

Phytochemical constituents and biological properties of *Scutellaria condensata* subsp. *pyncotricha*

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Abstract

Scutellaria is widely used in traditional medicine as a medical plant, in Asian countries, especially in China. It has been applied in treatment because of its sedative, antithrombotic, anti-inflammatory, antioxidant, and antiviral effects. *Scutellaria condensata* Rech. f. subsp. *pyncotricha* (Rech. f.) Rech. f. is a wild perennial plant which grows in Iran. In this study, GC-FID and GC-MS were used to examine the hydro-distilled volatile oil from the aerial parts of *S. condensata*. The total phenolic and flavonoids contents were determined by spectrophotometer. Also, the evaluation of antioxidant activity of essential oil and the methanolic extract was carried out using DPPH assay. The antibacterial activity of *S. condensata* essential oil was examined against four Gram-negative and five Gram-positive bacteria. Additionally, the review evaluation of volatile oil compounds of *Scutellaria* species was done. The process led to the identification of thirty-two compounds constituting 96.5% of the volatile oil. The major constituents were found to be linalool (25.3%), carvacrol (16.3%), and (E)-caryophyllene (13.4%). As a result, the highest scavenging activity belonged to methanolic extract (IC₅₀= 38.2 µg/ml), followed by essential oil (IC₅₀= 93.2 µg/ml). The total phenolic and flavonoid content were (120.7 mg GAE/g sample) and (78.6 mg QE /g sample), respectively for the methanolic extract. The essential oil showed moderate to high inhibitory activity against the *Bacillus cereus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Bacillus pumilus*. The study indicates *S. condensata* potential for being a prospective antioxidant and antibacterial source in pharmaceutical and food industries.

Keywords: *Scutellaria condensata* subsp. *Pyncotricha*; phytochemical screening; antibacterial; antioxidant; chemotaxonomy.

Introduction

The *Scutellaria*, belonging to Lamiaceae family, includes nearly 350 species. Being anti-inflammatory, antioxidant, sedative, antiviral and antithrombotic, some *Scutellaria*

species are widely used in traditional medicine in Asian countries, especially in China, Korea, and Japan [1]. Twenty *Scutellaria* species including *S. fragillima*, *S. xylorrhiza*, *S.*

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farsistanica, *S. luteo-coerulea*, *S. glechomoides*, *S. nepetifolia*, *S. theobromina* and *S. persica* as an endemic species currently exist in Iran [2]. Phytochemicals, the secondary metabolites, are the outcome of secondary metabolism in the plant, which are created for defenses and used for determination of taxonomy of plant species. Thus, evaluating the compositions of medicinal plants is a measure for the biological activities of medical plants. Thus, phytochemicals represent the biological activity of medical plants. Natural products have been the source of most of the active ingredients of medicines and drug discovery using natural products is a challenging task for designing new leads [3-6]. *Scutellaria* is a source of biological active compounds such as volatile oils, flavonoids, phenolic compounds, dihydropyrano coumarins, polyhydroxy flavonoids, stilbenes, terpenoids, phenethyl alcohol glycosides, and polysaccharides [7-15]. It is indicated that flavonoids and terpenoids extracted from *Scutellaria* show insect antifeedant, cytotoxic and anticancer activity [16-18]. Extracts and volatile oils of *Scutellaria* species had antimicrobial, antioxidant and phytotoxic activity. Linalool, (E)-caryophyllene, germacrene D and caryophyllene oxide are the most volatile oil constituents [19-21]. The goal of this study is to evaluate the medical effect of chemical constituents, antioxidant and antibacterial activity of essential oil and methanolic extract of the aerial part of *S. condensata subspecies pycnotricha*. According to the impact of chemotaxonomy on prediction the biological activity of medicinal species, the diversity on essential oil composition of *Scutellaria* species was reviewed.

Experimental

Plant material

S. condensata subspecies *pycnotricha* (Boshghabi, Persian name) was founded in Northwest of Sanandaj, Kurdistan Province, Iran, at an altitude of 2400 m in July 2016 (flower stage) and botanist Hossein Maroofi (voucher specimen no 13340) confirmed it in the herbarium of the Institute of Forests and Rangelands Researches, Sanandaj, Iran.

Isolation of essential oil

The air-dried sample of essential oil (120 g) was extracted by hydrodistillation. This process that lasted 4 hours, was completed by Clevenger-type apparatus. The volatile oil was dried by (Na₂SO₄) and held in the closed dark vial at 4 °C before the analysis.

GC and GC/MS analyses

Volatile oil was analyzed qualitatively and quantitatively by GC/Mass and GC/FID. GC/FID was used as an optimum separation method. The injection of the diluted volatile oil in chloroform to Thermoquest GC/FID was done by DB-5 column (60 m × 0.25 mm), so that the temperature of injector was 250 °C, and that of detector was 300 °C. The nitrogen with a flow rate of 1 mL/min was used as carrier gas. The oven temperature was between 60 to 250 °C at the rate of 5 °C/min. At last, it was held for 2 min in isothermal elution situation. Carrier gas Helium was used for GC/MS (Thermoquest-TRACE) and ionization voltage was at 70eV. The mass range in detector was from 43 to 456 m/z. The injection of a mixture of normal alkanes including C₆-C₃₀ to GC/FID was done as method of volatile oil for determination of experimental retention index of compounds. The constituent of volatile oil was specified based on

factors such as comparing the mass spectra by a library (Adams and Wiley), retention index and retention time, then the quantity of specified constituents was determined by relative area percentage of GC/FID (without correction factors) [22].

Preparation of the extract

The maceration methods were used for the methanolic extract of *S. condensata* subsp. *pycnotricha* (200g) for 72 h triplicate in room temperature. The extract was concentrated by a rotary evaporator in 50 °C and reserved in 4 °C.

Phytochemical screening

Qualitative identification of the constituents of *S. condensata* subsp. *pycnotricha* methanolic extract was carried out using the procedures that explained by Kumar Bargah [23].

Measurement of free radical-scavenging activities (DPPH assay)

The capacity of *S. condensata* subsp. *pycnotricha* essential oil and methanolic extract to scavenge DPPH was specified based on the technique reported by Euch et al. [24]. The absorbance of the mixture containing sample and reagent was measured at 517 nm and BHT was used as a control for its compurgation. Quantification of free radical-scavenging activity of the sample was carried out according to the evaluation of the IC₅₀ (sample concentration providing 50% inhibition), by plotting the inhibition percentage against sample concentrations.

Determination of total phenolics content

The Folin–Ciocalteu procedure defined the total phenolics content (TPC) of the *S. condensata* subsp. *pycnotricha* extract [25]. Total phenolic content was reported as milligram gallic acid

equivalents per gram of plant extract (mg (GAE)/g).

Determination of total flavonoid

Total flavonoid content of the methanolic extract of *S. condensata* subsp. *pycnotricha* was determined based on the method reported by Ghasemzadeh et al. [26]. The absorbance of mixture solution was measured to be 430 nm by spectrophotometer. Then, quercetin flavonoid was used to plot a standard curve and the result was represented in mg quercetin/g dry weight.

Antibacterial activities

An experiment was done on essential oil of *S. condensata* subsp. *pycnotricha* against 9 bacteria, including *Bacillus cereus* PTCC1015 (Persian Type Culture Collection number), *Escherichia coli* ATCC25922 (American Type Culture Collection number), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC10031, *Bacillus subtilis* ATCC 465, *Pseudomonas aeruginosa* ATCC85327, *Bacillus pumilus* PTCC1274, *Staphylococcus epidermidis* ATCC12228, and *Enterococcus faecalis* ATCC 29737. The antibacterial activity of essential oil was specified by the disk diffusion method and using Mueller–Hinton Agar plates, then, the results were expressed as inhibition zones. Meanwhile, the MIC values were reported by the broth microdilution assay [27].

Results and discussion

S. condensate has two subspecies in Iran: *S. condensata* Rech.f. ssp. *condensate* and *S. condensata* Rech.f. ssp. *pycnotricha*, but no study on them is available. Kurdistan Province, in the west of Iran, is an appropriate place for finding medicinal plants and introducing their traditional medicine. According to a dry weight of plant and

using hydrodistillation method, the yellow essential oil of *S. condensata* subsp. *pycnotricha* by 0.4% yield (w/w%) was obtained from Clevenger apparatus. The 96.47% of *S. condensata* subsp. *pycnotricha* essential oil constituents were determined by examination of GC/MS and GC/FID spectrum. Based on GC/MS spectrum, thirty-two compounds were recognized and GC/FID was used to examine their quantity. The *S. condensata* subsp. *pycnotricha* compounds were listed in Table 1 which is based on the retention

time of in DB-5 column. Linalool (25.3%), carvacrol (16.26%), (E)-caryophyllene (13.43%), α -terpineol (5.03%), geraniol (4.86%) and caryophylla-4(12), 8(13)-dien-5 α -ol (4.08%) were the major compounds. The oxygenated monoterpenes (37.03%), including Linalool, α -terpineol and geraniol, were the main compounds in *S. condensata* essential oil. There was not any monoterpene hydrocarbon in the essential oil (Figures 1, 2, and 3).

Table 1. Chemical composition of the essential oils of *S. condensata* subsp. *pycnotricha*

	Compound	RI ^a	RI ^b	%Area
1	1-Octen-3-ol	975	974	0.4
2	3-Octanol	987	988	0.2
3	Linalool	1096	1095	25.3
4	α -Terpineol	1185	1186	5.0
5	Nerol	1228	1227	1.3
6	Carvacrol methyl ether	1242	1241	0.5
7	Geraniol	1252	1249	4.9
8	carvacrol	1298	1298	16.3
9	β -Longipinene	1399	1400	0.5
10	(Z)-Caryophyllene	1409	1408	0.2
11	(E)-Caryophyllene	1415	1417	13.4
12	α -Humulene	1452	1452	1.7
13	(E)- β -Ionone	1488	1487	0.3
14	(E,E)- α -Farnesene	1505	1505	0.2
15	Farnal	1507	1508	0.1
16	6-Methyl- α -ionone	1522	1520	0.2
17	<i>cis</i> -Sesquisabinene hydrate	1542	1542	0.2
18	Hedycaryol	1544	1546	1.1
19	E-Nerolidol	1560	1561	2.6
20	Caryophyllene oxide	1584	1582	2.1
21	Fokienol	1594	1596	0.4
22	Humulene epoxide II	1606	1608	0.1
23	Caryophylla-4(12),8(13)-dien-5 α -ol	1638	1639	4.1
24	allo-Aromadendren epoxide	1639	1639	9.8
25	Khusilal	1644	1647	0.1
26	α -Bisabolol	1684	1685	0.3
27	Nonadecane	1902	1900	0.1

28	Methyl hexadecanoate	1919	1921	0.2
29	(Z)-Falcarinol	2031	2035	2.2
30	Heneicosane	2102	2100	0.2
31	(E)-Phytol acetate	2217	2218	0.3
32	Trtriacontane	3304	3300	2.3
total			96.5	

^aRI: retention indices relative to C₆-C₂₄ n-alkanes.

^bRI: retention indices from literature (DB-5 column).

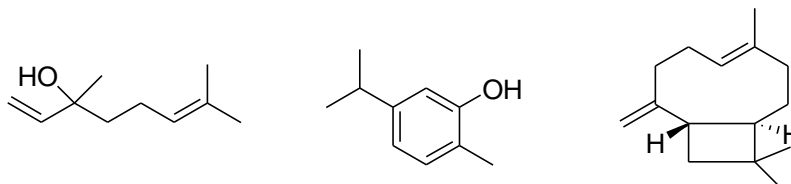


Figure 1. The structure of major compounds from *S. condensata subsp. pycnotricha* essential oil

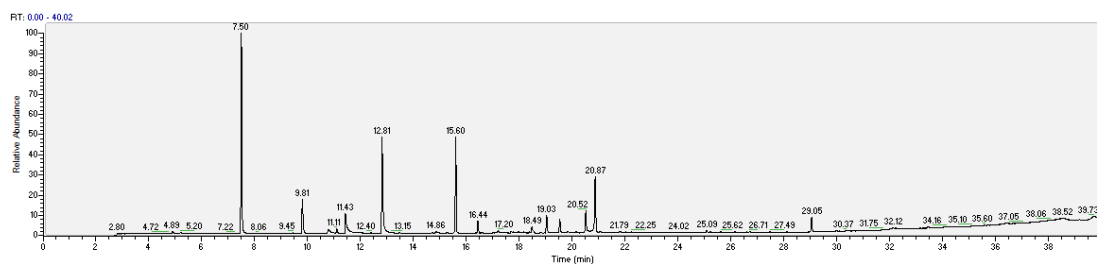


Figure 2. GC-MS chromatogram of *S. condensata subsp. pycnotricha* essential oil

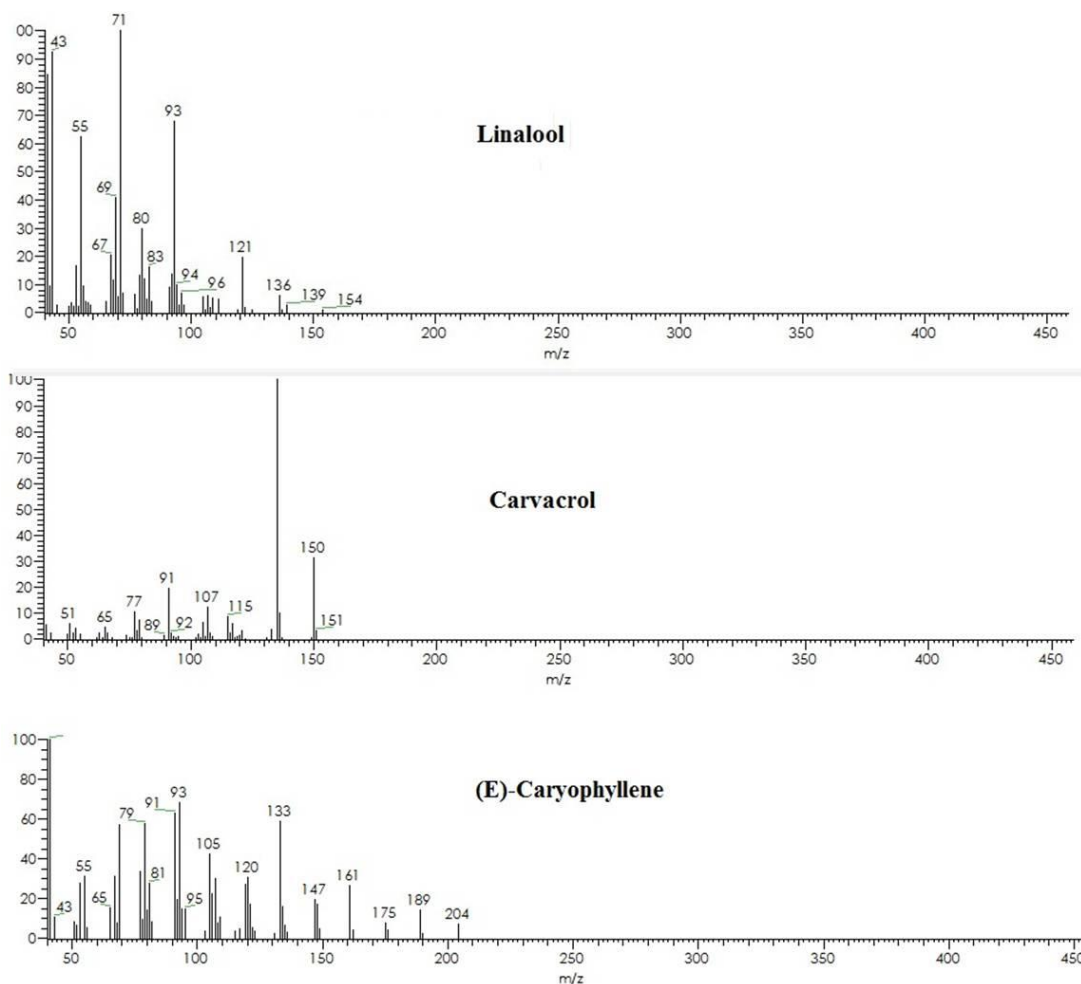


Figure 3. Mass spectra of *S. condensata subsp. pycnotricha* essential oil major constituents

Given to the same metabolism pathway in different species and chemotaxonomy, we can use comparison of essential oil compounds of *Scutellaria* in the taxonomy of *Scutellaria* genus. This comparison is

useful in the prediction of species biological activity. In Table 2, you can find the major compounds, extraction method and biological activity of *Scutellaria* species' essential oils.

Table 2. Volatile oil compounds of *Scutellaria* species

Plant name	Method of extraction	Main compounds and percentage	Biological activity	reference
<i>S. condensata</i> Rech. f. subsp. <i>pycnotricha</i>	hydrodistillation	Linalool (25.3%) Carvacrol (16.26%) (E)-Caryophyllene (13.43%)	antibacterial and antioxidant activity	This study
<i>S. lateriflora</i>	hydrodistillation	δ -cadinene (27%) calamenene (15.2%) β -elemene (9.2%),	-	[14]
<i>S. barbata</i>	hydrodistillation	Hexahydrofarnesylacetone (11.0%) 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (7.8%) Menthol (7.7%)	Antimicrobial activity	[20]

<i>S. pinnatifida</i> a. Hamilt. Subsp. <i>Mucida</i>	hydrodistillation	Germacrene D (9.56 %) α -Pinene (5.37 %) Bornyl Cinnamate (4.09%)	-	[29]
<i>S. pinnatifida</i> subsp. <i>pinnatifida</i>	hydrodistillation	Methyl chavicol (Z)- β -Ocimene (E)- β -Ocimene	-	[29]
<i>S. pinnatifida</i> subsp. <i>alpina</i>	hydrodistillation	Germacrene D (39.7%) β -Caryophyllene (15.0%) δ -Cadinene (5.3%)	-	[29]
<i>S. laeteviolacea</i>	hydrodistillation	1-Octen-3-ol (27.72%) Germacrene D (21.67%) β -Caryophyllene (9.18%)	-	[30]
<i>S. baicalensis</i>	steam distillation	Germacrene D (19.44%) Caryophyllene (18.9%) γ -Elemene (6.23 %)	-	[31]
<i>S. orientalis</i> L. subsp. <i>virens</i>	hydrodistillation	β -Caryophyllene (22.08%) γ -Cadinene (19.92%) Camphene (6.00%)	-	[32]
<i>S. orientalis</i> ssp. <i>alpina</i>	hydrodistillation	hexahydrofarnesylacetone (11.7%) hexadecanoic acid (7.6%) caryophyllene (7.4%)	-	[33]
<i>S. utriculata</i>	hydrodistillation	linalool (20.1%) 4-vinyl guaiacol (15.5%) α -terpineol (8.9%)	-	[33]
<i>S. immaculata</i>	hydrodistillation	Acetophenone (30.39%) Eugenol (20.61%) Thymol (10.04%),	antioxidant activity	[34]
<i>S. schachristanica</i>	hydrodistillation	Acetophenone (34.74%) Linalool (26.98%) Eugenol (20.67%)	antioxidant activity	[34]
<i>S. ramosissima</i>	hydrodistillation	Germacrene D (23.96%) β -Caryophyllene (11.09%) Linalool (9.63%)	antioxidant activity	[34]
<i>S. repens</i>	steam distillation	aromadendrene(30.7%) β -funebrene (15.0%) β -gurjunene(8.0%)	antimicrobial activity	[35]
<i>S. grossa</i>	steam distillation	Linalool (37.0 %) 1-Octen-3-ol (32.0 %)	antimicrobial activity	[36]
<i>S. albida</i> ssp. <i>abida</i>	steam distillation	Linalool (52.63 %) trans-Nerolidol (9.03 %)	antimicrobial activity	[37]
<i>S. litwinowii</i>	hydrodistillation	(E)- β -Farnesene (20.3 %) Germacrene D (16.9 %)	-	[38]
<i>S. californica</i>	Headspace solid phase microextraction	β -Caryophyllene (56.6%) Germacrene D (6.9%) Methyl-2-metylbutyrate (4.9%)	-	[39]
<i>S. brevibractetata</i> subsp. <i>brevibracteata</i>	hydrodistillation	β -caryophyllene (22.8%) caryophyllene oxide (16.0%)	-	[40]
<i>S. brevibractetata</i> subsp. <i>subvelutina</i>	hydrodistillation	β -Caryophyllene (28.3%), Linalool (12.4%) Hexadecanoic acid (10.8%)	-	[40]

<i>S. brevibractetata</i> subsp. <i>pannosula</i>	hydrodistillation	β -Caryophyllene (36.4%) α -Cadinol (9.8%) δ -Cadinene (7.0%)	-	[40]
<i>S. rupestris</i> ssp. <i>adenotricha</i>	hydrodistillation	Linalool (38.8%) Geraniol (8.1%) α -Terpineol (7.1%)	Antimicrobial activity	[41]
<i>S. sieberi</i>	hydrodistillation	Linalool (22.7%) β -Caryophyllene (14.2%) (2R, 5E)-Caryophyll-5-en-12-al (6.3%)	Antimicrobial activity	[41]
<i>S. luteo-caerulea</i>	hydrodistillation	trans-Caryophyllene (25.4%) Germacrene D (7.9%) Linalool (7.4%).	-	[42]
<i>S. volubilis</i>	hydrodistillation	Germacrene D (20.4%) B-caryophyllene (17.5%) α -humulene (14.7%)		[43]
<i>S. strigillosa</i>	hydrodistillation	Germacrene D (37.78 %) 1-octen-3-ol (8.96 %) bicyclogermacrene (3.67 %)	Phytotoxic and Antimicrobial Activities	[21]
<i>S. havanensis</i>	hydrodistillation	β -caryophyllene (75.6 %), α -humulene (11.6 %) caryophyllene oxide (2.6 %)	-	[44]

It is reported that according to preliminary phytochemical tests, the secondary metabolite in methanolic extract of *S. condensata* subsp. *pyncotricha* were phenolic, tannin, saponin, flavonoid, phlobatannin and steroids (Table 3). Investigations indicated that flavonoids, tannins, saponins, alkaloids, coumarins and steroids were the main secondary metabolite of *Scutellaria* species. As the result of such examination, the phenolic compounds were the major secondary metabolite of *Scutellaria* species. It is also revealed that the antioxidant activity of the plant was due to the phenolic compounds [28]. So, free radical scavenging capacities of essential oil and methanolic extract of *S. condensata* subsp. *pyncotricha* were measured by DPPH assay (Table 4). As a result, the highest scavenging activity

belonged to methanolic extract ($IC_{50}=38.2 \mu\text{g/mL}$), followed by essential oil ($IC_{50}=93.2 \mu\text{g/ml}$), compared with BHT as a positive control ($IC_{50}=24.0 \mu\text{g/mL}$). According to the spectrophotometer results, the total phenolic and total flavonoid content of methanolic extract of *S. condensata* were 120.7 mg GAE/g sample and 78.6 mg QE /g sample, respectively (Table 4). The high antioxidant activity of methanol extract of *S. condensata* subsp. *pyncotricha* was related to its phenolic content. The essential oil of *S. condensata* subsp. *pyncotricha* was tested against four Gram-negative and five Gram-positive bacteria. As a result, the essential oil showed moderate to high inhibitory activity against the *Bacillus cereus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Bacillus pumilus* (Table 5).

Table 3. Preliminary phytochemical screening of *S. condensata subsp. pycnotricha* methanolic extract

phytochemical constituents	Test methods	result
Carbohydrates	Fehling's solutions	-
Glycosides	keller-kilani	-
Pheolics	ferric chloride	+
Tannins	ferric chloride	+
Alkaloids	Dragendorff's	-
Proteins & Amino acids	Ninhydrin test	+
Saponins	Foam test	+
Flavonoids	Alkaline reagent	+
Phlobatannins	Precipitate test	-
Terpenoids	-	+
Steroids	Salkowski,s test	+

+ Presence; - Absence

Table 4. Antioxidant activity, total phenolic content and total flavonoid content of the essential oil and methanolic extract of *S. condensata subsp. pycnotricha*

Extracts	DPPH assay	TPC	TFC
	IC ₅₀ (µg/mL)	mg gallic acid /g Sample	mg quercetin /g Sample
Essential Oil	92.2±0.6	-	-
Methanol extract	38.2±0.3	120.7±0.4	78.6±0.9
BHT	24±0.4	-	-

Values were the means of three replicates ± standard deviation

Table 5. *In vitro* antibacterial activity of *S. condensata subsp. pycnotricha* essential oil

Sample	Microorganism								
	<i>Bacillus pumilus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>
Essential Oil	14 ^a (15) ^b	11 (>15)	12 (15)	18 (7.5)	11 (15)	11 (15)	14 (15)	14 (15)	-
Tetracycline ^c	nt	21 (3.2)	20 (3.2)	nt	nt	nt	- (nt)	34 (1.6)	nt
Gentamicin ^d	nt	- (nt)	- (nt)	nt	nt	nt	23 (3.2)	- (nt)	nt
Ampicillin ^e	15 (15)	14 (15)	13 (15)	nt	nt	nt	12 (15)	19 (15)	nt

^aZone of inhibition (in mm) includes diameter of the disc (6 mm), ^bMinimum inhibitory concentration values as mg ml⁻¹, (-): Inactive, (7 – 13): moderately active, (> 14): highly active, nt: not tested, ^cTested at 30 µg/disc, ^dTested at 10 µg/disc, ^e: Tested at 10 µg/disc.

Conclusion

The present study is the first one on the phytochemical analysis as well as antioxidant and antibacterial activities of *S. condensata subsp. pycnotricha* essential oil and extract. The study on volatile oil compounds of *Scutellaria* genus can be used for the further study on the taxonomy of *Scutellaria* genus and their biological activity prediction. The high antioxidant activity and the medium antibacterial inhibitory effect of

the *S. condensata subsp. pycnotricha* indicate its potential for being a prospective antioxidant and antibacterial source in pharmaceutical and food industries.

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