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Molecular Profiling of the Essential Oil of *Teucrium stocksianum* Collected at Three Different Stages

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Abstract: A plethora of research studies indicated that most of the synthetic drugs are associated with cumbersome effects.Due to high efficacy and safety profile of plant based natural products, numerous researchers are considering it a good alternate of the synthetic drugs. In the current studyessential oil was extracted from *Teucrium stocksianum* collected at three stages i.e. before bloom (BB), full bloom (FB) and after bloom (AB). Qualitative and quantitative variation in the chemical composition of essential oil of *T.stocksianum* was recorded. Highest numbers (38) of compounds were observed in the essential oil of FB. While 28 components with different percentages were detected in each sample extracted from the aerial parts collected before and after full bloom of the plant. The major component of each samples was, caryophylleneoxid (37%), santalol,cis,alpha- (16.26%) and component No 22 (38.17%) in BB, FB and AB respectively.

Key words: *Teucrium stocksianum* · Essential oil composition

INTRODUCTION

Plant kingdom gathers multiple importance and are playing diversified role in the survival and prosperity of human beings. Plants are used as food, shelter, spices, cosmetics and medicines. The medicinal values of the plants are due to the presence of different phytochemical constituents, known as secondary metabolites [1]. Medicinal plants are most frequently used in indigenous medicines around the globe. Plants based remedies are effective, economical possesses strong safety profile and are associated with minimal side effects [2].

Family Lamiaceae have cosmic commercial and pharmacological importance. Different species of this family are used from time immemorial i.e. *Ajuga* species are traditionally used as analgesic [3-4], *Calamintha* is used for skin diseases [5], *Dracocephalum* is used as anti-fever [6], *Mentha*is used as stomachic antimicrobial [7], *Nepeta* species for gastro-diseases [8-9], *Ocimum* for infections [10] and *Salvia* are used for its insecticidal and antimicrobial activities [5, 11]. Most of the herbs of this family are used as remedy for different diseases. *Ocimum basilicum* shows antimicrobial effect and is use in the periodontal diseases [12-13]. Some clinical evidences indicate the use of this herb as anti-plaque agent in humans. *Stachys officinalis* have high value in folk medicines due to its astringent,anti-diarrhea propertiesand for relief of gums, mouth and throat irradiations [14]. *Hyssopus officinalis*(hyssop) contains volatile oils which have been used with tea to treat cough, cold, fever and sore throat [15]. *Scutellaria latiliflora* has been reputed to have tranquillizing activity [16]. Aqueous extract of oregano (*Origanum vulgaris*), rosemary (*Rosmarinus officinalis*), sega (*Salvia officinalis*) and thyme (*Thymus vulgaris*) have shown strong antioxidant activities [17-19].

One of the predominant features of the family Lamiaceae is the essential oil, which are the typical component of most of the members of this family which imparts massive medicinal and commercial importance [20]. The essential oils are composed of copious useful compounds having significant medicinal value such as

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menthol and thymol. These plants are mostly used in various medicinal preparations, food and cosmetics. likewise, *spearmint, pennyroyal, mint, peppermint* and *thyme* oils are also important in many ways [21]. Most of the essential oils of this family have revealed insecticidal activities [22]. In our previous research project we have reported the analgesic activity of the methanolic extract and essential oil of *T. stocksianum* [20]. The aim of our present work was to determine the variation in the chemical composition of the essential oil of *T. stocksianum* collected at three different stages in-order to ascertain the most suitable time for collection of the plant for the extraction of essential oil.

MATERIALS AND METHODS

Plant Material: The aerial parts *T. stocksianum* were collected from District Swat, in three different stages of plant growth cycle i.e before flowering (BF), in full bloom (FB) and after flowering (AF) stage. Plant was cleaned from dirt by washed with tap water followed by washing with distilled water. Plant specimens were identified by Dr. Nisar Professor Department of Botany, University of Malakand, Pakistan. An authenticated whole plant voucher specimen (H.UOM.BG.199b) was deposited in the herbarium of the same University.

Essential Oil Extraction: Fresh aerial parts of the *T. stocksianum* were chopped in to small pieces. About 400 g of the plant material collected at three different stages were subjected to hydro-distillation for 3-4 h using Clevenger type apparatus. After completion of this process light yellowish essential oil (EO) of 0.52%, 0.76 and 0.64% (v/w) were obtained from BF, FB and AF stages of *T. stocksianum* respectively. All the three samples of essential oils were dried with anhydrous Sodium sulfide (Na_2SO_4) and were stored in refrigerator at 4°C before analysis. Then EO was qualitatively and quantitatively analyzed with GC-MS.

Gas Chromatography-Mass Spectrometry: Chemical composition of the essential oils of *T. stocksianum* were determined with GC-MS (GC-MSQP 2010, Tokyo, Japan) connected with auto-sampler (AOC-20s) and auto-injector (AOC-20i). Helium gas was used for the elution of sample. Separation of the components was carried out in capillary column (DB-5MS manufactured by Agilent Technology USA) with 0.250 mm internal diameter, 0.25 µm thickness and of 30 m length. Sample injector was operated at 250°C in split mode with 1ml/min. The capillary column was initially operated at 50°C for 1min and then raised to 150°C at a rate of 15°C/min and was maintained for 15 min. Then the temperature of the column was raised to 280°C at a rate of 2.5°C/min for 3 min. Electron impact ionization mode having 70 ev energy, at a 250°C ion source temperature, interface temperature 240°C with 80 KPa pressure, solvent cut time 1.8 min. Mass spectra were obtained in the range of 40 to 650 *m/z*. To estimate the Retention Indices the E. oil, a series of normal alkanes was also injected under similar experimental conditions. Components of the E. oil were identified by comparing the mass spectra acquire with those of standard mass spectra from the NIST library (NIST 05). Relative concentrations of the individual components were estimated from the peak areas of the total ion chromatograms.

RESULTS

Molecular profiling of Essential oils: Essential oils were obtained from the aerial parts of *T. stocksianum* through hydro-distillation process. The E. oils of all the three stages were of pale yellow colour with pleasant aromatic odor.

Chemical Composition of the E. Oil of Aerial Parts Before Bloom (BB): GC-MS analysis of the first stage i.e. before bloom (BB) revealed the presence of 28 components. The caryophylleneoxid was found in predominant percentage (37%), followed by o-cymene (18.64), germacrene B (5.63%), caryophyllene (4.2%) and alpha-caryophyllene (3.16%). While the percentages of the rest of the components were less than 3%, depicted in Table 1.

Chemical Composition of the *E. oil* of Aerial Parts at Full Bloom (FB): In this analysis 38 different components were identified. The components found in high percentages were santalol,cis,alpha- (16.26%), D-limonene (12.78%), o-cymene (12.44%), (Z)-beta-farnesene (8.49%), alpha- phellandrene (7.29%), bicyclo[3.1.0] hexane,4-methylene-1-(1-methyl) (5.28%), santalol, cis, alpha- (4.73%), caryophyllene (3.51%), p-cymen-8-ol (3.14%) and Alpha-caryophyllene (3.13%), as shown in Table 2.

Chemical Composition of the *E. oil* of Aerial Parts after Full Bloom (AFB): The E.oil obtained after full bloom, revealed the presence of 28 components. In this analysis component No 22 was found in highest percentage (38.17%) followed by compound No 26 with 26.28%. o-cymene was in 10.97%, germacrene B 4.46%, p-cymen-8-ol 2.54% and compound No, 20 in 2.24%. The percentages of the other components were less than 2%, shown n Table 3.

Table 1: Qualitative and quantitative composition

S. No	Compounds	RI	Percentages
1	Alpha- Phellandrene	9.554	0.63
2	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methyl)	12.071	0.88
3	Beta-Myrcene	13.176	0.87
4	o-Cymene	15.228	18.64
5	D-Limonene	15.482	1.44
6	Eucalyptol	15.688	0.44
7	Gamma- Terpinen	17.394	1.25
8	Beta-Linalool	20.419	1.14
9	Thujone	21.382	1.83
10	p-Cymen-8-ol	26.698	0.56
11	Propanal, 2-methyl-3-phenyl	29.476	1.09
12	p-Cymen-3-ol	32.554	2.06
13	Nerol acetate	36.752	0.83
14	Cyclohexane, 2,4-diisopropenyl-1	37.018	0.81
15	Caryophyllene	38.294	4.20
16	Alpha-Caryophyllene	40.053	3.16
17	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl-,(R)	41.410	1.10
18	Germacrene D	41.562	0.28
19	Eudesma-4(14),11-diene	41.410	1.12
20	Germacrene B	41.867	5.63
21	Cycloheptane, 4-methylene-1-methyl-2 -(2-methyl-1-propan-1-yl)-1-vinyl-	44.023	2.62
22	Nerolidol	44.624	2.64
23	Caryophylleneoxid	45.278	37.10
24	Epiglobulol	45.455	1.16
25	Dihydrocurcumene	46.173	1.07
26	Ar-tumerone	48.580	1.36
27	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl	52.341	0.69
28	Heneicosane	63.348	1.79
	Total	95.26%	

RI = Retention indices.

Table 2: Qualitative and quantitative composition

S. No	Compounds	RI	Percentages
1	Alpha- Phellandrene	9.561	7.29
2	Alpha – Pinene	9.886	0.95
3	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methyl)	12.079	5.28
4	B – Pinene	13.188	0.95
5	Beta-Myrcene	13.176	1.9
6	o-Cymene	15.241	12.44
7	D-Limonene	15.501	12.78
8	B-cis-Ocimene	16.780	0.08
9	Gamma- Terpinen	17.4	0.17
10	Beta-Linalool	20.435	2.12
11	Thujone	21.403	0.92
12	p-Cymen-8-ol	26.267	3.14
13	p-menth-1-en-8-ol	26.711	1.82
14	cis-Geraniol	26.651	0.26
15	Propanal, 2-methyl-3-phenyl	29.497	0.38
16	p-Mentha-6,8-dien-2-1,(R) -(-)-	29.616	0.74
17	p-Cymen-3-ol	32.575	0.85
18	p-Meth-1-en-8-ol,acetate	35.079	0.09
19	Copaene	36.301	0.14
20	Nerol acetate	36.761	0.22
21	Cyclohexane, 2,4-diisopropenyl-1	37.023	0.22
22	Caryophyllene	38.308	3.51

Table 2: Continued

S. No	Compounds	RI	Percentages
23	Alpha-Caryophyllene	39.930	3.13
24	(Z)-beta-Farnesene	40.067	8.49
25	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl-,(R)	41.426	0.16
26	Germacrene B	41.876	2.48
27	Santalol,cis,alpha-	41.875	4.73
28	Beta- Sesquiphellandrene	43.008	0.48
29	Cycloheptane, 4-methylene-1-methyl-2 -(2-methyl-1-propan-1-yl)-1-vinyl-	44.030	0.36
30	Humulen-(v1)	44.301	0.15
31	Nerolidol	44.625	1.14
32	1H-Cycloprop[e]azulen-7-ol,decahydro -1,1, 7-trimethyl-4-methylene-	45.273	3.89
33	Dihydrocurcumene	46.390	0.23
34	Ar-tumerone	48.584	1.28
35	1-Cycloheptene,1,4-dimethyl-3-(2-methyl--propene-1-yl)-4-vinyl-	48.697	0.60
36	Santalol,cis,alpha-	49.441	16.26
37	Santalol, E-cis,epi-beta	50.575	0.10
38	Alpha-santalol	50.592	0.15
	Total	99.02%	

RI = Retention indices.

Table 3: Qualitative and quantitative composition

S. No	Compounds	RI	Percentages
1	Alpha- Phellandrene	9.568	0.23
2	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methyl)	12.086	0.41
3	B – Pinene	12.086	0.41
4	o-Cymene	15.242	10.97
5	D-Limonene	15.496	0.68
6	Eucalyptol	15.693	0.29
7	Beta-Linalool	20.427	0.80
8	Thujone	21.391	1.07
9	p-Cymen-8-ol	26.255	2.54
10	p-menth-1-en-8-ol	26.696	0.40
11	Propanal, 2-methyl-3-phenyl	29.487	0.67
12	p-Cymen-3-ol	32.408	0.48
13	Copaene	36.299	0.28
14	Nerol acetate	36.750	0.59
15	Cyclohexane, 2,4-diisopropenyl-1	37.022	0.62
16	Caryophyllene	38.296	1.07
17	Alpha-Caryophyllene	40.058	1.76
18	Eudesma-4(14),11-dinene	41.416	0.96
19	Germacrene B	41.878	4.46
20	Cycloheptane, 4-methylene-1-methyl-2 -(2-methyl-1-propan-1-yl)-1-vinyl-	44.021	2.24
21	Nerolidol	44.6259	1.97
22	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.al	45.292	38.17
23	Caryophylleneoxid	45.455	0.53
24	Dihydrocurcumene	46.171	0.18
25	3-methyl-2-butanoic acid, cyclobutyl ester	48.548	0.47
26	Santalol,cis,alpha-	49.429	26.28
27	1H-Indene,2,3,3a,4,7,7a-hexahydro-2,2,4,4,	52.340	0.33
28	Heneicosane	63.359	0.14
	Total	99%	

RI = Retention indices.

DISCUSSION

The antioxidant and antimicrobial potentials of essential oils and plant extracts have created the basis of many pharmaceuticals, food preservatives and natural therapies [23]. Therefore, the search for natural additives, particularly of plant origin, has markedly increased [24]. The antioxidant potential of phenolic and other constituents present in the essential oils and organic extracts of the plants has been widely studied by numerous researchers [25]. Variation in the chemical composition of phytochemicals occurs not only with change in geographical location but also depends upon the stage of collection [26-27]. In the current study we determined the qualitative and quantitative variation in the chemical composition of the essential oils of *T. stocksianum* collected at three different stage of the plant growth. In our study we found significant qualitative and quantitative differences in the chemical composition of compounds. Highest numbers (38) of compounds have been recorded in the essential oil extracted from the sample collected at full bloom of the plant (Table 1). While 28 components with different percentages were detected in each E. oils extracted from the aerial parts collected before and after full bloom of the plant, as shown in Table 2 and 3 respectively. Same was the findings of Yousuf *et al*.[28] how determined the variation in the concentrations and numbers of components in the essential oil of *T. stocksianum* collected from the same geographic location in two different seasons. The major components were of the three samples were, caryophylleneoxid (37%), santalol,cis,alpha- (16.26%) and component No 22 (38.17%) in BB, FB and AB respectively, which is totally different from the results of Yousuf *et al* who recorded α-cadinol as major compound in the essential oil of *T. stocksianum* collected from United Arab Emirate [28]. While Jaimand *et al* have reported camphene (20.6%) as major constituent from the same specie collected from Iran [29].

A plethora of research studies are available which shows the significant and diversified biological and pharmacological potentials of the essential oils. Abdollahi *et al* have reported antinociceptive activity of the E. oil of *T. polium*. In another study the E. oil of *T. royleanum* has shown profound *in vitro* antioxidant potential [30]. Numerous authors have reported the antioxidant potential of the E. oil and organic extract of different species the genus *Teucrium*. Salah *et al* reported antioxidant activity of *Teucrium sauvagei*, while Yildirim *et al* reported the antioxidant potential of the ethanolic extract of *T. polium* [31-32].

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