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Comparison of the Volatile Composition of *Stachys pubescence* Oils Obtained by Hydro Distillation and Steam Distillation

[1]Mahmoud Biglar, [2,3]Mohammad Reza Shams Ardekani, [2,3]Mahnaz Khanavi, [4]Abbas Shafiee, [5]Abdolhossein Rustaiyan, [6]Fahimeh Salimpour and [2,7]Fatemeh Farjadmand
[1]Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Traditional Pharmacy, School of Traditional Iranian Medicine and Persian Medicine, Pharmacy Research Center,
[3]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran
[4]Department of Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[5]Sciences and Research Campus, Islamic Azad University, Tehran, Iran
[6]Department of Biology, Faculty of Science, Islamic Azad University, North Branch of Tehran, Tehran 19585-936, Iran
[7]Pharmaceutical Division Staff, Food and Drug Organization, Ministry of Health and Medical Education, Tehran, Iran

Abstract: The oils obtained by hydrodistillation and steam distillation of the aerial part of *Stachys pubescence* Ten. was analyzed by GC and GC/MS. Water distilled essential oil of the aerial part of *S. pubescence*, was rich in fatty acids like hexadecanoic acid and linoleic acid and also benzaldehyde and spathulenol whereas the steam distilled oil of the plant contained hexadecanoic acid, spathulenol and eugenol. Both of oils were rich in fatty acids (36.6 and 27.9%, respectively). Moreover, the content of oxygenated mono and sesquiterpenss were defined higher in steam distilled oil than hydrodistiled oil (24.5, 17.2 and 6.1, 15.5%, respectively). In conclusion it seems that oxygenated terpenoids were trended to steam distillation method more than hydrodistilation, respectively.

Key words: *Stachys pubescence*, Lamiaceae, hydrodistillation, steam distillation, essential oil composition, eugenol, spathulenol, hexadecanoic acid, linoleic acid

INTRODUCTION

The genus *Stachys* L. (Lamiaceae) comprises more than 300 species in the world. In Iran, Stachys genus is represented by about 34 species including *Stachys pubescence* as an endemic species (Mozaffarian, 1998; Rechinger and Hedge, 1982). Flavonoids (Skaltsa *et al*., 2007; Delazar *et al*., 2005), Phenyl ethanoid glycosides (Nazemiyeh *et al*., 2006; Miyase *et al*., 1996; Nishimura *et al*., 1991), terpenes (Paternostro *et al*., 2000; Khanavi *et al*., 2003, 2004, 2008), iridoids (Meremeti *et al*., 2004) and saponins (Yamamoto *et al*., 1994) are the main compounds occurring in this genus.

There are also several reports about the major compounds of the oil of various *Stachys* species, for instance the oil of *S. turcomanica* is rich in germacrene D (17.4%), 7-epi-α-selinene (10.5%) and β-elemene (9.2%) (Firouznia *et al*., 2009). Moreover, main compounds of *S. trinervis* oil were reported previously as α-pinene (42.68%) and δ-2-carene (31.90%) while in *S. subaphylla* oil major constituents were identified as δ-2-carene (23.93%), α-pinene (19.29%) and sabinene (19.11% (Khanavi *et al*., 2008).

In Iran, the aerial parts of *S. inflata* are traditionally used for the treatment of infection, asthma, rheumatic and other inflammatory disorders (Maleki *et al*., 2001). Several studies have shown that *Stachys* species have identified with various effects such as anti-inflammatory (Khanavi *et al*., 2005; Kukic *et al*., 2007; Rezazadeh *et al*., 2005; Sharifzadeh *et al*., 2005), antinephritic (Hayashi *et al*., 1994), hypotensive (Takeda *et al*., 1997), anxyolytic (Rabbani *et al*., 2003), antimicrobial (Dulger *et al*., 2005; Skaltsa *et al*., 1999), antiallergic

Corresponding Author: Fatemeh Farjadmand, Pharmaceutical Division Staff, Food and Drug Organization, Ministry of Health and Medical Education, Tehran, Iran

reactions (Shin, 2004; Kim *et al*., 2003), antioxidant (Meremeti *et al*., 2004; Haznagy-Radnai *et al*., 2006; Vundac *et al*., 2007), cytotoxic effects (Khanavi *et al*., 2012) as well as an effect on hyaluronidase activity (Takeda *et al*., 1985).

The aim of this study is to determine the hydrodistilled and steam-distilled essential oils composition of *S. pubescence* and carry out a comparative evaluation between these two methods.

MATERIALS AND METHODS

Plant material: The aerial part of *Stachys pubescence* was collected from Khalkhal, Province of Ardabil, Iran in June 2009 during the flowering stage. Voucher specimen has been deposited at the Institute of Medicinal Plants (ACECR), Karaj, Iran.

Isolation of the volatile oils: The air-dried aerial part of this species was subjected to separate hydrodistillation using a Clevenger-type apparatus for 3 h and also was submitted for 3 h to steam distillation. After decanting and drying of the oils on anhydrous sodium sulfate, they were sealed in dark vials until usage.

Gas chromatography/mass spectrometry: Analysis of the water and steam distilled oils from *S. pubescence* were analyzed by means of GC and GC/MS. The GC analysis was performed on a Shimadzu 15 A gas chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 mL min^{-1}) and the capillary column used was DB-5 (50 m×0.2 mm; film thickness 0.32 μm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C min^{-1} rate and kept constant at 220°C for 10 min.

GC/MS analysis was performed using a Hewlett-Packard 5973 mass selective detector connected with a HP 6890 gas chromatograph. The separation was achieved by use of a HP5MS (5% Phenylmethylsiloxane) capillary column (60 m×0.25 mm; film thickness 0.25 μm). The column temperature was held at 60°C for 3 min and programmed up to 220°C at a rate of 5°C min^{-1} and then kept constant at 220°C for 3 min. Helium was used as the carrier gas (1 mL min^{-1}). MS were taken at 70 eV. Identification of oils compounds were made by comparing their mass spectra and Retention Indices (RI) with those given in the literature and those authentic samples (Adams, 1995). Relative percentage amounts were calculated from peak area using a Simadzu CR4A chromatopac software which was adjusted for that.

Identification of the compounds: Retention indices of components were calculated by using retention times of n-alkans that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of their mass spectra and Retention Indices (RI) with those reported in the literature (Adams, 1995; Davies, 1990) and of the authentic samples or by comparison with those held in a computer library (Wiley 275.L).

RESULTS AND DISCUSSION

Table 1 shows the constituents of hydrodistilled and steam distilled essential oils of *S. pubescence*. Both oils were light yellow with a distinct sharp odour in a yielding of 0.2% (w/w) for hydrodistillation and 0.3% (w/w) for steam distillation, respectively.

The 36 components were detected in the hydrodistilled oil of *S. pubescence* representing 89.1% of the total oil. The major constituents were hexadecanoic acid (24.6%) and linoleic acid (12.0%), whereas the steam distilled oil of the plant contained 23 compounds (90.2%), with hexadecanoic acid (21.5%), spathulenol (11.6%) and eugenol (11.1%) as the main constituents.

From Table 1 it is evident that the composition of the oils obtained by hydrodistillation and steam distillation of *S. pubescence* are different quantitatively but the total amount of the non-terpenoid fraction in the hydrodistilled and steam distilled oils of the plant (53.9 and 37.4%) were higher than monoterpenes (10 and 24.5%) and sesquiterpenes (22.1 and 17.2%) and some of the identified components in steam distilled oil were not found in the water distilled oil. Also, oxygenated sesquiterpenes were higher in steam distilled oil (17.2% against 15.5%). This pattern was observed in oxygenated monoterpenes (24.5 against 6.1%) too.

Previous investigation on the oil of some species of *Stachys* showed various compositions. Sesquiterpene hydrocarbons were the predominant fraction in the oils of *S. scardica*, *S. cretica* ssp. *cretica*, *S. germanica* ssp. *heldreichii* and *S. laxa* with germacrene D as the major compound (Skaltsa *et al*., 2003; Sajjadi and Mehregan, 2003) where as spathulenol is the main compound of *S. spinolosa* and *S. byzantina* and α-copaene and β-caryophyllene in *S. euboica* were the main components (Skaltsa *et al*., 2003; Khanavi *et al*., 2004). Also, the results of our previous researches showed that monoterperne hydrocarbons with δ-2-carene and α-pinene are major compounds of *S. trinervis* and *S. subaphylla* (Khanavi *et al*., 2008).

Although, most species of *Stachys* have rather low amounts of aliphatic and non- terpenoid fractions, some

Table 1: Camparative chemical composition (%) of *Stachys pubescence* oil obtained by hydrodistillation and steam distillation

Compounds	RI	Hydrodistillation (%)	Steam distillation (%)
α-pinene	939	0.9	-
Benzaldehyde	961	7.6	2.7
1-octen-3-ol	978	1.3	1.7
β-pinene	980	3.0	-
3-octanol	993	0.2	-
Linalool	1098	3.7	8.2
n-nonanal	1099	0.5	-
1-octen-3-yl acetate	1110	0.8	0.9
Trans pinocarveol	1139	-	1.5
Trans verbenol	1144	-	0.6
α-terpineol	1189	0.6	2.6
Myrtenol	1194	-	4.5
Geraniol	1255	0.3	0.1
(E)-cinamaldehyde	1266	-	1.3
Nonanoic acid	1280	-	3.5
Thymol	1290	-	0.6
Carvacrol	1298	0.4	4.5
(E,E),2,4,decadienal	1314	1.1	-
4-methoxy acetophenone	1348	1.1	-
Eugenol	1356	3.1	11.1
β-bourbonene	1384	3.7	-
α-cedrene	1409	2.0	-
β-cedrene	1418	0.2	-
Geranyl acetone	1453	0.3	0.3
Germacrene D	1480	0.6	-
(E)-β-ionone	1485	0.8	1.6
n-pentadecane	1500	0.1	-
β-bisabolene	1509	0.1	-
Spathulenol	1576	7.1	11.6
Dodecanoic acid	1580	-	0.9
Epi-α-muurolol	1641	-	1.7
Cubenol	1642	0.4	-
α-cadinol	1653	1.3	-
Valeranone	1672	0.9	-
α-bisabolol	1683	5.5	3.9
Cedryl acetate	1762	0.3	-
Nonadecane	1900	0.1	-
Methyl hexadecanoate	1927	0.5	-
Hexadecanoic acid	1978	24.6	21.5
Eicosane	2000	0.2	-
Methyl linoleate	2092	0.9	2.9
Henicosane	2100	2.6	-
Linoleic acid	2159	12.0	2.0
Tricosane	2300	0.3	-
Total		89.1	90.2
Non-terpenoids			
Alkanes		3.3	-
Alcohols, aldehydes, ketones, esters		14.0	9.5
Fatty acids		36.6	27.9
Terpenoids			
Monoterpene hydrocarbons		3.9	-
Oxygenated monoterpenes		6.1	24.5
Sesquiterpene hydrocarbons		6.6	-
Oxygenated sesquiterpenes		15.5	17.2
Phenyl propanoides		3.1	11.1

other species of *Stachys*, as the same of our results, have shown relatively high amounts of fatty acids and aliphatic esters (Skaltsa *et al*., 2003).

According to our result it is evident that the composition of the oils obtained by hydrodistillation and steam distillation of *S. pubescence* are different qualitatively and quantitatively. Some of the identified components in water distilld oil were not found in the steam distilled oil. Also it seems that in steam distillated oils, oxygenated terpenoids are higher than in hydrodistilled whereas non-terpenoids are the main fraction in hydrodistilled oil of this species.

CONCLUSION

According to mentioned results, two different ways of essential oil isolation caused definite variations in major terpenoids. It seems that oxygenated terpenoids are trended toward steam distillation more than hydrodistilation, respectively. Further investigation is needed to confirm these issues but if this pattern of compound isolation were repeated in some other plants it might be a new way of producing some essential oils enriched oxygenated compounds.

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