Chemical Composition, Total Phenolic Content, and Antioxidant Activity of *Salvia officinalis*, *Satureja hortensis*, and *Achillea millefolium* Essential Oils

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ABSTRACT

Objective: This study investigated the chemical composition, phenolic content, and antioxidant activity of essential oils extracted from three distinct medicinal plants: *Salvia* spp., *Satureja* spp. (Satureja hortensis), and *Achillea* spp. (Yarrow).

Methods: **:** Essential oils were isolated from the aerial parts of each plant using hydrodistillation via a Clevenger apparatus. Gas chromatography-mass spectrometry (GC-MS) was employed for the identification and quantification of volatile compounds. Phenolic content was determined using the Folin-Ciocalteu assay, and antioxidant activity was assessed by measuring the percentage of free radical scavenging capacity against 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Results: GC-MS analysis identified α-pinene as a common compound in all three plant species. Salvia essential oil exhibited the highest total phenolic content (8.1 mg gallic acid equivalents [GAE]/mL), while Satureja hortensis had the lowest (4.0 mg GAE/mL). In terms of antioxidant capacity, Salvia essential oil demonstrated the strongest DPPH radical scavenging activity, followed by Yarrow, with Satureja hortensis showing the least effect. Notably, Yarrow essential oil was rich in 1,8-cineole (24.37%) and carvacrol (15.57%), Salvia officinalis contained high levels of camphor (33.60%) and 1,8-cineole (13.83%), and Satureja hortensis was characterized by a predominant presence of carvacrol (54.16%) and γ-terpinene (28.87%).

Conclusions: The findings highlight that the essential oils of Salvia officinalis, Satureja hortensis, and Yarrow possess significant phenolic content and antioxidant activity, suggesting their potential application as natural antioxidants in the food and pharmaceutical industries.

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1. Introduction

The essence and extract can be considered as a rich source for producing medicinal plants and as a preservative and antioxidant in food materials, pharmaceuticals, and cosmetics items. In the future, due to higher safety, natural compounds such as these combinations may be substituted by other chemical factors. It should also be noted that even natural materials such as extract and essential oils may also be higher in certain allergic doses than prescribed, toxicity, and even Mutagenic to some cells. It can be concluded that critical oils presented toxic effects compared with extracts in lower concentrations. Therefore, it is suggested that in the utilization of essential oils in food products, pharmaceuticals, and cosmetics pay more attention to its safety. In order to eliminate or reduction of the chemical and synthetic compounds in foodstuffs, a lot of research has been done to replace the natural chemicals. Herbal essential oils are one of the essential natural preservatives. It comes from various organs of plants, such as seeds, roots, buds, bark, shoots, leaves, buds, and flowers. In general, the extraction of volatile oils from the aromatic and medicinal plants to reach their active ingredients in different ways such as distillation, extraction with solvent, extraction with ultra-critical fluid, mechanical methods, and…Each of these methods for a particular plant has its drawbacks and advantages, and it has a different efficiency for various plants. The classical and relative plan to achieve the volatile oils of aromatic and medicinal plants is the same distillation that has been carried out from the distant past to distilled by water. Research on healthy and natural antioxidants, and especially antioxidants from plant resources, has increased in recent years. Natural antioxidants are widespread in many foods, such as oilseeds, kernels, nuts, legumes, vegetables, fruits, fruit plants, spices, tea, and meat. The study was aimed at examining the chemical compounds and the essential antioxidant activity of tri plants (Salvia officinalis, Satureja hortensis and Achillea millefolium).

2.Materials and Methods

All chemical reagents utilized in this investigation, including methanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium sulfate, were acquired from Merck (Germany). Solvents were of the highest purity grade, ensuring minimal interference with experimental outcomes.

2.1. Plant Material Collection and Preparation:

The study focused on three distinct medicinal plant species: Satureja hortensis , Achillea millefolium , and Salvia officinalis. Plant samples were randomly harvested during their optimal growth phase, specifically in late spring and early summer, to ensure maximum accumulation of desired secondary metabolites. The aerial parts of the plants were meticulously separated and subjected to a controlled drying process, carried out in the shade at ambient temperature, to prevent degradation of thermolabile compounds and prepare them for subsequent essential oil extraction.

2.2. Essential Oil Extraction:

For each plant species, approximately 100 g of the dried plant material was subjected to hydrodistillation using a Clevenger-type apparatus. The distillation process was maintained for a duration of 4 hours, ensuring comprehensive extraction of volatile compounds. The essential oil yield, calculated as milliliters of essential oil per 100 g of dry plant material, varied among the species: Achillea millefolium exhibited a 0.4% yield, Satureja hortensis 2.1%, and Salvia officinalis 1.1%. Immediately following extraction, the obtained essential oils were carefully collected into airtight, dark-colored tubes, which were then wrapped in aluminum foil to protect them from light-induced degradation. These samples were subsequently stored at 4°C until further analysis.

2.3. Chemical Composition Analysis of Essential Oils (GC-MS):

The qualitative and quantitative characterization of the essential oil constituents was performed using a Shimadzu (Japan) Gas Chromatograph coupled with a Mass Spectrometer (GC/MS). A capillary column (30 m length, 250 µm internal diameter, 0.25 µm film thickness) was employed for chromatographic separation. The oven temperature program commenced at 60°C, ramped up to 275°C at a rate of 5°C per minute, and then held isothermally at 265°C

for 30 minutes. Helium served as the carrier gas, maintaining a constant flow rate. The injector temperature was set at 230°C. Following sample injection, the resulting chromatograms were analyzed. Individual compounds were identified by comparing their retention times (RT) and mass spectral data with those contained within the instrument's integrated mass spectral library (NIST database). This allowed for both the identification of key essential oil components and the determination of their relative percentage abundance.

2.4. Determination of Total Phenolic Content (TPC):

The total phenolic content of the essential oils was quantified colorimetrically using the Folin-Ciocalteu method. Briefly, 20 µL of each essential oil sample was thoroughly mixed with 1.6 mL of distilled water. Subsequently, 100 µL of Folin-Ciocalteu reagent was added to the mixture. After an incubation period of 8-10 minutes, 300 µL of a 20% (w/v) sodium carbonate solution was introduced. The samples were then vortexed and incubated in a water bath (Ben Marie) at 40°C for 30 minutes. Finally, the absorbance of the resulting solutions was measured at 760 nm using a spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per milliliter of essential oil, calculated against a standard curve prepared with gallic acid.

2.5. Measurement of Antioxidant Activity via DPPH Radical Scavenging Assay:

The antioxidant capacity of the essential oils, specifically their ability to scavenge DPPH free radicals, was assessed according to the methodology described by Ebrahimzadeh et al. Initially, serial dilutions of the essential oils were prepared in methanol to achieve final concentrations of 50, 100, 250, 500, and 1000 µg/mL. For each concentration, 3 mL of the essential oil solution (in methanol) was mixed with 1 mL of a 1 mM DPPH methanolic solution. The mixtures were then vigorously vortexed and incubated in the dark at room temperature for 35 minutes to allow the scavenging reaction to proceed. The absorbance of each sample was subsequently measured at 517 nm using a spectrophotometer. The DPPH radical scavenging activity was calculated as a percentage relative to a control (DPPH solution without essential oil) using the provided equation:

$$\text{Percentage} = \frac{\text{Percentage of sampling absorption-percentage of evidence absorption DPPH}}{\text{Percentage of evidence absorption}} \times 10$$

3.Results

3.1. Chemical Composition of Essential Oils:

The detailed chemical profiles of the volatile essential oils extracted from the three plant species (*Satureja hortensis, Achillea millefolium,* and *Salvia officinalis*) were meticulously determined through Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The identified compounds and their relative percentages are comprehensively presented in Tables 1, 2, and 3 for Achillea millefolium, Satureja hortensis, and Salvia officinalis essential oils, respectively.

Table 1: Chemical Composition of Achillea millefolium Essential (Yarrow)Oil as Determined by GC-MS.

Row	Detention time	Amount of composition	Chemical formula	Composition name
1	10.555	1.09	$C_{10}H_{16}$	α.-Pinene
2	120012	1.24	$C_{10}H_{16}$	Sabinene
3	12.168	0.47	$C_8H_{16}O$	1-Octen-3-ol
4	12.142	2.34	$C_{10}H_{16}$	β-Pinene
5	12.551	0.61	$C_{10}H_{16}$	β-Myrcene
6	13.662	1.29	$C_{10}H_{16}$	α.-Terpinene
7	13.959	5.75	$C_{10}H_{16}$	Cymene
8	14.127	0.54	$C_{10}H_{16}$	Limonene
9	14.305	24.37	$C_{10}H_{18}O$	1,8-Cineole
10	15.195	9.63	$C_{10}H_{16}$	γ -Terpinene
11	15.639	2.23	$C_{10}H_{18}O$	cis-Sabinenehydrate
12	16.603	0.89	$C_{10}H_{18}O$	Linalool

13	16.750	1.36	$C_{10}H_{18}O$	
14	18.215	0.63	$C_{10}H_{18}O$	trans-Pinocarveol
15	18.465	2.58	$C_{10}H_{16}O$	Camphor
16	18.69	0.7	$C_{10}H_{18}O$	Menthone
17	18.870	1.34	$C_{10}H_{16}O$	cis-Verbenol
18	19.168	0.56	$C_{10}H_{18}O$	α-Terpineol
19	19.289	0.93	$C_{10}H_{18}O$	Borneol
20	19.418	1.1	$C_{10}H_{16}O$	Methol
21	19.550	4.3	$C_{10}H_{18}O$	Terpineol-4
22	20.017	4.55	$C_{10}H_{18}O$	A-Terpineol
23	20.067	0.93	$C_{10}H_{16}O$	Myrtenol
24	20.940	1.27	$C_{10}H_{16}O$	Chrysanthyl acetate
25	23.198	15.57	$C_{10}H_{16}O$	Carvacrol
26	25.469	1.72	$C_{12}H_{20}O_2$	Nerol acetate
27	31.303	0.82	$C_{15}H_{24}O$	Spathulenol
28	31.505	0.93	$C_{15}H_{24}O$	Caryophylene Oxide
29	31.889	1.62	$C_{15}H_{26}O$	Delta- Cadinol
30	33.242	0.42	$C_{15}H_{26}O$	Muurolol
31	33.322	1.04	$C_{15}H_{26}O$	Eudesmol
32	33.405	3.21	$C_{15}H_{24}$	Armadendrene
33	33.998	0.96	-	
35	35.256	2.99	$C_{12}H_{22}$	2,5-Octadiene, 3,4,5,6-tetramethyl
		100		

The GC-MS analysis of Achillea millefolium essential oil revealed a complex mixture of compounds, with **1,8-Cineole (24.37%)** and **Carvacrol (15.57%)** being the most abundant constituents, followed by γ -Terpinene (9.63%).

Table 2: Chemical Composition of Satureja hortensis (Savory) Essential Oil as Determined by GC-MS.

Row	Detention time	Amount of composition	Chemical formula	Composition name
1	10.212	0.37	$C_{10}H_{16}$	1-phellandrene
2	10.526	1.44	$C_{10}H_{16}$	α-Pinene
3	12.222	0.56	$C_{10}H_{16}$	β-Pinene
4	12.528	1.63	$C_{10}H_{16}$	Myrcene
5	13.645	2.61	$C_{10}H_{16}$	α-Terpinene
6	13.942	8.74	$C_{10}H_{14}$	Cymene
7	14.103	0.3	$C_{10}H_{16}$	limonene
8	15.207	28.87	$C_{10}H_{16}$	γ-Terpinene
9	19.526	0.36	$C_{10}H_{18}O$	4-Terpineol
10	23.272	54.16	$C_{10}H_{14}O$	Carvacrol
11	25.167	0.48	$C_{10}H_{14}O$	Thymol
12	27.041	0.47	$C_{15}H_{24}$	Caryophyllene
		100		

As shown in Table 2, a total of 12 compounds were identified in the essential oil of Satureja hortensis,

accounting for 100% of the total essential oil composition. Among these, **Carvacrol (54.16%)** was the predominant constituent, followed by γ -Terpinene (28.87%).

Table 3: Chemical Composition of Salvia officinalis Essential Oil as Determined by GC-MS.

Row	Detention time	Amount of composition	Chemical formula	Composition name
1	10.528	7.01	$C_{10}H_{16}$	α-pinene
2	11.228	6.49	$C_{10}H_{16}$	Camphene
3	12.257	2.08	$C_{10}H_{16}$	β-pinene
4	12.563	0.91	$C_{10}H_{16}$	Myrcene
5	13.958	0.52	$C_{10}H_{14}$	-
6	14.139	2.23	$C_{10}H_{16}$	Limonene
7	14.303	13.83	$C_{10}H_{18}O$	1,8-Cineole
8	17.042	25.45	$C_{10}H_{16}O$	α-Ttujone
9	17.383	2.64	$C_{10}H_{16}O$	β-Thujone
10	18.549	33.60	$C_{10}H_{16}O$	Camphor
11	19.302	1.50	$C_{10}H_{18}O$	Borneol
12	22.872	1.29	$C_{12}H_{20}O_2$	bornyl acetate
13	27.056	0.46	$C_{15}H_{24}$	trans-Caryophyllene
14	28.067	1.32	$C_{15}H_{24}$	α-Caryophyllen
15	31.814	0.69	$C_{15}H_{24}$	Alloaromadendrene
		100		

he analysis of Salvia officinalis essential oil revealed **Camphor (33.60%)**, α-Thujone (25.45%), and 1,8-Cineole (13.83%) as the most abundant constituent compounds, indicating a distinct chemical profile for this species.

3.2. Total Phenolic Content (TPC):

The total phenolic compounds in the essential oils of the three plant species were quantified using the Folin-Ciocalteu method. Figure 1 illustrates the total phenolic content, expressed as milligrams of gallic acid equivalents (GAE) per milliliter of essential oil, for each of the studied plant extracts.

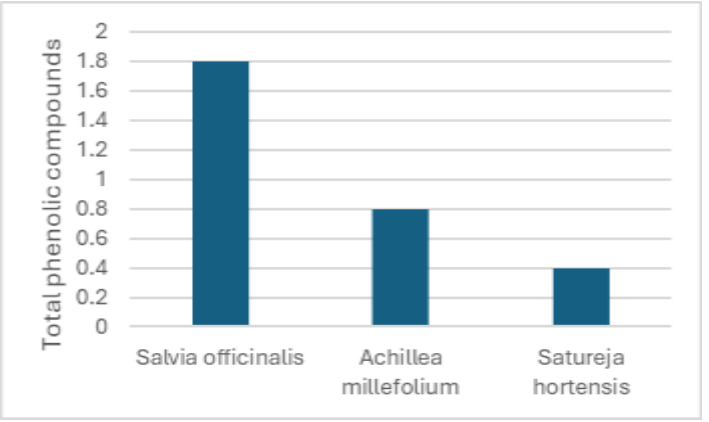

Figure 1: Total phenolic compounds (mg GAE/mL) in the essential oils of Achillea millefolium , Satureja hortensis , and Salvia officinalis.

Numbers have no significant differences with each other ($p>0.05$). As shown in Figure 1, Salvia officinalis essence has the highest amount of total phenolic compounds with 8.1 mg Gallic acid per ml essential oil. The lowest amount of the total phenolic compounds associated with the Satureja hortensis plant with 0.4 mg Gallic acid per ml. There is no significant difference in phenolic compounds between peppermint and Salvia officinalis and also Achillea millefolium ($p>0.05$), but there is a significant difference in the other essential oils ($p<0.05$). Table 4 shows the results for the percentage of DPPH free radical elimination of 3 plants studied at different concentrations.

3.3. DPPH Free Radical Scavenging Activity:

Table 4 presents the percentage of DPPH free radical scavenging activity for the essential oils of Achillea millefolium, Salvia officinalis, and Satureja hortensis at various concentrations (50, 100, 250, 500, and 1000 ppm).

Table 4 - Percentage of DPPH free radical elimination of 3 essential plants studied at different concentrations

Concentration of essential ppm (ppm)	Satureja hortensis	Salvia officinalis	Achillea millefolium
50	9.8d	67.36b	50.37c
100	18.43c	79.72a	62.71b
250	22.15b	85.81a	75.23ab
500	33.32b	90.5a	86.2a
1000	66.2a	98.39a	87.45a

As evident from Table 4, the DPPH free radical scavenging activity generally increased with increasing concentrations of all essential oils, reaching its maximum at 1000 ppm. Salvia officinalis essential oil demonstrated the highest scavenging activity across all concentrations, culminating in 98.39% at 1000 ppm. In contrast, Satureja hortensis essential oil exhibited the lowest antioxidant activity, even at the highest concentration tested. While there were significant differences in activity at lower concentrations, at 1000 ppm, Achillea millefolium , Salvia officinalis, and Satureja hortensis essential oils showed no significant differences from each other ($p > 0.05$).

4. Discussion

The current research meticulously investigated the chemical composition and antioxidant potential of essential oils derived from Satureja hortensis, Salvia officinalis, and Achillea millefolium. Our findings concerning the significant constituents of Satureja hortensis essential oil are largely in agreement with previous studies. For instance, Kamkar et al. identified 32 compounds in Satureja hortensis essential oil, with Thymol, Carvacrol, and γ -Terpinene being the primary components. Furthermore, Pfefferkorn et al. reported Carvacrol, γ -Terpinene, α-Terpinene, and p-Cymene as dominant compounds in Satureja hortensis essential oil across different regions and seasons in Germany, which aligns well with our present results (Table 2). Our study revealed that the essential oil compounds possess both phenolic components and demonstrable antioxidant activity, suggesting their potential utility as natural antioxidants in the food and pharmaceutical industries (Figure 1). A direct correlation has been consistently reported between free radical inhibition activities and the total phenolic content in plants, where increased extract concentration typically leads to enhanced antioxidant function (Table 4). The observed increase in antioxidant activity at higher concentrations is likely due to an elevated number of hydroxyl groups in the reaction environment, which enhances the capacity for hydrogen donation to free radicals, thereby strengthening their inhibitory effect. Regarding Salvia officinalis essential oil, our results identified α-Thujone, Camphor, and 1,8-Cineole as the major chemical compounds under natural irrigation conditions. This aligns partially with previous work; Hedayati et al. reported β\betaβ-Pinene, Mannool, Abietatriene, α\α-Pinene, trans-meta-mentha-8,2-diene, Linalool acetate, and Bornyl acetate as main components in the aerial parts of Sahandi Salvia officinalis essential oil. In our investigation, the volatile Achillea millefolium essential oil was predominantly characterized by 1,8-Cineole, Carvacrol, and γ-Terpinene (Table 1). This finding shows some variations compared to other studies. The observed discrepancies in chemical compounds reported across various studies can be attributed to a multitude of factors, including genetic variations within plant species (chemotypes), diverse climatic conditions, cultivation practices, harvesting periods, and preservation methods. Such variations in compositional profiles can significantly influence the antioxidant properties of the essential oils. Consistent with these observations, our study found Salvia officinalis essential oil to exhibit the highest percentage of DPPH free radical inhibition across all tested concentrations, aligning with its highest total phenolic content (Figure 1). This strong correlation underscores that the abundance of phenolic compounds directly contributes to a higher capacity for scavenging free radicals. Conversely, Satureja hortensis essential oil, with the lowest phenolic content, demonstrated the least DPPH radical scavenging activity. This strong inverse relationship between phenolic content and DPPH inhibition has been well-documented in previous research. The antioxidant activity of all three plant essential oils studied increased in a dose-dependent manner with increasing concentration. This is because higher concentrations lead to a greater availability of phenolic compounds, which are known to significantly contribute to free radical inhibition. The variations in antioxidant properties among the essential oils are thus closely linked to their distinct chemical compositions.

5. Conclusion:

The diverse chemical profiles and varying antioxidant activities of these essential oils underscore their potential as valuable natural antioxidants. Particularly, the high phenolic content and potent radical scavenging activity of *Salvia officinalis officinalis* highlights its promising application in the food and pharmaceutical industries

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