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*Corresponding author

Mahdi Hashim Ibrahim

mahdi.ibrahim@su.edu.krd

RECEIVED : 21/12 /2023

ACCEPTED : 03/08/ 2024

PUBLISHED : 31/10/ 2024

KEYWORDS:

Anti-candida
properties, antioxidant
activity, phytochemical
screening, Salvia
verticillata extract, total
tannin quantification

The Anti-Candidal Activity of Salvia verticillata subsp. verticillata Against Several Candida Species

Mahdi Hashim Ibrahim¹, Badr Qader Surchi¹, Safiya A.
Azeez², M. Hakkı Alma³, Ekrem Kireççi⁴, Mustafa A Yılmaz⁵,
Metin Tansu Uguz⁶, Tufan Salan⁷ and Huseyin Hüseyin
Taniş⁸

1. Department of Biology, College of Science, Salahaddin University-Erbil,
Erbil, Kurdistan Region, Iraq.

2. Director of General Education of Erbil, Director of Central Education in Erbil, Sabat
Preparatory School for Girls

3. Department of Environmental Engineering, Faculty of Engineering, University of
Igdir, Igdir, Turkey

4. Department of Basic Medical Sciences, Faculty of Medicine, Microbiology,
Kahramanmaraş Sütçü İmam University, Turkey

5. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dicle University,
Diyarbakir, 21280, Turkey

6. Kahramanmaraş Sutcu Imam University Vocational School of Health Services,
Turkey

7. Department of Materials Science and Engineering, Kahramanmaraş Sutcu Imam
University, Kahramanmaraş, Turkey

8. Department of Biology, Faculty of Arts and Sciences, Kahramanmaraş Sutcu Imam
University, Kahramanmaraş, Turkey

ABSTRACT

The examination of Salvia verticillata extract encompassed an exploration of its anti-Candida properties, phytochemical composition, antioxidant activity, and the quantification of total tannins. The use of ethanol as a solvent yielded the highest extraction efficiency at 26.1%. The plant exhibited a substantial tannin content of 6.5 mg/kg, signifying a noteworthy concentration. The inhibitory zones against Candida tropicalis reached a minimum diameter of 17.6 mm, while Candida guilliermondii displayed the most significant inhibition with a zone diameter of 21.8 mm. The minor inhibitory concentration (MIC) findings for all Candida species ranged from 6.25 to 12.5 g/ml. In the case of Candida guilliermondii, the synthetic antifungal activity FLU/25 demonstrated a maximal inhibition zone measuring 39.30 mm. Additionally, the maximum antioxidant activity, recorded at 0.3 ml, reached a value of 98.65. Phytochemical screening unveiled elevated concentrations of phenols and flavonoids, with malic acid (1901.1 g/g), hesperidin (302.4 g/g), and rosmarinic acid (30619.93 g/g) all experiencing an increase in concentration. These findings provide a comprehensive understanding of the diverse bioactive components and properties associated with Salvia verticillata extract.

1.Introduction

Salvia, the well-known genus of the Lamiaceae family, encompasses over a thousand plant species distributed globally. Derived from the Latin word for sage, *Salvia* encompasses annual, biennial, or perennial herbaceous plants, often incorporating woody subshrubs, and these plants produce flowers in clusters, presenting a striking display with colors ranging from blue to red, while white and yellow are less commonly observed (Uysal et al., 2023). The genus *Salvia* L. (Lamiaceae) is distinguished by 98 species, four subspecies, and three varieties, with fifty-six of them being endemic to Turkey. *Salvia* species in Anatolia are commonly employed for alleviating conditions such as sore throats, abdominal pains, and colds (Katanić Stanković et al., 2020). *Salvia* is widely cultivated for flavoring and traditional medicine, undergoing extensive chemical studies (Avula et al., 2022) and (Larit and León, 2023). It serves as a rich source of polyphenols, boasting over 160 recognized, including some unique to the genus (Ismael and Sciences, 2021) and (Morzel et al., 2022). Several polyphenolic compounds in *Salvia* originate from caffeic acid through various condensation reactions. These polyphenols exhibit rapid healing properties, contributing to the swift advancement in *Salvia* species' phytochemistry (Askari et al., 2021).

S. officinalis, in traditional medicine, addresses inflammation, dizziness, tremors, paralysis, seizures, ulcers, gout, rheumatism, diarrhea, and hyperglycemia. Recent studies document both traditional uses and new biological effects of this plant (Cardile et al., 2009, Ghorbani and Esmaeilzadeh, 2017)). Traditionally, it has treated oral, gingival, and throat issues, fought bacterial and fungal infections, aided wound healing, enhanced memory, alleviated the common cold, and addressed sweating, stomach inflammation, and ulcer formation. Its essential oil is utilized in food preservation and as a spice for distinct aromas and improved digestion (Sharifi-Rad et al., 2018).

The pharmacological effects of *Salvia* L. can be attributed to its diverse and intricate chemical composition, which encompasses a broad spectrum of specialized metabolites. These

include, but are not limited to, terpenoids, phenolic acids, lignans, flavonoids, and alkaloids. The intricate interplay of these compounds within the plant contributes to the multifaceted pharmacological profile exhibited by *Salvia* L., highlighting the significance of its rich and varied chemical makeup in influencing the physiological responses and therapeutic properties associated with this botanical genus (Hafez Ghoran et al., 2022). Additionally, *Salvia* spp. exhibit antioxidant activity, containing high levels of phenolic content, steroids, alkaloids, and to a lesser extent, saponins. Notably, its extract is rich in phenolic compounds, with caffeic acid and rosmarinic acid identified as the most abundant (Uysal et al., 2023). Additionally, substances such as tanshinones, camphor, caryophyllene, borneol, α - and β -thujone contribute to the extensive range of documented bioactivities associated with *Salvia* plants (Zaccardelli et al., 2020). Additionally, *Salvia* serves as a natural antibacterial in the meat and fish industry, a fragrance and soap additive in cosmetics, a herbal dye in textile and landscape architecture, and for medical purposes in treating oral inflammations, gum diseases, and regulating digestive systems (Uysal et al., 2023).

This study targeted to determine isolated phytochemicals compounds using LC-MS/MS, total yield, and antioxidant activity. Finally, compare some artificial antifungals with plant anti-candidal extracts by anti-candidal activity by disc diffusion technique and MIC. *Salvia verticillata* was extracted using a microwave technique.

2. MATERIALS AND METHODS

Materials

2.1 The Study Designs

This study includes five steps which are plant collection, ethanol extraction, determination of yield percentage, tannin analysis, and determining antioxidant analysis. Additionally, phytochemical profile of the target plant investigated. Finally, minimum inhibitory concentration (MIC) was used for determining Anti-candida activity of extracted *Salvia verticillata* against several candida sp. All the laboratory analysis was performed in the laboratory of Kahramanmaraş Sütçü İmam

University. Additionally, phytochemical analyses were carried out at DICILA University in Turkey.

2.2 Studied Samples

Plant specimens, specifically *Salvia verticillata*, were gathered during the flowering period in June 2021. The taxonomic identification and authentication processes were conducted at Salahaddin University, specifically in the College of Sciences-Biology. Subsequently, the collected *Salvia verticillata* samples were preserved and given the herbarium code for species 7473, as documented by Arslan et al. in 2021. Furthermore, the Media Diagnostic Centre in Erbil, accredited by the College of American Pathology (CAP), supplied various *Candida* species, including *Candida albicans* (ATCC 1023), *C. glabrata*, *C. parapsilosis*, *C. famatta*, *Candida krusei*, *C. tropicalis*, and *C. guilliermondii*.

2.3 Ethanol Extract and its Efficiency

The comprehensive analysis and extraction procedures were meticulously carried out at DICILA University in Turkey. In a concise overview, the plant material, comprising 25 grams of powder, underwent extraction using 250 milliliters of 90% ethanol. This extraction process adhered to a consistent ratio of plant material to solvent, maintaining a precise proportion of 1:10. The implementation of the advanced Microwave milestone NEOS system played a pivotal role in optimizing and expediting the extraction procedure. To elaborate, this state-of-the-art system ensured a controlled and efficient extraction by maintaining the specified ratio of plant material to solvent, thereby enhancing the overall efficacy of the extraction process. Following the extraction, the concentrated plant extract underwent a filtration process utilizing a rotary evaporator, executed at a temperature of 45°C to further refine and concentrate the extract. As part of the subsequent steps, the concentrated extract was dissolved in DMSO to achieve an initial screening concentration of 1g/mL. This strategic dissolution allowed for a standardized and controlled concentration for subsequent analyses and assessments. The detailed and meticulous approach employed in these extraction and screening processes at DICILA University

reflects a commitment to precision and rigor in scientific methodologies. The study utilized a combined extract, maintaining a 1:1 ratio (Salari et al., 2016, Remok et al., 2023). Finally, extract efficiency of extracted plant was determined according to (Ghavam et al., 2020).

2.4 Tannin Analysis

The root samples, having undergone a comprehensive drying process, were subjected to grinding to attain a particle size suitable for passing through a 1 mm sieve. In the subsequent phase, a meticulous procedure involved blending 10 mg of the prepared samples with diethyl ether, enriched with 5% acetic acid. This augmented solvent facilitated the extraction of pigments and fats from the samples. Following the extraction process, the quantification of the total tannin content was meticulously conducted using a spectrophotometer, with readings precisely taken at a wavelength of 580 nm. This analytical method enabled a detailed and accurate determination of the concentration of tannins present in the root samples, ensuring a comprehensive assessment of their chemical composition (Remok et al., 2023).

2.5 DPPH Assay

The experimental procedure, as outlined by (Krzeminska et al., 2022), involved conducting an assay to assess antioxidant activity. In this process, a 1 ml solution containing free radicals was combined with an antioxidant solution, resulting in a total volume of 3 ml. The amalgamated mixture underwent incubation for varying durations, including both shorter and longer time intervals, specifically for half an hour, and this incubation was conducted in complete darkness to ensure accurate evaluation. The results of the assay were subsequently measured using a spectrophotometer, with readings taken at a wavelength of 517 nm. To enhance the reliability of the findings, the experiment was repeated in triplicate, emphasizing the importance of reproducibility in determining antioxidant activity. Moreover, the establishment of a standard curve played a crucial role in increasing the accuracy of the results, providing a reference point for calibration and precise quantification.

2.6 Phytochemical Analysis

The Mass Spectrometry analysis was meticulously conducted by employing a state-of-the-art Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer, which was equipped with an Electrospray Ionization (ESI) source capable of functioning in both positive and negative ionization modes. This cutting-edge instrumentation allowed for a comprehensive exploration of molecular structures and compositions. The data obtained from the LC-MS/MS analyses were systematically collected and processed using the Lab Solutions software developed by Shimadzu, based in Kyoto, Japan, as referenced in the works of (Ertas et al., 2015, Bayrakçeken Güven et al., 2023).

To ensure precision and accuracy in quantification, the analyses were executed in a multitude of response monitoring (MRM) modes, encompassing both quantitative and confirmation measures. This approach aimed to enhance the reliability of the results by incorporating a dual assessment strategy, reinforcing the robustness of the analytical procedures employed in the

Mass Spectrometry analysis. The utilization of advanced technology and comprehensive data processing software underscored the commitment to a thorough and meticulous investigation of the molecular components under scrutiny.

2.7 Liquid Chromatography-Tandem Mass Spectrometry Analysis

Thirty-seven organic acids, both phenolic and non-phenolic, commonly found in plant resources, were identified and measured. Linear regression equations and the linear ranges of the standard compounds under investigation are illustrated in Figure 1 (Ismael et al., 2019). Correlation coefficients consistently exceeded 0.99. Table 5 presents the results demonstrating the limit of detection (LOD) and quantitation (LOQ) for the proposed analytical method. The LOD for the analyzed compounds varied from 0.05 to 25.8 g/L, while the LOQ ranged from 0.17 to 85.9 g/L. Furthermore, the recovery rates for the phenolic compounds fell within the range of 96.9% to 106.2%.

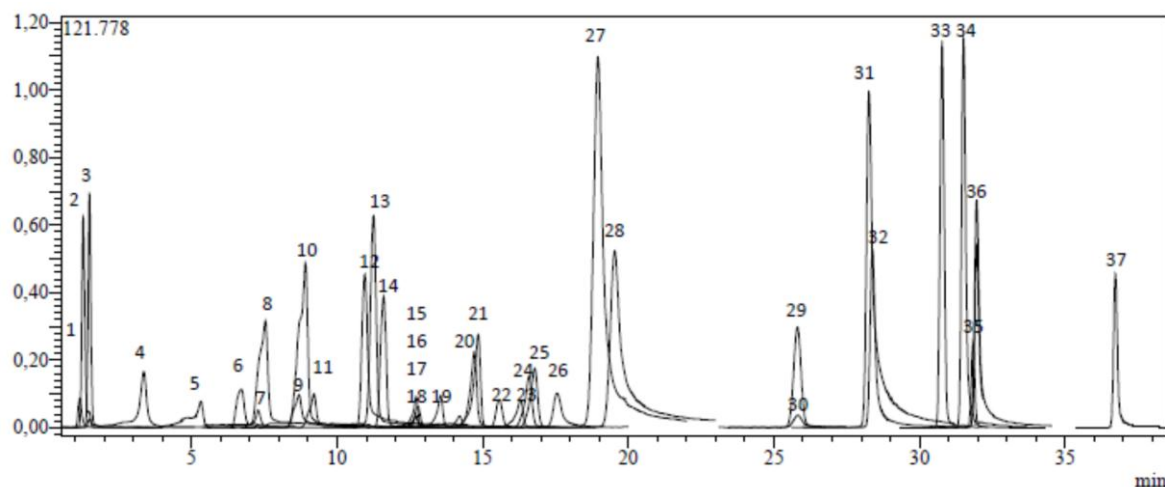


Figure 1: Chromatogram illustrations

3. Anti-Candida Activity

3.1 Disc Diffusion Method

The disc diffusion method was employed to determine the antifungal activity of the extracted plant's active compounds, based on previous studies (Abdallah et al., 2017) and (Z Sahin et

In a succinct overview of the experimental procedure, a suspension of *Candida* species (0.1 ml) was evenly distributed on solid media plates. Subsequently, filter paper discs with a diameter of 6 mm were saturated with a 50 µl aliquot of the plant extract and strategically placed on the

inoculated plates. Following this application, the plates were subjected to a chilling process at 4°C for a duration of three hours, after which they were transferred to an incubator set at 35°C for a period of 48 hours. The inhibition zones resulting from the interaction between the *Candida* species and the plant extract were then measured in millimeters to gauge the extent of antifungal activity. It is noteworthy that all tests were conducted in triplicate, ensuring the reliability and reproducibility of the experimental outcomes. As part of the comparative analysis, standard antifungal agents, namely Fluconazole 25 mcg (FLU), Ketoconazole 10 mcg (KTZ), Fluconazole 10 mcg (FLC), Nystatin 100 units (NYS), Clotrimazole 10 mcg (CLT), and Miconazole 10 mcg (MCZ), were incorporated into the experiments. This inclusion served as a reference point to evaluate the efficacy of the plant extract in relation to established antifungal agents. The meticulous design of these experiments, along with the integration of standard controls, aimed to provide a comprehensive understanding of the antifungal properties of the plant extract against *Candida* species.

3.2 The Minimum Inhibitory Concentration

The determination of the Minimum Inhibitory Concentration (MIC) for the plant extract brought to light its noteworthy antifungal activity, particularly at lower concentrations. The methodology employed for this assessment encompassed broth microdilution, where the Sabouraud dextrose broth (SDB) medium was meticulously blended with the plant extracts at a ratio of 9 to 1, resulting in a total volume of 10 ml. The experimental setup ensured a thorough integration of the extract into the growth medium, allowing for a comprehensive evaluation of its impact on fungal growth. Following the incubation period, which spanned 48 and 72 hours, the outcomes were gauged by employing a spectrophotometer. This analytical instrument provided quantitative insights into the fungal growth inhibition facilitated by the plant extract. To enhance the reliability and robustness of the findings, the entire experimentation process was meticulously conducted in triplicate, thereby

ensuring consistency and reproducibility.

It is crucial to highlight that a standardized method was adhered to for the adjustment of variables during the experimentation process. This procedural consistency aimed to establish a reliable baseline for comparison, facilitating a systematic evaluation of the plant extract's antifungal efficacy. In essence, the careful orchestration of these experimental procedures and analytical assessments contributes to a more comprehensive understanding of the plant extract's potential as an antifungal agent. (Ghavam et al., 2020, Krzeminska et al., 2022).

4. Statistical analysis

Data underwent statistical analysis using GraphPad (Prism 6), with ANOVA utilized to evaluate uptake values. A significance threshold of $p < 0.05$ was implemented, ensuring a rigorous standard for identifying statistically significant differences. Visual representation was achieved through the use of histograms and a pie chart (Khudhur et al., 2019).

5. RESULTS AND DISCUSSION

5.1 Yield Assessment

Table 1 displays the maximum extraction yield achieved with ethanol solvent, reaching 26.1%. The variation in essential oil composition and output is notably influenced by various factors such as genotype, environmental conditions, phenological stage, and plant segments utilized in the drying process. This finding aligns with observations from several studies (Kerkoub et al., 2018, Ghavam et al., 2020), while (Fatma Ebru et al., 2017) reported extraction yield *Salvia officinalis* which was (of 36.72 %) with methanol.

Table 1: *Salvia verticillata* yield extract.

	Replicate 1	Replicate 2	Replicate 3	Yield (%)
Mean	26.2 ±	25.9 ±	26.2±	26.1
Standard Deviation	0.006	0.007	0.008	± 0.007
Standard Error	0.002	0.003	0.004	0.003

5.2 Tannin Analysis Insights

Table 2 presents the outcomes of tannin measurement utilizing (n-butanol-HCl-iron)

method. Mimosa-tannin, measured under standard reaction/conditions using the regression equation ($y=66.357x+0.4117$) derived from the linear standardization curve, served as the n-butanol/HCl assay standard. The considerable tannin content in the *Salvia* extract was employed to calculate the tannin percentage.

In our investigation, the tannin content exhibited significant diversity, yielding a result expressed as absorbance (A580/mg). Obtained result was 6.5 mg/kg, As outlined in (Table 2). These findings, in alignment with other studies, indicate that the tannin content in *S. officinalis* extract was determined to be 19.1 ± 0.7 mg catechin Eq/g dry matter (Salari et al., 2016, Remok et al., 2023).

Table 2: Calculation of Tannin

	Replicate 1	Replicate 2	Replicate 3	Mean
Extract (mg/kg)	6.3 $\pm$	6.8 $\pm$	6.4 $\pm$	6.5 $\pm$
Stander Division	0.11	0.15	0.13	0.13
Stander Error	0.1	0.16	0.18	0.14

5.3 Anti-Candida Activity

The inhibition zones of *Salvia verticillata* plant extracts displayed significant variations against the tested *Candida* species, as outlined (Table 3). According to the results of the disc diffusion agar plate, the inhibition zone for *Candida tropicalis* was (17.6 mm) which considered as the lowest and the highest inhibition zone was (21.8 mm) for *Candida guilliermondii*. Additionally, MIC results across all *Candida* species ranged from 6.25 (lower) to 12.5 (higher) $\mu\text{g/ml}$. Regarding artificial antifungal activity of FLU/25, was 39.30 mm against *Candida guilliermondii*, as detailed in

Table 3. This underscores that the antimicrobial effects of mentioned plant are related to chemical valuable composition (Uysal et al., 2023). Extracts of *S. lavandulifolia* exhibited the most potent anti-candidal activity, followed by the extracts or active compounds of *S. sclarea* and *S. officinalis*. It was observed that the *Candida albicans* strain responsible for oropharyngeal infections demonstrated the highest resistance, whereas *C. albicans* ATCC 1023 showed the most susceptibility (Jirovetz et al., 2007). MIC values varied from 1.56 to 25.00 mg/mL, indicating the pFfiguotential anti-candidal effects of the extracts on yeast cultures. Remarkably, the amalgamated plant extracts from leaves and rootstock exhibited increased antifungal potency against *C. neoformans*, *C. laurentii*, and *G. capitatum* (Dulger and Dulger, 2021). After a 14-day period, the minimum inhibitory concentration (MIC) value was determined, and the regeneration of fungi hyphae and mycelium was confirmed. *Salvia officinalis* L. EO exhibited an MIC value of 10 mg·L⁻¹ against *Verticillium dahliae*. Similarly, *Penicillium aurantiogriseum* displayed comparable sensitivity to *Salvia officinalis* L. EO, with the MIC value of 10 mg·L⁻¹ being sustained for the entire 14-day duration (Rus et al., 2015). MIC results showed varied effects from 3.12 to 25 mg/mL, indicating a notable antifungal impact across all extracts on the fungal cultures. Particularly, the extracts demonstrated a more pronounced antifungal effect against *C. albicans*, *C. neoformans*, and *B. cinerea*. These results align with those of other researchers (Jirovetz et al., 2007, Salari et al., 2016, Dulger and DÜLger, 2021).

Table 3: summarizes the inhibition zones (in mm) and minimum inhibitory concentrations (MIC) of the extracts, along with their artificial antifungal activities.

Species	Inhibition	MIC	FLU	MCZ	FLU	KTC	NY	CLT
	Zone	($\mu\text{g/ml}$)	10mcg	10mcg	25mcg	10mcg	$\frac{100}{U}$	10mcg
<i>Candida albicans</i>	Mean	19	6.25	6	14.61	10.55	14.05	20.21
	(mm)	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$

	SD	0.2	0.11	0	0.1	0.13	0.1	0.24	0.18
	SE	0.15	0.09	0	0.04	0.05	0.04	0.1	0.07
<i>Candida glabrata</i>	Mean	20.1	6.25	16.71	14.54	15.68	13.33	24.72	12.29
	(mm)	±	±	±	±	±	±	±	±
	SD	0.01	0.43	0.16	0.2	0.21	0.11	0.31	0.22
	SE	0.018	0.381	0.06	0.08	0.09	0.04	0.12	0.09
<i>Candida krusei</i>	Mean	18.2	12.5	15.32	13.52	24.63	18.79	14.49	25.91
	(mm)	±	±	±	±	±	±	±	±
	SD	0.13	0.01	0.15	0.2	0.66	0.09	0.17	0.22
	SE	0.015	0.015	0.06	0.08	0.27	0.04	0.07	0.09
<i>Candida tropicalis</i>	Mean	17.6	12.5	20.52	12.46	27.67	23.96	21.23	19.95
	(mm)	±	±	±	±	±	±	±	±
	SD	0.01	0.16	0.08	0.27	0.17	0.1	0.15	0.14
	SE	0.12	0.18	0.03	0.11	0.07	0.04	0.06	0.06
<i>Candida famata</i>	Mean	20.16	6.25	6	6	6	6	24.75	20.26
	(mm)	±	±	±	±	±	±	±	±
	SD	0.022	0.35	0	0	0	0	0.13	0.16
	SE	0.18	0.3	0	0	0	0	0.05	0.07
<i>Candida parapsilosis</i>	Mean	19.3	6.25	21.43	11.53	26.36	27.48	24.47	22.42
	(mm)	±	±	±	±	±	±	±	±
	SD	0.021	0.021	0.16	0.17	0.17	0.32	0.15	0.28
	SE	0.01	0.012	0.07	0.07	0.07	0.13	0.06	0.11
<i>Candida guilliermondii</i>	Mean	21.8	6.25	37.48	24.19	38.4	37.61	21.45	31.26
	(mm)	±	±	±	±	±	±	±	±
	SD	0.23	0.14	0.26	0.15	0.42	0.32	0.18	0.19
	SE	0.22	0.11	0.11	0.06	0.17	0.13	0.07	0.08

5.4 Antioxidant Activity

Table 4 illustrates that the plant extract derived from *Salvia verticillata* exhibited notable antioxidant effects against DPPH radicals. The highest antioxidant activity was observed at a volume of 0.2 mL, reaching 98.68%, and gradually declined to 96.60% at 0.1 mL. The findings suggest that the plant extracts displayed superior antioxidant properties in comparison to the synthetic antioxidant BHT. The hydromethanolic extract from the induced culture demonstrated robust antioxidant activity with IC₅₀ values of 11.1 µg/mL, 6.5 µg/mL, and 69.5 µg/mL for DPPH, ABTS, and superoxide anion radical, respectively.

The potential antioxidant activity of the extracts was evaluated using two complementary test systems: β-carotene/linoleic acid and DPPH free radical-scavenging. In the β-carotene/linoleic acid test system, *S. verticillata* subsp. *verticillata*

exhibited an inhibitory capability of $74.4 \pm 1.29\%$. Radical removing of other compounds also reported, for instance, synthetic antioxidant (BHT) in parallel for the purpose of the comparison. The results find support from various sources corroborating the antioxidant activity (Vergine et al., 2019), (Poulios et al., 2020), (Francik et al., 2020), and (Gecer et al., 2021).

Table 4. Percentages of DPPH Radical Inhibition

	MWE	MWE	MWE	BHT	BHT	BHT
	0.1ml	0.2ml	0.3ml	0.1ml	0.2ml	0.3ml
Mean (%)	96.50 ±	97.67 ±	98.65 ±	68.76 ±	69.11 ±	74.8 ±
SD	0.11	0.13	0.11	0.21	0.2	0.32
SE	0.11	0.08	0.09	0.12	0.14	0.11

5.5 Chemical Analysis Insights

This study employed UHPLC-ESI-MS/MS to investigate the phenolic acids in the plant extract of *Salvia verticillata*. Ten different phytochemicals were identified in the ethanol extract. Investigations were done on 37 root ethanol extract non-phenolic, phenolic, and flavonoid components. Table 5 and Figure 2 present the analytical parameters and findings. High concentrations of rosmarinic acid (30619.93 g/g), malic acid (1901.1 g/g), and hesperidin (302.4 g/g) were found, as shown in the table. The smallest concentrations of salicylic acid (2.88 g/g) and p-coumaric acid (36.23 g/g) were found. These results agree with other works by (Stanković et al., 2020), (Karami et al., 2020), (Juee, 2022), and (Balkır et al., 2023).

As a valuable source of chemicals with exceptional health qualities, *S. verticillata* can open up new opportunities for use as a food ingredient in the cosmetic and pharmaceutical industries, with a particular focus on rosmarinic acid (Katanić Stanković et al., 2020). The investigation into the efficacy of rosmarinic acid was conducted to further elucidate the correlation between the levels of rosmarinic acid and the antioxidant activity within the plant extracts. Notably, among the tested specimens, *S. verticillata* subsp. *verticillata* exhibited the highest concentration of rosmarinic acid, thereby suggesting a potential link between elevated rosmarinic acid levels and heightened antioxidant

activity in the studied plant extracts. This exploration aims to provide a more comprehensive understanding of the role of rosmarinic acid in contributing to the overall antioxidant potential of *S. verticillata* subsp. *verticillata* (Tepe et al., 2007). HPLC further separated two significant active fractions (F-31 and F-39), resulting in several active chromatographic peaks. Carnosol and 12-methoxy-trans-carnosic acid were isolated as two primary active compounds and identified by a combination of NMR and mass spectrometry (Kerkoub et al., 2018). Many natural products are produced usually by plants which establish a significant source of microbicides, pesticides and numerous pharmacological medicines (Tekeli et al., 2014, Ghorbani and Esmaeilzadeh, 2017, Remok et al., 2023). In the realm of folk medicine, *Salvia* species, traditionally valued for therapeutic properties and a rich pharmacological profile encompassing anti-inflammatory, antioxidant, and antimicrobial attributes, are sought after in healing practices across cultures, addressing diverse health concerns (Uysal et al., 2023). Additionally, these plants significantly contribute to the cosmetic industry, with their aromatic extracts and essential oils incorporated into skincare and haircare formulations, providing a popular choice for enhancing sensory appeal and potential skin and hair benefits (Alves-Silva et al., 2023) and (Ertas et al., 2023).

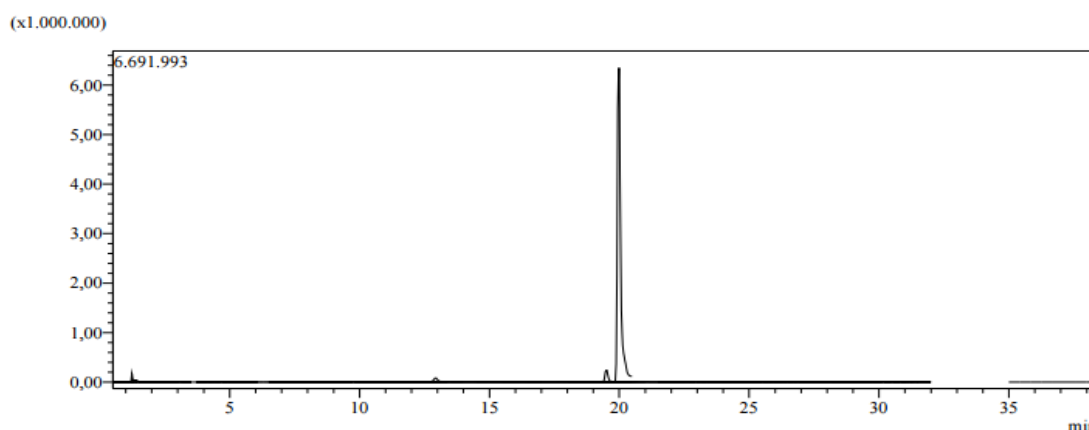


Figure 2 illustrate the LC-MS/MS chromatogram.

Table 5: LC-MS/MS Analytical Parameters

No	Analytes	RT ^a	Mother ion (m/z) ^b	Fragment ions	Ion. mode	Equation	R ^{2c}	RSD% ^d		Linearity Range (µg/L)	LOD/LOQ (µg/L) ^e	Recovery (%)		U ^f
								Interday	Intraday			Interday	Intraday	
1	Coumarin	17. 40	147. 05	91.0-103.2	Poz	y=33.64×-89700	0.994	0.01306	0.01239	1000-20000	208.4/228.4	0.99947	1.00081	ND.
2	Hesperidin	12. 67	610. 90	303.1-465.1	Poz	y=1340.27×-43769	0.998	0.00945	0.01126	25-1000	3.4/4.2	1.01733	1.01263	302.54
3	p-Coumaric acid	11. 53	162. 95	119.3-93.3	Neg	y=3199.20×+13002	0.992	0.01820	0.01727	25-1000	7.3/9.1	1.00617	1.01224	36.23
4	o-Coumaric acid	15. 45	162. 95	119.4-93.3	Neg	y=1219.34×-10915	0.999	0.02730	0.02566	25-1000	24.4/31.1	0.98344	0.99061	ND.
5	Gallic acid	3. 00	168. 85	125.2-79.2	Neg	y=226.76×+38152	0.998	0.01601	0.01443	250-10000	95.5/106.9	1.00004	1.00454	----
6	Caffeic acid	8. 80	178. 95	135.2-134.3	Neg	y=3963.32×+178156	0.998	0.01454	0.01469	25-1000	18.4/22.4	1.00917	0.98826	63.32
7	Vanillic acid	8. 57	166. 90	152.3-108.3	Neg	y=35.84×-12097	0.999	0.00528	0.00619	1000-20000	122.2/139.7	1.00093	1.04095	----
8	Salicylic acid	11. 16	136. 95	93.3-65.3	Neg	y=5286.26×+309192	0.989	0.01016	0.01242	25-1000	5.0/6.5	1.00989	0.99013	2.88
9	Quinic acid	1. 13	190. 95	85.3-93.3	Neg	y=41.06×+10671	0.996	0.00259	0.00274	250-10000	75.8/79.4	1.00288	0.98778	----
10	4-OH-benzoic acid	7. 39	136. 95	93.3-65.3	Neg	y=409.03×+112079	0.998	0.01284	0.01538	250-10000	33.2/38.1	0.99662	1.00058	ND.
11	Ferulic acid	12. 62	192. 95	178.3	Neg	y=80.45×-31782	0.997	0.00708	0.00619	250-10000	36.6/42.0	0.99987	1.00289	197.56
12	Chlorogenic acid	7. 13	353. 15	191.2	Neg	y=781.36×-18697	0.998	0.00058	0.00076	25-1000	6.2/8.1	1.00806	0.99965	19.01
13	Rosmarinic acid	14. 54	359. 00	161.2-197.2	Neg	y=909.67×-201692	0.994	0.02014	0.01751	100-5000	6.6/8.8	0.99206	1.03431	30619.93
14	Protocatechuic acid	4. 93	152. 95	108.3	Neg	y=297.75×+30590	0.995	0.01236	0.01296	100-5000	28.2/31.4	0.99404	1.01070	----
15	Cinnamic acid	25. 61	147. 00	103.15-77.3	Neg	y=9.06×-12403	0.996	0.00648	0.00816	5000-20000	821.8/859.7	1.00051	0.99927	ND.
16	Sinapinic acid	12. 66	222. 95	208.3-149.2	Neg	y=141.96×-73294	0.992	0.01446	0.01517	250-10000	78.7/86.1	1.00164	0.99962	ND.
17	Fumaric acid	1. 48	115. 00	71.4	Neg	y=64.99×-11592	0.997	0.00536	0.00460	100-5000	28.1/34.5	0.99748	0.99867	ND.
18	Vanillin	10. 87	151. 00	136.3-92.2	Neg	y=446.10×+70934	0.998	0.00696	0.00793	250-10000	44.3/53.1	0.99679	0.99611	ND.

Table. 5 Analytical parameters (Continuing)

No	Analytes	RT ^a	Mother ion (m/z) ^b	Fragment ions	Ion. mode	Equation	R ^{2c}	RSD% ^d		Linearity Range (µg/L)	LOD/LOQ (µg/L) ^e	Recovery (%)		U ^f
								Interday	Intraday			Interday	Intraday	
19	Pyrocatechol	6. 48	109. 00	108.35-91.3	Neg	y=30.61x+14735	0.996	0.01313	0.01339	1000-20000	261.1/278.4	0.99987	0.99936	ND.
20	Malic acid	1. 23	133. 00	115.2-71.3	Neg	y=316.95x-42041	0.999	0.00477	0.00527	250-10000	55.3/67.5	1.01266	0.99836	1909.1
21	Syringic acid	9. 02	196. 95	182.2-167.3	Neg	y=42.33x-52547	0.996	0.01049	0.01345	1000-20000	212.5/233.3	0.99922	0.99977	ND.
22	Hesperetin	31. 76	300. 95	164.2-136.2	Neg	y=876.67x+48916	0.997	0.03209	0.02605	25-1000	5.6/6.9	0.98850	0.99435	ND.
23	Naringenin	30. 68	270. 95	151.2-119.3	Neg	y=4315.1x+178410	0.995	0.02054	0.02019	25-1000	5.4/6.4	0.99883	1.01002	ND.
24	Rutin	12. 61	609. 05	300.1-271.1	Neg	y=561.91x-16879	0.997	0.00473	0.00624	25-1000	5.5/6.5	1.00994	0.98017	40.66
25	Quercetin	28. 17	300. 90	151.2-179.2	Neg	y=1198.48x+480562	0.990	0.01589	0.01360	100-5000	23.3/28.9	0.98470	1.00103	ND.
26	Quercitrin	16. 41	447. 15	301.1-255.1	Neg	y=339.39x+38910	0.999	0.01528	0.02320	100-5000	22.0/25.2	0.99726	1.00620	ND.
27	Apigenin	31. 43	268. 95	117.3-151.2	Neg	y=4548.36x+295252	0.990	0.02304	0.02204	25-1000	5.4/6.3	1.01444	1.01331	ND.
28	Chrysin	36. 65	252. 95	143.3-119.4	Neg	y=2032.13x+95593	0.993	0.00490	0.00630	25-1000	5.4/6.2	1.00338	1.00437	ND.
29	Liquiritigenin	25. 62	254. 95	119.3-135.1	Neg	y=2384.96x+59141	0.996	0.01849	0.01738	25-1000	5.5/6.6	1.00333	0.99957	ND.
30	Isoquercitrin	13. 42	463. 00	300.1-271.1	Neg	y=803.23x+4981	0.999	0.00682	0.00515	25-1000	5.4/6.3	1.00594	1.00722	ND.
31	Apigetrin	16. 59	431. 00	268.2-239.2	Neg	y=1775.55x+91121	0.993	0.01797	0.01607	25-1000	5.4/6.1	1.01394	1.00419	ND.
32	Rhoifolin	16. 11	577. 05	269.2-211.1	Neg	y=237.15x+11887	0.999	0.00747	0.01528	100-5000	23.1/27.9	1.01046	1.01739	ND.
33	Nicotiflorin	14. 68	593. 05	285.1-255.2	Neg	y=498.38x+79274	0.991	0.00737	0.00875	100-5000	22.4/25.5	1.02558	1.00970	ND.
34	Fisetin	19. 30	284. 95	135.2-121.3	Neg	y=547.46x+274791	0.991	0.00557	0.00820	250-10000	54.4/61.4	0.99877	1.00031	ND.
35	Luteolin	28. 27	284. 75	133.2-151.2	Neg	y=3272.65x+150557	0.997	0.00575	0.00696	25-1000	5.4/6.5	1.00772	0.99524	ND.
36	Myricetin	18. 72	317. 00	179.2-151.3	Neg	y=583.55x+205727	0.999	0.00652	0.00711	250-10000	53.2/57.2	0.99982	1.00042	ND.
37	Kaempferol	31. 88	284. 75	255.1-117.3	Neg	y=26.29x+87558	0.992	0.01436	0.01070	1000-20000	206.6/214.3	0.99971	0.99851	ND.

aRT: Time of retention, bMother ion(m/z): Molecular ions corresponding to standard compounds (m/z ratio), cR2: Coefficient of determination, dRSD: Relative standard deviation, eLOD/LOQ (µg/L): Detection/quantification limits, fU (%): Percent relative uncertainty at a 95% confidence level (k=2).

CONCLUSION

This study highlights *Salvia verticillata* as a promising source of valuable compounds with versatile applications, concluding insights from the present study:

- The ethanol extraction of *Salvia verticillata* yielded a maximum of 26.1%, showcasing the impact of genotype and environmental factors, consistent with previous research.
- Tannin content in the *Salvia* extract, determined through the n-butanol-HCl-iron method, revealed a substantial amount of 6.5 mg/kg, aligning with findings from other studies.
- *Salvia verticillata* extracts exhibited significant variations in inhibition zones against *Candida* species, indicating promising anti-candidal efficacy.
- The plant extract demonstrated robust DPPH radical scavenging activity, surpassing the control (BHT) in antioxidant potency.
- UHPLC-ESI-MS/MS analysis uncovered a rich chemical profile in *Salvia verticillata*, supporting its potential applications in the food, cosmetic, and pharmaceutical industries.

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