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Full Length Research Paper

Chemical composition and antimicrobial activity of essential oil of Salvia potentillifolia Boiss. & Heldr. ex Benth. from Turkey

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The present study describes the chemical composition and antimicrobial activity of essential oil from *Salvia potentillifolia* Boiss. & Heldr. ex Benth., which is endemic for Turkey. The essential oil was obtained by hydrodistillation and components of the essential oil were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The major components of *S. potentillifolia* essential oil were 1.8-cineole (19.33%), β -pinene (11.97%) and α -pinene (8.99%). Antimicrobial activity of the essential oil was investigated against standard control and resistant strains, using disc diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The essential oil of *S. potentillifolia* showed relatively low levels of antimicrobial activity against the bacteria tested. However, the oil was as effective as the antibiotic tested against *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 51299 [vancomycin resistant *Enterococcus faecalis* (VRE)], *Enterococcus faecalis* ATCC 29212, *Haemophilus influenzae* ATCC 49247 strains. The most bactericidal activity was obtained against *Haemophilus* species (4 µg/ml).

Key words: Salvia, lamiaceae, essential oil, GC-MS analysis, antimicrobial effect, MRSA, VRE.

INTRODUCTION

The genus *Salvia* (common name: sage) is the largest and the most important aromatic and medicinal member of the Lamiaceae family and is represented by more than 900 species spread throughout the world (Tenore et al., 2010). The word *Salvia* was derived from the Latin salvare, meaning "to heal or to be safe and unharmed" referring to the medicinal value of the plant (Gali-Muhtasıb, 2006). *Salvia* species have long been used in folk medicine against colic, diarrhea, colds, cough, flu, stomach problems, tuberculosis, chronic bronchitis, bacterial infections, febrile attacks, rheumatism and sexual debility and in the treatment of mental and nervous conditions (Kamatou et al., 2005, 2008). Some *Salvia* species have been studied various biological and pharmacological properties, including antibacterial (Akin et al., 2010; Delamare et al., 2007), antifungal (Fraternale et al., 2005), antioxidant (Bozin et al., 2007; Miguel et al., 2011), anticholinesterase (Kıvrak et al., 2009; Orhan et al., 2007), anti-inflammatory (Chan et al., 2011; Kamatou et al., 2005) properties in many parts of the world. Some members of this genus have economically important due to use as spices and flavoring agents in the perfumery and cosmetics (Delamare et al., 2007).

The flora of Turkey includes 88 species and 93 taxa of

which 45 are endemic (Davis et al., 1988; Duman, 2000; Hedge, 1982). Several *Salvia* species are widely distributed in Anatolia and Mediterranean region where they are known locally as "adaçayı" and are consumed as herbal tea. They have been traditionally used as antiseptic, antibacterial, diuretic, spasmolytic, stomachic, stimulants, wound-healer and carminative agents in Turkish folk medicine (Baytop, 1999; Tabanca et al., 2006). There are many reports on antibacterial (Akin et al., 2010), antioxidant (Kelen and Tepe, 2008; Tepe et al., 2006), anticholinesterase (Orhan et al., 2007; Tel et al., 2010), insecticidal activities (Kotan et al., 2008) of Turkish *Salvia* species.

Salvia potentillifolia Boiss and Heldr. ex Bentham is an endemic in Turkey, where it grows only in Antalya, Konya, Burdur, Afyon and Denizli provinces. This species, which is a perennial suffruticose herb with yellowish flower, prefers dry rocky slopes and areas of scrub in altitudes from 900-1700 m above sea level (Hedge, 1982). The essential oil of *S. potentillifolia* was previously studied by Sarer (1990). Kivrak et al. (2009) reported the antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *S. potentillifolia* collected in Burdur. In the present study, we investigated the antimicrobial activity on resistant and sensitive bacterial species of essential oil of *S. potentillifolia* collected from Antalya.

MATERIALS AND METHODS

Plant material

S. potentillifolia was collected during the plant's flowering period (August) from (1300-1350 m) Elmalı Cedar Research Forest, Antalya, Turkey. The taxonomic identification of plant material was confirmed by plant taxonomist, İ. Gökhan Deniz (Department of Biology, Akdeniz University, Antalya, Turkey).

Collected plant material was dried in shade and aerial parts of the plant were ground. The voucher specimen was preserved in the Herbarium of the Biology Department of Akdeniz University, Antalya, Turkey.

Isolation of essential oil

The essential oil sample was isolated from dried and ground aerial parts by hydrodistillation for 3 h using a Clevenger-type apparatus (Ildam Cam, Turkey). The oil was stored in tightly closed dark vials at -20°C prior to further analysis.

Gas Chromotography-Mass Spectrometry (GC-MS) analysis

The analysis of the essential oil was performed using a Agilent 6890 GC series 5973 MSD system equipped with a HP 5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness). For GC-MS detection an electron ionisation (EI) system was used with ionisation energy of 70 eV. Helium (He) was used as a carrier gas, with a flow rate of 1 ml/min. The injector and the transfer line temperature were set to 250 and 280°C. respectively. The column temperature gradually increased from 40 to 150°C at a 2°C/min, and was held for 10 min at 150°C. The injected volume was 1 µl.

The split ratio was 1:60. Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in Wiley/NIST mass spectral library of the GC/MS data system and/or by visual inspection of published spectral daata (Adams, 2001).

Antimicrobial activity test

Reference strains

ATCC (American Type Culture Collection) guality control strains, recommended by CLSI (Clinical and Laboratory Standards Institute), were used to test the antimicrobial activity of Salvia species. The reference strains were as follows: Staphylococcus aureus ATCC 25923 (β-lactamase negative), Staphylococcus aureus ATCC 29213 (β-lactamase positive), Staphylococcus aureus ATCC 43300 [Methicillin resistant (MRSA)], Staphylococcus epidermidis ATCC 12228 (Quality control strain, Enterococcus faecalis ATCC 29212 (Vancomycin sensitive), Enterococcus faecalis ATCC 51299 [Vancomycin resistant (VRE)], Streptococcus pyogenes ATCC 19615 (Quality control strain), Escherichia coli ATCC 25922 (βlactamase negative), Escherichia coli ATCC 35218 (β-lactamase positive), Klebsiella pneumoniae ATCC 13883 (Quality control strain), Klebsiella pneumoniae ATCC 700603 [Extended spectrum β-lactamase (ESBL) positive], Enterobacter cloacae ATCC 23355 (Cefalosporinase positive), Serratia marcescens ATCC 8100 (Quality control strain), Proteus vulgaris ATCC 13315 (Quality control strain), Salmonella typhimurium ATCC 14028 (Quality control strain), Pseudomonas aeruginosa ATCC 27853 (Quality control strain). Haemophilus influenzae ATCC 49247 (β-lactamase negative), Haemophilus influenzae ATCC 49766 (β-lactamase positive).

Disc diffusion method

The standard disc diffusion method recommended by CLSI (CLSI, 2006a) was used to determine antimicrobial property of the essential oil. Haemophilus Test Medium Agar (BD Diagnostics, Heidelberg, Germany) was used for H. influenzae strains. 5% sheep-blood Mueller Hinton Agar (BD Diagnostics, Heidelberg, Germany) was used for S. pyogenes ATCC 19615 and Mueller Hinton Agar (Merck KGaA, Darmstadt, Germany) was used for all other bacteria. Bacteria strains were first inoculated into Blood Agar (Merck KGaA, Darmstadt, Germany) and incubated over-night at 37°C for 24 h and checked for purity. Then all bacteria suspensions were prepared in 0.9% NaCl solution as per 0.5 McFarland (1x 10⁸ cells per ml, BioMérieux, Marcy l'Etoile, France) standard density. Prepared bacteria suspensions were spread on media by sterile swab. A 10 µl of the essential oil was impregnated into each standard empty disc (6 mm in diameter), then the discs were placed on the plates one by one. Standard antibiotic discs, recommended by CLSI, which were suitable for microorganisms, were placed into the same plates as positive controls. An empty disc was also used to test if discs were sterile or not. S. pyogenes ATCC 19615, Haemophilus species were incubated in 5% CO_2 at 37°C for 24 h. Others were incubated in normal atmosphere at 37°C for 24 h. The diameters of the inhibition zones were calculated in millimeters. Each assay was performed four times.

Determinations of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The microdilution broth susceptibility assay for bacteria was used, as recommended by CLSI, for determination of the MIC (CLSI, 2006b). The essential oil dissolved in Mueller Hinton Broth (MHB,

Merck KGaA, Darmstadt, Germany) added 0.5% Tween 80 (Sigma Ultra, MO, USA) was first diluted to the highest concentration (512 μ g/ml) to be tested, and then serial two-fold dilutions were made in the concentration range of 128-0.0625 µg/ml in a microtitre plate (96 wells). Haemophilus Test Medium Broth was used for H. influenzae strains. Lysed horse blood added Cation-adjusted Mueller Hinton Broth (CAMHB) was used for S. pyogenes ATCC 19615 and CAMHB was used for all other bacteria. Bacterial strains were cultured over-night at 37°C in Blood Agar (Merck KGaA, Darmstadt, Germany) and checked for purity. Then all bacteria suspensions were prepared in 0.9% NaCl solution as 0.5 McFarland standard density (1x 10^8 cells per ml, BioMérieux, Marcy l'Etoile, France). The wells of 96 well microplates were filled with 50 μl of medium. A 50 μl from the stock solution of the essential oil (512 µg/ml) was added into the first well and serial dilutions were transferred into 12 consecutive wells. Then, each well was inoculated with 50 μ l of each bacteria strain (5x10⁵ cfu/ml). The growth conditions (medium+microorganisms+Tween 80) and the sterility of the medium (MHB) were checked in two control wells for each strain tested. The same procedure was also applied to each control antibiotic. The microtitration plates were incubated under normal atmospheric conditions at 37 °C for 24 h. The bacterial growth was indicated by the presence of a white "pellet" in the bottom of the wells. The MIC was defined as the lowest antimicrobial concentration which prevented visible growth. Each assay was performed four times.

To evaluated MBC [recommended by CLSI, (2006b)], 10 μ l from wells containing concentrations of the essential oil equal to and greater than the MIC was subcultured on blood agar to determine whether the initial inoculum was inhibited from multiplying (static action) or was killed (bactericidal action). Plates were then incubated at 37°C for 24 h for fast growing Gram (-) bacillus, 48 h for staphylococci and enterococci, and finally 72 h for all other bacteria. MBC was defined as the lowest concentration of the antimicrobial agent which could kill 99.9% of microorganisms.

Statistical analysis

To compare the effects of essential oils and antibiotics based on disc-diffusion test. each bacteria group [Gram (+) and Gram (-)] was evaluated with Fischers Ki Square Test (χ 2). Comparisons of antibiotic and essential oil results obtained as consequences of four repetitions is shown in Table 2. Results were considered significant at P<0.05. The sample size (n) for every test result (significant two-tailed), degrees of freedom (df) and the level of significance (p) are also given. The standard version of SPSS for Windows 11.0.0. SPSS Inc. 1989-2001 and Microsoft Excel XP programs were used in the preparation and evaluation of the data.

RESULTS AND DISCUSSION

Thirty-three components, representing 83.69% of the total oil, were identified from the essential oil of *S. potentillifolia*. The list of detected compounds with their relative percentage, retention time are given in Table 1, where the components are listed in order of elution from a HP 5 MS column. The essential oil of *S. potentillifolia* was characterized by 1,8-cineole (19.33%), β -pinene (11.97%), α -pinene (8.99%) as the major compounds, followed by caryophyllene oxide (5.79%), p-cymene (4.35%), borneol (3.91%), bornyl acetate (3.33), γ-cadinene (2.80%), β -caryophyllene (2.58%) and γ-terpinene (2.13%). According

to the study of Kıvrak et al. (2009), the main compounds of essential oils obtained from *S. potentillifolia* by steam and hydrodistillation methods were reported as α -pinene (29.2 and 31.3%), β -pinene (14.9 and 14.6%), 1,8-cineole (7.44 and 7.27%), terpinen-4-ol (3.53 and 1.76%), β myrcene (2.83 and 3.13%), limonene (2.64 and 2.77%), sabinene (2.53 and 2.78%), caryophyllene oxide (2.44 and 2.47%) and camphor (2.34 and 1.67%). There were major differences in the percentage composition of essential oil when compared to the results of our study. These differences may be due to the different growth habitat.

The analysis of the essential oil composition of *Salvia* species indicated that essential oils of these species showed significant variations in the concentration of compounds (Flamini et al., 2007; Liang et al., 2009; Ozer et al., 2007; Oztürk et al., 2009; Pitarokili et al., 2006; Salehi et al., 2008). Although, 1.8-cineole was found to be the major compound in the essential oil of some species such as *S. fruticosa* (Sivropoulou et al., 1997), *S. tomentosa* (Haznedaroglu et al., 2001), *S. aramiensis* (Demirci et al., 2002), *S. apiana* (Borek et al., 2006), *S. aucheri* (Özcan et al., 2003).

The essential oil of S. potentillifolia showed relatively low levels of antimicrobial activity against the bacteria tested. According to disc diffusion test results, given in Table 2, S. aureus ATCC 25923 and H. influenzae ATCC 49247 were determined as the most sensitive strains with a 16 mm and 15.75 mm inhibition zones, respectively. As shown in Table 3, S. pyogenes ATCC 19615, H. influenzae ATCC 49247 and 49766 were evaluated as the most sensitive species against the oil with the lowest MIC of 4 µg/ml. The results of both disc diffusion and MIC tests indicated that P. aeruginosa ATCC 27853, K. pneumoniae ATCC 700603 and S. typhimurium ATCC 14028 were the most resistant strains to the essential oil of S. potentillifolia. According to the results of many Salvia studies, Gram (-) bacteria are more resistant to essential oils than Gram (+) bacteria (Delamare et al., 2007; Khalil et al., 2011; Kıvrak et al., 2009). On contrary to these results, S. suffruticosa oil showed significant activity against all Gram (-) bacteria (Norouzi-Arasi et al., 2005). In the study of Bozin et al. (2007), all tested E. coli strains, S. sonei and S. typhi showed high sensitivity to S. officinalis essential oil.

When compared with the antimicrobial activity of *S. potentillifolia* essential oil and antibiotics as statistical, the antibiotics tested against *S. aureus* ATCC 25923, *S. aureus* ATCC 12228, *S. pyogenes* ATCC 19615, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 35218, *E. coli* ATCC 25922, *E. cloacae* ATCC 23355, *S. marcescens* ATCC 8100, *P. vulgaris* ATCC 13315, *H. influenzae* ATCC 49766 strains were determined more effective than the essential oil. However, the oil was as effective as the antibiotic tested against *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (MRSA), *E. faecalis* ATCC 51299 (VRE), *E. faecalis* ATCC 29212, *H. influenzae* ATCC 49247 strains. Some pathogenic microorganisms including Gram -positive (MRSA and VRE) and Gram-negative bacteria

Component ^a	RI	Compositions ^c (%)
Tricyclene	927	0.07
lpha-Thujene	931	1.03
α-Pinene	939	8.99
Camphene	949	1.86
β-Pinene	979	11.97
α-Phellandrene	1005	2.12
Δ ³ -Carene	1011	0.41
ρ-Cymene	1028	4.35
1.8-Cineol	1032	19.33
(Z)-β-Ocimene	1040	0.07
(E)-β-Ocimene	1051	0.03
γ-Terpinene	1062	2.13
cis-Sabinene hydrate	1070	0.53
Terpinolene	1088	0.24
Linalool	1105	0.49
β-Thujone	1115	0.20
Trans-p-Menth-2-en-1-ol	1117	0.16
Camphor	1142	1.69
Trans-verbenol	1144	0.55
Borneol	1165	3.91
Terpinen-4-ol	1177	1.60
α -Terpineol	1189	1.43
Linalyl acetate	1257	0.18
Bornyl acetate	1286	3.33
β-Caryophyllene	1418	2.58
α -Humulene	1453	1.04
β-Farnesene	1461	0.07
Germacrene-D	1481	1.10
Δ-Cadinene	1513	2.80
Spathulenol	1576	1.29
Caryophyllene oxide	1584	5.79
Humulene epoxide-II	1605	1.60
β-Eudesmol	1650	0.75
Total		83.69

Table 1. Chemical composition of S. potentillifolia essential oil.

^a Compounds are generally listed following their elution order.

^b Retention indices on HP 5 MS column. ^c Percentages obtained by GC peak area

like *P. aeruginosa* have developed resistance against antibiotics and have become a serious therapeutic problem worldwide. These bacteria are the major causes of nosocomial infections (Taubes, 2008). To prevent spread of resistance, new antimicrobial agents have been investtigated and essential oils are also one of the alternative agents which have an increased interest among researchers (Kalemba and Kunicka, 2003). Therefore, we thought that these results obtained against resistant bacteria like MRSA and VRE are rather important.

S. potentillifolia essential oil observed a weak bactericidal effect. The most bactericidal activity was obtained against Haemophilus species (4 µg/ml). However, their sensitivities against *S. potentillifolia* essential oil of *H. influenzae* species in Gram (-) bacteria group were unexpected. Inouye et al. (2001) thought that, this might be ascribed hydrophobic outer membrane of *H. influenzae* forming rough colonies.

The antimicrobial activity results obtained in our study showed some discrepancy with those of Kıvrak et al (2009). In this study, *S. aureus*, *M. luteus*, *B. subtilis* and *B. cereus* were the most susceptible species and the essential oil was also inhibited the growth of Gram (-) bacteria such as *K. pneumoniae*, *P. vulgaris*, *S. enteritidis* and *E. coli*. Furthermore, the essential oil was found more active than those of the reference discs. Some other

Bacterial specie	E (mm)	A (mm)	Р	χ²	Ν
S. aureus ATCC 25923 [#]	16.25±0.53	32.50±1.38 (P)	0.004		-
S. aureus ATCC 29213*	12.75±0.40	21.75±0.67 (P)	0.06	12.17	-
S. aureus ATCC 43300*	12.75±0.40	15.50±0.56 (FOX)	0.5		-
S. epidermidis ATCC 12228 [#]	9.25±0.16	20.75±0.01 (VA)	0.01	6.37	-
S. pyogenes ATCC 19615 [#]	14.00±0.00	39.50±1.67 (P)	0.00005	16.46	-
E. faecalis ATCC 51299*	11.75±0.29	14.25±0.40 (VA)	0.5	3.84	-
E. faecalis ATCC 29212*	11.75±0.28	20.00±0.00 (VA)	0.07		-
K. pneumoniae ATCC 13883 [#]	8.00±0.14	26.50±0.79 (CAZ)	0.0003	22.92	-
K. pneumoniae ATCC 700603	-	10.00±0.00 (CAZ)	-		-
<i>E. coli</i> ATCC 35218 [#]	8.00±0.14	20.00±0.00 (AMC)	0.007	14.43	-
E. coli ATCC 25922 [#]	8.50±0.16	20.75±0.72 (AMC)	0.007		-
<i>E. cloacae</i> ATCC 23355 [#]	8.50±0.16	32.50±1.38 (MEM)	0.00003	17.72	-
S. marcescens ATCC 8100 [#]	9.25±0.14	30.75±1.30 (MEM)	0.0001	15.03	-
<i>P. vulgaris</i> ATCC 13315 [#]	9.50±0.18	36.00±0.00 (FEP)	0.00001	19.51	-
P. aeruginosa ATCC 27853	-	31.50±1.12 (MEM)	-	-	-
S. typhimurium ATCC 14028	-	20.00±0.00 (AMP)	-	-	-
<i>H. influenzae</i> ATCC 49766 [#]	12.50±0.00	34.50±1.50 (CXM)	0.0002	14.07	-
H. influenzae ATCC 49247*	15.75±0.58	15.00±0.00 (AMP)	0.8		-

Table 2. Antimicrobial activity of *S. potentillifolia* essential oil against the bacterial strains tested based on disc diffusion method.

E: Essential oil, A: Antibiotics, N: Negative control (empty disc), P: Penicillin G (10 units). FOX: Cefoxitin (30 µg), VA: Vancomycin (30µg), CAZ: Ceftazidime (30µg). AMC: Amoxycillin/clavulanic acid 2:1 (30µg), MEM: Meropenem (10µg), FEP: Cefepime (30µg). AMP: Ampicillin (10 µg), CXM: Cefuroxime sodium (30µg). * Essential oil is as effective as antibiotic. [#] Antibiotic is more effective than essential oil. (±) Standard deviation.

Table 3. Antimicrobial activity of *S. potentillifolia* essential oil against the bacterial strains tested based on MIC and MBC methods.

Bacterial specie		MIC		MBC		
	E (μg/ml)	A (μg/ml)	Ν	E (μg/ml)	A (μg/ml)	
S. aureus ATCC 25923	8	<0.0625 (P)	-	64	0.5	
S. aureus ATCC 29213	16	2 (P)	-	128	2	
S. aureus ATCC 43300	16	128 (OX)	-	128	128	
S. epidermidis ATCC 12228	32	1 (VA)	-	>128	2	
S. pyogenes ATCC 19615	4	<0.0625 (P)	-	8	<0.0625	
E. faecalis ATCC 51299	64	32 (VA)	-	128	>128	
E. faecalis ATCC 29212	64	2 (VA)	-	>128	>128	
K. pneumoniae ATCC 13883	64	0.25 (CAZ)	-	64	0.25	
K. pneumoniae ATCC 700603	>128	64 (CAZ)	-	>128	64	
E. coli ATCC 35218	128	16 (AMC)	-	>128	16	
E. coli ATCC 25922	128	8 (AMC)	-	>128	8	
E. cloacae ATCC 23355	128	<0.0625 (MEM)	-	>128	<0.0625	
S. marcescens ATCC 8100	64	<0.0625 (MEM)	-	64	<0.0625	
P. vulgaris ATCC 13315	16	<0.0625 (FEP)	-	16	<0.0625	
P. aeruginosa ATCC 27853	>128	0.25 (MEM)	-	>128	0.25	
S. typhimurium ATCC 14028	>128	1 (AMP)	-	>128	1	
H. influenzae ATCC 49766	4	0.5 (CXM)	-	4	0.5	
H. influenzae ATCC 49247	4	4 (AMP)	-	4	4	

E: Essential oil, A: Antibiotics, N: Negative control, P: Penicillin G, OX: Oxacillin. VA: Vancomyci, CAZ: Ceftazidime, AMC: Amoxycillin/clavulanic acid 2:1, MEM: Meropenem. FEP: Cefepime, AMP: Ampicillin, CXM: Cefuroxime sodium.

members of the Salvia genus have been subjected to antimicrobial activity evaluation (Akin et al., 2010; Cardile et al., 2009; Kelen and Tepe, 2008; Kotan et al., 2008; Miguel et al., 2011; Ozkan et al., 2010). If compared with our results, S. officinalis from Tunusia (Bouaziz et al., 2009), S. officinalis and S. triloba from South Brazil (Delamare et al., 2007), S. officinalis from Syria (Khalil and Li, 2011), S. lanigera from Cyprus (Tenore et al., 2010) showed higher antimicrobial activity against tested bacteria. Generally, the antimicrobial activity of the essential oils is due to its major compounds. The minor compounds possess also an important role on antimicrobial activity (Ceylan and Fung, 2004). In most of the studies, the antimicrobial activity of Salvia species was considered to be related to 1,8-cineole, β-caryophyllene, thujone, camphor, borneol in their contents (Akin et al., 2010; Delamare et al., 2007; Kelen and Tepe, 2008; Tenore et al., 2010). However, synergistic or antagonistic effects between some components may affect the antimicrobial activity of the oils (Cosentino et al., 1999). Low antimicrobial potential of S. potentillifolia could be associated with the interaction between some compounds in its content.

In conclusion, all of the bacteria tested in this study are pathogenic for humans and cause serious disease. *S. potentillifolia* essential oil was found to be as effective as the antibiotic against MRSA and VRE as statistical. Also, the oil showed high bactericidal activity against *Haemophilus* species. The results obtained at the end of this study can provide support for research of new antimicrobial agents. But, further researches are necessary for the identification and the isolation of microbiologically effective molecules present in *S. potentillifolia* essential oil and its use in clinical therapy.

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