Antioxidant and Antibacterial Properties of *Alpinia galanga*, *Curcuma longa*, and *Etlingera elatior* (Zingiberaceae)

Eric WC Chan, Voon Pei Ng, Vi Vian Tan, Yin Yin Low
Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia

**ABSTRACT**

Antioxidant and antibacterial properties of methanolic extracts, non-polymeric phenolic fractions, and polymeric tannin fractions of leaves and rhizomes of *Alpinia galanga* and *Curcuma longa*, and leaves and inflorescences of *Etlingera elatior* were investigated. Antioxidant properties based on total phenolic content (tPc) and ascorbic acid equivalent capacity (AEAC) were screened using the Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, respectively. Antibacterial activity based on minimum inhibitory dose (MID) was tested against Gram-positive *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus cereus* using the disc-diffusion method. The effect of ethylenediamine tetraacetic acid (EDTA) on the antibacterial properties of extracts and fractions was also studied. Extraction yields ranged from 4.1-6.0%. Yields of non-polymeric phenolic (nP) fractions (66-92%) were much higher than that of polymeric tannin (Pt) fractions (0.5-10%), suggesting that the former were the major compounds. Highest tPc and AEAC were observed in the Pt fraction of *A. galanga* rhizomes, in the crude extract and nP fraction of *C. longa* rhizomes, and in the Pt fraction of *E. elatior* leaves. Leaf extracts and fractions of *A. galanga* and *C. longa* did not show any antibacterial activity against *S. aureus*, *M. luteus*, and *B. cereus*. Rhizome extracts and fractions of *A. galanga* and *C. longa* had no inhibitory effect on *M. luteus* and *S. aureus*, respectively. PT fractions of *E. elatior* leaves and inflorescences displayed no antibacterial activity. With the addition of 0.01 mg/ml of EDTA, extracts and fractions of *A. galanga*, *C. longa*, and *E. elatior* showed moderate, weak, and strong responses, respectively. Strongest antibacterial activity was observed in the PT fraction of *A. galanga* rhizomes with MID of 0.06 mg/disc against all three bacterial species. PT fractions of *E. elatior* leaves and inflorescences displayed antibacterial activity with MID of 0.13 mg/disc, which showed no activity prior to the addition of EDTA. The effect of EDTA on the antibacterial activity of extracts and fractions of these three ginger species warrants further investigation.

**Key words:** Crude extracts, fractions, non-polymeric phenolic, polymeric tannin, leaves, rhizomes, inflorescences

**INTRODUCTION**

Gingers of the family Zingiberaceae are perennial herbs that produce aromatic rhizomes.[1] Ginger plants are widely used as spice, condiment, and traditional medicine. The ethno-medicinal uses of rhizomes and leaves of gingers have been reviewed.[2,3] Rhizomes of ginger plants are eaten raw or cooked as vegetables and used for flavouring food. Species that are widely cultivated are *Alpinia galanga*, *Curcuma longa*, *Etlingera elatior*, and *Zingiber officinale*. Rhizomes of *Z. officinale* are used as additives and flavouring in the food and beverage industry. They are used in the production of beverages such as ginger beer, ginger ale, and ginger wine.[4] They are also widely used to make ginger bread, biscuits, cakes, puddings, and pickle. Rhizomes of *C. longa* are popular as a spice used in curries both for flavouring and colouring.[1] Rhizomes of *A. galanga* are used as spice for meat dishes. As traditional medicine, ginger rhizomes are consumed by women during ailment, illness, and confinement. Rhizomes are also taken as a carminative for relieving flatulence.

Leaves of ginger plants have also been used for food flavouring and in traditional medicine.[1] In Malaysia, leaves of *C. longa* are used to wrap fish before steaming or baking and as spice for curries. Leaves of *E. elatior*, mixed with other aromatic herbs, are used by post-partum women for bathing to remove body odour.[3] They are also used for cleaning wounds. A decoction of leaves of *A. galanga* is consumed to treat diarrhea.[4]
Beside rhizomes and leaves, other plant parts of gingers are also consumed as food, spice, and condiment. Young inflorescences of *E. elatior* are an essential ingredient of sour curry dishes. In recent years, gingers have become popular ornamental plants as their flowers and foliage are colourful and attractive. Species of *Alpinia*, *Curcuma*, *Etlingera*, *Hedychium*, *Kaempferia*, and *Zingiber* have been cultivated as horticultural plants for their attractive leaves and/or flowers.

Previous studies on the antioxidant and antibacterial properties of ginger species are confined to rhizomes. Although leaves of ginger species have been used for food flavouring and in traditional medicine, little research has been done on their antioxidant properties until recent years. The antioxidant properties of ginger leaves have recently been reviewed. Studies have shown that leaves of gingers have stronger antioxidant activity than rhizomes and that leaves of highland ginger populations have higher antioxidant activity than lowland populations. Drying of ginger leaves using thermal methods resulted in drastic declines in antioxidant activity.

Screening of antioxidant properties of leaves of 26 species and nine genera of gingers showed that *Etlingera* species had the highest values followed by *Alpinia* species. Of leaves of five *Etlingera* species studied, *E. elatior* had the strongest antioxidant properties. Antioxidant properties of leaves of *E. elatior* were significantly higher than inflorescences and rhizomes. Leaves of *Etlingera* exhibited moderate inhibition against Gram-positive bacteria with no activity against Gram-negative bacteria. However, it is not known if the antioxidant and antibacterial activities of ginger plants are due to their non-polymeric phenolic (NP) or polymeric tannin (PT) constituents.

In this study, the antioxidant and antibacterial properties of methanolic extracts, NP fractions, and PT fractions of leaves and rhizomes of *A. galanga* and *C. longa*, and leaves and inflorescences of *E. elatior* were investigated. The effect of ethylenediamine tetraacetic acid (EDTA) on the antibacterial properties of extracts and fractions was also studied.

### MATERIALS AND METHODS

#### Plant materials

Ginger species studied were *A. galanga*, *C. longa*, and *E. elatior*. Fresh leaves and rhizomes of *A. galanga* and *C. longa*, and inflorescences of *E. elatior* were purchased from the market. Leaves of *E. elatior* were collected beside the campus of UCSI University. Materials are wrapped in a plastic bag, kept in the refrigerator and brought to the laboratory for analysis the next day. Leaves, rhizomes, and inflorescences (100 g each) were cleaned and shredded into 0.2 cm strips using a pasta maker (IKEA Malaysia). Brief botanical descriptions and uses of the ginger species studied are shown in Table 1.

#### Extraction

Leaf, rhizome, and flower strips (100 g each) were transferred into a 1000 ml extraction flask and extracted with 500 ml of methanol, successively three times for 1 h each time. The mixture was swirled continuously at 120 rpm with an orbital shaker. Samples were then filtered using a vacuum filter. After filtration, the residues were transferred back into the extraction flask and extracted again with 500 ml methanol. After drying at 50°C using a rotary evaporator, the dried extracts were kept at −20°C in freezer for further analysis.

#### Fractionation

Tannins were fractionated following the procedure of column chromatography previously described. Crude extract (2 g) dissolved in methanol (16 ml) was applied onto a chromatographic column (40 × 3 cm) packed with Sephadex LH-20 (GE Health, Sweden) and equilibrated with 100% (v/v) methanol. To obtain the NP constituents, the column was washed with 250 ml of 100% methanol. PT constituents were eluted from the column using 250 ml of 70% acetone. After evaporating at 50°C using a rotary evaporator, the fractions were tested for total phenolic content, and for free radical scavenging and antibacterial activities.

#### Antioxidant properties

Total phenolic content (TPC) of extracts and fractions was determined using the Folin-Ciocalteu (FC) assay. Samples (300 μl in triplicate) were introduced into test tubes wrapped with aluminium foil, followed by 1.5 ml of FC reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min in the dark before absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in milligram per gram of extract. The calibration equation for GA (Fluka) was $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$) where $y$ is absorbance and $x$ is mg/ml of GA.

Free radical scavenging (FRS) activity of extracts and fractions was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different dilutions of samples (1 ml in triplicate) were added to 2 ml of DPPH (5.9 mg in 100 ml of methanol) in test tubes wrapped with aluminium foil. Absorbance ($A$) was measured at 517 nm after 30 min of incubation in the dark at room temperature. All measurements were made in triplicate using distilled water as blank. FRS activity of samples (%) was calculated...
Table 1: Brief botanical descriptions and uses of ginger species studied

<table>
<thead>
<tr>
<th>Species</th>
<th>Botanical description and use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpinia galanga</strong> (L.) Sw. (Greater galangal)**</td>
<td>Plants can grow up to 3 m tall. Rhizomes (2-4 cm diameter) are branched, light yellow, fibrous, and fragrant. Leaves are alternate, pubescent and oblong-lanceolate. Flowers are terminal, white, and fragrant. The species is widely cultivated in Southeast Asia for its rhizomes as spice. Shoots and inflorescences are eaten raw, and rhizomes are used in traditional medicine.</td>
</tr>
<tr>
<td><strong>Curcuma longa</strong> L. (Tumeric)**</td>
<td>Plants can grow up to 1 m tall. Rhizomes (1-2 cm diameter) are branched, bright orange, and strongly aromatic. Leaves are oblong-lanceolate with an acute apex and aromatic. Inflorescences are terminal and erect, with pale green bracts and white flowers. The species is extensively cultivated in tropical Asia for its rhizomes as spice. Rhizomes are the source of turmeric which has a long tradition of medicinal use.</td>
</tr>
<tr>
<td><strong>Etlingera elatior</strong> (Jack) Sm. (Torch ginger)**</td>
<td>Plants can grow up to 5-6 m tall, forming dense clumps. Rhizomes (3-4 cm diameter) are stout and pungent. Leaves (100-150 cm long) are lanceolate and green. Crushed leaves emit a pleasant sour fragrance. The species is native to Malaysia and Indonesia, and is widely cultivated in the tropics for its inflorescences as spice.</td>
</tr>
</tbody>
</table>

as \((A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100\) and calculated as IC_{50}, the concentration of sample needed to scavenge 50\% of the DPPH free radical. IC_{50} was then expressed as ascorbic acid (AA) equivalent antioxidant capacity (AEAC) using the equation of AEAC (mg AA/g of extract) = IC_{50AA}/IC_{50sample} \times 10^5. IC_{50} of AA used for calculation of AEAC was 0.00387 mg/ml.

**Antibacterial activity**
Antibacterial activity of extracts and fractions was measured using the disc-diffusion method.\[^{12,14}\] Bacterial species tested were Gram-positive *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus cereus*. Inoculums (100 \(\mu\)l) were spread evenly onto 20 ml Mueller-Hinton agar set in 90 mm Petri dishes using a sterile cotton swab. Sterilized paper discs (6 mm diameter) were impregnated with plant samples (2 mg per disc) using a micropipette and firmly placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. Streptomycin susceptibility discs (10 \(\mu\)g) were used as positive controls. After incubation overnight at 37\(^\circ\)C, the minimum inhibitory dose (MID) or minimum concentration of extract or fraction in mg/disc required to show a zone of inhibition was noted. The disc-diffusion method was repeated by adding 0.01 mg/ml of EDTA to the agar to enhance the antibacterial activity of plant samples.

**Statistical analysis**
All experiments were done in triplicate \((n = 3)\) and results were expressed as means ± standard deviation (SD). Results were analyzed using the Turkey Honestly Significant Difference (HSD) one-way analysis of variance (ANOVA) software developed by Vassar College, New York State, USA. The significant difference was based on \(P < 0.05\).

**RESULTS AND DISCUSSION**

**Percentage yield**
The percentage yield of methanolic extracts from leaves and rhizomes of *A. galanga* was 6.0 and 5.5\%, from leaves and rhizomes of *C. longa* was 4.3 and 4.1\%, and from leaves...
and inflorescences of *E. elatior* was 4.1 and 4.8%, respectively [Table 2].

Methanol was chosen as the solvent for extraction as it is most suitable for extracting phenolic compounds from plant tissues and is able to inhibit the action of polyphenol oxidase. Methanol is efficient in cell wall degradation, easy to evaporate, and able to prevent microbial growth. Based on TPC and AEAC, the extraction efficiency of four different solvents on leaves of *C. longa* and *E. elatior* showed that methanol was the most efficient followed by 50% aqueous methanol, ethyl acetate, and dichloromethane. The extraction yield of three successive extractions of *A. galanga* leaves using methanol was 79, 15, and 6%, respectively. TPC values were 492, 95, and 40 mg GAE/100 g.

The percentage yield of NP and PT fractions from leaves and rhizomes of *A. galanga* was 74, 0.5, 66, and 6.0%; from leaves and rhizomes of *C. longa* was 92, 7.4, 86, and 2.0%; and from leaves and inflorescences of *E. elatior* was 85, 10, 80, and 9.0%; respectively [Table 2].

All three species yielded much higher percentage of NP (66-92%) than PT (0.5-10%) constituents, suggesting that the former were the major compounds. An earlier study reported the tannin content in methanol rhizome extracts of eight ginger species varied from 1.2-18%. Rhizomes of *Curcuma barthia* contained 1.8% tannin content.

**Antioxidant activity**

Results of antioxidant properties (AOP) of crude extracts of *A. galanga*, *C. longa*, and *E. elatior* are shown in Table 2. In *A. galanga*, TPC of leaves was significantly higher than that of rhizomes. However, AEAC values were comparable. In *C. longa*, both TPC and AEAC of rhizomes were significantly higher than those of leaves. Values were 1.6 and 1.5 times higher in rhizomes than leaves. In *E. elatior*, both TPC and AEAC of leaves were significantly higher than those of inflorescences. Values of leaves were 7.7 and 5.3 times higher than inflorescences, respectively.

Earlier studies have reported that leaves of *A. galanga* had significantly higher TPC but significantly lower AEAC than rhizomes. This study found that leaves and rhizomes of *A. galanga* had comparable AEAC. Comparing the AOP of leaves of five *Alpinia* species, TPC and AEAC values of *A. galanga* were the lowest. The significantly higher TPC and AEAC of *C. longa* rhizomes than leaves have earlier been reported. It is interesting to note that this trend in *C. longa* was unique among *Curcuma* as leaves of *C. aromatica*, *C. mangga*, and *C. zanthorrhiza* have significantly stronger AOP than rhizomes. An earlier study has reported that TPC and AEAC values were significantly higher in leaves than inflorescences of *E. elatior*. Values were 12 and 14 times higher in leaves than in inflorescences, respectively.

AOP of NP and PT fractions of *A. galanga*, *C. longa*, and *E. elatior* are shown in Table 2. In leaves of *A. galanga*, TPC and AEAC of the NP fraction were comparable to those of the PT fraction. In rhizomes, values of the PT fraction were significantly higher than the NP fraction. In leaves of *C. longa*, TPC and AEAC of the PT fraction were significantly higher than the NP fraction and vice versa for rhizomes where values of the NP fraction were significantly higher than the PT fraction. In both leaves and inflorescences

---

**Table 2: Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of extracts and fractions from Alpinia galanga, Curcuma longa, and Etlingera elatior**

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant part</th>
<th>Extract/fraction</th>
<th>Yield (%)</th>
<th>TPC</th>
<th>AEAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. galanga</em></td>
<td>Leaves</td>
<td>Crude extract</td>
<td>6.0</td>
<td>62 ± 6.9ab</td>
<td>29 ± 1.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP fraction</td>
<td>74</td>
<td>52 ± 3.4ab</td>
<td>17 ± 0.1ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT fraction</td>
<td>0.5</td>
<td>48 ± 6.2b</td>
<td>17 ± 1.1b</td>
</tr>
<tr>
<td></td>
<td>Rhizomes</td>
<td>Crude extract</td>
<td>5.5</td>
<td>39 ± 3.4a</td>
<td>31 ± 2.8a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP fraction</td>
<td>66</td>
<td>32 ± 5.4a</td>
<td>8.0 ± 0.8a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT fraction</td>
<td>6.0</td>
<td>155 ± 16d</td>
<td>143 ± 6.0d</td>
</tr>
<tr>
<td><em>C. longa</em></td>
<td>Leaves</td>
<td>Crude extract</td>
<td>4.3</td>
<td>59 ± 2.1a</td>
<td>39 ± 1.6a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP fraction</td>
<td>92</td>
<td>57 ± 2.1a</td>
<td>39 ± 2.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT fraction</td>
<td>7.4</td>
<td>68 ± 1.0c</td>
<td>60 ± 0.3c</td>
</tr>
<tr>
<td></td>
<td>Rhizomes</td>
<td>Crude extract</td>
<td>4.1</td>
<td>94 ± 3.9bc</td>
<td>59 ± 5.6bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP fraction</td>
<td>86</td>
<td>92 ± 6.3bc</td>
<td>53 ± 1.0bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT fraction</td>
<td>2.0</td>
<td>28 ± 2.9d</td>
<td>21 ± 3.4d</td>
</tr>
<tr>
<td><em>E. elatior</em></td>
<td>Leaves</td>
<td>Crude extract</td>
<td>4.1</td>
<td>192 ± 16a</td>
<td>242 ± 27a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP fraction</td>
<td>85</td>
<td>204 ± 20a</td>
<td>229 ± 5.3a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT fraction</td>
<td>10</td>
<td>575 ± 79b</td>
<td>616 ± 13a</td>
</tr>
<tr>
<td></td>
<td>Inflorescences</td>
<td>Crude extract</td>
<td>4.8</td>
<td>25 ± 2.2c</td>
<td>46 ± 13c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP fraction</td>
<td>80</td>
<td>22 ± 3.6d</td>
<td>45 ± 4.9d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT fraction</td>
<td>9.0</td>
<td>191 ± 14a</td>
<td>236 ± 28a</td>
</tr>
</tbody>
</table>

TPC in mg GAE/g extract and AEAC in mg AA/g extract are means ± SD (n = 3). ANOVA was analysed using the Tukey HSD test. For each column, values followed by the same letter (a–d) are not statistically different at *P* < 0.05. ANOVA does not apply between species. Abbreviations: NP = non-polymeric phenolic, PT = polymeric tannin, GAE = gallic acid equivalent, and AA = ascorbic acid.
of *E. elatior*, TPC and AEAC of PT fractions were significantly higher than NP fractions.

In leaves of *C. longa* and *E. elatior*, AOP values of PT fractions were significantly higher than NP fractions. In leaves of *A. galanga*, both fractions had comparable values. AOP values of fractions of the other plant parts were somewhat varied. Values of the PT fraction were significantly higher in rhizomes of *A. galanga* and in inflorescences of *E. elatior*. In rhizomes of *C. longa*, values of the NP fraction were significantly higher. Generally, PT fractions have stronger AOP than NP fractions with the exception leaves of *A. galanga* and rhizomes of *C. longa*.

Tannins are polymeric phenolic compounds of intermediate to high molecular weight, ranging between 500 and 3000 Da.[25,26] Due to their higher molecular weight and greater degree of hydroxylation of aromatic rings, tannins exhibit strong antioxidant potential.[26] Their antioxidant potency depends on the number of phenolic hydroxyl groups and the degree of hydroxylation of aromatic rings. Polymeric tannins have been reported to be much more potent antioxidants than simple monomeric phenolics.[27] Tannins were 15-30 times more effective at quenching peroxyl radicals than simple phenolics, suggesting that they are important biological antioxidants. The basic mechanisms of antioxidant activity of tannins are free radical scavenging activity, chelation of transition metals, inhibition of prooxidative enzymes, and lipid peroxidation.[26,28]

It is generally believed that tannins are not absorbed by the gastro-intestinal tract in the human body due to their high molecular weight and their ability to form insoluble complexes with components of food such as proteins.[25,28] The molecular weight of a compound should be less than 500 Da to be considered orally active.[29] Though some studies have shown that the absorption of tannins is higher than it was assumed, there are still many questions concerning their bioavailability. Generally, absorption of tannins decreases with increasing polymerization.

Stronger AOP of the NP fraction of *C. longa* rhizomes may be attributed to curcuminoids, the major phenolic compounds.[30,31] Curcuminoids notably curcumin and demethoxycurcumin have been reported to possess potent DPPH radical scavenging activity.[32] The wide ranging radical scavenging, ferric ion reducing, and ferrous ion chelating activities of curcumin have also been studied. The presence of hydroxy and β-diketone groups in curcuminoids contributes to their potent AOP.[33,34] Other phenolic compounds with AOP found in rhizomes of *C. longa* included β-coumaric acid, vanillin, ferulic acid, and quercetin.[33,35]

### Antibacterial activity

Results of antibacterial activity of crude extracts of *A. galanga*, *C. longa*, and *E. elatior* are shown in Table 3. Leaves of *A. galanga* and *C. longa* did not show any antibacterial activity against *S. aureus*, *M. luteus*, and *B. cereus*. Rhizomes of *A. galanga* showed inhibition against *S. aureus* and *B. cereus* with MID of 0.50 and 1.00 mg/disc, respectively. Rhizomes of *C. longa* strongly inhibited the growth of *M. luteus* and *B. cereus* with MID of 0.13 mg/disc. It is interesting to note that leaf and rhizome extracts of *A. galanga* and *C. longa*
were ineffective against *M. luteus* and *S. aureus*, respectively. Leaves and inflorescences of *E. elatior* inhibited the growth of all three bacterial species with MID ranging from 0.50 to 2.00 mg/disc. Strongest antibacterial activity was observed in the rhizome extract of *C. longa* with MID of 0.13 mg/disc against *M. luteus* and *B. cereus*.

Antibacterial activity of NP and PT fractions of *A. galanga*, *C. longa*, and *E. elatior* is shown in Table 3. Leaves of *A. galanga* and *C. longa* did not show any antibacterial activity. The PT fraction of rhizomes of *A. galanga* showed inhibition against *S. aureus* and *B. cereus* while the NP fraction had no activity. On the contrary, the NP fraction of rhizomes of *C. longa* showed inhibition against *M. luteus* and *B. cereus* while the PT fraction had no activity. The observation that all fractions of *A. galanga* and *C. longa* were ineffective against *M. luteus* and *S. aureus*, respectively, is noteworthy. NP fractions of leaves and inflorescences of *E. elatior* showed antibacterial activity with the exception of the PT fraction against *S. aureus*. Surprisingly, PT fractions showed no antibacterial activity. Strongest antibacterial activity was observed in the PT fraction of *A. galanga* rhizomes with MID of 0.13 mg/disc against *B. cereus*.

In support of this study, it was earlier reported that leaf extract of *A. galanga* did not have inhibitory effect on seven bacterial species including *S. aureus*, *M. luteus*, and *B. cereus*. The antibacterial activity of *A. galanga* rhizomes has been well documented. Rhizomes of *A. galanga* had strong inhibitory effect against *S. aureus*. Using transmission electron microscopy, it was observed that the rhizome extract of *A. galanga* caused membrane damage and cytoplasm coagulation in *S. aureus*. In this study, it was observed that the rhizome extract and fraction of *A. galanga* inhibited *S. aureus* but not *M. luteus*. On the contrary, an earlier study reported that *A. galanga* rhizome extract was non-inhibitive against *S. aureus* but had inhibitory effect against *M. luteus*. Results of this study showed that the growth of *S. aureus* was not inhibited by extracts and fractions of *C. longa* which contradicted earlier findings that *C. longa* rhizome extracts had inhibitory effect against *S. aureus*. The disparity in findings could be due to different strains of bacteria used.

Leaves and inflorescences of *E. elatior* are known to have antibacterial activity. Leaves of *E. elatior* exhibited moderate inhibition against *S. aureus*, *M. luteus*, and *B. cereus* with no activity against Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis*. Extracts of *E. elatior* flowers displayed antibacterial activity against *Bacillus megaterium*, *E. coli*, and *P. aeruginosa* and against *Bacillus thuringiensis*, *Bacillus subtilis*, *Proteus mirabilis*, *E. coli*, and *S. aureus*.

Results of antibacterial activity of extracts and fractions using the disc-diffusion method with 0.01 mg/ml of EDTA added to the agar are shown in Table 3. This concentration was chosen because at 0.1 mg/ml of EDTA, there was no lawn culture of *M. luteus*. The addition of EDTA was to improve the antibacterial efficacy of extracts and fractions. Leaf extract of *A. galanga* showed antibacterial activity against *S. aureus* and *B. cereus*, with MID of 1.00 mg/disc, although the fractions remained inactive. Extract and the PT fraction of rhizomes inhibited the growth of all three bacterial species while the NP fraction of rhizomes inhibited *B. cereus*. EDTA did not have any effect on extracts and fractions of leaves and rhizomes of *C. longa*. Leaf extract and PT fraction of rhizomes remained inactive. Again, *S. aureus* is not susceptible to all extracts and fractions. All extracts and fractions of *E. elatior* inhibited the growth of all three bacterial species. Antibacterial activity of PT fractions of leaves and inflorescences, which showed no activity without EDTA, had been greatly enhanced with MID of 0.13 mg/disc. With the addition of 0.01 mg/ml of EDTA, strongest antibacterial activity was observed in the PT fraction of *A. galanga* rhizomes with MID of 0.16 mg/disc against *M. luteus*, *S. aureus*, and *B. cereus*.

Findings of this study showed that different plant and bacterial species respond differently to EDTA. With 0.01 mg/ml of EDTA, extracts and fractions of *A. galanga*, *C. longa*, and *E. elatior* showed moderate, weak, and strong response, respectively. Noteworthy are the PT fractions of *E. elatior* which showed strong inhibitory effects. It can be seen that EDTA greatly enhanced the antibacterial activity of PT fractions of *E. elatior*.

Previous studies have also reported varying effects of EDTA on the antibacterial activity of plant samples. Adding 2 mM EDTA caused *P. aeruginosa* to be susceptible to leaf extracts of *Etlingera* species but the chelating agent inhibited the culture of *E. coli* and *S. choleraesuis*. Adding 1 mM of EDTA rendered streptomycin ineffective against *V. choleraesuis* but enhanced the efficiency of the antibiotic against *P. aeruginosa*. EDTA inhibited the culture of *B. cereus* but *S. aureus* grew prolifically. It has bactericidal effect on *P. aeruginosa* and *Staphylococcus epidermidis*. The chelating agent is known to enhance the effectiveness of antimicrobials and antibiotics, especially against Gram-negative bacteria. It alters the structure of the outer membrane of Gram-negative bacteria by chelating divalent cations, weakening the outer membrane, and renders the entry of antibiotics.

**CONCLUSION**

All three ginger species had much higher percentage yield of NP than PT constituents, suggesting that the former
were the major compounds. AOP of methanolic extracts and fractions from various plant parts of each species showed variable trends. Highest TPC and AEAC were observed in the PT fraction of *A. galanga* rhizomes, in the extract and NP fraction of *C. longa* rhizomes, and in the PT fraction of *E. elatior* leaves. Leaf extracts and fractions of *A. galanga* and *C. longa* did not show any antibacterial activity against *S. aureus*, *M. luteus*, and *B. cereus*. Rhizome extracts and fractions of *A. galanga* and *C. longa* had no inhibitory effect on *M. luteus* and *S. aureus*, respectively. PT fractions of leaves and florescences of *E. elatior* displayed no antibacterial activity. With the addition of 0.01 mg/ml of EDTA, extracts and fractions of *A. galanga*, *C. longa*, and *E. elatior* showed moderate, weak, and strong responses, respectively. Strongest antibacterial activity was observed in the PT fraction of rhizomes of *A. galanga* with MID of 0.16 mg/disc against all three bacterial species. Noteworthy was the strong inhibition of PT fractions of *E. elatior* leaves and flowers which showed no antibacterial activity prior to the addition of EDTA. The effect of EDTA on the antibacterial activity of these three ginger species warrants further investigation. A range of EDTA concentrations and more bacterial species including Gram-negative bacteria should be tested.

**ACKNOWLEDGEMENTS**

The authors would like to thank the Faculty of Applied Sciences, UCSI University for the support in conducting this study. The assistance provided by the laboratory staff is gratefully acknowledged.

**REFERENCES**

Chan, et al.: Antioxidant and Antibacterial Properties of Alpinia galanga, Curcuma longa, and Etlingera elatior (Zingiberaceae)


