



## Phytochemical Screening and Antibacterial Properties of Silverbush (*Peperomia pellucida*) against Selected Cultured Bacteria

Jessica O. Tablang<sup>1</sup>, Ron Patrick C. Campos<sup>1\*</sup> and James Kennard S. Jacob<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, College of Arts and Sciences, Isabela State University-Echague, Isabela, Philippines, 3309

**Correspondence Author:** Ron Patrick C. Campos, Department of Biological Sciences, College of Arts and Sciences, Isabela State University, San Fabian, Echague, Isabela, Philippines,  
Phone number: (+639)21-884-8521, E-mail: rpcampos023@gmail.com

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### ABSTRACT

**Background:** Medicinal plants are great repository of natural products that can be exploited for pharmaceutical development. *Peperomia pellucida* (L.) Kunth is a widely utilized plant that is traditionally used as treatment for various illnesses. **Objective:** This study aimed to determine the phytochemistry and antimicrobial activity of the crude extracts of *P. pellucida*. **Results:** Phytochemical tests revealed that *P. pellucida* contains valuable phytochemical constituents namely alkaloids, flavonoids, glycosides, terpenoids and steroids. Moreover, ethanolic extracts of *P. pellucida* showed antibacterial activity against *E. coli* and *S. aureus* with a notable average inhibition of 15.43±0.67 mm and 13.22±0.34 mm respectively. **Conclusion:** These results affirm that *P. pellucida* possesses bioactive compounds that can be potentially developed as novel drugs.

**Key words:** medicinal plant, *Peperomia pellucida*, phytochemistry, secondary metabolites, shiny bush

### INTRODUCTION

The study of medicinal plants has increasingly become the focus of extensive research throughout the world mainly because of the diversity and potential that these plants offer as source of novel natural products (Lee *et al.*, 2008; Street, 2012). *Peperomia pellucida*, also called Silverbush, belongs to the family Piperaceae. It is an herbaceous plant found in many South American and Asian countries. The plant is distinguished by its heart-shaped and fleshy leaves with lush and succulent stems, shallow roots and small flowers, which eventually develop into numerous tiny seeds attached on cord-like spikes (Mosango, 2008). The plant has been widely utilized in various medicinal practices and is used traditionally for treatment of different ailments such as convulsions, conjunctivitis, headache, fever, gout, skin diseases and rheumatic pains (Mosango, 2008; Ghani, 1998).

Natural products of plant origin remain the most important sources of new drugs (Odugbemi, 2006). Studies regarding plant phytochemistry and antimicrobial efficacy are therefore important because of their historical significance and also due to the fact that a portion of the world still relies on plants for the treatment of different diseases (Martinez *et al.*, 1996; Jadeja *et al.*, 2005). With this, the present study was carried out to determine the phytochemistry and antimicrobial activity of the crude extracts of *P. pellucida* against several selected cultured microorganisms.

### MATERIALS AND METHODS

#### Collection of plant samples

Plant samples of *P. pellucida* were collected from various areas within the Isabela State University-Main Campus, Isabela, Philippines. The shoot parts of the plant were obtained, and the roots were discarded. The samples were rinsed with distilled water and dried in open air under a shade in order to prevent the ultra violet rays from inactivating the chemical substances present in the plant. The dried leaves were then pulverized using mechanical grinder to a fine powder and was stored.

#### Preparation of Crude Extract

Fifty (50) grams of powdered sample of *P. pellucida* was soaked on 500 mL laboratory grade 95% ethanol for 48 hours. It was then filtered using a Whatman filter paper No. 1 in an Erlenmeyer flask. The filtrate was refluxed in a rotary evaporator until a sticky residue is obtained. The extracted product was stored and maintained in a flask.

### **Qualitative Phytochemical Screening**

The crude extracts of *P. pellucida* were qualitatively screened for the presence of phytochemicals based on standard protocols described by Khandelwal (2002) and Sofowara (1993). Results were determined based on the color and intensity of the reaction and was interpreted as (+) if chemical is present in traceable amount and (-) if chemical is absent.

#### **Test for alkaloids**

Wagner's test: A few ml of the extract was added with a few drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide dissolved in 100 ml of distilled water). The formation of reddish-brown precipitate confirms the presence of alkaloids.

#### **Test for saponins**

Foam test: A portion of the extract was diluted with distilled water until the volume was made up to 20 ml. The solution was vigorously shaken and the formation of persistent froth indicates presence of saponins.

#### **Test for tannins**

Braymer's test: A fraction of the extract was added with 10% alcoholic FeCl<sub>3</sub> solution and the formation of blue or brown to dark green coloration indicates the presence of tannins.

#### **Test for glycosides**

Keller-Kelliani test: Five (5) ml of the extract was mixed with two (2) ml of glacial acetic acid (CH<sub>3</sub>CO<sub>2</sub>H) containing one (1) drop of FeCl<sub>3</sub>. The mixture was carefully added to a prepared one (1) ml of concentrated H<sub>2</sub>SO<sub>4</sub> to form a lower layer. The presence of a brown ring at the interface indicates presence of glycosides.

#### **Test for terpenoids**

Salkowski's test: Two (2) ml of the extract was added with one (1) ml of chloroform followed by a few drops of concentrated sulfuric acid. The immediate presence of reddish-brown layer indicates the presence of terpenoids.

#### **Test for flavonoids**

Alkaline Reagent test: A few drops of sodium hydroxide solution (aqueous NaOH and HCl) were added to a small amount of the extract and observed for the formation of yellow to orange color.

#### **Test for sterols**

Liebermann-Burchard test: One (1) ml of the extract was dropped with a few ml of acetic anhydride and chloroform, then carefully added with drops of H<sub>2</sub>SO<sub>4</sub>. The formation of dark green to red color indicates presence of sterols.

### **Source of test bacteria**

Bacterial isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from the culture collections of the Microbiology and Bio-Industry Laboratory, College of Arts and Sciences, Isabela State University-Echague. The bacterial isolates were maintained in Nutrient Broth medium for the antibacterial assay.

### **Antibacterial Assay**

The antibacterial analysis was carried out using disc diffusion method. This involved the use of filter paper discs as carrier for the antimicrobial agents. Circular sterilized discs with 6 mm diameter was cut from Whatman no. 1 filter paper and impregnated with liquid treatments. Cultures of *E. coli* and *S. aureus* were aseptically inoculated onto Petri plates containing Mueller-Hinton Agar using sterile cotton swab and then the impregnated discs were placed on the surface of the medium afterwards. The plates were incubated at 37°C and turned upside-down to prevent contamination. The zone of inhibition of each paper disc was observed and recorded every eight (8) hours within a 24-hour incubation period. Zone of inhibition was measured using a calibrated digital Vernier caliper. The assay was conducted in triplicates with the following treatments: distilled water (T<sub>1</sub>), streptomycin sulfate (T<sub>2</sub>), laboratory grade 95% ethanol (T<sub>3</sub>) and ethanol extract (T<sub>4</sub>).

### **Statistical Analysis**

Statistical analysis was laid out in a Completely Randomized Design (CRD) with three replicates per treatment. The recorded data were treated statistically using one-way analysis of variance (ANOVA). The means were compared by Least Significant Difference test at p < 0.05 using IBM™ SPSS v23.

## RESULTS AND DISCUSSION

The ethanolic extract of *P. pellucida* was used to determine the phytochemical constituents of the plant. The ethanolic extracts of the plant indicated presence of the different phytochemical constituents (Table 1). The results indicate that *P. pellucida* contains valuable phytochemical constituents namely alkaloids, flavonoids, glycosides, terpenoids and steroids. However, the results also suggest the absence of saponins and tannins.

**Table 1. Phytochemical constituents of *P. pellucida* crude extract.**

PHYTOCHEMICAL SCREENING	
Constituents	Result
Alkaloids	+
Saponins	-
Flavonoids	+
Glycosides	+
Terpenoids	+
Tannins	-
Steroids	+

**Legend:** (+) means presence of the chemical; (-) means absence of the chemical

A handful of studies have elucidated the several phytochemical constituents of the plant. One study has isolated four chemicals from *P. pellucida* namely alkaloids, cardenolides, saponins and tannins by using dried powdered samples of the plant (Egwuche *et al.*, 2011). On the other hand, saponins were believed to be selectively present only in the leaf extracts of the plant (Majumder, 2012). Discrepancy of results obtained in the phytochemical screening can be attributed to the difference in the extraction method. The results show that *P. pellucida* contains pharmacologically active compounds which can be utilized in a wide array of pharmaceutical applications.

The presence of bioactive compounds in *P. pellucida* provides the plant with various effects on biological activities. The zones of inhibition of the different treatments against *E. coli* and *S. aureus* is presented in Table 2.

**Table 2. Antibacterial activities of *P. pellucida* crude extract against *E. coli* and *S. aureus*.**

Treatment	Zone of Inhibition (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
Distilled water	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>
Streptomycin	33.07±2.98 <sup>b</sup>	32.20±1.72 <sup>b</sup>
Ethanol	6.97±0.42 <sup>a</sup>	8.13±0.47 <sup>a</sup>
<i>P. pellucida</i> extract	15.43±0.67 <sup>c</sup>	13.22±0.34 <sup>c</sup>

**Note:** Means in the same column not sharing the same superscript are significantly different at 5% significance level.

The results indicate the presence of bioactive compounds in the *P. pellucida* extracts that can inhibit the growth of both *E. coli* and *S. aureus*. The antibacterial drug streptomycin, treated as positive control, inhibited the *E. coli* with a diameter of 33.07±2.99 mm and *S. aureus* with 32.20±1.72 mm. The ethanolic extracts of *P. pellucida* recorded an average diameter of 15.43±0.67 for the *E. coli* and 13.22±0.34 for the *S. aureus*. Ethanol exuded small zones of inhibition with 6.97±0.42 and 8.13±0.47 for the *E. coli* and *S. aureus* respectively. The negative control distilled water produced no zone of inhibition. Statistical analysis revealed that the ethanolic extracts of *P. pellucida* were significantly different with the zones of inhibition produced by distilled water, ethanol and streptomycin.

The antibacterial properties of *P. pellucida* has been investigated by several authors and results indicated the presence of inhibiting properties in the plant. The plant has been determined to possess broad spectrum of antimicrobial activity against different strains of pathogenic bacteria and fungi namely *E. coli*, *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*,

*Klebsiellae pneumoniae*, *Salmonellae typhi*, *Candida albicans*, *Penicillium notatum*, *Rhizopus stolonifer* and *Aspergillus niger* (Oleyede *et al.*, 2011). These results are further corroborated by multiple studies involving the antibacterial potential of the plant (Wei *et al.*, 2011). It has also been claimed that the chemical constituent dillapiol may be one of the agents that are responsible for its antimicrobial activity (Rafael *et al.*, 2008; Brazao *et al.*, 2014). Phytol was believed to be a major compound present in the tissues of *P. pellucida* and is one of the most important diterpenes that possesses antimicrobial properties (Kumar *et al.*, 2010). The results indicate the presence of antibacterial agents in the plant despite the variance in the method of extraction of the chemical constituents. It is clear that the ethanolic extract of *P. pellucida* possessed antibacterial properties which can be tapped for exploitation in different pharmacological applications.

## CONCLUSIONS

Based on the foregoing results, the ethanolic extract of *P. pellucida* possessed phytochemical constituents that can be utilized for the isolation of important bioactive compounds. The isolation of bioactive compounds can provide a novel and economic way of obtaining important chemicals that possess various pharmaceutical applications. The presence of antibacterial properties within the plant can also be of importance for the development of new therapeutic agents.

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