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

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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.[1] 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.[5] They are also used for cleaning wounds. A decoction of leaves of *A. galanga* is consumed to treat diarrhea.[6]

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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.[1] 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.[7-9] 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.[10] Studies have shown that leaves of gingers have stronger antioxidant activity than rhizomes[11] and that leaves of highland ginger populations have higher antioxidant activity than lowland populations.[12] Drying of ginger leaves using thermal methods resulted in drastic declines in antioxidant activity.[13]

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.[11] Of leaves of five *Etlingera* species studied, *E. elatior* had the strongest antioxidant properties.[12] 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.[15,16] 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.[11,12] 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.[11,12] 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