DETERMINATION OF ANTIOXIDANT ACTIVITY, INHIBITORY EFFECT TO GLUCOSE ABSORPTION AND ACUTE TOXICITY OF *SCAPHIUM SCAPHIGERUM* FRUIT GEL POWDER

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ABSTRACT:

Background: Samrong or malva nut (*Scaphium scaphigerum* (G.Don) Guib and Planch) is a Thai plant in Sterculiaceae family. Gel from the fruits of Samrong has been traditionally used as dessert and drink. In this study, Samrong fruit gel powder was determined for *in vitro* antioxidant activity, glucose absorption inhibitory effect, and acute toxicity in animal model.

Methods: Samrong fruit gel powder was prepared by boiling the dry mature fruits with water and the swollen gel was dried in a hot air oven and powdered. The powder was determined for physical properties including swell volume, total acidity, loss on drying, total ash, viscosity and microbial limits. Its chemical properties including infrared and thin layer chromatographic (TLC) fingerprints were investigated. The powder was tested for *in vitro* antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay, ferric reducing antioxidant power assay and thiobarbituric acid reactive substances method. It was determined for glucose absorption inhibitory effect through the intestinal wall of the animal and acute toxicity in animal models was studied.

Results: Samrong fruit gel powder which was qualitatively controlled for physical and chemical properties exhibited glucose absorption inhibitory effect, low antioxidant activity, and low acute toxicity.

Conclusion: The quality controlled Samrong fruit gel powder promoted the activity related to weight control with low acute toxicity. This first report shows that the fruit gel powder could be developed as a weight controlling food supplement.

Keywords: Samrong; *Scaphium scaphigerum*; Glucose absorption; Acute toxicity; DPPH; TLC fingerprint; IR fingerprint

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INTRODUCTION

Dietary fibers are the indigestible portion of plant that could move the intake food through the digestive system, absorb water and ease the defecation. Dietary fiber consists of non-starch polysaccharides such as cellulose and many other plant components such as dextrins, inulin lignin, waxes, chitins, pectins, beta-glucans and oligosaccharides [1]. Previous clinical and metabolic studies indicated that some fiber supplements can control glycemic response in diabetics [2]. In general, water-soluble fibers such as guar gum and pectin are more effective in reducing the postprandial rise in serum glucose after

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mixed meals or glucose load than water insoluble fibers such as wheat, corn bran, soy hulls, and cellulose [2]. Fiber-rich foods are also effective in reducing fasting serum glucose levels, insulin requirements, and urinary excretion of glucose in insulin-dependent and non-insulin dependent diabetics [2]. Fiber rich diets stimulate chewing and increase the time required for consumption of meal. Foods remain longer in the mouth, allowing more time to develop a feeling of satisfaction, thereby interrupting food intake [2]. The gut-filling properties and chewing required during ingestion of fiber may trigger afferent signals inducing satiety. The effect of fiber slowing the rate of gastric emptying may reduce hunger and prolong the feeling of satiety [2]. Dietary fiber has received a great deal of attention from researchers, the food industry, and consumers, due to the health benefits that are associated with the consumption of fiber rich foods [3].

Samrong or malva nut (*Scaphium Scaphigerum* (G.Don) Guib and Planch) is a Thai tropical plant in Sterculiaceae family. This plant commonly distributes in Vietnam, China, Indonesia, Cambodia, Mynmar, Malaysia as well as in the tropical rain forest in the eastern parts of Thailand, especially Chanthaburi and Trat provinces [2, 4]. The mature fruits which have wings become brown during December to January and fall during March to April. The mature seeds have an ellipsoid shape and glabrous with a rough brown skin. Samrong seed coats contain high amount of dietary fibers which can promote swelling brown glassy gel after soaking in water [5, 6]. This gel composed of polysaccharide PP-III, which the main substances are arabinose, galactose, uronic acid and small amounts of glucose, xylose, and mannose [5, 7]. Samrong fruit gel can be consumed as a dessert and drink to relief apthouns ulcer and cough [5, 6]. Consumption of Samrong fruit gel at a dose of 0.08 percent of body weight for 6 and 8 weeks resulting in reduction of body weight and body mass index (BMI) in Thai overweight people [8]. Even though there are several formulations of Samrong fruit gel products in the market, however, there is no report concerning the specification, the quality control, the possible mechanism of action related to weight control effect and the acute toxicity of Samrong fruit gel powder. Therefore, this experiment was set up in order to evaluate the physical and chemical properties, antioxidant activity and microbial limits of Samrong fruit gel powder. Then the studies of acute toxicity and inhibitory effect to glucose absorption in animal models were performed.

MATERIALS AND METHODS

Plant material

Dried Samrong mature fruits were purchased from Chantaburi province, Thailand in April, 2013. The specimens were identified by comparison with the authentic plant materials at the Forest Herbarium, Wildlife and Plant Conservation Department, Bangkok, Thailand. The fruits were cleaned and cut off the upper and lower parts. Psyllium (*Plantago ovata*) seed powder and konjac (*Amorphophallus campanulatus*) root powder, which were used as positive controls, were purchased in a local market in Bangkok, Thailand in April 2015.

Preparation of Samrong fruit gel

Dried Samrong fruits were soaked in distilled water at room temperature for 15 minutes, then the contaminations were separated. The swollen Samrong fruit gel was rinsed and boiled with distilled water at 100ºC for 60 minutes. The swollen fruit gel was drained on a sieve, then squeezed through a fabric filter. The gel was spread on a stainless steel tray and was dried in a hot air oven at 65–70ºC for 8 h. The dried gel was powdered using an electronic mill, and passed through a sieve number 20 to obtain the powder with the particle size of 841 μm.

Quality control of Samrong fruit gel powder

Evaluation of physical properties

The selected Samrong fruit gel powder was evaluated for its physical properties including swell volume, total acidity, loss on drying, total ash, and viscosity as mentioned in United States Pharmacopoeia (USP) 36 for a monograph on psyllium hemicellulose [9].

Evaluation of microbial limits

Samrong fruit gel powder was evaluated for total aerobic microbial count, specific bacteria and total yeast and mold count followed the methods according to Association of Southeast Asian Nations (ASEAN) guideline on stability study of drug product, 2005 [10].

Infrared spectrometric (IR) fingerprint

The infrared spectra of Samrong fruit gel powder was analyzed by fourier transform infrared (FT-IR) (potassium bromide, KBr disc) techniques using FT-IR Nicolett Magna, USA.

Thin layer chromatographic (TLC) fingerprint

According to the method from Skaska-kaminska, et al. [11] Samrong fruit gel was prepared using hydrolysis method to hydrolyze the sugar bonds in polysaccharide structures to obtain monosacharides and compared with the reference monosaccharide standards. Ninety five percent ethanol (210 ml) was added to the fruit gel (5 g) and the mixture was left at the temperature of 4ºC for 12 h, then filtered. Fifteen millilitres of 5% sulfuric acid (H_2SO_4) in water was added to the precipitate part. The mixture was heated on a water baht at the temperature of 90ºC for 2 h, then stayed at room temperature until it was cooled down. The potential of hydrogen (pH) of the solution was adjusted to ventral (pH =7) using barium carbonate ($BaCO_3$) and pH indicator paper. After adjusting the pH, the mixture was filtered. The supernatant was evaporated on a water bath. The dried hydrolysis fraction was dissolved in distilled water (1 ml) and used for TLC analysis [11].

TLC condition

System 1 1-propanol: ethyl acetate: water (4:0.5:0.5 v/v/v)

System 2 1-butanol: 1 - propanol: ethyl acetate: water (3:1:1:1 v/v/v/v)

Detector

15% aniline in 30% orthophophoric acid and methanol was detected in white light and under ultraviolet (UV) at the wavelength of 366 nm.

Antioxidant activity determination

Samrong fruit gel was tested for *in vitro* antioxidant activity using methods as follows:

DPPH scavenging assay

A solution of DPPH (1.52×10^{-4} M) was prepared by dissolving 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in methanol. The reaction was generated by adding an equal volume of DPPH into Samrong fruit gel powder solutions (0.5 – 20 mg/ml) then mixed together. After 15 min, the absorbance of the reaction solution was measured at the wavelength of 517 nm using microplate reader [12]. The percentage of inhibition was calculated. The determination was carried out in triplicate. The average concentration of sample required for 50% scavenging of the DPPH free radical (EC_{50}) was then calculated. DPPH scavenging activity of Samrong fruit gel powder was expressed as the mean of EC_{50} ± standard deviation (SD).

Ferric reducing antioxidant power assay (FRAP)

Samrong fruit gel powder solution was prepared at the concentration of 5,000 µg/ml. Sample solution (100 µl) was mixed with 100 ml of 0.1 M sodium phosphate buffer (pH 6.6) and 100 µl of 1% w/v potassium ferricyanide solution. The solution was incubated at 50°C for 20 min. Then 10% w/v trichloroacetic acid (400 ml) was added and centrifuged at 650 rpm for 20 min. The supernatant (100 µl) was mixed with distilled water (100 µl) and 0.1% w/v ferric chloride solution (20 µl). The absorbance of the solution was measured at the wavelength of 700 nm. The amount of ferrous ion (Fe^{2+}) was calculated using ferrous sulphate ($FeSO_4$) standard curve [13]. The determination was carried out in triplicate. The average amount of ferrous ion was then calculated. Ferric reducing antioxidant power of Samrong fruit gel powder was expressed as the mean of ferrous ion amount per 100 g sample ± SD.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances assay (TBARS)

Emulsion of 0.6% linoleic acid in methanol was prepared. The linoleic acid emulsion (50 µl) was added to Samrong fruit gel powder solution at the concentration range of 0.5 – 2.0 mg/ml (50 µl) then 0.2 mM ferric chloride solution (50 µl) was added. The solution was incubated on a water bath at a temperature of 37°C for 40 min. Then 20% acetic acid solution (350 µl) and 0.5% thiobarbituric acid (TBA) in 20% acetic acid (pH 3.5) (600 µl) were added, respectively. The solution was incubated on a water bath at 85°C for 60 min and cooled down. Then 10% sodium dodecyl sulfate solution (50 µl) was added and the solution was centrifuged at 5,000 rpm for 10 min. The absorbance of the supernatant was measured at the wavelength of 532 nm [14, 15]. The percentage of inhibition was then calculated. The determination was carried out in triplicate and the average concentration of sample required for 50% scavenging of the linoleic acid oxidation (EC_{50}) was calculated. Inhibitory effect to lipid peroxidation of Samrong fruit gel was expressed as the mean of EC_{50} ± SD.

Evaluation of acute toxicity of Samrong fruit gel powder

Adult male and female Wistar rats (200 – 300 g) and adult male and female ICR mice with a weight range of 25 – 40 g were purchased from National Laboratory Center, Mahidol University. Three animals of the same sex were housed in a cage under controlled conditions (25 ± 2ºC, humanity 55 ± 10% RH) with free access to food and water for 1 week.

Acute toxicity of Samrong fruit gel was studied. According to the organization for economic co-operation and development (OECD) guideline [16], fifteen male and female rats and mice were individually separated into 6 groups of 3 animals which were treated as follow;

Group 1: Control group (administration of distilled water) of Wistar rats

Group 2: Control group (administration of distilled water) of ICR mice

Group 3: Oral administration with Samrong fruit gel powder swollen in water at the dose of 600 mg/kg body weight in male Wistar rats

Group 4: Oral administration with Samrong fruit gel powder swollen in water at the dose of 600 mg/kg body weight in female Wistar rats

Group 5: Oral administration with Samrong fruit gel powder swollen in water at the dose of 600 mg/kg body weight in male ICR mice

Group 6: Oral administration with Samrong fruit gel powder swollen in water at the dose of 600 mg/kg body weight in female ICR mice

The amount of dead animal was observed within 7 days after the administration. Then the animals were sacrificed. The visceral organs and any pathological lesions were observed. The evaluation of acute toxicity of Samrong fruit gel powder was started at the dose of 600 mg/kg. If there was no death animal, then the experiment was finished and lethal dose that kills 50 percent of a test sample (LD_{50}) of Samrong fruit gel powder was concluded to be higher than 600 mg/kg without the experiments of lower doses. The method was approved by Institute of Animal Care and Use Committes, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand (No. PYR007/2556).

Evaluation of glucose absorption inhibitory effect of Samrong fruit gel powder

Samrong fruit gel powder was evaluated for inhibitory effect to glucose absorption through the intestinal wall *in vitro* [17]. The intestinal sacs were prepared from male mice and used for the evaluations. Male fasting mice were sacrificed by carbon dioxide (CO_2) inhalation, then the abdomen was opened and the upper part of mice small intestine was taken. The selected small intestine was cleaned, turned inside out and then it was tied at the both ends as a sac (1 cm long). The sac was filled with Kreb-hanseleit solution with glucose 140 mg% and incubated in Kreb-hanseleit mucosal fluid adding Samrong fruit gel powder at the concentrations of 0.05 to 0.2 mg/ml at the temperature of 37ºC with 95% oxygen (O_2) and 5% CO_2 for 30 min. After 30 min of incubation, serosal fluid of each group was analyzed for glucose concentration. The intestinal sac was incubated in various conditions as follow:

Group 1: Control group (administration of distilled water)

Groups 2-4: Administration of Samrong fruit gel powder solutions at the concentrations of 0.05, 0.1 and 0.2 mg/ml, respectively

Group 5: Administration of psyllium seed powder solution at the concentration of 0.1 mg/ml.

Group 6: Administration of glucomanan powder solution at the concentration of 0.1 mg/ml.

Serosal fluid of each group was analyzed for glucose concentration compared with control group after 30 min of incubation [16]. The method was approved by Institute of animal Care and Use committes, faculty of pharmacy, Mahidol University, Bangkok, Thailand. The obtained data was expressed as the mean ± SEM. The data was analyzed by Student's *t*-test using SPSS for Windows, version 16.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

Preparation and quality control of Samrong fruit gel powder

The obtained Samrong fruits gel powder appeared as dark brown powder with specific odor. From the quality control, the physical and chemical properties of Samrong fruit gel powder were summarized in Table 1. From the results, the physical characteristics of Samrong fruit gel powder including swell volume, total acidity, loss on drying and total ash are in acceptable ranges accordingly USP 36 phyllium hemicellulose. The viscosity of Samrong fruit gel powder is also in acceptable criteria of USP 36 xanthan gum viscosity.

The hydrolyzed fraction of Samrong fruit gel powder exhibited specific TLC fingerprint with major chromatographic bands corresponded to standard monosaccharides including galactose, arabinose, rhamnose, glucose and mannose as shown in Figure 1. Samrong fruit gel powder showed IR fingerprint with the peaks at 10 specific positions as shown in Figure 2 and Table 3.

Samrong fruit gel powder exhibited low DPPH scavenging and ferric reducing effects. However, it exhibited moderate inhibitory effect to lipid peroxidation tested by TBARS assay (Table 2).

From microbial limit test, there was no pathogenic bacteria and fungi including

Table 1 Physical and chemical properties of Samrong fruit gel powder.

Property	Result
Swell volume (mg/g dried powder)	158.05 ± 0.00
Total acidity (mL of 0.3N sodium hydroxide, NaOH)	0.57 ± 0.06
Loss on drying (% weight)	7.31 ± 0.07
Total ash (% weight)	5.49 ± 0.01
Viscosity (cps.)	15.13×10^3
TLC fingerprint (Figure 1)	The hydrolysed fraction of samrong fruit gel shows specific TLC fingerprint with the bands corresponded to galactose, arabinose and rhamnose
IR fingerprint (KBr disc)	IR fingerprint of samrong fruit gel powder shows the peaks at wave number corresponded to the functional groups of hydroxyl (OH), ketone (C=O), C-O, CH_3 and CH
Microbial limit	The gel powder promotes negative results to the pathological bacterial including *Bacillus cereus*, *Clostridium* spp., *E. coli*, *P. aeruginosa*, *S. aureus*, *Salmonella* spp. and *C. Albicans* contamination tests

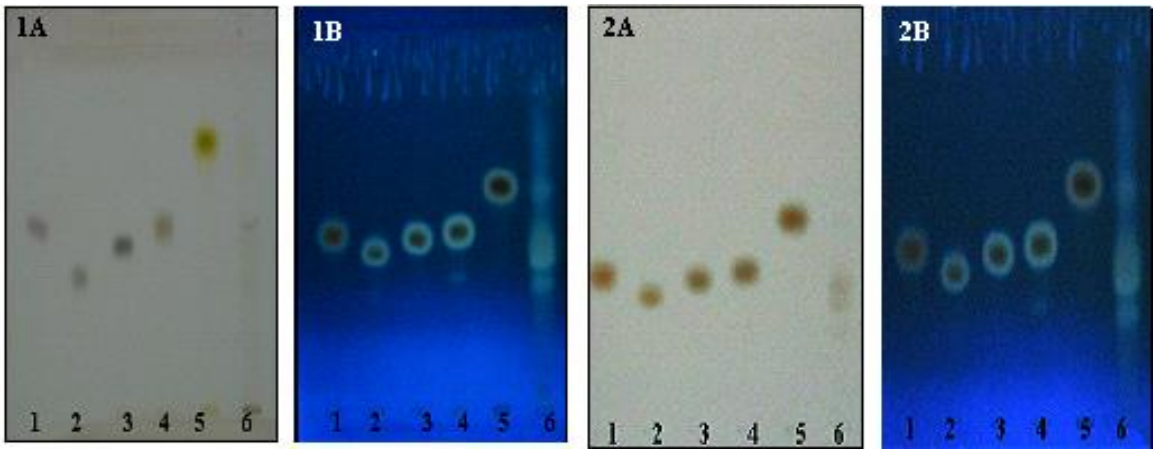

Figure 1 Thin layer chromatography of hydrolyzed fraction of Samrong fruit gel powder; 1 = arabinose, 2 = galactose, 3 = glucose, 4 = mannose, 5 = rhamnose, 6 = hydrolyzed fraction of Samrong fruit gel powder; adsorbent = silica gel GF_{254}, solvent system 1 = 1-proponol : ethyl acetate : water (7:2:1 v/v/v), detection = 15% aniline in 30% orthophospholic acid in methanol detect in white light (1A), 15% aniline in 30% orthophospholic acid in methanol detect under 366 nm (1B); solvent system 2 = 1-butanol:1-proponol:ethyl acetate:water (3:1:1:1 v/v/v/v), detection = 15% aniline in 30% orthophospholic acid in methanol detect in white light (2A), 15% aniline in 30% orthophospholic acid in methanol detect under 366 nm (2B).

Table 2 Antioxidant activities of Samrong fruit gel powder

Sample	Antioxidant activity		
	DPPH assay (EC_{50}, mg/ml)	FRAP method (g $FeSO_4$/100 g sample)	TBARS method (EC_{50}, µg/ml)
Samrong fruit gel powder	5.12 ± 0.51	1.16 ± 0.01	42.42 ± 6.71
Trolox	0.03 ± 0.00	-	4.66 ± 0.37
Gallic acid	-	2565 ± 52.71	-

Bacillus cereus, *Clostridium* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella aureus*, *Salmonella* spp. and *Candida albicans* found in Samrong fruit gel powder.

Evaluation of acute toxicity of Samrong fruit gel powder

The controls of each group of male and female rats and mice composed of 3 animal/group which were orally administered distilled water. The maximum administered dose of Samrong fruit gel powder that was swollen in water was 600 mg/kg animal body weight (1g of Samrong fruit powder was dissolved in 15 ml of distilled water). Each group of 3 animals was orally administered with

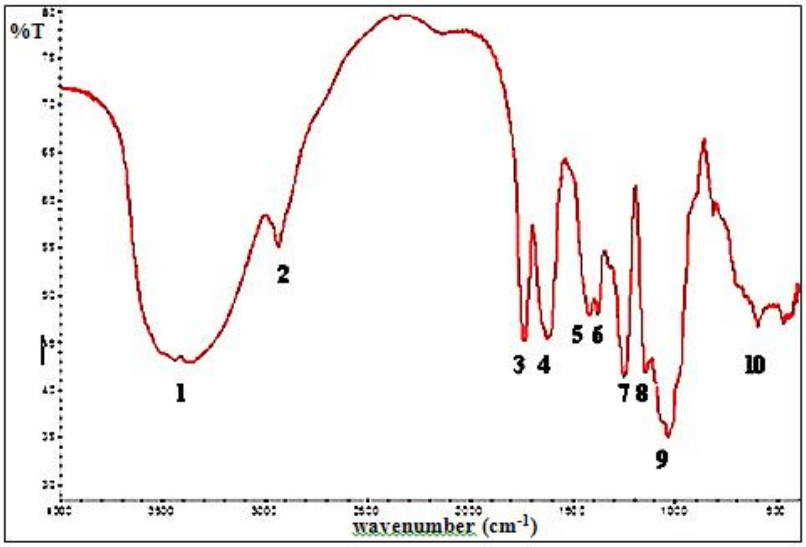

Figure 2 IR spectra (KBr disc) of Samrong fruit gel powder

Table 3 IR spectra (KBr disc) and functional groups of Samrong fruit gel powder

Wavenumber (cm^{-1})	Functional group	Wavenumber (cm^{-1})	Functional group
3412.86 (1)	OH stretching	1378.81 (6)	$-CH_3$ bending
2940.63 (2)	CH stretching	1249.81 (7)	C-O stretching (ester, ether)
1737.62 (3)	C=O stretching	1148.12 (8)	C-O stretching (secondary, tertiary alcohols)
1624.24 (4)	C=C stretching	1036.80 (9)	C-O stretching (primary alcohol)
1429.03 (5)	C=C stretching	601.44 (10)	OH bending (out of plane)

Table 4 Glucose level in mice intestine sac incubated in the residue of Samrong fruit gel powder, glucomannan and phyllium seed and inhibitory effect (% inhibition)

Groups	n	Sugar level in intestine sac (mg/mL) (mean ± SEM)	% inhibition
Control	7	309.7 ± 20.1	0
Samrong 0.05 mg/ml	5	283.2 ± 50.4	8.6
Samrong 0.1 mg/ml	5	195.2 ± 26.1*	37.0
Samrong 0.2 mg/ml	6	187.2 ± 16.9*	39.6
Phyllium seed 0.1 mg/ml	5	198.1 ± 20.2*	36
Glucomannan 0.1 mg/ml	5	201.2 ± 18.5*	35

*significant difference when compared with control group ($p < 0.05$)

Samrong fruit gel powder in water at the maximum dose with the liquid volume not more than 0.5 and 2 ml for mice and rats, respectively.

After observing of animal behaviors, neurological signs, food consuming and the movement within 24 h, no abnormality was found in every group of animals and no animal died within 7 days after the administration suggesting that Samrong fruit gel powder had LD_{50} value higher than 600 mg/kg. Then the animals were sacrificed, the visceral organs pathologies were observed. No lesion abnormality of visceral organs of the animals which received Samrong fruit gel powder was found, compared to the control groups. Therefore, Samrong fruit gel promoted low acute toxicity in animal model (LD_{50} > 600 mg/kg).

Evaluation of glucose absorption inhibitory effect of Samrong fruit gel powder

Glucose absorption inhibitory effect of Samrong fruit gel powder compared with other weight control herbs including glucomannan and psyllium seed was tested by the determination of glucose absorption into mice intestine sac which might show the same absorptive activity as in human. It was found that Samrong fruit gel powder at the concentrations of 0.1 and 0.2 mg/ml

significantly inhibited the glucose absorption in comparison with the control group in dose dependent manner but showed the similar inhibitory activity to psyllium seed and glucomannan at the same dose (Table 4).

Samrong is a local tree of some parts of Thailand. The fruits of this plant promoted gel after soaking in water. However, there is no report concerning the physical and chemical qualities of the gel from the fruits of this plant and also no report about the toxicity and biological activities related to weight control. In this study, Samrong fruit gel powder was prepared and quality controlled for physical and chemical properties. This fruit gel powder promoted low *in vitro* antioxidant effects. There is scientific evidence proved that some phenolic compounds associated with the cell wall of plants such as flavonoids, hydroxycinnamic acids and tannins promoted antioxidant activities. These compounds along with dietary fiber were recognized to have an effect in the prevention of chronic diseases and in reducing the risk of developing cancer [18-20]. Moreover, the associated phenolic compounds which are not absorbed in the small intestine, can reach the colon intact and become fermentable substrates for bacterial microflora, along with indigestible carbohydrates and proteins. This produced metabolites and promoting an antioxidant environment [20]. Previous reports suggest that dietary fibers exhibited various biological activities including lowering blood lipid levels, specifically triglycerides and low-density lipoprotein cholesterol. It could reduce cardiovascular diseases, increase in satiety, consequently decrease in obesity trends, enhanced gastrointestinal immunity and overall colonic health, and also decrease blood glucose levels [3, 21-24]. Fabek, et al., [3] reported that among six selected dietary fibers including guar gum, locust bean gum, fenugreek gum, flaxseed gum, xanthan gum, and soy-soluble polysaccharide, xanthan gum (1% w/w) showed the highest attenuation in glucose diffusion, in comparison to the other gums and the control in dialysis system experiment. From the experiment, Samrong fruit gel powder solution significantly inhibited the glucose absorption comparing with the control group in dose dependent manner similar to the effects of psyllium seed powder and glucomannan root powder solutions at the same dose (0.1 mg/ml). The results suggest the mechanism of action related to weight control support previous study from Latainin reported the decreasing effects to body weight and body mass index of Thai overweight people by Samrong fruit gel [9]. For the determination of the safety of Samrong fruit gel powder, acute toxicity test was performed in male and female rats and mice at the dose of 600 mg/kg body weight. Administration of Samrong fruit gel powder solution promoted no abnormality in every group of animals and no animal died within 7 days suggesting the low toxicity in animal model. There is a report of soluble soybean fiber at the doses of 2.43 and 2.91 g/kg body weight/day in male and female rats showed no toxicity change after 3 months of administration which was then considered as no-observed adverse-effect level (NOAEL) [25]. Another study reported that resistant glucan mixture was also indicated in NOAEL at the dose of 3.3 and 3.9 g/kg body weight/day in male and female rats in 90 days of subchronic toxicity test [26]. Therefore, long term studies such as subchronic or chronic toxicity tests of Samrong fruit gel powder are still needed to ensure the safety of human consumption of Samrong fruit gel powder in the future. It was also suggested that despite minor effects of pH, the dilutions were primarily responsible for the viscosity of the fiber which could promote the effect to the function of the fiber in physiological conditions [3]. Therefore, the mechanism of action in digestive system, the physical and chemical stabilities of the Samrong fruit gel powder and the development of food supplement products should be further studied.

Quality controlled Samrong fruit gel powder for their physical and chemical properties promoted low *in vitro* antioxidant activity. It exhibited glucose absorption inhibitory effects comparing to the effects of psyllium seed powder and glucomannan root powder. This Samrong fruit gel powder showed low acute toxicity in animal models.

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