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Original Article

The Volatile Constituents and Antimicrobial Analysis of *Parietaria Officinalis* from the Northern Part of Iran, Guilan Province

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Abstract

The antimicrobial activity of the methanol extract of *Parietaria officinalis* from Guilan Province in northern Iran was evaluated against *Escherichia coli* (*E. coli*) (Gram-negative) and *Staphylococcus aureus* (*S. aureus*) (Gram-positive) using the microtitre plate method to determine the Minimum Inhibitory Concentration (MIC). The Folin-Ciocalteu method was used to analyze the total phenolic compounds. The amount of flavonoids present was determined using the colorimetric aluminum chloride technique. The essential oil of *P. officinalis* was analyzed to identify its primary components through chemical analysis. Gas Chromatography with a flame ionization detector (GC-FID) and Mass Spectrometry (MS) using a gas chromatograph (GC) (GC-MS) were utilized to analyze the components of the essential oil from wild-grown *P. officinalis* in Iran. The lowest concentration that inhibits growth for *Staphylococcus aureus* is 0.01 mg/µL, while for *Escherichia coli* is 5 mg/µL. The extract showed greater effectiveness against *S. aureus* than the gram-negative bacteria, *E. coli*. The total phenolic content was measured at 72.06 (SD=1.48), equivalent to micrograms of phenolic compounds in terms of gallic acid per milligram of dry methanolic extract. The total flavonoid content was recorded at 48.14 (SD=5.05), equivalent to micrograms of flavonoid compounds per milligram of dry methanolic extract. Among 65 observed compounds, 62 components were identified, constituting approximately 98.2%. The oil was rich in geranyl acetate (15.0 %), Viridiflorol (8.9%), trans-β-Ionone (4.8%), Caryophyllene oxide (4.7%), Hexahydrofarnesyl acetone (4.2%), 2,3-Epoxygeranial (4.2%), Bornyl angelate (2.3%) and (-)-Spathulenol (2.2 %). This research found that *P. officinalis* could be a promising natural substitute for antibiotics in treating *S. aureus* infections. The antibacterial effects are likely due to its phytochemical components. Results indicated that the *P. methanolic* extract possessed significant potential for both flavonoid and phenolic content. The extract obtained by the proposed procedure is enriched with flavonoids and is a candidate for a wide range of pharmacological properties.

Keywords: *Parietaria officinalis*, Urticaceae, Essential oil, Geranyl acetate, Viridiflorol, trans-β-Ionone, Caryophyllene oxide, Hexahydrofarnesyl acetone, 2,3-Epoxygeranial, Phenolic content, Flavonoid content, MIC.

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1. Introduction

Parietaria is a plant that belongs to the family Urticaceae [1]. *Parietaria. officinalis*, *Parietaria. judaica*, *Parietaria. lusitanica*, *Parietaria. Mauritanica*, *Parietaria. ramiflora*, and *Parietaria. cretica* are some of the different species of Parietaria [2, 3]. The genus Officinalis includes four perennial or annual herbaceous species in Iran [4]. This plant has the shape of a bush and looks like a chamomile. It grows in Europe and the Indian subcontinent. Parietaria officinalis L. is a medicinal plant used as a diuretic and blood purifier [5]. It is also a remedy for urinary tract problems such as urinary tract infections (urinary tract infections), dropsy, and bladder stones [5]. In addition, the plant is suitable for treating bronchitis, gallstones, and rheumatism [5].

Parietaria pollen triggers allergies, and many studies have been conducted on plant allergens [6- 9]. A study of cross-reactivity between the pollen of the *P. judaica* and *P. officinalis* species found that the sensitivity was caused by contact with these two species [10-14]. In addition, a major allergen from *P. officinalis* (Pol) was isolated and characterized [2]. Other studies have also reported identifying and characterizing the allergens *P. judaica* [10-14] and *P. officinalis* [15- 16]. The allergens have molecular weights between 10 and 14 kDa and are highly cross-reactive [15–16]. Chemical components such as flavonoids [17], caffeoylmalic acid, and pyrrolic acids [18] have also been identified in the leaves and flowers of Parietaria officinalis.

Using plant volatiles as one of the influential factors for modifying the behavior of insects can be an important step in pest control, thereby reducing the use of pesticides and the risk to humans and the environment [19]. Unlike chemical pesticides with similar chronic and acute toxicity profiles, insects quickly become resistant, causing environmental damage and adversely impacting the environment and sustainability. Chemical messengers (semiochemicals) are non-toxic and can be used with insecticides or as suitable replacements in pest control programs [20].

The active components in herbs are created through genetic mechanisms, but their levels in plants can vary due to certain environmental factors. Although the Parietaria species has been extensively studied, there are no reports of volatile components of Iranian *P. officinalis* and the other *Parietaria species*. To the best of our knowledge, it is the first time that the main components of the essential oil of this plant have been studied. Elucidating the composition of *P. officinalis* essential oil and studying its extracts in experimental models can help expand the knowledge of ancient ethnopharmacological use in the northern part of Iran, Guilan, to identify the species as a candidate for herbs to evaluate medicinal products or phytopharmaceutical therapy. This data may be helpful to semiochemical-based management strategies in *P. officinalis*, such as capturing or attracting natural enemies.

2. Materials and Methods

2.1. Plant material

The aerial portions of Parietaria Officinalis were collected in May at Eshkevar, Roodsar, in the Iranian province of Guilan. Examples of vouchers (no. The Herbarium of the Research

Institute of Forests and Rangelands (TARI), located in Tehran, Iran, has been deposited with 5486 specimens.

2.2. Isolation of the Essential oil

A Clevenger-style apparatus and hydrodistillation method were used to extract the essential oil for three and a half hours, and 120 g of the air-dried plant material was used. Following decanting and anhydrous sodium sulfate drying, the yellowish oil was recovered in yields of 0.08% w/w.

2.3. Gas Chromatography

GC-FID analysis of the oil was performed using a Shimadzu l5A gas chromatograph equipped with a spilt/spilt less (ratio l:30), injector (250ºC), and a flame ionization detector (250ºC). DB-5 was used as the capillary column (50 m x 0.2 mm, film thickness 0.32 µm), and the carrier gas was N_2 (1 mL/min). The column was initially maintained at 60°C for three minutes. Next, for five minutes, it was heated to 220°C at a rate of 5°C per minute. Relative percentage amounts were calculated with a Shimadzu C-R4A chromatopac using only peak area and no correction factors.

2.4. Gas Chromatography-Mass Spectrometry

GC-MS analysis was conducted using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column (30 m x 0.25 mm, film thickness 0.25 µm). The carrier gas utilized was helium at a 1 ml/min flow rate. Initially, the column temperature was held at 60 degrees Celsius for three minutes before being ramped up to 220 degrees Celsius at a rate of 5 degrees Celsius per minute. Upon reaching 220 degrees Celsius, the temperature was maintained for 5 minutes. Under conditions where the energy is less than 70 eV and the scan rate is two scans per second, the mass spectrometers were utilized to measure masses ranging from 30 to 350 atomic mass units.

2.5. Identification of components

The components of essential oils were identified by comparing their mass spectra with those in a computer library and with authentic compounds. Identification is performed by comparing their retention indices with authentic compounds or literature data. The retention index was calculated using the homologous n-alkane series for all volatile compounds.

2.6. Folin-Ciocalteu method for total phenolic compounds assay

In short, 25 µl of the sample (at concentrations of 1, 10, and 1000 µg/ml) was added to each well of a 96-well multiwell plate. Additionally, 125 µl of Folin–Ciocalteu's reagent (10% v/v in distilled water) and 100 µl of 7.5% (w/v) Na2CO3 were pipetted into each well. Phenolic compounds undergo oxidation, reducing phosphomolybdic and phosphotungstic acid present in the Folin–Ciocalteu's reagent to form blue-colored molybdenum and tungsten oxides. The blue coloration's absorbance was measured at 765 nm against a blank sample after an hour and a half. The results were compared to a standard curve of gallic acid solutions at various concentrations (10, 50, 100, 250, 500, and 1000 mg L–1) and reported as micrograms of gallic acid equivalents per milliliter of the sample. The measurements were carried out three times to ensure accuracy [21].

2.7. Determination of total flavonoid content using colorimetric aluminum chloride method

The colorimetric aluminum chloride method was utilized to detect flavonoids. 20 µl of the sample solutions (1, 10, and 1000 µg/ml) were combined with 80 µl of distilled water and 6 µl of NaNO2 (15% w/v), followed by a six-minute incubation period. Later, six microliters of aluminum chloride solution containing 10% concentration, 80 microliters of NaOH with a 4% weight/volume ratio, and eight microliters of distilled water were added to individual wells of a 96-well multiwell plate. The mixture was then left to sit at room temperature for 3 minutes. The absorbance of the mixture was then measured at 510 nm. Calculating the Rutin content using a calibration curve determined the amount of flavonoids present. This curve was created by making Rutin solutions containing 10, 50, 100, 250, 500, and 1000 mg L-1 in methanol [22].

2.8. Antimicrobial determination

The antibacterial activities were analyzed by initially concentrating the methanol extracts using a rotary evaporator while the aqueous extracts were freeze-dried. The desiccated extracts were then preserved in sterilized, sealed-culture tubes at 4°C until each dry extract was dissolved in 1 mL of distilled water.

The methanolic extract from various plant parts, such as the flower, stem, root, and leaf, was tested for its antimicrobial properties. Two microbial species, *S. aureus* and *E. coli*, were subjected to the minimum inhibitory concentration (MIC) method using liquid dilution. To establish the minimum inhibitory concentration Growth (MIC), we prepared 100 mg concentrations of each extract using a DMSO solvent and Mueller Hinton culture medium Broth (MHB). The final ratio of DMSO to the culture medium was 1 to 16. 200µL concentration was placed into the houses of a plate of 96 houses. As a negative control, DMSO solvent was included. It was prepared with an MHB culture medium (with a ratio of 1 to 16). A 10 µL gentamicin antibiotic was added to 200 µL of culture medium as a positive control. 20 µL of microbial suspension was transferred to all wells of each plate, including those containing extract, positive control, and negative control. After 24 hours of placing the plates in a greenhouse at a temperature of 37 degrees Celsius, 50µL of Triphenyl Tetrazolium Chloride (TTC) solution with a concentration of 5 mg/ml was added to each plate, and they were placed back in the greenhouse for an additional 3 hours. Then, the microplates were checked for producing red color. A higher concentration of the last concentration that took the red color of tetrazolium was used as the MIC of the extract for the examined bacteria considered.

3. Results and Discussion

Studies have shown that medicinal plants contain at least one special secondary active ingredient in addition to general and basic compounds. These special active ingredients, of which thousands of types, are called natural plant substances. Nowadays, most medicinal plants are classified according to the active ingredients they contain. The chemical components extracted from the upper parts of Parietaria officinalis and their respective percentage and retention indices are detailed in

Table 1. Table 1 indicates that out of the 65 compounds identified, 62 compounds comprising 98.2% of the essential oil were identified.

Table 1. Components identified[a,b,c] in the oil of the aerial parts of *P. Officinalis***.**

Compound	RI*	Percentage	Compound	RI*	Percentage
α-pinene[a,b,c]	934	0.1	Viridiflorol[a,b,c]	1597	8.9
Sabinene[a,b,c]	973	0.2	Humulene epoxide II[a,b,c]	1612	1.0
β-pinene[a,b,c]	978	0.3	1-epi-Cubenol[a,b,c]	1630	0.4
6-Methyl-5-heptene-2-one[a,b,c]	984	0.3	epi-alpha- Murrolol[a,b,c]	1645	1.7
p-cymene[a,b,c]	1024	0.3	Torreyol[a,b,c]	1649	0.4
L-Limonene[a,b,c]	1028	0.7	α-Cadinol[a,b,c]	1658	1.0
1,8-cineol[a,b,c]	1031	0.2	14-hydroxy-9-epi-(E)-Caryophyllene[a,b,c]	1674	0.6
Acetophenone[a,b,c]	1067	0.4	Heptadecane[a,b,c]	1694	0.6
Linalool[a,b,c]	1098	0.2	Anthracene[a,b,c]	1780	0.5
Nonanal[a,b,c]	1108	0.3	Octadecene[a,b,c]	1794	1.0
Pinocamphone[a,b,c]	1167	3.3	Phytan[a,b,c]	1803	0.7
2,3-Epoxyneral[a,b,c]	1225	2.5	Hexahydrofarnesyl acetone[a,b,c]	1842	4.2
2,3-Epoxygeranial[a,b,c]	1236	4.2	Diisobutyl phthalate[a,b,c]	1868	0.8
Neral[a,b,c]	1243	3.3	Nonadecane[a,b,c]	1895	1.4
Geraniol[a,b,c]	1256	2.6	(E,E)-Farnesyl acetone[a,b,c]	1916	0.8
Geranial[a,b,c]	1272	2.8	Methyl palmitate[a,b,c]	1921	0.3
Menthyl acetate[a,b,c]	1294	1.2	Palmitic acid[a,b,c]	1961	0.9
Methyl geranate[a,b,c]	1323	0.5	Eicosane[a,b,c]	1992	1.0
Dihydrocarvyl acetate[a,b,c]	1328	0.4	Verticellol[a,b,c]	2014	0.9
Neryl acetate[a,b,c]	1363	1.6	Epimanool[a,b,c]	2049	0.5
Geranic acid[a,b,c]	1370	0.7	Heneicosane[a,b,c]	2079	0.7
α-Copaene[a,b,c]	1378	0.3	Phytol[a,b,c]	2092	3.4
geranyl acetate[a,b,c]	1386	15.0	Incensole[a,b,c]	2148	1.4
trans-Caryophyllene[a,b,c]	1423	1.1	Incensole acetate[a,b,c]	2178	1.5
α-Bergamotene[a,b,c]	1436	0.4	Docosane[a,b,c]	2189	0.8
Geranyl acetone[a,b,c]	1451	0.7	Tricosane[a,b,c]	2288	0.8
cis-β-Farnesene[a,b,c]	1455	0.4	Tetracosane[a,b,c]	2385	0.4
α-Curcumene[a,b,c]	1482	1.0	**Total**	1597	**98.2**
trans-β-Ionone[a,b,c]	1488	4.8	Viridiflorol[a,b,c]	1612	8.9
α-Muurolene[a,b,c]	1501	0.5	Humulene epoxide II[a,b,c]	1630	1.0
γ-Cadinene[a,b,c]	1516	0.9	1-epi-Cubenol[a,b,c]	1645	0.4
cis-Calamenene[a,b,c]	1524	1.2	epi-alpha- Murrolol[a,b,c]	1649	1.7
fbornyl[a,b,c]	1562	2.3	Torreyol[a,b,c]	1658	0.4
(-)-Spathulenol[a,b,c]	1582	2.2	α-Cadinol[a,b,c]	1674	1.0
Caryophyllene oxide[a,b,c]	1587	4.7	14-hydroxy-9-epi-(E)-Caryophyllene[a,b,c]	1694	0.6

Group components	RI*
Monoterpene hydrocarbons	1.6
Oxygen-containing monoterpene	44.7
Sesquiterpene hydrocarbons	5.8
Oxygen-containing Sesquiterpene	25.9
Diterpene hydrocarbons	0.7
Oxygen-containing diterpene	7.7
Non-terpene	11.8

R.I. = retention indices. *In elution order from DB-5 column. Identified with: [a] K.I. (Kovats Index); [b] Eight Peak Index of Mass Spectra; [c] GC-MS Library.

Table 1 shows that the essential oil of this plant contains five monoterpenes hydrocarbons (1.6%), fifteen oxygenated monoterpenes (44.7%), eight sesquiterpenes hydrocarbons (5.8%), eleven oxygenated sesquiterpenes (25.9%), one diterpene hydrocarbons (0.7%), five oxygenated diterpene (7.7%), seventeen nonterpene compounds (11.8%) such as aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and esters. According to the data, oxygenated sesquiterpenes comprise a comparatively large percentage of the oil, while oxygenated monoterpenes comprise most of the mixture. The primary components of the essential oil are geranyl acetate (15.0%), trans-β-Ionone (4.8%), 2,3-Epoxygeranial (4.2%), Pinocamphone (3.3%), Neral (3.3%), Geranial (2.8%), Geraniol (2.6%), 2,3-Epoxyneral (2.5%) and Bornyl angelate (2.3%) as oxygenated monoterpenes, Viridiflorol (8.9%), Caryophyllene oxide (4.7%), hexahydrofarnesyl acetone (4.2%), (-)-Spathulenol (2.2%) as oxygenated sesquiterpenes and Phytol (3.4%) as oxygenated diterpenes. The highest proportion is geranyl acetate (15%). Geranyl acetate is primarily used as a component of perfumes for creams and soaps and as a flavoring agent. It is mainly used in rose, lavender, and geranium formulations with a desired sweet, fruity or citrus aroma. The next known active ingredient is Viridiflorol. Viridiflorol has demonstrated moderate antibacterial activity against Mycobacterium tuberculosis, the causative agent of tuberculosis, in an in vitro test [23]. It is also produced by the endophytic root fungus Serendipita indica and shows antifungal activity against Colletotrichum truncatum [24]. β-Ionone (4.8%) was also identified. The ionones are formed when carotenoids are broken down. The carotenes β-carotene, and the xanthophyll -cryptoxanthin can all be metabolized to β-ionone and thus have vitamin A activity as they can be converted into retinol and retinal by herbivorous animals. Carotenoids that do not contain the β -ionone moiety cannot be converted to retinol and, therefore, have no vitamin A activity. Therapeutic uses of caryophyllene oxide could exploit the antifungal efficacy observed in clinical trials for onychomycosis compared to ciclopiroxalamine and sulconazole, with a concentration of 8% causing eradication within 15 days [25]. This agent also shows anti-platelet properties in vitro [26]. Caryophyllene oxide (4.7%) was also identified. Natural bicyclic sesquiterpenes, β-caryophyllene (BCP), and β -caryophyllene oxide (BCPO) are found in many plants worldwide. Both BCP and BCPO (BCP(O)) possess significant anti-cancer activities and influence the growth and proliferation of numerous cancer cells [27]. Neral (3.3%) was identified. Natural analogs such as Neral have been studied. The synergism of these terpenes with compounds used clinically and non-clinically has been observed as potential candidates for treating various types of cancer. The anti-cancer potential of this compound is described in a review [28].

The main components are oxygen-containing monoterpenes. Oxygenated monoterpenes can also exist in alcoholic ketone aldehyde and forms. One of the most commonly known monoterpenes is limonene. Terpenoids such as limonene (0.7%) and phytol (3.4%) share a common precursor with phytocannabinoids.

They are common flavorings and fragrances in the human diet generally recognized by the U.S. Food and Drug Administration and other regulatory authorities as Agencies were certainly recognized. Oxygenated monoterpene-rich essential oils are potential antifungal agents for dermatophytes [29]. Due to these components, adding this essential oil to some dermatological preparations is recommended to relieve skin problems, especially facial bruises, eczema, and dermatitis. The proportion of limonene is 0.7%. Studies have shown that it has anti-cancer properties. Limonene contains some biological enzymes that fight off cancer [30]. The smell of pine is an example of L-limonene, and the smell of orange is an example of D-limonene.

In a comparative study of the essential oil compounds of this plant in northern Iran and northeast Algeria [31], it is clear that there is a large difference between the type and percentage of compounds of this identical species from two different parts of the world. While the highest composition of the Iranian sample is geranyl acetate (15%), hexenol (53%) was reported as the main composition of the Algerian species. The proportion of linalool in the Algerian sample is 3.23%, while it is only 0.2% in the Iranian sample. What is interesting is that camphor (0.13%), eucalyptus (0.35%), and methyl salicylate (0.325) were found in the Algerian sample. However, these potent therapeutic compounds were not detected in the Iranian sample.

For this reason, more effective therapeutic agents appeared in the Algerian sample. From the composition table of both samples, the composition of the Iranian sample is higher but with a lower percentage. For example, the nonanal percentage of the Algerian sample is twice that of the Iranian one. For this reason, it can be stated that the components of essential oils depend on factors such as genetics, climate, and plant habitat. A comparison of the average and types of compounds showed that there could be a significant difference in the quantity and quality of the essential oil, even for a particular type. This edition highlights the influence of genetics on the quantity and quality of essential oils.

Approximately 8000 plant phenolics are found in nature, with half being flavonoids. Phenolics exhibit a variety of biochemical functions, including antioxidant, antimutagenic, and anti-carcinogenic properties and the ability to regulate gene expression. Most of the antioxidant activity in plants or plant-based products is attributed to this most prevalent class of phytochemicals [32-33]. The total phenolic content of the plant's methanolic extract was measured based on the standard composition of gallic acid and the Folin-Ciocalciteu method was 72.06±1.48. The results were presented as micrograms per milligram (µg/mg) of gallic acid equivalents in the one-milligram dry methanolic extract. The experiments were repeated three times to ensure the validity of the results. The number of phenolic compounds in plant tissues can range from 0.5 to 5 micrograms per milligram of dry weight. These compounds are typically considered secondary products of plant metabolism, having minimal impact on the plant's physiological or ecological processes. Total phenolic content was measured at 72.06. Phenolic compounds play a significant role as

plant components due to their redox properties, contributing to antioxidant activity. The presence of hydroxyl groups in plant extracts aids in free radical scavenging. The phenolic content values in this study showed minor differences compared to those found in existing literature. These variations could be attributed to various factors such as varying amounts of sugars, carotenoids, or ascorbic acid, as well as differences in duration, geographical location, or extraction methods, all of which can affect the number of phenolics [34-35].

Flavonoids are the most abundant natural phenolic compounds found in various parts of plants, either as free molecules or as glycosides. They exhibit numerous biological activities such as antimicrobial, inhibition of mitochondrial adhesion, antiulcer, antiarthritic, antiangiogenic, anti-cancer, and inhibition of protein kinase. Flavonoids consist of two benzene rings with a propane unit separating them. Among all phenolics, flavones and flavonols are the most commonly found [36-37]. Total flavonoid content was measured using the standard microgram equivalent per milligram of dry methanolic extract was 48.14±5.05. The experiments were repeated three times to ensure the validity of the results. Flavonoids have many health benefits, as they work as antioxidants and help protect against cardiovascular disease, certain types of cancer, and age-related cell damage. Thanks to their polyphenolic properties, they are able to neutralize harmful free radicals like superoxide and hydroxyl radicals [38].

Table 2 shows that the methanolic extract has a stronger effect on Gram-positive bacteria than Gram-negative bacteria, particularly *E. coli*. Gram-negative bacteria have an outer membrane as a protective barrier, limiting water entry and large hydrophobic molecules. This may explain why the methanolic extract is less effective against *E. coli*. This indicates that the plant's primary compounds, which are mostly hydrophobic, could be the reason for this. In essence, the extract from this plant exhibits powerful antimicrobial properties against gram-positive bacteria. The primary antimicrobial components in plant extracts are aromatic and saturated organic compounds, which are highly soluble in alcoholic solvents, specifically methanol. Because this plant contains various compounds with varying polarities, methanol was employed to extract and analyze its activities. Moreover, most compounds found in this plant have low polarity and molecular weight.

The study found that the methanolic extracts of *P. officinalis* effectively inhibited the growth of the bacteria tested. This suggests that the extract from this plant could be a valuable tool for controlling food poisoning and as a natural alternative to chemical preservatives in food preservation.

Table 2. Antimicrobial activity (MIC *mg/µL*) of the methanolic extract of the flower, stem, root and leaf organs of *P. officinalis***.**

Organ	*E. coli*	*S. aureus*
Leaves	32	<0.01
Stems	14	1
Roots	5	0.5
Flowers	8	0.5

4. Conclusion

The methanolic extracts from *P. officinalis* showed potent inhibition against the bacteria,

particularly *S. aureus*. The extract had a high total phenolic content (72.06 µg GAE/mg) and a high total flavonoid content (48.14 µg/mg). Medicinal plants are usually classified according to their ingredients. Considering this plant's wide variety of compounds using the GC-FID method, this technique seems to be a suitable and rapid method for checking plant volatiles.

Conflict of interest

The authors declare to have no conflict of interest.

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