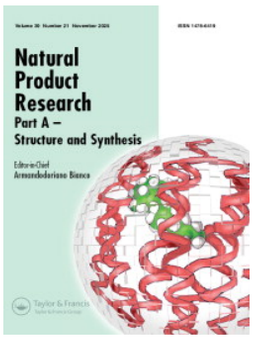

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ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: www.tandfonline.com/journals/gnpl20

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To cite this article: Turqui Tarik, Benaiche Ghania, Debih Hadi , Benkouider Imen & Khellaf Rebbas (17 Nov 2025): The first investigation of the chemical composition antioxidant and antibacterial activities of the aerial parts aqueous methanolic extract of the Algerian endemic plant *Psychine stylosa* desf, Natural Product Research, DOI: 10.1080/14786419.2025.2588798

To link to this article: https://doi.org/10.1080/14786419.2025.2588798

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2025.2588798

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The first investigation of the chemical composition antioxidant and antibacterial activities of the aerial parts aqueous methanolic extract of the Algerian endemic plant *Psychine stylosa* desf

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ABSTRACT

The phytochemical profile of the aqueous methanolic extract from the aerial parts of *Psychin stylosa* Desf., an Algerian endemic species belonging to the Brassicaceae family and previously unstudied, was analysed using the HPLC-MS/MS technique, allowing for the identification of 65 compounds from diverse chemical classes, including phenolic compounds, flavonoids, sugars, alkaloids, sulphur compounds, peptides, terpenoids, and steroids, as well as 13 unidentified compounds. The extract showed a high total polyphenol content of 64.39 ± 1.63 mg GAE/g extract and a flavonoid content of 20.39 ± 0.53 mg QE/g extract, indicating its richness in bioactive phenolic constituents. It also exhibited strong antioxidant activity, with an IC_{50} value of 9.82 ± 1.70 µg/mL in the DPPH assay, while no antibacterial effect was observed against the tested bacterial strains. This study represents the first report combining phytochemical characterisation and biological evaluation of this plant species, highlighting its potential as a natural source of antioxidant compounds.

ARTICLE HISTORY
Received 9 August 2025
Accepted 8 November 2025

KEYWORDS
Psychine stylosa; extract; endemic; HPLC-MS/ms; antioxidant

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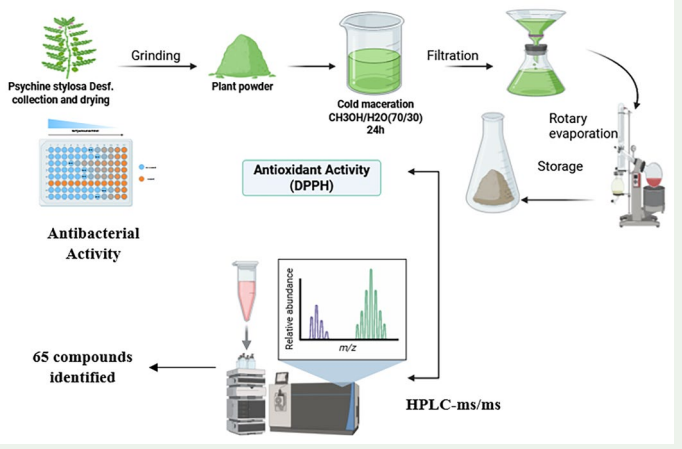
Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2025.2588798.



1. Introduction

Through numerous experiments, herbalists are able to identify the therapeutic benefits of various plants. By applying a trial-and-error approach, they refine their methods to achieve the desired outcomes, leading to the continuous expansion of the list of known medicinal herbs. Many modern pharmaceutical drugs are derived from plants, originally discovered through the traditional practices of indigenous peoples (Pan et al. 2014). Due to their complete biodegradability, minimal or no toxicity, natural availability, and generally lower cost compared to molecules synthesised through complex chemical processes (Nasim, et al. 2022), natural sources are gaining increasing popularity as alternative medicines. Given their potential as rich and natural sources of antioxidants, research on the antioxidant properties of medicinal plants has significantly increased in recent years(Chaouche et al. 2013; Haddouchi et al. 2014).

The Brassicaceae family comprises around 25 tribes, 338 genera, and over 3,700 plant species (OECD 2012). One of the endemic species of this family is *Psychine stylosa* (Synonyms: *Psychine numidica* Spreng., *Thlaspi psychine* Willd.) (APD 2024; POWO 2024) (see Figure S1). This hardy, bristly, and erect-branching annual herb features oval-oblong stem leaves that clasp the stem with sinuate margins. It produces relatively large (20–25 mm), whitish-yellow flowers that gradually turn reddish. The central stipe is accompanied by semicircular lateral petals. The ovary is covered in fine hairs and spines, and contains 24–32 ovules. The fruit is lomentaceous, consisting of two distinct segments: the upper segment forms a subulate beak, while the lower segment is ellipsoidal, bilocular, laterally compressed, and bears auriculate, leaf-like wings along its back (Tison et al. 2014). This plant thrives in the high plateaus and the A.C. section of the Tell region of Algeria, particularly in clayey-gypsiferous soils.This study aims to investigate the phytochemical composition of the aqueous methanolic extract (PS) from the aerial parts of *Psychine stylosa*, using High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (HPLC-MS/MS). A total of 65 compounds

belonging to various chemical classes were identified, including polyphenols, flavonoids, sugars, alkaloids, sulphur compounds, peptides, and other organic constituents. The total polyphenol content was determined to be 64.39 ± 1.63 mg GAE/g extract, and the total flavonoid content was 20.39 ± 0.53 mg QE/g extract. The extract also exhibited strong antioxidant activity as evidenced by the DPPH assay, showing an IC_{50} value of 9.82 ± 1.70 µg/mL. Furthermore, the antibacterial activity was tested against three Gram-positive strains (*Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*) and three Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*), but the extract did not demonstrate any inhibitory effects against these bacterial strains. This study highlights the promising biological potential of *P. stylosa*, owing to its rich content of bioactive compounds

2. Results and discussion

To identify the components of the Psychine stylosa (PS) extract, a comprehensive analysis was conducted using HPLC-MS/MS technology. The aim was to explore the chemical compounds present in the extract and to gain a deeper understanding of this plant species, which is being studied for the first time. The analytical results were further supported by mass spectrometry techniques (ESI-MS and ESI-MS/MS) in positive ion mode, providing additional accuracy and reliability in compound identification. (Figure S2) presents a TIC analysis of a sample examined without applying fragmentor voltage or collision energy, reflecting the full ion spectrum of the positively charged ions in the sample. (Figure S3) displays an Extracted Ion Chromatogram (EIC) within a mass range of 50 to 3200 m/z, allowing for more precise and selective compound detection. (Figure S4) shows the relationship between response intensity and acquisition time during the MS/MS experiment, using a defined solvent ratio, offering detailed insights into the fragmentation behaviour of the compounds. The HPLC-MS/MS analysis resulted in the identification of 65 compounds, as illustrated in the chromatogram (Figure S5). The findings revealed the presence of 13 unknown compounds (Table S1), along with 11 phenolic compounds, including polyphenols and flavonoids (Table S2). Among this group, six phenolic compounds and five flavonoids were identified and ranked in order of abundance as follows: Phenolic compounds: *Oxyquinoline, Piscidic Acid, Di-O-methylcrenatin, 4-(2-hydroxypropoxy)-3,5-dimethyl-Phenol, 5-Hydroxyindol-2-carboxylic acid, Vanillyl alcohol*.

Flavonoids: *6-C-Galactosylisoscutellarein, 6-C-Chinovopyranosylluteolin, Robinetin 3-rutinoside, Resveratrol 4'-(6-galloylglucoside), 3,4,7-Trihydroxy-5-methoxy-8-prenylflavan 4-O-(β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside)*. The molecular structures of these phenolic compounds and flavonoids identified in the aqueous-methanol extract of *Psychine stylosa* are illustrated in (Figure S6).

Among the phenolic compounds identified, (compound 1, Figure S6) Oxyquinoline (8-Hydroxyquinoline) (8-OHQ) stands out as a privileged scaffold present in many biologically active molecules and marketed drugs (Song et al. 2015; Saadeh, et al. 2020). Derivatives of 8-OHQ are utilised in the treatment of infectious diseases (e.g. 5-nitro-8-OHQ), neuropathies (e.g. 5-chloro-7-iodo-8-OHQ or clioquinol), and various cancers. Therapeutic strategies involving 8-OHQ mainly target essential enzymes such as iron-dependent ribonucleotide reductase, which plays a key role in DNA synthesis

(Shen, et al. 1999; Heffeter et al. 2019), as well as matrix metalloproteinases implicated in cancer metastasis (Chen, Yang et al. 2019). Other metalloenzyme targets include cytosolic and nuclear oxygenases (Hopkinson et al. 2013), histone demethylases (King et al. 2010), and HIF prolyl hydroxylase (Smirnova et al. 2010).

Moreover, metal complexes formed with 8-OHQ ligands have demonstrated intrinsic anticancer properties through disruption of cellular metal and redox balance (Adlard et al. 2008; Pape et al. 2021). A broad body of literature indicates that the biological activities of 8-OHQ derivatives can be fine-tuned through specific substitutions on the quinoline ring. For example, substitution at position 2 with aromatic amides (R2) enhances lipophilicity and antiviral activity due to the electron-withdrawing nature of anilide groups (Kos et al. 2019). The incorporation of glucoconjugates has also been proposed as a prodrug strategy (Oliveri et al. 2012), leading to improved anticancer activity in some cell lines (Krawczyk et al. 2019). Similarly, position 5 substitution (R5) with electron-withdrawing groups increases anticancer effects (Shaw et al. 2010), while sulphonic acid substitution (as in sulfoxine: 8-hydroxy-5-quinolinesulfonic acid) appears to reduce cytotoxicity, likely due to diminished cell permeability (Dömötör et al. 2017).

Similarly, piscidic acid was identified as one of the major constituents in considerable abundance, demonstrating particular significance according to the study by Ressaissi. This compound exhibited notable permeability across Caco-2 cell monolayers, suggesting its ability to reach the liver and modulate cholesterol metabolism. Although its inhibitory activity against HMG-CoA reductase was modest compared to standard statins, piscidic acid showed low cytotoxicity and contributed to the cholesterol-lowering effect of the aqueous extract, highlighting its therapeutic potential in managing hypercholesterolaemia (Ressaissi et al. 2017).

6-C-Galactosylisoscutellarein (compound 7, Figure S6), a rare C-glycosyl flavonoid derived from isoscutellarein, was identified as the most abundant flavonoid among the discovered compounds. It may contribute to antioxidant and anti-inflammatory activities, warranting further investigation into its biological role. Likewise, the results of spectroscopic chemical analyses using HPLC-MS/MS revealed a complex and rich chemical composition of the aqueous-methanolic extract of *Psychine stylosa*. In addition to the identification of several major phenolic compounds, 28 additional compounds were detected, distributed among various bioactive chemical classes. These included three alkaloids, known for their diverse pharmacological effects such as antimicrobial and anticancer activities (Cushnie et al. 2014), and six peptides, which may contribute to antioxidant activities or the regulation of cellular processes (Udenigwe and Aluko 2012). In addition, four terpenoids were identified, which are compounds with anti-inflammatory and antibacterial properties (Gershenzon and Dudareva 2007), along with four amino acid derivatives, which may influence metabolic pathways and cellular functions (Li et al. 2020). Furthermore, three sulphur compounds and five sugar derivatives were detected, in addition to one organic acid, one steroid, and one fatty acid (Table S3). Moreover, the analysis revealed the presence of 12 structurally diverse organic compounds, as shown in (Table S4), highlighting the significant chemical diversity of the extract. This rich variety of chemical classes strongly supports the potential of this plant species as a natural source of bioactive compounds and reinforces the medicinal value of *Psychine stylosa*. It also calls for

further studies to elucidate the physiological mechanisms associated with each chemical class.

The aqueous-methanolic extract of *Psychine stylosa* (PS) exhibited a high total polyphenol content, reaching 64.39 ± 1.63 mg gallic acid equivalents per gram of extract (mg GAE/g extract), as determined using a gallic acid calibration curve (Figure S7). Additionally, the flavonoid content was substantial, measured at 20.39 ± 0.53 mg quercetin equivalents per gram of extract (mg QE/g extract), based on a quercetin calibration curve (Figure S8). These findings highlight the plant's richness in bioactive phenolic constituents with potential antioxidant properties.

In the DPPH free radical scavenging assay, the extract demonstrated strong antioxidant activity, with an IC_{50} value of 9.82 ± 1.70 µg/mL. This activity was significantly higher than that of ascorbic acid, which showed an IC_{50} of 28.38 ± 1.40 µg/mL. The superior antioxidant capacity of the extract is likely attributed to its high content of polyphenols and flavonoids.

To the best of our knowledge, this study represents the first scientific investigation into the phytochemical profile and antioxidant activity of *Psychine stylosa*. These results emphasise its potential as a promising natural source of antioxidants and support further research to isolate active constituents and evaluate their pharmaceutical or nutraceutical potential.

The antibacterial potential of the aqueous-methanolic extract of *Psychine stylosa* was assessed using the microdilution method to determine the minimum inhibitory concentration (MIC), against a panel of clinically relevant bacterial strains, including both Gram-negative and Gram-positive species. The assay followed standardised protocols, with ampicillin used as a reference antibiotic for comparison.

As shown in **(**Table S5**)**, the extract did not exhibit significant inhibitory activity against the tested Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), and *Klebsiella pneumoniae* (ATCC 10031) with all MIC values exceeding 512 µg/mL. These results reflect the low efficacy of the extract against Gram-negative bacteria, which are known to possess an outer membrane barrier that limits the penetration of many plant-derived compounds, particularly those with high molecular weight or polarity. In contrast, ampicillin showed good activity against *E. coli* (MIC = 8 µg/mL), but limited or no effect against *P. aeruginosa* and *K. pneumoniae* (MIC = 512 µg/mL and >512 µg/mL, respectively). For Gram-positive bacteria, the *P. stylosa* extract exhibited weak activity against *Enterococcus faecalis* (ATCC 29212), with a MIC value of 512 µg/mL, while no activity was observed against *Bacillus cereus* (CCM 99) and *Staphylococcus aureus* (ATCC 25213). By comparison, ampicillin showed significantly higher activity, especially against *E. faecalis* (MIC = 2 µg/mL) and *S. aureus* (MIC = 32 µg/mL). Although the extract did not display strong antibacterial effects in its crude form, the limited inhibition observed against *E. faecalis* may suggest the presence of bioactive compounds with selective antimicrobial potential. The weak overall performance could be attributed to low concentrations of active constituents or to the chemical nature of the compounds, which may not exert strong effects in their crude, unpurified form. To the best of our knowledge, this is the first scientific study investigating the antibacterial activity of *Psychine stylosa*. These findings lay the groundwork for future phytochemical research focused on isolating and characterising active fractions or pure compounds

with enhanced antibacterial properties. Additionally, synergistic effects with antibiotics or other natural extracts may be explored to enhance its therapeutic potential.

3. Materials and methods

See Supplementary Material

4. Conclusion

This study represents the first comprehensive phytochemical analysis of *Psychine stylosa*, an endemic plant species from Algeria, using advanced HPLC-MS/MS techniques. The analysis revealed 65 chemical compounds, including phenolic compounds, flavonoids, alkaloids, peptides, and sulphur-containing molecules, highlighting its significant chemical diversity. The extract showed high total polyphenol (64.39 mg GAE/g) and flavonoid content (20.39 mg QE/g), with potent antioxidant activity surpassing that of ascorbic acid (IC_{50} = 9.82 µg/mL). Although the crude extract exhibited weak antibacterial activity, particularly against Gram-negative strains, limited inhibition against *Enterococcus faecalis* suggests the presence of selectively active compounds. The identification of bioactive constituents such as 8-hydroxyquinoline and 6-C-galactosylisoscutellarein provides a strong basis for future research into their pharmaceutical potential. These findings underscore the therapeutic promise of this endemic species and support further studies on compound isolation, biological evaluation, and synergistic effects. Overall, this work opens new avenues for valorising Algeria's botanical heritage in drug discovery and nutraceutical development.

Author contributions

CRediT: **Turqui Tarik**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Benaiche Ghania**: Supervision; **Benkouider Imen**: Formal analysis; **Khellaf Rebbas**: Resources.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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