ANNALES HORTICULTURAE
Formerly: ANNALES UMCS SECTIO EEE HORTICULTURA
VOL. XXVII (1) 2017

 DOI: 10.24326/ah.2017.1.2

[1] Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology in Szczecin, Słowackiego str. 17, 71-434 Szczecin, Poland
e-mail: pjadczak@zut.edu.pl
[2] Department of Horticulture, West Pomeranian University of Technology in Szczecin, Słowackiego str. 17, 71-434 Szczecin, Poland
[3] Institute of Chemistry and Environmental Protection, West Pomeranian University of Technology, al. Piastów 42, 71-065 Szczecin, Poland

PAULA JADCZAK[1], KAMILA BOJKO[2], ANETA WESOŁOWSKA[3]

Chemical composition of essential oils isolated from Mexican giant hyssop [*Agastache mexicana* (Kunth.) Link. & Epling.] via hydrodistillation in Deryng and Clevenger apparatuses

Porównanie składu chemicznego olejku eterycznego pozyskanego przez hydrodestylację ziela kłosowca meksykańskiego [*Agastache mexicana* (Kunth.) Link. & Epling.] z wykorzystaniem aparatów Derynga oraz Clevengera

Summary. Flowering herb of *Agastache mexicana* (Kunth.) Link. & Epling. was collected, dried in natural conditions and distilled in order to extract the essential oils. The extraction method involved hydrodistillation in either Deryng or Clevenger apparatus. The extracted oils were subjected to GC-MS analysis. The most abundant compounds identified in the extracts were pulegone (47.77–49.48%), limonene (15.45–15.93%) and *cis*-menthone (12.25–12.89%). The study showed that the distillation method did not significantly affect either qualitative or quantitative composition of the essential oil of *Agastache mexicana* (Kunth.) Link. & Epling.

Key words: *Agastache mexicana* (Kunth.) Link. & Epling., extraction method, essential oil

INTRODUCTION

Mexican giant hyssop [*Agastache mexicana* (Kunth.) Link. & Epling.] is one of 20 species belonging to *Agastache* genus of Lamiaceae family [González-Ramírez *et al.* 2012]. It is a perennial plant native to Asia and South America. In Mexico, Mexican giant hyssop is commonly called *toronil*, and its herb is widely used in folk medicine to treat *empacho* (digestive system disorders), *mal de ojo* (anxiety, high fever, headaches) and during soul cleansing rituals. Herbal raw material of Mexican giant hyssop is its herb used in the form of decoctions, infusions and ethanol extracts. Mexican giant hyssop is

helpful in phytotherapeutic treatment of insomnia and anxiety and cardiovascular system disorders [Linares *et al.* 1995]. Its inflorescences are recommended for their analgesic properties in soothing pain of variable origin (rheumatic, headache, stomach ache), and the herb collected prior to flowering is used as a tranquilizer [Madaleno 2007]. Recent studies have confirmed pharmacological activity of Mexican giant hyssop towards blood vessels and its antioxidant and anti-inflammatory properties [Ibarra-Alvadaro *et al.* 2010, Gonzalez-Ramirez *et al.* 2012]. As a typical genus of Lamiaceae family, *Agastache* plants are rich in biologically active substances, such as phenylpropanoids (flavonoids, free phenolic acids, lignans, polyphenols) or terpenoids [Svoboda *et al.* 1995, Suchorska-Tropiło and Pióro-Jabrucka 2004, Zielińska and Matkowski 2014].

The main and most abundant components of the essential oil extracted from *Agastache mexicana* include limonene, methyl chavicol (estragol) and linalool [Estrada--Reyes *et al.* 2010]. Composition of the essential oil depends on the time of herb collection, agrotechnical procedures employed during cultivation, as well as the climate, weather conditions and soil [Omidbaigi and Mahmoodi 2004, Suchorska-Tropiło and Pióro-Jabrucka 2004]. Essential oils present in aromatic plants are usually extracted via distillation (aqueous, vapor or aqueous-vapor distillation). Distillation may cause chemical changes in the natural components (isomerization, saponification, polymerization with less stable components) and affect the content and composition of the essential oil, including percentage share of its basic components.

The aim of the study was to determine the effect of the distillation method on the content and composition of the essential oil in *Agastache mexicana* (Kunth.) Link. & Epling.

MATERIAL AND METHODS

Plant material. The aerial parts of *Agastache mexicana* (Kunth.) Link. & Epling. were collected at the flowering stage from the Horticultural Experimental Station, West Pomeranian University of Technology in Szczecin (north-western Poland) in July 2015. The herb material was dried in ambient shade conditions and stored in paper bags in darkness until analysis. The plants were grown in an unheated greenhouse from the seeds sown in the last decade of April. The seeds were sown into seeding boxes filled with garden soil in rows at a spacing of 10 cm. During vegetation, the plants were fertilized two times (at 14 days interval) with a liquid fertilizer Ekolist (0.5%).

Isolation of essential oils. 10 grams of dried aerial parts of *A. mexicana* in a 1000 ml round-bottomed flask along with 500 ml of distilled water were subjected to hydrodistillation for 4 h (after which no more essential oil was obtained) using Deryng apparatus [Farmakopea Polska VI]. The hydrodistillation was also performed in Clevenger apparatus [Farmakopea Polska VIII] for 4 h. The extracted essential oils were separated from water, dried over anhydrous sodium sulfate and stored in dark sealed vials at 4°C until GC-MS analysis.

The qualitative GC-MS analysis of the essential oils was carried out using HP 6890 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm) and directly coupled to a mass selective detector MSD 5973. Helium (1 ml/min) was used as a carrier gas. Samples of 2μl (40 mg of oil dissolved in

1.5 ml of dichloromethane) were injected in the split mode at 5 : 1 ratio. The injector and the transfer line were kept at 280°C. The ion source temperature was 230°C. The initial temperature of the column was kept at 40°C for 5 min, then increased to 60°C at a rate of 30°C/min, then to 230°C at a rate of 6°C/min (kept constant for 10 min), and then increased to a final temperature of 280°C at a rate of 30°C/min. The oven was held at this temperature for 5 min. Mass spectra were taken at 70eV. Mass range was from 40 to 550 m/z. Solvent delay time was 4 min. Total running time for a sample was about 51 min. The relative percentage of the essential oil constituents was evaluated from the total peak area (TIC) by the apparatus software.

Compound identification. The components of the isolated essential oils were identified by comparison of their mass spectra with those stored in NIST 2002 and Wiley NBS75K.L mass spectra libraries and confirmed by comparison of their calculated retention indices with data available online in the NIST Chemistry WebBook [http://webbook.nist.gov/chemistry/].

The retention indices (RI) were calculated for all volatile constituents using a homologous series of n-alkanes (C_7-C_{40}) under the same chromatographic conditions used for the essential oil analysis.

Statistical analysis presented in Tables 1 and 3 was performed using AWAR software created by the Department of Applied Informatics, Institute of Soil Science and Plant Cultivation, Puławy, Poland. The means were separated using Tukey's test at p = 0.05. To show the influence of the distillation method on the Mexican giant hyssop oil composition, 20 compounds accounting for more than 2.89% of the essential oil content were selected for statistical analysis given in Table 3.

RESULTS AND DISCUSSION

Statistical analysis presented in Table 1 revealed that the distillation method had no significant effect on percentage content of the essential oil extracted from Mexican giant hyssop herb. The oils isolated from the aerial parts of *A. mexicana* were yellow liquids and their yield was 2.255% (v/w) for Deryng and 2.261% (v/w) for Clevenger apparatus. Mean content of the essential oil was 2.258%. Irrespective of the type of the distillation device, a total of 44 compounds were identified, which for the oil extracted in Deryng apparatus accounted for 99.23%, and for the oil obtained in Clevenger apparatus for 99.18% of the identified compounds.

Table 1. Essential oil content in the herbs of Mexican giant hyssop [*Agastache mexicana* (Kunth.) Link. & Epling.]
Tabela 1. Zawartość olejku eterycznego w zielu kłosowca meksykańskiego [*Agastache mexicana* (Kunth.) Link. & Epling.]

Distillation apparatus Aparat do destylacji	Essential oil content Zawartość olejku eterycznego (%)
Deryng	2.255
Clevenger	2.261
Mean	2.258
LSD $_{\alpha=0.05}$ / NIR $_{\alpha=0,05}$	n.s.

n.s. – not significant

Table 2. Chemical omposition of essential oils from Mexican giant hyssop [*Agastache mexicana* (Kunth.)Link. & Epling.] obtained by different distillation methods (%)
Tabela 2. Skład chemiczny olejku eterycznego kłosowca meksykańskiego [*Agastache mexicana* (Kunth.) Link. & Epling.] pozyskanego różnymi metodami destylacji (%)

Compounds Składniki	Retention index (RI) Indeks retencji	Deryng apparatus Aparat Derynga	Clevenger apparatus Aparat Clevengera
α-pinene/ α-pinen	933	0.10	0.08
β-thujene/ β-tujen	973	0.29	0.30
1-okten-3-ol/ 1-octen-3-ol	980	0.70	0.69
3-octanone/ 3-oktanone	987	0.16	0.16
β-myrcene/ β-mircen	991	1.16	1.22
α-phellandrene/ α-fellandren	1004	0.10	0.09
p-cymene/ p-cymen	1025	0.07	0.05
limonene/ limonen	**1030**	**15.93**	**15.45**
linalool/ linalol	1101	0.14	0.15
thujene/ tujon	1112	0.25	0.30
trans-p-2,8-menthadien-1-ol/ *trans*-p-2,8-mentadien-1-ol	1122	0.99	0.80
cis-p-2,8-menthadien-1-ol/ *cis*-p-2,8-mentadien-1-ol	1137	1.07	0.89
isopulegol/ izopulegol	1147	0.07	0.06
***trans*-menthone/ *trans*-menton**	**1155**	**2.89**	**3.17**
***cis*-menthone/ *cis*-menton**	**1168**	**12.89**	**13.25**
isopulegon/ izopulegon	1178	1.60	1.62
p-cymen-8-ol	1191	0.16	0.13
α-terpineol	1194	0.18	0.18
dihydrocarvone/ dihydrokarwon	1199	0.13	0.11
estragole (methyl chavicol)/ estragol (metylochawikol)	1203	0.69	0.51
verbenone/ werbenon	1213	0.21	0.17
cis-carveol/ *cis*-karweol	1220	0.79	0.50
trans-carveol/ *trans*-karweol	1227	0.38	0.25
β-citronellol/ β-cytronelol	1233	0.37	0.26
pulegone/ pulegon	**1249**	**47.77**	**49.48**
geraniol	1258	0.44	0.51
α-citral (geranial)/ α-cytral (geranial)	1267	0.21	0.24
thymol/ tymol	1294	0.57	0.62
carvacrol/ karwakrol	1318	0.18	0.17
δ-elemen	1334	0.28	0.30
bicycloelemene/ bicykloelemen	1340	0.17	0.17
α-cubebene/ α-kubeben	1356	1.06	0.85
β-burbonen	1390	0.17	0.11
β-elemen	1395	0.20	0.07
β-caryophyllene/ β-kariofilen	1426	1.62	1.40
α-caryophyllene (α-humulene)/ α-kariofilen (α-humulen)	1460	0.09	0.09
γ-muurolene/ γ-murolen	1488	0.66	0.61

bicyclo germacrene/ bicyklogermakren	1503	0.49	0.44
δ-cadinene/ δ-kadinen	1529	0.20	0.15
spatulenole/ spatulenol	1586	1.36	1.45
caryphyllene oxide/ tlenek kariofilenu	1592	1.70	1.60
10-epi-γ-eudesmol	1619	0.11	0.12
τ-muurolol	1649	0.35	0.41
α-kadinol/ α-cadinol	1665	0.28	0.31
Total/Łącznie	–	99.23	99.18

RI – retention indices relative to n-alkanes (C_7-C_{40}) on HP-5MS column/ wskaźniki retencji względem n-alkanów (C_7-C_{40}) na kolumnie HP-5MS

Table 3. Statistical analysis of main constituents of the essential oil from Mexican giant hyssop [*Agastache mexicana* (Kunth.) Link. & Epling.] depending on the type of the distillation apparatus (%)

Tabela 3. Analiza statystyczna głównych składników olejku eterycznego kłosowca meksykańskiego [*Agastache mexicana* (Kunth.) Link. & Epling.] w zależności od aparatu użytego do destylacji (%)

Essential oil constituent Składnik olejku eterycznego	Distillation apparatus Aparat do destylacji		Mean Średnia	$LSD_{\alpha = 0.05}$ $NIR_{\alpha = 0,05}$
	Deryng	Clevenger		
limonene/ limonen	15,93	15,45	15,69	n.s.
trans-menthone/ *trans*-menton	2,89	3,17	3,03	n.s.
cis-menthone/ *cis*-menton	12,89	13,25	13,07	n.s.
pulegone/ pulegon	47,77	49,48	48,63	n.s.

n.s. – not significant

Main components of oil were pulegone (47.77–49.48%), limonene (15.45–15.93%), *cis*-menthone (12.25–12.89%), and *trans*-menthone (2.89–3.17%). Similar findings were reported by Svoboda *et al.* [1995] who identified pulegone (75%), menthone (14%) and limonene (3%) as the basic ingredients of the essential oil in Mexican giant hyssop grown in Scotland. According to Myadelet *et al.* [2013], the essential oil from *Agastache mexicana* grown in Russia is rich in menthone (42.2%), isomenthone (18.8%), pulegone (7.3%), and methyl chavicol (3.8%). Juárez *et al.* [2015] reported methyl eugenol (36.41%), estragole (27.92%), linalool (10.66%), and pulegone (6.46%) to be the main components of the essential oil from *A. mexicana*. Statistical analysis of the study outcomes indicated no significant differences in the content of basic compounds between the oils extracted with different apparatuses (Tab. 3). Similar results were published by Wesołowska *et al.* [2015], who studied the effect of a distillation method on the chemical profile of essential oil obtained from summer savory (*Satureja hortensis* L.) herb and found no significant effect of the apparatus used for distillation on the qualitative and quantitative composition of the essential oil.

CONCLUSIONS

1. The distillation method had no significant effect on percentage content of the essential oil extracted from Mexican giant hyssop herb.

2. Irrespective of the method of distillation in the essential oil obtained from the herbs, 44 chemical compounds were identified and the main compounds were: pulegone (47.77–49.48%), limonene (15.45–15.93%), *cis*-menthone (12.25–12.89%), and *trans*-menthone (2.89–3.17%).

3. The method of distillation of essential oil did not significantly affect the content of basic chemical compounds.

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Streszczenie. Kwitnące ziele *Agastache mexicana* (Kunth.) Link. & Epling. zostało zebrane, wysuszone w warunkach naturalnych i poddane destylacji w celu pozyskania olejku eterycznego. Zostały użyte metody destylacji: hydrodestylacja w aparacie Derynga i w aparacie Clevengera. Wyizolowane olejki poddano analizie GC-MS. Do głównych związków w analizowanych olejkach należały: pulegon (47,77–49,48%), limonen (15,45–15,93%) i *cis*-menton (12,25–12,89%). Stwierdzono, że metoda destylacji nie miała istotnego wpływu na skład jakościowy i ilościowy olejku eterycznego *Agastache mexicana* (Kunth.) Link. & Epling.

Słowa kluczowe: *Agastache mexicana* (Kunth.) Link. & Epling., metoda destylacji, olejek eteryczny

Orzymano:/ Received: 8.02.2017
Zaakceptowano:/ Accepted: 28.07.2017