



Original Article

Phytochemical Composition and Antifungal Potential of *Salvia palaestina* Populations from Kurdistan Against Dermatophytes

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ABSTRACT

The paper studied the phytochemical compounds and antifungal activity of *Salvia palaestina* (Lamiaceae) populations growing in the Kurdistan Region of Iraq from March to July 2025. The plants were tested against the dermatophytes *Trichophyton rubrum* and *Microsporum canis* using 80% methanol extracts from different populations. Antifungal activity was measured by inhibition zones, which differed significantly among groups. While SP9 and SP13 showed moderate activity, SP1 was the most active, particularly against *Microsporum canis*, which showed 55.44 mm inhibition at 100% concentration. Overall, *Microsporum canis* was less sensitive than *Trichophyton rubrum*. Phytochemical analysis revealed distinctive patterns of phenolics and flavonoids. The most common compound was salvigenin, detected in seven populations. The highest diversity of chemical compositions was observed in populations SP1, SP2, SP3, and SP4, which contained significant compounds including ferulic acid and caffeoylquinic acids. These findings suggest that *Salvia palaestina* has potential antifungal properties, particularly in populations rich in phenolic and flavonoid compounds. Population-specific variations in phytochemicals, likely influenced by environmental factors, appear to strongly affect antifungal efficacy.

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Keywords: *Salvia palaestina*, Antifungal properties, phytochemical analysis, Environmental conditions, Dermatophytes.

1 Introduction

The genus *Salvia* L., frequently referred to as "sage," is the most extensive genus in the Lamiaceae family, including more than 1,000 species found worldwide in both tropical and temperate areas ^[1]. *Salvia palaestina* Benth is a remarkable species native to the Eastern Mediterranean and the Middle East, with distinct populations found in the mountainous regions of Iraq and the Kurdistan Region ^[2]. Although *S. palaestina* is known for its culinary applications and therapeutic advantages, its precise pharmacological roles are still being studied, especially with regard to its secondary metabolites ^[3]. These metabolites include polyphenols with multiple phenolic hydroxyl groups, such as flavonoids, phenolic acids, tannins, and stilbenes ^[4]. Research shows that these substances, especially flavonoids and phenolic acids, have a major impact on human health by acting as antimicrobials, anti-inflammatory agents, and antioxidants ^[5]. Additionally, these active compounds have been found in higher concentrations in alcoholic extracts of *Salvia* species

^[6]. Recent studies highlight the urgent need for new antifungal medications because fungal infections are becoming more common and dermatophytes are becoming more resistant to conventional antifungal treatments ^[7,8]. In both humans and animals, dermatophytes like *Microsporum canis* and *Trichophyton rubrum* are the main causes of superficial fungal infections, which frequently result in chronic and recurrent issues ^[9]. There is an urgent need for efficient, natural antifungal substitutes because current treatments are often limited by significant toxicity, adverse effects, and the emergence of resistant fungal strains. ^[10] Although a number of *Salvia* species have shown antimicrobial qualities, little is known about the particular antifungal effectiveness of *S. palaestina* populations that exist in the particular environmental circumstances of the Kurdistan Region of Iraq ^[11]. Even within the same species, there can be differences in the medicinal potency of plants due to factors like altitude, climate, and soil composition that have a significant impact on phytochemical production ^[12]. The objective of this study is to assess the antifungal effectiveness of 80% methanolic extracts from various Iraqi *S. palaestina* populations against the pathogenic fungi *Trichophyton rubrum* and *Microsporum canis* ^[13]. In order to establish a connection between chemical composition and antifungal effectiveness, this

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study also attempts to characterize the phytochemical traits of these populations, focusing on the distribution of crucial bioactive compounds ^[14].

2 Methods and Materials

2.1 Plant Samples

Aerial samples from fifteen different *Salvia palaestina* populations were collected between March and July 2025 in the Kurdistan Region of Iraq during their peak flowering period. The sampling sites ranged in altitude from 229 to 1280 meters and represented diverse ecosystems, including Mountain Rowanduz, Mountain Suleimany, Foothill Persian, and Foothill Kirkuk areas " Table 1 ". These sites, spanning the northern, central, and southern parts of the region, reflect the ecological and climatic variations of the area " Plate 2 ". The selected populations provide

a representative sample of the species, as Northern Kurdistan is a major center of diversity for the *Salvia* L. genus " Plate1". Although *S. palaestina* is not considered endangered, collections were conducted solely for educational purposes and with institutional approval. Fieldwork adhered to national and international regulations, including the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the IUCN Policy Statement on Research Related to Species at Risk of Extinction. For each sample, field data were recorded, including growth stage, geographic coordinates, and ecological characteristics. Collected samples were dried, compressed, and stored under refrigeration. Taxonomic identification was performed using the *Flora of Turkey*, and voucher specimens were deposited in the herbarium of the University of Garmian, Kurdistan.



Plate 1: plant *Salvia palaestina* in their habitat.

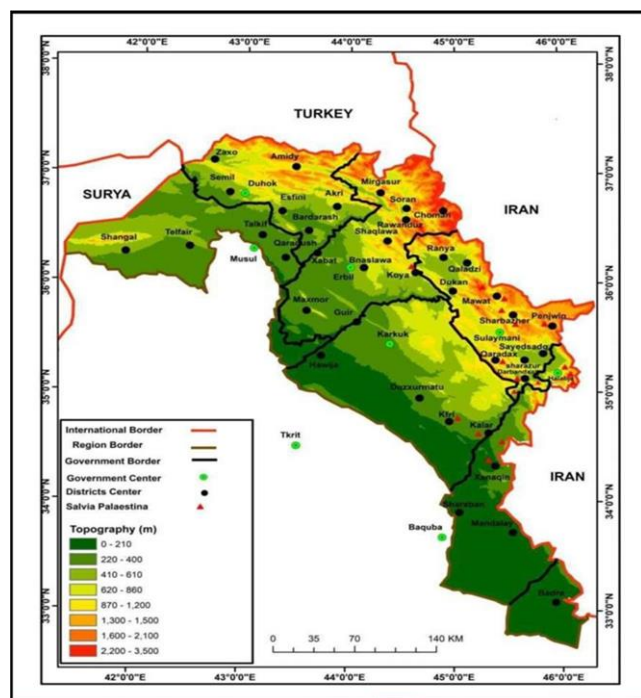


Plate 2: Distribution map of surveyed studied plant *S. palaestina*.

2. 2 Method of Extraction

Upon arrival at the laboratory, the aerial parts of *S. palaestina* were air-dried at room temperature. The dried material was then ground into a fine powder using a laboratory mill.

2. 2. 1 Antifungal Assay Extraction

To evaluate antifungal activity, 10 g of powdered plant material was mixed with 100 mL of 80% methanol, yielding a 1:10 weight-to-volume ratio. The mixture was stirred in a shaker incubator at 25–30 °C for 24 hours to facilitate maceration [15]. For phytochemical profiling and HPLC analysis, 1 g of the dried, ground aerial parts was extracted with 80% methanol (v/v) [16]. The extraction was performed in an ultrasonic bath (Elmasonic, Germany) at 25 °C for 30 minutes. The resulting mixture was centrifuged to obtain the supernatant. The final extracts were stored in dark glass flasks at 4 °C until further phytochemical examination and chromatographic analysis [17].

2. 3 Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content of the extracts was measured using the Folin-Ciocalteu method [18,19]. Total flavonoid content was determined using a colorimetric assay based on aluminum chloride [20,21].

2. 4 Analysis of Phytochemical Composition by HPLC

High-Performance Liquid Chromatography (HPLC) was employed to analyze the *S. palaestina* extracts using a Shimadzu LC-20AD system (Shimadzu, Japan) [22].

2. 5 Evaluation of Antifungal Activity

2. 5. 1 Preparation of Fungal Culture

The colonies taken to form a culture of 7-10 days old were used to prepare fungal suspensions. The colonies that were separated were soaked in a sterile saline solution. In order to achieve a uniform density of the cells, the turbidity of the suspension was adjusted to match the standard McFarland solution [23].

2. 5. 2 Inoculation and Testing Procedures

Susceptibility testing was conducted using the disk diffusion method. The standardized fungal suspension was applied onto Sabouraud Dextrose Agar (SDA) plates. Following the application of the extracts and incubation, the antifungal efficacy was evaluated by determining the size of the inhibition zone [24,25].

Both controls were established for the control treatment: positive for fungi and negative for fungi.

- Add 50µL/well of Fluconazole (100mg/ml), serving as an antifungal (positive control for the fungi under investigation). Three replicates for a single treatment of the examined pathogenic fungi (*Microsporum canis* and *Trichophyton rubrum*) were utilized.
- In the earlier approach, a well was created, and a micropipette (50µL/well) of sterilized distilled water (thenegative control) was added to the designated well. Three repetitions for each treatment examined fungal species [26].

3 Statistical Evaluations

Analysis of data was conducted using the R software environment. The Analysis of Variance (ANOVA) was utilized to assess statistical variations among experimental groups. Following that, Tukey's test was utilized to identify particular pairwise differences between phytochemical composition, controls, fungal strains, and concentrations of the extract.

4 Results and Discussions

4. 1 Phytochemicals Present in *S. palaestina* Varieties (TFC and TPC):

The results of *S. palaestina* populations under study in terms of the total phenolic content (TPC) and total flavonoid content (TFC) are shown in "Table 1" below. The quantitative range of flavonoid measured ranged between 7.94 and 18.59 milligrams of quercetin equivalents per gram of dry weight (mg QUE/g DW). The population SP4 (obtained at Chami Sarqala) had the greatest values of flavonoids, whereas population SP13 (obtained at Chwarta) had the least values of flavonoids. Significant differences were statistically determined between the 15 groups that were studied ($p < 0.05$), indicating that there is a large variable of metabolic activity in the species. The TPC had high and low values of 27.54mg Gallic Acid Equivalents per gram of dry weight (mg GAE/g DW). According to "Table 2", population SP1 (obtained from Zhalay Safar) showed the greatest accumulation of phenolic chemicals, whilst population SP15 (acquired from Mawat) showed the lowest concentration. The significant differences in TPC and TFC found in the 15 *S. palaestina* populations indicate that the phytochemical composition varies throughout the species and is heavily impacted by the specific

environment. This result lends credence to the theory of "phytochemical accumulation caused by stress" [27]. The differences seen between populations (such as the higher TPC in SP1 compared to the lower TPC in SP15) can be attributed to the interaction between the growing environment and the genotype of the plant [28,29]. Previous research on the Lamiaceae family suggests that environmental conditions, including precipitation, temperature, and UV radiation, significantly affect the synthesis of secondary metabolites [30,31]. Studies indicate that plants grown at higher elevations or in regions with elevated sun radiation often produce bigger quantities of flavonoids (similar to those present in SP4) [32,33]. Plants often increase the synthesis of different secondary metabolites that act as protective compounds during drought circumstances. The greatest amount of phenolic chemicals can be produced under drought stress [34]. Some populations may have higher phenolic levels as a physiological response to drought stress [35]. Water scarcity is a well-documented abiotic stressor that causes oxidative stress in plant

tissues through the production of Reactive Oxygen Species (ROS) [36]. To minimize oxidative damage, *Salvia* species often boost the phenylpropanoids pathway [37]. This improves the operation of important enzymes such as phenylalanine ammonia-lyase (PAL), resulting in the formation of phenolic acids and flavonoids, functioning as potent antioxidants and free radical neutralizers [38,39]. The results show that populations in possibly more stressful or arid microclimates (probably SP1 and SP4) appear to have produced more of these protective chemicals in order to survive [40]. This is consistent with findings from other *Salvia* species, which indicate that increased phenolic levels and essential oil output are favorably correlated with drought stress [41]. However, this response is not linear. While moderate stress usually increases the production of metabolites, extreme stress might totally impede metabolic activities [42]. This explains why the response is not consistent; genetic variations allow certain populations to be "high-responders" to stress, while others may prefer vegetative growth over chemical defense [43,44].

Table 1: Sampling locations of *S. palaestina* plants.

Code	Sample Locations	Height by meter	Longitude	Latitude
SP1	Zhalay safar	325	45.8792788	35.5150117
SP2	Esayi	300	45.8538412	35.5665524
SP3	Kanimasi	273	45.3596366	34.65082488
SP4	Chami sarqala	229	45.341	35.4355
SP5	Zhalanaw	335	45.354882	34.6512117
SP6	Shameran	380	46.68482030	35.08135492
SP7	Beerke	980	45.43214	35.640329
SP8	Goshan	1200	45.3422678	35.23225560
SP9	Sarswraw	1250	45.963523	35.6066896
SP10	Kani kawa	1100	45.9630349	35.6055013
SP11	Byara	1000	46.0183557950	35.016931830
SP12	Haibat sultan mountain	1245	44.095294365	35.22855060
SP13	Chwarta	830	45.7270425	35.03199275
SP14	Surqlat	1195	45.5233997	36.719943
SP15	Mawat	1280	45.9967999	36.7285233

Table 2: lists the phytochemical composition of various populations of *S. palaestina* plants.

Code	TPC (mg GAE/g DW)	TFC (mg QUE/g DW)
SP1	78.50 ± 0.39 a	8.60 ± 0.21 GH
SP2	42.35 ± 5.93 f	9.55 ± 0.17 EF
SP3	70.73 ± 2.67 b	15.72 ± 0.81 B
SP4	62.14 ± 1.91 c	18.59 ± 0.69 A
SP5	68.91 ± 1.13 b	8.63 ± 0.11 GH
SP6	36.91 ± 4.37 fg	12.79 ± 0.55 C
SP7	55.76 ± 3.41 d	18.22 ± 0.30 A
SP8	39.50 ± 2.35 f	9.55 ± 0.18 EF
SP9	32.40 ± 0.21 g	9.74 ± 0.26 E
SP10	47.33 ± 3.33 e	9.66 ± 0.22 EF
SP11	72.76 ± 2.65 b	10.23 ± 0.51 DF
SP12	51.22 ± 1.23 de	13.23 ± 0.21 C
SP13	47.50 ± 2.65 e	7.94 ± 1.22 H
SP14	38.50 ± 4.46 fg	11.33 ± 0.17 D
SP15	27.54 ± 3.46 h	10.63 ± 0.32 D

*Read the little letters (a to h) in a vertical manner. The TPC values ought to be compared across populations. Read the capital letters (A -H) in a vertical manner. There should be comparison of TFC values across populations. R1: no significant difference in the means that contain the same letter ($p > 0.05$). Most notably different means are those that differ completely in letters ($p < 0.05$).

4. 2 Profiling of Flavonoids and Phenolic Acids' Phytochemicals

4. 2. 1 Distribution of Flavonoids

It is possible to distinguish flavonoid fraction because of the existence of both glycosides and aglycones. Salvigenin is the most common flavonoid which was detected in nearly half of the samples (SP1, SP2, SP4, SP5, SP7, SP11, and SP14), showing that it can be used as a good chemotaxonomic signature of this species. Hispidulin and tetramethoxeflavone were two examples of methylated flavones having similar quantities in SP1 and different distributions elsewhere. The occurrence of luteolin 7-glucuronide (SP1, SP3, SP10, SP12), luteolin 7-glucoside (SP2, SP4, SP7, SP11) and apigenin 7-glucoside (SP2, SP3, SP9, SP15) indicated the existence of glycosidic variation.

4. 2. 2 Phenolic Acids' Composition

The detected phenolic acids include ferulic acid and caffeoylquinic acids, which have a specific distribution that notably differentiates populations SP1, SP2, SP7, and SP11. The research was discriminating between rosmarinic acid (RA) and its methylated form, 3-O-methylrosmarinic acid. Its methylated form is found only in SP1, SP3, SP11 and SP15 whereas in SP3, SP8, SP10 and SP14, the primary marker was RA. This deviation

presents the different enzymatic activity associated with the process of methylation in these groups.

Salvianolic acid A was exclusively in 4 populations (SP2, SP5, SP10 and SP11) as compared to Syringic acid which was in SP4, SP6, SP13 and SP15 exhibiting varied metabolic groups.

4. 2. 3 Implications for Chemotaxonomy and the Environment

Metabolic profile indicates that SP1 and SP2 are different chemical groups that exhibit the maximum diversity of phenols acids and flavonoids. The mixture of methyl rosmarinate, ferulic acid, caffeoylquinic acids, salvigenin, hispidulin and tetramethoxeflavone in SP1 was a complex ^[45]. The population of SP8 and SP12, however, possessed less extensive chemical signatures. The observed intraspecific variability indicates the occurrence of distinct chemotypes which are most probably influenced by the genetic diversity or by evolutionary adaptations to environmental factors (such as soil structure, height, or UV radiation) to that effect ^[46]. It is the presence of the specific chemotypes (probably influenced by genetic diversity or adaptive evolution to environmental conditions (soil composition, elevation, UV exposure, etc.) which is reflected in the observed intraspecific variability ^[47]. These observations highlight the imperativeness of selecting certain populations, especially SP1 and SP2, to therapeutic uses or breeding initiatives to capitalize on bioactive compounds productions as much as possible ^[48].

Table 3: General classification of the chemical compounds identified by HPLC from wild *Salvia palaestina* populations.

Chemical composition	plant populations
Phenolic acid	
methyl rosmarinate or 3-O-methylrosmarinic acid	SP1- SP3- SP11- SP15
Syringic acid	SP4- SP6- SP13- SP15
Rosmarinic acid	SP3- SP8- SP10- SP14
Salvianolic acid A	SP2- SP5- SP10- SP11
Ferulic acid	SP1-SP2- SP7-SP9- SP12
Caffeoylquinic acids	SP1- SP2- SP7- SP11
Flavonoids	
Salvigenin	SP1-SP2- SP4- SP5- SP7- SP11- SP14
Luteolin 7-glucuronide	SP1 -SP3-SP10- SP12
Luteolin 7- glucoside	SP2-SP4-SP7- SP11
Apigenin 7-glucoside	SP2- SP3- SP9- SP15
Hispidulin	SP1-SP4- SP5- SP13
tetramethoxeflavone	SP1- SP2- SP6-SP7- SP15

4. 3 Efficacy against Fungi

The antifungal effectiveness of *S. palaestina* extracts was strongly influenced by plant population and concentration "Figure 17". SP1 was the most active population. With an

inhibition zone of 55.44 mm against *M. canis* at 100% concentration, SP1 considerably outperformed the controls "Figure1". Similarly, SP7 demonstrated significant effectiveness against *T. rubrum* at a concentration of 100% (40.22 mm) "Figure 7". *M. canis* typically showed the most sensitivity and

vulnerability in the most effective experiments ^[49]. Moderate to low effectiveness was observed in certain populations. SP4 showed moderate efficacy with an average inhibition of 26.333333 mm in *T. rubrum* "Figure 4", although it was not as effective as the control (38.33 mm) "Figure 16". In population SP3, where inhibition was uniform across fungal species, there was no appreciable difference between *M. canis* (25.48 mm) and *T. rubrum* (25.62666667 mm) "Figure 4". Conversely, populations SP14 and SP15 showed very little effectiveness "Figures 14, 15". Many preparations failed to halt the growth of fungus. Populations SP9 in *T. rubrum* and SP12 in *M. canis* had minimal activity against the two fungal species at all concentrations "Figures 9, 12" ^[50]. Similarly, SP6 was ineffective at both 50% and 100% concentrations "Figure 6". Similarly, at lower concentrations (25% and 50%), SP11 did not inhibit *M.*

canis "Figure 11" ^[51]. The observed antifungal effects vary by population and depend on concentration "Figure 16" ^[52]. These compounds most likely function by disrupting fungal cell membranes or preventing the synthesis of enzymes ^[53]. These findings align with previous research on the Lamiaceae family, namely the genus *Salvia*, which is known to have antibacterial chemicals ^[54,55]. The phytochemical content and antibacterial efficacy were significantly impacted by environmental variability ^[56,57]. Bioactive metabolite levels were higher in populations under drought stress, such as SP1 and SP2 ^[58]. Furthermore, samples collected between March and July of 2025 showed that seasonal weather and the plant's growth stage are important factors in phytochemical accumulation. This suggests that the plant's defense mechanisms are activated by environmental stress, resulting in potent antifungal properties ^[59].

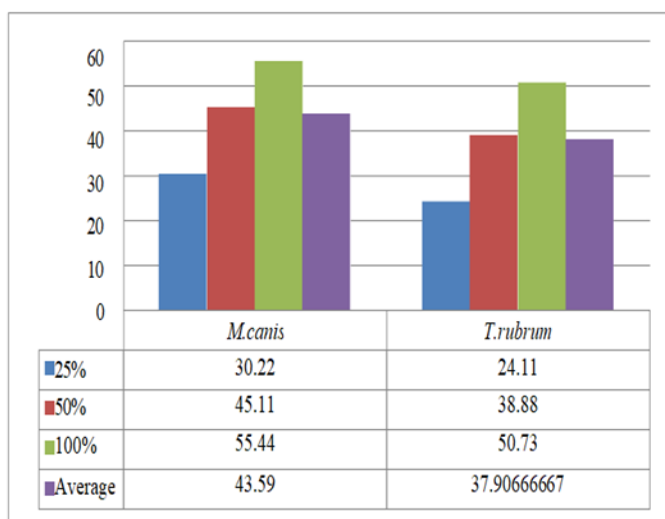


Figure 1: The antifungal effectiveness of SP1

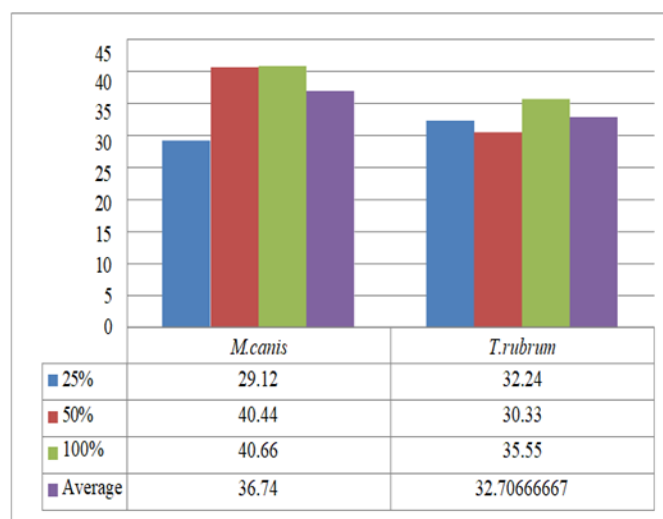


Figure 2: The antifungal effectiveness of SP2

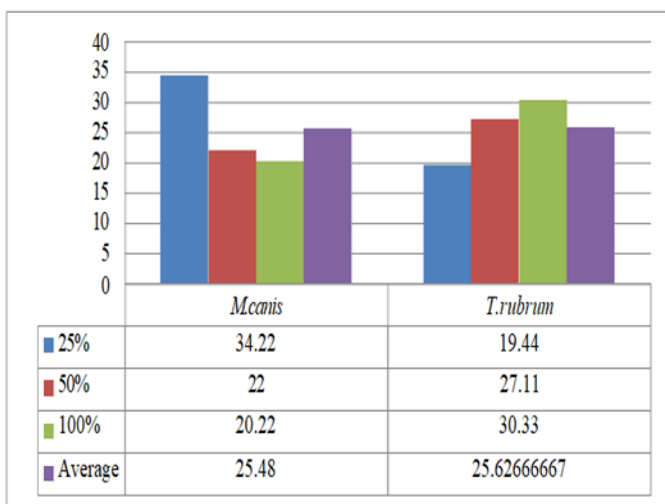


Figure 3: The antifungal effectiveness of SP3

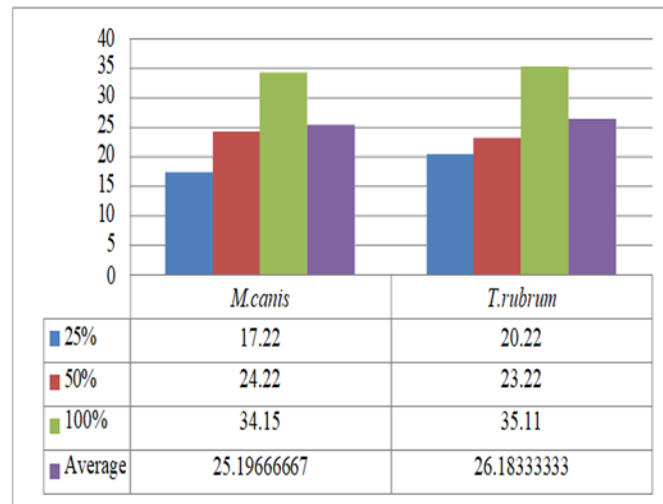


Figure 4: The antifungal effectiveness of SP4

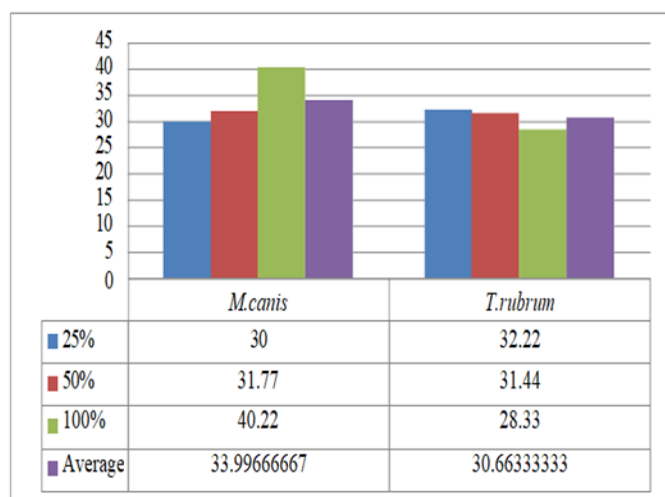


Figure 5: The antifungal effectiveness of SP5

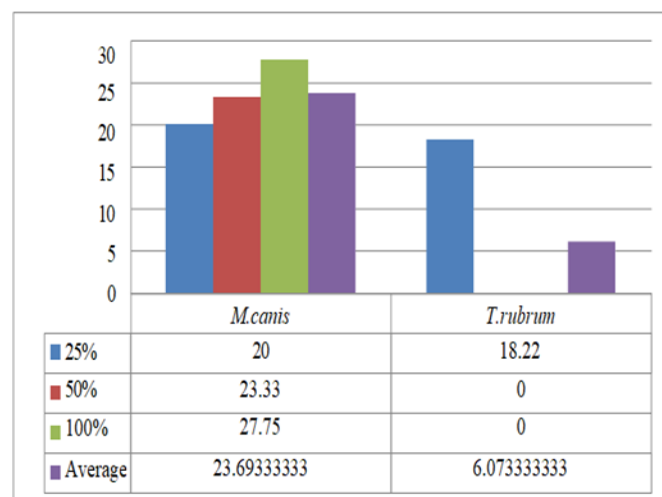


Figure 6: The antifungal effectiveness of SP6

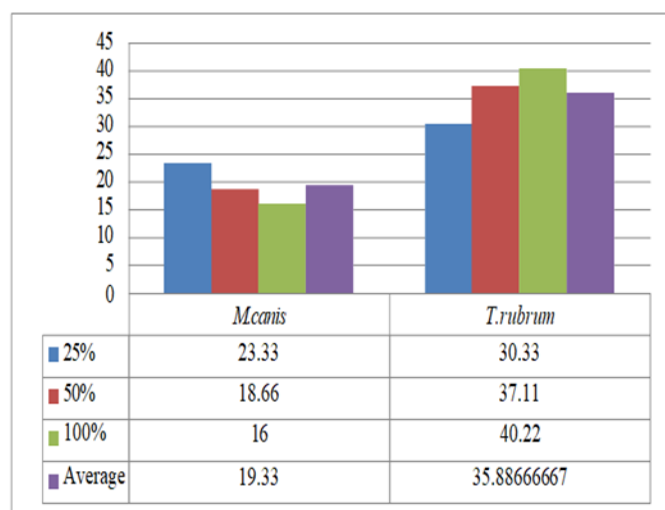


Figure 7: The antifungal effectiveness of SP7

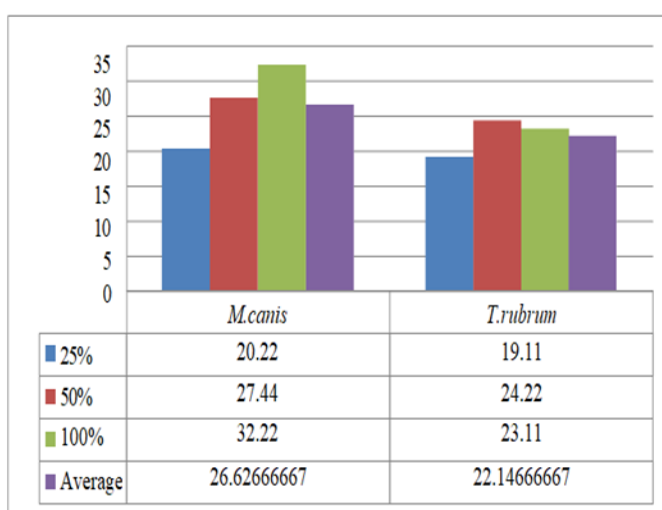


Figure 8: The antifungal effectiveness of SP8

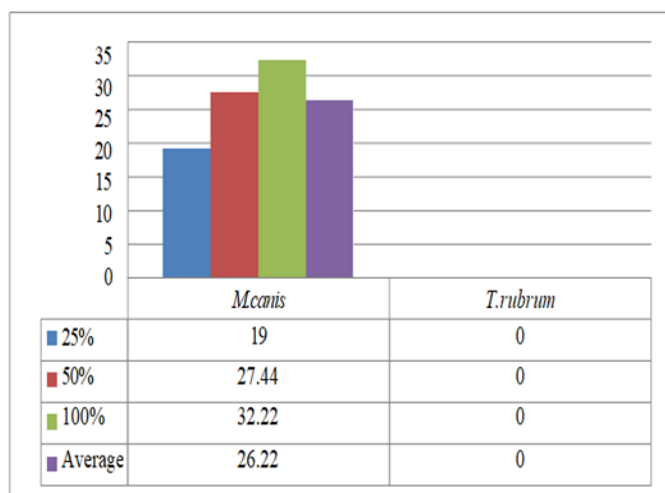


Figure 9: The antifungal effectiveness of SP9

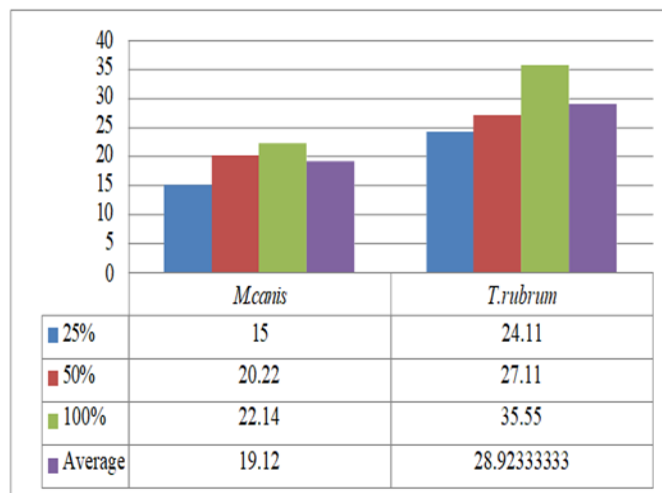


Figure 10: The antifungal effectiveness of SP10

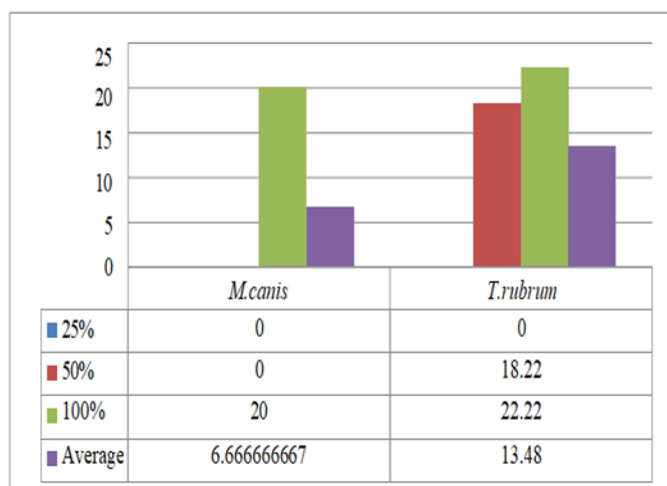


Figure 11: The antifungal effectiveness of SP11

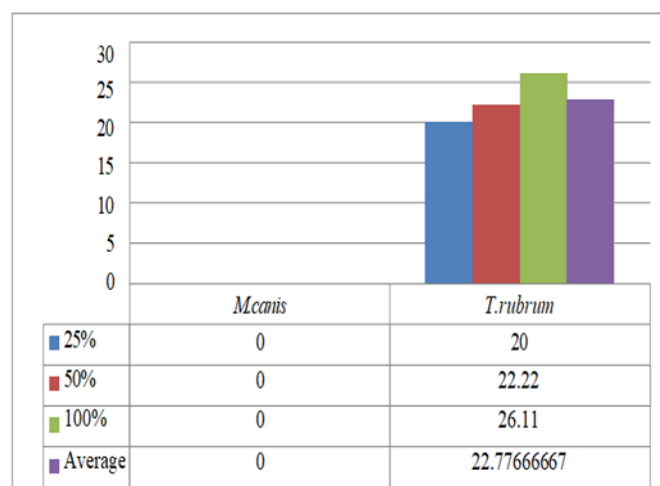


Figure 12: The antifungal effectiveness of SP12

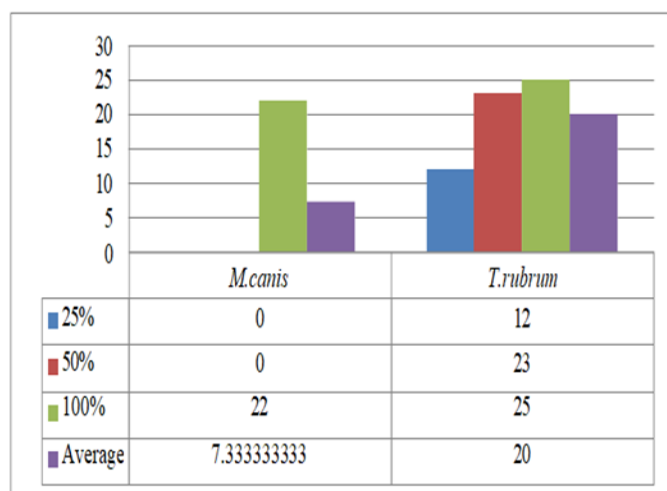


Figure 13: The antifungal effectiveness of SP13

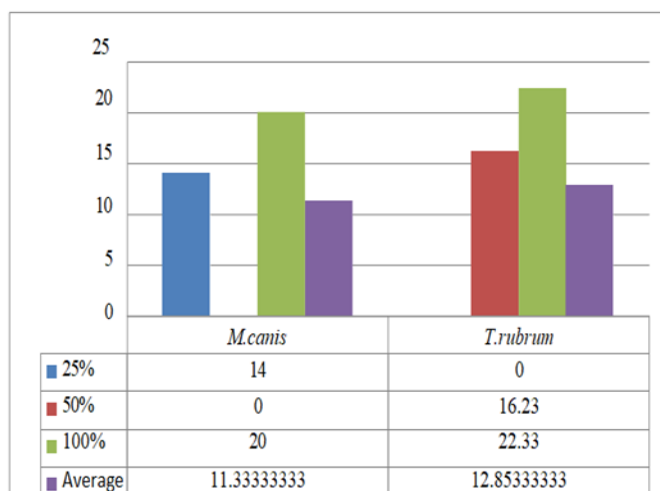


Figure 14: The antifungal effectiveness of SP14

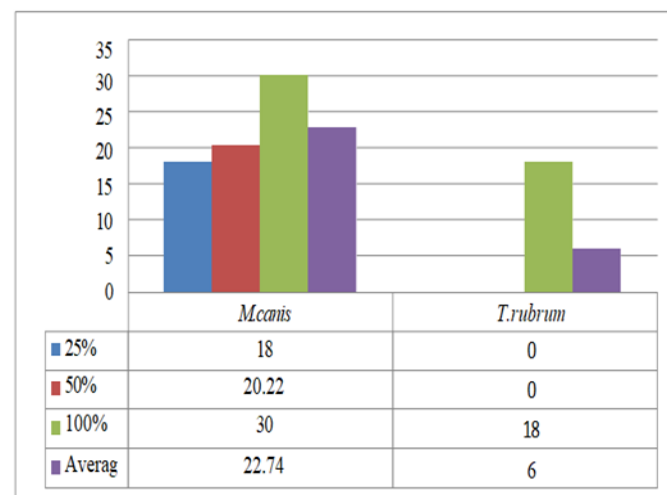


Figure 15: The antifungal effectiveness of SP15

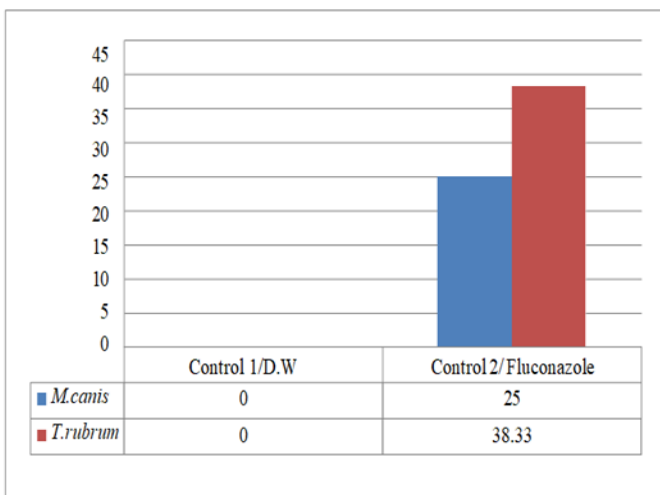


Figure 16: All studied controls

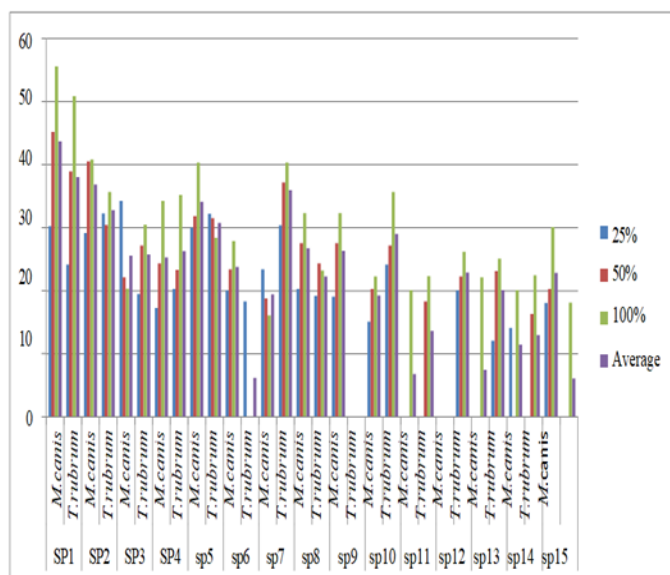


Figure 17: The antifungal effectiveness of all plant populations

5 Conclusions

This study demonstrates that *Salvia palaestina* leaves contain bioactive compounds with significant antifungal activity, particularly against *Microsporium canis*. However, the plant's efficacy strongly depends on its geographic and environmental context. Based on the results, populations SP1, SP2, SP3, and SP4 exhibited the highest diversity of phenolic acids and flavonoids. The flavonoid salvigenin was the most widely distributed compound, occurring in SP1, SP2, SP4, SP5, SP7, SP11, and SP14, highlighting its importance as a diverse phytochemical. Populations SP1 and SP4 are particularly promising for pharmaceutical or nutraceutical applications, as they were identified as rich sources of phenolics and flavonoids, respectively. These findings support the hypothesis that environmental stressors such as drought and specific climatic conditions promote the production of secondary metabolites as an adaptive defense strategy. Future studies should aim to map these biochemical processes more precisely, linking soil and climate data with the observed chemical profiles to better understand the mechanisms underlying metabolite variation.

Table of Symbols

Symbol	Definition
SPN1-15	<i>S. palaestina</i> plants in different populations
TFC	Total flavonoid content
TPC	Total phenolic content

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Conflicts of Interest: None.

Authors' Contribution Statement

The field trips that collected the populations of *Salvia palaestina* (SP1–SP15) were organized and carried out by Dina and Sirwan, who also recorded the study locations in their geographic and environmental context. The phytochemical extraction and antifungal tests were carried out by Dina. Dina wrote the manuscript and examined the data pertaining to phenolic and flavonoid diversity. The completed manuscript was reviewed by all authors and approved.

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