

## Anti-Inflammatory and Antioxidant Properties of Crude Methanol Extracts from the Flowers and Peels of Khlong Seng Local Durian (*Durio Zibethinus* Murr.)

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### Abstract

The agricultural byproduct, represented by durian peels during the season, needs to be converted into economic value within the local area. This study aimed to assess the pharmaceutical properties, specifically antioxidant and anti-inflammatory effects, of methanol extracts from both flowers and peels of the local durian in the district. Additionally, *in vitro* testing was conducted to evaluate the impact on cells and the inhibition of nitric oxide induced by LPS. Results indicated that the flowers' extract exhibited a higher concentration of antioxidants compared to the peels. The FRAP assay, using Trolox and Fe(II) as standards, showed statistically significant differences ( $p$ -value < 0.05) with values of  $427.40 \pm 7.64$   $\mu\text{mole TE/g}$  extract and  $3,568.47 \pm 77.55$   $\mu\text{mole Fe(II) equivalent/g}$  extract, along with a DPPH scavenging of 85.19 % and an  $\text{IC}_{50}$  of  $114.50 \pm 7.27$   $\mu\text{g/mL}$ . Concerning the inhibition of LPS-induced nitric oxide content, the peels' extract, excluding KS7, demonstrated a response. KS10 exhibited the highest statistically significant difference ( $p$ -value < 0.05) in inhibition at concentrations of 0.6, 0.8 and 10  $\mu\text{g/mL}$ , while others showed inhibition at 1.0  $\mu\text{g/mL}$ . The outcomes of this research suggest the potential to enhance the value of agricultural waste, contributing to both the development of durian agriculture and public health at the local level.

**Keywords:** Free radical scavenging, Flavonoid content, MTT assay, Phenolic content

### Introduction

Plant extracts, comprising active compounds, are derived from diverse plant components such as bark, leaves, stems, flowers, fruits, roots, tubers, and rhizomes [1]. These bioactive substances display unique properties inherent to specific plant species. Current research endeavors are focused on investigating the capacity of various plant extracts to inhibit bacterial proliferation and the growth of microorganisms [2]. This includes analysis of both edible and residual plant parts, aimed at identifying alternative bioactive compounds that could supplant conventional chemicals. Predominantly, plant extracts are gaining traction in numerous industries, including the food and pharmaceutical sectors, primarily due to their potential in combating infectious diseases and addressing the growing concern of drug resistance [3]. A multitude of studies have concentrated on the anti-inflammatory properties inherent in these extracts. For example, investigations have revealed that extracts obtained from the bark of *Pseudobombax marginatum* demonstrate significant anti-inflammatory effects. These studies have primarily focused on their methods of extraction and efficacy in mitigating inflammation. In a similar vein, *Veronicastrum latifolium* has been recognized for its properties as an inflammation inhibitor. Inflammation, a natural defensive response of the body to injury, infection, or irritation, involves physiological processes such as vasodilation, increased

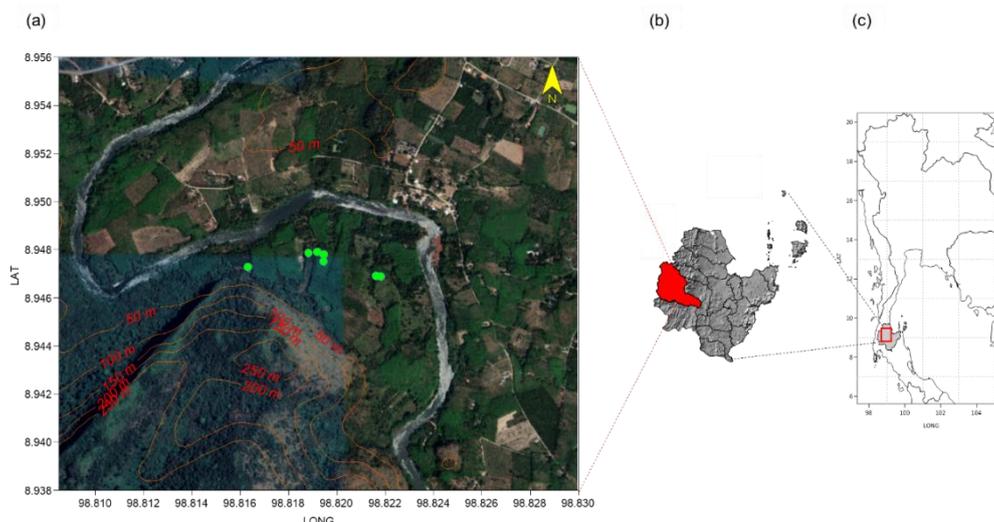
blood flow and the mobilization of immune cells [2,4,5]. This response manifests clinically as redness, swelling, heat and pain. While essential for immunity, chronic or excessive inflammation can precipitate various health complications and diseases [6]. A prevalent cause of inflammation is infections triggered by bacteria, viruses, or fungi [7]. For instance, *Staphylococcus aureus* can induce skin conditions marked by inflammation in both the skin and subcutaneous tissues [8]. This includes disorders such as facial acne. Similarly, *Pseudomonas aeruginosa*, typically originating in the lower respiratory system, possesses the capacity to infect the urinary tract, skin and soft tissues. It is particularly notorious for causing infections in burn wounds, a common occurrence in clinical settings [9]. The healing process of such inflammatory conditions can be augmented through medications designed to suppress the production of pro-inflammatory substances [9]. This treatment regime may encompass both modern pharmacological interventions and antibiotics. However, it is imperative to note that prolonged use of these medications may result in adverse health effects and the development of resistance [10]. Antioxidant properties in natural sources frequently overlap with anti-inflammatory benefits. For example, certain foods and herbs are rich not only in antioxidants but also demonstrate significant anti-inflammatory capabilities [11]. Turmeric, containing curcumin, serves as a prime example. This compound is renowned for its potent antioxidant and robust anti-inflammatory effects. Similarly, fruits like blueberries and oranges, while being rich in antioxidants, also provide anti-inflammatory advantages upon consumption [12,13]. These natural sources offer a dual benefit, effectively combating oxidative stress while fostering a healthier inflammatory response within the body. In essence, the interplay between anti-inflammatory and antioxidant properties is pivotal in sustaining health and averting a variety of diseases. Thus, incorporating foods and supplements endowed with both these attributes is integral to a balanced and health-supportive diet [14].

The southern region of Thailand is renowned for its significant role in both local and international fruit exports [15]. Among the fruits cultivated, durian, prevalent in this area, is found in various species, with the original type being particularly prominent. However, a concern has arisen over the increasing preference for replanting favored species, consequently leading to a diminished popularity of the original varieties [16]. Notably, durian peels constitute approximately 68 % of the total fruit weight, resulting in a considerable volume of waste produced annually in Thailand, especially during the durian season spanning April to September [17]. Traditionally, these agricultural residues have been managed through disposal methods such as burning and burial, practices that could potentially harm the environment. Burning, for instance, can exacerbate air pollution, posing health risks to local communities and contributing to broader global warming issues. Chemical analysis of durian peels has revealed the presence of pectin, a substance renowned for its skin moisturizing properties [18]. Intriguingly, these extracts from durian peels exhibit industrial characteristics akin to pectin. Furthermore, research has shown that discarded durian peels have exceptional qualities for enhancing soil in agricultural practices [19,20]. In the realm of public health, durian peels have been found to contain antioxidants and anti-inflammatory agents, attributable to phytochemicals like anthraquinones, flavonoids, phenols, saponins, and terpenoids [21]. Additionally, these peels demonstrate antimicrobial efficacy against both bacteria and fungi, underscoring their potential as a valuable natural resource with diverse health benefits [22]. This study was aimed to evaluate the *in vitro* antioxidant activity of crude extracts obtained from the peels and flowers of durian, later the cytotoxicity and anti-inflammatory effects was examined on RAW264.7 mouse macrophage cell line. These endeavors seek to augment the economic value of agricultural waste from durian peels in Khlong Seng, potentially paving the way for applications across various sectors, including industry, agriculture, and public health.

## Materials and methods

### Sampling site and sample collection

The study took place in Ban Khao Thep Phithak, a distinct area in the Khao Phang Sub-district of Ban Ta Khun District, Surat Thani Province. The region features a unique valley-like topography with water storage facilities, including the Rajjaprabha Dam and a comprehensive drainage system for hydrological connectivity. The study site has a single access point, as shown in **Figure 1**, with geographical coordinates ranging from latitude 8.915 to 9.365 and longitude from 98.4767 to 98.8433. Random sampling was employed to collect specimens from local durian trees (Khlong Seng variety) in Villages 1, 2 and 3. The sampling specifically targeted durian trees that were in the active phase of fruit production within the specified region. The mean elevation above sea level stands at 41 m (MASL), with an average air temperature of 29 °C. The typical soil moisture content registers at 11 %, while the average air humidity is 82 %. Additionally, the average soil pH is measured at 6.13.



**Figure 1** Illustrates the sample site map. Panel (a) marks the sampling location at Ban Khao Thep Phithak, while panel (b) highlights the specific area within Surat Thani Province. Panel (c) provides an overview of the province's southern location in Thailand.

#### Crude extract preparation

The crude extracts from durian flowers and peels were prepared through a systematic process. Initially, the samples were chopped into smaller pieces and subjected to drying at 50 °C in an incubator for 24 h. Following this drying phase, the samples underwent reflux with methanol, maintaining a 1:10 sample-to-methanol ratio, at a controlled temperature of 70 °C for 2 h. Post-reflux, the supernatant was carefully evaporated using a rotary evaporator (Rotavapor R-300, BUCHI), resulting in the crude extract. This extract was subsequently reconstituted in DMSO to facilitate further analysis.

#### Determination of total phenolic content

The total phenolic contents (TPC) in durian flower and peel extracts were determined using the Folin-Ciocalteu colorimetric method, as modified from the procedure described by Pang *et al.* [23]. A standard gallic acid solution was prepared by dissolving it to a concentration of 2 mg/mL in DMSO. For the assay, 10  $\mu$ L of the sample was added to a 96-well microplate, followed by the addition of 90  $\mu$ L of 10 % Folin-Ciocalteu reagent and 100  $\mu$ L of 7 %  $\text{Na}_2\text{CO}_3$ , bringing the final volume to 200  $\mu$ L. The resultant, blue-colored mixture was thoroughly shaken and incubated for 30 min. Subsequently, the absorbance of this mixture was measured at a wavelength of 765 nm, with all experiments conducted in triplicate, and the average absorbance values obtained at different concentrations of gallic acid were used to construct a calibration curve. This curve was then utilized to determine the quantity of total phenolic compounds in the sample, measured in equivalent milligrams of gallic acid per 1 g of the crude extract (mg GAE/1 g extract), through a calculation based on a linear equation derived from the gallic acid standard curve.

#### Total flavonoid contents

The total flavonoid content was determined using the aluminum chloride colorimetric technique [24]. In a 96-well microplate, 10  $\mu$ L of the crude extract (2 mg/mL) was dispensed, followed by the addition of 40  $\mu$ L of 0.25 M  $\text{NaNO}_2$ . Subsequently, 50  $\mu$ L of 0.15 M aluminum chloride ( $\text{AlCl}_3$ ) was added. The mixture was allowed to stand at room temperature for 5 min, followed by the addition of 100  $\mu$ L of 1M NaOH. The absorbance of the resulting reaction was measured at 506 nm using a spectrophotometric method, with each sample tested in triplicate. Various concentrations of quercetin were used to construct a calibration curve, and quantification was expressed as milligrams of quercetin equivalent per 1 g of the crude extract (mg QE/1 g extract).

#### 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Following the procedures outlined by Promden *et al.* [25] free radical scavenging activity was evaluated using the DPPH assay, with certain modifications. Ten microliters of varying sample concentrations, DMSO (employed as the control), or ascorbic acid (utilized as a positive control) were combined with 190  $\mu$ L of a 100  $\mu$ M DPPH methanolic solution in a 96-well microplate. Subsequently, the

microplate was incubated at 37 °C for 30 min, and absorbance was measured at 515 nm. The calculation of the DPPH radical reduction activity of the test sample was based on the following equation: Scavenging effect (%) =  $[(A_0 - A_1)/A_0] \times 100$ , where  $A_0$  signifies the absorbance of the control reaction and  $A_1$  denotes the absorbance in the presence of the tested compound. The  $SC_{50}$ , which represents the concentration at which 50 % inhibition occurred, was determined through graphical analysis. This involved creating a curve within the linear range by plotting the concentration of the compound against its associated scavenging effect.

#### **Ferric reducing antioxidant power (FRAP) assay**

The FRAP assay was conducted following the method described by Benzie and Strain [26] with minor adjustments. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid and 20 mM iron (III) chloride in a 10:1:1 ratio. In a 96-well plate, 10  $\mu$ L of the sample (2 mg/mL in DMSO) was combined with 190  $\mu$ L of the FRAP reagent. The mixture was allowed to react for 30 min at 37 °C, away from light and the absorbance of each sample was measured at 593 nm. All measurements were conducted in triplicate. The results were quantified as micromoles of  $Fe^{2+}$  equivalent/g of extract using a calibration curve prepared analogously with an aqueous solution of  $FeSO_4$  at final concentrations ranging from 62.5 to 500  $\mu$ M. Additionally, the results were also expressed as micromoles of Trolox equivalent/g of extract, determined using a calibration curve prepared in a similar manner with an aqueous solution of Trolox at final concentrations ranging from 6.25 to 200  $\mu$ M.

#### **Cell culture**

RAW 264.7 cell line (a murine macrophage-like cell line, ATCC No. TIB-71) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, US) supplemented with 10 % heat-inactivated fetal bovine serum (FBS) (Gibco, US) and 100 units/mL of antibiotic and anti-mycotic (Gibco, US). Cells were cultured in a petri dish at 37 °C with 5 % carbon dioxide ( $CO_2$ ). The cells were harvested when they reached 80 - 90 % confluence.

#### **Cell viability testing**

RAW 264.7 cell line at a concentration of  $1 \times 10^5$  cells/100  $\mu$ L was seed into each well of a 96-well culture plate overnight. Cells were treated with 100  $\mu$ L of crude extracts at the concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1  $\mu$ g/mL, respectively. Methanol (1 %) was used as a vehicle control. After 20 h of incubation, 50  $\mu$ L of culture supernatant was collected to measure nitric oxide. Ten microliters of 5 mg/mL of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well and incubated in  $CO_2$  incubator for 4 h. Culture supernatant was removed. The formazan crystal was dissolved with 0.04 N of HCl and measured the absorbance at 570 nm. The cell viability was calculated according to the following equation.

$$\text{Cell viability (\%)} = (\text{OD}_{570} \text{ of sample} - \text{OD}_{570} \text{ blank}) / (\text{OD}_{570} \text{ of control} - \text{OD}_{570} \text{ blank}) \times 100. \quad (1)$$

The experiment was performed in duplicate. The result was summarized from the 3 independent experiments.

#### **Nitric oxide assay**

RAW 264.7 cell line was treated with crude extract as previously described before stimulation with 100 ng/mL of lipopolysaccharide (LPS) from *Escherichia coli* (Sigma, US) for 20 h. The 50  $\mu$ L of supernatant were collected to measure nitric oxide by Griess's assay. In brief, 50  $\mu$ L of 1 % w/v of sulfanilamide and incubated in dark for 5 min. Next, 0.1 % N-1-naphthyl ethylenediamine dihydrochloride in 2 % phosphoric acid were added and incubated in dark for 5 min. The absorbance was measured at 570 nm. The concentration of nitric oxide in culture supernatants were calculated from standard curve generated from sodium nitrate solution.

#### **Statistical analysis**

The result was reported as mean  $\pm$  standard deviation (SD). The independent experiment was done, each carried out in triplicate. The statistical analysis, which included one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. The  $p$ -values less than or equal to 0.05 were assigned significance.

## Results and discussion

### Total phenolic, total flavonoid, DPPH scavenging, DPPH assay and FRAP assay

The samples obtained for this study were labeled under the code “Klong Seng (KS).” Specifically, KS1-KS6 represented the collection of flowers, while KS7-KS13 comprised the conventional Klong-Sang durian peels, originating from durian trees aged between 30 and 60 years. The crude methanol extracts showed parallel trends in both total phenolic and flavonoid content, with KS4 registering the highest values, followed by KS1 and KS3 durian flowers. The 3 extracts with the highest content demonstrated a statistically significant difference ( $p$ -value < 0.05) compared to other crude extracts from durian flowers and peels, as outlined in **Table 1**. The phenolic content had values of  $33.28 \pm 1.24$  mg GAE/g extract,  $26.08 \pm 0.14$  mg. GAE/g extract and  $18.69 \pm 0.55$  mg GAE/g extract for KS4, KS1, and KS3 respectively, the linear equation of the gallic acid standard curve was  $y = 0.036x + 0.044$ ,  $R^2 = 0.9929$  (**Figure 2(a)**). For flavonoid contents the highest values were  $99.19 \pm 1.54$  mg QE/g extract,  $69.49 \pm 2.85$  mg QE/g extract and  $52.79 \pm 0.89$  mg QE/g extract calculated from the linear equation value of the standard curve QE was  $y = 0.254x + 0.011$ ,  $R^2 = 0.9947$  (**Figure 2(b)**) in KS4, KS1, and KS3, respectively. The quantity of both substances exhibits a distinct correlation with the % free radical DPPH scavenging activity, surpassing 50 % in the crude extract from durian flowers. The highest percentage was 85 % in KS4, followed by 74 % in KS1 and 69 % in KS3, while the remaining 10 crude extracts ranged from 10 to 38 %, as indicated in Table 1. The  $IC_{50}$  was further done for the 3 crude extract which show the high percentage and found that the KS4 was  $114.50 \pm 7.27$   $\mu$ g/ml, KS1 was  $146.18 \pm 2.69$   $\mu$ g/mL and KS3 was  $219.80 \pm 19.67$   $\mu$ g/ml (**Table 1**). The inhibition assay using the FRAP of the crude extracts using the linear equation of the Trolox standard curve was  $y = 7.140x + 0.069$ ,  $R^2 = 0.9954$  (**Figure 2(c)**) and Fe(II) standard curve was  $y = 0.824x - 0.003$ ,  $R^2 = 0.9964$  (**Figure 2(d)**) found the highest in KS4, KS1, KS3 from durian flowers, and KS7 from durian peel and have a statistically significantly different ( $p$ -value < 0.05) from other 9 crude extracts listed in **Table 1**.

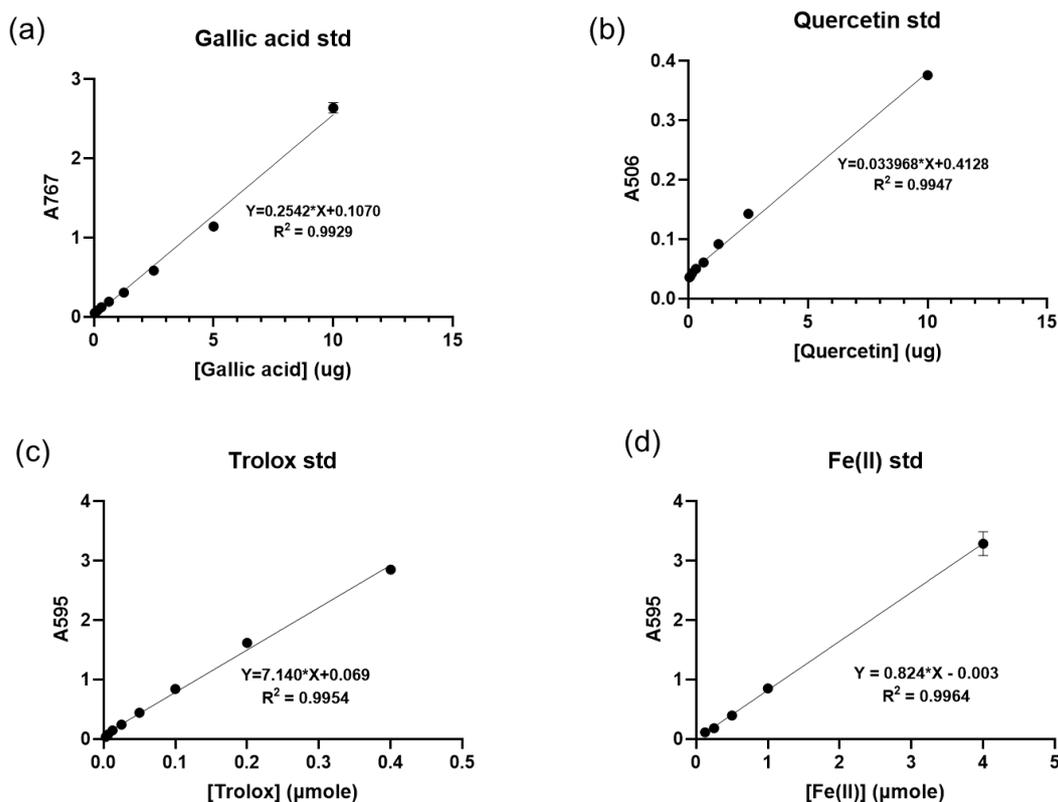
**Table 1** The total phenolic and flavonoid content, as well as the antioxidant activity assay, for both durian flowers and durian peels crude extracts.

Sample	TPC (mg GAE/ g extract)	TFC (mg QE/ g extract)	Antioxidant activity			
			% DPPH Scavenging at 250 $\mu$ g/mL	DPPH $IC_{50}$ of DPPH ( $\mu$ g/mL)	FRAP ( $\mu$ mole TE/g extract)	FRAP ( $\mu$ mole Fe (II) equivalent/g extract)
KS1	$26.08 \pm 0.14^{**}$	$69.49 \pm 2.85^{**}$	74.80	$146.18 \pm 2.69$	$415.54 \pm 19.88^{**}$	$3,474.99 \pm 280.43^{**}$
KS2	$9.34 \pm 0.29$	$21.24 \pm 0.74$	37.01	N.D.	$173.04 \pm 7.86$	$1,507.84 \pm 151.58$
KS3	$18.69 \pm 0.55^{**}$	$52.79 \pm 0.89^{**}$	65.69	$219.80 \pm 19.67$	$327.44 \pm 9.75^{**}$	$2,759.82 \pm 191.54^{**}$
KS4	$33.28 \pm 1.24^{**}$	$99.19 \pm 1.54^{**}$	85.19	$114.50 \pm 7.27$	$427.40 \pm 7.64^{**}$	$3,568.47 \pm 77.55^{**}$
KS5	$13.95 \pm 0.18$	$20.24 \pm 1.50$	36.27	N.D.	$194.85 \pm 6.66$	$1,684.14 \pm 125.52$
KS6	$15.85 \pm 0.05$	$38.94 \pm 2.36$	37.41	N.D.	$240.51 \pm 15.31$	$2,055.29 \pm 212.15$
KS7	$18.50 \pm 0.92$	$33.66 \pm 3.81$	38.88	N.D.	$313.96 \pm 22.45^{**}$	$2,651.55 \pm 281.47^{**}$
KS8	$8.21 \pm 0.61$	$13.68 \pm 0.89$	21.80	N.D.	$177.54 \pm 11.03$	$1,544.11 \pm 154.19$
KS9	$10.90 \pm 0.69$	$10.96 \pm 0.43$	17.17	N.D.	$185.26 \pm 10.97$	$1,586.19 \pm 161.91$
KS10	$13.19 \pm 0.37$	$15.39 \pm 0.25$	23.47	N.D.	$222.40 \pm 8.71$	$1,908.19 \pm 168.17$
KS11	$16.34 \pm 0.87$	$19.81 \pm 1.50$	27.02	N.D.	$245.79 \pm 13.78$	$2,117.76 \pm 203.03$
KS12	$11.05 \pm 0.83$	$14.68 \pm 0.25$	19.58	N.D.	$205.45 \pm 12.89$	$1,770.30 \pm 163.22$
KS13	$13.31 \pm 0.64$	$16.96 \pm 0.74$	20.39	N.D.	$214.82 \pm 6.69$	$1,846.24 \pm 137.61$

\*\* : Statistically significant difference at  $p$ -value < 0.01, N.D. = not determined.

Durian flower and peel crude methanol extracts were analyzed to determine their total phenolic compounds and total flavonoid content. The durian peel contains a high concentration of biologically active substances, particularly phenolic compounds. There are numerous phenolic compounds found in nature, each with its own chemical structure formula. The structures of these compounds range from simple to complicated [27,28]. For this study, the KS4 and KS7 crude extracts exhibited the highest levels of both total phenolic and total flavonoid content in durian flowers and peels, recording values of  $33.28 \pm 1.24$ ,  $18.50 \pm 0.92$ ,  $99.19 \pm 1.54$  and  $33.66 \pm 3.81$  mg QE/g extract, respectively. The comparison of the levels of total phenolic compounds and total flavonoids in crude methanol extracts of durian flowers and peels obtained in this study, it was observed that they were lower than those in the commercial durian peel extract from Sumatra, Indonesia. It's worth noting that the extraction methods differed; our research utilized the reflux method, whereas the Indonesian durian crude extract was obtained through the maceration method using 96 % ethanol, revealing a value of 148.34 mg GAE/100 g DW and 310.30 mg RE/g sample [29]. The phenolic compound and flavonoid compounds exhibit antioxidant properties, functioning through the mechanism of scavenging free radicals. This entails contributing hydrogen atoms, removing electron-deficient oxygen, and binding with metals [30]. In this study, the antioxidant activity of the chosen crude extracts was primarily assessed through the FRAP method, employing Trolox equivalent and Fe(II) as standard solutions [26]. Additionally, the DPPH method, commonly utilized in preliminary tests for antioxidant properties, was employed. This method offers a quick, convenient, and easily executable means of evaluating antioxidant mechanisms, ensuring accuracy in the results. DPPH serves as a widely employed chemical for evaluating antioxidant capacity due to its stability as a free radical [31]. In contrast, the FRAP method employs distinct concepts from the previously mentioned approaches to showcase the potential of compounds in acting as reducing agents [32]. The outcomes are expressed as FRAP assay, representing the capacity to convert  $\text{Fe}^{3+}$ -TPTZ to  $\text{Fe}^{2+}$ -TPTZ. A higher FRAP value indicates a greater antioxidant capacity, as highlighted by Huang *et al.* [32]. The antioxidant activity of the crude durian flower extract KS4 was found to be the highest, measuring  $427.40 \pm 7.64$   $\mu\text{mole TE/g extract}$  and  $3,568.47 \pm 77.55$   $\mu\text{mole Fe(II) equivalent/g extract}$ . In comparison, the crude durian peel extract KS7 exhibited antioxidant activity with values of  $313.96 \pm 22.45$   $\mu\text{mole TE/g extract}$  (Trolox equivalent) and  $2,651.55 \pm 281.47$   $\mu\text{mole Fe(II) equivalent/g extract}$ . These measurements were conducted using the FRAP method with Trolox equivalent and Fe (II) as standard solutions.

In the % DPPH scavenging method, the crude durian flower extract KS4 and the crude durian peel extract KS7 exhibited scavenging percentages of 85.19 and 38.88 %, respectively. The  $\text{IC}_{50}$  value for the crude extract KS4 was the lowest, measuring  $114.50 \pm 7.27$   $\mu\text{g/mL}$ . The  $\text{IC}_{50}$  value for each sample of durian peel crude extract was not analyzed, as their % radical scavenging was less than 50 % according to **Table 1**. This association correlates with the overall phenolic and flavonoid content in this study, when compared to methanol crude extracts of durian flowers and durian peels from other experiments, the  $\text{IC}_{50}$  values were in line with those of the remaining economical durian peel crude extracts discarded from the fresh market in Yangtze University (Jingzhou, China), which measured  $71.28 \pm 2.01$   $\mu\text{g/mL}$  [33]. The antioxidant evaluation conducted in this research suggests a higher efficacy in flowers compared to peels. Despite this, the community traditionally utilizes durian peels, following the wisdom of local sages, for treating wounds with inflammatory conditions. In terms of overall fruit quantity, as much as 70 - 85 % of residual peels are likely to remain [34,35].

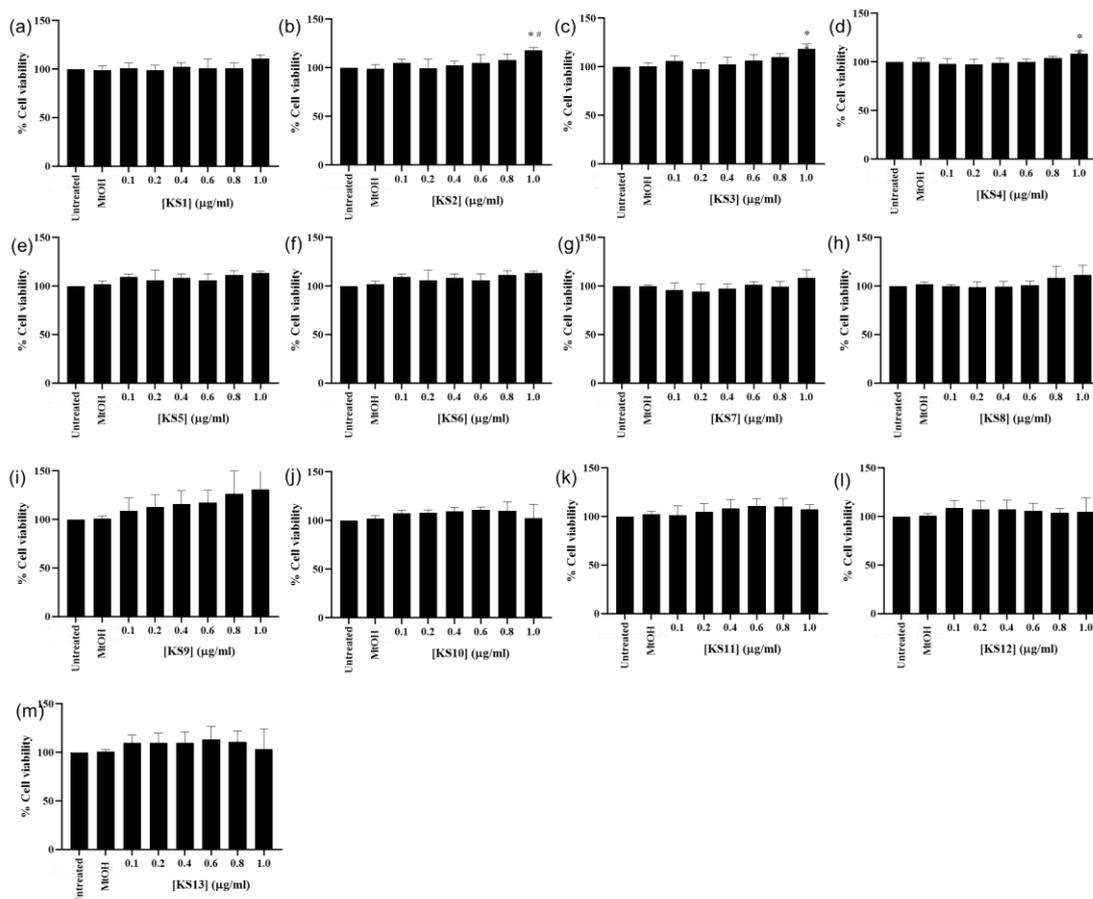


**Figure 2** The standard calibration curves for various substances. Panel (a) depicts the Gallic acid standard calibration curve, panel (b) shows the quercetin standard curve, panel (c) represents the Trolox calibration curve and panel (d) illustrates the Fe(II) calibration curve.

In the % DPPH scavenging method, the crude durian flower extract KS4 and the crude durian peel extract KS7 exhibited scavenging percentages of 85.19 and 38.88 %, respectively. The  $IC_{50}$  value for the crude extract KS4 was the lowest, measuring  $114.50 \pm 7.27 \mu\text{g/mL}$ . The  $IC_{50}$  value for each sample of durian peel crude extract was not analyzed, as their % radical scavenging was less than 50 % according to **Table 1**. This association correlates with the overall phenolic and flavonoid content in this study, when compared to methanol crude extracts of durian flowers and durian peels from other experiments, the  $IC_{50}$  values were in line with those of the remaining economical durian peel crude extracts discarded from the fresh market in Yangtze University (Jingzhou, China), which measured  $71.28 \pm 2.01 \mu\text{g/mL}$  [33]. The antioxidant evaluation conducted in this research suggests a higher efficacy in flowers compared to peels. Despite this, the community traditionally utilizes durian peels, following the wisdom of local sages, for treating wounds with inflammatory conditions. In terms of overall fruit quantity, as much as 70 - 85 % of residual peels are likely to remain [34,35].

#### MTT assay and nitric oxide assay

Crude methanol extracts from durian flowers, KS2, KS3, and KS4, at a concentration of  $1.0 \mu\text{g/mL}$ , significantly decreased RAW264.7 cells viability ( $p$ -value  $< 0.05$ ), suggesting that these crude extract toxic to the cell at the highest tested concentration. In contrast, the other crude extracts did not show impact to the cell viability at all tested concentrations when compared to both the untreated or vehicle (methanol) control (**Figure 3**).

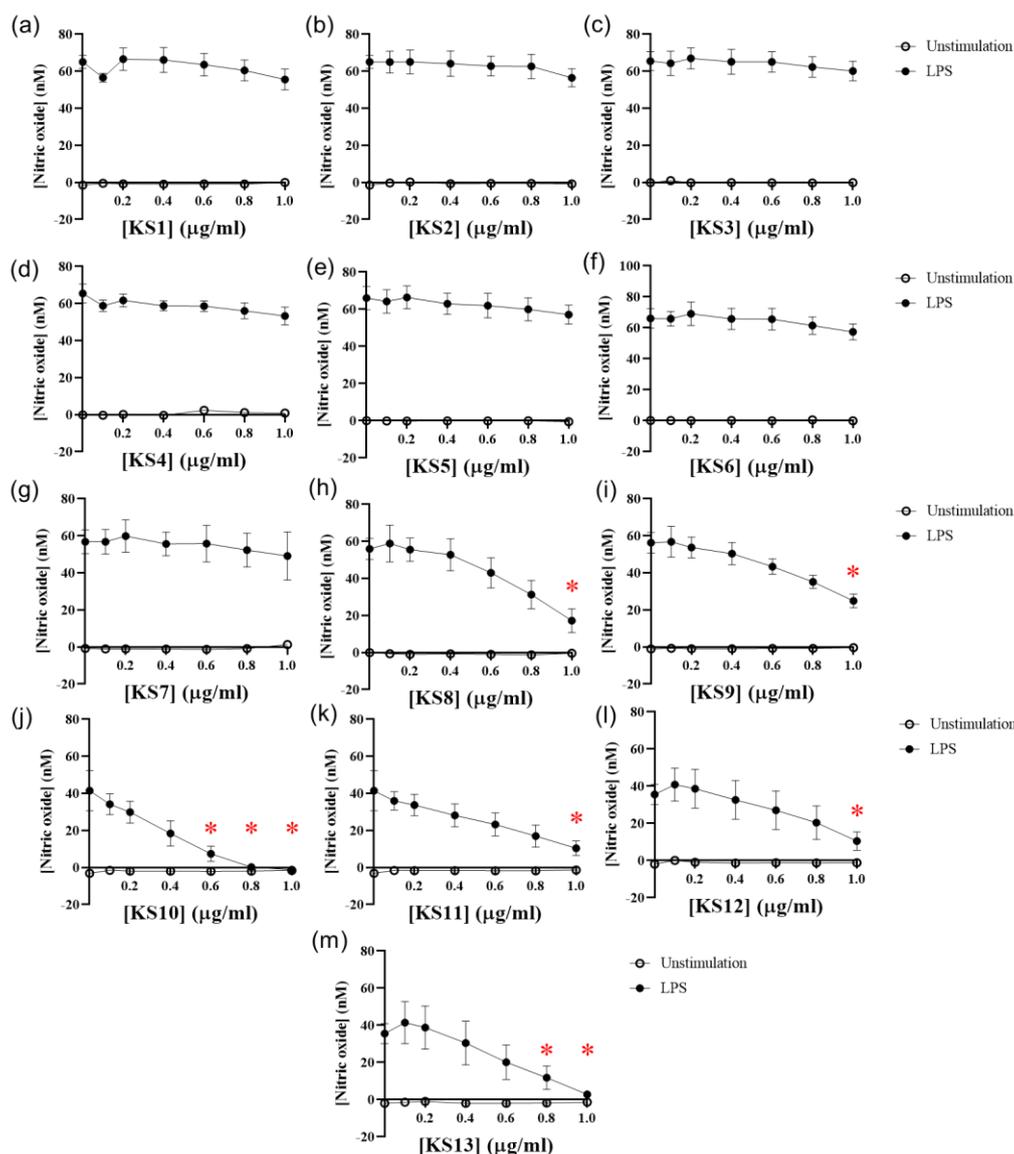


**Figure 3** The comparison between an unstimulated control group and the concentrations of nitric oxide released from RAW 264.7 macrophage culture cells. These cells were stimulated with lipopolysaccharide (LPS) along with various extract concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL) for 24 h. The RAW 264.7 cells were induced by LPS to produce nitric oxide, and the results indicate the concentrations of nitric oxide secreted by these macrophage cells.

The durian peel extract from KS10 demonstrated notable inhibition of NO production, particularly at concentrations of 0.6, 0.8 and 1.0 µg/mL, resulting in nitric oxide levels of  $7.363 \pm 7.097$ ,  $0.249 \pm 2.327$  and  $-1.187 \pm 1.019$  nM, respectively (**Figure 4(j)**). Additionally, the durian peel extract from KS13 exhibited significant inhibition of NO production at concentrations of 0.8 and 1.0 µg/mL, with corresponding nitric oxide levels of  $11.773 \pm 11.020$  nM and  $2.776 \pm 3.089$  nM, respectively (**Figure 4(m)**). Durian peel extracts from KS8, KS9, KS11, and KS12, each at a concentration of 1 µg/mL, exhibited significant inhibition, with nitric oxide levels of  $17.207 \pm 11.103$ ,  $24.835 \pm 6.412$ ,  $10.371 \pm 6.847$  and  $10.499 \pm 8.555$  nM, respectively (**Figures 4(h) - 4(l)**). A statistically significant difference ( $p$ -value < 0.05) was observed when compared to crude extracts from durian peels KS6, as well as crude extracts from all durian flowers, which were ineffective in inhibiting NO production.

The results revealed that crude extracts from durian flowers KS2, KS3 and KS4, at a concentration of 1.0 µg/mL, exhibited toxicity towards macrophage cells, impacting cell viability. In contrast, crude extracts from durian flowers KS1, KS5, KS6, as well as crude extracts from durian peels KS7, KS8, KS9, KS10, KS11, KS12 and KS13, at all concentrations tested, showed no adverse effects on cell viability. Subsequently, the extracts were tested to evaluate its anti-inflammatory impact on macrophage cells, specifically in reducing the secretion of nitric oxide induced by lipopolysaccharide (LPS). LPS, a toxin found in various microbial cells, particularly in Gram-negative bacteria, serves as the stimulant for this assessment [36]. The assessment involved quantifying the secretion of nitric oxide is inherently unstable with a short half-life in seconds [37]. To assess the efficacy of LPS in stimulating macrophage cells to release nitric oxide, the researchers compared the nitric oxide secretion levels of macrophage cells under LPS stimulation at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL with those not stimulated by LPS.

The results indicated a significant increase in nitric oxide secretion from macrophage cells in the vehicle control compared to the cell control, highlighting the impact of LPS concentration. Inhibiting the production or secretion of nitric oxide is recognized as a mechanism capable of mitigating the progression and severity of various diseases. This study revealed that the crude methanol extract from durian peels, specifically KS8, KS9, KS10, KS11, KS12 and KS13 at a concentration of 1  $\mu\text{g}/\text{mL}$ , effectively inhibited NO production. The extracts demonstrated maximum effectiveness at the highest concentration tested (1  $\text{g}/\text{mL}$ ). To elucidate and establish a dose-dependent relationship, future research involving concentrations twice as high, such as 5 and 10  $\text{g}/\text{mL}$ , would enhance understanding of the extract's effects and reinforce evidence for dose-dependent outcomes. The study demonstrated that crude extracts from durian flowers and durian peels, the byproduct of local Khlong Saeng durian, possess anti-inflammatory properties by diminishing the secretion of nitric oxide in LPS-stimulated macrophages. However, the anti-inflammatory effectiveness of crude extracts from durian flowers and durian peels was found to be insufficient.



**Figure 4** The anti-inflammatory potential of methanol crude extracts from both durian flowers and peels was investigated concerning the production of nitric oxide (NO) in LPS-stimulated macrophage culture cells (RAW 264.7). Panels (a) to (m) present graphs illustrating the anti-inflammatory activity based on the concentration of the crude extracts. Specifically, panels (a) to (f) depict results from the crude extracts derived from durian flowers, while panels (e) to (m) showcase outcomes from the crude extracts obtained from durian peels.

It may be necessary to compare their efficacy with modern anti-inflammatory drugs, such as dexamethasone, naproxen, etc., which are known for their ability to reduce nitric oxide levels [38]. As per earlier findings, modern anti-inflammatory drugs like NSAIDs, including naproxen (0.1 - 100  $\mu$ M) and flurbiprofen (1 - 100  $\mu$ M), have demonstrated effectiveness in reducing nitric oxide secretion. The capability to inhibit nitric oxide secretion in LPS-stimulated macrophage cells is dependent on the concentration of the drug [39]. Therefore, it is evident that the reduction in nitric oxide secretion observed with methanol crude extracts from durian flowers and durian peels in this study is comparable to the effect of modern anti-inflammatory drugs.

## Conclusions

This investigation revealed pharmaceutical activity in extracts derived from both the flowers and peels of durians, which are produced at a volume of 1,321,648 tons in Thailand. Notably, durian peels constitute a significant 68 % of the total yield. Enhancing the value of Khlong Seng durian peels holds promise for elevating both production levels and income within the community. The findings of this study imply the potential to transform discarded durian peels into valuable resources, providing advantages to individuals in both the agricultural and industrial sectors. This endeavor aligns seamlessly with the ongoing advancements in agriculture and public health.

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