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Essential oil composition and glandular trichome structure of the weather prophet *Dimorphoteca pluvialis*

Jorge M. S. Faria [1,2,*]

[1] INIAV, I.P., National Institute for Agrarian and Veterinary Research, Quinta do Marquês, 2780-159 Oeiras, Portugal
[2] MED, Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Évora University, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal
* Correspondence: fariajms@gmail.com
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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 (Δ9-hydroxy,10t,12t-octadecadienoic acid), a highly reactive C_{18} fatty acid with value for the manufacture 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 analysing the EO composition of shoots and flowers of *D. pluvialis*, extracted by hydrodistillation for 15, 30 or 60 minutes. Shoot surface displayed sharp and elongated non-glandular protection trichomes, while the sepals additionally showed shorter and wider 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, terpenic and phenolic compounds. The extracted EOs showed high amounts of trans-2-hexenal, a C_6 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

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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 raining. 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 makes them potential substitutes of 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 by 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 group, two conjugated double bonds relative to the α-carbon of the hydroxy group (Δ10,Δ12) and a trans-Δ12 unsaturation, setting this compound apart from other plant hydroxy fatty acids and granting it the potential for a wide range of new applications [3,4].

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 0.05 M sodium cacodylate buffer, pH 7.0 for 45 min at room temperature. After 1-2 min under vacuum (26 mm Hg, 3.46 kPA), the fixative was substituted with 3% glutaraldehyde in 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 (OsO_4) 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 cross sections 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 ($FeCl_3$) and potassium dichromate for phenolic compounds and Ruthenium red for pectins ([5] 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 hydrodistillation of shoots or flowers of the aerial parts of *D. pluvialis*, in a Clevenger-type apparatus according to the European Pharmacopoeia [6] for 15, 30 or 60 min at a distillation rate of 3 mL/min. When 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 μm; 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 μm; J & W Scientific

Inc., Rancho Cordova, CA, USA). Oven temperature was programmed to increase from 45 to 175°C, at 3°C / min increments, then up to 300°C at 15°C / min increments, and finally held isothermal for 10 min. Gas chromatographic settings were as follows: injector and detectors temperatures, 280°C and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using a split sampling technique, ratio 1:50. The volume of injection was 0.1 µL 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 DB 1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific, Inc., Rancho Cordova, CA, USA) interfaced with 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; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70eV; scan range, 40-300 u; 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 upon 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 statistics software. Statistical significance was determined with one-way ANOVA, and individual means were compared using the Tukey's Post-Hoc test with $p < 0.05$. Results were presented as mean ± 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, non-glandular trichomes were long and uniseriate (with 2 to 7 stacked cells), slightly pointing towards the sepal tip, with an average height of 160.3±12.0 µm and width of 14.5±1.0 µm, preferentially distributed in the margins of the sepal. In the stem, trichomes were multiseriate with three columns of 2 to 6 stacked cells and showed an average 78.3±2.6 µm in height and 57.6±1.2 µm 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 (Fig. 1 and 2). These secretory structures were 141.9±8.7 µm in height (4 to 7 stacked cells) and 43.4±1.4 µm in width (Fig. 1a and b). In the stems, the same type of capitate glandular trichome was observed, however, with lower height (101.2±3.2 µm) and width (37.1±1.6 µm) (Fig. 1c and d). The glandular head was made up of 2 to 3 cell layers, where the second and third cell layers showed a high chloroplast content, in contrast to the apical cells (Fig. 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 uniquely adapted to the physiology of secretory cells [7]. 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 [8].

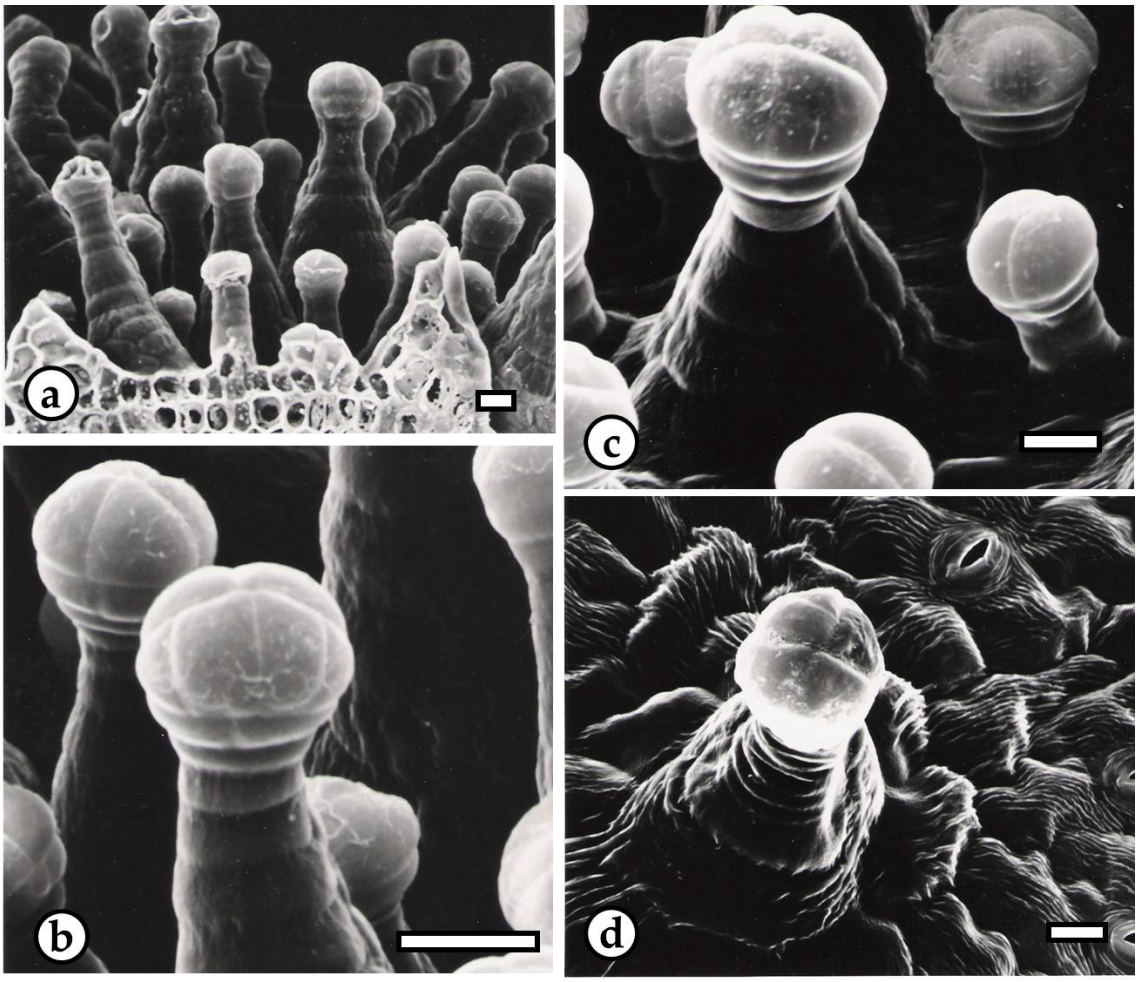

Figure 1. Scanning electron micrographs depicting the distribution of glandular trichomes in sepals (a), detail of glandular capitate-type trichomes of sepals (b) and vegetative shoots (c and d) of *Dimorphotheca pluvialis*. Bar = 15 μm.

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 the Sudan IV (Fig. 2b) or Sudan Black B (Fig. 2c) staining, with higher incidence in the first cell layer. Through the Nile blue A staining, a strong blue colour in the cells of the glandular head indicate 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 (Fig. 2d). The use of Nadi reagent allowed the identification of terpenic compounds in the exudate of the subcuticular space (Fig. 2e). The presence of phenolic compounds was confirmed with the iron (III) trichloride ($FeCl_3$) or potassium dichromate staining. The first stained intensely the cells of the glandular head (Fig. 2f) while the second stained mainly the apical cells where the metabolites are accumulated before secretion to the subcuticular space (Fig. 2g). The Periodic Acid-Schiff reagent (PAS), used to detect polysaccharides, stained of the trichome cells (stem and glandular head) but not the subcuticular space. Additionally, the 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 (≤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±3.3 % (60 min), for the shoots, and from 82.0±3.8 (60 min) to 91.5±1.5 % (15 min), for the flowers (Table 1). This fraction contained the main EO compounds (≥10 %), namely, the aldehyde 2-*trans*-hexenal, that varied from 23.0±0.1 (15 min) to 28.9±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, that varied from 16.4±1.0 (30 min) to 26.7±0.3 % (60 min), in the shoots, and 3.5±1.7 (15 min) to 18.6±2.4 (60 min), in the flowers; and the alcohol hexanol, that varied from 16.2±1.6 (60 min) to 21.6±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 ($C_6H_{10}O$) 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 [9,10].

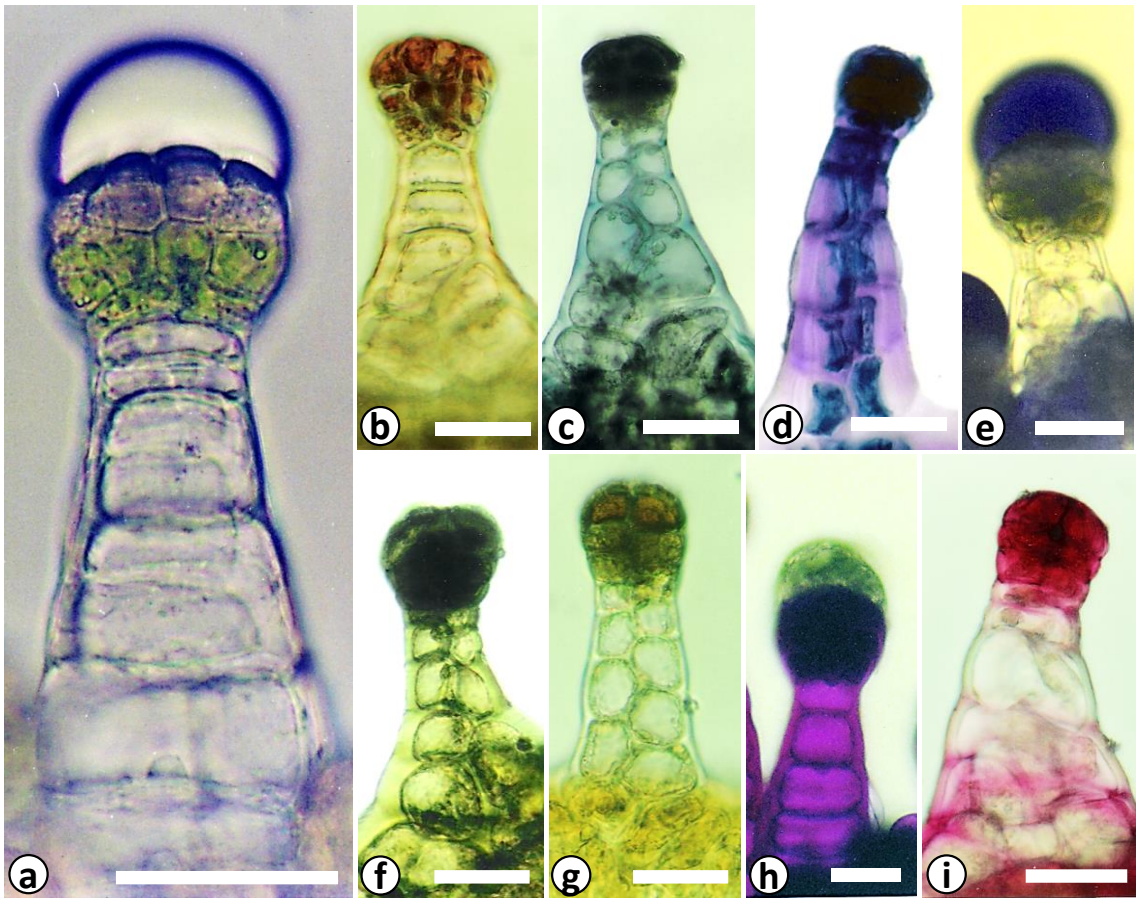

Figure 2. Light microghaphs of glandular capitate-type trichomes (a) stained with Sudan IV (b) and Sudan Black B (c), for the detection of total lipids; Nile blue A (d), for acidic lipids; Nadi reagent (e), for the identification of terpenes; iron (III) trichloride (f) and potassium dichromate (g), for phenolic compounds; Periodic Acid-Schiff reagent (h), for the detection of polysaccharides; and Ruthenium Red (i) for pectins. Bar = 25 μm.

Together with *cis*-3-hexen-1-ol and hexenol, 2-*trans*-hexenal belongs to the green leaf volatiles (GLV), 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 [9]. The terpene fraction occurred in low relative amounts, that varied from 8.1±0.3 (60 min) to 10.6±1.0 % (15 min), in the shoots, and 8.9±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±0.2 %, at 15 min, in the shoots; and from 6.4±0.9, at 15 min, to 12.5±1.9 %, at 60 min, in the flowers) than the oxygen-containing monoterpenes (from 0.1±0.1, at 30 min, to 0.2±0.0 %, at 60 min, in the shoots; and from 0.3±0.1, at 15 min, to 0.6±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±0.4 % (60 min), in the shoots, and 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±0.4 (30 min) to 2.3±0.0 % (15 min), in the shoots, and 1.3±0.3 (30 min) to 1.6±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±0.1 %, at 15 min, in the shoots; and from 0.9±0.1, at 60 min, to 3.1±0.7 %, 30 min, in the flowers) than oxygen containing molecules (from 2.7±0.1, at 60 min, to 6.0±1.9 %, at 30 min, in the shoots; and from 0.5±0.1, at 15 min, to 8.9±1.3 %, 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±0.5 % (15 min), in the shoots, and 0.1±0.1 (15 min) to 1.7±0.5 (30 min), in the flower; and α-eudesmol with proportions that varied from 1.1±0.2 (60 min) to 2.3±0.7 % (30 min), in the shoots, and 0.0±0.0 (15 min) to 2.0±0.7 (30 min), in the flowers.

Table 1. Composition of the essential oils extracted from the shoots or flowers of *Dimorphoteca pluvialis* through hydrodistillation with the duration of 15, 30 or 60 minutes. For each compound at each parameter, values are presented as mean ± standard error of 6 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±0.1a	1.7±0.1b	1.0±0.4b	1.6±0.2a	1.7±0.4a	1.5±0.4a
Hexanal	800	0.4±0.1b	1.7±0.2a	1.7±0.1a	1.3±0.1a	0.9±0.1a	1.2±0.2a
Octane	800	0.7±0.1b	1.3±0.0a	1.7±0.1a	1.2±0.2a	1.7±0.6a	1.1±0.2a
2-*trans*-Hexenal	866	23.0±0.1a	25.3±3.3a	28.9±0.8a	56.3±4.1a	34.2±3.5b	22.3±3.7b
***cis*-3-Hexen-1-ol**	868	24.0±1.6a	16.4±1.0b	26.7±0.3a	3.5±1.7b	16.8±3.5a	18.6±2.4a
Hexanol	882	21.6±0.2a	18.8±1.1b	16.2±1.6b	18.4±0.9a	11.4±2.0a	18.4±2.9a
α-Thujene	924	0.2±0.1a	0.0±0.0b[1]	0.0±0.0b	0.3±0.1a	1.0±0.5a	1.5±0.7a
α-Pinene	930	0.2±0.0a	0.2±0.0a	0.2±0.0a	0.1±0.1a	0.5±0.1a	1.1±0.5a
Camphene	938	0.4±0.0a	0.2±0.0b	0.2±0.0b	0.1±0.0a	0.1±0.0a	1.7±1.2a
Sabinene	958	2.3±0.0a	2.1±0.3a	2.4±0.4a	3.9±0.3a	1.5±0.4b	4.1±0.2a
β-Pinene	963	2.3±0.0a	0.9±0.4b	1.0±0.4b	1.6±0.3a	1.3±0.3a	1.4±0.3a
Dehydro-1.8-cineole	973	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.1±0.0a	0.1±0.1a
2-Pentyl furan	973	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.1±0.0b	0.1±0.0b	0.7±0.3a
***n*-Octanal**	973	0.0±0.0a	0.1±0.1a	1.1±0.6a	0.0±0.0a	0.2±0.1a	0.3±0.1a
β-Myrcene	975	0.0±0.0a	0.2±0.1a	0.1±0.1a	0.1±0.0a	0.6±0.2a	0.5±0.2a
α-Phellandrene	995	0.1±0.1b	0.5±0.1a	0.0±0.0b	0.0±0.0a	0.4±0.2a	0.5±0.1a
Benzene acetaldehyde	1002	0.1±0.0a	0.0±0.0a	0.1±0.1a	0.1±0.0a	0.1±0.1a	0.1±0.1a
α-Terpinene	1002	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
***p*-Cymene**	1003	0.1±0.0a	0.1±0.1a	0.1±0.1a	0.0±0.0b	0.2±0.1a	0.3±0.1a
β-Phellandrene	1005	0.0±0.0c	0.2±0.0a	0.1±0.0b	0.1±0.0b	0.2±0.1a	0.5±0.1a
Limonene	1009	0.1±0.1b	0.4±0.0a	0.5±0.0a	0.0±0.0b	0.5±0.2a	0.5±0.1a
γ-Terpinene	1035	0.1±0.0b	0.1±0.0b	0.2±0.0a	0.2±0.0a	0.2±0.1a	0.3±0.0a
Terpinolene	1064	0.0±0.0b	0.0±0.0b	0.2±0.1a	0.1±0.1a	0.3±0.1a	0.2±0.1a
***n*-Nonanal**	1073	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.3±0.1a	0.2±0.1a
Terpinen-4-ol	1148	0.2±0.0a	0.2±0.1a	0.1±0.1a	0.3±0.1a	0.3±0.1a	0.5±0.1a
α-Terpineol	1159	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
***n*-Decanal**	1180	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.4±0.1a	0.4±0.1a
β-Damascenone	1356	0.0±0.0a	0.3±0.2a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.1±0.0a
***n*-Dodecanal**	1397	0.0±0.0a	0.2±0.1a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
β-Caryophyllene	1414	0.0±0.0a	0.0±0.0a	0.4±0.2a	0.8±0.2a	1.0±0.3a	0.6±0.2a

α-Humulene	1447	0.6±0.1a	0.5±0.3a	0.0±0.0a	0.4±0.3a	0.2±0.1a	0.1±0.0a
Bicyclogermacrene	1487	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.2±0.1a	1.9±0.9a	0.2±0.0a
Elemol	1530	1.3±0.5a	1.3±0.3a	0.9±0.2a	0.1±0.1b	1.7±0.5a	0.8±0.1a
Spathulenol	1551	0.2±0.1a	0.3±0.2a	0.0±0.0a	0.3±0.2b	0.9±0.2a	0.4±0.1b
β-Caryophyllene oxide	1561	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.1±0.0a
γ-Eudesmol	1609	0.7±0.2a	0.8±0.5a	0.3±0.1a	0.1±0.1b	1.0±0.2a	0.5±0.1a
β-Eudesmol	1620	0.7±0.1a	1.3±0.3a	0.5±0.0b	0.0±0.0b	1.4±0.2a	0.5±0.1a
α-Eudesmol	1634	1.3±0.4a	2.3±0.7a	1.1±0.2a	0.0±0.0b	2.0±0.7a	0.8±0.2b
% Identification		84.1±1.1a	77.3±3.5a	85.6±2.9a	91.5±1.5a	87.2±0.6ab	82.0±3.8b
Grouped compounds							
Monoterpene hydrocarbons		5.7±0.2a	4.8±0.1b	5.0±0.1b	6.4±0.9b	7.0±1.3b	12.5±1.9a
Oxygen-containing monoterpenes		0.2±0.0a	0.2±0.1a	0.1±0.0a	0.3±0.1a	0.4±0.1a	0.6±0.1a
Sesquiterpene hydrocarbons		0.6±0.1a	0.5±0.3a	0.4±0.2a	1.5±0.4ab	3.1±0.7a	0.9±0.1b
Oxygen-containing sesquiterpenes		4.2±1.1a	6.0±1.9a	2.7±0.1a	0.5±0.1b	8.9±1.3a	3.1±0.5b
C13 Norisoprenoid		0±0.0a	0.3±0.2a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.1±0.0a
Others		73.5±2.0b	65.5±1.4b	77.5±3.3a	82.5±2.4a	67.8±2.1b	64.9±5.5b
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 (≤0.01%).

4. Conclusion

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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