Herbal Medicines Journal **Summer 2017; Vol. 2, No. 3:97-104**
DOI: https://doi.org/10.22087/hmj.v0i0.623

Original Article

The Antioxidant Properties and Chemical Composition of *Stachys inflata Benth* Leaves Grown Wild in Lorestan Province

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Received: 05.07.2017; Accepted: 12.08.2017

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Abstract

Background and Aim: In recent years there has been an increasing interest in herbal medicines and natural antioxidant products to be used as medicines and food additives. The present research identified the various antioxidative activities of ethanol and methanol extracts, and chemical composition of *Stachys inflate Benth* leaves essential oil.

Materials and Methods: This experimental study was carried out in 2015 in Lorestan University of Medical Sciences. Ethanol and methanol extracts of *Stachys inflata Benth* leaves were primarily prepared. The total antioxidant capacity of samples was assessed by phosphomolybdate method. Total phenol content and total flavonoid content were determined by Folin-Ciocalteu and Zhishen methods. The components of *Stachys inflate Benth* leaves essential oil were analyzed with gas chromatography/mass spectrometry (GC/MS).

Results: It was demonstrated that the total antioxidant capacity of ethanol and methanol extracts of Stachys inflata Benth leaves were 4.30±0.17; 3.63±0.23 mg of ascorbic acid equivalents /g of extract. Moreover, the total phenol content ethanol and methanol extracts of Stachys inflata Benth leaves were 830.334±26.41; 384.29±13.37mg of gallic acid equivalents /g of extract, and flavonoid content ethanol and methanol extracts of Stachys inflata Benth leaves were 17.09±0.154; 31.18±1.03 mg of quercetin equivalents /g of extract. GC/MS data and retention indices of *Stachys inflate Benth* leaves essential oil samples were used to identify 54 constituents. These compounds make up a total of 48.87 percent essential oil. Phenol, 2-methyl-5(1-methyleth; Phenol, 5-methyl-2-(1methyleyth; 2-pentadecanone and hexadecanoic acid are the major compounds of *Stachys inflate Benth* leaves essential oil. This study indicated that *Stachys inflate Benth* leaves essential oil has remarkable antioxidant properties. Furthermore, *Stachys inflate Benth* leaves essential oil was identified as an easily accessible source of natural antioxidants such as Phenol,2-methyl-5(1-methyleth; Phenol,5-methyl-2-(1methyleyth; 2-pentadecanone,6,10,14-trimet; Veridi florol; 2-cycohexen-1-on,2-methyl-5- and 1H-cycloprop[e]azulen-7-ol,dec. It may also be suitable to be used in food and pharmaceutical applications.

Conclusion: This study demonstrated that Stachys inflata Benth leaves are easily accessible sources of natural antioxidants. Therefore, they may be suitable to be used in food and pharmaceutical applications.

Keywords: Antioxidant activity, Chemical composition, Stachys inflata Benth

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Please cite this article as: Ahmadvand H, Farajollahi S, Amiri H, Amiri A. The Antioxidant Properties and Chemical Composition of *Stachys inflata Benth* Leaves Grown Wild in Lorestan Province. Herb. Med. J. 2017;2(3):97-104.

Introduction

Oxidative stress is the main cause and accelerating factor of some diseases such as nephrotoxicity, diabetes, cancer, coronary heart disease etc. (1, 2). An imbalance between oxidants and antioxidants causes oxidative stress (3). The free radical theory of aging holds that organisms grow old due to the accumulation of free radical damage that occurs in cells by the passage of time (4). A free radical is considered to be any atom or molecule containing a single unpaired electron in an outer shell (5). Free radicals are chemically reactive. In most biological systems, free radical damage is linked to oxidative damage (6). Antioxidants play an important role in scavenging free radicals and lipid peroxidation, and also in preventing the break of a chain of oxidative reaction (7), therefore, antioxidants protect humans against different diseases related to free radicals (8). Inhibition of oxidative reactions in foods, pharmaceuticals and cosmetics, and the capacity to prevent diseases that cause oxidative stress in the human body are some of the positive potential uses of the antioxidants (9).

Various materials have been proposed as antioxidants. Phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes and procyanidins that clean up free radicals were recently introduced in dose-dependent methods (10). Phenolic compounds have a broad spectrum of usage as compounds that act against free radicals and play a significant role in curbing them (11). *Stachys inflata Benth* (the Family Lamiaceae, the Subfamily Laminoideae) is one of the greatest genera of the Lamiaceae. The genus includes between 275 to 300 species worldwide (12). Iran, as a territory with diversity of *Stachys*, hosts about 35 species (12, 13). The main taxonomic problem of *Stachys* is the delimitation of taxa within natural groups defined as formal sections (12). Stachys inflata Benth is an Iranian traditional medicine which is used as a remedy to treat infective, rheumatic and other inflammatory disorders (14). *Stachys inflata Benth* is one of the species that is native to Iran. Its Persian name is *poulk, or gole arghavan* (14). The aerial parts of this plant are used to make a kind of herbal tea to treat various infections and many inflammatory disorders such as asthma and rheumatoid arthritis (14). A hydro-alcoholic extract from the aerial parts of Stachys inflata Benth showed anti-inflammatory activities (14). *Stachys inflata Benth* has been dealt with in several phytochemical analyses in Iran (15).

Due to the utile properties of this plant, particularly in therapeutic uses, and also since its chemical composition and antioxidant properties have not been studied in Lorestan province before, we decided to determine the chemical composition of essential oil and measure the antioxidant properties of ethanol and methanol extracts of this plant in Lorestan province.

Materials and Methods

Isolation of the Essential Oil of *Stachys inflata Benth* Leaves:

Stachys inflata Benth leaves were prepared in July 2014 from farms located in Aleshtar around Garin in Lorestan province (western Iran). SIBL were collected during flowering stage and were air-dried at ambient temperature in the shade separately. The volatile oil of the SIBL was obtained by hydrodistillation using a Clevenger-type apparatus for 3 h giving yellow oil in 1% yield. The oil was decanted and dried over anhydrous sodium sulfate. The oil was dried over anhydrous sodium sulfate and stored at 4ºC. The voucher specimen was deposited at Herbarium of the Agriculture and Natural Resources Research Center of Lorestan Province, Khorramabad, Iran.

Isolation of the Ethanol and Methanol Extracts of *Stachys inflata Benth* Leaves:

After removing the impurities, they were washed to remove contaminants and then spread in shade to dry. Leaves of the plants were collected during flowering stage and were air-dried at ambient temperature in the shade separately. The powder obtained from leaves was taken in a Soxhlet extractor and extracted with ethanol

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Table 1: Total antioxidant activity, total phenols content and total flavonoids content of *Stachys inflata Benth* leaves.

	SILEE	SILME	P value
Total antioxidant activity (nmol ascorbic acid equivalents/g extract)	4.30±0.17	3.63±0.23	0.031
Total phenols (mg of gallic acid equivalents (GAE)/g extract)	830.334±26.41	384.29±13.37	0.02
Total flavonoids (mg of of quercetin equivalents/g extract)	17.09±0.154	31.18±1.03	0.001

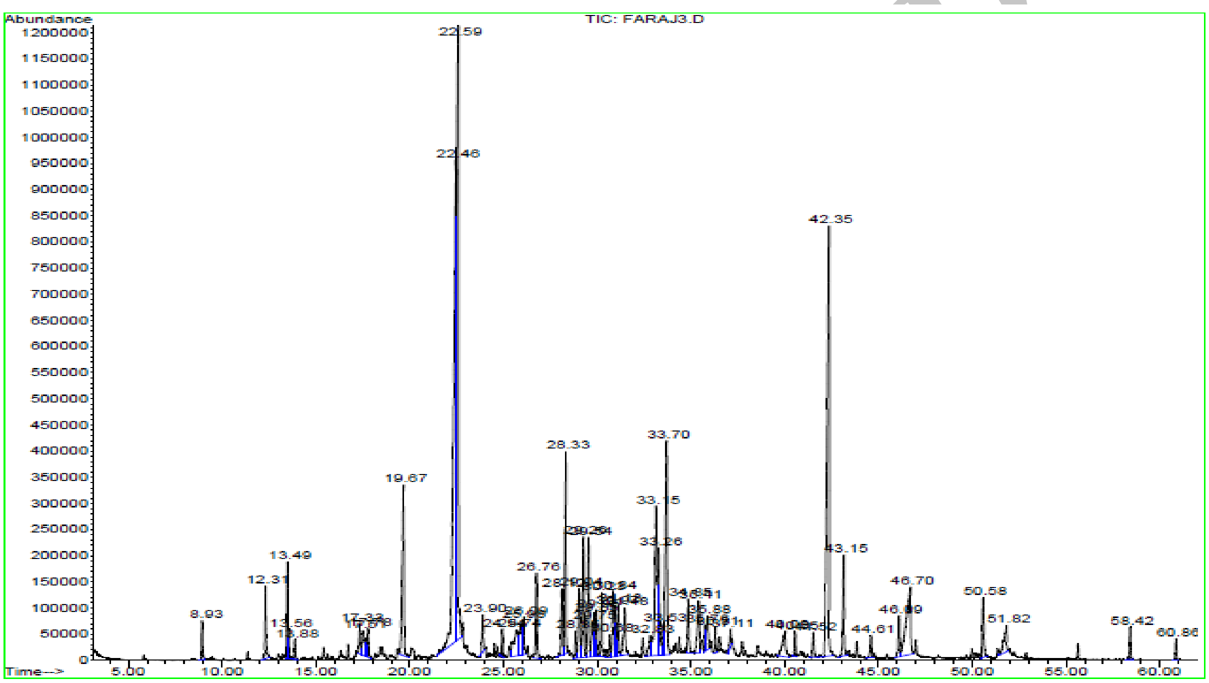

Figure 1. Typical gas chromatogram of *the Stachys inflata Benth* leaves essential oil.

or methanol. The solvent was recovered by distillation. The residue was concentrated, dried and stored for further experiment and analysis.

Total Flavonoid Content:

The total flavonoid content was measured in accordance with our previous study (Ahmadvand, 2014). Standard curve of flavonoid was prepared (y=o.o45x+o.o309 Standard formula). The results (mean±SD) were expressed as mg of quercetin equivalents /g of extract (16).

Total Phenol Content:

The total phenol content was measured in accordance with the previous study (17). Seven hundred and fifty microliter Folin-Ciocalteu was poured in all pipes, and then 100 µl of the extract of plant was added. Subsequently, it was vortexed and after being kept for 5 minutes in room temperature, 750 microliter sodium carbonate was added. Again, solutions were stored at room temperature for 40 minutes and then sample absorbance at 725 nm was read. Phenol standard curve was prepared (y=o.oo8x+o.1649 standard formula). Results (mean±SD) were reported in milligram equivalent by Gallic acid per gram of dry weight.

Total Antioxidant Capacity:

The Total antioxidant capacity was measured in accordance with previous the study (17). One ml

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reagent and 0.1ml of the extract or essential oil were added to all pipes. After vortexing the obtained solutions, they were placed in boiling water for 90 minutes, and then, sample absorption at wavelength of 695 nm was read. Standard curve of antioxidants was prepared (y=o.7633x+o.o149 standard formula).

Table 2: Chemical composition of the *Stachys inflata Benth* leaves essential oil (SIBL).

Compound	Retention Time (min)	Yield%
1-octen-3-oL	8.92	0.43
1-octanol	12.31	1.16
Linalool	13.49	1.31
Ninanal	13.57	0.31
Cyclopentanol,1-methyl	13.89	0.23
Linalylpropionate	17.33	0.49
Cyclohexanol2-methyl-5(1-met)	17.51	0.64
Benzoic acid	17.77	0.54
2-cycohexen-1-on,2-methyl-5-	19.67	4.10
Phenol,2-methyl-5-(1-methleth	22.46	15.27
Phend,5-methyl-2-(1-methyleth	22.59	14.41
2-cy clohexen-1-one,3-methyl-6-	23.91	0.55
Alpha-copaene	24.94	0.31
Trans – caryophyllene	26.76	1.31
Alpha.-caryophyllene	28.11	0.96
Hexadecana ,2,6,10.14,tetramethyl	28.32	3.55
Azulene,1,2,3,3a,4,5,6,7-octan	28.85	0.45
Naphthalene,1,2,3,4,4a.5,6.,8a	29.04	1.45
Germacrene D	29.26	2.01
3-Buten-2-one,4-2,6,6,-trimeth	29.54	2.17
1H-cycloprep[e]azulene 1a,2,3,	29.75	0.87
Germacrene B(CAS)	29.85	0.60
Naphthlene,1,2,4,5,6,8a-hexa	29.95	0.60
Beta-Bisabolene	30.32	0.99
Naphthalee,1,2,3,5,6,8a-hexan	30.85	1.17
5-Amino-1-ethylprazole	30.97	0.81
Naphthalene ,1,2,3,4,4a,5,6,9a-	30.68	0.36
(1s*,6s*,7s*)-Tricyclo[5.3.2.o	31.13	1.05
2(4H)-Benzofuranon,5,6,7,7a-t	31.48	1.13
Caryophylla-3,8(13)-dien-5-beta	32.83	0.54
1H-cycloprop[e]azulen-7-ol,dec	33.15	3.97
Caryophllene oxide	33.26	2.03
Hexadecane (CAS)	33.53	0.48
Veridhflorl	33.07	4.47
meGastigmarienine 2	34.85	0.97
Naphthalene ,1,2,3,4,4a,5,6,8a-	35.41	1.41
2-Napthalenemethanol,decahydr	35.75	0.44
t-muurolol	35.88	0.67

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Trans-7-tetradecene	36.31	0.35
Heptadecan	37.11	0.28
Tetradecanoic acid	40.03	1.09
Octade3cane	40.55	0.30
Isopropyi myristate	41.52	0.34
2-pentacanone,6,10,14,trimet	42.36	11.2
1,2Benznedicarboxylic acide .b	43.15	1.68
5,9,13,-pentadecatrien-2-one,G,	44.60	0.36
Dibuty phthalate	46.09	0.70
Hexadecanoic acid (CAS)	46.96	3.70
Phytol	50.58	1
Ethyl linoleate	51.82	0.97
Hexanedieic acid ,bis (2-ethyhe)	58.42	0.48
Pentacosane (CAS)	60.86	0.27
Decanoic acid (CAS)	77.81	3.28
Hexadecana ,2,6,10.14,tetramethyl	28.32	3.55

Results (mean±SD) were reported in milligram equivalent by ascorbic acid per gram of dry weight

Identification of the Components of *Stachys inflate Benth* Leaves Essential Oil by Gas Chromatography/ mass Spectrometry

Chemical composition of *Stachys inflates Benth* leaves essential oil was identified by Gas chromatography/ mass spectrometry in accordance with the previous study (17).

Statistical Analyses

Data were analyzed using unpaired Student's t-tests. Statistical analyses were performed using the software package used for statistical analysis version 13 (SPSS) for windows software. A *P* value of < 0.05 was considered statistically significant.

Results and Discussion

The phenolic compounds have many beneficial effects in biological systems such as antidiabetic (18), antibacterial (19), immune stimulating (20), anti-allergic (21), antihypertensive (22), antithrombotic (23), hepatoprotective (24), anti-inflammatory (25), anticarcinogenic (26) systems. Flavonoids constitute an important group of polyphenols with antioxidant, antiviral, antibacterial (27, 28) and anti-inflammatory activities (29).

The present study has confirmed a strong correlation between antioxidant activities and chemical composition of *Stachys inflata Benth* leaves.

Total Antioxidant Activity

The total antioxidant activity of *Stachys inflata Benth* leaves ethanol extract (SILEE) and *Stachys inflata Benth* leaves methanol extract (SILME) were measured to be (4.30±0.17; 3.63±0.23) nmol of ascorbic acid equivalents/g SILEE or SILME. The total antioxidant activity of SILEE was significantly (1.18-fold) higher than that of SILME (P=0.03١) (Table 1).

Khanavi et al. reported the total antioxidant capacity of various *Stachys* species such as *Stachys inflata Benth*, *Stachys* **tnnevis**, *Stachys byzantina*, *Stachys subaphylla*, *Stachys laxa* and *Stachys persica* aerial parts extract prepared were 31.08±0.53, 9.11±0.69, 9.33±1.03, 17.11±0.98, 35.06±1.06 and 61.43±4.35of Gallic acid equivalents/g extract (30). The difference in antioxidant activity of extracts may be attributed to the differences in the amount and kind of existing antioxidant compounds in them. Our recent results and another studies indicated that Stachys inflata Benth leaves possess a good antioxidant activity.

Total phenols

The total phenol of SILEE and SILME were (830.33±26.41) and (384.29±13.337) Gallic acid equivalents/g SILEE or SILME (Table 1). The total phenol of SILEE was significantly (2.16-fold) higher than that of SILME (P=0.02) (Table 1).

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Total phenol shown by the SILEE or SILME may be due to the presence of different phenolic compounds. Khanavi et al. reported that the total antioxidant capacity of various Stachys species such as Stachys inflata Benth (1478.81±44.32), *Stachys tnnevis* (430.58±29.85), *Stachys byzantine* (638.30±30.61), *Stachys subaphylla* (1016.04±76.60), *Stachys laxa* (2089.99±157.13) and *Stachys persica* (3294.96±313.87) (30). Mohammadi et al. reported that the total phenol of *Stachys inflata Benth* aerial parts extract prepared was 129.96±5.6 of Gallic acid equivalents/g extract (31). Salar Bashi et al. reported that the total phenol of *Stachys parriflora L* aerial parts extract prepared was 20.89 of Gallic acid equivalents/g extract (32). Also, Ebrahimabadi et al. reported that the total phenol of *Stachys inflata Benth* aerial parts different extracts prepared varied from 28.08±0.87 to 54.08±1.62 of Gallic acid equivalents/g extract (33).

Total flavonoids

The total flavonoid of SILEE and SILME were (17.09±0.154) and (31.18±1.03) mg of quercetin equivalents/g SILEE or SILME. The total antioxidant activity of SILEE was significantly (1.82-fold) lower than that of SILME (P=0.00۱) (Table 1).

Total flavonoid shown by the SILEE or SILME might be due to the presence of different flavonoids such as catechin, epicatechin and myricetin. Mohammadi et al. reported that the total flavonoid of *Stachys inflata* Benth aerial parts extract prepared was 29.62±1.4 of quercetin equivalents/g extract (31). They also reported that the total flavonoid of *Stachys inflata Benth* aerial parts extract prepared was 29.62±1.4 of Gallic acid equivalents/g extract (31). Moreover, Salar Bashi et al. reported that the total phenol of *Stachys parriflora L* airal parts extract prepared was 6.22 of Gallic acid equivalents/g extract (32). The difference in the total flavonoid content of extracts might be attributed to the differences in the amount and kind of existing antioxidant compounds in them.

The Chemical composition of the *Stachys inflata Benth* Leaves *Essential* Oil (SIBL)

The amount of the essential oil obtained from SIBL was 1% (W/W). The results of the GC-MS analysis of the oils have been indicated in table 2.

A typical gas Chromatogram of *Stachys inflata Benth* leaves essential oil has been indicated in Fig 1.

Fifty-four compounds of SIBL were identified. The main constituents found in the SIBL were Phenol,2-methyl-5(1-methylethyl (Carvacrol) (15.27%); Phenol,5-methyl-2-(1methyleyth (14.41%); 2-pentadecanone,6,10,14-trimet (11.02%); Veridi florol (4.47%); 2-cycohexen-1-on,2-methyl-5- (4.10%); 1H-cycloprop[e]azulen-7-ol,dec (Spathulenol) (3.97%); Hexadecanoic acid (3.70%); Decanoic acid (CAS) (3.28%); 3-Buten-2-one,4-2,6,6,-trimeth (Ionone) (2.17%); Caryophllene oxide (2.03%); Germacrene D (2.01%) and Naphthalene,1,2,3,4,4a.5,6.,8a (1.45%).

Yavari and Shahgolzari reported that the main constituents found in the *Stachys inflata Benth* aerial parts prepared from Touyserkan, Hamedan Province, Iran were Caryophyllene oxide, E-citral, Z-citral, Spathulenol, ar-Curcumine, 1,8-Cineol, limonene and α-Terpineol (34). Yavari and Shahgolzari reported that the main constituents found in the different populations of *Stachys inflata Benth* aerial parts prepared from Khan-gormaz protected area, in Touyserkan, Hamedan Province, Iran were Caryophyllene oxide (0-22.49%), Dibutylphthalate (0.47-34.66%), Spathulenol (20.15-41.64) and caryophyllene (2.15-24.81%) (34). Samanehsadat Mahzooni-kachapi et al. reported that the main constituents found in the S. lavandulifolia Vahl. aerial parts prepared from Baladeh Mountain area (Mazandaran province, Iran) were Hexadecanoic acid (3.9%), α-cadinol (2.6%), α-pinene (13.7%), germacrene-D (8.9%), β-pinene (7.0%), myrcene (4.5%), β-phellandrene (5.7%), spathulenol (3.5%), δ-cadinene (2.0%) and Z-ocimene (3.4%), (35). The results of this study indicate that the composition of volatile oil of *Stachys inflata Benth* is similar to those with different quantities but the composition of volatile oil of *Stachys inflata Benth* is not similar to those which are reported from another study. The observed differences might be due to using different parts of the plant for analysis, as well as various environmental and genetic factors, different chemotypes and the nutritional status of the plants and also other factors that can influence the oil composition.

Conclusion

This study indicated that the essential oil of *Stachys inflata Benth* leaves is an easily accessible source of natural antioxidants such as Carvacrol, Hexadecanoic

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acid, Decanoic acid, Caryophllene oxide, Germacrene D and Naphthalene that might be suitable to be used in food and pharmaceutical applications.

Acknowledgment

The authors wish to thank the vice-chancellor for research in Lorestan University of Medical Sciences, Lorestan. Iran, and Razi Herbal Medicines Research Center in the same university.

Conflict of Interest

The authors declare that they have no conflict of interest.

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