

# Taxonomy, phytochemical and bioactive compounds and potential use as material with different drying methods of *Alpinia latilabris* Ridl. new record from Thailand

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## Abstract

*Alpinia latilabris* Ridl., a new record from Thailand, has great potential for use as a material for food and traditional medicine. Dried samples preserve the quality and avoid the degradation of phytochemicals. The aim here was to determine the taxonomy and changes in the phytochemical and bioactive compounds when using different drying methods as well as the antioxidant properties in this first report for this species. The results show that freeze-dried samples had greater quality volatile compounds, bioactive compounds, organic acid, phenolic acid, flavonoids and antioxidants compared with a fresh sample while having a microstructure similar to that of the fresh sample. The major volatile compounds were 1,8-cineole in fresh and dried samples, as confirmed by FTIR spectra. The bioactive chemicals are sensitive to thermal drying and sunlight due to degradation of the phytochemicals. This result can be useful information and be applied to the preparation of material for further development of functional foods, medicinal plants or cosmetics.

**Keywords:** antioxidants; flavonoids; organic acids; phenolic acids; volatile compounds

## Introduction

*Alpinia* Roxb. is the largest genus in the tribe Alpinieae with more than 250 species belonging to subfamily Alpinoideae, family Zingiberaceae or ginger family (Kress *et al.*, 2002; Leong-Škorničková *et al.*, 2019). The genus is distributed in tropical Asia to Australia and the tropical Pacific islands, including the Solomon Islands, New Hebrides, New Caledonia, Fiji and Samoa. Whereas, the center of diversity of the genus *Alpinia* is the Malesian region, where about 160 species are found (Larsen and Larsen, 2006). The first attempt to enumerate the *Alpinia* species number in Thailand was recorded by Larsen (1996) with 13 species. Later, Saensouk *et al.* (2003) reported two new records. During an ongoing revision study of the genus *Alpinia* for the Flora of Thailand account, we have found a species belong to genus *Alpinia* which is called Kha-Khom by local people in the northeast part of Thailand. After that we compared the morphological characteristics of this species with the original description of *Alpinia latilabris* Ridl. in J. Straits Branch Roy. Asiat. Soc. (1899, 32:

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168), and this species matched with *Alpinia latilabris*. This species is accepted, and its native range is Borneo, Malaya, Myanmar and Vietnam (Kew Science, 2021). Therefore, this species is a new record for Thailand. The local people used the young inflorescence, young pseudostem and young rhizomes of this species as vegetables. The microstructure, phytochemical (volatile compounds and organic acids), bioactive compounds (phenolic acids and flavonoid compounds) along with the antioxidant properties (DPPH radical scavenging activity: DPPH and Ferric Reducing Antioxidant Power: FRAP) have never been reported in Thailand. Even more, dried samples are used as material in food or traditional medicine, which have never been studied previously.

*Alpinia latilabris* Ridl. is a medicinal plant and species that is widely used. Both in food, herbs, spices, for aesthetics and active substances. The few reported chemical compounds in *A. latilabris* were 1,8-cineole and  $\beta$ -pinene (19.0%) as the most abundant components in the fruit oil (unripe and ripe) found in Malaysia (Ibrahim *et al.*, 2014). Most have been reported in galangal (*Alpinia galanga*), *A. conchigera*, *A. zerumbet* and *A. malaccensis* (Chumroenphat *et al.*, 2019; Zhou *et al.*, 2021). The main substances found in genus *Alpinia* were essential oils, which comprise most of the active ingredients at more than 70% (terpene alcohols, ketone esters, aldehydes and their derivatives) found in the rhizome, about 0.2-1.5% (dry weight). The most active ingredients in the rhizome essential oil were 1,8-Cineole (64.2%) followed by limonene 3.7%, which has antioxidant activity (DPPH and FRAP). Galangal is a yellow crystal spicy and pungent smelling agent, which is generally a component of essential oils. Many reports found phenolic compounds in many plants. Phenolic compounds collectively known as simple polyphenols are predominantly found in tubers or root crops. This substance performs a role as an antioxidant. Almost all parts are used (leaves, stems, flowers and rhizomes), especially the rhizomes that are widely used in both for food, medicine and cosmetics.

Each of the various drying procedures has its own set of benefits and disadvantages. Freeze drying (FD) is good for preserving quality, but it needs a long time for the drying period, high energy usage and expensive capital costs. Hot air drying (HD) is the most common method of drying. However, the low temperature for drying with HD takes a long time to remove moisture from the material. Instead, at high temperatures, there is a possibility of color, flavor and bioactive chemicals being degraded. Sun drying (SD) is a conventional method at a low temperature due to the long time required for drying the sample and may possibly result in product quality and bioactive ingredients (Prathapan *et al.*, 2009; Chumroenphat *et al.*, 2021). In this research, we reported different drying methods for *Alpinia*.

Consequently, we aimed to find the taxonomy, volatile compounds, organic acids, phenolic acids, flavonoids and antioxidants with DPPH and FRAP of *A. latilabris* and compared drying methods as potential usage for food and traditional medicine. This research will mainly provide useful information for a wider application of these plants. This study effort should provide a reliable framework for development studies on the usage as a material for functional foods and traditional medicines.

## Materials and Methods

All the chemicals for phenolic content, flavonoid content, vitamin c content and other high-purity solvents (AR and HPLC grade) were provided from Merck (Darmstadt, Germany). Standards for analysis with HPLC, including organic acid (oxalic acid, malic acid, citric acid, succinic acid, quinic acid and fumaric acid), flavonoids (myricetin, rutin, apigenin, quercetin and kaempferol) and phenolic acids (gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, genistic acid, vanillic acid, caffeic acid, syringic acid, vanillin, *p*-coumaric acid, cinnamic acid, ferulic acid and sinapinic acid) were purchased from Sigma–Aldrich Co. (St. Louis, MO., U.S.A.).

### *Taxonomy*

Specimens for research were collected from Sakon Nakhon Province. Voucher specimens were deposited at Mahasarakham University Herbarium and Khon Kaen University Herbarium (KKU). The morphological

characteristics of specimens were described using a stereo microscope. Identification and classification of this species was based on morphological data and available literature. Distribution data and ecological data were also provided. Utilization data of the Kha-Khom was obtained through interviewing local villagers who were living in Phu Phan District, Sakon Nakhon Province. The conservation status of this plant in Thailand was based on the evaluation criteria of the distribution area and author during a survey for collection of specimens.

*Plant material and sample preparation for drying*

The fresh samples were collected during April-May 2021 in the northeastern region of Thailand. The voucher specimen number was AZCT021 deposited in the herbarium. The rhizomes were cleaned of all physical contaminants (gravel and soil) and washed with water. Then samples were cut into slices before drying with different drying methods and compared between fresh and dried samples maintained at 4 °C. Rhizome drying: each 1000 g of *A. latilabris* sliced sample were dried with different drying test methods (freeze drying (FD), sun drying (SD) and hot air drying with 40, 60 and 80 °C (HD40, HD60 and HD80, respectively). The final dried samples must have a lower moisture content than 7% by each drying method. For FD, *A. latilabris* rhizome slices were freeze dried (Scanvac Cool Safe; 100-9 Pro, Labo Gene ApS, Denmark) and cooled to -100 °C and the vacuum was under 20 Pa absolute pressure. HD involved a hot-air oven dryer that was electric thermo-static drying (model FED 115, WTB Binder, Germany) for drying at 40, 60 and 80 °C. For SD, sample slices were revealed to the sun at 35-45 °C for 2-3 days. The samples were ground after each drying process and then sieved through a 60-mesh wire sieve. The samples were kept at -20 °C before analysis.

*Microstructure of fresh and dried Alpinia latilabris* Ridl.

The microstructure was observed by scanning electron microscope (SEM) (Hitachi, TM-4000plus, Japan) with the TM 4000plus program used to examine section slides of fresh and dried samples. For SEM analysis in vacuum mode, samples were coated with gold (Hitachi, MC1000, Japan).

*Identification of volatile compounds using gas chromatography/mass spectrometry (GCMS) with headspace solid-phase microextraction (HS-SPME)*

Extraction of rhizome volatile constituents used a HS-SPME coupled to GC/MS instrument with methods according to Chen *et al.* (2018) with some modified. The SPME fibre uses divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS/10, Agilent Technologies; Switzerland), which was prepared at 200 °C for 1 h. The finely powdered rhizome (1.0 g) was placed in a 10 mL bottle and equilibrated for 10 minutes at 80 °C. The samples were extracted for 40 min with an agitator (speed 250 rpm) and then the SPME fibre was inserted into the GC injector for desorption at 250 °C for 5 min.

The volatile components of the rhizome were measured using a GC/MS series QP2010 of Shimadzu (Tokyo, Japan). The GC/MS analysis used a fused silica capillary column (Rtx-5MS; Restek Co., Bellefonte, PA, USA) with 30 m x 0.25 mm id x 0.25 µm film thickness. The temperature of the injection port was 250 °C and transfer line at 280 °C, and the ion source was 230 °C. The oven temperature of the GC program followed the method of Chen *et al.* (2018) with some modification of initially 50 °C for 5 min, then to 100 °C (3 °C/min) hold for 5 min, then to 250 °C (5 °C/min) constant for 3 min and a final ramp to 280 °C (50 °C/min) hold time 5 min. The MS condition was operated in the full-scan range: m/z 40-550 amu with electron ionisation mode, 70 eV. The blank control was run without sample using the same procedure. The peaks were identified by comparing their mass fragmentation pattern to data available in the spectral with NIST14 libraries (NIST). The n-alkanes standard solution (C7-C23, Sigma-Aldrich) was analysed using the same procedure to obtain the retention index (RI). The relative content of each compound was displayed as a percent peak area.

#### *Total phenolic contents (TPC) and Total flavonoid contents (TFC)*

The TPC and TFC methods were done according to Chumroenphat *et al.* (2019). The reaction of the experiment was measured using a Varioskan Lux Multimode microplate reader (Thermo Fisher Scientific, USA) at 725 nm and results were expressed in gallic acid equivalents (mg GAE/g db) with dry basis. For TFC absorbance measured at 510 nm, the results were shown in rutin equivalents (mg RE/g db) with a dry basis.

#### *Vitamin C by HPLC*

The method of extraction and analysis for vitamin C followed Siriamornpun and Kaewseejan (2017). The identified used a Shimadzu LC-20AC series HPLC system (Shimadzu, Tokyo, Japan) with diode array detector at 280 nm, and the result was shown on a dry basis.

#### *Organic acid by HPLC*

The extraction of organic acid follows Moreno-Ortega *et al.* (2020). The identification method was determined previously and described by Zhang *et al.* (2022) with some modifications using a HPLC system including an anion exchange column, Aminex HPX-87H (300 mm × 7.8 mm, Bio-Rad, Richmond CA, USA), at 65 °C and detected with a photodiode array at 210 nm. The concentrations were calculated using calibration curves constructed previously with appropriate chromatographic standards. The result was expressed on a dry basis.

#### *Phenolic acids and flavonoid compounds by HPLC*

The extraction method for phenolic acid and flavonoid compounds follows Chumroenphat *et al.* (2021). Identification used HPLC apparatus (Shimadzu, Tokyo, Japan) and analysis conditions were according to Chumroenphat *et al.* (2019). The phenolic acids and flavonoids in the extracts were identified by comparing their relative retention times with external standards. The result was expressed on a dry basis.

#### *FTIR measurements*

The measured spectrum was applied by a FTIR instrument (Frontier) with a UATR accessory (Perkin Elmer, USA) and a Diamond/ KRS-5 crystal composite were used to get the FTIR spectra of *A. latilabris* samples (one bounce). The examination of *A. latilabris* powders was possible without any special preparation due to the direct use of UATR-FTIR spectra. Thirty-two scans with a spectral resolution of 4 cm<sup>-1</sup> were used to generate the spectral data, which covered the spectral range 4000-400 cm<sup>-1</sup>. A force gauge of 110 units was used to measure all *A. latilabris* powders. The software also recorded a background spectrum, which was automatically removed by the software

#### *Antioxidant activities*

##### DPPH free radical scavenging assay

The scavenging DPPH radicals of the extracts were studied using a previously published method by Chumroenphat *et al.* (2019). Results were expressed as mg Trolox equivalents (TE) per one gram on a dried basis (mg TE/g db).

##### Ferric reducing/antioxidant power assay (FRAP)

The procedures used for this assay were according to Chumroenphat *et al.* (2019), which were measured using a Varioskan Lux Multimode microplate reader (Thermo Fisher Scientific, USA) at 593 nm, and reported as mg FeSO<sub>4</sub> in 1 g on a dry basis (mg FeSO<sub>4</sub>/g db).

### *Statistical analysis*

All data were analysed using a statistical program. These were described as mean  $\pm$  one standard deviation (SD) of three replicates and data were analysed using a one-way analysis of variance (ANOVA). The significance was relative to the control.

## Results and Discussion

### *Taxonomy of *Alpinia latilabris* Ridl. - new records from Thailand*

Inst. Bot. Buitenzorg 20:81. 1904. - *Alpinia sericea* Ridl., J. Linn. Soc., Bot. 42:163. 1914. - *Languas hookeriana* (Valeton) Merr., Univ. Calif. Publ. Bot. 15: 34. 1929. - *Languas sericea* (Ridl.) Merr., Univ. Calif. Publ. Bot. 15:35. 1929. - *Catimbium latilabre* (Ridl.) Holttum, Gard. Bull. Singapore 13(1):153. 1950.

Herb, 2-3 m tall. Leaf sheaths pubescent near base of ligule and petiole; ligule ovate, ca 1.2 by 1 cm, apex acute, pubescent; blade lanceolate-oblong, 30-70 by 8-10 cm, base cuneate-attenuate, apex acuminate-caudate, cauda sometimes spiral, 1.8-2 cm long, margin pubescent, midrib or all of lower surface pubescent; petiole ca 2.5 cm long, pubescent. Inflorescence paniculate, semi-erect, 20-30 cm long; subtended by 2 long sheaths when young; peduncle 10-15 cm long, densely pubescent; rachis densely pubescent, bearing 25 or more cincinni; bracts absent; peduncle of cincinnus ca 1 cm long, densely pubescent with 2 flowers on cincinnus; bracteoles ca 2.5 by 2-2.5 cm, white, pink-tipped; pedicel ca 1.5 cm long, densely pubescent. Flower milky white; ovary ovoid, densely pubescent; style slender; stigma curved upwards, cup-shaped, white, ciliate; epigynous glands 2, flat, ca 5 mm long; calyx tubular, 2-2.2 by 0.5-0.8 cm, apex trilobed, split on one side, glabrous except apices of lobes; floral tube shorter than calyx, 1.8-2 cm long, corolla lobes milky white with pink apices, oblong, ca 2.5-3 by 1.5-2 cm, dorsal corolla lobe longer than lateral lobes, margin pubescent; lateral staminodes subulate, 2-8 by 2 mm; labellum yellow with purple-red stripes, broadly ovate and spatulate, 4-6 by 5-6 cm, apex distinctly trilobed; stamen: filament ca 20 by 5 mm, pink and slightly speckled; anther ca 12 by 4 mm, yellow; anther crest not prolonged at apex. Fruit globose, ca 2 cm in diam., orange to red when mature, slightly pubescent, ribbed, apex with persistent calyx; seeds angled, aril white.

Thailand. - north-eastern: Sakon Nakhon (coll. no. Saensouk, 2800).

Distribution. - Peninsular Malaysia (Pahang, type), Sabah (type of *Alpinia sericea*), Indonesia (Java, type of *Alpinia hookeriana*).

Ecology. - Dry evergreen forests, 50-500 m alt.

Phenology. - Flowering February - March and fruiting March - June.

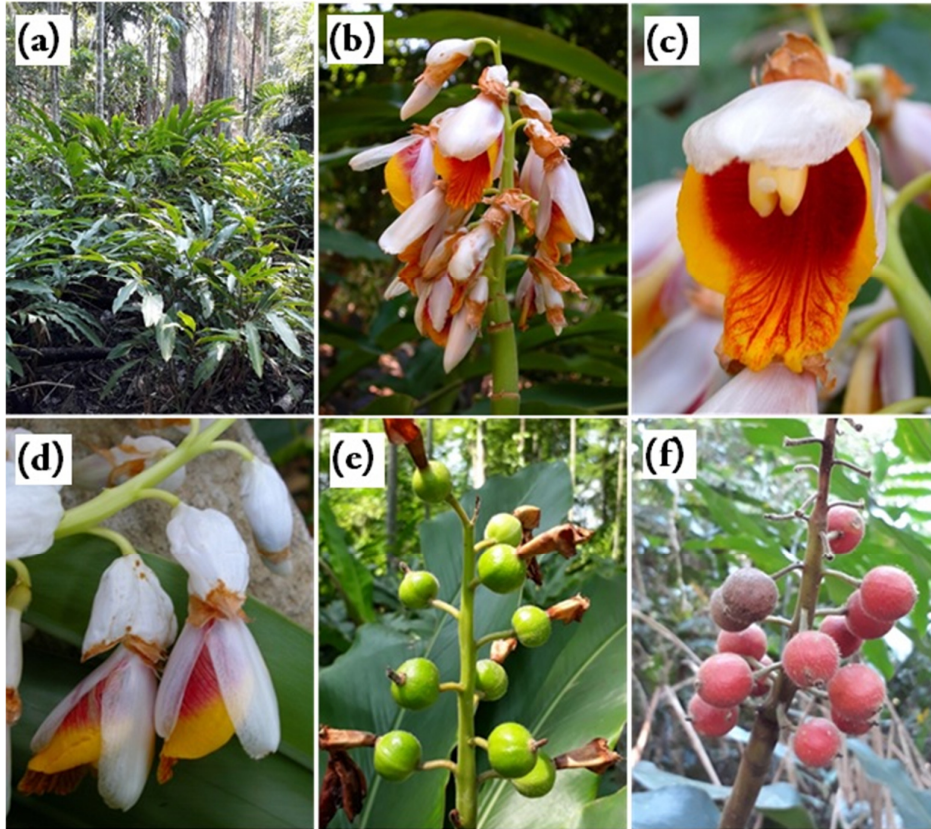
Conservation status: This species is reported in a few localities in Thailand. Therefore, it is reported to be a rare species.

Vernacular. - Kha-Khom.

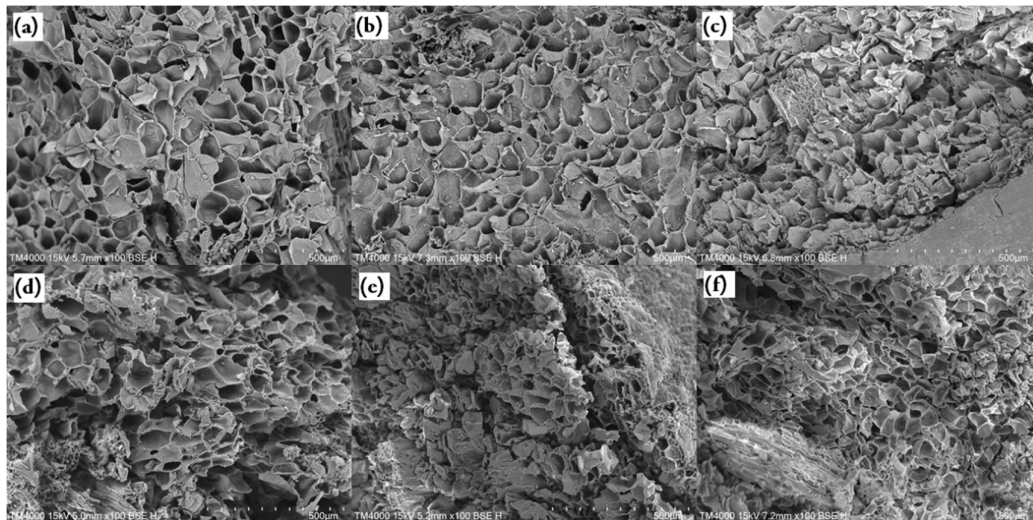
### *Change in microstructure of *Alpinia latilabris* Ridl. with different drying methods*

The microstructures of fresh and dried *A. latilabris* Ridl. were determined by scanning electron microscope (SEM) as shown in Figure 2. The results show that the microstructure of the freeze dried (FD), hot air dried at 40 °C (HD40) and 60 °C tissues were comparable to that of the fresh samples. This is because water is sublimated via the direct transfer of water from the frozen substance to vapour in the freeze-drying process, which maintains the shape of the microstructure (An *et al.*, 2016), also the hot air drying at 40 °C and 60 °C has a stable temperature and water transfer. Whereas the sun dried (SD) and 80 °C dried samples (HD80) had more damaged microstructure compared with FD, HD40 and HD60. The microstructure of the parenchyma cell was seriously affected, and starch grains were dispersed throughout the tissue, as shown in Figure 2. In addition, the SD and HD80 samples demonstrated different microstructures due to temperature and moisture gradients that produced cell-wall rupture, folding and deformation throughout the drying process. The SD has

a more damaged microstructure due to variations in sun-induced temperatures during the drying process (Deng and Zhao, 2008; Chumroenphat *et al.*, 2021).



**Figure 1.** Inflorescence, flowers and fruits of *Alpinia latilabris* Ridl., a new record from Thailand (a): Habitat; (b): Inflorescence; (c): Flower showing labellum; (d): lateral view of cinchus; (e): Young fruits (green); (f): Mature fruits (red)



**Figure 2.** Microstructure of fresh and dried *Alpinia latilabris* Ridl. in scanning electron micrographs after using different drying methods (fresh, FD, HD, SD;  $\times 200$ ): (a): fresh sample; (b): freeze drying; (c): sun drying; (d): hot air drying at 40 °C; (e): hot air drying at 60 °C; (f): HD80: hot air drying at 80 °C

*Effect of drying method on volatile compounds of Alpinia latilabris*

In the fresh and dried rhizomes of *A. latilabris*, numerous volatile compounds were found. Total compounds identified were 44 as presented in Table 1. The number of compounds found in *A. latilabris* rhizome samples, fresh and dried, ordered by SD, HD60, FD, HD40, HD80 and fresh was 36, 30, 29, 28, 24 and 14 compounds, respectively. The relatively high contents were 1,8-cineole, (-)- $\beta$ -pinene and  $\alpha$ -pinene. These data support previous studies of predominant volatile compounds found in *Alpinia* spices, which were 1,8-cineole by more than 60% (Rana *et al.*, 2010; Victório *et al.*, 2011; Raj *et al.*, 2013). However, Raj *et al.* (2013) reported that the major compounds of *A. calcarata* grown in India were 1,8-cineole,  $\beta$ -fenchyl acetate,  $\beta$ -pinene, camphene and  $\alpha$ -terpineol, and *A. malaccensis* had different compounds, which were  $\alpha$ -phellandrene,  $\beta$ -pinene and p-cymene. The main volatile component discrepancies can be attributable to the diverse origins of ginger, extraction processes and solvent types applied (Ding *et al.*, 2012). In the case of different drying methods, all drying methods decreased the levels of all 1,8-cineole contents, compared with the fresh sample. The loss of 1,8-cineole content was in the following decreasing order: HD40>HD60>SD>HD80>FD. However, FD was greater and maintained 1,8-cineole due to not using temperature in the drying process. Whereas, the thermal drying methods drastically decreased after hot-air dried in HD40 (72%), HD60 (71%), HD80 (43%) and sun-dried (66%). In the case of low temperature (HD40, HD60 and SD) there was a decreased content of 1,8-cineole, indicating that chemical component degradation, such as curcumin degradation to vanillin, vanillic acid by direct oxidative and/or through the action of an oxidizing enzyme such as polyphenoloxidase (PPO), causes more degraded smaller molecules (Chumroenphat *et al.*, 2021). Particularly in the case of SD, when UV and light are also implicated, the losses of volatile compounds in the HD80 sample were due to higher drying temperatures. In more detail, several volatiles in plant samples have a higher affinity for the water fraction and were lost along with the evaporating water during the drying process (Sellami *et al.*, 2011).

Moreover, the variability of the relative content in different drying methods may be explained by transformation of terpinotene, limonene,  $\alpha$ -terpinene,  $\gamma$ -terpinene,  $\alpha$ -terpineol and 1,8-cineole in the drying processes with reaction of ring expansion and ring opening of pinenes and their metabolic pathways (Narushima *et al.*, 1982). Comparable findings were reported by Mohammed *et al.* 2020, who found chemical transformations occurring during the drying processes in rosemary (*Rosmarinus Officinalis* L.) resulted in a significant decrease in major components (1,8-cineole and camphor). Whereas, other constituents were either at par or at increased levels due to the chemical content depending on the drying periods and other factors like temperature, mode of heating, condensation apparatus, cooling gradient, duration of extraction and the presence of water in the extraction assembly, which were kept constant. Finally, different drying methods resulted in varying volatile components. FD, on the other hand, considerably preserved the volatile compounds, whereas drying with heat resulted in volatile compound losses.

**Table 1.** Main volatile compounds in *Alpinia latilabris* with different drying methods

No	Constituent <sup>a</sup>	Ident. RI <sup>b</sup>	Ref. RI	Ref.	Relative content (%)					
					Fresh	FD	SD	HD40	HD60	HD80
1	Acetic acid	679	-	-	-	-	1.11	-	-	1.5
2	2-methylbutanal	682	-	-	-	-	3.82	3.32	5.19	4.83
3	Hydroxyacetone	689	-	-	-	-	5.27	7.95	14.31	7.85
4	2,3-Pentanedione	704	-	-	-	-	2.45	2.5	4.67	2.41
5	Hexanal	805	860	d	-	-	1.81	-	-	-
6	$\alpha$ -Thujene	927	924	e	0.23	0.21	0.52	0.41	-	0.42
7	$\alpha$ -Pinene	933	939	c	8.6	7.65	9.3	13.5	13.4	8.39
8	Camphene	948	954	c	2.24	2.37	3.54	3.46	2.16	2.76
9	$\beta$ -Thujene	954	-	-	-	0.24	0.33	0.2	-	0.27
10	(-)- $\beta$ -Pinene	976	976	c	18.27	15.91	16.36	24.91	25.02	17.31
11	$\alpha$ -Phellandrene	1005	1002	e	-	-	0.41	0.21	-	-
12	3-Carene	1010	-	-	-	-	0.19	-	0.32	-

No	Constituent <sup>a</sup>	Ident. RI <sup>b</sup>	Ref. RI	Ref.	Relative content (%)					
					Fresh	FD	SD	HD40	HD60	HD80
13	terpinolene	1017	1088	c	-	-	-	-	0.17	-
14	(+)-4-Carene	1017	-	-	-	0.3	1.68	0.67	-	0.48
15	o-Cymene	1026	1023	d	-	0.49	2.08	-	1.15	0.86
16	1,8-Cineole	1032	1031	c	58.53	52.07	19.87	16.18	16.62	33.18
17	$\gamma$ -Terpinene	1061	1059	c	0.11	0.31	1.37	0.9	-	-
18	$\alpha$ -Terpineol	1075	1188	c	-	-	0.45	-	0.51	-
19	L-Fenchone	1090	1114	e	1.14	0.99	2.41	2.22	1.95	1.78
20	M-Cymene	1096	1020	e	-	-	1.35	-	-	-
21	Fenchol	1115	1117	c	0.56	0.41	1.07	0.62	0.34	0.45
22	Pinocarveol	1140	-	-	0.17	1.03	1.41	1.12	0.76	0.89
23	(+)-2-Bornanone	1146	-	-	0.37	0.56	0.82	0.83	0.47	0.66
24	Pinocarvone	1165	-	-	-	0.52	0.59	0.48	0.35	-
25	endo-Borneol	1169	1119	c	0.47	0.94	0.99	0.73	0.51	0.57
26	(1R)-(-)-Myrtenal	1201	-	-	-	1.61	0.81	-	0.73	-
27	Bornyl acetate	1222	1285	c	4.16	7.33	12.52	10.5	4.48	10.23
28	exo-2-hydroxycineole	1230	-	-	0.33	-	0.09	-	-	-
29	Fenchyl acetate	1237	1220	c	-	-	0.32	0.6	0.31	-
30	Isobornyl acetate	1289	1287	e	-	-	0.25	-	-	-
31	Copaene	1380	1272	c	-	-	-	0.27	0.36	-
32	Caryophyllene	1425	1425	c	-	0.37	0.64	1.51	1.08	0.67
33	$\gamma$ -Muurolene	1482	1497	c	-	-	-	0	0.16	-
34	Sclarene	1497	-	-	-	0.21	0.44	0.32	0.26	0.37
35	$\beta$ -copaene	1520	-	-	-	0.16	-	0.5	0.26	-
36	Tau-Cadinol acetate	1530	-	-	-	-	-	0.32	-	-
37	2(1H)-Naphthalenone, octahydro-4a,7,7-trimethyl-, cis-	1536	-	-	-	0.32	0.55	0.35	0.29	0.41
38	9,11-Dimethyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane	1610	-	-	-	0.17	0.35	0.2	-	-
39	solongifolol	1636	-	-	-	0.15	-	-	-	-
40	But-3-enal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexenyl)-	1642	-	-	-	0.55	0.71	0.63	0.42	0.53
41	Neoisolongifolene, 8-bromo-	1649	-	-	-	0.58	0.66	-	0.61	0.61
42	Longifolenaldehyde	1685	-	-	-	0.08	-	-	0.44	-
43	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	1757	-	-	-	0.27	-	-	-	-
44	Ambrial	1815	-	-	0.39	0.71	2.7	-	1.97	2.2

- : Not detected; FD: Freeze dried; HD: Hot air dried; SD: Sun dried.

a Compounds listed in order of elution from capillary column.

b Retention indices (RI) relative to n-alkanes (C7-C30) on elite-5MS capillary column.

c Retention indices from Rana *et al.* (2010)

d Retention indices from Victório *et al.* (2011)

e Retention indices from Raj *et al.* (2013)

#### *Effect of drying method on total phenolic content (TPC) and total flavonoid content (TFC)*

TPC, TFC and vitamin c contents were determined as indicative bioactive compounds found in samples. The effect of drying method on the TPC, TFC and vitamin c of *A. latilabris* extracts are shown in Table 2. Comparing all samples, the concentration of TPC decreased in all dried samples ( $p < 0.05$ ). The highest TPC content was found in the fresh sample followed by FD, SD, HD40, HD60 and HD80, respectively. According to Chumroenphat *et al.* (2021), TPC in fresh samples was decreased by freeze-drying, sun-drying and hot air-drying. In terms of TFC, the fresh sample had the highest contents compared with all drying methods. The lowest was found in hot drying methods (HD40, HD60 and HD80) with no significant

differences ( $p < 0.05$ ). Similar for vitamin c content, the highest level was found in fresh, while other drying methods significantly decreased it ( $p < 0.05$ ). Vitamin c was decreased most by SD (88%), followed by HD80 (81%), HD40/HD60 (79%) and FD (76%). This supports the findings of a previous study, reporting that FD preserved more TPC, TFC and vitamin c contents than other drying methods (Chumroenphat *et al.*, 2021). These findings may be explained by decreases of TPC, TFC and vitamin c due to polyphenol oxidase (PPO) activity at low temperature. Sun-drying was apparently unable to inhibit PPO activity as much, as the enzyme was activated at the temperature of sun drying (35-40 °C). While heat drying at HD40 was a low temperature for PPO activity combined with oxygen that is overflowing in hot air oven which is too low for TPC, TFC and vitamin c. In addition, the high temperature 60 and 80 °C had degradation of the polyphenol in the sample, leading to a lower level of TPC, TFC and vitamin c. On the other hand, FD had the highest level compared with the other drying methods due to the low temperature used for freeze drying, polyphenol oxidase was inhibited, supporting previous studies (An *et al.*, 2016; Lakshmi *et al.*, 2018; Chumronephat *et al.*, 2021). The findings of this study should be valuable in understanding the *A. latilabris* rhizome drying process, which will help to preserve food quality and bioactivity by avoiding phytochemical degradation.

**Table 2.** Total phenolics, total flavonoid content and vitamin c content in *A. latilabris* with drying methods

Sample name	TPC (mg GEA /g db)	TFC (mg RE/ g db)	Vitamin c (mg /100 g db)
Fresh	51.56±1.27 a	8.80±0.26 a	242.30±1.88 a
FD	18.64±0.65 b	4.91±0.12 b	56.29±0.92 b
SD	18.52±0.39 b	3.57±0.16 c	28.70±0.32 e
HD40	14.83±1.21 c	1.77±0.07 d	49.30±0.81 c
HD60	14.83±1.20 c	1.13±0.02 d	49.10±0.61 c
HD80	9.86±0.55 d	1.74±0.10 d	46.02±0.54 d

TPC: Total phenolic content; TFC: Total flavonoid content FD: Freeze dried; SD: Sun dried. HD: Hot air dried. Values are display as mean ± SD of triplicate measurements (n = 3). Means with different letters (a, b, c, d) are significantly different at  $p < 0.05$  within the same column.

#### *Effects of drying method on phenolic acids and flavonoid compounds of Alpinia latilabris*

The identification of the phenolic compounds was by comparing their retention times with individual standards, as shown in Table 3. For the dried sample, FD drying had the highest total phenolic acids (553 µg/g db) (losses 51% compared to fresh sample) followed by SD (280 µg/g db), HD80 (257 µg/g db), HD40 (248 µg/g db) and HD60 (206 µg/g db). The highest individual phenolic acids in *A. latilabris* were caffeic acid (fresh), vanillin (FD, HD40 and HD80) and gentisic acid (SD and HD60). In these studies, vanillin was at highest levels in dried samples caused by the metabolic pathways that reduced ferulic acid production to vanillic acid, while vanillic acid can be reduced to vanillin, causing an increase in the level of vanillin. According to a previous study of dried turmeric (Zingiberaceae family), at low temperatures from both HD samples (50 °C) and SD sample (35-40 °C), vanillin levels increased, whereas curcumin levels declined dramatically, particularly when UV and light were present (Suresh *et al.*, 2009; Agnihotri and Mishra, 2011; Chumroenphat *et al.*, 2021) as shown in the pathway supported by Gallage and Møller (2015). Elzaawely *et al.* (2007) found that vanillin and cinnamic acid were significantly higher in chloroform and ethyl acetate extracts of *A. zerumbet* following exposure to copper. It was observed that other phenolic acids were found to be significantly less sensitive to low temperatures when FD and HD40 were used, but highly sensitive when SD was used. Thermal dried samples (HD60 and HD80), similar to a previous report, may not be the major cause of these effects; other factors, such as polyphenol oxidases (PPO), may also play a role in the degradation of phenolic acids during the preparation process prior to drying (Prathapan *et al.*, 2009). Our results suggest that the fresh sample of *A. latilabris* mostly had total phenolic acid rather than the whole sample with drying methods. As a conclusion, we suggest that

depending on the type of compound regarded as the most important, each drying method may be appropriate for different products.

The identified flavonoid compounds in *A. latilabris* with different drying methods are shown in Table 3. Four flavonoids, namely rutin, catechin, quercetin and apigenin, were identified and quantified by HPLC. The overall and individual quantities of flavonoid compounds differed significantly. In general, catechin and apigenin were found to be the most predominant flavonoid compounds in all *A. latilabris* samples studied. In addition, we found significant differences in total and individual contents of flavonoids, comparing jujube varieties and various tissues. When comparing the amount of total and individual contents of flavonoids with different drying methods, it was found that the fresh sample had the highest level of flavonoids. While, FD and SD had higher levels of total flavonoids than HD40, HD60 and HD80, respectively.

**Table 3.** Contents of phenolic acid and flavonoid compound analyses in *Alpinia latilabris* with different drying methods

Parameter	Drying method					
	Fresh	FD	SD	HD40	HD60	HD80
<i>Phenolic acid content (mg/100g db)</i>						
Gallic acid	107.85±0.78 d	20.61±0.04 g	19.15±0.12 e	27.47±0.24 d	20.66±0.20 e	22.47±0.50 e
Protocatechuic acid	18.86±0.31 j	5.42±0.38 i	3.95±0.13 k	4.12±0.02 i	3.72±0.03 j	4.06±0.07j
<i>p</i> -hydroxybenzoic acid	36.01±0.73 h	42.98±0.97 e	17.54±0.42 f	4.62±0.10 i	10.32±0.10 h	9.89±0.08 h
Gentisic acid	120.04±0.79 c	65.08±0.99 c	61.51±1.01 a	35.24±0.61 c	41.45±0.19 a	54.19±0.50 b
Vanillic acid	8.81±0.16 k	3.40±0.17 j	12.63±0.11h	6.33±0.20 h	8.67±0.23i	10.23±0.32 h
Caffeic acid	454.88±5.18 a	49.63±1.43 d	36.66±1.80d	21.51±0.44 e	26.32±0.43 d	28.44±1.30 d
Syringic acid	0.74±0.01 l	0.87±0.07 k	2.09±0.02 j	0.95±0.04 j	1.37±0.04 k	1.66±0.06 k
Vanillin	206.85±1.06 b	238.15±1.29 a	47.41±0.35 b	56.60±0.65 a	28.65±0.49 c	60.57±0.67 a
<i>p</i> -coumaric acid	57.89±0.14 e	22.51±0.19 f	8.95±0.28i	10.01±0.19 g	8.97±0.03i	8.57±0.08i
Ferulic acid	55.22±0.20 f	11.85±0.30 h	11.62±0.63 h	13.28±0.17 f	12.49±0.05 g	11.14±0.30 g
Cinnamic acid	54.32±0.28 g	73.06±0.68 b	43.61±0.88 c	48.32±0.71 b	30.07±0.73 b	34.44±0.36 c
Sinapinic acid	21.70±0.81 i	19.58±0.73 g	15.11±0.44 g	20.12±0.53 e	14.26±1.33 f	12.29±0.29 f
<b>Total phenolic acids</b>	1143.17±10.45 a	553.14±7.24 b	280.23±6.90 c	248.57±3.90 e	206.95±3.85 f	257.95±4.53 d
<i>Flavonoid content (mg/100g db)</i>						
Rutin	4.34±0.16 d	10.69±0.14 d	1.99±0.02 d	2.50±0.07 d	2.39±0.08 d	2.06±0.04 d
Catechin	856.62±12.00 a	572.24±10.27 a	740.53±3.38 a	619.84±8.70 a	554.69±1.85 a	565.25±4.17 a
Quercetin	61.21±2.42 c	146.60±2.34 b	34.91±0.37 c	29.75±1.45 b	25.93±0.71 c	26.96±0.47 c
Apigenin	124.27±5.18 b	117.15±0.31 c	71.93±0.68 b	18.60±0.59 c	55.70±1.36 b	57.17±0.87 b
<b>Total flavonoid compounds</b>	1046.44±4.94 a	846.68±2.61 c	849.36±1.11 b	670.69±2.70 d	638.71±1.00 f	651.44±1.39 e

Means with different letters (a, b, c, d) are significantly different at  $p < 0.05$  within the same column with drying method. While total phenolic acid and total flavonoid compounds compared within the same row ( $p < 0.05$ ). Values are expressed as mean  $\pm$  SD of triplicate measurements ( $n = 3$ ). ND: Not detected; FD: Freeze dried; HD: Hot air dried; SD: Sun dried

#### *Effects of drying method on organic acids of A. latilabris with HPLC*

Organic acids have a variety of beneficial effects on human health and metabolism, including fumaric acid that has effectiveness in the prevention of cardiovascular disease, citric acid that has potential for diabetic protection and malic acid that has potential as an antimicrobial agent against a variety of harmful microorganisms (Gao *et al.*, 2012; Delgado *et al.*, 2018). Several studies have also found that the main organic acid in fruits, and that fruits contain a lot of organic acid. This study describes the first report of organic acids in *A. latilabris*. The result shows that we have found total organic acid contents for all samples with different drying methods for *A. latilabris* in the following order – HD80 > SD > HD60 > Fresh > FD > HD40, as shown

in Table 4. Oxalic acid was found to be the main organic acid in all dried samples, which ranged from 13 to 50 mg/g db. However, contents of citric acid (0.3-1.7 mg/g db), malic acid (0.6-15.0 mg/g db), quinic acid (1.3-8.6 mg/g db), succinic acid (1.3-3.6 mg/g db) and fumaric acid (0.1-0.6 mg/g db) were far lower than oxalic acid in our study. In the case of an increase in the amount of all organic acids (oxalic acid, citric acid, malic acid, quinic acid, succinic acid and fumaric acid) due to different drying methods, the dehydration, decarboxylation and Maillard reactions may be responsible for the increase in most of the organic acids. On the other hand, a thermal treatment for the fruit resulted in a decrease in the organic acids (Barretto *et al.*, 2013; Sarkar *et al.*, 2020).

**Table 4.** Contents of organic acid analyses in *Alpinia latilabris* with different drying methods

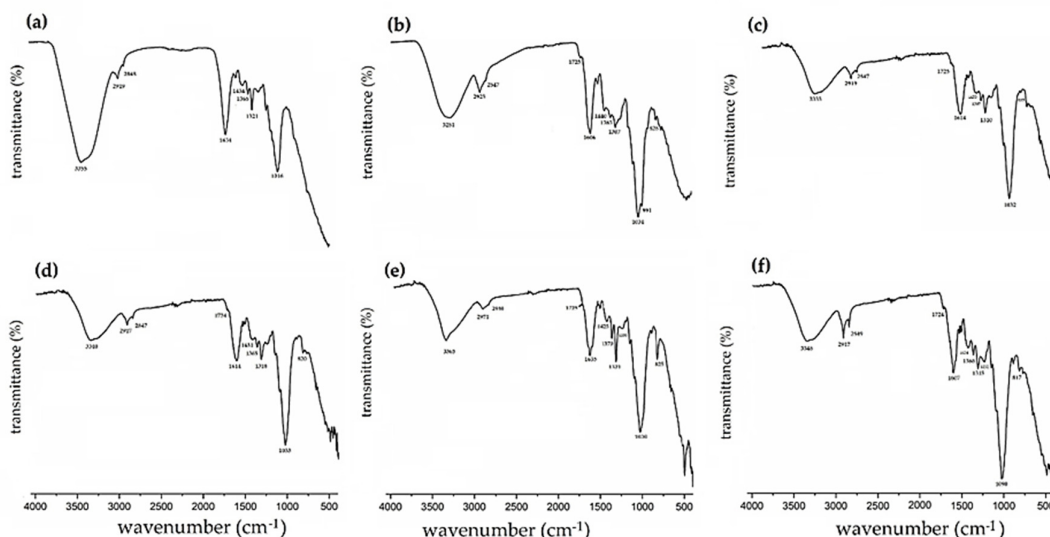
Individual organic acids	Organic acid contents (mg/g db)					
	Fresh	FD	SD	HD40	HD60	HD80
Oxalic acid	38.41±0.69 c	17.60±0.27 e	45.03±0.19 b	13.02±0.86 f	29.65±1.44 d	50.00±0.22 a
Citric acid	ND	0.34±0.01 e	1.00±0.01 c	0.52±0.02 d	1.50±0.01 b	1.72±0.02 a
Malic acid	0.96±0.02 d	0.60±0.01 f	8.12±0.04 b	0.64±0.01 e	7.44±0.04 c	15.03±0.14 a
Quinic acid	2.93±0.05 b	2.12±0.02 c	ND	1.33±0.02 d	8.62±0.05 a	ND
Succinic acid	ND	3.60±0.10 a	1.36±0.03 d	1.07±0.02 e	1.57±0.04 c	1.83±0.02 b
Fumaric acid	ND	0.06±0.01e	0.39±0.01 b	0.25±0.01c	0.09±0.01d	0.57±0.01a
<b>Total</b>	42.30±0.25 d	24.32±0.07e	55.90±0.06 b	16.83±0.16 f	48.87±0.27 c	69.15±0.32 a

Means with different letters (a, b, c, d) are significantly different at  $p < 0.05$  within the same row. Values are expressed as mean  $\pm$  SD of triplicate measurements ( $n = 3$ ). ND: Not detected; FD: Freeze dried; HD: Hot air dried; SD: Sun dried

#### *Relationship of chemical compounds degradation by FTIR analysis*

Figure 3 shows FTIR spectra of *A. latilabris* from different drying methods. Each peak comes from the absorption of functional groups in *A. latilabris* with different drying methods. Several previous studies reported the main compounds found in rhizome of *Alpinia galnaga* (Mallavarapu *et al.*, 2002) and other species in the genus *Alpinia*, for example, *Alpinia calcarata* K. Schum (Paldia *et al.*, 2010), *Alpinia zerumbet* (Murakami *et al.*, 2009), *Alpinia* species K. Schum (Indrayan *et al.*, 2011) and *A. officinarum* Hance (Rana *et al.*, 2010; Wu *et al.*, 2012). Nevertheless, the volatile compounds found in *Alpinia* sp. contained camphor, pinene and thujone as well. The spectrum of signature peaks for 1,8-cineole were 1016-1080  $\text{cm}^{-1}$  (C-O stretching vibrations), 1164-1042  $\text{cm}^{-1}$  (C-O-C) and 2854-2968  $\text{cm}^{-1}$  (C-H stretching vibrations) (Ciko *et al.*, 2016; Rashed *et al.*, 2019; Yin *et al.*, 2021).  $\beta$ -pinene was 817 - 828  $\text{cm}^{-1}$ , Camphor,  $\alpha$ - and  $\beta$ -thujone were both present, resulting in a distinctive stretch vibration for the C=O bond at around 1740  $\text{cm}^{-1}$ , present in all samples. Interestingly, the variability in the relative content from different drying methods transformed the chemical constituents in the samples as described by a previous study (Mohammed *et al.* 2020). The deformation of 1,8-cineole was present in the spectral peaks present at 1016-1080  $\text{cm}^{-1}$  and 2854-2968  $\text{cm}^{-1}$ , which are at different intensities. In the comparison with fresh and dried samples, we found that the relative contents of 1,8-cineole decreased as shown in Table 1. While  $\beta$ ,  $\alpha$ -Pinene and camphene increased significantly in the dry compared to the fresh sample. This supports the previous study of Shen *et al.* (2020) who reported the amount of released  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, camphene and limonene increased after samples were dried. The spectra shown in the band as 817 -828  $\text{cm}^{-1}$  had significantly increased intensity in the HD60 samples.

For the relationship of phenolic compounds, as previously reported (Staurt, 2004; Chumroenphat *et al.*, 2021), ferulic acid can be degraded into smaller molecules with different functional groups, such as vanillic acid and vanillin, as shown in Figure 3 with FTIR spectra of aldehyde group (C-O stretching) peaks at 1724  $\text{cm}^{-1}$  and 1725  $\text{cm}^{-1}$ , which could indicate that vanillic acid and vanillin were the predominant compounds found in fresh and dried samples, as shown in Table 3.



**Figure 3.** FTIR spectra of fresh and dried *A. latilabris*. (a): fresh, (b): FD, (c) SD, (d): HD40, (e): HD60, (f): HD80

#### *Effects of drying method on Antioxidant Activity of Alpinia latilabris*

The results of antioxidant capacity with different drying methods are shown in Table 5. The antioxidant activities were to determine DPPH radical scavenging activity shown by trolox equivalents on concentration (TE) and FRAP value as mg FeSO<sub>4</sub>/ g db. DPPH is a free-radical molecule. The free-radical scavenging ability has long been measured using the color of DPPH. Antioxidants produce a color change from purple to yellow and a decrease in absorbance at 517 nm when they interact with the DPPH radical measured using a microplate reader. There were significant decreases, compared with fresh samples. DPPH radical-scavenging activity was found to be highest in the fresh sample (33 mg TE /g db) and lowest found in HD80 (0.93 mg TE/g db). The DPPH radical-scavenging activity of the HD80 sample was 33 times lower than the fresh sample. Moreover, all samples that were dried lost activity for DPPH radical-scavenging due to the temperature of the SD and HD40, which was 35-40 °C; this is the temperature at which PPO will be stable while performing at maximum capacity (Prathapan *et al.*, 2009). While, the temperatures of HD60 and HD80 degraded the bioactive compounds and gave color losses (Summen and Erge, 2014). This study supports the previous report that found that FD preserved more bioactive compounds than other drying methods (Chen *et al.*, 2020). Additionally, to analyze the anti-oxidation capabilities, the FRAP assay was used to evaluate the reducing properties of compounds that function by breaking the free-radical chain by donating a hydrogen atom (Benzie and Strain, 1996). Results shown in Table 5 have similar values for DPPH antioxidant activity to the highest FRAP value found in the fresh sample (24 mg FeSO<sub>4</sub>/ g db) and lowest found in HD80 (7 mg FeSO<sub>4</sub>/ g db). When compared to the dried samples, it was found that FD (16 mg FeSO<sub>4</sub>/g db) had a high FRAP value and losses in the FRAP value of 33% from the fresh sample were caused by PPO activity, which occurs during preparation prior to drying and is inhibited in the freeze-dried process due to the low temperature. These results followed a similar trend as TPC and TFC. Based on the findings of previous research, the relationship between bioactive content (TPC and TFC) and antioxidant activity (DPPH and FRAP activity) has a strong positive correlation (Wan-Ibrahim *et al.*, 2010; Chumroenphat *et al.*, 2019; Kainama *et al.*, 2020).

**Table 5.** Antioxidant activities measured by means of DPPH radical scavenging and FRAP analyses with drying method

Drying method	DPPH (mg TE /g db)	FRAP (mg FeSO <sub>4</sub> / g db)
Fresh	33.66±0.35 a	24.41±1.30 a
FD	6.40±0.12 b	16.34±1.09 b
SD	2.38±0.08 c	10.80±0.12 c
HD40	2.18±0.06 c	9.97±0.56 c
HD60	2.18±0.09 c	9.20±0.56 c
HD80	0.93±0.01 d	7.47±0.41 d

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters (a, b, c, d) are significantly different at p < 0.05 within the same column. FRAP: Ferric reducing antioxidant activities; DPPH radical scavenging activities; FD: Freeze dried; SD: Sun dried. HD: Hot air dried

## Conclusions

In conclusion, *Alpinia latilabris* Ridl. (local name: Kha-Khom from Sakon Nakhon Province, northeastern Thailand) was reported as a new record for Thailand. The local people used this species for food and in traditional medicine due to several phytochemical and bioactive compounds and biologic activities. Our present study on the effects of different drying methods differed in the conditions that affect the microstructure, volatile compounds, bioactives and antioxidant activities. Freeze-dried samples had a microstructure that was similar to that of the fresh sample. Furthermore, when compared to all other samples dried by different methods, the freeze-dried sample had a higher amount of bioactive compounds and antioxidant activity. 1,8-cineole was the predominant volatile compound found in *Alpinia* spices. Thermal drying can lead to deformation and decreased the 1,8-cineole relative contents. On the other hand, (-)- $\beta$ -pinene and  $\alpha$ -pinene were increased with thermal drying, as confirmed by FTIR spectra. All these bioactive chemicals are sensitive to thermal drying and sunlight (the combination of UV, visible light and air being especially damaging). This justifies freeze drying providing the highest amounts of TPC, TFC and vitamin C. The organic acids were increased by drying samples. Alternatively, there were losses in the phenolic compounds in dried compared to fresh samples. The predominant phenolic acid and flavonoid were vanillin and catechin, respectively.

Each drying method could be appropriate for certain products depending on the targeted compounds, such as volatiles, phenolics, flavonoids or organic acids. This study should provide useful information about the design of the drying method for producing *A. latilabris* Ridl rhizome dried material, thus preserving the food quality, great bioactivity and high antioxidants by avoiding the degradation of the phytochemicals.

## Authors' Contributions

The study was conceptualized and designed by TC and SS. TC: designed the research study and/or evaluation. TC and SS drafted the manuscript and reviewed it before submitting it to the journal. Both authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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