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Product Quality and Safety

A new type of laboratory microdevice for distillation of lavender and herb raw materials

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Abstract. *Bulgaria is known for its essential oils and medicinal plants, which occupy increasing parts in the agricultural areas. In recent years, the boom in lavender production has made our country the largest producer of lavender oil in the world. This intensified the scientific work with it and led to the creation of new lavender varieties and lines that are used in production. All research and tests on an industrial scale go through the laboratory distillation of small amounts of plant material to determine the quantity and quality of essential oil. In laboratory practice, micro-apparatus for water distillation is widely used, which does not reflect the real results for the yield and quality of the product. This necessitated the creation of a new laboratory device for determining the content of essential oil in raw materials with labile (rich in esters) oil by the application of steam distillation. The design of this apparatus mirrors industrial installations, thus the results it produces accurately simulate those of industrial production. Tests with herbal raw materials, like Lavandula angustifolia Mill., Salva sclarea L. and Helichrysum italicum (Roth) G. Don showed that the model is effective and applicable. The oils obtained by the new apparatus retained an ester content of 40.2%, 75.0% and 4.8%, respectively; these figures represent a significant improvement over previous methods.*

Keywords: essential oil crops, content, quality, apparatus

Introduction

Essential oil plants are distributed in all climatic zones of the globe and are diverse in terms of taxonomy, location of essential oil, its method of deposition, the optimal time of vegetation and technical maturity. Although essential oils are complex mixtures of chemically diverse compounds, they share common properties: volatility and insolubility in water. It is these qualities that are used to separate them from the raw material in industrial or laboratory conditions and are enshrined in the principles of the distillation method. This is the main way to obtain natural aromatic products and in particular - essential oils. In turn, distillation can be water or steam - depending on the environment in which it takes place. Hydrodistillation is the oldest way in which the material is immersed in water and boils with it. It provides very good conditions for hydrodiffusion and is indispensable for highly aggregated raw materials such as flowers (roses, orange blossom, ylang-ylang) and finely ground plant parts, as it provides continuous stirring and contact with water vapor. On an industrial scale, however, steam distillation has been required, in which steam is passed through a layer of material, which can be generated in the apparatus itself or supplied by a steam generator. Practically on an industrial scale, water distillation is used only in the production of rose oil. Steam distillation has been widely adopted not just for its simplicity and effectiveness, but also because it prevents the loss of certain ingredients. Hydrolysis of esters to alcohols and acids can occur during water distillation. This can have serious implications in the case of ester-rich oils and special precautions have to be taken to prevent or at least to limit the extent of ester degradation. The most important examples of this are lavender (lavandin) or clary sage oils rich in linalyl acetate and cardamom oil rich in a-terpinyl acetate. Especially with

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lavender, it is well known that the quality of the oil drops dramatically with the combination of high temperature and water, there are studies that show that even the presence of water during storage leads to hydrolysis processes and loss of valuable components. At the same time, most laboratory micro-devices for determining the content of essential oil in plant and animal raw materials use the principle of hydrodistillation, with reflux of the distillate in the distillation flask. Such are the devices of the European Pharmacopoeia (corresponds to the type described in ISO 6571 (www.iso.org.)), Dering microapparatus (Baj et al., 2015), the American Spice Trade Association (ASTA, 2021) or other modifications (Krüger, 1995; Ferhat et al., 2006). In recent years, the boom in lavender production has made our country the largest producer of lavender oil in the world. This intensified the scientific work with it and led to the creation of new varieties and lines that are used in production (Grebenicharski, 2016). All research and tests on an industrial scale go through the laboratory distillation of small amounts of plant material to determine the quantity and quality of essential oil. This suggests that the laboratory facility must also use steam distillation to make their results comparable and real. Moreover, national and international standards explicitly state that they apply to oils obtained by steam distillation (BDS 17 399:1998; ISO 3515:2002). The aim of this study was to construct a new micro-apparatus for lavender laboratory distillation that could also be applied to other herbal raw materials. The design uses models from previous achievement developments of the Institute for roses (IRAP).

Material and methods

Plant material

The main material was *Lavandula angustifolia* Mill. We used lavender herbs, variety "Hemus", from the experimental field of IRAP and lavender from fields of private farms, located in the west area of the Kazanlak valley. The details of the lavender fields are given in Table 1.

Table 1. Description and geographical data of lavender material

№	Farm	Localization		Variety
1	IRAP	42°6350' N	25°3885' E	Hemus
2	Asen 1	42°6432' N	25°1762' E	Population
3	Asen 2	42°6480' N	25°1951' E	Population
4	Yasenovo	42°6933' N	25°2792' E	Population
5	Gabarevo	42°5187' N	25°1588' E	Population
6	Koprinka	42°6363' N	25°3249' E	Population
7	Krun	42°6848' N	25°3802' E	Population

As an additional raw material we used inflorescences of *Salvia sclarea* L. (clary sage) and the whole stems of *Helichrysum italicum* Roth. (helichrysum). Both were grown in the experimental field of IRAP. All raw materials were harvested in the period June-July, 2020, at the specific moment of their technical maturity, i.e. in the phase of full flowering.

Studied traits

Parallel distillation was carried out using the new model of steam distillation apparatus and an approved micro-apparatus for water distillation, which was developed by IRAP (Balinova-Tsvetkova and Dyakov, 1974), which is Clevenger type (Clevenger, 1928). The new design is shown on Figure 1. It was made of processable glass. Description and method of operation: The device is connected to the charging cartridge and the steam source via a standard inlet (40/45 mm). The steam mixture is directed upwards through a tube that has three degrees of height/diameter ratio (40/50, 25/ and 10/130 mm). The vapor then enters the cooler, where it condenses and turns into distillate. The cooling agent is tap water, which is fed counter currently in the direction of the distillate, i.e. bottom to top. The mixture of essential oil and water flows into a separator, where the oil is decanted on the upper surface. As the receiver works on the principle of connected vessels, the oil is collected in the expanded part until its final accumulation, and the excess distillate is discharged through the outlet pipe. At the end of the process, the steam supply is stopped and the essential oil is drained through a tap at the bottom of the appliance. Meanwhile, it passes through a graduated part, where the volume is measured in milliliters. The reading accuracy is 0.01 ml and the measuring range is 0.0 - 0.7 ml. After collecting, the oil was treated with anhydrous Na_2SO_4 and stored in tightly closed vials ant 4°C till analysis.

Chemical analysis

The essential oil was analyzed using gas chromatography, performed on a Agilent 7820A GC System, coupled with flame ionization detector (GC/FID) and 5977B MS detector (GC/MS). The protocols were made according to gas chromatographic analysis of lavender, clary sage and helichrysum oils (BDS 17

399, 1998; ISO 3515, 2002; Saint-Lary et al., 2018). The capillary column EconoCapTM ECTM (30 m x 0.32 mm ID, 0.25 µm film thickness) was used. It was operated with oven program from 40°C (5 min held) to 240°C at a rate of 10°C /min, 10 min held at the final temperature was applied. Hydrogen (99.999%) was used as a carrier gas at a constant flow rate of 1.0 ml/min. The split ratio was 1:50, the inlet temperature was set to 200°C and the FID temperature was set to 300°C. The GC/MS analysis was performed at all the conditions described above. The ingredients were quantified by area of FID peaks without any correction factor. The oil constituents were identified by their mass spectra, matching with the NIST and MS library, as well as whenever possible, the authentic substances were used.

Statistical analyses

The data were expressed as a mean ± standard deviation (SD) from three replicates for each sample. ANOVA (one way) and Tukey (HSD) statistical analysis of the differences between the two types of distillation were performed. The statistical tests were established in XLSTAT 2023.1. 2 (1406).

Results and discussion

The model with dimensions of the new type laboratory apparatus is shown on Figure 1.

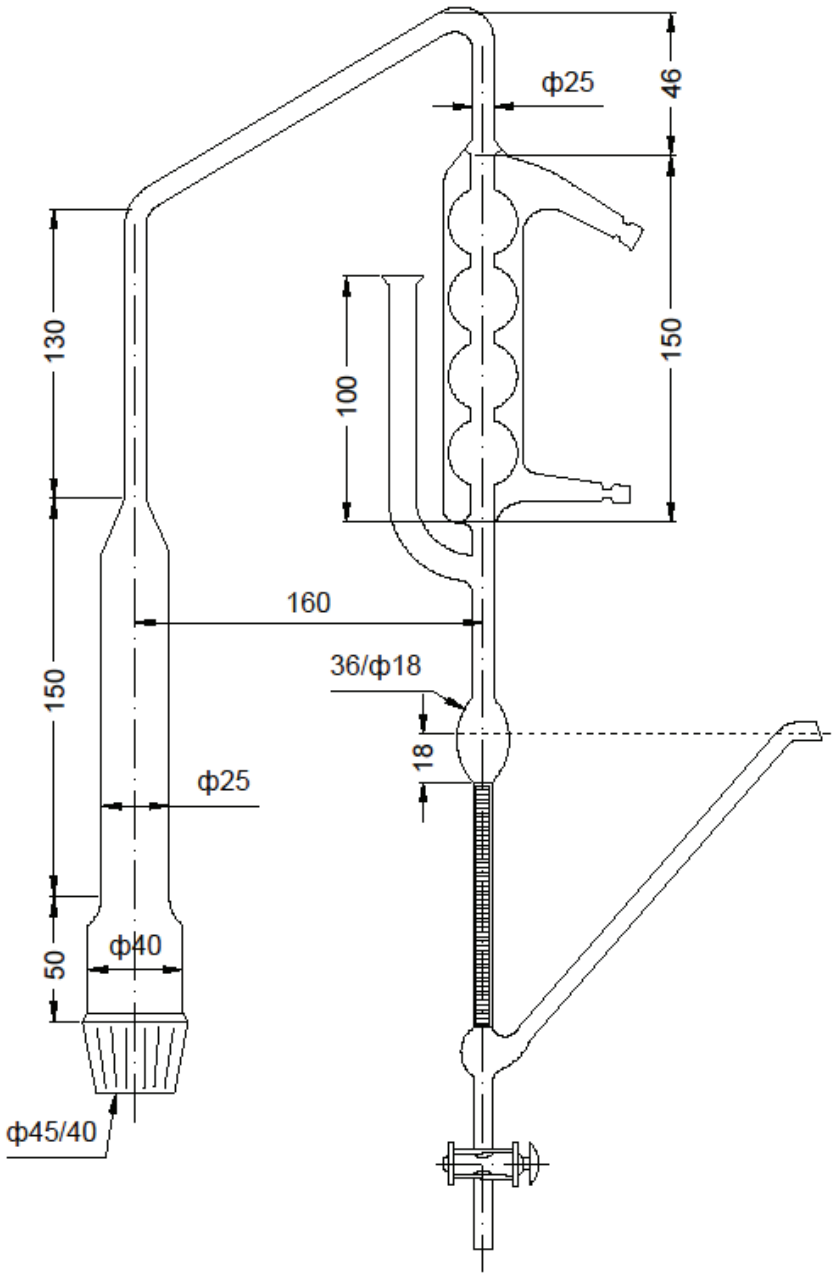

Figure 1. The new model of laboratory apparatus for microdistillation, M 1:2

The apparatus is constructed from processable glass. The diameter of the inlet section is kept as standard (45/40 mm), which allows the use of cartridges for loading raw material with different volumes, depending on the oil content. The measuring part can read the volume of the essential oil from 0 to 0.7 ml, with an accuracy of 0.01 ml. The appliance can use different steam sources.

Principle of operation: the vapors of the essential oil and water vapor move up the inlet pipe and are directed to the ball cooler. In the intertubular space of the latter cold water is supplied, in countercurrent. The liquefied distillate enters the separator expansion (pre-filled with water). There, the mixture separates and the essential oil is retained, and the distillation water moves and separates through the outlet pipe. After termination of the process, the essential oil is drained through the tap at the bottom of the florentine and the amount is measured by the volumetric measuring part.

The raw materials are selected by ester content in the essential oil - in clary sage it is over 58%, in lavender - from 30% to 50%, in helichrysum - up to 20%. The yield results are shown on Figure 2.

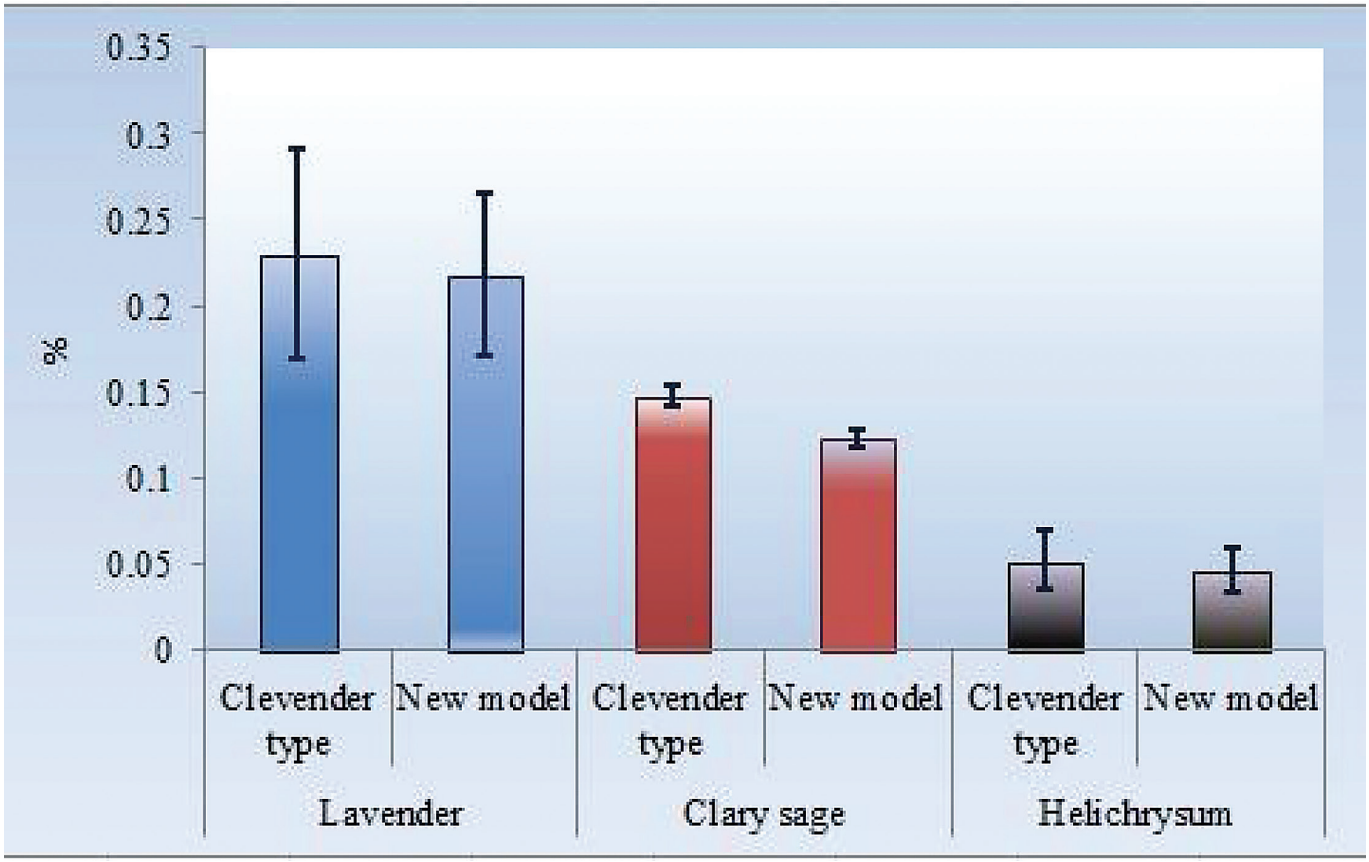

Figure 2. The results of the essential oil content (%, w/v) for lavender, clary sage and helichrysum, distilled with traditional (Clevenger) type and with new model apparatuses

The results were compared with those obtained from a Clevenger micro-apparatus under the same conditions: sample weight, distillation duration, temperature, and speed.

The measured values for the essential oil content are comparable to those reported in the literature (Saharkhiz et al., 2009; Lawrence, 2015; Saint-Lary et al., 2018). The results indicated that the new device produced lower oil yields for all three raw materials. The average values of the model with water distillation were 6%, 13% and 20% higher, respectively, for lavender, helichrysum and clary sage. This is reasonable given the oil content of each of them. The error values show very good reproducibility levels. In our case, the reported quantities more realistically reflect the production results, and this is very important for the practice. Statistical analysis of the rose oil yield showed no significant difference between the two studied methods.

The quality of the essential oil is a major priority in the production and its laboratory simulation. The chemical composition of lavender, clary sage and helichrysum distillation products, using the new apparatus is shown in Table 2, Table 3 and Table 4.

Table 2. Chemical composition of lavender essential oil obtained by the Clevenger type and by the new model apparatus for microdistillation

№	Compound, Rel. %	Clevenger type apparatus (water distillation)									
		Asen L_R	Asen new	Yasenovo	Krun	Gabarevo	Koprinka	Hemus	min	max	mean±SD
1	Alpha_pinene	0.14±0.04	0.17±0.06	0.14±0.0	0.06±0.02	0.08 ± 0.0	0.07±0.0	0.07±0.02	0.06	0.17	0.10±0.05[a]*
2	Camphen	0.06±0.06	0.16±0.06	0.08±0.0	0.10±0.04	0.11 ± 0.0	0.14±0.0	0.16±0.03	0.06	0.16	0.13±0.05[a]
3	3-octanone	0.61±0.25	1.19±0.91	0.78±0.0	1.77±0.64	0.43 ± 0.0	1.98±0.0	1.31±0.54	0.43	1.98	1.22±0.60
4	Cis-ocimene	6.45±1.56	6.49±3.70	4.48±0.0	1.33±0.16	3.38 ± 0.0	1.25±0.0	1.80±0.72	1.25	6.49	3.20±2.47[a]
5	Limonene	1.32±0.51	1.09±0.08	0.93±0.0	1.73±0.17	1.90 ± 0.0	1.97±0.0	2.79±0.66	0.93	2.79	2.05±0.92[a]
6	Trans-ocimene	2.79±0.53	2.51±0.30	2.14±0.0	1.93±0.17	1.54 ± 0.0	1.83±0.0	1.40±0.29	1.40	2.79	1.88±0.62[a]
7	Linalool	28.59±5.32	29.3±1.87	29.00±0.0	39.26±2.45	28.49 ± 0.0	39.33±0.0	37.42±4.07	28.49	39.33	34.66±5.52[a]
8	Camphor	0.15±0.05	0.10±0.02	0.10±0.0	0.18±0.08	0.20 ± 0.0	0.11±0.0	0.14±0.04	0.10	0.20	0.14±0.04[a]
9	Borneol	0.74±0.11	0.72±0.03	0.97±0.0	1.58±0.05	0.48 ± 0.0	1.60±0.0	0.50±0.50	0.48	1.60	0.75±0.51[a]
10	Lavandulool	0.65±0.01	0.60±0.14	0.66±0.0	0.69±0.09	0.80 ± 0.0	0.69±0.0	0.84±0.14	0.60	0.84	0.75±0.15[a]
11	Terpinen-4-ol	5.05±0.83	4.97±2.16	5.60±0.0	3.63±0.08	2.89 ± 0.0	3.19±0.0	1.72±0.93	1.72	5.60	3.21±1.86[a]
12	Alpha- terpineol	1.14±0.18	0.95±0.38	1.25±0.0	1.64±0.06	1.26 ± 0.0	1.22±0.0	2.50±0.94	0.95	2.50	1.87±0.94[a]
13	Linnalyl acetate	30.36±4.46	31.16±2.81	32.58±0.0	23.14±0.42	38.39 ± 0.0	22.03±0.0	32.50±5.97	22.03	38.39	30.67±5.43[a]
14	Lavandulil acetate	3.80±1.17	3.94±1.25	4.93±0.0	4.28±0.07	2.86 ± 0.0	3.92±0.0	2.68±0.60	2.68	4.93	3.41±1.02[a]
15	Beta- cariophylene	9.45±0.65	9.26±0.86	9.27±0.0	8.71±0.41	6.79 ± 0.0	10.54±0.0	4.24±3.24	4.24	10.54	6.70±3.39[a]
		New type apparatus (steam distillation)									
1	Alpha_pinene	0.24±0.02	0.23±0.09	0.18±0.0	0.11±0.01	0.17±0.0	0.11±0.0	0.06±0.01	0.06	0.24	0.13±0.08[b]
2	Camphen	0.14±0.0	0.12±0.03	0.15±0.0	0.11±0.01	0.09±0.0	0.12±0.0	0.13±0.02	0.09	0.15	0.13±0.02[b]
3	3-octanone	0.58±0.42	1.37±1.12	0.69±0.0	2.02±0.10	0.29±0.0	1.78±0.0	1.27±0.18	0.58	2.02	1.22±0.61[b]
4	Cis-ocimene	9.29±2.27	6.92±4.26	6.56±0.0	1.45±0.07	7.24±0.0	1.48±0.0	1.89±0.14	1.45	9.29	4.13±3.37[b]
5	Limonene	1.17±0.12	1.32±0.60	1.35±0.0	2.19±0.16	0.90±0.0	2.21±0.0	3.17±0.29	0.90	3.17	2.19±0.95[b]
6	Trans-ocimene	2.62±0.35	2.89±0.09	1.82±0.0	2.13±0.04	2.78±0.0	2.05±0.0	0.93±0.06	0.93	2.89	1.83±0.83[b]
7	Linalool	21.28±2.78	23.45±1.50	18.87±0.0	31.58±0.22	23.0±0.0	32.16±0.0	24.47±2.38	18.87	32.16	24.90±4.24[a]
8	Camphor	0.09±0.01	0.08±0.01	0.07±0.0	0.10±0.01	0.21±0.0	0.09±0.0	0.09±0.02	0.09	0.21	0.10±0.04[a]
9	Borneol	0.49±0.11	0.54±0.04	0.46±0.0	1.12±0.03	0.71±0.0	1.18±0.0	0.18±0.03	0.18	1.18	0.51±0.37[b]
10	Lavandulool	0.54±0.08	0.49±0.11	0.48±0.0	0.47±0.01	0.59±0.0	0.48±0.0	0.64±0.15	0.47	0.64	0.56±0.12[a]
11	Terpinen-4-ol	4.14±0.65	3.63±3.04	2.59±0.0	1.87±0.03	4.64±0.0	1.61±0.0	0.80±0.15	0.80	4.64	2.19±1.69[b]
12	Alpha- terpineol	0.29±0.0	0.19±0.03	0.22±0.0	0.35±0.01	0.77±0.0	0.33±0.0	0.20±0.11	0.19	0.77	0.28±0.16[a]
13	Linnalyl acetate	35.08±0.13	36.75±0.87	39.91±0.0	29.42±0.39	32.8±0.0	27.95±0.0	51.63±4.22	27.95	51.63	40.68±9.70[a]
14	Lavandulil acetate	4.02±0.43	3.69±0.66	5.79±0.0	3.64±0.07	4.48±0.0	3.66±0.0	2.32±0.31	2.32	5.79	3.37±1.07[b]
15	Beta- cariophylene	11.66±2.08	11.47±3.68	13.65±0.0	14.77±0.11	10.8±0.0	16.07±0.0	5.44±0.77	5.44	16.07	9.93±4.24[a]

*a,a same superscripts within the same column represent significant differences at the level of significance $p < 0.05$

Table 3. Chemical composition of clary sage essential oil (Rel.%) , obtained by the Clevenger type and by the new model apparatus for microdistillation

№	Compound	Clevenger type apparatus (water distillation)	New type apparatus (steam distillation)
1	Alpha- pinene	0.01±0.01[a]*	0.01±0.0[b]**
2	Camphen	0.02±0.02[a]	0.01±0.01[a]
3	Myrcene	1.18±0.28[a]	0.23±0.06[a]
4	Cis-ocimene	0.51±0.08[a]	0.14±0.07[a]
5	Limonene	0.28±0.04[a]	0.07±0.0[a]
6	trans-ocimene	1.01±0.16[a]	0.23±0.08[a]
7	Linalool	20.61±1.13[a]	7.22±2.67[a]
8	Camphor	0.04±0.02[a]	0.02±0.02[b]
9	a-terpineol	4.61±0.07[a]	0.38±0.15[a]
10	Nerol	1.17±0.09[a]	1.05±0.17[b]
11	Linalyl acetate	36.57±6.81[a]	64.02±2.01[a]
12	Neryl acetate	1.49±0.08[a]	0.33±0.04[a]
13	Geranyl acetate	2.77±0.21[a]	0.57±0.09[a]
14	Beta-kariofilen	1.12±0.27[a]	1.24±0.11[b]
15	Germacrene	1.85±0.59[a]	2.11±0.90[b]
16	Sclareol	13.52±4.86[a]	10.37±5.02[b]

*The data are presented as mean ± SD,
**a,a same superscripts within the same row represent significant differences at the level of significance $p < 0.05$

Table 4. Chemical composition of helichrysum essential oil (Rel.%), obtained by the Clevenger type and by the new model apparatus for microdistillation

№	Compound	Clevenger type apparatus (water distillation)	New type apparatus (steam distillation)
1	Alpha- pinene	7.35±2.59[a]	2.96±0.59[a]
2	Camphen	0.34±0.17[a]*	0.10±0.04 [b]**
3	Beta- pinene	0.47±0.11[a]	0.26±0.02[a]
4	Limonene + 1,8 cineole	2.21±0.39 [a]	1.38±0.22[a]
5	Linalool	2.30±0.57[b]	1.18±0.60[a]
6	Camphor	0.33±0.20[a]	0.31±0.17[b]
7	Borneol	0.09±0.11[a]	0.03±0.02[b]
8	Terpinen-4-ol	0.72±0.07[a]	0.23±0.09[a]
9	a-terpineol	0.76±0.05[a]	0.24±0.05[a]
10	Nerol	1.80±0.62[a]	0.68±0.05[a]
11	Neral	1.12±0.8[a]	1.15±0.82[b]
12	Isoitalicene	0.29±0.24[a]	0.33±0.21[b]
13	Neryl acetate	13.07±1.10[a]	13.37±1.85[b]
14	Italicene+ beta-cariofilen	11.95±1.20[a]	10.15±1.74[b]
15	curcumene	13.84±0.74[a]	15.60±1.22[b]
16	Beta- selinene	6.03±0.69 [a]	8.92±1.75[b]
17	Alpha- selinene	3.97±0.43[a]	5.93±1.17[b]

*The data are presented as mean ± SD,

**a,a same superscripts within the same row represent significant differences at the level of significance $p < 0.05$

Lavender has been studied in detail as a target crop.

The results revealed a typical composition for Bulgarian lavender oil, in particular - for the "Hemus" variety (Konakchiev, 2015). Sixteen components have been identified, all listed in international standard (www.iso.org/standard/36253.html). The most important - linalyl acetate and linalool ranged from 22.03% to 51.63% and 18.87% to 39.33%, respectively. The composition of oils from different points of the rose valley is lower levels of esters, but this is reasonable and proves the importance of varietal material. The comparison between the two devices shows that linalylacetate is on average 33% more in the oil than the new device – by 25% on average for local lavenders and by 55% on average for Hemus variety. At the same time, the amount of alcohol (linalool) is on average 39% less (by 28% on average for local and by 42% for the variety). It is obvious that water medium and high temperature lead to large-scale hydrolysis processes and the result is a deterioration of oil quality. The other components do not have such differences in content, but for example in the case of cis-ocimene there are also higher levels (by 27% on average) in the oil from the new device. In this case, higher average levels (37%) were observed in local lavender samples, and only 9% for the Hemus variety.

Lavandulyl acetate is also an ester formula, but it is a minor component and it is difficult to draw conclusions from its values. On average, its levels were the same for both apparatuses.

It is noteworthy that the β-caryophyllene content was on average 51% higher in the oil produced by the new apparatus. This sesquiterpene has proven healing benefits (Fidyt et al., 2016) and the results can be interpreted as an additional advantage.

In the essential oil produced from clary sage (see Table 3), seventeen distinct components were identified. The composition of the oil replicates the literature data (Saharkhiz et al., 2009). The results showed even more the advantages of the new laboratory device. According to Bulgarian standards, the linalyl acetate content should exceed 45% (https://bds-bg.org/bg/project/list). All the essential oils obtained with the Clevenger apparatus fell outside this limit. On average, this compound was 75% more in the samples than the new model. The opposite trend was observed for linalool, which had decreased threefold. This fact proves that the ester decomposes in an aqueous medium. The other ester formations – neryl acetate and geranyl acetate, were in minor content. This is why their discussion poses some difficulties. The progenitor nerol was also with minor values, although a declining trend was observed there.

The analysis of the helichrysum essential oil revealed a composition typical for the local area. (Saint-Lary et al., 2008). Seventeen compounds were identified, with the principal ester being neryl acetate, which ranged from 13.07% to 13.77%. Unlike the basic ester of lavender and sage, neryl acetate did not show a clear tendency to increase or decrease. The results correlated with another

study for Corsican origin, introduced in south Bulgaria (Tzanova et al., 2018). It has been found that the flowering stage is more important than the distillation type, while laboratory (water) and industrial (vapor) samples showed almost equal neryl acetate content (20.6% and 18.01%, respectively). This could be due to specific differences in the chemical composition, which may account for its stability in the aquatic environment. On the other hand, the quantitative levels in the oil are lower. The influence of these factors can be proven by further investigations and statistical analysis.

It is very interesting that in all the experiments the content of α-terpineol was over 30% less in the essential oils from the new laboratory device. A similar trend was also observed in the study by Tzanova et al. (2018). The fact could be subject of other investigations.

Conclusion

The newly developed laboratory apparatus demonstrates promising efficacy in determining both the content and quality of essential oils in lavender, as well as other herbal raw materials. The reliability and repeatability of the obtained results make this model particularly advantageous. Importantly, it displays exceptional performance with raw materials possessing high ester content, effectively preserving the quality of the extracted essential oils. Thus, this model can contribute significantly to enhancing the precision and reliability of essential oil analysis in diverse herbal raw materials.

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