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CHEMICAL COMPOSITION, ANTIOXIDANT AND ALLELOPATHIC ACTIVITIES OF ESSENTIAL OIL OF SALVIA *OFFICINALIS* L.

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ABSTRACT

This study assessed the chemical composition, antioxidant and allelopathic activities of essential oil of *Salvia officinalis* L. Twenty-eight compounds were found (corresponding to 98.82% of the essential oil). The majors compounds were: camphor (27.59%), camphene (23.70%), α-pinene (13.75%), β-pinene (6.28%), and limonene (5.38%). Monoterpenes (68%) predominated in the essential oil. The essential oil exhibited 85.3% of DPPH radical scavenging activity and the IC_{50} value was 3.67 µg/mL, which characterized this oil as a great antioxidant.The essential oil was tested at concentrations of 0, 100, 200, 400, 600, 800, 1000, and 2000 mg/L (w/v) to assess the allelopathic effect on germination of tomato (*Lycopersicum esculentum* Mill.), guinea grass (*Panicum maximum* Jacq.), and chia (*Salvia hispanica* L.). The final germination percentage of the three species was not inhibited by the essential oil at the concentrations tested. However, the germination speed index, time, and germination rate were adversely affected in most concentrations tested, with a significant difference in comparison with the control treatment for tomato and chia seeds. The same behavior was not observed for guinea grass seeds.

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INTRODUCTION

Essential oils are volatile substances characterized by a strong odour and formed by secondary metabolites produced in aromatic plants (Bakkali *et al*., 2008). Being mainly found in the plant families Asteraceae, Lamiaceae, Lauraceae, Myrtaceae, Rutaceae, and Apiaceae (Alonso, 1998). The multiple mechanism of action of essential oils combined with their broad spectrum of activity make these secondary metabolites (and originating aromatic plants) suitable material for soil improvement and pest management (weeds, insects, fungi, bacteria, nematodes). Their use in agriculture could offer many advantages since their natural origin makes them less harmful than synthetic chemicals. In addition, volatility implies less residues after application and their composite nature involves multiple mechanisms of action that prevent pathogens from developing resistance to all the participating compounds (Chalkos *et al*., 2010). They have generally displayed low toxicity, relatively low cost, and rapid degradation (Miranda *et al*., 2015). The family Lamiaceae contains about 300 genera and about 7,500 herbaceous or shrub species, some genera have exhibited some medicinal properties and many species are used as spices, ornamentals, and for oil extraction (Joly, 2002; Sousa and Lorenzi, 2005). The genus *Salvia* spp. is the largest member of the family Lamiaceae, with over 900 species around the world (Çadirci *et al*., 2012). In Brazil, this genus contains 68 species, distributed in the South, Southeast, Midwest, and Northeast regions, being *Salvia officinalis* L. (sage) is an important cultivated species (Santos, 2015). *S. officinalis* extracts are widely used in the drug, beverage, cosmetic, and fragrance industries (Darwish, 2014). The essential oil extracted from this species has exhibited allelopathic, antifungal, antimicrobial, anticancer,

and antioxidant properties (Hamidpour *et al*., 2013; Miranda *et al*., 2015). In addition, there are reports of its insecticidal activity on *Thrips tabaci* Lindeman (Koschier and Sedy, 2003), *Sitophilus oryzae* L. (Popovića *et al*., 2006), *Tetranychus urticae* Konch (Laborda *et al*., 2013), *Aedes aegypti* L., and *Anopheles quadrimaculatus* Say (Ali *et al*., 2015). Due to its medicinals properties, studies referring to the antioxidant potential of *S. officinalis* are essential to verify its possible free radical scavenging action on substrates. In addition, the allelochemical compounds produced and released into the environment by the plant directly influence the biological systems and may be responsible for the biological activity found in many species. In this aspect, the objective of this work was to determine the chemical constituents of the essential oil of leaves of *S. officinalis* and to evaluate its antioxidant and allelopathic potential.

MATERIALS AND METHODS

Plant material

We purchased dried leaves of *S. officinalis* from a commercial supplier. These leaves were crushed and converted into powder using a Wiley mill and, subsequently, stored protected from light until they were used to extract the essential oil.

Distillation of essential oil

The essential oil was obtained by standard water steam dragging methodology using a Clevenger-type apparatus. About 70 g of dried sage leaves were submitted to extraction by hydro-distillation in 750 mL distilled water for three to four hours. After extraction, the essential oil was stored in the dark at 4 °C.

Analysis of chemical composition by gas chromatography coupled with mass spectrometry (GC-MS)

The constituents of the essential oil were identified through gas chromatography coupled to mass spectrometry (GC-MS). The analysis of essential oil of sage was carried out using a Thermo-Finnigan GC-MS system, composed of a FOCUS GC (Thermo Electron) and coupled to a DSQ II (Thermo Electron) and a TriPlus AS automatic injector (Thermo Electron). The identification of the components was performed by comparing their retention times essential oil with those retrieved from the literature (Adams, 2007) for the same compounds analyzed using Kovats retention index.

Antioxidant activity

The measurement of free radicals scavenging activity (2.2-diphenyl-1-picrylhydrazyl, DPPH) was performed as suggested by Scherer and Godoy (2009) and Rufino *et al*. (2007) with modifications. For the analysis, 0.1 mL of pure essential oil was placed in test tubes containing 3.9 mL DPPH radical (0.2 mM), diluted in methanol and homogenized in a test-tube mixer. For control, we used 0.1 mL control solution (methyl alcohol, acetone, and water) with 3.9 mL DPPH radical, which were homogenized. Methyl alcohol was used as whitening agent in order to calibrate the spectrophotometer (UV mini-1240, Shimadzu Co., Japan). The mixtures were incubated protected from light at room temperature until measurement.

Subsequently, the absorbance at 515 nm was measured using a spectrophotometer and monitored every 30 minutes until stabilization. The DPPH index was calculated using the antioxidant activity equation (%) = [(Abs0 -Abs1) /Abs0] × 100, where Abs0 is the absorbance of the whitening agent and Abs1 the absorbance of the sample. The concentration of the essential oil responsible for 50% decrease in the initial activity of DPPH free radical (IC_{50}) was calculated through the linear regression of the antioxidant activity, using the equation $y = 0.0337x + 0.0943$, with a $R^2 = 0.9917$.

Germination bioassays

Germination bioassays for assessing the essential oil of *S. officinalis* were carried out using tomato (*Lycopersicum esculentum* L.), guinea grass (*Panicum maximum* Jacq. var. Mombasa), and chia (*Salvia hispanica* L.) as target species. The treatments included control (distilled water with 1% dimethyl sulfoxide [DMSO]) and concentrations of 100, 200, 400, 600, 800, 1000, and 2000 mg/L (w/v) of essential oil of *S. officinalis* (Souza-Filho, 2009) using 1% DMSO as eluent. Four replications of 25 seeds from each species were sown in Petri dishes (9 cm diameter) and two sheets of filter paper moistened with 3 mL of solutions containing different concentrations of the essential oil were used as substrate. Oil solutions were added only at the start of the bioassays, and, when necessary, only distilled water was added. Then, these plates were placed in a germination chamber at 25 °C with a 24-hour photoperiod. Germination speed index (GSI), germination time (GT), and germination rate (GR) were determined by daily counts of seed germination (Maguire, 1962). The seeds were considered germinated when the radical protrusions were 2 mm long (Borghetti and Ferreira, 2004). The total number of germinated seeds was calculated after seven days.

Experimental design and statistical analysis

A completely randomized design was the strategy used for the experiments involving eight concentrations of essential oil of *S. officinalis* tested for the three species. The data were submitted to normality (Shapiro-Wilk) and homoscedasticity (Bartlett) tests through analysis of variance (ANOVA). Means were compared by Tukey's test at 5% probability using the R software (R Development Core Team, 2014).

RESULTS AND DISCUSSION

Essential oil obtained from dried leaves of *S. officinalis* exhibited yellow color, with intense odor and twenty-eight compounds were identified, accounting for 98.82% of the total oil (Table 1). The major constituents were camphor (27.59%), camphene (23.70%), α-pinene (13.75%), and β-pinene (6.28%); followed by limonene (5.38%), borneol (3.58%), 1.8-cineole (3.54%), and caryophyllene oxide (2.24%). Other compounds were present in amounts less than 2%. On the whole, the classes of compounds identified belonged to terpenes. The most representative fraction of the essential oil was constituted by monoterpenes (68%), which were also quantitatively greater, corresponding to 93% of the essential oil. The other 32% was characterized as sesquiterpenes, making up 7% of the essential oil. Porte *et al*. (2013) identified 47 compounds in sage oil and the main one was α-tujone (40.90%), followed by camphor (26.12%). Awen *et al*. (2011) also indicated camphor as the main compound, which

Table 1. Chemical composition of essential oil of *Salvia officinalis* L.

RT[(a)]	Compound[(b)]	Area (%)	KI[(c)]
12.44	Tricyclene	0.92	918
13.11	α-Pineno	13.75	929
14.07	Camphene	23.70	944
15.57	β – Sabinene	0.39	968
15.80	β – Pineno	6.28	972
16.80	β -Myrcene	1.73	988
18.48	α -Terpinene	0.38	1013
19.00	*p*-Cymene	1.39	1021
19.31	Limonene	5.38	1025
19.48	1,8 cineol	3.54	1028
21.34	- Terpinene	0.95	1054
23.18	Terpinolene	0.25	1081
27.44	Camphor	27.59	1141
29.21	Borneol	3.58	1166
29.84	4-Terpineol	0.53	1175
30.91	α -Terpineol	0.15	1191
36.98	Bornyl acetate	1.29	1281
37.08	Anethole	0.29	1283
38.01	Carvacrol	0.16	1297
45.44	β -Caryophyllene	1.19	1410
47.65	α -Humulene	0.81	1446
50.36	α -Zingiberene	0.22	1490
51.59	γ-Cadinene	0.15	1510
54.86	Spatulenol	0.71	1566
55.10	Caryophylene oxide	2.24	1570
55.84	Globulol	0.45	1583
56.70	1,2-Epoxide-humulene	0.71	1597
59.35	α -Cadinol	0.24	1645

Note. [a] = Retention time in minutes; [b] = Compounds identified in the DB-5ms capillary column; [c] = Literature retention indices.

corresponded to 27.3% of essential oil of *S. officinalis* among 13 identified compounds, followed by (-)thujone (24.3%), which was not found in our study. Lakušić *et al*. (2013) found that sesquiterpenes were dominant (50.7 - 57.0%) in theessential oils extracted from young leaves of *S. officinalis*, whereas the development of leaves increased the amount of monoterpenes (55.4-88.4%), realizing that leaves of the same plant in different stages of development formed different types of essential oils. According to these reports, it is observed that there was a variation in the composition of the essential oil of *S. officinalis*. According to Lamien-Meda *et al*. (2010), this significant variation in the composition depends on plant genotypes, organ age and environmental influences (fertilization, light intensity, climatic conditions, season and growth site). The essential oil of *S. officinalis* assessed in the present study had 85.3% of DPPH radical scavenging activity and an IC_{50} value of 3.67 µg/mL. The result of IC_{50} corresponds to the quantity required to decrease the initial concentration of DPPH by 50%. The essential oil exhibited the same standard observed for butyl hydroxy toluene (BHT), which is a synthetic antioxidant. In similar tests, BHT had IC_{50} values of 5.37, 9.27 and 9.5 µg/mL, and DPPH radical scavenging activity was 83.16 and 95.85% (Cansian *et al*., 2012; Pandini *et al*., 2015; Sá *et al*., 2012). Bozin *et al*. (2007) found that the essential oil of *S. officinalis* was able to reduce the stable purple-colored DPPH radical into yellow-colored DPPH–H, reaching 50% of reduction with IC_{50} values of 1.78 µg/mL, thus obtaining better results than BHT (74% e 5.37 µg/mL, respectively). The final germination percentages of the species assessed were not influenced by the essential oil of sage in the concentrations tested in the present study (Table 2, 3 and 4). However, the other parameters of germination exhibited negative effect, with a significant difference for tomato and chia seeds in comparison to the control treatment.

Ferreira (2004) explains that many times there is no allelopathic effect on germinability, but it influences other parameters, such as GSI and/or plant development, as observed in our study. The GSI and germination rate of chia seeds were influenced by the essential oil of *S. officinalis* at the lowest concentration (100 mg/L) (Table 2). The GSI was reduced to 35% at 800 mg/L. There were 10-12 seedling germinated per day in comparison with 15-55 in the control treatment. Germination time was greater from concentration of 400 mg/L, with the greatest effect at the highest concentration (2000 mg/L) that increased 37% in comparison with seeds germinated in water. Although the GSI and germination time of chia seeds were not affected negatively by the concentrations of the oil, the germination rate was lower in all concentrations of the essential oil in comparison with the control treatment. GSI, time, and germination rate of tomato seeds were affected by the essential oil of *S. officinalis* at concentrations of 600, 400, and 100 mg/L, respectively.

Table 2. Parameters of germination of *Salvia hispanica* L. submmited to *Salvia officinalis* L. essential oil.

Treatment	*Salvia hispânica*			
mg/L	% G ns	GSI	GT	GR
0	83	15.55a	1.56b	0.67a
100	73	10.53b	1.90ab	0.53b
200	77	10.87b	1.93ab	0.52b
400	82	10.37b	2.10a	0.48b
600	81	11.97b	1.95ab	0.52b
800	80	10.12b	2.11a	0.48b
1000	78	11.15b	2.04a	0.50b
2000	83	10.25b	2.13a	0.47b
Average	80	11.35b	1.96	0.52
Minimum	56	8.53	1.30	0.40
Maximum	96	17.0	2.50	0.77
SD	9.22	2.15	0.25	0.08
CV%	11.58	18.98	12.79	15.11

Note. %G: germination percentage; GSI: germination speed index (seedlilng per day); GT: germination time (days) and GR:germination rate (days). SD: standard deviation; CV%: coeficiente de variação. Averages followed by the distinct letters in the column indicate significant differences according to Tukey test at 5% probability level. ns.: non significant.

Table 3. Parameters of germination of *Lycopersicum esculentum* L. submitted to essential oil of *Salvia officinalis* L.

Treatment	*Lycopersicum esculentum* var. Cerasiforme		
mg/L	% G ns	GSI	GT
0	81	5.91 a	3.65 b
100	85	5.39 ab	4.19 ab
200	89	5.65 ab	4.15 ab
400	77	4.67 abc	4.32 a
600	76	4.36 bc	4.52 a
800	63	3.44 c	4.69 a
1000	74	4.32 bc	4.45 a
2000	80	4.71 abc	4.44 a
Average	78	4.81	4.3
Minimum	48	2.68	3.2
Maximum	96	6.42	5.0
SD	11.85	0.94	0.36
CV%	15.16	19.59	8.48

Note. % G = germination percentage; GSI = germination speed index (seedling per day); GT = germination time (days); GR = germination rate (days); SD = standard deviation; CV% = coefficient of variation; ns = non-significant. Averages followed by different letters in the same column indicate significant differences according to Tukey's test at 5% probability level.

The GSI was reduced to 42% at 800 mg/L concentration, with 3.44 seedlings germinated by day, in comparison with the control test that had 5.91 seedlings germinated.

In addition, the germination of tomato seeds was slow at this concentration, with 28% delay in germination time. The germination rate was lower for every essential oil concentration in comparison with the control treatment (Table 3). Guinea grass seeds did not influence the parameters of germination when submitted to the essential oil of *S. officinalis* (Table 4). These seeds were not sensitive as tomato and chia seeds.

Table 4. Parameters of germination of *Panicum maximum* Jacq. var. Mombaça submmited to essential oil *Salvia officinalis* L.

Treatment		*Panicum maximum* var. mombaca		
mg/L	% G ns	GSI ns	GT ns	GR ns
0	82	8.29	2.58	0.39
100	87	8.73	2.61	0.39
200	88	8.89	2.59	0.39
400	87	8.68	2.62	0.39
600	81	7.66	2.78	0.39
800	89	8.24	2.82	0.37
1000	82	7.83	2.76	0.36
2000	89	8.31	2.83	0.36
Average	86	8.33	2.70	0.37
Minimum	68	5.75	2.32	0.31
Maximum	96	10.50	3.22	0.43
SD	6.95	1.05	0.25	0.03
CV%	8.12	12.67	9.13	8.98

Note. %G: germination percentage; GSI: germination speed index (seedlilng per day); GT: germination time (days) and GR:germination rate (days). SD: standard deviation; CV%: coeficiente de variação. Averages followed by the distinct letters in the column indicate significant differences according to Tukey test at 5% probability level. ns.: non significant.

Like our results, Almeida *et al*. (2010) tested essential oil of *S. officinalis* and the major compounds were thujone, camphor, and borneol. The authors observed inhibition in germination and initial growth roots of cress, radish, and lettuce. However, the oil exhibited different level of activity. It is worth noting that the response of the same compound, extract or essential oil can perform in different manners according to the target species. Viecelli and Cruz-Silva, (2009) tested aqueous extracts of *S. officinalis* and observed changes in the response pattern of germination percentage and GSI of lettuce seeds. The differences were related to concentration, method of obtaining the extract, and season of leaf collection. In this context, the tested species may respond differently to the chemical compounds, since they have different sensitivity (Bajalan *et al*., 2013). According to Ferreira and Áquila (2000), the resistance or tolerance to allelochemicals is more or less specific, and there are botanical species which are more sensitive than others.

The effect of essential oil of *S. officinalis* may be associated with camphor, which was the major component found in the present analysis. Chen *et al*. (2013) reported that camphor was the major essential oil component of many aromatic plant species. It exhibited a number of biological properties, such as insecticidal, antimicrobial, antiviral, anticoccidial, antinociceptive, anticancer, and antitussive activities, in addition to being used as a skin penetration enhancer. However, allelopathic activity is an uncommon result of a single constituent, generally associated with a group of compounds (Miranda *et al*., 2014). Loizzo *et al*. (2010) assessed the chemical composition and antiproliferative activity of two Salvia species and the results clearly showed that the activity could not be related to the major abundant compounds. Consequently, the minor components may be involved in some type of synergism with the other active compounds. Vokou *et al*. (2003) emphasized that essential oils are a mixture of many compounds in different proportions and it is often not known whether and how they might interact. The authors assessed the potential allelopathic activity of 47 monoterpenes — individually or in pairs — in lettuce germination and seedling growth and concluded that in some cases compounds act independently, whereas in other cases they act synergistically or antagonistically. Nevertheless, in general, the character of the interaction cannot be predicted on the basis of individual compounds acting individually. According to Bakkali *et al*. (2008), in most cases, only the main constituents of certain essential oils have been assessed. Generally, the major components reflect rather well the biophysical and biological features of the essential oils from which they were isolated. The amplitude of their effects depends on their concentration when they were tested individually or in essential oils. Thus, functions of the various molecules contained in an essential oil seem questionable in comparison with the action of one or two main components of the oil. However, it is possible to affirm that the activity of the main components is modulated by other minor molecules. Therefore, the authors suggested that, for biological purposes, studying an entire essential oil rather than some of its components is more informative, because the concept of synergism seems to be more meaningful. Finally, it is concluded that the chemical composition of the essential oil of *S. officinalis* is diversified. The major components identified were camphor, camphene, α-and β-pinene, and limonene, with 68% of monoterpenes comprising 93% of the total essential oil. The oil has 85.3% of DPPH radical scavenging activity and the IC_{50} was 3.67 µg/mL, characterizing it as a great antioxidant. The final germination of the three species tested was not influenced by the essential oil. However, the GSI, time, and germination rate of tomato and chia seeds were adversely affected in most concentrations tested. The same behavior was not observed for guinea grass seeds. It is worth noting that botanical species respond differently and many times there is no allelopathic effect on germinability.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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