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Antioxidative, anti-inflammatory, antibacterial, and anti-adhesive activities of different solvent extracts from various parts of *Basella rubra* L.

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Abstract *Basella rubra* is a green leafy vegetable commonly grown in a tropical region with high nutritional value. The antioxidative, anti-inflammatory, antibacterial, and anti-adhesive activities of crude extracts of *B. rubra* from leaves, stems, and fruits extracted with hexane, ethyl acetate (EtOAc), methanol (MeOH), and water were investigated. The results showed that MeOH extract from leaves had the highest yield of 22.62%. EtOAc extract from leaves presented the highest total phenolic contents and antioxidant activity. Furthermore, EtOAc extract from fruits exhibited the highest % nitric oxide (NO) inhibition with IC_{50} of 53.2 ± 3.3 μg/ml, followed by EtOAc extract from leaves with IC_{50} of 74.4 ± 4.3 μg/ml in LPS-activated RAW 264.7 cells. The antibacterial activity revealed that EtOAc and MeOH extracts from fruits inhibited *Staphylococcus aureus* DMST 8840, *Salmonella typhimurium* DMST 562, and *Escherichia coli* DMST 4212 with MIC of 3.91 mg/ml and 15.63 mg/ml, respectively. EtOAc extract from fruits was the most effective in inhibiting *E. coli* DMST 4212 and *S. aureus* DMST 8840 with adhesive inhibition of 75.8% and 55.9% to human colonic epithelial Coco-2 cells, respectively. Comparatively, MeOH extract from fruits showed 53.1% inhibition of *S. typhimurium* DMST 562. However, EtOAc extract from leaves was the least effective in inhibiting the adhesion of Gram-negative bacteria but moderately inhibited Gram-positive bacteria. Gas chromatography-mass spectroscopy (GC-MS) analysis revealed that EtOAc and MeOH extracts from fruits contained ergoline, sulfamide derivatives, and saturated fatty acids. The present results indicate that it might be possible to exploit EtOAc or MeOH extracts from fruits and EtOAc extract from leaves of *B. rubra* as novel functional food products.

Keywords: Antimicrobial activity, Anti-adhesion, Cell toxicity, Malabar spinach

Introduction

Basella rubra (Basellaceae) is a green leafy and edible vegetable in tropical and sub-tropical areas, commonly known as Malabar spinach, Cyelon

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spinach or Indian spinach (Roy *et al*., 2010). It is a succulent, branched, twining, and herbaceous vine, growing several meters in length with red-purplish stems. *B. rubra* fruit is a small, dark purple with a single black seed (Singh *et al*., 2016). In Thailand, *B. rubra* leaves have regularly been consumed; however, stems and seeds are discarded as waste. Various bioactive compounds are presented in the leaves, stems, and fruits of *B. rubra*, including vitamins, minerals, dietary fiber, flavonoids, betalains, and phenolic compounds (Deshmukh and Gaikwad, 2014). This plant has been widely used as traditional medicine for treating several diseases, including wound healing, antioxidant, anti-inflammation, anti-ulcer, and antimicrobial activities (Deshmukh and Gaikwad, 2014; Singh *et al*., 2016). Methanol extract from stems, leaves, and fruits of *B. alba* and *B. rubr*a have shown to be a potent antioxidant and antimicrobial agent (Adegoke and Ojo, 2017). However, various extraction solvents have been reported to affect plant extracts' yield and biological activities and result in different pharmacological properties (Mehmood *et al*., 2021; Truong *et al*., 2019). A previous study reported that the methanol extract of *B. rubra* leaves had intense anti-inflammatory activity against oxazolone-induced colitis in rats (Bhanu Priya *et al*., 2014).

Up to date, no information has been reported on the effect of different extraction solvents on the phenolic compounds, antioxidant, anti-inflammation, and antimicrobial activity from various parts of *B. rubra* (stems, leaves, and fruits). Therefore, further investigations are necessary to provide suitable extraction solvent and essential information about the phenolic contents and biological activities of *B. rubra*, supporting potential applications in the food and pharmaceutical industries. This study aimed to evaluate the effect of different solvents on the phenolic contents, antioxidant, anti-inflammation, and antibacterial activity of stems, leaves, and fruits of *B. rubra*. Additionally, the impact of the resulting extracts on the anti-adherence activities of pathogenic bacteria to intestinal human epithelial Caco-2 cells was investigated.

Materials and methods

Chemical reagents

Dulbecco's Modified Eagle's Medium (DMEM), Minimum Essential Media (MEM), L-glutamine, Non-Essential Amino Acids (NEAA), fetal bovine serum (FBS), and antibiotics (penicillin/streptomycin) were brought from Gibco (New York, USA). 2,4,6-tripyridyl-S-triazine (TPTZ)**,** 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and lipopolysaccharide (LPS) were obtained from

Sigma-Aldrich (MO, USA). Other chemicals used in the experiments were of analytical grade.

Cell lines, media, and bacterial strains

The mouse macrophage cell line RAW 264.7 and human colonic epithelial cell line Caco-2 were obtained from RIKEN BRC Cell Bank (Ibaraki, Japan). RAW 264.7 cells were cultured in DMEM with 10% FBS, 1% L-glutamine, and 1% antibiotics. Caco-2 cells were cultured in MEM with 20% FBS, 1% L-glutamine, 1% NEAA and 1% antibiotics. Cells were incubated at 37 ℃ with 5% CO_2.

Escherichia coli DMST 4242, *Salmonella typhimurium* DMST 562, and *Staphylococcus aureus* DMST 8840 were obtained from the National Institute of Health of Thailand. These strains were cultivated in Brain Heart Infusion (BHI) broth (Himedia, India) for 18 h at 37 °C before antibacterial assays.

Preparation and extraction of B. rubra leaves, stems, and fruits

Fresh stems, leaves, and mature fruits (with seed) of *B. rubra* were collected from a local supplier, in Chonburi province, Thailand. Various parts of *B. rubra* were separated and washed with distilled water. Each part was dried using a tray dryer (OFM, Thailand) at 55 ℃ and ground using a grinder (Schmersal, Germany). Samples were stored at 4 ℃ in a plastic bag covered with aluminum foil before being extracted within one month.

The extraction method was adapted from Adegoke and Ojo, (2017). Ground samples were extracted using the sequential Soxhlet extraction method with hexane, ethyl acetate (EtOAc), methanol (MeOH), and water. Then, the extracted samples were evaporated using a rotary evaporator (Buchi, Switzerland). Crude extracts were stored at 4 ℃ until analysis.

Determination of total phenolic content (TPC)

TPC was followe as described previously (Nitteranon, 2018). Five hundred microliters of sample was adding with 250 µl Folin-Ciocalteu reagent and 2.5 ml sodium carbonate (7%). The samples were incubated at room temperature for 30 min and spectrophotometrically analyzed at 765 nm. TPC contents were calculated as mg gallic acid equivalents (GAE)/g extract

Determination of antioxidant activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

DPPH method was used to evaluate the antioxidant activity of different solvent extracts of stems, leaves, and fruits of *B. rubra* as previously described (Braca *et al*., 2001). Briefly, 150 mM of DPPH radical solution was prepared and added to a microplate of 200 µl with a sample of 22 µl. Then, the samples were incubated at room temperature for 30 min and spectrophotometrically determined at 520 nm. The results were calculated at percentage DPPH inhibition.

FRAP (Ferric reducing ability power) assay

FRAP assay was performed as previously described (Benzie and Strain, 1996). One hundred microliters of extract were added with 3 ml FRAP reagent (300 mM acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$). The samples were incubated at room temperature for 15 min and spectrophotometrically evaluated at 593 nm. The antioxidant activity was calculated as mmol Trolox/g of extract.

Determination of in vitro anti-inflammatory activity using nitric oxide (NO) inhibition assay

Mouse macrophage (RAW 264.7) cells were seeded at 5 x 10^5 cells/ml for 200 µl in a 96-well plate and incubated at 37 ℃ with 5% CO_2 for 16–24 h as previously described (Nitteranon *et al*., 2011) Then, samples with different concentrations (62.5-1000.0 µg/ml) were added to the 96 well-plate with 1 µg/ml LPS to induce inflammation in RAW 264.7 cells. The samples were incubated for 24 h. After that, 100 µl of supernatant was mixed with 100 µl of Griess reagent (0.1% N-(1-naphathyl) ethylenediamine in phosphoric acid and 1% sulfanilamide) and incubated at room temperature for 10 min. The samples were spectrophotometrically determined at 542 nm, and the result was expressed as the half-maximal inhibitory concentration (IC_{50}).

Determination of cell viability

Cell viability was determined using 3-(4,5-dimethythiazol-2-yl)-2,5 diphenyltetrasodium bromide (MTT) assay as previously described (Nitteranon *et al*., 2011). Cells were seeded in a 96-well plate and incubated at 37 ℃ for 16-24 h. Then, the extracts were added to the cells, incubated for 72 h, and compared to the control without the extract. Then, 20 µl of MTT solution was added and incubated further at 37 ℃ for 15 min. After that, the supernatant was discarded and 200 µl of dimethylsulfoxide (DMSO) was added. The samples were spectrophotometrically determined at 550 mm.

Determination of antibacterial activity

Agar diffusion assay

The antibacterial activity of sample extracts against three pathogenic strains was determined by the agar diffusion method (Rauha *et al*., 2000). Pathogenic bacteria were incubated with BHI broth with or without sample extracts at 37 ℃ for 18 h. One hundred microliters of sample extract solution (1 mg/ml) were added to each well. Plates were incubated at 37 ℃ for 24 h. The diameter of the inhibition zone was measured and recorded. Streptomycin and penicillin solutions at 100 µg/ml were tested against targeted bacteria as a positive control.

Minimal inhibitory concentration (MIC) assay

Mininal inhibitory concentration (MIC) was performed as previously described (Srisukong *et al*., 2016). The crude extracts of *B. rubra* were diluted using two-folded serial dilutions. The initial microbial samples were at 1 x 10^8 CFU/ml. Pathogenic bacteria were cultured in BHI broth containing sample extracts at 37 ℃ for 24 h. Changes in the turbidity were compared with the control. Streptomycin and penicillin solutions at 100 µg/ml were used against targeted bacteria as a positive control The first dilution with no microbial growth was recorded as the MIC.

Determination of anti-adhesive activity

The anti-adhesive activity was performing as previously described (Zhao and Shah, 2015) with modifications. Caco-2 cells were seeded at the concentration of 5 x 10^5 cells/well in a 24-well plate and incubated at 37 ℃ with 5% CO_2 for 24 h. Meanwhile, the pathogenic bacteria were cultivated for 16–18 h; then, the bacteria were collected with a centrifuge at 3500 *x*g for 10 min at 4 ℃ and washed twice with phosphate-buffered saline (PBS) buffer (pH 7.4). Bacterial cells were resuspended in an antibiotic-free MEM medium to obtain a bacterial density of 10^8 CFU/ml. Sample extracts of EtOAc fruit, MeOH fruit, and EtOAc leave from *B. rubra* were dissolved in antibiotic-free MEM with 0.5% DMSO. Before adding the samples, Caco-2 cells were washed twice with PBS to remove antibiotics. Then, the sample extracts were added to Caco-2 cells and incubated for 1 h at 37 ℃, followed by adding pathogenic bacteria in the ratio of 100:1 (bacteria: Caco-2 cells) and further incubated for 4 h. For the control group, pathogenic bacteria were added to the cells without the extracts. After incubation, the supernatant was discarded, and Caco-2 cells were washed twice with PBS to remove non-adhered bacteria. Then, Caco-2 cells

were trypsinized by adding 100 µl of 0.25% trypsin-EDTA. Viable bacterial cells were further cultivated with an appropriate medium. The relative percentage of bacterial adhesion to Caco-2 cells was calculated as follows.

$$\text{Relative percentage adhesion (\%)} = \frac{\text{CFU of sample}}{\text{CFU of control}} \times 100$$

Gas chromatography-mass spectroscopy (GC-MS) analysis

EtOAc and MeOH extracts from leaves and fruits of *B. rubra* were subjected to GC-MS analysis using a 6890n Gas chromatography system coupled to VL/MSD 5973 mass spectropmeter (Agilent Technologies, USA), attached with a capillary column HP-5 with 0.25 mm i. d. × 30 m length. Helium gas (99.9%) was used as the carrier gas at a flow rate of 1 ml/min and injection volumn of 1 µl was employed with an injector temperature of 260 ℃. The oven temperature was programmed initially from 40 ℃ (held for 3 minutes) with an increase of 10 ℃/min to 190 ℃, then 10 ℃/min to 260 ℃, to hold for 15 min. The total analysis time for each sample was 45 min. The area under a peak represents the quantity of the compound present in each extract. Major constituents were identified by using mass spectrum library (wiley7n.1).

Statistical analysis

All experiments were performing at least in triplicate, representing the mean ± standard deviation. One-way analysis of variance (ANOVA) was used for data analysis. Duncan's test was performed at $p < 0.05$ using SPSS statistics program version 17.0 to evaluate the statistically significant differences.

Results

Determination of percent yield

The yields for stems, leaves and fruits of *B. rubra* with different solvents were show in Table 1. The used solvents were hexane, EtOAc, MeOH, and water to get the extracts for testing their effects on the yield of *B. rubra*. The maximum yield (9.86 %) was obtained with MeOH extracts from stem of *B. rubra*, while the minimum (0.89 %) was with EtOAc extract. The maximum yield of leaves was shown in MeOH extract (18.50 %), while the minimum was shown in EtOAc extract (3.66 %). The extract from fruits of *B. rubra* gave the highest yield in MeOH extract (22.62 %) and the lowest was shown in EtOAc extract (8.28%). Therefore, the maximum yield was obtained from MeOH extract.

Determination of total phenolic content

The total phenolic contents of stems, leaves, and fruits of *B. rubra* from different extracting solvents were show in Table 1. The results indicated substantial variation in the total phenolic content in stems, leaves, and fruits, ranging from 0.2 to 44.0 mg GAE/g extract**.** Furthermore, using solvent extracts showed a significant difference ($p < 0.05$) in total phenolic content. *B. rubra* leaves were found rich in phenolic compounds, which varied from 8.2 ± 0.5 to 44.0 ± 2.1 mg GAE/g, followed extract from stems which varied from 11.2 ± 0.4 to 35.8 ± 0.1 mg GAE/g, and the extract from fruits varied from 0.2 ± 0.1 to 29.8 ± 2.3 mg GAE/g. The EtOAc was more efficient than the others for extracting phenolic compounds from the stems and leaves, followed by aqueous extracting phenolic compounds from fruits. EtOAc extract from leaves showed significantly ($p < 0.05$) the highest total phenolic content.

Determination of antioxidant activities

The antioxidant activity of stems, leaves, and fruits of *B. rubra* was determined using DPPH and FRAP assays**.** Results showed significant difference **(**$p < 0.05$) in the antioxidant activities of all extracts of *B. rubra* **(**Table 1**)**. The EtOAc extract from leaves exhibited the highest DPPH scavenging activity (68.4 ± 2.3 %), followed by EtOAc extract from stems (54.6 ± 6.8 %), and aqueous extract from fruits (45.1 ± 1.0 %) at 2.5 mg/ml concentration.

Table 1. Percentage of extract yield, TPC, DPPH, FRAP, and IC_{50} of NO with different solvents from the stems, leaves, and fruits of *B. rubra*

Samples	Solvent	% yield	TPC (mg GAE/g of extract)	% radical scavenging of DPPH	FRAP (mmol Trolox/g of extract)	IC_{50} of NO (µg/ml)
Stem	Hexane	2.07	11.2 ±0.4^{b}	44.2 ±0.8^{e}	17.5 ±2.5^{b}	407.6 ±39.9^{d}
	EtOAc	0.89	35.8 ±0.1^{ef}	54.6 ±6.8^{f}	86.1 ±1.9^{g}	182.9 ±8.2^{b}
	MeOH	9.86	22.7 ±0.2^{cd}	28.4 ±1.5^{cd}	30.7 ±1.6^{cd}	292.0 ±6.5^{c}
	Water	9.46	22.0 ±0.2^{cd}	41.0 ±2.6^{e}	45.8 ±3.1^{e}	-
Leave	Hexane	6.63	8.2 ±0.5^{ab}	37.1 ±2.5^{de}	63.9 ±2.7^{f}	150.3 ±19.2^{b}
	EtOAc	3.66	44.0 ±2.1^{f}	68.4 ±2.3^{g}	99.9 ±4.2^{h}	74.4 ±4.3^{a}
	MeOH	18.50	25.7 ±1.7^{d}	18.2 ±5.2^{b}	22.7 ±5.4^{bc}	417.9 ± 16.4^{d}
	Water	14.62	9.6 ±1.2^{b}	17.4 ±1.2^{b}	31.6 ±0.4^{cd}	-
Fruit	Hexane	20.03	0.2 ± 0.1^{a}	5.4 ±1.6^{a}	0 ±0.0^{a}	187.6 ± 18.5^{b}
	EtOAc	8.28	22.6 ±0.7^{cd}	30.6 ±2.0^{cd}	39.1 ±4.0^{de}	53.2 ± 3.3^{a}
	MeOH	22.62	14.0 ±0.8^{bc}	25.8 ±2.2^{bc}	26.9 ±4.7^{c}	415.8.4 ± 1^{d}
	Water	11.69	29.8 ±2.3^{de}	45.1 ±1.0^{e}	95.0 ±1.9^{gh}	490.5 ±52.7^{e}

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

FRAP assay yielded a similar antioxidant activity**.** The highest ferric ion reduction was observed by the EtOAc extract from leaves, followed by EtOAc extract from stems, and aqueous extract from fruits. However, hexane extract from fruits exhibited the lowest antioxidant activity**.**

EtOAc was an excellent solvent in extracting antioxidants from the stems and leaves of ***B. rubra***, followed by the aqueous solvent in extracting antioxidant compounds from the fruits of *B. rubra*.

Determination of in vitro anti-inflammatory activity

The results of the *in vitro* anti-inflammatory activity of the stems, leaves, and fruits of ***B. rubra*** on LPS-induced RAW 264**.**7 cells were present in Table 1. Among these extracts, EtOAc extract from fruits exhibited the highest inhibitory activity against NO with an IC_{50} value of 53.2 ± 3.3 µg/ml, followed by EtOAc extract from leaves with an IC_{50} value of 74.4 ± 4.3 µg/ml, while cell viability was maintained > 90 % (data not shown). Other extracts exhibited weak NO inhibiting activity, whereas aqueous extract from stems and leaves did not show NO inhibitory activity on LPS-activated RAW 264.7 cells.

Determination of antibacterial activity

The agar diffusion method first measured the antibacterial activity of various parts of *B. rubra* with different solvents. The antibacterial activity was observed in EtOAc and MeOH extracts from fruits tested against *E. coli* DMST 4212, *S. typhimurium* DMST 562, and *S. aureus* DMST 8840 (Table 2). EtOAc and MeOH extracts from fruits showed a zone of inhibition against *E. coli* DMST 4212, *S. typhimurium* DMST 562, and *S. aureus* DMST 8840, with the inhibition zone ranging from 30.0 ± 2.00 to 35.3±0.58 mm and from 12.3 ± 2.31 to 18.0 ± 0.00 mm, respectively. EtOAc extract from fruits was more efficient in inhibiting pathogenic organisms than MeOH extract from fruits. Nonetheless, stem and leave extracts of *B. rubra* could not inhibit pathogenic bacteria in this study.

In MIC test, the lowest concentrations inhibiting pathogenic bacteria of EtOAc and MeOH extracts from fruits were 3.91 and 15.63 mg/ml, respectively (Table 3).

Table 2. Antibacterial activity of EtOAc and MeOH extracts from fruits of *B. rubra* against pathogenic bacteria

Samples	Inhibition zone diameter (mm)		
	E. coli DMST 4212	*S. typhimurium* DMST 562	*S. aureus* DMST 8840
Fruit-EtOAc	31.0 ± 1.00[b]	30.0 ± 2.00[b]	35.3 ± 0.58[a]
Fruit-MeOH	18.0 ± 0.00[a]	12.3 ± 2.31[b]	16.7 ± 0.58[a]
Streptomycin	20.7 ± 0.58	21.3 ± 0.58	ND
Penicillin	ND	ND	25.7 ± 1.15

Different letters in the same column indicate significant differences at $p < 0.05$.
ND = Not determined

Determination of anti-adhesive activity

Based on the results of total phenolic content, antioxidant activity, anti-inflammation, and antibacterial assay, EtOAc extract from leaves (0.25 mg/ml), EtOAc from fruits (1 mg/ml), and MeOH from fruits (1 mg/ml) extracts were selected to determine the anti-adhesive activity to Caco-2 cells. No significant cytotoxicity was observed as > 90**%** of cell viability was viable after 24 h incubation (Figure 1A). Then, these three extracts were further determined for their anti-adhesive activity. EtOAc extract from fruits was the most active against the adhesion of ***E. coli*** DMST 4212 and *S. aureus* DMST 8840 to Caco-2 cells, with inhibitory activity of 75.8 and 55.9 %, respectively. MeOH extract from fruits had an inhibitory effect against the adhesion of ***S. typhimurium*** DMST 562 to Caco-2 cells with a percentage of 53.1 %**.** Additionally, EtOAc extract from leaves inhibited the adhesion of ***S. aureus*** DMST 8840, while it was the least effective in inhibiting *E. coli* DMST 4212 and *S. aureus* DMST 8840 to Caco-2 cells (Figure 1B).

Table 3. Minimum inhibitory concentration (MIC) of EtOAc and MeOH extracts from fruits of *B. rubra* against pathogenic bacteria

Samples	Minimum inhibitory concentration: MIC (mg/ml)		
	E. coli DMST 4212	*S. typhimurium* DMST 562	*S. aureus* DMST 8840
Fruit-EtOAc	3.91	3.91	3.91
Fruit-MeOH	15.63	15.63	15.63

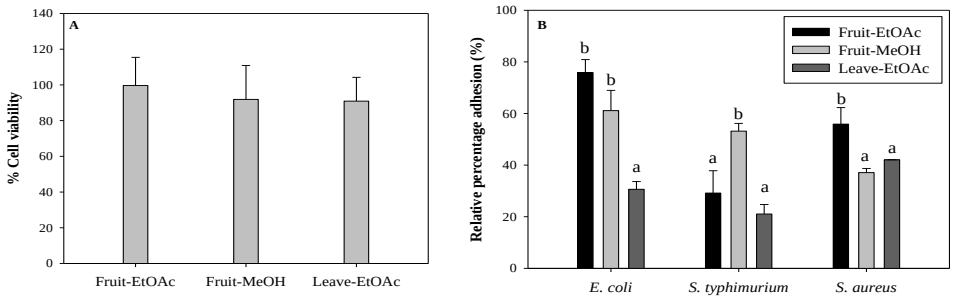

Figure 1. Effects of fruit EtOAc, fruit MeOH, and leave EtOAc extracts of *B. rubra* on A) the cell viability of Caco-2 cells and B) the adhesion of pathogenic bacteria to Caco-2 cells**.** Different letters indicate significant differences ($p <$ 0**.**05) at the same pathogenic strains

GC-MS analysis

GC-MS profile of EtAOc extract from fruits exhibited the occurrence of 34 chemical compounds. The identified compounds with their retention time (min) and peak area (%) are presented in Table 4. Three compounds were identified. The major compounds of EtOAc extract from fruits included 9-octadecanoic acid, methyl ester (14.2%), hexadecanoic acid, methyl ester (12.1%), and hexadecanoic acid (11.4%). GC-MS profile of MeOH extract from fruits showed three major compounds and identified as ergoline sulfamide derivative (24.91%), phenol, 2, 4-bis (1,1-dimethylethyl) (16.78%), and hexadecanoic acid methyl ester (10.35%). In addition, the identified major compounds in EtOAc extract from leaves were phytol (29.9%), Phenol, 2, 4-bis (1,1-dimethylethyl)- (6.4%), and tetracecane (3.1%).

Table 4. GC-MS analysis of fruit EtOAc, fruit MeOH, and leave EtOAc extracts of *B. rubra*

Samples	Solvents	Retention time (min)	Peak area (%)	Compounds
Fruit	EtOAc	32.049	12.12	Hexadecanoic acid, methyl ester
		32.820	14.21	9-octadecanoic acid, methyl ester
		35.356	11.40	Hexadecanoic acid
Fruit	MeOH	22.844	16.78	(Phenol,2,4-bis)1,1-dimethylethyl-
		32.038	10.35	Hexadecanoic acid, methyl ester
		32.254	24.91	Ergoline, sulfamide derivative
Leave	EtOAc	22.876	6.43	(Phenol,2,4-bis)1,1-dimethylethyl-
		28.256	3.07	Tetracecane
		35.523	29.92	Phytol

Discussion

The extraction efficiency depends on several factors, including the nature of phytochemical components, extraction methods, and the solvent system (Truong *et al*., 2019). A significant difference ($p < 0.05$) in extraction yield between stems, leaves, and fruits of *B. rubra* might be due to the varying polarity of the solvents and availability of extracted compounds in each part of the plant. Previous studies have reported that methanol is the common solvent in the *B. rubra* extraction from leaves (Adegoke and Ojo, 2017; Kumar *et al*., 2018). No previous studies exist to compare solvents and parts of *B. rubra*.

This study observed that the extracts from the stem, leave, and fruit of *B. rubra* have substantial amounts of polyphenol compounds most effectively extractable by EtOAc extract, comparatively by MeOH and aqueous extracts, and very poorly by hexane extract (Table 1). Our findings indicated that antioxidant activities exhibited a positive correlation with polyphenol contents. The extract of leaves using EtOAc showed the highest antioxidant activity compared to fruit and stem, which is relevant to the previous study (Adegoke and Ojo, 2017), depicting that leave from *B. rubra* and *B. alba* have comparable DPPH scavenging activity to fruit and stem. GC-MS analysis revealed that the leave extract of *B. rubra* had phytol, illustrating the potential for antioxidant activity (McGinty *et al*., 2010). However, Kuma *et al*. (2018) found that the aqueous stem of *B. rubra* and *B. alba* extracts showed the highest phenolic (caffeic acid and *p*-coumaric acid), flavonoids, and antioxidant activities. Therefore, the difference may be due to the variety of *B. rubra*, plant harvest duration, and extraction method. In this study, aqueous extract of *B. rubra* fruits exhibited the highest content of phenolic and antioxidant properties compared with other organic solvents used in fruit extraction. Kumar *et al*. (2015) found that the extraction of *B. rubra* fruit could obtain betacyanin and possess antioxidant activity. These results have also revealed that the extraction solvent significantly ($p < 0.05$) affected total phenolic contents and scavenging activities from the stems, leaves, and fruits. Thus, EtOAc extract proved to be the most effective solvent for extracting phenolic compounds compared with the aqueous and MeOH extracts, showing moderate extractability of these compounds.

Inflammation is a complex process associated with the reaction of body tissues to infection and is involved in various diseases, including cancer (Chen *et al*., 2018). The results illustrated that EtOAc extract from leaves and EtOAc extract from fruits of *B. rubra* effectively inhibited NO production in LPS-activated RAW 264.7 cells. The anti-inflammatory activities of these compounds may be due to the high level of phenolics in the extract. In a

previous study, MeOH extract from *B. rubra* leaves exhibited anti-inflammatory activity in oxazolone-induced colitis in rats (Bhanu Priya *et al*., 2014). The phytochemicals found in these extracts were phytol and saturated fatty acids, including hexadecanoic acid (palmitic acid, C16:0) and octadecanoic acid (stearic acid, C18:0), impacting inflammation related to a previous study by Mah *et al*. (2017). They found that fatty acids from leave extracted from *Sida rhombifolia* have anti-inflammatory activities. Rodda *et al*. (2012) found that *B. rubra* leave extract in 50% ethanol could lower inflammation in rat-induced with carrageenan. These findings suggest that EtOAc extract of leaves and fruits of *B. rubra* is the most potent extract, a promising source of anti-inflammatory and antioxidant agents.

Remarkably, EtOAc and MeOH extracts of *B. rubra* fruit are potential antibacterial agents for further natural antibiotics development. The antibacterial activity of fruit extracts from *B. rubra* may be linked to the presence of phytochemicals such as 9-octadecanoic acid methyl ester, hexadecanoic acid methyl ester, and hexadecanoic acid. Previous studies have found that 9-octadecanoic acid can inhibit several pathogenic bacteria, including *Lactococcus garviae*, *Vibrio anguillarium*, *V. harveyi*, and *V. alginocolyticus* (Desbois and Smith, 2010). Furthermore, hexadecanoic acid extracted from *Salvia lanigera* could inhibit *S. aureus* (Tenore *et al*., 2011). Therefore, hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid) exhibit antibacterial activity toward Gram-positive and Gram-negative bacteria (Casillas-Vargas *et al*., 2021). The interaction between fatty acids and bacterial membranes might create transient or permanent pores and cause bacterial growth inhibition (Desbois and Smith, 2010). In addition, the MeOH extract of *B. rubra* fruit contained the essential phytochemical compounds, including ergoline, sulfamide derivatives and phenol, 2, 4-bis (1,1-dimethylethyl) with antibacterial properties (Belakhdar *et al*., 2015). Moreover, other phytochemical compounds present in *B. rubra* fruits such as flavonoids, saponin, tannin, carotenoids, ghomphrenedin I, II, and III could inhibit the growth of *S. aureus*, *B. cereus, Klebsiella pneumonia* (Adegoke and Ojo, 2017; Singh *et al*., 2016).

Bacterial adhesion is the process of infection at the cell surface or epithelial cells. Using antibiotics to control and treat bacterial infections, including *Salmonella* or *Listeria*, can cause drug resistance, making it more difficult for a patient to be cured. Therefore, using natural extract is the alternative way to inhibit the attachment and growth of those pathogenic bacteria. Treating intestinal Caco-2 cells using fruit EtOAc extract could inhibit Gram-positive and Gram-negative bacteria. However, fruit MeOH extract could effectively inhibit only Gram-negative bacteria. These anti-adhesive results

correspond with antibacterial activities. In addition, EtOAc extract from leaves of *B. rubra* inhibited Gram-negative bacteria least effectively but Gram-positive bacteria moderately. It might depend on the difference in cell wall composition because Gram-negative bacteria have an outer membrane that might halt hydrophobic compounds into the cell wall. Saturated fatty acid compounds and hydrophobic phenolic compounds from *B. rubra* extract could inhibit the adhesion of bacteria. Stenz *et al*. (2008) reported that oleic acid (C18:1 (n-9)) inhibited *S. aureus* attachment through a mechanism involving bacterial lipids. Therefore, using fruits and leaves extract from *B. rubra* to treat pathogenic bacterial infections is possible and might further replace synthetic antibiotics. More studies on compound isolation need to be accomplished.

In conclusion, this study revealed the effect of different solvents on the biological activities of stems, leaves, and fruits of *B. rubra*. These findings have demonstrated that the leaves and fruits of *B. rubra* could be a good source of antioxidative, anti-inflammatory, antimicrobial, and anti-adhesive activities. The present results indicate that exploiting fruit and leave extracts from *B. rubra* may prevent pathogenic infection as a new alternative for antibiotics. Therefore, *B. rubra* plants have great potential as functional foods.

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