample was diluted and filtered using a 0.45µm PTFE filter, resulting in a concentration of 0.01 mg/mL (10 ppm).

Chromatographic analysis using UPLC-PDA-ESI-MS

The analysis was performed using Ultra-Performance Liquid Chromatography (UPLC) coupled with a Photodiode Array Detector (PDA) and a Quadrupole Mass Analyzer (QDa). The system used was the Acquity UPLC H-Class system (Waters, USA), controlled by Empower software. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm I.D., 1.7 µm) along with an Acquity BEH C18 1.7 µm guard column (Vanguard 2.1 × 5 mm, Waters, USA). The solvents used for analysis were ultrapure water (LC-MS grade, Milli-Q Millipore), acetonitrile (Lichrosolv hypergrade, J.T. Baker), and formic acid (LC-MS grade, Merck). The column and sample temperatures were set at 40°C and 20°C, respectively. The mobile phase consisted of two components: 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient elution started at 98% A and decreased to 50% A over a 30-minute period. A 7-minute delay injection time was applied to allow for column equilibration. The flow rate was set to 0.3 mL/min. The PDA detector operated across a range of 210-400 nm. Mass spectrometry (MS) analysis was conducted using both negative and positive Electrospray Ionization (ESI) modes. The mass spectrometer detected ions within a mass range of 150-1000 Da.

Results

UPLC-PDA-ESi-MS of infused water extract of wild *Achillea fragrantissima* leaves

The phytochemical composition of wild *A. fragrantissima* leaves was comprehensively analyzed through UPLC-PDA-ESi-MS machine using a freshly collected sample. This analytical approach enabled the identification and characterization of a wide range of bioactive compounds present in the leaves. Detection was performed with the PDA detector measuring the absorption maxima across multiple wavelengths and the single wavelength was set to 240 nm, while the mass spectrometer was operated in both positive and negative ESi modes to capture a broad spectrum of molecular ions. Figure 1 through Figure 3 illustrates the chromatographic profiles obtained from the PDA and MS analysis.

Table 1 presents a detailed summary of some of the key active compounds detected in the leaf extract, including their elution times, molecular weights and their *m/z* in the negative ESi mode. Figure 4 provides a representation of the chemical structures of the major polyphenolic constituents identified in the leaves. These structures highlight the diversity of flavonoids, phenolic acids, and other active compounds that contribute to the plant's bioactivity. Further investigation into the water extract of *A. fragrantissima* reveals the presence of bioactive N-alkylamides, which are illustrated in the MS profile shown in Figure 5. These compounds were only detected in the positive ESi mode, offering insight into their molecular characteristics and potential pharmacological effects. In contrast, Figure 6 shows the MS profiles of active compounds detected in the negative ESi mode, providing a complementary view of the plant's chemical diversity.

Citation: Salama S, Abu-Lafi S. UPLC-PDA-ESi-MS based profiling of phytochemicals in wild *Achillea fragrantissima* from Palestine. *Pharm Pharmacol Int J.* 2024;12(6):202–208. DOI: 10.15406/ppij.2024.12.00453

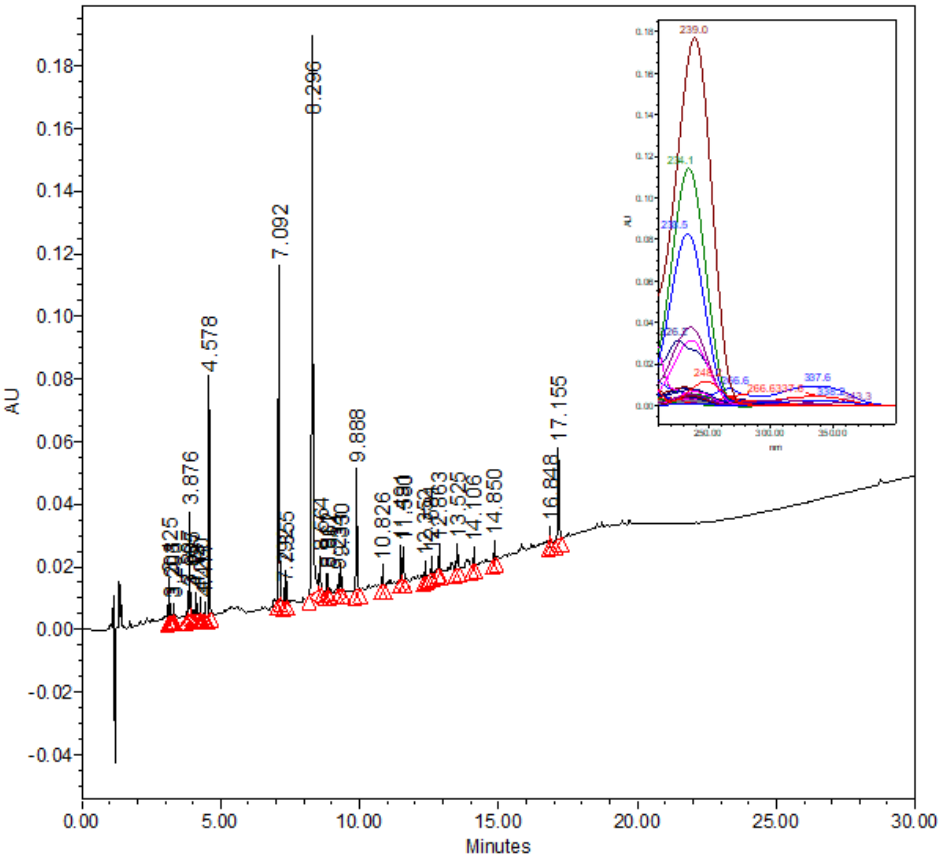

Figure 1 UPLC-PDA chromatogram of the water infusion extract from wild *A. fragrantissima* leaves at 240 nm. On the right side, the overlaid UV-Vis spectra of all the peaks are presented.

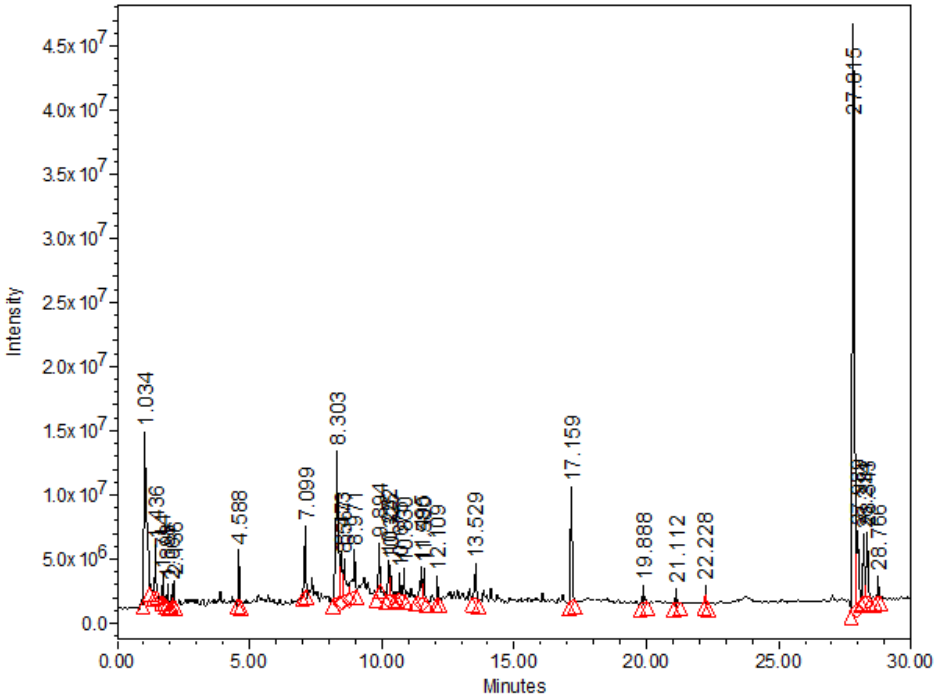

Figure 2 TIC (Total Ion Chromatogram) of the water-infused extract from wild *A. fragrantissima* leaves using UPLC-MS-ESi in the negative ion mode, with a full scan range from 150 to 1000 Da.

Citation: Salama S, Abu-Lafi S. UPLC-PDA-ESi-MS based profiling of phytochemicals in wild *Achillea fragrantissima* from Palestine. *Pharm Pharmacol Int J.* 2024;12(6):202–208. DOI: 10.15406/ppij.2024.12.00453

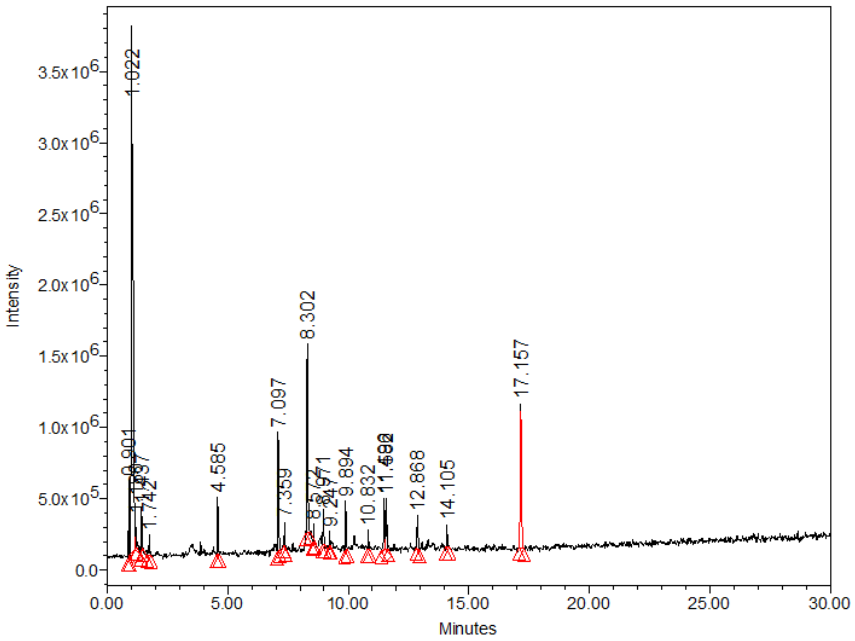

Figure 3 TIC (Total Ion Chromatogram) of the water-infused extract from wild *A. fragrantissima* leaves using UPLC-MS-ESi in the positive ion mode, with a full scan range from 150 to 1000 Da.

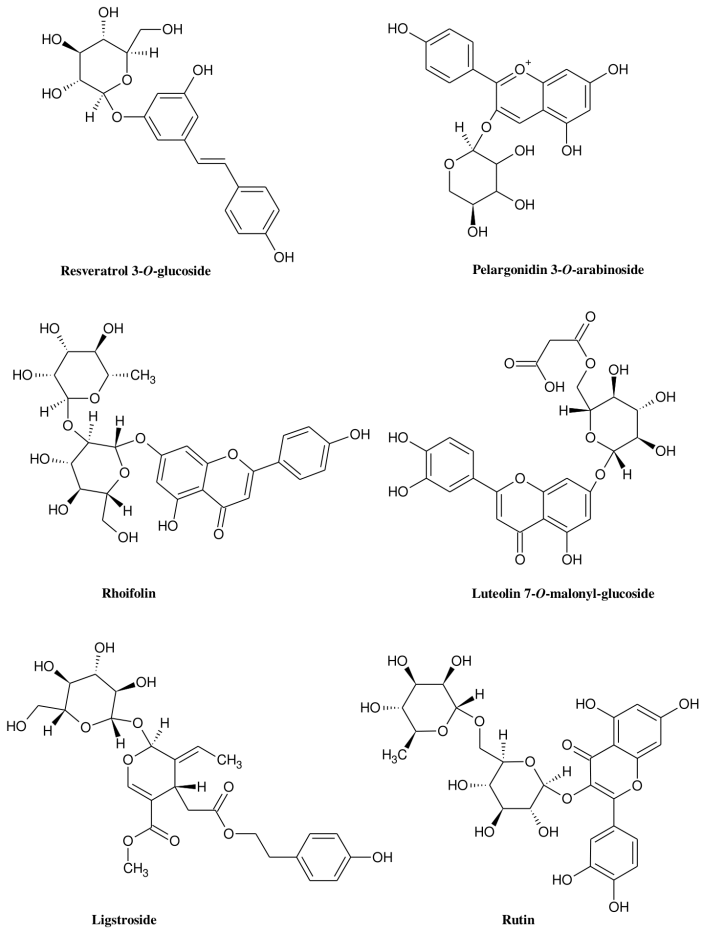

Figure 4 The chemical structures of the major polyphenolic constituents in the wild leaves of *A. fragrantissima*

Citation: Salama S, Abu-Lafi S. UPLC-PDA-ESi-MS based profiling of phytochemicals in wild *Achillea fragrantissima* from Palestine. *Pharm Pharmacol Int J.* 2024;12(6):202–208. DOI: 10.15406/ppij.2024.12.00453

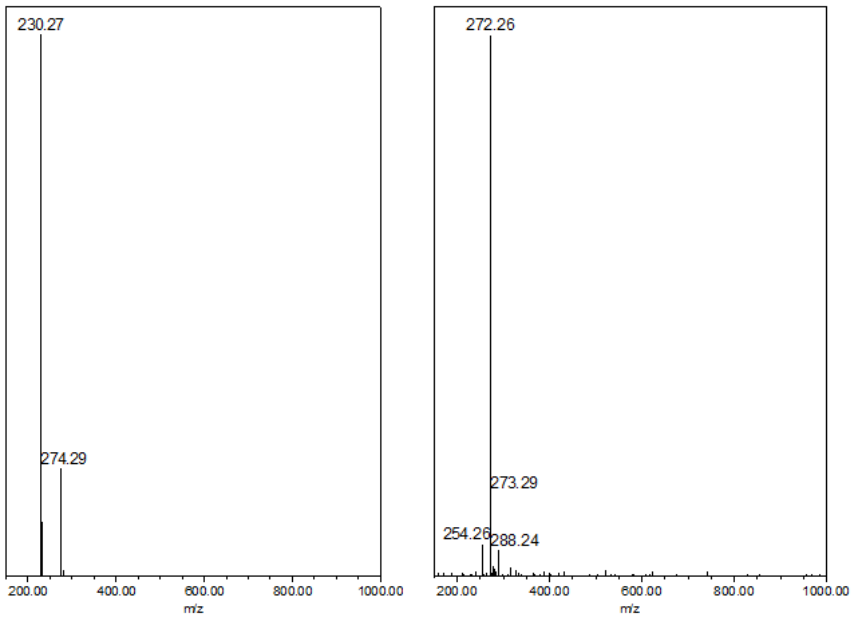

Figure 5 MS spectra of bioactive N-alkylamides present in the water extract of wild *A. fragrantissima*, utilizing positive ESi mode.

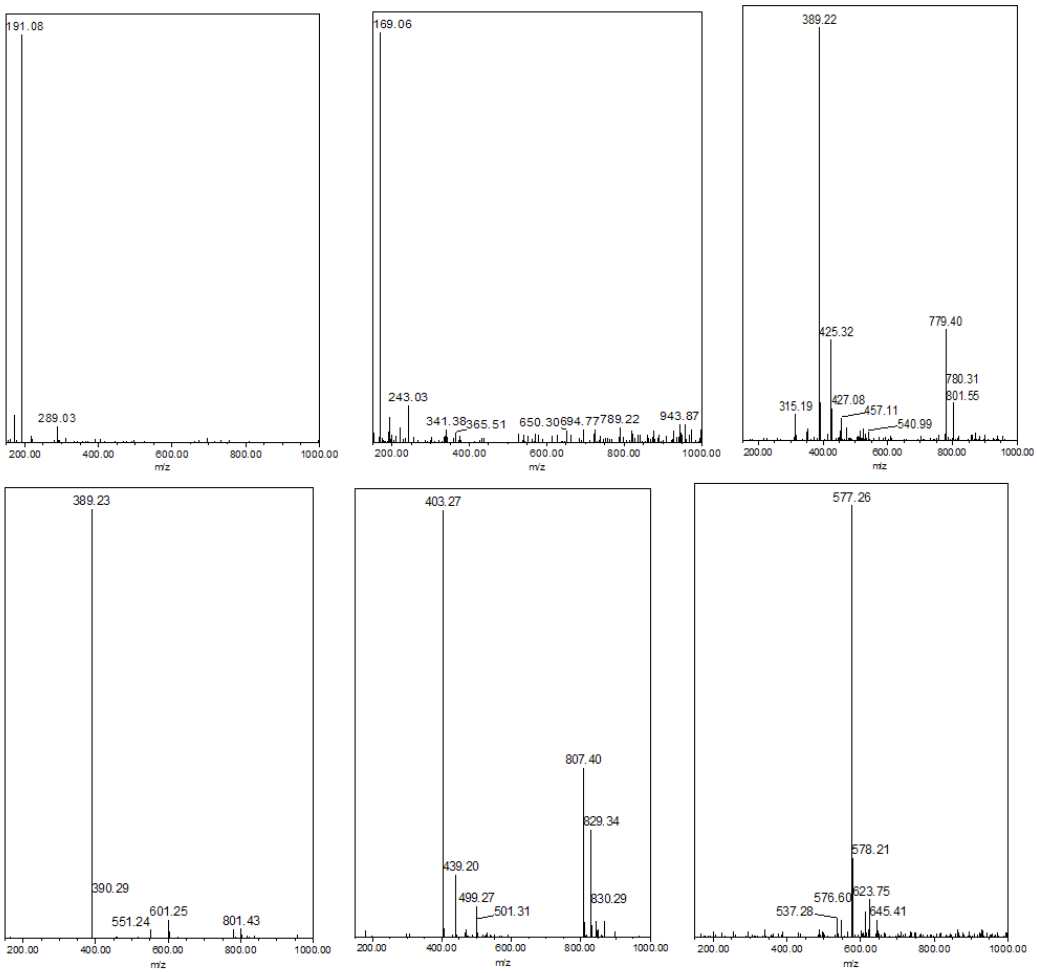

Figure 6 MS spectra of selected polyphenols found in the water extract of wild *A. fragrantissima* leaves, recorded in negative ESI mode, includes, scopoletin, p-coumaric acid ethyl ester, resveratrol 3-*O*-glucoside, resveratrol 5-*O*-glucoside, pelargonidin 3-*O*-arabinoside and rhoifolin respectively.

Citation: Salama S, Abu-Lafi S. UPLC-PDA-ESi-MS based profiling of phytochemicals in wild *Achillea fragrantissima* from Palestine. *Pharm Pharmacol Int J.* 2024;12(6):202–208. DOI: 10.15406/ppij.2024.12.00453

Table 1 Phenolic compounds names and MS from wild *A. fragrantissima* leaves extracted by water infusion. The polyphenol class and the sub-class are indicated in parenthesis

#	Name	*RT	*Mwt	*m/z* [M-H]-
1	Kaempferol 3-*O*-(6"-malonyl-glucoside) (*Flavonoids/ Flavonols*)	0.901	534	533
2	Phloretin (Flavonoids/ Dihydrochalcones)	0.901	274	273
3	Catechine (Flavonoids/Flavanols)	0.901	290	289
4	Hydroxycaffeic acid (Phenolic acids/ Hydroxycinnamic acids)	1.022	196	195
5	Dihydrocaffeic acid (Phenolic acids/ Hydroxyphenylpropanoic acids)	1.022	182	181
6	Ferulic acid 4-*O*-glucoside (Phenolic acids/ Hydroxycinnamic acids)	1.022	356	355
7	5-5'-Dehydrodiferulic acid (Phenolic acids/ Hydroxycinnamic acids)	1.022	386	385
8	Scopoletin (Polyphenol/ Hydroxycoumarins)	1.18	192	191
9	p-Coumaric acid ethyl ester (Phenolic acids/ Hydroxycinnamic acids)	1.44	192	191
10	Gallic acid (Phenolic acids/ Hydroxybenzoic acids)	1.74	170	169
11	Resveratrol 3-*O*-glucoside (Stilbenes)	4.58	390	389
12	Resveratrol 5-*O*-glucoside (Stilbenes)	7.09	390	389
13	Pelargonidin 3-*O*-arabinoside (Flavonoids/ Anthocyanins)	8.3	403	403
14	Rutin (Flavonoids/ Flavonols)	9.25	610	609
15	Lariciresinol-sesquilignan (Lignans)	11.58	556	555
16	Rhoifolin (Flavonoids/ Flavones)	12.86	578	577
17	Luteolin 7-*O*-malonyl-glucoside (Flavonoids/ Flavones)	14.11	534	533
18	Ligstroside (Polyphenol/ Tyrosols)	17.16	524	523

*RT, Retention time in minutes; Mwt, molecular weight in Da

Discussion

Achillea species, compared to many other plant species, are particularly rich in a broad variety of specialized metabolites. In our study, we aimed to replicate a traditional tea infusion of *A. fragrantissima* by extracting the plant in boiled water, as commonly practiced. As anticipated, this method resulted in a lower number and concentration of active compounds compared to previous extractions of *A. fragrantissima* using ethyl acetate, which we had analyzed using HPLC-PDA.[3] This observation was consistent with our expectations. Nevertheless, the chemical complexity of the *A. fragrantissima* metabolome remains remarkable, encompassing a wide variety of compounds, including flavonoids, anthocyanins, phenolic acids, and tyrosol derivatives (Figure 1 & Table 1). This diversity suggests the presence of numerous bioactive compounds that may contribute to the plant's potential pharmacological effects.

Using UPLC-PDA, the compounds exhibit absorption peaks around 236-248 nm, 266-277 nm, and 311-338 nm, which are characteristic of the polyphenol class (Figure 1). Positive ESi-MS analysis (Figure 3) revealed several bioactive N-alkylamides, such as dodeca-2Z,4E-diene-8,10-diynoic acid isobutylamide (RT 21.11 min, molecular weight 243 Da, a protonated [M+H]+ ion at *m/z* 244 Da), undeca-2E,4E-diene-8,10-diynoic acid isobutylamide (RT 27.98 min, molecular weight 229 Da, a protonated [M+H]+ ion at *m/z* 230 Da), and anacycline (RT 28.76 min, molecular weight 271 Da, a protonated [M+H] + ion at *m/z* 272 Da). Figure 5 illustrates the MS protonated [M+H]+ ions at *m/z* values of 230 and 272 Da, respectively. The structures of several N-alkylamides in the ethanolic extracts of *Achillea* species, namely *A. millefolium* and *A. ptarmica*, which are thought to play a role in their pharmacological effects, were characterized using HPLC-ESi-MS/MS and GC-EI-MS.[8]

In contrast, phenolic compounds were more effectively detected in the negative ESi mode. Eighteen compounds vary in RT, ranging from 0.901 to 17.16 minutes were detected (Figure 2). Table 1 provides the information on various active compounds identified in the extract, including their retention times (RT), molecular weights (Mwt), and the observed masses (*m/z*) corresponding to the $[M-H]^-$ ions, indicating the molecular species after ionization in the negative mode. The chemical structures of some of these compounds are shown in Figure 4. As depicted in Table 1, a naturally occurring polyphenolic compounds, Kaempferol 3-*O*-(6"-malonyl-glucoside, phloretin and catechin were eluted near to the void at 0.901 minutes. They showed typical deprotonated pseudo molecular ion $[M-1]^-$ of 533, 304, 273 Da respectively. Kaempferol 3-*O*-(6"-malonyl-glucoside) is a natural glycosylated flavonol with a malonyl group attached to the glucose moiety. Kaempferol in general is known for its potent antioxidant, anti-inflammatory, and anticancer properties. Kaempferol 3-*O*-(6"-malonyl-glucoside), with its additional malonylation, might show enhanced bioactivity and solubility, which could improve its pharmacological potential.[17,18] Phloretin is a flavonoid that has demonstrated antioxidant, anti-inflammatory, and anti-diabetic properties. It is particularly noted for its ability to inhibit the absorption of glucose, thus helping regulate blood sugar levels.[19]

Moreover, catechine is a flavonol with powerful antioxidants with a wide range of health benefits, including anti-cancer and anti-inflammatory properties.[20] Another cluster of phenolic acids that belongs to hydroxycinnamic acids (hydroxycaffeic acid, dihydrocaffeic acid, ferulic acid 4-*O*-glucoside and 5-5'-dehydrodiferulic acid) were seen at 1.022 minutes showing a deprotonated $[M-H]^-$ ion at *m/z* of 195, 181, 355 and 385 Da respectively. Hydroxycinnamic acids are a naturally occurring phenolic acid found in many plants, has demonstrated strong antioxidant and anti-inflammatory properties.[21] Another hydroxycinnamic acid, p-coumaric acid ethyl ester, was detected at a retention time of 1.44 minutes, with a deprotonated $[M-H]^-$ ion at *m/z* 191 Da. This was followed by the phenolic acid gallic acid, which had a $[M-H]^-$ ion at m/z 169 Da (Figure 6). Two isomers of resveratrol, resveratrol 3-*O*-glucoside and resveratrol 5-*O*-glucoside, both belonging to the stilbene class, were eluted at retention times of 4.58 and 7.09 minutes, respectively (Table 1).

Citation: Salama S, Abu-Lafi S. UPLC-PDA-ESi-MS based profiling of phytochemicals in wild *Achillea fragrantissima* from Palestine. *Pharm Pharmacol Int J.* 2024;12(6):202–208. DOI: 10.15406/ppij.2024.12.00453

The MS spectra (Figure 6) show similar deprotonated $[M-H]^-$ ions at *m/z* 389 Da for both isomers. Additionally, a dimer adduct peak at *m/z* 779 Da, corresponding to $[2M-1]^-$, was observed for resveratrol 3-*O*-glucoside, which is likely due to the high concentration of the sample injected. Resveratrol is known for its antiangiogenic, immunomodulatory, antimicrobial, and neuroprotective properties.[22] Oleuropein aglycone and ligstroside polyphenols were detected at retention times of 8.97 and 17.16 minutes, with deprotonated $[M-H]^-$ peaks at *m/z* 377 and 523 Da, respectively. These values correspond to molecular weights of 378 and 524 Da, respectively. Pelargonidin 3-*O*-arabinoside was detected at a retention time of 8.3 minutes, exhibiting a deprotonated $[M-H]^-$ ion at m/z 403 Da (Figure 6). Additionally, a dimer peak at *m/z* 807 Da, corresponding to the formation of adduct $[2M-1]^-$, was observed. Rutin, a well-known flavonol, was detected at 9.25 minutes with a deprotonated ion at *m/z* 609 Da. The flavones rhoifolin and luteolin 7-*O*-malonyl-glucoside were identified at retention times of 12.86 and 14.11 minutes, with respective deprotonated ions at *m/z* 577 and 533 Da (Figure 6). These flavonoids are widely recognized for their various health-promoting properties, including antioxidant, anti-inflammatory, and cardiovascular benefits.

Conclusion

The study confirms that *A. fragrantissima* contains a diverse array of bioactive compounds, highlighting its pharmacological potential. Although the traditional tea infusion method yielded fewer metabolites compared to more concentrated extraction methods, it still produced a wide range of active compounds mainly flavonoids, phenolic acids, many known for their antioxidant and anti-inflammatory properties. UPLC-PDA and ESi-MS analyses identified key bioactive compounds such as kaempferol 3-*O*-(6”-malonyl-glucoside), phloretin, catechin, and resveratrol isomers. These findings suggest that even traditional preparation methods like boiled water infusions can retain significant bioactive potential. Although the concentration and diversity of compounds were lower, the chemical complexity of *A. fragrantissima* remains noteworthy, supporting its traditional and modern therapeutic applications. Further studies are needed to explore the use of different solvents and extraction techniques to better capture the full range of bioactive compounds present in the plant.

Acknowledgments

None.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

None.

References

1. Sabbobeh R, Hejaz H, Al-Jaas H, et al. Phytochemical analysis of cultivated and wild Salvia palaestina using GC-MS: A comparative study. *World Journal of Pharmaceutical Sciences*. 2015;3(12):2348–2356.
2. Bremer K, Humphries CJ. Generic monograph of the Asteraceae-Anthemideae. *Bulletin of the British Museum (Natural History) Botany*. 1993;23(2):71–177.
3. Patocka J, Navratilova Z. *Achillea fragrantissima*: pharmacology review. *Clinics in Oncology*. 2019;4:1–5.
4. Al-Rimawi F, Alakhras F, Al-Zereini WA, et al. HPLC analysis of chemical composition of selected Jordanian medicinal plants and their bioactive properties. *Oriental Journal of Chemistry*. 2018:34(5):2397–2403.
5. Almuhanna Y, Alqasmi MH, AlSudais H, et al. Effect of *Achillea fragrantissima* extract on excision wound biofilms of MRSA and Pseudomonas aeruginosa in diabetic mice. Int J Mol Sci. 2023;24(11):9774.
6. Mudawi MM, El-Wahab MFA, Yassin AY, et al. Evaluation of anticonvulsant activity and HPLC–DAD profiling of *Achillea fragrantissima* (Gaisoom) extracts growing in Saudi Arabia. *Asian Journal of Pharmaceutical Research & Health Care*. 2017;9(3):92–100.
7. Eissa TAF, Gonzalez-Burgos E, Carretero ME, et al. Chemical characterization of polyphenols of Egyptian Achillea fragrantissima with in vitro antioxidant study. *Chiang Mai Journal of Science*. 2018;45(2):897–904.
8. Veryser L, Taevernier L, Wynendaele E, et al. N-alkylamide profiling of *Achillea ptarmica* and *Achillea millefolium* extracts by liquid and gas chromatography-mass spectrometry. *J Pharm Anal*. 2017;7(1):34–47.
9. Apel L, Lorenz P , Urban S, et al. Phytochemical characterization of different yarrow species (*Achillea sp.*) and investigations into their antimicrobial activity. *Z Naturforsch C J Biosci*. 2021;76(1–2):55–65.
10. Barda C, Grafakou ME, TomouEM, et al. Phytochemistry and evidence-based traditional uses of the genus *Achillea* L.: An update (2011-2021). *Sci Pharm*. 2021;89(50):1–36.
11. Dikpinar T, Göger F, Bitiş L. LC-MS/MS analysis and biological activities of endemic *Achillea sieheana* Stapf from Türkiye. *J Res Pharm*. 2022;26(6):1796–1804.
12. Smelcerovic A, Lamshoeft M, Radulovic N, et al. LC-MS analysis of the essential oils of *Achillea millefolium* and *Achillea crithmifolia*. *Chromatographia*. 2010;71(1/2):37–42.
13. Bader A, Flamini G, Cioni PL, et al. Essential oil composition of *Achillea santolina* L. and *Achillea biebersteinii Afan*. collected in Jordan. *Flavour Fragr J*. 2003;18(1):36–38.
14. Tsiftsoglou OS, Krigas N, Gounaris C, et al. Isolation of secondary metabolites from *Achillea grandifolia* Friv. (Asteraceae) and main compounds’ effects on a glioblastoma cellular model. *Pharmaceutics*. 2023;15(5):1383.
15. Kürkçüoğlu M, Demirci B, Tabanca N, et al. The essential oil of *Achillea falcata* L. *Flavour Fragr J.* 2003;18:192–194.
16. Cirak C, Radusiene J, Raudone L, et al. Phenolic compounds and antioxidant activity of *Achillea arabica* populations. *South African Journal of Botany*. 2022;147:425–433.
17. Imran M, Salehi B, Sharifi-Rad J, et al. Kaempferol: A key emphasis to its anticancer potential. *Molecules.* 2019;24(12):2277.
18. Hussain MS, Altamimi ASA, Afzal M, et al. Kaempferol: paving the path for advanced treatments in aging-related diseases. *Exp Gerontol*. 2024;188:112389.
19. Behzad S, Sureda A, Barreca D, et al. Health effects of phloretin: from chemistry to medicine. *Phytochem Rev*. 2017;16(3):527–533.
20. Kim JM, Heo HJ. The roles of catechins in regulation of systemic inflammation. *Food Sci Biotechnol.* 2022;31(8):957–970.
21. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol Rep.* 2019;(24):e00370.
22. Vikal A, Maurya R, Bhowmik S, et al. Resveratrol: A comprehensive review of its multifaceted health benefits, mechanisms of action, and potential therapeutic applications in chronic disease. *Pharmacological Research - Natural Products*. 2024;3:100047.