ORIGINAL ARTICLE

Antidiabetic and anticancer activities of *Mangifera indica* cv. Okrong leaves

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ABSTRACT

Diabetes and cancer are a major global public health problem. Plant-derived agents with undesirable side-effects were required. This study aimed to evaluate antidiabetic and anticancer activities of the ethanolic leaf extract of *Mangifera indica* cv. Okrong and its active phytochemical compound, mangiferin. Antidiabetic activities against yeast α-glucosidase and rat intestinal α-glucosidase were determined using 1 mM of p-nitro phenyl-α-D-glucopyranoside as substrate. Inhibitory activity against porcine pancreatic α-amylase was performed using 1 mM of 2-chloro-4 nitrophenol-α-D-maltotroside-3 as substrate. Nitrophenol product was spectrophotometrically measured at 405 nm. Anticancer activity was evaluated against five human cancer cell lines compared to two human normal cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Mango leaf extract and mangiferin exhibited dose-dependent inhibition against yeast α-glucosidase with the IC_{50} of 0.0503 and 0.5813 mg/ml, respectively, against rat α-glucosidase with the IC_{50} of 1.4528 and 0.4333 mg/ml, respectively, compared to acarbose with the IC_{50} of 11.9285 and 0.4493 mg/ml, respectively. For anticancer activity, mango leaf extract, at ≥200 μg/ml showed cytotoxic potential against all tested cancer cell lines. In conclusion, mango leaf possessed antidiabetic and anticancer potential *in vitro*.

Key words: Anticancer, antidiabetic, *Mangifera indica L.*, mangiferin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by an uncontrolled increase in blood glucose level.[1-3] Key enzymes for hydrolysis of carbohydrates are α-amylase, which is participating in hydrolysis of polysaccharides and oligosaccharides and α-glucosidase which further hydrolysis di-and tri-saccharides to glucose and other monosaccharides.[4] Cancer is a group of diseases differentiated by the uncontrolled growth and spread of abnormal cells. There are many occurrences common cancer types, that is, breast, lung, liver and colorectal cancers.[5] Currently, chemical agents such as acarbose and doxorubicin are available for treatment of diabetes and cancer, respectively. However, all of these treatments are related with undesirable side effect[1-4,6,7] leading to increase in ethnobotanical use of medicinal plants which may be safer and less destructive to human body.

Mango (*Mangifera indica* L.), a long living large evergreen tree, belong to the Anacardiaceae family.[8] It possesses pharmacological effects, that is, antidiabetic, antioxidant, antimicrobial, anticancer, and anti-inflammatory

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properties.[9] Mango is a rich source of various polyphenolic compounds, especially mangiferin [Figure 1], which is the major component that can be detected in all parts of the mango. This compound is a xanthone derivative that referred as super antioxidant. It also has been found for pharmacological effects including antioxidant, radioprotective, antiallergic, antidiabetic, anticancer, antimicrobial, immunomodulatory, and anti-inflammatory activities.[10,11] This study aimed to evaluate antidiabetic activity of mango leaf extract and mangiferin on *in vitro* inhibition of α-amylase and α-glucosidase enzymes and to demonstrate inhibitory activity against selected cancer cell lines.

MATERIALS AND METHODS

Materials and chemicals

M. indica "Okrong" leaves were collected in Thailand. They were authenticated by Assoc. Prof. Dr. Nijsiri Ruangrungsi. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Leaf samples were washed with water and dried in hot air oven at 50°C. The dried leaves were pulverized and exhaustively extracted with ethanol by Soxhlet apparatus. The extract was filtered through Whatman number 1 filter paper and evaporated to dryness *in vacuo*. The yield was recorded and the extract was stored at −20°C.

Antidiabetic activities

Inhibition of yeast alpha-glucosidase activity

The enzyme inhibition activity against yeast α-glucosidase (Sigma-Aldrich, USA) were determined using 1 mM of p-nitrophenyl-α-D-glucopyranoside (PNPG) as substrate according to Wan *et al*.[12] with minor modifications. In 96 well plate, 30 µl of enzyme solution (0.5 U/ml), 30 µl of 0.1 M sodium phosphate buffer (pH 6.9) and 30 µl of tested inhibitors (mango, mangiferin [MIRA, China], or acarbose [Sigma-Aldrich, USA]) in dimethyl sulfoxide (DMSO) were mixed and incubated at 37°C for 10 min. Next, 30 µl of substrate were added and incubated again at 37°C for 20 min. After incubation, 80 µL of 0.2 µM Na_2CO_3 was added to stop the reaction. The absorbance was measured at 405 nm using Anthos Zenyth 200 RT microplate reader (Biochrom, England). All tested inhibitors were analyzed in triplicate. The percent inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(\text{OD405 control} - \text{OD405 inhibitor})}{\text{OD405 control}} \times 100$$

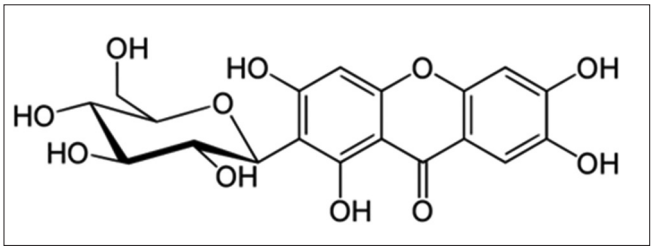

Figure 1: Chemical structure of mangiferin (1,3,6,7-Tetrahydroxyxanthone C2-ß-D-glucoside)

Inhibition of rat alpha-glucosidase activity

The enzyme inhibition activity against intestinal acetone powders from rat (Sigma-Aldrich, USA) were determined using 1 mM of PNPG as substrate, according to Lordan *et al*.[13] and Hemalatha *et al*.[14] with minor modifications. Intestinal acetone powders from rat (30 mg/ml) in 0.1 M sodium phosphate buffer (pH 6.9) was sonicated for 20 min. The suspension was centrifuged at 3500 rpm for 30 min to remove particulate matter. In 96 well plate, 50 µl of tested inhibitors in DMSO, 100 µl of substrate and 50 µl of enzyme solution (0.5 U/ml) were mixed and incubated at 37°C for 30 min. The absorbance was measured at 405 nm using microplate reader. All tested inhibitors were analyzed in triplicate. The percent inhibition was calculated as aforementioned formula.

Inhibition of pancreatic alpha-amylase activity

The enzyme inhibition activity against α-amylase from porcine pancreas (Sigma-Aldrich, USA) were determined using 1 mM of 2-chloro-4 nitrophenol-α-D-maltotroside-3 as substrate, following a method as described previously by Yonemoto *et al*.[15] with modifications. In 96 well plate, 30 µl of enzyme solution (25 U/ml) and 30 µl of tested inhibitors in DMSO were mixed and preincubated at 37°C for 10 min. Then, 30 µl of substrate were added and incubated again at 37°C for 20 min. The absorbance was measured at 405 nm using microplate reader. All tests were analyzed in triplicate. The percent inhibition was calculated as aforementioned formula.

Anticancer activity

Cell cultures

The human cancer cell lines; ductal carcinoma (BT474, ATCC HTB20), bronchogenic carcinoma (Chago K-1, ATCC HTB-168TB), liver hepatoblastoma (Hep-G2, ATCC HB8065), gastric carcinoma (Kato-III, ATCC HTB103), and colon adenocarcinoma (SW 620, ATCC CCL227); The human normal cell lines; skin fibroblast (CCD-986SK, ATCC CRL1947) and lung fibroblast (WI-38 VA-13 subline 2RA, ATCC CLS 300421) were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University. BT474, Chago K-1, Hep-G2, Kato-III, SW 620, and WI-38 cell lines were cultured in RPMI 1640 medium containing 5% fetal calf serum and CCD-986SK cell line was cultured in Dulbecco's modified Eagle's medium. They were incubated at 37°C in a 5% (v/v) CO_2 atmosphere.

Colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

(MTT; Sigma-Aldrich, USA) were determined as described previously by Mosmann[16] with minor modifications. In 96 well plate, 198 µl of 5,000 cells in culture medium were added and incubated at 37°C in a 5% (v/v) CO_2 atmosphere for 24 h. Then, 2 µl of tested inhibitors (mango extract, mangiferin, or doxorubicin), or 2 µl of negative control (DMSO for mango extract and mangiferin; or water for doxorubicin) were added and incubated at 37°C for 48 h. Ten microliter of MTT solution (5 mg/ml) were added into each well and incubated at 37°C for 4 h. The media were removed. A mixture of 150 µl of DMSO and 25 µl of glycine (0.1 mol/l) were added into each well and mixed thoroughly to dissolve the formazan crystals. The absorbance was measured at 540 nm using microplate reader. All tests were analyzed in quadruplicate. The percent survival was calculated as follows:

$$\% \text{ Survival} = \frac{\text{Absorbance intensity of tested sample}}{\text{Absorbance intensity of negative control}} \times 100$$

RESULTS

Antidiabetic activities

Antidiabetic activities of mango leaf extract, mangiferin, and acarbose showed a dose-response relationship [Figure 2]. For yeast α-glucosidase, mango leaf extract showed the greatest inhibition with the IC_{50} of 0.05 mg/ml [Table 1]. Mango leaf extract, mangiferin, and acarbose revealed 86.6, 93.3, and 73.9% inhibition at the concentration of 0.156, 2.5, and 40 mg/ml, respectively [Figure 2]. For rat α-glucosidase, mangiferin showed the greatest inhibition with the IC_{50} of 0.43 mg/ml [Table 1]. They revealed 86.0, 92.6, and 58.7% inhibition at the concentration of 2.5, 1.25, and 1.25 mg/ml, respectively [Figure 1]. For pancreatic α-amylase, mangiferin also showed the most inhibition with the IC_{50} of 1.0485 mg/ml [Table 1]. They revealed 97.2, 95.6, and 73.9% inhibition at the concentration of 10, 5 and 0.156 mg/ml, respectively. Acarbose was used as a positive control in this study.

Anticancer activity

Mango leaf extract, at 200 µg/ml, showed cytotoxicity against all tested cancer cell lines. Mangiferin did not significantly affect % survival of tested cancer cells [Figure 3]. Doxorubicin was used as a positive control; normal skin fibroblast (CCD) and normal lung fibroblast (Wi-38) were comparable cell lines in this study. Mango leaf extract, at high dose, also showed toxicity on lung fibroblast. On the contrary, the extract increased %survival of skin fibroblast. At high dose, mangiferin tended to increase the survival of skin and lung fibroblasts [Figure 3]. The IC_{50} for cytotoxic activities of the extract, mangiferin and doxorubicin are shown in Table 2.

DISCUSSION

α-Glucosidase may be largely divided into two types due to the difference in primary structure, Types I (yeast) and

Table 1: Antidiabetic activities of mango leaf extract, mangiferin, and acarbose*

	IC_{50} (mg/ml)		
	Yeast α-glucosidase	Rat α-glucosidase	Pancreatic α-amylase
Mango leaf extract	0.0503	1.4528	2.2840
Mangiferin	0.5813	0.4333	1.0485
Acarbose	11.9285	0.4493	0.0511

*The tests were done in triplicate

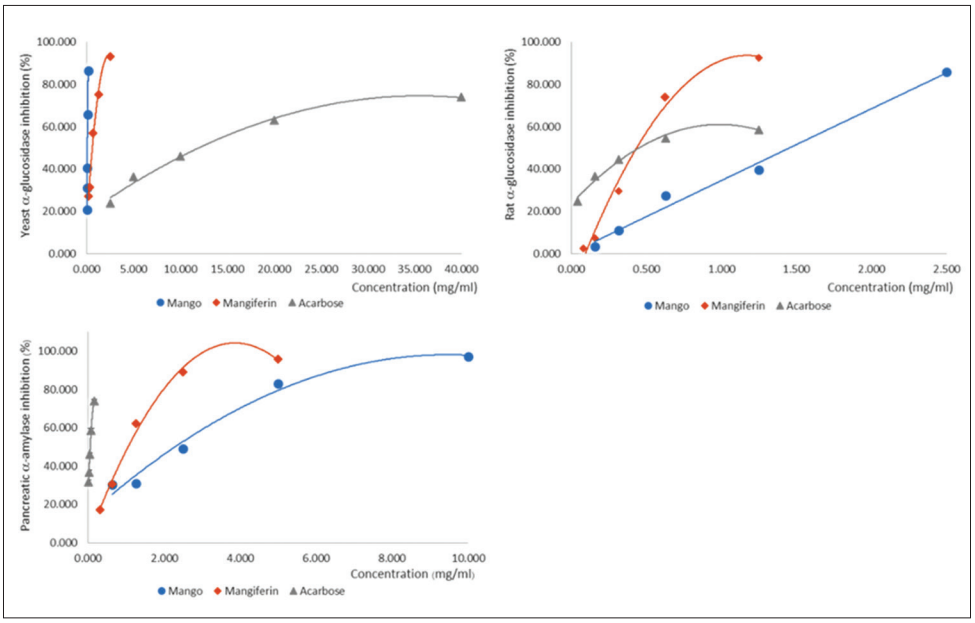

Figure 2: Yeast α-glucosidase, rat α-glucosidase and pancreatic α-amylase inhibitions of mango leaf extract, mangiferin, and acarbose at different concentrations

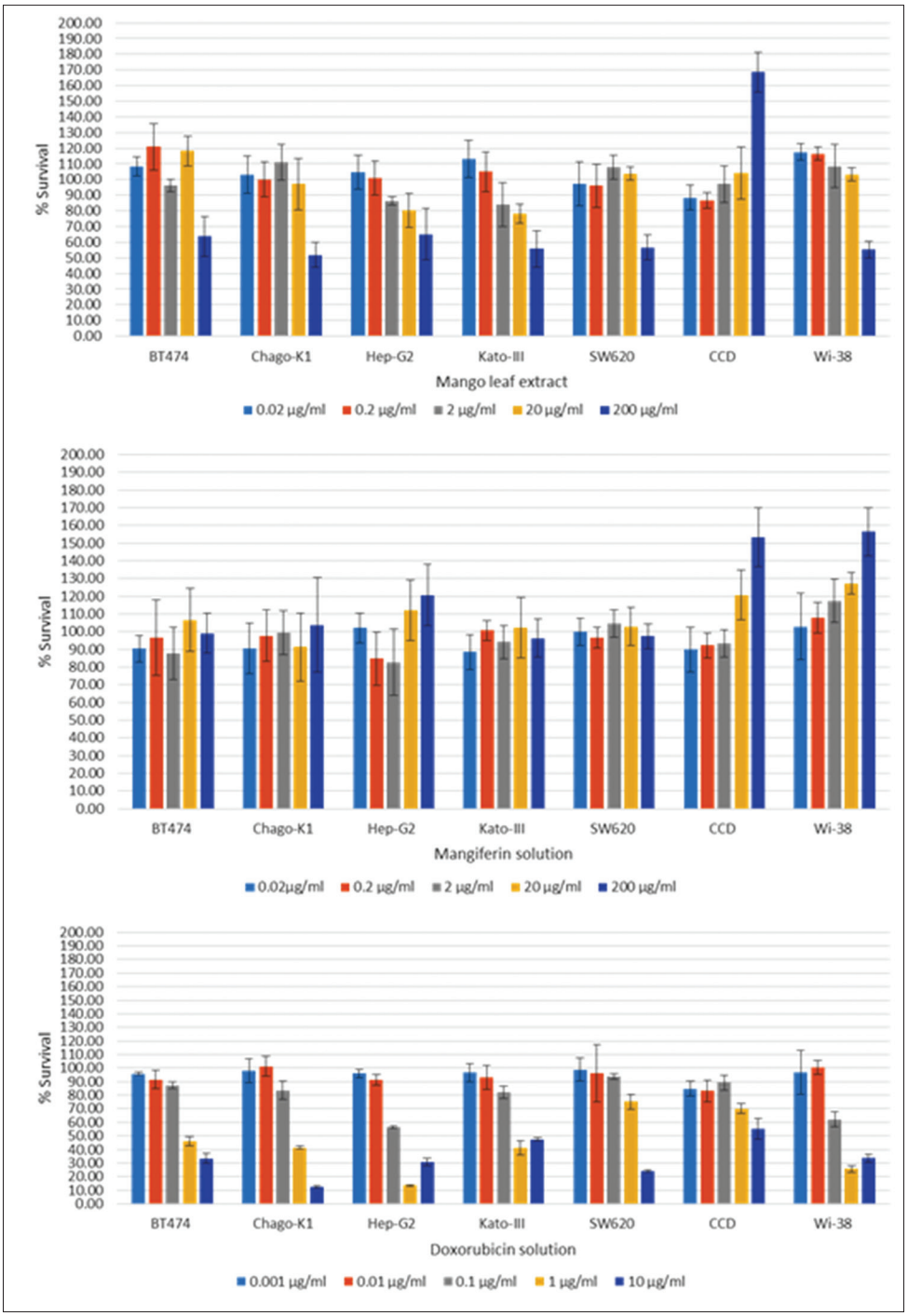

Figure 3: Inhibition of cancer cell growth by mango leaf extract, mangiferin and doxorubicin

Table 2: Cytotoxic activities of mango leaf extract, mangiferin, and doxorubicin

	IC_{50} (µg/ml)						
	BT474	**Chago-K1**	**Hep-G2**	**Kato-III**	**SW620**	**CCD**	**Wi-38**
Mango leaf extract	>200	>200	>200	>200	>200	>200	>200
Mangiferin	>200	>200	>200	>200	>200	>200	>200
Doxorubicin	0.80	0.65	0.12	0.71	2.57	>10	0.22

II (mammals).[17] Previous studies reported that various foods were active for yeast α-glucosidase, they had the potential to inhibit yeast α-glucosidase more than rat α-glucosidase and had inhibited those α-glucosidase more

than α-amylase. On the contrary, acarbose which was anti-diabetic drug, had more potential to inhibit α-amylase than α-glucosidase and had slightly or no ability to inhibit yeast α-glucosidase relative to rat α-glucosidase.[6,17] The similar results were found that both mango peels and mango seeds extracts had potential to inhibit α-glucosidase more than α-amylase with the IC_{50} of 3.5, 4.0 and 0.34, 0.71 μg/ml, respectively.[1,6] Their leaf extract inhibited α-glucosidase with the IC_{50} of 59.0 μg/ml. They were active for yeast α-glucosidase, these dose-dependent inhibitory activity were significantly higher than acarbose.[3,6] Different solvent extractions gave different inhibited potency. As an example, mango stem barks ethanolic extract showed the maximum inhibitory effects with the IC_{50} of 37.86 μg/ml; hexane extract showed moderate inhibitory effects with the IC_{50} of 114.13 μg/ml; petroleum ether, chloroform and aqueous showed no inhibitory effects on alpha-amylase activities.[18] However, the low IC_{50} value may be because the occurrence of other phenolic acids, flavonoids and carotenoids.[1] Previous study compared antidiabetic potential of mature and tender mango leaves aqueous methanolic extracts. Mature leaves extract inhibited α-glucosidase and α-amylase with the IC_{50} of 21.03 and 35.73 μg/ml, respectively due to their higher saponin, polyphenol, flavonoid contents. Tender leaves extract inhibited α-glucosidase and α-amylase with the IC_{50} of 27.16 and 22.01 μg/ml, respectively. They concluded that mango mature leaf had more potential to inhibit α-glucosidase; whereas, mango tender leaf had potential to inhibit α-amylase when compared to each other.[2] Mangiferin had more potent to inhibit α-glucosidase than α-amylase with the IC_{50} of 41.88 and 74.35 μg/ml, respectively.[19] In addition, many flavonoids were weakly inhibiting rat α-glucosidase. Our findings, mango leaf extract had strong potential to inhibit yeast α-glucosidase when compared to acarbose and mangiferin. It had the potential to inhibit α-glucosidase more than α-amylase. Mangiferin had strong potential for rat α-glucosidase when compared to acarbose and mango leaves extract. It also had more potent to inhibit α-glucosidase than α-amylase. Acarbose had strong potential to inhibit α-amylase compared to α-glucosidase.

MTT assay, an accurate and uncomplicated method, provides a useful quantitative data on the antiproliferative and anticancer potential of natural extracts.[20,21] Whole fruit, fruit juice, or fruit peel extracts from several mango cultivars showed toxic effect on cancer cell lines, including breast (MDA-MB-231 and MCF 7), cervix (HeLa cells), colon (SW-480 and SW-620), leukemia (Molt-4), lung (A-549), prostate (LnCap), and renal (786-0) cancer cell line. They had low cytotoxicity against normal cell lines, including breast (MCF-10A) and colon (CCD-18Co) normal cell lines, and no toxicity effect on lung fibroblast normal cell line (CCD-25 Lu).[7,20-22] In this study, mango leaf was used. The leaf extract at high dose (IC_{50} >200 μg/ml) possessed cytotoxic activities against all tested cancer cell lines (ductal carcinoma, bronchogenic carcinoma, liver hepatoblastoma, gastric carcinoma, and colon adenocarcinoma). However, at that high dose, the toxicity on lung fibroblast normal cell line was also shown; whereas there was no toxic effect, especially enhancing effect toward skin fibroblast normal cell line. The antiproliferative potential of mango extracts might be due to their bioactive compounds synergistic actions.[21] Mangiferin is one of the natural xanthone, which was extracted from mango tree. It was shown to inhibit cancer cell lines, including liver, breast, prostate, colon and nasopharyngeal cancer cell lines.[23,24] From the findings, mangiferin did not show significantly toxicity against all tested cancer cell lines. The previous study also reported that only high dose of mangiferin inhibited tested cancer cell lines.[23] This study found that mangiferin also had the potential on increasing the survival of skin and lung normal cell lines.

CONCLUSION

Mango leaf extract and its active compound, mangiferin showed *in vitro* inhibitory potential on key enzymes involving glucose metabolism, that is, α-amylase and α-glucosidase. Mango leaf extract, ≥200 μg/ml, showed cytotoxicity against tested cancer cell lines. Both mango leaf extract and mangiferin increased % survival of skin fibroblast.

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Conflicts of interest

There are no conflicts of interest.

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