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Therapeutic Potential of *Salvia absconditiflora* Greuter&Burdet: From Polyphenol Screening to Antimicrobial, Antibiofilm and Anti-inflammatory Applications

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HIGHLIGHTS

- The aqueous extract of *Salvia absconditiflora* Greuter&Burdet significantly reduced nitrite levels in RAW 264.7 macrophage cells.
- It showed a pronounced concentration-dependent effect on the biofilm formation of *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 and *S. epidermidis* ATCC 35984.
- It also performed a significant inhibitory effect on the violacein production of *C. violaceum* ATCC 12472, indicating a disruption of the quorum-sensing pathways.

Abstract: Plant extracts contain an extensive chemical profile that offers several benefits for well-being. Microbial burden and inflammation impair immune function and play key roles in the development of chronic diseases. This study investigated the phenolic profile and biological activities of an aqueous extract of *Salvia absconditiflora* Greuter&Burdet obtained by ultrasound-assisted extraction (UAE), a green and eco-friendly method. Phenolic characterization by LC–MS/MS identified luteolin-7-*O*-glucoside as the major compound. The extract demonstrated strong antioxidant activity, with an IC_{50} value of 0.0705 mg/mL in the DPPH assay and a FRAP value of 119.0877 mg Trolox/g. Anti-inflammatory effects were evaluated in LPS/IFNγ-stimulated RAW 264.7 macrophages. The extract significantly reduced nitrite levels at concentrations of 0.1–100 µg/mL and showed anti-inflammatory activity at 0.1–50 µg/mL without markedly affecting cell viability. Cytotoxicity assessments revealed selective anticancer potential. The extract significantly reduced cell viability in HCT-116 colon cancer cells across the 0.1–100 µg/mL range, while no significant cytotoxic effects were observed

in HL60 acute myeloid leukemia cells or in healthy L929 mouse fibroblasts, indicating moderate selectivity toward colon cancer cells. Moreover, the aqueous extract exhibited selective antimicrobial activity, particularly against Gram-positive bacteria and certain *Candida* species. It showed a pronounced concentration-dependent effect on biofilm formation in *Staphylococcus aureus* (ATCC 29213 and ATCC 43300) and *Staphylococcus epidermidis* (ATCC 35984). Additionally, inhibition of violacein production in *Chromobacterium violaceum* ATCC 12472 suggested disruption of quorum-sensing pathways. Our research demonstrates the promise of *S. absconditiflora* as a source of novel antimicrobial agents and therapeutic leads.

Keywords: *Salvia absconditiflora* Greuter&Burdet; ultrasound-assisted extraction; antibiofilm; anti-quorum sensing; anti-inflammatory activity.

INTRODUCTION

Herbal extracts contain an extensive chemical profile that provides several advantages to well-being. Well-being is linked to favorable physical health, longevity, and a positive mental state [1]. To address these conditions, new solutions must be developed that promote healthy ageing through prevention and medication. Microbial burden and inflammation are both problems that affect the immune system and thus well-being and, if left untreated, can lead to serious chronic diseases.

Increasing antibiotic resistance is one of the factors complicating the treatment of biofilm infections, especially those associated with pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis*. Biofilms are extracellular layers of substances formed by bacteria to protect themselves from external influences and are highly resistant to antibiotics and the immune system [2,3]. Biofilm-associated diseases are a major issue in modern medicine as they are resistant to traditional antibiotics and the human immune system [4].

The genus *Salvia* belongs to the Lamiaceae family. Lamiaceae is the fourth largest family, based on the number of species in Turkiye. Among them, *Salvia* is an ancient plant known worldwide to alleviate common viral diseases, soothe physical ailments, fight harmful microorganisms and treat parasitic diseases, coughs, bronchitis, abnormal cell growth, cognitive decline, and circulatory problems. They are consumed as beverages and traditionally used to heal wounds, reduce swelling, and treat skin problems [5-9]. The name *Salvia* comes from the Latin word "salvere", which means "to heal" [10]. *Salvia* is widely distributed in the Mediterranean, South Africa, Central and South America, and Asia [11].

As traditional thermal extraction procedures have disadvantages such as extended extraction times, thermal degradation, and excessive solvent use, new-generation, environmentally friendly extraction processes have been developed, one of which is ultrasound-assisted extraction. Ultrasound-assisted extraction with water may align with green chemistry principles, which aim to protect the environment and human health. This method encourages industries to be environmentally friendly, and it is also beneficial to adopt green methods in the production of herbal preparations/medicines [12,13].

Salvia absconditiflora Greuter & Burdet (=*Salvia cryptantha* Montbret & Aucher ex Benth.) is a species endemic to Turkiye and is called “yakışalba, kara ot”. The aerial parts of the plant are consumed as tea to treat stomach problems, common cold, bronchitis, and shortness of breath [14,15].

Natural sources are a major focus of scientific studies due to their complex chemical composition, which is associated with a wide range of biological activities and therapeutic potential. *Salvia* species are widely recognized for their antimicrobial properties. A limited number of studies appear to have examined the antibacterial and antioxidant potentials of the endemic species *S. absconditiflora*, whereas studies addressing its anti-quorum sensing and antibiofilm activities have not yet been found in the literature review [16-19].

Previous studies indicate that anti-inflammatory activity varies among different *Salvia* species. Extracts of *S. ceratophylla*, *S. dominica*, *S. multicaulis*, *S. palaestina*, *S. spinosa* and *S. syriaca* species have been reported to exhibit anti-inflammatory activity in the croton oil-induced inflammation model [20]. Additionally, *S. fruticosa*, *S. trichoclada*, and *S. verticillata* demonstrated anti-inflammatory effects, with the n-butanol extract of *S. fruticosa* being the most effective [21]. *Salvia plebeia* has also been shown to reduce TNF-α, IL-6, and nitric oxide levels in LPS-stimulated RAW 264.7 macrophages [22].

In a study examining the cytotoxic effects of *Salvia* species, including *S. dominica*, *S. lanigera*, *S. menthaefolia*, *S. palaestina*, *S. sclarea* and *S. spinosa* in different cancer cell lines (DBTRG-05MG, T98G, U-87MG, WiDr, HT-29, MDA Pca2b, JEG-3, HEC-1A, CIR). *S. menthaefolia* was identified as the species with the most pronounced cytotoxic activity among those tested species [23]. Ethanol extracts of *S. lanigera* were not cytotoxic even at 200 µg/mL (the highest dose) in HCT-116 and LoVo cell lines [24]. Other *Salvia*

species in HL60 cell lines are available in the literature. In the study by Tayarani-Najaran and coauthors [25], the IC_{50} values of the methanol, n-hexane, and dichloromethane fractions of *S. chorassanica* ranged from 90.37 to 151.6 µg/mL, whereas the IC_{50} values of the ethyl acetate and aqueous fractions were reported to be >300 µg/mL [25]. Kawabe and coauthors reported that compounds isolated from *S. grandifolia* did not exhibit strong cytotoxic activity in HL60 and HeLa cell lines [26].

To the best of our knowledge, there are no studies describing either the anti-inflammatory activity of *S. absconditiflora* in LPS/IFN-γ–stimulated RAW 264.7 macrophages or its cytotoxic activity in HCT-116 and HL-60 cell lines.

In light of the plant's historical use in Turkiye and the growing adoption of ultrasound-assisted extraction as a green technology in recent years, this study aims to comprehensively analyze the plant's phenolic profile via LC–MS/MS and evaluate its antimicrobial, anti-quorum sensing, antibiofilm, and anti-inflammatory activities, which, to the best of our knowledge, are investigated together for the first time, to elucidate its potential health benefits.

MATERIAL AND METHODS

Plant Material

The flowering aerial parts of *S. absconditiflora* were collected in Ankara on 27 May 2022. The voucher specimen was deposited in the herbarium of the Faculty of Pharmacy of Ankara University (AEF) under the number AEF30956, and this species was identified by Prof. Dr. Hayri Duman (Gazi University).

Extraction

10 g of the flowering aerial parts of the plant were mixed with 300 ml of distilled water. A Hydra-800 ultrasonic system (Türkiye) with a frequency of 24 KHz and ultrasonic power of 800 watts was used for the extraction. The ultrasonic power was maintained continuously at 20% for 5 min. It was filtered through filter paper. The extracts were then freeze-dried. The extract was stored at +4 °C until the analyses were performed.

Total Phenolic Content

The plant extract sample (1 mg/mL) was prepared and reacted with Folin-Ciocalteau's reagent. The tubes were then filled with 7% Na_2CO_3 and incubated for 30 min. The absorbance value of the samples was determined at 750 nm. The same procedure was performed with gallic acid, and the results were expressed as mg gallic acid equivalent (GAE) per g of crude extract for each sample. The experiments were performed in triplicate [27].

Total Flavonoid Content

The aluminum chloride method, with slight modifications, was used to determine the total flavonoid content of the extract. Briefly, a plant extract sample (1 mg/mL) was prepared, and 1 M sodium acetate and 10% $AlCl_3$ were added to each tube. After 6 min of incubation, 2 mL of distilled water was added, and incubation was carried out for 30 min. The absorbance was recorded at 415 nm. The tests were performed in triplicate. The same procedure was performed with quercetin, and the results were expressed as mg quercetin equivalent (QE) per g of crude extract for each sample [28].

LC-MS/MS Analysis

The phenolic content of the water extract of *S. absconditiflora* was identified and quantified by Thermo Fischer Scientific Dionex Ultimate 3000 model UHPLC coupled to a tandem MS instrument (TSQ Quantum Access Max) with the Xcalibur 2.2 software programme at Hitit University Scientific Technical Application and Research Center [29]. An ODS Hypersil (4.6 × 250 mm, 5µm) column at 30 °C was used for chromatographic separation. 0.1% formic solution in water (A) and methanol (B) was employed in gradient elution as the mobile phase. The gradient program was fixed as follows: 0– 1 min, 0% B; 1 –22 min, 95% B; 22–25 min, 95% B; 25–30 min, 100% B. The total evaluation time was 34 min. with conditioning time. The flow rate of the solvent was 0.7 ml/min. The injection volume was 20 µL.

The MS/MS parameters were set as follows: Capillary temperature 300°C, vaporizer temperature 350°C, sheath gas pressure 30 Arb, aux gas pressure 13 Arb, spray voltage (V) (positive polarity) 4000, spray voltage (V) (negative polarity) 2500, discharge current 4.0 µA.

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity of the plant extract was investigated by measuring ferric-reducing antioxidant potential. The method is performed by the conversion of [Fe(III)-TPTZ] complex, which is formed as a result of the reaction of iron (III) with Tripyridyltriazine (TPTZ), to [Fe(II)-TPTZ]. 10 mM TPZT, 20 mM $FeCl_3$ $6H_2O$, and 300 mM sodium acetate buffer (pH 3.6) were reacted to prepare the FRAP reagent. This reagent was applied to the prepared plant extracts, which were then left to incubate at 37°C for 30 min. The absorbance value of the samples was measured at 593 nm. The experiments were performed in triplicate. The findings were presented as Trolox equivalent per gram crude extract for each sample [30,31].

DPPH Free Radical Scavenging Activity

The radical scavenging activity of the extract was tested using a 1, 1- diphenyl-2-picryl hydrazyl (DPPH) technique. Briefly, a 100μM solution of DPPH radical in methanol was mixed with different concentrations of the extract. After 30 min. of incubation in the dark and at room temperature, the absorbance(A) was measured at 517 nm. The tests were performed in triplicate. Trolox was used as a reference substance [32].

The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = [(\text{Absorbance control} - \text{Absorbance test sample}) / \text{Absorbance control})] \times 100$$

Cell Viability

HCT-116 (human colon cancer cell line), HL60 (human acute promyelocytic leukemia cell line), and L929 (normal mouse fibroblast cell line) were grown in RPMI 1640 medium containing 1% penicillin-streptomycin, 1% L-glutamine, and 10% heat-inactivated fetal bovine serum.

The effects of *S. absconditiflora* on the viability of HCT-116, HL60, L929, and RAW 264.7 cells were evaluated by MTT assay [33]. Equal amounts of cells were seeded in 96-well plates and then incubated with different concentrations of the plant extracts (0.1-100 μg/mL) for 72h. After the incubation period, MTT solution (5mg/ml) was added to the wells. After a 4-h incubation with the MTT solution at 37°C, the SDS-HCl solution was added to the HL-60 cells to dissolve the formazan crystals and incubated overnight at 37°C. Absorbance measurement was performed at 550 nm using a microplate spectrophotometer (Spectramax, Molecular Device, USA). For HCT-116, L929, and RAW 264.7 cells, a mixture of MTT and medium was removed. DMSO was used to dissolve the formazan crystals. The cell viability of the untreated cells was used as a control group. Cell viability analyses were performed with four replicates in HCT-116, L929, and HL60 cell lines for *S. absconditiflora*, and with five replicates in HCT-116 cell lines and seven replicates in HL60 cell lines for docetaxel.

Anti-inflammatory Activity

RAW 264.7 macrophage cells were seeded to the 96-well plates. The next day, they were incubated with the water extract of *S. absconditiflora* (0.1-100 μg/ml) for 1 hour, and then LPS (10ng/ml) /IFN γ (1ng/ml) was applied to the wells. Following 20h incubation, equal amounts of cell supernatants were mixed with Griess reagent and absorbance at 550nm was measured with a microplate spectrophotometer (Spectramax, Molecular Device, USA) [34]. Nitrite levels were determined by the sodium nitrite standard graph. Nitrite determination for *S. absconditiflora* was performed with six replicates, and for aminoguanidine with four replicates.

Antimicrobial activity

A variety of bacterial and fungal strains were employed to assess the antibacterial capabilities of the aqueous extract. The bacterial strains used were: methicillin-susceptible *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, and *Pseudomonas aeruginosa* ATCC 27853. The fungal species *Candida parapsilosis* ATCC 22019, *Candida glabrata* RSKK 4019, *Candida krusei* RSKK 3016, and *Candida albicans* ATCC 10231 were preferred for the analysis. *In vitro* antimicrobial efficiency of the extract was determined using the minimum inhibitory concentration by the CLSI guidelines [35,36].

The extract was prepared for various tests on antibacterial and antifungal activities by dissolving it in sterile distilled water. Stock solutions of final concentrations that ranged from 0.05 to 25 mg/mL were obtained by performing a twofold serial dilution. Mueller-Hinton broth (MHB, Difco, Difco Laboratories, Detroit, MI, USA) was used as the diluent. The control groups were the microtiter plate wells containing only the inoculum

and growth medium. Thereafter, bacterial suspensions were standardized to 5 × 10^5 CFU/mL from 24-h cultures grown on MHA agar plates. Following inoculation, the plates were incubated at 35°C for 24 h for microbial growth. Chloramphenicol (Sigma, St. Louis, MO, USA) was included as the reference antibacterial agent.

The antifungal activity test was performed using an extract prepared in RPMI 1640 broth (ICN-Flow, Aurora, OH, USA) with glutamine and without bicarbonate and pH indicator. Serial dilutions were made to get a concentration range from 25 to 0.05 mg/mL. The control wells contained the modified culture medium together with the fungal inoculum but without the extract. The fungal suspensions were inoculated to achieve a final concentration of 0.5–2.5 × 10^3 CFU/mL per well. The microtiter plates were then incubated at 35 °C for 48 h. The minimum inhibitory concentration (MIC) was determined as the lowest extract concentration (mg/mL) that completely prevented visible fungal growth. Amphotericin B (Sigma, USA) was used as the reference antifungal compound. Sterile distilled water was used as a negative control for all antibacterial and antifungal assays.

The antimicrobial activities of luteolin-7-*O*-glucoside and apigenin, identified by LC-MS/MS as major constituents of the extract of *S. absconditiflora*, were evaluated against the same listed bacterial and fungal strains according to the CLSI guidelines for broth microdilution. Luteolin-7-*O*-glucoside (Merck; CAS No. 5373-11-5) and apigenin (Alfa Aesar; CAS No. 520-36-5) were dissolved in sterile distilled water to prepare stock solutions of 50 µg/mL. Two-fold serial dilutions were performed in appropriate culture media, and the final concentrations ranged from 25 to 0.025 µg/mL.

Antibiofilm activity

In this context, the antibiofilm activity of the extract against the susceptible strains *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, and *S. epidermidis* ATCC 35984, which were selected as test microorganisms, was evaluated. To achieve this, the concentrations between 25 and 0.05 mg/mL were adjusted in special media designed for specific strains for the dilution process.

The appropriate media formulations to promote biofilm formation in *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 strains were determined before testing. Specifically, *S. aureus* strains were cultured in Tryptic Soy Broth supplemented with 2.5% NaCl and 1% glucose, while *S. epidermidis* ATCC 35984 was cultured in Standard Tryptic Soy Broth (Merck, Darmstadt, Germany). *In vitro* biofilm activity of the extract was evaluated using a modified crystal violet microtiter plate assay, as outlined by Onbas and coauthors [37].

A single colony from the actively proliferating culture was chosen and inoculated into 5 mL of the appropriate medium, then cultured at 37°C overnight. After incubation, each culture was diluted 1:100 in the same medium. Then 140 µL of the modified medium was added to the wells of a 96-well U-bottom microtiter plate (LP Italiana, Italy). Serial dilutions were performed according to the predetermined concentration ranges and 10 µL of each diluted culture suspension was added to the corresponding wells. Wells containing only the medium and inoculum served as positive controls, while wells containing only the medium served as negative controls.

After 24 h of static incubation at 37 °C, biofilm formation was assessed using a crystal violet binding test. After staining, the bound crystal violet was resolved, and the absorbance was measured at 595nm using a microplate reader (µQuant, BioTek Inc., Winooski, VT, USA).

In addition, the optical density (ODc) was determined by adding three standard deviations to the mean OD value of the negative control wells. Biofilm formation was classified according to whether the mean OD value exceeded this threshold, as described by Stepanović and coauthors [38].

To further evaluate the contribution of the individual phenolics, luteolin-7-*O*-glucoside and apigenin were tested for their biofilm activities against *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, and *S. epidermidis* ATCC 35984. The stock solutions were prepared in distilled water and serially diluted in the corresponding biofilm-inducing media to obtain a final concentration range of 25–0.05 µg/mL. The experimental setup, including preparation of the inoculum, incubation conditions, and the modified crystal violet assay to quantify the biofilm, was identical to that described for the crude extract.

Anti-quorum sensing activity

The anti-quorum sensing activity of the extract was screened by using the reporter strain *Chromobacterium violaceum* ATCC 12472. Before the assay, the MIC of the extract was determined. The modified protocol conducted in the experiment was adapted from Batohi and coauthors [39]. *C. violaceum* ATCC 12472 was cultured in Luria-Bertani (LB) broth (Merck, Darmstadt, Germany) as a control and in LB broth supplemented with the extract concentrations below the MIC. The cultures were incubated at 30 °C for 24 h.

After incubation, 1 mL of each culture suspension was removed and centrifuged at 10,000× g for 5 min. The resulting supernatant was discarded, and the pellets were resuspended in 1 mL DMSO. Violacein production was determined by measuring absorbance at 585 nm. The percentage inhibition of violacein production was calculated using the formula:

Violacein inhibition (%) = [(OD585 nm Control - OD585 nm Test) / OD585 nm Control)] × 100

To determine whether the major flavonoids of *S. absconditiflora* contribute to the inhibition of quorum sensing, luteolin-7-*O*-glucoside and apigenin were tested against *C. violaceum* ATCC 12472 using the same protocol as described above. The stock solutions were diluted in LB broth to achieve final concentrations of 25 to 1.56 µg/ml, all of which were below the MIC of these compounds for the strain.

Statistical Analysis

One-way ANOVA was performed using GraphPad Prism 9 to compare differences between groups. All experiments, including phytochemical screenings and biological activity assays, were conducted in triplicate. A p-value less than 0.05, 0.01, 0.001, and 0.0001 were considered statistically significant. Data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

This study characterizes the phenolic composition and investigates the antimicrobial, antibiofilm, anti-quorum sensing, cytotoxic, and anti-inflammatory potential of an ultrasound-assisted water extract of the endemic species *Salvia absconditiflora*.

The yield of the water extract was found as 12.8%. The total phenolic content of the extract was calculated using the calibration curve of gallic acid. ($y=0.017x+0.0278$, $R^2=0.9870$) and expressed in GAE per gram of dry extract. The total content of flavonoids in the extract was derived from the calibration curve of quercetin ($y=0.0047x+0.0332$, $R^2 = 0.9992$) and expressed in QE per gram of dry extract (Table 1).

Table 1. Total phenolic and flavonoid content of water extract of *Salvia absconditiflora*

	Total phenolic content mg GAE/g extract	**Total flavonoid content mg QE/g extract**
Aqueous extract	87.570± 0.0012	20.241± 0.0031

The results of Onder and coauthors [40] showed that the total phenolic and total flavonoid content of the ethyl acetate extract of *S. absconditiflora* was 74.16 mg GAE/extract and 60.62 mg RE/extract, respectively. This shows that the total phenolic content is similar, but the total flavonoid content is lower in this study. This variation may be attributed to the polarity and selectivity of the extraction solvent, as ethyl acetate is known to be more likely to dissolve free flavonoids. Consequently, both the solvent type and the extraction method play a crucial role in the phytochemical profile of the extract.

In this study, the phenolic profile of the water extract was screened, and the contents of luteolin-7-*O*-glucoside, hesperidin and apigenin were determined by LC-MS/MS. The primary constituent was identified as luteolin-7-*O*-glucoside with a value of 97.127 µg/g dry extract. Linearity ($R^2 > 0.99$ for most compounds), RSD % values (typically <5%), detection and quantification limits were mentioned in Table 2. The main compound detected in the aqueous extract of *S. absconditiflora* is luteolin-7-*O*-glucoside, a pharmacologically active compound with a good safety profile, reasonable bioavailability, and diverse therapeutic potential. As the presence of sugar increases the polarity of the compound, it is more soluble in water than the aglycone form of luteolin. On the other hand, rosmarinic acid, which is commonly regarded as a chemotaxonomic marker for the Lamiaceae family, was not detected in the aqueous extract due to its low water solubility. Our results differed from those of Onder and coauthors [40]. The deviating results could be due to using different solvents and extraction procedures.

The IC_{50} value of DPPH activity was measured as 0.0705 ± 0.0018 mg/mL, while IC_{50} was determined as 0.0156 ± 0.0001 mg/mL for Trolox as a control. FRAP value was observed as 119.0877 ± 0.0057 mg Trolox/g. Our results on antioxidant activities show that *S. absconditiflora* has a moderate free radical scavenging activity and a remarkable ability to reduce ferric ions. Earlier studies indicated that *Salvi*a sp. extracts have potential anti-inflammatory and antioxidant properties in connection with phenolic content [41-43]. The aqueous extract of S. *absconditiflora* was found to significantly reduce the viability of RAW264.7 cells at a concentration of 100 µg/mL ($p<0.05$) and also significantly reduce LPS/IFNγ-induced nitrite levels without affecting cell viability at concentrations ranging from 0.1 to 50 µg/ml ($p<0.0001$). In RAW 264.7 macrophages, the LPS/IFNγ-induced inflammatory response was significantly suppressed by *S. absconditiflora* at 0.1 to 50 µg/ml, and this suppression was not due to a decrease in cell viability. When

evaluating its cytotoxic effects, it was determined that the aqueous extract of S. *absconditiflora* significantly reduced the viability of HCT-116 colon cancer cells in the concentration range of 0.1-100 µg/mL but did not show a statistically notable cytotoxic effect in the HL60 cell line in the same concentration range. It also showed no significant cytotoxic effect on the L929 cell line, a normal mouse fibroblast cell line. While it markedly reduced the viability of the colon cancer cell line without showing cytotoxic effects on healthy cells, it did not show cytotoxic effects on the acute myeloid leukemia cell line. Further studies are needed to elucidate the cellular pathways responsible for the selectivity of the cytotoxic effect in the colon cancer cell line.

When comparing earlier reports on luteolin-7-*O*-glucoside and apigenin with the findings of this study, luteolin-7-*O*-glucoside and apigenin may have contributed to the anti-inflammatory activity of *S. absconditiflora*. Park and coauthors reported that luteolin-7-*O*-glucoside inhibited nitric oxide levels in RAW264.7 cells, with an IC_{50} value of 22.7 µM [44]. Karki and coauthors reported the IC_{50} value of LPS-stimulated luteolin-7-*O*-glucoside as 30 µM [45]. This value suggests that the maximum concentration of *S. absconditiflora* extract applied to the cell culture,100 µg/mL, may contain luteolin-7-*O*-glucoside at a level lower than the effective concentration reported in the literature. Furthermore, apigenin concentration was detected in the *S. absconditiflora* extract at a lower concentration than luteolin-7-*O*-glucoside, suggesting that the contribution of this compound to the mild anti-inflammatory effect of the extract is limited. Park and coauthors reported that apigenin did not affect LPS-induced nitric oxide production at concentrations of 20 and 40 µM, while it significantly reduced at concentrations of 60, 80, and 100 µM [46]. Since only a modest anti-inflammatory effect was observed in the nitric oxide-dependent model employed in this study, and considering the diversity of inflammatory pathways, further studies with different models are expected to provide more information on the anti-inflammatory effect (Figures 1 and 2).

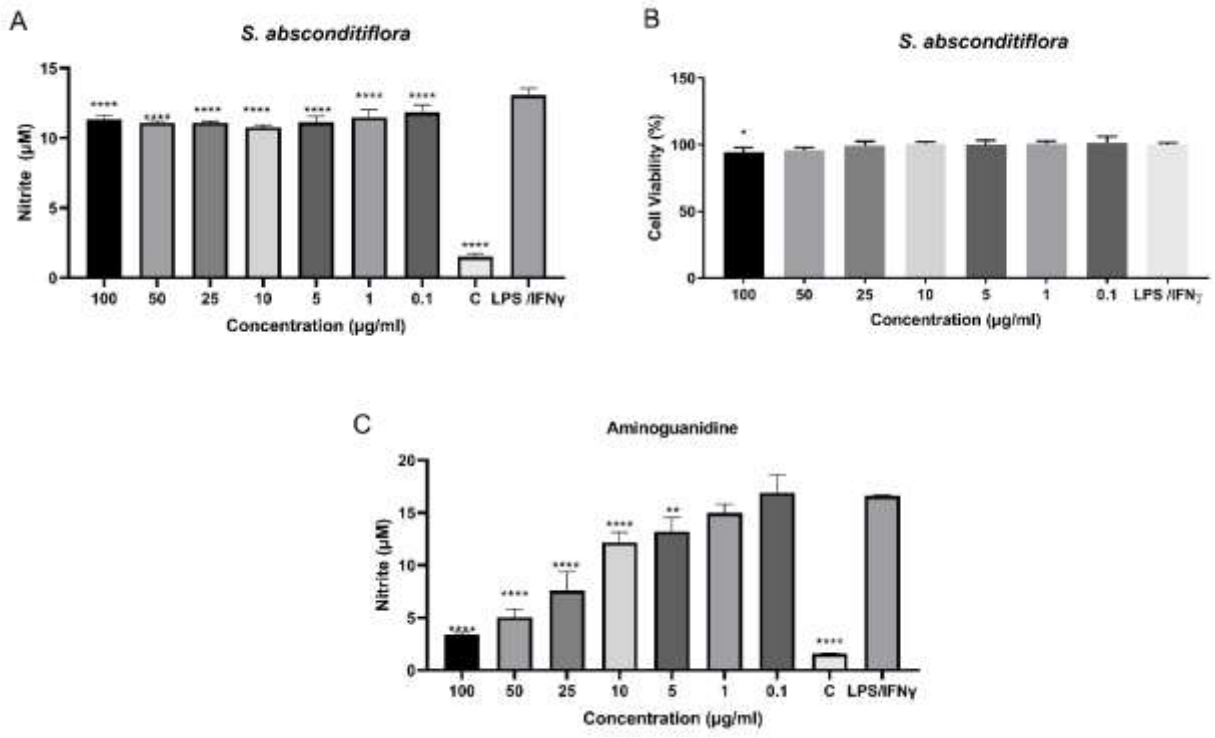


Figure 1. (A) Effects of the aqueous extract of *Salvia absconditiflora* on the nitrite levels of LPS/IFNγ-stimulated RAW 264.7 macrophage cells. C: Control group (n=6). **(B)** Effects of the aqueous extract of *S. absconditiflora* on the cell viability of LPS/IFNγ-stimulated RAW 264.7 macrophage cells. C: Control group (n=6). **(C)** Aminoguanidine (AG) was used as positive control (n=4) (*p<0.05;**p<0.01;***p<0.001;****p<0.0001; statistically significant according to the LPS/IFNγ group).

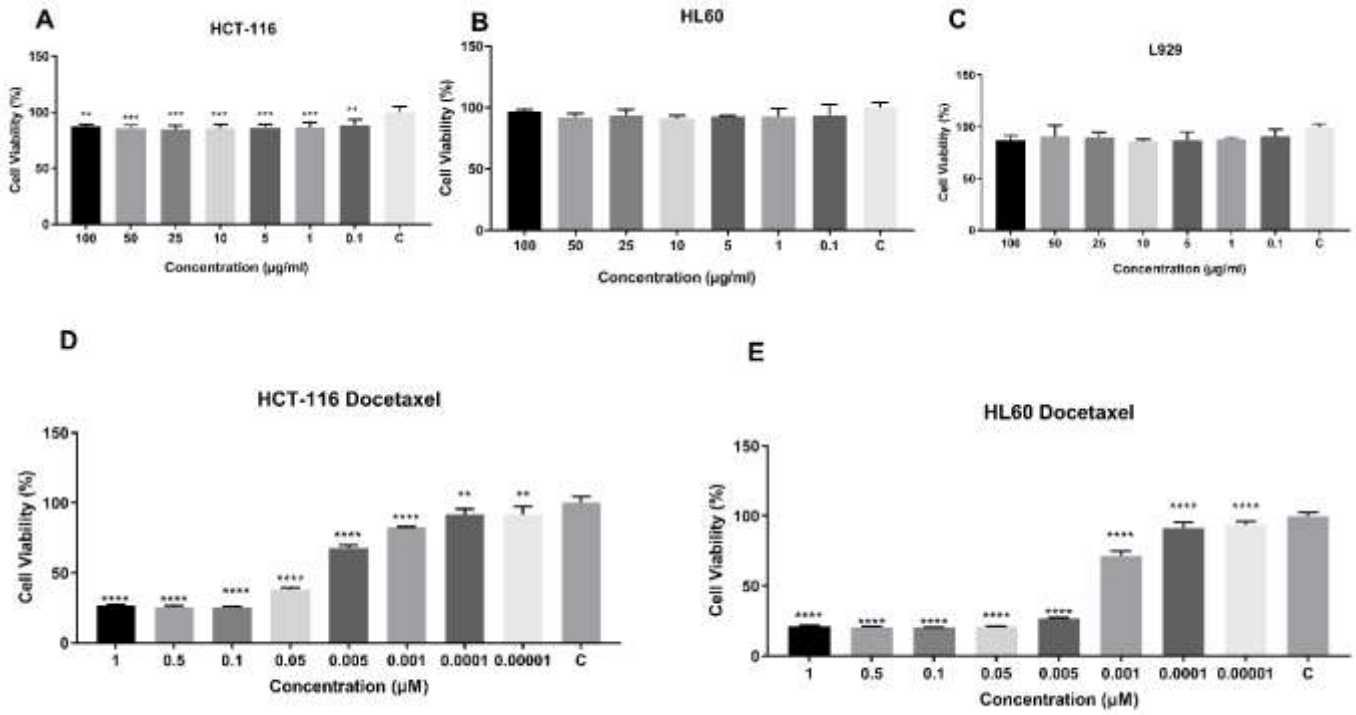


Figure 2. Effects of the aqueous extract of *Salvia absconditiflora* on the cell viability of HCT-116 (n=4) **(A)**, HL60 (n=4) **(B)**, and L929 cells (n=4) **(C)**. C: Control group. Docetaxel was used as a positive control on HCT-116 cell line (n=5) **(D)** and HL60 cell line (n=7) **(E).** (*p<0.05;**p<0.01;***p<0.001;****p<0.0001; statistically significant according to the control group).

Table 3 demonstrates that the aqueous extract of *S. absconditiflora* effectively inhibited some bacterial and fungal strains. Among Gram-positive bacteria, *S. aureus* ATCC 29213 (methicillin-susceptible, MSSA) and *S. aureus* ATCC 43300 (methicillin-resistant, MRSA) exhibited a minimum inhibitory concentration of 25 mg/mL, indicating that the extract has moderate action against the tested strains. For the *S. epidermidis* ATCC 35984 strain, the MIC was 6.25 mg/mL, while this Gram-positive strain is more sensitive than strains of *S. aureus*. For *E. faecalis* ATCC 29212, no inhibition was observed up to the tested concentrations. In the Gram-negative bacterial strains, no antimicrobial activity was observed against *A. baumannii* ATCC 19606, *E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853.

Among the fungal strains, the extract showed inhibitory activity against *C. parapsilosis* ATCC 22019, *C. glabrata* RSKK 4019 and *C. krusei* RSKK 3016, with MIC values of 25 mg/ml for each strain. The present finding indicates the antifungal action of the extract against these non-*C. albicans* species. On the other hand, there was no observed inhibitory activity against *C. albicans* ATCC 10231, showing strain-specific susceptibility.

Overall, the aqueous extract of *S. absconditiflora* presents selective action, more pronounced against Gram-positive bacteria and certain *Candida* species. Both luteolin-7-*O*-glucoside and apigenin showed remarkable antimicrobial effects against Gram-positive bacteria, especially *S. aureus* ATCC 29213 (MSSA), *S. aureus* ATCC 43300 (MRSA) and *S. epidermidis* ATCC 35984 (Table 3). Luteolin-7-*O*-glucoside showed MIC values of 6.25 µg/mL for MSSA and MRSA and 3.12 µg/mL for *S. epidermidis*, while apigenin inhibited MSSA at 12.5 µg/mL, MRSA at 6.25 µg/mL and *S. epidermidis* at 3.12 µg/mL. Remarkably, both compounds also inhibited *E. faecalis* ATCC 29212 at low micromolar concentrations (12.5 µg/mL for luteolin, 6.25 µg/mL for apigenin), although the aqueous extract showed no activity against this strain, suggesting that these flavonoids contribute substantially to the antimicrobial spectrum.

Neither luteolin-7-*O*-glucoside nor apigenin inhibited the growth of the tested Gram-negative bacteria (*A. baumannii*, *E. coli*, *P. aeruginosa*) or *Candida* strains at the tested concentrations, indicating a selective activity profile in favor of Gram-positive bacteria.

It was demonstrated that the aqueous extract performed good inhibitory action against MSSA and MRSA, with the MIC value obtained at a concentration of 25 mg/mL (Table 3). According to Walencka and coauthors [47] and Demirpolat [48], these results support earlier reports of *Salvia* species, which have also been shown to be effective against Gram-positive bacteria due to their bioactive components, specifically terpenoids and phenolic acids. Diterpenoids from *Salvia sclarea*, such as salvipisone and aethiopinone, enhance the action

of antibiotics against MRSA and *S. epidermidis* biofilms, and thus similar mechanisms might account for the activity of *Salvia absconditiflora*, too [47].

Table 3. Antimicrobial activity of the extract and the main components (luteolin-7-*O*-glucoside and apigenin) detected in the extract by LC-MS/MS against the tested bacterial and fungal strains. The MIC values are given in mg/mL for the extract and in µg/mL for the components.

Gram-positive bacteria	The extract (mg/mL)	Luteolin-7-*O*-glucoside (µg/mL)	Apigenin (µg/mL)	Chloramphenicol (µg/mL)
S. aureus ATCC 29213 (MSSA)	25	6.25	12.5	8
S. aureus ATCC 43300 (MRSA)	25	6.25	6.25	16
S. epidermidis ATCC 35984	6.25	3.12	3.12	8
E. faecalis ATCC 29212	-	12.5	6.25	256
Gram-negative bacteria				
A. baumannii ATCC 19606	-	-	-	128
E. coli ATCC 25922	-	-	-	4
P. aeruginosa ATCC 27853	-	-	-	512
Fungal strains				Amphotericin B (µg/mL)
C. albicans ATCC 10231	-	-	-	0.25
C. parapsilosis ATCC 22019	25	-	-	0.25
C. glabrata RSKK 4019	25	-	-	0.5
C. krusei RSKK 3016	25	-	-	1

ATCC: American Type Culture Collection; RSKK: Refik Saydam National Type Culture Collection; –: represents no activity. –: No antimicrobial activity was detected in the tested concentrations

The extract showed higher activity against *S. epidermidis* (MIC: 6.25 mg/mL), and this might simply reflect strain-specific differences in cell wall permeability or the development of biofilm (Table 3). The lack of activity against *E. faecalis* is consistent with studies indicating intrinsic resistance of some Gram-positive strains to plant extracts, possibly due to efflux pumps or cell wall adaptations [49].

The extract inhibited *C. parapsilosis*, *C. glabrata* and *C. krusei* (MIC: 25 mg/mL), but not *C. albicans* (Table 3). This specific antifungal action parallels the findings for *Salvia officinalis* extracts, which exhibit differing effectiveness against *Candida* species based on secondary metabolites, including thujone and camphor. The resistance of *C. albicans* may result from the development of drug transporters, as noted in clinical isolates [49].

No detectable inhibition was observed in Gram-negative strains (*A. baumannii*, *E. coli* and *P. aeruginosa*), which may be explained by the innate or acquired resistance to many plant-derived antimicrobials in Gram-negative species. These results emphasise the intrinsic differences in susceptibility profiles, which are largely influenced by the structural characteristics of the bacterial cell envelope [50].

Luteolin-7-*O*-glucoside and apigenin showed potent, selective anti-Gram-positive activity, with inhibition of *S. aureus* ATCC 29213 (MSSA), *S. aureus* ATCC 43300 and the highest potency against *S. epidermidis* ATCC 35984; both also suppressed *E. faecalis* ATCC 29212, while no measurable activity against *E. coli*, *P. aeruginosa*, *A. baumannii* or *Candida* was observed at the concentrations tested. These results are consistent with reports that luteolin directly inhibits the growth of MRSA/MSSA and the bioactivity of *staphylococci* and that apigenin exhibits bacteriostatic activity against quinolone-resistant *S. aureus* and broader antibacterial activity in staphylococci [51,52].

The antimicrobial evaluation showed that while the crude extract of *Salvia absconditiflora* exhibited broad-spectrum activity, its potency was significantly lower than that of the pure phenolic components and clinical standards. The extract produced moderate inhibition across the tested strains, with MIC values generally in the mg/mL range. In contrast, the pure compounds, specifically luteolin-7-*O*-glucoside and apigenin, demonstrated concentrated bioactivity, yielding lower MIC values in the µg/mL range, indicating they are the primary contributors to the extract's phytotherapeutic potential. Compared to the positive controls, chloramphenicol exhibited superior antibacterial activity against both Gram-positive and Gram-negative bacteria. Similarly, although the extract showed measurable antifungal effects against *C. parapsilosis* ATCC 22019, *C. glabrata* RSKK 4019, and *C. krusei* RSKK 3016 it did not reach the high-sensitivity threshold established by Amphotericin B.

The aqueous *S. absconditiflora* extract showed a pronounced concentration-dependent effect on the biofilm formation of *S. aureus* ATCC 29213 (Figure 3a). At the MIC (25 mg/mL), biofilm formation was completely inhibited (***$p < 0.001$). The data shown in Figure 3a at the concentration of 25 mg/mL can be omitted as the value obtained was below the cut-off. Interestingly, at certain high subinhibitory concentrations, particularly between 3.13 and 12.50 mg/mL, a slight but reproducible increase in biofilm formation was observed, and this was not statistically significant (ns). It is noteworthy that at lower concentrations (1.56-0.05 mg/mL), biofilm formation remained below control values, indicating strong inhibition. Taken together, these results demonstrate a biphasic response to the *S. absconditiflora* extract, where optimal antibiofilm efficacy is achieved at the MIC or relatively low concentrations.

As shown in Figure 3b, the aqueous *S. absconditiflora* extract significantly inhibited the biofilm formation of *S. aureus* ATCC 43300 in a concentration-dependent manner. At 25 mg/mL (the MIC), biofilm formation was completely inhibited compared to the control (***$p < 0.001$), indicating strong anti-antibiofilm activity at this completely inhibitory level. The data shown in Figure 3b at the concentration of 25 mg/mL can be omitted as the value obtained was below the cut-off. Interestingly, a slight increase in biofilm formation was again observed at certain sub-inhibitory concentrations (e.g., 12.50 mg/mL). Such a biphasic response must also be considered for the *S. aureus* ATCC 43300 strain. Overall, these data show a clear concentration-dependent antibiofilm effect of *S. absconditiflora* extract against *S. aureus* ATCC 43300, with optimal inhibition occurring at the MIC and the potential for a subtle paradoxical increase in biofilm formation at exposure below the MIC.

As shown in Figure 3c, biofilm formation of *S. epidermidis* ATCC 35984 is totally suppressed by the aqueous *S. absconditiflora* extract in a concentration-dependent manner, with MICs at 6.25 mg/mL and higher concentrations. The data shown in Figure 3c at the concentrations of 25-6.25 mg/mL can be omitted as the value obtained was below the cut-off. A significant reduction in biofilm formation was also observed at concentrations of 3.13-0.78 mg/mL. Subinhibitory concentrations also only caused an inhibition of biofilm formation compared to the control.

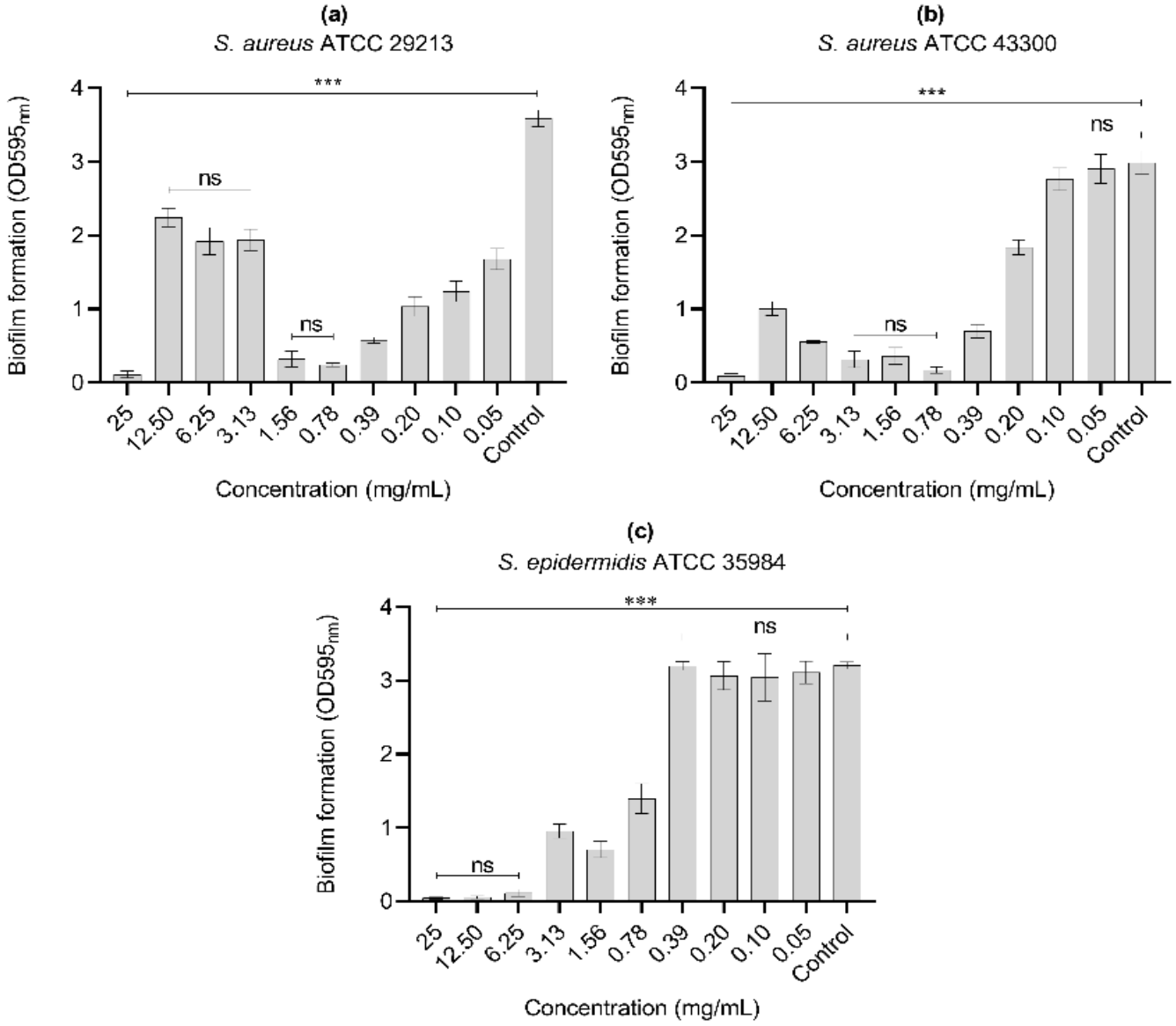


Figure 3. Antibiofilm activity of *Salvia absconditiflora* aqueous extract against **(a)** *S. aureus* ATCC 29213, **(b)** *S. aureus* ATCC 43300 and **(c)** *S. epidermidis* ATCC 35984. Biofilm formation (OD595) was quantified after incubation with extract concentrations from 25 mg/mL down to 0.05 mg/mL. The control represents untreated cells. Data are expressed as mean ± SD (n = 3). Statistically significant reductions in biofilm mass compared to control are indicated (***p < 0.001), while "ns" means no significant difference.

Luteolin-7-*O*-glucoside and apigenin both showed concentration-dependent inhibition of biofilm formation in *S. aureus* ATCC 29213 (Figure 4a and b). Luteolin-7-*O*-glucoside significantly suppressed biofilm development at all concentrations tested. At the concentrations of 25 and 12.5 µg/mL, biofilm formation was significantly reduced. Apigenin similarly suppressed biofilm formation only at concentrations below 1.56 µg/mL. Luteolin-7-*O*-glucoside and apigenin significantly inhibited biofilm formation of *S. aureus* ATCC 43300 in a concentration-dependent manner (Figure 4c and d). Luteolin-7-*O*-glucoside almost completely suppressed biofilm formation at concentrations of 6.25, 12.50, and 25 µg/mL. Apigenin showed a similar pattern and strongly suppressed biofilm formation at these concentrations. Both luteolin -7-*O*-glucoside and apigenin showed a strong effect on the biofilm of *S. epidermidis* ATCC 35984 (Figure 4e and f). Luteolin-7-*O*-glucoside completely eliminated biofilm formation at concentrations ≥3.13 µg/mL. Similarly, apigenin inhibited biofilm development with a similar pattern at these concentrations. Compared to the *S. aureus* strains, *S. epidermidis* showed greater susceptibility to both compounds. Both luteolin-7-*O*-glucoside and apigenin are phenolic compounds with antibiofilm activity. In the present study, both compounds selectively inhibited Gram-positive bacteria and effectively interrupted the biofilm formation of *S. aureus* and *S. epidermidis*. Luteolin-7-*O*-glucoside consistently showed lower concentrations than apigenin, indicating a stronger antibiofilm efficacy.

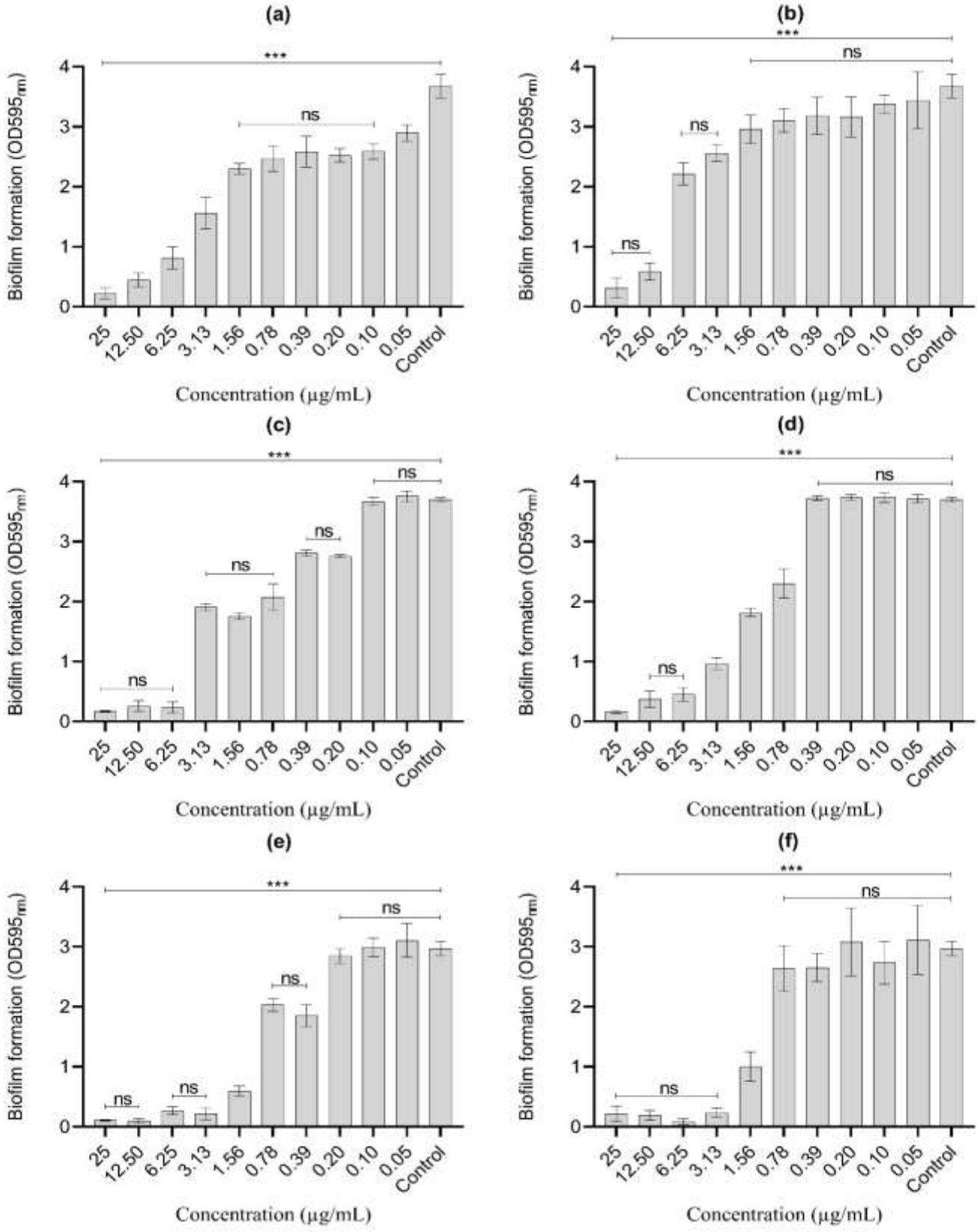


Figure 4. Antibiofilm activity of luteolin-7-*O*-glucoside and apigenin against **(a)** *S. aureus* ATCC 29213 (luteolin-7-*O*-glucoside), **(b)** *S. aureus* ATCC 29213 (apigenin) **(c)** *S. aureus* ATCC 43300 (luteolin-7-*O*-glucoside), **(d)** *S. aureus* ATCC 43300 (apigenin), **(e)** *S. epidermidis* ATCC 35984 (luteolin-7-*O*-glucoside), **(f)** *S. epidermidis* ATCC 35984 (apigenin). Biofilm formation (OD595) was quantified after incubation with the content concentrations from 25 µg/mL down to 0.05 µg/mL. The control represents untreated cells. Data are expressed as mean ± SD (n = 3). Statistically significant reductions in biofilm mass compared to control are indicated (***p < 0.001), while "ns" means no significant difference.

The results presented in this study show a concentration-dependent biofilm activity of the aqueous *S. absconditiflora* extract against *S. aureus* (ATCC 29213 and ATCC 43300) and *Staphylococcus epidermidis* (ATCC 35984). The extract showed a biphasic response characterised by a complete inhibition of the biofilm at the minimum inhibitory concentration (MIC) and a slight increase in biofilm formation at certain sub-inhibitory concentrations. This biphasic response is in agreement with previous studies of plant extracts and suggests that subinhibitory concentrations of antimicrobial plant extracts paradoxically enhance biofilm formation in some bacterial strains. Similar observations were made, for example, with *E. coli* treated with subinhibitory concentrations of various medicinal herbs. Biofilm development was triggered by stress-driven adaptive responses [53].

The antibiofilm activity of flavonoids, particularly luteolin and apigenin, has been increasingly documented, with recent studies providing mechanistic insights. Liu and coauthors [54] demonstrated that luteolin significantly suppresses biofilm formation in Gram-positive pathogens, including *S. aureus*, by impairing initial adhesion and destabilizing mature biofilms, likely through interference with quorum-sensing pathways and extracellular polymeric substance (EPS) production. In our study, the extract tested in the antibiofilm assays was not a purified compound but a complex extract in which luteolin-7-*O*-glucoside was identified as one of the predominant constituents. Since luteolin-7-*O*-glucoside is the glucoside derivative of luteolin, it may plausibly contribute to the overall effect. Consistently, Qian and coauthors [55] also reported marked antibiofilm activity for luteolin, partly associated with disruption of *S. aureus* cell integrity.

The aqueous *S. absconditiflora* extract showed a pronounced inhibitory effect on the production of violacein by *C. violaceum* ATCC 12472, indicating a disruption of the quorum-sensing pathways (Figure 5). At 12.50 mg/mL, still below the MIC (25 mg/mL), the extract achieved a reduction in violacein production by more than 80, indicating strong anti-quorum sensing (anti-QS) activity at sub-inhibitory levels. This inhibitory effect decreased in a dose-dependent manner, with moderate (56.37%) and minimal (17.05%) violacein inhibition at 6.25 mg/mL and 3.13 mg/mL, respectively (Figure 5).

Several studies have emphasised the anti-quorum sensing (anti-QS) properties of *Salvia* species and support the strong QS inhibitory activity also observed in this study for *S. absconditiflora*. *S. officinalis* and *S. sclarea* have been documented to diminish quorum sensing regulated virulence factors in *P. aeruginosa* and *C. violaceum*, indicating that the bioactive compounds in these species disrupt bacterial communication pathways [56,57]. The significant reduction of violacein production at subinhibitory levels of the aqueous extract of *S. absconditiflora* corresponds with previous studies on *Salvia* sp., which identified phenolic acids and terpenoids as important quorum-sensing inhibitors. These chemicals likely interfere with quorum sensing by inhibiting the synthesis of signalling molecules or receptor binding, thereby reducing the expression of virulence factors mediated by quorum sensing [58]. Since no difference in violacein production was found between the control and test groups that would have allowed the percentage reduction to be calculated, the results were expressed in optical density. The effect of luteolin-7-*O*-glucoside and apigenin on quorum sensing regulated violacein production in *C. violaceum* ATCC 12472 is shown in Figure 6. Neither luteolin-7-*O*-glucoside nor apigenin showed significant inhibition of violacein biosynthesis at the concentrations tested (25–1.56 µg/mL), as the absorbance values at 585 nm remained comparable to those of the untreated control. These results indicate that, none of the flavonoids exhibited measurable anti-quorum sensing activity against *C. violaceum* within the range tested.

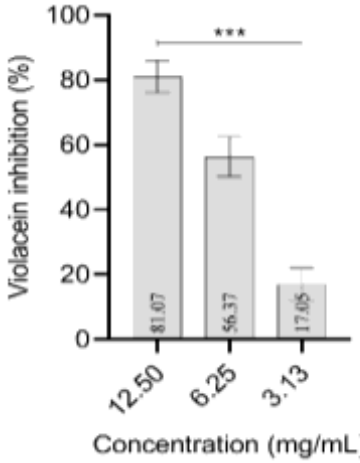


Figure 5. Anti-quorum sensing activity of the aqueous *Salvia absconditiflora* extract against *C. violaceum* ATCC 12472. The bars represent the mean percent violacein inhibition (± SD) at concentrations of 12.50, 6.25 and 3.13 mg/mL, with a significant reduction observed at higher doses (***$p < 0.001$). The MIC for *C. violaceum* is 25 mg/mL.

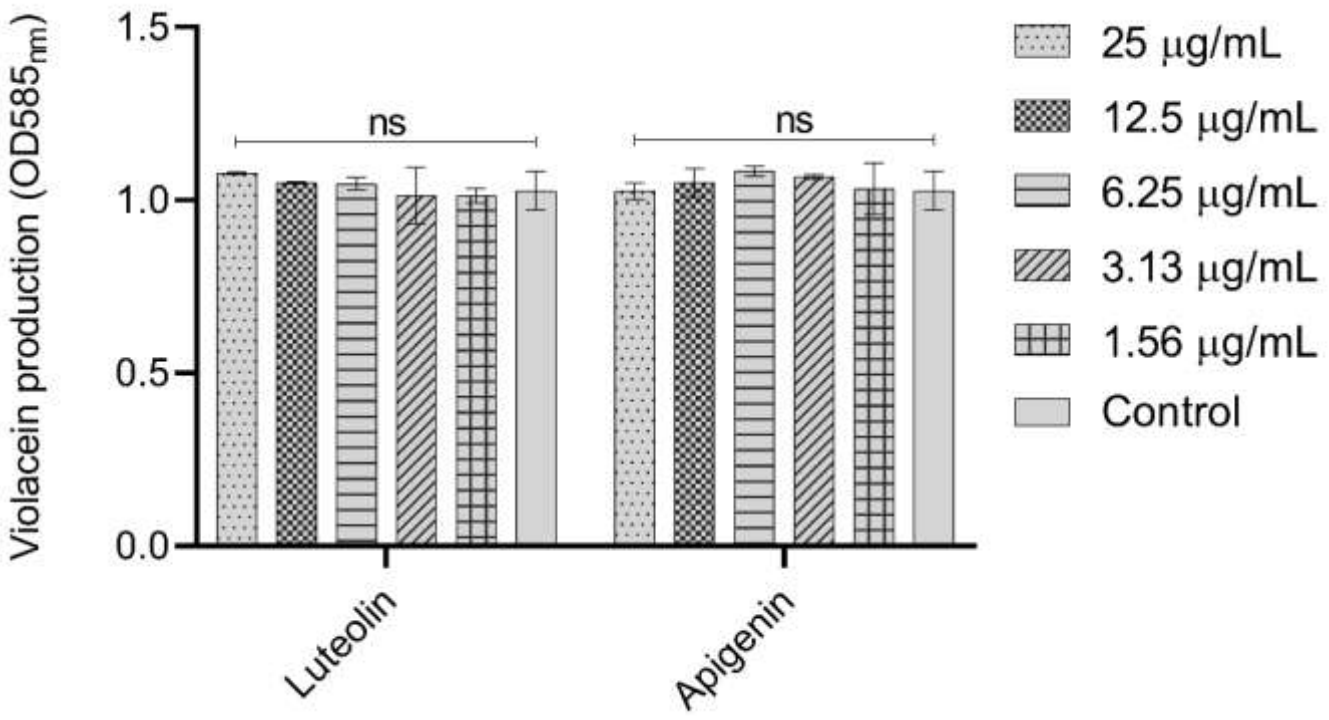


Figure 6. Effect of luteolin-7-*O*-glucoside and apigenin on quorum–sensing–regulated violacein production in *C. violaceum* ATCC 12472. Cultures were treated with different sub-MIC concentrations of luteolin-7-*O*-glucoside and apigenin (25–1.56 µg/mL), and violacein production was quantified spectrophotometrically at 585 nm after 24 hours of incubation. No statistically significant decrease in violacein concentration was observed compared to the untreated control (ns, not significant), indicating that neither compound exhibited anti-quorum-sensing activity within the concentration range tested. Error bars represent the mean ± standard deviation of triplicate experiments.

Table 2. Phenolic and flavonoid compounds identified and quantified in aqueous extract of *Salvia absconditiflora* (µg/g dry extract)

Compound	Rt	Parent ion (m/z)	Ion. Mode	MS/MS CE	RSD%	Linearity Range (ppm)	Linear Regression Equation	R^2	LOD/LOQ mg/L	Aqueous extract
Gallic Acid	9.19	169.7	Neg.	80.50 (25)-126.20(16)	4.5	0.125-10	y = -1772.12+66065.7X	0.9949	0.061/0.203	nd
Protocatechuic Acid	11.11	155.01	Pos.	65.40 (22)-93.20 (13)	4.9	0.125-10	y = 27640.8+35692.1X	0.9926	0.049/0.162	nd
Protocatechuic aldehyde	12.07	136.9	Neg.	92.25 (25)-108.20 (25)	2.2	0.125-10	y = 506291+900489X	0.9963	0.026/0.087	nd
Sesamol	12.07	137.18	Neg.	108.17 (15)-109.29 (14)	2.1	0.125-10	y = 190429+294285X	0.9965	0.031/0.103	nd
Gentisic Acid	12.34	153.7	Neg.	109.50 (21)	2.3	0.125-10	y = 81035.3X	0.9979	0.013/0.043	nd
Catechin	12.58	289.2	Neg.	203.90 (22)-245.70 (17)	2.5	0.125-10	y = -46484.8+91012.1X	0.9987	0.042/0.058	nd
Chlorogenic Acid	13.61	353.4	Neg.	86.50 (43)-192.10 (21)	3.5	0.125-10	y = 21653.3X	0.9937	0.106/0.353	nd
Epicatechin	13.99	291.5	Pos.	123.30 (15)-139.30 (16)	2.5	0.125-10	y = -137604+217920X	0.9951	0.003/0.006	nd
Caffeic Acid	14.19	179.7	Neg.	135.20 (27)-136.20 (18)	2.5	0.125-10	y = 166126+470479X	0.9981	0.042/0.058	nd
Vanilin	14.71	150.91	Neg.	92.30 (23)-136.10 (16)	3.7	0.125-10	y = 18407.3+4851.4X	0.9937	0.023/0.076	nd
Syringic Acid	15.21	183.07	Neg.	77.30 (23)-123.20 (13)	4.6	0.125-10	y = 150673-55037.7X	0.9149	0.194/0.647	nd
Taxifolin	15.71	303	Neg.	126.20 (23)-285.50 (15)	3.8	0.125-10	y = 8374.89+42570.1X	0.9979	0.001/0.005	nd
p-Coumaric Acid	15.86	163.9	Neg.	94.30 (33)-120.20 (17)	3.5	0.125-10	y = 79931.9+73318.2X	0.9927	0.069/0.109	nd
Ferulic Acid	16.13	195.022	Pos.	89.40 (30)-177.40 (7)	4.5	0.125-10	y = 96528.3+177434X	0.9941	0.063/0.118	nd
p-Hydroxybenzoic Acid	16.37	137.9	Neg.	66.60 (38)-94.60 (17)	4.7	0.125-10	y= 537544+881649X	0.9951	0.243/0.809	nd
Salicylic Acid	16.38	137.136	Neg.	65.50 (35)-93.26 (18)	4.8	0.125-10	y= 485398+972318X	0.9960	0.030/0.099	nd

Cont. Table 2

Hesperidin	16.78	608.78	Neg.	301.34 (32)-164.01 (60)	4.3	0.125-10	y= 1.43184e+006X	0.9977	0.104/0.347	0.207
Rosmarinic Acid	16.86	359.18	Neg.	134.30 (44)-162.20 (20)	2.6	0.125-10	y = -127182+253431X	0.9980	0.003/0.005	nd
Oleuropein	16.95	539.1	Neg.	275.80 (22)-377.50 (16)	2.0	0.125-10	y= 375327+1.76005e+006X	0.9969	0.009/0.029	nd
Luteolin-7-*O*-glucoside	17.30	446.89	Neg.	284.00 (45)-285.00 (40)	4.1	0.125-10	y= 135610X	0.9985	0.117/0.390	97.127
Rutin	17.48	609.37	Neg.	300.60 (38)-301.70 (34)	1.6	0.125-10	y=2.20056e+006+1.00456e+007X	0.9977	0.022/0.073	nd
Resveratrol	17.59	228.98	Pos.	107.20 (22)-135.10 (14)	4.8	0.125-10	y= 97778.9+63601.2X	0.9988	0.019/0.062	nd
Ellagic Acid	18.61	301.669	Neg.	284.80 (30)-174.15 (34)	5.0	0.125-10	y= 745549+1.07598e+006X	0.9977	0.087/0.289	nd
Naringenin	19.08	273	Pos.	147.10 (20)-153.00 (24)	4.8	0.125-10	y= 2.35935e+006X	0.9986	0.005/0.017	nd
Quercetin	19.75	301	Neg.	152.10 (23)-179.90 (20)	2.9	0.125-10	y=111352+2.43427e+006X	0.9941	0.001/0.005	nd
Luteolin	20.23	287.02	Pos.	135.10 (32)-153.00 (31)	3.9	0.125-10	y = 1.48908e+007X	0.9981	0.222/0.741	nd
Apigenin	21.15	268.86	Neg.	117.19 (40)-149.11 (27)	3.9	0.125-10	y = 831953X	0.9987	0.114/0.380	1.711
Pinocembrin	21.19	257.07	Pos.	153.11 (23)-103.27 (34)	1.4	0.125-10	y = 789059X	0.9979	0.095/0.316	nd
Chrysin	22.59	254.97	Neg.	153.10 (30)-103.28 (33)	1.5	0.125-10	y= 1.33937e+007X	0.9972	0.049/0.163	nd
Galangin	22.64	268.85	Neg.	169.15 (29)-171.08 (31)	5.0	0.125-10	y= 1.66971e+006X	0.9929	0.034/0.113	nd
Flavone	22.94	222.9	Pos.	77.28 (35)-121.15 (26)	1.7	0.125-10	y= 1.57469e+007+3.51252e+007X	0.9954	0.037/0.124	nd

Parent ion (m/z): Molecular ions of the standard compounds, MS2(CE): Collision energy, R2: coefficient of determination, Rt: Retention Time, LOD: limit of detection, LOQ: Limit of Quantification, RSD: Relative Standard Deviation, Neg: Negative ionization mode, Pos: Positive ionization mode

CONCLUSION

In this study, we investigated the phenolic profile, antimicrobial, and anti-inflammatory activities of *Salvia absconditiflora*, which belongs to the *Salvia* species traditionally used for treating various diseases. Our research demonstrates the promise of *S. absconditiflora* as a source of novel antimicrobial agents and therapeutic leads. Although apigenin and luteolin-7-*O*-glucoside have been reported to be cytotoxic and anti-inflammatory, *S. absconditiflora* extract containing apigenin and luteolin-7-*O*-glucoside exhibits mild anti-inflammatory and cytotoxic effects. Interactions with other compounds may have resulted in different and less pronounced cytotoxic or anti-inflammatory effects than those exhibited individually. Such interactions may contribute to the mild cytotoxic and anti-inflammatory effects of *S. absconditiflora* extract. Accordingly, cytotoxicity was evaluated based on % viability values, as IC_{50} values could not be determined. We also recommend additional study into different anti-inflammatory pathways to clarify the therapeutic potential. Importantly, in this study, ultrasound-assisted extraction (UAE) was used as an extraction procedure regarded as practical and effective. UAE emerges as a valuable technique for extracting secondary metabolites and a promising method of investigations, but it should be kept in mind that while prolonging ultrasound-assisted extraction time may enhance the recovery of phenolic compounds, excessively long extraction durations can also lead to thermal or oxidative degradation of these bioactive constituents. The integration of traditional ethnobotanical knowledge with modern green extraction and pharmacological evaluation highlights the potential of natural active compounds for drug development studies.

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Conflicts of Interest: The authors declare no conflict of interest.

Use of Generative Artificial Intelligence

The authors declare that large language models and other generative artificial intelligence (AI) or AI-assisted technologies cannot be credited as authors and have not been listed as authors of this paper.

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