ISSN No:-2456-2165

Antimicrobial Activity of Essential Oil and Crude Organic Extracts of Salvia officinalis L. Leaves from Nepal

Susan Joshi*[1], Ram Darash Pandey[1], Rabin Bhattarai[1] and Bhim Bahadur Gharti[2]
[1]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2]Department of Chemistry, Tri-Chandra Multiple College, T U, Ghantaghar, Kathmandu, Nepal

Abstract:-The chemical constituents of essential oil from air-dried leaves of *Salvia officinalis* L. growing in Nepal was determined by gas chromatography couple with mass spectrometry (GC/MS) technique. A total number of twenty-one phytoconstituents were identified representing 99.95% of total oil composition. The major chemical components were camphor (65.18%), camphane (9.73%), eucalyptol or 1,8-cineole (4.72%), 2-nepthalenementhanol (3.85%) and α-pienene (2.33%) whereas the minor chemical components were boreneol (1.45%), eucarvone (1.44%), benezenemethanol (1.36%), viridiflorol (1.23%), o-cymene (1.22%), limonene (1.07%), trans-pinocarveol (0.90%), 2-(3-oxobutyl)-cyclohexonone(0.84%), β-caryophyllene oxide (0.78%), β-pienene (0.78%), m-cymene (0.72%), β-Ocimene (0.71%), 8-hydroxy-p-cymene (0.67%), bornylacetate (0.49%), valerenal (0.40%) and tricylene (0.11%). Since our *S. officinalis* lacks thujone content that poses higher risk of toxicity at high doses, it becomes more favorable to use for the treatment of several diseases in human. On the other hand, bacterial inhibitory activity of essential oil and the three different extracts was determined to evaluate its antibiotic potential using agar well disc diffusion method. The essential oil of *S. officinalis* L. leaves revealed strong antimicrobial potential against both Gram-positive *Staphylococcus aureus*, Gram-negative *Klebsiella pueunmoniae* and *Escherichia coli* bacteria. Among three extracts, the ether extract of *S. officinalis* L. leaves showed strong antimicrobial activity against *Klebsiella pueunmoniae* and *Escherichia coli* but weak against *Staphylococcus aureus*.

Keywords:- *Salvia Officinalis, Camphor, Camphene, Antimicrobial, Ether, Essential Oil.*

I. INTRODUCTION

The genus *Salvia*, commonly known as sage, has numerous common names including garden sage, common sage, kitchen sage, golden sage, culinary sage which comprises more than 1000 species all over the world [1]. *Salvia officinalis* L. belongs to Lamiaceae family and is native to Middle East and Mediterranean areas [1-3]. This medicinal plant is a woody evergreen shrub with strong aroma growing up to 30 cm to 1 m in height. The leaves are greenish grey color, rugose on the upper side, white underneath due to many short soft hairs (trichomes), oblong, ranging in size up to 6.4cm long and 2.5cm wide [4].

The botanical name of sage refers to its medicinal properties as *Salvia* is derived from Latin world *Salvare* which means to heal or care [5]. This plant has been used by tribal communities of Nepal, China, India and Europe as folk medicine for the treatment of bronchitis, cough, asthma, angina, mouth and throat inflammations, depression, excessive sweating, skin diseases, and many other diseases [6-8]. *Salvia* essential oils have been used in the treatment of a wide range of diseases like those of the nervous system, heart and blood circulation, respiratory system, digestive system, and metabolic and endocrine diseases. In addition, sage essential oil has been shown to have carminative, antispasmodic, antiseptic, and astringent properties [9,10]. The essential oil of *Salvia* species has various compositions depending on the genetic, climatic, seasonal, and environmental factors [11]. The essential oil of this species issued as an anticholinesterase [12], antidiabetic [13], anti-inflammatory [14], spasmolytic, antiseptic and astringent [15], antioxidant, antimicrobial, anticancer [16-19], antimutagenic [20], and antiviral [21]. It is also used traditionally in herbal tea food flavoring, flavoring agents in perfumery and cosmetics [22]. Literature evidence have shown that commercial sage oil is generally characterized by thujones, with α-thujone usually predominating (18–43%) over β-thujone (3–8.5%), camphor (4.5–24.5%), 1,8-cineole (5.5–13%), α-humulene (0–12%), α-pinene (1–6.5%), camphene (1.5–7%), and bornyl acetate (2.5% maximum) [23].

Infectious diseases are the major health problem nowadays which account for approximately one half of all loss of human life worldwide. The efficiency of many used antibiotics is being threatened by the emergence of multi drug resistant microorganisms and superbugs [24]. This resistance is largely due to indiscriminate use of antibiotic commonly applied for the treatment of infectious diseases [25]. Some antibiotics have much side effects which limit their uses. Thus, there is urgent need to develop new antimicrobial agents with no or less side effects. Although many synthetic antibiotics have been producing in labs, natural products still cover about 50% of modern antibiotics. Hence, natural products play avital role in new drug development in pharmaceutical areas [26]. Accordingly, *S. officinalis* is a good source of bioactive compounds such as camphor, 1,8-cineole, α-thujone, and β-thujone [27]. However, only very few research works had been carried on *S. officinalis* species grown in Nepal. So, there is no information about its secondary metabolites (essential

oil, organic extracts) and antimicrobial activity. Therefore, the present study aimed to investigate the chemical compositions of essential oil and organic extracts of *S. officinalis* L. leaves grown in Nepal and examine their antimicrobial activities.

Fig. 1: Leaves of Salvia officinalis L. plant

II. MATERIAL AND METHODS

2.1 Plant Material

The cultivated leaves of *S. officinalis* L. were collected from Sanobharyang, Kathmandu district of Nepal, which is located in 27^{O} 43'23" N and 85^{O} 17'22" E at about 1300 m above sea level. This medicinal plant was taxonomically identified, and a voucher specimen has been deposited in the Central Department of Botany, Tribhuvan University, Kirtipur, Nepal. The shade dried powdered leaves of *S. officinalis* L. were successively extracted with hexane, ether, ethylacetate and ethanol solvents by cold percolation method to prepare the corresponding organic extracts.

2.2 Phytochemical Profiling

The phytochemicals present in different extracts were subjected to chemical test using the standard protocol following Ciulei I. [28].

1.3 Isolation of Essential Oils

The essential oil was isolated by hydro-distillation method using Clevenger-type distillation apparatus. An amount of 50 gm in three portions of fresh leaves were kept into around bottom flask along with distilled water and round bottom flask was fitted with Clevenger-type distillation apparatus. The content of the flask was heated in heating mantle at boiling temperature and the process was allowed to continue for 4.5 hours and followed by standing for one hour at room temperature. The *S. officinal is* L. leaves essential oil obtained was kept in anhydrous sodium sulphate to remove water and filtered to store in airtight reagent bottle at 4ºC for further use.

2.4 Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The chemical composition of the leaves of *S. officinalis* L. essential oils were analyzed by a gas chromatography (Shimadzu GC 2010) having an Rtx-5 MS column (30m X 0.25mm X 0.25µm) using helium as carrier gas. An amount of 10 µL of the sample diluted with spectroscopic grade acetone (1:100) was injected into the GC inlet under pressure flow control mode maintaining purge flow 3 mL/min after fixing the split ratio at 100. The initial column oven temperature was set at 90ºC and the injection temperature was 200ºC. The chemical constituents separated were detected and identified by a mass spectrometer (Shimadzu QP 2010 Plus). During the analysis, the ion source and the interface temperature was set at 200ºC and 250ºC respectively. The detector voltage was 0.70 kV, scanning time was from 0 to 48 min and scan speed was 2000 with m/z range of 40 to 400. Identification of the oil components was based on their retention indices determined by reference to a homologous series of n-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in NIST 05 and FFNSC 1.3 libraries as references.

2.5 Antimicrobial activity

Bacterial inhibitory activity of the extracts is determined to evaluate its antibiotic potential. Inhibition of bacterial growth was tested using agar well disc diffusion method following Dingel et al. [29]. The test microorganisms were used in this antibacterial assay included Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherechia coli* (ATCC 25922) and *Klebsiella pueumoniae* (ATCC 20063). These standard bacteria were collected from Sukra Raj Tropical Infectious Hospital, Teku, Kathmandu, Nepal. The bacteria strains were grown in a nutrient broth at 37°C, maintained nutrient agar slants at 10°C and standardized to 0.5 Mc Farland (10^{6} CFU/ml). The different extracts of *S. officinalis* L. leaves (100 mg) were dissolved in 1mL dimethyl sulphoxide (DMSO) for this activity test. The inoculums containing10^{6} CFU/ml bacteria spread on the solid Muller Hinton Agar (MHA) plates with a sterile cotton swabs moistened with bacteria suspension and dried it. The wells were made in the incubated petri plates with the help of sterile cork borer of diameter of 6 mm and were labeled properly and the plant extracts were loaded into the respective wells with the help of micropipette. An antibiotic erythromycin (100 µg/ml working concentration) was used as a positive control in the separate well. The petri plates were left for few minutes to diffuse plant extracts in media and incubated overnight at 37°C for 24 hours. The diameter of the resulting zone of inhibition (mm) of growth was measured using measuring scale. All the experiments were carried out in triplicate.

III. RESULT AND DISCUSSION

3.1 Phytochemical Profiling

The different classes of phytochemicals present in hexane, ether, ethyl acetate and ethanol extracts of *S. officinalis* leaves were tabulated in the Table 1. A wide range of constituents include alkaloids, reducing sugar, glycosides, saponins, coumarins, flavonoids, quinones, tannins, steroids, terpenes/terpenoids were found. Volatile oils were absent in ethylacetate and ethanol extracts of *S. officinalis* leaves.

Table 1: Classes of Phytochemicals from Ether, Ethylacetate and Ethanol Extracts of *S. officinalis* Leaves

Note: + sign indicates presence and - sign indicates absence

Phytochemical screening revealed that hexane extract of *S. officinalis* leaves had volatile oils, polyphenols and tannins but lacked reducing sugars and glycosides. Ether extract

contained volatile oils, polyphenols, terpenoids, quinones, flavonoids and tannins. Hence, phytochemical profiling ascertained the presence of the different types of potential bioactive phyto-constituents from this medicinal plant.

3.2 Gas Chromatography-Mass Spectrometry Analysis

The percentage yield of essential oil obtained by hydro-distillation of *S. officinalis* L. leaves was quantified and the amount of essential oil was found to be 1.57 % (v/w). The essential oil obtained from *S. officinalis* L. leaves was pale yellow color, slightly viscous with sweet strong camphoraceous odour. The gas chromatography-mass spectrometry (GC/MS) of the essential oil of *S. officinalis* L. leaves from Nepal revealed the presence of twenty-one phytoconstituents in chromatogram representing 99.95% of the total oil composition shown in Fig. 2. The main phytoconstituents components were camphor (65.18%), camphane (9.73%), eucalyptol or 1,8-Cineole (4.72%), 2-napthalenemethanol (3.85%) and α-pienene (2.33%). The minor phytoconstituents were boreneol (1.45%), eucarvone (1.44%), benezenemethanol (1.36%), viridiflorol (1.23%), o-cymene (1.22%), Limonene (1.07%), trans-pinocarveol (0.90%) ,2-(3-oxobutyl) cyclohexanone (0.84%), β-caryophyllene oxide (0.78%), β-pienene (0.78%), m-cymene (0.72%), β-Ocimene (0.71%), 8-hydroxy-p-cymene (0.67%), bornyl acetate (0.49%), valerenal (0.40%) and tricylene (0.11%). The phytoconstituents present in the essential oils of leaves were tabulated below with their gram molecular weight, % peak area, retention time and identification method in Table 2.

As shown in Table 2, the identified compounds were eight monoterpene hydrocarbons, five oxygenated monoterpene hydrocarbons, two monoterpene alcohols, one sesquiterpene alcohol, two monoterpeneketones, one monoterpene ester, one sesquiterpene aldehyde and one sesquiterpenoid epooxide. In this chemical profile, camphor was found as the major component with an abundance of65.18%. The dominant chemical compound like thujone from other varieties of *S. officinalis* was not detected in our essential oil from *S. officinalis* L. leaves. As compared to the literature evidences, our chemical profile results were different in the percentage compositions of phytoconstituents and chemical compounds from previously published works on GC/MS analysis of *S. officinalis* L. leaves essential oil from many other different country region such as Tunicia [30], Abha (Saudi Arabia) [31] , Kashmir (India) [32], Syria [33], Albania I [23], Mexico [23], California [23] and Albania II [34]as tabulated in Table3 .The most abundant principal phytochemical components were camphor, camphene, eucalyptol (1,8-cineole), α-pinene, bornyl acetate, boreneol and β-pinene in *S. officinalis* L. leaves essential oils from these different country regions. But the phytoconstituents like 2- napthalenemethanol, benezenemethanol, trans-pinocarveol, 2-(3-oxobutyl) cyclohexanone, 8-hydroxy-p-cymene, and valerenal are not detected in those essential oils from above mentioned different country regions. The phytoconstituents like *α* –and *β*–thujone were present in the essential oils from these different country regions but thujones were absent in our chemical profile of *S. officinalis* essential oil.

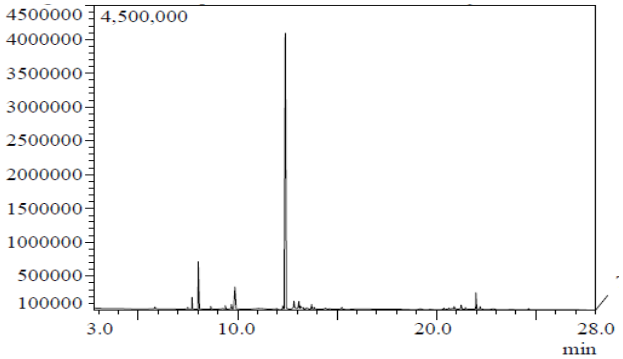

Fig.2: GC/MS chromatogram of essential oil of S. officinalis L. leaves

Figure 3: GC/MS chromatogram of essential oil of S. officinalis L. leaves (magnified).

Table 2: Chemical Constituents of Essential oils of S. officinalis L. leaves from Nepal.

S. No.	Chemical constituents	Molecular Formula	Retention Time	Area %	IM
1	Tricyclene	$C_{10}H_{16}$	7.433	0.11	RT, MS
2	α-pienene	$C_{10}H_{16}$	7.692	2.33	RT, MS
3	Camphene	$C_{10}H_{16}$	8.033	9.73	RT, MS
4	β-pinene	$C_{10}H_{16}$	8.667	0.78	RT, MS
5	β-ocimene	$C_{10}H_{16}$	9.383	0.71	RT, MS
6	p-cymene	$C_{10}H_{14}$	9.633	0.72	RT, MS
7	o-cymene	$C_{10}H_{14}$	9.700	1.22	RT, MS
8	Limonene	$C_{10}H_{16}$	9.792	1.07	RT, MS
9	Eucalyptol(1,8-Cineole)	$C_{10}H_{18}O$	9.875	4.74	RT, MS
10	trans-Pinocarveol	$C_{10}H_{16}O$	12.300	0.90	RT, MS
11	Camphor	$C_{10}H_{16}O$	12.442	65.18	RT, MS
12	Borneol	$C_{10}H_{18}O$	12.875	1.40	RT, MS
13	Benzenemethanol	$C_{10}H_{14}O$	13.142	1.36	RT, MS
14	8-hydroxy-p-cymene	$C_{10}H_{14}O$	13.217	0.67	RT, MS
15	Eucarvone	$C_{10}H_{14}O$	13.783	1.44	RT, MS
16	Bornyl acetate	$C_{12}H_{20}O_2$	15.308	0.49	RT, MS

ISSN No:-2456-2165

17	β-caryophyllene oxide	$C_{15}H_{24}O$	20.925	0.78	RT, MS
18	Viridiflorol	$C_{15}H_{56}O$	21.25	1.23	RT, MS
19	Valerenal	$C_{15}H_{22}O$	21.492	0.40	RT, MS
20	2- naphthalenemethanol	$C_{15}H_{26}O$	22.008	3.85	RT, MS
21	2-(3-oxobutyl)-cyclohexanone	$C_{10}H_{16}O_2$	22.217	0.84	RT, MS
Total				99.95	

RT=Retention Time, MS=Mass Spectrometry, IM = Identification Method

Table 3: Chemical Constituents of Essential oil of S. officinalis L. leaves from different country regions.

Chemical constituents	Area %[a]	Area %[b]	Area %[c]	Area %[d]	Area %[e]	Area %[f]	Area %[g]	Area %[h]	Area %[i]
Tricyclene	0.11	-	-	-	-	0.2	0.1	0.2	0.3
α-pienene	2.33	0.84	1.15	7.69	3.70	5.0	2.4	5.2	6.2
Camphene	9.73	0.78	2.58	6.11	2.60	5.2	3.5	5.3	4.2
β-pinene	0.78	0. 85	2.06	28.33	6.00	4.1	2.6	1.2	2.6
β-ocimene	0.71	0.71	-	-	-	0. 1	0. 1	-	-
p-cymene	0.72	-	0.36	1.19	0.60	0.6	0.2	0.2	trace
o-cymene	1.22	-	-	-	-	1.22	1.22	1.3	-
Limonene	1.07	1.43	-	-	-	1.5	1.7	2.2	-
Eucalyptol(1,8-Cineole)	4.74	14.14	15.0	7.76	62.0	26.9	15.5	11.9	27.5
trans-Pinocarveol	0.90	-	-	-	-	-	-	-	-
Camphor	65.18	25.14	20.3	36.97	8.00	12.8	14.8	21.4	11.5
Borneol	1.40	2.81	2.16	18.38	5.00	1.2	1.00	1.7	trace
Benzenemethanol	1.36	-	-	-	-	-	-	-	-
8-hydroxy-p-cymene	0.67	-	-	-	-	-	-	-	-
Eucarvone	1.44	1.44	-	-	-	-	-	-	-
Bornyl acetate	0.49	1.05	1.6	2.1	-	1.1	0.5	1.8	-
β-caryophyllene oxide	0.78	0.06	-	-	-	0.1	0.2	-	-
Viridiflorol	1.23	7.98	9.9	2.16	-	2.0	7.4	1.5	-
Valerenal	0.40	-	-	-	-	-	-	-	-
2- naphthalenemethanol	3.85	-	-	-	-	-	-	-	-
2-(3-oxobutyl)-cyclohexanone	0.84	-	-	-	-	-	-	-	-
α-thujone	-	18.83	14.9	40.45	1.38	17.2	18.8	27.4	1.1
β-thujone	-	4.46	5.68	4.97	0.72	3.8	4.4	6.0	1.2

Note: [a]=Nepal, [b]=Tunisia, [c]= Abha(Saudi Arabia, [d]=Kashmir, [e]=Syria, [f]= AlbaniaI, [g]=Mexico, [h]=California, and [i]= Albania II

According to the literature, the yield and the chemical composition of *S. officinalis* essential oils depend on various factors such as region, environmental conditions, season, genetic background and plant parts used for essential oil extraction [35-38]. The proportion of oxygenated monoterpenes, such as camphor, 1,8-cineole, *α*-thujone, and *β*-thujone, is considered to indicate the quality of the extracted oil [24]. According to ISO 9909, the phytoconstituents such as α-pinene, camphene, limonene, 1,8-cineole, thujone isomers, camphor, bornyl acetate, and a-humulene are standardized in the essential oils of *S. officinalis* L. for its medicinal application. [24].

One previous report investigated the chemical diversity of southeast European populations of Dalmatian sage, identified four distinct chemotypes (A-D) group. Chemotype A was represented by the high content of α-thujone and camphor, along with the low levels of β-thujone, while Chemotype B was characterized by the high levels of α-thujone and α-humulene, but camphor was detected only in low amounts. Chemotype C was represented by high concentration of both isomers of thujone, along with camphor whereas chemotype D was characterized by the high percentage of camphor and β-pinene, and by the low abundance of α- and β-thujone [39]. Hence, it was clear that our sample of *S. officinalis* complied with the chemotypes D of Dalmatian sage, as both isomers of thujone were not detectable while the content of camphor was dominant (65%). However, it is expected that the plant's response to the different climatic and environmental conditions leads to the huge variance in their secondary metabolites. The essential oil shows a very good medicinal potential due to presence of high concentration of camphor. Camphor is today mostly used in the form of inhalants and of camphorated oil, a preparation of 19% or 20%camphor in a carrier oil, for the home treatment of cold sand as a major active ingredient of liniments and balms used as topical analgesics [40].

Besides, standardized essential oil extract of *Salvia* (sage)have shown many health benefits to humans including cognitive effect in healthy young adults [41]. Although the normal usage of sage is regarded as safe, the use of sage in an inordinate amount might induce harmful effect due to the high content of thujone [42]. Many varieties of *S. officinalis* found around the globe contain relatively much higher concentration of thujone posing a greater risk of toxicity [43]. Even that the

excessive usage of sage as a treatment of human health diseases is greatly concerned, our *S. officinalis* without thujone content, becomes more favorable to use.

3.3 Antimicrobial Analysis

The capability of the essential oil and organic extracts of *S. officinalis* L. leaves to inhibit bacteria was given in Table 4. The result obtained from the agar disc diffusion method demonstrated that the essential oil of *S. officinalis* L. leaves had high bioactivity against Gram-positive *Staphylococcus aureus*, Gram-negative *Klebsiella pueumoniae* and Gram-negative *Escherichia coli* with large inhibition zones (11, 10, and 10mm respectively) compared with positive control erythromycin (6mm). The ether extract of *S. officinalis* L. leaves showed strong antimicrobial activity against *K. pueunmoniae* and *E. coli* with large inhibition zones (10 and 10mm, respectively) and moderate activity against *S. aureus* microorganism (7 mm). Ethyl acetate extract showed weak activity towards Gram-positive as well as Gram-negative bacteria. Ethanol extract showed moderate to low activity against *S. aureus, K. pueumoniae* and *E. coli* with inhibition zones (8, 7 and 6mm, respectively). The antibacterial activity showed by the *S. officinalis* leaves extracts could be attributed to the presence of some classes of secondary metabolites such as volatile oil, polyphenols, terpenoids, quinones, flavonoids and

Table 4: Antimicrobial Analysis of Different Extracts and Essential Oil of S. officinalis against Microorganisms

S.N.	Plant Extracts	Micro organisms	Antimicrobial Activity, ZOI (mm)	
			Erythromycin	*S. officinalis*
1	Ether	*S. aureus*	6	7
		K. pueumoniae	6	10
		E. coli	6	10
2	Ethyl acetate	*S. aureus*	6	7
		K .pueumoniae	6	6
		E. coli	6	6
3	Ethanol	*S. aureus*	6	8
		K. pueumoniae	6	7
		E .coli	6	6
4	Essential oil	*S. aureus*	6	11
		K. pueumoniae	6	10
		E.coli	6	10

IV. CONCLUSIONS

In the present study, we have extracted essential oil from the leaves of a medicinal plant, *S. officinalis* located in Nepal, analyzed their chemical compositions and assessed their antimicrobial activity. Comparing the results of our study with other samples collected from different parts of world revealed some significant similarities and differences in dominant compositions of the medicinal plants. We infer that the geographical features and environmental conditions influence the compositions of secondary metabolites in the plants. Our study revealed that camphor is dominant bioactive phytoconstituents of *S. officinalis* leaves while thujones are missing. Due to this reason, *S. officinalis* medicinal plant may represent a natural, safe, and effective source for the treatment of many human diseases. With the increase in pharmacological knowledge on the beneficial effects of phytoconstituents of *S. officinalis*, we believe the possibility in the development of novel natural drugs to prevent, control, and treat many health problems such as diabetes, Alzheimer and cancer.

ACKNOWLEDGEMENT

We are thankful to [2]Department of Chemistry, Tri-Chandra Multiple College, T. U., Ghantaghar, Kathmandu and for providing laboratory facilities and Department of Plant Resources, Thapathali, Kathmandu, Nepal for GC/MS analysis. The authors wish to thank Professor Bijay Sing for his support.

REFERENCES

1. Bisset N.G., Wichtl M. 2nd ed. CRC Press; Boca Raton. Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis with Reference to German Commission E Monographs; FI: 2001. pp. 440–443.
2. Physicians' Desk Reference (PDR) for Herbal Medicines. 3rd ed. Thompson; Montvale, NJ: 2004. pp. 698–701.
3. Miura K., Kikuzaki H., Nakatani N. Apianane terpenoids from *Salvia officinalis*. *Phytochemistry*. 2001, 58:1171–1175.
4. Clebsch, Betsy; Carol D. Barner. *The New Book of Salvias*. Timber Press (2003). p. 216. ISBN 978-0-88192-560-9.
5. Miura K., Kikuzaki H., Nakatani N. Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *J Agric Food Chem*. 2002, 50:1845–185.
6. Rami K, Li Z. Antimicrobial activity of essential oil of Salvia officinalis L. collected in Syria. *Afr J Biotech* 2011; 10:8397-402.
7. Walch S, Tinzoh L, Zimmerman B, Stuhlinger W, Lachenmeier D. Antioxidant capacity and polyphenolic composition as quality indicators for aqueous infusions of *Salvia officinalis* L. *Front Pharmacol* 2011,2:29.
8. Khan A, Najeeb-ur- Rahman, Alkharfy K, Gilani A. Antidiarrheal and antispasmodic activities of Salvia officinalis are mediated through activation of K+channels. *J Bangladesh Pharmacol Soc* 2011, 6:111-6.
9. Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA, Menichini F. Cytotoxic activity of essential oils from Labiatae and Lauraceae families against *in vitro* human tumor models. *Anticancer Res* 2007, 27:3293-9.
10. Radulescu V, Chiliment S, Oprea E. Capillary gas chromatography-mass spectrometry of volatile and semi-volatile compounds of Salvia officinalis. *J Chromatogr* 2004, 1027:121-6.

11. Hadri A, Gomez Del Rio M, Sanz J, Coloma A, Idaomar M, Ozanas B, *et al*. Cytotoxic activity of α-humulene and transcaryo-phyllene from Salvia officinalis in animal and human tumor cells. *An R Acad Nac Farm* 2010, 76:343-56.
12. Croteau R, Felton M, Karp F, Kjonaas R. Relationship of camphor biosynthesis to leaf development in sage (Salvia officinalis). *Plant Physiol* 1981, 67:820-4.
13. Kennedy, D.O., Pace, S., Haskell, C., Okello, E. J., Milne, A., Scholey, A.B. Effects of cholinesterase inhibiting sage (Salvia officinalis) on mood, anxiety and performance on a psychological stressor battery. *Neuropsychopharmacology*, 200631, 845–852.
14. Eidi, A., Eidi, M. Antidiabetic effects of sage (Salvia officinalis L.) leaves in normal and streptozotocin-induced diabetic rats. Diabetes and metabolic syndrome. *Clinical Research and Reviews* 2009, 3: 40–44.
15. Abu-Darwish, M.S., Cabral, C., Ferreira, I.V., Gonçalves, M.J., Cavaleiro, C., Cruz, M.T., Al-Bdour, T.H., Salgueiro, L., Essential oil of common sage (Salvia officinalis L.) from Jordan: assessment of safety in mammalian cells and its antifungal and anti-inflammatory potential. *Bio Med Research International* 2013, 1–9.
16. Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., Statti, G.A., Menichini, F. Cytotoxic activity of essential oils from Labiatae and Lauraceae families against in vitro human tumor models. *Anticancer Research* 2007, 27: 3293–3299.
17. Sertel, S., Eichhorn, T., Plinkert, P.K., Efferth, T., Chemical composition and antiproliferative activity of essential oil from the leaves of a medicinal herb, Levisticum officinale, against UMSCC1 head and neck squamous carcinoma cells. *Anticancer Research* 2011, 31: 185–191.
18. Kontogianni, V.G., Tomic, G., Nikolic, I., Nerantzaki, A.A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerothanassis, I.P., Tzakos, A.G. Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. *Food Chemistry* 2013, 136: 120–129, F., Delfine, S., Cardile, V., Rosselli, S., Bruno, M. Chemical composition and anticancer activity of essential oils of Mediterranean sage (Salvia officinalis L.) grown in different environmental conditions. *Food and Chemical Toxicology* 2003, 55: 42–47.
19. Vuković-Gacić, B., Nikcević, S., Berić-Bjedov, T., Knezević-Vukcević, J., Simić, D., Antimutagenic effect of essential oil of sage (Salvia officinalis L.) and its monoterpenes against UV-induced mutations in Escherichia coli and Saccharomyces cerevisiae. *Food and Chemical Toxicology* 2006, 44: 1730–1738.
20. Loizzo, M.R., Saab, A.M., Tundis, R., Statti, G.A., Menichini, F., Lampronti, I., Gambari, R., Cinatl, J., Doerr, H.W. Phytochemical analysis and in vitro antiviral activities of the essential oils of seven Lebanon species. *Chemistry & Biodiversity* 2008, 5: 461–470.
21. Longaray D, A.P.; Moschen-Pistorello, I.T.; Artico, L.; Atti-Serafini, L.; Echeverrigaray, S. Antibacterial activity of the essential oils of Salvia officinalis L. and Salvia triloba L. cultivated in South Brazil. *Food Chem*. 2007, 100: 603–608.
22. Jonathan D. Craft, Prabodh Satyal and William N. Setzer, The Chemotaxonomy of Common Sage (Salvia officinalis) Based on the Volatile Constituents. *Medicines* 2017**,** 4, 47: doi:10.3390/medicines 4030047.
23. Abdullah N., Zulkifri K.S., Abdullah A., Aziman N., Kamarudin WSSW. Assessment on the antioxidant anti-bacterial activities of selected fruit peels. *Int. J. Chem Tech Res* 2012 4(4): 1534-1542.
24. Hammuel C., abdullahi M.S., anyim B.P., Adesina O.B., Inekwe U.V. The pytochemical and antimicrobial activities of oil from the seed of Thevetia peruviana plant. *J. Appl Environ. Biol Sci* 2011,1(12):597-601
25. Baker J.T., Barries R.P., Carte B Natural product drug discovery: New perspective on international collaboration. *J. Nat Prod.*1995, 58:1325-1357.
26. Raal A, Orav A, Arak E. Composition of the essential oil of *Salvia officinalis* L. from various European countries. *Nat. Prod. Res.* 2007, 21:406-41.
27. Culie I. Mythology for analysis of vegetable drugs, Practical manuals on industrial utilization of medicinal and aromatic plant, Bucharest. *Phytochemistry, 1982* 63 (1): 97-104.
28. Dingel J., W.W. Red and G.L. Solomons, *Journal of Science Food and Agriculture* 1953, 4(3): 149-153. DOI:10.1002/jsfa.2740040305
29. Khedher MRB, Khedher SB, Chaieb I., Tounsi S., Hammami M., Chemical composition and biological activities of Salvia officinalis essential oil from Tunisia, *EXCLI Journal*, 2017,16:160-173-ISSN1611-2156.
30. Jery A. E., Hasan M., Rashid M.M., Al Mesfer M. K., Danish M., Rebah F.B., Phytochemical characterization, and antioxidant and antimicrobial activities of essential oil from leaves of the common sage *Salvia officinalis* L. from Abha, Saudi Arabia, *Asian Biomed* (Res Rev News) 2020, 14(6):261–270, DOI 10.1515/abm-2020-0035.
31. Bhat G, Rasool S, Shakeel-u-Rehman, Ganaie M, Qazi PH, Shawl AS. Seasonal variation in chemical composition, antibacterial and antioxidant activities of the essential oil of leaves of Salvia officinalis (sage) from Kashmir, India. *TEOP*. 2016, 19:1129–40.
32. Rami K, Li Z. Antimicrobial activity of essential oil of Salvia officinalis L. collected in Syria. *Afr J Biotech* 2011, 10:8397-402.
33. Couladis M, Koutsaviti A, chemical composition of essential oil of *Salvia officinalis, S fruticosa, Melissa officinalis,* and their infusions, *Ratar. Povrt.* 2017, 54(1):36-41 doi:10.5937/ratpov54-12365
34. Rezaeieh KAP, Gurbuz B. Volatile oil yield and constituents of Salvia officinalis, S. tomentosa Mill. and S. glutinosa growing in Ankara, Turkey. *Asian J Agric Res.* 2017, 11:43–47.
35. Bernotiené G, Nivinskiené O, Butkiené R, Mockuté D. Essential oil composition variability in sage (*Salvia officinalis* L.). *Chemija*. 2007, 18:38–43.
36. de Hadri A, del Rio MG, Sanz J, Coloma AG, Idaomar M, Ozanas BR. Cytotoxic activity of α-humulene and transcaryophyllene from *Salvia officinalis* in animal and human tumor cells. *An R Acad Nac Farm*. 2010, 76:343–56.
37. Santos-Gomes PC, Fernandes-Ferreira M. Organ and season-dependent variation in the essential oil

composition of *Salvia officinalis* L. cultivated in two different sites. *J Agri Food Chem*. 2001, 49:2908–16.
38. Cvetkovikj I., Stefkov, G., Karapandzova, M., Kulevanova S., Satović Z. Essential Oils and chemical diversity of southeast European populations of Salvia officinalis L. *Chemistry and Biodiversity* 2015, 12: 1025-1039. doi: 10.1002/cbdv.201400273
39. Xu H, Blair NT, Clapham DE. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid in dependent mechanism. *J Neurosci.* 2005, 25(39):8924-37.
40. Leung, Albert Y. 1980. Encyclopedia of common natural ingredients used in food, drugs, and cosmetics: Wiley.
41. Tildesley N. T. J., Kennedy D. O., Perry E. K., Ballard C. G., Savelev S., Wesnes K. A., and Scholey A. B. "Salvia lavandulaefolia (Spanish Sage) enhances memory in healthy young volunteers." *Pharmacology Biochemistry and Behavior* 2003, 75 (3):669-674. doi: https://doi.org/10.1016/S0091-3057(03)00122-9.
42. Walch S. G., Tinzoh L. N., Zimmermann, W. Stühlinger B. F., and Lachenmeier D. W. "Antioxidant Capacity and Polyphenolic Composition as Quality Indicators for Aqueous Infusions of Salvia officinalis L. (sage tea)." *Front Pharmacol* 2011, 2:79. doi: 10.3389/fphar.2011.00079.