Original article P11

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 EC₅₀ 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 IC₅₀ value of 7.90 ± 1.90 µg/ml but Zingiber ligulatum showed less activity (IC₅₀ =42.27 \pm 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 identied as Zingiber officinale Rosc. and Khing-Haeng as Zingiber ligulatum Roxb.

The previous reported about of *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 Diccussion

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)

Plant or Sample	Part of used	Extract	% Yield	Antioxidant	Cytotoxicity
				$EC_{50} \pm SEM (\mu g/ml)$	$IC_{50} \pm SEM (\mu g/ml)$
Zingiber officinale (Khing)	Rhizome	Water (ZO1)	8.15	> 100	> 100
		EtOH (ZO2)	4.29	15.10 ± 2.50	7.90 ± 1.90
Zingiber ligulatum (Khing-Haeng)	Rhizome	Water (ZL1)	15.84	> 100	> 100
		EtOH (ZL2)	10.70	15.89 ± 2.92	42.27 ± 2.28
BHT	-	-	-	11.36 ± 0.21	-

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. Its confirmed using Zingiber officinale instead Zingiber ligulatum of Thai folk doctors for treatment cancer.

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References

- 1. Aeschbach R, Loliger J, Scott BC, Murcia A, Butler B, Halliwell B, Aruoma OI. Antioxidant actions of thymol, carbacrol, 6-gingerol, zingerone and hydroxytyrosol. Food and Chemical Toxicology 1994; 32:31–6.
- 2. Bode AM, Ma WY, Surh YJ, Dong Z. Inhibition of epidermal growth factor-induced cell transformation and activator protein activation by [6]-gingerol. Cancer Research 2001; 61: 850–3.
- 3. Chung WY, Jung YJ, Surh YJ, Lee SS, Park KK. Antioxidative and antitumor promoting effects of [6]-paradol and its homologs. Mutation Research 2001; 496: 199–206.
- 4. Ficker C, Smith ML, Akpagana K, Gbeassor M, Zhang J, Durstm T, Assabgui, R, Arnason JT. Bioassay-guided isolation and identification of antifungal compounds from ginger. Phytotherapy Research 2003; 17: 897–902.
- 5. Habsah M, Amran M, Mackeen MM, Lajis NH, Kikuzaki H, Nakatani N, Rahman AA., Ghafar Ali, A.M.Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. Journal of Ethnopharmcology 2000; 72: 403–10.
- 6. Katiyar, S.K., Agarwal, R., Makhtar, H. Inhibition of tumor promotion in SENKAR mouse skin by ethanol extract of *Zingiber officinale* rhizome. Cancer Research 1996; 56: 1023–30.
- 7. Keum, YS, Kim J, Lee KH, Park KK, Surh YL, Lee JM, Lee SS, Yoon JH, Joo SY, Cha IH, Yook JI. Induction of apoptosis and caspase-3 activation by chemopreventive [6]-paradol and structurally related compounds in KB cells. Cancer Letters 2002; 177: 41–7.
- 8. Leal PF, Braga MEM, Sato DN, Carvalho JE, Marques MOM, Meireles MAA. Functional properties of spice extracts obtained via supercritical fluid extraction. Journal of Agricultural and Food Chemistry 2003; 51: 2520–25.
- 9. Lee E, Surh YJ. Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. Cancer Letters 1998; 134: 163–8.
- 10. Miyoshi N, Nakamura Y, Ueda Y, Abe M, Ozawa Y, Uchida K, Osawa T. Dietary ginger constituents, galanals A and B, are potent apoptosis inducers in Human T lymphoma Jurkat cells. Cancer Letters 2003; 199: 113–9.
- 11. Skehan P, Storeng, R, Scudier D, Monks A, Mc Mahon J, Vistica D, Warren JT, Bokesch H, Kenney S, and Boyd MR. New colorimetric cytotoxicity Assay for anticancer-drug screening. J. Natl. Cancer Inst.1990; 82: 1107-12.
- 12. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer 2003; 3: 768–80
- 13. Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH, Duke CC. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thrombosis Research 2003; 111: 259–65.

- 14. Wang CC, Chen LG, Lee LT, Yang LL. Effect of 6- gingerol, an antioxidant from ginger, on inducing apoptosis in human leukemic HL-60 cells. In Vivo 2003; 17:641–645.
- 15. Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effects of methanol extract of crude drugs. Chem. Pharm. Bull. 1994; 42: 1663-1665.