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Journal of Atlantic Research and Technology

Chemical Profile and Antioxidant Properties of Essential Oils and Solvent Extracts of *Warionia Saharae* from Morocco

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ARTICLE INFO

Article history:
Received 27th January, 2025
Received in revised form 29th March, 2025
Accepted 4th April, 2025
Available online 30th April, 2025

Keywords:
Warionia saharae
Essential oil
Solvent extract
Antioxidant activity
Chemical composition

ABSTRACT

The objective of this study was to investigate the chemical composition and antioxidant potential of the essential oil and solvent extracts from *Warionia Saharae* leaves, a medicinal plant from Morocco. The chemical analysis of the essential oil was performed using GC and GC-MS, revealing a dominance of β-eudesmol (36.33%), linalool (15.23%), and nerolidol (11.44%). The solvent extracts (aqueous, ethanol/water 50:50 v/v, and ethanol/water 80:20 v/v) were analyzed for their total phenolic, flavonoid, and tannin contents using colorimetric methods. The ethanol/water (50:50) extract exhibited the highest phenolic content (37.00 mg EAG/g MS), followed by ethanol/water (80:20) and aqueous extracts. Antioxidant activity was assessed using the DPPH assay, and a positive correlation was observed between the antioxidant potential and the phenolic content of the extracts. The ethanol/water (50:50) extract showed the strongest antioxidant activity, suggesting that *Warionia Saharae* could be a valuable source of natural antioxidants. These findings highlight the plant's potential for further exploration as a source of bioactive compounds for pharmaceutical or nutraceutical applications.

1. Introduction

Morocco's diverse geography, extending from the Mediterranean Sea to the Atlantic Ocean, creates a unique natural environment with a wide range of Mediterranean bioclimates. This diversity supports a rich and varied flora, including a significant number of endemic species. With 4,200 species and subspecies, Morocco holds a prominent position in the Mediterranean region for its medicinal plants, 500 of which are aromatic or medicinal, and 250 are commonly used by the local population [1]. The country's long-standing medical tradition and traditional knowledge in medicinal plants further highlight its importance in this field.

These natural compounds play a crucial role as a source of pharmaceuticals in various pharmacopeias around the world. Numerous active substances derived from plants are used to produce essential medicines. Moreover, many modern formulations incorporate plant-based raw materials. Medicinal plants can be utilized directly in their fresh or dried forms, as well as in extract form, often combined with other plants or synthetic excipients [2].

Among these medicinal plants is Warionia saharae Benth. & Coss, a monotypic genus within the Asteraceae family. This plant is considered endemic to the northwestern Sahara, specifically in Morocco and western Algeria. The aromatic species was first reported in the Beni Ounif region, located in southwestern Algeria, by J.P. Adrien Warion (1837-1880), a French military physician and botanist who amassed extensive collections of species during his time in North Africa [3].

In the context of valorizing endemic aromatic and medicinal plants, this study was conducted to examine the chemical composition and antioxidant activity of the leaves of Warionia saharae.

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2. Materials and Methods

Plant material

The plant used in this study was purchased from a herbalist in Zagora, located in southeastern Morocco, who specializes in the sale of medicinal plants. According to the herbalist, the plant was harvested in the same region, then dried in a dark, dry, and well-ventilated area. It was subsequently stored away from light until it was utilized for this study. The species was later identified by Professor Raouane Mohamed, a biology professor at the École Normale Supérieure in Rabat.

Essential oil isolation

The essential oil was extracted from dried leaves using hydro-distillation with a Clevenger apparatus. 200 g of ground plant material were placed in a 2000 mL glass flask filled with water and boiled for three hours. The essential oil yield was 0,415% (w/w). The essential oil was then stored in amber vials at 4 to 6 °C, and protected from light until analysis.

Preparation of the extracts

Ultrasound-assisted maceration was used to prepare the extracts. A quantity of 2 g of plant powder was mixed with 100 mL of three different solvent systems: water (100%), ethanol/water (50:50 v/v), and ethanol/water (80:20 v/v). The ultrasonic system was set to operate for 30 minutes at a temperature of 30 °C. The mixture was then filtered using filter paper. The filtrate was collected and subjected to a second filtration to remove any remaining solid plant material. The three filtrates were placed in flasks for solvent removal, which was carried out under reduced pressure at 60 °C using a rotary evaporator. The resulting extracts were dried in an oven at 50 °C until a dry extract was obtained. This dry extract was then stored in a refrigerator for future testing.

GC analysis

The chemical composition of the essential oil was analyzed using GC-MS. The analysis was performed with a Hewlett-Packard gas chromatograph (HP 6890) coupled to a mass spectrometer (HP 5973). The sample was fragmented by electron impact at 70 eV, using an HP-5 MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness). The column temperature was programmed from 50°C to 250°C at 4°C/min, with helium as the carrier gas at a flow rate of 1.5 mL/min.

Phytochemical Screening

Phytochemical screening is a qualitative test used to identify the presence or absence of various families of secondary metabolites in plants. In this study, four tests were conducted to detect the presence of coumarins, tannins, saponins, and free quinones in the leaves of *W. saharae*.

Detection of Coumarins

To detect coumarins, 3 mL of 10% NaOH was added to 2 mL of a 5% infusion in a test tube. After shaking, the presence of coumarins was indicated by a yellow coloration [4].

Detection of Tannins with FeCl3

Tannins were detected by mixing 1 mL of 5% aqueous extract with 2 mL of water and 2-3 drops of 1% ferric chloride (FeCl3). The appearance of a blue-black or blue-green color confirmed the presence of tannins [5].

Detection of Saponins

Saponins were identified based on foam height. 2 mL of hot water was added to 1 mL of aqueous extract, shaken vigorously, and left to stand for 20 minutes. The foam height indicated the presence of saponins:

- No foam: Negative result (no saponins)
- Foam less than 1 cm: Weakly positive
- Foam between 1-2 cm: Positive
- Foam over 2 cm: Strongly positive [5].

Detection of Free Quinones

1 g of powdered leaves was mixed with 15-30 mL of petroleum ether in a tube, shaken, and left to stand for 24 hours. The extract was then filtered and concentrated using a rotary evaporator. The presence of free quinones was indicated by a color change (yellow, violet, or red) in the aqueous phase upon adding a few drops of 10% NaOH [4].

Determination of Total Polyphenols

Total polyphenols were quantified using Folin–Ciocalteu reagent according to the method previously reported by Singleton & Rossi, and Ragaee et al, with some modifications [6,7]. First, 200 µL of the sample was pipetted into a test tube and mixed with 1 mL of Folin-Ciocalteu reagent (FCR) diluted tenfold in distilled water. After allowing the mixture to react for 4 minutes, 800 µL of sodium carbonate (Na2CO3) solution, with a concentration of 75 mg/mL, was added, and the mixture was vortexed. The reaction was then incubated for 2 hours at room temperature in the dark. The absorbance of the resulting solution was measured at 765 nm using a UV-VIS spectrophotometer, with all measurements being performed in duplicate. Three control samples were prepared by mixing 200 µL of each solvent system with 1 mL of Folin-Ciocalteu reagent and 800 µL of sodium carbonate solution. The total polyphenol concentration was calculated using the regression equation from a calibration curve created with gallic acid and expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM). The calibration curve was generated with varying concentrations of gallic acid (0-150 µg/mL) under the same assay conditions.

Flavonoid Quantification

Total flavonoids in W. saharae extracts were quantified using the aluminum chloride (AlCl3) method with some modifications [8,9,10].

1 mL of each extract, diluted in its original solvent, was mixed with 1 mL of 2% AlCl3 dissolved in methanol. The mixture was allowed to stand for 10 minutes, after which the absorbance was measured at 430 nm using a UV-VIS spectrophotometer. The flavonoid concentrations were reported as milligrams of quercetin equivalents per gram of dry matter (mg EQC/g DM), calculated from the regression equation of the calibration curve established with quercetin standards, with concentrations ranging from 0 to 150 µg/mL.

Condensed Tannin Quantification

The quantification of condensed tannins in the three extracts of *W. saharae* was performed using vanillin, following the method described by [11]. For each sample or standard, 400 µL was mixed with 3 mL of a 4% vanillin solution in methanol and 1.5 mL of concentrated hydrochloric acid (HCl). The mixture was incubated for 15 minutes, and the absorbance was measured at 500 nm against a blank. The concentration of condensed tannins was calculated from the regression equation of the calibration curve established with catechin standards, with concentrations ranging from 0 to 600 µg/mL. The results were expressed as milligrams of catechin equivalents per gram of dry matter (mg ECT/g DM).

Evaluation of Antioxidant Activity

The free radical-scavenging activities of essential oil and solvent extracts were measured using DPPH as described by Sharififar et al [12]. This technique is based on the reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl), a purple compound that forms stable free radicals. When exposed to an antioxidant, DPPH is reduced to DPPH-H (2,2-diphenyl-1-picrylhydrazine), a yellow non-radical compound. The reduction reaction can be monitored by measuring the decrease in absorbance. 200 µL of each extract and essential oil (diluted in methanol) at various concentrations were added to 1800 µL of a methanolic DPPH solution (4 mg/100 mL). The mixture was thoroughly mixed and allowed to stand at room temperature in the dark for 60 minutes. Absorbance was then measured at 517 nm against a blank. The percentage inhibition of the DPPH radical (I%) was calculated using the formula:

$$\%I = [(Abs0 - Abs1) / Abs0] \times 100$$

where Abs0 is the absorbance of the control reaction (containing all reagents except the tested compound), and Abs1 is the absorbance of the tested compound. The concentration of the sample required to achieve 50% inhibition (IC50) was determined from the inhibition percentage versus concentration graph. All tests were conducted in triplicate, with ascorbic acid used as a positive control.

3. Results and discussion

3.1. Chemical Composition

The chemical composition of the essential oil extracted from W. saharae leaves is detailed in Table 1. The primary constituents were identified as β-eudesmol (36.33%), linalool (15.23%), and nerolidol (11.44%), which dominated the essential oil profile. These results are consistent with previously published studies. For instance, Ramaut et al. (1985) reported a similar composition in W. saharae, with β-eudesmol making up 42.25%, nerolidol 17.26%, and linalool 8.63%. Gherib et al also found similar dominant compounds in the W. saharae leaves from Algeria, with (E)-nerolidol (25.7%), β-eudesmol (25.0%), and linalool (17.1%) being the major constituents [13]. However, Amezouar et al observed a different chemical profile in the Tata region of Morocco, with β-eudesmol (38.12%) and (Trans) nerolidol (25.95%) as the main components, but linalool was absent [14].

The variation in essential oil composition across different regions and studies can be attributed to factors such as genetic variability, population and origin of plant material, plant part used for extraction, plant developmental stage, and environmental conditions (e.g., climate, soil properties, habitat). Moreover, factors such as cultivation practices (timing of planting, irrigation, fertilization), post-harvest methods (drying, distillation), and analytical conditions can also impact the chemical profile [15].

Table 1. Chemical Composition of W. saharae Essential Oil

Compound	P (%)	Tr (min)
3-Eicosanone	0,64	10,872
Oxalic acid, tetradecyl ester	0,23	11,656
1,8 Cineole	0,22	12,887
Trans-Linalool oxide	0,28	13,537
Linalool	15,23	14,018
Hotrienol	0,32	14,061
Terpinen-4-ol	1,05	15,302
α-Terpineol	5,91	15,525
Cis-Geraniol	2,22	15,939
Geraniol	6,48	16,319
Thymol	0,61	16,95
β-Damascenone	0,54	18,214
Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	0,32	18,511
Phenol, 3,5-bis(1,1-dimethylethyl)-	0,32	19,718
1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene	2,22	19,958
CIS-α-Copaene-8-ol	0,22	20,085
3,4-Seco-dammar-4(28)-ene-3-oic acid, 20,24-epoxy-25-hydroxy-, (24S)-	1,51	20,218
Nerolidol	11,44	20,398
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	0,21	20,448
1-Cyclohexyl-1-(4-ethylcyclohexyl)ethane	1,45	20,542
β-Santalol	0,18	20,689
1-Methylene-2B-hydroxymethyl-3,3-dimethyl-4B-(3-methylbut-2-	0,54	20,949

enyl)-cyclohexane		
cis-6, trans-8, cis-12-Methyl octadecatrienoate	0,58	21,209
Selina-3,7(11)-diene	3,71	21,446
β-Eudesmol	36,33	21,883
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	0,44	21,933
Myristic acid	0,29	22,426
1,2,3,4-Tetramethylnaphthalene	0,16	22,617
2-(4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-o	0,18	22,687
Glaucyl alcohol	0,99	22,977
(Z)-25-Tetratriacontan-2-one	0,16	23,327
11,14-Eicosadienoate methyl ester	0,44	23,731
2-Pentacosanone	0,54	23,897
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	0,42	23,947
Phytol	0,32	25,925

P (%): Percentage in the essential oil, Tr (min): Retention time in minutes

3.2. Phytochemical Screening

Phytochemical analysis of W. saharae leaves revealed the presence of tannins, coumarins, and saponins, while free quinones were absent (Table 2). These results are consistent with previous studies concerning the presence of tannins and coumarins [16,17]. However, discrepancies were observed when compared to Cheriti et al, who reported no tannins in W. saharae plants from Algeria [18], and Bounegta et al, who detected free quinones [17]. These variations may be due to differences in species, genotype, plant physiology, developmental stage, and environmental conditions [19].

Table 2. Phytochemical Screening of W. saharae Leaves

Components	Observations	Results
Coumarins	Appearance of a yellow color	+++
Tannins	Appearance of a blue-green color	+++
Saponins	Foam less than 1 cm	+
Free Quinones	No appearance of yellow, red, or violet color	-

(+): low presence, (++): middle presence, (+++): high presence

3.3. Determination of Total Phenolic Contents

The phenolic content of the extracts, expressed as mg GAE/g DW for polyphenols, mg QE/g DW for flavonoids, and mg CE/g DW for tannins, showed significant variation depending on the solvent used (Table 3). The ethanol/water (50:50) extract had the highest concentrations of polyphenols (37.00 mg GAE/g DW), flavonoids (15.16 mg QE/g DW), and tannins (20.64 mg CE/g DW). In contrast, the aqueous extract showed the lowest concentrations of these compounds. The intermediate values were observed for the ethanol/water (80:20) extract. The differences in extraction efficiency can be attributed to solvent polarity, with more polar solvents, such as ethanol, proving more effective at extracting a broader range of phenolic compounds [20].

Table 3. Total Phenolic, Flavonoid, and Tannin Contents of W. saharae Extracts

Extract	Polyphenols (1)	Flavonoids (2)	Tannins (3)
Aqueous	14.87 ±0.2339	5.63 ±0.3217	7.57 ±0.270
Ethanol/Water (50:50 v/v)	37.00 ±0.5895	15.16 ±0.2199	20.64 ±0.879
Ethanol/Water (80:20 v/v)	24.25 ±1.1933	9.703 ±0.0334	14.08 ±1.34

3.4. Antioxidant Activity

The antioxidant activity of the extracts was evaluated using the DPPH method, with the IC50 values presented in Table 4. The essential oil showed the highest radical scavenging activity, with an IC50 of 0.152 mg/mL, which was lower than that of ascorbic acid (0.217 mg/mL), indicating stronger antioxidant activity. Among the extracts, the ethanol/water (50:50) extract was the most effective, with an IC50 of 0.9452 mg/mL, followed by the aqueous and ethanol/water (80:20) extracts with IC50 values of 1.0206 mg/mL and 1.2993 mg/mL, respectively. These results are in agreement with previous studies, such as Amezouar et al, who reported an IC50 of 21.49 µg/mL for W. saharae essential oil [14].

Table 4. Antioxidant Activity of W. saharae Essential Oil and Extracts

Extract	Aqueous	Ethanol/ Water (50:50 v/v)	Ethanol/ Water (80:20 v/v)	E.O	Ascorbic Acid
IC50	1,0206	0,9452	1,2993	0,152	0,2175

4. Conclusion

This study explored Warionia saharae's valorization by characterizing its phytochemicals and evaluating its antioxidant activity. Phytochemical screening identified tannins, coumarins, and saponins in the leaves, with no free quinones. Essential oil extraction yielded 0.415%, a moderate amount influenced by various factors.

Ultrasonic-assisted maceration produced three extracts, with the aqueous extract having the highest yield, followed by hydroethanolic extracts at 80% and 50%. The hydroethanolic (50/50) extract had the highest concentrations of polyphenols, flavonoids, and tannins.

The essential oil exhibited an IC50 of 0.152 mg/mL, indicating superior antioxidant activity compared to ascorbic acid. The hydroethanolic (50/50) extract also demonstrated strong antioxidant activity with an IC50 of 0.9452 mg/mL. These results highlight W. saharae's potential as a source of bioactive compounds.

Further research is needed to fully characterize and isolate the chemical compounds in W. saharae. Exploring other biological activities and optimizing extraction methods could enhance its application in pharmaceuticals and natural product industries. Additionally, investigating the impact of environmental factors on phytochemical content and antioxidant activity could provide insights into maximizing its potential.

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