

SELECTIVE ANTICANCER ACTIVITY OF *CURCUMA XANTHORRHIZA* FERMENTED JUICEWARINTORN RUKSIRIWANICH^{1*}, HIROYUKI AKAZAWA², PENSAK JANTRAWUT¹

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ABSTRACT

Objective: The objective of this study was to investigate the anticancer activity and toxicity of fermented juices from six Thai plants, *Garcinia mangostana* Linn., *Tinospora crispa* Linn., *Litchi chinensis* Sonn., *Dimocarpus longan* Lour., *Curcuma xanthorrhiza* (CX) Roxb., and *Averrhoa bilimbi* Linn., in cancer cell lines and normal cells as compared with common commercial fermented juices.

Methods: The plants were fermented for 3 months, and their biological activities including 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging, metal chelating, lipid peroxidation inhibition, and tyrosinase inhibition activities were subsequently evaluated. Furthermore, their *in vitro* anticancer activities in human colon adenocarcinoma (HT-29), cervical cancer (HeLa), hepatocellular carcinoma (HepG2), and murine melanoma (B16F10) cell lines and their toxicities in normal human skin fibroblasts were assessed.

Results: The fermented juice of CX showed a higher antioxidant activity than those of the other plants, with an SC₅₀ (DPPH scavenging) value of 0.011 mg/mL, an IPC₅₀ (lipid peroxidation inhibition) of 0.027 mg/mL, an MC₅₀ (metal chelating) value of 0.170 mg/mL, and an IC₅₀ (tyrosinase inhibition) of 0.027 mg/mL. Moreover, the fermented juice of CX displayed selective toxicity in cancer cell lines (HeLa, HepG2, and B16F10), with a significantly lower toxicity in normal human skin fibroblasts as compared with an expensive commercial fermented juice product.

Conclusion: The present study suggests that the fermented juice of CX can be developed as a functional food supplement with antioxidant properties or as an anticancer product with low toxicity to normal human skin fibroblasts.

Keywords: Fermented juice, *Curcuma xanthorrhiza*, Anticancer, HeLa, HT-29, HepG2, B16F10.

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INTRODUCTION

Fermentation is a cheap and energy efficient means of preserving perishable raw materials. When fruit and vegetables are harvested, they undergo rapid deterioration, especially in the humid tropics where the prevailing environmental conditions accelerate the process of decomposition [1]. Fermentation requires very little sophisticated equipment for either the fermentation process or for subsequent storage of the fermented product [2]. It is a technique that has been employed for generations to preserve food for consumption at a later date and to improve food security [3,4].

Molasses is an agro-industrial by-product of the processing of sugarcane and sugar beet into sugar. It is often used as a microbiological energy source in a wide range of fermentation processes to grow yeasts, molds, and bacteria that transform sugars into alcohol, yeast, citric acid, and the food additives monosodium glutamate and lysine. Molasses is the most cost-effective of the main energy sources available for such industries, is easy to incorporate into fermentation processes, and represents a stable and predictable substrate for storage and handling [5]. The development of foods and beverages that promote health and well-being is one of the current research priorities. The trend of consumption of food enriched in probiotics is increasing due to scientific evidence that they help to maintain healthy gut microflora [6,7]. It has been suggested that fruit or vegetable juices could serve as a suitable media for cultivating probiotic bacteria [8]. Various studies have been carried out to explore the suitability of fermented fruit or vegetable juices such as tomato, beetroot, and cabbage as raw materials for the production of fermented drinks.

Many medicinal plants have shown antioxidant and other applications to promote vitality and well-being. *Garcinia mangostana* (GM) (Clusiaceae), commonly known as mangosteen, is a slow-growing tropical evergreen tree found mainly in India, Myanmar, Sri Lanka, and Thailand. Recently, products manufactured from GM have begun to be used as botanical dietary supplements in the United States due to their potent antioxidant potential [9]. Moreover, GM, ethyl acetate extract was reported to have cytotoxic effect on T47D breast cancer cell line [10]. *Tinospora crispa* (TC) (Menispermaceae) is an herbaceous climbing plant that is widely distributed in Southeast Asia, particularly in Vietnam, Thailand, and Malaysia [11]. Oral administration of the stem decoction of this medicinal herb has been used in traditional Thai medicine due to its antipyretic, antidiabetic, anti-inflammatory, antioxidant, and health-maintaining properties [12-14]. Lychee (*Litchi chinensis* [LC]) and Longan (*Dimocarpus longan* [DL]) are members of the Sapindaceae family, which are highly attractive subtropical fruits widely distributed in Northern Thailand. These fruits are well received by consumers due to their delicious taste and possible health benefits. They contain significant amounts of phenolic compounds that have been confirmed to have antioxidant and anticancer activities [15-18]. *Curcuma xanthorrhiza* (CX), a member of the Zingiberaceae family and a native Indonesian plant, is grown in Thailand, the Philippines, Sri Lanka, and Malaysia. CX is a low-growing plant with a rhizome similar to ginger and has an aromatic, pungent odor, and bitter taste [19] with antibacterial activity [20]. *Averrhoa bilimbi* (AB), also called Bilimbi, is found throughout Indonesia, the Philippines, Bangladesh, and other Southeastern Asian countries. Bilimbi has been used in traditional medicine to control obesity and diabetes mellitus [21] and also possesses antihyperlipidemic properties [22]. Moreover, some Thais believe that the fermented juice of these six plants or expensive

commercial fermented juice (10 USD/mL) can be used as anticancer remedies by drinking once daily. However, there exists no scientific evidence of the anticancer activities or the safety profiles in normal cells of the fermented juices of these six plants.

In the present study, these six plants were fermented for 3 months, and their biological activities including 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging, metal chelating, lipid peroxidation inhibition, and tyrosinase inhibition were evaluated. Furthermore, their *in vitro* anticancer activities in HeLa, HT-29, HepG2, and B16F10 cell lines and their toxicity in normal cells were assessed.

MATERIALS AND METHODS

Materials

Ascorbic acid, α -tocopherol, DPPH, ammonium thiocyanate (NH_4SCN), ethylenediaminetetraacetic acid (EDTA), ferrozine, ferric chloride (FeCl_2), potassium persulfate, linoleic acid, sulfurhodamine B (SRB), tris(hydroxymethyl)-methylamine, and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tyrosinase from mushroom (4187 U/mg) and L-tyrosine were purchased from Fluka (Switzerland). Dulbecco's modified Eagle's medium (DMEM), α -modified Eagle's culture medium (MEM-alpha), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from GIBCO (Grand Island, NY, USA). Absolute ethanol was purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals and reagents were of analytical grade.

Plant selection

Six medicinal plants, GM, TC, LC, DL, CX, and AB, which have scientific evidence of antioxidant activity, were selected. These plants were collected from Chiang Mai Province in Thailand during January–February 2014 (Table 1). The specimens were authenticated by a botanist and deposited at the Faculty of Pharmacy, Chiang Mai University, Thailand.

Preparation of fermented juices

The six plants were washed, cut into pieces, and air-dried. For the fermentation process of CX and TC, each plant was mixed with water (reverse osmosis) and molasses in a 1:10:1 weight ratio, while a ratio of 3:10:1 was used for the other plants (GM, LC, DL, and AB). The fermented juices were sampled at 0, 1, 2, and 3 months for antioxidant and tyrosinase inhibition activity determination.

Antioxidant assays

DPPH radical scavenging activity

The DPPH radical scavenging activities of the fermented juices were determined using a previously described method [23], with slight modifications. Briefly, five serially diluted concentrations of each sample, along with 50 μL distilled water and 50 μL ethanolic solution of DPPH, were added to the wells of a 24-well plate (Nalge Nunc International, NY, USA). The reaction mixture was allowed to incubate for 30 min at $25 \pm 2^\circ\text{C}$, after which the solution was separated, transferred to a 96-well microplate, and the absorbance was measured at 515 nm against a blank (ethanol) using a plate reader (Bio-Rad, model 680 microplate reader, USA). Ascorbic acid was used as the positive control. All experiments were performed in triplicate. The percentages of DPPH radical scavenging activity were calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Where, A_{sample} is the absorbance of the DPPH radical with the extract and A_{control} is the absorbance of the DPPH radical alone. The SC_{50} value, which is the concentration of the sample that scavenged 50% of the DPPH radical, was determined. A histogram of the DPPH scavenging activity (%) of 0.01 mg/mL samples following 0, 1, 2, and 3 months fermentation is presented.

Lipid peroxidase inhibition activity

The lipid peroxidase inhibition activities of the fermented juices were assayed using a modified ferric thiocyanate method [24,25]. Five serially diluted concentrations of each sample, 100 μL distilled water and 50 μL linoleic acid in 50% (v/v) DMSO, were added to a 24-well plate. The reaction was initiated by the addition of 50 μL NH_4SCN (5 mM) and 50 μL FeCl_2 (2 mM). The mixture was incubated at $37 \pm 2^\circ\text{C}$ for 1 h. During the oxidation of linoleic acid, peroxides are formed that lead to the oxidation of Fe^{2+} to Fe^{3+} . The latter ions form a complex with thiocyanate, which can be detected at 490 nm using a plate reader following solution separation and transfer to a 96 microplate. Distilled water without sample was used as a negative control, and α -tocopherol was used as a positive control. All experiments were performed in triplicate. The inhibition percentages of the lipid peroxidation of linoleic acid were calculated using the following equation:

$$\text{Inhibition of lipid peroxidation activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Where, A_{sample} is the absorbance of NH_4SCN , FeCl_2 , and linoleic acid with the extract and A_{control} is the absorbance of NH_4SCN , FeCl_2 , and linoleic acid alone. The IPC_{50} value, which is the concentration of the sample that inhibited 50% of the peroxidation, was determined. A histogram of the inhibition of lipid peroxidation activity (%) of 0.01 mg/mL samples following 0, 1, 2, and 3 months fermentation is presented.

Chelating ability

The Fe^{2+} chelating abilities of the fermented juices were measured using the ferrous iron-ferrozine complex method [26]. Briefly, the reaction mixture containing 2 mM FeCl_2 (10 μL), 5 mM ferrozine (10 μL), and five serially diluted concentrations of each sample were mixed in a 24-well plate and incubated for 10 min at $25 \pm 2^\circ\text{C}$. Subsequently, the solution was separated, transferred to a 96 microplate, and the absorbance was measured at 570 nm using a plate reader. The absorbance of the control was determined by replacing the samples with ethanol. EDTA (0.001–10 mg/mL) was used as a positive control. All experiments were performed in triplicate. The MC_{50} value, which is the concentration of the sample that chelated 50% of the ferrous iron, was determined. The abilities of the samples to chelate ferrous ions were calculated using the following equation:

$$\text{Metal chelation activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Where, A_{sample} is the absorbance of FeCl_2 and ferrozine with the extract and A_{control} is the absorbance of FeCl_2 and ferrozine alone. A histogram of

Table 1: Scientific name, family, common name, and abbreviation of the six selected medicinal plants for fermentation

Scientific name	Family	Common name	Abbreviation
<i>Garcinia mangostana</i> L.	Clusiaceae	Mangosteen	GM
<i>Tinospora crispa</i> L.	Menispermaceae	Heartleaf moonseed	TC
<i>Litchi chinensis</i> Sonn.	Sapindaceae	Lychee	LC
<i>Dimocarpus longan</i> Lour.	Sapindaceae	Longan	DL
<i>Curcuma xanthorrhiza</i> Roxb.	Zingiberaceae	Temulawak	CX
<i>Averrhoa bilimbi</i> Linn.	Oxalidaceae	Bilimbi	AB

the metal chelation activity (%) of 0.01 mg/mL samples following 0, 1, 2, and 3 months fermentation is presented.

Tyrosinase inhibition assay

The fermented juices were assayed using a previously described tyrosinase inhibition method [27], with slight modifications. Briefly, 120 µL 1.66 mM tyrosine solution in 0.1 M phosphate buffer (pH 6.8), 60 µL five serially diluted concentrations of each sample, and 60 µL phosphate buffer were mixed in a 96-well plate and incubated at 37±2°C for 60 min. Subsequently, 60 µL tyrosinase enzyme solution (0.6 mg/mL) in phosphate buffer was added. The enzyme activity at 37±2°C was measured at 450 nm using a plate reader. Kojic acid was used as a positive control. All experiments were performed in triplicate. The IC₅₀ value, which is the concentration of the sample that inhibited 50% of the enzyme activity, was determined. The inhibition percentages of tyrosinase were calculated using the following equation:

$$\text{Tyrosinase inhibition activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Where, A_{sample} is the absorbance of tyrosine and tyrosinase with the extract and A_{control} is the absorbance of tyrosine and tyrosinase alone. A histogram of the tyrosinase inhibition activity (%) of 0.01 mg/mL samples following 0, 1, 2, and 3 months fermentation is presented.

Cell culture

Human colon adenocarcinoma (HT-29), cervical cancer (HeLa), hepatocellular carcinoma (HepG2), and murine melanoma (B16F10) cells were cultured under the standard conditions in complete DMEM, and normal human skin fibroblasts were cultured in complete MEM-alpha, both supplemented with 10% (v/v) FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL). Cells were incubated in a temperature controlled, humidified incubator (Shel Lab, model 2123TC, USA) with 5% CO₂ at 37°C.

Cytotoxic activity measured by the SRB assay

The selected fermented juices at five serially diluted concentrations, the standard anticancer drugs, cisplatin and doxorubicin, and a commercial fermented juice were tested for cytotoxic activity on four cancer cell lines and normal skin fibroblasts using the SRB assay according to the method reported by Papazisis et al. [28]. Briefly, cells were plated at a density of 1.0×10⁴ cells/well on 96-well plates and incubated for 24 h at 5% CO₂ and 37°C. Cells were subsequently exposed to five serially diluted concentrations of each sample for 24 h. Following incubation, cells were fixed in 50% TCA solution at 4°C for 1 h and washed 5 times with distilled water. The excess water was drained and the plates were air-dried for 24 h. Cells were subsequently stained with 50 µL 0.4% SRB solution in 1% acetic acid for 30 min at room temperature (25±2°C). Following staining, the SRB solution was decanted and the plates were washed with 1% acetic acid until only the adhered dye remained. The plates were air-dried and 100 µL 10 mM tris-base solution was added to solubilize the dye. The plates were shaken for 30 min at room temperature (25±2°C),

and the absorbance was measured at 540 nm using a plate reader. All experiments were performed in triplicate. The percentages of cell viability were calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100\%$$

Where, A_{sample} is the optical density of the cells treated with the extract, A_{control} is the optical density of the non-treated cells, and A_{blank} is the optical density of the tris-base solution at time zero. The IC₅₀ value, which is the concentration that inhibits 50% of cell viability, was obtained by plotting the percentages of cell viability versus the concentrations of the samples.

Statistical analysis

The results are presented as the mean ± standard deviation (SD) of three independent experiments. The data were analyzed using ANOVA with an least SD test; p<0.05 is considered statistically significant.

RESULTS AND DISCUSSION

Antioxidant effect of the fermented juices

DPPH scavenging activity

The percentages of DPPH scavenging activity of the fermented juices at 0.01 mg/mL at time 0, and following 1, 2, and 3 months fermentation and their SC₅₀ values are shown in Fig. 1 and Table 2. Ascorbic acid (the standard antioxidant) had an SC₅₀ value of 0.025 mg/mL. The fermented juice from CX exhibited a higher DPPH scavenging activity than those of the other plants. The highest

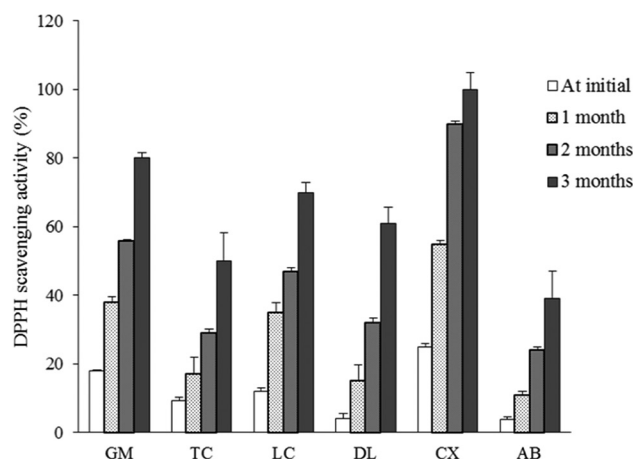


Fig. 1: Comparison of the percentages of DPPH scavenging activity of fermented juices at time 0, and following 1, 2, and 3 months fermentation

Table 2: DPPH radical scavenging (SC₅₀), lipid peroxidation inhibition (IPC₅₀), metal chelation (MC₅₀), and tyrosinase inhibition (IC₅₀) activities of fermented juices at 3 months and reference compounds

Scientific name	Abv	SC ₅₀ (mg/mL)	IPC ₅₀ (mg/mL)	MC ₅₀ (mg/mL)	IC ₅₀ (mg/mL)
<i>Garcinia mangostana</i>	GM	0.015±0.003	0.015±0.005	n	0.027±0.002
<i>Tinospora crispa</i>	TC	0.081±0.012	0.051±0.001	n	0.043±0.001
<i>Litchi chinensis</i>	LC	0.029±0.011	0.015±0.012	n	0.008±0.001
<i>Dimocarpus longan</i>	DL	0.046±0.013	0.078±0.001	n	0.041±0.001
<i>Curcuma xanthorrhiza</i>	CX	0.011±0.008	0.027±0.001	0.170±0.090	0.027±0.005
<i>Averrhoa bilimbi</i>	AB	0.095±0.009	0.028±0.001	n	0.510±0.065
Ascorbic acid ^{a)}	-	0.025±0.008	-	-	-
α-Tocopherol ^{a)}	-	-	0.0005±0.0001	-	-
EDTA ^{a)}	-	-	-	0.0002±0.0001	-
Kojic acid ^{a)}	-	-	-	-	0.0008±0.0001

a): Reference compound, n=No activity, -: Did not detected, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical

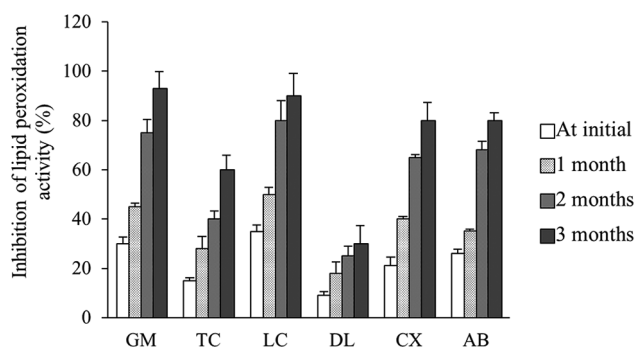


Fig. 2: Comparison of the percentages of lipid peroxidation inhibition activity of fermented juices at 0.01 mg/mL at time 0, and following 1, 2, and 3 months fermentation

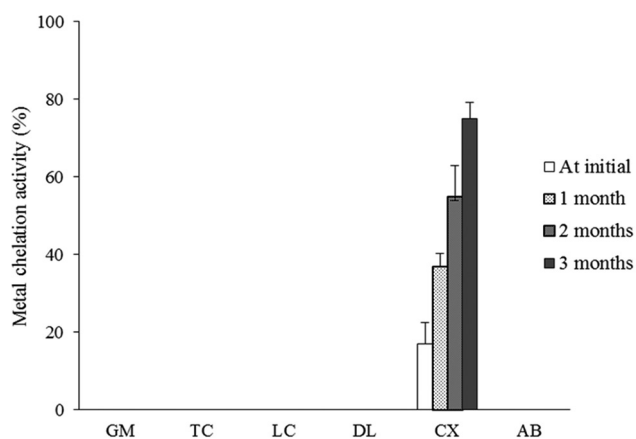


Fig. 3: Comparison of the percentages of metal chelation activity of fermented juices at 0.01 mg/mL at time 0, and following 1, 2, and 3 months fermentation

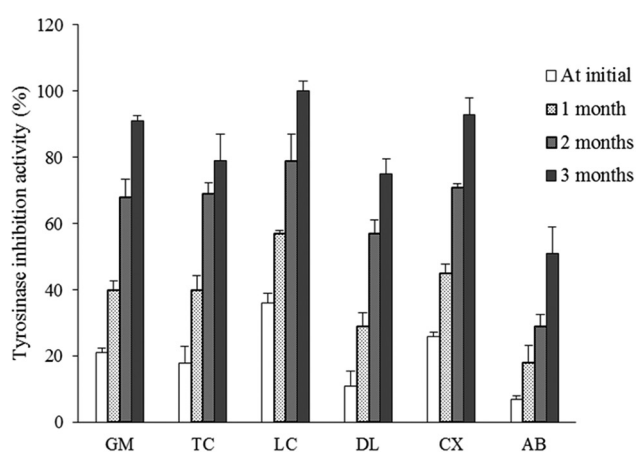


Fig. 4: Comparison of the percentages of tyrosinase inhibition activity of fermented juices at 0.01 mg/mL at time 0, and following 1, 2, and 3 months fermentation

scavenging activity, 98.64%, of CX fermented juice was found at 0.01 mg/mL and following 3 months fermentation, with an SC_{50} value of 0.011 mg/mL, which was 2.3 times more potent than ascorbic acid. The DPPH scavenging activity of every fermented juice was increased with increasing fermentation time. The effect of fermentation on the antioxidant properties of various plant materials

has been previously investigated, with an increase in phenolic content following fermentation being shown to be responsible for the enhanced antioxidant activity [29,30]. Previous studies have indicated that the strong antioxidant activity of the rhizome oil of CX may be related to the high level of xanthorrhizol (32%); however, there are other relevant constituents present such as curcuminoid, which is the main yellow bioactive substance [31,32]. The radical scavenging activity of curcuminoids from the rhizomes of CX has been investigated and confirmed by many researchers [33-35]. The root of CX also contains a mixture of dicinnamoylmethane derivatives such as curcumin (diferuloylmethane), monodemethoxycurcumin (feruloyl-p-hydroxycinnamoylmethane), and bisdesmethoxycurcumin (bis-(p-hydroxycinnamoyl) methane) [36].

Lipid peroxidation inhibition activity

None of the fermented juices had a greater lipid peroxidation inhibition activity than α -tocopherol. At 0.01 mg/mL, standard α -tocopherol had an IPC_{50} value of 0.0005 mg/mL (Table 2). Among the six plants, GM and LC fermented juices showed the highest peroxidation inhibition activities, with IPC_{50} values of 0.015 mg/mL, whereas DL fermented juice had the lowest activity, with an IPC_{50} value of 0.078 mg/mL. The fermented juice of CX had a moderate lipid peroxidation inhibition activity, with an IPC_{50} value of 0.027 mg/mL (Table 2 and Fig. 2). Curcumin, which is also one of the components of CX, has been found to inhibit liposomal peroxidation and peroxide-induced DNA damage [37].

Metal chelating activity

The MC_{50} values of the metal chelating activities of the six fermented juices are shown in Table 2. None of the fermented juices except CX showed any chelating activity. The fermented juice of CX had an MC_{50} value of 0.170 mg/mL, whereas the standard compound, EDTA, had a value of 0.0002 mg/mL. Fig. 3 shows the comparison of the metal chelating percentages of CX fermented juices at 0.01 mg/mL at time 0, and following 1, 2, and 3 months fermentation. The metal chelating activity of CX fermented juice increased with increasing fermentation time. This may be due to the fact that CX contains an enormous amount of polyphenolic compounds [38], which are potent antioxidants as a result of their metal chelating properties [39]. Moreover, the high chelating effect may be due to the ability of xanthorrhizol, the major active compound in CX, to chelate Cu^{2+} ions and inhibits the initiation of free radical formation [40], which could be attributed to tyrosinase inhibition activity [41].

Tyrosinase inhibition activity

Fig. 4 and Table 2 show the comparison of the tyrosinase inhibition activities (%) of the fermented juices at 0.01 mg/mL at time 0, and following 1, 2, and 3 months fermentation and the IC_{50} values of the tyrosinase inhibition activity of the fermented juices at 3 months. The tyrosinase inhibition activity of every fermented juice was increased with increasing fermentation time. The fermented juice of LC showed the highest tyrosinase inhibition activity of 97.47%, with an IC_{50} value of 0.008 mg/mL following 3 months fermentation; however, this value was lower than that of Kojic acid, which is a strong tyrosinase enzyme inhibitor. The CX fermented juice showed a tyrosinase inhibition activity of 93.11%, with an IC_{50} value of 0.027 mg/mL following 3 months fermentation, which was 3.4 times lower than that of the LC fermented juice. Evaluation of tyrosinase inhibition is the most common approach for the elucidation of skin lightening agents since this enzyme catalyzes the rate-limiting step of pigmentation [42]. Recently, it has been shown that fermented rice extract has an enhanced tyrosinase inhibition activity as compared with the unfermented rice extract. Moreover, fungi are known to have great potential for the production of bioactive compounds which lead to the enhancement of various biological activities in fermented substrates. Fungi produce many enzymes during fermentation, such as glycoside, hydrolase, cellulase, or xylan-degrading enzymes

Table 3: The cytotoxicity (IC₅₀) and cell viability (%) of the fermented juices, the commercial fermented juice product, and the standard anticancer drugs, cisplatin and doxorubicin, in four cancer cell lines, and normal fibroblast cells, respectively

Scientific name	Abv	IC ₅₀ (mg/mL)				Cell viability in normal cells (%)
		HeLa	HT-29	HepG2	B ₁₆ F ₁₀	
<i>Garcinia mangostana</i>	GM	736±11	451±38	>	655±41	112.5±8.4
<i>Tinospora crispa</i>	TC	862±23	>	615±18	>	39.6±1.3
<i>Litchi chinensis</i>	LC	>	>	>	>	104.3±11.1
<i>Dimocarpus longan</i>	DL	>	>	>	>	68.6±7.4
<i>Curcuma xanthorrhiza</i>	CX	370±23	446±27	386±8	470±14	59.6±3.6
<i>Averrhoa bilimbi</i>	AB	>	>	>	>	110±9.8
Commercial fermented juice product		>	293±11	>	>	44.4±4.6
Cisplatin ^{a)}		0.28±0.02	0.19±0.01	26.40±1.20	-	-
Doxorubicin ^{a)}		7.59±0.03	8.21±0.05	0.70±0.02	8.29±0.10	-

a): Reference compound, >: IC₅₀> 1000 mg/mL, -: Not detected

and esterase [43], which may suggest enhancement of tyrosinase inhibition activity [44].

Cytotoxic activity in human cancer cell lines

The cytotoxicity (IC₅₀) and cell viability (%) of the fermented juices, a commercial fermented juice product, and standard anticancer drugs in four cancer cell lines and normal skin fibroblasts, respectively, are shown in Table 3. The fermented juice of CX showed greater cytotoxicity than those of the other fermented juices with IC₅₀ values of 370, 446, 386, and 470 mg/mL in HeLa, HT-29, HepG2, and B16F10, respectively, whereas the fermented juices of LC, DL, and AB showed no activity in these four cancer cell lines. GM and TC fermented juices showed cytotoxicity in certain cancer cell lines; HeLa (IC₅₀ value of 736 mg/mL), HT-29 (IC₅₀ value of 451 mg/mL), and B16F10 (IC₅₀ value of 655 mg/mL) for GM; and HeLa (IC₅₀ value of 862 mg/mL) and HepG2 (IC₅₀ value of 615 mg/mL) for TC. The expensive commercial fermented juice product exhibited cytotoxicity only in HT-29 cells, with an IC₅₀ value of 293 mg/mL, and also showed some toxicity in normal skin fibroblasts, with a cell viability of 44.4%. As expected, the standard anticancer drugs, cisplatin and doxorubicin, showed greater anticancer activity than all fermented juices, with IC₅₀ values in the µg/mL range (Table 3). Xanthorrhizol, a sesquiterpenoid compound isolated from the rhizome of CX, has been reported to possess a variety of biological properties including antibacterial, antifungal, and anticancer activities [45-47]. With respect to anticancer activity, Choi *et al.* [48] observed that injection of 0.2–1.0 mg xanthorrhizol/kg bw had an antimetastatic effect in a mouse lung metastasis model. *In vitro* experiments have shown the induction of apoptosis through the upregulation of Bax and p53 in HeLa and HCT116 human colon cancer cells [49]; thus, the phytochemicals found in CX, especially xanthorrhizol, may be synergistic and responsible for the cytotoxicity of CX fermented juice. Furthermore, the results of the present study show that CX fermented juice had a lower toxicity in normal skin fibroblast cells (59.6%) and a higher anticancer activity in HeLa, HepG2, and B16F10 cells than the expensive commercial fermented juice product that some Thais drink to prevent cancer. Moreover, CX fermented juice may be preferential to standard potent anticancer drugs that have a higher toxicity in normal cells [50].

CONCLUSION

The present study demonstrates the *in vitro* antioxidant and anticancer activities, as well as the toxicity in normal cells, of various fermented plant juices. The fermented juice of CX showed the highest DPPH scavenging activity and relatively high lipid peroxidation inhibition, metal chelating, and tyrosinase inhibition activities as compared with those of other fermented juices. Moreover, the fermented juice of CX exhibited cytotoxicity in four cancer cell lines including HeLa, HT-29, HepG2, and B16F10 cells, with low toxicity in normal fibroblast cells. Further assessment of the underlying molecular mechanism of this fermented juice should be carried out for its development as a functional food supplement or anticancer product.

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AUTHOR'S CONTRIBUTION

1. Study conception and design: Ruksiriwanich and Jantrawat
2. Acquisition of data: Akazawa
3. Analysis and interpretation of data: Ruksiriwanich
4. Drafting of manuscript: Jantrawat
5. Critical revision: Ruksiriwanich

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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