ORIGINAL PAPER

BIOLOGICAL ACTIVITY OF ESSENTIAL SAGE OIL

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Abstract. *Essential sage oil (ESO) was isolated from dry leaves by steam distillation with Neo-Clevenger apparatus. The chemical analysis of the ESO consisted in the assessment of the total polyphenolic content (Folin-Ciocâlteu method) and of the radical scavenging activity with DPPH. The antioxidant activity was measured using two standard compounds: gallic acid and ascorbic acid. Phenolic content of essential oil of S. officinalis was significant (15.465 mg GAE/g oil) and antioxidant activity was high. Antibacterial activity of essential sage oil was evaluated against Gram positive and Gram-negative bacterial strains isolated from clinical specimens. The sage oil showed significant but variable antibacterial activity with inhibition zones ranging from 4 mm to 9.5.mm. The effect was stronger on Gram positive (Enterococcus, Staplylococcus) than Gram negative bacteria (Escherichia sp, Proteus sp, Klebsiella sp).*

Keywords: *sage oil, phenolic compounds, antioxidant activity, antibacterial activity, Staphylococcus sp, Enterococcus sp*

1. INTRODUCTION

The genus *Salvia* L. (Lamiaceae) includes about 900 species, spread all over the world, from which the most studied species are common sage (*Salvia officinalis* L.), trilobed sage (*Salvia fruticosa* Mill.), and spanish sage (*Salvia lavandulifolia* Vahl) [1-3]. In traditional medicine, sage has been used for inflammation of the mouth and throat, headache, abdominal pain (ulcers, diarrhea), dyspepsia, seizure, gout, rheumatism, atherosclerosis, hyperglycemia, excessive sweating [1-4]. Most of the bioactive compounds which are reported from *S. officinalis* have been isolated from its essential oil, alcoholic extract, aqueous extract, or infusion preparation [2-6]. It was found that *S. officinalis* contains many constituents including phenolic compounds, alkaloids, carbohydrate, glycosidic derivatives, terpenes/terpenoids and waxes [2-4]. In the essential oil of *S. officinalis* were identified more than 120 components, the main components being: borneol, camphor, caryophyllene, cineole, elemene, humulene, ledene, pinene, and thujone [3, 4], while the alcoholic extracts of *S. officinalis* exhibit a high content of rosmarinic acid and luteolin-7-glucoside but also caffeic acid and 3-caffeoylquinic acid. In the aqueous extract of *S. officinalis* were found high contens of rosmarinic acid and ellagic acid, followed by rutin, chlorogenic acid and quercetin [4, 6-10].

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The environmental conditions such as temperature, day length, day light, soil, humidity, or species variety influence the quantitative compositions of essential oils [1, 3, 4, 11-19]. Sage essential oil (ESO) can be used for many purposes (pharmaceuticals, cosmetic products, food etc.) having considerable economic potential but also important therapeutic properties [1-4, 9, 10, 15-19].

Several studies data indicates that sage essential oil contents monoterpenes (α- and β-thujone, 1,8-cineole, camphor), diterpenes (e.g., carnosic acid) triterpenes (oleanoic and ursolic acids) and some concentrations of phenolic compounds from which rosmarinic acid is the most abundant [2, 3-5, 8, 9]. It is well-known that high doses of α-thujone in plants can be responsible to causes convulsions [4] and chronic exposure which can lead to neurotoxicity [1, 2] and carcinogenicity [4], while optimal concentration of α-thujone is effective in digestion stimulation, menstrual problems, as well as in the pain realise associated with rheumatism [4, 6, 8-10, 20-24]. Although the sage oil has a high thujone content, it has been estimated that it takes between 2 and 20 cups of sage tea to reach the acceptable daily intake of thujone [3, 4]. Therefore, it is unlikely to rich the overdose of α-thujone responsable of the toxicity effects, but for medical consideration the consumption of *S. officinalis* is not recommended in pregnancy and lactation [3, 4].

The antimicrobial resistance to available antibiotics is an emerging problem and stimulated the interest in natural alternatives [25-29]. Therefore, plant-derived medicines are considered to be safe alternatives compared to synthetic drugs or in some cases the combination with synthetic compounds can improve their efficacy in some diseases difficult to cure [25]. Considering the literature, was found interesting to determine total phenolic compounds and antioxidant capacity of *S. officinalis* essential oil from Dobrogea area and to test it against both Gram-negative bacteria and Gram-positive bacteria in order to estimate his antimicrobial effect.

2. MATERIALS AND METHODS

2.1. MATERIALS

All used reagents for chemical determinations were of analytical reagent grade. Gallic acid was purchased from Fluka (Buchs, Switzerland) and Folin-Ciocâlteau reagent from Merck (Darmstadt, Germany). The solution of gallic acid (standard phenolic compound) 1×10^{-2} mol/L was prepared by dissolving 0.1881g of gallic acid in 100 mL ethanol. Folin-Ciocâlteau reagent was diluted with distilled water 1:2 (v:v). DPPH (2,2-difenil-1-picrililhidrazil) was purchased from Aldrich (Germany). The standard compound solution 0.0063% (1.268 mM) was prepared in a 200 mL calibrated flask by dissolving 0.0010 g of 2,2-difenil-1-picrililhidrazil in methanol. Ascorbic acid was purchased from Aldrich (Germany). The stock solution 0.5 mg/mL was prepared in a 250 mL calibrated flask by dissolving 0.125 g of ascorbic acid in distillated water. For calibration curve and determinations, the standard solution of ascorbic acid was prepared by diluted with distilled water 1:10 (v:v) from the stock solution. Spectrometric measurements were carried out using a UV-VIS JASCO V550 scanning spectrophotometer.

2.2. METHODS

2.2.1. Isolation of Essential Sage Oil

Leaves of sage (*Salvia officinalis* L.) were harvested in June 2021, from organic cultures in Topraisar, Constanta County, Romania. The sage leaves were dried at ambient temperature and grinded to powder.

The extraction of Essential Sage Oil (ESO) was performed by steam distillation of 1000g dry plant using Neo-Clevenger apparatus for 4 hours. Resulted ESO was dried with anhydrous sodium sulphate and stored in sealed smoked vials at 4°C for further analysis. The yield of the resulted ESO was 2.45%.

2.2.2. Determination of total phenolic content

For Total Phenols Content (TPC) and antioxidant activity determinations a quantity of 0.1165 g of *Salvia officinalis* L. essential oil (ESO) obtained was brought to mark with ethanol to 10 mL volume. The total phenols content was estimated according to the Folin-Ciocâlteau method [9, 10, 20-24]. The absorbance was measured at 681 nm. Total phenols content of essential sage oil was expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100g d.w). The determinations were performed in triplicate and the mean value was reported.

The calibration curve was plot using a standard solution of gallic acid and the absorbance measurements were performed at 681 nm. In a series of 50 mL volumetric flasks, volumes of 1, 2, 3, 4, 5, 6, 7, and 8 mL of gallic acid standard solution were introduced and after that, was added 1 mL of Folin-Ciocâlteau-reagent 1:2 (v:v) and 1 mL of 20% (w/v) aqueous Na_2CO_3 aswell. Finally, in 10 minutes the volume was brought to the sign with distilled water. After another 30 min. of incubation at 25ºC the absorbance was measured at 681 nm.

The calibration curve was linear in the range of 0.68 – 5.44 mg GAE/L, (R^2 =0.9977) (Fig. 1). To measure the total phenols content, 1 mL ESO solution was added in 50 mL calibrated flasks, then 1 mL Folin-Ciocâlteau-reagent 1:2; 1 mL sodium carbonate solution 20% and the process was the same as those used for calibration.

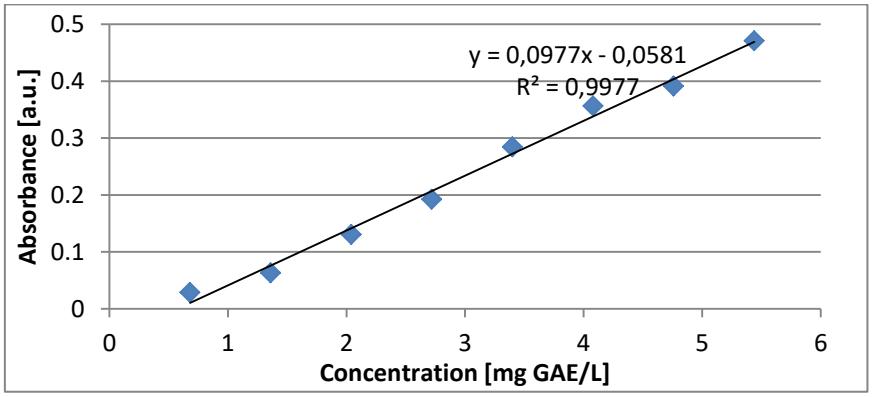

Figure 1. Calibration curve of gallic acid in the range of 0.68 – 5.44 mg GAE/L at 681 nm.

2.2.3. Antioxidant activity

The antioxidant activity was evaluated using DPPH Radical Scavenging test using two standard compounds to plot calibration curves: gallic acid (GA) and ascorbic acid (AA) and the results are expressed as equivalents (mg GAE and mg AAE, respectively). Absorbance measurements were carried out at 530 nm [9, 20, 22].

In 25 mL calibrated flasks, different volume of gallic acid or ascorbic acid solutions were added, then 5 mL DPPH 1.268 mM in methanol, filled up to the mark with methanol and let in the dark, to the room temperature for 45 minutes before the absorbance was determined at 530 nm using methanol as blank.

The solutions absorbance' decrease due to the antioxidant capacity of standard compounds determined the downward allure of calibration curves. The calibration curve with gallic acid as standard was linear in the range of 0 – 4.76 mg GAE/L and the correlation coefficient was 0.99832 (Fig. 2).

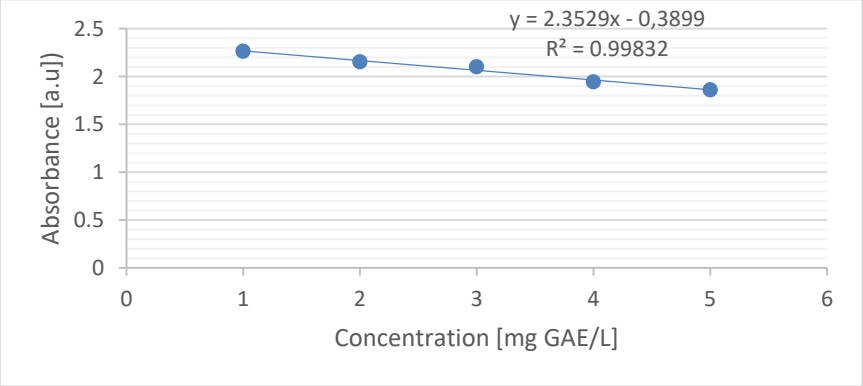

Figure 2. Calibration curve of gallic acid in the range of 0.68 – 4.76 mg GAE/L at 530 nm.

The calibration curve with ascorbic acid as standard was linear in 0 – 5 AAE/L range and the correlation coefficient was 0.9911, according to Fig. 3.

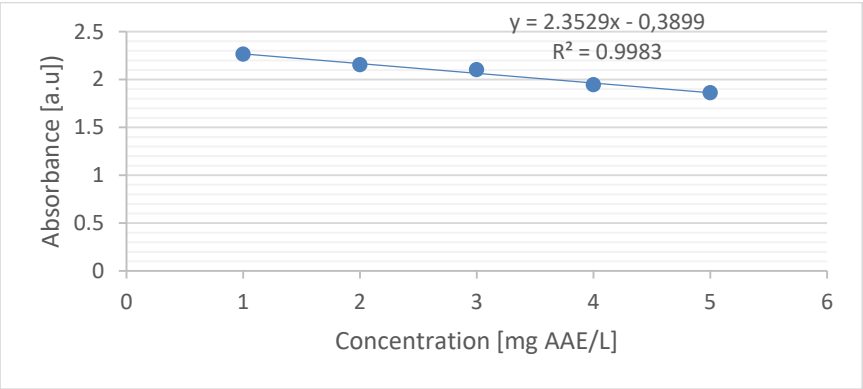

Figure 3. Calibration curve of ascorbic acid in the range of 1 – 5 mg AAE/L at 530 nm.

2.2.4. Antibacterial activity

Twenty bacterial strains, both Gram-positive and Gram-negative (Table 1) against essential sage oil (ESO) were tested.

The evaluation of inhibitory activity of extracts and essential oil of sage was performed by the well plate method [30]. Bacterial strains were inoculated into Mueller Hinton broth and incubated overnight at 37°C for 18 hours. After dilution, the inoculation was carried out by spreading the culture (with density between $9 \cdot 10^5$-and $1 \cdot 10^6$ UFC/mL) with a pharyngeal swab onto Mueller Hinton agar surface. After inoculation, the media were kept for 1 hour to room temperature to allow the surface to dry.

Subsequently, wells (d = 6 mm) were performed using a sterile test tube. 100 µL of each extract and ESO were pipetted into wells and media were left at room temperature to allow the diffusion of extracts. Inoculated media were thereafter incubated at 37°C for 48 h. The inhibitory effect was assessed as the size of inhibition zone (mm).

Table 1. Bacterial strains used to assess the effect of essential sage oil (ESO)

Crt. No.	Bacterial strain	Observations
1.	*Escherichia coli*	Isolated from urinary tract infection (UTI)
2.	*Klebsiella*	Isolated UTI
3.	*Proteus*	Isolated UTI
4.	*Enterococcus 1*	Isolated UTI
5.	*Enterococcus 2*	Isolated UTI
6.	*Enterococcus 3*	Isolated UTI
7.	*Enterococcus 4*	Isolated UTI
8.	*Staphylococcus 1*	Isolated from skin infection (SI)
9.	*Staphylococcus 2*	Isolated (SI)
10.	*Staphylococcus 3*	Isolated (SI)
11.	*Staphylococcus 4*	Isolated (SI)
12.	*Staphylococcus 5*	Isolated (SI)
13.	*Staphylococcus 6*	Coagulase positive
14.	*Staphylococcus 7*	Hemolytic, isolated from UTI
15.	*Staphylococcus 8*	Hemolytic, isolated from UTI
16.	*Staphylococcus 9*	Hemolytic, isolated from UTI
17.	*Staphylococcus 10*	Coagulase negative
18.	*Staphylococcus 11*	Coagulase negative
19.	*Staphylococcus 12*	Hemolytic, isolated from UTI
20.	*Staphylococcus 13*	Hemolytic, isolated from UTI

3. RESULTS AND DISCUSSION

3.1. RESULTS

Results of total phenolic content and antioxidant activity of sage oil are expressed as mg GAE/g oil are presented in Table 2. From the obtained data it can be observed that *Salvia officinalis* L. has a high phenolic content and a good antioxidant activity which indicates that ESO is a good natural source of antioxidants.

The obtained results are similar with previous published studies concerning the total phenols concentration in essential sage oils from dry plants [1-9].

Table 2. Chemical characterization of essential sage oil

Sample	Yield ESO [%]*	Total phenolic content [mg GAE/g oil]	DPPH Assay	
			Gallic acid [mg GAE/g oil]	Ascorbic acid [mg AAE/g oil]
Salvia officinalis L. essential oil	2.45	15.465	1.08	4.41

**Yield percentage was determined by volume/weight ratio.*

Difusimetric assessment of antibacterial activity of essential sage oil (ESO)

Generally, all strains were more or less sensitive to essential oil of sage (Table 3). However, the extent to which the individual bacteria responded to extracts was variable. In this respect, there was also a significant difference of the ability of extracts and essential oil to inhibit Gram-positive and Gram-negative bacteria. The effect of extracts was generally

weaker on Gram-negative bacteria compared to Gram positive. In the case of Gram-negative bacteria, the most pronounced inhibitory effect had ESO (inhibition zone = 7.33 mm).

The results of this study were comparable to other findings showing significant inhibitory effect of sage essential oil on enterococci [31].

Table 3. Difusimetric assessment of antibacterial activity of essential sage oil (ESO)

Bacterial strain extract	ESO Inhibition zone [mm]	Bacterial strain extract	ESO Inhibition zone [mm]
Escherichia coli	6	*Staphylococcus* 1	7
Klebsiella	10	*Staphylococcus* 2	10
Proteus	6	*Staphylococcus* 3	5
Average activity	***7.33***	*Staphylococcus* 4	8
Enterococcus 1	12	*Staphylococcus* 5	12
Enterococcus 2	8	*Staphylococcus* 6	12
Enterococcus 3	4	*Staphylococcus* 7	10
Enterococcus 4	14	*Staphylococcus* 8	5
Average activity	***9.5***	*Staphylococcus* 9	10
		Staphylococcus 10	12
		Staphylococcus 11	10
		Staphylococcus 12	10
		Staphylococcus 13	12
		Average activity	***9.46***

ESO showed significant but variable antibacterial activity with inhibition zones ranging from 4.0 mm to 9.5 mm. The effect was stronger on Gram-positive (*Enterococcus, Staplylococcus*) than Gram-negative bacteria (*Escherichia sp, Proteus sp, Kleksiella sp*).

3.2. DISCUSSION

The total polyphenolic content determined by Folin-Ciocalteu method indicated a high concentration of polyphenolic compounds in investigated sage essential oil (15.465 mg GAE/g oil). Regarding the different values of sage essential oil antioxidant activity determined by DPPH method using gallic acid and ascorbic acid the differences can be explained by the different references used for determinations. The combination of TAC assays using different references provides a better picture of the antioxidant status of the analyzed sage oil. The types and quantities of phenolic compounds contribute to the varying antioxidant activity of sage oil [3, 7, 8].

Difusimetric assessment of antibacterial activity of essential sage oil

Enterococci can cause a range of infectious diseases (urinary tract infections, bacteraemia, endocarditis), and they are regarded as opportunistic pathogens in the hospital environment [31]. Therefore, sage components might be view as valuable alternatives to antibiotics against especially vancomycin resistant strains [32]. Important effects of growth inhibition were also recorded in *Staphylococcus* strains, when large inhibition zones were recorded, ranging from 9.46 mm to 7.31 mm.

Significant effects of ESO have also been observed against *Staphylococcus aureus* in other studies [33-35]. Importantly, the sage oil was efficient against methicillin resistant strains of *S. aureus* that cause difficult to treat infections [36-39]. A higher sensitivity of *Enterococcus* and *Stapphylococcus* groups was also recorded by others [31, 40], findings more or less similar to our results.

4. CONCLUSIONS

The evaluated ESO sample presents a high phenolic content and a very good antioxidant activity. Sage extracts and ESO exhibited variable inhibitory effect on the bacterial growth depending on the extract type, on the one hand and bacterial species, on the other. Most sensitive groups were *Enterococcus* and *Staplylococcus* while the growth of Gram-negative bacteria was less affected by essential sage oil. Considering all obtained data can be concluded that ESO has a significant antioxidant and antibacterial activity and can be considered a good source of natural antioxidants, useful for therapeutical, cosmetic, or other commercial purposes.

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