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*Corresponding author

Mahdi Hashim Ibrahim

mahdi.ibrahim@su.edu.krd

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

Mahdi Hashim Ibrahim[1], Badr Qader Surchi[1], Safiya A. Azeez[2], M. Hakkı Alma[3], Ekrem Kireçci[4], Mustafa A Yılmaz[5], Metin Tansu Uguz[6] , Tufan Salan[7] and Huseyin Hüseyin Tanış[8]

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. Kahramanmaras Sutcu Imam University Vocational School of Health Services, Turkey
7. Department of Materials Science and Engineering, Kahramanmaras Sutcu Imam University, Kahramanmaras, 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 µg/g), hesperidin (302.4 µg/g), and rosmarinic acid (30619.93 µg/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 Esmaeilizadeh, 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 verticillate 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 verticillate 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 (leaves), 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 leaves 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%.

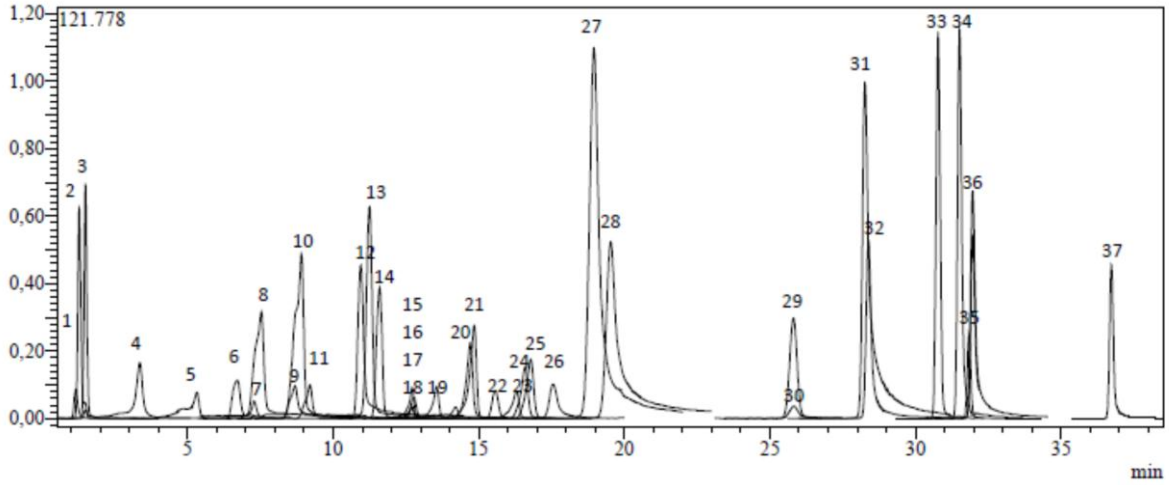

Figure 1: Chromatogram illustrations

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 ±	6.8 ±	6.4 ±	6.5 ±
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) µg/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	(µg/ml)	10mcg	10mcg	25mcg	10mcg	100 U	10mcg
Candida albicans	Mean (mm)	19 ±	6.25 ±	6 ±	14.61 ±	10.55 ±	14.05 ±	20.21 ±	18.15 ±

	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
Candida glabatra	Mean (mm) ± SD	20.1 ± 0.01	6.25 ± 0.43	16.71 ± 0.16	14.54 ± 0.2	15.68 ± 0.21	13.33 ± 0.11	24.72 ± 0.31	12.29 ± 0.22
	SE	0.018	0.381	0.06	0.08	0.09	0.04	0.12	0.09
Candida krusei	Mean (mm) ± SD	18.2 ± 0.13	12.5 ± 0.01	15.32 ± 0.15	13.52 ± 0.2	24.63 ± 0.66	18.79 ± 0.09	14.49 ± 0.17	25.91 ± 0.22
	SE	0.015	0.015	0.06	0.08	0.27	0.04	0.07	0.09
Candida tropicalis	Mean (mm) ± SD	17.6 ± 0.01	12.5 ± 0.16	20.52 ± 0.08	12.46 ± 0.27	27.67 ± 0.17	23.96 ± 0.1	21.23 ± 0.15	19.95 ± 0.14
	SE	0.12	0.18	0.03	0.11	0.07	0.04	0.06	0.06
Candida famata	Mean (mm) ± SD	20.16 ± 0.022	6.25 ± 0.35	6 ± 0	6 ± 0	6 ± 0	6 ± 0	24.75 ± 0.13	20.26 ± 0.16
	SE	0.18	0.3	0	0	0	0	0.05	0.07
Candida parapsilosis	Mean (mm) ± SD	19. 3 ± 0.021	6.25 ± 0.021	21.43 ± 0.16	11.53 ± 0.17	26.36 ± 0.17	27.48 ± 0.32	24.47 ± 0.15	22.42 ± 0.28
	SE	0.01	0.012	0.07	0.07	0.07	0.13	0.06	0.11
Candida guilliermondii	Mean (mm) ± SD	21.8 ± 0.23	6.25 ± 0.14	37.48 ± 0.26	24.19 ± 0.15	38.4 ± 0.42	37.61 ± 0.32	21.45 ± 0.18	31.26 ± 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 ± 1.29%. Radical removing of other compunds also reported, for instance, synthetic antioxidant (BHT) in parallel for the purpose of the comparision. 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 0.1ml	MWE 0.2ml	MWE 0.3ml	BHT 0.1ml	BHT 0.2ml	BHT 0.3ml
Mean (%) ± SD	96.50 ± 0.11	97.67 ± 0.13	98.65 ± 0.11	68.76 ± 0.21	69.11 ± 0.2	74.8 ± 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 Esmaeilizadeh, 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).

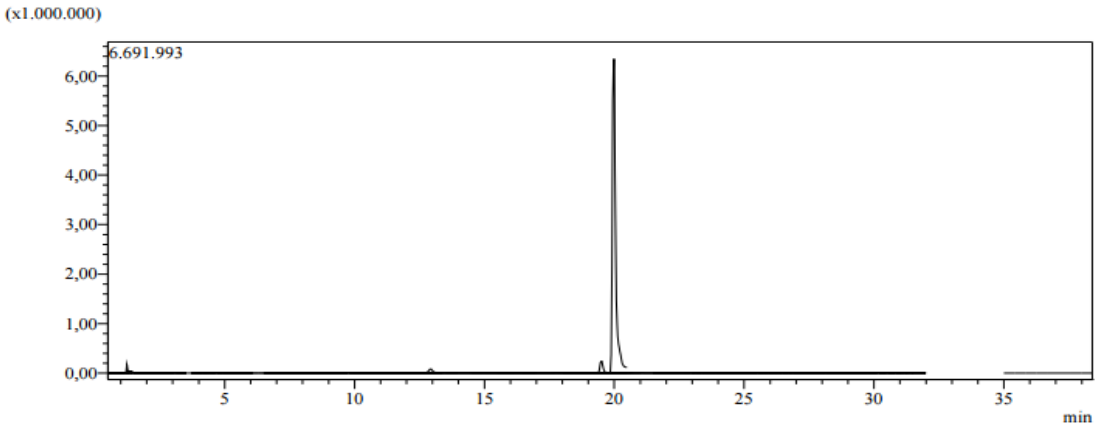

Figure 2 illustrate the LC-MS/MS chromatogram.

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	R²[c]	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=30.61×+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=316.95×-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=42.33×-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=876.67×+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=4315.1×+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=561.91×-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=1198.48×+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=339.39×+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=4548.36×+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=2032.13×+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=2384.96×+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=803.23×+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=1775.55×+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=237.15×+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=498.38×+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=547.46×+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=3272.65×+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=583.55×+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=26.29×+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.

REFERENCES

ALVES-SILVA, J. M., MACCIONI, D., COCCO, E., GONÇALVES, M. J., PORCEDDA, S., PIRAS, A., CRUZ, M. T., SALGUEIRO, L. & MAXIA, A. J. P. 2023. Advances in the Phytochemical Characterisation and Bioactivities of Salvia aurea L. Essential Oil. 12**,** 1247.

ASKARI, S. F., AVAN, R., TAYARANI-NAJARAN, Z., SAHEBKAR, A. & EGHBALI, S. J. P. 2021. Iranian Salvia species: A phytochemical and pharmacological update. 183**,** 112619.

AVULA, B., BAE, J.-Y., CHITTIBOYINA, A. G., WANG, Y.-H., WANG, M., SRIVEDAVYASASRI, R., ALI, Z., LI, J., WU, C., KHAN, I. A. J. J. O. P. & ANALYSIS, B. 2022. Comparative analysis of five Salvia species using LC-DAD-QToF. 209**,** 114520.

BALKıR, Ş., HAZMAN, Ö., AKSOY, L., YıLMAZ, M. A., CAKIR, O., KARA, R. & EROL, İ. J. A. C. S. 2023. Phytochemical Profile, Antioxidant and Antimicrobial Potency of Aerial Parts of Salvia Tomentosa Miller. 70.

BAYRAKÇEKEN GÜVEN, Z., ALSHEHRI, O., YÜCE, N., BAKAN, E., DEMIRCI, B., YILMAZ, M. A., ERTAS, A. & BASARAN, A. A. 2023. Chemical composition, nutritional values, elemental analysis and biological properties of Prunus mahaleb L.: From waste to new potential sources for food, cosmetic and drug industry. *Food Bioscience,* 53**,** 102632.

CARDILE, V., RUSSO, A., FORMISANO, C., RIGANO, D., SENATORE, F., ARNOLD, N. A. & PIOZZI, F. 2009. Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. *J Ethnopharmacol,* 126**,** 265-72.

DULGER, G. & DÜLGER, B. 2021. Antifungal activity of Salvia verticillata subsp. verticillata against fungal pathogens. *Düzce Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi*.

ERTAS, A., BOGA, M., YILMAZ, M. A., YESIL, Y., TEL, G., TEMEL, H., HASIMI, N., GAZIOGLU, I., OZTURK, M. & UGURLU, P. 2015. A detailed study on the chemical and biological profiles of essential oil and methanol extract of Thymus nummularius (Anzer tea): Rosmarinic acid. *Industrial Crops and Products,* 67**,** 336-345.

ERTAS, A., YIGITKAN, S. & ORHAN, I. E. J. P. 2023. A Focused Review on Cognitive Improvement by the Genus Salvia L.(Sage)—From Ethnopharmacology to Clinical Evidence. 16**,** 171.

FATMA EBRU, K., AYSE, A. & CAGLAR, K. J. N. P. C. R. 2017. Extraction and HPLC analysis of sage (Salvia officinalis) plant. 5**,** 8-10.

FRANCIK, S., FRANCIK, R., SADOWSKA, U., BYSTROWSKA, B., ZAWIŚLAK, A., KNAPCZYK, A. & NZEYIMANA, A. J. M. 2020. Identification of phenolic compounds and determination of antioxidant activity in extracts and infusions of salvia leaves. 13**,** 5811.

GECER, E. N. J. J. O. I., POLYMERS, O. & MATERIALS 2021. Green synthesis of silver nanoparticles from Salvia aethiopis L. and their antioxidant activity. 31**,** 4402-4409.

GHAVAM, M., MANCA, M. L., MANCONI, M. & BACCHETTA, G. 2020. Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of Salvia hydrangea DC. ex Benth. *Sci Rep,* 10**,** 15647.

GHORBANI, A. & ESMAEILIZADEH, M. 2017. Pharmacological properties of Salvia officinalis and its components. *J Tradit Complement Med,* 7**,** 433-440.

HAFEZ GHORAN, S., TAKTAZ, F., MOZAFARI, A. A., TUNÇTÜRK, M., SEKEROGLU, N. & KIJJOA, A. J. M. 2022. Uncommon Terpenoids from Salvia Species: Chemistry, Biosynthesis and Biological Activities. 27**,** 1128.

ISMAEL, B. Q. J. Z. J. O. P. & SCIENCES, A. 2021. Phytochemical screening and anti-candida activities of Crocus cancellatus herb. Ethanol extract. 33.

JIROVETZ, L., WLCEK, K., BUCHBAUER, G., GOCHEV, V., GIROVA, T., STOYANOVA, A., SCHMIDT, E. & GEISSLER, M. 2007. Antifungal Activities of Essential Oils ofSalvia lavandulifolia,Salvia officinalisandSalvia sclareaagainst Various PathogenicCandidaspecies. *Journal of Essential Oil Bearing Plants,* 10**,** 430-439.

JUEE, L. J. J. P. P. R. 2022. Phytochemical characterization and mushroom tyrosinase inhibition of different extracts from Salvia officinalis L. leaves. 10**,** 605-615.

KARAMI, F., JABERI, S., AFSARI, A. & MOEIN, M. J. T. I. P. S. 2020. Quantitation of Rosmarinic Acid and Caffeic Acid in Various Salvia Genera by High Performance Thin Layer Chromatography. 6**,** 205-212.

KATANIĆ STANKOVIĆ, J. S., SREĆKOVIĆ, N., MIŠIĆ, D., GAŠIĆ, U., IMBIMBO, P., MONTI, D. M. & MIHAILOVIĆ, V. 2020. Bioactivity, biocompatibility and phytochemical assessment of lilac sage, Salvia verticillata L. (Lamiaceae) - A plant rich in rosmarinic acid. *Industrial Crops and Products,* 143**,** 111932.

KERKOUB, N., PANDA, S. K., YANG, M. R., LU, J. G., JIANG, Z. H., NASRI, H. & LUYTEN, W. 2018. Bioassay-Guided Isolation of Anti-Candida Biofilm Compounds From Methanol Extracts of the Aerial Parts of Salvia officinalis (Annaba, Algeria). *Front Pharmacol,* 9**,** 1418.

KHUDHUR, P., BAKIR, A., RAHMAN, L. & ISMAEL, B. 2019. Anethumgraveolens and Apiumgraveolens leaf-extract and their antifungal effects on pathogenic Candida species: In vitro study. *Zanco Journal of Medical Sciences,* 23**,** 135-142.

KRZEMINSKA, M., OWCZAREK, A., GONCIARZ, W., CHMIELA, M., OLSZEWSKA, M. A. & GRZEGORCZYK-KAROLAK, I. 2022. The Antioxidant, Cytotoxic and Antimicrobial Potential of Phenolic Acids-Enriched Extract of Elicited Hairy Roots of Salvia bulleyana. *Molecules,* 27.

LARIT, F. & LEÓN, F. J. P. 2023. Therapeutics to Treat Psychiatric and Neurological Disorders: A Promising Perspective from Algerian Traditional Medicine. 12**,** 3860.

MORZEL, M., CANON, F., GUYOT, S. J. J. O. A. & CHEMISTRY, F. 2022. Interactions between salivary proteins and dietary polyphenols: potential consequences on gastrointestinal digestive events. 70**,** 6317-6327.

NISHIDA, A., HIDAKA, K., KANDA, T., IMAEDA, H., SHIOYA, M., INATOMI, O., BAMBA, S., KITOH, K., SUGIMOTO, M. & ANDOH, A. J. I. B. D. 2016. Increased expression of interleukin-36, a member of the interleukin-1 cytokine family, in inflammatory bowel disease. 22**,** 303-314.

POULIOS, E., GIAGINIS, C. & VASIOS, G. K. J. P. M. 2020. Current state of the art on the antioxidant activity of sage (Salvia spp.) and its bioactive components. 86**,** 224-238.

REMOK, F., SAIDI, S., GOURICH, A. A., ZIBOUH, K., MAOULOUA, M., MAKHOUKHI, F. E., MENYIY, N. E., TOUIJER, H., BOUHRIM, M., SAHPAZ, S., SALAMATULLAH, A. M., BOURHIA, M. & ZAIR, T. 2023. Phenolic Content, Antioxidant, Antibacterial, Antihyperglycemic, and alpha-Amylase Inhibitory Activities of Aqueous Extract of Salvia lavandulifolia Vahl. *Pharmaceuticals (Basel),* 16.

SALARI, S., BAKHSHI, T., SHARIFIFAR, F., NASERI, A. & GHASEMI NEJAD ALMANI, P. 2016. Evaluation of antifungal activity of standardized extract of Salvia rhytidea Benth. (Lamiaceae) against various Candida isolates. *J Mycol Med,* 26**,** 323-330.

SHARIFI-RAD, M., OZCELIK, B., ALTıN, G., DAŞKAYA-DIKMEN, C., MARTORELL, M., RAMÍREZ-ALARCÓN, K., ALARCÓN-ZAPATA, P., MORAIS-BRAGA, M. F. B., CARNEIRO, J. N. P., ALVES BORGES LEAL, A. L., COUTINHO, H. D. M., GYAWALI, R., TAHERGORABI, R., IBRAHIM, S. A., SAHRIFI-RAD, R., SHAROPOV, F., SALEHI, B., DEL MAR CONTRERAS, M., SEGURA-CARRETERO, A., SEN, S., ACHARYA, K. & SHARIFI-RAD, J. 2018. Salvia spp. plants-from farm to food applications and phytopharmacotherapy. *Trends in Food Science & Technology,* 80**,** 242-263.

STANKOVIĆ, J. S. K., SREĆKOVIĆ, N., MIŠIĆ, D., GAŠIĆ, U., IMBIMBO, P., MONTI, D. M., MIHAILOVIĆ, V. J. I. C. & PRODUCTS 2020. Bioactivity, biocompatibility and phytochemical assessment of lilac sage, Salvia verticillata L.(Lamiaceae)-A plant rich in rosmarinic acid. 143**,** 111932.

TEKELI, Y., KARPUZ, E., DANAHALILOGLU, H., BUCAK, S., GUZEL, Y. & ERDMANN, H. 2014. Phenolic composition, antioxidant capacity of Salvia verticcilata and effect on multidrug resistant bacteria by flow-cytometry. *Afr J Tradit Complement Altern Med,* 11**,** 147-52.

TEPE, B., EMINAGAOGLU, O., AKPULAT, H. A. & AYDIN, E. 2007. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of Salvia verticillata (L.) subsp. verticillata and S. verticillata (L.) subsp. amasiaca (Freyn & Bornm.) Bornm. *Food Chemistry,* 100**,** 985-989.

UYSAL, I., KOÇER, O., MOHAMMED, F. S., LEKESIZ, Ö., DOĞAN, M., ŞABIK, A. E., SEVINDIK, E., GERÇEKER, F. Ö., SEVINDIK, M. J. A. I. P. & PHARMACY 2023. Pharmacological and nutritional properties: Genus Salvia. 11**,** 140-155.

VERGINE, M., NICOLÌ, F., NEGRO, C., LUVISI, A., NUTRICATI, E., ACCOGLI, R. A., SABELLA, E. & MICELI, A. J. R. O. N. P. 2019. Phytochemical profiles and antioxidant activity of Salvia species from southern Italy. 13**,** 215.

ZACCARDELLI, M., PANE, C., CAPUTO, M., DURAZZO, A., LUCARINI, M., SILVA, A. M., SEVERINO, P., SOUTO, E. B., SANTINI, A. & DE FEO, V. J. F. 2020. Sage species case study on a spontaneous Mediterranean plant to control phytopathogenic fungi and bacteria. 11**,** 704.