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Product development of functional beverage from mangosteen juice supplemented with high anti-inflammatory activity herbal plants from Thailand

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Abstract The anti-inflammatory activity effect of eight Thai herbal plant extracts were investigated using nitric oxide assay. The significant lowest IC_{50} was exhibited in Sappan wood (*Caesalpinia sappan* Linn) with the value 5.11±0.51 µg/ml. Therefore, this herbal plant was selected to further product development of mangosteen juice supplemented with high anti-inflammatory activities herbal plants. The based on the criterion used for selecting was amount of anti-inflammatory activities and the preference score acceptability. Mangosteen juice supplemented with 0.20%w/v Sappan wood extract was chosen in term of high nutritional value. Then, the futher formular of mangosteen beverage with high anti-inflammatory activities was improved by using Ratio Profile Test (RPT). Results revealed that the formula contained mangosteen juice 55% w/v and 0.20%w/v Sappan wood extracted, had potential antioxidant activity of 91.15 ± 0.47 DPPH %, total phenolic compound of 5.34± 0.34 mg gallic acid/ml sample, tannin content 6.92± 0.27 mg/ml, anthocyanin content of 4.59± 0.53 mg/l which being shown to be higher than the control formula. Interestingly, the low concentration of developed mangosteen beverage exhibited higher toxicity to breast cancer cell (MDA-MB-231) than the control formula. This finding suggested that these developed functional mangosteen beverage could be served as a healthy alternative functional beverage for consumers.

Keywords: Mangosteen, herbal plants, Sappan wood, Product development

Introduction

Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit of the Guttiferae family cultivated in Southeast Asia countries including Thailand, Vietnam, China and others. This fruit is very popular due to their positive acceptance of taste in juicy, sweet, slightly acid taste, a pleasant aroma and their role in improving human health (Garrity *et al*., 2004). Therefore, it is very

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popular which has also been known as the “queen of fruits” or “super fruit”and medicinal medicine in Thailand (Ovalle-Magallanes *et al*., 2017, Kaur *et al*., 2020). There are many phytochemicals such as xanthones, phenolic compound, anthocyanin and procyanidins which found in difference part of mangosteen. Especially α- xanthones and ɣ-mangosteen are major bioactive ingredients having anti-proliferative, pro-apoptotic, anti-cancer, anti diabetes, anti-microbes, anti-inflammatory and protection against damages in various human organ (Chen *et al*., 2018, Aizat *et al*., 2019).

Remarkably, recent reviews have reported that mangosteen juice had high nutritional supplement such as flavonoids, tannins, sugars, dietary fibers and antioxidant vitamins (B2, B5 or E) (Ovalle-Magallanes *et al*., 2017). Additional ly, the hydrolysis of mangosteen aril for 6 hours were reported as prebiotic activity for *Lactobacillus acidophilus* and *Bacillus lactis* (Anprung and Sangthawan, 2012). Furthermore, antioxidant capacity and possesses anti-inflammatory benefits with no side effects on immune and renal function for long-term consumption has been exhibited in mangosteen–based formula (Xie *et al*., 2015).

In Thailand, there are many medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or pure compounds for treating various disease conditions (Arif *et al*., 2009). Herbal plants play an important role in the development of potent therapeutic agent (Kumar *et al*., 2013). Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered (Padnanabhan and Tangle, 2012). Inflammation is an essential component of immune-mediated protection against infection, pathogens and tissue damage and relate to contribute for some diseases including cancer (Mantovani *et al*., 2008).

From the interesting properties of mangosteen juice and herbs as mentioned above, product development of mangosteen juice supplemented with potential high anti-inflammatory activity has been evaluated in order to increase nutritional value. In addition, no research of product development of mangosteen juice supplemented with potential herb has been statemented. Therefore, the aim of this study was to develop and evaluate the mangosteen beverage with chosen high anti-inflammatory activity herbs.

Materials and methods

Materials

Eight Thai herbal plants such as Turmeric (*Curcuma longa* Linn), Jewel Vine (*Derris scandens* (Roxb.) Benth), Sappan wood (*Caesalpinia sappan*

Linn), Rosy leadwort (*Plumbago indica* L.), Kariyat (*Andrographis paniculata* (Burm.f.) Wall.ex Nees), Birch (*Betula alnoides*), Lingzhi (*Ganoderma lucidum* (Curtis) P. Karst) and Faise calumba (*Coscinium fenestratum* (Goetgh.) Colebr.) were purchased from the local market in Koa Kitchakoot distric, Chanthaburi province, Thailand. Maturing mangosteen fruits were also purchased from local farmers in Chanthaburi province, Thailand. It was harvested in June which is full blooming season and were then transported to the laboratory.

Herbal plants preparation

All herbal plants were prepared by slicing into small sizes (1 cm^3). Next, the sample was boilded by mixing with distilled water with the ratio for 1:8 in the pot and sterilized at 100 ℃ for 30 min and then continued to evaporated at 70 ℃ for 2 hours. The obtained herbal plant solutions were stored in the freezer at -80 ℃ for 12 hours in aluminum bags and then freezed dried at -50 ℃ for 72 hours. The powder herbs were stored at room temperature in 500 g aluminum bags for the chemical properties determination as following mention.

Chemical properties determination of Thai herbal plants

Eight Thai herbal plants as mention above were analyzed the chemical properties such as nitrix oxide assay, DPPH scavenging assay, phenolic compound, tannin content and pH as described below.

Nitrix oxide assay determination

Mouse macrophage cells (RAW 264.7 cells) were cultured DMEM medium (10% FBS, 1% Glutamine and 1% Streptomycin/peniciilin) at the concentrations $5x10^5$cells/ml per well for 24 hours in 96-well plates. Then, the cells were incubated in an incubator at 37℃ at 5% CO_2 concentration (Thermoscientific, USA) for 16-24 hours. After that, the various concentration of herbal plant extracts were placed in a cell culture with lipopolysaccharide (LPS) at a concentration of 1 µg/ml to induce inflammatory conditions. Then, 100 µl of the sample was taken and 100 µl of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added to 100 µl to determine the resulting nitric oxide (NO), and the absorbance reaction were assessed using a microplate reader at 542 nm. (SpectraMR, DYNEX Technologies, USA). Results were expressed as %inhibition relative to control. The level of isolated required to inhibit NO

evolution by 50% was defined as an IC50 value and was interpolated from the dose reponse results (Nitteranon *et al*., 2011).

DPPH scavenging assay

The DPPH radical scavenging activity was the ability to reduce the free radical 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, Sigma). The DPPH radical scavenging activity was modified from the assay method of Gulcin *et al.* (2003). Briefly, 2 ml of the samples solution were mixed with 2 ml of 0.16 mM DPPH in methanol and kept in dark at room temperature for 30 min to complete the reaction. Next, 2 ml of 80 %v/v methanol mixed with 2 ml of 0.16 mM DPPH. All Thai herbal plant extracts were kept in dark at room temperature. The absorbance was measured at 517 nm. The scavenging effect of DPPH free radicals was calculated as follows:

DPPH scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where $A_{control}$ is the absorbance of control reaction and A_{sample} is the absorbance of the samples.

Total phenolic compounds determination

The total phenolic content was analyzed with a modified method from Iqbal *et al.* (2005). Briefly, 3 g of herbal plants extract were mixed with 30 ml of 80% ethanol (v/v). Then, the supernatant was filtered through Whatman filter paper No. 1. The reaction mixture contained 50 µl of clear soluble in 950 µl of deionized water, 200 µl of newly prepared diluted Folin-Ciocalteu reagent from Merck, and 0.5 ml of 7.5 % sodium carbonate. The final mixtures were incubated in dark at room temperature for 2 h to complete the reaction, and then, the absorbance was measured at 760 nm. The gallic acid was used as a standard. The total phenolic content of the sample was calculated as gallic acid equivalents per g dry weight of extraction. The reaction was conducted in triplicate and the results were averaged.

Tannin content and pH determination

The tannin concentration was modified by the method of Hou *et al*. (2003). One ml of Thai herbal plants extract were mixed with the solution of distilled water and 95% ethanol with a ratio of 1:1 at 80 ℃ for 2 hours. The volume of 0.10 ml extraction with distilled water was adjusted until 1 ml. Then, folin-ciocateu phenol reagent 10%w/v and 7.5%w/v $NaCO_3$ were added with 5 and 10 ml, respectively. The solution was incubated for 10 minutes. The absorbance was detected at 760 nm. The concentration of tannic acid was

calculated by using standard curve of gallic acid. And pH determination by using a pH-meter (Subtex, Taiwan)

Product development of mangosteen juice supplemented with high potential anti-inflammatory activity herbal plant

In this section, high anti-inflammatory activity herbal plant was chosen to mix into mangosteen juice. For mangosteen juice preparation was modified by the method of our previously research (Manurakchinakorn *et al*., 2016). In brief, after peeling, the maturing mangosteen fruits were cleaned with distilled water and the juice was prepared. Firstly, the fruit was separated from its pulp (aril) and pericarp. Then, the juice from aril part was extracted by a hydraulic press (Thai sakaya-A2). The obtained juice was mixed with distilled water at a ratio of mangosteen juice to water; 45:55. The juice was adjusted the total soluble solid to 13 °Brix with sucrose and 0.30% (w/v) salt was added. Then, it was pasteurized at 60 ℃ for 30 minutes and filled in a small glass bottle (50 ml.). For develop the formulation of mangosteen juice, there were two steps. The first step was selected of suitable formula. Six formulas of mangosteen juice supplemented with various concentration of high potential of chosen anti-inflammatory activity herbal plant such as 0.00, 0.20, 0.40, 0.60, 0.80 and 1.00 %w/v, respectively. Then, the mangosteen beverage from six formulas were evaluated for sensory evaluation, some physical properties investigation, chemical properties determination and microbiological properties determination, respectively. The second step was developed for mangosteen juice supplemented with high anti-inflammatory activity herbal plant by using Ratio Profile Test (RPT) as following mention.

Sensory evaluation of mangosteen juice supplemented with high anti-inflammatory activity herbal plant

The mangosteen juice supplemented with six various concentration of high potential anti-inflammatory activity herbal plants were evaluated for sensory attributes (colour, aroma, taste and overall liking) by using a 9-point hedonic scales (Watts *et al*., 1989) with 50 untrained panelists from the staffs and students of Department of Food Innovation and Business, Faculty of Agro-industrial Technology, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus, Chanthaburi, Thailand.

Physical properties determination

The mangosteen juice supplemented with various concentration of high potential anti-inflammatory activity herbs samples were evaluated for colour

using a colour meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with a standard plate. Colour measurements were expressed in: L* indicating the lightness on a 0 to 100 scale from black to white; a* (+,-) indicating the redness or greenness, respectively; b* (+,-) indicating yellowness and blueness, respectively.

Chemical properties determination

The total soluble solid was observed by using a hand refractometer (Atago, Japan) while pH by a pH-meter as previously mention. For anthocyanin content determination, the amount of the anthocyanin content was investigated by an established procedure from Lee *et al.* (2005). Briefly, 1N of HCl was mixed with the samples at the ratio of 1:10. The 4N of HCl used to adjusted pH to 1 of the solution and then shaking (100 rpm) for 24 hours at room temperature. The supernatant was centrifuged (5,000 xg for 10 minute) and was measured the absorbance at 535 nm. The total anthocyanin content was calculated as following the equation:

$$\text{Total anthocyanin content (mg/l)} = (A \times MW \times DF \times 10^3)/(\varepsilon \times l)$$

when A = (A520-A700) pH1- (A520- A700) pH4.5

MW = 499.20 g/mol (cyanidine-3-glucoside)

DF = dilution factor

ε = molar extinction coefficient 26,900 ($l.mol^{-1}.cm^{-1}$) (cyanidine-3-glucoside)

l = the width of cuvettes (cm)

For tannin content and DPPH antioxidant activity were assessed as explained previously method.

Microbiological properties determination

The mangosteen juice with supplemented with various concentration of high anti-inflammatory activity herbal plant samples were assessed for its microbiological properties for total microorganism using total plate count on Plate Count Agar (PCA).

Improvement of mangosteen juice supplemented with high potential anti-inflammatory activity herbal plant

The second step, the suitable formula from the step 1 was selected for continuing improvement of the product so that it can be accepted by the consumers as much as possible, based on the ideal of each factor preferred by

the consumer as the guidelines for the formula development. The formula was evaluated by 30 consumers who are familiar with the product using Ratio Profile Test (RPT). The physical properties, chemical characteristic, and microbiological properties of the developted mangosteen juice were done as described previously.

Cytotoxicity of developed mangosteen beverage determination

The final developted mangosteen juice supplemented with high anti-inflammatory activity herbal plants and control (without herbal plant) were assessed for cytotoxicity assay by using MTT assay. This method was measured by the Microbiology department, Faculty of Science, Chulalongkorn University in Bangkok, Thailand. This method was evaluated following the method reported by Senthilraja and Kethiresan (2015) with the slight modifications. In brief, seeding cell MDA-MB-231 cell was seeded at 1.5 X 10^4 in 96 well plate for overnight (total volume 100 µl/well). For the cell treatment, mangosteen beverage was prepared by using six difference concentrations of bioactive compounds which diluted in completed media and added to the well that contain the cell (100 µl/well). Supernatant was removed, mixed complete media that contain with sample solution or DMSO (vehicle control) and incubated for 24 h. For the measurement the cell cytotoxicity, MTT solution (conc. 5 mg/ml) 10 µl/well was added and incubated at 37 ℃ for 4 h in CO_2 incubator. The purple formazan was dissolved by using isopropanol with HCl (100 µl/well) and mixed. Finally, the absorbance was monitored at wavenumber 540 nm by microplate reader.

Data analysis

Property analysis was carried out in three replicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$) (Steel *et al*., 1997). Mean with significant differences was separated by Duncan's multiple range test (DMRT) using the computer software.

Results

Chemical properties determination of Thai herbal plants extract

From chemical properties determination of eight herbal plants extract, significantly highest activity of anti-inflammatory activity herbs was exhibited in Sappan wood (*C. sappan* Linn) with the IC_{50} value 5.11±0.51 µg/ml while significantly ($\rho \leq 0.05$) lowest was founded in Faise calumba (*C. fenestratum*

(Goetgh.) Colebr.) with the data 581.60±15.90 µg/ml. For DPPH antioxidant activity, significantly highest was showed in Turmeric (*C. longa* Linn) with the value 92.89±0.45%, however, this data did not significantly with Jewel Vine (*D. scandens* (Roxb.) Benth) and Rosy leadwort (*P. indica* L.) with the data 92.48±0.13 and 92.45±0.44%, respectively. For total phenolic content and tannin content, significantly ($\rho \leq 0.05$) highest was also founded in Sappan wood extract with the data 18.35±0.92 mg gallic acid/ml sample, 122.97±2.49 mg/ml. For pH, significantly highest was founded in Kariyat (*A. paniculata* (Burm.f.) Wall.ex Nees) with the value 9.17±0.0 as present in Table1. In term of high anti-inflammatory activity, therefore, Sappan wood extract was selected to further product development of mangosteen juice mixed with Thai herbal plant extract.

Table 1. Chemical properties of eight species of Thai herbal plants extract

Herbal plants species	IC_{50}(µg/ml)	DPPH (%)	Total phenolic content (mg gallic acid/ml sample)	Tannin content (mg/ml)	pH
Turmeric (*C. longa* Linn)	405.00±40.60^{e}	92.89±0.45^{a}	13.67±0.95^{c}	72.83±0.93^{e}	6.58±0.01^{d}
Jewel Vine (*D.scandens* (Roxb.) Benth)	564.50±75.40^{b}	92.48±0.13^{ab}	15.17±0.43^{b}	97.78±1.22^{c}	6.20±0.01^{e}
Sappan wood (*C. sappan* Linn)	5.11±0.51^{h}	90.59±0.51^{bc}	18.35±0.92^{a}	122.97±2.49^{a}	6.86±0.01^{b}
Rosy leadwort (*P.indica* L.)	431.40±36.90^{d}	92.45±0.44^{ab}	17.56±0.33^{a}	113.39±1.67^{b}	5.02±0.01^{h}
Kariyat (*A.paniculata* (Burm.f.) Wall.ex Nees)	514.50±123.50^{c}	57.23±1.42^{d}	10.35±0.74^{d}	70.25±1.00^{f}	9.17±0.01^{a}
Birch (*B. alnoides*)	260.50±8.30^{f}	89.59±1.92^{c}	8.41±0.77^{e}	45.06±1.51^{g}	5.05±0.02^{g}
Lingzhi (*G. lucidum* (Curtis) P. Karst)	166.70±70.80^{g}	88.80±0.77^{c}	14.68±0.52^{bc}	76.19±0.89^{d}	5.65±0.01^{f}
Faise calumba (*C. fenestratum* (Goetgh.) Colebr.)	581.60±15.90^{a}	88.95±0.40^{c}	17.66±0.34^{a}	122.78±0.79^{a}	6.74±0.01^{c}

Mean with different letters are statistically different ($\rho \leq 0.05$) according to Duncan's multiple range test.

Product development of healthy mangosteen beverage supplemented with high anti-inflammatory herbal plant

In the first step, The 6 formulas of mangosteen juice supplemented with various concentration of Sappan wood extract including 0.00, 0.20, 0.40, 0.60, 0.80 and 1.00 %w/v, respectively were investigated. Result revealed that the high concentration of Sappan wood extract tended to be decreased the preference score of colour, aroma, taste and overall acceptability. Significanlty ($\rho \leq 0.05$) highest preference score for colour, aroma, taste and overall acceptability were showed in control mangosteen juice (without herbal plant) with the value 7.77 ±0.97, 7.23 ±0.86, 7.47 ±1.01 and 7.60 ±0.97, respectively. In contrast, this datas did not significantly difference with mangosteen juice mixed with 0.20 % w/v Sappan wood extract. Therefore, in term of high potential anti-inflammatory activities herbal plant adding, mangosteen juice mixed with 0.20% w/v Sappan wood extract was chosen to further study in second step to develop mangosteen juice formula.

Table 2. Sensory evaluation of six formulas of mangosteen juice supplemented with Sappan wood extract (Based on 9-point hedonic scores)

Concentration of Sappan wood extract (%)	Preference Scores ±Standard deviation			
	Colour	Aroma	Taste	Overall Acceptability
0.00	7.77 ±0.97 [a]	7.23 ±0.86 [a]	7.47 ±1.01 [a]	7.60 ±0.97 [a]
0.20	7.53 ±0.78 [a]	7.20 ±1.03 [a]	7.37 ±0.85 [a]	7.43 ±1.04 [ab]
0.40	6.67 ±1.03 [b]	6.83 ±1.12 [ab]	7.33 ±.061 [a]	7.17 ±1.18 [ab]
0.60	6.63 ±0.85 [b]	6.67 ±1.06 [ab]	7.30 ±1.88 [a]	6.90 ±1.09 [b]
0.80	6.27 ±0.98 [b]	6.67 ±1.03 [ab]	7.27 ±1.14 [a]	6.97 ±1.00 [b]
1.00	6.30 ±1.21 [b]	6.53 ±1.14 [b]	6.28 ±1.19 [b]	6.33 ±1.03 [c]

Likewise, the supplementation of the amount of Sappan wood extract tended to be decreased L*, while, a* and b* tended to be increase. Overall, the colore of mangoteen juice mixed with Sappan wood extract in all of samples were purple. For the microbiological properties, mangosteen juice mixed with Sappan wood extract did not effect for the viable cell count as presents in Table 3. All of treatments were founded the number of microorganisms of <10 ±0.00 CFU/ml.

Table 3. Physical and microbiological properties of six formulas of mangosteen juice supplemented with Sappan wood extract

Concentration of Sappan wood extract (%w/v)	Colour			Total viable count (CFU/ml)^{ns}
	L*	a*	b*	
0.00	2.35 ±0.01^{a}	2.03 ±0.03^{d}	3.26 ±0.04^{e}	<10 ±0.00
0.20	2.22 ±0.02^{b}	2.04 ±0.02^{d}	3.37 ±0.03^{d}	<10 ±0.00
0.40	2.16 ±0.02^{c}	2.07 ±0.04^{c}	3.40 ±0.03^{c}	<10 ±0.00
0.60	2.07 ±0.02^{d}	2.06 ±0.02^{c}	3.43 ±0.03^{b}	<10 ±0.00
0.80	1.96 ±0.02^{e}	2.10 ±0.01^{b}	3.44 ±0.01^{ab}	<10 ±0.00
1.00	1.87 ±0.01^{f}	2.13 ±0.01^{a}	3.47 ±0.02^{a}	<10 ±0.00

L* (lightness) 0 = black, 100 = white
a*(redness/greenness) + = redness, - = greenness
b*(yellowness/blueness) + = yellowness, - = blueness
Each data represents the mean of three replications.
Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.

Table 4. Chemical properties of six formulas of mangosteen juice supplemented with Sappan wood extract

Concentration of Sappan wood extract (%w/v)	DPPH (%)	Total phenolic content (mg gallic acid/ml sample)	Tannin content (mg/ml)	Anthocyanin content (mg/l)	Total soluble solid^{ns}	pH
0.00	89.61 ±0.53^{e}	4.57 ±0.58^{f}	4.51 ±0.44^{f}	4.40 ±0.25^{d}	13.00 ±0.00	3.74 ±0.00^{c}
0.20	90.10 ±0.27^{d}	5.24 ±0.47^{e}	6.89 ±0.36^{e}	4.45 ±0.24^{cd}	13.00 ±0.00	3.75 ±0.01^{c}
0.40	91.70 ±0.54^{c}	6.69 ±0.12^{d}	7.79 ±0.28^{d}	4.56 ±0.21^{bcd}	13.00 ±0.00	3.75 ±0.01^{c}
0.60	92.06 ±0.41^{bc}	7.65 ±0.26^{c}	9.04 ±0.19^{c}	4.68 ±0.28^{abc}	13.00 ±0.00	3.76 ±0.01^{b}
0.80	92.15 ±0.27^{b}	8.87 ±0.37^{b}	10.34 ±0.36^{b}	4.76 ±0.22^{ab}	13.00 ±0.00	3.76 ±0.01^{b}
1.00	92.83 ±0.38^{a}	10.46 ±0.30^{a}	10.74 ±0.18^{a}	4.87 ±0.28^{a}	13.00 ±0.00	3.77 ±0.00^{a}

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.

From Table 4, the addition of Sappan wood extract significantly($\rho \leq 0.05$) increased the DPPH antioxidant activity, total phenolic content, tannin content,

anthocyanin content and pH. Significantly (ρ≤0.05) highest of those chemical parameter were founded in mangosteen juice mixed with 1.00%w/v Sappan wood extract. Nevertheless, the addition of Sappan wood extract did not effect in the value of total soluble solid.

In the second step, from the selection of the mangosteen juice formula in the step 1, it was founded that the optimum mangosteen juice supplemented with 0.20%w/v Sappan wood extract. The researcher applied the formula to further product development by conducting a descriptive sensory test, Ratio Profile Test (RPT) by using 30 consumers who are familiar with the product. The result was showed in Table 5 and Figure1.

Table 5. The results of the organoleptic quality test of the selected standard formula mangosteen juice supplemented with Sappan wood extract by using Radio Profile Test method

Sensory characteristics	Ideal value (I/I)	Aceptance score (S/I)
Colour	1.00 ^{b}	1.70 ±0.49 ^{a}
Aroma of mangosteen	1.00 ^{b}	1.62 ±0.43 ^{a}
Aroma of herb	1.00 ^{a}	0.80 ±0.13 ^{b}
Sweet taste	1.00 ^{b}	1.08 ±0.20 ^{a}
Sour taste	1.00 ^{a}	0.83 ±0.11 ^{b}
texture	1.00 ^{a}	0.89 ±0.15 ^{b}

Mean with different letters are statistically different (p≤0.05) according to Duncan's multiple range test.

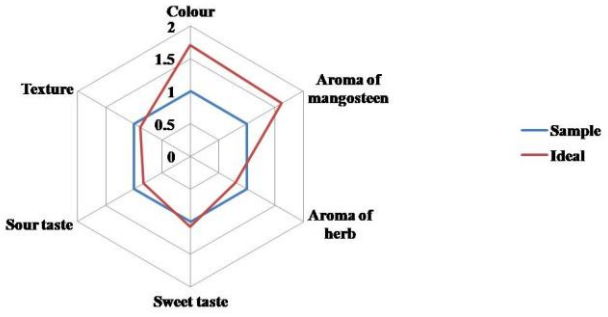

Figure 1. The spider web graph shows the outline of the selected standard formula mangosteen juice supplemented with Sappan wood extract by using Radio Profile Test method

From the Radio Profile Test method, the acceptance score from the familiar consumers showed that, they want to add the colour, aroma of

mangosteen with the score 1.70±0.49 and 1.62 ±0.43, respectively as shown in Table 5 and Figure 1.

Consequently, we were further developed by increasing the concentration of mangosteen juice from 45 %v/v to 55%v/v in order to increase the colour and aroma of mangosteen as the consumers request. Then the developed mangosteen juice supplemented with Sappan wood extract was evaluated in sensory evaluation as shown in Table 6. Also, the properties of some physical, microbiological and chemical properties were determined as showed in Table 7-8, respectively.

Table 6. Sensory evaluation of developted mangosteen beverage supplemented with Sappan wood extract (Based on 9- point hedonic scores)

Concentration of Sappan wood extract (%w/v)	Preference Scores±Standard deviation				
	Colour	Aroma^{ns}	Taste^{ns}	Texture	Overall Linking
0.00(Control)	7.80±0.55^{a}	7.43±0.63	7.67±0.84	7.63±0.67^{a}	7.67±0.48^{a}
0.20	7.50±0.51^{b}	7.40±0.72	7.63±.0.96	7.60±.0.86^{b}	7.57±0.50^{b}

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.
^{ns} mean no significant difference ($p \geq 0.05$)

The highest of colour, aroma, taste, texture and overall linking were still founded in control mangosteen juice (without Sappan wood extract) which the datas 7.80±0.55,7.43±0.63,7.67±0.84, 7.63±0.67 and 7.67±0.48, respectively. However, non significantly for the preference of aroma, taste and overall acceptability were exhibited when compared between control and mangosteen beverage supplemented with 0.20%w/v Sappan wood extract (Table 6).

Table 7. Physical and microbiological properties of developed mangosteen beverage supplemented with Sappan wood extract

Concentration of Sappan wood extract (%w/v)	Colour			Total viable count (CFU/ml) ^{ns}
	L*^{ns}	a*	b*	
0.00(Control)	2.25±0.08	2.04±0.02^{b}	3.26±0.01^{b}	<10±0.00
0.20	2.24±0.02	2.06±0.02^{a}	3.34±0.02^{a}	<10±0.00

L* (lightness) 0 = black, 100 = white
a*(redness/greenness) + = redness, - = greenness
b*(yellowness/blueness) + = yellowness, - = blueness
Each data represents the mean of three replications.
Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.
^{ns} mean no significant difference ($p \geq 0.05$)

In developed mangosteen beverage supplemented with Sappan wood extract, the supplementation of the amount of Sappan wood extract also tended to be decreased L*, while, a* and b* significantly ($\rho \leq 0.05$) increased. For the

microbiological properties, mangosteen beverage mixed with Sappan wood extract still did not affect for the viable cell count (Table 7).

Table 8. Chemical properties of developed mangosteen beverage supplemented with Sappan wood extract

Concentration of Sappan wood extract (%)	DPPH (%)	Total phenolic content (mg gallic acid/ml sample)	Tannin content (mg/ml)	Anthocyanin content(mg/l)	Total soluble solid^{ns}	pH^{ns}
0.00(Control)	90.00±0.59^{b}	4.63±0.46^{b}	4.56±0.26^{b}	4.48±0.40^{b}	15.00±0.00	3.72±0.02
0.20	91.15±0.47^{a}	5.34±0.34^{a}	6.92±0.27^{a}	4.59±0.53^{a}	15.00±0.00	3.71±0.01

^{ns} mean no significant difference ($p \geq 0.05$)

The developed mangosteen beverage supplemented with 0.20%w/v Sappan wood extract was significantly($\rho \leq 0.05$) higher of DPPH antioxidant activity, total phenolic content, tannin content and anthocyanin content than those control mangosteen beverage. Those values were 91.15±0.47%, 5.34±0.34 mg gallic acid/ml sample, 6.92±0.27 mg/ml and 4.59±0.53 mg/l, respectively. However, the addition of 0.20%w/v Sappan wood extract did not significantly effect to total soluble solid and pH (Table 8).

Then, the developed high anti-inflammatory activity mangosteen beverage was observed for cytotoxicity to the breast cancer cell MDA-MB-231. Our result exhibited that the increasing of the concentration of mangosteen beverage both control and developed formula tended to be toxic to the MDA-MB-231 cell. Interestingly, the developed mangosteen beverage product is more effective in inhibility of breast cancer cell than the control formula in the range 5.00-25.00% (v/v) concentration as shown in Figure 2.

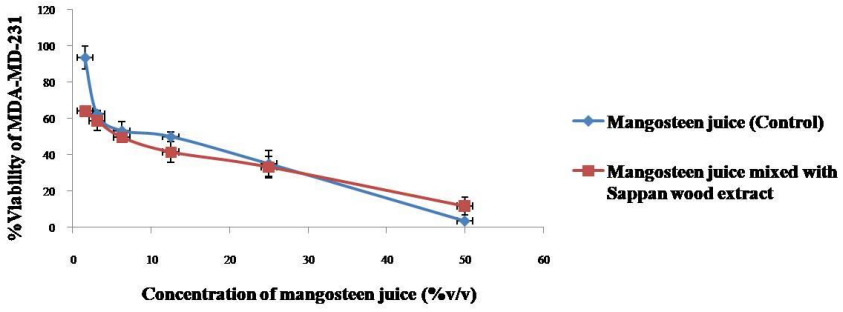

Figure 2. Effect of mangosteen juice supplemented with Sappan wood extract to %viability of breast cancer cell MDA-MB-231

Discussion

Eight herbal plant species were hot water extracted and then investigated some chemical properties. Results demonstrated that significantly ($\rho \leq 0.05$) highest activity of anti-inflammatory (lowest IC_{50}) was revealed in Sappan wood extract (*C. sappan* Linn). This result agree with Badami *et al.* (2003) who also founded this wood exhibited strong antioxidant activity by the low IC_{50} value in both nitric oxide assay and DPPH scavenging activity mothod. So, this herb was further chosen in the development of healthy mangosteen juice supplemented with high anti-inflammatory activity herbal plant.

In addition, best on several triterpenoids, flavonoids, oxygen heterocycles, lipids, steroids and amino acids were reported in this wood (Badami *et al.*, 2004). Therefore, this herbal plant was also showed significantly ($\rho \leq 0.05$) highest in amont of total phenolic content and tannin content, wherease, this datas were did not significantly with Faise calumba (*C. fenestratum* (Goetgh.) Colebr.).

However, the increasing amount of Sappan wood extract tended to decrease the preference score of all parameters in sensory evaluation. This could be the Sappan wood extract had high content of phenolic compound and tannin content. Therefore, the bitter flavor was occurred depending on the amount of Sappan wood extracted adding. So, the most of consumers did not like those flavour then the preference scores in all parameters were decreased. Nevertheless, based on the high anti-inflammatory activity and the flavor preference acceptability was still acceptance by the consumers. Mangosteen beverage supplemented with 0.20 %w/v Sappan wood extract was selected in term of the preference score of colour, aroma, taste and overall acceptability which did not significantly from the control mangosteen juice formula.

For some physical properties, due to the colour of Sappan wood extract powder is orange-red. So, the lightness (L*) was decreased while, the redness (a*) and yellowness (b*) were increased with depending on the amount of Sappan wood extract adding. Moreover, due to this wood shows red colour and has been used for plant dye in wines and meat and other products (Badami *et al*., 2004).

For the chemical properties, due to Sappan wood extract showed highest amount of tannin and total phenolic compound. Therefore, the total phenolic compound, tannin content, DPPH antioxidant activity and anthocyanin content were increased when adding more amount of herb. While, this herb did not

have sugar and neutral pH (6.86±0.01), therefore, the amount of herb adding did not effect to increase much amount of the level of total soluble solid and pH, respectively.

From the Radio Profile Test method, the aceptance score from the familiar consumers need to add more amount of colour and aroma of mangosteen. Therefore, the researcher developed a healthy mangosteen beverage by increasing the amount of mangosteen juice form 45%v/v to 55% v/v. However, from sensory evaluation, the developed a healthy mangosteen beverage was showed little lower significantly in the preference of colour, texture and overall acceptability. In contrast, for the aroma and taste were founded did not significantly difference. Overall, the developed mangosteen beverage was founed higher significantly difference in total phenolic compound, tannin content, DPPH antioxidant activity and anthocyanin content than those control formula. This could be the amount of Sappan wood extract added, so, those chemical properties were increased.

Then, the final developed a healthy mangosteen product and used it to test the viability of breast cancer cell MDA-MB-231, as shown in Figure 2. According to mangosteen juice was suppresses the growth factor and TNF-alpha (Priya *et al*., 2018). Moreover, mangosteen xanthones (gartanin and α-mangosteen) inhibited the growth of cancer cell lines from different stage of human arinary bladder cancer (Lui *et al*., 2013). In addition, Sappan wood extract has been reported to possess immunosuppressive activities, anti-inflammatory, antioxidant, anticancer and antibacterial (Badami *et al*., 2004; Pawar *et al*., 2008). Thus, our developed mangosteen beverage were reduced the number of breast cancer cell MDA-MB-231 as founded in this research. Similarly, it was founded the high concentration of mangosteen juice tended to be increase toxicity to the breast cancer cells and cancer cells respond well to developed mangosteen beverage at 5.00-25.00% v/v concentrations because the synergistic effect of the effect of mangosteen and Sappan wood extract, resulted in increased sensitivity to the extracts of developed mangosteen beverage in this concentration range (Pérez-Rojas *et al*., 2016). However, the cytotoxicity of mangosteen beverage to the normal cell line could be evaluated for comparing inthe further research.

In conclusion, the generated data are strongly suggested that this investigation would be the new product development of mangosteen drink with high anti-inflamatory activity from Sappan wood extract. Expectively, this beverage could be produced as a healthy drink for consumers in the future. On

the orther hand, for the health beneficial effect and futheractual production, the shelf life and the production cost could be monitored. For improving the nutritional value, the addition of probiotic microorganism, dietary fiber and others phytochemicals in the mangosteen beverage would be the alternative way.

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