Antioxidant and antibacterial activities of flavonoids and curcuminoids from *Zingiber spectabile* Griff.

Yasodha Sivasothy a, Shaida Fariza Sulaiman b, Kheng Leong Ooi b, Halijah Ibrahim c, Khalijah Awanga,⁎

a Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia
b School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
c Institute of Biological Sciences, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia

**Abstract**

The antioxidant potential of spectaflavoside A (1) along with kaempferol and its four acetylhamnosides (2–6), demethoxycurcumin (7) and curcumin (8), isolated from the rhizomes of *Zingiber spectabile* was evaluated using three different assays; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power assay (FRAP) and β-carotene bleaching assay, while their antibacterial activities against eight different food-borne bacteria; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *Bacillus cereus*, *Bacillus licheniformis* and *Staphylococcus aureus* were determined using broth microdilution assay. The highest antioxidant activity in all assays was demonstrated by kaempferol (6), with more that 89% of activity. This was followed by curcumin (8) and demethoxycurcumin (7). These two curcuminoids were found to have the potential in extending the shelf-life of different food products as compared with other tested compounds due to the higher antioxidant activities that are ranging from 56.27% in FRAP assay to 77.27% in β-carotene bleaching assay, and minimum inhibitory concentrations against six food-borne bacteria are ranged from 62.50 μg/mL to 500 μg/mL. Hence, this would suggest that the rhizomes of *Z. spectabile* may be a promising source of natural preservative in the food industry.

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**1. Introduction**

Microbial contamination is also an important issue leading to the deterioration and poisoning of food products by food-borne pathogens (Ebrahimabadi et al., 2010; Ebrahimim, Hadian, Mirjalilii, Sonboli, & Yousefzadi, 2008). Food additives with antioxidant and antimicrobial properties can be applied to extend the shelf-life of food and maintain their safety, nutritional quality, functionality, and palatability. The preference for naturally and biologically produced antioxidants and antimicrobials in protecting the human body from diseases and reducing food spoilage has become increasingly popular rather than those of synthetic origin which have undesirable side effects (Bounatirou et al., 2007; Ebrahimabadi et al., 2010).

The plant kingdom with a remarkable diversity in producing natural compounds has attained special interest and, today, accessing to plant materials with dual antioxidant and antimicrobial capabilities is an ideal goal in the field of research on food additives (Bounatirou et al., 2007; Ebrahimabadi et al., 2010). Essential oils and nonvolatile secondary metabolites especially those rich in phenolic secondary metabolites, in particular the flavonoids, from edible and medicinal plants, culinary herbs and spices have been effectively employed for this purpose (Ho, Noryati, Sulaiman, & Rosma, 2010; Wong & Kitts, 2006). They must be non-toxic, inexpensive, effective, have a carry-through effect during processing and not alter the quality and sensory property of the end-product (Jayaprakasha, Rao, & Sakariah, 2006).

*Zingiber spectabile* Griff., locally known as *tepus tanah*, is native in the moist lowland forests of Peninsular Malaysia. The leaves and rhizomes are used to flavour food. Its young rhizomes are sliced, soaked in vinegar and used as an appetizer (Jones, 1993). They must be non-toxic, inexpensive, effective, have a carry-through effect during processing and not alter the quality and sensory property of the end-product (Jayaprakasha, Rao, & Sakariah, 2006).

*Zingiber spectabile* Griff, along with kaempferol-3-O-(3′,4′-di-O-acetyl)-α-L-rhamnopyranoside (2), kaempferol-3-O-(2′,3′-di-O-acetyl)-α-L-rhamnopyranoside (3), kaempferol-3-O-(2′,4′-di-O-acetyl)-α-L-rhamnopyranoside (4),...
kaempferol-3-O-(4″-O-acetyl)-α-L-rhamnopyranoside (5), kaempferol (6), demethoxycurcumin (7) and curcumin (8), from the dichloromethane and ethyl extracts of its rhizomes (Fig. 1) (Sivasothy et al., 2012). Since the rhizomes of Z. spectabile are found to be rich in phenolic secondary metabolites, this prompted us to investigate their antioxidant and antibacterial properties as alternative sources of natural preservative agents to increase the shelf-life of food. Thus the antioxidant activities of the isolates from the rhizomes of Z. spectabile were investigated via three different assays; DPPH-radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and β-carotene bleaching assay. Their antibacterial activities against Gram-negative strains; Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhimurium and Vibrio parahaemolyticus, and Gram-positive strains; Bacillus cereus, Bacillus licheniformis and Staphylococcus aureus, known to cause infections and food poisoning, were also evaluated.

2. Materials and method

2.1. Plant material and chemicals

Z. spectabile was collected from Negeri Sembilan in November 2009 and a voucher specimen was deposited with the University Malaya herbarium (KU 0108). β-carotene, 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), 4,4′-(3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl) bisbenzenesulfonic acid (ferrozine), linoleic acid and 2,4,6-tripryidyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (Springfield, NJ). Dimethyl sulphoxide (DMSO) and p-iodonitrotetrazolium chloride (INT) were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Ferric chloride hexahydrate, ferrous chloride hexahydrate, ferrous chloride hexahydrate, and Tween 80 were obtained from Fluka (Switzerland), while acetic acid and hydrochloric acid (HCl) were purchased from Fisher Scientific (Springfield, NJ). Dimethyl sulphoxide (DMSO) and p-iodonitrotetrazolium chloride (INT) were purchased from Sigma-Aldrich Chemical (St. Louis, MO) while Tween 80 and the nutrient broth were purchased from Fluka (Switzerland) and Oxoid (England), respectively.

2.2. Isolation of compounds 1–8

Compounds 1–8 were isolated and characterized according to the methods described previously (Sivasothy et al., 2012).

2.3. Antioxidant assay

2.3.1. DPPH radical scavenging assay

The free radical scavenging activity of the isolates was measured using DPPH assay as described by Ramos et al. (2003) with some modifications. In a well of a 96-well plate, 50 μL of each compound (with an initial concentration of 1 mg/mL) was added to 150 μL of Ethanolic DPPH solution (300 μM). For the negative control, only 50 μL of DMSO was added to the DPPH solution. The decrease in absorbance was determined at 515 nm using a Multiskan EX microplate reader (Thermo Scientific, Finland) after 30 min of incubation at 37 °C. The capacity of the sample to prevent the oxidation of DPPH scavenging activity was measured as follows: % inhibition = ([absorbance of negative control at 0 min – absorbance of sample at 0 min]/absorbance of negative control at 0 min) × 100%. All experiments were performed in three replicates.

2.3.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay developed by Benzie and Strain (1996) was modified to be performed in a 96-well plate. The FRAP solution was prepared by adding 10 mL of acetate buffer 300 mM (which was adjusted to pH 3.6 by the addition of acetic acid) to 1 mL of ferric chloride hexahydrate 20 mM (dissolved in distilled water) and 1 mL of TPTZ 10 mM (dissolved in HCl 40 mM). The FRAP solution was then incubated at 37 °C for 5 min. In a well of a 96-well plate, 50 μL of each compound (with an initial concentration of 1 mg/mL) was added to react with 150 μL of the FRAP solution. The increase in absorbance at 593 nm was measured using a Multiskan EX microplate reader (Thermo Scientific, Finland) after 20 min of incubation at 37 °C. The FRAP percentage was calculated as follows: % inhibition = [absorbance of sample/maximum absorbance (3.0)] × 100%. All experiments were performed in three replicates.

2.3.3. β-Carotene bleaching assay

The β-carotene bleaching activity was determined according to a minor modification of the procedure described by Miller (1971). Briefly, 1 mL of β-carotene solution (2 mg/mL in chloroform) was transferred into a round bottom flask (50 mL) containing 20 μL of linoleic acid and 200 μL of Tween 80 solution. After evaporating to dryness under vacuum at room temperature, 50 μL of oxygenated distilled water was added to the residue with energetic agitation to form an emulsion. The emulsion (150 μL) was mixed with 50 μL of each compound (with an initial concentration of 1 mg/mL) in each well of the 96-well plate and the absorbance was immediately measured at 470 nm using a Multiskan EX microplate reader (Thermo Scientific, Finland). The reaction mixture was incubated at 50 °C for 120 min to induce auto-oxidation and the absorbance was measured again. A negative control mixture was prepared similar to the sample mixture with DMSO instead of the tested compounds. The capacity of the sample to prevent the oxidation of β-carotene was determined as follows: % inhibition = ([absorbance of negative control at 0 min–120 min) – (absorbance of sample at 0 min–120 min)]/[absorbance of control at 0 min] × 100%. All measurements were performed in three replicates.

2.3.4. Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD). Analysis of variance was determined by one-way ANOVA using GraphPadPrism (San Diego, CA). Significant differences between the means were calculated according to Duncan’s multiple range tests. Differences at p < 0.05 were considered statistically significant.

2.4. Broth microdilution assay

Minimum inhibitory concentration (MIC) values of all the isolates were evaluated using micro-well dilution method as described by Eloff (1998) with some modifications. Overnight cultures of eight food-borne bacteria i.e. three Gram-positive strains; B. cereus (ATCC 10876) B. licheniformis (ATCC 12759) and S. aureus (ATCC 12600), and five Gram-negative strains; E. coli (ATCC 25922), K. pneumoniae (ATCC 13883), P. vulgaris (ATCC 6380), S. typhimurium (ATCC 14028) and V. parahaemolyticus (ATCC 17802), were adjusted to 0.5 McFarland turbidity standard (108 CFU/mL) and then diluted 1:100 with sterile nutrient broth. The compounds were dissolved in 99.5% (v/v) DMSO to obtain an initial concentration of stock solution of 10 mg/mL. Eight serial twofold dilutions of the stock solution were prepared (to obtain a final concentration of 500 to 3.906 μg/mL). Briefly, each well of 96-well plate was filled with 5 μL of compound solution, 90 μL of nutrient broth and 5 μL of bacteria inoculum. The antibiotic tetacycline and DMSO (in similar volume with test sample) were respectively included as positive and negative controls in each assay. The plates were covered and incubated overnight at 37 °C. As an indicator of bacteria growth, 40 μL of 0.4 mg/mL p-iodonitrotetrazolium chloride (INT) dissolved in distilled water was then added to the wells and incubated at 37 °C for 30 min. Bacteria growth in the wells was indicated by a reddish-pink colour.
Fig. 1. Structures of compounds 1–8.
whereas clear/colourless wells indicated inhibition by the tested samples. MIC value was defined as the lowest concentration of samples showing clear wells or with complete inhibition of bacteria growth. The assay was performed in triplicate.

3. Results and discussion

3.1. Antioxidant activity

Despite the extensive studies on antioxidant potentials of curcuminoids and kaempferol derivatives, the emphasis is mostly to verify their medicinal values in healing various degenerative diseases. Thus, their roles as food preservatives still remain obscured. Since a combination of antioxidant and antibacterial (food-borne pathogens) effects is well recognized as an important attribute for natural preservatives, this is apparently the first comparative analysis among the major compounds isolated from the rhizomes of Z. spectabile towards these bioactivities. The antioxidant capacities were systematically assessed using three different assays at an initial concentration of 1 mg/mL. The interrelationships between different mechanisms of antioxidant reactions of these assays would be useful for comparing the capabilities of the isolated compounds in intercepting the radical chain propagation, restoring the redox potential and suppressing the lipid peroxidation process (Bursal & Gülçin, 2011). The results obtained from these three assays have led to the similar finding of the marked activity of kaempferol (6), followed by curcumin (8) and demethoxycurcumin (7) with a significant difference (p < 0.05) in the β-carotene assay and no significant differences (p > 0.05) in the DPPH and FRAP assays (Figs. 2–4). This strongly implied the direct involvement of these compounds in enhancing the primary antioxidant activity with the highest ferric reducing ability of kaempferol at 100% of inhibition. The highest antioxidant activity of kaempferol could be related to its flavonol structural configuration having the required antioxidant functional groups such as the hydroxyl group at the 3-position and the 2,3-double bond in conjugation with the 4-oxo function (Rice-Evans, Miller, & Paganga, 1997). Hence, the compound can be described as a potent reductant as it assists in reducing the redox reactions.

The percentage of DPPH scavenging activity of curcumin (8) (75.01 ± 0.67%) (Fig. 2) was comparable to its β-carotene bleaching activity (77.27 ± 1.29%) (Fig. 4). Although the DPPH scavenging activity of demethoxycurcumin (7) (73.62 ± 0.17%) was found to be significantly lower than that of kaempferol (6) (Fig. 2), this compound was found to demonstrate the greatest activity in this assay. This was followed by its β-carotene bleaching activity (62.08 ± 4.75%) (Fig. 4), and ferric reducing activity (56.27 ± 0.16%) (Fig. 3). As indicated in Fig. 2, no significant difference was observed between the DPPH radical scavenging activity of demethoxycurcumin (7) and its parent compound (curcumin) (8). This finding was in agreement with the results reported by Jayaprakasha et al. (2006), stipulating the equal effectiveness of these two compounds in scavenging free radicals. Mounting evidences have pointed out that the radical scavenging mechanisms of the natural antioxidants play a vital role in terminating and delaying lipid oxidation chain reactions in food during storage and processing (Sun, Zhang, Lu, Zhang, & Zhang, 2011; Zhang, Li, & Zhou, 2010). Therefore, the antiradical properties of kaempferol and curcuminoids might be accountable for their lipid peroxidation inhibitory capacities, which facilitates to impede the lipid radical oxidation and thereby prevents food rancidity. Moreover, the H-atom donation from the β-diketone moiety to a lipid alkyl or a lipid peroxyl radical was also described as a potential antioxidant action of curcuminoids (Ak & Gülçin, 2008).

Meanwhile, the glycosylation at the 3-position was found to decrease the primary antioxidant activities of spectafloavoside A (1), kaempferol-3-O-(3",4"-di-O-acetyl)-α-L-rhamnopyranoside (2), kaempferol-3-O-(2",3",4"-tri-O-acetyl)-α-L-rhamnopyranoside (3), kaempferol-3-O-(2",4"-di-O-acetyl)-α-L-rhamnopyranoside (4) and kaempferol-3-O-(4"-O-acetyl)-α-L-rhamnopyranoside (5) as compared to their corresponding aglycone, kaempferol (6) (Soobrattee, Neerghen, Luximon-Ramma, Aruoma, & Bahorun, 2005). This might be due to the steric hindrance after the substitution of glycosidic residues (Gao et al., 2011). In compliance with their observation, the higher primary antioxidant activity of the tested curcuminoids (7 and 8) in comparison to the acylated kaempferol-3-O-rhamnosides (2–5) could be associated with the phenolic hydroxyl and methoxyl groups and the conjugated diene moiety (Portes, Gardrat, & Castellan, 2007; Ruby, Kuttan, Dinesh Babu, Rajasekharan, & Kuttan, 1995). The antioxidant activity is known to increase when the phenolic hydroxyl group is at the ortho position with respect to the methoxyl group, which is crucial for stabilizing the phenoxy radical after hydrogen transfer (Portes et al., 2007). Thus, the differences in the antioxidant capacities of these compounds may be explained by their structural conformations, particularly by the distribution of hydroxyl groups and their position in relation to the other substitutions.

![Graph](https://example.com/graph.png)  
*Fig. 2. DPPH scavenging activities of compounds 1–8. Values are mean ± standard deviation of triplicate analyses. Values followed by different letters mean significant differences (p < 0.05).*
2.3. Antibacterial activity

As all isolated compounds possess lipid peroxidation inhibition effect (atleast 25% of inhibition), their potential roles in the prevention of food deterioration were further evaluated on different food-borne pathogenic bacteria. During the initial stages of antibacterial screening, eight different food-borne bacteria were selected, however only six were found to be susceptible to the compounds at a final concentration of 1 mg/mL. As indicated in Table 1, all the six tested bacteria were inhibited by demethoxycurcumin (7) with the lowest minimum inhibition concentration (MIC) against B. licheniformis (62.50 μg/mL). This compound is relatively more effective towards Gram-positive bacteria than Gram-negative bacteria, which could be the reason of their differences in cell membrane constituents and structures. With exception of E. coli, curcumin (8) was found to inhibit the other five bacteria and it could be ranked the second active antibacterial compound. Besides, the MIC values of this compound against P. vulgaris and B. cereus were comparable to that of demethoxycurcumin (7). Hence, the demethoxylation of curcumin (8) is strongly suggested to enhance its bioavailability (cellular uptake) and facilitate its binding at endocytosis receptor through a free phenolic moiety. Wang, Lu, Wu, and Lv (2009) had improved the stability and solubility of curcumin (8) by microencapsulation technique. When our results were compared with the microcapsule curcumin and different strains of bacteria from China General Microbiological Culture Collection Center, similar MIC against B. cereus at 125 μg/mL was obtained. However their microcapsule curcumin exhibited greater activities on E. coli (MIC = 250 μg/mL) and S. aureus (MIC = 62.5 μg/mL) than curcumin (8) used in this study.

The curcuminoids revealed higher antibacterial activity than that of kaempferol (6), the acylated kaempferol-3-O-rhamnosides (2–5) and specataflavoside A (1). The permeability of the bacterial cells to the tested compounds is one of the determining factors of their antibacterial activity. The presence of a higher number of hydroxyl groups in kaempferol (6) and its acylated rhamnose derivatives as well as in specataflavoside A (1) makes them more hydrophilic compared to the curcuminoids, thus causing the penetration into the cell membrane of the bacteria to be more difficult, hence, resulting in
them being less active than the curcuminoïds. The presence of a diene ketone system also provides lipophlicity to the curcuminoïds and thus enhances penetration into target organisms (Jayaprakasha et al., 2006). E. coli, could be considered as the most resistant bacterium used in this study as it was only inhibited by demethoxycurcumin (7) with a MIC of 500 μg/mL. The results therefore highlighted the remarkable effects of this curcuminoïd in inhibiting a broader spectrum of Gram-positive and Gram-negative food-borne bacteria. Furthermore, V. parahaemolyticus and S. aureus were identified as the two most sensitive bacteria. The inhibitory abilities of all the tested compounds excepting spectaflavoside A (1) and kaempferol-3-O-(“3”,“4”-di-O-acetyl)-α-L-rhamnopyranoside (2) against V. parahaemolyticus, were found higher than a natural seafood preservative, 5% lactic acid (MIC = 1.56 mg/mL) (Ho et al., 2010). Thus, these compounds might be potential alternatives of seafood preservatives.

In line with the results obtained from this study, many earlier studies have also demonstrated the low antioxidant activities of kaempferol and its derivatives (2–6) (Basile et al., 2000). Moreover, a study by Liu, Orjala, Sticher, and Rali (1999) using 10-acetylated kaempferol glycosides, showed higher antibacterial activity in compounds having cis-p-coumaroyl groups in their structures. The relative amount of kaempferol-3-O-(“2”-di-“E”-p-coumaroyl)-rhamnosoïd in extracts of some Epacridaceae species was also found to be correlated with their antibacterial activities (Bloor, 1995).

4. Conclusion

The food preservation effects of Z. speciable are mainly attributed to the curcuminoïds that display higher antioxidant and antibacterial activities. For the first time, the marked inhibitory activity of demethoxycurcumin (7) was verified against a broad spectrum of food-borne pathogens in the present study. Based on the discussion above, the demethoxycurcumin (7) can be utilized as a potent candidate for suppressing oxidative chain propagation and minimizing lipid oxidation in food systems, maintaining nutritional quality and prolonging the shelf-life of food products by controlling microorganism spoilage processes.

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References


Table 1

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<th>Compounds</th>
<th>Minimum inhibition concentration (µg/mL)</th>
<th>Gram-positive bacteria</th>
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<tr>
<td></td>
<td>Protex vulgaris (ATCC 6380)</td>
<td>Vibrio parahaemolyticus (ATCC 17802)</td>
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<tr>
<td>Spectaflavoside A (1)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Kaempferol-3-O-(“3”,“4”-di-O-acetyl)-α-L-rhamnopyranoside (2)</td>
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<td>Kaempferol (6)</td>
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</tr>
<tr>
<td>Demethoxycurcumin (7)</td>
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