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Research article

Drying-dependent changes in phytochemicals and bioactivities of *Curcuma stahlianthoides* Škorničk. & Soonthornk., a vulnerable endemic Thai species

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Abstract

Importance of the work: There is a lack of documentation regarding the nutritional and phytochemical composition and bioactivities of *Curcuma stahlianthoides* Škorničk. & Soonthornk., an endemic species in Thailand, as well as the effects of drying methods on these properties.
Objectives: To evaluate the effects of sun drying and freeze drying on the phytochemicals and bioactivities of *C. stahlianthoides* rhizomes.
Materials and Methods: Rhizomes were sun-dried or freeze-dried, ground into powder and analyzed for proximate composition, total curcuminoid content (using high-performance liquid chromatography), total phenolic and flavonoid contents, antioxidant activities (2,2-diphenyl-1-picrylhydrazyl, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid and ferric ion reducing antioxidant power assays) and antibacterial activity based on minimum inhibitory concentration determination.
Results: The freeze-dried samples contained higher fat (4.16%) and protein (3.44%) contents than the sun-dried samples (3.28% and 2.93%, respectively), while the carbohydrate content was comparable at approximately 78%. The freeze-dried samples had a higher mean ± SD total phenolic content (1.67 ± 0.08 mg gallic acid equivalents/g) and total flavonoid content (4.82 ± 0.17 mg quercetin equivalents/g) than the sun-dried extracts ($p < 0.05$). Antioxidant activities and antibacterial effects were also enhanced, particularly against Gram-positive bacteria, with minimum inhibitory concentrations of 12.5–25 mg/mL. Extraction yields were 2.75% for the sun-dried and 2.18% for the freeze-dried samples, while the total curcuminoid content was 12.95 µg/g dry weight and 14.11 µg/g dry weight, respectively. The total phenolic content had a strong positive correlation with antioxidant activities (Pearson's correlation coefficient > 0.90, $p < 0.05$).
Main finding: Drying methods influenced phytochemical retention and related antioxidant and antibacterial activities of *C. stahlianthoides*, offering new insight into post-harvest processing and utilization of this rare Thai species.

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Introduction

The genus *Curcuma* is one of the largest genera of the family Zingiberaceae, comprising more than 170 species worldwide (Soonthornkalump, 2022). The genus occurs in South and Southeast Asia and South China, with some species extending further eastward to Northern Australia and the Pacific archipelago (Záveská et al., 2012). Among these, currently, 68 *Curcuma* species have been recorded in Thailand (Soonthornkalump et al., 2025). Many *Curcuma* species are utilized widely in food, traditional medicine and cosmetic applications (Burapan et al., 2020). *Curcuma* species, such as *C. longa* and *C. comosa*, have been reported widely to exhibit antioxidant, anti-inflammatory and antimicrobial activities that are related mainly to the presence of curcuminoids, phenolic compounds, flavonoids, terpenoids and essential oils (Masuda et al., 1992; Prasad et al., 2014; Hewlings and Kalman, 2017; Pintatum et al., 2020).

Curcuma stahlianthoides Škorničk. & Soonthornk., commonly known as Bussarin, is an endemic Thai species found in northeastern Thailand and exhibits ant-mediated seed dispersal (myrmecochory) and has been assessed as "Vulnerable", according to IUCN criteria due to its limited distribution and restricted habitat (Soonthornkalump et al., 2022). Although *C. stahlianthoides* is related closely to several bioactive *Curcuma* species, information on its nutritional composition and biological properties remains unknown. This lack of scientific information highlights a major knowledge gap in the fields of food science and ethnopharmacology (Chumroenphat et al., 2019; Burapan et al., 2020).

Drying is an important post-harvest process that can affect the quality of plant materials because the drying method may influence the retention of nutrients and bioactive compounds, especially compounds that are sensitive to heat and oxidation (Ghafoor et al., 2020; Komonsing et al., 2022). Sun-drying is a traditional and low-cost method applied commonly in local processing; however, prolonged drying time and direct exposure to sunlight may lead to degradation of heat-sensitive compounds (Komonsing et al., 2022; Llano et al., 2022). On the other hand, freeze drying has been used to improve the retention of aromatic volatiles and thermos-labile phytochemicals, although it involves higher costs (Latinović et al., 2024; Kaur et al., 2025). Comparative evaluation of drying methods is essential to identify optimal processing for the preservation of bioactive compounds in *C. stahlianthoides.*

Although the phytochemical composition and antimicrobial activity of *C. stahlianthoides* have not yet been reported, several *Curcuma* species, including *C. zedoaria* Roxb., *C. aeruginosa* Roxb. and *C. caesia* Roxb., have been studied extensively for their bioactive properties. For example, studies have shown that rhizome extracts and essential oils from these species contain phenolic compounds, flavonoids and terpenoid-derived constituents, which are associated with antioxidant capacity and antimicrobial activity, particularly against Gram-positive bacteria (Akarchariya et al., 2017; Dosoky and Setzer, 2018; Budiansyah et al., 2023). In addition, post-harvest processing, especially drying, has been recognized as a critical factor affecting the retention and stability of sensitive phytochemicals in *Curcuma* rhizomes (Llano et al., 2022).

Therefore, the present study aimed to investigate the nutritional composition, phytochemical content, antioxidant activity and antimicrobial properties of extracts from *C. stahlianthoides* rhizomes prepared by sun drying or freeze drying, as evidence regarding these properties remains scarce. The results are expected to provide basic scientific evidence on the functional potential of this species and to support its potential application in the development of functional food and natural antimicrobial products.

Material and Methods

Plant materials and sample preparation

Fresh rhizomes of *C. stahlianthoides* (Fig. 1) were collected from a type locality in the flowering season in 2023 (Soonthornkalump et al., 2022). Botanical identification was confirmed by comparison with voucher specimens at the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand (voucher no. SK-2023-CS01). The rhizomes were washed thoroughly, sliced into approximately 3–5 mm thick pieces and divided into two treatments: sun drying (BD) under natural sunlight (average temperature 35–38°C for 48 hr) and freeze drying (BF) using a lyophilizer (−50°C, 0.04 mbar, for 72 hr). The dried samples were ground into fine powder using a laboratory grinder, passed through a 60-mesh sieve and stored in sealed aluminum foil bags at 4°C prior to further analysis.

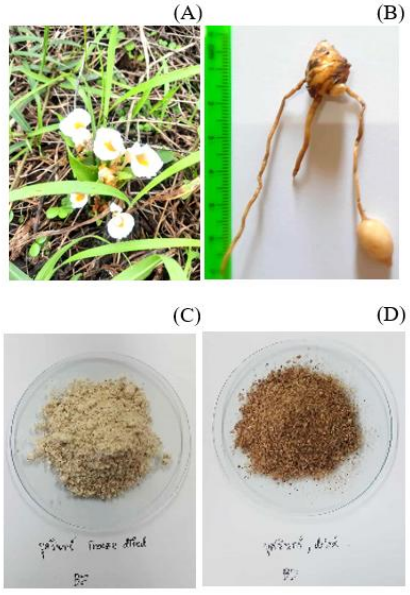


Fig. 1 *Curcuma stahlianthoides* samples from plants collected in northeastern Thailand during June–August 2023: (A) flowering plant in its natural habitat; (B) rhizome and root structure. where scale bar = 10 mm; (C) freeze-dried rhizome powder; and (D) sun-dried rhizome powder.

Proximate analysis

The proximate composition of the dried rhizome powders (moisture, ash, crude fat, crude protein, crude fiber and carbohydrate contents) was analyzed according to Association of Official Analytical Chemists International (2019). The moisture content was measured based on oven-drying at 105°C until a constant weight. The ash content was determined based on incineration at 550°C in a muffle furnace. The crude fat was extracted using the Soxhlet method with petroleum ether as solvent. The crude protein content was calculated from total nitrogen determined using the Kjeldahl method (conversion factor ×6.25). All measurements were conducted in triplicate and expressed as mean ± SD values on a dry weight (DW) basis. The crude fiber content was analyzed using sequential acid-alkali digestion and the percentage of carbohydrate was calculated based on the difference, using Equation 1:

Carbohydrate = 100 - (Moisture + Ash + Fat + Protein + Fiber) (1)

Retention ratio (%)

The retention ratio percentage was calculated to evaluate the preservation efficiency of nutrients and bioactive compounds after drying. The calculation was adapted from the apparent retention concept proposed by Murphy et al. (1975) and later applied in nutrient retention studies (Lee et al., 2018). Since fresh rhizomes were not analyzed in the present study, the sun-dried sample (BD) was used as the reference. The retention ratio percentage was calculated using Equation 2:

Retention ratio = (Value of freeze-dried sample) / (Value of sun-dried sample) × 100 (2)

A retention ratio greater than 100% indicates improved preservation or increased extractability of bioactive compounds in the BF compared with BD.

Preparation of crude extracts

Powdered rhizome samples (each 20 g) from each drying method were extracted with 200 mL of 95% ethanol based on maceration at room temperature (25°C) for 48 hr with continuous shaking (150 revolutions per minute). The mixtures were filtered and filtrates were concentrated under reduced pressure using a rotary evaporator (40°C). The concentrated extracts were dried further to obtain viscous crude extracts. The extraction yield as a percentage was calculated based on the dry weight of the extract relative to the initial sample weight. The crude extracts were stored at 4°C in the dark until further analyses of phytochemical content, antioxidant activity and antimicrobial activity.

Determination of total curcuminoid content

The total curcuminoid content was determined using a reversed-phase high-performance liquid chromatography (HPLC) system (Shimadzu LC-20A; Japan) with ultraviolet (UV) detection at 425 nm, following the method of Jayaprakasha et al. (2002), with minor modifications. Briefly, 0.50 g of rhizome powder was extracted with 10 mL methanol based on ultrasonication (at 25°C for 30 min) and then passed through a 0.45 µm polytetrafluoroethylene (PTFE) membrane filter. Separation was achieved using a C18 column (4.6 × 250 mm, 5 µm diameter) with an isocratic mobile phase consisting of an acetonitrile-to-water-to-acetic acid ratio of 70:29:1 (volume per volume per volume) at 1.0 mL/min. The total curcuminoid content was quantified using an external calibration curve prepared from analytical-grade standards and results were expressed as micrograms per gram DW.

Determination of total phenolic content

The total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). An aliquot of 0.5 mL of extract solution (1 mg/mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and allowed to react for 5 min. Subsequently, 2 mL of 7.5% Na_2CO_3 solution was added and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV-visible light spectrophotometer. Gallic acid was used as a standard and the TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

Determination of total flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Zhishen et al., 1999). In brief, 0.5 mL of extract (1 mg/mL) was mixed with 2 mL of distilled water and 0.15 mL of 5% $NaNO_2$. After 5 min, 0.15 mL of 10% $AlCl_3$ solution was added, followed by 1 mL of 1 M NaOH after 6 min of incubation. The final volume was adjusted to 5 mL with distilled water. Absorbance was measured at 510 nm. Quercetin was used as the reference standard and the TFC was expressed as milligrams of quercetin equivalent (QE) per gram of extract.

Determination of antioxidant activities

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extracts was evaluated according to the method described by Brand-Williams et al. (1995), with minor modifications. A 0.1 mM sample of DPPH methanolic solution was mixed with the extract at different concentrations. The reaction mixture was incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging was calculated relative to control.

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation decolorization assay

The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay was performed according to Re et al. (1999), with some modifications. $ABTS^+$ radicals were generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark for 16 hr at room temperature. The solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then, an aliquot of 0.1 mL of sample was mixed with 3.9 mL of the $ABTS^+$ solution and incubated for 6 min. Absorbance was measured at 734 nm.

Results were expressed as milligrams of Trolox equivalents (TE) per gram of extract.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) values were determined according to Benzie and Strain (1996), with slight modifications. The FRAP reagent was prepared freshly by mixing acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. A 0.1 mL sample extract was added to 3 mL of FRAP reagent and incubated at 37°C for 30 min. Absorbance was measured at 593 nm and expressed as milligrams Fe^{2+} equivalents per gram of extract.

Antimicrobial activity test (disc diffusion method)

Using the disc diffusion method (Mostafa et al., 2018), the antimicrobial potential of the crude extracts was evaluated against four foodborne bacteria: *Staphylococcus aureus* TISTR 2329, *Bacillus cereus* TISTR 1527, *Escherichia coli* TISTR 074 and *Salmonella enterica* serovar Typhimurium ATCC 11331. The crude ethanolic extracts from rhizomes prepared by BD and BF were separately re-dissolved in dimethyl sulfoxide (DMSO) to obtain a final concentration of 250 mg/mL, sterilized by passing through a 0.22 µm Millipore filter and then 20 µL of 250 mg/mL of the sample was loaded over a sterile filter paper disc (6 mm in diameter) to obtain a final concentration of 5 mg/disc.

Each bacterial strain was cultured on Mueller-Hinton agar (MHA) plates. A single loopful of each bacterial colony was transferred into 10 mL of Mueller-Hinton broth and incubated at 35°C for 18 hr. Then, the bacterial suspension (approximately 1×10^8 colony-forming units (CFU)/mL) was measured and adjusted to an optical density of 0.6 at 600 nm using a spectrophotometer. A sterile cotton swab was dipped into the standardized bacterial suspension and spread evenly over the surface of an MHA plate. Then, sterile filter paper discs loaded with each sample were placed on the top of MHA plates. Filter paper discs loaded with DMSO were used as a negative control and 30 µg chloramphenicol discs were used as a positive control. Next, the plate was incubated at 35°C for 24 hr. Antimicrobial activity was determined by the presence of an inhibition zone surrounding the paper disc. The diameters of the inhibition zones were measured using a Vernier caliper and recorded in millimeters, including the 6 mm diameter of the paper disc.

Determination of minimum inhibitory concentration

The crude ethanolic extracts sample that showed antimicrobial activity were investigated further for the minimum inhibitory concentration (MIC) against Gram-positive bacteria (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli* and *S.* Typhimurium). The MIC values were determined using the disc diffusion method following Mostafa et al. (2018), with some modifications. Each crude extract solution (250 mg/mL in DMSO) was diluted serially with DMSO to obtain final concentrations of 12.5 mg/mL, 25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL. Subsequently, 20 µL of each dilution was loaded onto sterile filter paper discs (6 mm in diameter).

A sterile cotton swab was dipped into the standardized bacterial suspension of each bacterial strain (approximately 1×10^8 CFU/mL) and spread evenly over the surface of an MHA plate. Then, sterile filter paper discs loaded with each sample were placed on the top of MHA plates. A filter paper disc loaded with 20 µL of DMSO was used as a negative control and 30 µg chloramphenicol discs were used as a positive control. Then, the plate was incubated at 35°C for 24 hr. The inhibition zones diameters were measured by Vernier caliper and recorded against the concentrations of the effective extracts. The lowest concentration of the extract resulting in the presence of an inhibition zones was considered as the MIC.

Ethics statements

The present study involved only plant materials and did not include human participants or experimental animals. Therefore, ethical approval was not required.

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean ± SD. Comparisons between the two drying methods (BD and BF) were analyzed using an independent samples t test. Statistical significance was tested at $p < 0.05$. All statistical analyses were carried out using the SPSS Statistics program (IBM Corp.; USA).

Results and discussion

The extraction yields of *C. stahlianthoides* were 2.75% for BD and 2.18% for BF. Both extracts appeared as dark brown, viscous crude extracts and were obtained under the same ethanolic extraction conditions. Although BD yielded a slightly higher extract quantity than BF, both extracts were considered suitable for the subsequent antimicrobial assays.

Proximate composition and total curcuminoid content

The proximate composition results for the *C. stahlianthoides* rhizomes prepared using the different drying methods are presented in Table 1. BF contained significantly higher fat (4.16%) and protein (3.44%) contents than the BD sample (3.28% and 2.93%, respectively), whereas the carbohydrate content was similar in both samples (approximately 78% of dry weight). Based on these results, freezing-drying better retained certain nutritional components by reducing thermal degradation during processing. Similar observations have been documented in *C. longa*, where freeze drying was found to better preserve nutritional and phytochemical stability than sun drying or hot-air drying (Chumroenphat et al., 2021; Llano et al., 2022). The moisture contents of both BD and BF samples were below 9%, indicating effective dehydration under both drying conditions. The ash and crude fiber contents were not significantly different between the samples of the two drying conditions. Overall, the proximate composition of the *C. stahlianthoides* rhizomes was characterized by a high carbohydrate content with moderate levels of protein and fat, comparable to the profiles reported for *C. longa* and other *Curcuma* species, although

variations may occur depending on cultivar, geographical origin and analytical methodology (Burapan et al., 2020; El-Saadony et al., 2022). The nutritional profile of *C. stahlianthoides* was comparable to that reported for other medicinal *Curcuma* species (Chumroenphat et al., 2021; Llano et al., 2022), indicating its relevance among rhizomatous plants commonly studied in food and herbal research.

Table 1 Proximate composition and total curcuminoid content of *Curcuma stahlianthoides* rhizomes prepared by different drying methods, with retention ratios.

Parameter	BD	BF	Retention ratio (%)
Moisture (%)	8.76±0.24[a]	8.01±0.31[a]	91.4
Ash (%)	2.88±0.31[a]	2.91±0.11[a]	101.0
Crude fat (%)	3.28±0.58[b]	4.16±0.72[a]	126.8
Crude protein (%)	2.93±0.08[b]	3.44±0.02[a]	117.4
Crude fiber (%)	3.12±0.47[a]	3.35±0.02[a]	107.4
Carbohydrate (%)	79.03±0.31[a]	78.13±0.42[a]	98.9
Total curcuminoid (μg/g DW)	12.95±0.18[b]	14.11±0.22[a]	108.9

BF = freeze-dried; BD = sun-dried; Retention ratio (%) = (BF / BD) × 100; DW = dry weight. Values are presented as mean ± SD (n = 3). Different lowercase superscript letters within each row indicate significant differences ($p < 0.05$) based on independent samples t-test.

Although the difference was relatively small, freeze drying showed consistently higher total curcuminoid content (14.11 μg/g DW) than sun drying (12.95 μg/g DW) under the conditions evaluated (Table 1). This difference may have been related to the sensitivity of curcuminoids to processing conditions, particularly exposure to heat, oxygen and light. Curcuminoids are known to possess phenolic hydroxyl groups and conjugated diketone structures, which readily participate in oxidative reactions when exposed to thermal and photochemical stress (Masuda et al., 2001). During the sun drying, prolonged exposure to moderate temperatures and UV radiation may have promoted photo-oxidation and oxidative cleavage of curcuminoid structures. In addition, the presence of oxygen during open-air drying could have accelerate further radical-mediated degradation reactions. These mechanisms have been described in *Curcuma* rhizomes, where sun drying and hot-air drying were associated with greater curcuminoid losses than freeze drying (Chumroenphat et al., 2021). In contrast, freeze drying is conducted under low temperature and reduced pressure conditions, which may limit enzymatic activity and oxidative degradation. These factors are likely to reduce oxidative stress and slow down degradation reactions of curcuminoids during processing. Consequently, higher curcuminoid retention was observed in the freeze-dried *C. stahlianthoides* sample. The total curcuminoid content of *C. stahlianthoides* observed in the present study was lower than the values commonly reported for *C. longa* and *C. comosa*, where such interspecific variation has been attributed widely to genetic differences, environmental conditions and post-harvest handling practices (Li et al., 2011; Burapan et al., 2020).

The retention ratios of the total phenolic and flavonoid compounds exceeded 150%, indicating higher values in the BF samples than in the BD samples. This apparent increase may have been influenced by the structural changes in the plant tissues caused by freeze drying. Ice crystal formation during freezing can disrupt cellular matrices, potentially enhancing solvent accessibility and extractability of phenolic compounds, with similar effects being reported in ginger and turmeric species, where freeze drying improved phenolic retention and reduced oxidative losses compared with conventional drying methods (Ghafoor et al., 2020). In contrast, sun drying involves continuous exposure to UV radiation and ambient oxygen, which may promote photo-oxidation, polymerization and enzymatic browning of phenolic compounds, leading to reduced antioxidant functionality (Crozier et al., 2009). Together, these factors contributed to the higher retention of phenolic and flavonoid compounds in the freeze-dried *C. stahlianthoides* rhizomes under the conditions examined.

Total phenolic and flavonoid contents

The TPC and TFC values of the *C. stahlianthoides* extracts are presented in Table 2. The BF samples had significantly higher TPC (1.67 ± 0.08 mg GAE/g) and TFC (4.82 ± 0.17 mg QE/g) values than the BD extract (1.08 ± 0.11 mg GAE/g and 2.61 ± 0.33 mg QE /g, respectively), suggesting that freeze drying better preserved the phenolic and flavonoid compounds than sun drying. The higher phenolic and flavonoid contents in the BF samples may have been related to the low-temperature and oxygen-limited conditions of freeze drying, which helped to reduce the enzymatic oxidation and degradation of the phenolic compounds (Ghasemzadeh et al., 2016b; Latinović et al., 2024). In contrast, sun drying exposed plant tissues to UV radiation and moderate heat, which can accelerate degradation reactions affecting phenolic hydroxyl groups and glycosidic structures (Li et al., 2021).

Table 2 Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of *Curcuma stahlianthoides* rhizomes prepared by different drying methods, with retention ratios

Parameter	BD	BF	Retention ratio (%)
TPC (mg GAE/g DW)	1.08±0.11[b]	1.67±0.08[a]	154.6
TFC (mg QE/g DW)	2.61±0.33[b]	4.82±0.17[a]	184.7
DPPH (mg TE/g DW)	6.84±0.28[b]	9.35±0.24[a]	136.7
ABTS (mg TE/g DW)	7.11±0.25[b]	10.64±0.41[a]	149.6
FRAP (mg Fe^{2+}/g DW)	5.48±0.33[b]	8.22±0.26[a]	150.0

BF = freeze-dried; BD = sun-dried; Retention ratio (%) = (BF / BD) × 100; DPPH = 2,2-dphenyl-1-picrylhydrazyl; ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP = (ferric reducing antioxidant power); DW = dry weight. Values are mean ± SD (n = 3). Different lowercase superscript letters within each row indicate significant differences ($p < 0.05$) based on independent samples t-test.

Several degradation pathways may occur during sun drying. UV radiation has been reported to promote photo-oxidation of phenolic rings, leading to the formation of quinone intermediates and subsequent polymerization reactions (Crozier et al., 2009) . In addition, heat exposure in the range 35–38°C may enhance the activities of polyphenol oxidase and peroxidase, resulting in enzymatic browning and the conversion of phenolics into brown-colored pigments. Residual moisture present during sun drying can further facilitate Maillard

reactions between reducing sugars and amino acids, which may indirectly reduce the availability of reactive phenolic compounds. In contrast, freeze drying conducted at low temperature effectively limits enzymatic activity and oxidative reactions, thereby maintaining the native phenolic profile of plant tissues (Shofian et al., 2011). In addition, ice crystal formation during freezing may disrupt cellular structures, improving solvent accessibility during extraction and contributing to the higher phenolic and flavonoid content observed in the BF samples.

Although the TPC and TFC values of *C. stahlianthoides* were lower than those reported for *C. longa*, which is recognized for its high phenolic content in ethanolic extracts (Wu et al., 2024), the levels observed in the present study were within the range reported for *Zingiberaceae* species with moderate antioxidant capacity, such as *Zingiber zerumbet* (Ghasemzadeh et al., 2016a), suggesting that *C. stahlianthoides* contained phenolic and flavonoid compounds at levels that may have contributed to its biological activity.

Antioxidant activities (2,2-diphenyl-1-picrylhydrazyl, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, ferric reducing antioxidant power)

The antioxidant capacities of the extracts, evaluated based on the DPPH, ABTS and FRAP assays, are shown in Table 2. The BF extract had consistently higher antioxidant activities across all assays: DPPH (9.35 ± 0.24 versus 6.84 ± 0.28 mg TE g^{-1} DW); ABTS (10.64 ± 0.41 versus 7.11 ± 0.25 mg TE g^{-1} DW); and FRAP (8.22 ± 0.26 versus. 5.48 ± 0.33 mg Fe^{2+} g^{-1} DW). The higher antioxidant activities in the BF samples were associated with their higher total phenolic and flavonoid contents. Based on the analysis of the Pearson's correlation coefficients there were strong positive relationships between antioxidant activities and both the TPC and TFC ($r > 0.90$, $p < 0.05$), suggesting that phenolic and flavonoid compounds played important roles in the antioxidant properties of *C. stahlianthoides* (Carocho and Ferreira, 2013).

The DPPH and ABTS activities in the BF samples suggested improved radical-scavenging and electron-transfer capacities, whereas the elevated FRAP values reflected greater reducing power of preserved phytochemicals. The different antioxidant assays applied in the present study reflected distinct antioxidant mechanisms. Primarily, DPPH evaluates hydrogen atom donation and ABTS measures both hydrogen- and electron-transfer capacities, whereas FRAP reflects ferric ion reducing power (Rice-Evans et al., 1996; Priyadarsini, 2014). These findings were consistent with the ability of phenolic hydroxyl groups and curcuminoid structures to donate electrons and stabilize free radicals. In addition, the antioxidant activity in the crude extracts may have been influenced by interactions among multiple phytochemical constituents, including curcuminoids, flavonoids and other reducing compounds naturally present in the rhizomes. Similar findings have been reported in *Zingiberaceae* species, including *Zingiber officinale* (Ghasemzadeh et al., 2016b) and *Curcuma* spp. (Latinović et al., 2024).

Fig. 2 shows that the BF rhizome extract had higher retention ratios of the TPC, TFC and antioxidant capacities (DPPH, ABTS, FRAP) than the BD samples. Retention values exceeding 100% suggest that freeze drying was more effective in retaining heat- and light-sensitive phytochemicals during lyophilization. However, high retention values may not necessarily indicate an actual increase in the phytochemical content but could partly reflect improved extractability and reduced analytical loss during freeze drying. This observation was consistent with the principle that freeze drying minimizes enzymatic oxidation and thermal degradation by operating at low temperature and reduced pressure, thereby helping to maintain the structural integrity and redox potential of phenolic hydroxyl groups (Shofian et al., 2011). Furthermore, ice crystal formation during freezing may disrupt plant cellular structures, which can improve solvent penetration and extraction efficiency. By comparison, sun drying exposes plant tissues to UV radiation and elevated temperatures, which may promote the oxidative degradation of phenolic compounds (Li et al., 2021).

However, the antioxidant activities evaluated in the present study were based on *in vitro* chemical assays and may not have represented the full effects of biological antioxidants *in vivo*. Although the freeze-dried sample had a slightly lower extraction yield than the sun-dried sample, it had higher phenolic, flavonoid, curcuminoid and antioxidant activities, suggesting that there had been improved retention of antioxidant-related phytochemicals under low-temperature conditions.

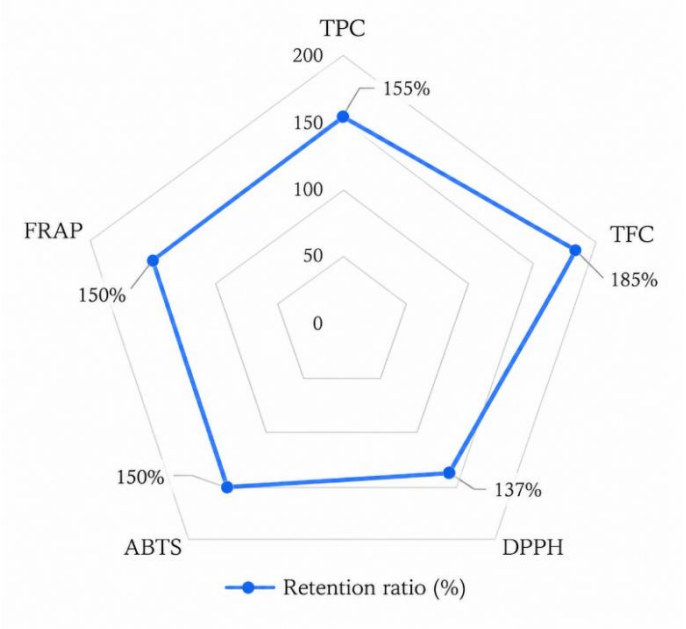


Fig. 2 Retention ratios of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities—(2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power FRAP)—of sun-dried (BD) and freeze-dried (BF) samples.

Antimicrobial activity

The ethanolic extracts of *C. stahlianthoides* had broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 3). However, larger inhibition zones were observed for the Gram-positive bacteria, *S. aureus* and *B. cereus* (16.33 ± 1.15–21.67 ± 1.15 mm) than against the Gram-negative bacteria, *E. coli* and *S.* Typhimurium (7.33 ± 0.58–11.67 ± 0.58 mm). The larger inhibition

zones against Gram-positive bacteria than Gram-negative bacteria were consistent with previous reports on ethanolic extracts from *C. longa* (Bhavsar et al., 2022). This selective activity aligned with the structural differences between Gram-positive and Gram-negative bacteria, as the outer lipopolysaccharide membrane in Gram-negative strains restricts the penetration of hydrophobic phenolic and terpenoid compounds (Nikaido, 2003). The inhibition zones of *C. stahlianthoides* ethanolic extract against *S. aureus*, *B. cereus* and *E. coli* in the present study were greater than those reported for *C. longa* ethanolic extract (Bhavsar et al., 2022). Notably, variations in antibacterial activity may be influenced by several factors, including plant cultivar, geographical origin, extraction conditions, phytochemical composition and differences in analytical methodologies.

Table 3 Antimicrobial activity of crude ethanolic extracts of *C. stahlianthoides* prepared using two drying methods against Gram-positive and Gram-negative bacteria

Extract	Inhibition zone (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. cereus*	*E. coli*	*S.* Typhimurium
BD	21.67±1.15[a]	16.33±1.15[a]	7.33±0.58[a]	11.67±0.58[a]
BF	21.33±1.53[a]	16.67±0.58[a]	7.67±0.58[a]	7 .67±0.58[b]
Negative control	NZ	NZ	NZ	NZ
Positive control	29.67±0.58	24.33±0.58	21.67±1.15	19.33±1.15

Crude ethanolic extracts = 250 mg/mL or 5 mg/disc; BF = freeze-dried, BD = sun-dried; Negative control = DMSO; Positive control = 30 µg chloramphenicol disc; NZ = no zone of inhibition. Diameters of inhibition zones include 6 mm diameter paper disc. Values = mean ± SD (n = 3). Different lowercase superscript within each bacterial strain indicate significant differences between drying methods ($p < 0.05$, t-test).

However, with the BF extract, there was no significant difference in inhibitory effect against *S. aureus* (21.33 ± 1.53 mm), *B. cereus* (16.67 ± 0.58 mm) and *E. coli* (7.67 ± 0.58 mm) compared to the BD extract (21.67 ± 1.15 mm, 16.33 ± 1.15 mm and 7.33 ± 0.58 mm, respectively), despite containing higher TPC and TFC levels (Table 2). Furthermore, the BD extract had significantly higher inhibitory effects against *S.* Typhimurium (11.67 ± 0.58 mm) than the BF extract (7.67 ± 0.58 mm) suggesting that higher TPC and TFC values did not necessarily correspond to stronger antimicrobial activity, as antimicrobial effectiveness depends on the type, structure, bioavailability and interactions of bioactive compounds in the extract (Farhadi et al., 2019). Freeze-drying preserves heat-sensitive phenolic and flavonoid compounds under low-temperature and vacuum conditions, resulting in higher phytochemical retention (Chan et al., 2009).

Nevertheless, many phenolic and flavonoid compounds contribute to antioxidant rather than antimicrobial activity (Daglia, 2012). In addition, antimicrobial activity is influenced more by the chemical structure and polarity of individual compounds than by the TPC alone (Cushnie and Lamb, 2011). Although sun drying may reduce overall phenolic and flavonoid levels, in addition, it can promote the formation of smaller or more bioactive compounds with comparable antibacterial effects (Vega-Galvez et al., 2009). Furthermore, bacterial inhibition may reach a saturation point at certain extract concentrations, limiting further enhancement of antimicrobial activity despite higher TPC and TFC values in freeze-dried extracts.

The MIC results are presented in Table 4 and Fig. 3. The lower MIC values for the Gram-positive bacteria (12.5–25 mg/mL) than for the Gram-negative bacteria (50–200 mg/mL) indicated higher sensitivity, consistent with other reports on *C. longa* and turmeric extracts (Burapan et al., 2020; Bhavsar et al., 2022; Odo et al., 2023). The MIC values of the *C. stahlianthoides* ethanolic extract against *S. aureus* and *B. cereus* in the present study (12.5–25 mg/mL) were relatively low compared to other *Curcuma* species such as *C. longa* (20.8–36.43 mg/mL), as reported by Bhavsar et al. (2022). Table 4 shows that the BF and BD extracts had similar MIC values against *B. cereus* (12.5 mg/mL), *S. aureus* (25 mg/mL) and *E. coli* (200 mg/mL). However, there was a lower MIC value for the BF extract against *S.* Typhimurium (50 mg/mL) compared to the BD extract (100 mg/mL), indicating the greater antibacterial potency of the BF extract against this bacterium. Although the BD extract produced larger inhibition zones against *S.* Typhimurium at concentrations of 100 mg/mL, 200 mg/mL and 250 mg/mL than the BF extract, no inhibitory zone was observed for the BD extract at concentrations below 100 mg/mL. These findings suggested that the BD extract may have stronger antibacterial activity at higher concentrations, whereas the BF extract had antibacterial effectiveness against *S.* Typhimurium even at lower concentrations.

The enhanced antimicrobial activity of the BF extract may be linked to its higher phenolic and curcuminoid contents, which have been shown to disrupt bacterial membranes, inhibit Adenosine triphosphate synthesis and interfere with quorum sensing mechanisms (Prasad et al., 2014; Dai et al., 2022). Similar antibacterial effects have been described for essential oils and ethanolic extracts from other *Curcuma* species, including *C. aeruginosa*, *C. mangga* and *Zingiber cassumunar*, where activity was related to sesquiterpene and curcuminoid composition (Kamazeri et al., 2012). These observations in the present study were consistent with the phytochemical profile of the freeze-dried extract, which retained higher levels of thermo-labile bioactive compounds.

Although the crude ethanolic extracts of *C. stahlianthoides* had moderate antibacterial potency compared to purified curcumin, which has been reported to show substantially lower MIC values (Dai et al., 2022), the antibacterial effects observed in the present study suggested their potential relevance for applications as natural preservatives or functional food ingredients, particularly in formulations requiring mild preservative activity.

Table 4 Minimum inhibitory concentration (MIC) of crude ethanolic extracts of *C. stahlianthoides* prepared using two drying methods against Gram-positive and Gram-negative bacteria

Extract	Concentration (mg/mL)	Inhibition zone (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*B. cereus*	*E. coli*	*S.* Typhimurium
BD	12.5	NZ	7.04±0.05	NZ	NZ
	25	7.12±0.12	8.21±0.81	NZ	NZ
	50	9.04±0.22	9.50±0.54	NZ	NZ
	100	13.32±1.05	10.36±0.69	NZ	8.21±0.23
	200	19.50±1.33	15.02±1.20	7.20±0.18	9.24±0.25
	250	23.15±1.05	17.33±1.04	7.32±0.11	12.17±0.84
	MIC	25	12.5	200	100
BF	12.5	NZ	7.05±0.01	NZ	NZ
	25	7.01±0.04	7.02±0.01	NZ	NZ
	50	8.34±0.09	8.26±0.05	NZ	7.02±0.01
	100	10.41±0.07	12.02±0.04	NZ	7.05±0.02
	200	17.62±0.89	14.60±0.57	7.01±0.02	8.12±0.07
	250	20.22±1.02	16.28±0.09	7.05±0.01	9.24±0.04
	MIC	25	12.5	200	50
Negative control		NZ	NZ	NZ	NZ
Positive control		29.68±0.16	24.18±0.37	21.13±0.99	19.23±0.64

BF = freeze-dried, BD = sun-dried. NZ = no zone of inhibition; MIC = lowest concentration of extract producing visible inhibition zone. Diameters of inhibition zones include 6 mm diameter paper disc. Values = mean ± SD (n = 3).

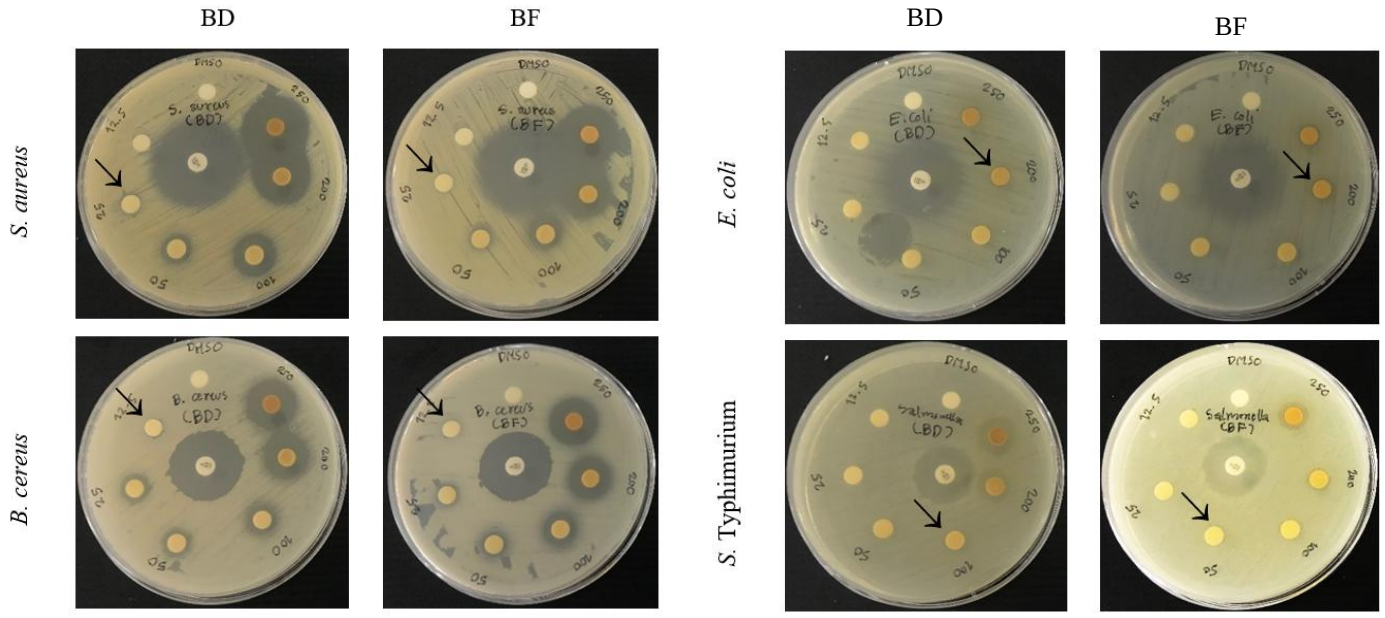


Fig. 3 Minimum inhibitory concentration (MIC) of *C. stahlianthoides* crude ethanolic extracts prepared using sun drying (BD) and freeze drying (BF) against Gram-positive bacteria (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli* and *S.* Typhimurium), where arrows (→) indicate MICs showing visible inhibition zones

Application potential

Based on the combined results from the proximate, phytochemical and biological analyses, *C. stahlianthoides* shared several biochemical characteristics with its better-known relatives, *C. longa* and *C. comosa*. However, the moderate levels of phenolic compounds and curcuminoids in *C. stahlianthoides* suggest a relatively milder antioxidant capacity. This characteristic was accompanied by a favorable nutritional profile and detectable antimicrobial activity.

From a practical standpoint, freeze drying appeared to be more effective for maintaining antioxidant-related bioactive compounds in *C. stahlianthoides*. The low-temperature conditions applied during freeze drying helped to protect thermo-labile curcuminoids and phenolic compounds, thereby contributing to improved retention of functional properties in the resulting extracts. Based on these findings, *C. stahlianthoides* may be considered a potential candidate for further investigation as a functional food ingredient or a source of natural preservatives compounds.

Future studies should focus on optimizing the extraction efficiency using advanced techniques, such as ultrasonic-assisted extraction or natural deep eutectic solvent systems (Dai et al., 2022), as well as on detailed structural characterization of curcuminoid derivatives using HPLC–mass spectrometry. In addition, *in vivo* studies are required to evaluate bioavailability, efficacy and safety prior to potential commercial applications.

Conclusions

This study examined the nutritional composition, phytochemical contents, antioxidant capacity and antimicrobial activity of *C. stahlianthoides*, a rare endemic species from Thailand. Based on the results, freeze drying was more effective than sun drying in retaining macronutrients and bioactive compounds, resulting in higher phenolic and flavonoid contents, curcuminoid levels and antioxidant and antibacterial activities, particularly against Gram-positive bacteria. Although the antioxidant capacity of *C. stahlianthoides* was lower than that reported for *C. longa*, the present findings suggested that this species may have potential for use as a natural ingredient in functional foods or related products. Further studies are needed to identify active compounds, optimize extraction conditions and evaluate bioavailability and safety.

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Declaration of generative AI in scientific writing

In this work, the authors used AI tools only to help check grammar and make the writing clearer. AI was not used to create any scientific content or analyze the data. All results, interpretations and conclusions come directly from the authors. The authors take full responsibility for the accuracy, veracity and originality of all information presented in this article.

CRediT author statement

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