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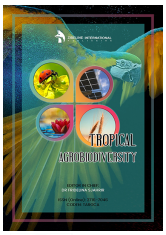

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY DETERMINATION OF *PEPEROMIA PELLUCIDA* EXTRACT

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ABSTRACT

Antibiotics play a vital role in combating diseases caused by pathogenic bacteria. Consumption of antibiotics may emit certain undesirable side effects. Hence, there is an initiative to discover new novel natural products with therapeutic potential, which is safer and can combat various ailments. *Peperomia pellucida,* which belongs to Piperaceae family, is a herbal plant that can be found in many regions of tropical Asia. This study aimed to extract *P. pellucida* and screen its phytochemical compositions, and finally test its antibacterial activity on gram positive and gram-negative bacteria. Ethanolic extraction was conducted to obtain a crude extract of the leaf sample. Phytochemical test screenings were also carried out. Antibacterial assay by agar well diffusion was done for the antibacterial activity to determine the minimum inhibitory concentration (MIC). The results showed that the ethanolic leave extract of *P. pellucida* contains steroids and tannins. The extract exhibited the maximum inhibition zones of 15.67 ± 0.58 mm, 9.67 ± 1.58 mm and 10.67 ± 0.58 mm against *E. coli*, *P. aeruginosa* and *S. aureus* respectively. Based on the MIC test, the ethanolic leaf extracts have shown potential antibacterial activity.

KEYWORDS

Piper pellucida, antibacterial, extraction, minimum inhibition concentration.

1. INTRODUCTION

The emergence of novel diseases caused by pathogenic bacteria has increased over the years (Vouge and Greub, 2016). These harmful bacteria may produce toxins that can deteriorate human health. For this reason, antibiotics play a vital role in combating diseases. However, the consumption of current antibiotics may emit certain undesirable side effects, and this has become a recent predicament in the medical field (Langdon et al., 2016).

Hence, scientists continuously seek alternatives, especially from medicinal plant products, which could enhance the effectiveness of present antibiotics. *Peperomia pellucida* or locally known as "sireh cina," is believed to exhibit antimicrobial properties (Fernandez et al., 2012; Mutee et al., 2010; De Fatima et al., 2004). It is very crucial to conduct extensive research on medicinal plants to evaluate the promising biological properties that are valuable in the pharmaceutical industry. Thus, this study aimed to determine the phytochemical compositions of *P. pellucida* ethanolic extract and to determine its antibacterial activity on selected gram-positive and gram-negative bacteria.

2. MATERIAL AND METHODS

2.1 Collection and preparation of plant material

Peperomia pellucida was collected from the Glasshouse and Nursery Complex (GNC), International Islamic University Malaysia (IIUM) Kuantan, Pahang. The leaves were washed with distilled water and oven-dried (45°C) for three days. The dried leaves were then grounded with an electrical blender to a fine powder and stored in an airtight glass jar. 40 g of the powdered leaves of each plant sample was macerated with 400 mL of 95% ethanol for 72 hours. The extract of *P. pellucida* was filtered and concentrated by evaporation in a vacuum rotavapor at 40 °C. The dried plant crude extracts were kept in a closed dark container in the refrigerator at 2-8°C until further use.

2.2 Phytochemical screening of the extract

The ethanolic extract of *P. pellucida* was screened for the presence of alkaloids, saponins, terpenoids, flavonoids, coumarins, steroids, and tannins.

2.2.1 Test for Alkaloids

Mayer's test: Mayer's reagent was added drop by drop to a test tube containing 1 mL of the ethanolic extract. The formation of a creamy precipitate indicates the presence of alkaloids.

2.2.2 Test for Saponins

Foam test: A small amount of extract was added in a test tube and shaken well with 5 mL of water. If foam produced persists for 10 minutes, this indicates the presence of saponins.

2.2.3 Test for Terpenoids

Salkowaski test: A small amount of crude extract was dissolved in chloroform, and concentrated sulphuric acid was added slowly drop by drop. The formation of reddish-brown colour shows the presence of terpenoids.

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2.2.4 Test for Flavonoids

Shinoda test: Magnesium ribbons were added, followed with few drops of concentrated hydrochloric acid to a test tube containing crude extract dissolve in 5 mL of 95 % ethanol. The mixture was heated until the pink coloured scarlet was observed, which indicates the presence of flavonoids.

2.2.5 Test for Coumarins

Small amounts of extract were added in a test tube with 3 mL of 10 % sodium hydroxide. Yellow colour indicates the presence of coumarin.

2.2.6 Test for Steroids

Liberman-Burchard test: 2 mL of acetic anhydride was added to a test tube containing small amounts of crude extract. The mixture was then boiled and cooled. Few drops of concentrated sulphuric acid were added. Green colour shows the presence of steroids.

2.2.7 Test for Tannins

Ferric chloride test: The crude extracts were dissolved in distilled water and few drops of 5 % ferric chloride solution were added. Green colour shows the presence of gallotannin, while brown colour indicates the presence of pseudotannin.

2.3 Antibacterial assay

The bacterial strains used in this study were *Staphylococcus aureus* (ATCC 25923), which is a gram-positive bacteria. Meanwhile, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were selected for the gram-negative bacteria. All bacterial were grown in the Muller-hinton agar (MHA) plate and maintained in Muller-hinton broth (MHB) at 4°C. A 100 mg of each extract was dissolved in 1 mL each of dimethylsulphoxide (DMSO), making a stock concentration of 100 mg/mL. Two antibiotics, namely tetracycline, and ciprofloxacin, were used as the positive control with a concentration of 10 µg/mL while sterile DMSO was used as the negative control.

2.3.1 Antibacterial Susceptibility Testing

The antibacterial activity of the ethanolic extract of *P. pellucida* was determined by the agar well diffusion method. The density of each microbial suspension was adjusted equal to that of 10^8 cfu/mL (standardized by 0.5 McFarland) and used as inoculums for the assay. The agar plate surface was inoculated by spreading 100 µL of bacterial inoculums over the entire agar surface.

The agar plates were allowed to dry, and 8 mm diameter wells were made with sterile micropipette tips in the inoculated agar. Each of the holes (3 in number) was filled with 50 µL of the extracts (100 mg/mL, 50 mg/mL, 20 mg/mL). The plates were allowed to stand for 1 hour for full diffusion of the extracts and incubated overnight (24 hrs) at 37 °C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition surrounding the well. The tests were performed in triplicates.

2.3.2 Minimum Inhibitory Concentration (MIC) Determination

The MIC for ethanolic extracts of *P. pellucida* was determined by a modified agar well diffusion method (Kamal et al., 2010). The serial-diluted concentrations of crude extracts (100 µL) were prepared habitually using serial two-fold dilutions to achieve a decreasing concentration range of 100 mg/mL to 3.125 mg/mL. A 50 µL volume of each dilution was propelled directly into the wells of MHA plates, which already inoculated with 100 µL of standardized bacterial inoculums (10^8 cfu/mL).

All plates were incubated at 37 °C for 24 hours and observed the inhibition zones. The minimum inhibitory concentration (MIC) endpoint was recorded as the lowest concentration of the crude extracts that shows a clear zone of inhibition (> 8 mm). The tests were performed in triplicates.

3. Results and Discussion

3.1 Phytochemical compounds in *P. pellucida*

Phytochemical constituents of the ethanolic leaf extract of *P. pellucida* were identified based on phytochemical screening tests. The result of the preliminary phytochemical screening of the plant crude extract is presented in Table 1.

Table 1: Phytochemical screening of ethanolic leaf extracts of *P. pellucida*

Phytochemicals	Chemical tests	*P. pellucida*
Alkaloids	Mayer's test	–
Steroids	Libermann-Burchard test	+
Saponins	Foam test	–
Flavonoids	Shinoda test	–
Tannins	Ferric chloride test	+
Coumarin	Coumarin test	–
Terpenoids	Acetic anhydride test	–

(+) Positive; (–) Negative

The phytochemical analysis showed the presence of steroids and tannins only in the ethanolic leaf extracts of *P. pellucida*. Alkaloids, saponins, flavonoids, coumarins, and terpenoids were observed to be absent in this extract. In a previous study, the phytochemical analysis showed the presence of alkaloids and flavonoids while steroids, tannins, and saponins were absent in the methanolic extracts of *P. pellucida*. Meanwhile, in a different study, an ethanolic extract of *P. pellucida* was found to contain tannins, saponins, and flavonoids (Theresa, 2012; Awe et al., 2013). The results may be different due to several reasons, such as the method of extraction, the difference in geographical locations, and environmental conditions. The chemical composition of the plants may be altered in response to climate change or its surrounding environment (Elgawad et al., 2014). Besides that, variation in the extraction method may influence the chemical composition in plants as each solvent has the capability of dissolving or extracting specific phytochemical constituents (Tiwari et al., 2011).

3.2 Antibacterial activity of *P. pellucida*

3.2.1 Antibacterial susceptibility testing

The antibacterial activity, which is indicated by a zone of inhibition for different concentrations of *P. pellucida* ethanolic extract on the tested bacteria, is shown in Figure 1 and Table 2.

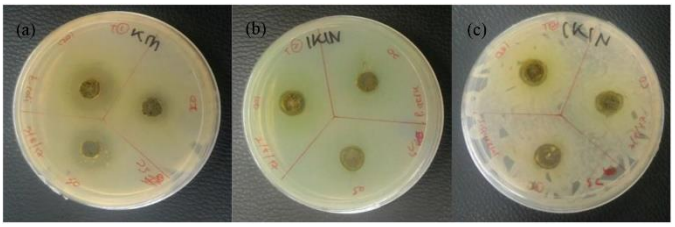

Figure 1: Antibacterial activity of different concentrations in ethanolic leaf extracts of *Peperomia pellucida* on (a) *E. coli*, (b) *P. aeruginosa* and (c) *S. aureus*

Table 2: Antibacterial activity of ethanolic leaf extract of *P. pellucida* by agar well diffusion method

Bacteria	Extract concentration (mg/mL)	Inhibition zone diameter (mean ± S.D) (mm)
		P. pellucida
E. coli	100	15.67±0.58
	50	14.00±1.00
	20	10.67±0.58
P. aeruginosa	100	9.67±1.58
	50	8.67±1.15
	20	NI
S. aureus	100	10.67±0.58
	50	9.33±1.15
	20	9.00±1.00

NI = No Inhibition

Based on the result, the ethanolic leaf extract of *P. pellucida* was found to be effective against *E. coli, P. aeruginosa,* and *S. aureus* at different extract

concentrations. However, the ethanolic extract of *P. pellucida* has no effect on *P. aeruginosa* at a concentration of 20 mg/mL. The data in Table 2 reveals that the extract was most effective against *E. coli* compared to the other tested bacteria. At a concentration of 100 mg/ml, the extract showed the largest inhibition zone at 15.67±0.58 mm against *E. coli*, followed by inhibition zones at 10.67±0.58 mm and 9.67±1.58 mm against *S. aureus* and *P. aeruginosa* respectively. The inhibition zone diameter decreases with the decrease of the extract concentration.

This result is in agreement with the findings observed an antibacterial activity from ethanolic leaf extracts of *P. pellucida* against *E. coli* (12 mm) and *P. aeruginosa* (19.6 mm) (Akinnibosun et al., 2008). Besides, the extract of *P. pellucida* also possessed antibacterial activity against *E. coli* (10 mm), *S. aureus* (10 mm), and *P. aeruginosa* (12 mm) (Oloyede et al., 2011).

Table 3: The control plate

Bacteria	Inhibition zone diameter (mm)		
	Tetracycline (+)	Ciprofloxacin (+)	DMSO (–)
E. coli	ND	34	NI
P. aeruginosa	18	ND	NI
S. aureus	ND	28	NI

ND = Not Determined, NI= No Inhibition, (+) = Positive control, (–) = Negative control

Tetracycline was used as an antimicrobial agent or antibiotic, particularly for *P. aeruginosa,* as it was reported that *P. aeruginosa* was susceptible to tetracycline with a maximum zone of inhibition (Sajjan et al., 2010). Meanwhile, for *E. coli* and S. *aureus*, ciprofloxacin was used as antimicrobial agent. *E. coli* and *S. aureus* was found to possess a high degree of sensitivity rates to ciprofloxacin (Bukhari et al., 2011).

3.2.2 Minimum Inhibitory Concentration Determination

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of compounds or extracts that is capable of inhibiting any microbial growth, and there is no further inhibition below that concentration. The MIC value the extract of *P. pellucida* against *E. coli, P. aeruginosa,* and *S. aureus* are given in Table 4.

Table 4: Minimum inhibition concentration of ethanolic leaf extract of *P. pellucida*

Bacteria	MIC (mg/mL)
E. coli	6.25
P. aeruginosa	25
S. aureus	12.5

E. coli was found to be the most sensitive bacteria on *P. pellucida*, thus having a MIC of 6.25 mg/mL. *P. aeruginosa* was found to be comparatively more resistant in the extract with the recorded MIC at 25 mg/mL. While for the *S. aureus*, the MIC was 12.5 mg/mL. In another study, the methanolic extract of *P. pellucida* showed antibacterial activity against *E. coli, S. aureus,* and *P. aeruginosa* with MIC values of 200, 200, and 100 mg/mL respectively (Oloyede et al., 2011). In contrast, a study reported the MIC of the methanolic leaf extract of *P. pellucida* against *P. aeruginosa* was 2.50 mg/mL while there was no MIC value recorded on *S. aureus* and *E. coli* (Theresa, 2012).

4. Conclusion

In conclusion, the phytochemical screening of ethanolic leaf extract of *Peperomia pellucida* in this study has shown the existence of steroids and tannins. It was found that there was a potential antibacterial activity of ethanolic leaf extracts of the plant against *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa*.

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