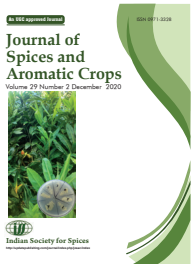
Journal of Spices and Aromatic Crops
Vol. 29 (2) : 140-147 (2020)
doi : 10.25081/josac.2020.v29.i2.6452

Indian Society for Spices



Evaluation of harvesting time and standardization of distillation duration for higher essential oil content and quality in German chamomile (*Chamomilla recutita* L.)

D Kumar[1]*, P Suryavanshi[2], R C Padalia[1], A Chauhan[1], K T Venkatesha[1], A K Tiwari[1], V R Singh[1], S Singh[1] & R K Upadhyay[1]

[1]*CSIR-Central Institute of Medicinal & Aromatic Plants (CIMAP), Research Centre, Pantnagar-263 149, Uttarakhand.*
[2]*CSIR-Central Institute of Medicinal & Aromatic Plants (CIMAP), Lucknow-226 015, Uttar Pradesh.*
**E-mail: dkonarya@gmail.com*

Received 31 August 2020; Revised 03 November 2020; Accepted 24 November 2020

Abstract

Essential oil yield and composition in aromatic crops might be affected by genetic, agronomical and environmental factors but till date there is no clear information about the harvesting time and distillation for higher essential oil content without affecting quality. The current study was carried out to evaluate harvesting of chamomile flowers without herb and with herb part at three different times (6 A.M., 12 P.M. and 6 P.M.) and four distillation treatments (3 h, 4 h, 5 h & 6 h) for dried chamomile flowers. Results indicated that essential oil content was more in chamomile flowers without herb (0.15-0.18%) as compared to flowers with herb (0.06-0.09%). Essential oil content in chamomile flowers without herb was found statistically at par at harvest time of 12 P.M. (0.18%) and 6 P.M. (0.18%) and significantly higher than harvesting time of 6 A.M. (0.15%). Essential oil of chamomile flowers without herb contained maximum α-bisabolol oxide-B, (*Z*)-spiroether, and chamazulene at 12 P.M. and 6 P.M. while, α-bisabolone oxide-A and α-bisabolol oxide-A were maximum at 6 A.M. and (*E*)-β-farnesene was more at 12 P.M. Similarly, in distillation experiment, higher oil content was observed in chamomile dried flowers which were hydro-distilled for 6 h (1.20%) compared to other hydro-distillation durations. Marker compounds i.e. α-bisabolol oxide-A, α-bisabolone oxide-A, α-bisabolol oxide-B, (*E*)-β-farnesene and chamazulene were more at 5 h and 6 h distillation duration while (*Z*)-spiroether was more at 3 h distillation duration. The present study showed that in order to obtain higher essential oil, flowers without herb harvested at 12 P.M or 6 P.M. should be subjected to 5-6 h hydro-distillation.

Keywords: aromatic crop, bisabolol oxide, chamazulene, chemical composition, diurnal variation, hydro-distillation

Introduction

Chamomile (*Chamomilla recutita* L.) is one of the oldest and very well known medicinal herb which belongs to Asteraceae family. *C. recutita* is also known as German chamomile. Botanically, it is annual herb with widespread roots. Its height varies from 40 to 70 cm, depending on the location, climate and

soil type. Chamomile is a native crop to Iran and Europe where it grows as a wild plant (Reichinger 1977; Pourohit & Vyas 2004). Chamomile is very well known for its medicinal properties like anti-inflammatory, anti-spasmodic, anti-bacterial and anti-septic etc. (Franke & Schilcher 2007). Chamomile contains about 0.24-1.9% essential oil in which more than 120 compounds have been identified (Kumar *et al*. 2016). Chamomile essential oil is also known as blue oil in national and international markets. Marker compounds of chamomile oil are bisabolol oxides, farnesene, α-bisabolone oxide-A, α-bisabolol and chamazulene which are actively involved in reducing inflammation and other medicinal properties (Jakolev *et al*. 1983). Nationally and internationally, presence of marker compound in chamomile essential oil makes it a commodity of great demand which is increasing day-by-day besides its medicinal properties. Chamazulene, one of the most important component responsible for blue coloration of chamomile essential oil was derived from matricine by the process of pH changes during distillation process (Singh *et al*. 2016). Essential oil content and its composition vary due to genetic variability or environmental factors. Single or a combination of environmental factors influence the content of essential oil and also its composition. In general, temperature and light fluctuation throughout the day affect the essential oil content and composition in aromatic crops (Singh *et al*. 2016; Asghari *et al*. 2014). In chamomile, harvesting of flowers is cumbersome which increases the production cost. According to Salamon (2007), maximum chamomile flowers production may be achieved by efficient and effective collection of flowers at right time and stage. It is also essential to know how essential oil content and composition changes during daytime and seasons. Seasonal variations of chamomile essential oil yield have been previously studied by several researchers (Pino *et al*. 2002). Diurnal variation of chamomile essential oil has not been extensively studied yet.

Besides genetic, environmental and agri-practices, distillation duration during essential oil extraction also plays an important role in yield and composition of essential oils. Till date, no literature is available on optimization of distillation duration for essential oil extraction to increase yield. Hence, the objectives of the present study were to find the most appropriate harvesting time of chamomile flowers for higher essential oil content without affecting quality and also to standardize the distillation duration to get maximum essential oil content.

Materials and methods

Plant sample

Plant material for experiment was collected from chamomile plant variety CIM-Sammohak available at the Experimental Farm of CSIR-Central Institute of Medicinal & Aromatic Plants, Research Centre, Pantnagar. Harvesting of chamomile flowers was done in full blooming stage at three times during the day (6 A.M, 12 P.M and 6 P.M). Required amount of fresh flowers were harvested in two ways i.e. flowers with herb part and flowers without herb part on the same day and for optimization of harvesting time and plant part with respect to essential oil content.

Essential oil extraction

Samples were subjected to hydro-distillation just after harvesting on the same day in March, 2020. Rest of the samples were air dried at room temperature ($<25^0$C) for 10–15 days to determine essential oil content in dried chamomile flowers. As per experimental design, the extracted oil from samples were used for optimising the distillation duration for maximum recovery of essential oils, the dried flowers were hydro-distilled for 3 h, 4 h, 5 h and 6 h by using glass Clevenger apparatus to produce oil by recommended method. The oil volume was measured directly in the extraction burette. The essential oil content (%, v/w) was calculated as volume of oil (mL) per 100 g of fresh and dried plant material. The obtained oil was dried over anhydrous sodium sulphate in sealed vial at low temperature.

GC-MS analysis

The essential oil samples were analysed by GC-FID and GC-MS techniques. GC-FID analysis of essential oil was carried out on Thermo Fisher Trace GC-1300

equipped with TG-5 (30 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column and with flame ionization detector (FID). Nitrogen was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The oven temperature programming was done from 60–230°C with an increase of 3°C min^{-1}. The injector and detector temperatures were 230°C and 250°C, respectively. The injection volume was 0.02 μL neat with a split ratio of 1:40. Gas chromatography-mass spectrometry (GC-MS) analysis of essential oil was performed on Perkin-Elmer Turbomass Quadrupole Mass Spectrophotometer fitted with PE-5 (Perkin Elmer) fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven column temperature was programmed from 60 to 240°C with an increase of 3°C min^{-1} with initial and final hold time of 2 min, using helium as carrier gas at a flow rate of 1.0 mL min^{-1}. The injector and ion source temperatures were maintained constant at 250°C. The injection volume was 0.02 μL neat with split ratio 1:30. MS were taken at 70 eV (EI). The essential oil constituents were identified by comparing retention indices (RI), mass spectra library search and by comparing their mass spectra (MS) data with literature data (Adams 2007).

Statistical analysis

The experiments were arranged as completely randomized design with eight replications of each treatment and all the statistical analyses were performed by using OPSTAT statistical software package (Sheoran *et al*. 1998) at 0.05 probability level.

Results and discussion

Essential oil content (%) in flowers with herb and without herb

Essential oil content (%) was found to be more in flowers without herb (0.15-0.18%) compared to flowers with herb (0.06-0.09%) during the day (Table 1). Data showed that in both cases the essential oil content increased as the days proceeded. There was a significant difference between harvesting time treatments in terms of essential oil content (%) (Table 1). Essential oil content varied from 0.06%-0.09% and 0.15%-0.18% in fresh flowers with herb and without herb, respectively. Essential oil content was significantly influenced by harvesting time and significantly highest essential oil content (0.09 & 0.09% and 0.18 & 0.18%) was obtained when flowers with herb and without herb were harvested at 1 P.M. and 6 P.M compared to 6 A.M. (0.06 & 0.15%). In general, the results showed that the essential oil content in chamomile flowers both with herb and without herb increased from morning to evening. Higher essential oil content at 12 P.M. and 6 P.M. might be due to increased temperature and light intensity; higher light intensity and higher temperature might have enhanced metabolic reactions leading to the increased synthesis of secondary metabolites in the plant. Harvesting time is an important aspect for maximum oil recovery from the flowers (Oliveira *et al*. 2012). Salehi & Hazrati (2017) also reported similar results. Variation in oil yield at different harvesting time has been reported by various researchers in several plants (Marcum & Hanson 2006; Callan *et al*. 2007; Ebrahimi *et al*. 2008).

Influence of hydro-distillation duration on essential oil content in dried chamomile flowers

Distillation duration had significant effect on essential oil content which varied from 0.6%–1.2% (Table 2). The maximum essential oil content was recorded at 6 h distillation duration (1.2%) which was significantly higher as compared to hydro-distillation durations of 5 h (0.88%), 4 h (0.70%) and

Table 1. Essential oil content (%) in fresh flower with herb and without herb harvested at different time

Harvesting time	Essential oil content (%)	
	Fresh flower without herb	Fresh flower with herb
6 A.M.	0.15	0.06
12 P.M.	0.18	0.09
6 P.M.	0.18	0.09
C.D (P=0.05)	0.01	0.01

3 h (0.60%). 3 h and 4 h distillation durations were statistically at par with each other; 6 h distillation duration recorded almost double amount of essential oil content compared to 3 h distillation duration. The reason might be that in this study we used dried chamomile flowers which shows higher essential oil content due to lower moisture level. Several previous studies have reported that the distilled material containing both stem and leaves/flowers yield less essential oil content compared to only flower, as the oil glands are located in flowers and not in other plant parts (Topalov 1989). These results are in line with the findings of Cannon *et al*. (2013) who reported that by altering the distillation duration, essential oil production can be increased. Similarly, Valtcho *et al*. (2013) reported that the content of essential oil is affected by distillation duration. Bruce *et al*. (2001) also reported that distillation duration influences essential oil yield and its composition.

Essential oil composition as influenced by harvesting time

The qualitative and quantitative composition of the essential oil of chamomile flower was analysed by GC and GC-MS techniques. A total of 24 constituents were identified in chamomile essential oil samples extracted from flowers without herb and with herb at different harvesting times (Table 3). α-bisabolol oxide-A, α-bisabolone oxide-A, α-bisabolol oxide-B, (*Z*)-spiroether, chamazulene, and (*E*)-β-farnesene were marker compounds which were detected in the chamomile essential oil. Most representative fractions were dominated by oxygen containing sesquiterpenes which include most characteristic chamomile essential oil compounds such as α-bisabolol oxide-B, α-bisabolone oxide-A and α-bisabolol oxide-A. The results indicated that only flowers must be harvested for recovery of major compounds and herb part with flower should be avoided during harvesting. In comparison to chamomile oil in fresh flowers with herb, only chamomile oil of fresh flowers contains higher content of marker compounds. α-bisabolol oxide-B, (*Z*)-spiroether, and chamazulene were higher in the samples harvested at 12 P.M. (23.41%, 7.77%, 3.34%) and 6 P.M. (37.32%, 10.06%, 3.09%) and α-bisabolone oxide-A, α-bisabolol oxide-A were maximum in the samples harvested at 6 A.M. (27.46%, 32.62%) and (*E*)-β-farnesene was found highest in the samples harvested at 12 P.M. (4.43%) (Table 3). The variation in essential composition may be due to environmental factors such as light intensity, temperature and abiotic stresses (Tounekti & Khemira 2015). The results are in line with the findings of Salehi & Hazrati (2017). Chamazulene, α-bisabolol and its oxidised metabolites have powerful role in controlling the aroma of essential in chamomile which contribute to anti-inflammatory, anti-bacterial, insecticidal and anti-ulcer properties (Farhoudi *et al*. 2014). The presence and content of these compounds in chamomile essential oil indicate its drug value.

Table 2. Essential oil content in dried flowers at different hydro-distillation duration

Hydro-distillation duration	Essential oil content (%)
3 h	0.600
4 h	0.700
5 h	0.875
6 h	1.200
C.D (P=0.05)	0.106

Essential oil composition as influenced by distillation duration

The essential oil obtained from German chamomile at different distillation duration (3, 4, 5 and 6 h.) was analyzed by GC-FID and GC-MS techniques. Altogether, 24 compounds represented 90.51 to 92.60% of the total essential oil (Table 4). α-bisabolol oxide-A (25.24% & 24.12%), α-bisabolol oxide-B (18.53% & 17.17%), (*E*)-β-farnesene (9.65% & 12.60%) and chamazulene (2.79% & 3.27%) were more in the essential oil samples which were hydro-distilled for 5 h and 6 h and (*Z*)-spiroether (10.32%) was more in those samples which were hydro-distilled only for 3 h. α-bisabolone oxide-A remained unchanged with increase in distillation duration. The maximum content of α-bisabolol oxide-A was obtained at 5 h distillation duration and further distillation decreased its content in the essential oil. Similar trend

Table 3. Essential oil composition of German chamomile as influenced by harvesting time

S.No.	Retention index		Compound	Harvesting time					
				Concentration (%)					
				Fresh flowers without herb			Fresh flowers with herb		
	$RI_{Cal.}$	$RI_{Lit.}$		T_1	T_2	T_3	T_1	T_2	T_3
1	942	946	Camphene	0.07	-	-	-	-	-
2	1018	1021	6-Methyl-5-hepten-2-one	0.13	0.09	0.05	0.12	-	-
3	1024	1026	1,8-Cineole	0.05	0.06	-	-	-	-
4	1046	1044	(*E*)-β-Ocimene	0.09	0.11	-	-	-	-
5	1050	1056	Artemesia ketone	1.47	2.06	0.55	0.34	0.67	0.21
6	1073	1071	p-Cresol	0.34	0.52	0.31	0.10	0.06	0.21
7	1142	1145	Camphor	0.95	0.55	0.10	-	0.10	0.12
8	1150	1155	Isoborneol	0.35	0.20	0.23	-	0.15	0.15
9	1232	1235	Neral	0.08	0.13	-	-	-	-
10	1262	1264	Geranial	-	-	-	-	-	-
11	1390	1389	β -Elemene	0.29	0.27	0.21	0.20	0.25	0.15
12	1418	1417	β -Caryophyllene	0.05	0.07	0.04	-	-	-
13	1456	1454	(*E*)-β-Farnesene	2.93	4.43	2.58	5.16	7.31	2.10
14	1488	1484	Germacrene-D	-	0.93	0.60	0.18	0.65	ND
15	1504	1500	Bicyclogermacrene	-	0.51	0.20	-	0.05	0.15
16	1507	1505	(*E*,*E*)-Farnesene	0.27	0.34	0.26	1.44	2.54	1.05
17	1618	1622	10-*epi*-γ-Eudesmol	0.36	0.28	0.35	0.02	0.09	0.32
18	1655	1656	α-Bisabolol oxide-B	17.49	23.41	37.32	3.37	7.73	9.32
19	1682	1684	α -Bisabolone oxide-A	27.46	17.73	18.37	14.85	20.23	10.71
20	1686	1685	α -Bisabolol	0.16	0.17	0.05	0.34	0.23	0.07
21	1726	1730	Chamazulene	2.41	3.34	3.09	0.23	0.23	0.34
22	1745	1748	α -Bisabolol oxide-A	32.62	29.3	19.18	42.43	38.64	31.93
23	1874	1879	(*Z*)-Spiroether	5.70	7.77	10.06	16.04	12.04	29.69
24	1888	1890	(*E*)-Spiroether	0.76	0.62	0.44	1.27	1.48	0.46
			TOTAL	94.03	92.89	93.99	86.09	92.45	86.98

$RI_{Cal.}$=Retention Index calculated; $RI_{Lit.}$=Retention Index literature; (-)=Absent

T_1=Harvesting at 6:00 A.M.; T_2=Harvesting at 12:00 P.M.; T_3=Harvesting at 6:00 P.M.

was noticed in α-bisabolol oxide-B and α-bisabolone oxide-A. (*E*)-β-farnesene content increased as the distillation duration increased, it varied from 8.85% to 12.6%, maximum content was obtained at 6 h distillation duration. Chamazulene content was also maximum at 6 h (3.27%) and minimum at 3 h (2.27%) distillation duration. (*Z*)-spiroether showed decreasing trend with increased distillation duration, content decreased from 10.32% to 7.01% from 3 h to 6 h distillation duration, respectively. Verma *et al*. (2016) reported that distillation duration influenced the essential oil composition in *Acorus calamus*. Distillation duration influenced the essential oil yield and its composition in coriander spp. (Bruce *et al*. 2001).

The present study concluded that harvesting of

Table 4. Chemical composition of German chamomile influenced by distillation duration

S.No.	Retention index		Compound	Distillation duration (Hrs.) Concentration (%)			
	$RI_{Cal.}$	$RI_{Lit.}$		T_1	T_2	T_3	T_4
1	942	946	Camphene	0.1	0.12	0.05	-
2	1018	1021	6-Methyl-5-hepten-2-one	0.39	0.51	0.27	0.33
3	1024	1026	1,8-Cineole	0.11	0.1	0.04	0.05
4	1046	1044	(*E*)- β-Ocimene	0.16	0.21	0.07	0.1
5	1050	1056	Artemesia ketone	2.66	3.02	1.06	1.37
6	1073	1071	p-Cresol	0.42	0.4	0.15	0.25
7	1142	1145	Camphor	0.7	0.65	0.27	0.4
8	1150	1155	Isoborneol	0.11	0.14	0.12	0.16
9	1232	1235	Neral	0.1	0.09	-	-
10	1262	1264	Geranial	0.1	0.47	-	-
11	1390	1389	β-Elemene	0.36	0.38	0.32	0.33
12	1418	1417	β -Caryophyllene	0.11	0.1	0.09	0.12
13	1456	1454	(*E*)- β-Farnesene	8.9	8.85	9.65	12.6
14	1488	1484	Germacrene-D	1.28	1.18	1.3	1.66
15	1504	1500	Bicyclogermacrene	0.46	0.45	0.46	0.55
16	1507	1505	(*E*,*E*)-farnesene	0.26	0.25	0.43	0.33
17	1618	1622	10-*epi*-γ-Eudesmol	0.65	0.86	0.95	0.65
18	1655	1656	α-Bisabolol oxide-B	16.23	15.84	18.53	17.17
19	1682	1684	α -Bisabolone oxide-A	22.14	21.55	21.64	21.32
20	1686	1685	α -Bisabolol	0.12	0.4	0.14	0.1
21	1726	1730	Chamazulene	2.27	3.04	2.79	3.27
22	1745	1748	α-Bisabolol oxide-A	23.88	24.04	25.24	24.12
23	1874	1879	(*Z*)-Spiroether	10.32	7.31	7.19	7.01
24	1888	1890	(*E*)-Spiroether	0.77	0.55	0.87	0.57
			TOTAL	92.60	90.51	91.63	92.46

$RI_{Cal.}$=Retention Index calculated; $RI_{Lit.}$=Retention Index literature; (-)=Absent

T_1= hydro-distillation for 3h; T_2=hydro-distillation for 4h; T_3=hydro-distillation for 5h; T_4=hydro-distillation for 6h

flowers without herb part should be done during mid-day (12 P.M.) or in evening (6 P.M.) to get higher essential oil yield without affecting its quality and composition. In addition, hydro-distillation duration also showed positive effect on essential oil content. The maximum essential oil content was found in 6 h distillation followed by 5 h. Therefore, up to 5-6 h distillation of dried chamomile flowers is recommended for higher essential oil content. Distillation duration must be taken into consideration when comparing the essential oil yield and its composition.

Acknowledgments

The authors are grateful to Director, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and Scientist In-Charge, CSIR-CIMAP, Research Centre, Pantnagar for encouragement and necessary facilities to perform this work.

References

Adams R P 2007 Identification of essential oil components by gas chromatography/ mass spectrometry, Allured Publ. Corp., Carol Stream, IL, USA.

Argyropoulou C, Daferera D, Tarantilis P A, Fasseas C & Polissiou M 2007 Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (Verbenaceae) at two developmental stages. Biochem. System Ecol. 35: 831–837.

Asghari G, Gholamali H, Mahmoudi Z & Asghari M 2014 Diurnal variation of essential of the oil components of *Pycnocycla spinosa Decne.* exBoiss. Jundishapur J. Nat. Pharm. Prod. 9: 35–38.

Bruce Smallfield M, Van Klink John W, Perry Nigel B & Dodds Kenneth G 2001 Coriander spice oil: Effects of fruit crushing and distillation duration on yield and composition. J. Agric. Food Chem. 49: 118–123.

Callan N W, Johnson D L, Westcott M P, Lopes P N, Kato J M, Andrade A H E, Maia S G J & Welty L E 2007 Herb and oil composition of dill (*Anethum graveolens* L.): Effects of crop maturity and plant density. Ind. Crops Prod. 25: 282–287.

Cannon Jeffrey B, Charles Cantrell L, Astatkie T & Valtcho Zheljazkov D 2013 Modification of yield and composition of essential oils by distillation duration. Ind. Crops Prod. 41: 214–220.

Ebrahimi S N, Hadian J, Mirjalili M H, Sonboli A & Yousefzadi M 2008 Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. Food Chem. 110: 927–931.

Farhoudi R, Lee D J & Hussain M 2014 Mild drought improves growth and flower oil productivity of German chamomile (*Matricaria recutita* L.). J. Ess. Oil Bearing Plants 17: 26–31.

Franke R & Schilcher H 2007 Relevance and use of chamomile (*Matricaria recutita* L.). Acta. Hort. 749: 29–43.

Jakolev V, Issac O & Flaskamp E 1983 Pharmacological investigations with compounds of chamomile. VI Investigations on the anti-phlogistic effects of chamazulene and matricine. Planta Med. 49: 67–73.

Kumar Amit, Singh O P & Soni S 2016 Studies of effect of FYM and Urea on vegetative growth of German chamomile (*Matricaria chamomilla* L.). Res. Environ. Life Sci. 9: 368–370.

Marcum D B & Hanson B R 2006 Effect of irrigation and harvest timing on peppermint oil yield in California. Agric. Water Mgt. 82: 118–128.

Oliveira A R M F, Jezler C N, Oliveira R A, Mielke M S & Costa L C B 2012 Determination of hydro-distillation duration and harvest moment on the essential oil of peppermint. Hort. Bras. 30: 155–159.

Pino J A, Bayat F, Marbot R & Aguero J 2002 Essential oil of chamomile *Chamomilla recutita* (L.) Rausch from Iran. J. Ess. Oil Res. 14: 407–408.

Pourohit S S & Vyas S P 2004 Medicinal Plants Cultivation. *Agrobios*, India.

Reichinger K H & Flora Iranica 1977 Akademische Verlagsgesellschaft 22: 82–88.

Salamon I 2007 Effect of the internal and external factors on yield and qualitative-quantitative characteristics of chamomile essential oil. In: Proceedings of the first international symposium on Chamomile research,

development and production. Presov, Slovakia, pp.45–64.

Salehi A & Hazrati S 2017 How essential oil content and composition fluctuate in German chamomile flowers during the day? J. Ess. Oil Bearing Plants 20: 622–631.

Sheoran O P, Tonk D S, Kaushik L S, Hasija R C & Pannu R S 1998 Statistical Software Package for Agricultural Research Workers. In: Hooda D S & Hasija R C (Eds.) Recent Advances in information theory, Statistics & Computer Applications (pp.139–143). Department of Mathematics Statistics, CCS HAU, Hisar.

Singh O, Khanam Z, Misra N & Srivastava M K 2011 Chamomile (*Matricaria chamomilla* L.): An overview. Pharmacog. J. 5: 82–95.

Topalov V D 1987 Lavender. In: Topalov V D, Dechev I I & Pehlivanov M S (Eds.), Plant production (pp.359–367). Zemizdat, Sofia, Bulgaria.

Tounekti T & Khemira H 2015 NaCl stress-induced changes in the essential oil quality and abietane diterpene yield and composition in common sage. J. Intercult. Ethnopharmacol. 4: 208–216.

Valtcho Zheljazkov D, Charles Cantrell L, Astatkie T & Jeliazkova Ekaterina 2013 Distillation duration effect on Lavender essential oil yield and composition. J. Oleo Sci. 62: 195–199.

Verma R S, Padalia R C, Chauhan Amit, Upadhyay R K & Singh V R 2016 Optimization of distillation conditions for better recovery of *Acorus calamus* L. essential oil. J. Essent. Oil Res. DOI: http://dx.doi.org/10.1080/10412905.2016.1222973.