applied sciences

MDPI

*Article*

# Volatile Organic Compounds of the Glandular Trichomes of *Ocimum basilicum* and Artifacts during the Distillation of the Leaves

**Bruno Tirillini [1,*] and Filippo Maggi [2]**

[1] Department of Biomolecular Sciences, University of Urbino, 61029 Urbino, Italy
[2] School of Pharmacy, University of Camerino, 62032 Camerino, Italy; filippo.maggi@unicam.it
* Correspondence: bruno.tirillini@uniurb.it

**Abstract:** Focusing on volatile organic compounds (VOC) of *Ocimum basilicum*, this study aims to determine the chemical composition of VOC in secretory trichomes and compare it with that of essential oil obtained by hydrodistillation of leaves. The technique of extracting the content of glandular trichomes refers to the microneedle shuttle analysis. Hydrodistillation of fresh leaves was done with a Clevenger distiller (EO). The chemical compositions were determined by GC/FID and GC/MS. The head of the capitate trichomes does not contain volatile compounds. Fifty volatile compounds were detected in the EO, and twenty-four volatile compounds were detected in the VOC; the main components were eugenol (from 15.47% ± 1.05% to 41.89% ± 2.83%) and linalool (from 32.05% ± 2.57% to 28.99% ± 2.32%), respectively. During the distillation of the basil leaves 26 artifacts are formed. The composition of the essential oil of *O. basilicum* therefore depends not only on the plant but also on the method used to obtain it.

**Keywords:** *Ocimum basilicum*; secretory trichomes composition; essential oil; artifacts



**Citation:** Tirillini, B.; Maggi, F. Volatile Organic Compounds of the Glandular Trichomes of *Ocimum basilicum* and Artifacts during the Distillation of the Leaves. *Appl. Sci.* **2021**, *11*, 7312. https://doi.org/10.3390/app11167312

Academic Editor: Natália Martins

Received: 10 June 2021
Accepted: 6 August 2021
Published: 9 August 2021



## 1. Introduction

*Ocimum basilicum* L. (sweet basil) belonging to the family Lamiaceae, the genus includes about 35 species of aromatic annual and perennial herbs and shrubs [1]. *O. basilicum* is a multipurpose herb characterized by its rich and aromatic essential oil content [2,3]. The aromatic leaves, flowers, and seeds are added to foods and beverages for flavor; extracted as active ingredient for use in perfumes, soaps, cosmetics, and dental products; they are included in traditional herbal medicines to treat fevers, headaches, kidney problems, gum ulcers, childbirths, rheumatoid arthritis, and menstrual irregularities [4–6]. Beside these traditional medical uses, recent scientific studies have demonstrated potent antioxidant [7], antiviral [8], and anti-proliferative activities [9] of some compounds occurring in *O. basilicum* leaf essential oil and extract [10].

Basil oil presents remarkable differences in composition, and some chemotypes from different geographical origins have been reported [1,2]. *O. basilicum* is rich in essential oils and have been the subject of numerous chemical studies [11,12]. Four major essential oil chemotypes in *O. basilicum* were recognized, each with a number of small variants: (1) methyl chavicol-rich; (2) linalool-rich; (3) methyl eugenol-rich; and (4) methyl cinnamate-rich [13].

In basil, as in the other species of the Lamiaceae family, essential oil is stored in glandular trichomes [14]. Although the essential oil composition of a great number of Lamiaceae species is well known, little information is available on the secretion products of the various types of trichomes. Most of previous works concerned the morphology and structure of the glands and involved microscopical observations [15–19]. Only in a few cases the morphological study was accompanied and supported by chemical analyses of secreted oils: in *Mentha piperita* [20,21], *Salvia officinalis* [22–24], *Thymus vulgaris* [25], *Rosmarinus officinalis* [26,27], and *Ocimum basilicum* [28].

*O. basilicum* bears capitate and peltate glandular trichomes, distributed on both sides of the leaf. The subcuticular space of peltate trichomes appeared intensely colored with lipophilic stain, thus indicating the presence of VOC [28].

On the contrary, capitate trichomes did not present any staining with lipophilic stain, indicating the presence of polysaccharides [28–32].

Volatile plant constituents have different ecological functions. They play an important role in the process of plant growth, such as plant–plant competition and cooperative co-evolution, in the attraction of pollinators, in defense against insects and against the attraction of herbivores [33–35].

In recent years, micro-extraction methods for determining the composition of VOCs from plant material have developed considerably. These techniques have high sensitivity, and can be applied to matrices of gas, liquid, and solid samples.

In summary, microextraction techniques include solid phase microextraction (SPME), stir-bar sorptive extraction (SBSE), single drop microextraction (SDME), hollow fiber liquid phase microextraction (HF-LPME), dispersive liquid liquid microextraction (DLLME), and the gas purge microsyringe extraction (GP-MSE). These techniques, however, requires appropriate calibration with the use of standard substances.

Furthermore, the absorption of the fibers depends on the chemical class of the products present in the matrix [36–38].

Another approach is to isolate the secreting structures and to study their contents [36–40]. The method used by us refers to microneedles shuttle analysis [41].

The aim of this work is to compare the VOCs content directly sampled in capitate and peltate trichomes with the composition of the VOCs determined by hydrodistillation (essential oil) of the leaves of *O. basilicum*. It has been chosen to take the contents of the VOCs from the glandular hairs with the method of microneedle shuttle analysis since the possibility of forming artifacts during the collection is minimal.

## 2. Materials and Methods

### 2.1. Plant Materials

Commercial plants of *O. basilicum* var. *Italia* were bought at flower market and cultivated in pots at the Botanical Garden of Urbino. Fresh mature leaves were collected, before blossom, for chemical analyses. Voucher specimens were deposited in the Herbarium Urbinate (Botany Institute, Urbino University), under the acquisition number OB2234/20.

### 2.2. Sampling from Capitate and Peltate Trichomes

The trichome secretion was sampled by perforating with microneedles peltate and capitate trichomes as reported before [41]; the secretion was accumulated in microvials containing n-hexane and stored in sealed vials under refrigeration prior to analysis.

Samples were taken from three plants by sampling about 500 trichomes of both types per plant.

### 2.3. Isolation of the Essential Oil

Fresh leaves were subjected to hydrodistillation using a Clevenger-type apparatus for 4-h yielding 0.5% ± 0.1% of a yellowish oil. The oils were dried over anhydrous sodium sulfate and stored in sealed vials under refrigeration prior to analysis.

The same three plants sampled with microneedles were hydrodistilled separately.

### 2.4. GC-FID e GC-MS Analysis

GC–FID analysis of the volatile components was carried out using an Agilent 4890D instrument coupled to an ionization flame detector (FID). Compounds were separated on a HP-5 capillary column (5% phenylmethylpolysiloxane, 25 m × 0.32 mm i.d.; 0.17 mm film thickness; J&W Scientific, Folsom, CA, USA), working with the following temperature program: 5 min at 60 °C, rising at 4 °C/min to 220 °C, then at 11 °C/min to 280 °C, then held for 15 min; injector and detector temperatures, 280 °C; carrier gas, helium

(1.4 mL/min); injection volume, 1 µL; split ratio, 1:34. A mixture of aliphatic hydrocarbons (C8–C30; Sigma, Milan, Italy) in hexane was directly injected into the GC injector under the above temperature program in order to calculate the retention indices of each compound.

GC–MS analysis was performed using an Agilent 6890N gas chromatograph coupled to a 5973N mass spectrometer equipped with a HP-5MS capillary column (5% phenylmethylpolysiloxane, 30 m × 0.25 mm i.d., 0.1 mm film thickness; J&W Scientific). The same was programmed at 60 °C for 5 min, rising at 4 °C/min to 220 °C, then at 11 °C/min to 280 °C, then held for 15 min, and finally at 11 °C/min to 300 °C and held for 5 min; carrier gas, helium; flow rate, 1.0 mL/min; injector and transfer line temperatures, 280 °C; injection volume, 2 µL; split ratio, 1:50; scan time, 75 min; acquisition mass range, 29–400 *m*/*z*. All mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 eV.

The identification of volatile components was based on computer matching with the WILEY 275, NIST 05 and ADAMS libraries. A home-made library was used as well. Whenever possible, components were identified by comparing the retention times and mass spectra with those of authentic compounds using the program MSD Chemstation G1701 EA (Agilent).

## 3. Results

The head of the capitate trichomes does not contain volatile compounds and this in accordance with the numerous histochemical observations made on this type of trichomes [25,26].

The observed composition of the volatile compounds of *O. basilicum* hydrodistilled leaf oils (LEO) is presented in Table 1 and a chromatogram is shown in Figure 1.

**Table 1.** Chemical composition (as percentages) of *O. basilicum* leaf essential oil (LEO%) and peltate trichomes (VOC %).

| Peak | [a] Compounds | LEO% | VOC% | RI | RI * |
|---|---|---|---|---|---|
| 1 | alpha-pinene | 0.19 ± 0.02 | 0.02 ± 0.01 | 938 | 939 |
| 2 | camphene | 0.02 ± 0.01 | —— | 954 | 954 |
| 3 | sabinene | 0.23 ± 0.02 | 0.02 ± 0.01 | 976 | 975 |
| 4 | beta-pinene | 0.54 ± 0.05 | 0.04 ± 0.01 | 979 | 979 |
| 5 | octan-3-one | 0.03 ± 0.01 | —— | 983 | 984 |
| 6 | myrcene | 0.17 ± 0.02 | 0.03 ± 0.01 | 990 | 991 |
| 7 | delta-2-carene | 0.01 ± 0.01 | —— | 1003 | 1002 |
| 8 | 1.8-cineole | 5.84 ± 0.54 | 5.71 ± 0.53 | 1031 | 1031 |
| 9 | (E)-beta-ocimene | 1.06 ± 0.12 | 0.16 ± 0.02 | 1050 | 1050 |
| 10 | gamma-terpinene | 0.03 ± 0.01 | —— | 1060 | 1060 |
| 11 | n-octanol | 0.04 ± 0.01 | —— | 1067 | 1068 |
| 12 | terpinolene | 0.27 ± 0.02 | —— | 1087 | 1089 |
| 13 | linalool | 32.05 ± 2.57 | 28.99 ± 2.32 | 1097 | 1097 |
| 14 | camphor | 0.07 ± 0.01 | 0.93 ± 0.09 | 1144 | 1146 |
| 15 | borneol | 0.76 ± 0.06 | —— | 1169 | 1169 |
| 16 | terpinen-4-ol | 0.03 ± 0.01 | —— | 1179 | 1177 |
| 17 | alpha-terpineol | 0.67 ± 0.07 | 0.55 ± 0.05 | 1187 | 1189 |
| 18 | bornyl acetate | 2.77 ± 0.22 | —— | 1289 | 1289 |
| 19 | alpha-terpinyl acetate | 0.14 ± 0.01 | —— | 1349 | 1349 |
| 20 | eugenol | 15.47 ± 1.05 | 41.89 ± 2.83 | 1359 | 1359 |
| 21 | alpha-copaene | 0.18 ± 0.02 | 0.17 ± 0.02 | 1378 | 1377 |
| 22 | beta-cubebene | 0.21 ± 0.02 | —— | 1388 | 1388 |
| 23 | beta-elemene | 0.69 ± 0.06 | 1.7 ± 0.15 | 1391 | 1391 |
| 24 | beta-longipinene | 0.04 ± 0.01 | —— | 1401 | 1401 |
| 25 | methyl eugenol | 0.3 ± 0.01 | 0.45 ± 0.05 | 1404 | 1404 |
| 26 | (Z)-caryophyllene | 0.22 ± 0.02 | —— | 1409 | 1409 |
| 27 | cis-alpha-bergamotene | 9.35 ± 0.79 | 6.34 ± 0.54 | 1414 | 1413 |

**Table 1.** *Cont.*

| Peak | [a] Compounds | LEO% | VOC% | RI | RI * |
|---|---|---|---|---|---|
| 28 | cadina-3.5-diene | 0.47 ± 0.04 | —— | 1452 | 1452 |
| 29 | alpha-humulene | 0.72 ± 0.07 | 0.41 ± 0.04 | 1454 | 1455 |
| 30 | cis-muurola-4(14).5-diene | 0.86 ± 0.08 | 0.32 ± 0.03 | 1467 | 1467 |
| 31 | (Z)-beta-bergamotene | 0.35 ± 0.03 | 0.28 ± 0.02 | 1482 | 1483 |
| 32 | alpha-amorphene | 4.24 ± 0.36 | 2.91 ± 0.24 | 1491 | 1490 |
| 33 | cis-beta-guaiene | 0.02 ± 0.01 | —— | 1494 | 1493 |
| 34 | gamma-amorphene | 0.47 ± 0.04 | —— | 1496 | 1496 |
| 35 | bicyclogermacrene | 1.51 ± 0.13 | 1.15 ± 0.14 | 1500 | 1500 |
| 36 | gamma-patchoulene | 0.11 ± 0.01 | —— | 1506 | 1506 |
| 37 | germacrene A | 2.26 ± 0.22 | 0.07 ± 0.01 | 1508 | 1509 |
| 38 | alpha-bulnesene | 1.41 ± 0.09 | 0.14 ± 0.01 | 1510 | 1510 |
| 39 | gamma-cadinene | 3.88 ± 0.35 | 2.64 ± 0.24 | 1514 | 1514 |
| 40 | trans-cycloisolongifol-5-ol | 0.06 ± 0.01 | —— | 1516 | 1515 |
| 41 | delta-cadinene | 0.11 ± 0.01 | —— | 1523 | 1523 |
| 42 | beta-sesquiphellandrene | 0.24 ± 0.02 | —— | 1525 | 1524 |
| 43 | 10-epi-cubebol | 0.06 ± 0.01 | —— | 1535 | 1535 |
| 44 | cis-muurol-5-en-4-beta-ol | 0.02 ± 0.01 | —— | 1552 | 1552 |
| 45 | cis-muurol-5-en-4-alpha-ol | 0.03 ± 0.01 | —— | 1561 | 1561 |
| 46 | spathulenol | 0.02 ± 0.01 | —— | 1579 | 1578 |
| 47 | 1.10-di-epi-cubenol | 1.29 ± 0.14 | 0.18 ± 0.02 | 1620 | 1619 |
| 48 | epi-alpha-cadinol | 9.97 ± 0.85 | 4.9 ± 0.41 | 1640 | 1640 |
| 49 | alpha-cadinol | 0.14 ± 0.01 | —— | 1654 | 1654 |
| 50 | 7-epi-alpha-eudesmol | 0.19 ± 0.02 | —— | 1665 | 1664 |
| | Class of compounds | | | | |
| | Oxygenated monoterpenes | 39.42 ± 3.02 | 36.18 ± 2.77 | | |
| | Sesquiterpenes | 27.34 ± 2.12 | 16.13 ± 1.25 | | |
| | Allylbenzenes | 15.77 ± 1.34 | 42.34 ± 3.34 | | |
| | Oxygenated sesquiterpenes | 11.78 ± 0.96 | 5.08 ± 0.41 | | |
| | Monoterpenes | 2.52 ± 0.23 | 0.27 ± 0.02 | | |
| | Esters | 2.91 ± 0.27 | —— | | |
| | Ketones | 0.03 ± 0.01 | —— | | |
| | Alcohol | 0.04 ± 0.01 | —— | | |

[a] Compounds are listed in order of their elution from a HP-5 column. Values are means of three determinations ± SD. RI: retention indices on HP-5 column, experimentally determined using homologous series of $C_8$-$C_{30}$ alkanes. RI *: retention indices from the literature.

The compounds are listed in order of elution on the HP5 column. Fifty-nine volatile compounds were detected in the LEO and 50 were identified with peak weight percentages of 99.81 ± 0.11% (mean ± S.E.).

Oxygenated monoterpene hydrocarbons accounting for 39.42% ± 3.02% of the total composition of oil were the predominant volatile group in LEO, followed by sesquiterpene hydrocarbons (27.34 ± 2.12%). Among the six oxygenated monoterpene hydrocarbons identified in LEO, linalool, was the most prominent (32.05 ± 2.57%). The relatively abundant oxygenated monoterpene in LEO was 1,8-cineole (5.84 ± 0.54%).

Twenty sesquiterpene hydrocarbons were identified in LEO. Among them, cis-alpha-bergamotene was the most abundant, accounting for 9.35% ± 0.79%, followed by alpha-amorphene (4.24 ± 0.36%) and gamma-cadinene (3.88 ± 0.35%).

In addition, amounts of allylbenzenes such as eugenol (15.47 ± 1.05%) and oxygenated sesquiterpenes such as epi-alpha-cadinol (9.97 ± 0.85%) were detected in LEO, along with nine monoterpenes, one ketone, and one alcohol. The ketone and the alcohol were present at trace levels. The composition of LEO was compared with that of trichomes peltate heads (VOC).

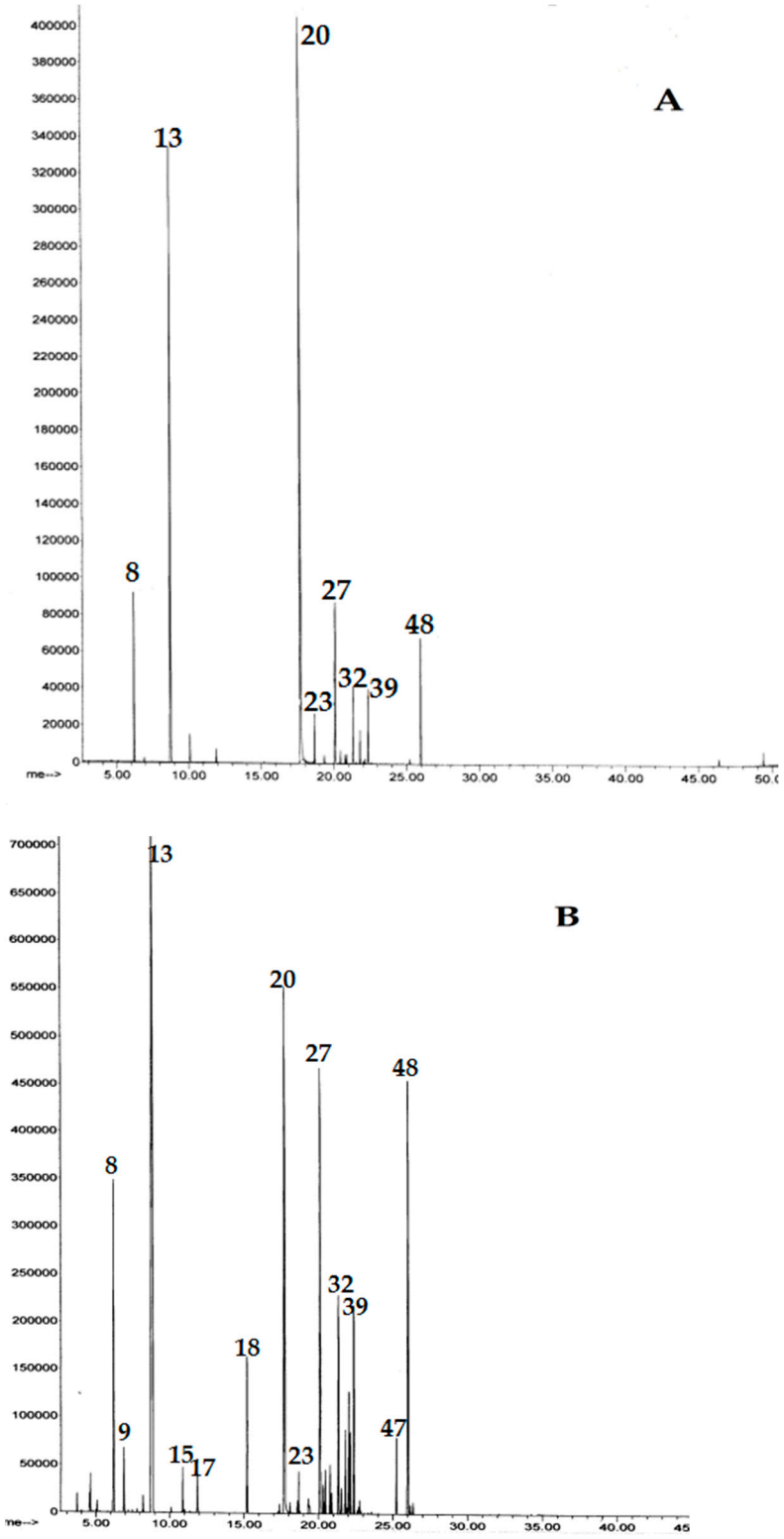


**Figure 1.** Chromatographic profiles of *O. basilicum* VOC (**A**) and LEO (**B**). The numbers correspond to the compounds detected as described in Table 1.

The observed composition of VOC is presented in Table 1 and a chromatogram is shown in Figure 1.

Twenty-four volatile compounds were detected in the VOC and all were identified with peak weight percentages of 100 ± 0.01%.

Allylbenzenes accounting for 42.34% ± 3.34% of the total composition of oil were the predominant volatile group in VOC. Among them, eugenol was the most abundant, accounting for 41.89% ± 2.83%. Oxygenated monoterpene hydrocarbons accounting for 36.18% ± 2.77% of the total composition of oil were also important as volatile group in VOC, followed by sesquiterpene hydrocarbons (16.13% ± 1.25%). Among the four oxygenated monoterpene hydrocarbons identified in VOC, linalool, was the most prominent (28.99% ± 2.32%). The relatively abundant oxygenated monoterpene hydrocarbons in VOC were 1,8-cineole (5.71% ± 0.53%).

Eleven sesquiterpene hydrocarbons were identified in VOC. Among them, cis-alpha-bergamotene was the most abundant, accounting for 6.34% ± 0.54%, followed by alpha-amorphene (2.91% ± 0.24%) and gamma-cadinene (2.64% ± 0.24%).

In addition, amounts of oxygenated sesquiterpenes such as epi-alpha-cadinol (4.9% ± 0.41%) and monoterpenes such as (E)-beta-ocimene (0.16% ± 0.02%) were detected in VOC. Four monoterpenes were present at trace levels.

The proportion of allylbenzenes in the VOC (42.34% ± 3.34%) was higher than that in the LEO (15.77% ± 1.34%).

LEO contains monoterpenes about ten times higher than those contained in VOC (2.52% ± 0.23% and 0.27% ± 0.02%, respectively).

Similarly, sesquiterpenes are about twice as abundant in LEO as in VOC.

During the distillation of the basil leaves 26 compounds are formed that are not present in the heads of the peltate trichomes. These compounds are present in modest quantities from 0.01% to 0.27% for monoterpenes, from 0.03 to 0.76 for oxygenated monoterpenes. Bornyl acetate is formed in appreciable quantities (2.77% ± 0.22%) and is the most abundant neo-formed compound.

## 4. Discussion

Hydrodistillation at atmospheric pressure is the most frequently used method of essential oils isolation. The advantage with respect to other isolation methods, e.g., extraction by organic solvents and supercritical $CO_2$, is that isolates by hydrodistillation do not include non-volatile compounds. The main disadvantage is the formation of artefacts, thermal degradation reactions and hydrolysis, especially for aromatic plants that contain unstable volatile compounds as the main constituents of their essential oils. During hydrodistillation, water as polar solvent accelerates many reactions, especially reactions via carbocations as intermediates. The pH can fall as low as 2.8 during such extraction of the oil [42].

Many of the trace components of essential oils that are detected could well arise during the isolation procedures. Such oils are steam distilled under conditions where organic acids can be liberated from the plant material, and cyclizations of aldehydes and other monoterpenes may occur [43].

The action of phosphatases must be inhibited. The latter cleave phosphate esters to give the free alcohols characteristic of isolated plant oils. The distribution of terpene alcohols between free and esterified forms in vivo is not known in any particular oil, but considerable amounts of the latter and other bonded forms (e.g., glucosides and esters) are probably present [44].

Oils that have been obtained from plant material which has been gathered and stored may also contain products of photolysis, oxidation, and other chemical modification. Such contaminants are often extremely important as regards odor and flavor for commercial use [45].

The distilled oil of *Citrus deliciosa Tenore* var. *Caí* was characterized by aromatic nuances making the oil less appreciated (inferior quality) than the cold-pressed oil. Probably as consequence of artifacts formation during the distillation process [46].

The artifact compounds that formed during distillation of *O. basilicum* leave were: camphene, octan-3-one, delta-2-carene, gamma-terpinene, n-octanol, terpinolene, borneol, terpinen-4-ol, bornyl acetate, alpha-terpinyl acetate, beta-cubebene, beta-longipinene, (Z)-caryophyllene, cadina-3,5-diene, cis-beta-guaiene, gamma-amorphene, gamma-patchoulene,

trans-cycloisolongifol-5-ol, delta-cadinene, beta-sesquiphellandrene, 10-epi-cubebol, cis-muurol-5-en-4-beta-ol, cis-muurol-5-en-4-alpha-ol, spathulenol, α-cadinol, 7-epi-α-eudesmol.

These compounds are present in very low concentrations from 0.01 to 0.76 except for bornyl acetate which was formed in significant quantities (2.77% ± 0.22%).

Some of these compounds have already been reported in the literature as artifacts produced during distillation or during the storage of essential oil.

Hofmann [47] reported that several mono- and sesquiterpene derivatives (e.g., linalool oxides, p-cymene, (Z)-caryophyllene) might originate as artefacts during distillation.

Germacrenes readily converts to cadinenes, muurolenes, and elemene-type sesquiterpene hydrocarbons [48].

During the distillation of *Abies x arnoldiana* Nitz., and *A. veitchii* Lindl. various artifacts, including a derivative of borneol, were formed [49].

Moreover, during the distillation time, acid-catalyzed hydrolysis of bornyl acetate takes place. This hydrolysis only takes place to a small extent during distillation. On the basis of hydrodiffusion, the oxygenated compounds are available for distillation much faster than the hydrocarbons. Thus, bornyl acetate is in contact with the acidic medium for a relatively short period, whereby extreme hydrolysis of this compound is prevented [49].

Distilled EO generally increase their proportion in oxygenated monoterpenes (alpha-terpineol, 4-terpineol and sabinene hydrates) that could originate as a result of the process (artifacts).

Structural rearrangements of limonene, sabinene, gamma-terpinene, and beta-pinene influenced by heat and oxidation would lead to p-cymene and related compounds of the p-menthadiene skeleton, such as terpinolene and beta-phellandrene [46].

The bicyclogermacrene could be partially transformed to spathulenol. A mechanism for the transformation of bicyclogermacrene into spathulenol seems to be a possible reaction sequence which may explain the transformation by autoxidation [50–52].

The chemical changes and artifact formation in (*Citrus aurantium* L. var. *cyathifera* Y. Tanaka) cold-pressed peel oil upon storage at 20, 5, and 21 °C for 3, 6, and 12 months were investigated by Njoroge et al. Notable decreases of germacrene D, myrcene, linalyl acetate, and limonene occurred. Prominent increases of cis-carveol, trans-beta-farnesene, trans-p-2,8-menthadien-1-ol, linalool, and beta-caryophyllene were found. Thirty-four artifact compounds were formed upon 12 months at 20 °C. The artifacts consisted of alcohols, carbonyl compounds, esters, epoxides, and hydrocarbons. The prominent artifact compounds were (±)-carvone, trans,trans-farnesyl acetate, sabinene hydrate, 1-octen-3-ol, cis,cis-farnesyl acetate, and dihydrocarveol acetate [51].

Changes in 44 compounds of *Citrus junos* Sieb. ex Tanaka steam-distilled peel oil and possible artifacts that accrue during storage at 25 °C were investigated by Kashiwagi, et al. Total monoterpene hydrocarbons decreased markedly, with major losses of limonene and gamma-terpinene and notable increases in p-cymene, as well as alcohols [52].

Beta-elemene cyclization and structural rearrangement could lead to beta-selinene, which upon oxidation could form eudesmol derivatives [53].

The increase in the relative percentages of the sesquiterpene alcohols could to some extent be attributed to possible reactions with the germacrene hydrocarbons. It is suggested that the possible formation of the tricyclic sesquiterpene alcohols, globulol, viridiflorol, and spathulenol, as artifacts in the oil could be attributed to oxidation of the germacrene type hydrocarbons, aromadendrene, allo-aromadendrene, and bicyclogermacrene to tricyclic sesquiterpenes [50].

## 5. Conclusions

Compounds biosynthesized from the plant can be identified with the shuttle analysis that is with the withdrawal of the secretion directly from the head of the secretor trichomes by means of microneedles followed by GC/MS analysis. As reported by numerous works, during hydrodistillation new compounds are formed (artifacts) and in the case of hydrodistillation of the leaves of *O. basilicum* have formed 26 new compounds. Some of these

artifacts were not reported in the literature. Some compounds reported as artifacts may also be present as primary compounds. Further studies are needed to explain the kinetics of artifact formation from compounds really biosynthesized by the plant.

**Author Contributions:** Conceptualization, B.T.; methodology, B.T.; software, B.T.; validation, B.T. and F.M.; formal analysis, B.T.; investigation, B.T.; data curation, B.T.; writing—original draft preparation, B.T.; writing—review and editing, F.M.; visualization, B.T. and F.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## References

1. Chikkaswamy, B.K.; Paramanik, R.C.; Varadaraj, N.; Paramanik, A.; Ramesh, H.L.; Shivashankar, M.; Sivaram, V.R. Determination of genetic variation in Piper species using 4C nuclear DNA and RAPD marker. *Int. J. Res. Pharm. Sci.* **2013**, *4*, 58–64. [CrossRef]
2. Simon, J.E.; Morales, M.R.; Phippen, W.B.; Vieira, R.F.; Hao, Z. Basil: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herb. In *Perspectives on New Crops and New Uses*; Janick, J., Ed.; ASHS Press: Alexandria, VA, USA, 1999; pp. 499–505.
3. Carović-Stanko, K.; Liber, Z.; Besendorfer, V.; Javornik, B.; Bohanec, B.; Kolak, I.; Satovic, Z. Genetic relations among basil taxa (Ocimum L.) based on molecular markers, nuclear DNA content, and chromosome number. *Plant Syst. Evol.* **2010**, *285*, 13–22. [CrossRef]
4. Meyers, M. *Basil: An Herb Society of America Guide*; The Herb Society of America: Kirtland, OH, USA, 2003.
5. Purushothaman, B.; Prasannasrinivasan, R.; Suganthi, P.; Ranganathan, B.; Gimbun, J.; Shanmugam, K. A comprehensive review on Ocimum basilicum. *J. Nat. Remedies* **2018**, *18*, 71–85. [CrossRef]
6. Dhama, K.; Sharun, K.; Gugjoo, M.B.; Tiwari, R.; Alagawany, M.; Iqbal Yatoo, M.; Thakur, P.; Iqbal, H.M.N.; Chaicumpa, W.; Michalak, I.; et al. A Comprehensive Review on Chemical Profile and Pharmacological Activities of Ocimum basilicum. *Food Rev. Int.* **2021**, *37*, 1–29. [CrossRef]
7. Jayasinghe, C.; Gotoh, N.; Aoki, T.; Wada, S. Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* **2003**, *51*, 4442–4449. [CrossRef]
8. Chiang, L.C.; Ng, L.T.; Cheng, P.W.; Chiang, W.; Lin, C.C. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clin. Exp. Pharmacol. Physiol.* **2005**, *32*, 811–816. [CrossRef] [PubMed]
9. Manosroi, J.; Dhumtanom, P.; Manosroi, A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett.* **2006**, *235*, 114–120. [CrossRef]
10. Shahrajabian, M.H.; Sun, W.; Cheng, Q. Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): A review. *Int. J. Food Prop.* **2020**, *23*, 1961–1970. [CrossRef]
11. Hegnauer, R. (Ed.) *Chemotaxonomie der Pflanzen*; Birkhiuser: Basel, Switzerland, 1989; Volume 8.
12. Grayer, R.J.; Kite, G.C.; Goldstone, F.J.; Bryan, S.E.; Paton, A.; Putievsky, E. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochemistry* **1996**, *43*, 1033–1039. [CrossRef]
13. Ravid, U.; Putievsky, E.; Katzir, I.; Lewinsohn, E. Enantiomeric composition of linalol in the essential oils of *Ocimum* species and in commercial basil oils. *Flavour Fragr. J.* **1997**, *12*, 293–296. [CrossRef]
14. Fahn, A. (Ed.) *Secretory Tissues in Plants*; Academic Press: London, UK, 1979.
15. Antunes, T.; Sevinate-Pinto, I. Glandular Trichomes of *Teucrium scorodonia* L. Morphology and Histochemistry. *Flora* **1991**, *185*, 65–70. [CrossRef]
16. Ascensao, L.; Marques, N.; Pais, M.S. Peltate glandular trichomes of *Leonotis leonurus* leaves: Ultrastructure and histochemical characterization of secretions. *Int. J. Plant Sci.* **1997**, *158*, 249–258. [CrossRef]
17. Ascensão, L.; Marques, N.; Pais, M.S. Glandular trichomes on vegetative and reproductive organs of *Leonotis leonurus* (Lamiaceæ). *Ann. Bot.* **1995**, *75*, 619–626. [CrossRef]
18. Ascensão, L.; Pais, M.S. The leaf capitate trichomes of *Leonotis leonurus*: Histochemistry, ultrastructure and secretion. *Ann. Bot.* **1998**, *81*, 263–271. [CrossRef]
19. Bouret, T.M.; Howard, R.J.; O'Keefe, D.P.; Hallahan, D.L. Gland Development on Leaf Surfaces of *Nepeta racemosa*. *Int. J. Plant Sci.* **1994**, *155*, 623–632. [CrossRef]
20. Voirin, B.; Bayet, C. Developmental changes in the monoterpene composition of *Mentha* x *piperita* leaves from individual peltate trichomes. *Phytochemistry* **1996**, *43*, 573–580. [CrossRef]
21. McCaskill, D.; Croteau, R. Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha* x *piperita*) rely exclusively on plastid-derived isopentenyl diphosphate. *Planta* **1995**, *197*, 49–56. [CrossRef]

22. Tirillini, B.; Ricci, A.; Pellegrino, R. Secretion constituents of leaf glandular trichome of *Salvia officinalis* L. *J. Essent. Oil Res.* **1999**, *11*, 565–569. [CrossRef]
23. Falk, K.L.; Gershenzon, J.; Croteau, R. Metabolism of monoterpenes in cell cultures of common sage (*Salvia officinalis*): Biochemical rationale for the lack of monoterpene accumulation. *Plant Physiol.* **1990**, *93*, 1559–1567. [CrossRef]
24. Venkatachalam, K.V.; Kionaas, R.; Croteau, R. Development and essential oil content of secretory glands of sage (*Salvia officinalis*). *Plant Physiol.* **1984**, *76*, 148–150. [CrossRef]
25. Yamaura, T.; Tanaka, S.; Tabata, M. Localization of the biosynthesis and accumulation of monoterpenoids in glandular trichomes of thyme. *Planta Med.* **1992**, *58*, 153–158. [CrossRef] [PubMed]
26. Boix, Y.F.; Victório, C.P.; Defaveri, A.C.A.; do Carmo de Oliveira Arruda, R.; Sato, A.; Lage, C.L.S. Glandular trichomes of *Rosmarinus officinalis* L.: Anatomical and phytochemical analyses of leaf volatiles. *Plant Biosyst.* **2011**, *145*, 848–856. [CrossRef]
27. Sagawa, T.; Ikeda, H.; Hiraoka, T.; Hayakawa, K. Study of rosemary peltate glandular trichomes using combined morphological and chemical approach. *Food Sci. Technol. Res.* **2013**, *19*, 491–495. [CrossRef]
28. Maleci Bini, L.; Gentili, L.; Tirillini, B.; Pellegrino, R. Secretion Constituents of Leaf Glandular Trichomes of *Ocìmum basilicum* L. In *Flavour and Fragrance Chemistry*; Lanzotti, V., Taglialatela-Scafati, O., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp. 143–150.
29. Tozin, L.R.S.; Marques, M.O.M.; Rodrigues, T.M. Herbivory by leaf-cutter ants changes the glandular trichomes density and the volatile components in an aromatic plant model. *AoB Plants* **2017**, *9*, plx057. [CrossRef]
30. Manan, A.A.; Taha, R.M.; Mubarak, E.E.; Elias, H. In vitro flowering, glandular trichomes ultrastructure, and essential oil accumulation in micropropagated *Ocimum basilicum* L. *Vitr. Cell. Dev. Biol. Plant* **2016**, *52*, 303–314. [CrossRef]
31. Werker, E.; Putievsky, E.; Ravid, U.; Dudai, N.; Katzir, I. Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L. (Lamiaceae). *Ann. Bot.* **1993**, *71*, 43–50. [CrossRef]
32. Schnepf, E. Glands Cells. In *Dynamic Ultrastructure*; Robards, A.W., Ed.; McGraw-Hill: New York, NY, USA, 1974.
33. Dong, F.; Fu, X.; Watanabe, N.; Su, X.; Yang, Z. Recent advances in the emission and functions of plant vegetative volatiles. *Molecules* **2016**, *21*, 124. [CrossRef] [PubMed]
34. Boncan, D.A.T.; Tsang, S.S.K.; Li, C.; Lee, I.H.T.; Lam, H.-M.; Chan, T.-F.; Hui, J.H.L. Terpenes and terpenoids in plants: Interactions with environment and insects. *Int. J. Mol. Sci.* **2020**, *21*, 7382. [CrossRef]
35. Abbas, F.; Ke, Y.; Yu, R.; Yue, Y.; Amanullah, S.; Jahangir, M.M.; Fan, Y. Volatile terpenoids: Multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta Med.* **2017**, *246*, 803–816. [CrossRef]
36. Yang, C.; Wang, J.; Li, D. Microextraction techniques for the determination of volatile and semivolatile organic compounds from plants: A review. *Anal. Chim. Acta* **2013**, *799*, 8–22. [CrossRef] [PubMed]
37. Zhu, F.; Xu, J.; Ke, Y.; Huang, S.; Zeng, F.; Luan, T.; Ouyang, G. Applications of in vivo and in vitro solid-phase microextraction techniques in plant analysis: A review. *Anal. Chim. Acta* **2013**, *794*, 1–14. [CrossRef] [PubMed]
38. Keene, C.K.; Wagner, G.J. Direct demonstration of duvatrienediol biosynthesis in glandular heads of tobacco trichomes. *Plant Physiol.* **1985**, *79*, 1026–1032. [CrossRef]
39. Gershenzon, J.; Duffy, M.A.; Karp, F.; Croteau, R. Mechanized techniques for the selective extraction of enzymes from plant epidermal glands. *Anal. Biochem.* **1987**, *163*, 159–164. [CrossRef]
40. Slone, J.H.; Kelsey, R.G. Isolation and purification of glandular secretory cells from *Artemisia tridentata* (ssp. vaseyana) by percoll density gradient centrifugation. *Amer. J. Bot.* **1985**, *72*, 1445–1451. [CrossRef]
41. Tirillini, B.; Stoppini, A.M. Injection of a sample by means of microneedles followed by capillary gas chromatography. *J. Chromatogr. Sci.* **1995**, *33*, 139–142. [CrossRef]
42. Mastelic, J.; Jerkovic, I. Application of co-distillation with superheated pentane vapour to the isolation of unstable essential oils. *Flavour Fragr. J.* **2003**, *18*, 521–526. [CrossRef]
43. Baines, D.A.; Jones, R.A.; Webb, T.C.; Campion-Smith, I.H. The chemistry of terpenes-I. The effect of hydrogen ion concentration and oxygen upon the acid catalysed cyclization of citral. *Tetrahedron* **1970**, *26*, 4901–4913. [CrossRef]
44. Banthorpe, D.V.; Charlwood, V.; Francis, M.J.O. The biosynthesis of monoterpenes. *Chem. Rev.* **1972**, *72*, 115–155. [CrossRef]
45. Schratz, E.; Wahlig, T. Gaschromatographische analyse aetherischer ole aus pflanzenextrakten. *Planta Med.* **1965**, *13*, 218–225. [CrossRef]
46. Minteguiaga, M.A.; Frizzo, C.D.; Dellacassa, E.S. Odour-active compounds of Citrus deliciosa Tenore var. Caí essential oils detected by gas chromatography-mass spectrometry and gas chromatography-olfactometry. *J. Pharm. Pharmacogn. Res.* **2017**, *5*, 345–353.
47. Hofmann, L. *Einfluss Von Genotyp, Ontogenese Und Äusseren Faktoren Auf Pflanzenbauliche Merkmale Sowie Ätherische Öle und Flavonoide von Klonen der Schafgarbe (Achillea millefolium Aggregat)*; Technische Universität: München, Germany, 1993.
48. Tressl, R.; Engel, K.H.; Kossa, M.; Koppler, H. Characterization of tricyclic sesquiterpenes in hop (*Humulus lupulus*, var Hersbrucker Spat). *J. Agric. Food Chem.* **1983**, *31*, 892–897. [CrossRef]
49. Koedam, A.; Scheffer, J.J.C.; Svendsen, A.B. Monoterpenes in the volatile leaf oil of Abies X Arnoldiana Nitz. *J. Agric. Food Chem.* **1980**, *28*, 862–866. [CrossRef]
50. Toyota, M.; Koyama, H.; Mizljtani, M.; Asakawa, Y. (−)-Ent-spathulenol isolated from liverworts is an artefact. *Phytochemistry* **1996**, *41*, 1347–1350. [CrossRef]

51. Njoroge, S.M.; Ukeda, H.; Sawamura, M. Changes of the volatile profile and artifact formation in daidai (Citrus aurantium) cold-pressed peel oil on storage. *J. Agric. Food Chem.* **2003**, *51*, 4029–4035. [CrossRef]
52. Kashiwagi, T.; Phi, N.T.L.; Sawamura, M. Compositional changes in yuzu (*Citrus junos*) steam-distilled oil and effects of antioxidants on oil quality during storage. *Food Sci. Technol. Res.* **2010**, *16*, 51–58. [CrossRef]
53. Bryant, R. The Sesquiterpenoids. In *Rodd's Chemistry of Carbon Compounds*; Coffey, S., Ed.; Elsevier: Amsterdam, The Netherlands, 1969; pp. 256–366.