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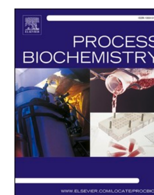

In vitro evaluation of antioxidant, α-glucosidase inhibitor, and antibacterial activities of frangipani flower and the principal component analysis of its constituents

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ABSTRACT

Frangipani flower (*Plumeria rubra*) plants are commonly found and grown in tropical and subtropical regions. The present study explored the antioxidant, α-glucosidase inhibitor, and antibacterial activities of *Plumeria rubra* flower extracts. *P. rubra* flower was extracted by maceration using hexane, ethyl acetate, methanol, and ethanol. The capability of *P. rubra* flower extract to scavenge radicals was evaluated using a β-carotene bleaching assay and DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay. The total phenolic content (TPC) was assessed using the Folin-Ciocalteau method. Its antibacterial activity was assessed using the disc diffusion method against *Salmonella typhimurium* and *Escherichia coli*. The α-glucosidase inhibitor assay was conducted using α-glucosidase from *Saccharomyces cerevisiae*. The results revealed that the ethanolic 50 % extract of *P. rubra* flower showed the highest antioxidant activity of 66.49 % at 1000 μg/mL in the β-carotene bleaching assay, and the ethanolic 70 % extract had the highest antioxidant activity with an IC_{50} of 93.2 μg/mL in DPPH assay. Meanwhile, for α-glucosidase inhibitory activity, the methanolic extract showed the highest activity of 94.05 % at 1000 μg/mL. These results were in accordance with the total phenolic content (TPC) of the extract, in which the highest TPC was obtained from 50 % ethanol extract (1405.33 mg gallic acid equivalent (GAE)/g). *P. rubra* extracts at 5 mg/mL showed good antibacterial activity against *E. coli* and moderate activity against *S. typhimurium* with inhibition zones of 8.14 mm and 6.88 mm, respectively. Moreover, the liquid chromatography-high-resolution mass spectrometry (LC-HRMS) analysis suggested that several phenolic compounds were presented in the *P. rubra* flower extracts. The constituents of *the P. rubra* flower were analyzed using principal component analysis (PCA). This study revealed that *P. rubra* flower extract could be used as a natural antidiabetic, antibacterial, and antioxidant agent in food additives, functional food industries, and pharmaceutical industries.

1. Introduction

More than 50 million people die each year globally [1]. The top leading causes of death in the past decade include non-communicable diseases (e.g., cardiovascular diseases (CVDs), chronic obstructive pulmonary disease, cancers, and neurodegenerative diseases), infectious diseases (especially COVID since 2019), and road injuries [2]. Non-communicable diseases (NCDs) account for almost 70 % of total deaths. One of the significant risk factors for the disease is poor diet. Unhealthy diets are associated with many cardiometabolic deaths [3]. Therefore, maintaining a healthy lifestyle, including consuming balanced and nutritious food, exercising, and having an adequate sleep, is critical to prevent diseases.

Natural products constitute a significant source of nutrition and have been shown to possess therapeutical effects. Plant-based food and supplements are packed with antioxidant phytochemicals that could reduce CVD risks from oxidative stress and inflammation [4,5]. Polyphenols and carotenoids are two major antioxidant phytochemicals found in plants. In addition to having anti-inflammatory effects, polyphenols such as catechins, anthocyanins, and chlorogenic acid have been previously shown to inhibit platelet aggregation [6], which plays a crucial role in the pathophysiology of CVDs. Polyphenols, saponins, and dietary

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fibers from plants can prevent type 2 diabetes and obesity (other CVD risk factors) by reducing glucose absorption from the digestive tract, modulating insulin secretion, and affecting lipid and carbohydrate metabolism in adipocytes [7]. Many phytochemicals exhibit antibacterial, antiviral, antifungal, and anthelmintic effects [8–11]. Compounds such as berberine, reserpine, conessine, chalcones, and eugenol have been reported to possess antimicrobial activity without inducing resistance from the target microorganism and show synergistic effects with existing antibacterial agents [12]. Hence, exploring the biological activities of medicinal plants and extending previous investigations to better understand their mechanisms of action could pave the way to take the full benefits of the plant products.

Frangipani flower or Plumeria plants are commonly found and grown in tropical and subtropical regions. This flower grows in gardens or cemeteries as an ornamental plant in Indonesia. This plant is adored because of its beauty and pleasant fragrance. Several studies have reported the practical use of Plumeria species to treat diabetes mellitus, ulcers, leprosy, and toothache [13–15]. Essential oil from Plumeria species has been used in aromatherapy, cosmetics ingredients, and massage oil [16].

The extract of Plumeria species was previously reported to contain several constituents, such as flavonoids, alkaloids, triterpenes, iridoids, glycosides, phenolics, alkaloids, amino acids, and fatty acid esters [17, 18]. Previous studies showed that Plumeria extract was proven to have antioxidant, antimicrobial, anti-inflammatory, antipyretic, antinociceptive, antidiabetic, antimalarial and anticancer activities [17, 19–21]. However, compared to other plant parts, there needs to be more in-depth information about the study of *Plumeria rubra* flower extract and its constituents concurrent with its biological activity.

The primary purpose of the present study was to evaluate some biological activities (i.e., antioxidant, antibacterial, and α-glucosidase inhibitory activities) of *P. rubra* flower and its chemical composition using Fourier transform infrared spectroscopy (FTIR) and liquid chromatography-high-resolution mass spectrometry (LC-HRMS). To our knowledge, this study is the most comprehensive study about *P. rubra* flower extract and its constituents concurrent with its biological activity.

2. Materials and methods

2.1. Plant materials, chemicals, bacterial strains, and general instrumentations

Flowers of *P. rubra* (pink variant) were collected from Gunungkidul Regency, Yogyakarta Province, Indonesia, in December 2020. Voucher specimens have been deposited in Research Center for Food Technology and Processing, National Research and Innovation Agency, Indonesia. Folin-Ciocalteu reagent, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), α-glucosidase [(EC 3.2.1.20)] type I from *Saccharomyces cerevisiae*, gallic acid, *p*-nitrophenyl α-D-glucopyranoside (p-NPG), β-carotene, ascorbic acid and (-)-epicatechin were purchased from Sigma-Aldrich (Tokyo, Japan). Amoxycillin was purchased from Oxoid (Hampshire, UK). *Escherichia coli* (strain FNCC-0091) and *Salmonella typhimurium* (strain FNCC-0050) were obtained from Gadjah Mada University (Yogyakarta, Indonesia). This research purchased Solvents from Merck (Darmstadt, Germany). Gas chromatography–mass spectrometry (GC-MS) analysis was performed using GCMS-QP 2010 (Shimadzu, Japan) with an Rtx-5 column (30 m × 0.25 mm × 0.25 μm). The column oven temperature program started at 50 ℃, held for 5 min, and continued to 240 ℃ and held for 7 min. The injection temperature was at 300 ℃. The compound similarity was performed using the NIST library. FTIR analysis was performed using Vertex 80 (Bruker, Germany) in the wavenumber of 4000–600 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans for each sample. Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) was performed using Thermo Scientific™ Vanquish™ UHPLC Binary Pump and Orbitrap high-resolution mass spectrometry (Thermo Scientific, USA) with an analytical column Phenyl-Hexyl 100 mm × 2.1 mm ID × 2.6 μm. The samples were dissolved in methanol, and the solvent elution was acetonitrile-water containing 0.1 % formic acid, with an injection volume was 3 μL at the flow rate of 0.3 mL/min. The ion source was 3.30 kV, either positive or negative mode.

2.2. Preparation of extracts

P. rubra flower was dried, powdered, and extracted by maceration using several solvents, i.e., hexane (Hx), ethyl acetate (EtOAc), methanol (MeOH), and ethanol (EtOH). The ethanol varied at 100 %, 70 %, and 50 % concentrations, while other solvents were used at 100 %. The solid-to-liquid ratio was 1:10 w/v, and the maceration was performed at room temperature for 24 h. The final mixture was filtered and evaporated to extract the *P. rubra* flower.

2.3. Antioxidant activity

The in-vitro antioxidant capacity of the extracts was assessed by (a) β-carotene-bleaching assay, which determines the ability of antioxidants to inhibit lipid peroxidation, and (b) DPPH assay, which evaluates the radical scavenging activity.

2.3.1. β-carotene-bleaching assay

The antioxidant activity of *P. rubra* flower extracts in the β-carotene-linoleate model system was determined according to a previous method [22] with a slight adjustment. The assay was conducted by reacting the β-carotene reagent (consisting of 0.2 mg of β-carotene, 1 mL of linoleic acid, 20 mg of Tween 40, and 5 mL of distilled water) with the extract (1000 μg/mL in methanol). The solution was incubated at 50 ℃, and the absorbance of the solution was measured at 470 nm at 20-minute intervals using an ELISA reader. The reaction was finished after 120 min. The positive control used were gallic acid and ascorbic acid at a concentration of 1000 μg/mL. The antioxidant activity of *P. rubra* flower extracts was calculated using the following equation:

$$\text{Antioxidant activity }(\%) = 100[1 - \frac{(\text{As0} - \text{As120})}{(\text{Ab0} - \text{Ab120})}] \quad (1)$$

where A_{s0} and A_{s120} are the absorbances of β-carotene in the presence of extract at 0 min and 120 min, respectively, while A_{b0} and A_{b120} is the absorbance of β-carotene without the extract at 0 min and 120 min, respectively.

2.3.2. DPPH assay

The DPPH assay was conducted using a previous method [23] with minor adjustments. Extracts of *P. rubra* flower (at a series concentration from 100 μg/mL to 800 μg/mL) were dissolved in methanol and reacted with one mM DPPH. After 30 min of reaction in the dark at room temperature, the absorbance of the final solution was measured at 517 nm using an ELISA reader. The positive control was ascorbic acid at a series concentration of 10–100 μg/mL. The radical scavenging activity of *P. rubra* flower extracts was then calculated using the following equation:

$$\text{DPPH scavenging activity}(\%) = (\frac{A_0 - A_1}{A_0} x100) \quad (2)$$

where A_0 and A_1 are absorbances of the DPPH solution in the absence and presence of the extract, respectively.

2.4. α-Glucosidase inhibitory activity

The inhibitory activity of *P. rubra* flower extract against α-glucosidase was determined using a previously reported method [24]. The extract (1000 μg/mL in 10 % of dimethyl sulfoxide) was reacted with p-NPG (3 mM) in a phosphate buffer solution (pH 7). After pre-incubated at 37 °C for 5 min, the solution was reacted with

α-glucosidase enzyme (0.065 U/mL) at the same temperature for 15 min. Sodium carbonate (0.2 M) was then added to the final solution, and the absorbance of the solution was measured at 400 nm using an ELISA reader. The positive controls were epicatechin at 625 μg/mL and acarbose at 312.5 μg/mL.

2.5. Total phenolic content (TPC) analysis

TPC analysis of the *P. rubra* extracts was performed using the Folin-Ciocalteu method [25]. The extract (1000 μg/mL in methanol) was reacted with Folin-Ciocalteu reagent and mixed with Na_2CO_3 (20 %) and water. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using an ELISA reader. Gallic acid (GA) was used as a standard, and the TPC was expressed as GA equivalent (mg GA/g of extract).

2.6. Antibacterial activity assay

The *P. rubra* flower extracts were evaluated for their antibacterial activity against *E. coli* and *S. typhimurium* using the agar disc diffusion method [26]. Filter paper disks (6 mm in diameter) were each impregnated with extracts (dissolved in 10 % dimethyl sulfoxide) and control. Amoxycillin was used as the positive control. The disks were then put onto the agar medium, which had been inoculated with bacteria at 1×10^8 CFU/mL. After incubation for 24 h at 37 °C, the inhibition zone formed was measured using a caliper. The positive control used was Amoxycillin 10 μg.

2.7. Statistical analysis

Statistical evaluation of the biological activity of the extracts was conducted by one-way analysis of variance (ANOVA) followed by Duncan's test [27]. Differences at $p < 0.05$ were considered statistically significant.

2.8. PCA analysis

Principal component analysis (PCA) was performed using SIMCA 14.0 software (Umetrics, Umea, Sweden). The data of metabolites (121 metabolites) obtained from LC-HRMS measurement and their area were used as the variables to build the PCA model. The result was observed using the PCA score plot obtained from the principal component (PC) 1 and PC2. In addition, the quality of the PCA model was evaluated using the R^2 value and Q^2 value. Further analysis using partial least square-discriminant analysis (PLS-DA) was carried out using the same variables used in the PCA model. The PLS-DA model was evaluated using the score plot, R^2X value, R^2Y value, and Q^2 value. Analysis of the variable importance for projections (VIP) was performed for the identification of discriminating metabolites.

3. Results and discussion

3.1. Antioxidant activity

A correlation between the antioxidant activity of plants with their hypoglycemic activities has been reported [28]. Medicinal plants possessing antioxidant activities are considered to be used for diabetic treatment since oxidative stress has a role in the progression of diabetes disease. The antioxidant activities of *P. rubra* flower extracts were performed using β-carotene-bleaching and DPPH assay. The β-carotene bleaching assay was conducted to evaluate the extract's capability in the system, which consists of water, lipid, and emulsifier. Hydroperoxyl radicals produced by the oxidation of linoleic acid in the system will attack the β-carotene, and the antioxidant present in the system will inhibit the rapid discoloration of β-carotene [29]. Thus, this study's bleaching rate of β-carotene will depend on the antioxidant activity of

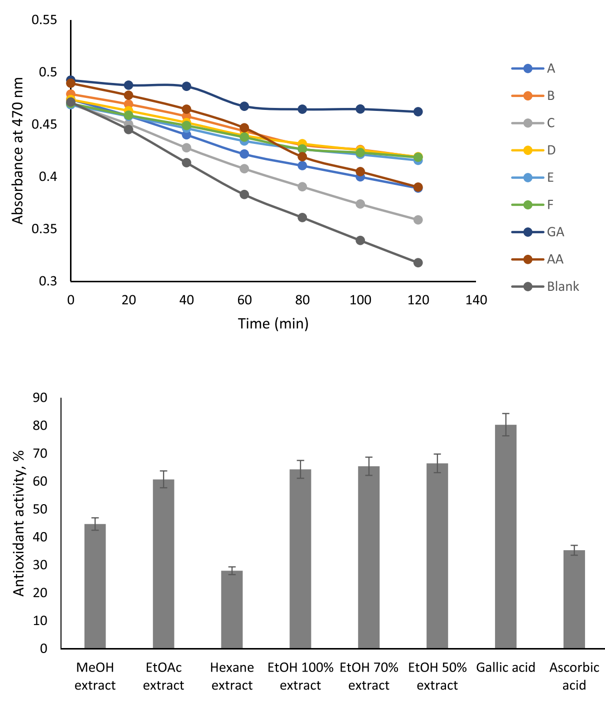

Fig. 1. The antioxidant activity of *P. rubra* flower extract using β-carotene bleaching assay: (a) The change of absorbance of *P. rubra* flower extract, which is A: MeOH extract, B: EtOAc extract, C: Hx extract, D: EtOH 100 % extract, E: EtOH 70 % extract, F: EtOH 50 % extract, GA: gallic acid, and AA: ascorbic acid. All at a concentration of 1000 μg/mL. (b) Antioxidant activities of *P. rubra* flower extracts at 1000 μg/mL.

P. rubra flower extract. The results of the *P. rubra* flower extracts to prevent the oxidation of β-carotene are presented in Fig. 1. a). and Fig. 1. b).

Fig. 1. (a) showed that all the tested *P. rubra* flower extracts exhibited antioxidant activity in inhibiting β-carotene bleaching, compared with the blank solution (negative control). After 120 min of reaction, ethanol 50 % extract retained the highest antioxidant activity of 66.49 %, followed by an extract of ethanol 70 %, ethanol 100 %, ethyl acetate extract, methanol extract, and hexane extract as 65.43 %, 64.30 %, 60.72 %, 44.70 %, and 27.95 %, respectively (Fig. 1.(b)) The ethanol, methanol, and ethyl acetate extracts showed remarkable antioxidant activity in a lipid emulsion system by outperforming ascorbic acid (35.21 %), which is known as a potent antioxidant. A previous study showed that lipophilic antioxidants were more efficient at protecting against oxidation in a lipid emulsion system than hydrophilic antioxidants [30]. More lipophilic molecules tend to be oriented in the oil-water interface to protect the oil efficiently.

In contrast, water-soluble molecules preferentially stay dissolved in the water phase and, thus, do not play a significant role in inhibiting lipid peroxidation [31]. While gallic acid is also water-soluble, it is not as hydrophilic as ascorbic acid and, therefore, could be more active at the oil-water interface, which may explain its highest antioxidant activity (80.33 %) among all tested samples. Although the hexane extract theoretically would contain lipophilic antioxidants, its activity during the β-carotene bleaching assay was the lowest of all. This discrepancy could arise from the limited number of compounds extracted using hexane, compared with ethanol which can dissolve compounds with a wide range of polarity. Compounds with antioxidant activity in the hexane extract include 7,8-dihydroxy-4-methyl coumarin (Table 3). This compound was also present in the ethanol and methanol extracts. Other antioxidants such as rutin, kaempferol, and quercetin derivatives were only present in ethanol, methanol, and ethyl acetate extract (Table 3), which could contribute to the higher activity of antioxidants.

Table 1
Antioxidant activity of *P. rubra* flower extract using DPPH assay and its α-glucosidase inhibitory activity.

Samples	IC_{50} of DPPH test, μg/mL	% Scavenging activity using the DPPH test at 400 μg/mL	α-Glucosidase inhibition (%) at 1000 μg/mL
Hx extract	> 500[g]	0.09[a]	58.21[b]
EtOAc extract	286.68[f]	62.33[b]	ND
MeOH extract	255.9[e]	70.52[c]	94.05[e]
EtOH 100 % extract	165.4[d]	78.7[e]	82.81[c]
EtOH 70 % extract	93.2[b]	79.6[e]	83.36[c]
EtOH 50 % extract	107.6[c]	76.5[d]	89.24[d]
Ascorbic acid	8.2[a]	-	-
Epicatechin at 625 μg/mL	-	-	48.50[a]
Acarbose at 312.5 μg/mL	-	-	92.49[e]

Different letters in the same column indicate significant differences ($p < 0.05$).
ND: not detected

The antioxidant activity of *P. rubra* flower extracts using the DPPH assay is presented in Table 1. The IC_{50} of *P. rubra* flower extract from the DPPH assay ranged between 93.2 to > 500 μg/mL. The values of antioxidant activity among *P. rubra* flower extracts were significantly different at $p < 0.05$. Hexane extract (Hx) exhibited the lowest antioxidant activity with an $IC_{50\ of}$ more than 500 μg/mL, which could be due to the poor solubility of antioxidants from the Hx extract in methanol (solvent used in the DPPH assay). Among the tested extracts, the EtOH 70 % extract showed the highest antioxidant activity (IC_{50} = 93.2 μg/mL), followed by EtOH 50 % and EtOH 100% (IC_{50} of 107.6 μg/mL and 165.4 μg/mL, respectively). Ascorbic acid showed an order of magnitude higher activity than the ethanolic extracts with an IC_{50} of 8.2 μg/mL. Although their activity was comparatively lower than ascorbic acid, the IC_{50} of the ethanolic extract was lower than 100 μg/mL; thus, ethanolic extracts of *P. rubra* flower may serve as a good source of natural antioxidants.

Overall, the extract of *P. rubra* flower, especially the ethanolic extract, showed significant antioxidant activities compared to others. These results are probably because of the phenolic compounds present in the extracts, such as rutin, kaempferol, coumarin derivatives, quercetin derivatives, and caffeic acid derivatives (Table 3). Several mechanisms on how phenolic compounds react with the DPPH radical have been proposed, such as that the DPPH radical triggers the oxidation attacked the A ring of phenolic compound, forms a hydrophilic dimer, and is further oxidized to oligomers [32]. DPPH reagent was commonly used in antioxidant assay since it is rapid, sensitive, and easy [33]. Comparing antioxidant activities performed by β-carotene bleaching assay and DPPH assay, the extracts of *P. rubra* flower showed similar results. Thus, these methods consistently evaluated the antioxidant activities of *P. rubra* flower extracts.

3.2. α-Glucosidase inhibitory activity

An alpha-glucosidase enzyme in the intestine is responsible for carbohydrate digestion into glucose molecules [34]. However, for type 2 diabetes mellitus patients, this enzyme inhibitor is needed to delay the digestion of carbohydrates [22]. Synthetic agents such as miglitol, acarbose, and voglibose are commonly used to treat diabetes; however, they cause side effects such as abdomen distention, diarrhea, and intestinal pain [35]. Thus, α-glucosidase inhibitory agents from nature are urgently needed.

The α-glucosidase inhibitory activities of the *P. rubra* flower extracts are shown in Table 1. Methanol extract of *P. rubra* flower had the highest α-glucosidase inhibitory activity of 94.05 % at 1000 μg/mL, followed by EtOH 50 % extract, EtOH 70 % extract, EtOH 100 % extract, and hexane extract with α-glucosidase inhibitory activity of 89.24 %, 83.36 %, 82.81 %, and 58.21 %, respectively. Meanwhile, the ethyl acetate extract of *P. rubra* flower did not show any α-glucosidase inhibitory activities at 1000 μg/mL. This study used epicatechin and acarbose as positive control and showed the α-glucosidase inhibitory activity of 48.50 % and 92.49 %, respectively, at a concentration of 625 μg/mL and 312.5 μg/mL. According to a previous study, the total phenolic compound correlates positively with the α-glucosidase inhibitory and antioxidant activity [36]. Another study also revealed the potential antidiabetic activity of *P. alba* on diabetic mice. It was shown that the *P. alba* extract from ethyl acetate fraction and supernatant fraction at 250 mg/kg reduce hyperglycemia in mice. Moreover, the treatment of that dose for 14 days, significantly reduce the lipid parameters including triglycerides, HDL and total cholesterol [52]. The extract of *P. rubra* flower had a good α-glucosidase inhibitory activity. It indicated that these extracts could be used as a natural source in antidiabetic treatment since α-glucosidase inhibitors can delay glucose absorption in the intestine and prevent increasing blood glucose levels.

Table 2
Antibacterial activity of *P. rubra* flower extract against *S. typhimurium* and *E. coli* using the disc diffusion method.

Samples	C (mg/mL)	Inhibition zone (mm) *S. Typhimurium*	Inhibition zone (mm) *E. coli*
Hx extract	1.25	6.34 ± 0.13[b]	6.51 ± 0.19[b]
	2.5	6.15 ± 0.07[a]	6.85 ± 0.48[c]
	5	6.40 ± 0.19[b]	6.48 ± 0.33[b]
EtOAc extract	1.25	6.46 ± 0.42[b]	6.32 ± 0.11[b]
	2.5	6.18 ± 0.09[a]	7.11 ± 0.38[c]
	5	6.40 ± 0.26[b]	8.14 ± 0.33[d]
MeOH extract	1.25	6.24 ± 0.06[a]	6.30 ± 0.26[b]
	2.5	6.17 ± 0.11[a]	7.29 ± 0.11[c]
	5	6.38 ± 0.11[ab]	7.60 ± 0.97[c]
EtOH 100 % extract	1.25	6.86 ± 0.01[c]	6.81 ± 0.08[c]
	2.5	6.13 ± 0.01[a]	6.09 ± 0.02[a]
	5	6.88 ± 0.41[c]	6.41 ± 0.25[b]
EtOH 70 % extract	1.25	6.82 ± 0.24[b]	6.82 ± 0.03[c]
	2.5	6.07 ± 0.05[a]	6.07 ± 0.02[a]
	5	6.39 ± 0.08[b]	6.31 ± 0.01[b]
EtOH 50 % extract	1.25	6.87 ± 0.35[c]	6.44 ± 0.08[b]
	2.5	6.13 ± 0.01[a]	6.06 ± 0.04[a]
	5	6.81 ± 0.28[c]	6.47 ± 0.03[b]
DMSO	10 %	6.00 ± 0.00[a]	6.00 ± 0.00[a]
Amoxycillin	10 μg	30.67 ± 0.22[d]	32.35 ± 0.15[e]

Different letters in the same column indicate significant differences ($p < 0.05$).

3.3. Antibacterial activity

This study used the disc diffusion method to investigate *P. rubra* flower extract's antibacterial activity. The antibacterial activity of *P. rubra flower* extract was assessed against *S. typhimurium* and *E. coli*; the results are presented in Table 2. Positive control in this study was using amoxicillin. Generally, *P. rubra* flower extract showed low and moderate antibacterial activity against *S. typhimurium* and *E. coli*. The antibacterial activity of *P. rubra* flower extract against *S. typhimurium* ranged from 6.07 to 6.88 mm. The 10 % dimethyl sulfoxide as a negative control showed no inhibition zone in this study. The highest antibacterial activity against *S. typhimurium* was ethanol 100 % extract of 6.88 mm at 5 mg/mL and significantly different with negative control ($p < 0.05$). The antibacterial activity of *P. rubra* flower extract against *E. coli* ranged from 6.06 mm to 8.14 mm. The highest antibacterial activity against *E. coli* was an ethyl acetate extract of 8.14 mm at 5 mg/mL and significantly different with negative control ($p < 0.05$).

On the other hand, Amoxycillin as the positive control showed high inhibition against *S. typhimurium* and *E. coli* as of 30.67 and 32.35 mm, respectively. A previous study reported that leaves of ethanolic extract and chloroform extract of *P. rubra* have good antibacterial activity

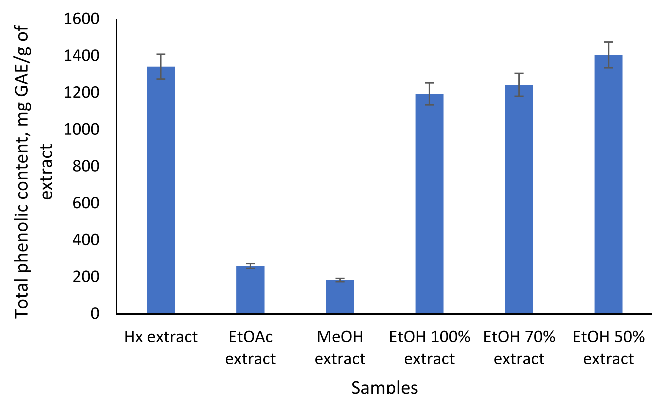

Fig. 2. Total phenolic content of *P. rubra* flower extracts at 1000 μg/mL using Folin-Ciocalteau reagent.

against *S. epidermidis* and *E. coli* at a concentration of 1500 μg/mL [37]. Another report also revealed the antibacterial activities of *Plumeria alba* against foodborne pathogens such as *B. cereus, S. aureus, E. coli, S. typhimurium, S. flexneri, B. subtilis,* and *S. pneumoniae* [38,39]. Several kinds of literature report on the mechanism of active compounds from plants against bacterial cells, including corrupting the cell wall [40], attacking the cytoplasmic membrane [41], and increasing the membrane permeability followed by leakage of the cell [42].

3.4. Total phenolic content

Plants, including flavonoids, anthocyanins, and phenolic acids, commonly produce phenolic constituents. Gallic acid, coumaric acid, quercetin, caffeic acid, and resveratrol are phenolic compounds usually obtained from plants [43]. The phenolic content of the *P. rubra* flower extract was performed using the Folin-Ciocalteau reagent. The result was presented in Fig. 2, and the total phenolic content of *P. rubra* flower extracts ranged from 182.99 to 1405.33 mg GAE (gallic acid equivalent)/g of extract. The highest total phenolic content was ethanol 50 % extract of 1405. 33 mg GAE/g followed by hexane extract, ethanol 70 % extract, ethanol 100 % extract, ethyl acetate extract, and methanol extract with 1341.15, 1242.67, 1193.33, 259.31, and 182.99 mg GAE/g, respectively. In this study, the hexane extract showed a relatively high value of TPC, and it probably came from the volatile compound from the hexane extract of the *P. rubra* flower. The GC-MS analysis of *P. rubra* flower extracts (hexane extract) showed that the *P. rubra* flower contained several volatile compounds. The five main constituents of the volatile compound of P. rubra flower extract were (-)-caryophyllene oxide (17.04 %), n-hexacosanol (9.27 %), estran-3-one, 17-(acetyloxy) 2-methyl- (8.31 %), methyl linolelaidate (6.18 %) and stearyl vinyl ether (5.21 %). The other volatile compound of *P. rubra* flower extracts were tetradecane (4.62 %), methyl 9, 12, 15-octadecatrienoate (3.07 %), pentadecane (4.84 %), 2-methyltricosane (3.24 %), n-heptanal (2.06%), 2-methyl-undecane (1.99 %), methyl 14-methyl-pentadecanoate (2.75 %), squalene (2.23 %), allyl hexanoate (2.08 %), methyl tridecyl ketone (1.92 %), and cyclohexanol (1.01 %). A previous study showed that plant extract's high antioxidant activity could result from their phenolic constituents [22,44]. Phenolic constituents also contributed to reducing the risk of metabolic disorders and complications caused by diabetes mellitus [43].

3.5. FTIR (Fourier transform infrared) spectra

The FTIR spectra of *P. rubra* flower extracts are shown in Fig. 3. It showed the profile of functional groups in the extract's constituents. Several of the extracts showed similar patterns, while some also showed different spectra patterns. FTIR was used in this study because of its quick analysis and ease of preparing the analysis. Another study showed that FTIR was a helpful tool in the qualitative analysis of the sample [45]. Thus, this analysis would indicate the critical constituents in the *P. rubra* flower extracts that have suitable biological activities.

In this study, the extract of methanol, ethanol 70 %, and ethanol 50 % demonstrated very similar FTIR spectra, whereas the extract of hexane showed quite different FTIR spectra among others. Peaks around 3197–3351 cm^{-1} indicated the hydroxyl groups, possibly coming from phenol or alcohol. This region was still observed in the hexane extract of the *P. rubra* flower, indicating the presence of phenolic compounds in the hexane extract. The stretching vibration from aliphatic CH_3, CH_2, and CH was shown at peaks of 2924 cm^{-1} and 2856 cm^{-1}. The peak at 1738 cm^{-1} was associated with the carbonyl (C=O) group's stretching vibration at 1061, 1026, 998, and 973 cm^{-1} corresponded to the C-O group. The vibrations at 1641 cm^{-1} and 1603 cm^{-1} could be from the stretching vibration of C=C. The bending vibration of aliphatic CH_3, CH_2, and CH was demonstrated from the vibration at 1453, 1377, 1363,

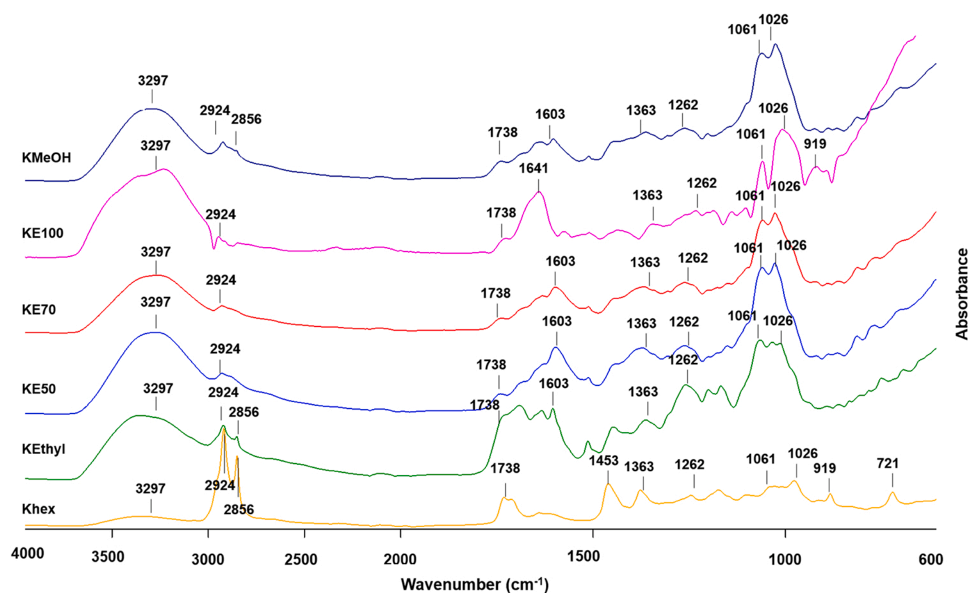

Fig. 3. FTIR spectra of *P. rubra* flower extracts (purple = methanol, pink = ethanol 100 %, red = ethanol 70 %, blue = ethanol 50 %, green = ethyl acetate, and orange = hexane).

Table 3
Metabolites compounds of methanol extract, ethanol extract, ethyl acetate extract and hexane extract of *P. rubra* obtained from LC-HRMS analysis.

No.	Compounds name	Formula	Calculated MW	RT (min)	Area	Ionization
	Methanol extract					
1	α,α-Trehalose	$C_{12}H_{22}O_{11}$	342.11578	0.784	1.17E + 08	ESI-
2	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06282	0.798	7.35E + 08	ESI-
3	4-Coumaric acid	$C_9H_8O_3$	164.04683	4.33	35,078,111	ESI+
4	2-(1-benzothiophene-3-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one	$C_{19}H_{14}OS$	290.07591	4.694	16,678,306	ESI+
5	Benzyl 6-O-beta-D-glucopyranosyl-beta-D-glucopyranoside	$C_{19}H_{28}O_{11}$	432.16268	5.524	91,236,332	ESI-
6	2,3-Dihydro-1-benzofuran-2-carboxylic acid	$C_9H_8O_3$	164.04683	5.742	49,419,531	ESI-
7	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09435	6.618	3.87E + 08	ESI+
8	7,8-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192.04134	6.785	7.18E + 08	ESI+
9	5,7-dihydroxy-2-(2,3,4-trihydroxyphenyl)-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04186	7.924	18,861,523	ESI+
10	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chrome-3-yl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside	$C_{26}H_{28}O_{16}$	596.13621	7.924	26,692,827	ESI+
11	Rutin	$C_{27}H_{30}O_{16}$	610.15212	8.586	6.12E + 08	ESI+
12	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04128	8.593	2.19E + 08	ESI+
13	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	464.09466	8.638	3.77E + 08	ESI-
14	Citral	$C_{10}H_{16}O$	152.11963	9.072	12,833,884	ESI+
15	Kaempferol	$C_{15}H_{10}O_6$	286.04717	9.463	18,013,870	ESI+
16	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.12642	9.737	27,389,235	ESI-
17	Corchorifatty acid F	$C_{18}H_{32}O_5$	328.22474	13.24	11,236,083	ESI-
18	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_5$	330.24034	13.696	43,759,645	ESI-
19	(+/-)9-HpODE	$C_{18}H_{32}O_4$	312.2299	15.578	13,278,136	ESI-
20	Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27635	15.671	17,943,326	ESI+
21	Asiatic acid	$C_{30}H_{48}O_5$	488.34978	15.868	7,025,073	ESI-
22	Octadecanamine	$C_{18}H_{39}N$	269.30773	16.442	38,823,224	ESI+
	Ethanol 100 % extract					
1	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06277	0.806	5.14E + 08	ESI-
2	2-Mercaptoethanol	C_2H_6OS	78.01385	0.898	19,413,862	ESI+
3	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09411	6.624	3.06E + 08	ESI+
4	7,8-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192.0414	6.791	4.26E + 08	ESI+
5	2,3-Dihydro-1-benzofuran-2-carboxylic acid	$C_9H_8O_3$	164.04678	7.317	10,294,351	ESI-
6	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	464.0952	8.463	12,186,360	ESI-
7	Rutin	$C_{27}H_{30}O_{16}$	610.1519	8.584	4.29E + 08	ESI+
8	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04146	8.598	1.18E + 08	ESI+
9	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.12623	10.749	12,481,155	ESI-
10	N,N-Diethyldodecanamide	$C_{16}H_{33}NO$	255.25545	13.236	14,755,829	ESI+
11	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_5$	330.24031	13.705	19,128,769	ESI-
12	(+/-)9-HpODE	$C_{18}H_{32}O_4$	312.2299	15.583	13,977,661	ESI-
13	Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27617	15.672	13,912,047	ESI+
14	Asiatic acid	$C_{30}H_{48}O_5$	488.34965	15.874	13,089,463	ESI-
15	Octadecanamine	$C_{18}H_{39}N$	269.3077	16.438	38,225,038	ESI+
16	9-Oxo-10(E),12(*E*)-octadecadienoic acid	$C_{18}H_{30}O_3$	294.21844	16.567	37,861,209	ESI+
17	Choline	$C_5H_{13}NO$	103.09947	17.489	9,204,869	ESI+
18	Stearamide	$C_{18}H_{37}NO$	283.28685	18.329	2.88E + 08	ESI+
19	cis-12-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.27062	19.408	6243640	ESI+
	Ethanol 70 % extract					
1	Gluconic acid	$C_6H_{12}O_7$	196.05745	0.806	1.9E + 08	ESI-
2	2-Mercaptoethanol	C_2H_6OS	78.01385	0.93	18,956,293	ESI+
3	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06271	5.319	2.05E + 08	ESI-
4	Benzyl 6-O-beta-D-glucopyranosyl-beta-D-glucopyranoside	$C_{19}H_{28}O_{11}$	432.16269	5.564	35,358,681	ESI-
5	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09404	6.66	7.23E + 08	ESI+
6	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chrome-3-yl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside	$C_{26}H_{28}O_{16}$	596.13722	7.961	19,388,672	ESI-
7	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04137	7.962	13,508,821	ESI+
8	Rutin	$C_{27}H_{30}O_{16}$	610.1526	8.617	2.72E + 08	ESI+
9	5,7-dihydroxy-2-(2,3,4-trihydroxyphenyl)-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04137	8.645	1.6E + 08	ESI+
10	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	464.09453	8.676	2.9E + 08	ESI-
11	Citral	$C_{10}H_{16}O$	152.11958	9.115	6,826,024	ESI+
12	Kaempferol	$C_{15}H_{10}O_6$	286.04668	9.495	11,331,790	ESI+
13	Trifolin	$C_{21}H_{20}O_{11}$	448.10014	9.545	7,043,409	ESI-
14	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.1263	9.765	44,166,981	ESI-
15	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	317.29177	13.275	1.28E + 08	ESI+
16	N,N-Diethyldodecanamide	$C_{16}H_{33}NO$	255.25554	13.279	12,867,466	ESI+
17	Asiatic acid	$C_{30}H_{48}O_5$	488.34974	15.503	15,500,136	ESI+
18	Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27634	15.71	20,556,699	ESI+
19	Octadecanamine	$C_{18}H_{39}N$	269.3077	16.48	45,687,683	ESI+
20	α-Eleostearic acid	$C_{18}H_{30}O_2$	278.22377	16.579	34,773,823	ESI+
21	9(Z),11(E),13(*E*)-Octadecatrienoic Acid methyl ester	$C_{19}H_{32}O_2$	292.23934	17.439	54,559,884	ESI+
22	Stearamide	$C_{18}H_{37}NO$	283.28685	18.363	2.06E + 08	ESI+
	Ethanol 50 % extract					
1	D-(-)-Fructose	$C_6H_{12}O_6$	180.06277	0.806	68,904,529	ESI-
2	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06277	0.837	1.5E + 09	ESI-
3	4-Coumaric acid	$C_9H_8O_3$	164.04666	4.369	14,989,353	ESI+
4	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09393	5.324	1.26E + 09	ESI+

(*continued on next page*)

Table 3 (*continued*)

No.	Compounds name	Formula	Calculated MW	RT (min)	Area	Ionization
5	7,8-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192.04137	6.832	1.21E + 09	ESI+
6	2,3-Dihydro-1-benzofuran-2-carboxylic acid	$C_9H_8O_3$	164.0468	7.363	12,555,495	ESI-
7	2-(acetylamino)-3-(1H-indol-3-yl)propanoic acid	$C_{13}H_{14}N_2O_3$	246.10017	7.839	5,477,078	ESI-
8	Rutin	$C_{27}H_{30}O_{16}$	610.1526	8.446	26,899,113	ESI+
9	5,7-dihydroxy-2-(2,3,4-trihydroxyphenyl)-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04134	8.655	1.58E + 08	ESI+
10	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	464.09447	8.685	3.05E+ 08	ESI-
11	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04134	9.253	6,303,548	ESI+
12	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.12617	9.778	42,827,907	ESI-
13	N,N-Diethyldodecanamide	$C_{16}H_{33}NO$	255.25551	13.286	15,648,270	ESI+
14	Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27634	15.715	21,782,358	ESI+
15	Octadecanamine	$C_{18}H_{39}N$	269.30773	16.495	37,859,985	ESI+
	Ethyl acetate extract					
1	2-Mercaptoethanol	C_2H_6OS	78.01386	0.992	20,362,447	ESI+
2	2-(1-benzothiophene-3-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one	$C_{19}H_{14}OS$	290.07585	5.292	26,699,839	ESI+
3	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09422	5.4	1.7E + 08	ESI+
4	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06285	5.402	33,008,057	ESI-
5	Caffeic acid	$C_9H_8O_4$	180.04158	5.566	39,712,300	ESI-
6	8-Hydroxyquinoline	C_9H_7NO	145.05237	7.17	14,223,984	ESI+
7	2,3-Dihydro-1-benzofuran-2-carboxylic acid	$C_9H_8O_3$	164.04676	7.814	1.67E + 08	ESI-
8	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chrome-3-yl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside	$C_{26}H_{28}O_{16}$	596.1371	8.017	5,593,175	ESI-
9	Rutin	$C_{27}H_{30}O_{16}$	610.15217	8.491	10,032,872	ESI+
10	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	464.09498	8.553	17,729,937	ESI-
11	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chrome-4-one	$C_{15}H_{10}O_7$	302.04146	8.722	1.09E + 08	ESI+
12	Kaempferol	$C_{15}H_{10}O_6$	286.04708	9.582	17,613,931	ESI+
13	Trifolin	$C_{21}H_{20}O_{11}$	448.09972	9.6	27,849,041	ESI-
14	7-Hydroxycoumarin	$C_9H_6O_3$	162.03121	10.596	20,023,162	ESI+
15	Quercetin	$C_{15}H_{10}O_7$	302.04225	10.596	53,560,015	ESI-
16	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.12605	10.832	15,830,865	ESI-
17	Genistein	$C_{15}H_{10}O_5$	270.05253	11.942	10,459,344	ESI-
18	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	317.29173	13.329	1.59E + 08	ESI+
19	Glycitein	$C_{16}H_{12}O_5$	284.06809	13.769	56,098,768	ESI-
20	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_5$	330.24028	13.789	1.22E + 08	ESI-
21	5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chrome-4-one	$C_{17}H_{14}O_5$	298.08335	15.156	21,862,678	ESI+
22	(+/-)9-HpODE	$C_{18}H_{32}O_4$	312.22978	15.71	22,690,234	ESI-
23	Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27631	15.764	16,735,201	ESI+
24	Octadecanamine	$C_{18}H_{39}N$	269.30773	16.536	36,994,714	ESI+
25	Linolenic acid ethyl ester	$C_{20}H_{34}O_2$	306.25453	17.825	30,756,434	ESI+
	Hexane extract					
1	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06299	0.801	7,328,251	ESI-
2	7,8-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192.04158	6.792	19,979,510	ESI+
3	Dibenzylamine	$C_{14}H_{15}N$	197.11966	7.531	91,375,348	ESI+
4	N,N-Diethyldodecanamide	$C_{16}H_{33}NO$	255.25545	13.234	16,576,405	ESI+
5	5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chrome-4-one	$C_{17}H_{14}O_5$	298.08336	15.071	34,824,045	ESI+
6	Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27634	15.686	13,629,163	ESI+
7	Octadecanamine	$C_{18}H_{39}N$	269.30776	16.447	32,444,013	ESI+
8	Avobenzone	$C_{20}H_{22}O_3$	310.15599	17.319	12,859,789	ESI+
9	Lupeol	$C_{30}H_{50}O$	426.38475	21.363	86,664,498	ESI+

1262, 1167, 919, 841 cm^{-1}, and 721 cm^{-1}. The results of the FTIR study supported the presence of phenolic compounds in methanol, ethanol (100 %, 70 %, and 50 %), ethyl acetate, and hexane extracts of *P. rubra* flower that support its antioxidant, antidiabetic, and antibacterial activities.

3.6. Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) analysis

Constituents of the methanol, ethanol, ethyl acetate, and hexane extracts of *P. rubra* flower as analyzed by LC-HRMS are presented in Table 3. The compounds were identified using database compounds, including ChemSpider and Mz cloud, with similarity above 80 %. It was revealed that the *P. rubra* flower extracts contain several phenolic compounds such as coumaric acid and its derivatives, rutin, kaempferol, quercetin and its derivatives, chlorogenic acid, and caffeic acid. Besides phenolic compounds, the *P. rubra* flower extracts also contain carboxylic acids, terpenes, terpenoids, amines, alcohols, amides, sugars, glycosides, and esters.

It is interesting that 4-coumaric acid (4-CA) was only detected in the methanol and 50% ethanol extracts, given a higher solubility of the compound in ethanol than that in either water or methanol [46]. Simple sugars such as trehalose and fructose were only present in the methanol and 50 % ethanol extracts due to their high solubility in polar solvents via the formation of intermolecular hydrogen bonds, while glycosides such as quercetin-3β-D-glucoside were detected in all extracts except in the hexane extract. The aglycon form (i.e., quercetin) was only present in the ethyl acetate extract. A previous study showed that in solvent phases, the antioxidant capacity of quercetin and its glycosides depends on their proton affinity, and thus, quercetin-3β-D-glucoside would exhibit higher activity than quercetin [47]. This tendency may explain why the ethyl acetate extract provided a slightly lower radical scavenging activity than the methanol, ethanol, and ethanol-water extracts, despite the presence of quercetin. It is also worth noted that significantly higher quantities of phenolic compounds such as quinic acid, chlorogenic acid, and 7,8-dihydroxy-4-methylcoumarin were found in these extracts, which could contribute to their higher antioxidant capacity.

While the antibacterial activity of all extracts against *S. typhi* was generally on par, slightly higher activity was shown by the ethyl acetate and methanol extracts when tested against *E. coli* (different letter after inhibition zone value in Table 2 showed significant difference at $p < 0.05$). Coumarins exhibit diverse antibacterial activity against

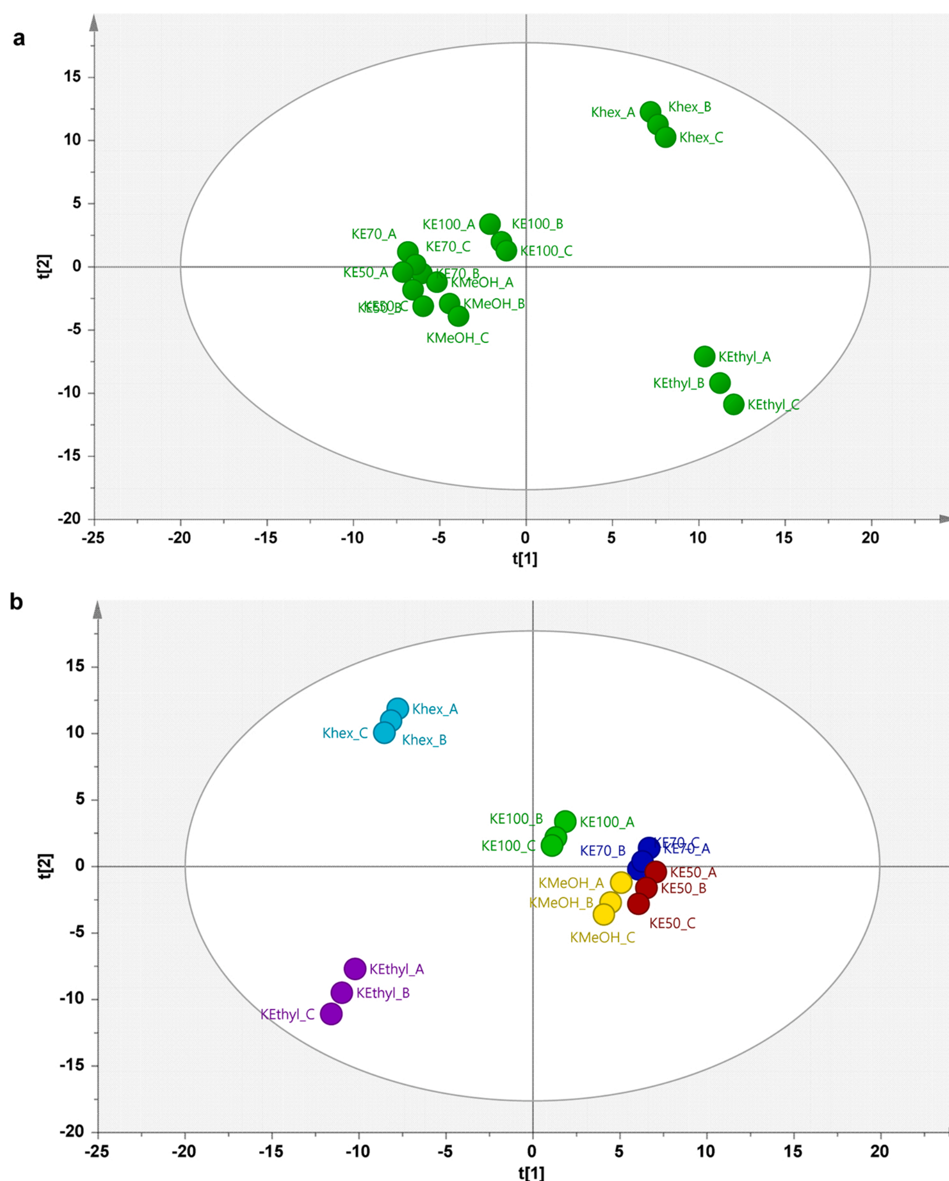

Fig. 4. Analysis of different *P. rubra* flower extracts (KMeOH = methanol, KE100 = ethanol 100 %, KE70 = ethanol 70 %, KE50 = ethanol 50 %, KEthyl = ethyl acetate, and Khex = hexane) using (a) principal component analysis (PCA) and (b) partial least square-discriminant analysis PLS-DA.

Gram-positive and Gram-negative bacteria. A previous study revealed 7-hydroxycoumarin (7-HC) as an effective antimicrobial agent against *E. coli* and *S. aureus* by affecting their motility and quorum-sensing activity [48]. 7-HC was present in the ethyl acetate extract and absent in others. 4-CA, which was mainly found in the methanol extract, also has bactericidal activity against *E. coli* by disrupting cell membranes and binding to the genomic DNA [49]. Simple phenolic acids have shown antibacterial activities against some Gram-negative bacteria [50]. However, their potency in inhibiting *E. coli* growth is significantly reduced by the number of substituents in the benzene ring [51]. This may explain why the ethanol (100 %, 70 %, and 50 %) extracts did not give stronger inhibition toward *E. coli*, despite their higher total phenolic contents in comparison with the methanol and ethyl acetate extracts.

Further analysis of metabolites in *P. rubra* flower extract using principal component analysis (PCA) was also performed. Principal component analysis using four principal components (PC) toward six types of *P. rubra* flower extract showed that extracts of methanol, ethanol 100 %, ethanol 70 %, and ethanol 50 % were clustered in one region. In contrast, ethyl acetate extract and hexane appeared in different regions, as depicted in the PCA score plot (Fig. 4. a). It means that the *P. rubra* flower extract of methanol, ethanol 100 %, ethanol 70 %, and ethanol 50 % had high similarity in their metabolite composition. Meanwhile, the ethyl acetate extract and hexane had more differences in their metabolite compositions; therefore, they appeared in the area far from those of methanol and ethanol extracts. The PCA model had R^2 of 0.936, indicating goodness of fit, with Q^2 of 0.823, indicating good model predictivity. Further analysis using PLS-DA confirmed the differentiation result from PCA, as shown in the PLS-DA score plot (Fig. 4.b.). Methanol and all ethanol extract of *P. rubra* flower appeared in one cluster, whereas ethyl acetate and hexane extract appeared in different areas. The PLS-DA model created using four latent variables provided a high accuracy ($R^2X = 0.935$, $R^2Y = 0.792$) and good predictivity ($Q^2 = 0.603$). The results from PCA and PLS-DA supported that methanol and ethanol have similar polarity and tend to be polar; thus, the composition of extracted compounds is similar. Meanwhile, ethyl acetate and hexane tend to be non-polar solvents. Therefore, the extracted compounds will significantly differ from methanol and ethanol. The metabolites' essential roles in classification from the PLS-DA model could be observed using variable importance for projections

Table 4
The discriminating metabolites obtained from VIP analysis of *P. rubra* flower extract.

No.	Compounds	VIP	Formula	Calculated MW	RT (min)	Ionization	Source of extracts
1	α,α-Trehalose	1.17	$C_{12}H_{22}O_{11}$	342.1158	0.878	ESI -	KE100, KE70, KE50, KMeOH
2	2-Mercaptoethanol	1.54	C_2H_6OS	78.01385	0.993	ESI +	KE100, KE70, KE50, KEthyl
3	2-(1-benzothiophene-3-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one	1.16	$C_{19}H_{14}OS$	290.0759	4.806	ESI +	KE100, KE70, KE50, KMeOH, KEthyl
4	Phthaldialdehyde	1.00	$C_8H_6O_2$	134.0364	5.378	ESI +	KE100, KE70, KE50, KMeOH, KEthyl
5	Melilotoside	1.10	$C_{15}H_{18}O_8$	326.1	5.84	ESI -	KE100, KE70, KE50, KMeOH, KEthyl
6	Methyl (1S,4aS,7R,7aS)-4'-[(1S)-1-hydroxyethyl]-5'-oxo-1-{[(2S,3R,4 S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-4a,7a-dihydro-1H,5'H-spiro [cyclopenta[*c*]pyran-7,2'-furan]-4-carboxylate	1.10	$C_{21}H_{26}O_{12}$	470.1418	7.509	ESI -	KE100, KE70, KE50, KMeOH
7	Cianidanol	1.25	$C_{15}H_{14}O_6$	290.078	7.51	ESI +	KE100, KE70, KE50, KMeOH
8	Dibenzylamine	1.33	$C_{14}H_{15}N$	197.1197	7.62	ESI +	KE100, KE70, KHex
9	Coumarone	1.01	C_8H_6O	118.0415	9.502	ESI +	KE100, KE70, KE50, KMeOH, KEthyl
10	4,5-Dicaffeoylquinic acid	1.05	$C_{25}H_{24}O_{12}$	516.1262	9.823	ESI -	KE100, KE70, KE50, KMeOH, KEthyl
11	Methyl (1S,4aS,7S,7aS)-1-(β-D-glucopyranosyloxy)-4'-[(1 S)-1-{[(2E)-3-(4-hydroxyphenyl)-2-propenoyl]oxy}	1.12	$C_{30}H_{32}O_{14}$	616.1783	12.275	ESI -	KE100, KE70, KE50, KMeOH, KEthyl
12	Methyl (1S,4aS,7R,7aS)-4'-[(1 S)-1-hydroxyethyl]-5'-oxo-1-{[(2 S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-4a,7a-dihydro-1H,5'H-spiro [cyclopenta[*c*]pyran-7,2'-furan]-4-carboxylate						KE100, KE70, KE50, KMeOH
13	N-acetyl-beta-D-glucosaminylamine	1.23	$C_8H_{16}N_2O_5$	220.1069	12.661	ESI +	KE100, KE70, KHex, KEthyl
14	Navenone A	1.34	$C_{15}H_{15}NO$	225.1145	13.869	ESI +	KE100, KE70, KHex
15	Asiatic acid	1.18	$C_{30}H_{48}O_5$	488.3497	15.555	ESI -	KE100, KE70, KE50, KMeOH, KHex, KEthyl
16	(*Z*)-?-amylcinnamyl alcohol = (2Z)-2-Benzylidene-1-heptanol	1.17	$C_{14}H_{20}O$	204.1505	15.559	ESI +	KE100, KE70, KE50, KMeOH, KEthyl
17	(1S,4S,5R,10S,13S,17S,19S,20R)-10-hydroxy-4,5,9,9,13,19,20-heptamethyl-24-oxahexacyclo[15.5.2.0Âa,Âaâ¸.0â´,Âaâ·.0âµ,Âaâ´.0â¸,ÂaÂl]tetracos-15-en-23-one	1.22	$C_{30}H_{46}O_3$	454.3436	16.817	ESI +	KE100, KE70, KE50, KMeOH, KHex, KEthyl
18	1-Linoleoyl-2-Hydroxy-sn-glycero-3-PC	1.43	$C_{26}H_{50}NO_7P$	519.3313	17.138	ESI +	KE100, KE70, KMeOH, KEthyl
19	Choline	1.43	$C_5H_{13}NO$	103.0995	17.575	ESI +	KE100, KE70, KMeOH
20	Stearamide	1.37	$C_{18}H_{37}NO$	283.2869	18.418	ESI +	KE100, KE70, KHex
21	1-Stearoylglycerol	1.31	$C_{21}H_{42}O_4$	358.3069	18.591	ESI +	KE100, KE70, KE50, KMeOH, KHex, KEthyl
22	Dromostanolone tetrahydropyranyl ether	1.11	$C_{25}H_{40}O_3$	388.2974	19.506	ESI -	KE100, KMeOH, KHex, KEthyl
23	10,12-Heptacosanedione	1.43	$C_{27}H_{52}O_2$	408.3955	19.664	ESI +	KMeOH, KHex
24	10-Hydroxy-16-hentriacontanone	1.44	$C_{31}H_{62}O_2$	466.4738	19.962	ESI +	KMeOH, KHex
25	16,18-Tritriacontanedione	1.32	$C_{33}H_{64}O_2$	492.4894	21.043	ESI +	KE100
26	(9E)-9-Nonacosen-2-one	1.46	$C_{29}H_{56}O$	420.4318	21.942	ESI +	KE50, KMeOH, KHex

Note: KE 100 = ethanol 100 % extract, KE 70 = ethanol 70 %, KE 50 = ethanol 50 %, KHex = hexane extract, KMeOH = methanol extract; KEthyl = ethyl acetate extract.

(VIP) value. Variables with more than one VIP value are considered discriminating metabolites that play essential roles in sample classification. The discriminating metabolites of *P. rubra* flower extract of methanol, ethanol (100 %, 70 %, 50 %), ethyl acetate, and hexane are shown in Table 4.

4. Conclusion

The α-glucosidase inhibitory, antioxidant, and antibacterial activity of *P. rubra* flower extract was performed. β-Carotene bleaching and DPPH assay were applied to evaluate the antioxidant activity of *P. rubra* flower extracts and showed coherent results. It was revealed that the ethanolic extract of *P. rubra* flower showed good antioxidant activity. This was in line with the high total phenolic content in the ethanol extract of *P. rubra* flower. Meanwhile, the methanolic extract showed promising activity as an α-glucosidase inhibitor. The antibacterial activity of the ethyl acetate extract of *P. rubra* flower was excellent against *E. coli*. The principal component analysis revealed that methanol and ethanol extracts have similar polarity and tend to be polar. Thus, the composition of extracted compounds is similar. The present study showed that *the P. rubra* flower is a potent source of natural antioxidants and antidiabetic medicines in functional foods and pharmaceutical industries.

Declaration of Competing Interest

The authors declare that there are no conflicts in relation to this work.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.procbio.2023.04.025.

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