

## Article

# Influence of Harvest Time, Method of Preparation and Method of Distillation on the Qualitative Properties of Organically Grown and Wild *Helichrysum italicum* Immortelle Essential Oil

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**Abstract:** The most important process in immortelle is the extraction of the essential oil by distillation. The chemical properties of the plant depend largely on the cultivation type, climatic conditions and agrotechnical measures. In this work, studies were carried out on organically grown and wild immortelle harvested during the summer (July) and autumn (October) seasons of 2020. Immortelle samples were prepared by drying naturally in two ways: in direct sunlight and in shade. To extract the essential oil, immortelle was distilled in two ways: on an industrial and a laboratory scale. The essential oil was analyzed by gas chromatography to determine the composition and quality of the essential oil. The results of this work showed that the qualitative properties of the essential oil were influenced by the cultivation type, harvesting time, preparation method and distillation method. A higher yield ( $0.21\% \pm 0.03$ ) of essential oil was obtained from wild immortelle whereas a better utilization rate ( $0.38\% \pm 0.09$ ) of essential oil was obtained from the organically grown immortelle. It was also found that a higher yield of essential oil was obtained from the summer harvest ( $0.19\% \pm 0.01$ ) and a higher utilization rate from the autumn harvest ( $0.33\% \pm 0.13$ ) with the industrial scale distillation and shade drying. In general, better qualitative properties of immortelle essential oil were obtained from wild immortelle, which was confirmed by a chromatographic analysis and better content of  $\gamma$ -curcumene ( $16.64\% \pm 0.30$ ) and neryl acetate ( $8.15\% \pm 0.19$ ) and other constituents except  $\alpha$ -pinene where organically grown had a higher share ( $24.27\% \pm 0.97$ ).

**Keywords:** immortelle; harvest; drying; qualitative properties; essential oil;  $\alpha$ -pinene; neryl acetate



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## 1. Introduction

Immortelle (*Helichrysum italicum*) is a typical Mediterranean plant belonging to the composite family *Asteraceae*, which includes more than 600 different species. Immortelle grows at altitudes up to 1700 m above sea level, usually on sandy or acid clay soils [1]. It consists of three subspecies: *Helichrysum italicum* (Roth) G. Don subsp. *microphyllum* (Willd.) Nyman, *Helichrysum italicum* subsp. *serotinum* (Boiss.) P. Fourn. and *Helichrysum italicum* (Roth) G. Don subsp. *italicum* [1–3].

Immortelle is native to Mediterranean countries (Cyprus, Greece, Croatia, Italy, France, Spain, Algeria, Morocco) and the best-known natural location is on the island of Corsica [4].

Immortelle is a low aromatic shrub with yellow flowers, 50 to 70 cm tall, and grows on rocky and sandy soils [5]. It is resistant to low temperatures and can survive long periods without water [6]. Success in growing immortelle depends on agro-ecological conditions and agrotechnical measures. The yield can vary from 8 to 20 tons per hectare, depending on the plant spacing, irrigation and harvest timing. However, at high yields, the quality and concentration of the oil are not satisfactory.

Today, immortelle is in high demand in the world market because of its wide and varied uses. It is used in traditional human medicine for its anti-inflammatory [7–9] and antibacterial [10–18] properties. There are data in the literature reporting the use of immortelle as an antiparasitic agent in animals [19,20] but not as an insecticidal agent [21]. It is also used as a seasoning for food and in the cosmetic and pharmaceutical industries [22].

Immortelle is harvested for essential oil during its flowering period; that is, when 50% of the flowers are open. It is harvested in the early morning hours when the concentration of essential oil in the inflorescences is highest [22]. Essential oils are aromatic molecules found in plants. The composition and content of the essential oil in a plant depends on the developmental stage of the plant, climatic and soil conditions, method of isolation and chemotype of a plant as well as the geographical origin and vegetative cycle of the vegetation [5,23]. The fragrance of aromatic plants comes from terpene compounds (isoprenoids), which are the main constituents of essential oils. In addition, essential oils contain a large number of compounds with similar structures and physicochemical properties, which further complicates their identification [24]. The chemical composition of immortelle essential oils from different European countries has been studied by numerous authors [11,13,25,26]. The main components of essential oil are  $\alpha$ -pinene, neryl acetate,  $\alpha$ -cedrene, nerol,  $\alpha$ -curcumin,  $\gamma$ -curcumene and geranyl acetate. After the immortelle is harvested, the essential oil is distilled within 24 h usually, most commonly by evaporation steam. The reason for this is because the concentration of the essential oil drops rapidly from the moment the flowers are harvested due to the biochemical processes that occur in them. On average, 0.1–0.17% of the essential oil is contained in the flowers, so 1 kg of essential oil requires 700 to 1000 kg of fresh immortelle [4,27]. From 1 hectare of land, 3500–4000 kg of dry flowers or 7000–8000 kg of fresh flowers can be harvested, which means that the amount of essential oil obtained from 1000 kg of plant material can vary from 400 g to more than 3500 g. Depending on the production conditions, the yield of essential oil ranges from 8 to 12 kg/ha [22,27].

The rest of the distilled herb can also be used in organic farming as a fertilizer. Depending on the capacity, a certain amount of oil may remain in the hydrolate so that the hydrolate is subjected to a simple distillation if the value of the oil thus obtained justifies the cost of distillation.

However, the problem in Croatia is that the existing distillation capacities are too small and do not offer the possibility of distilling immortelle at the optimal time.

Therefore, in the processing of wild plants including immortelle, certain technological procedures are allowed that do not change the natural structure of the parts of the plant that constitute the yield and also allow the preservation of the biological value of the stock or product. In this way, the deterioration of the collected material is prevented and the losses of the essential oil are minimal with only a slight deterioration in the oil quality. Such processes can be mechanical, thermal or fermentative and also a combination of these processes. The technological processes used in the primary processing of aromatic plants include drying and mechanical processing [28]. The drying process increases the values of the commodity and decreases the enzymatic and microbial activity processes [29–31]. Apart from natural drying, which is the simplest and most common process, there are other methods used in Mediterranean countries (Cyprus, Greece, Croatia, Italy, France, Spain, Algeria, Morocco) to bring the material to moisture content [32]. The drying of immortelle is carried out at temperatures of 35–40 °C. In the natural drying of immortelle, drying can be done in the sun, in the shade or by a combination of these two ways, exposing the material to the sun only on the first day, turning the material over several times during the day and then drying in the shade.

Therefore, the aim of this work is to determine the qualitative properties of the essential oil of organically grown and wild immortelle; that is, whether the essential oil yield, utilization rate of the essential oil and quality of the essential oil depends on the harvest (summer and autumn), the method of preparation (immediately after harvest

and dried by sunlight and in the shade) and the method of distillation (comparison of an industrial scale relative to a laboratory scale).

## 2. Materials and Methods

### 2.1. Samples Used in the Investigation

Organically grown immortelle and wild immortelle from the area of Central Dalmatia, Croatia, were used for this work. The samples were collected during the summer (July) and autumn (October) harvest seasons in 2020.

The samples of organically grown and wild immortelle were harvested from an area of approximately 1500 square meters for each cultivation type. The coordinates of the organically grown immortelle were 44.120131, 15.497642 and the wild immortelle 43.978788, 15.661878. The soils were of a similar chemical composition and the weather conditions were identical given the proximity of the harvest site.

The sampling was performed according to the regulation on the methods of sampling and quality control 99/2008 [33]. About 1000 kg of each sample (organically grown and wild immortelle) in each season (summer and autumn harvest) was collected. Organically grown and wild immortelle were collected when at least 50% of the flowers were open. Special attention was paid to the collection of the organically grown immortelle (propagation by dropping seeds during the collection). Both types of immortelle were harvested with hand shears at 20 cm, cutting a few cm above the woody part in the case of the organically grown immortelle and leaving 30% of the young shoots for the further growth of the plant.

### 2.2. Immortelle Preparation Methods

Each sample was first quartered into two parts: a larger part for the industrial scale (300 kg) and a smaller part for the laboratory scale (12 kg). Each of these parts was then divided into three equal mass quantities. One part was immediately distilled and the other two were either sun or shade-dried.

Drying to achieve a mass equal to 30% of the initial mass took up to 12 days, depending on the humidity and when the immortelle was harvested.

### 2.3. Methods of Distillation

The distillation was carried out on an industrial scale, which had a capacity of 8000 L and was equipped with a steam generator (custom made, Mahovina d.o.o., Jastrebarsko, Croatia) producing 500 kg of steam per hour and in a laboratory scale still, which had a capacity of 30 L and a steam flow rate of 5 kg per hour. The main difference between the industrial scale and the laboratory scale is the steam generator; on an industrial scale, it is a separate part of the whole scale whereas on a laboratory scale, the water is heated under the teapots in the same vessel in which the teapots are located. In order to reach a pre-pressure on an industrial scale, the water must exceed a temperature of 100 °C (105 °C), i.e., it must be completely dry (the plant material comes out dry after the distillation) whereas on a laboratory scale, the temperature is up to 100 °C and the plant material comes out moist. The duration of the distillation was 90 min for both plants and for all samples, for both fresh and dried immortelle. In this time, almost 100% of the essential oil was distilled and the composition of the essential oil was desirable.

After the distillation, the yield and utilization rate of the essential oil were determined. The utilization rate is the essential oil yield multiplied by the efficiency of the scale.

### 2.4. Chromatographic Analysis of the Essential Oil

The purified essential oil for each sample was mixed to obtain a representative sample. The samples were subjected to a gas chromatographic analysis according to method HRN EN ISO 5509:2004. Gas chromatographic analyses were performed using a CP-3800, Varian (Palo Alto, CA, USA) equipped with a flame ionization detector (FID CP-3800, Varian, Palo Alto, CA, USA) and a DB-23 fused silica capillary column (60 m × 0.25 mm; film

thickness 0.25 μm, CP-3800, Varian, Palo Alto, CA, USA). Helium was the carrier gas (1.5 mL/min) and the hydrogen flow was 30 and the air flow was 300 mL/min.

The injector temperature was maintained at 250 °C and the oven (CP-3800, Varian, Palo Alto, CA, USA) temperature program was from 60 to 220 °C at a rate of 7 °C/min. The detector (FID) was operated at 250 °C. The injection volume was 1.0 μL. The retention time (Rt) and retention indices (RI) for all components were verified.

### 2.5. Statistical Analysis

In this study, a set of 2 × 2 × 3 × 2 factorial experiments with three replicates was performed to investigate the effects of the factors on the essential oil yield and utilization rate in immortelle. The factors observed to have a positive influence on the measured parameters in previous studies are: (a) the cultivation type (organically grown in relation to wild immortelle), (b) the harvesting time (summer and autumn), (c) the method of preparation (immediately after harvest and dried by sunlight and in the shade) and (d) the method of distillation (comparison of an industrial scale relative to a laboratory scale).

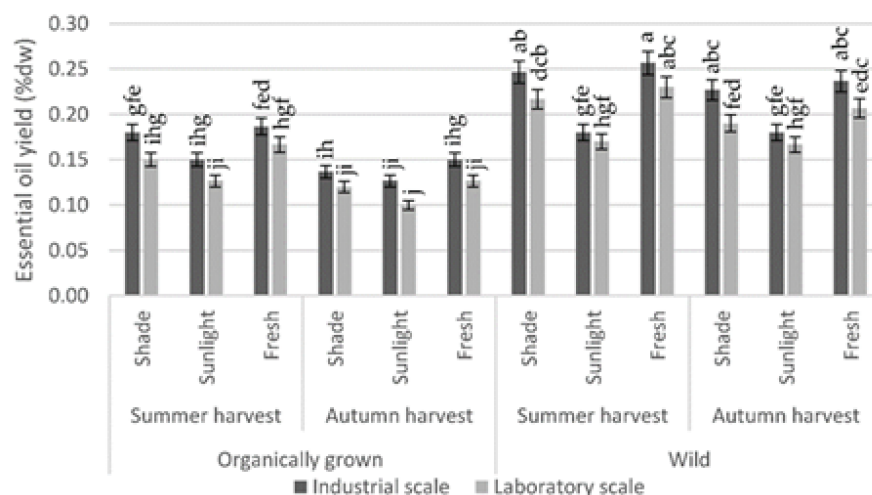
For investigating the influence of the cultivation type on the content of α-pinene, linalool, nerol, neryl acetate, γ-curcumene and italdiones, a set of 2 × 2 factorial experiments with three replicates was performed. The factors observed to have a positive influence on the measured parameters in previous studies are: a) the cultivation type (organically grown in relation to wild immortelle) and b) the harvesting time (summer and autumn).

For both factorial experiments, an analysis of variance (ANOVA) was used to evaluate the influence of the factors and their interactions on the measured parameters at *p* < 0.05. A Duncan’s multiple range test was also performed and significant differences were observed at *p* < 0.05.

For the statistical analyses, the software package R (R Development Core Team, Vienna, Austria, 2008), the interface RStudio (RStudio, Inc., Boston, MA, USA, 2018) and the additional software packages multcomp, MASS and pwr were used. The Excel 2016 software package (Microsoft Corporation, Redmond, DC, USA, 2016) was used for the figure design.

### 3. Results

Based on the obtained results, the qualitative properties of the essential oil of organically grown and wild immortelle are shown in Figure 1 and Table 1, i.e., the influence of harvest time, the cultivation type, the method of preparation and the method of distillation on the yield of the essential oil.



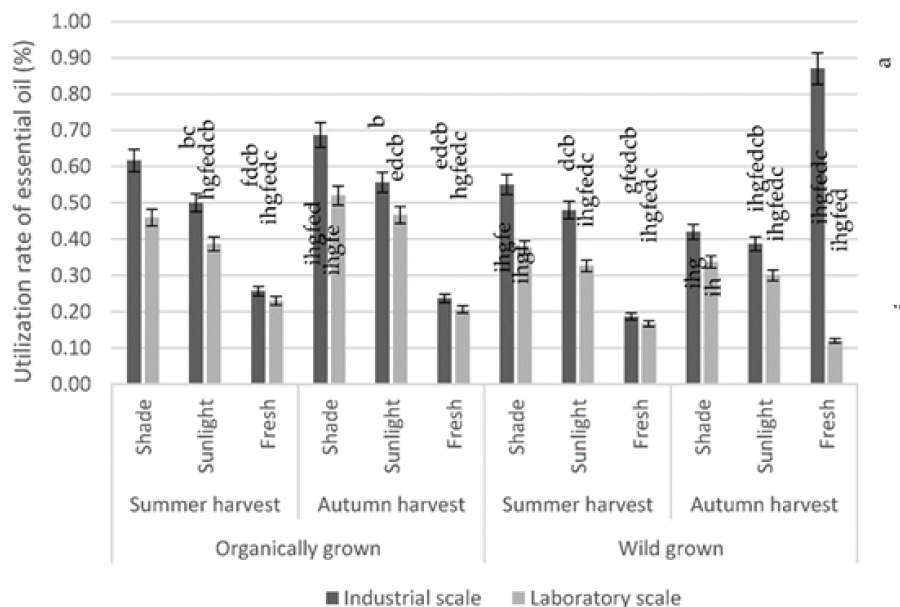
**Figure 1.** The influence of the analyzed factors on the essential oil yield of immortelle. (The bars designated by different small letters (a, b, c, d, e, f, g, h, i, j) are significantly different (*p* < 0.05)).

**Table 1.** Results of the analysis of the variance of the influence of the analyzed factors on the content of the essential oils in immortelle at a significance level of  $p < 0.05$ .

Influencing Factors	Statistical Indicators				
	Sum of Squares	Degree of Freedom	Mean of Square	F	p-Value
Intercept	2.233089	1	2.233089	6207.815	0.000000
Harvest	0.010756	1	0.010756	29.900	0.000002
Type	0.077356	1	0.077356	215.042	0.000000
Preparation	0.026178	2	0.013089	36.386	0.000000
Distillation	0.010272	1	0.010272	28.556	0.000002
Harvest* Type	0.001422	1	0.001422	3.954	0.052488
Harvest* Preparation	0.001111	2	0.000556	1.544	0.223858
Type* Preparation	0.002678	2	0.001339	3.722	0.031425
Harvest* Distillation	0.000006	1	0.000006	0.015	0.901617
Type* Distillation	0.000006	1	0.000006	0.015	0.901617
Preparation* Distillation	0.000311	2	0.000156	0.432	0.651432
Harvest* Type* Preparation	0.000078	2	0.000039	0.108	0.897748
Harvest* Type* Distillation	0.000050	1	0.000050	0.139	0.710922
Harvest* Preparation* Distillation	0.000044	2	0.000022	0.062	0.940168
Type* Preparation* Distillation	0.000478	2	0.000239	0.664	0.519405
Harvest* Type* Preparation* Distillation	0.000100	2	0.000050	0.139	0.870580
Error	0.017267	48	0.000360	-	-

\* Statistical interaction.

Figure 2 and Table 2 show the qualitative properties of the essential oil of organically grown and wild immortelle, i.e., the influence of harvest time, the cultivation type, the method of preparation and the method of distillation on the utilization rate of the essential oil.



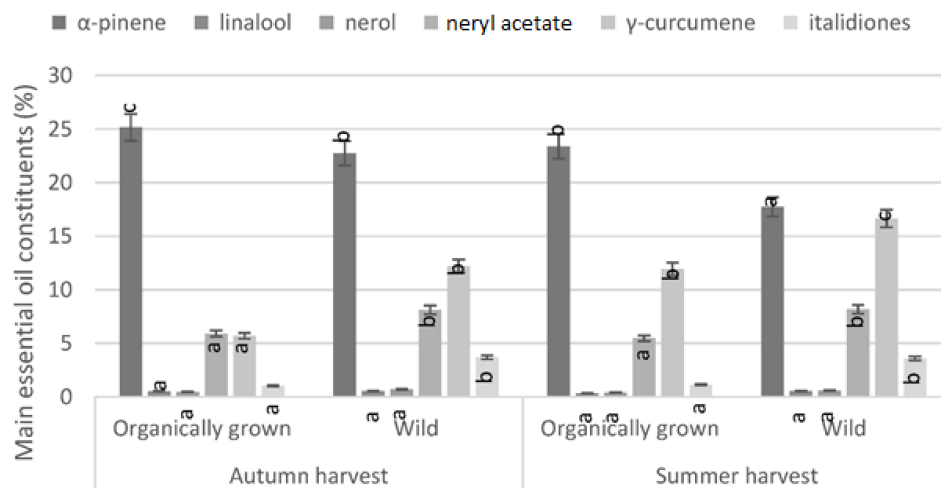
**Figure 2.** The influence of the analyzed factors on the utilization rate of the essential oil. (The bars designated by different small letters (a, b, c, d, e, f, g, h, i, j) are significantly different ( $p < 0.05$ )).

**Table 2.** Results of the analysis of the variance of the influence of the analyzed factors on the utilization rate of the essential oils in immortelle at a significance level of  $p < 0.05$ .

Influencing Factors	Statistical Indicators				
	Sum of Squares	Degree of Freedom	Mean of Square	F	p-Value
Intercept	11.62423	1	11.62423	492.4944	0.000000
Harvest	0.04061	1	0.04061	1.7207	0.195847
Type	0.04550	1	0.04550	1.9278	0.171410
Preparation	0.55770	2	0.27885	11.8143	0.000067
Distillation	0.42781	1	0.42781	18.1255	0.000096
Harvest* Type	0.00190	1	0.00190	0.0806	0.777764
Harvest* Preparation	0.09211	2	0.04605	1.9512	0.153209
Type* Preparation	0.21867	2	0.10933	4.6323	0.014472
Harvest* Distillation	0.03967	1	0.03967	1.6807	0.201036
Type* Distillation	0.05837	1	0.05837	2.4729	0.122391
Preparation* Distillation	0.02831	2	0.01415	0.5997	0.553047
Harvest* Type* Preparation	0.22995	2	0.11498	4.8713	0.011854
Harvest* Type* Distillation	0.04253	1	0.04253	1.8021	0.185773
Harvest* Preparation* Distillation	0.16744	2	0.08372	3.5470	0.036585
Type* Preparation* Distillation	0.13462	2	0.06731	2.8518	0.067565
Harvest* Type* Preparation* Distillation	0.15994	2	0.07997	3.3881	0.042033
Error	1.13293	48	0.02360	-	-

\* Statistical interaction.

The influence of the harvest (autumn and summer harvest) and cultivation type (organically grown and wild) on the content of the essential oil as well as their interaction were the main factors influencing the oil components, as shown in Figure 3 and Table 3.



**Figure 3.** The influence of the analyzed factors on the essential oil constituents. (The bars designated by different small letters (a, b, c) are significantly different ( $p < 0.05$ )).



**Table 3.** Results of the analysis of the variance of the influence of the analyzed factors on the immortelle essential oil constituent at a significance level of  $p < 0.05$ .

Constituent	Influencing Factors	Statistical Indicators				
		Sum of Squares	Degree of Freedom	Mean of Square	F	p-Value
$\alpha$ -Pinene	Harvest	34.10	1	34.10	1099.3	0.000001
	Type	48.68	1	48.68	1569.1	0.000001
	Harvest* Type	7.86	1	7.86	253.2	0.000002
	Error	0.25	8	0.03		
Linalool	Harvest	0.0280	1	0.02803	0.478	0.509
	Type	0.0385	1	0.03853	0.657	0.441
	Harvest* Type	0.0261	1	0.02613	0.445	0.523
	Error	0.4695	8	0.05868		
Nerol	Harvest	0.0184	1	0.01841	0.252	0.629
	Type	0.1519	1	0.15187	2.077	0.187
	Harvest* Type	0.0010	1	0.00101	0.014	0.909
	Error	0.5849	8	0.07312		
Neryl Acetate	Harvest	0.118	1	0.118	2.317	0.1665
	Type	18.229	1	18.229	357.893	0.000003
	Harvest* Type	0.180	1	0.180	3.536	0.0969
	Error	0.407	8	0.051		
$\gamma$ -Curcumene	Harvest	85.28	1	85.28	1412.5	0.000002
	Type	94.58	1	94.58	1566.6	0.000003
	Harvest* Type	2.42	1	2.42	40.1	0.000225
	Error	0.48	8	0.06		
Italidiones	Harvest	0.000	1	0.000	0.000	0.992
	Type	19.431	1	19.431	280.931	0.000002
	Harvest* Type	0.025	1	0.025	0.364	0.563
	Error	0.553	8	0.069	-	-

\* Statistical interaction.

#### 4. Discussion

All results in this section are indirectly commented as an average value of the shown results. According to the results of the statistical analysis (Figure 1 and Table 1), it can be seen that the harvest time, cultivation type, method of preparation, method of distillation and cultivation type  $\times$  the harvest time of immortelle had an impact on the essential oil yield.

One of the most important components in the essential oil yield is moisture. Organically grown immortelle generally has a slightly higher percentage of moisture than wild immortelle. The reason for this is that cultivated immortelle has more soil to grow in and a larger root so it can draw moisture from greater depths whereas wild immortelle grows in karst, occasionally literally from pieces of soil blown by the wind into a crevice. As a rule, about 65% of the mass is lost in drying. Part of the mass was lost from the harvest to the scale so the moisture content at harvest time was from 75% to 80% of the mass; after drying, the moisture content was from 2% to 5%. As the essential oil protects the plant from drought and because the organically grown immortelle has access to water, this is one of the factors why wild immortelle has a higher essential oil content. Accordingly, in the organically grown immortelle essential oil, the yield was  $0.14\% \pm 0.02$  and in the wild immortelle it was significantly higher at  $0.21\% \pm 0.03$ . The higher yield of the essential oil was in the summer harvest and amounted to  $0.19\% \pm 0.01$  whereas in the autumn it was  $0.16\% \pm 0.04$ .

These values also depended on the method of preparation and it was found that shade-dried immortelle had a significantly higher oil yield of  $0.18\% \pm 0.04$  compared with the sunlight-dried immortelle ( $0.15\% \pm 0.03$ ). This proved that the sunlight drying not only lost moisture but also lost active ingredients, which is not desirable and rules out sun drying as a drying method. A significantly higher yield of essential oil was produced on an industrial scale of  $0.19\% \pm 0.04$  compared with the laboratory scale of  $0.16\% \pm 0.04$ .

Tzanova et al. [34] obtained an essential oil yield of 0.19% to 0.26% by distillation on an industrial scale. Similar results were obtained in this study. According to Maksimović et al. [35], yields of essential oil ranging from 0.02% to 0.78% were obtained. Mollova et al. [36], obtained yields from 0.10% to 0.14% in their study and Kladar et al. [26], obtained only a 0.03% yield, which was the lowest yield among all the cited results.

These results are comparable with the results obtained because the method of preparation was the same (everything was collected manually during the flowering period, placed in a distillery and distilled). The laboratory and industrial distillations with evaporation steam were carried out on the same principle where water vapor extracts the oil from the plant. Occasionally, there can be a distillation of wet steam on a laboratory scale due to the absence of a steam generator where the plant is soaked in water and the water is heated until it evaporates.

The obtained results of the statistical analysis (Figure 2 and Table 2) showed that the utilization rate of the essential oil was influenced by the harvest time, cultivation time, method of preparation, method of distillation and cultivation type  $\times$  the method of preparation.

The essential oil utilization rate in the organically grown immortelle was significantly higher at  $0.38\% \pm 0.09$  and at  $0.27\% \pm 0.11$  in the wild immortelle. In the autumn harvest, the utilization rate was slightly higher at  $0.33\% \pm 0.13$  whereas in the summer it was  $0.32 \pm 0.09$ . The method of preparation with drying in the shade led to better utilization of the essential oil at  $0.42\% \pm 0.08$  whereas drying in sunlight was  $0.37\% \pm 0.07$  and the fresh samples were only  $0.18\% \pm 0.11$ .

A higher essential oil utilization rate, considering all the parameters studied, was obtained on an industrial scale at  $0.48\% \pm 0.16$  whereas on a laboratory scale it was  $0.32\% \pm 0.11$ .

For the final price of an essential oil, the recovery rate is important in addition to the quantity. It is closely related to the cost of distillation. On an industrial scale, the operating cost of the plant is known (from energy to human labor) and it is unprofitable to continue distillation if the amount of oil recovered in that minute (or in 5 min) does not cover the operating cost of the timescale. Therefore, laboratory scale distillation, which is much cheaper, can be carried out for several hours if necessary to determine the exact proportion of the essential oil in immortelle. The utilization rate has also been studied in other works [23,37–40] where it was found that the difference in the utilization rate can also be defined by agrotechnical measures, the soil quality and the harvest time.

Geographical, climatic and other environmental factors influence the composition of essential oil in immortelle [41]. Laboratory and industrial distillation are carried out according to the same principle, i.e., water vapor extracts oil from the plant. The quality of the oil depends on the plant and not on the method of distillation, assuming that the distillation is performed to the end. In addition, by drying, the plant loses water and not the essential oil. The reason for this is that the volatile compounds are extracted first and it is only toward the end of the distillation (in our experience, in the last 15 to 20 min) that the volatile compounds are heavier, which are the most desirable compounds in the oil. Without them, the volatile compounds, a few of which are undesirable, will have a higher proportion in the oil composition, meaning they will be above the limits where they are desirable. Over time, the oil will be of a diminished quality, often resulting in a lack of consumer interest. The proportion of each component of the essential oil can vary depending on when it is harvested and grown.

Croatian essential oil is classified by major components, which are  $\alpha$ -pinene, neryl acetate and  $\gamma$ -curcumene [13,17,42,43]. The esters, of which neryl acetate is the most abundant, are responsible for the regenerative power of immortelle essential oil. According to the results, monoterpene was the dominant group of chemical constituents in the essential oil followed by sesquiterpene.

The analysis of the variance of the influence of the analyzed factors on the immortelle essential oil showed that the content of monoterpene  $\alpha$ -pinene was dependent on both



factors as well as their interaction. Its content was significantly higher in organically grown immortelle in the autumn harvest ( $25.14\% \pm 0.18$ ) whereas the lowest content was in wild immortelle in the summer harvest ( $17.74\% \pm 0.24$ ). In general, organically grown immortelle contained  $24.27\% \pm 0.97$  of  $\alpha$ -pinene whereas wild immortelle contained  $20.24\% \pm 2.74$ . Immortelle harvested in the autumn contained  $23.94\% \pm 1.33$  of  $\alpha$ -pinene whereas immortelle from the summer harvest contained  $20.57\% \pm 3.10$ .

Monoterpenic alcohols linalool ( $0.55\% \pm 0.07$ ) and nerol ( $0.69\% \pm 0.27$ ), both desirable components, were present in a highest content in the essential oil from the wild immortelle in the autumn harvest whereas the lowest content of both linalool ( $0.34\% \pm 0.30$ ) and nerol ( $0.39\% \pm 0.29$ ) was obtained in the organically grown immortelle from the summer harvest. For both monoterpenic alcohols, the content was not influenced either by the harvest or the type of cultivation.

Monoterpene esters, of which neryl acetate is the most desirable compound, were significantly higher in the wild immortelle from the summer harvest ( $8.1\% \pm 0.21$ ) whereas it was significantly lowest in the organically grown immortelle from the summer harvest ( $5.46\% \pm 0.20$ ). A statistical analysis showed that the neryl acetate content was influenced by the type of cultivation whereas the harvesting period or interaction did not have any significant influence. On average, the wild type of immortelle contained  $8.15\% \pm 0.19$  of neryl acetate whereas organically grown contained  $5.69\% \pm 0.32$ .

Sesquiterpene ( $\gamma$ -curcumene) was higher in the wild immortelle from the summer harvest ( $16.64\% \pm 0.30$ ) whereas the lowest was the organically grown from the autumn harvest ( $5.70\% \pm 0.14$ ). As for monoterpene, both factors significantly influenced the content of  $\gamma$ -curcumene as well as their interaction. In general, the wild immortelle contained  $14.43\% \pm 2.44$  of  $\gamma$ -curcumene whereas the organically grown contained  $8.81\% \pm 3.41$ . Immortelle harvested in the summer contained  $14.29\% \pm 2.59$  of  $\gamma$ -curcumene whereas immortelle from the autumn harvest contained  $8.95\% \pm 3.57$ .

Sesquiterpene ketones (italidiones) were higher in the wild immortelle oil from the autumn harvest ( $3.69\% \pm 0.30$ ). Due to their biological activity, italidiones (a group of isomeric  $\beta$ -diketones) deserve special attention as they contribute to the specific scent of immortelle and its remarkable anti-hematoma properties [44]. The cultivation type had a statistically significant influence on the content of italidiones. On average, the wild type of immortelle contained  $3.67\% \pm 0.22$  of italidiones whereas the organically grown contained  $1.10\% \pm 0.26$ .

The color of both samples distilled in both distillation processes was light yellow to golden and the odor of immortelle is specific and very intense and long lasting.

Similar results for  $\alpha$ -pinene (12.8%) and neryl acetate (10.4%) in immortelle cultivated in Croatia have been obtained in the literature [17].

Studies by Peršić et al. [45] indicated that the preferred component of immortelle essential oil has an  $\alpha$ -pinene content of less than 25% and a neryl acetate content of more than 5%. Other studies [39] found similar values of the observed components.

Thus, according to studies in France, the proportions of neryl acetate (17.6–56.1%),  $\gamma$ -curcumene (8.6–18.2%), linalool (14.9%) and nerol (3.7–14.4%) were recorded [23,37,38]. The composition of immortelle essential oil grown in Italy consisted of neryl acetate (31%) and  $\gamma$ -curcumene (10.7%) [25] whereas in Portugal  $\alpha$ -pinene was 53.5% and  $\gamma$ -curcumene was 27.4% [39]. In the Western Balkans,  $\alpha$ -pinene was 10.2–29.9% and neryl acetate was 4.1–13.5%; there were lower proportions of  $\gamma$ -curcumene (0–22%) [13,46]. Mediterranean essential oils are characterized by a high content of  $\alpha$ -pinene (22%) and lower proportions of  $\gamma$ -curcumene (10%),  $\beta$ -selin (6%), neryl acetate (6%) and  $\beta$ -caryophyllene (5%) [17,47,48].

## 5. Conclusions

Based on the results obtained on organically grown and wild immortelle in the area of Central Dalmatia during summer and autumn harvesting, it can be concluded that the timing of harvesting affects the yield and the degree of utilization of the essential oil as well as the  $\alpha$ -pinene and  $\gamma$ -curcumene content. Moreover, the yield and utilization rate

were higher in the autumn harvest. Shade drying of immortelle led to a higher yield of essential oil. It was found that distillation on an industrial scale gave better results, i.e., a higher amount of essential oil per kg of immortelle mass. According to the results, better qualitative properties were found in the wild immortelle essential oil, which was confirmed by a chromatographic analysis. Monoterpenic hydrocarbons were the dominant group of chemical constituents in the essential oil followed by sesquiterpene hydrocarbons.

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