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ORIGINAL PAPER

Phytochemical composition and biological activity of native Australian ginger (*Alpinia caerulea*)

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Abstract
The edible, endemic Australian species *Alpinia caerulea* belongs to the same family as ginger and turmeric. The rhizome and fruit have a mild ginger-like flavour, but there is very little information on its chemical composition or potential biological activities. This study found low levels of ascorbic acid in the fruit and fruit capsule (2.3–3.4 mg/100 g fresh weight), as well as detecting six polyphenols (rutin, quercetin-3-glucoside, quercetin, vanillic acid, kaempferol and chlorogenic acid) across all of the plant parts. The volatile profile of the rhizome was also explored for the first time. The volatiles were dominated (91.7%) by (*E*)-8(17),12-labdadiene-15,16-dial, but included 20 other minor constituents; mainly monoterpenes and sesquiterpenes. Extracts showed no inhibitory activity against tyrosinase or cyclooxygenase-2 (COX-2), but moderate anti-acetylcholinesterase was found for the rhizome and stem extracts. Further investigation into *A. caerulea* and other species from this genus will help to elucidate their full nutritional and bioactive potential.

Keywords *Alpinia caerulea* · Rhizome · Acetylcholinesterase inhibition · Volatile profile

Introduction

The Zingiberaceae family contains over 1600 species [1], including the commercially important species ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*); both of which are known for their pungent-like flavours. *Alpinia* is the largest genus in the Zingiberaceae family, comprising approximately 230 species found across the Asia–Pacific region—with many of these species known for their bioactive compounds and traditional pharmaceutical applications [2]. Studies on various *Alpinia* species have reported high levels of 1′-acetoxychavicol acetate (galangal acetate) and hydroxychavicol acetate [3, 4], the flavonoid galangin, and a number of as well as common polyphenols including gallic acid and ellagic acid [5, 6]. However, the chemical composition and potential uses of many other *Alpinia* species remains unexplored.

Three *Alpinia* species are native to Australia: *A. caerulea* (found along most of the eastern coastline), *A. arundelliana* (found in south-eastern Queensland and north-eastern New South Wales) and *A. modesta* (found only in far north-eastern Queensland) [7–9]. The rhizome of all three species reportedly have a ginger-like pungent flavour [10]; however, the principal pungent component(s) has not been identified. It may bear structural similarities to gingerols, the class of compounds which provide the strong pungent flavour of commercial ginger. On the other hand, galangal acetate and dihydrogalangal acetate were found as the main pungent compounds of the Asian species *A. galangal* [3]. Nevertheless, almost all aspects of the chemical composition or bioactivity of Australian *Alpinia* remain virtually unknown.

The best-known of the Australian species is *Alpinia caerulea* (native Australian ginger), which is a large perennial herb reaching 2–3 m in height [11]. Both the rhizomes and the small (1 cm diameter) blue fruit have a mild ginger flavour [7]. Indigenous Australians from northern Queensland ate the crisp white pulp surrounding the seeds to moisten the mouth, and baked snake or fish meat in the leaves to impart

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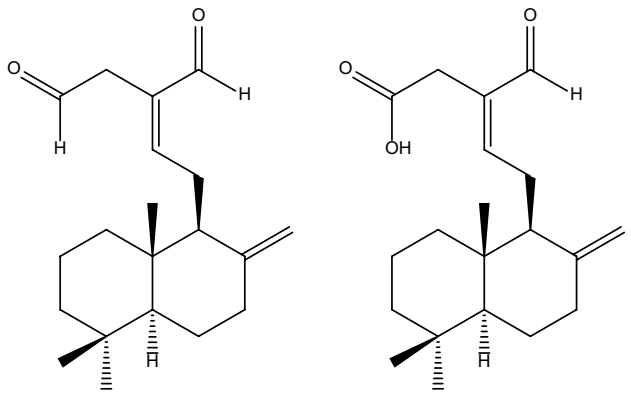
a ginger-like flavour [12]. Similarly, early European settlers are reported to have used this species as a ginger replacement in cooking [13]. More recently, the species has been widely used as a landscaping plant and has been anecdotally proposed as a potential 'bushfood' crop [14]. Exploring potential ginger substitutes is particularly important in light of emerging pathogens of *Z. officinale* such as bacterial wilt (*Ralstonia pseudosolanacearum*), which currently has no successful treatment options [15].

However, despite the advantageous characteristics of *A. caerulea* and its historical importance, there have been very few studies investigating the chemical composition or biological activity of this species. In one previous study, *A. caerulea* fruit was found to contain minimal fat (0–5% by weight) and low levels of protein (1–4%), but higher levels of carbohydrates (58%) [16]. In another study, He et al., [17] isolated and characterised the diterpenoids (*E*)-8(17),12-labdadiene-15,16-dial and zerumin A (Fig. 1) from *A. caerulea* fruit, rhizomes and leaves. These authors also showed that *A. caerulea* fruits showed potent anti-angiogenic properties (i.e., they prevented new blood vessels from forming), which could be beneficial for tumour prevention. The only other study on this species found that the methanol extracts of *A. caerulea* leaves showed moderate antimicrobial activity, including inhibiting the growth of *Serratia marcescens*, *Proteus mirabilis* and *Klebsiella pneumoniae* [13]. More information on the composition and bioactive properties of *A. caerulea* could support its potential uptake as a ginger-replacement crop, particularly for use in boutique or niche applications.

This study expands on the results of a brief preliminary phytochemical investigation presented by Johnson [18], to explore the phytochemical constituents, vitamin C content, volatile compounds and polyphenols found in *A. caerulea*. A second aim was to investigate the potential bioactivity of this species using three different enzyme inhibition assays: acetylcholinesterase inhibition, tyrosinase (polyphenol oxidase) inhibition, and cyclooxygenase-2 (COX-2) inhibition. This could indicate whether the species has potential anti-Alzheimer activity, skin-whitening or anti-browning activity, and anti-inflammatory activity, respectively.

These assays were selected based on reported bioactivities in other Zingiberaceae species [19, 20], including anti-Alzheimer activity in *Z. officinale* [21–23] and *Alpinia oxyphylla* [24, 25], tyrosinase inhibition in *Alpinia zerumbet* and *Etlingera* species [26, 27], and anti-inflammatory activity in *Z. officinale* [28, 29] and numerous *Alpinia* species [30–34].



8(17),12-Labdadiene-15,16-dial Zerumin A

Fig. 1 The structures of (E)-8(17),12-labdadiene-15,16-dial and zerumin A

Materials and methods

Sample collection and preparation

Samples of *Alpinia caerulea* were collected from Rockhampton, Central Queensland during early autumn (16th March 2022) when the fruit were at a mature stage. Mature plants (> 1 m height; likely several years of age) were sampled. The species identity was confirmed using the online edition of the *Flora of Australia* (www.ausflora.org.au; accessed 17th Mar 2022). It should be noted that *A. caerulea* is classified as "least concern" by the Queensland Government.

Following collection, the sample was rinsed in distilled water, patted dry, and separated into various parts (rhizome, leaves, stems, fruit and fruit capsule; Fig. 2). Following extraction and analysis for ascorbic acid, the remaining portion of each sample was frozen at − 80 °C and freeze-dried (− 50 °C; 35 mT). Moisture contents were determined from the mass loss upon freeze-drying.

The freeze-dried samples were finely ground (Breville Coffee & Spice Grinder; Botany, NSW, Australia) for use in subsequent analysis.

Reagents

Metaphosphoric acid, ascorbic acid and rutin were obtained from Chem-Supply Australia (Port Adelaide, SA, Australia). All other chemicals and reagents were sourced from Sigma-Aldrich Australia (Castle Hill, NSW, Australia), including gallic acid, Trolox and the remaining polyphenol standards used in the LC–MS/MS analyses. All reagents were of analytical grade or higher.

HPLC analysis of ascorbic acid

Analysis of ascorbic acid was only conducted on the main parts of the plant that would be expected to be eaten: the rhizome, fruit (flesh only) and fruit capsule. Extraction in 3% metaphosphoric acid and analysis by HPLC followed the

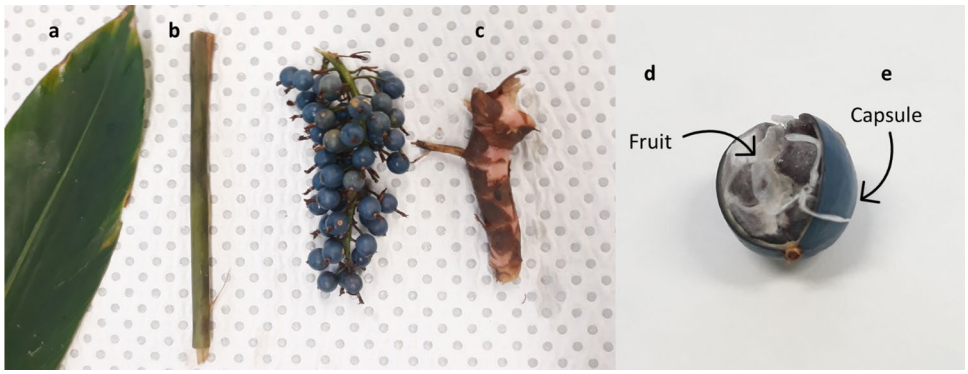


Fig. 2 Parts of the native ginger plant. **a** Leaves, **b** stem, **c** rhizome, **d** fruit, **e** fruit capsule (Color figure online)

methods previously reported by our laboratory [35]. Briefly, approximately 1 g of fresh sample was homogenised in 14 mL of 3% metaphosphoric acid and sonicated for 20 min, before the supernatant was collected and syringe filtered (Livingstone 0.45 µm PTFE membrane) after centrifugation (1000 rcf for 5 min). Ascorbic acid was quantified on an Agilent 1100 HPLC system and an Agilent Eclipse XDB-C18 column, with an injection volume of 5 µL and detection wavelength of 245 nm. An isocratic gradient of 0.01 M phosphoric acid (1 mL/min) was used for 3 min, followed by a 5 min methanol ramp to wash the column [35]. The ascorbic acid content of the samples was calculated using an external calibration (0.5–20 mg/L) and expressed in mg/100 g on a fresh weight basis. Analysis was conducted in triplicate for each sample type.

Extraction and analysis of phytochemical constituents

Polar phenolic compounds were extracted in triplicate with 90% v/v methanol, as previously described by Johnson et al., [36]. Briefly, approximately 1 g of freeze-dried sample was combined with 10 mL of 90% methanol, shaken end-over-end at 50 rpm for 60 min, and centrifuged (1000 rcf; 10 min) to collect the supernatant. The extraction was repeated with another 10 mL of 90% methanol, 20 min shaking, and centrifugation (1000 rcf; 10 min), to give a combined extract volume of 20 mL.

To conduct the TPC assay, 20 µL of sample extract (previously diluted with 90% methanol if required) was combined with 100 µL of 1:10 diluted Folin–Ciocalteu reagent, and incubated in darkness for 10 min before adding 100 µL of 7.5% aqueous Na_2CO_3. After a further incubation in darkness for 10 min, the 96-well plate was shaken for 300 s and the absorbance was measured at 750 nm.

The FRAP microplate assay was performed by combining 10 µL of sample extract (diluted if necessary) with 200 µL of FRAP reagent. The FRAP reagent comprised 300 mM acetate buffer at pH 3.56, 20 mM aqueous ferric chloride and 10 mM TPTZ (made in 40 mM HCl); combined in a 10:1:1 ratio. After shaking for 300 s, the absorbance was measured at 593 nm.

The CUPRAC assay was conducted by combining 10 µL of sample extract (diluted if necessary) with 50 µL each of 10 mM aqueous copper (II) chloride, 1 M aqueous ammonium acetate, 7.5 mM neocuproine ethanol solution and Milli-Q water in a 96-well plate. After incubating in darkness for 30 min, the plate was shaken for 60 secs and the absorbance measured at 450 nm.

Finally, the TMAC was measured by combining 40 µL of sample extract in separate wells with either 160 µL of pH 1 buffer (containing 0.025 M KCl) or 160 µL of pH 4.5 buffer (containing 0.4 M sodium acetate). After shaking the plate for 300 s, the absorbance was measured at both 510 nm and 700 nm. The TMAC was then calculated using the formula:

$$\text{Anthocyanin content}\left(\text{mg cyd-3-glu L}^{-1}\right) = \left(\text{A} \times 449.38\ \text{g mol}^{-1} \times \text{Dilution Factor} \times 1000\right) / \left(26900\ \text{M}^{-1}\ \text{cm}^{-1} \times 0.6\ \text{cm}\right)$$

The total phenolic content (TPC), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC) and total monomeric anthocyanin content (TMAC) of the methanolic extracts were also analysed using microplate-based versions of the methods described by Johnson et al., [36]. A Bio-Rad iMark microplate reader (Bio-Rad, Hercules, California, USA) was used for the absorbance readings.

where $A = (pH_1: Absorbance_{510\,nm} - Absorbance_{700\,nm}) - (pH_{4.5}: Absorbance_{510\,nm} - Absorbance_{700\,nm})$ and the dilution factor, following the methods as described above, was 5.

Assays were performed in duplicate for each extract, with the average well absorbance used in subsequent calculations. TPC results were expressed as mg of gallic acid equivalents (GAE) per 100 g; FRAP and CUPRAC results as mg of Trolox equivalents (TE) per 100 g; and TMAC

results in cyanidin-3-glucoside (cyd-3-glu) equivalents per 100 g. All phytochemical results are presented on a dry weight basis.

Extraction and analysis of volatile compounds by GC–MS

Volatile compounds were extracted and analysed from the rhizome samples only, as these were perceived to contain the most volatile compounds from their aroma. A mass of 1.00 ± 0.01 g of rhizome (fresh weight) was homogenised in 5 mL of dichloromethane (DCM) and sonicated for 30 min (Soniclean 160TD ultrasonic cleaner; Soniclean, Dudley Park, SA, Australia), before the supernatant was collected and syringe filtered (0.45 µm PTFE; Livingstone, Mascot, NSW, Australia) into a GC–MS vial [37]. Extractions and subsequent analyses were performed in triplicate.

The volatile compounds were analysed on a single quadrupole Shimadzu QP2010 Plus system with a Shimadzu SH-Rxi-5Sil MS column (29 m × 0.25 mm i.d. × 0.25 µm thickness; Shimadzu, Kyoto, Japan), following the methods described by Johnson et al., [38]. Compounds were identified by comparison of their mass spectra to the NIST14 and NIST14s libraries, and the similarities between their Kovat's linear retention indices (LRIs) and literature LRI values. Additionally, the exported total ion chromatograms (TICs) were interrogated using the MS-DIAL software (RIKEN Center for Sustainable Resource Science, Yokohama City, Japan) [39], to ensure that all compounds present were detected and identified.

All major peaks in the TIC (defined as those having a slope of > 1000 counts/min) were automatically integrated by the software; this was adjusted with manual integration using a point-to-point baseline where the automatic integration results could be improved. The peak area (area between the curve and the baseline) was then calculated for each peak. To calculate the proportion of each compound, these peak areas were then divided by the summed area of all chromatogram peaks, giving an estimate of the relative abundance of each compound.

Targeted LC–MS/MS analysis of selected phenolic compounds

Targeted profiling of the phenolic compounds in all methanolic extracts was performed using a NexeraX2 liquid chromatography system coupled with a Shimadzu LCMS-8040 system (Shimadzu, Kyoto, Japan). The method followed that reported by Johnson et al., [40]. It used a Raptor biphenyl column (100 mm × 2.1 mm, 2.7 µm; Shimadzu, Kyoto, Japan) and water/methanol for the mobile phases, each containing 5 mM ammonium formate and 0.1% formic acid.

The mass spectrometry module used an ESI source, with the following conditions: interface temperature of 350 °C, DL temperature 250 °C, heat block temperature 400 °C, interface voltage of 4.50 kV. Nitrogen was used as the nebulizing gas and drying gas, at flow rates of 3 L/min and 15 L/min, respectively. Analysis was performed in both negative and positive ionization modes (depending on the compound).

The method included a total of 30 polyphenol compounds [40], namely: gallic acid, protocatechuic acid, gentisic acid, neochlorogenic acid, 4-hydroxybenzoic acid, (+)-catechin, caffeic acid, chlorogenic acid, salicylic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, ellagic acid, vitexin, rutin, quercetin 3-glucoside, quercetin, myricetin, luteolin, kaempferol, naringenin, apigenin, resveratrol, cyanidin 3-glucoside, malvidin 3-glucoside, pelargonidin, delphinidin and cyanidin.

The precursor *m/z*, product *m/z*, collision energy, and Q1 and Q3 pre-biases were optimised for each targeted compound, as described in Johnson et al., [40]. Two transitions were used for most compounds; the first for quantitation, and the second for identity confirmation. The identification details for the detected compounds are provided in Table 3.

For each standard, a 5- or 6-point external calibration curve was created between concentrations of approximately 0.01–10 mg/L. All samples were analysed in triplicate and results were expressed as mg/100 g on a dry weight basis.

Determination of anti-acetylcholinesterase activity

The anti-acetylcholinesterase (AChE) activity of the *A. caerulea* methanolic extracts was assessed using an in vitro enzyme inhibition assay, as reported by Zheng et al., [41]. Firstly, 40 µL of different concentrations of each methanol extract was placed in a 96-well plate along with 160 µL of 0.2 M phosphate buffer (at pH 7.7). To each well, 80 µL of 1 mM DTNB and 10 µL of 2 U/mL AChE solution was added, before being left to equilibrate for 5 min. Following this, 15 µL of 8 mM acetylthiocholine iodide was added, before being left to react for 5 min. The absorbance at 405 nm was measured using a Bio-Rad iMark microplate reader (as described in section "Extraction and analysis of phytochemical constituents"). The inhibitory activity of the extracts (as IC_{50} values) was calculated by comparing the absorbance of the sample wells to the blank (no AChE added) and negative control (no inhibitor) wells. The snythetic AChE inhibitor donepezil and natural AChE inhibitor caffeine [42] were used as positive controls.

Concentration of methanol extracts for further bioactivity testing

To further concentrate and semi-purify the polar extracts for further bioactivity testing, the remainder of the 90% methanol extracts (from section "Extraction and analysis of phytochemical constituents") were concentrated to a semi-solid consistency using a rotary evaporator (temperature limited to 27 °C). After re-dissolving the soluble material in 30 mL of Milli-Q water and vacuum filtration (0.45-µm Advantec filter paper), the solution was freeze-dried at − 50 °C and 40 mTorr (Flexi-Dry MP system, FTS Systems; Stone Ridge, NY, USA). The lyophilized plant extract resulting from this process were then weighed out and dissolved in Milli-Q water to obtain stock solutions of ~ 1000 mg/L lyophilized plant extract. Where necessary, this was further diluted using Milli-Q water to obtain the required concentration.

Determination of anti-tyrosinase (polyphenol oxidase) activity

To perform the anti-tyrosinase assay, we used a protocol adapted from Nile et al., [43]. Firstly, 50 µL of the lyophilized plant extract solution was combined with 50 µL of 200 U/mL tyrosinase solution (made up in 0.05 M phosphate buffer, adjusted to pH 6.5) in a 96-well plate. After incubating at room temperature (25 °C) for 5 min, 100 µL of 12 mM L-Dopa was added. The absorbance of the wells was measured at 475 nm every minute for 30 min, using the Bio-Rad iMark microplate reader described in section "Extraction and analysis of phytochemical constituents". The slope of the change in absorbance was used to quantify the % inhibition of tyrosinase, as per the following formula:

$$\%inhibition = 100 - \left(\frac{\Delta extract}{\Delta no\ inhib - \Delta no\ enzyme} \times 100 \right)$$

where $\Delta_{extract}$, $\Delta_{no\ inhibitor}$ and $\Delta_{no\ enzyme}$ indicate the average slope of the test extract well, no inhibitor well, and no enzyme well, respectively.

Determination of anti-inflammatory activity (COX inhibition)

The lyophilized plant extract solutions were also tested for their inhibitory activity against the cyclooxygenase-2 (COX-2) enzyme, which plays an important role in the inflammatory process [44]. COX-2 inhibition screening was conducted using a commercial inhibitor screening assay kit (item no. 701080; Cayman Chemical, Ann Arbor, Michigan, USA), following the manufacturer's instructions.

Briefly, 10 µL of the test solution was mixed with 10 µL of working concentration COX-2, 10 µL of heme solution and 160 µL of reaction buffer in a 96-well plate and incubated for 10 min at 37 °C. Then 10 µL of arachidonic acid substrate was added, followed by gentle mixing, incubation for another 2 min at 37 °C, and addition of 30 µL of saturated stannous chloride solution. After a final incubation for 15 min at 37 °C, the reaction substrate was diluted 2000× using ELISA buffer, and 50 µL of the diluted sample placed in a mouse anti-rabbit IgG-coated 96-well plate, along with 50 µL of $PGF_{2\alpha}$ AChE tracer solution and 50 µL of ELISA antiserum.

After 18 h incubation at 4 °C, the wells were emptied, rinsed five times with ELISA wash buffer, and 200 µL of Ellman's Reagent added to each well. After allowing colour development for 40 min, the absorbance of the wells was measured at 415 nm (using the Bio-Rad iMark microplate reader described in section "Extraction and analysis of phytochemical constituents"), with results quantified against a series of 8 $PGF_{2\alpha}$ standards (3.9–500 pg/mL). After subtracting the $PGF_{2\alpha}$ concentration of the background control wells, the percent COX inhibition was calculated using the following equation:

$$\%\text{inhibition} = \frac{\text{Av conc. of initial activity well} - \text{conc. of sample well}}{\text{Av conc. of initial activity well}} \times 100$$

where conc. refers to the concentration of the $PGF_{2\alpha}$ in the sample/control.

Corresponding controls were prepared using 10 µL of inhibitor vehicle (either water or DMSO) in the place of the inhibitor. The negative control (corresponding to no COX-2 inhibition) was prepared containing 10 µL of COX-2 with no inhibitor. Background activity samples were prepared by placing a small amount of COX-2 enzyme in boiling water for 3 min; 10 µL of this was taken for the corresponding COX reactions. These samples correspond to complete COX-2 inhibition.

Data analysis

GC–MS and LC–MS/MS data were collected and analysed using Shimadzu LabSolutions software (Kyoto, Japan). Statistical analysis was conducted in R Studio running R 4.0.5 [45]. Where applicable, results are presented as mean ± 1

Table 1 Moisture contents, total phenolic content, antioxidant capacity, and anthocyanin content of various parts of the native ginger plant

Plant part	Moisture (%)	TPC (mg GAE/100 g)	FRAP (mg TE/100 g)	CUPRAC (mg TE/100 g)	TMAC (mg cyd-3-glu/100 g)
Leaves	74.7	2053 ± 35^{a}	2132 ± 41^{b}	6702 ± 267^{b}	ND^{b}
Stems	89.2	2463 ± 183^{a}	3002 ± 154^{a}	8393 ± 443^{a}	20 ± 14^{b}
Rhizome	90.9	1227 ± 157^{b}	532 ± 26^{d}	3548 ± 108^{c}	ND^{b}
Fruit	49.0	557 ± 34^{c}	378 ± 23^{d}	2100 ± 122^{d}	ND^{b}
Fruit capsule	68.3	2496 ± 529^{a}	897 ± 44^{c}	3680 ± 124^{c}	65 ± 30^{a}
ANOVA	–	***	***	***	**

All TPC, FRAP, CUPRAC and TMAC values are given in mg/100 g, on a dry-weight basis (mean ± SD of n = 3 replicates where applicable). Entries in the same row followed by different superscript letters were significantly different from one another according to a one-way ANOVA followed by post hoc Tukey testing at $\alpha = 0.05$

ND not detected

**P < 0.01; ***P < 0.001

standard deviation. A p-value < 0.05 was considered as statistically significant.

Results and discussion

Physical characteristics, phytochemical composition and ascorbic acid content

The average diameter of the mature whole fruit was 12.5 ± 0.5 mm, while the average mass was 1.31 ± 0.08 g (n = 12 for both). The outer capsule made up 23.4% of the whole fruit by mass. The moisture content ranged from 49% in the fruit flesh to 91% in the rhizome (Table 1). This fell within the range of moisture contents previously reported by Brand Miller et al., [16] in *A. caerulea* fruit (40–89%).

The stems showed the highest antioxidant capacity (as measured by both the FRAP and CUPRAC assays), as well as the second-highest total phenolic content (Table 1). The highest TPC was seen in the fruit capsule; however, compared to the other samples, it showed a much lower relative antioxidant capacity (for both FRAP and CUPRAC). This indicates that the specific phenolic compounds found in the fruit capsule were not particularly antioxidant-active, in contrast to the phenolics found in the other plant parts. Numerous previous studies have indicated that polyphenols can vary widely in their individual antioxidant capacities [40, 46, 47]. For example, in one study vanillic and ellagic acid showed very similar responses on the TPC assay, but ferulic acid showed almost no activity in the FRAP assay [40]. Future studies, using LC–MS with online-ABTS or a similar system [48, 49], would be beneficial for identifying the antioxidant activities of specific phenolic compounds from this species.

The leaves showed a moderately high TPC and antioxidant capacity, while the rhizome contained a moderate TPC and a correspondingly low total antioxidant capacity. The TPC found in *A. caerulea* rhizome was somewhat lower than the average TPC previously found in dried ginger (*Z. officinale*) samples (1713 ± 235 mg GAE/100 g) [50] and *A. officinarum* rhizome [51]. Anthocyanins were only detected in the fruit capsule and stem samples. It is possible that anthocyanins may be at least partially responsible for the dark blue colour of the fruit capsule (see Fig. 2e).

Ascorbic acid was detected in low concentrations in the fruit (2.28 ± 0.33 mg/100 g fresh weight) and the fruit capsule (3.44 ± 0.20 mg/100 g FW), but it was not detected in the rhizome. This was similar to the mean value of 4 mg/100 g ascorbic acid reported for two samples of *A. caerulea* fruit [16]. However, the ascorbic acid content of the rhizome and fruit capsule does not appear to have been previously reported. Testing was not performed here on the 'non-edible' plant parts (leaves and stems). In contrast to our results, Devi et al., [52] reported a relatively higher ascorbic acid content in *A. galanga* rhizome (54 mg/100 g DW). Assuming a similar moisture content, this would be approximately comparable to the ascorbic content found in *A. caerulea* fruit on a fresh-weight basis. However, Nampoothiri et al., [5] found no ascorbic acid in *A. galanga* rhizomes and only 1 mg/100 g DW in *A. calcarata* rhizomes.

Volatile profiling by GC–MS

Unlike other Alpinia species [53, 54], the volatile composition of the native ginger DCM extracts was dominated by a single compound (Fig. 3), identified as (*E*)-8(17),12-labdadiene-15,16-dial from its mass spectra. It comprised an average of 91.7 ± 0.5% of the total volatile constituents.

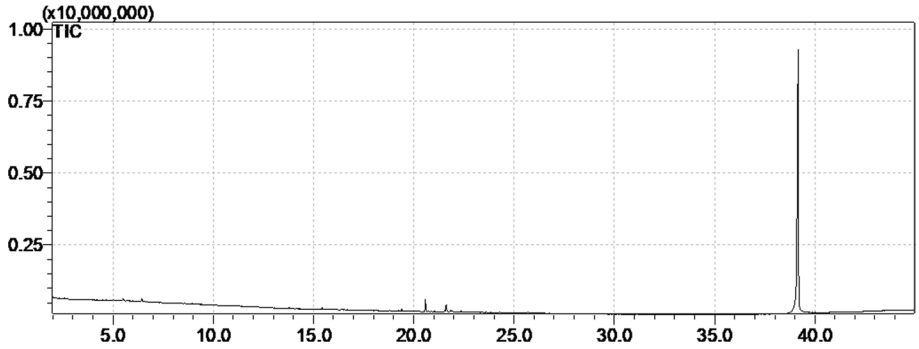


Fig. 3 Representative total ion chromatogram of one of the native ginger DCM extracts

Along with zerumin A, this diterpenoid is one of the major compounds previously reported from *A. caerulea* [17]. The likely reason for the absence of Zerumin A in the chromatogram is its additional carboxylic acid functionality, which would reduce its volatility and may make it unsuitable for detection by gas chromatography.

In addition to its previous report from *A. caerulea*, (*E*)-8(17),12-labdadiene-15,16-dial has been found in other Zingiberaceae species, including *Alpinia zerumbet* rhizomes [55] and *Curcuma longa* leaves [56], *Zingiber montanum* rhizomes [57], and *Etlingera sessilanthera* rhizomes [58], amongst other species. It has a number of reported bioactive properties, including antifungal activity [56], antibacterial activity [57, 58], cytotoxic activity against specific cell lines [59], and antiglycation activity [55]. Although this compound does not currently have any commercial applications, some authors have suggested that it may be useful in preventing glycation complications associated with diabetes [55], while others have suggested that its chemical structure could be modified to improve its anti-microbial activity and specificity [58].

The specific aroma and flavour of (*E*)-8(17),12-labdadiene-15,16-dial remain unknown, limiting any uses as an aromatic or flavouring agent. Investigation on this topic—as

Table 2 The major volatile compounds found in the rhizome DCM extracts, along with their proportion of the total volatile content

No.	Compound	Class	Ret time (min)	LRI	Lit. LRI	Proportion of total volatiles (%)[a]
1	2,4-Dimethyl-heptane	Alkane	2.58	785	822	0.13 ± 0.03
2	α-Pinene	Monoterpene	5.03	892	933	0.14 ± 0.02
3	Camphene	Monoterpene	5.51	909	953	0.63 ± 0.18
4	β-Pinene	Monoterpene	6.44	936	980	0.56 ± 0.03
5	*Trans*-β-ocimene	Monoterpene	8.59	999	1050	0.16 ± 0.04
6	*Cis*-β-ocimene	Monoterpene	8.93	1010	1040	0.13 ± 0.03
7	3,7-Dimethyldecane	Alkane	9.18	1017	1086	0.18 ± 0.05
8	Dodecane	Alkane	13.79	1155	1199	0.22 ± 0.06
9	1,3-Di-tert-butylbenzene	Phenylpropane	15.43	1207	1249	0.30 ± 0.03
10	β-Elemene	Sesquiterpene	19.25	1340	1393	0.13 ± 0.03
11	Tetradecane	Alkane	19.40	1346	1399	0.35 ± 0.07
12	Selina-5,11-diene	Sesquiterpene	20.58	1389	1447	2.48 ± 0.21
13	Germacrene D	Sesquiterpene	21.54	1425	1480	0.22 ± 0.01
14	Aristolochene	Sesquiterpene	21.62	1429	1481	1.57 ± 0.10
15	Eremophilene	Sesquiterpene	21.84	1437	1486	0.33 ± 0.05
16	Bicyclogermacrene	Sesquiterpene	21.93	1441	1494	0.21 ± 0.05
17	2,4-Di-tert-butylphenol	Phenylpropane	22.37	1458	1502	0.22 ± 0.02
18	Elemol	Sesquiterpene	23.25	1493	1547	0.07 ± 0.06
19	Hexadecane	Alkane	24.29	1536	1600	0.12 ± 0.05
20	Neointermedeol	Sesquiterpene	25.71	1595	1656	0.14 ± 0.05
21	(*E*)-8(17),12-labdadiene-15,16-dial	Diterpenoid	39.17	2188	2220	91.72 ± 0.51

[a]Values given as percentages of the total peak area (n = 3 replicates)

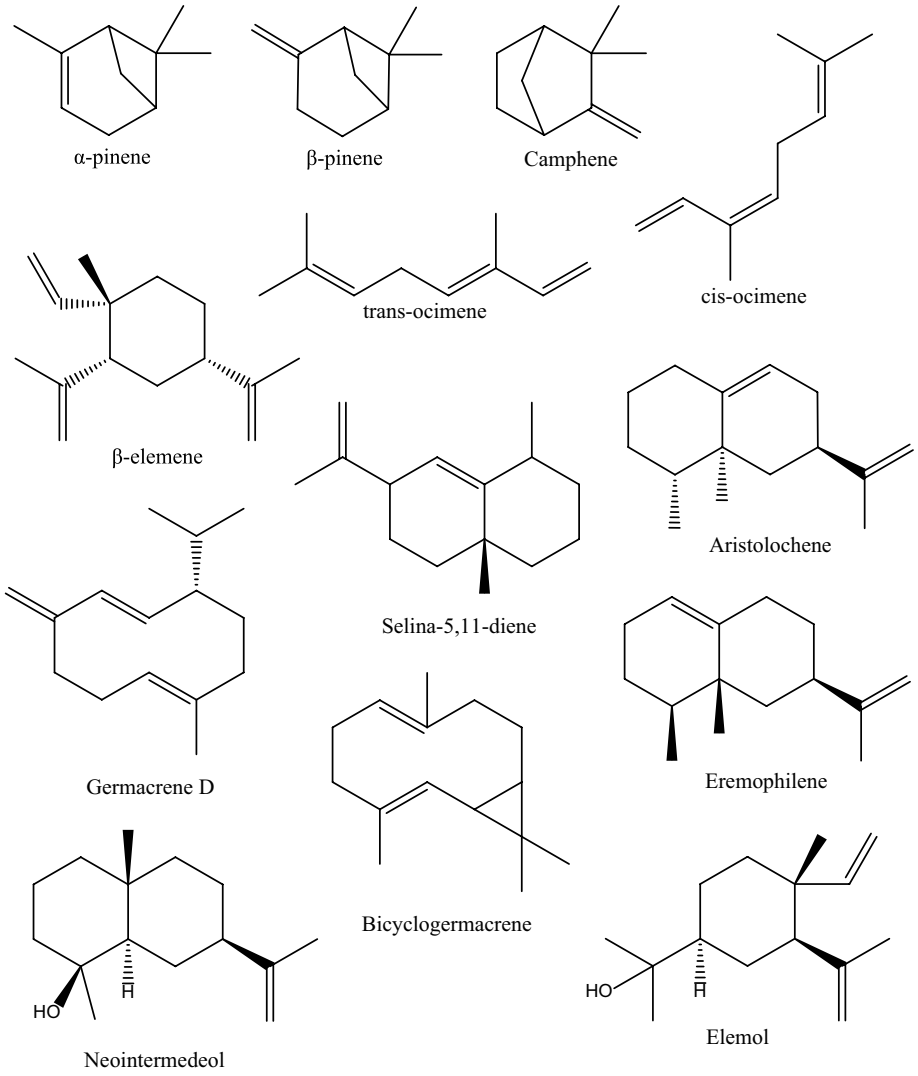


Fig. 4 Structures of the monoterpenes and sesquiterpenes identified from the *A. caerulea* rhizome

well as the safety of this compound—is an important step toward exploring future potential uses for which it could be exploited.

In addition to (*E*)-8(17),12-labdadiene-15,16-dial, a further 20 volatile compounds were identified from their mass spectra fragmentation patterns and linear retention indices (Table 2). These comprised 5 monoterpenes and 8 sesquiterpenes (Fig. 4), along with 2 phenylpropanes and 5 alkanes. The most abundant compounds, following (*E*)-8(17),12-labdadiene-15,16-dial (91.7% of total peak area), were selina-5,11-diene (2.5%), aristolochene (1.6%), camphene (0.6%) and β-pinene (0.6%). Notably, 1,8-cineole (eucalyptol) was not detected in the samples, although it typically tends to be a major constituent (10–40%) in other *Alpina* species [53, 54].

Amongst the terpenoid constituents, α-pinene, camphene and elemol have also been previously identified from ginger (*Zingiber officinale*) [37, 60]. However, most of the other volatiles are not found in *Z. officinale* at significant levels, if at all. Consequently, it is somewhat of a surprise that *A. caerulea* has a similar (albeit weaker) odour to *Z. officinale*.

One recent study identified the major odour-active volatiles from fresh ginger (*Z. officinale*) as (*E*)-isoeugenol, 1,8-cineole, vanillin, geranial, and linalool [61]; other studies suggest that β-myrcene, citronellal, bornyl acetate and α-pinene also play a part [60, 62]. However, the only one of these volatiles which is also found in *A. caerulea* in this study was α-pinene (at rather low levels). Consequently, the compound(s) which provide the ginger-like aroma in *A. caerulea* remain unconfirmed.

Quantitative profiling of phenolic compounds by LC–MS

Targeted LC–MS profiling revealed the presence of 6 polyphenols (Fig. 5), out of the 30 polyphenol compounds included in the method (Table 3). Rutin (0.16–21.4 mg/100 g dry weight) and quercetin-3-glucoside (0.88–9.15 mg/100 g

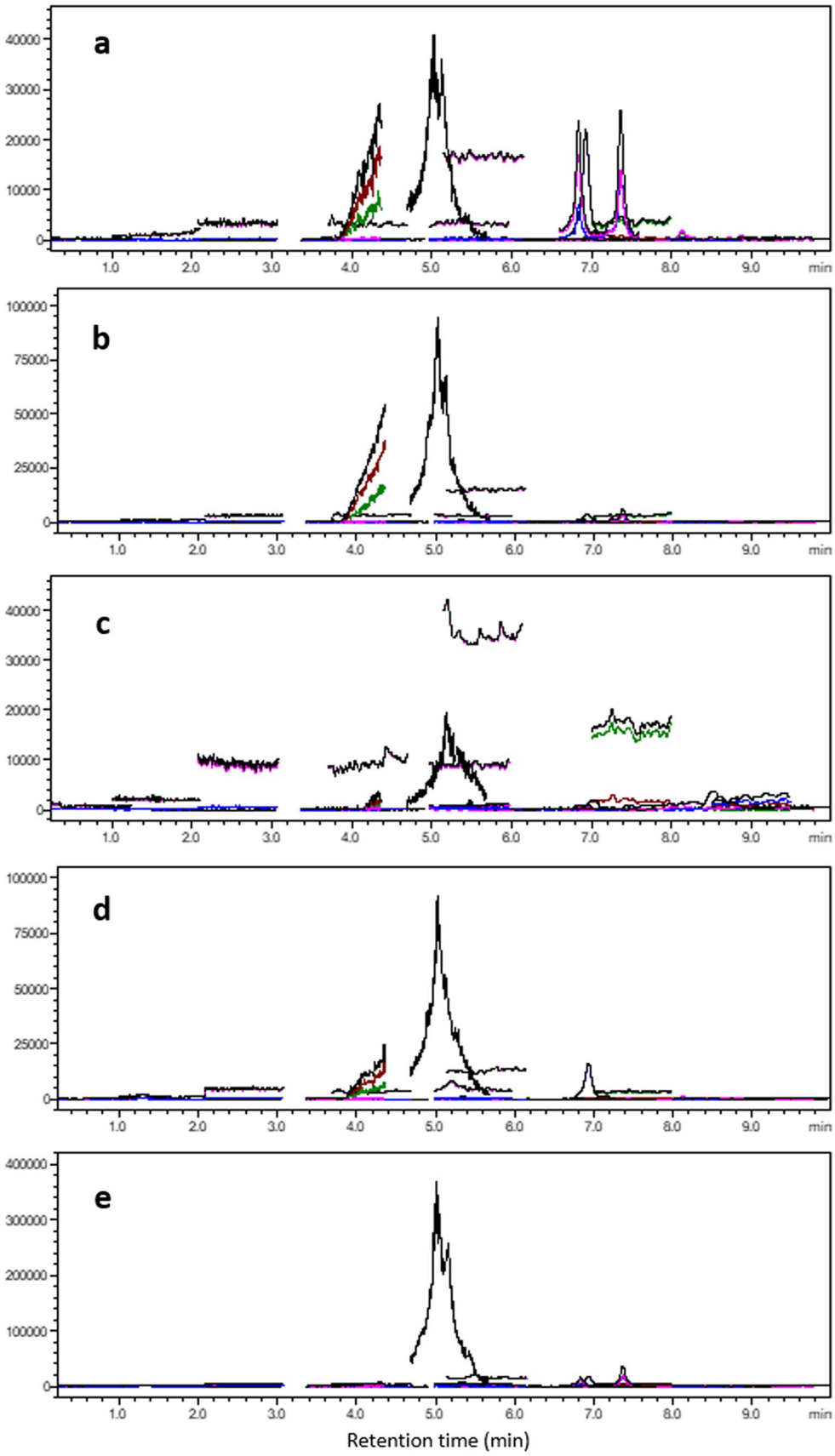


Fig. 5 LC–MS total ion chromatograms showing the major polyphenol peaks found in different parts of *A. caerulea*: **a** leaf, **b** stem, **c** rhizome, **d** fruit, **e** fruit capsule

Table 3 Identification details and concentrations of selected polyphenols which were detected in different parts of the native ginger samples using targeted LC–MS/MS analysis (results given as mg/100 g on a dry-weight basis; n = 3 replicates)

Analyte	Retention time (min)	Ionisation mode	Ion transitions (collision energy)	Rhizome	Fruit	Capsule	Leaves	Stem	ANOVA
Chlorogenic acid	4.48	Negative	353.2 -> 191.1 (16)	ND	ND	0.03 ± 0.01	ND	ND	–
Vanillic acid	5.56	Negative	167.1 -> 108.1 (20) 167.1 -> 152.1 (16)	ND^{c}	0.40 ± 0.07^{bc}	5.51 ± 0.70^{a}	ND^{c}	1.27 ± 0.26^{b}	***
Rutin	7.03	Negative	609.2 -> 300.0 (41) 609.2 -> 271.1 (61)	0.36 ± 0.22^{d}	0.16 ± 0.03^{d}	5.75 ± 0.41^{b}	21.4 ± 0.59^{a}	1.37 ± 0.23^{c}	***
Quercetin 3-glucoside	7.14	Negative	463.1 -> 300.0 (29) 463.1 -> 151.2 (36)	0.88 ± 0.03^{d}	2.91 ± 0.08^{b}	2.87 ± 0.07^{b}	9.15 ± 0.63^{a}	1.89 ± 0.04^{c}	***
Quercetin	8.41	Negative	301.1 -> 151.0 (21) 301.1 -> 179.0 (19)	ND^{b}	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	0.24 ± 0.05^{a}	0.06 ± 0.01^{b}	***
Kaempferol	9.19	Negative	285.1 -> 187.2 (30) 285.1 -> 239.1 (27)	ND	ND	ND	0.43 ± 0.07	ND	–

Entries in the same row followed by different superscript letters were significantly different from one another according to a one-way ANOVA followed by post hoc Tukey testing at $\alpha = 0.05$

ND not detected

***$p < 0.001$

DW) were the most abundant polyphenols; and were also detected in all sample types. The corresponding aglycone, quercetin, was absent from the rhizome but found in all other sample types, albeit at much lower concentrations (0.04–0.24 mg/100 g DW). Vanillic acid was detected in the fruit, fruit capsule and stem samples (0.40–5.51 mg/100 g DW), but notably not in the leaf samples. Finally, kaempferol (0.43 mg/100 g DW) and chlorogenic acid (0.03 mg/100 g DW) were only found in the leaf and fruit capsule samples respectively. In contrast to the results of Nampoothiri et al., [5] in *A. galanga* and *A. calcarata*, gallic acid, ellagic acid and ferulic acid were not detected here. However, chlorogenic acid and rutin were both previously reported from *A. pricei* rhizomes [34].

The leaves generally had the highest polyphenol concentrations, followed by the capsules and fruit. The low TPC levels seen for the rhizome and fruit samples were generally mirrored in the lower number/concentration of individual phenolic compounds (Table 3). Notably, the fruit capsule—which showed the highest TPC:antioxidant capacity ratio (section "Physical characteristics, phytochemical composition and ascorbic acid content")—contained high levels of vanillic acid, which has previously been found to possess very low FRAP activity [40]. On the other hand, the leaves contained high levels of rutin, which shows much higher FRAP (unpublished data from our laboratory). However, the total number and concentration of phenolic compounds identified from the samples using LC–MS/MS was generally quite low, indicating that the majority of polyphenols present did not correspond to common polyphenols for which authentic standards were available (see the list provided in section "Targeted LC–MS/MS analysis of selected phenolic compounds"). Consequently, further studies using high resolution mass spectrometry are required to fully characterise the polyphenols present in this species.

Anti-acetylcholinesterase activity

The rhizome showed the strongest anti-AChE activity out of all the plant parts tested (IC_{50} value of 4252 mg/L), followed by the stems and leaves (6256 and 9485 mg/L, respectively). The fruit and fruit capsule showed lower anti-AChE activity (11,074 and 17,566 mg/L, respectively). The level of AChE inhibition afforded by the rhizome was comparable to that recently found by our laboratory for the seedcoat material from the best adzuki bean genotype tested (4483 mg/L) [63].

Additionally, all plant parts showed greater inhibitory activity against AChE compared to commercial ginger (*Z. officinale*; 75,081 mg/L according to previously unpublished data from our laboratory), which has documented anti-Alzheimer activity [22, 23]. This highlights that further investigation should be conducted into identifying and characterising the anti-AChE agent(s) in *A. caerulea*.

Chen et al., [64] attributed the AChE inhibitory activity of *Alpinia oxyphylla* to the sesquiterpenoids, with (9*E*)-humulene-2,3;6,7-diepoxide found to show the strongest anti-AChE activity. On the other hand, the flavonoid galangin was identified as a potent AChE inhibitor from *A. officinarum* [65], while 5-hydroxymethylfurfural from *A. oxyphylla* provided neuroprotective effects against Alzheimer's disease [66]. The AChE inhibitory activity of (*E*)-8(17),12-labdadiene-15,16-dial or zerumin A does not appear to be reported, although zerumin A does show neuroprotective activity by preventing oxidative damage to cells [67]. Zerumin A is also reportedly a positive modulator of $GABA_A$ receptors, providing it with potential therapeutic activity against Alzheimer's disease [68]. Consequently, it is possible that one of these diterpenoids may be responsible for the AChE inhibition of *A. caerulea*.

Anti-tyrosinase activity

The highest concentrations of the lyophilized plant extract tested (~ 1000 mg/L) showed only moderate inhibitory activity against tyrosinase. The strongest inhibition was shown by the stems (29.2% inhibition), followed by the leaves (13.4%). The fruit, fruit capsule and rhizome extracts each provided < 10% inhibition of tyrosinase at 1000 mg/L. Consequently, further anti-tyrosinase testing was not conducted. Tyrosinase inhibition has not been widely tested across *Alpinia* species, although Tu and Tawata [26] found that *A. zerumbet* extracts provided moderately high anti-tyrosinase activity (2–3 × less than kojic acid).

Anti-inflammatory activity

As this study was exploratory only and aimed to screen for anti-inflammatory activity, a selection of the lyophilized plant extracts (rhizome, fruit capsule and leaf) were tested only at a single concentration (~ 1000 mg/L). None of the extracts tested showed any inhibitory activity against COX-2, therefore further in-depth testing was not pursued. This contrasted with work on several other *Alpinia* species, which reported anti-inflammatory activity [30–34], including COX-2 inhibition in *A. officinarum* [32]. Consequently, this demonstrates the wide range of bioactivities (and lack thereof) found within the *Alpinia* genus.

Conclusion

To the best of our knowledge, this study provided the first detailed insight into the polyphenol content and volatile composition of the native Australian ginger *A. caerulea*. It found a total of six phenolic compounds across various plant parts, in addition to low concentrations of ascorbic acid in the fruit and fruit capsule. The volatile profile was dominated by a single diterpene; (*E*)-8(17),12-labdadiene-15,16-dial.

No inhibitory activity was found against COX-2, while the extracts showed low to moderate inhibition of tyrosinase. Both the rhizome and stems showed moderate anti-AChE activity, but were nevertheless superior to *Z. offinale*, which has well-documented anti-Alzheimer activity. Although these results suggest that *A. caerulea* may not contain potent AChE inhibitors which could be developed into pharmaceutical drugs, future research could consider the potential role of native ginger as part of a healthy and diverse diet. It is also worth noting that this species may show other biological activities—such as antibacterial, anti-cancer or cytotoxic activity—which were not considered in this study.

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Data Availability The datasets supporting this study are available upon request from the corresponding author.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

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