

Microwave-assisted extraction as an alternative tool for extraction of *Stachys aegyptiaca* essential oil

Alaa M. Shaheen^a, Ibrahim A. Saleh^a, El-Sayed A. El-Kashoury^c, Wafaa A. Tawfik^a, Elsayed A. Omar^b, Mohamed-Elamir F. Hegazy^a, Essam Abdel-Sattar^c

^aDepartments of Phytochemistry, ^bMedicinal and Aromatic Plants Researches, National Research Centre, Giza, ^cDepartment of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Correspondence to Mohamed-Elamir F. Hegazy, PhD, Department of Phytochemistry, National Research Centre, 33 El-Bohouth St., Dokki, Giza - 12622, Egypt;
Tel: +201022900036; fax: +20-233370931;
e-mail: elamir77@live.com

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Background and objectives

Stachys aegyptiaca Pers. (family Lamiaceae) is a perennial aromatic wild plant collected from Saint Catherine Protectorate, Sinai. The essential oil of *S. aegyptiaca* was obtained using two different techniques, conventional hydrodistillation (HD) and microwave-assisted extraction (MAE). The aim of the present study was to compare the effect of the two techniques on oil yield and oil composition. MAE offered reduction in the extraction time with better oil yield compared with HD.

Materials and methods

Two different techniques, conventional HD and MAE, were used for the extraction of essential oil from *S. aegyptiaca*. The chemical composition of the essential oil was analyzed using the gas chromatography–mass spectrometry technique.

Results and conclusion

Gas chromatography–mass spectrometry of the essential oils obtained revealed the presence of 48 and 30 components constituting 99.31 and 99.82% of the total composition of the oils obtained using; MAE and HD, respectively. Variations in the percentage yield and chemical composition were observed. The major component found in the extracted oils was α -pinene (24.65% HD and 41.14% MAE). MAE offered reduction in the extraction time (60 min vs. 3 h) with better oil yield (1.4% w/v) when compared with HD (0.9% w/v). MAE could be used as an alternative tool for the isolation of essential oils from their natural sources.

Keywords:

hydrodistillation, microwave-assisted extraction, *Stachys aegyptiaca*

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Introduction

Genus *Stachys* is one of the largest genera of family Lamiaceae containing about 300 species and centered in the Mediterranean region and south western Asia [1]. *Stachys* spp., known as mountain tea, were used in folk medicine to treat several complaints. Teas prepared from the plants of this genus were used as sedative, diuretic, antispasmodic, and emmenagogue [2]. They were used in traditional medicine in the treatment of diarrhea, sore throat, internal bleeding, and weakness of the heart and liver [2]. The different species of the genus were also investigated for their antimicrobial [1,3,4], antioxidant [5–7], anxiolytic [8–10], and anti-inflammatory properties [11–13]. Phytochemical investigations of the genus *Stachys* revealed the presence of essential oils [14–16], terpenoids [17–19], flavonoids [20–22], phenylethanoid glycosides [23–26], iridoids [27,28], and saponins [29–31]. Halim *et al.* [14] studied the chemical constituents of essential oil. The results revealed the presence of 14 monoterpene hydrocarbons (75%), four oxygenated monoterpenes (1.16%), and seven

sesquiterpene hydrocarbons (17%), and the dominant compound was α -pinene (54.46%).

Microwave extraction technology is considered as a greener method for medicinal and aromatic plant extraction when compared with the conventional extraction technique, as it offers shorter extraction time, great reduction in the power used, high extraction selectivity and increased production [32]. Conventional techniques run a severe risk for thermal degradation of most of the phytoconstituents with a high risk for increased pollution concerns. Microwave-assisted extraction (MAE) is a new concept for natural product extraction methodologies and is considered as a green sustainable innovative extraction technology that meets the challenges of the 21st century [33].

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The objective of this research was to compare the potential of MAE for the extraction of essential oil from *S. aegyptiaca* with the conventional hydrodistillation (HD) method.

Materials and methods

Plant material

Air-dried aerial parts of *S. aegyptiaca* Pers. were collected in May 2013 from Wadi Jibaal in Saint Catherine Protectorate. A voucher specimen (ID 213) has been deposited in the herbarium of National Research Centre. The collection was carried out under the permission of Saint Catherine Protectorate for scientific purposes. The plant was kindly authenticated by Dr Mona Marzouk, Associate Professor of Taxonomy, National Research Center, Cairo, Egypt.

Microwave-assisted extraction

MAE was carried out using a focused microwave apparatus (model MARS 240/50, no. 907511; CEM Corporation, Matthews, North Carolina, USA), frequency 2450 MHz operating at 2450 MHz with a maximum power of 1600 W. Hundred grams of the dried aerial parts was placed in a 5000 ml round bottomed flask that was connected to Clevenger-type apparatus outside of a microwave oven. The extraction was carried out in 800 W power for 60 min. Temperature was adjusted at 100°C. The essential oil was recovered and its volume was determined using a micropipette. The obtained yield was calculated as percentage (volume of recovered oil per weight of the sample). The obtained oil was dried using anhydrous sodium sulfate and saved in a refrigerator until analysis.

Hydrodistillation extraction

For comparison, HD extraction of the essential oil was carried out using 100 g of *S. aegyptiaca*. Extraction was carried out for 3 h using a Clevenger-type apparatus according to the Egyptian Pharmacopoeia procedures [34]. After HD extraction, the same post extraction procedures for MAE were applied to the recovered oil.

Investigation of the essential oil composition using gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC–MS) analysis of the essential oil samples was carried out using GC–MS instrument at the Department of Medicinal and Aromatic Plants Research, National Research Center, with the following specifications. A TRACE GC Ultra Gas Chromatographs (Thermo Scientific Corp., Waltham, MA, USA)

coupled with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) was used. The GC–MS system was equipped with a TraceGOLD™ TG-WaxMS, Waltham, MA USA; column (30 m×0.25 mm internal diameter, 0.25 μm film thickness). Analyses were carried out using helium as a carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1 : 10 using the following temperature program: 40°C for 1 min; increasing at 4.0°C/min to 160°C and maintaining for 6 min, followed by increasing at 6°C/min to 210°C and maintaining for 1 min. The injector and detector were both maintained at 210°C. Oil samples were diluted with hexane (1 : 10, v/v) and 0.2 μl of the mixtures was injected. Mass spectra were obtained by means of electron ionization at 70 eV, using a spectral range of *m/z* 40–450. Most of the components were identified using mass spectra (authentic chemicals, Wiley spectral library collection, and NSIT library) as well as using comparison of their retention indices and mass spectra with those published [35].

Results and discussion

The components identified in the essential oil extracted using HD and MAE techniques from the aerial parts of *S. aegyptiaca* and their relative percentages are given in Table 1 in the order of their elution from the column.

Forty-eight components were identified in microwave-extracted oil, representing 99.31% of the oil composition, whereas 30 components were identified in hydrodistilled oil, representing 99.82% of the oil composition. Figure 1 shows the total ion chromatograms of *S. aegyptiaca* essential oils extracted using HD and MAE, respectively.

The main components found in the oil were α -pinene (24.65 and 41.14%), *trans*-caryophyllene (14.65 and 8.63%), 6-epi-shyobunol (14.61 and 11.15%), α -cadinol (11.08 and 5.55%), δ -cadinene (5.75 and 2.39%), and (–)-spathulenol (5.41 and 4.32%) extracted by means of HD and MAE, respectively. Oxygenated sesquiterpenes and monoterpene hydrocarbons are the main classes of compounds of the extracted *S. aegyptiaca* essential oils, representing 37.91 and 49.56% of the oil composition using HD and MAE, respectively.

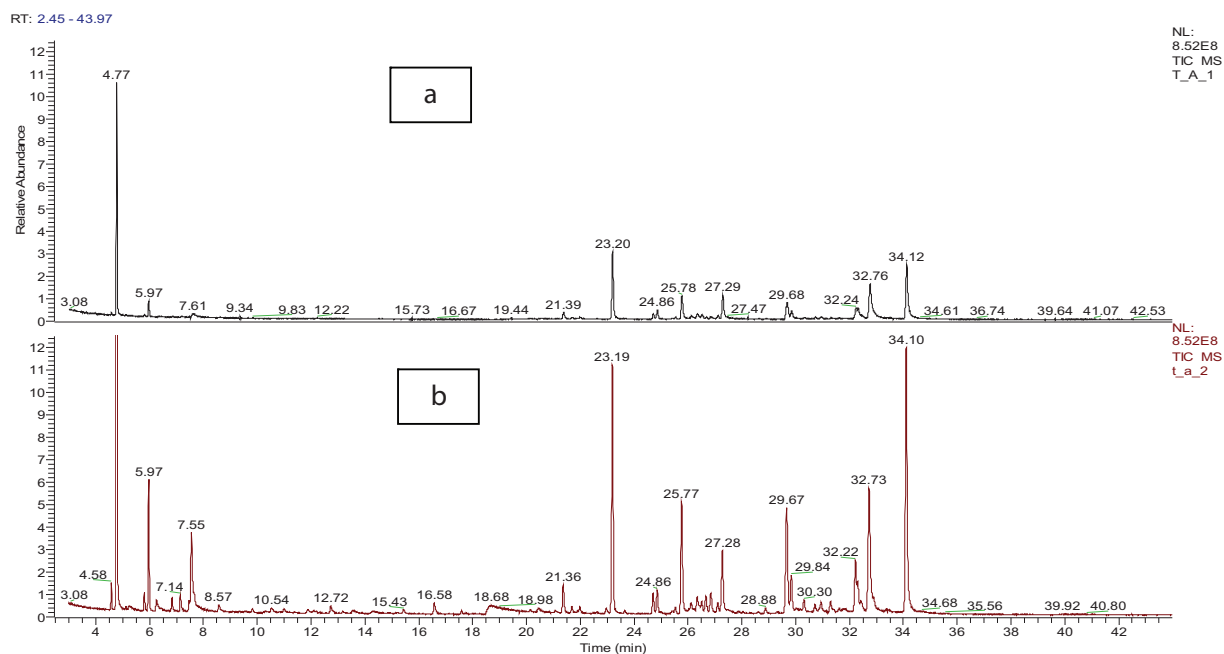
Although α -pinene has been similarly reported as a major component in the essential oil of the leaves [14], a qualitative and quantitative variation among other constituents is obvious.

Table 1 Identified components in *Stachys aegyptiaca* essential oil, extracted using hydrodistillation and microwave-assisted extraction and their relative percentages

Classed	Components	RT	LRI	Relative percentage	
				HD	MAE
mh	α -Thujene	4.58	901.6	0.05	0.48
mh	α -Pinene	4.77	908.8	24.65	41.14
mh	Sabinene	5.80	945.8	0.24	0.36
mh	β -Pinene	5.97	951.7	2.06	2.8
mh	β -Myrcene	6.26	962.3	–	0.35
mh	3-Carene	6.83	982.7	–	0.34
mh	α -Terpinene	7.14	993.9	–	0.5
mh	o-Cymene	7.48	1004.2	–	0.22
mh	Limonene	7.55	1006.3	–	3.1
om	α -Terpineol	7.61	1007.7	1.23	–
mh	γ -Terpinene	8.57	1033.4	–	0.27
om	Fenchone	9.82	1066.3	–	0.09
om	α -Thujone	10.53	1085.2	–	0.14
om	β -Thujone	11.00	1098.1	–	0.12
om	Isopinocarveol	11.88	1118.4	–	0.11
om	Cis-verbenol	12.11	1124.1	–	0.05
om	Trans-verbenol	12.72	1137.2	–	0.21
om	Terpinene-4-ol	13.53	1156.1	–	0.12
om	Cis-p-mentha-1(7),8-dien-2-ol	15.43	1199.5	–	0.13
om	Chrysanthenyl acetate	16.58	1225	–	0.41
om	Carvacrol	18.68	1271.4	–	1.31
sh	α -Copaene	21.39	1331.6	1.53	1.05
sh	β -Bourbonene	21.69	1338.2	0.23	0.17
sh	β -Elemene	21.98	1344.8	0.3	0.14
sh	Trans-caryophyllene	23.20	1372	14.65	8.63
sh	Humulene	24.71	1406	0.88	0.52
sh	Aromadendrene	24.86	1409.5	1.68	0.83
sh	Valencene	25.42	1422.8	0.22	0.1
sh	γ -Muurolene	25.54	1425.1	0.42	0.2
sh	Germacrene-D	25.78	1430.8	5.1	4.19
sh	β -Selinene	26.12	1438.9	0.44	0.22
sh	α -Selinene	26.35	1444	0.72	0.35
sh	α -Muurolene	26.53	1448.3	0.62	0.05
os	Limonen-6-ol, pivalate	26.66	1451.2	–	0.55
sh	E-farnesene	26.86	1455.8	0.56	0.7
sh	γ -Cadinene	27.11	1461.7	0.58	0.29
sh	δ -Cadinene	27.29	1465.8	5.75	2.39
os	(-)-Spathulenol	29.68	1522.1	5.41	4.32
os	(-)-Caryophyllene oxide	29.84	1526.1	2.05	1.38
os	Salvial-4(14)-en-1-one	30.34	1538.1	0.33	0.44
os	Veridiflorol	30.74	1547.6	0.3	0.27
os	α -acorenil	30.96	1553.2	0.25	0.36
os	Ledene oxide-(II)	31.29	1563.7	–	0.36
os	Cubenol	31.63	1569.2	0.18	0.11
os	τ -Cadinol	32.24	1584.2	1.95	1.81
os	τ -Muurolol	32.33	1586.3	1.75	0.63
os	β -Selinenol	32.43	1588.7	–	0.3
os	α -Cadinol	32.76	1596.7	11.08	5.55
os	6-epi-Shyobunol	34.12	1631.3	14.61	11.15
Total mh				27	49.56
Total om				1.23	2.69
Total sh				33.68	19.83
Total os				37.91	27.23
Total identified				99.82	99.31

HD, hydrodistillation; LRI, linear relative index; mh, monoterpene hydrocarbon; MAE, microwave-assisted extraction; om, oxygenated monoterpene; os, oxygenated sesquiterpene; RT, retention time; sh, sesquiterpene hydrocarbon.

Figure 1



Total ion chromatograms of the essential oils obtained from the dried aerial part of *Stachys aegyptiaca*: (a) Hydrodistillation extraction and (b) microwave-assisted extraction

Comparison of both methods revealed that α -terpineol (1.23%) extracted by means of HD was missing in microwave-extracted oil. However, some components were detected in MAE oil that were missing in HD: β -myrcene (0.35%), 3-carene (0.34%), α -terpinene (0.5%), *o*-cymene (0.22%), limonene (3.1%), γ -terpinene (0.27%), fenchone (0.09%), α -thujone (0.14%), β -thujone (0.12%), isopinocarveol (0.11%), *cis*-verbenol (0.05%), *trans*-verbenol (0.21%), terpinen-4-ol (0.12%), *cis*-*p*-mentha-1(7),8-dien-2-ol (0.13%), chrysanthenyl acetate (0.41%), carvacrol (1.31%), limonen-6-ol, pivalate (0.55%), ledene oxide-(II) (0.36%), and β -selinenol (0.3%).

MAE not only gives better yield (1.4 vs. 0.9%) but also shortens the extraction time when compared with HD (60 min vs. 3 h). The unique MAE mechanism, which provides non contact heat production and delivery to the extraction matrix, is able to heat up the intracellular contents of plant cells, which produces vapor and generates tremendous pressure on the plant cell wall, causing complete rupture of the cell wall, which causes complete and fast discharge of the active constituents from the ruptured cells to the surrounding extraction solvent [36]. The percentage of α -pinene in the oil obtained by means of MAE is much higher than that obtained by means of HD (41.14 vs. 24.65%). This gives an additional advantage to this method due to the biological activities (antibacterial, antiproliferative,

anti-inflammatory, and antioxidant) reported for this compound [37–39]. These results are in agreement with those previously reported for a number of *Stachys* spp., which revealed the presence of α -pinene as the major constituent in their essential oils [15,40–44].

Conclusion

MAE, as an alternative green extraction method to conventional HD, showed better extraction yield (1.4 vs. 0.9%) and shorter extraction time (60 min vs. 3 h). α -Pinene was the dominant component extracted using both methods. In addition, the results revealed qualitative and quantitative variability in the composition of the oils obtained.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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