Antioxidant and Antimicrobial Activity of Zingiberaceae Plants in Taiwan

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Abstract The rhizomes of the Zingiberaceae family are a vegetable widely used in many Asian countries, and their medicinal functions have been broadly discussed and accepted in many traditional recipes. In this study, 18 species of five genus of Zingiberaceae plants from Taiwan area were collected and analyzed for their functional properties. Methanolic extracts of the plants were analyzed for their total phenol compounds, α, α -diphenyl- β -picrylhydrazyl (DPPH) scavenging activity, and reducing power. Antimicrobial activity of these samples was also determined. The results showed that the total phenol compounds of the *Alpinia* genus averaged 17, 30 mg/g for *Curcumas*, and the highest, 36.5 mg/g for *Vanoverberghia sasakiana*. Antioxidant performances were best observed in *Vanoverberghia* and *Hedychium*, both 89%, and DPPH scavenging activity followed similar trends. Particularly, *Zingiber oligophyllum*, considered as a traditional medicinal plant used in Taiwan exhibited low DPPH scavenging activity and reducing power. Most Zingiberaceae plant extracts exhibited antimicrobial activity against all tested food microorganisms. *Hedychium* and *Vanoverberghia*, did not show antimicrobial activities on *Escherichia coli* and *Vibrio parahaemolyticus*. This study is a positive demonstration of the utility of screening Taiwan's endemic Zingiberaceous plants for their food and medicinal uses.

Keywords Antimicrobial · Antioxidant · Ginger · Reducing power · Zingiberaceae

Introduction

The Zingiberaceous plants are characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties. It is commonly known as ginger, and exists in about 50 genera and 1,300 species worldwide, distributed mainly in South and Southeast Asia [1]. Turmeric of Zingiberaceous plants in powder form is widely applied as a food additive in many Asian countries. Medicinal functions for treatment of diseases such as diarrhea, coryza, dermatosis disorders and rheumatism are also widely mentioned in traditional remedies [2–5].

Zingiberaceae plants contain many essential oils, including terpenes, alcohols, ketones, flavonoids, carotenoids and phytoestrogens [6–8]. For instance, the water extract of *Zingiber officinale* exhibits 6-Gingerol, and is mostly found in the rhizome in concentrations of 130–7,138 ppm. The major functional compound in *Curcuma* turmeric is curcumin, which has concentrations as high as 38,000 ppm in certain species [8]. Less-polar constituents including curcuminoids, kava pyrones and gingerols isolated from Zingiberaceous plants, which have been reported for their biological activities in antifungal, antioxidant, insecticidal, and anti-inflammatory activities [9–11] are particularly important and relevant for these applications.

This study reveals the bioactive and medicinal potentials of ginger species in Taiwan. Six genera and 25 species of Zingiberaceae plants are native to Taiwan. Among these, *Alpinia* is the largest genus, with 14 species recorded so far [12]. The antioxidant and antimicrobial properties of Taiwan's endemic and imported Zingiberaceae plants have

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not yet been thoroughly explored [7]. This work evaluated the antioxidant and antimicrobial properties of 18 Taiwan endemic species collected from all over the island. The concentrations of phenolic compounds, antioxidant capacity, α, α -diphenyl- β -picrylhydrazyl (DPPH) scavenging activity and reducing power of the samples were determined. The activities of ginger rhizomes against different food-related microorganism were also measured. This investigation evaluates the potential medicinal applications of Taiwan's endemic Zingiberaceae plants.

Materials and Methods

Rhizome Collection

Plant materials were collected and purchased from the Taiwan Endemic Species Research Institute, the Council of Agriculture, traditional markets, and local growers from whole Taiwan Island. The rhizomes were removed from the plant and cleaned for use.

Sample Treatment and Extraction

Peeled and washed samples were further chopped into 2×2 cm dice and dried with freeze-dryer (Freeze dryer Alpha 1-2/LD-2, Vacuum pump RZ-5, Christ, Germany) for 48 h. Dried samples were subsequently milled using commercial hand-carry milling machine. For methanol extraction, sample powders (1.25, 2.5, 3.75, or 5 g) were mixed with 20 ml methanol (Sigma, St. Louis, MO, USA) in a rotary shaker for 12 h. The mixtures were then filtered (Whatman No.1, Middlesex, England). The filtrate was then concentrated in a rotary evaporator (Büchi R-210, Flawil, Switzerland) until dried. The weight of extract was then measured and recorded. The yield of methanolic extract was expressed as milligram of extract per gram of ginger fresh weight, calculated as follows:

Yield = weight of Rotavapor dried sample (mg)/fresh sample (g) $\times$ 100%

Total Phenolics Determination

The total phenolics content of ginger extracts was determined by the Folin–Ciocalteu method [13]. Briefly, 0.5 ml diluted extract solution was shaken for 1 min with 100 μ l of Folin–Ciocalteu reagent and 6 ml of distilled water. The mixture was shaken and 2 ml of 15% Na_2CO_3 were added and shaken once again for 30 s. Finally, the solution was brought up to 10 ml by adding distilled water. After 1.5 h, the absorbance at 750 nm was evaluated using a spectrophotometer (Beckman Coulter DU-640, CA, USA). The results were expressed as gallic acid equivalents.

Antioxidant Activity Determination

The antioxidant capacity of samples was measured by the method described by Miller and Rice-Evans, and Arnao et al. [14, 15] with little modifications. Peroxidase (4.4 units/ml, Sigma, MO, USA), H_2O_2 (50 μ M, Merck, Germany), 2, 2-azino-bis (3-ethylbenz-thiazoline-6-sulphonic acid) [100 μ M, Sigma, MO, USA] and distilled water (1 ml) were mixed and kept in dark for 1 h for reaction. One ml of plant extract was subsequently added and determined by absorbance at 734 nm. The antioxidant capacity was calculated by the following formula:

Antioxidant activity (%) = $[1 - (\text{A}_{734 \text{ nm, sample}} / \text{A}_{734 \text{ nm, control}})] \times 100$

DPPH Free Radical Scavenging Activity Test

The capacity of Zingiberaceae to remove 1,1-diphenyl-2-picryl-hydrazyl radical (Sigma, MO, USA) was determined by the method described by Shimada et al. [16]. Briefly, 1 ml of methanolic extract and 5 ml of freshly prepared 0.1 mM DPPH methanolic solution were thoroughly mixed and kept in the dark for 60 min. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer. The blank was prepared by replacing the extract with methanol (1 ml). The percentage of free radical scavenging activity was calculated as follows:

Scavenging activity (%) = $[1 - (\text{A}_{517 \text{ nm, sample}} / \text{A}_{517 \text{ nm, blank}})] \times 100$

Reducing Power Test

Reducing power was measured according to the method described by Duh and Yen [17]. One ml of plant extract, phosphate buffer (0.2 M, pH 6.6, 0.5 ml), and potassium hexacyanoferrate solution (1% v/w, 2.5 ml; both from Merck) were placed in a test tube and heated at 50 °C for 20 min. The tube was cooled on ice and 0.5 ml 10% trichloroacetic acid (Merck) were added. After centrifugation at 3,000 $\times$ g for 10 min, a 1 ml of aliquot supernatant was mixed with 1 ml distilled water and 0.1 ml ferric chloride (0.1%) and reacted for 10 min. Finally, the absorbance at 700 nm was measured, increased absorbance of the reaction mixture indicated increased reducing power.

Antimicrobial Activity

To screen the activity of methanolic extracts of Zingiberaceae plants against food related microorganism, a filter paper disc method was used as described by Gülçin et al. [18]. *Escherichia coli* Bioresources Collection and Research Center (BCRC) 10675, *Salmonella enterica* BCRC 10242, *Staphylococcus aureus* spp. aureus BCRC 10451 and *Vibrio*

parahaemolyticus BCRC 12863 were purchased from BCRC, Republic of China. *E. coli* stock cultures were maintained on potato dextrose agar (Difco, Detroit, MI, USA) and the other three were kept on nutrient agar (Difco, Detroit, MI, USA) according to the culture manual. Culture concentrations of 1×10^8 colony-forming unit/ml were plated on their respective agar plates. Sterile filter paper discs with 6 mm diameter (Whatman) were immersed with 500 μ l of Zingiberaceae extract. After thorough absorption, they were then placed on agar plate for cultivation. *Salmonella* plates were incubated at 30 °C, while *E. coli*, *Staphylococcus* and *Vibrio* plates were kept at 37 °C for 72 h. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. Amoxicillin–clavulanic acid (ACA; 20 μ g /1 ml per disc); ofloxacin (20 μ g /1 ml per disc; Sigma) were used as references. Ginger extracts with antimicrobial activity were serially diluted into concentration ranging from 1.0–0.01 mg/ml for determination of their minimum inhibitory concentration (MIC) according to Habsah et al. [6] and Wilson et al. [19].

Results

Extraction Yield

Extraction yield was expressed as weight (mg) of crude extract per gram of vacuum-dried plant material as shown in

Table 1. Extraction yield ranged from lowest 41 mg/g (*Alpinia pricei* Hayata) to highest 127 mg/g (*Alpinia zerumbet* (Pers.) Burt & Smith), notably *Vanoverberghia sasakiana*, the endemic species Taiwan has second high in extraction rate of 177 mg/g. Average extraction rate was found to be 0.084% (84 mg/g). Habsah et al. [6] has displayed the methanolic extraction rate of *Alpinia*, *Costus* and *Zingiber*, which ranged 0.09–0.57% in various samples. In general, freeze drying preserves more reducing power of plant extract, such as yam (*Dioscorea* sp.) extract as compared to hot-air drying and drum drying [20]. The color and appearance of our vacuum-dried samples remained unchanged and intact. *V. sasakiana* showed a 36.5 mg/g of total phenol content (Table 1), highest in Taiwan endemic species.

Antioxidant Capacity, DPPH Scavenging and Reducing Power

On average, antioxidant activity of our nine *Alpinia* samples was 67%, which was the lowest of the total antioxidant performance. The activity was highest in *Vanoverberghia* and *Hedychium*, both 89% (Table 2). In DPPH scavenging activity and reducing power, *Vanoverberghia* and *Hedychium* extract exhibited similar levels as those of total antioxidant contents. Particularly, *Zingiber* sp. exhibited poorest performances in DPPH and reducing power. The rest of the Zingiberaceae plant extracts displayed an average of 70–78% in DPPH scavenging properties and 0.87 (*Alpinia*) to 1.13 (*Curcuma*) in absorbance A_{700} (Table 2).

Table 1 Extraction yield and contents of total phenolics of gingers

	Species	Collection site	Extraction yield (mg/g)	Total phenols (mg/g)
1	<i>Alpinia japonica</i> (Thunb.) Mig	Hsinchu county	58.8 $\pm$ 7.7	18.3 $\pm$ 6.3
2	<i>Alpinia kawakamii</i> Hayata	TESRI, Jiji township, Nantou county	79.3 $\pm$ 15.6	18.7 $\pm$ 3.4
3	<i>Alpinia kusshakuensis</i> Hayata	Sindian city, Taipei county	64.3 $\pm$ 6.4	19.5 $\pm$ 1.45
4	<i>Alpinia mesanthera</i>	Fusing township, Taoyuan county	82.6 $\pm$ 5.8	19.5 $\pm$ 4.3
5	<i>Alpinia officinarum</i>	Sansia township, Taipei county	52.2 $\pm$ 6.3	19.3 $\pm$ 2.5
6	<i>Alpinia pricei</i> Hayata	Hengchun, Pingtung county	41.1 $\pm$ 11.7	19.2 $\pm$ 5.1
7	<i>Alpinia shimadai</i>	Jhudong township, Hsinchu county	107.5 $\pm$ 19.4	20.2 $\pm$ 5.3
8	<i>Alpinia uraiensis</i> Hayata	Yilan county	89.5 $\pm$ 13.9	17.6 $\pm$ 1.5
9	<i>Alpinia zerumbet</i> (Pers.) Burt & Smith	TESRI ^a , Jiji township, Nantou county	127.5 $\pm$ 3.8	15.3 $\pm$ 2.2
10	<i>Costus speciosus</i> (Koenig) Smith	Sioushuei township, Changhua county	93.2 $\pm$ 9.7	22.9 $\pm$ 2.3
11	<i>Curcuma domestica</i>	Jhongpu township, Chiayi county	87.1 $\pm$ 6.3	35.6 $\pm$ 5.5
12	<i>Curcuma longa</i> L.	Ren-ai township, Nantou county	94.2 $\pm$ 7.5	21.4 $\pm$ 1.7
13	<i>Curcuma viridiflora</i>	Fongshan city, Kaohsiung county	91.4 $\pm$ 5.8	29.4 $\pm$ 3.7
14	<i>Curcuma zedoaria</i>	Pingtung city, Pingtung county	92.5 $\pm$ 7.6	33.4 $\pm$ 5.7
15	<i>Hedychium coronarium</i> Koenig	Jinshan county, Taipei county	32.6 $\pm$ 5.8	25.8 $\pm$ 4.4
16	<i>Vanoverberghia sasakiana</i>	Lanyu township, Taitung county	117 $\pm$ 12.6	36.5 $\pm$ 8.9
17	<i>Zingiber kawagooi</i> Hayata	Beinan township, Taitung county	95.6 $\pm$ 7.4	28 $\pm$ 2.9
18	<i>Zingiber oligophyllum</i> K. Schumann	Jhongpu township, Chiayi county	74 $\pm$ 4.3	20 $\pm$ 4.5

^a All value in this table represent the mean $\pm$ SD ($n=3$)

Table 2 Multiple antioxidant capacity of gingers

Ginger sample	Antioxidant capacity (%)	DPPH free radical scavenging activity (%)	Reducing power (absorbance 700 nm)
1 <i>Alpinia japonica</i> (Thunb.) Mig	69.5 $\pm$ 6.7 ^b	71.8 $\pm$ 6.4	0.8 $\pm$ 0.2
2 <i>Alpinia kawakamii</i> Hayata	73.5 $\pm$ 4.6	76.7 $\pm$ 5.3	0.9 $\pm$ 0.1
3 <i>Alpinia kushshakuensis</i> Hayata	70.9 $\pm$ 5.67	76.7 $\pm$ 6.3	0.8 $\pm$ 0.1
4 <i>Alpinia mesanthera</i> Hayata	62.3 $\pm$ 7.6	64.6 $\pm$ 7.4	0.8 $\pm$ 0.3
5 <i>Alpinia officinarum</i>	75.4 $\pm$ 9.6	80.5 $\pm$ 9.3	1.4 $\pm$ 0.2
6 <i>Alpinia pricei</i> Hayata	70.6 $\pm$ 7.9	74.9 $\pm$ 5.4	0.7 $\pm$ 0.15
7 <i>Alpinia shimadai</i>	72.2 $\pm$ 1.3	69.3 $\pm$ 7.3	1.0 $\pm$ 0.3
8 <i>Alpinia uraiensis</i> Hayata	62.5 $\pm$ 5.8	68.6 $\pm$ 4.3	0.8 $\pm$ 0.4
9 <i>Alpinia zerumbet</i> (Pers.) Burt & Smith	53.8 $\pm$ 8.6	59.4 $\pm$ 3.5	0.47 $\pm$ 0.2
10 <i>Costus speciosus</i> (Koenig) Smith	81.3 $\pm$ 6.7	78.5 $\pm$ 4.4	1.3 $\pm$ 0.3
11 <i>Curcuma domestica</i>	89 $\pm$ 7.4	81.3 $\pm$ 6.3	1.6 $\pm$ 0.4
12 <i>Curcuma longa</i> L.	76.1 $\pm$ 5.1	72.1 $\pm$ 5.2	1.2 $\pm$ 0.5
13 <i>Curcuma viridiflora</i>	53 $\pm$ 5.3	76.4 $\pm$ 5.0	1.1 $\pm$ 0.2
14 <i>Curcuma zedoaria</i>	76 $\pm$ 6.4	65.4 $\pm$ 6.1	0.9 $\pm$ 0.2
15 <i>Hedychium coronarium</i> Koenig	89.6 $\pm$ 11.6	90.1 $\pm$ 7.2	1.6 $\pm$ 0.3
16 <i>Vanoverberghia sasakiana</i> Funak. & H. Ohashi	89 $\pm$ 6.9	89.5 $\pm$ 7.1	1.01 $\pm$ 0.2
17 <i>Zingiber kawagooi</i> Hayata	79 $\pm$ 4.8	42 $\pm$ 7.8	0.9 $\pm$ 0.08
18 <i>Zingiber oligophyllum</i> K. Schumann	88 $\pm$ 5.9	32 $\pm$ 9.3	0.34 $\pm$ 0.08

^a Concentration of each methanolic extract was adjusted to 100 mg/ml

^b All values in this table represent the mean $\pm$ SD ($n=3$)

Antimicrobial Activity

Most Zingiberaceae plant extracts exhibited antimicrobial activity against all tested microorganisms (Table 3). Few

Alpinia sp. showed no inhibition zone against *Vibrio*. Three cultivars *Alpinia japonica*, *Alpinia kawakamii* and *Alpinia kushshakuensis*, now acclimated cultivars in Taiwan, showed resistance to all of the four microorganisms (Table 3).

Table 3 Antimicrobial activities of gingers, and amoxicillin–clavulanic acid, ofloxacin (parenthesis indicates minimum inhibitory concentration (mg/ml) for antimicrobial activity of Zingiberaceae extracts)

Species	<i>Escherichia coli</i>	<i>Salmonella enterica</i>	<i>Staphylococcus aureus</i>	<i>Vibrio parahaemolyticus</i>
1 <i>Alpinia japonica</i> (Thunb.) Mig	13 ^a (0.05)	8 (0.05)	8 (0.08)	15 (0.05)
2 <i>Alpinia kawakamii</i> Hayata	12.5 (0.05)	9 (0.05)	7 (0.05)	12 (0.05)
3 <i>Alpinia kushshakuensis</i> Hayata	7 (0.05)	10 (0.08)	9 (0.05)	8 (0.05)
4 <i>Alpinia mesanthera</i>	9 (0.05)	10 (0.05)	13 (0.08)	11 (0.05)
5 <i>Alpinia officinarum</i>	11 (0.05)	–	5 (0.05)	–
6 <i>Alpinia pricei</i> Hayata	4 (0.05)	9 (0.05)	10 (0.05)	–
7 <i>Alpinia shimadai</i>	9 (0.08)	11 (0.05)	9 (0.05)	–
8 <i>Alpinia uraiensis</i> Hayata	12 (0.05)	12 (0.05)	14 (0.08)	–
9 <i>Alpinia zerumbet</i> (Pers.) Burt & Smith	6 (0.05)	7 (0.08)	9 (0.05)	8 (0.05)
10 <i>Costus speciosus</i> (Koenig) Smith	11 (0.05)	8 (0.05)	11 (0.05)	–
11 <i>Curcuma domestica</i>	15 (0.05)	17 (0.05)	20 (0.05)	18 (0.05)
12 <i>Curcuma longa</i> L.	17 (0.05)	6 (0.05)	–	13 (1.0)
13 <i>Curcuma viridiflora</i>	15.6 (0.05)	19 (1.0)	18 (0.05)	20 (0.05)
14 <i>Curcuma zedoaria</i>	16 (0.05)	13.5 (0.05)	11 (0.05)	–
15 <i>Hedychium coronarium</i> Koenig	–	9 (0.05)	14 (0.05)	–
16 <i>Vanoverberghia sasakiana</i>	–	8 (0.05)	9 (0.05)	–
17 <i>Zingiber kawagooi</i> Hayata	11 (0.05)	9 (0.02)	11 (0.05)	8 (0.05)
18 <i>Zingiber oligophyllum</i> K. Schumann	9 (0.08)	–	6.5 (0.05)	8 (0.08)
19 ACA ^b	20	18	15	15
20 Ofloxacin ^c	18	15	–	12

^a Diameter of zone (mm)

^b Amoxicillin–clavulanic acid (20 μ g /1 ml per disc)

^c Ofloxacin (20 μ g /1 ml per disc)

Fig. 1 Effect of *Curcuma* extracts on the growth of *E. coli* (a), *Salmonella enterica* (b), *Staphylococcus aureus* (c) and *Vibrio parahaemolyticus* (d)

Curcuma sp., of which the antimicrobial properties have been widely studied [3, 6, 8], also exhibited strong inhibition in this study. However, *Hedychium* and *Vanoverberghia*, both with high antioxidant activities did not showed antimicrobial activities on *E. coli* and *V. parahaemolyticus* (Table 3). The species which performed best in the inhibition zone tests, *Curcuma domestica* and *Curcuma viridiflora* were chosen for the further test of inhibition in culture broths. Figure 1 shows that two of the *Curcuma* species have similar antimicrobial activities as ACA and ofloxacin. Wilson et al. [19] compared the antimicrobial effects of *Curcuma zedoaria* and *Curcuma malabarica* against *S. aureus*. Their finding indicated that *C. zedoaria* does not inhibit growth of this food bacteria and our results were similar to it. Three of the other *Curcuma* species exhibited strong inhibition against above food bacteria. Generally, most of the plant extracts gave a MIC of 0.05 mg/ml (Table 3). Notably, *C. zedoaria* showed MIC of 0.05 mg/ml against *Staphylococcus*, the results were not similar to that of Wilson et al. [19]. This could be explained because different microorganisms were used and different extraction procedures were used. In general, methanolic extracts of samples were almost completely

active against *E. coli*, *S. enterica* and *S. aureus*, but not for *V. parahaemolyticus*. The MIC ranged from 0.05 to 1.0 mg/ml (Table 3), among these, *A. japonica*, *A. mesanthera* and *A. uraiensis* need higher concentration to inhibit *Staphylococcus* (0.08 mg/ml). However, in the findings of Habsah et al. [6], most potent inhibitory activity against *Bacillus subtilis* and *Staphylococcus* was brought by *Alpinia mutica* and *Aspergillus ochraceous*. Inconsistence of concentration may due to species variation.

Discussion

Zingiberaceae plants have received much attention, since they produce many complex compounds that are useful in food as herbs and spices, flavoring and seasoning, and in the cosmetics and medicinal industries as antioxidant and antimicrobial agents. The theory of oxygen-stress on aging and age-degenerated diseases places an importance on daily use of natural phytochemicals and compounds [21]. Bhandari et al. [22] administered an ethanolic extract of *Z. officinale* abstract for diabetic rats. A dosage of 200 mg/kg produced effects such as lowered serum total cholesterol

and triglycerides, and increased high-density lipoproteins-cholesterol levels. The *Zingiber* extract also significantly reduced lipid peroxidation in tissues [23]. However, they also found that another *Zingiber* species, *Zingiber oligophyllum*, does not support medicinal effects of Bernd's reports in the *in vitro* antioxidative test system. The total phenol, DPPH and reducing power of *Z. oligophyllum* were found to be less than those of other genera. Most of the Zingiberaceae plants were consumed fresh and some were concentrated for external use. Different cultivars have complicated contents and will contain unidentified components. Taiwan has a diversity of flora environment and subtropical climate pattern, after their adaptation to this natural environment, the antioxidant and antimicrobial activities of the imported-species and native Zingiberaceae plants in Taiwan remain unknown. This work is the first to investigate thoroughly the antioxidant and antimicrobial activity of 18 species of commonly consumed native Zingiberaceae plants in five genera in Taiwan. All *in vitro* system of Taiwan Zingiberaceae plants is reported. The average antioxidant activity of various species of *Alpinia*, the most widely distributed and diversified genus, is reported as 67%. *Curcuma* exhibits rather strong antioxidant properties, as described in past studies. Interestingly, *Vanoverberghia*, is a new species discovered in year 2000 [24], currently is an endemic species in Orchid Island, Taitung, Taiwan. *Hedychium* is used as a traditional remedy for flu and fever in aborigine Taiwan. Two of these genres were found to have the highest antioxidation performance, 89% for each. The search for novel natural compounds to suppress body oxidation in modern society will always continue. The high antioxidant and antimicrobial effects of species endemic to Taiwan, including *Vanoverberghia* and *Hedychium*, has also expanded our focus beyond *Curcuma* sp.

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