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RESEARCH ARTICLES

Analysis of phenolic compounds in the methanol extract of *Salvia candidissima* subsp. *candidissima* by LC-HESI-MS/MS and evaluation of its antioxidant and antifungal properties

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Abstract
This study evaluated the methanolic extract of *Salvia candidissima* subsp. *candidissima* (*Salvia candidissima*) for its phenolic composition, total phenolics content and total flavonoid content (TPC and TFC), antioxidant capacity, and antifungal potential. UHPLC-LC–MS/MS screening identified 11 phenolic compounds (out of 29 analyzed), with luteolin-7-glucoside (1578.279 µg/g extract), rosmarinic acid (776.977 µg/g), apigenin (118.250 µg/g), and luteolin (41.267 µg/g) being the most abundant. The extract contained 94.41 ± 0.46 mg GAE/g extract of TPC and 40.57 ± 1.03 mg QE/g extract of TFC. In the DPPH assay, the extract exhibited strong free radical scavenging with an IC50 of 91.79 ± 1.12 µg/mL, while FRAP indicated substantial reducing power at 88.41 ± 1.17 mg TE/g extract. Antifungal activity was tested against several phytopathogens, including *Phytophthora infestans* (FF), *Sclerotinia sclerotiorum* (SS), *Rhizoctonia solani* (RS), *Fusarium oxysporum* f. sp. *melonis* (FOM), *Fusarium oxysporum* f. sp. *niveum* (FON), *Verticillium dahliae* (VD), and *Monilinia fructigena* (MF). At 8 mg/mL, growth inhibition varied: FF 75.26%, FON 56.65%, RS 52.26%, MF 49.16%, VD 47.28%, FOM 27.16%. The antifungal effects were linked to the presence of phenolics and flavonoids such as luteolin-7-glucoside, rosmarinic acid, and apigenin, which are known for antifungal activity. Overall, the methanolic extract of *S. candidissima* emerges as a promising candidate for developing plant-derived natural antifungal agents.

Keywords Antioxidant activity · Antifungal activity · Phytochemical composition · *Salvia candidissima* subsp. *candidissima*

Abbreviations

TPC	Total phenolic contents
TFC	Total flavonoid contents
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
LOD	Limits of detection
LOQ	Limits of quantification
PDA	Potato dextrose agar
DMSO	Dimethyl sulfoxide

Introduction

The genus *Salvia* (Lamiaceae) comprises approximately 1000 species distributed across diverse geographical regions, particularly in the Americas and Asia (Walker and Sytsma 2007; Özler et al. 2013). Within the flora of Türkiye, 95 *Salvia* species have been reported, with an exceptionally high endemism rate of 51%. Salvia species are widely recognized for their medicinal and economic importance, being traditionally used for the treatment of various ailments (Davis 1982; Poyraz and Koca 2006; Celep et al. 2009).

Phytochemically, *Salvia* species are rich in secondary metabolites such as flavonoids, terpenoids, and essential oils. The aerial parts generally contain abundant flavonoids, triterpenoids, and monoterpenoids, whereas the roots are primarily rich in diterpenoids (Ulubelen 2000). These metabolites contribute to the broad pharmacological spectrum of the genus, including antimicrobial, antidiabetic,

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antioxidant, and antitumor activities (Hitokato et al. 1980; Ulubelen 2003; Tosun et al. 2009). Moreover, *Salvia* extracts and essential oils are utilized in cosmetics, perfumery, pharmaceuticals, herbal teas, and as flavoring agents (Demirci et al. 2003; Marin et al. 1996).

Türkiye is a significant biodiversity center for *Salvia*, hosting 96 species and four subspecies (Doğan et al. 2008). Among them, *Salvia candidissima* subsp. *candidissima* is a perennial herbaceous species, 30–60 cm tall, with erect, branched stems and densely hairy, oblong–ovate leaves. The corolla is white, while the bracts are yellowish-green. The plant typically grows on calcareous or schist slopes at altitudes between 700 and 2000 m (Ozer 2016) and flowers from May to September (Karabacak 2009). Previous studies have reported the presence of phenolics, terpenoids, essential oils, fatty acids, and volatile organic compounds in its aerial and root parts, along with antioxidant, antidiabetic, and antimicrobial activities (Ulubelen et al. 1997; Bakoglu et al. 2016; Bayar and Genç 2018; Adımcılar et al. 2019; Güzel Kara et al. 2023).

The present study aimed to (i) characterize the phytochemical composition of *S. candidissima* methanolic extract via LC-MS/MS, (ii) determine its total phenolic and flavonoid contents, (iii) evaluate its antioxidant potential through DPPH and FRAP assays, and (iv) investigate its in vitro antifungal activity against major phytopathogenic fungi.

Materials and methods

Plant material

Specimens of the species *S. candidissima* subsp. *candidissima* were collected from natural populations in the Sarız region (Kayseri, Turkey) at coordinates 38°33'29.40"N 36°26'48.98"E during the flowering period and under optimal ecological conditions. Taxonomic verification was conducted by Assoc. Prof. Dr. Melih Yılar from the Department of Plant Protection. The plant specimen (Accession No.: MY-1002) is preserved in the Herbology Laboratory.

Preparation of the methanolic extract

Aerial parts of the plant were cleaned, shade-dried, and pulverized using a stainless-steel mill. A 100 g sample was extracted with 600 mL of methanol on an orbital shaker for 24 h. The resulting extract was filtered (Whatman No. 1) and concentrated under reduced pressure at 32 °C. The final residue was weighed and stored in amber vials at 4 °C for subsequent ICP–MS, antioxidant, and antifungal analyses.

Reagents and solutions

All solutions were prepared using analytical grade reagents and ultrapure water (Milli-Q, Millipore, Bedford, MA, USA). LC/MS grade methanol (MeOH) and chloroform ($CHCl_3$) used for the analysis of individual phenolic compounds were obtained from Merck (Darmstadt, Germany), while HPLC grade formic acid was purchased from J.T. Baker (Phillipsburg, NJ, USA).

All standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of each phenolic compound were prepared in MeOH at a concentration of 1000 mg/L and stored at 4±2 °C. Quantitative analysis of phenolic compounds was performed using the external standard calibration method.

Phytochemical characterization by LC–MS/MS

Phenolic compounds in *Salvia candidissima* were analyzed using a Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled with a TSQ Quantum Access Max triple quadrupole mass spectrometer (LC–MS/MS). Separation was performed on an Inertsil ODS HYPERSIL C18 column (dimensions: 250 mm × 4.6 mm, particle size: 5 µm) using a gradient of 0.1% formic acid in water (A) and methanol (B). The elution profile was: 0–1 min (0% B), 1–22 min (95% B), 22–25 min (95% B), and 25–30 min (100% B). The flow rate was 0.7 mL/min with a 20 mu injection volume and a 34-min total run time.

The method was adapted from Doğan et al. (2025). Quantification was based on six-point linear calibration curves. Limits of detection (LOD) and quantification (LOQ) were defined as LOD = 3 × (S/N) and LOQ = 10 × (S/N), respectively. Relevant chromatograms are provided in the supplementary materials (Supplementary material 1).

Determination of total flavonoid content (TFC)

Total flavonoid content was assessed via a colorimetric method modified from Yılar et al. (2020). Briefly, aliquots of the extract and quercetin standard solutions were diluted to 4.8 mL with methanol, followed by addition of 100 µL of 1 M ammonium acetate and 100 µL of 10% aluminum chloride solution. The mixture was then thoroughly mixed. The reaction mixture was then left to incubate at room temperature for 45 min for complex formation. Spectrophotometric measurement of absorbance was performed at 415 nm, and flavonoid levels were calculated as milligrams of quercetin equivalents per gram of extract (mg QE g^{-1}).

Determination of total phenolic content (TPC)

The total phenolic content was quantified using the Folin–Ciocalteu colorimetric procedure described by Akman et al. (2023), with slight modifications. To summarize, a mixture of 100 µL extract, 4.5 mL distilled water, 100 µL Folin–Ciocalteu reagent, and 300 µL 2% (w/v) sodium carbonate solution was prepared. The reaction was carried out for 2 h at room temperature in subdued light. Absorbance readings were taken at 760 nm using a UV–Vis spectrophotometer, and phenolic content was reported as milligrams of gallic acid equivalents per gram of extract (mg GAE g^{-1}).

DPPH radical scavenging activity

Antioxidant capacity was evaluated via the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, modified from Şimşek et al. (2025). Briefly, 1 mL of 0.26 mM methanolic DPPH was mixed with extracts at concentrations ranging from 20 to 1000 µg/mL. Following a 30-minute dark incubation at room temperature, absorbance was measured at 517 nm. The IC_{50} values (concentration for 50% inhibition) were calculated from döşe-response curves. Results were compared against standard antioxidants: Trolox, ascorbic acid, BHA, and BHT.

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of the extract was analyzed using the modified FRAP assay as reported by Şimşek et al. (2025). A 1 mL aliquot of the extract solution (1 mg/mL) was combined with.

Phosphate buffer (pH 6.6) and potassium ferricyanide. This mixture was incubated at 50 °C for 20 min. Subsequently, trichloroacetic acid and ferric chloride were added. The absorbance of the resulting Prussian blue complex was measured at 700 nm. The results were reported as milligrams of Trolox equivalents per gram of extract (mg TE g^{-1}). The reducing efficiency of the extract was compared to that of ascorbic acid, BHA, and BHT standards.

In vitro antifungal bioassay

The fungi of plant pathogen used in the study *Phytophthora infestans*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *melonis*, *Fusarium oxysporum* f. sp. *niveum*, *Verticillium dahliae*, and *Monilinia fructigena* were obtained from the stock cultures in Phytopathology laboratories of Department of Plant protection, Faculty of agriculture, Ahi Evran University.

Antifungal activity was evaluated using the modified agar dilution method. The methanolic extract was dissolved in 1% (v/v) Dimethyl sulfoxide (DMSO) and incorporated into sterilized PDA to reach final concentrations of 0.25–8 mg/mL. Five-millimeter mycelial discs from 7-day-old cultures were inoculated onto 60 mm Petri dishes and incubated at 23 ± 2 °C for 10 days The applications were made in 3 sets of 2 repetitions each.

Pandey et al. (1982): I (%) = 100×(dc − dt) /dc where I = inhibition percentage, dc = mycelial growth in control, and dt = mycelial growth in treatment.

A 1% DMSO solution served as the negative control, while the commercial fungicide thiram (80%) was used as the positive control. All treatments were carried out in triplicate and independently repeated twice to ensure reproducibility.

Statistical analysis

SPSS version 15.0 was used to perform ANOVA on all experimental data. Differences among treatment means were evaluated by Duncan's multiple range test at $p < 0.05$. All results are shown as mean ± SD.

Results and discussion

Phytochemical composition (LC–MS/MS analysis)

The quantitative LC–MS/MS profiling of the methanolic extract of *Salvia candidissima* revealed the presence of 11 phenolic compounds out of the 29 standards analyzed (Table 1). The chromatographic fingerprints demonstrated a chemically diverse profile dominated by luteolin-7-glucoside (1578.279 µg g^{-1} extract), followed by rosmarinic acid (776.977 µg g^{-1}), apigenin (118.250 µg g^{-1}), and luteolin (41.267 µg g^{-1}). Minor constituents included caffeic acid, ferulic acid, and vanillic acid, each occurring in trace amounts.

These findings are in partial agreement with previous studies that reported phenolic diversity in S. candidissima extracts. For example, Llorent-Martínez et al. (2025) identified 35 phenolic compounds within methanolic extracts of *S. candidissima*, highlighting rutin and hyperoside as the dominant flavonoids. Similarly, Emre et al. (2021) characterized both phenolic acids and flavonoids in *S. candidissima*, noting caffeic acid, vanillic acid, rosmarinic acid, and ferulic acid as major components. Topçu et al. (1995) further described several terpenoids such as spathulenol, salvigenin, and diosmetin in aerial tissues of the same species.

Table 1 Quantitative analysis of MeOH extracts of *Salvia candidissima* subsp. *candidissima* determined by LC-ESI-MS/MS

No	RT	Phenolic compound	*Salvia candidissima* subsp. *candidissima* amount (µg/g extract)
1	9.19	Gallic acid	n.d.*
2	11.11	Protocatechuic acid	n.d.
3	12.06	Protocatechuic Aldehyde	13.424
4	12.07	Sesamol	8.761
5	12.34	Gentisic acid	2.517
6	12.58	Catechin	n.d.
7	13.61	Chlorogenic acid	15.653
8	13.99	Epicatechin	0.196
9	14.19	Caffeic acid	20.131
10	14.71	Vanillin	n.d.
11	15.71	p-Coumaric acid	n.d.
12	15.86	Taxifolin	n.d.
13	16.13	Ferulic acid	n.d.
14	16.36	Salicylic acid	n.d.
15	16.37	*p*-Hydroxybenzoic acid	n.d.
16	16.78	Hesperidin	n.d.
17	16.86	Rosmarinic acid	776.977
18	16.95	Oleuropein	n.d.
19	17.30	Luteolin-7-Glucoside	1578.279
20	17.48	Rutin	3.317
21	17.59	Rezveratrol	n.d.
22	19.08	Ellagic acid	n.d.
23	19.16	Naringenin	n.d.
24	19.75	Quercetin	n.d.
25	20.23	Luteolin	41.267
26	21.15	Apigenin	118.250
27	21.19	Pinocembrin	n.d.
28	22.59	Chrysin	n.d.
29	22.64	Galangin	n.d.

*n.d.: Not detected

Table 2 Total phenolic and total flavonoid content of *Salvia candidissima* subsp. *candidissima*

Sample	Total phenolic contents (mg GAE g^{-1} extract)	Total flavnoid contens (mg QE g^{-1} extract)
MeOH extract	94.41 ± 0.46	40.57 ± 1.03

Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extract were determined as 94.41 ± 0.46 mg GAE g^{-1} and 40.57 ± 1.03 mg QE g^{-1} extract, respectively (Table 2). These values demonstrate that *S. candidissima* is a significant source of phenolic antioxidants, corroborating its traditional use as a medicinal plant.

When compared with earlier studies, the results fall within the reported range but exhibit notable quantitative differences. Llorent-Martínez et al. (2025) reported TPC and TFC values of 66.51 ± 1.69 mg GAE g^{-1} and 23.23 ± 0.37 mg RE g^{-1}, respectively, whereas Bayar and Genç (2018) found higher concentrations (TPC: 83.53 ± 5.92 mg GAE g^{-1}; TFC: 59.02 ± 3.59 mg QE g^{-1}) for *S. candidissima* Vahl. Stankov et al. (2020) observed TPC values of 25.16 ± 0.74 mg GAE g^{-1} in ethanol extracts (95%) and 22.86 ± 1.47 mg GAE g^{-1} in 70% ethanol extracts, highlighting the influence of solvent polarity on extraction efficiency.

DPPH radical scavenging activity

The DPPH free radical scavenging assay demonstrated strong antioxidant potential for the methanolic extract, with an IC_{50} value of 91.79 ± 1.12 µg mL^{-1} (Fig. 1). Although the radical-scavenging capacity of this extract is lower than that of standard antioxidants such as Trolox, BHT, and ascorbic acid, it has demonstrated a notable effect.

For instance, Llorent-Martínez et al. (2025) reported a radical scavenging activity of 226.41 ± 1.03 mg TE g^{-1} for methanolic extracts, whereas Stankov et al. (2020) recorded values of 275.67 ± 4.06 and 237.12 ± 2.57 mM TE g^{-1} for 95% and 70% ethanol extracts, respectively. Similarly, Tepe et al. (2006) and Tosun et al. (2009) recorded IC_{50} values of 49.7 µg mL^{-1} and 33.4 µg mL^{-1}, respectively, for different

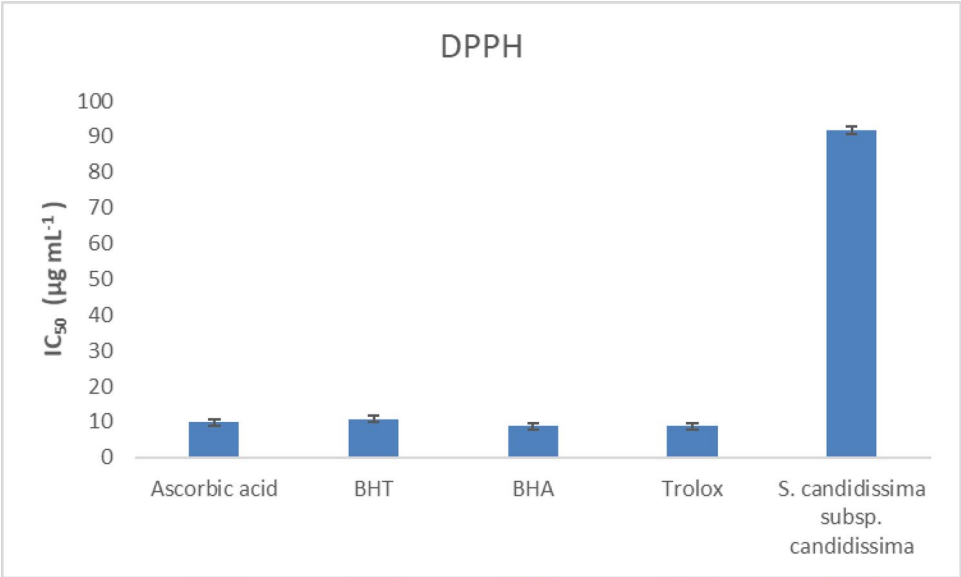

Fig. 1 DPPH test results of the methanol extract of *Salvia candidissima* subsp. *candidissima*

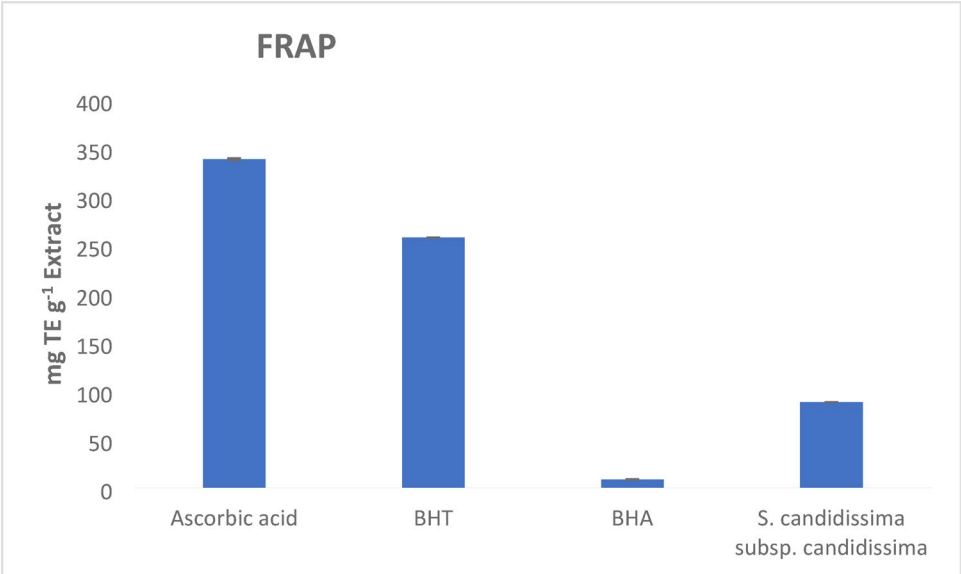

Fig. 2 FRAP results of the methanol extract of *Salvia candidissima* subsp. *candidissima*

S. candidissima extracts. Diri (2006) observed the highest scavenging efficiency in ethanol (94.7%), hexane (93.82%), and ethyl acetate (92.3%) extracts.

Nonetheless, the extract's significant activity indicates a synergistic contribution of polyphenols, particularly luteolin-7-glucoside and rosmarinic acid, which are well-documented free radical scavengers (Kishore et al. 2007; Aydın et al. 2004).

Ferric-reducing antioxidant power (FRAP)

The ferric-reducing capacity of the methanolic extract was measured as 88.41 ± 1.17 mg TE g^{-1} (Fig. 2). When benchmarked against reference antioxidants, the reducing activity ranked in the following order: ascorbic acid > BHT > methanolic extract > BHA. These results indicate that the extract exhibits moderate yet consistent electron-donating potential, which is characteristic of phenolic-rich matrices.

Previous investigations have reported varying FRAP values for *S. candidissima*. Llorent-Martínez et al. (2025) measured 124.57 ± 3.07 mg TE g^{-1}, while Bayar and Genç (2018) documented 1.20 ± 0.16 mmol TE g^{-1}. The observed discrepancy likely stems from the extraction system, solvent polarity, and temperature conditions employed.

Antifungal activity

The antifungal potential of the methanolic extract was assessed against a range of phytopathogenic fungi, including FF, SS, RS, FOM, FON, VD, and MF. The inhibitory

Table 3 Antifungal activity of *Salvia candidissima* subsp. *candidissima* MeOH extract against major plant pathogens (percent mycelial growth inhibition, % ± SE)

Concentration (mg/mL)	SS**	RS	FF	FON	FOM	VD	MF
N-	0.00 ± 0.00b*	0.00 ± 0.00e	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00d
0,25	0.00 ± 0.00b	0.00 ± 0.00e	0.00 ± 0.00f	38.13 ± 0.93e	0.00 ± 0.00f	6.50 ± 6.50e	0.00 ± 0.00d
0,50	0.00 ± 0.00b	0.00 ± 0.00e	18.31 ± 0.69e	39.35 ± 0.46e	1.847 ± 1.47e	23.40 ± 0.34d	0.00 ± 0.00d
1	0.00 ± 0.00b	0.00 ± 0.00e	29.27 ± 0.91d	41.46 ± 0.98d	24.56 ± 2.87d	23.69 ± 0.48d	0.00 ± 0.00d
2	0.00 ± 0.00b	35.77 ± 2.17d	55.87 ± 0.73c	51.26 ± 0.15c	29.47 ± 3.52 cd	33.25 ± 1.92c	31.32 ± 1.12c
4	0.00 ± 0.00b	41.44 ± 4.27c	56.98 ± 0.57c	56.25 ± 0.44b	32.97 ± 1.28c	38.75 ± 1.75c	37.02 ± 0.41c
8	0.00 ± 0.00b	52.26 ± 0.34b	75.26 ± 0.32b	56.65 ± 0.30b	40.11 ± 2.17b	47.28 ± 1.06b	49.16 ± 0.51b
P+	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a

*Values represent mean ± standard error (SE) of three replicates. **Different superscript letters within the same column indicate statistically significant differences among treatments according to ANOVA followed by Duncan's multiple range test ($p < 0.05$)

effects were dose-dependent and statistically significant ($p < 0.05$).

At the highest concentration (8 mg mL^{-1}), the extract inhibited mycelial growth by 75.26% in FF, 56.65% in FON, 52.26% in RS, 49.16% in MF, 47.28% in VD, and 40.11% in FOM, while no inhibition was observed in SS cultures (Table 3). These results demonstrate a selective antifungal spectrum. The commercial fungicide thiram (80%) exhibited complete growth inhibition, serving as a positive control.

Comparable antifungal activity was reported by Bayar and Genç (2018), who found that essential oils from *S. candidissima* suppressed *R. solani* and *A. solani* growth by 57.92% and 51.87%, respectively. The antifungal properties observed in the present study can be ascribed to secondary metabolites such as luteolin-7-glucoside, rosmarinic acid, and apigenin compounds known to disrupt fungal cell wall integrity, inhibit spore germination, and interfere with membrane permeability (Lee et al. 2019; Aydın et al. 2024).

Conclusion

The LC–MS/MS profiling revealed a complex phenolic matrix dominated by luteolin-7-glucoside, rosmarinic acid, apigenin, and luteolin, indicating that the plant is a valuable natural reservoir of bioactive flavonoids and phenolic acids. These compounds are well-known for their strong antioxidant and antimicrobial properties, which collectively contribute to the overall biological potency of the extract. The relatively high total phenolic (94.41 ± 0.46 mg GAE g^{-1}) and total flavonoid (40.57 ± 1.03 mg QE g^{-1}) contents, along with the marked DPPH scavenging (IC_{50} = 91.79 ± 1.12 µg mL^{-1}) and ferric-reducing (88.41 ± 1.17 mg TE g^{-1}) capacities, highlight the plant's substantial redox potential. These results underscore the importance of *S. candidissima* as a promising source of natural antioxidants capable of mitigating oxidative stress through multiple electron-donating and radical-scavenging mechanisms. Furthermore, the extract exhibited moderate-to-strong antifungal effects against several economically significant phytopathogens, including FF, SS, RS, FOM, FON, VD, and MF species. Overall, this study not only strengthens the phytochemical and pharmacological understanding of *S. candidissima* but also establishes a foundation for its potential.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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