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Evaluation of Antidiabetic, Antioxidant and Other Phytochemical Properties of Thai Fruits, Vegetables and Some Local Food Plants

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Abstract

Antidiabetic, antioxidant, anti-acetylcholinesterase and prebiotic activities, total phenolics and flavonoids of 33 crude ethanolic extracts of Thai local fruits, vegetables and some local food plants were determined. Mangosteen (*Garcinia mangostana*) fruit peel and Indian gooseberry (*Phyllanthus emblica*) fruit extracts had highest antioxidant activity. Bamboo grass (*Tiliacora triandra*) leaf extract had strongest α-amylase inhibitory activity (78.28 % inhibition), while mulberry (*Morus alba*) fruit extract had strongest α-glucosidase inhibitory activity (59.63 % inhibition). Star cactus (*Aloe vera*) leaf pulp extract had strongest anti-acetylcholinesterase activity (31.55 % inhibition). Indian gooseberry fruit and mangosteen fruit peel extracts had highest content of total phenolics and flavonoids, respectively. The extract with highest indigestible polysaccharide content was the extract of mangosteen fruit peels (188.62 mg/g extract), while those with relatively high indigestible polysaccharides were the extracts of pineapple fruits (*Ananas comosus*), lotus seeds (*Nelumbo nucifera*), black rice grains (*Oryza sativa*) and pisang mas banana fruits (*Musa sapientum*). Based on these properties, 5 plant extracts were selected to study for their prebiotic effect on growth and fermentation of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yogurt at 42 °C. Addition of lotus seed extract resulted in highest proliferation of these bacteria with 2.24 logCFU/g increase of total lactic acid bacterial counts in yogurt after 24 h fermentation, while addition of black rice grain, pisang mas banana fruit and pineapple fruit extracts caused good growth of these bacteria with 2.12 - 2.19 logCFU/g increase of total counts.

Keywords: Antidiabetic, antioxidant, anti-acetylcholinesterase, prebiotics, edible plants

Introduction

Fruits and vegetables are rich in many micronutrients including vitamins C and vitamin A and some electrolytes. They provides low calorie, and are good sources of phytochemicals functioning as antioxidants, phytoestrogens and anti-inflammatory compounds. More than 5,000 phytochemical compounds have been isolated from fruits, vegetables and cereals. Main phytochemical antioxidant compounds in foods that have been reported were i) polyphenols such as flavonoids (isoflavones, flavones, flavonols, anthocyanins, flavanols, and flavanones), phenolic acids, stilbenes and lignans, ii) glucosynolates and iii) carotenoids [1]. As beneficial effect of antioxidants on disease prevention is well known, there are an increase awareness on the role of nutrients on defence against oxidative stress and damages induced by free radicals. Oxidative stress is an imbalance between formation of reactive oxygen/nitrogen species and ability of organisms to fight against reaction occurred by using antioxidant system. Oxidative stress reduces ability of internal system to fight against targeted biological molecules. Excessive production of free radicals leads to damage to lipid, protein, carbohydrate, and DNA molecules. Damage induced by oxidative stress has been confirmed to be related to pathogenesis, pathophysiology of some chronic diseases affecting health problems [1,2]. Certain nutrients in food are

important for prevention of oxidative stress injury or free radical induced diseases. There were evidences that intake of fruits and vegetables could reduce risk of oxidative stress related diseases such as diabetes, Alzheimer's disease and other diseases [3].

Diabetes mellitus is a common form of chronic metabolic disorder, characterized by disruptions in metabolism of carbohydrate, proteins and fat, and caused by defects of insulin secretion and/or insulin action. Among the 2 types of diabetes (type 1 and type 2 diabetes mellitus), type 2 diabetes mellitus (non-insulin dependent diabetes mellitus) is more common form and accounts for 90 - 95 % of all diabetic cases. Most diabetes patients will eventually suffer from multiple complications including neuropathy, nephropathy, retinopathy, ketoacidosis, diabetic foot, heart attack, stroke and cardiovascular disease [4,5]. Decreasing post-prandial hyperglycemia is a therapeutic approach for treating diabetes in the early state. This is accomplished by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase in the digestive tract. These enzyme inhibitors can delay carbohydrate digestion and prolong overall carbohydrate digestion time causing delayed glucose absorption rate. Some drugs such as biguanides, sulfonylureas, meglitinides, thiazolidinediones, α-glucosidase inhibitors, incretinmimetics, dipeptityl peptidase-IV inhibitors and insulin have been used in control and treatment of diabetes. However, most of these drugs usually result in some severe side effects [5]. Therefore, use of plant derived drug is alternative way to treat diabetes. Several plants have been reported to possess anti-diabetic activity in experimental rat [5]. However, only few information regarding α-amylase and α-glucosidase inhibitory activity of Thai fruits and vegetables has been reported.

Alzheimer's disease (AD) is a neurodegenerative disease, the most common form of dementia among the elderly. This disease is characterized by progressive decline in memory which affects brain function, neuronal loss in regions associated with memory and cognition, as well as accumulation of extracellular β-amyloid and intraneuronal neurofibrillary tangles. AD affects important parts of brain as well as the cortex and limbic system [6]. In addition, AD patients' brains were found to loss acetylcholine which is an important neurotransmitter of central nervous system responsible for increased memory and enhanced learning. Acetylcholine is related to signal transport in synapse. After transporting signal in the synapse, it will be hydrolyzed to choline and acetate by enzymatic reaction. Use of acetylcholinesterase inhibitor is one strategy to treat this disease as it can protect the cells from free radical toxicity and injury induced by β-amyloid [7]. Some acetylcholinesterase inhibitors such as physostigmine and tacrine have been used for symptomatic treatment of AD. Athough these drugs can obstruct the progress of AD, there are some disadvantages. For example, physotigmine has weak activity against oral cavity, poor penetration to brain and pharmacokinetics, while tacrine has hepatotoxic [8]. Thus, there is the need to search for natural, safe and effective anti-Alzheimer's disease and anti-diabetic drug with antioxidant activity. The use of natural antioxidants plays an important role in prevention of oxidative stress related diseases. Some researches have used free radical scavengers from plants with glutathione containing antioxidants and substances with RH groups, vitamin, polyphenol and other compounds to decrease the effect of oxidative stress [9].

Thai herbs, fruits and vegetables are a major source of natural antioxidants such as polyphenol (phenolics, flavonoids), vitamin C and other compounds [10,11]. Some Thai local plants including fruits of Indian gooseberry (*Phyllathus emblica*) [11,12] and black rice (*Oryza sativa*) [13], fruit peels of mangosteen (*Garcinia mangostana*) [14], and leaves of lopea tree (*Acanthopanax trifoliatus*) [15] were reported to have very high phenolic content and strong antioxidant activity. So far, only few plants with strong anti-diabetic and anti-acetylcholinesterase activities have been reported [12,16-18]. For instance, *Alium sativum* (garlic) bulbs [19], *Ginkgo biloba* leaves [19], *Terminalia chebula* fruits [5], *Aloe vera* leaves and *Morinda citrifolia* barks/leaves [16] have good anti-diabetic effect, while *Stephania pierrei* tubers [18] and *Nelumbo nucifera* (lotus) petals [12] have strong anti-acetylcholinesterase activity. However, there are still many interesting Thai local plants that have never been investigated. Therefore, the search for new acetylcholinesterase, α-amylase and α-glucosidase inhibitors with strong antioxidant activity from natural sources is of great interest.

Fruits and vegetables not only provides phytochemicals for preventing chronic diseases, but they are good source of dietary fiber [10,11]. Dietary fiber includes non-digestible carbohydrates (resistant starch,

resistant maltodextrins, fructo-oligosaccharides and galacto-oligosaccharides). These are prebiotics which are able to resist digestion in small intestine and then will be fermented in the large intestine [20]. Prebiotics not only stimulate the growth of probiotic bacteria, a beneficial natural flora, but improve function of intestine wall and host immune system [21]. Some plants such as banana, onion, garlic and Jerusalem artichoke (*Helianthus tuberosus*) have been reported to contain fructo-oligosaccharide and inulin. Inulin is a small molecule of fructo-oligosaccharide that plants store as a nutrient [22].

In the current study, 33 crude ethanolic extracts of fruits, vegetables and some Thai local food plants were screened for their antioxidant, antidiabetic and anti-acetylcholinesterase activities as well as their phenolic, flavonoid and indigestible polysaccharide contents for use to study the prebiotic property, a growth enhancement of probiotic bacteria in yogurt. The plants selected were the plants known to possess either one of those potential activities and the plants that have never or rarely been studied. The studied plants listed in **Table 1** are Thai local edible plants widely consumed as fresh fruits and vegetables, spices and herbs, or used as ingredients in Thai traditional cuisine, especially seven interesting plants collecting from Chiang Mai in northern Thailand. These were lopea tree (*A. trifoliatus*), Pak Chiangda (*Gymnema inodorum*) and Pak huanmoo (*Dregea volubilis*) consumed as fresh vegetables or used as an ingredient for cooking Thai curry in northern Thailand, Malodge (*Elaeagnus latifolia*) consumed as fresh fruits and sometimes used to relieve constipation, local apple (*Chrysophyllum cainito*) consumed as fresh fruits, mulberry (*Morus alba*) consumed as raw or cooked fruits or used as an ingredient in mulberry wine making, and Jeekuk (*Zingiber zerumbet*, a tuberous root herb plant) wildly used in foods and beverages and used traditionally as herbal medicine for treatment of many aliments due to its potential biological and pharmacological properties [23].

Materials and methods

Extraction of plant materials

Thirty three species of fruit, vegetable and some local Thai food plants (**Table 1**) were used in this study. These plant materials were washed, cut, dried at 40 °C, and ground to a fine powder. Then, 10 g of each were soaked in 100 mL of 80 % ethanol, and shaken at 150 rpm for 48 h. The mixtures were filtered. The filtrates were evaporated using vacuum rotary evaporator and air dried at 40 °C. Then, these dried extracts were ready for use to prepare the stock solution.

α-amylase inhibition assay

Alpha-amylase inhibition assay was analyzed according to the method as described by Sancheti *et al.* [24] with little modification. Briefly, 240 µL of 1 % starch solution (Starch soluble, 14418, Sisco Research Laboratories Pvt. Ltd., India) was mixed with 120 µL plant extract sample, and incubated at 37 °C for 5 min. Then, 240 µL of α-amylase (from porcine pancreas, A6255, Sigma-Aldrich, USA) solution (1 unit/mL in 0.5 M Tris HCl buffer) was added into this mixture, and incubated at 37 °C for 3 min, followed by addition of 240 µL color reagent: the mixture of i) 12 g sodium potassium tartrate dissolved in 8 mL of 2 M NaOH and ii) 96 mM 3,5-dinitrosalicylic acid in the ratio of 1:1, Loizzo *et al.* [25]. The mixture was boiled in a water bath for 15 min, cooled down to ambient temperature, and diluted by adding 2,160 µL distilled water. The absorbance of this reaction mixture was measured at 540 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). Acarbose solution (1 mg/mL) (A9890-1G, Sigma, China) was used as a positive control, while 30 % ethanol was used as a negative control. The α-amylase inhibitory activity was calculated using the following equation;

$$\% \text{ α-amylase inhibition} = \frac{\text{A } control - \text{A } sample}{\text{A } control} \times 100 \quad (1)$$

where A *control* is the absorbance of control and A *sample* is the absorbance of sample.

Table 1 Fruits, vegetables and some Thai local food plants used in this study.

Scientific name	Thai name/ Common name	Family	Plant part
Acanthopanax trifoliatus Merr.	Pak pam/ Lopea tree	Araliaceae	Whole plant
Alium sativum Linn.	Thai garlic/ Common garlic	Amaryllidaceae	Bulb
Allium oschaninii	Hom Dang Thai/ Shallot	Amaryllidaceae	Bulb
Aloe vera (Linn.) Burm.f.	Wan HangJarake/ Star Cactus	Aloaceae	Leaf pulp
Ananas comosus (L.) Merr.	Subparos-sriracha/ Pineapple	Bromeliaceae	Fruit pulp
Annona squamosa Linn.	Noina/ Sugar apple	Annonaceae	Fruit pulp
Carissa carandas Linn.	Mamuanghow ma now ho/ Karunda	Apocynaceae	Fruit
Centella asiatica Linn. Urban.	Buabok/ Asiatic pennywort	Apiaceae	Whole plant
Chrysophyllum cainito Linn.	Local apple/ Star apple	Sapotaceae	Fruit
Dregea volubilis Stapf.	Pak huan moo/ -[a]	Asclepiadaceae	Flower
Elaeagnus latifolia Linn.	Malodge/ Bastard Oleaster	Elaeagnaceae	Fruit
Garcinia mangostana Linn.	Mungkut/ Mangosteen	Clusiaceae	Fruit peel
Ginkgo biloba L.	Pagkuaw/ Maidenhair tree	Ginkgoaceae	Leaf
Gymnema inodorum Lour.	Pak chiangda/ Gymnema	Asclepiadaceae	Whole plant
Helianthus tuberosus Linn.	Kantawan/ Jerusalem artichoke	Asteraceae	Tuber
Ipomoea batatas (L.) Lam.	Mantorpuek/ Purple sweet potato	Comvolvulaceae	Tuber
Ipomoea batatas (L.)Lam.	Manted/ Orange Sweet potato	Comvolvulaceae	Tuber
Malus domestica Borkh.	Appledang/ Red apple	Rosaceae	Fruit
Morinda citrifolia Linn.	Yaw/Great morinda	Rubiaceae	Fruit
Morus alba Linn.	Mon/ Mulberry	Moraceae	Fruit
Momordica cochinchinensis (Lour.) Spreng.	Fugkawn/ Gac fruit	Cucurbitaceae	Fruit
Musa sapientum Linn. (AA group)	Kluai Lep Mu Nang/ Lep Mu Nang banana	Musaceae	Fruit pulp
Musa sapientum Linn. (AA Khai)	Kluai khai/ Pisang Mas banana	Musaceae	Fruit pulp
Musa sapientum Linn.(*Musa* ABB cv. Kluai 'Namwa')	Kluai Namwa/ Cultivated banana	Musaceae	Fruit pulp
Nelumbo nucifera Gaertn.	Medbua/ Lotus seed	Nelumbonaceae	Seed
Oryza sativa	Kawdam/ Black rice	Poaceae	Fruit
Passiflora laurifolia Linn.	Saowaros/ Passion fruit	Passifloraceae	Fruit pulp and seed
Phyllanthus emblica Linn.	Makampom/ Indian gooseberry	Phyllanthaceae	Fruit
Salacca zalacca (Gaerth.) Voss	Sara/ Salak plum	Arecaceae	Fruit pulp
Tiliacora triandra (Colebr.) Diels	Yanang/ Bamboo grass	Menispermaceae	Leaf
Zanthoxylum limonella Alston.	Maquand/ Kamchat ton	Rutaceae	Seed
Zea mays Linn. var. saccharata	Kawpodwan/ Sweet corn	Poaceae	Seed
Zingiber zerumbet (Linn) Smith.	Jeekuk/ Shampoo ginger	Zingiberaceae	Rhizome

[a] Common name is not available.

α-glucosidase inhibition assay

Rat intestinal α-glucosidase inhibition assay of all plant extracts were determined according to the method of Kim *et al.* [26] with slightly modification. Rat-intestinal acetone powder (0.5 g) (I1630, Sigma-Aldrich, USA) was suspended in 10 mL of 0.9 % saline. The suspension was sonicated 15 times, 30 s each time at 4 °C, centrifuged at 10000×g for 15 min at 4 °C. The resulting supernatant was used in this assay. To perform this assay, solution of plant extract sample (50 µL, 1 mg/mL in 30 % ethanol) or acarbose (1 mg/mL, positive control) was pre-incubated with 100 µL of rat intestinal α-glucosidase at 37 °C for 10 min. Then, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (Calbiochem, Switzerland) in 0.1 M phosphate buffer (pH 6.9) was added, and incubated at 37 °C for 10 min before measuring the absorbance at 405 nm using the microplate reader (IEMS Reader MF Type 1401, Thermo Labsystem, Finland). The 30 % ethanol was used as a negative control. The α-glucosidase inhibitory activity was calculated as follows;

$$\% \text{ α-glucosidase inhibition} = \frac{\text{A } control - \text{A } sample}{\text{A } control} \times 100 \quad (2)$$

where A *control* is the absorbance of control and A *sample* is the absorbance of sample.

Antioxidant activity assay

Antioxidant activity of 33 plant ethanolic extracts was determined by 2 different methods including DPPH radical scavenging activity assay and ferric reducing antioxidant power assay.

DPPH radical scavenging activity assay

The free radical scavenging activity of the plant extracts was measured according to the method of Brand-Williams *et al.* [27]. Each stock solution of extracts and α-tocopherol (95240; Fluka, Switzerland), a positive control were prepared and diluted to the concentrations of 1 - 1,000 µg/mL in methanol. Each diluted extract (75 µL) at 5 concentrations was added to 2.925 mL of a 0.025 g/L 2, 2-diphenyl-1-picrylhydrazyl (DPPH; D9132; Sigma-Aldrich, Germany) solution in methanol. The reaction mixtures were then incubated in the dark for 30 min. The absorbance at 515 nm was measured at 0 and 30 min of incubation using the UV-Visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). To prepare a standard curve of DPPH, the absorbance of DPPH at different concentrations (0.025 - 0.0008 g/L) was measured at 515 nm. The remaining $DPPH^{\cdot}$ concentration in the reaction mixture was calculated from the DPPH standard curve, and the percentage of the remaining $DPPH^{\cdot}$ was calculated using the following equation;

$$\% \text{ DPPH}^{\cdot}_{\text{REM}} = [\text{DPPH}^{\cdot}]_T / [\text{DPPH}^{\cdot}]_{T=0} \times 100 \quad (3)$$

where $[DPPH^{\cdot}]_T$ and $[DPPH^{\cdot}]_{T=0}$ were the concentration of $DPPH^{\cdot}$ at steady state (30 min) and zero time, respectively.

The percentage of the remaining $DPPH^{\cdot}$ in each reaction mixture of 5 different concentrations of all extracts was then plotted against µg of extract / mg of $DPPH^{\cdot}$ to obtain the amount of antioxidant or extract necessary to decrease the initial $DPPH^{\cdot}$ by 50 % (EC_{50}). The EC_{50} values of all extracts were calculated by the following linear regression of plots, and the antiradical efficiency ($AE=1/ EC_{50}$) values were also calculated.

$$[\% \text{ DPPH}^{\cdot}_{\text{REM}}] = b\,[\text{µg antioxidant/ mg DPPH}^{\cdot}] + a \quad (4)$$

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of the plant extracts by the FRAP method was analyzed according to the procedure as previously described by Lado *et al.* [28]. To do FRAP assay, 1 mg/mL extract (100 µL) was mixed with 3.0 mL FRAP reagent (25 mL of 300 mM acetate buffer, 2.5 mL of 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ; 93285; Fluka, Switzerland) in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$), and incubated at 37 °C for 5 min. The absorbance was measured at 594 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia) against blank (FRAP reagent without the sample). The concentration of Fe^{2+}-TPTZ (reducing capacity) was calculated by comparing the absorbance at 594 nm with the standard curve of the Fe (II) standard solutions (ferrous sulfate heptahydrate) at the concentration of 3 - 0.0469 mM. Alpha-tocopherol was used as a positive control. The reducing power of each extract was expressed as mmol Fe(II)/g extract.

Acetylcholinesterase inhibitory activity assay

The anti-acetylcholinesterase activity of all plant extracts was determined according to the method previously reported by Ellman *et al*. [29] and Sancheti *et al*. [30] with slightly modification. Acetylcholinesterase from electric eel (E.C. 3.1.1.7, Sigma, Sigma-Aldrich, USA), acetylcholine iodide

(ATCI, Fluka, Sigma-Aldrich, United Kingdom), 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB, Sigma, Sigma-Aldrich, USA) were employed. Galanthamine hydrobromide from *Lycoris* sp. (Sigma, Sigma-Aldrich, USA) was used as the standard drug. In this method, 240 µL acetylcholinesterase solution (0.025 U/mL), 120 µL sample (0.1 and 1 mg/mL of the plant extract in 30 % ethanol), 2,160 µL Tris-HCl buffer (50 mM Tris-HCl, pH 8) were mixed and incubated at 4 °C for 30 min. Then, 240 µL DTNB (0.3 mM) and 240 µL ATCI (1.8 mM) were added. The reaction mixture was incubated at 37 °C for 20 min. Then, the absorbance was measured at 412 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). The blank was prepared for correcting the background absorbance, in which the acetylcholinesterase enzyme was replaced by buffer. Control was performed in the same manner by replacing the sample with 30 % methanol. The percentage of inhibition was calculated using the following formula;

$$\% \text{ inhibition} = \frac{\text{A } control - \text{A } sample}{\text{A } control} \times 100 \quad (5)$$

where A *control* and A *sample* are the absorbance values of the control and the sample, respectively.

Determination of total phenolic content

Total phenolic content of crude ethanolic extract of each plant sample was determined according to the method as described by Singleton *et al.* [31]. The sample (0.1 mL of 1 mg/mL crude extract in 30 % ethanol) was transferred to a test tube, then 6 mL ultra-pure water was added. Folin-Ciocalteu's phenol reagent (UN3264, VWR chemical, European Commission) (500 µL) was added, shaken thoroughly, and allowed to stand for 1 min. Then, 1.5 mL of 20 % Na_2CO_3 and 1.9 mL of ultra-pure water were added, mixed well and incubated for 30 min at 25 °C. After incubation, the absorbance was measured at 760 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). Standard curve of gallic acid (48630; Fluka, Spain) at 10 - 1,000 µg/mL concentration was prepared. The total phenolic contents in each plant extracts were calculated from the linear equation of the gallic acid standard curve. The results were expressed as mg gallic acid equivalents (GAE) / g extract.

Determination of total flavonoid content

Total flavonoid contents in all samples were analyzed according to the method of Kathirvel and Sujatha [32]. The plant extract (250 µL of 1 mg/mL extract in 30 % ethanol) was transferred to a test tube and mixed with 1.25 mL distilled water and 75 µL of 5 %$NaNO_2$ (w/w). After 5 min, 150 µL of 10 % $AlCl_3$ (w/w) was added and allowed to stand for 6 min at room temperature. Then, 500 µL of 1 M NaOH and 275 µL of distilled water were added. The mixture was mixed well, and the absorbance was measured at 510 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty). Catechin (Catechin hydrate, 22110, Aldrich, Germany) at 10 - 1,000 µg/mL final concentration was used to plot a standard curve. The 30 % ethanol was used as a blank sample. Total flavonoid contents in all samples were expressed as mg catechin eqivalents (CE) /g extract.

Determination of prebiotic properties

The indigestible polysaccharide content in all plant extracts were analyzed according to the method of Wichienchot *et al*. [33]. The acidic digestion was performed by mixing 3 mL of each plant extract (1 mg/mL in 30 % ethanol) with HCl buffer (pH 1). Then, 1 mL of this mixture was analyzed for the total sugar content before digestion using dinitrosalicylic acid method, modified from the method of Miller [34]. The rest of this mixture was incubated at 37 °C for 4 h before the reaction was terminated by adding 1 N NaOH. This acid-digested solution was further digested by 2 unit/mL α-amylase (A6255; from porcine pancreas; Fluka; Switzerland) in phosphate buffer (pH 7) for 6 h. After heating to 80 °C for 10 min, the total sugar content was analyzed using phenol-sulfuric method [35]. The indigestible polysaccharide content (mg/g extract) in the sample was calculated by subtracting the total sugar after digestion (mg/g extract) with the total sugar before digestion (mg/g extract).

Effect of fruit and vegetable extracts on growth of lactic acid bacteria during yogurt fermentation

Bacterial strains and starter culture preparation

Three species of lactic acid bacteria (LAB) were used in this study (*Lactobacillus acidophilus* TISTR 1034 and *Lactobacillus bulgaricus* TISTR 451, obtained from the Microbiological Resources Centre for Southeast Asian Region (Bangkok MIRCEN), Thailand and *Streptococcus thermophilus* BCC 5366, obtained from BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand). They were subcultured twice onto deMan Rogosa Sharpe (MRS) agar (Difco Laboratories, USA) and incubated for 24 h at 37 °C. Then, a loopful of each LAB strain was inoculated into 5 mL of MRS broth. After incubation, cells were collected by centrifugation at 3000 ×g for 15 min, washed twice and resuspended in 0.1 % peptone water. Turbidity was adjusted to match the turbidity of 5 McFarland standard to obtain an inoculum concentration of 10^8 CFU/mL.

Fermentation of yogurt supplemented with fruit and vegetable extracts

In the current study, 5 plant extracts with high indigestible polysaccharide content were selected to study their effect on the growth of the starter culture according to the modified method of Agil *et al.* [36]. In brief, seven treatments of yogurt were produced: treatment 1 - 5, yogurt made from milk supplemented with each of 5 plant extracts; treatment 6 (positive control), yogurt made from milk supplemented with 4 % inulin (I2255; from chicory; Sigma-Aldrich; St. Louis, MO, USA) and treatment 7 (negative control), yogurt made from milk only. To produce yogurt, full fat milk with 3.56 % fat and 12.32 % total solid was used. Skim milk powder (5 %) was added into this milk to get 18 % total solid. The mixture was warmed up to 50 °C and homogenized. Then, all treatment mixtures were pasteurized at 85 °C for 15 min, and cooled down to 42 °C before inoculating with 2 % starter culture (24 h old mixed culture of *L. acidophilus* TISTR 1034, *L. bulgaricus* TISTR 451 and *S. thermophilus* BCC 5366 (1:1:1, v/v/v) in whole milk at 42 °C). All samples were incubated at 42 °C for 24 h. At 0, 12 and 24 h fermentation, the yogurt samples of each treatment were taken for measurement of pH value, total acidity [37] and evaluation of total LAB counts using spiral plate technique by spiral plater (Autoplate 4000, Spiral Biotech, USA) on MRS agar. The experiments were done in triplicate. All data were analyzed by ANOVA and Duncan's multiple range test at 95 % confidence level using the IBM SPSS 22.0 version statistical package, USA.

Results and discussion

Antidiabetic activity

Bamboo grass (*T. triandra*) leaf extract had strongest α-amylase inhibitory activity (78.28 % inhibition), followed by the extracts of common garlic, salak plum, Indian gooseberry, sweet corn, star apple, Jerusalem artichoke, pineapple, karunda, gac fruit, lopea tree and great morinda (21.33 - 37.12 % inhibition), but other plant extracts had relatively low α-amylase inhibitory activity. For α-glucosidase inhibitory activity, only mulberry (*M. alba*) fruit extract had strongest α-glucosidase inhibitory activity (59.63 % inhibition) among all extracts (**Table 2**).

Bamboo grass leaf extract has been reported to contain some polyphenol compounds such as *p*-hydroxybenzoic acid, minecoside, flavone glycoside cinnamic acids derivative, monoepoxybetacarotene, santonin, protopseudohypericin, 3-*O*-methylluteolin glucoside malonylated, 3-demethoxy-9α-hydroxyligballinol-*O*-glucoside and flavanone glycoside [38]. Flavonoids including flavonols, flavones, catechins, flavanones and some other flavonoids are natural compounds having potential hypoglycemic properties. Flavonoids (flavonols, flavones, catechins, quercetins, flavanones and others) help to improve altered glucose and oxidative metabolism [1]. Vessal *et al.* [39] reported that intraperitoncal administration of quercetin in streptozocin-induced diabetic rats could decrease glucose level in their blood stream. Plant extracts with α-amylase inhibitory activity may be antidiabetic as α-amylase is an enzyme hydrolysed α-(1,4)-glucosidic linkage of starch into maltose and glucose. Inhibition of this enzyme may delay absorption of glucose into blood stream and help to alleviate the symptom of type 2-

diabetes. Therefore, it is possible that bamboo grass leaves with high flavonoids could have antidiabetic activity.

For antidiabetic activity of garlic extract, it has been reported that garlic extract administration in rats (0.1 - 0.5 g/ kg body weight) resulted in reducing of serum glucose level, total cholesterol, triglycerides, urea, uric acid, aspartate amino transferase and alanine amino transferase in rats as well as increasing of insulin level in blood serum of streptozotocin-induced diabetic rats [40]. Garlic contains some active compounds including sulfur compounds (allicin and S-allylcysteine) and organic sulfur compounds (thiosulfinates ajoenes and allicin, diallyl thiosulphate) which are good for health. S-allylcysteine is one of the active compounds in garlic that will help to maintain the serum glucose level in blood, insulin level in plasma and growth rate of streptozotocin-induced diabetic rats to normal level [41].

The extract of Pak Chiangda (*G. inodorum*) had not too strong α-amylase and α-glucosidase inhibitory activity (13.94 and 3.72 %, respectively), but Shimizu *et al.* [42] reported that crude saponin mixture extracted from *G. inodorum* leaves could inhibit glucose absorption in the isolated intestinal tract and suppressed the increased blood glucose in rats. Pak Chiangda is a vegetable widely used in Northern Thai food. Chiabchalard *et al*. [43]. reported that significant decrease of peak plasma glucose was observed in healthy human after comsumption of *G. inodorum* tea with meal or 15 min after meal.

Mulberry (*M. alba*) fruit extract had strongest α-glucosidase inhibitory activity. The α-glucosidase, an enzyme in lumen of intestine and in brush border membrane, hydrolyses starch and oligosaccharide into monosaccharide before being absorbed. Inhibition of this enzyme delays carbohydrate digestion, thereby controlling blood glucose levels in patients [44]. Strong α-glucosidase inhibitory activity in mulberry fruits is probably due to the effect of its active compound such as 1-deoxynojirimycin which is the hypoglycemic constituent found in *Morus alba* tree [45]. Jiao *et al.* [46] reported that *M. alba* fruit polysaccharide had antihyperglycemic effect and could relieve diabetic symptoms in Type 2 diabetes mellitus rat model. Moreover, the polysaccharide isolated from leaves of *M. alba* has been reported to enhance pancreatic β-cell regeneration and insulin secretion in streptozotocin-induced diabetic rats [47].

Antioxidant and anti-acetylcholinesterase activities, phenolics and flavonoids

Of all, the extracts of mangosteen (*G. mangostana*) fruit peels and Indian gooseberry (*P. emblica*) fruits had strongest antioxidant activity by DPPH radical scavenging activity (2.20 - 2.27 antiradical efficiency) and FRAP (2.99 - 3.51 mmol Fe(II)/g extract) methods. These 2 plant extracts contained high amount of total phenolics (416.07 mg GAE/g extract of Indian gooseberry fruits and 397.36 mg GAE/g extract of mangosteen fruit peels). In addition, the extracts which possessed relatively strong antioxidant activity were the extracts of lotus seed (*N. nucifera*), Kamchat ton (*Z. limonella*) and lopea tree (*A. trifoliatus*) (0.50 - 0.74 antiradical efficiency by DPPH method and 0.80 - 1.19 mmol Fe(II)/g extract by FRAP method). There were some correlations between the antioxidant activity, total phenolic and flavonoid contents of these extracts (**Tables 2** and **3**).

Strong antioxidant activities, and high phenolics and flavonoids in Indian gooseberry fruits, mangosteen fruit peels, lotus seeds, kamchat ton seeds and whole plants of lopea tree may be due to the action of their active compounds. Indian gooseberry extract has been reported to contain gallic acid, ellagic acid, mucic acid 1,4-lactone 3-*O*-gallate, isocorilagin, chebulanin, chebulagic acid and mallotusinin [48], while *m*-hydroxybenzoic acid and 3,4-dihydroxymandelic were found in the peels and rinds of mangosteen fruits, respectively [49]. Kredy *et al.* [50] identified the flavonoid compounds in fresh lotus seed epicarp, and found 6 glycosylate flavonols and 1 aglycone flavonol, while Yen *et al.* [51] reported that lotus seed extract contained some of the active components such as phenolic acid, gallic acid, caffeic acid, chlorogenic acid and *p*-hydroxybenzoic acid. In addition, some compounds isolated from kamchat ton were limonellone, lupeol, alkaloid rutaecarpine and coumarins (xanthoxyletin, osthol and scopoletin) [52].

In addition, main compounds in lopea tree leaf extract were identified as chlorogenic acid and rutin [15]. Phuong *et al.* [53] reported that diterpenoids (continentalic acid and kaurenoic acid) were found in roots and stems of lopea tree (*A. trifoliatus*). Lopea tree, an interesting medicinal plants is a shrub that usually thrives in roadsides, valleys or on mountain slopes. The stem barks and leaves of *A. trifoliatus* have been used as an antiflatulent agent and a tonic to improve general weakness, respectively. In

northern of Thailand, young leaves and the shoots of this plant are usually consumed as vegetable [54]. For antioxidant activity of kamchat ton (*Z. limonella*), it was probably due to its active compounds. Tangjitjaroenkun *et al.* [55] reported the antioxidant activity of *Z. limonella* stem extract and found that it contained quinolone alkaloid, limonellone and other compounds previously isolated including ubiquitous lupeol, alkaloid rutaecarpine and coumarins (xanthoxyletin, osthol and scopoletin) [52].

Table 2 Antioxidant, antidiabetic and anti-acetylcholinesterase activities of fruit, vegetable and some Thai local food plant extracts.

Scientific name	Common name	Antioxidant activity [a]			Antidiabetic activity		Anti-acetylcholinesterase activity (%) ± SD	
		DPPH assay		FRAP assay (mmol Fe(II)/ g extract ± SD	α-amylase inhibition (%) ± SD	α- glucosidase inhibition (%) ± SD	1 mg/mL	0.1 mg/mL
		EC_{50} (µg extract / mg DPPH) ± SD	AE (10^{-3}) ± SD					
A. trifoliatus	Lopea tree	1,982 ± 3.31	0.50 ± 0.01	0.93 ± 0.03	23.25 ± 1.30	8.07 ± 1.60	6.78 ± 0.75	2.89 ± 0.38
A. sativum	Common garlic	74,386.33 ± 3.48	0.01 ± 0.00	0.003 ± 0.00	37.12 ± 0.90	9.38 ± 1.10	12.86 ± 1.15	4.55 ± 1.81
A. oschaninii	Shallot	55,355.19 ± 0.85	0.02 ± 0.00	0.02 ± 0.01	15.43 ± 1.20	12.83 ± 5.90	8.61 ± 1.83	2.93 ± 0.89
A. vera	Star cactus	23,733.02 ± 3.85	0.04 ± 0.00	0.07 ± 0.00	18.24 ± 1.90	7.85± 2.40	31.55 ± 1.44	14.04 ± 2.84
A. comosus	Pineapple	62,907.35 ± 1.86	0.02 ± 0.00	0.01± 0.01	26.61 ± 1.10	12.16±2.50	9.30 ± 1.54	2.54 ± 0.19
A. squamosa	Sugar apple	3,385.10 ± 2.72	0.29 ± 0.01	0.28 ± 0.01	2.30 ± 1.80	12.43 ± 2.20	9.67 ± 1.12	3.03 ± 0.24
C. carandas	Karunda	3,075.84 ± 1.54	0.33 ± 0.01	0.38 ± 0.00	24.51 ± 1.50	12.26 ± 4.00	8.58 ± 1.50	1.79 ± 0.61
C. asiatica	Asiatic pennywort	4,393.94 ± 2.43	0.23 ± 0.00	0.38 ± 0.02	14.30 ± 2.10	2.12 ± 2.00	3.24 ± 0.78	1.67 ± 0.13
C. cainito	Star apple	3,258.62 ± 5.44	0.31 ± 0.00	0.40 ± 0.00	30.10 ± 1.40	13.19 ± 1.90	9.24 ± 1.75	2.12 ± 0.88
D. volubilis	-[b]	11,604.20 ± 0.91	0.09 ± 0.00	0.14 ± 0.02	5.68 ± 1.60	7.98 ± 2.40	5.29 ± 1.94	2.63 ± 1.35
E. latifolia	Bastard Oleaster	27,465.56 ± 1.11	0.04 ± 0.00	0.07 ± 0.01	7.52 ± 1.50	12.59 ± 3.40	8.51 ± 0.87	7.30 ± 1.42
G. mangostana	Mangosteen	440.73 ± 1.31	2.27 ± 0.01	2.99 ± 0.08	19.01 ± 1.20	5.43 ± 5.70	13.79 ± 1.71	2.93 ± 0.49
G. biloba	Maidenhair tree	6,229.66 ± 1.35	0.16 ± 0.00	0.37 ± 0.02	16.43 ± 1.20	14.47 ± 3.30	8.08 ± 1.07	2.48 ± 0.89
G. inodorum	Gymnema	8,366.34 ± 4.55	0.12 ± 0.00	0.23 ± 0.01	13.94 ± 1.30	3.72 ± 2.40	9.02 ± 1.57	4.96 ±1.42
H. tuberosus	Jerusalem articheoke	112,047.80 ± 4.55	0.04 ± 0.05	0.01 ±0.01	28.84 ± 1.50	6.45 ± 2.40	5.51 ± 1.09	2.63 ± 0.81
I. batatas	Purple sweet potato	34,250.15 ± 2.90	0.03 ± 0.00	0.10 ± 0.01	2.82 ± 1.20	12.01 ± 3.20	9.16 ± 1.76	3.48 ± 1.40
I. batatas	Orange sweet potato	103,368.80 ± 3.31	0.01 ± 0.00	0.02 ± 0.01	5.32 ± 1.00	14.41 ± 1.80	9.75 ± 1.82	3.51 ± 1.32
M. domestica	Red apple	26,825.12 ± 1.68	0.04 ± 0.00	0.03 ± 0.01	19.04 ± 0.90	13.50 ± 3.30	5.87 ± 0.49	1.45 ± 0.41
M. citrifolia	Great morida	23,035.48 ± 4.87	0.04 ± 0.00	0.06 ± 0.01	21.33 ± 1.40	11.35 ± 2.40	10.45 ± 0.11	4.42 ± 0.79
M. alba	Mulberry	8,020.26 ± 5.65	0.12 ± 0.01	0.21 ± 0.02	18.44 ±0.90	59.63 ± 0.80	4.24 ± 0.65	2.06 ± 0.19
M. cochinchinensis	Gac fruit	76,993.93 ±1.95	0.01 ± 0.00	0.02 ± 0.00	23.31 ± 1.20	9.78 ± 5.20	9.17 ± 0.88	3.05 ± 0.34
M. sapientum (AA group)	Lep Mu Nang banana	58,732.48 ± 5.18	0.02 ± 0.00	0.01 ± 0.00	1.76 ± 1.20	10.61 ± 4.10	9.17 ± 1.50	2.09 ± 0.35
M. sapientum (AA Khai)	Pisang Mas banana	7,690.25 ± 2.59	0.13 ± 0.00	0.38 ± 0.01	3.74 ± 1.30	5.55 ± 0.30	4.26 ± 1.14	1.51 ±0.51
M. sapientum (*Musa* ABB cv. Kluai 'Namwa')	Cultivated banana	6,978.51 ± 4.34	0.14 ± 0.00	0.19 ± 0.02	2.67 ± 2.00	11.50 ± 2.40	5.36 ± 0.71	1.63 ± 0.63
N. nucifera	Lotus seed	1,340.86 ± 4.56	0.74 ± 0.00	0.80 ± 0.23	10.22 ± 1.70	4.01 ± 1.10	11.81 ± 0.95	5.60 ± 1.08
O. sativa	Black rice	4,294.95 ± 4.38	0.23 ± 0.00	0.44 ± 0.02	7.84 ± 1.60	4.57 ± 3.20	13.27 ± 1.64	5.51 ± 0.87
P. laurifolia	Passion fruit	8,644.88 ± 5.81	0.12 ± 0.00	0.15 ± 0.01	18.24 ± 1.40	15.09 ± 3.30	10.99 ± 0.45	4.15 ± 0.87
P. emblica	Indian gooseberry	453.50 ± 2.15	2.20 ± 0.01	3.51 ± 0.03	31.31 ± 1.00	11.04 ± 2.50	17.36 ± 2.34	5.57 ± 1.10
S. zalacca	Salak plum	53,427.08 ± 4.27	0.02 ± 0.00	0.01 ±0.01	32.53 ± 1.50	12.07 ± 2.20	6.66 ± 0.83	2.27 ± 0.53
T. triandra	Bamboo grass	6,346.05 ± 1.17	0.16 ± 0.00	0.27 ± 0.02	78.28 ± 0.30	10.30 ± 1.80	4.60 ± 0.85	2.18 ± 0.61
Z. limonella	Kamchat ton	1,511.89 ± 2.31	0.66 ± 0.00	1.19 ± 0.02	1.39 ± 1.20	5.69 ± 4.20	8.96 ± 1.33	6.33 ± 0.56
Z. mays	Sweet corn	144,948.80 ± 6.31	0.01 ± 0.00	0.01 ± 0.01	30.73 ± 0.90	10.90 ± 1.00	5.34 ± 1.05	2.00 ± 0.57
Z. zerumbet	Shampoo ginger	11,579.44 ± 5.41	0.09 ± 0.00	0.07 ± 0.02	9.41 ± 1.80	7.27 ± 4.70	10.50 ± 1.16	7.42 ± 0.56
Galanthamine	Galanthamine	-[c]	-	-	-	-	82.40 ± 0.83	77.34 ± 1.73
α-Tocopherol	Vitamin E	505.35 ± 4.25	1.98 ± 0.02	0.59 ± 0.04	-	-	-	-
Acarbose	Positive control	-	-	-	96.02 ± 0.4	96.56 ± 1.10	-	-

[a]Data are mean of 3 replications.
[b]Common name is not available.

The extract of star apple (*C. cainito*) had moderate antioxidant activity (0.31 antiradical efficiency and 0.40 mmol Fe(II)/g extract by DPPH and FRAP method, respectively), but not the extract of malodge (*E. latifolia* fruit) (0.04 antiradical efficiency and 0.07 mmol Fe(II)/g extract by DPPH and FRAP method, respectively). Star apple is a local plant in central America and can be found in northern Thailand. Its fruits look like a pear fruit. The color of fruit pulp is purple red or pale green, sweet taste and fragrance odor. Morton *et al.* [56] reported that the fruits of star apple contained 67.2 calories which came from 0.72 - 2.33 g protein, 14.7 g carbohydrate and 0.55 - 3.33 g dietary fiber, and also contained carotene, thiamine, riboflavin, niacin and vitamin C. Kubola *et al.* [11] reported the antioxidant activity of this fruit extract, and found that its ripe fruits contained phenolic acids (hydrobenzoic acid, gallic acid and protocatechuic acid), hydrocinnamic acid (chlorogenic acid and ferulic acid) and flavonoids (rutin, myricetin, luteotin, quercetin, apigenin and kaempferol). Differently, malodge (*E. latifolia*) is a fruit with sour taste. The ripe fruits are orange or red in color. It has been used for treatment of laxative. This plant has rarely been studied. Seal [57] revealed that fruits of *E. latifolia* contained crude protein (148.2 g/kg), carbohydrate (743 g/kg), crude fiber (7 g/kg) and nutritive value (3,702.73 kcal/kg).

However, only star cactus (*Aloe vera*) gel extract had higher anti-acetylcholinesterase activity (31.55 % inhibition), compared to other plant extracts. Bawankar *et al.* [58] identified active compounds in star cactus and found that it contained 22.22 % hexadecanoic acid, 16.2 % 9-octadecenoic acid, 5.59 % tricosane, 5.2 % 1-octadecanol and other compounds in small amount. In the current study, lotus (*N. nucifera*) seed extract had anti-acetylcholinesterase activity of 11.81 % inhibition, even though the extract of lotus seed embryos has been found to relieve memory loss in rat by inhibiting acetylcholinesterase in hippocampus [59]. This was probably due to the action of active compounds in lotus seed. Lotus is a water plant with strong pharmacological activity. The stamens of *N. nucifera* have been demonstrated an improvement in memory in rat [60].

Indigestible polysaccharide contents

The extracts with relatively high indigestible polysaccharides (120.34 - 188.62 mg/mL) were mangosteen, shallot, bastard oleaster, pineapple, lotus seed, purple sweet potato, black rice, Jerusalem artichoke, pisang mas banana, salak plum, orange sweet potato, cultivated banana and lep mu nang banana extracts (**Table 3**). Based on their indigestible polysaccharide content, antioxidant activity and flavor, the extracts of lotus seed, black rice, pisang mas banana, cultivated banana and pineapple were selected to study their effect on growth and fermentation of *L. acidophilus*, *L. bulgaricus* and *S. thermophilus* during yogurt fermentation.

Effect of plant extracts on growth and fermentation of probiotic bacteria in yogurt

At the beginning of fermentation, all treatments had slightly different viable counts (6.46 - 6.54 log CFU/g) of lactic acid bacteria (LAB). After 12 h fermentation, total LAB counts in all treatments increased by 1.29 - 1.77 log unit. The samples added with the extract of lotus seed, pisang mas banana, cultivated banana or pineapple had significantly higher total LAB counts than the control treatment ($P < 0.05$). After 24 h fermentation, yogurt fortified with lotus seed extract had highest number of total LAB (2.24 log CFU/g increase), followed by those of black rice, pisang mas banana and pineapple extracts. Addition of black rice, pisang mas banana and pineapple extracts caused good growth of LAB (2.12 - 2.19 log CFU/g increase) which may relate to their high indigestible polysaccharides (**Tables 3** and **4**). The pH values of all yogurt samples decreased to 3.84 - 4.84 after 24 h fermentation, while the total acidity increased to 0.41 - 1.29 %. The pH values of the yogurt samples fortified with each of plant extracts had significantly lower pH values (pH 3.84 - 4.54) at the end of fermentation, compared to the sample fortified with inulin (pH 4.51) and the control treatment (pH 4.84) ($P < 0.05$). Fortification with the extracts of pisang mas banana, cultivated banana and pineapple may result in more sour taste of yogurt, compared to others (**Table 5**).

Table 3 Phenolic, flavonoid and indigestible polysaccharide contents in fruits, vegetables and some Thai local food plant extracts.

Scientific name	Common name	Total Phenolics[a] (mg GAE / g extract) ± SD	Total flavonoids[a] (mg CE / g extract) ± SD	Indigestible polysaccharide[a] (mg / g extract) ± SD
A. trifoliatus	Lopea tree	149.86 ± 0.64	113.25 ± 2.40	3.27 ± 1.50
A. sativum	Common garlic	61.86 ± 2.73	1.51 ± 1.47	95.05 ± 4.40
A. oschaninii	Shallot	66.62 ± 1.30	1.60 ± 1.47	168.69 ± 3.10
A. vera	Star cactus	72.49 ± 2.66	5.01 ± 1.39	70.75 ± 4.70
A. comosus	Pineapple	63.40 ± 1.86	1.68 ± 1.34	155.23 ± 1.00
A. squamosal	Sugar apple	98.74 ± 0.73	30.58 ± 0.66	87.32 ± 5.70
C. carandas	Karunda	98.46 ± 0.49	43.75 ± 1.43	57.39 ± 1.80
C. asiatica	Asiatic pennywort	82.11 ± 3.22	18.01 ± 1.62	35.81 ± 2.20
C. cainito	Star apple	92.73 ± 3.78	9.21 ± 0.97	25.91 ± 4.70
D. volubilis	-[b]	87.70 ± 1.34	13.65 ± 0.93	7.80 ± 3.80
E. latifolia	Bastard Oleaster	70.24 ± 2.68	2.62 ± 1.33	155.51 ± 4.80
G. mangostana	Mangosteen	397.36 ± 2.77	351.60 ± 3.71	188.62 ± 3.60
G. biloba	Maidenhair tree	123.60 ± 2.34	24.57 ± 3.23	71.81 ± 2.70
G. inodorum	Gymnema	105.16 ± 2.92	23.98 ± 2.33	13.47 ± 4.20
H. tuberosus	Jerusalem articheoke	62.00 ± 1.92	3.90 ± 1.32	130.13 ± 1.00
I batatas	Purple sweet potato	69.69 ± 1.17	8.43 ± 1.12	137.13 ± 3.00
I. batatas	Orange Sweet potato	63.82 ± 2.10	5.95 ± 1.69	125.91 ± 1.40
M. domestica	Red apple	66.20 ± 2.30	5.36 ± 1.16	109.04 ± 1.30
M. citrifolia	Great morinda	72.48 ± 3.27	8.09 ± 1.13	17.77 ± 1.60
M. alba	Mulberry	87.84 ± 2.26	16.56 ± 0.57	100.09 ± 2.20
M. cochinchinensis	Gac fruit	66.48 ± 2.33	3.82 ± 1.58	6.81 ± 3.00
M. sapientum (AAgroup)	Lep Mu Nang banana	61.16 ± 1.92	1.51 ± 1.21	120.34 ± 2.80
M. sapientum (AA Khai)	Pisang Mas banana	98.46 ± 0.49	33.23 ± 0.27	129.22 ± 3.10
M. sapientum (*Musa* ABB cv. Kluai Namwa)	Cultivated banana	73.73 ± 2.97	8.77 ± 1.13	124.85 ± 1.90
N. nucifera	Lotus seed	122.75 ± 3.84	89.37 ± 2.46	147.49 ± 4.90
O. sativa	Black rice	113.12 ± 2.25	38.34 ± 2.64	136.91 ± 4.30
P. laurifolia	Passion fruit	85.33 ± 1.14	16.98 ± 1.55	55.31 ± 4.90
P. emblica	Indian gooseberry	416.07 ± 6.11	48.88 ± 3.06	31.90 ± 1.90
S. zalacca	Salak plum	62.57 ± 1.63	2.54 ±0.84	127.76 ± 4.60
T. triandra	Bamboo grass	101.25 ± 1.81	22.63 ± 1.53	18.18 ± 1.60
Z. limonella	Kamchat ton	190.52 ± 3.94	135.36 ± 1.63	2.28 ± 0.60
Z. mays var. *saccharata*	Sweet corn	62.99 ± 0.66	1.17 ± 1.23	74.54 ± 3.30
Z. zerumbet	Shampoo ginger	82.25 ± 3.27	4.41 ± 1.82	69.41 ± 2.20
Inulin	-	-	-	396.57 ± 1.30

[a]Data are mean of 3 replications.
[b]Common name is not available.

Table 4 Change of total lactic acid bacteria in yogurt fortified with different plant extract during fermentation at 42 °C.

Treatment [x]	Total lactic acid bacteria (log CFU/ g)[y] ± SD		
	0 h	12 h	24 h
Lotus seed	6.54±0.02[a]	8.00±0.01[b]	8.78±0.03[a]
Black rice	6.51±0.08[a]	7.92±0.05[bc]	8.70±0.07[ab]
Pisang mas banana	6.52±0.04[a]	8.28±0.12[a]	8.66±0.10[ab]
Cutivated banana	6.49±0.02[a]	8.20±0.11[a]	8.51±0.02[cd]
Pineapple	6.46±0.02[a]	8.23±0.09[a]	8.58±0.03[bc]
Inulin	6.53±0.03[a]	7.82±0.02[c]	8.52±0.15[cd]
Control[z]	6.52±0.06[a]	7.82±0.04[c]	8.41±0.03[d]

[a,b,c,d]Different letter in different row of the same column indicates significant different ($P < 0.05$).; [x]yogurt sample fortified with each of plant extracts; [y]Data are mean of 3 replications.; [z] yogurt sample without fortification of plant extract.

Table 5 Change of pH value and total acidity in yogurt fortified with different plant extract during fermentation at 42 °C

Treatment [x]	pH value and total acidity (%) [y] ± SD					
	0 h		12 h		24 h	
	pH	total acidity	pH	total acidity	pH	total acidity
Lotus seed	6.26 ± 0.02[b]	0.18 ± 0.02[b]	5.24 ± 0.04[b]	0.34 ±0.05[d]	4.42 ± 0.01[c]	0.55 ± 0.01[c]
Black rice	6.28 ± 0.01[b]	0.20 ± 0.01[b]	5.27 ± 0.03[b]	0.31 ± 0.02[d]	4.54 ± 0.02[b]	0.51 ± 0.00[d]
Pisang mas Banana	6.19 ± 0.02[c]	0.20 ± 0.01[b]	4.79 ± 0.06[d]	0.47 ± 0.01[b]	3.84 ± 0.03[f]	0.49 ± 0.01[e]
Cultivated Banana	6.25 ± 0.03[b]	0.18 ± 0.00[b]	5.02 ± 0.02[c]	0.39 ± 0.01[c]	4.04 ± 0.04[d]	0.57 ± 0.01[b]
Pineapple	5.86 ± 0.03[d]	0.31 ± 0.03[a]	4.98 ± 0.01[c]	0.70 ± 0.02[a]	3.91 ± 0.04[e]	1.29 ± 0.01[a]
Inulin	6.45 ± 0.02[a]	0.17 ± 0.01[b]	5.64 ± 0.01[a]	0.29 ± 0.04[d]	4.51 ± 0.02[b]	0.55 ± 0.00[c]
Control[z]	6.44 ± 0.03[a]	0.17 ± 0.02[b]	5.67 ± 0.03[a]	0.33 ± 0.00[d]	4.84 ± 0.01[a]	0.41 ± 0.00[f]

[a,b,c,d,e]Different letter in different row of the same column indicates significant different ($P < 0.05$).; [x]yogurt sample fortified with each of plant extracts; [y]Data are mean of 3 replications.; [z]yogurt sample without fortification of plant extract.

Growth stimulation effect of lotus seed extract may relate to its prebiotic property. Prebiotics are short chain indigestible carbohydrates or non-digestible oligosaccharide (oligosaccharide, polysaccharide, resistance starch and sugar polyols). Resistant starch is a part of starch which will not be hydrolysed to D-glucose within 120 min after food is ingested, but it will be fermented in large intestine [61]. Zhang *et al.* [62] reported that resistant starch type 3 from lotus seed could enhance the growth of bifidobacteria.

The extracts of black rice, pisang mas banana, pineapple and cultivated banana enhanced the growth of starter bacteria in yogurt. This may result from their high indigestible polysaccharides. Black rice has been reported to contain high amount of insoluble dietary fiber [63,64], while pisang mas banana and cultivated banana contain high amount of resistant starch [65]. In addition, van Loo *et al*. [66] reported that banana contained prebiotics, inulin and oligofructose (0.3 - 0.7 g/100 g). These substances tolerate hydrolyzing in upper part of small intestine and pass through large intestine to be the substrate for the beneficial bacteria (bifidobacteria and lactobacilli). These bacteria use this substrate for growth and

fermentation. This will be beneficial for health of human and animals such as anti-infection in digestive system, improvement of motility of food in the intestine, production of short chain fatty acids for intestinal cancer prevention, appetite control by peptide secretion in the digestive tract, enhancing mineral absorption (calcium, iron and magnesium), decreasing of lipid and cholesterol in some occasion as well as enhancing immune system [64].

Conclusions

Of all 33 plant extracts, the most potent inhibitors against α-amylase, α-glucosidase and acetylcholinesterase were bamboo grass leaves, mulberry fruits and star cactus leaf pulp, respectively, while the plants with the highest antioxidant activity, flavonoid and indigestible polysaccharide contents was mangosteen fruit peel. Regular consumption of these plants may associate with reduced risks of diabetes, Alzheimer's disease and some other oxidative stress related diseases, and gain health benefit from prebiotic substances in these food plants.

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References

[1] M Blasa, L Gennari, D Angelino and P Ninfali. *Fruit and Vegetable Antioxidants in Health*. *In*: RR Watson and VR Preedy (Eds.). Bioactive Food in Promoting Health: Fruits and vegetables. Elsevier, New York, USA, 2010, p. 37-58.
[2] AM Pisoschi and A Pop. The role of antioxidants in the chemistry of oxidative stress: A review. *Eur. J. Med. Chem*. 2015; **97**, 55-74.
[3] P Rajendran, N Nandakumar, T Rengarajan, R Palaniswami, EN Gnanadhas, U Lakshminarasaiah, J Gopas and I Nishigaki. Antioxidants and human diseases. *Clin. Chim. Acta* 2014; **436**, 332-47.
[4] DK Patel, R Kumar, D Laloo and S Hemalatha. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac. J. Trop. Biomed*. 2012; **2**, 411-20.
[5] P Arulselvan, HAA Ghofar, G Karthivashan, MFA Halim, MSA Ghafar and S Fakurazi. Antidiabetic therapeutics from natural source: A systematic review. *Biomed. Prev. Nutr*. 2014; **4**, 607-17.
[6] M Heinrich and HL Teoh. Galanthamine from snowdrop-the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *J. Ethnopharmacol*. 2004; **92**, 147-62.
[7] N Tabet. Acetylcholinesterase inhibitors for Alzheimer's disease: Anti-inflammatories in acetylcholine clothing! *Age. Ageing* 2006; **35**, 336-8.
[8] H Sugimoto. The new approach in development of anti-Alzheimer's disease drugs via the cholinergic hypothesis. *Chem. Biol. Interact*. 2008; **175**, 204-8.
[9] DA Butterfield, A Castegna, CB Pocernich, J Drake, G Scapagnini and V Calabrese. Nutritional approaches to combat oxidative stress in Alzheimer's disease. *J. Nutr. Biochem*. 2002; **13**, 444-61.
[10] P Maisuthisakul, S Pasuk and P Ritthiruangdej. Relationship between antioxidant properties and chemical composition of some Thai plants. *J. Food Compos. Anal*. 2008; **21**, 229-40.
[11] J Kubola, S Siriamornpun and N Meeso. Phytochemicals, vitamin C and sugar content of Thai wild fruits. *Food Chem*. 2011; **126**, 972-81.
[12] S Nanasombat, T Bubpasawan, N Tamaput and Y Srimakhan. Antimicrobial activity of Thai medicinal plants against beverage spoilage microorganisms and their potential in retarding Alzheimer's disease progression. *Pharmacog. Commun*. 2014; **4**, 77-87.
[13] K Sadabpod, K Kangsadalampai and L Tongyonk. Antioxidant activity and antimutagenicity of Hom Nil rice and black glutinous rice. *J. Health Res*. 2010; **24**, 49-54.

[14] W Pothirat, MT Chomnawang, R Supabphol and W Gritsanapan. Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stage of maturity. *Fitoterapia* 2009; **80**, 442-7.
[15] P Sithisarn and S Jarikasem. Antioxidant activity of *Acanthopanax trifoliatus*. *Med. Princ. Pract*. 2009; **18**, 393-8.
[16] O Neamsuvan, N Madeebing, L Mah and W Lateh. A survey of medicinal plants for diabetes treating from Chana and Nathawee district, Songkhla province, Thailand. *J. Ethnopharmacol*. 2015; **174**; 82-90.
[17] K Ingkaninan, P Temkitthawon, K Chuenchom, T Yuyaem and W Thongnoi. Screening for acetycholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *J. Ethnopharmacol*. 2003; **89**, 261-4.
[18] P Tappayuthpijarn, A Itharat and S Makchuchit. Acetylcholinesterase inhibitory activity of Thai traditional nootropic remedy and its herbal ingredients. *J. Med. Assoc. Thai*. 2011; **94**, S183-9.
[19] M Bahmani, H Golshahi, K Saki, M Rafieian-Kopaei, B Delfan and T Mohammadi. Medicinal plants and secondary metabolites for diabetes mellitus control. *Asian Pac. J. Trop. Disease*. 2014; **4**, S687-92.
[20] D Mudgil and S Barak. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. *Int. J. Biol. Macromol*. 2013; **61**, 1-6.
[21] JG Muir, SJ Shepherd, O Rosella, R Rose, JS Barrett and PR Gibson. Fructan and free fructose content of common Australian vegetables and fruits. *J. Agr. Food Chem*. 2007; **55**, 6619-27.
[22] P Thammarutwasik, T Hongpattarakere, S Chantachum, K Kijroongrojana, A Itharat, W Reanmongkol, S Tewtrakul and B Ooraikul. Prebiotics: A review. *Songklanakarin J. Sci. Tech*. 2009; **31**, 401-8.
[23] AY Koka, FL Beltrame and AV Pereira. Several aspects of *Zingiber zerumbet*: A review. *Rev. Bras. Farmacogn*. 2016; **26**, 385-91.
[24] S Sancheti, S Sancheti and SY Seo. Antidiabetic and antiacetylcholinesterase effects of ethyl acetate fraction of *Chaenomeles sinensis* (Thouin) Koehne fruits in streptozotocin-induced diabetic rats. *Exp. Toxicol. Pathol*. 2013; **65**, 55-60.
[25] MR Loizzo, AM Saab, R Tundis, F Menichini, M Bonesi, V Piccolo, GA Statti, BD Cindio, PJ Houghton and F Menichini. *In vitro* inhibitory activities of plants used in Lebanon traditional medicine against angiotensin converting enzyme (ACE) and digestive enzymes related to diabetes. *J. Ethnopharmacol.* 2008; **119**, 109-16.
[26] GN Kim, JG Shin and HD Jang. Antioxidant and antidiabetic activity of *Dangyuja* (*Citrus grandis* Osbeck) extract treated with *Aspergillus saitoi*. *Food Chem.* 2009; **117**, 35-41.
[27] W Brand-Williams, ME Cuvelier and C Berset. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Tech.* 1995; **28**, 25-30.
[28] C Lado, M Then, I Varga, E Szoke and K Szentmihályi. Antioxidant property of volatile oils determined by the ferric reducing ability. *Z. Naturforsch. C* 2004; **59**, 354-8.
[29] GL Ellman, KD Courtney, VJ Andres and RM Featherstone. A new rapid colorimetric determination of acetylcholinesterase activity. *Biochem*. *Pharmacol.* 1961; **7**, 88-95.
[30] S Sancheti, S Sancheti, BH Um and SY Seo. 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose: A cholinesterase inhibitor from *Terminalia chebula*. *South Afr. J. Bot.* 2010; **76**, 285-8.
[31] VL Singleton, R Orthofer and RM Lamuela-Raventós. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocateu reagent. *Meth. Enzymol.* 1999; **299**, 152-78.
[32] A Kathirvel and V Sujatha. *In vitro* assessment of antioxidant and antibacterial properties of *Terminalia chebula* Retz. leaves. *Asian Pac. J. Trop. Biomed.* 2012; **2**, S788-95.
[33] S Wichienchot, P Thammarutwasik, A Jongjareonrak, W Chansuwan, P Hmadhlu, T Hongpattarakere, A Itharat and B Ooraikul. Extraction and analysis of prebiotics from selected plants from southern Thailand. *Songklanakarin J. Sci. Tech.* 2011; **33**, 517-23.
[34] GL Miller. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959; **31**, 426-8.

[35] M Dubois, KA Gilles, JK Hamilton, PA Rebers and F Smith. Colorimetric method for determination of sugars and related substances. *Anal*. *Chem.* 1956; **28**, 350-6.
[36] R Agil, A Gaget, J Gliwa, TJ Avis,WG Willmore and F Hosseinian. Lentils enhance probiotic growth in yogurt and provide added benefit of antioxidant protection. *LWT Food Sci. Tech.* 2013; **50**, 45-9.
[37] AOAC. *Official Methods of Analysis: AOAC Official Method*. Association of Official Chemists, Washington DC, USA, 2005.
[38] P Boonsong, N Laohakunjit and O Kerdchoechuen. Identification of polyphenolic compounds and colorants from *Tiliacora triandra* (Diels) leaves. *Agr. Sci. J*. 2009; **40**, 13-16.
[39] M Vessal, M Hemmati and M Vasei. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol*. 2003; **135**, 357-64.
[40] A Eidi, M Eidi and E Esmaeili. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine* 2006; **13**, 624-9.
[41] G Saravanan, P Ponmurugan, GPS Kumar and T Rajarajan. Antidiabetic properties of *S*-allyl cysteine, a garlic component on streptozotocin-induced diabetes in rats. *J. Appl. Biomed*. 2009; **7**, 151-9.
[42] K Shimizu, M Ozeki, A Iino, S Nakajyo, N Urakawa and M Atsuchi. Structure-activity relationships of triterpenoid derivatives extracted from *Gymnema inodorum* leaves on glucose absorption. *Jpn. J. Pharmacol*. 2001; **86**, 223-9.
[43] A Chiabchalard, T Tencomnao and R Santiyanont. Effect of *Gymnema inodorum* on postprandial peak plasma glucose levels in healthy human. *Afr. J. Biotechnol*. 2010; **9**, 1079-85.
[44] Z Yang, Y Wang, Y Wang and Y Zhang. Bioassay-guided screening and isolation of α-glucosidase and tyrosinase inhibitors from leaves of *Morus alba*. *Food Chem*. 2012; **131**, 617-25.
[45] C Liu,W Xiang, Y Yu, ZQ Shi, XZ Huang and L Xu. Comparative analysis of 1-deoxynojirimycin contribution degree to α-glucosidase inhibitory activity and physiological distribution in *Morus alba* L. *Ind. Crop. Prod.* 2015; **70**, 309-15.
[46] Y Jiao, X Wang, X Jiang, F Kong, S Wang and C Yan. Antidiabetic effects of *Morus alba* fruit polysaccharides on high-fat diet-and streptozotocin-induced type 2 diabetic rats. *J. Ethnopharmacol*. 2017; **199**, 119-27.
[47] Y Zhang, C Ren, G Lu, W Cui, Z Mu, H Gao and Y Wang. Purification, characterization and anti-diabetic activity of a polysaccharide from mulberry leaf. *Regul. Toxicol. Pharm.* 2014; **70**, 687-95.
[48] W Luo, M Zhao, B Yang, J Ren, G Shen and G Rao. Antioxidant and antiproliferative capacities of phenolics purified from *Phyllanthus emblica* L. fruit. *Food Chem*. 2011; **126**, 277-82.
[49] R Zadernowski, S Czaplicki and M Naczk. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). *Food Chem*. 2009; **112**, 685-9.
[50] HM Kredy, D Huang, B Xie, H He, E Yang, B Tian and D Xiao. Flavonols of lotus (*Nelumbo nucifera,* Gaertn.) seed epicarp and their antioxidant potential. *Eur. Food Res. Tech*. 2010; **231**, 387-94.
[51] GC Yen, PD Duh and HJ Su. Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes. *Food Chem*. 2005; **89**, 379-85.
[52] A Somanabandhu, N Ruangrungsi, GL Lange and MG Organ. Constituents of the stem bark of *Zanthoxylum limonella*. *J. Sci. Soc. Thailand* 1992; **18**, 181-5.
[53] NT Phuong, KA Lee, SJ Jeong, CX Fu, JK Choi, YH Kim and JS Kang. Capillary electrophoretic method for the determination of diterpenoid isomers in *Acanthopanax* species. *J. Pharm. Biomed. Anal.* 2006; **40**, 56-61.
[54] P Sithisarn, S Jarikasem and K Thisayakorn. *Acanthopanax trifoliatus*, *a Potential Adaptogenic Thai Vegetable for Health Supplement*. *In*: I Rasooli (Ed.). Phytochemicals-Bioactivities and Impact on Health. Intech, Rijeka, Croatia, 2011, p. 253-68.
[55] J Tangjitjaroenkun, O Chantarasriwong and W Chavasiri. Chemical constituents of the stems of *Zanthoxylum limonella* Alston. *Phytochem. Lett.* 2012; **5**, 443-5.
[56] JF Morton. *Chrysophyllum cainito* L. Sapotaceae. *In*: JF Morton (Ed.). Fruit of Warm Climates. Creative Resource Systems, Miami, USA, 1987, p. 408-10.

[57] T Seal. Evaluation of nutritional potential of wild edible plants, traditionally used by the tribal people of Meghalaya state in India. *Am. J. Plants Nutr. Fertilization Tech*. 2012; **2**, 19-26.
[58] R Bawankar, VC Deepti, P Singh, R Subashkumar, G Vivekanandhan and S Babu. Evaluation of bioactive potential of an *Aloe vera* sterol extract. *Phytother. Res.* 2013; **27**, 864-8.
[59] ES Kim, JB Weon, BR Yun, J Lee, MR Eom, KH Oh and CJ Ma. Cognitive enhancing and neuroprotective effect of the embryo of the *Nelumbo nucifera* seed. *Evid. Based Compl. Alternat. Med*. 2014; **2014**, 869831.
[60] M Mehta, A Adem and M Sabbagh. New acetylcholinesterase inhibitors for Alzheimer's disease. *Int. J. Alzheimer's Disease* 2012; **2012**, 728983.
[61] SH Al-Sheraji, A Ismail, MY Manap, S Mustafa, RM Yusof and FA Hassan. Prebiotics as functional foods: A review. *J. Funct. Food.* 2013; **5**, 1542-53.
[62] Y Zhang, H Zeng, Y Wang, S Zeng and B Zheng. Structural characteristics and crystalline properties of lotus seed resistant starch and its prebiotic effects. *Food Chem.* 2014, **155**, 311-8.
[63] D Sumczynski, Z Bubelová and M Fišera. Determination of chemical, insoluble dietary fibre, neutral-detergent fibre and *in vitro* digestibility in rice types commercialized in Czech markets. *J Food Compos. Anal*. 2015; **40**, 8-13.
[64] P Hołownia, B Jaworska-Łuczak, I Wiśniewska, P Biliński and A Wojtyła. The benefits & potential health hazards posed by the prebiotic inulin: A review. *Polish J. Food Nutr. Sci.* 2010; **60**, 201-11.
[65] N Vatanasuchart, B Niyomwit and K Wongkrajang. Resistant starch content, *in vitro* starch digestibility and physico-chemical properties of flour and starch from Thai bananas. *Maejo Int. J. Sci. Tech*. 2012; **6**, 259-71.
[66] J van Loo, P Coussement, LD Leenheer, H Hoebregs and G Smits. On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit. Rev. Food Sci. Nutr.* 1995; **35**, 525-52.