



Proceeding Paper Essential Oil Composition and Glandular Trichome Structure of the Weather Prophet Dimorphoteca pluvialis ⁺

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Abstract: *Dimorphoteca pluvialis* (L.) Moench, usually known as weather prophet, African daisy, or Cape marigold, is an Asteraceae commonly found in gardens due to its appealing white to yellowish flowers. Recently, its use as a non-food oilseed crop has been investigated due to the high amounts of dimorphecolic acid (Δ 9-hydroxy,10t,12t-octadecadienoic acid), a highly reactive C₁₈ fatty acid with value for the manufacturing of paints, inks, lubricants, plastic, and nylon. However, information on the essential oil (EO) composition of its plant tissues is scarce. The present work focused on characterizing the glandular trichomes, the main site for secretion of natural products, of shoots and sepals, and analyzing the EO composition of shoots and flowers of *D. pluvialis* extracted by hydrodistillation for 15, 30, or 60 min. The shoot surface displayed sharp and elongated non-glandular trichomes. A capitate trichome with a biseriate peduncle and a multiseriate head was the only type of glandular trichome identified. A histochemical analysis of the glandular head revealed the presence of acid lipids and terpenic and phenolic compounds. The extracted EOs showed high amounts of trans-2-hexenal, a C₆ aldehyde that protects plants against harmful substances but is considered toxic for humans. This study described, for the first time, the composition of EOs of *D. pluvialis* plants.

Keywords: Dimorphoteca pluvialis; essential oil; non-food crops; trans-2-hexenal; trichomes

1. Introduction

Dimorphoteca pluvialis Moench, usually known as weather prophet, African daisy, or Cape marigold, is an Asteraceae believed to be native to South Africa and Namibia. It owes its name to the fact that its flowers close at night and on cloudy days before rain. The aerial parts of this annual species form a bushy plant (up to 30 cm), with shoots branching from the base and holding oblanceolate leaves (ca. 7 cm long), lobed to toothed, that are numerous at the base of the stems and fewer and smaller near the top. The plant is covered with large flower heads that blossom at the same level with a white appearance except near the base, where they have a dark purple or violet section. The flower heads are composed of fertile female and sterile male ray florets and hermaphrodite disk florets. The seeds (achenes) developing from disk florets have flattened margins (wings), while those produced by the ray florets are unwinged [1,2]. In the past decades, non-food oilseed crops have garnered interest for industrial use due to the extracted oils containing compounds with functional groups that make them potential substitutes for the mineral oils used to produce, e.g., lubricants, surfactants, coatings, or polymers, with the added advantage that these can be supplied at a constant and more economical rate. This is the case for *D. pluvialis*, whose seed oil can be composed of more than 60% of dimorphecolic acid (9-hydroxy-trans, *trans*-10, 12-octadecadienoic acid), a valuable C_{18} fatty acid that contains a C9 hydroxyl



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Copyright: © 2023 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). group, two conjugated double bonds relative to the α -carbon of the hydroxy group ($\Delta 10$, $\Delta 12$), and a *trans*- $\Delta 12$ unsaturation, setting this compound apart from other plant hydroxy fatty acids and granting it the potential for a wide range of new applications [2,3].

In the present work, *D. pluvialis* flowers and vegetative shoots were analyzed for the structural and chemical characterization of their glandular trichomes and the chemical profiling of their essential oils.

2. Material and Methods

2.1. Plant Material

Aerial parts of *D. pluvialis* in the flowering stage were collected from the vicinity of Campo Grande, Lisbon, in the spring. The flowers were isolated from the shoot tissues and immediately processed to be used for structural analysis and essential oil extraction. A voucher specimen is kept in the Herbarium of the Botanical Garden of Lisbon University, Lisbon, Portugal.

2.2. Structural and Chemical Characterization of Glandular Trichomes

Longitudinal- and cross-sections were obtained from shoots and sepals. Structural characterization of glandular trichomes was performed through scanning electron microscopy (SEM) and light microscopy (LM). For SEM, samples were fixed with 1.5% (v/v) glutaraldehyde in a 0.05 M sodium cacodylate buffer, pH 7.0, for 45 min at room temperature. After 1–2 min under a vacuum (26 mm Hg, 3.46 kPA), the fixative was substituted with 3% glutaraldehyde in a 0.1 M sodium cacodylate buffer, pH 7.0, for 2 h at room temperature. The material was rinsed thoroughly in the same buffer, post-fixed with a 2% osmium tetroxide (OsO₄) aqueous solution for 2 h at room temperature, dehydrated in a graded acetone series and critical point dried in a Polaron E 3500. Dried specimens were mounted on stubs coated with gold in a Polaron E 5350. Observations were carried out on a JEOL T220 SEM (JEOL Ltd., Tokyo, Japan) at 15 kV.

For the chemical characterization of the glandular trichomes, longitudinal- and crosssections of the aerial parts were stained with Sudan black B, Sudan IV, and Nile blue A for total lipids, Nadi reagent for terpenoids, periodic acid–Schiff (PAS) reagent for polysaccharides with vicinal glycol groups, iron (III) trichloride (FeCl₃) and potassium dichromate for phenolic compounds, and Ruthenium red for pectins ([4] and references therein). Observations were made under a Leica DM-2500 microscope (Leica Microsystems CMS GmbH, Wetzlar. Germany). The images were digitally obtained using a Leica DFC-420 camera (Leica Microsystems Ltd., Heerbrugg, Switzerland) and the Leica Application Suite software (version 2.8.1).

2.3. Essential Oil Extraction and Analysis

Essential oils (EOs) were obtained by the hydrodistillation of shoots or flowers of the aerial parts of *D. pluvialis* in a Clevenger-type apparatus according to the European Pharmacopoeia [5] for 15, 30, or 60 min at a distillation rate of 3 mL/min. When the EO yield was below 0.05%, distilled *n*-pentane was used to collect the volatiles. Samples were stored in glass vials at -20 °C until analysis.

Samples were analyzed by gas chromatography (GC), for component quantification, and gas chromatography coupled to mass spectrometry (GC-MS) for component identification. Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL gas chromatograph (Perkin Elmer, Shelton, CT, USA) equipped with two flame ionization detectors (FIDs), a data handling system, and a vaporizing injector port into which two columns of different polarities were installed: a DB 1 fused silica column (30 m × 0.25 mm, i.d., film thickness 0.25 μ m; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused silica column (30 m × 0.25 mm, i.d., film thickness 0.15 μ m; J & W Scientific Inc., Rancho Cordova, CA, USA). The oven temperature was programmed to increase from 45 to 175 °C at 3 °C/min increments and then up to 300 °C at 15 °C/min increments and was finally held isothermal for 10 min. Gas chromatographic settings were as follows: injector

and detector temperatures, 280 °C and 300 °C, respectively, and the carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using a split sampling technique ratio of 1:50. The volume of injection was 0.1 μ L of a pentane EO solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as a mean value of two injections from each volatile oil, without response factors.

The GC-MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph, equipped with a DB 1 fused silica column (30 m \times 0.25 mm, i.d., film thickness 0.25 µm; J & W Scientific, Inc., Rancho Cordova, CA, USA) interfaced with a Perkin Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer). GC-MS settings were as follows: injector and oven temperatures were as above; transfer line temperature, 280 °C; ion source temperature, 220 °C; the carrier gas, helium, was adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; scan range, 40–300 u; and scan time, 1 s. The identity of the components was assigned by comparison of their retention indices relative to C8-C25 *n*-alkane indices, and GC-MS spectra from a laboratory-made library based on the analyses of reference EOs, laboratory-synthesized components, and commercially available standards.

2.4. Data Treatment and Statistical Analysis

Statistical analysis was performed with SPSS version 29 statistical software. Statistical significance was determined with one-way ANOVA, and individual means were compared using Tukey's post hoc test with p < 0.05. Results were presented as mean \pm standard error (SE) of 6 samples.

3. Results and Discussion

In D. pluvialis, the indumentum showed mostly a uniform distribution of glandular and non-glandular trichomes in the floral and vegetative parts (including the abaxial and adaxial leaf surfaces). Non-glandular trichomes were singular multicellular structures with a pointed tip, devoid of any pigmentation. Morphometric differences were found between non-glandular trichomes of the sepals and the shoot stems. In the sepals, nonglandular trichomes were long and uniseriate (with two to seven stacked cells), slightly pointing toward the sepal tip, with an average height of 160.3 \pm 12.0 μ m and a width of 14.5 \pm 1.0 μ m, preferentially distributed in the margins of the sepal. In the stem, trichomes were multiseriate with three columns of two to six stacked cells and showed an average of 78.3 \pm 2.6 μ m in height and 57.6 \pm 1.2 μ m in width. The glandular trichomes observed in sepals were capitate-type multicellular structures composed of a biseriate stalk and a multiseriate globoid glandular head, capping the products of secretion under the subcuticular space (Figures 1 and 2). These secretory structures were 141.9 \pm 8.7 μ m in height (4 to 7 stacked cells) and $43.4 \pm 1.4 \,\mu\text{m}$ in width (Figure 1a and b). In the stems, the same type of capitate glandular trichome was observed; however, with a lower height $(101.2 \pm 3.2 \ \mu\text{m})$ and width $(37.1 \pm 1.6 \ \mu\text{m})$ (Figure 1c,d). The glandular head was made up of two to three cell layers, where the second and third cell layers showed a high chloroplast content, in contrast to the apical cells (Figure 2a), whose function was, probably, to help provide the carbon and energy needs for specialized metabolite production since trichomes are believed to harbor specific Rubisco isoforms that uniquely adapt to the physiology of secretory cells [6]. The produced secondary metabolites are, most likely, accumulated in the apical cells (first cell layer) from where they can be exuded into the subcuticular space and then volatilized through micropores in the cuticle surface or released after cuticle rupture. Glandular trichomes are sites of substantial production in secondary metabolites whose functions can span from the regulation of plant growth to the defense of the plant against pathogens or even other plants [7].



Figure 1. Scanning electron micrographs depicting the distribution of glandular trichomes in sepals: (a) detail of glandular capitate-type trichomes of sepals (b) and vegetative shoots (c,d) of *Dimorphotheca pluvialis*. Bar = $15 \mu m$.

In the present study, the chemical nature of the exudate was assessed through histochemical assays. Lipidic compounds were detected in the glandular head after a positive result for Sudan IV (Figure 2b) or Sudan black B (Figure 2c) staining, with a higher incidence in the first cell layer. Through Nile blue A staining, a strong blue color in the cells of the glandular head indicates that some of the identified lipids can be considered acidic in nature, while the faint pink color in the trichome stem revealed the presence of neutral lipids (Figure 2d). The use of the Nadi reagent allowed the identification of terpenic compounds in the exudate of the subcuticular space (Figure 2e). The presence of phenolic compounds was confirmed with the iron (III) trichloride (FeCl₃) or potassium dichromate staining. The first intensely stained the cells of the glandular head (Figure 2f), while the second stained mainly the apical cells where the metabolites are accumulated before secretion to the subcuticular space (Figure 2g). The periodic acid–Schiff reagent (PAS), used to detect polysaccharides, stained the trichome cells (stem and glandular head) but not the subcuticular space. Additionally, Ruthenium red, differential staining for pectins, stained the glandular head, suggesting a mucilaginous nature for the compounds accumulated in the glandular head.

The EOs extracted from *D. pluvialis* shoots or flowers showed very low yields ($\leq 0.05\%$, v/f.w.), generally not reaching the lowest measure scale of the Clevenger apparatus. The

non-terpenic fraction was dominant in all extracted EOs, varying from 65.5 ± 1.4 (30 min) to $77.5 \pm 3.3\%$ (60 min) for the shoots and from 82.0 ± 3.8 (60 min) to $91.5 \pm 1.5\%$ (15 min) for the flowers (Table 1). This fraction contained the main EO compounds ($\geq 10\%$), namely, the aldehyde 2-*trans*-hexenal, which varied from 23.0 ± 0.1 (15 min) to $28.9 \pm 0.8\%$ (60 min) in the shoots and from 22.3 ± 3.7 (60 min) to 56.3 ± 4.1 (15 min) in the flowers; the alcohol *cis*-3-hexen-1-ol, which varied from 16.4 ± 1.0 (30 min) to $26.7 \pm 0.3\%$ (60 min) in the shoots, and from 3.5 ± 1.7 (15 min) to 18.6 ± 2.4 (60 min) in the flowers; and the alcohol hexanol, which varied from 16.2 ± 1.6 (60 min) to $21.6 \pm 0.2\%$ (15 min) in the shoots and from 11.4 ± 2.0 (30 min) to 18.4 ± 2.9 (60 min) in the flowers. Overall, the increase in distillation time led to a relative increase in the proportions of 2-*trans*-hexenal and *cis*-3-hexen-1-ol and a decrease in hexanol in the shoots, but in the flowers, 2-*trans*-hexenal steeply decreased while *cis*-3-hexen-1-ol increased. 2-*trans*-Hexenal is a medium-chain aldehyde (C₆H₁₀O) known as a potent odorant, generally the product of enzymatic oxidation of unsaturated fatty acids. In plants, its protective effect is linked to the induction of biological defense responses; however, it appears to be toxic to humans [8,9].



Figure 2. Light micrographs of glandular capitate-type trichomes (**a**) stained with Sudan IV (**b**) and Sudan black B (**c**) for the detection of total lipids; Nile blue A (**d**) for acidic lipids; a Nadi reagent (**e**) for the identification of terpenes; iron (III) trichloride (**f**) and potassium dichromate (**g**) for phenolic compounds; a periodic acid–Schiff reagent (**h**) for the detection of polysaccharides; and Ruthenium red (**i**) for pectins. Bar = $25 \mu m$.

Table 1. Composition of the essential oils extracted from the shoots or flowers of *Dimorphoteca pluvialis* through hydrodistillation with a duration of 15, 30, or 60 min. For each compound at each parameter, values are presented as mean \pm standard error of six samples, and the different letters indicate statistically significant differences (*p* < 0.05).

Components	RI		Shoots			Flowers	
Time (min)		15	30	60	15	30	60
Octene	799	3.9 ± 0.1 a	$1.7\pm0.1~\mathrm{b}$	$1.0\pm0.4~\mathrm{b}$	1.6 ± 0.2 a	1.7 ± 0.4 a	1.5 ± 0.4 a
Hexanal	800	$0.4\pm0.1b$	$1.7\pm0.2~\mathrm{a}$	$1.7\pm0.1~\mathrm{a}$	$1.3\pm0.1~\mathrm{a}$	$0.9\pm0.1~\mathrm{a}$	$1.2\pm0.2~\mathrm{a}$
Octane	800	$0.7\pm0.1\mathrm{b}$	$1.3\pm0.0~\mathrm{a}$	$1.7\pm0.1~\mathrm{a}$	1.2 ± 0.2 a	1.7 ± 0.6 a	1.1 ± 0.2 a
2-trans-Hexenal	866	$23.0\pm0.1~\mathrm{a}$	$25.3\pm3.3~\mathrm{a}$	$28.9\pm0.8~\mathrm{a}$	56.3 ± 4.1 a	$34.2\pm3.5b$	$22.3\pm3.7b$
cis-3-Hexen-1-ol	868	$24.0\pm1.6~\mathrm{a}$	$16.4\pm1.0~\mathrm{b}$	$26.7\pm0.3~\mathrm{a}$	$3.5\pm1.7~\mathrm{b}$	16.8 ± 3.5 a	18.6 ± 2.4 a
Hexanol	882	$21.6\pm0.2~\mathrm{a}$	$18.8\pm1.1~\mathrm{b}$	$16.2\pm1.6~\mathrm{b}$	$18.4\pm0.9~\mathrm{a}$	11.4 ± 2.0 a	$18.4\pm2.9~\mathrm{a}$
α-Thujene	924	$0.2\pm0.1~\mathrm{a}$	0.0 ± 0.0 b 1	$0.0\pm0.0~\mathrm{b}$	$0.3\pm0.1~\mathrm{a}$	$1.0\pm0.5~\mathrm{a}$	$1.5\pm0.7~\mathrm{a}$
α-Pinene	930	$0.2\pm0.0~\mathrm{a}$	$0.2\pm0.0~\mathrm{a}$	$0.2\pm0.0~\mathrm{a}$	0.1 ± 0.1 a	$0.5\pm0.1~\mathrm{a}$	$1.1\pm0.5~\mathrm{a}$
Camphene	938	$0.4\pm0.0~\mathrm{a}$	$0.2\pm0.0~\mathrm{b}$	$0.2\pm0.0~\mathrm{b}$	$0.1\pm0.0~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$	$1.7\pm1.2~\mathrm{a}$
Sabinene	958	$2.3\pm0.0~\mathrm{a}$	2.1 ± 0.3 a	2.4 ± 0.4 a	3.9 ± 0.3 a	1.5 ± 0.4 b	4.1 ± 0.2 a
β-Pinene	963	$2.3\pm0.0~\mathrm{a}$	0.9 ± 0.4 b	$1.0\pm0.4~\mathrm{b}$	1.6 ± 0.3 a	1.3 ± 0.3 a	1.4 ± 0.3 a
Dehydro-1.8-cineole	973	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$	0.1 ± 0.1 a
2-Pentyl furan	973	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.1\pm0.0~{ m b}$	$0.1\pm0.0~{ m b}$	0.7 ± 0.3 a
<i>n</i> -Octanal	973	$0.0\pm0.0~\mathrm{a}$	0.1 ± 0.1 a	1.1 ± 0.6 a	$0.0\pm0.0~\mathrm{a}$	0.2 ± 0.1 a	$0.3\pm0.1~\mathrm{a}$
β-Myrcene	975	$0.0\pm0.0~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$	0.1 ± 0.1 a	$0.1\pm0.0~\mathrm{a}$	0.6 ± 0.2 a	$0.5\pm0.2~\mathrm{a}$
α-Phellandrene	995	$0.1\pm0.1~{ m b}$	$0.5\pm0.1~\mathrm{a}$	$0.0\pm0.0~\mathrm{b}$	$0.0\pm0.0~\mathrm{a}$	$0.4\pm0.2~\mathrm{a}$	$0.5\pm0.1~\mathrm{a}$
Benzene acetaldehyde	1002	$0.1\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	0.1 ± 0.1 a	$0.1\pm0.0~\mathrm{a}$	0.1 ± 0.1 a	0.1 ± 0.1 a
α-Terpinene	1002	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	0.0 ± 0.0 a	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$
<i>p</i> -Cymene	1003	$0.1\pm0.0~\mathrm{a}$	0.1 ± 0.1 a	0.1 ± 0.1 a	$0.0\pm0.0~{ m b}$	0.2 ± 0.1 a	$0.3\pm0.1~\mathrm{a}$
β-Phellandrene	1005	$0.0\pm0.0\mathrm{c}$	$0.2\pm0.0~\mathrm{a}$	$0.1\pm0.0~\mathrm{b}$	$0.1\pm0.0~{ m b}$	0.2 ± 0.1 a	$0.5\pm0.1~\mathrm{a}$
Limonene	1009	$0.1\pm0.1\mathrm{b}$	$0.4\pm0.0~\mathrm{a}$	0.5 ± 0.0 a	$0.0\pm0.0~{ m b}$	0.5 ± 0.2 a	$0.5\pm0.1~\mathrm{a}$
γ-Terpinene	1035	$0.1\pm0.0\mathrm{b}$	$0.1\pm0.0~{ m b}$	$0.2\pm0.0~\mathrm{a}$	$0.2\pm0.0~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$	$0.3\pm0.0~\mathrm{a}$
Terpinolene	1064	$0.0\pm0.0\mathrm{b}$	$0.0\pm0.0~\mathrm{b}$	$0.2\pm0.1~\mathrm{a}$	0.1 ± 0.1 a	0.3 ± 0.1 a	$0.2\pm0.1~\mathrm{a}$
<i>n</i> -Nonanal	1073	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~{ m b}$	$0.3\pm0.1~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$
Terpinen-4-ol	1148	$0.2\pm0.0~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$	0.1 ± 0.1 a	$0.3\pm0.1~\mathrm{a}$	$0.3\pm0.1~\mathrm{a}$	$0.5\pm0.1~\mathrm{a}$
α-Terpineol	1159	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$
n-Decanal	1180	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.4\pm0.1~\mathrm{a}$	$0.4\pm0.1~\mathrm{a}$
β-Damascenone	1356	$0.0\pm0.0~\mathrm{a}$	$0.3\pm0.2~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$
<i>n</i> -Dodecanal	1397	$0.0\pm0.0~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$
β-Caryophyllene	1414	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.4\pm0.2~\mathrm{a}$	$0.8\pm0.2~\mathrm{a}$	1.0 ± 0.3 a	0.6 ± 0.2 a
α-Humulene	1447	$0.6\pm0.1~\mathrm{a}$	$0.5\pm0.3~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.4\pm0.3~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$
Bicyclogermacrene	1487	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.2\pm0.1~\mathrm{a}$	$1.9\pm0.9~\mathrm{a}$	$0.2\pm0.0~\mathrm{a}$
Elemol	1530	$1.3\pm0.5~\mathrm{a}$	1.3 ± 0.3 a	$0.9\pm0.2~\mathrm{a}$	$0.1\pm0.1~{ m b}$	1.7 ± 0.5 a	$0.8\pm0.1~\mathrm{a}$
Spathulenol	1551	$0.2\pm0.1~\mathrm{a}$	0.3 ± 0.2 a	$0.0\pm0.0~\mathrm{a}$	0.3 ± 0.2 b	$0.9\pm0.2~\mathrm{a}$	$0.4\pm0.1~{ m b}$
β-Caryophyllene oxide	1561	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$
γ-Eudesmol	1609	0.7 ± 0.2 a	$0.8\pm0.5~\mathrm{a}$	$0.3\pm0.1~\mathrm{a}$	$0.1\pm0.1~{ m b}$	1.0 ± 0.2 a	0.5 ± 0.1 a
β-Eudesmol	1620	$0.7\pm0.1~\mathrm{a}$	1.3 ± 0.3 a	$0.5\pm0.0~\mathrm{b}$	$0.0\pm0.0~{ m b}$	1.4 ± 0.2 a	0.5 ± 0.1 a
α-Eudesmol	1634	1.3 ± 0.4 a	2.3 ± 0.7 a	1.1 ± 0.2 a	$0.0\pm0.0~\mathrm{b}$	2.0 ± 0.7 a	0.8 ± 0.2 b
% Identification		$84.1\pm1.1~\mathrm{a}$	$77.3\pm3.5~\mathrm{a}$	$85.6\pm2.9~\mathrm{a}$	91.5 ± 1.5 a	$87.2\pm0.6~a~b$	$82.0\pm3.8\mathrm{b}$
Grouped compounds							
Monoterpene hydrocarbons		$5.7\pm0.2a$	$4.8\pm0.1~\text{b}$	$5.0\pm0.1~\mathrm{b}$	$6.4\pm0.9~\mathrm{b}$	7.0 ± 1.3 b	12.5 ± 1.9 a
Oxygen-containing monoterpenes		$0.2\pm0.0a$	$0.2\pm0.1~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$	$0.3\pm0.1~\mathrm{a}$	$0.4\pm0.1~\mathrm{a}$	$0.6\pm0.1~\mathrm{a}$
Sesquiterpene hydrocarbons		$0.6\pm0.1a$	0.5 ± 0.3 a	$0.4\pm0.2~\mathrm{a}$	$1.5\pm0.4~\mathrm{a}~\mathrm{b}$	$3.1\pm0.7~\mathrm{a}$	$0.9\pm0.1\mathrm{b}$
Oxygen-containing sesquiterpenes		$4.2\pm1.1a$	$6.0\pm1.9~\mathrm{a}$	$2.7\pm0.1~\mathrm{a}$	$0.5\pm0.1~\mathrm{b}$	8.9 ± 1.3 a	3.1 ± 0.5 b
C13 Norisoprenoid		$0\pm0.0~\mathrm{a}$	$0.3\pm0.2~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.0\pm0.0~\mathrm{a}$	$0.1\pm0.0~\mathrm{a}$
Others		$73.5\pm2.0b$	$65.5\pm1.4b$	$77.5\pm3.3~\mathrm{a}$	$82.5\pm2.4~\mathrm{a}$	$67.8\pm2.1~\mathrm{b}$	$64.9\pm5.5b$
Yield (%, V/fresh weight)		< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

¹ Values below quantification limits were considered as 0.0 ± 0.0 for statistical treatment and can be referred to as compounds in trace amounts ($\leq 0.01\%$).

Together with *cis*-3-hexen-1-ol and hexanol, 2-*trans*-hexenal belongs to the green leaf volatiles (GLVs), six carbon-long aldehydes, esters, and alcohols that are released by plants

upon attack and function on the activation of the biochemical mechanisms of biological defense and resistance [8,9]. The terpene fraction occurred in low relative amounts, which varied from 8.1 \pm 0.3 (60 min) to 10.6 \pm 1.0% (15 min) in the shoots and from 8.9 \pm 1.3 (15 min) to 19.4 ± 2.2 (30 min) in the flowers. The monoterpene hydrocarbon fraction showed higher proportions (from 4.8 ± 0.1 at 30 min to $5.7 \pm 0.2\%$ at 15 min in the shoots and from 6.4 \pm 0.9 at 15 min to 12.5 \pm 1.9% at 60 min in the flowers) than the oxygencontaining monoterpenes (from 0.1 \pm 0.1, at 30 min, to 0.2 \pm 0.0%, at 60 min, in the shoots; and from 0.3 \pm 0.1, at 15 min, to 0.6 \pm 0.1%, 60 min, in the flowers). The dominant monoterpene hydrocarbons were sabinene with proportions that varied from 2.1 ± 0.3 (30 min) to $2.4 \pm 0.4\%$ (60 min) in the shoots and from 1.5 ± 0.4 (30 min) to 4.1 ± 0.2 (60 min) in the flowers; and β -pinene with proportions that varied from 0.9 \pm 0.4 (30 min) to 2.3 \pm 0.0% (15 min) in the shoots and from 1.3 \pm 0.3 (30 min) to 1.6 \pm 0.3 (60 min) in the flowers. For the sesquiterpenes, hydrocarbon proportions had lower relative amounts (from 0.4 ± 0.2 at 60 min to $0.6 \pm 0.1\%$ at 15 min in the shoots and from 0.9 ± 0.1 at 60 min to $3.1 \pm 0.7\%$ at 30 min in the flowers) than oxygen-containing molecules (from 2.7 ± 0.1 , at 60 min to 6.0 \pm 1.9% at 30 min in the shoots and from 0.5 \pm 0.1 at 15 min to 8.9 \pm 1.3% at 30 min in the flowers). The dominant oxygen-containing sesquiterpenes were elemol with proportions that varied from 0.9 ± 0.2 (60 min) to $1.3 \pm 0.5\%$ (15 min) in the shoots and from 0.1 \pm 0.1 (15 min) to 1.7 \pm 0.5 (30 min) in the flowers and α -eudesmol with proportions that varied from 1.1 \pm 0.2 (60 min) to 2.3 \pm 0.7% (30 min) in the shoots and from 0.0 ± 0.0 (15 min) to 2.0 ± 0.7 (30 min) in the flowers.

4. Conclusions

The shoots of *D. pluvialis* are covered with glandular trichomes that produce and secrete mainly acid-lipidic substances, terpenes, and phenylpropanoids. The extracted essential oils did not show qualitative differences between shoots and flowers; however, the main compounds, 2-*trans*-hexenal, *cis*-3-hexen-1-ol, and hexanol, varied with the duration of the hydrodistillation. The presence of dimorphecolic acid was not detected, suggesting it is restricted to the seeds or non-extractable by hydrodistillation.

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