

Chemical Analysis and Biological Activities of *Salvia lavandulifolia* Vahl. Essential Oil

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Abstract

Genus *Salvia* is one of important genera belonging to family lamiaceae. Most of reported biological activities of *Salvia* usually attributed to its volatile oil. The chemical composition of essential oil from *Salvia lavandulifolia* was analyzed by GC/MS. A total of sixty seven components were identified in the oil of *S. lavandulifolia* representing 95.78% of the total oil. β -caryophyllene (11.87%), spathulenol (8.13%), neomenthol (7.75%), pulegone (6.97%), hexadecanoic acid (6.85%), germacrene-D (5.70%), bicyclogermacrene (4.53%), caryophyllene oxide (3.97%) and humulene (3.29%) were found to be the major constituents. The oil showed no antimicrobial and antileishmanial activities in a concentration up to 200 and 20 $\mu\text{g/mL}$, respectively. It displayed a weak antimalarial activity (47 % inhibition) against *P. falciparum*. The oil exhibited anti-inflammatory activity adopting iNOS inhibition assay with IC_{50} of 30 $\mu\text{g/mL}$, but there is no cytotoxicity demonstrated by the oil at tested concentration of 100 $\mu\text{g/mL}$.

Keywords: *S. lavandulifolia*, essential oil, antimalaria, antimicrobial, antiinflammmtory, anticancer.

1- Introduction

Family Lamiaceae is famous for volatile oil bearing plants, encompasses more than 200 genera (Topçu, 2006). The genus *Salvia* is the largest and most common genus of this family and widely distributed. It contains more than 700 species, world wide spread particularly through Mediterranean region, Asia, Central and South America.

Salvia as a word means "safe" derived from a Latin word "salvare" referring to the healing prosperities of main specie *Salvia officinalis*. The historical importance of *Salvia* is also shown in an old proverb "How can man die that has sage in the garden?" (Quer, 1999). In addition, Dioscorides; the Greek physician; reported the aqueous decoction of sage to stop wounds bleeding, sores and to clean ulcers. In folkloric medicine *Salvia* species are used extensively for treatment of epilepsy, colds, bronchitis, tuberculosis, hemorrhage, and menstrual disorders (Dweck et al., 2000; Tildesley et al., 2005; Topçu, 2006) and as antiseptic, astringent, depurative, digestive, febrifuge and tonic, and also externally as insecticide, throat infections and useful for hoarseness and cough (González et al., 2012; Jirovetz et al., 2007). Moreover, studied species of this genus showed other biological activities, such as antispasmodic, anti-inflammatory, antidiabetic, anti-bacterial, anti-fungal, antiparasitic, antitumor (Kelen and Tepe, 2008; Loizzo et al., 2009). Plants of genus *Salvia* are aromatic plants characterized by their essential oil production, the therapeutic activity in aromatherapy and phytotherapy of several *Salvia* species, in addition to manufacturing of perfumery and cosmetics is due to their essential oils. The use of *salvia* essential oils in food is also reported by many researchers as flavoring agent for alcoholic and non-alcoholic beverages, ice creams and chewing gums, besides their use as spices for meat and pickles.

Salvia lavandulifolia Vahl. (*S. rosmarinifolia*, spanish sage), native to the Iberian Peninsula and distributed through North and South America, Europe and Asia (Amalia and Giannouli, 2005; Kintzios, 2003). It is a small herb and grows up to 100 cm height with opposite green or gray-white leaves. Many secondary metabolites have been isolated from *S. lavandulifolia* including flavonoids, triterpenes and monoterpenes were reported in aerial parts, while diterpenoids are the main components for the root phytochemistry. Furthermore, it is a rich source of polyphenolics such as rosmarinic acid.

S. lavandulifolia has many biological activities were revealed and discussed before by researchers such as; antioxidant, anti-inflammatory, antimicrobial, spasmolytic activity which is mainly attributed to its content of camphor and bornyl acetate, hypoglycemic, memory-enhancing and treatment of different age related cognitive disorders related to ageing and to improve the cognitive function, which is due to their anticholinesterase activity, antiseptic, analgesic and sedative (Perry et al., 1996; Perry et al., 1997; Tildesley et al., 2003). It is believed traditionally that *S. lavandulifolia* is effective as gargles for mouth ulcers, common cold symptoms, menstrual symptoms, and menopause symptoms such as flushing and sweating (Quer, 1999). Furthermore, *S. lavandulifolia* is traditionally used as food preserving condiment due to its characteristic flavor and antioxidant properties (Guillén and Ibargoitia, 1995; Topçu, 2006) and to give a spicy flavor for meat, soups and cheese.

Regarding the safety of *S. lavandulifolia* as food preservative and flavor, long term use of showed no side effects (Perry et al., 2001). Herraiz-Peñalver et al., studied different populations of wild *S. lavandulifolia*

and they found the variation in certain compounds such as camphor from high percentage in some populations and its absence in others, they concluded the presence of *S. lavandulifolia* essential oil chemotypes (Herraiz-Peñalver et al., 2010). In this study we aim to characterize the chemical components and evaluate the antimicrobial, antiprotozoal, anti-inflammatory and anticancer activities of essential oil extracted from aerial parts of *S. lavandulifolia* collected in April from Peru and obtained from Missouri Botanical Garden.

2-Material and methods

2.1-Plant materials

The aerial parts *Salvia lavandulifolia* Vahl. were collected from Peru in April, 2012 and obtained through Missouri Botanical Garden. Voucher sample was deposited in National Center for Natural Products Research, university of Mississippi, USA.

2.2-Isolation of the essential oil

The dried and powdered aerial parts of *S. lavandulifolia* (270 g) were mixed with 2 L of water distilled for 4 h using a Clevenger type apparatus to obtain essential oil. On moisture-free basis the essential oil percentage was 1.33%. The essential oil was analyzed by GC and GC-MS.

2.3-Chemical Composition

2.3.1- Chemicals

GC-grade *n*-hexane (>99%) and the reference standards β -pinene, β -myrcene, cymene, limonene, γ -terpinene, linalool oxide, linalool, isoamylisovalerate, α -terpineol, terpinen-4-ol, *n*-decanal, geraniol, 1-decanol, β -elemene, caryophyllene oxide, alloaromadendrene and sclareol were purchased from Sigma-Aldrich. A mixture of *n*-alkanes (C₈H₁₈ – C₂₃H₄₈) was used for the determination of the retention indices, and the alkane standards were purchased from PolyScience Corp. (Niles, IN, USA).

2.3.2- Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography analysis was performed using an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5975C quadrupole mass spectrometer and a 7693 auto sampler. Separation was achieved using an Agilent J&W DB-5MS (5% Phenyl 95% dimethyl arylene siloxane) fused silica capillary column (30 m x 0.25 mm I.D. x 0.25 μ m film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was: 70 °C hold for 2 min, then 70-150 °C at 1.5 °C/min, 150-250 °C at 5 °C/min (hold for 5 min). The injector temperature was 250 °C. The injection volume was 0.5 μ L with a split ratio of 50:1. The essential oil sample was diluted in *n*-hexane (0.5% v/v) prior to the GC/MS analysis.

Mass spectra were recorded at 70 eV at a scan mode from *m/z* 40 to 500. The interface and the ion source temperatures were 280 °C and 230 °C, respectively. The MS quadrupole temperature was 150 °C. Data acquisition was performed with Agilent Mass Hunter software (version B.07.00).

2.3.3- Identification of individual components

Compound identification involved comparison of the spectra with the database (Wiley and NIST) using a probability-based matching algorithm. Further identification was based on the relative retention indices compared with the literature (Davies, 1990) and the reference standards purchased from commercial sources and isolated in-house.

2.4-Biological activities:

2.4.1-Antimicrobial activity

The essential oil was tested against microorganisms obtained from American Type Culture Collection, fungi including; *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *C. neoformans* ATCC 90113, and *A. fumigatus* ATCC 204305 and the bacteria; *S. aureus* ATCC 29213, MRSA ATCC 33591, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, and *M. intracellulare* ATCC 23068. The activities were evaluated following a previously reported method (Búfalo et al., 2015).

2.4.2-Antiplasmodial activity

The antimalarial activity was tested against chloroquine sensitive (D6) strain of *P. falciparum* by measuring of plasmodial LDH activity following the procedure of (Makler and Hinrichs, 1993).

2.4.3-Antiprotozoal activity

The oil was also evaluated for its antiprotozoal activities against *L. donovani* promastigotes, axenic amastigotes, intracellular amastigotes in THP1 cells and *T. brucei* trypanomastigote forms. The procedure used to record the activity was previously reported (Mohamed et al., 2016)

2.4.4-Anti-inflammatory

Inhibition of NF- κ B and iNOS Activities :The assay was performed as described earlier using parthenolide (Sigma-Aldrich) as a positive control (Zhang et al., 2015).

2.4.5-Anti-cell proliferative

The cytotoxicity of the oil was evaluated against four human cancer cell lines (SK-MEL, KB, BT-549, SKOV-3) and noncancerous pig kidney epithelial (LLC-PK1) and monkey kidney fibroblast (VERO) cells in terms of their anti-cell proliferation activity during a 48 h incubation with the cell lines following previously reported procedure (Zhang et al., 2015).

3-Results and discussions:

The water-distilled essential oil of *S. lavandulifolia* air dried areal parts was analyzed by the mean of GC and GC-MS systems (**Figure 1**). Chemical composition of the oil and relative components percentage are cited in **Table 1**. Sixty seven compounds were identified making up to 95.784% of the oil. β -Caryophyllene was characterized as major component (11.876%), followed by spathulenol (8.13%), neomenthol (7.75%), pulegone (6.97%), hexadecanoic acid (6.85%), Germacrene-D (5.70%), bicyclogermacrene (4.53%), caryophyllene oxide (3.97%) and humulene (3.29%). Many studies have characterized the chemical composition of *S. lavandulifolia* essential oil, the available chemical composition data on the essential oil of *S. lavandulifolia* showed camphor (7-39%), 1,8-cineol (14.4-34.5%), borneol (up to 9%) and camphene (up to 12%) as major compounds (Foray et al., 1999; Herraiz-Peñalver et al., 2010; Porres-Martínez et al., 2013; Savelev et al., 2004; Zrira et al., 2004). Herraiz-penalver et al reported α -pinene (23.2%), β - pinene (19.2%), limonene (16.6%), β -Caryophyllene (8.1%), caryophyllene oxide (5.6%) and viridiflorol (9.7%) as the major components of *S. lavandulifolia* essential oil obtained from plant material collected from Castilla-La Mancha, Spain. On the other hand, essential oil of *S. lavandulifolia* extracted from plant material cultivated in Solsona, Lerida (Spain) contained β -phellandrene (9.3%), terpineol (12%) and ledol (11%) (Porres-Martínez et al., 2013). Foray et al reported the linalyl acetate (10.2%) as major component in plant material grown in south France. Pirozan et al., found that main oil components *S. lavandulifolia* obtained from Brazil were β -thujone (19.96%), camphor (18.97%), α -thujone (18.95%), 1,8-cineole (8.13%), and β -pinene (3.96%) (Pirozan et al., 2009). In the present work, almost all major components reported before were not detected such as; camphor, 1,8-cineol, borneol, camphene, α -pinene, β - pinene, limonene, β -phellandrene, terpineol, ledol, linalyl acetate, β -thujone, α -thujone, viridiflorol and terpineol were found only in small concentrations 0.886 and 0.326%, respectively. This variation in chemical composition *S. lavandulifolia* oil is mainly attributed to the climatic conditions and stage of development; as a result it will affect the biological activities of the essential oil.

Table 1: Chemical composition of *S. lavandulifolia* essential oil

No.	RI ^a	RI ^b	Compounds	% ^c	Identification
1	1088	1506	Linalool	0.980	t_R , MS
2	1122	--	<i>cis</i> -2- <i>p</i> -Menthen-1-ol	0.486	MS
3	1126	1478	Menthone	2.935	t_R , MS
4	1133	1468	Isomenthone	0.548	t_R , MS
5	1137	1559	Neomenthol	7.754	MS
6	1141	--	Isopulegon	0.146	MS
7	1145	1601	Terpinen-4-ol	0.180	t_R , MS
8	1157	1685	α -Terpineol	0.356	t_R , MS
9	1193	1230	Pulegone	6.975	MS
10	1198	1223	Carvone	0.096	t_R , MS
11	1207	1231	Piperitone	0.618	MS
12	1237	1278	Bornyl Acetate	0.254	t_R , MS
13	1246	1270	Thymol	1.304	t_R , MS
14	1253	1297	Carvacrol	0.124	MS
15	1284	1482	Bicycloelemene	0.094	MS
16	1287	1315	Piperitenone	0.171	MS
17	1304	--	Eugenol	1.141	t_R , MS
18	1312	1997	Piperitenone Oxide	0.122	MS
19	1329	1493	α -Copaene	0.284	t_R , MS
20	1336	1526	β -Bourbonene	0.493	t_R , MS
21	1345	1591	β -Elemen	0.283	MS
22	1349	--	Cinerolon	0.159	MS
23	1362	1667	β -Guaiene	0.138	MS
24	1375	1617	Caryophyllene	11.876	t_R , MS
25	1387	1560	β -Cubebene	0.242	MS
26	1395	1650	Aromadendrene	0.411	MS
27	1414	1672	Humulene	3.297	t_R , MS

28	1427	--	(Z)-4-chloro-2,3-dimethyl-1,3-hexadiene	0.443	MS
29	1441	1692	γ -Muurolene	0.898	MS
30	1445	1712	Germacrene-D	5.709	t_R , MS
31	1457	--	Ledene	0.574	t_R , MS
32	1462	1738	bicyclogermacrene	4.533	MS
33	1469	1727	α -Muurolene	0.460	MS
34	1474	--	Cadina-3,9-diene	0.205	MS
35	1478	--	methyl 4,4,7-trimethyl-4,7-dihydroindan-6-carboxylate	0.432	MS
36	1484	1766	γ -Cadinene	0.678	MS
37	1493	1784	δ -Cadinene	1.979	MS
38	1509	1368	α -ylangene	0.373	MS
39	1549	--	Juniper camphor	0.332	MS
40	1560	2153	Spathulenol	8.133	t_R , MS
41	1564	1966	Caryophyllene Oxide	3.974	t_R , MS
42	1570	2104	Globulol	1.573	MS
43	1580	2103	Viridiflorol	1.163	MS
44	1585	--	Guaiol	0.414	MS
45	1593	--	Aromadendrene oxide	1.392	MS
46	1599	1482	β -Guaiene	0.682	MS
47	1612	--	Dillapiole	0.727	MS
48	1619	2037	4-epi-cubenol	0.312	MS
49	1626	--	isospathulenol	1.353	MS
50	1633	--	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	0.796	MS
51	1636	--	α -epi-Cadinol	0.740	MS
52	1646	--	α -epi-Muurolol	0.769	MS
53	1649	1723	γ -Himachalene	0.669	MS
54	1653	2224	α -Cadinol	2.693	MS
55	1683	--	Isoaromadendrene epoxide	0.995	MS
56	1687	--	Ledene oxide	0.505	MS
57	1701	--	Murolan-3,9(11)-diene-10-peroxy	0.369	MS
58	1709	--	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	0.664	MS
59	1735	--	Lauryl acrylate	0.320	MS
60	1894	--	Hexahydrofarnesyl acetone	0.657	MS
61	1945	--	Farnesyl acetone	0.279	MS
62	1959	--	Palmitic acid methyl ester	0.213	MS
63	1988	--	Hexadecanoic acid	6.850	MS
64	2078	--	Docosane	0.148	t_R , MS
65	2081	--	Phytol	0.167	MS
66	2094	--	Linoleic acid	0.408	t_R , MS
67	2099	--	Oleic acid	0.736	t_R , MS
Total				95.784	

Compounds listed in order to their elution on the DB-5MS column.

RI^a Relative retention indices calculated against C₈ – C₂₃n-alkanes on the apolarDB-5MS column.

RI^b Relative retention indices calculated against n-alkanes on the polar CW 20M column(Davies, 1990)

% ^c calculated from GC/MS data.

Identification method: t_R , identification based on the retention times (t_R) of genuine compounds on the HP-1 column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and NIST libraries and comparison with literature data.

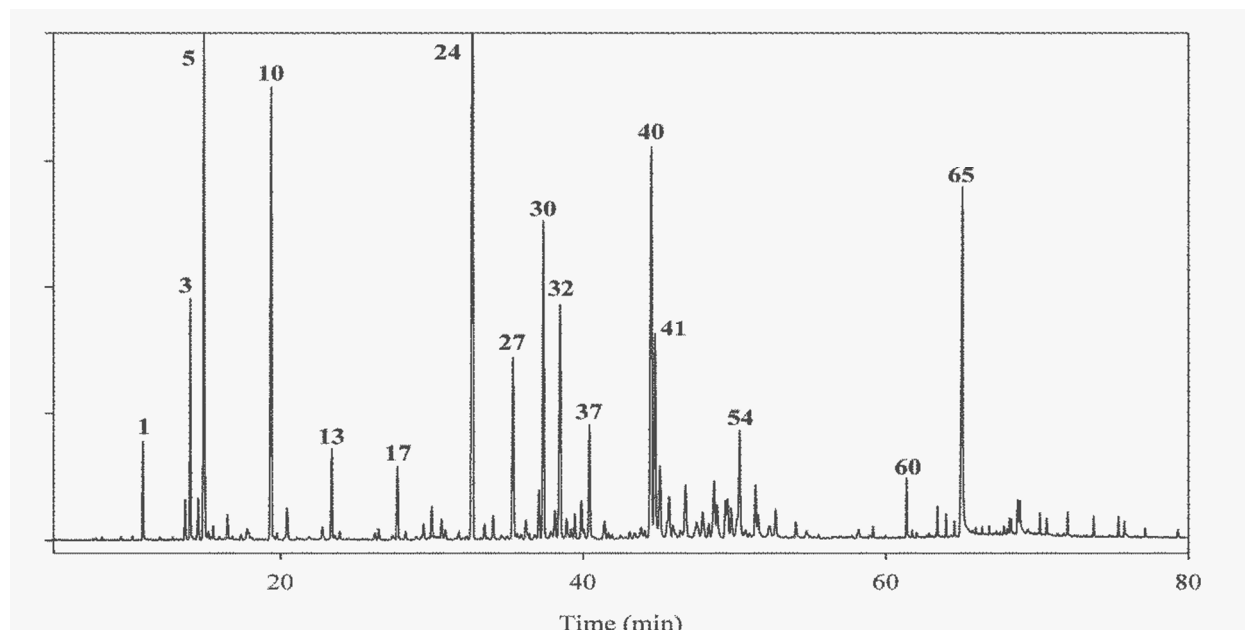


Figure 1. GC chromatogram of *S. lavandulifolia* essential oil. Peak identification was consistent with Table 1.

The essential oil was evaluated for antimicrobial, antimalarial, anti-inflammatory and anti-cancer activities (Tables 2 - 4). No antimicrobial activity was observed at the highest concentration of 200 $\mu\text{g/mL}$ against *Candida albicans*, *C. glabrata*, *C. krusei*, *Aspergillus fumigates*, *Cryptococcus neformans*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginos*, *Mycobacterium intracellulare* using previously reported method. In a published study for antimicrobial activity of *S. lavandulifolia* essential oil (plant material from Brazil) it was noted that the activity has been attributed to the presence of 1,8-cineol, β -thujone, camphor, borneol and cymene, but they are not detected in our essential oil (Pierozan et al., 2009). Jirovetz et al., studied the antifungal activity of essential oils from three *Salvia* species against *Candida* species, the study revealed that the most active oil is possessed by the *S. lavandulifolia* with MIC (5 $\mu\text{g/mL}$) and MFC (143 $\mu\text{g/mL}$) against *C. albicans*. Jirovetz et al., assumed the strong anticandidal activity of *S. lavandulifolia* essential oil was probably due to the presence of both components camphor and 1,8-cineol in the tested oil (Jirovetz et al., 2007). The chemical characterization of the oil under study showed the absence of camphor and 1,8-cineol, which suggested to be the main cause of weak antibacterial and antifungal activities. In the present study, *S. lavandulifolia* essential oil is also evaluated for the first time for antimalarial activity against D6 (chloroquine sensitive) strain of *Plasmodium falciparum*, it showed 47% inhibition at test concentration of 15.86 $\mu\text{g/mL}$. Milhau et al., investigated the antimalarial activity of eight essential oil and they observed the most efficient oil contained linalool and p-cymene as major components (Milhau et al., 1997). The antimalarial activity of our essential oil may be attributed to the presence of linalool traces, which was detected in concentration of 0.98%. The oil was tested for the activity against *L. donovani* and *Trypanosoma brucei*, it showed no activity in a concentration up to 20 $\mu\text{g/mL}$. In similar studies for evaluation of antiprotozoal activities, the diterpenes isolated from aerial parts of *S. gilliessi* and roots of *S. cilicica*, *Salvia deserta* (Búfalo et al., 2015; Sanchez et al., 2006; Tan et al., 2002) have been found to be active against *Leishmania* spp and *Trypanosoma cruzi* parasites. Another study revealed that the methanolic extract of *Salvia bucharica* leaves showed significant antileishmanial activity with IC_{50} value of 72.31 $\mu\text{g/mL}$. Many researchers documented the antiprotozoal activity for *Salvia* spp., is attributed to the diterpenoids isolated through chromatographic methods from the extracts and no activity recorded for the essential oil.

Table 2: Antimicrobial and antimalarial activities of *S. lavandulifolia* essential oil

Sample	Cancer cell lines, IC_{50}				Noncancer kidney cell lines, IC_{50}	
	SK-MEL	KB	BT-549	SK-OV-3	LLC-PK1	Vero
Essential oil	NA	NA	NA	NA	NA	NA
Doxorubicin	1	1.3	1.5	1.6	1.1	NA at 5 $\mu\text{g/mL}$

NA = no activity upto 100 $\mu\text{g/mL}$ of essential oil

The essential oil was tested for anti-inflammatory activity in terms of its interaction with cellular targets related to inflammation such as the nuclear transcription factor (NF- κ B) and inducible nitric oxide synthase (iNOS) to determine the possible mechanism in defeating the inflammation. The NF- κ B play important role in pro-inflammatory mediators expression including NO and PGE2. The NF- κ B is noticed highly activated at the sites of inflammation in many diseases. Therefore, the degree of suppression of NF- κ B activation will reveal the

potential anti-inflammatory. Nitric oxide (NO) is a significant mediator in various responses of inflammation. Inhibition of inducible nitric oxide synthase (iNOS) will reduce the NO production. The essential oil showed anti-inflammatory through iNOS inhibition with IC₅₀ of 30 µg/mL and showed no inhibition for NF-κB. Furthermore, the complexity of oil chemistry makes it difficult to evaluate the inhibitory effect of each single component. It is suggested that the activity is attributed to the presence of monoterpene; linalool, where similar studies reported that plants producing monoterpenes have potential anti-inflammatory (Durgha et al., 2015). Moreover, the presence of phenolic compound; eugenol plays a role in anti-inflammatory effect of the oil, it was reported that eugenol and isoeugenol inhibit the synthesis the protein of iNOS (LI et al., 2006).

Table 3: Anti-inflammatory activity of *S. Lavandulifolia* essential oil

	NF-κB IC ₅₀ µg/mL	iNOS IC ₅₀ µg/mL
Essential oil	NA	30
Parthenolide	0.33	0.3

NA = no activity up to 100 µg/mL of essential oil

The cytotoxicity of *S. lavandulifolia* oil documented in many studies; Foray et al., investigated the cytotoxicity of essential oils of three species of *Salvia*, they revealed that *S. lavandulifolia* less active than the control drug doxorubicin (about 100- 1000 times weak anticancer activity). Four human cancer cell lines (SK-MEL, KB, BT-549, SKOV-3) and noncancerous pig kidney epithelial (LLC-PK1) and monkey kidney fibroblast (VERO) cells were employed for testing the cytotoxicity in terms of anti-cell proliferation activity of the oil. The essential oil under investigation showed no anti-cancer activity when tested on cancer cell lines using doxorubicin as standard anticancer drug.

Porres-Martínez et al., also reported that two essential oil samples extracted from *S. lavandulifolia* showed no cytotoxicity for U373-MG cell line, in the same study they evaluated the protective effect of *S. lavandulifolia* oil on H₂O₂ treated cell lines. The protective effect of the oil was mainly due to the scavenging effect against produced reactive oxygen species (ROS). In addition, literature showed that the essential oil from plants of family Lamiaceae are effective in inhibition of lipid peroxidation (Bozin et al., 2006).

Table 4: Anticancer activity of *S. lavandulifolia* essential oil

M.O.	% inhibition	M.O.	% inhibition
<i>Candida albicans</i>	NA	<i>Staph. aureus</i>	NA
<i>C. glabrata</i>	NA	Methiciline-resistant <i>Staph.</i>	NA
<i>C. krusei</i>	NA	<i>E. coli</i>	5
<i>Aspergillus fumigatus</i>	10	<i>Pseudomonas aeruginosa</i>	18
<i>Cryptococcus neformans</i>	12	<i>Klebsiella pneumoniae</i>	NA
<i>M. intracellulare</i>	NA	Vancomycin resistant <i>Enterococcus faecium</i> ATCC 700221	6
<i>Plasmodium falciparum</i> D6	47		

4-Conclusion

This work included the isolation and analysis of essential oil components using GC-MS and evaluation of biological activities. This is first time to report essential oil from *Salvia* does not contain **cineol**, **borneol** and **camphor** compounds. These compounds play important role in reported activities of *Salvia* and their absence may justify the biological results. Therefore, they could be used as standards to evaluate the quality of essential oils isolated from *Salvia*. On the other hand literature documented that the total extracts of *Salvia* spp. showed more promising activities.

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