Cytotoxic and antioxidant activities of two species of ginger extracts

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Abstract
Ginger or Khing (Thailand), a plant that belongs to the family Zingiberaceae, is one of the herbs commonly used in Thai traditional medical formulas. From selective interviews with Thai folk doctors and review Thai traditional medicine textbooks found that the word ginger was classified in two terms, Khing and Khing-Haeng. Folk doctors described that Khing-Haeng is more pungent than Khing but Khing-Haeng is becoming extinct. Thus, nowadays they used Khing instead Khing-Haeng. Khing is identified as Zingiber officinale and Khing-Haeng as Zingiber ligulatum. Thus, the objectives of this research are the investigation and comparing biological activities, using in vitro cytotoxicity against lung cancer cell lines (COR-L23) and antioxidant activity. Only ethanolic extracts of Zingiber officinale and Zingiber ligulatum showed similar antioxidant activity with EC\textsubscript{50} value of 15.10 ± 2.50 and 15.89 ± 2.92 μg/ml, respectively. For cytotoxic activity, only the ethanolic extract of Zingiber officinale showed activity against COR-L23 with IC\textsubscript{50} value of 7.90 ± 1.90 μg/ml but Zingiber ligulatum showed less activity (IC\textsubscript{50} = 42.27 ± 2.28 μg/ml). These results revealed that Zingiber officinale possessed high cytotoxic activity against lung cancer cells and confirmed using Zingiber officinale replace Zingiber ligulatum which nearly disappear.

Keywords: Zingiber officinale (Khing), Zingiber ligulatum (Khing-Haeng), cytotoxicity, antioxidant activity.

Introduction
Ginger or Khing (Thailand), a plant that belongs to the family Zingiberaceae, is one of the herbs commonly used in Thai traditional medical formulas. Generally, the word Khing refers to the edible ginger, Zingiber officinale. From selective interviews with Thai folk doctors and review Thai traditional medicine textbooks found that the word ginger was classified in two terms, Khing and Khing-Haeng. Folk doctors described that Khing, refers to the edible ginger, King haeng is more pungent taste than Khing refers to be the medical ginger. Practically, they used Khing more than Khing-Haeng because Khing-Haeng is becoming extinct. Thus, nowadays they also used Khing instead Khing-Haeng as medical ginger. Khing was growing widely in Thailand so it find easily for commercial. Resulting from our investigated and identification, they are 2 species of the family Zingiberaceae; Khing was identified as Zingiber officinale Rosc. and Khing-Haeng as Zingiber ligulatum Roxb.

The previous reported about Z. officinale found that its have a variety of biological activities including anticancer (Katiyar et al., 1996; Lee and Surh, 1998; Bode et al., 2001; Chung et al., 2001; Keum et al., 2002; Leal et al., 2003; Miyoshi et al., 2003; Wang et al., 2003), antioxidation and anti-inflammation (Aeschbach et al., 1994; Habsah et al., 2000; Surh, 2003), anti-platelet aggregation (Tjendraputra et al., 2003), anti-fungal (Ficker et al., 2003) and neuroprotective (Kim and Kim, 2004). Surprisingly, there have been no reports on biological activities of Zingiber ligulatum. The objectives of this research are the investigation and comparing biological activities, using in vitro cytotoxicity against lung cancer cell lines (COR-L23) and antioxidant activity.
Methods
Plant materials
Khing (Z. officinale) was collected from amphor Khaoko, Phetchabun province, and Khing-Haeng (Z. ligulatum) was collected from amphor Saentum, Trad province. Their herbarium were collected for identification by expertee from Department of Forestry Bangkok, Thailand where the herbarium vouchers have been kept.

Extraction
The extraction procedures used were similar to those practiced by folk doctors (ethanolic extract and water extract). Dried ground material was marcerated with 95% ethanol, and then filtered and concentrated to dryness under reduced pressure. For water extract, plants were decocted, and then filtered and dried by freeze drying. The percentage yields of extracts were calculated.

In vitro assay for Antioxidant activity
Antioxidant activity determined using DPPH assay, according to modified method of Yamasaki et al. (1994). The mixture was incubated at 25°C for 30 min. Then the decrease in absorbance due to DPPH was measured at 540 nm using a micro-plate reader. The antioxidant activity of each extract expressed as IC50 (mg/ml).

In vitro assay for cytotoxic activity
Plant extracts were diluted and tested the cytotoxicity against COR-L23 using sulphorhodamine B (SRB) assay (Skehan et al., 1990). The monolayered of cell cultures in 96-well plate were treated with sample for 4 replications. The plates were incubated for an exposure time at of 72 hours, and then the medium was removed and washed. The plates were incubated for a recovery period of 3 days. The survival percentage was measured colorimetric using SRB assay and IC50 value was calculated by means of GraphPad Prism (version 4.0) program.

Results and Discussion
Table 1. The percentage yields, cytotoxicity against COR-L23 and Antioxidant activity by DPPH assay of the extracts of Khing and Khing-Haeng (n=3)

<table>
<thead>
<tr>
<th>Plant or Sample</th>
<th>Part of used</th>
<th>Extract</th>
<th>% Yield</th>
<th>Antioxidant</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EC50 ± SEM (µg/ml)</td>
<td>IC50 ± SEM (µg/ml)</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Rhizome</td>
<td>Water (ZO1)</td>
<td>8.15</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>(Khing)</td>
<td></td>
<td>EtOH (ZO2)</td>
<td>4.29</td>
<td>15.10 ± 2.50</td>
<td>7.90 ± 1.90</td>
</tr>
<tr>
<td>Zingiber ligulatum</td>
<td>Rhizome</td>
<td>Water (ZL1)</td>
<td>15.84</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>(Khing-Haeng)</td>
<td></td>
<td>EtOH (ZL2)</td>
<td>10.70</td>
<td>15.89 ± 2.92</td>
<td>42.27 ± 2.28</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.36 ± 0.21</td>
<td>-</td>
</tr>
</tbody>
</table>

The results of antioxidant activity and cytotoxic activity of the extracts from Zingiber officinale and Zingiber ligulatum are shown in Table 1. The ethanolic extract of Zingiber officinale and Zingiber ligulatum showed moderate antioxidant activity with EC50 values of 15.10 ± 2.50 and 15.89 ± 2.92 µg/ml, respectively. For cytotoxic activity, only ethanolic extracts of Zingiber officinale was exhibited against COR-L23 with IC50 values 7.90 ± 1.90 µg/ml.
Conclusion

The results from our testing showed that the ethanolic extracts of *Zingiber officinale* and *Zingiber ligulatum* have similar antioxidant activity while only the ethanolic extracts of *Zingiber officinale* possessed high cytotoxic activity against lung cancer cells. It's confirmed using *Zingiber officinale* instead *Zingiber ligulatum* of Thai folk doctors for treatment cancer.

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References