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Synergistic Effect of Pericarp of Mangosteen and Propolis from Stingless Bee Extracts on Nitric Oxide Scavenging Activity

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Abstract

The aim of this research is to study the synergistic effect on the nitric oxide scavenging activity of mangosteen pericarp and the stingless bee (*Tetragonula laviceps*) propolis extracts and their phytochemical constituents. The propolis and mangosteen pericarp were extracted by reflux method with ethanol. TPC and TFC of propolis extract were 123.73±2.80 mg GAE/g extract and 70.65±11.21 mg QE/g extract, respectively, and mangosteen pericarp extract was 387.93±15.10 mg GAE/g extract and 87.00±5.06 mg QE/g extract, respectively. The ESI-LC-MS data displayed that both extracts have a variety of phytochemical constituents, such as xanthones, flavonoids, and miscellaneous. The synergistic effect of Nitric oxide scavenging activities of propolis and mangosteen pericarp extracts showed higher activity than individual extracts with various concentrations. Thus, the synergistic effect of propolis and mangosteen pericarp extracts may be an alternative source of inflammatory drug development in the future.

Keywords: synergistic effect; nitric oxide scavenging activity; phytochemical constituents; stingless bee propolis; mangosteen pericarp

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1 Introduction

Nitric oxide (NO) is a reactive nitrogen species (RNS) that is produced by the biochemical system in the human body. It was synthesized from the amino acid, arginine, by nitric oxide synthase [1-3]. The important role of nitric oxide is a signaling molecule in inflammation and immunological responses and also influences pathology and physiology in cells such as blood vessel dilation and inhibits the proliferation of smooth muscle cells. However, the production of a very high level of nitric oxide can be led to inflammation and damage of cells and other diseases [1,3-6].

Phenolic compounds and flavonoids are secondary metabolites from natural recourses. It has been reported to have antioxidant activities that are related to cancer, Alzheimer's disease, and vascular disease [7]. Mangosteen (*Garcinia mangostana* L.) has been used as traditional medicine such as for diarrhea, dysentery, infected wounds, chronic ulcer, and food allergies. It also was reported that has anti-inflammation [8]. Widowati et al. analyze the anti-inflammatory effects of *Garcinia mangostana* L. peel extract, α-mangostin, and γ-mangostin in LPS-induced murine macrophage cell line (RAW 264.7). They have anti-inflammatory by reducing COX-2, IL-6, IL-1β, and NO production [9]. In addition, the propolis of a stingless bee was produced from a mixture of vegetable resins, saliva secretions, wax, and soil by a stingless bee [10]. The studies reported that it has anti-inflammatory activity [11]. Pahlavani et al. reported that Caffeic acid phenethyl ester, a major constituent of Propolis, inhibited gene expression of LOX and COX enzymes. Moreover, leukotriene and prostaglandin production was also inhibited by propolis ethanolic extract [12]. They have been declared a variety of phytochemical constituents such as polyphenols, flavonoids, xanthones, and phenolic acids [8,10-11,13].

The synergistic activity effect of drugs is important to the development and drug discovery and has been an important issue in the biomedical world for over a century [14-15]. Therefore, mangosteen pericarp and stingless bee propolis are interesting to evaluate the synergistic effect of nitric oxide scavenging activity and their phytochemical constituents. Moreover, the data may be used for considering drug development for anti-inflammatory activity in the future.

2 Experimental section

2.1 Samples and sample preparation

Propolis of stingless bee (*Tetragonula laviceps*) was collected from Tambon Paseyawo, Amphoe Sai Buri, Pattani province, Thailand in August 2020. It was cut into small pieces. Whereas, mangosteen pericarp was bought from Mai market, Yala province, Thailand in July 2020. It was cut into small pieces and dried at 40°C in a hot air oven. Both samples were kept at room temperature until extraction.

2.2 Extraction method

Both samples were extracted with 95% ethanol in a ratio of 1:10 by reflux method for 1 hr. Then they were centrifuged at 2500 rpm for 5 mins and filtrated with Whatman No.1. The extract solution was evaporated at 50-60°C in a water bath. Extracts were kept at 2-4°C until activities investigation and phytochemical contents analysis.

2.3 Nitric oxide scavenging activity and synergic effect investigation

Briefly, the different concentrations of the sample of 1 mL were mixed with 0.5 ml of 5 mM sodium nitroprusside in phosphate buffer pH 7.4. After incubation for 90 min at 25°C, 1.5 ml of Griess reagent was added. The solution was measured absorbance at 546 nm by a UV-Visible spectrophotometer. The ascorbic acid and ethanol were used as standard and control, respectively. The % radical scavenging activity was calculated by equation **(**1**)** [16].

$$\%\text{Nitric oxide radical scavenging activity} = [Abs_c\text{-}Abs_s/Abs_c]\times 100 \quad \text{(equation 1)}$$

Where,

Abs_c = Absorbance of control

Abs_s = Absorbance of sample or standard

Evaluation of synergistic effect was according to [17]. Combinations of equal concentrations of extracts were used as aliquots in the reaction mixture. the methods of nitric oxide scavenging activities were following to Nitric oxide scavenging method.

2.4 Determination of Total phenolic content (TPC)

Briefly, the 0.5 ml of 0.100 mg/ml sample in ethanol was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau's phenol reagent. Two ml of 7.5% w/v sodium carbonate was added after that 5 min and incubation for 120 min. The absorbance of the solution was measured at 750 nm by a UV-Visible spectrophotometer [18]. Gallic acid was used as standard. The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per g extract (mg GAE/g extract), which was calculated from the calibration curves of gallic acid **(**R^2=0.994; y=0.005x + 0.0406**)**.

2.5 Determination of Total flavonoid content (TFC)

Briefly, the 1.5 ml of 0.100 mg/ml sample in ethanol was mixed with 1.5 ml of 2% w/v aluminium chloride 1.5 ml. The solution was incubated for 10 min. Then, it was measured absorbance at 437 nm by a UV-Visible spectrophotometer [18]. The results of the total flavonoid content were expressed as mg of quercetin equivalent (QE) per g extract (mg QE/g extract), which was calculated from the calibration curves of quercetin **(**R^2=0.999; y=0.0116x + 0.0434**)**.

2.6 Analysis of phytochemicals of propolis and mangosteen extracts by using ESI-LC-MS

The 10 mg/ml of samples in ethanol were vortexed for 1 min, ultrasonicated for 20 min, centrifuged at 10,000 rpm for 10 min, and filtrated with a 0.22 µm membrane. Phytochemicals were analyzed using LC-QTOF-MS of 1290 Infinity II LC-6545 Quadrupole-TOF, Agilent Technologies, USA. The ionization mode of propolis and mangosteen pericarp extracts were used in positive mode and negative mode, respectively. The compounds are identified using MassHunter Metabolite PCD and PCDL version 8. Compound separation was performed on Zorbax Eclipse Plus C18 UHPLC column Rapid Resolution (HD 150 mm length x 2.1 mm inner-diameter, particle size 1.8 µm). The volume of the sample for injection was 2.0 µl. The mobile phase of the positive mode was performed with gradient elution using A: 0.1 % formic acid in the water, B: 0.1 % formic in methanol, whereas, the negative mode was performed with gradient elution using A: 0.1 % Formic acid in the water, B: Acetonitrile. Mass range was collected of 100-1000 m/z for Positive mode and 100-1200 m/z for Negative mode.

3 Results and Discussion

In this study, propolis and mangosteen pericarp extracts were determined for the total phenolic content and total flavonoid content which are shown in Table 1. The extracts of propolis and mangosteen pericarp were 33.83 and 8.99%, respectively. The TPC and TFC of propolis extract were 123.73 ± 2.80 mg GAE/g extract and 70.65 ± 11.21 mg QE/g extract, respectively. While, TPC and TFC of mangosteen pericarp extract were 387.93 ± 15.10 mg GAE/g extract and 87.00 ± 5.06 mg QE/g extract, respectively. The %nitric oxide scavenging activity examination showed that the mangosteen pericarp extract had higher potential activity than the propolis extract. Also, the effect of the synergistic activity displayed that it has a better effect than using a single extract as shown in Table 2. In this result, the

potential of nitric oxide scavenging activity might be involved with the amount of phenolic and flavonoid of extracts. [19] reported the correlation of phytochemical contents (total phenolic and total flavonoid contents) of propolis extract and antioxidant activities had a strong correlation. However, the study of [1] presented that the number of total flavonoid contents of propolis might be not affected by that nitric oxide scavenging activity which may be related to the structure-activity of the compound [20].

The data of LC-MS analysis indicated that the phytochemical constituents of mangosteen pericarp extract displayed the twenty-four compounds, as eight xanthones, five flavones, two isoflavones, and chalcones, and one of isoflavanone, flavan, benzoquinone, anthraquinone, and phloroglucinol. Whereas, propolis extract showed forty-one compounds, as ten flavones, nine flavanones, five isoflavones, and xanthones, two of furanocoumarins, anthraquinone, and chalcones, one of the isocoumarins, stilbene, lignan, flavonol, flavan and curcumin (shown in Supplementary Table 1 and 2). Mostly, the studies reported that flavonoids and xanthones had a potential antioxidant activity [20-23]. Hence, it is possible that the xanthones and flavonoids of the two extracts are the bioactive ingredients for nitric oxide scavenging activity.

Table 1. The percentages of yield and total phytochemical content of extracts

Extracts	% Yield of extract	TPC (mg GAE/g extract ± SD)	TFC (mg QE/g extract ± SD)
Propolis	33.83	123.73 ± 2.80^{b}	70.65 ± 11.21^{b}
Mangosteen pericarp	8.99	387.93 ± 15.10^{a}	87.00 ± 5.06^{a}

Results are presented as means ± SD (n = 3). $^{a \text{ and } b}$ mean in the same column with different superscripts were significantly different ($P < 0.05$) by paired T-test.

Table 2. % Nitric oxide scavenging activities and synergistic effect of extract

Concentration (mg/ml)	% Nitric oxide scavenging activities		
	Propolis extract	Mangosteen pericarp extract	Synergistic effect
0.200	6.80 ± 0.08^{c}	20.36 ± 0.02^{b}	30.98 ± 0.23^{a}
0.400	16.67 ± 0.03^{c}	27.42 ± 0.02^{b}	46.91 ± 0.14^{a}
0.600	21.43 ± 0.08^{c}	36.13 ± 0.14^{b}	54.73 ± 0.12^{a}
0.800	31.88 ± 0.01^{c}	39.75 ± 0.70^{b}	63.51 ± 0.36^{a}
1.000	39.82 ± 0.07^{c}	51.19 ± 0.03^{b}	72.40 ± 0.05^{a}

Results are presented as means ± SD (n = 3). $^{a, b, \text{and } c}$ mean in the same row with different superscripts were significantly different ($P < 0.05$) by paired T-test.

4 Conclusions

Both extracts displayed high total phytochemical contents and Nitric oxide scavenging activities. Their synergistic effect showed a potential activity than individual extract. Therefore, propolis of stingless bees and mangosteen pericarp may be considered for drug development for anti-inflammatory activity.

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6 Declarations

6.1 Author Contributions

The names of the authors listed in this journal contributed to this research.

6.2 Funding Statement

This research was not supported by any funding sources.

6.3 Conflicts of Interest

The authors declare no conflict of interest.

7 Supplementary Data

Supporting information article can be accessed online.

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