JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Evaluation of Postharvest Drying, Key Odorants, and Phytotoxins in Plai (*Zingiber montanum*) Essential Oil

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Cite This: https://doi.org/10.1021/acs.jafc.1c00256 Read Online

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ABSTRACT: Plai or cassumunar ginger (*Zingiber montanum*), mainly distributed in tropical Asia, is an essential oil-bearing rhizomatous crop belonging to the Zingiberaceae family. Rhizomes and essential oil of this herb are used in culinary as flavoring agents, traditional medicines, and aromatherapy. In this study, the effect of different postharvest drying methods (air-, oven-drying at 40 and 60 °C, sun-, microwave-, and freeze-drying) of its sliced rhizome on the essential oil yield, composition, and sensory quality was investigated. The major key odorants and phytotoxins in its essential oil were identified for the first time through sensory- or bioassay-guided fractionation. Although the drying methods did not alter the oil composition significantly, oven-drying at 40 °C and freeze-drying produced the highest oil yield (81.0% of fresh rhizome) while maintaining the sensory quality. 4-Terpineol was found to be the majorly abundant key odorant in its oil through detailed sensory analysis. This oxygenated monoterpene was also demonstrated to be the major phytotoxin negatively affecting seed germination and shoot and root growth of wheatgrass seeds with IC_{50} values of 0.67, 0.10, and 0.17 mM, respectively. The current study is beneficial for further value addition of this crop in food industries and the agricultural sector.

KEYWORDS: *aromatic crop, gas chromatography, sensory analysis, allelopathy*

INTRODUCTION

Zingiber montanum (Koenig) Link ex Dietr. (Syn. *Zingiber cassumunar* Roxb., *Zingiber purpureum* Rosc.), commonly known as plai or cassumunar ginger, is a rhizomatous aromatic herb.[1−4] It is majorly distributed in tropical Asia, including India, China, and Southeast Asian countries.[5−7] Due to its unique aroma and taste, plai is used as a flavoring agent in traditional cuisines in these regions.[1,5,8,9] This herb is also known for its diverse ethnomedicinal uses, primarily for digestive tract disorders and the management of pain. It is used to treat asthma, muscle pain, diarrhea, and colic in India.[3,10] The indigenous communities in northeast India use the rhizome paste to treat indigestion, diarrhea, and snakebite. It is also utilized as the main ingredient in massage oil to relieve muscle pain and treat asthma in Thailand.[11] Malaysian communities use rhizome as vermifuge and postpartum medication. Besides, recent scientific studies have demonstrated antimicrobial, anti-inflammatory, analgesic, antioxidant, and anti-hypercholesterolemic activities of this herb.[7,8,12] At present, the use of its essential oil in aromatherapy is gaining popularity for relieving pain and inflammation.

Considering the traditional practices and known scientific information, this species of ginger can be utilized in a sustainable way in foods, beverages, and herbal medicines. Good agricultural practices, quality control techniques, and diversification of its use may play a key role in this endeavor. Postharvest processing of the aromatic and medicinal plants is important to preserve the quality and market value of the crop and the related end products.[13,14] About 10−30% postharvest loss has been estimated for *Zingiber officinale* (ginger), the major spice crop within this family, due to mishandling, and microbial and enzymatic spoilage during storage.[15,16] Drying is one of the important means to increase the shelf life and minimize packaging and shipping costs while retaining the quality.[14,17] In a very recent report, three different drying techniques (hot-air-drying at 40−80 °C, solar-drying, and sun-drying) were tested for plai rhizome.[18] However, this study exclusively used thermal drying and lacked the scope of nonconventional techniques, such as freeze- or microwave-drying. In the current study, six different postharvest drying techniques were evaluated for plai rhizome through the quantitative estimation of oil yield and composition. The sensory quality of the oil samples obtained through high oil-yielding techniques was also assessed. Besides, the major key odorant(s) in this oil were identified for the first time through sensory-guided fractionation.

Secondary metabolites in the rhizome may exert phytotoxicity around its vicinity through leaching or degradation of rhizome in the soil.[19−21] Phytotoxins from natural sources bear immense potential for weed management in agricultural fields as an alternative to synthetic herbicides.[22−24] A previous study reported the phytotoxic activity of plai oil in lettuce with an LD_{50} of 3.58 μL per plate.[4] However, no detailed investigation

Received: January 14, 2021
Revised: April 19, 2021
Accepted: April 22, 2021

was performed on the active phytochemicals responsible for its activity. In pursuit of the chemical basis of its activity, major phytotoxins from plai essential oil were identified for the first time in this study.

EXPERIMENTAL SECTION

Chemicals and Reagents. Technical grade solvents were distilled for the chromatography experiments. Silica gel of 60−120 mesh (Avra Synthesis, India) was used for performing column chromatography. Silica gel G-coated aluminum plates (Merck, 0.25 mm) were employed to carry out analytical thin-layer chromatography (TLC). Anisaldehyde staining agent was used for the visualization of spots. For the preparative TLC purpose, glass-supported silica gel plates (Merck, 20 × 20 cm^2, 1.00 mm) were used. The bands were visualized under UV (254 nm) light. Dipropylene glycol (DPG) (99%), deuterated chloroform (99.8 atom %D), and high-performance liquid chromatography (HPLC)-grade solvents were purchased from Sigma-Aldrich, St. Louis, MO. Organic wheatgrass seeds were purchased locally.

Collection and Authentication of the Plant Material. Fresh rhizome of *Z. montanum* was collected from the campus of CSIR-North East Institute of Science and Technology, Jorhat, located at 26°44′15.6948″N and 94°9′25.4622″E, altitude 94 m, during January 2019. Botanical identification of the plant material was confirmed by the taxonomist Dr. D Banik, one of the authors of this article. The voucher specimen was deposited in the institutional herbarium (voucher specimen no. NEIST-1859). Fresh rhizome samples were thoroughly washed with water and stored at −20 °C for further experiments.

Drying Techniques for Plai Rhizome. Fresh rhizomes were chopped transversely into thin slices (2.5−3.5 cm) and further divided into seven batches, each containing three sets (30.0 g each). Six batches were subjected to different drying methods, including air-drying, oven-drying at 40 and 60 °C, sun-drying, microwave-drying, and freeze-drying. The drying conditions and the durations were maintained as per previous reports with slight modifications ensuring that the moisture content in the sample was reduced below 20%.[17,25−29] For air-drying, the rhizome slices were kept under shade at room temperature (25−30 °C) for 3 days. Sun-drying was also performed for 3 days under direct sunlight. Two batches of samples were oven-dried individually at 40 °C (15 h) and 60 °C (8 h). For freeze-drying, the frozen samples were subjected to lyophilization for 24 h. Microwave-drying was performed at 200 W (2.0 min × 6 with a gap of 1.0 min between consecutive treatments). The remaining batch was further processed without drying and considered as the reference (fresh). The following equation was used for the determination of the moisture content in the dried sample: [(sample weight − dry weight)/sample weight] × 100%.

Hydrodistillation of the Essential Oil. Fresh (30.0 g) and dried (equivalent to 30.0 g of fresh) rhizome slices were subjected to hydrodistillation in a Clevenger-type apparatus. Extraction was continued for 10 h.[30] A yellowish essential oil was collected, and the residual oil in the collection arm was obtained with an *n*-hexane wash. The collected sample was dried over anhydrous sodium sulfate, and the organic solvent was evaporated at ambient conditions. The oil yield was expressed as the percentage with respect to the fresh weight of the rhizome (w/w). The extracted oil was stored at 4 °C in the dark.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. GC-MS analysis was carried out on a Thermo Fisher TRACE GC Ultra gas chromatograph attached with a DSQ mass spectrometer (GC-MS) and a TR-5MS capillary column (5% phenyl polysilphenylene-siloxane, 30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. In the GC temperature program, the initial column temperature was kept at 50 °C for 1.0 min. Then, a temperature gradient of 8 °C/min was set till 200 °C and held for 2.0 min, followed by a ramp of 20 °C/min to 300 °C and held for 3 min (total run time of 29.75 min). GC-MS samples were prepared in HPLC-grade ethyl acetate (∼50 ppm), and 1.0 μL of the sample solution was injected with a split ratio of 10:1. The inlet temperature was maintained at 250 °C. The area under the peak was considered for the determination of the relative percent for the individual analytes. Xcalibur 3.0.63 software was used for data processing and integrated NIST Mass Spectral Library (version 2.0f) for structural prediction. Similarity index (SI) and reverse similarity index (RSI) values of the library hits were considered to assess the quality of prediction. The identity of individual oil constituents was also supported by their linear retention index (RI) values relative to C_8−C_{20} *n*-alkanes. The calculated RI values were compared with the previously reported data.

NMR Analysis. An AVANCE III FT-NMR Spectrometer (500 MHz), Bruker, was used to record ^1H and ^{13}C NMR spectra. Bruker TopSpin 4.0.7 software was used for the data analysis. Phase and baseline corrections were made prior to the data analysis. The samples were dissolved in deuterated chloroform. Chemical shift values were reported in parts per million, and the residual solvent peak at 7.26 ppm was used as the reference.

Chromatographic Fractionation and Purification. The essential oil (200 mg) from fresh rhizome was fractionated using the silica gel column (60−120 mesh, 65 mL) chromatographic technique. Elution was carried out with increasing polarity of the eluent to obtain three fractions (**A**−**C**). The eluent and the yield of the fractions were as follows: (**A**) hexane (500 mL), 18.0 mg, (**B**) 5.0% dichloromethane in hexane (500 mL), 140.0 mg, and (**C**) dichloromethane (500 mL), 32.0 mg. The solvent was removed from the individual fractions at 35 °C and 250 mbar. The dried fractions were stored at 4 °C in the dark. Further, preparative thin-layer chromatographic purification of fraction **B** (loading amount, 50.0 mg) using 1:1 (v/v) *n*-hexane/dichloromethane as the mobile phase yielded purified 4-terpineol (**2**, 6.0 mg) and *trans*-4-(3′,4′-dimethoxyphenyl)buta-1,3-diene (**3**, 30.0 mg).

Difference from Control (DFC) Test. The difference from control test was performed to obtain the perceived odor difference in the chromatographic fractions (**A**−**C**), **2**, and **3** in reference to the crude oil.[31] The test included seven samples: (i) crude oil as the control, (ii) fraction **A**, (iii) fraction **B**, (iv) fraction **C**, (v) **2**, (vi) **3**, and (vii) crude oil as the blind control as well. The samples were prepared in 2.0% (v/v) concentration in a 1:1 (v/v) water and dipropylene glycol (DPG) mixture. In total, 20 panelists (eight males and 12 females, age 24−45 years) were chosen for the test. They were instructed to perceive the odor difference in the individual samples (ii)−(vii) in reference to control (i) and score the difference (difference score) in a five-point "difference scale" as follows: 0 no difference, +1 slightly different, +2 moderately different, +3 largely different, and +4 no similarity. The average values of the difference scores for the individual samples were plotted graphically. The value of the blind control represented the placebo effect.

In another set of the DFC test, the perceivable sensory (odor) difference was assessed in the oil samples obtained from oven-dried (40 °C) and freeze-dried rhizome in reference to the crude oil from fresh rhizome (control). A total of 36 assessors took part in the analysis. The test included the control, the blind control (oil from fresh rhizome), and two coded test samples (oil from the 40 °C and freeze-dried samples). Another oil sample from *Z. officinale* was included as the out-group. The analysis was performed similarly.

Determination of the Odor Threshold and Odor Activity Values (OTV/OAV). The single ascending method was used for the determination of the odor threshold values (OTVs) for the crude oil, **2**, and **3** in a 1:1 (v/v) DPG/water mixture.[32,33] Sixteen healthy adults (12 males and four females, age 24−45 years) participated in the analysis. Subjects with a nasal disorder were not included in the panel. Six dilutions (1.0, 0.5, 0.1, 0.05, 0.01, and 0.005%) of the individual samples were presented to the panelists. They were instructed to detect the odor of the samples through smelling strips in the order of increasing concentration. The lowest concentration at which odor stimulus was detected was recorded as the OTV. Distribution of the OTVs among the panelists was plotted, and the average OTV was calculated. The odor activity values (OAVs) for **2** and **3** in 1:1 (v/v) DPG/water containing 1.0% oil were calculated (concentration in the

https://doi.org/10.1021/acs.jafc.1c00256
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oil solution/OTV). The absolute concentrations of **2** and **3** in the oil were determined by plotting the individual standard graphs (peak area vs concentration in the range of 0.005−0.05 mg/mL) using purified compounds as the external standards in GC-MS. Further, injection of the crude oil sample (0.05 mg/mL) followed by fitting the peak area in the linear regression equation representing the standard graphs resulted in the absolute quantities of **2** and **3**.

Descriptive Sensory Analysis. Sensorial comparison among the crude oil, **2** and **3** was performed through quantitative descriptive sensory analysis.[33,34] A panel of 11 healthy adults (seven males and four females, age 24−45 years) with no nasal disorders who work with flavors and fragrances in the laboratory was selected for this aroma profiling. Samples (2.0%) were prepared in 1:1 (v/v) DPG/water, and smelling strips were provided. Seven aroma descriptors (sweet, woody, rooty, camphoraceous, spicy, herbaceous, and warming) that are known for cassumunar ginger were chosen for this study. Mandarin oil, cedarwood oil, freshly cut roots (*Chromolaena odorata*), camphor, ginger oil, a freshly cut herb (*Cynodon dactylon*), and cinnamon oil were used to define the descriptors, respectively, and were presented to the panelists as the reference. The assessors were instructed to perceive the odor of the individual samples and score each descriptor on a six-point scale (0 = not perceived to 5 = in perfect accordance with the reference). Panelists were asked to repeat the whole set of experiments after 3 h. The mean value of the scores for each descriptor was plotted in a spider web diagram.

Phytotoxicity Assay. The phytotoxic activity of plai oil was evaluated on wheatgrass seeds. The experiment was performed following previously reported procedures with few modifications.[35,36] Seeds were surface-sterilized with 1.0% sodium hypochlorite solution for ca. 1.0 min. They were thoroughly washed with deionized water at least five times, and the residual water on the seed surface was soaked over blotting paper. The solutions of the crude oil, fractions (**A**−**C**), or purified oil constituents (**2**, **3**) of different concentrations (50.0, 100.0, 200.0, and 300.0 μg/mL) were prepared in sterile deionized water (10.0 mL) containing 0.5% methanol (HPLC grade), and they were individually taken onto sterile Petri dishes of 90 mm diameter. Water (10.0 mL) containing 0.5% methanol without any inhibitor was used as the control. Then, the surface-sterilized wheatgrass seeds (20 numbers) were evenly placed on each Petri dish and incubated at 22 ± 1 °C with a 12/12 h light/dark photoperiod. Each experiment was performed in triplicate. After 3 days when >80% seeds were found to be germinated in the control, the number of germinated seeds and their root and shoot lengths were measured in each Petri dish. Percent inhibition of germination was calculated as [(no. of germinated seeds in the control − no. of germinated seeds in treated)/no. of germinated seeds in the control] × 100%. Similarly, percent growth inhibition was determined as [(length of root or shoot in the control − length of root or shoot in treated)/length of root or shoot in the control] × 100%.

Statistical Analysis. The determination of oil yield, compositional analysis, and percent inhibition in the phytotoxicity assay was performed in triplicate, and the quantified values were expressed as mean ± standard deviation. A single-factor analysis of variance (ANOVA) was performed to test the significant statistical difference in the compositions of oil samples from differently dried rhizomes. A *t*-test (paired two sample) was conducted to check the statistical difference for each test sample in reference to the blind control in the DFC tests. Also, a *t*-test (two-sample assuming equal variances) was carried out to assess the statistical difference in the activity of individual chromatographic fractions in reference to the crude oil in the phytotoxicity assay. All of the statistical analyses were carried out in MS Office Excel 2007 (Microsoft Corporation, Redmond).

RESULTS AND DISCUSSION

Taxonomic Identification of *Z. montanum*. Northeast India is well-known for the diversity of Zingiberaceous plants. About 19 genera and 88 species from the Zingiberaceae family have been reported from this region.[10] Many of them are rare, endangered, and wild. A large number of species with similar morphological characters and extensive variability within the same species are the risk factors for their authentication. Therefore, critical taxonomic assessment is essential for the identification of lesser-known Zingiberaceous plant materials.

In this study, the identification of *Z. montanum* was confirmed on the basis of the following morphological traits (Figure 1).[37] **Description**: herb 102.0−160.0 cm, perennial;

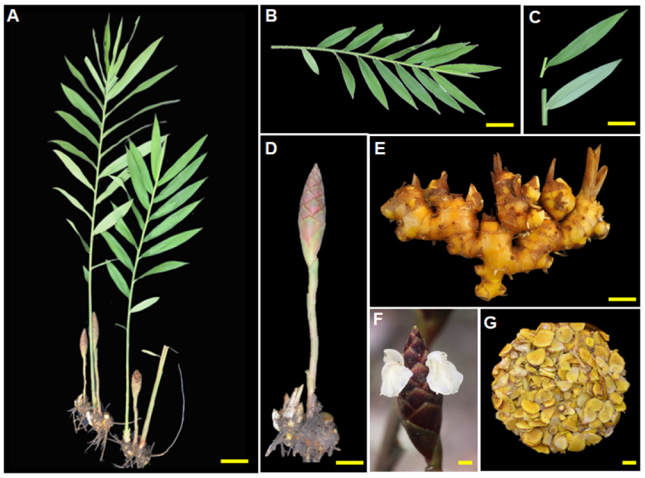

Figure 1. Taxonomic plate for *Z. montanum* (Koenig) Link ex Dietr. (A) Plant habit (scale bar: 9.7 cm), (B) leaves with pseudostem (scale bar: 9.7 cm), (C) leaf with abaxial and adaxial surfaces (scale bar: 5.1 cm), (D) inflorescence (scale bar: 3.2 cm), (E) rhizome (scale bar: 5.2 cm), (F) inflorescence axis with attached flowers (scale bar: 0.9 cm), and (G) rhizome slices (scale bar: 0.5 cm).

rhizomes aromatic, branched, yellowish-brown externally, light-yellow internally, scaly leaves 4−6, basal; pseudostem 2−3, 25.0−26.0 cm × 3.8−4.5 cm. **Leaves**: 15−23, median to pseudostem, alternate, subsessile, fronds 77.0−78.0 cm, leaf sheaths sparsely pubescent; ligules 0.2−0.3 cm long, bilobed, sparsely pubescent; lamina linear to broadly lanceolate, 16.6−28.2 cm × 3.8−5.0 cm, acute, entire, cuneate, pubescent below. **Inflorescence**: erect from pseudostem, peduncle 7.0−15.0 cm, spike ovate, 8.0−9.0 cm × 2.6−3.3 cm, apex acute; bracts 24−25, obovate, apex obtuse-acute, 4.0−4.1 cm × 2.4−2.5 cm, pubescent, maroon, basally whitish; bracteole obovate, apex obtuse, 2.5−2.6 cm × 1.4−1.8 cm, tridentate, whitish. **Flowers**: 24−25; calyx obovate, apex obtuse, glabrous, 1.0−1.1 cm × 0.9 cm, truncate, hyaline; corolla tubes slender, 4.0−4.5 cm long; dorsal lobe 1, lanceolate, pale-yellow to white, 3.0 cm × 0.7−0.8 cm; lateral lobes 2, lanceolate, basally united up to 0.8 cm, 2.2−2.5 cm long, 0.8−1.0 cm wide at base and 0.6−0.7 cm at apex; labellum 4-lobed, yellowish-white, 2.5 cm × 1.6−1.8 cm, basally clawed; claw 0.2−0.3 cm long, basal 2 lobes obtuse at apex, 1.0−1.2 cm × 0.5−0.6 cm, apical lobes obtuse-acute, 1.2−1.3 cm × 0.7−0.8 cm; stamen ca. 1.0 cm; filament 1.0−1.5 mm long; style 25.0−27.0 mm long, obconic stigma with ciliate margins; ovary 2.0−4.0 mm long, pubescent. **Phenology**: June−August.

This species shows morphological similarity with *Zingiber zerumbet* and *Zingiber kerrii*. *Z. montanum* possesses bifid pubescent ligule and obovate bracteole 2.5−2.6 × 1.4−1.8 cm^2 whereas *Z. zerumbet* has the entire ligule of 1.5−2.0 cm and bracteole ca. 1.5 cm long. *Z. kerrii* has 15−18 glabrous green bracts with a reddish-pink tip but *Z. montanum* has 24−25 pubescent maroon bracts.

https://doi.org/10.1021/acs.jafc.1c00256
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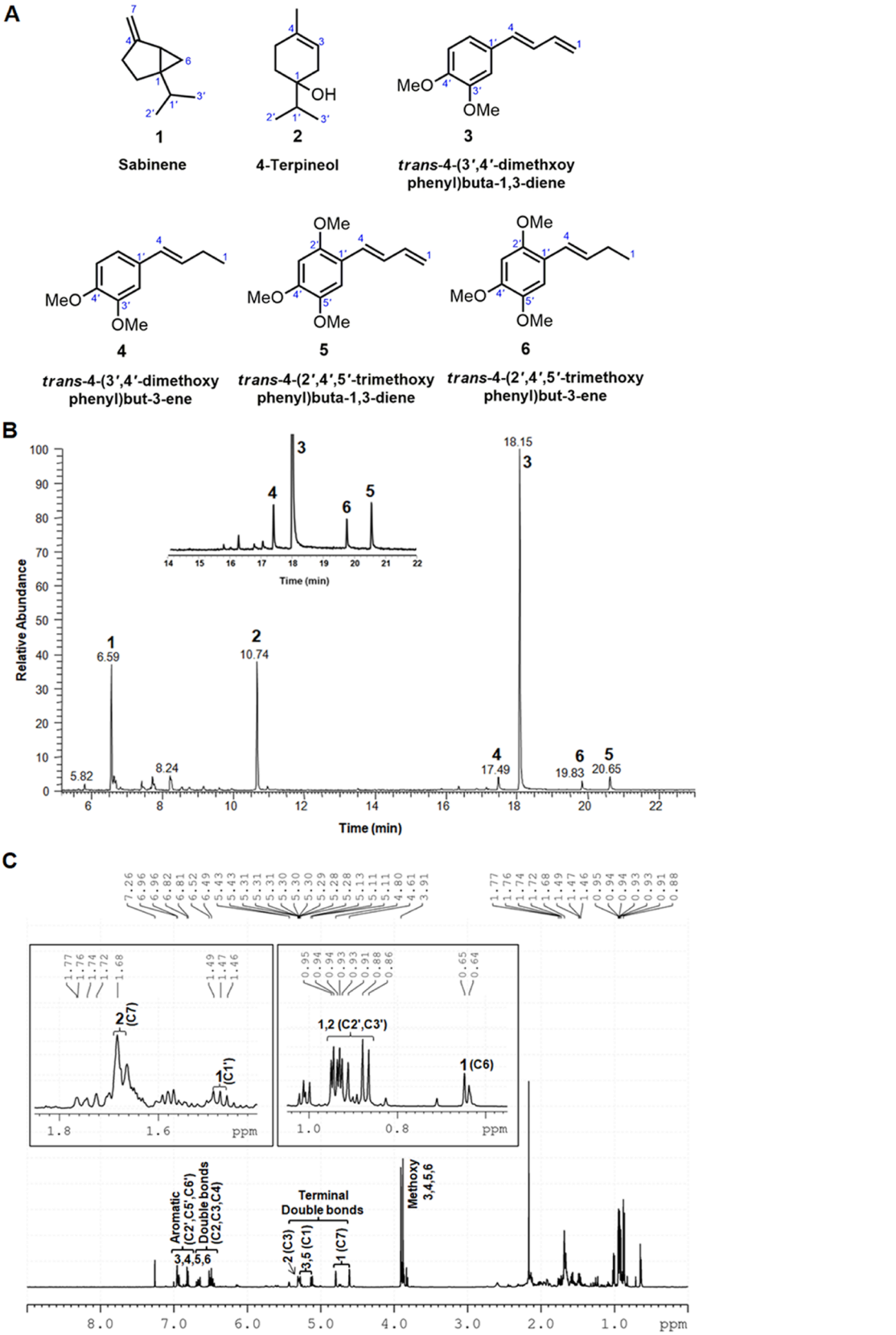

Figure 2. (A) Structures of the major chemical markers (**1**−**6**) in *Z. montanum* essential oil, (B) GC-MS profile (R_t 5.0−23.0 min), and (C) ^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of *Z. montanum* essential oil from fresh rhizome. Major peaks are labeled.

Characterization of the Chemical Markers. The major oil constituents in plai were identified using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy (Figure 2). Rhizome oil was majorly dominated by monoterpenes (~55%) and phenylbutenoids (~42%). Sabinene (**1**, 10.0%) and 4-terpeniol (**2**,

https://doi.org/10.1021/acs.jafc.1c00256
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Essential Oil Composition (Relative Area %) in the Fresh and Differently Dried Rhizome of *Z. Montanum*[a]

identity	R_t (min)	RI_{exp}	RI_{lit}	fresh rhizome	air-dried 3 days	oven-dried (40 °C) 15 h	oven-dried (60 °C) 8 h	sun-dried 3 days	MW-dried 2 min × 6	freeze-dried 24 h
α-pinene	5.82	937	937	0.40 ± 0.09	0.48 ± 0.26	0.70 ± 0.44	0.53 ± 0.22	0.29 ± 0.05	0.18 ± 0.04	0.89 ± 0.11
sabinene	6.59	976	974	10.0 ± 0.04	13.6 ± 0.36	9.46 ± 3.83	10.3 ± 5.04	13.6 ± 2.71	9.85 ± 4.38	15.0 ± 1.90
β-pinene	6.71	980	979	0.55 ± 0.10	0.84 ± 0.14	1.13 ± 0.40	0.78 ± 0.39	nd	0.36 ± 0.09	nd
β-myrcene	6.84	992	991	0.47 ± 0.08	0.42 ± 0.22	0.58 ± 0.33	0.47 ± 0.24	nd	0.38 ± 0.13	0.60 ± 0.15
α-terpinene	7.44	1019	1017	1.00 ± 0.10	1.12 ± 0.52	1.28 ± 0.57	1.14 ± 0.44	nd	1.07 ± 0.53	3.83 ± 0.76
β-phellandrene	7.75	1032	1031	1.65 ± 0.12	2.00 ± 0.84	2.07 ± 1.04	2.20 ± 1.00	2.03 ± 0.92	1.97 ± 1.24	2.70 ± 1.44
γ-terpinene	8.24	1062	1060	3.22 ± 0.11	3.64 ± 1.32	3.55 ± 1.04	3.85 ± 0.18	3.42 ± 1.31	3.78 ± 1.20	2.99 ± 0.98
4-terpineol	10.74	1182	1182	38.1 ± 0.38	24.6 ± 0.25	24.7 ± 5.59	23.1 ± 3.34	24.7 ± 0.46	17.0 ± 0.62	25.4 ± 1.25
trans-4-(3′,4′-dimethoxyphenyl)but-3-ene	17.49	1597	1595	1.25 ± 0.26	1.08 ± 0.32	1.81 ± 0.31	1.91 ± 0.39	4.17 ± 0.22	1.06 ± 0.31	3.53 ± 0.53
trans-4-(3′,4′-dimethxoyphenyl)buta-1,3-diene	18.15	1637	1636	35.3 ± 0.58	33.7 ± 2.09	38.8 ± 4.96	37.9 ± 8.23	34.7 ± 7.41	55.1 ± 1.41	31.1 ± 6.46
trans-4-(2′,4′,5′-trimethoxyphenyl)but-3-ene	19.83	1772	1770	2.16 ± 0.29	1.83 ± 0.21	3.71 ± 1.09	4.61 ± 0.82	4.73 ± 0.31	2.37 ± 0.90	4.34 ± 1.02
trans-4-(2′,4′,5′-trimethoxyphenyl)buta-1,3-diene	20.65	1820	1821	3.72 ± 0.24	3.99 ± 1.01	8.20 ± 2.57	11.3 ± 2.86	3.39 ± 0.82	5.55 ± 2.52	4.79 ± 1.09
total monoterpene				55.36	46.74	43.45	42.27	44.08	34.54	51.41
total phenylbutenoids				42.45	40.60	52.50	55.70	47.02	64.08	43.77

[a]nd, not detected.

38.1%) accounted for about 87% of the total monoterpenes (Table 1). The structural identity of these two constituents was confirmed on the basis of GC-MS library hits, NMR (^{1}H and ^{13}C) spectra, retention index values, and authentic standards. The GC-MS peak at R_t 10.74 min showed the library hit for 4-terpineol (**2**) with similarity index (SI) and reverse similarity index (RSI) values of 910 and 922, respectively. The retention index (RI 1182) was in perfect agreement with the reported value of **2** (Table 1). Also, a pure authentic standard of **2** was eluted at the same retention time, thus confirming its identity. The characteristic signals for **2** (δ_H 5.29, 1.68, 0.90−0.95 and δ_C 133.85, 118.40, and 71.77 ppm) were also visible in the ^{1}H and ^{13}C NMR spectra of the crude oil (Figure 2C and Supporting Information). The peak at R_t 6.59 min was predicted to be sabinene (**1**) through the GC-MS library search with reliable SI and RSI values of 902 and 918, respectively. The characteristic peaks at δ_H 4.80, 4.61, 0.87, and 0.64 and δ_C 154.74 and 101.69 ppm in the crude oil NMR spectra supported the identity of this constituent (Figure 2C and Supporting Information) and was further confirmed through the RI value and the injection of an authentic standard.

A portion of phenylbutenoid was exclusively dominated by *trans*-4-(3′,4′-dimethxoyphenyl)buta-1,3-diene (**3**, R_t 18.15 min, 35.3%) contributing about ∼83% of the total phenylbutenoids (Table 1). The structural identity of this major phenylbutenoid (**3**) was unambiguously confirmed through its retention index value (1637), electron ionization-mass spectrometry (EI-MS), NMR (^{1}H and ^{13}C) spectra of the crude oil, chromatographic fraction **B** (rich in **3**), and purified **3** (Figure 2C and Supporting Information). When the ^{1}H NMR spectra were analyzed, the signals for aromatic (δ_H 6.81−6.96 ppm), conjugated, and terminal double bonds (δ_H 6.45−6.70 and 5.11−5.32 ppm) and methoxy protons [δ_H 3.88 (s) and 3.91 (s) ppm] characteristic to **3** were visible. Ten signals within the range of δ_C 108−150 ppm also confirmed the presence of an aromatic ring and two unsaturations (conjugated and terminal). The signals at δ_C 149.02 and 148.89 ppm represented the oxygenated (methoxylated) aromatic carbons, whereas the peak at δ_C 116.64 ppm indicated the presence of terminal double bonds. The methylene on terminal unsaturation appeared at δ_H 5.30 (J = 16.7 Hz) and 5.12 (J = 10.0 Hz) ppm in the ^{1}H NMR spectrum. The high coupling value (J = 15.6 Hz) observed for the benzylic methine signal at δ_H 6.51 ppm confirmed the *trans* geometry of the internal double bond. The fragments in the EI-MS spectrum at m/z 190.01 $[M]^+$, 175.00 $[M - Me]^+$, 159.00 $[M - OMe]^+$, and 143.99 $[M - Me - OMe]^+$ also substantiated the presence of two methoxy groups in the structure.

EI mass spectral fragmentation was further supportive of the structural identification of minor phenylbutenoid constituents (Supporting Information). Minor peak eluting at R_t 17.49 min produced similar fragments with an increase of two mass units at m/z 192.04 $[M]^+$, 177.01 $[M - Me]^+$, 161.01 $[M - OMe]^+$, and 145.99 $[M - Me - OMe]^+$, indicating hydrogenation of unsaturation, and was characterized as *trans*-4-(3′,4′-dimethoxyphenyl)but-3-ene (**4**). NMR spectrum for fraction **B**, possessing a minor percentage of **4**, further confirmed its identity (Supporting Information). The signals at δ_H 3.87 (s) and 3.90 (s) ppm represented two methoxy groups and δ_C 25.97 and 13.75 ppm confirmed terminal saturation. The other two minor phenylbutenoids eluting at R_t 20.65 and 19.83 min (**5** and **6**), respectively, were found to be close structural analogues of **3** and **4**, respectively, with an additional methoxy group on the aromatic ring. The common presence of mass spectral fragments such as $[M]^+$ (m/z 220.00 for **5** and 222.05 for **6**), $[M - Me]^+$ (m/z 205.00 for **5** and 206.99 for **6**), $[M - OMe]^+$ (m/z 188.99 for **5** and 190.99 for **6**), $[M - Me - OMe]^+$ (m/z 173.99 for **5** and 176.00 for **6**), and $[M - 2Me - OMe]^+$ (m/z 158.99 for **5** and 161.01 for **6**) indicated the presence of three methoxy groups on the phenylbutenoid skeleton. The fraction **C**, rich in **5** and **6**, showed the presence of six methoxy signals (δ_H 3.90, 3.89, 3.88, 3.87, 3.84, and 3.82

https://doi.org/10.1021/acs.jafc.1c00256
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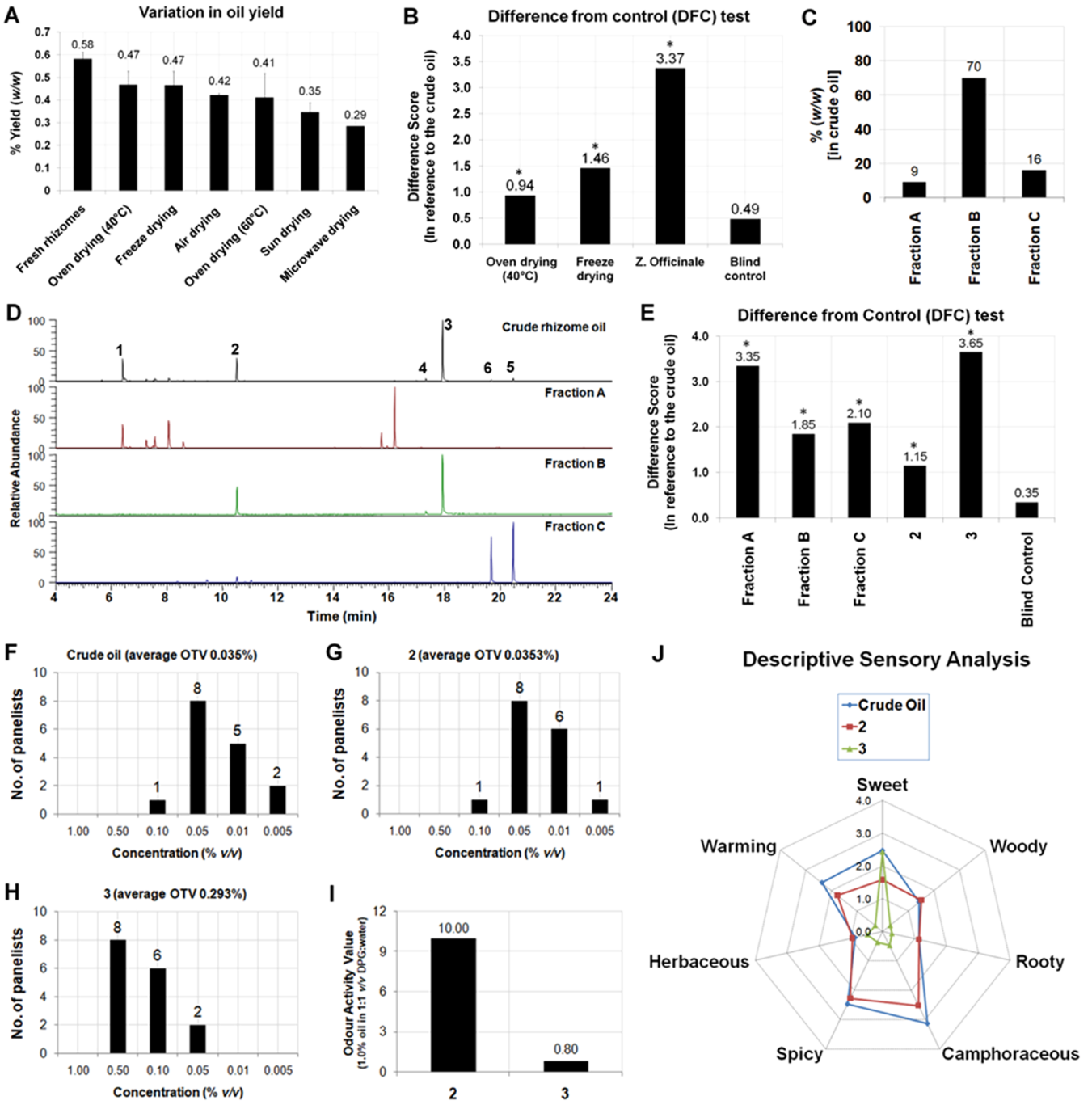

Figure 3. (A) Variation in the oil yield (% w/w) in differently dried *Z. montanum* rhizome with reference to the fresh rhizome and (B) difference from control (DFC) test for the oil samples from oven-dried (40 °C) and freeze-dried rhizome (*$P < 0.05$ vs the blind control). *Z. officinale* oil was included as the out-group. (C) Isolated % yield (w/w) of the individual fractions **A**–**C**; (D) stack plot of the GC-MS chromatograms for the crude oil and fractions **A**–**C** (R_t 4.0–24.0 min); and (E) difference from control (DFC) test for the fractions **A**–**C**, purified **2**, and **3** (*$P < 0.05$ vs the blind control). Fresh rhizome oil was used as the control and the blind control for the DFC tests; (F–H) distribution of odor threshold values (OTVs) for the crude oil, **2**, and **3**, respectively, in 1:1 (v/v) DPG/water for 16 panelists presented with six dilutions in the range of 0.005–1.00%; (I) odor activity values (OAVs) for **2** and **3** in 1.0% oil added in 1:1 (v/v) DPG/water; and (J) sensorial comparison among the crude oil, **2**, and **3** through descriptive sensory analysis.

ppm; each singlet) in the ^{1}H NMR spectrum, thus confirming the presence of these trimethoxy analogues. The doublets at δ_H 5.27 (J = 17.0 Hz) and 5.10 (J = 10.1 Hz) ppm corroborated to the terminal unsaturation in **5**. ^{13}C NMR signals at 56.72, 56.67, 56.42 (×2), 56.07, and 56.03 represented the methoxy carbons on **5** and **6**, whereas the peaks at 26.43 and 13.91 ppm indicated the reduction of terminal unsaturation in **6**. The RI values were also in good accordance with previously known data (Table 1).

Minor monoterpenoids, including α-pinene, β-pinene, β-myrcene, α-terpinene, β-phellandrene, and γ-terpinene, were characterized through GC-MS library hits and their RI values (Table 1). In addition, authentic standards were injected in GC-MS to confirm the identity of β-pinene, β-myrcene, and α-

https://doi.org/10.1021/acs.jafc.1c00256
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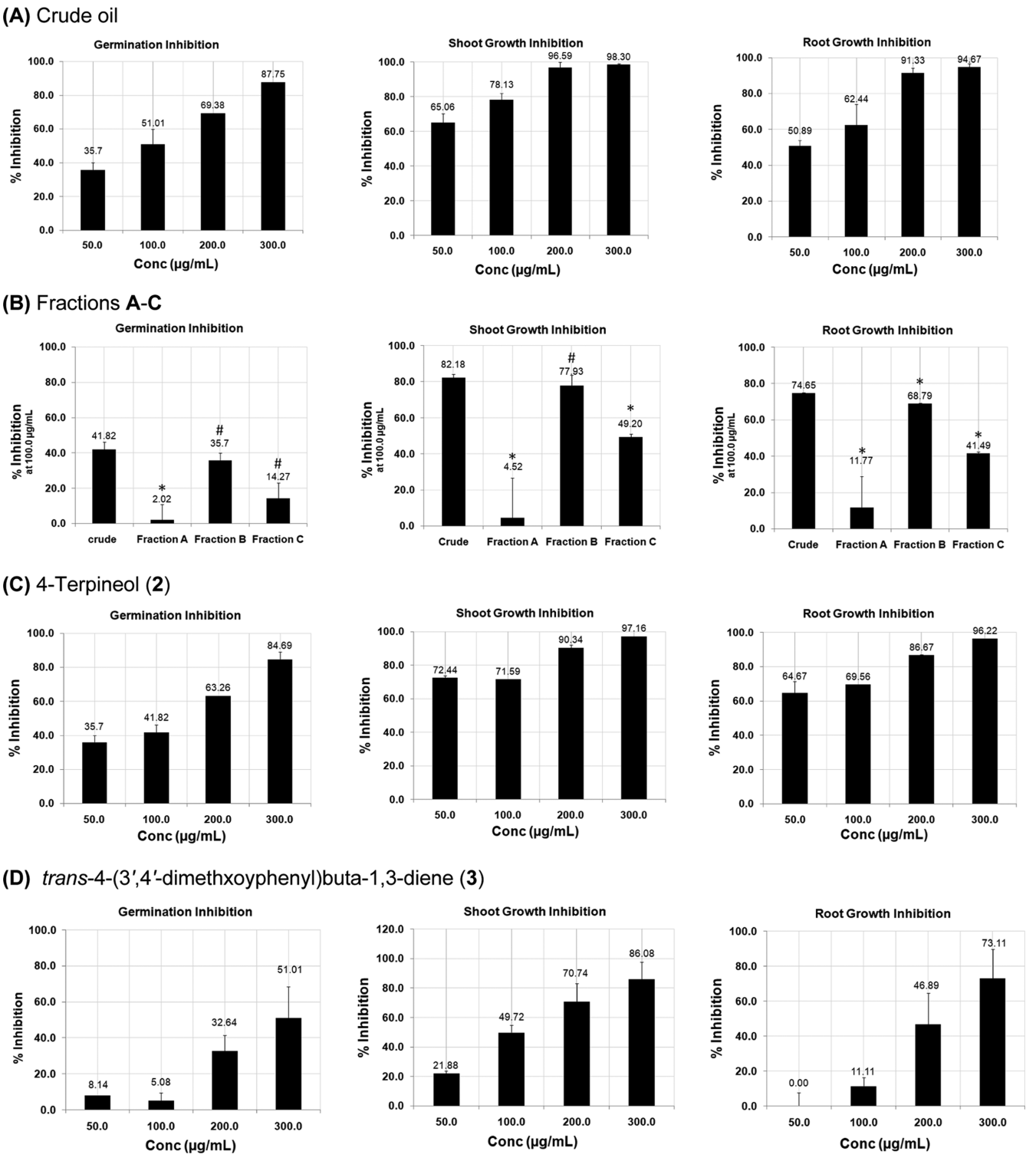

Figure 4. Inhibition of germination and shoot and root growth of wheatgrass seeds after 3 days by (A) the crude oil of *Z. montanum* (50.0−300.0 µg/mL), (B) fractions **A**−**C** (100.0 µg/mL) (*$P < 0.05$, #$P > 0.05$ vs crude oil), (C) **2** (50.0−300.0 µg/mL), and (D) **3** (50.0−300.0 µg/mL).

terpinene (Supporting Information). The identified chemical markers matched well with the previous reports on *Z. montanum* oil.[3,38,39]

Comparative Evaluation of the Postharvest Drying Techniques: Yield, Composition, and Sensory Quality. Postharvest drying to increase the shelf life of the aromatic crops often influences the essential oil yield, composition, and sensory quality. Sliced rhizome of plai was subjected to six different drying conditions (air-drying, oven-drying at 40 and 60 °C, sun-drying, microwave-drying, freeze-drying), and their oil yield and compositions were evaluated individually (Figure 3A). The drying processes reduced the moisture content in the rhizome to 7.8, 5.6, 6.3, 5.6, 17.0, and 2.5%, respectively, in comparison to 87.0% in the fresh sample. The oil yield (% w/w) was best retained through freeze-drying and oven-drying at 40 °C (0.47%) in reference to the fresh rhizome (0.58%). It

https://doi.org/10.1021/acs.jafc.1c00256
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was calculated to be 81.0% (i.e. [0.47/0.58] × 100%) of the oil yield obtained from the fresh sample. The yield reduced drastically in the case of sun-drying (0.35%) and microwave-drying (0.29%, i.e., 50% of the fresh rhizome). However, the oil composition was grossly similar across differently dried rhizome samples, except for some minor quantitative variations, especially in the case of monoterpenes (Table 1). A decrease in the percentage of total monoterpenes (hence causing a relative increase in the level of phenylbutenoids) was observed in different drying processes. For instance, in the case of the sun-dried sample, several minor monoterpenes, including β-pinene, β-myrcene, and α-terpinene, were not detectable. The volatility of monoterpenes probably led to a decrease in their levels during the drying conditions. The phenylbutenoid profiles were very similar across all of the tested drying methods. Single-factor ANOVA including the quantitative composition of the oil samples from differently dried rhizomes determined the p-value to be 0.99 (>0.05), indicating no significant constitutional difference across the samples.

The sensory quality of the oil samples obtained through high-yielding drying methods (i.e., freeze-drying and oven-drying at 40 °C) was tested through the difference from control (DFC) test in reference to the fresh rhizome. The difference scores were found to be 0.94 and 1.46 for oven (40 °C)- and freeze-dried samples, respectively (blind control, 0.49). This indicated a low degree of perceivable sensory difference in reference to the oil from a fresh rhizome. An oil sample from *Z. officinale* (out-group) was included for the validation of the experiment, which showed a very high difference score (3.37) (Figure 3B).

Identification of Key Odorants in Plai Essential Oil. The essential oil of *Z. montanum* possesses a spicy aroma with sweet, woody, rooty, and camphoraceous-type odor notes. Despite its use in aromatherapy and as a natural food flavoring agent, majorly abundant aroma active constituents are not known to date. To obtain the key odorants in this oil, different sensory analyses such as the difference from control (DFC) test, determination of odor threshold and odor activity values, and descriptive sensory analysis were adopted. A stepwise reduction in the complexity of the mixture containing key odorants through sensory (olfactory)-guided fractionation was the strategy for easy and confirmative identification of the major aroma active constituent(s).[40−42] Chromatographic fractionation of the oil followed by the sensory (odor) evaluation of the produced fractions in reference to the crude oil was performed with this view. The odor difference in the fractions **A**−**C** was assessed individually in reference to the crude oil through the DFC test (Figure 3E). The difference scores were obtained as 3.35, 1.85, and 2.10 (on a scale of 0−4) for the fractions **A**, **B**, and **C**, respectively, in reference to 0.35 for the blind control. This result revealed that fraction **A** containing nonpolar monoterpene hydrocarbons, such as sabinene, terpinolene, α-phellandrene, and γ-terpinene, had no significant contribution to its overall aroma (Figure 3D). However, fraction **B** (contributing 70% mass of the oil) that was mainly composed of **2** and **3** showed the lowest difference score, indicating that the abundance of the key aroma impact compounds in this fraction (Figure 3C,D). When purified **2** and **3** were subjected to the DFC test, the difference scores were found to be 1.15 and 3.65, respectively. It revealed 4-terpineol (**2**) as the major key odorant in the essential oil of plai. Fraction **C** also contained a small percentage of **2**, which was possibly responsible for its low difference score.

The average odor threshold values (OTVs) for the crude oil, **2**, and **3** were determined as 0.035, 0.035, and 0.290%, respectively, in 1:1 (v/v) DPG/water (Figure 3F−H). This showed that the lower limit of olfactory detection was very close for the oil and **2**. On the other hand, OTV for **3** was 8.3 times higher than those of the oil and **2**, indicating it to be a comparatively weak odorant, which was further confirmed when odor activity values (OAVs) were calculated for **2** and **3** in 1.0% oil in 1:1 (v/v) DPG/water. The OAV considers the concentration and OTV to quantify the contribution of individual constituents in creating the overall aroma of the mixture. The absolute quantity of **2** (35.3%) and **3** (23.5%) in the crude oil, as determined using their standard graphs, was applied to calculate the OAV. It was observed that 4-terpineol (**2**) had a 12.5 times higher OAV in comparison to that of the major phenylbutenoid (**3**), suggesting a strong influence of **2** in the overall aroma of the crude oil (Figure 3I).

Furthermore, the aroma profiles of **2** and **3** were compared in reference to that of the crude oil through descriptive sensory analysis (Figure 3J). Among seven aroma descriptors tested, high scores were obtained in the oil for camphoraceous, spicy, warming, and sweet. Interestingly, a very similar sensory profile was observed for **2** with high scores for camphoraceous and spicy notes. In contrast, **3** mainly had a sweet note with very low scores for all of the other descriptors. Previous studies have described the odor of 4-terpineol as spicy, woody, and green.[33,43−45] In a recent report, the minor constituents, viz., 4-terpineol and α-terpineol, were reported to be the key aroma active constituents in omija fruit, which were responsible for its characteristic spicy and wet-wood aromatics.[33] These findings further substantiated the key role of **2** in creating the overall impression of plai rhizome or its essential oil aroma.

Identification of Phytotoxins in Plai Essential Oil. The phytotoxic effect of fresh *Z. montanum* oil was assessed through seed germination and the growth (shoot and root) inhibition assay on wheatgrass seeds. The crude essential oil inhibited germination in a concentration-dependent manner after 3 days in the tested range of 50.0−300.0 μg/mL with the half-maximal inhibitory concentration (IC_{50}) value of 89.4 μg/mL (Figure 4A). Inhibition was found to be very high (87.8%) at 300.0 μg/mL. Also, concentration-dependent inhibition of shoot and root growth was observed for the germinated seeds with IC_{50} values of 23.1 and 52.1 μg/mL, respectively. Crude oil almost completely inhibited the growth of the shoot (96.6%) and the root (91.3%) at 200.0 μg/mL as measured after 3 days. These results indicated the phytotoxicity of plai oil through the inhibition of germination and growth.

To identify the active phytotoxins, bioassay-guided fractionation of the crude essential oil was performed. Single point (100.0 μg/mL, close to the IC_{50} value for the crude oil in the seed germination assay) inhibition was tested comparatively for the fractions **A**−**C** in reference to the crude (Figure 4B). No significant inhibition (germination or growth) was exerted by the hydrocarbon-rich fraction **A** at the tested concentration. However, fraction **B** mainly possessing **2** and **3** showed inhibition similar to that of the crude. The inhibition was observed to be 35.7, 77.9, and 68.8% at 100.0 μg/mL for germination and shoot and root growth, respectively. Fraction **C** containing minor phenylbutenoids (**5** and **6**) showed significant inhibition (14.3, 49.2, and 41.5%, respectively) though with a lower efficacy in comparison to the crude or

https://doi.org/10.1021/acs.jafc.1c00256
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fraction **B**. Therefore, fraction **B** was further considered in obtaining the major active phytotoxin(s). When purified **2** and **3** were individually tested for their phytotoxic potency, a dose-dependent inhibitory trend was observed for both the metabolites (Figure 4C,D). However, 4-terpineol (**2**) showed much higher inhibition efficacy that was comparable to the crude. Even at a low concentration (50.0 μg/mL), the percent inhibition was 35.7, 72.4, and 64.7% for germination and shoot and root growth, respectively. Extreme inhibition was noticed (84.7, 97.2, and 96.2%, respectively) at the highest concentration (300.0 μg/mL) tested. The IC_{50} values of **2** were determined to be 104.0 μg/mL (0.67 mM), 14.8 μg/mL (0.10 mM), and 26.3 μg/mL (0.17 mM) for the inhibition of seed germination and shoot and root growth, respectively. On the other hand, the IC_{50} values of **3** were found to be 371.2 μg/mL (1.95 mM), 107.5 μg/mL (0.56 mM), and 200.3 μg/mL (1.05 mM), respectively, indicating a lower phytotoxic efficacy. In a study by Vokou and co-workers, 4-terpineol was reported to be the most potent phytotoxin among 47 tested monoterpenoids.[46] It showed an extreme inhibition of seed germination and seedling growth (<15% of the control) of lettuce at 25.0 ppm through aerial contact in a closed Petri dish. These observations inferred 4-terpineol (**2**) as the major key phytotoxin in the essential oil of plai.

In conclusion, freeze-drying and oven-drying at 40 °C were shown to be the preferred drying techniques for plai rhizome slices, which were able to retain the oil yield, composition, and sensory quality. 4-Terpineol was found to be the major key odorant for producing the characteristic aromatics of the oil. The crude oil could exert phytotoxic activity on wheatgrass seed germination and growth in a concentration-dependent manner, and bioactivity-guided fractionation demonstrated 4-terpineol as the major phytotoxin in the oil. The current study provided a scientific basis for the selection of suitable postharvest drying of this aromatic crop. The knowledge generated on its aroma active constituents and phytotoxins is useful for producing valuable end products related to natural food additives or herbal formulations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c00256.

EI-MS spectra of the identified chemical markers; ^{1}H NMR spectra of crude oil and fractions **B** and **C**; ^{13}C NMR spectra of crude oil and fractions **B** and **C**; and GC-MS chromatograms of the oil and pure standards (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Phirose Kemprai acknowledges UGC, New Delhi, for the fellowship. The current work (manuscript no. NMN-202028) was supported by the CSIR-Nutritional and Nutraceutical Mission (HCP019) and the Director, CSIR-NEIST.

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https://doi.org/10.1021/acs.jafc.1c00256
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