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# Seasonal variation in the chemical composition and antioxidant activity of essential oils from four Salvia species in Baja California, Mexico: *Salvia apiana, Salvia clevelandii, Salvia munzii,* and *Salvia pachyphylla.*

# Variación estacional en la composición química y la actividad antioxidante de los aceites esenciales de cuatro especies de *Salvia* en Baja California, México: *Salvia apiana*, *Salvia clevelandii*, *Salvia munzii* y *Salvia pachyphylla*.

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## ABSTRACT

The present work investigates the seasonal variation in the chemical composition and antioxidant activity of essential oils extracted from the leaves of four Salvia species native to Baja California, Mexico: *Salvia apiana, Salvia clevelandii, Salvia munzii,* and *Salvia pachyphylla*. Camphor and 1,8-cineole were the secondary metabolites present in the highest concentrations across all four species throughout the seasons. Generally, the percentage of monoterpenoids was greater than that of sesquiterpenoids in the oil composition. The antioxidant activity was assessed using various methods: the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•), 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•+), and a β-carotene/linoleic acid bleaching assay. This evaluation of antioxidant activity revealed that it depended on the type of metabolites present in the oil and their concentrations, which varied throughout the seasons. These results suggest that the essential oil derived from these Salvias could represent a source of new products with potential applications in food preservation, pharmaceuticals, and cosmetics industries.

**KEY WORDS:** Essential oil, Seasonal variation, Antioxidant activity, Lamiaceae, *Salvia*.

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## RESUMEN

El presente trabajo investiga la variación estacional en la composición química y la actividad antioxidante de los aceites esenciales extraídos de las hojas de cuatro especies de Salvia nativas de Baja California, México: *Salvia apiana*, *Salvia clevelandii*, *Salvia munzii* y *Salvia pachyphylla*. El alcanfor y el 1,8-cineol fueron los metabolitos secundarios presentes en las concentraciones más altas en las cuatro especies a lo largo de las estaciones. En términos generales, el porcentaje de monoterpenoides fue superior al de sesquiterpenoides en la composición del aceite. La actividad antioxidante se evaluó mediante diversos métodos: el radical 1,1-difenil-2-picrilhidrazilo (DPPH•), el catión radical 2,2-azinobis (3-etilbenzotiazolina-6-sulfónico) (ABTS•+) y el ensayo de decoloración β-caroteno/ácido linoleico. Esta evaluación reveló que la actividad antioxidante dependía del tipo de metabolitos presentes en el aceite y de sus concentraciones, las cuales variaban a lo largo de las estaciones. Estos resultados sugieren que el aceite esencial derivado de estas especies de Salvia podría representar una fuente de nuevos productos con posibles aplicaciones en las industrias alimentaria, farmacéutica y cosmética.


**PALABRAS CLAVE:** Aceite esencial, Variación estacional, Actividad antioxidante, Lamiaceae, *Salvia*.

## Introduction

*Salvia* is the most diverse genus in the Lamiaceae family, with nearly 1,000 species distributed worldwide, exhibiting a remarkable diversity in growth forms, secondary compounds, floral morphology, and pollination biology (Walker *et al*., 2004; Wester & Claßen-Bockhoff, 2007). In Mexico, the genus is represented by 292 species, while 19 species are found in California and the California peninsula regions (Mediterranean ecosystem) (Villaseñor, 2004). The local ecosystem, particularly the California region where we collected the plant materials, is threatened by the growing human population and climate change, which could drastically reduce or eliminate several native species (Riordan & Rundel, 2014).

This genus has been commonly utilized in traditional medicine and has been the subject of extensive phytochemical research aimed at identifying the bioactive compounds present in both extracts and essential oils (Wu *et al*., 2012; Fu *et al*., 2013). Reports have demonstrated that this genus serves as a valuable source of antioxidants for the food and cosmetic industries (Tepe *et al*., 2007).

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In the genus *Salvia*, terpenes play a significant role in defense mechanisms against harmful chemical species, such as reactive oxygen species (ROS), and act as light-stress phytoalexins. This conclusion is further validated by the observation that the concentration of these metabolites is notably high in sagebrush species found in desert areas, which experience high levels of solar radiation. In contrast, other Salvia species from wetlands and fog forests exhibit lower concentrations of terpenes (Luis, 1991). Additionally, seasonal variations may influence the composition of the plant, as some compounds may accumulate at specific times in response to environmental changes. Consequently, material collected at different times of the year may contain new compounds with varying bioactivities, highlighting the importance of seasonal information for harvesting (Kamatou *et al*., 2008[a]).

This study describes the yield, chemical profile, and seasonal variation of the essential oil of four Baja California native Salvia species used by Indigenous communities for their medicinal properties, specifically *S. apiana* (white sage), *S. clevelandii* (blue sage), *S. pachyphylla* (Mojave sage), and *S. munzii* (Munz's sage), along with their antioxidant activity. This work aimed to evaluate the optimal time of year to obtain a high content of essential oils associated with high antioxidant activity, contributing to their potential applications in food, cosmetics, pharmaceuticals, and perfumery.

## Material and Methods

### Plant material

The leaves of the *Salvia clevelandii*, *Salvia munzii*, *Salvia apiana,* and *Salvia pachyphylla* were harvested at the beginning of each season in three sites from the northern region of Baja California, Mexico, in the municipality of Ensenada. Table 1 shows the GPS coordinates and altitude at which the different species were collected. The identification of the four plant species was confirmed by the Baja California University Herbarium of the Science School at the University of Baja California (Ensenada), and voucher specimens were housed at the same place.

**Table 1. Geographical locations and voucher specimens of *Salvia* species**

| Species | Voucher | Site collection | | |
|---|---|---|---|---|
| | | GPS coordinates | | Altitude (m) |
| ***S. apiana*** | BCMEX8716 | N 31° 59' 31'' | W 116° 38' 12'' | 320 |
| ***S. clevelandii*** | BCMEX8715 | N 31°58' 05'' | W 116° 37' 31'' | 345 |
| ***S. munzii*** | BCMEX8717 | N 31° 51' 55'' | W 116° 38' 34'' | 100 |
| ***S. pachyphylla*** | BCMEX8718 | N 32° 01' 41'' | W 115° 56' 11'' | 1630 |

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### Isolation of the volatile components

The air-dried leaf samples (500 g) from the four species were crushed and then extracted via hydrodistillation for 3 hours using a modified Clevenger-type system, following the recommendations in the European Pharmacopoeia (2014). This process yielded essential oils that were yellowish in color and had a pleasant odor. The oils obtained were dried over anhydrous sodium sulfate and stored under an $N_2$ atmosphere at 4 °C in the dark until tested and analyzed. The procedure was repeated for each season sample.

### Gas chromatography–Mass spectrometry

The analytical GC/MS system used was an Agilent 7890A GC paired with a 5975C Mass Detector from Agilent Technologies (Little Falls, CA, USA), featuring an HP-5MS capillary column (30 m × 0.25 mm × 0.25 micron) from Agilent Technologies, Inc. An Agilent Technologies 7693 autosampler was employed to inject a 0.5 µL sample solution. The ionization energy was set at 70 eV, with a mass range of 40 to 400 m/z. The initial temperature of the column was 70 ºC, gradually increasing to 100 ºC at a rate of 2 ºC/min, and subsequently up to 250 ºC at a rate of 10 ºC/min. The injector port temperature was maintained at 250 ºC. The carrier gas (helium) flow rate was 1.0 mL/min, with a gas dilution ratio of 1:2000. Gas chromatography identified most constituents by comparing their Kovats retention indices (RI). The Kovats retention indices were determined using a homologous series of n-alkanes (C8–C20) under the same operating conditions. Compound identification was supported by referencing the mass spectra library (NIST98). Volatile compounds were categorized into hydrocarbons and oxygenated monoterpenes, oxygenated sesquiterpenes, and hydrocarbon sesquiterpenes.

### Preparation of samples for biological assay

The essential oils were weighed and dissolved in methanol for testing. For the β-carotene bleaching assay, the essential oil was dissolved in ethanol. These standard solutions were prepared at room temperature (25 °C) under low light and airflow to prevent degradation of the oil oxidation.

### Free radical scavenging activity on DPPH

The radical-scavenging activity was conducted as described by Burda & Oleszek (2001) with minor modifications. For each sample oil, we prepared a stock solution (4 mg/mL) that was serially diluted two-fold (down to 0.003 mg/mL) with methanol. An aliquot of each dilution (1 mL) was combined with 1 mL of a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 0.03 mg/mL. At the same time, a control containing 1 mL of methanol and 1 mL of the DPPH solution was prepared. The mixtures were incubated at room temperature in the dark for 30 minutes. The absorbance was measured using methanol as a blank at 517 nm (A517). The radical-scavenging activity was calculated as the percentage of DPPH bleaching using the following formula:

$$DPPH\ (\%) = [1 - (B / A)] * 100$$

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Where *A* and *B* represent the absorbance values (517 nm) of the control and sample, respectively. All determinations were conducted in triplicate. For each oil, the percentage of DPPH decolorization was plotted against the concentration of each dilution. The concentration needed to reduce the absorbance of DPPH by 50 % was determined by interpolation from a linear regression analysis, resulting in the $EC_{50}$ value. Quercetin served as a reference compound.

### β-Carotene/linoleic acid antioxidant assay

The anti-lipid peroxidation properties of essential oils were evaluated using a β-carotene and linoleic acid bleaching model system, following the procedure outlined by Burda & Oleszek (2001). To achieve this, 1 mL of β-carotene solution (0.5 mg/mL in chloroform) was added to an Erlenmeyer flask containing 0.025 mL of linoleic acid and 0.2 mL of Tween 20. After evaporating the chloroform, 100 mL of distilled water, saturated with oxygen for 1 hour, was added to the flask.

From the resulting emulsion, 2.5 mL was taken and mixed with 0.35 mL of the corresponding oil solution in methanol (2 mg/mL). This mixture was shaken and held at 50 °C for 2 hours. The absorbance of the samples was measured using a spectrometer at 470 nm, immediately after preparation ($t_0$ min) and at the end of the experiment ($t_{120}$ min). The antioxidant activity (AA) was expressed as a percentage of inhibition of β-carotene bleaching compared to the control, calculated using the following formula:

$$AA\ (\%) = \{1 - [\ (AS_0 - AS_{120}) / (AC_0 - AC_{120})\ ]\ \} * 100$$

Where $AS_0$ is the absorbance of the sample at 0 min, $AS_{120}$ is the absorbance of the sample at 120 min, $AC_0$ is the absorbance of the control sample at 0 min, and $AC_{120}$ is the absorbance of the control sample at 120 min. α-tocopherol served as the reference compound.

### ABTS radical cation bleaching assay

The ABTS radical cation (ABTS•+) scavenging assay was conducted using the methodology developed by Re *et al*. (1999) and Kuskoski *et al*. (2004), with minor modifications. Eighteen hours before the experiments, the ABTS radical cation (ABTS•+) was generated by reacting an ABTS stock solution with 2.45 mM potassium persulfate, and the resulting mixture was stored in the dark at room temperature. The ABTS•+ solution (150 µL) was diluted with ethanol to achieve an absorbance of 0.7 ± 0.02, which was recorded as the initial absorbance.

From this solution, 980 µL was combined with 20 µL of the diluted oil for evaluation. The mixture was thoroughly shaken and measured at a wavelength of 754 nm, which was noted as the final absorbance. The results are reported as a percentage of inhibition using the following formula:

$$\%\ \text{of inhibition} = [\ (A_1 - A_2) / A1\ ] * 100$$

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Where $A_1$ is the initial absorbance of the ABTS solution, and $A_2$ is the final absorbance of the ABTS solution in the presence of the sample. All determinations were performed in triplicate, with quercetin serving as the reference compound.

### Statistical analysis

Data for yield and antioxidant activity tests represent averages of three analyses. The results were recorded as means ± standard deviation. An analysis of variance was performed using ANOVA procedures with Minitab v16.2.4.4 statistical software. Significant differences in means were evaluated using the Student's t-test, with *p*-values < 0.05 regarded as significant.

## Results and Discussion

### Seasonal variations in *Salvia* essential oil yields

The results of essential oil yield (w/w, dry weight) indicate that these varied by *Salvia* species and season, as shown in Figure 1. All species exhibited higher yields during the autumn season; however, the essential oil production did not differ significantly between the harvesting dates of summer and autumn for *S. apiana* and *S. clevelandii*. Compared to other species, S. apiana produced the highest yields across the four seasons, ranging from 4 % to 6 %. This Salvia species has the largest leaves, whereas *S. pachyphylla* and *S. munzii* are characterized by the lowest yields (0.3-3 %), and these two plants, unlike the others, have the smallest leaves, which likely affects the yields.

For all species, the dry season (from May to October) represents the optimal period for essential oil production. The harsher weather conditions (hot and dry summers) generally affect plant physiology, which utilizes essential oils for non-defensive purposes, such as temperature regulation and reduced water loss, among others (Davis *et al*., 1996; Sharifi-Rad *et al*., 2017).

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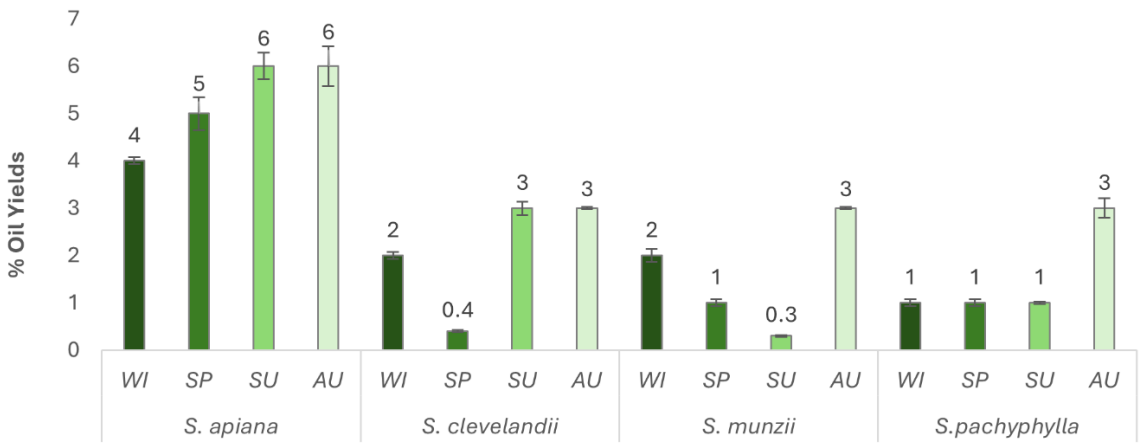


**Figure 1. Essential oil yields (w/w %) of *Salvia* species, collected during the four seasons. WI, SP, SU, and AU correspond respectively to winter, spring, summer, and autumn. Values are means of three replicates, and bars are mean ± SD (n=3)**

## Seasonal variations in *Salvia* essential oil composition

A total of 29 compounds were identified, representing 89-99 % of the essential oil's total composition. The results in Table 2 and the percentages of the main chemical classes of the compounds in the essential oils collected across the four seasons are shown in Table 3. In general, high percentages of hydrocarbons and oxygenated monoterpenes were observed in all four *Salvia* species.

The major compounds identified in these species during the different seasons included camphor (5-43 %), 1,8-cineole (3-38 %), caryophyllene (1-19 %), and limonene (4-20 %). However, camphor and 1,8-cineole are particularly notable as they are the only compounds consistently detected across all species throughout the four seasons, as displayed in Table 2.

*S. apiana* exhibited minor fluctuations in the oil components across the seasons, with two major components—camphor and 1,8-cineole—accounting for about 70 % of the total oil during these periods. The constituents observed in *S. apiana* align with previous reports indicating high levels of 1,8-cineole (Borek *et al.*, 2006), although camphor was more concentrated in these populations, likely due to the fact that the plants were collected from different environments influenced by a continental climate.

The other three species showed a wide variety of major compounds throughout the four seasons, with *S. clevelandii* demonstrating the most variation. Essential oil from *S. clevelandii* included six major compounds that varied significantly by season: phenylethyl phenylacetate (23.9 %), camphor (19.2 %), linalool (13.2 %), and δ-cadinane (10.9 %). These components made up more than 70 % of the total oil in winter and about 60 % and 35 % of the total oil in summer

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and autumn, respectively. This species exhibited drastic changes in autumn when phenylethyl alcohol appeared (43.1 %). These results differ from the data reported by Tucker *et al*. (1996), which identified camphor and 1,8-cineole as dominant components of *S. clevelandii* cultivated in Delaware. In our findings, although these compounds were consistently present throughout the seasons, others were identified in greater abundance, such as phenylethyl phenylacetate, phenylethyl alcohol, and linalool, aligning with the observations reported by Srinivas (1986).

The major components in *S. munzii* exhibited changes in the composition of α-pinene (8-13 %), limonene (12-19 %), 1,8-cineole (10-23 %), camphor (19-35 %), and caryophyllene (4-17 %) (Table 2). No discernible pattern in the fluctuations of the components was observed; however, significant variations were noted for caryophyllene, which decreased from summer (17.3 %) to autumn (4.8 %). Similar major components have been reported in S. munzii from a population collected in San Diego, California (Neisess *et al*., 1987).

There is only one phytochemical study of *S. pachyphylla* extracts (Guerrero *et al*., 2006). Nevertheless, this is the first report on the essential oils of *S. pachyphylla*. It is noteworthy that in this plant, the most abundant compounds are again 1,8-cineole, but surprisingly, 3-carene surpasses it (22.5-25.8 %), in contrast to the other *Salvias*, which do not contain this metabolite, except for S. apiana, but only in low levels (1-1.7 %).

The qualitative composition of seasonal variation in essential oils for *S. apiana* and *S. munzii* is comparable to *S. officinalis*, as described by Abu-Darwish *et al*. (2013), where the major components of the oil included 1,8-cineole (39-50 %) and camphor (8-25 %), fluctuating from one season to another. This study demonstrated that the essential oil yield and composition vary with season, climate, age, geographical region, and the genetic makeup of the plant (Kamatou *et al*., 2008[b]).

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## Table 2. Major compounds percentages (≥ 0.7 %) of the essential oils from *Salvia* specie

| Compound | KIC | KIL | *S. apiana* | | | | *S. clevelandii* | | | | *S. munzii* | | | | *S. pachyphylla* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | WI | SP | SU | AU | WI | SP | SU | AU | WI | SP | SU | AU | WI | SP | SU | AU |
| α-Pinene | 944 | 932 | 5.4 | 5.5 | 5.6 | 6.1 | 3.1 | 2.8 | 2.3 | | 8.2 | 8.7 | 13.4 | 9.3 | 6.8 | 5.2 | 6.4 | 5.6 |
| Camphene | 959 | 946 | 6.2 | 6.2 | 5.3 | 6.1 | 2.8 | | | | 3.9 | 3.6 | 5.6 | 4.6 | | | | |
| β-Pinene | 990 | 974 | 3.6 | 2.8 | 3.6 | 3.3 | | | | | 5.2 | 4.7 | 3.8 | 4.4 | | | | |
| β-Myrcene | 1002 | 988 | 1.3 | | 1.6 | 1.5 | | | | | 0.9 | 0.9 | | 1 | | 4 | 3.3 | 3.4 |
| o-Cymene | 1033 | 1020 | 0.9 | | | | | | | | | | | | 4.1 | 3.8 | 3.6 | |
| Limonene | 1037 | 1024 | 5.9 | 5.2 | 4.6 | 8.3 | | | | | 16.7 | 16.1 | 19.8 | 12.9 | 8.7 | 7.4 | 8.1 | 8.6 |
| 1,8-cineole | 1040 | 1026 | 37.5 | 27.9 | 28.7 | 26.5 | 3 | 4.7 | 9.8 | 18.4 | 20.8 | 23.8 | 10.8 | 23.4 | 11.9 | 15.1 | 18.8 | 20.6 |
| Trans-β-ocimene | 1044 | 1044 | | | | | | | | | | 0.9 | | | | | | |
| Camphor | 1143 | 1141 | 37.3 | 43.4 | 41.7 | 42.1 | 26.9 | 19.2 | 17.5 | 21.7 | 29.6 | 27.2 | 19.9 | 35.6 | 6.5 | 8.2 | 5.6 | 9.4 |
| 3-Carene | 1020 | 1004 | 1 | 1.7 | 1.4 | 1.1 | | | | | | | | | 25.8 | 25.1 | 24.6 | 22.5 |
| Caryophyllene | 1437 | 1416 | | 1.6 | 1.1 | | | | | | 9.8 | 11.9 | 17.3 | 4.8 | 15.2 | 10.1 | 18.5 | 11.9 |
| γ-Cadinene | 1545 | 1513 | | 2.8 | | | 2.3 | 2.6 | | | | | 2.2 | | | | 2.8 | |
| Tau-cadinol | 1670 | 1652 | | 1.4 | | | 4.5 | 7.3 | 5.7 | | | | | | | | | |
| δ-Cadinane | 1545 | 1520 | | | 1.7 | 1.8 | 7.2 | 10.9 | 10.1 | | 1.2 | | | | | | | |
| α-Cadinol | 1683 | 1671 | | | | 1.3 | 4.8 | 8.8 | 7.6 | | | | | | | | | |
| α-Terpineol | 1200 | 1186 | | | | | | | | 1.3 | | | | | | | | |
| α-Bisabolol | 1710 | 1701 | | | | | | | | | | | 2.6 | | | 6.7 | 3.9 | 6.7 |
| 4-Terpineol | 1185 | 1179 | 0.8 | | | | | | | | | | | | | | | |
| Linalool | 1111 | 1098 | | | | | 27.4 | 13.2 | 18.9 | 12.2 | | | | | | | | |
| Carotol | 1723 | 1592 | | | | | | 2.7 | 2.9 | | | | | | | | | |
| Phenylethyl alcohol | 1122 | 1115 | | | | | | | | 43.1 | | | | | | | | |
| Geraniol acetate | 1397 | 1384 | | | | | | | 2.6 | | | | | | | | | |
| Phenylethyl phenylacetate | 1954 | 1916 | | | | | 14.9 | 23.9 | 19.2 | | | | | | | | | |
| Borneol | 1173 | 1165 | | | | | | | | | 1.9 | 0.9 | | 1.9 | | | | |
| Borneol acetate | 1296 | 1288 | | | | | | | | | | | 2.2 | 1.1 | 4.5 | | | |
| Caryophyllene epoxide | 1608 | 1582 | | | | | | | | 0.7 | | | | | 2.6 | | | |
| β-Bisabolene | 1528 | 1508 | | | | | | | | | | | | | | | | 4.3 |
| β-Eudesmol | 1680 | 1626 | | | | | | | | | | | | | 3.1 | 4.3 | | |
| 2-Methyl-butanoic acid hexyl ester | 1249 | 1236 | | | | | | | | | | | | | | | | 2.4 |

WI, SP, SU, and AU represent winter, spring, summer, and autumn, respectively. KIC refers to Kovat's Index, which is calculated using the HP-5 MS capillary column. KIL denotes Kovat's Index Literature (Santos-Gómez & Fernández-Ferreira, 2001; Cvetkovikj *et al*., 2015; Ghalandarnejad *et al*., 2014).

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**Table 3. Presents the chemical fractions and total identified compounds of the essential oils from the leaves of four *Salvia* species collected during the four seasonal periods (%).**

| Salvia species | Season | Chemical class | | | | Others | Total of identified compounds |
|---|---|---|---|---|---|---|---|
| | | MH | MO | SH | SO | | |
| *S. apiana* | WI | 69.93 | 29.97 | 0 | 0 | 0 | 99.9 |
| | SP | 49.25 | 19.7 | 19.7 | 9.85 | 0 | 98.5 |
| | SU | 57.18 | 19.06 | 19.06 | 0 | 0 | 95.3 |
| | AU | 58.86 | 19.62 | 9.81 | 9.81 | 0 | 98.1 |
| *S. clevelandii* | WI | 19.38 | 29.07 | 19.38 | 19.38 | 9.69 | 96.9 |
| | SP | 9.61 | 28.83 | 19.22 | 28.83 | 9.61 | 96.1 |
| | SU | 9.66 | 38.64 | 28.98 | 9.66 | 9.66 | 96.6 |
| | AU | 0 | 64.28 | 0 | 16.56 | 16.56 | 97.4 |
| *S. munzii* | WI | 49.1 | 29.46 | 19.64 | 0 | 0 | 98.2 |
| | SP | 59.22 | 29.61 | 9.87 | 0 | 0 | 98.7 |
| | SU | 39.04 | 29.28 | 19.52 | 9.76 | 0 | 97.6 |
| | AU | 49.5 | 39.6 | 9.9 | 0 | 0 | 99 |
| *S. pachypylla* | WI | 35.68 | 26.76 | 8.92 | 17.84 | 0 | 89.2 |
| | SP | 44.95 | 17.98 | 8.99 | 17.98 | 0 | 89.9 |
| | SU | 47.8 | 19.12 | 19.12 | 9.56 | 0 | 95.6 |
| | AU | 38.16 | 19.08 | 19.08 | 9.54 | 9.54 | 95.4 |

WI, SP, SU, and AU represent winter, spring, summer, and autumn, respectively. MH stands for monoterpene hydrocarbons, MO denotes monoterpene oxygenated compounds, SH refers to sesquiterpene hydrocarbons, and SO signifies sesquiterpene oxygenated compounds.

## Seasonal variations in antioxidant activity

It is recommended to use methods based on different mechanistic principles to evaluate the antioxidant activities of extracts and essential oils (Müller *et al*., 2011). In this study, we used the decolorization of β-carotene, reduction of the DPPH free radical, and ABTS radical cation scavenging assays (Table 4).

In the oxidative decolorization of β-carotene assay, *S. apiana* and *S. clevelandii* exhibited

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low activity across all seasons, with percentages ranging from 1 to 13 %. *S. clevelandii* showed 26 % activity in spring, the season during which phenylethyl phenylacetate was observed as the predominant metabolite (23.9 %), potentially contributing to the increase in bioactivity in this result. *S. munzii* exhibited 27 % activity in spring, but in summer, its activity peaked at 93 %, surpassing the positive control α-tocopherol (60 %). During this season, a minor presence of γ-Cadinene (2.2 %), α-Bisabolol (2.6 %), and borneol acetate (2.2 %) was detected. The highest activity was recorded for *S. pachyphylla* at 98 % in autumn, with the minor presence of 2-methyl-butanoic acid hexyl ester (2.4 %) and β-Bisabolene (4.3 %).

**Table 4. Antioxidant activities over the seasons of four *Salvia* species**

| | | Antioxidant activity | | |
|---|---|---|---|---|
| Salvia species | Season | DPPH assay[a]* | ABTS assay[a]** | β–carotene assay[a]** |
| *S. apiana* | WI | 14.7 ± 1.26 | 92 ± 0.98 | 1 ± 0.61 |
| | SP | 9 ± 0.78 | 17 ± 0.77 | 13 ± 0.88 |
| | SU | 66.3 ± 0.67 | 10 ± 0.33 | 1 ± 0.42 |
| | AU | 4.8 ± 0.74 | 28 ± 0.53 | 1 ± 0.58 |
| *S. clevelandii* | WI | 9.5 ± 0.98 | 66 ± 1.04 | 4 ± 0.43 |
| | SP | 9.5 ± 1.97 | 51 ± 0.76 | 26 ± 1.14 |
| | SU | 23.1 ± 2.03 | 82 ± 0.89 | 3 ± 0.89 |
| | AU | 6.8 ± 0.46 | 76 ± 0.27 | 1 ± 0.25 |
| *S. munzii* | WI | 1.2 ± 0.70 | 8 ± 1.01 | 1 ± 0.04 |
| | SP | 10.3 ± 0.34 | 36 ± 0.41 | 27 ± 0.98 |
| | SU | 11.8 ± 0.54 | 24 ± 0.67 | 93 ± 1.21 |
| | AU | 17.3 ± 0.92 | 15 ± 0.15 | 3 ± 0.12 |
| *S. pachypylla* | WI | 7.8 ± 0.45 | 28 ± 2.45 | 11 ± 0.13 |
| | SP | 3.8 ± 0.62 | 8 ± 0.65 | 61 ± 1.78 |
| | SU | 3.4 ± 0.91 | 53 ± 0.34 | 1 ± 0.22 |
| | AU | 9.28 ± 0.90 | 40 ± 0.76 | 98 ± 0.93 |
| Quercetin | | 0.003 ± 2x10$^{-4}$ | 99 ± 4.1x10$^{-4}$ | ND |
| α-tocopherol | | ND | ND | 60 ± 7.1 |

WI, SP, SU, and AU correspond respectively to winter, spring, summer, and autumn. *in $EC_{50}$ (mg/mL), ** in % antioxidant activity, [a] Mean ± SD (n=3), ND: no determined.

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The DPPH free radical scavenging assay did not yield satisfactory results; the best results were for *S. munzii* in winter with an $EC_{50}$ of 1.2 mg/mL, followed by *S. pachyphylla* with concentrations of 3.4 and 3.8 mg/mL in summer and spring, respectively. None of the oils surpassed quercetin as the positive control (0.003 mg/mL).

In the ABTS radical cation decolorization assay, *S. apiana* in winter was more active at 92 %, followed by *S. clevelandii* in summer and autumn with 82 % and 76 %, respectively. The absence of the phenolic constituents in the oils, which are normally essential for potent antioxidant activity in the DPPH and ABTS assays, can explain the generally low activity of the oils in these assays. Moreover, the results showed no correlation between a single or predominant compound and antioxidant activity, explaining the activity as a synergy of the mixture of compounds.

## Conclusion

This study demonstrated significant seasonal variations in both the yield and chemical composition of leaf essential oils from four native Salvia species in Baja California, an ecologically threatened region. The results emphasize that environmental factors and seasonal dynamics significantly influence the profile of secondary metabolites, particularly monoterpenoids such as camphor and 1,8-cineole. These compositional changes, in turn, affect the antioxidant capacity of the oils, which varied among species and seasons.

Notably, some essential oils showed promising antioxidant potential, indicating their suitability as natural ingredients for applications in the food, cosmetics, and pharmaceutical industries. Further investigations are needed to validate their efficacy and safety in applied formulations and to explore their broader bioactivity properties.

## Author contributions

Vega-Granados, K., Montaño-Soto,M. performed the essential oil extractions; Vega-Granados, K., Díaz-Rubio, L. performed the antioxidant activity assays; Chávez-Velasco, D., Garcia-Flores, E. analyzed the essential oils by GC-MS; Vega-Granados, K., Córdova-Guerrero, I., Haro-Vázquez, Maria del Pilar. made the formal analysis of the results generated; Díaz-Rubio, L., Montaño-Soto, T. wrote the paper; Córdova-Guerrero, I., Delgadillo Rodríguez, J. managed the funding acquisition; Díaz-Rubio, L., made the review and editing of the paper; Córdova-Guerrero, I. made the conceptualization of the study and conducted the supervision of the project.

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## Conflict of interest

The authors declare no conflict of interest.

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