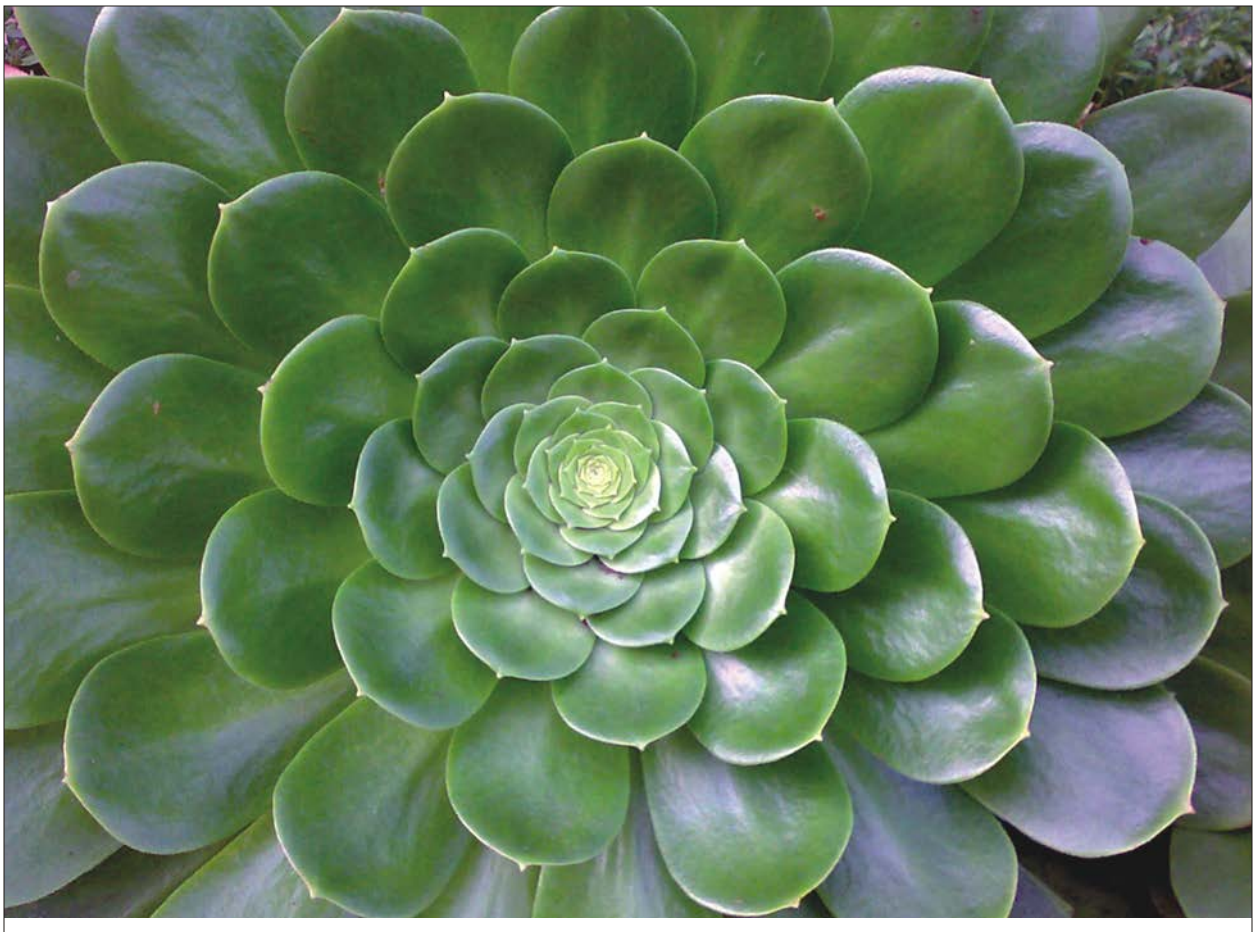

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Research Article
Antibacterial Activity of Buasbuas (*Premna pubescens* Blume) Leaf Extracts against *Bacillus cereus* and *Escherichia coli*

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Abstract

Background and Objective: *Premna pubescens* blume popularly known as buasbuas belonging to the family Lamiaceae, wide-spread in the forests of Sumatra and Malaya peninsula. The preliminary screening of the ethanol extract of *P. pubescens* revealed the presence of alkaloids, steroids, flavonoids and phenolic. Previous studies revealed that there is no systematic study regarding the antimicrobial activity of leaf extracts of *P. pubescens*. The aim of this study was to assess the *in vitro* effect of leaf extracts of *P. pubescens* against *Bacillus cereus* and *Escherichia coli* causative agent of diarrheal diseases. **Materials and Methods:** Fresh leaves were procured from personal *P. pubescens* plant collections aged 5-7 years. Crude ethanol extract of leaves from *P. pubescens* were tested *in vitro* against *B. cereus* and *E. coli* at concentrations 10, 20, 30, 40 and 50 μg μL^{-1}. **Results:** The *B. cereus* and *E. coli* showed concentration-dependent susceptibility towards the ethanol leaf extracts from *P. pubescens*. The degree of susceptibility varied depends on the concentrations, 50% concentration of *P. pubescens* leaf extracts showed the highest inhibition zone (12.5 and 13.6 mm diameter). **Conclusion:** Based on the current findings, it can be concluded that *P. pubescens* has antimicrobial activity which is as potent as standard antimicrobial drugs against *B. cereus* and *E. coli*. The antibacterial properties of *P. pubescens* leaves were not as effective as the commercial antibiotics chloramphenicol, enrofloxacin and penicillin. Nevertheless, future studies with higher extract concentrations, different method of extraction, properties from flowers, fruits, barks and roots extracts may be useful to evaluate the actual antibacterial performed to other pathogenic bacteria.

Key words: Secondary metabolites, antimicrobial, inhibition zone, flavonoids, active compound

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Premna pubescens blume is a plant belonging to the family Lamiaceae. *Premna pubescens* is native to Southeast Asia and is one of the most wide-spread large shrubs in the forests of Sumatra and Malaya peninsula (Leeratiwong *et al*., 2009). It grows as ornamental plant. It is popularly known as buasbuas in Malay ethnic system of culinary and medicine. Most of the plant parts of *P. pubescens* have been used in the traditional system of medicine in Malay ethnic to treat various infectious diseases. Leaf forms an ingredient in well-known bubur pedas formulation which is used for variety of food for sultan (Goud, 1995). The other uses of *P. pubescens* are as an antioxidant, anticancer, antiviral, antifungal and antibacterial (Saeed *et al*., 2015; Selvam *et al*., 2015).

Like the other *Premna*, *P. pubescens* are rich in a wide variety of secondary metabolites such as alkaloids, flavonoids and terpenoids (Shukri *et al*., 2011; Hasanah *et al*., 2015). Of these metabolites, phenolic compounds such as flavonoids contained in plants is usually beneficial as an antioxidant, anticancer, antiviral, antifungal and antibacterial (Singh *et al*., 2011; Saeed *et al*., 2015; Dongamanti *et al*., 2015). The *P. pubescens* predicted has anti-coagulant, anti-inflammatory, anti-parasitic, antioxidant and antimicrobial properties. Flavonoids have received increased attention as useful pharmaceuticals in managing diseases like diarrhea (Mustafa *et al*., 2010; Fratiwi, 2015).

A previous investigation revealed that ethanol extract from *P. pubescens* leaves contained potential anti-inflammation (Marbun and Restuati, 2015). Previous studies revealed that there is no systematic study regarding the antibacterial activity of the leaf of *P. pubescens*. In the present study, an attempt has been made to evaluate the anti-bacterial activity of the ethanol extract of leaves of *P. pubescens* againts *B. cereus* and *E. coli*. *Bacillus cereus* and also *E. coli* was able to produce an exotoxin that causes gastrointestinal disorders and diarrhea (Fratiwi, 2015; Lakshminarayanan *et al*., 2015). *Bacillus cereus* is a gram-positive bacteria and *Escherichia coli* is a gram-negative (Kirk *et al*., 2015). Thus, the aim of this current investigation is to evaluate the ethanol extracts of leaves from *P. pubescens* against a gram-positive bacteria (*B. cereus*) and a gram-negative (*E. coli*) *in vitro*.

MATERIALS AND METHODS

Plant materials and extraction: The whole plants of *P. pubescens* were collected from personal *P. pubescens* plant collections aged 5-7 years in Medan, North Sumatra, Indonesia (Fig. 1a). The botanical identification of the collected materials was done in herbarium of bogor botanical garden. The leaves were separated and oven dried at 28°C room for 1 week (Fig. 1b). The leaves were grounded into powder form using the grinder (Fig. 1c). Extraction using Soxhlet apparatus with 95% (v/v) ethanol as solvent for 12 h was performed. The resultant extraction was frozen and freeze dried for 24°C/48 h. Yield of ethanol leaves extracts: 30% (Villalobos *et al*., 2016).

Microorganisms and medium: The microorganisms used in this present study were bacteria (*Bacillus cereus* and *Escherichia coli*). The microorganisms were from clinical isolates. The *B. cereus* and *E. coli* were inoculated into in nutrient agar (NA, Merck, Germany), diluted 1:10 with sterile normal saline and then streak onto NA (Villalobos *et al*., 2016).

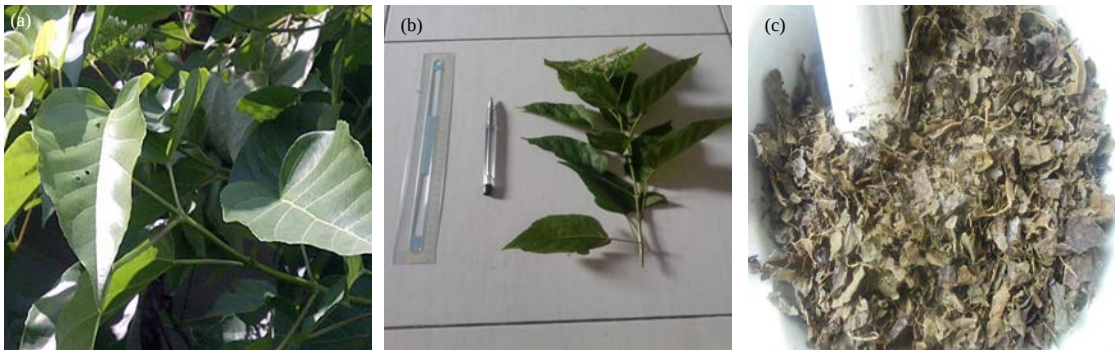

Fig. 1(a-c): Plant materials and extraction (a) The whole plants of *P. pubescens*, (b) *P. pubescens* leaves and (c) *P. pubescens* grounded leaves

Antimicrobial sensitivity test: Sterile 6.0 mm diameter blank disc (Becton Dickinson Microbiology System, USA) were used to impregnate 6 different dilutions of the extracts as follows: 0, 10, 20, 30, 40 and 50 μg μL^{-1} extract ethanol. Discs were stored at 5°C prior to use. Tests were performed by the disc diffusion method. Extract impregnated discs were placed on agar and incubated either at 37°C for 24-48 h. Antibacterial activities were then measured indicated by the clear zones of inhibition (Dettman *et al*., 2015).

Comparison with antibiotic drug: Antibiotic discs of chloramphenicol (50 μg μL^{-1}), enrofloxacin (50 μg μL^{-1}) and penicillin (10 U) were purchased from Oxoid Ltd., UK. All discs were stored at -5°C prior to use. The antibiotic discs were then placed onto the *B. cereus* and *E. coli* cultures for 24-48 h. Antibacterial activities were then measured indicated by the clear zones of inhibition (Bai *et al*., 2015; Mahboubi *et al*., 2015).

Statistical analysis: Results were expressed as Mean±SD of four separate experiments. Statistical significance was determined using analysis of variance or Student's t-test (Kuppusamy *et al*., 2016).

RESULTS

The results for the antibacterial activity test f various leaf extracts concentration of *P. pubescens* are shown in Table 1. The *in vitro* test on antibacterial activity revealed that ethanol of leaves of *P. pubescens* inhibited the growth of *B. cereus* and *E. coli* in a concentration dependent manner. The antibacterial activity was detected at 10 μg μL^{-1} leaves extract concentration for the ethanol extraction (Table 1). There was no antibacterial activity in control (Table 1). The growth of *B. cereus* and *E. coli* were inhibited by all concentrations of extracts. The *E. coli* was more sensitive to all various leaf extracts concentration of *P. pubescens* than *B. cereus* (Fig. 2).

The antibacterial activity of *P. pubescens* leaf extracts on *B. cereus* and *E. coli* were detected, the higher concentration exhibited higher antibacterial activity than the lower. The *B. cereus* was sensitive to all 3 commercial antibiotics tested, chlorampehenicol (50 μg μL^{-1}), enrofloxacin (50 μg μL^{-1}) and penicillin (10 U) (Table 2). Anyway, the growth of *E. coli* was inhibited only by chlorampehenicol and enrofloxacin but not penicillin. The *E. coli* was less sensitive to the commercial antibiotics chloramphenicol, enrofloxacin and penicillin than *B. cereus* (Table 2). The antibacterial properties of *P. pubescens* leaves (inhibition zones of 6.00-13.6 mm diameter) were not as effective as the commercial antibiotics chloramphenicol, enrofloxacin and penicillin (inhibition zones of 16.29-36.29 mm).

DISCUSSION

Almost all genus *premna* has been used to treat microbial infections (Sannomiya *et al*., 2015; Kchaou *et al*., 2016). Previous studies reported *P. schimperi* and *P. oligotricha* inhibited many types of bacteria including *E. coli* but not *B. cereus* (Mahboubi *et al*., 2015). There is no reported regarding the antibacterial activity of the leaf of *P. pubescens* against *B. cereus* and *E. coli*.

This study showed antimicrobial sensitivity testing was carried out *in vitro* using paper disc method against the following test organisms, *E. coli* and *B. cereus*. The zones of inhibition were measured after 24 h of incubation. The result of antimicrobial screening (sensitivity testing) using serial dilution method at varying concentrations (0, 10, 20, 30, 40 and 50 μg μL^{-1}) on the *E. coli* and *B. cereus* with zones of

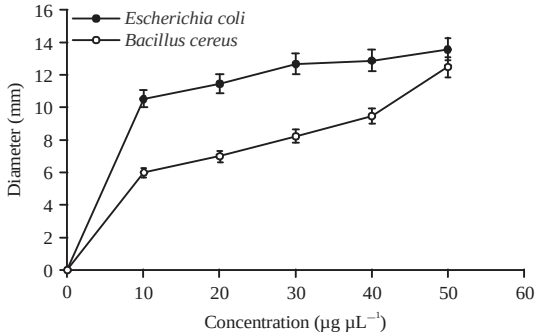

Fig. 2: Antibacterial activity of *P. pubescens* leaf extracts on *B. cereus* and *E. coli*

Table 1: *In vitro* antimicrobial activity of *P. pubescens* leaf extracts on *B. cereus* and *E. coli* (Determined by diameter of inhibition zones)

	Concentrations (μg μL^{-1})					
Microorganisms	0	10	20	30	40	50
Bacillus cereus	0.00±0.00	6.00±0.82^{ax}	7.00±1.41^{bx}	8.25±1.89^{cx}	9.50±1.29^{dx}	12.50±0.58^{ex}
Escherichia coli	0.00±0.00	10.55±0.16^{ay}	11.50±0.28^{by}	12.70±0.22^{cy}	12.91±0.37^{dy}	13.60±0.08^{ey}

Values are Mean±SD (mm) of four separate experiments. $^{a-d}$Means within a row where non-significant no common superscripts differ significantly ($p \leq 0.05$) and ^{x,y}Mean within a column no common differ significantly ($p \leq 0.05$)

Table 2: Inhibition zones of antibiotics

	Concentrations (50 μg μL^{-1})		
Microorganisms	Chloramphenicol	Enrofloxacin	Penicillin (10 U)
Bacillus cereus	27.99±2.30	23.79±2.60	36.29±4.80
Escherichia coli	16.29±2.10	25.69±3.20	0.00±0.00

Values are Mean±SD (mm) of four separate experiments

inhibition recorded in millimetre is as shown in Table 1. The choice of use of chloramphenicol, enrofloxacin and penicillin as clinical standards is based on the fact that at low concentrations, chloramphenicol only inhibits growth of the bacteria through induction of prokaryotic ribosomes to misread mRNA 56. Enrofloxacin prevents initiation of protein synthesis and leads to death of microbial cells. Penicillin inhibits bacterial growth by inhibiting protein biosynthesis (Lakshminarayanan *et al*., 2015).

In vitro of the leaf extracts of *P. pubescens* against against both gram-positive (*B. cereus*) and gram-negative (*E. coli*) bacteria resulted in growth inhibition lower susceptibility than the commercial antibiotics chloramphenicol, enrofloxacin and penicillin (Table 1, 2). It was noticed that the leaf extracts of *P. pubescens* was more potent than penicillin against *E. coli*. So, future studies with higher extract concentrations and the different method of extraction may be useful to evaluate the actual antibacterial properties of *P. pubescens* leaves extracts. Further investigations must be performed to examine the antibacterial properties from leaves, flowers, fruits, barks and roots extracts to other pathogenic bacteria at a higher concentration. In the present study, 50 μg μL^{-1} of extract has the antibacterial activity against *B. cereus* and *E. coli*. Toxicological studies on the extract must also be performed to ensure the safety of the extract. The finding of a potent herbal remedy that is safe will be an advancement opportunities in bacterial infection therapies.

CONCLUSION

In conclusion, *P. pubescens* leaves extracts display antimicrobial properties was able to inhibit moderately *B. cereus* and *E. coli.* Furthermore, this study showed that the antibacterial properties of *P. pubescens* leaves was not as effective as the commercial antibiotics chloramphenicol, enrofloxacin and penicillin, but it was more potent than penicillin against *E. coli*. Further investigations must be performed to examine the antibacterial properties from leaves, flowers, fruits, barks and roots extracts and the different method of extraction evaluate the actual antibacterial properties of *P. pubescens.*

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