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PHYTOCHEMICAL STUDY, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL TESTING OF LEAF EXTRACTS OF AKAPULKO (*Cassia alata* L.), PANSIT-PANSITAN (*Peperomia pellucida* L.) AND SAMBONG (*Blumea balsamifera* L.)

K.G.D. WAING[2*], M.A. MALUBAG[1], P.K. AYSON[1], L.J. QUINTERO[1], S.Y. TENORIO[1], AND D.S. CASTILLO[2]

[1)] Senior High School, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija

[2)] Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija

[*]**Corresponding Author: E Mail:** mailaalexamalubag@gmail.com

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ABSTRACT

Medicinal plants are important to our society as it provide the people the benefits it can give. This studied the phytochemicals present in the leaf extracts of *C. alata* L., *P. pellucida* L., and *B. balsamifera* L. and evaluated their antioxidant and antibacterial potential. In terms of antioxidant activity, DPPH radical scavenging assay was used while the disc diffusion method for the antibacterial testing. Saponins, tannins, terpenoids, and alkaloids were found to be present in the leaf extracts of *C. alata* L., *P. pellucida* L., and *B. balsamifera* L. Furthermore, *C. alata* L. *P. pellucida* L. and *B. balsamifera* L., were reported to have a potential as an antioxidant. The antibacterial testing of the plants resulted that *C. alata* L. has the ability to inhibit the growth of *E. coli* after 12, 18, and 24 hours of incubation.

Keywords: *Cassia alata*, *Peperomia pellucida*, *Blumea balsamifera*, Phytochemical, Antioxidant, and Antibacterial

INTRODUCTION

Medicinal plants play a vital role in people lives. These help individuals to cure or heal such diseases. Additionally, plant extracts are being used nowadays in terms of food, in

cosmetics and in pharmaceutical industries. In order to trace active compounds, a systematic study of medicinal plants is significant **[1]**. The study involves three medicinal plants namely akapulko (*Cassia alata* L.), pansit-pansitan (*Peperomia pellucida* L.) and sambong (*Blumea balsamifera* L.). *C. alata* L. is locally known as akapulko which belongs to the Fabaceae family **[2]** and is said to cure cough and are used as mouthwash and purgative. On the other hand, *P. pellucida* L. or commonly called as pansit-pansitan belongs to the Piperaceae family. It is an annual herb that is widely evident in some South-American and Asian countries **[3]**. The leaves are said to have a healing treatment in headache, fever, eczema, abdominal pains and convulsions. Lastly, *B. balsamifera* L. which is known as sambong, belongs to the family of Asteraceae that grows in Southeast Asia. The leaves are used as a tea and said to cure certain disorders such as rheumatism and hypertension **[4]**. There has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury **[5]** with the emergence of vaccines and antibiotics, infectious diseases that were previously fatal are now being controlled or eliminated. However, some strains of pathogens are becoming resistant to antibiotics like *Escherichia coli* thus, the demand for discovery of new antibiotics and alternative medications are on-going. As a result, antibiotic resistance is a global concern because of persistent developments of antibiotic resistant pathogens **[6]**.

The study generally aimed to evaluate the phytochemicals present in the leaves of the three plants namely *C. alata* L., *P. pellucida* L., and *B. balsamifera* L. Also, antibacterial and antioxidant activities of these plants were also tested and evaluated.

MATERIALS AND METHODS

Plant Materials

C. alata L., *P. pellucida* L., and *B. balsamifera* L. were collected at the vicinity of Quezon and Talavera, Nueva Ecija. The plants were picked by hands and put in polypropylene bags to serve as their containers before air drying. Gathering of the three plants were done in the morning to ensure that the plants were actively photosynthesizing.

Preparation of Extracts

This study used two types of extraction procedures which were the crude and hot water method. For crude extraction, 100 g of the leaves were disinfected using 70% ethyl alcohol. The leaves were then properly washed using distilled water and were crushed in pre-sterilized mortar and pestle by adding 5 ml of distilled water **[7].** The extract

was then filtered using a Whatman No.1 filter paper. On the other hand, hot water extraction was done using 20 g of powdered air dried plants. The functional components of the plant sample were obtained through distilled water extraction following the procedure of Eguchi *et al.* **[8].** Six hundred ml of distilled water was added to 20 g of powdered plant sample and was stirred in a 1000 ml capacity flask. Afterwards, the mixture was placed in a double boiler water bath for two hours at 80°C to 90°C. Then, the extracts were filtered using the Whatman No. 1 filter paper. The extracts were transferred in a sterile amber bottle and were refrigerated for prior use.

Phytochemical Screening

The test for different phytochemical constituents were carried out on the extracts of *C. alata* L., *P. pellucida* L., and B. *balsamifera* L. following the standard methods as given and described in Laboratory Manual for the UNESCO **[9]** utilizing the point scale + (present in trace amount),++ (present in appreciable amount) and - (absence of phytochemical). Alkaloids, flavonoids, saponins, steroids, tannins and terpenoids were the following phytochemical constituents that were tested.

Test for alkaloids

Five milliliters of the extract were prepared, and then 200 milliliters of 10% HCH_3CO_2 in C_2H_5OH was added. The mixture was filtered and the extract was allowed to become concentrated in a water bath until it reached one fourth of the original volume. Formation of the white precipitate or turbidity indicated the presence of alkaloids **[10].**

Test for flavonoids

Few drops of diluted sodium hydroxide were added in one milliliter of the extract. An intense yellow color was observed indicating the presence of flavonoids**[11].**

Test for saponins

The plant extract was diluted with 20 milliliters of distilled water and agitated for 15 minutes in a test tube. Presence of saponins were observed with the formed1cm layer of foam **[12].**

Test for steroids

Two milliliters of acetic anhydride was added to a 5 milliliters extract of plant sample with 2 milliliters of H_2SO_4. Presence of steroid was indicated by violet to blue or green precipitate **[12].**

Test for tannins

One percent of lead acetate was added in five milliliters of the extract. If yellow precipitate was formed, it indicated the presence of tannins **[11].**

Test for terpenoids

Five milliliters extract of plant sample was added with 2 milliliters $CHCl_3$ in a test tube. Then, 3 milliliters of H_2SO_4 was added

carefully to the mixture to form a layer. The formation of the reddish to brown color on the interface indicated the presence of terpenoids **[12].**

Antioxidant Assay using DPPH Radical Scavenging Assay and Total Phenolic Content

To determine the radical scavenging activity of the three plants *C. alata* L., *P. pellucida* L. and *B. balsamifera* L., the DPPH radical scavenging activity assay by Kolak *et al*. **[13]** was used. The crude culture extract was dissolved in methanol to a final concentration of 500 ppm. A 0.1 mM DPPH in methanol was freshly prepared by diluting 1 ml DPPH stock solution (3.49 mg DPPH in a 10ml methanol) to 100 ml methanol. Then, 1 ml of each of the crude extracts and 4ml of DPPH solution were mixed and incubated in the dark at 37^{o}C for 30 minutes. The reaction was done in triplicates. The absorbance reading was monitored at 517 nm using UV-Vis spectrophotometer (APEL-100). Finally, the ability to scavenge the DPPH radical was calculated using the equation: %DPPH scavenging effect = $[(A_{control}-A_{sample})/A_{contol}]x$ 100 where $A_{control}$ was the absorbance of the control which was the DPPH solution without crude culture extract, the A_{sample} was the absorbance of the test sample containing the mixture of DPPH and the crude culture extract. The synthetic antioxidant cathechin was used as positive control. In terms of evaluating the total phenolic content of *C. alata* L., *P. pellucida* L. and *B. balsamifera* L., the Folin – Ciocalteu method was used **[14]**. At first, a calibration curve was made at different concentrations (0.25, 0.5, 1.0, and 4.0 mg /ml) of ascorbic acid solutions using APEL-100 UVV is spectrophotometer (PD-303UV). These ascorbic acid solutions (volume of 20 mcL) were placed in vials. To each vial, 200mcL of Folin – Ciocalteu reagent was added and the vials were incubated at room temperature for 5 minutes. For this experiment, 20 ml of sodium carbonate with the concentration of 1 mg/ml was also prepared. Then, 1 ml sodium carbonate was added to the mixture of ascorbic acid and FC reagent was transferred to cuvettes and read using UV-Vis spectrophotometer at 680 nm wavelength. After standardization the calibration curve, 3 mg of the extracts were dissolved in 3ml distilled water. From each of the extracts, 200mcL was transferred to vials (in triplicates). To these, 200 mcL of DC reagent was added and incubated at room temperature for 5 minutes. Then, 1 ml sodium carbonate was added to the mixture of extracts and FC reagent and read using the APEL-100 UV-Vis spectrophotometer (PD-303UV) at 680 nm wavelength. Absorbance values of the extracts were compared with

the calibration curve using the ascorbic acid. The total phenolics were calculated based on the standard curve of the ascorbic acid and its linear regression as shown in the equation.

Experimental Treatments

As shown in Table 1, the different treatments were used in the study. Crude and hot water extracts of the three plants were tested against *E. coli*. These were *C. alata* L., *P. pellucida* L., and *B. balsamifera* L.

Table 1: Experimental treatments

TREATMENT	TREATMENT AND EXTRACTION PROCEDURE
T_1	*C. alata* L. (Crude Extract)
T_2	*C alata* L. (Hot Water Extract)
T_3	*P. pellucida* L. (Crude Extract)
T_4	*P. pellucida* L. (Hot Water Extract)
T_5	*B. balsamifera* L. (Crude Extract)
T_6	*B. balsamifera* L. (Hot Water Extract)
T_7	Streptomycin sulfate (Positive control)
T_8	Distilled water (Negative control)

Test Organisms

E. coli was used as bacterial test pathogen for the antibacterial activity screening of the three plants. Test organism was obtained from the Department of Biological Sciences, College of Arts and Sciences, Science City of Munoz, Nueva Ecija.

Evaluation of Antibacterial Property

Preparation of inoculum

The method in "Manual on Antimicrobial Susceptibility Testing: Disk Diffusion Test" **[15]** with minor modification was used as reference in the preparation of the inoculum. Five well-isolated colonies of the same morphological type were selected from an agar test tube culture. Isolates were transferred into a test tube containing 10 milliliters nutrient broth. The broth culture was incubated for 24 hours. The turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. The comparison was done visually comparing the inocula tube and the 0.5 McFarland standards against a printed paper with a white background and contrasting black lines under sufficient light condition.

Preparation of assay plates

Twenty eight grams of Nutrient Agar (NA) was dissolved in one liter of distilled water and sterilized by autoclaving for 30 minutes in 15 psi at 121^{o}C. Approximately 20 ml of sterilized media was poured at the sterilized petri plates and allowed to solidify. Digital pipettor was subjected on the nutrient broth which contains inocula and swab thoroughly on the base layer of the plate using a sterilized cotton swab.

Preparation of paper discs

Paper disc made from filter paper (Whatman No. 1) measuring approximately 6

millimeters in diameter were prepared with the use of a paper puncher. The paper discs were placed in petri plates and then sterilized in an autoclave for 30 minutes in 15 psi at 121 °C.

Statistical Analysis of Data

In terms of the antibacterial property of *C. alata* L., *P. pellucida* L. and *B. balsamifera* L., the experiments were laid out in a Complete Randomized Design (CRD). For the characteristics observed as affected by the different treatments, Analysis of Variance (ANOVA) was used. The comparisons among means were done using Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULT AND DISCUSSION

Phytochemical Screening

Table 2 shows the results obtained from the leaf extracts of *C. alata* L., *P. pellucida* L., and *B. balsamifera* L. Saponins, tannins and terpenoids were present in the *C. alata* L. CE while the said phytochemicals were absent on the hot water extract. In terms of *P. pellucida* L. the saponins and terpenoids were present in crude extract while alkaloids and terpenoids were also present in the hot water extract. Lastly, on the plant *B. balsamifera* L. alkaloids and saponins were both present in crude and hot water extracts.

Studies like El-Mahmood and Doughari **[16]** shows that *C. alata* L. contains saponins and tannins. Terpenoids were also found present in the study of Raji *et al*. **[17].** On the other hand, studies of Gini and Jothi **[18]** and Oloyede *et al.* **[19]** reported that alkaloids, tannins and terpenoids were present in *P. pellucida L.* In terms of *B. balsamifera* L., studies of Doctor & Manuel **[20],** reported that alkaloids are present in this plant.

Antioxidant Activity

Table 3 shows that *B. balsamifera* L. had the highest scavenging activity with 73.99%, followed by *C. alata* L. (45.96%), and lastly the *P. pellucida* L. (21.21%). Even though the results showed that they were lower than the Cathechin (positive control), the promising potential of these plants as antioxidant are still evident. In terms of the total phenolic content, *P. pellucida* L. shows the highest content (153.58 mg/GAE) followed by *C. alata* L. (43.17 mg/GAE) and the lowest which was *B. balsamifera* L. (26.92 mg/GAE).

Studies of Sarkar *et al,* **[21],** Mutee *et al*. **[22]** and Thach *et al*. **[23]** found that the three plants which were *C. alata* L., *P. pellucida* L. and *B. balsamifera* L. have a potential as an antioxidant.

Antibacterial Testing

Results on the antibacterial testing shows that *C. alata* L. inhibited *E. coli* after 12, 18 and 24 hours of incubation together with the positive control. The *C. alata* L. CE inhibited

growth of the *E. coli* after 12 hours (2.83 mm ± 2.56), 18 hours, (4.17 mm ± 3.82) and 24 hours (2.83mm ± 2.47) of incubation.

A study conducted by Karthika *et al.* **[24]** about the antimicrobial potential of *Senna alata* L. leaf extracts where in they used Streptomycin and tetracycline (50 µg/ml each) as reference drugs, and the corresponding solvents (ethanol, methanol, chloroform, acetone, benzene, petroleum ether, and aqueous) are used as positive controls, They reported that *E. coli* is found to be the fourth most sensitive organism with a measurement of 27 mm and considered *S. alata* L. with antimicrobial activity.

Studies suggest that the capacity of the extracts to inhibit such pathogens is because of the active secondary metabolites. Reports of Levin *et al.* **[25]** and El-Mahmood & Doughari **[16]** tend to agree with the kind of potential the extract exhibited. They reported that the antimicrobial properties of plants have a linked to the presence of bioactive secondary metabolites such as alkaloids, tannins, saponins, flavonoids, phenols, glycosides and diterpenes. From the results obtained from the phytochemical screening of this study, it was found out the tannins and saponins were present which may be one of the reason why the *C. alata* L. has the power to inhibit the *E. coli.*

Table 2: Phytochemical analysis of three plants namely *C. alata* L., *P. pellucida* L., and *B. balsamifera* L.

PHYTOCHEMICALS	*C. alata* L.		*P. pellucida* L.		*B. balsamifera* L.	
	CE	HWE	CE	HWE	CE	HWE
Alkaloids	-	-	-	++	+	++
Flavonoids	-	-	-	-	-	-
Saponins	++	-	++	-	++	++
Steroids	-	-	-	-	-	-
Tannins	++	-	-	-	-	-
Terpenoids	++	-	++	++	-	-

(+) present on trace amounts; (++) present on appreciable amount; (-) absent

Table 3: Radical scavenging assay and total phenolic content of *C. alata* L., *P. pellucida* L., and *B. balsamifera* L.

PLANT	Radical Scavenging Assay (%)	Total Phenolic Content (mg/GAE)
C. alata L.	45.96%	43.17 mg/GAE
P. pellucida L.	21.21%	153.58 mg/GAE
B. balsamifera L.	73.99%	26.92 mg/GAE
Cathechin (positive control)	78.91%	

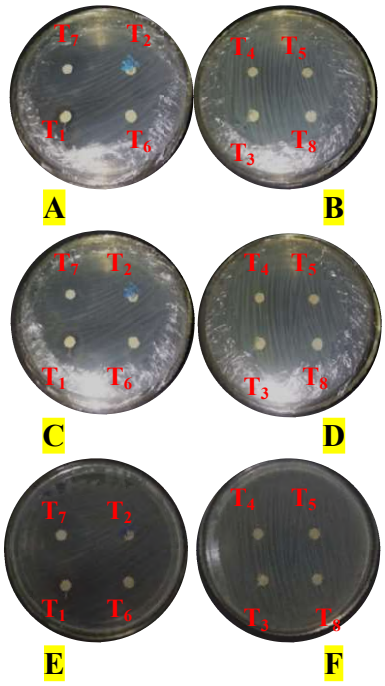

Figure 1: Zone of inhibition exhibited by different treatments against *E. coli* after (A and B) 12 hours, (C and D) 18 hours, and (E and F) 24 hours of incubation; T_1 (*C. alata* L. CE), T_2 (*C. alata* L. HWE) T_3 (*P. pellucida* L. CE), T_4 (*P. pellucida* L. HWE), T_5 (*B. balsamifera* L. CE), T_6 (*B. balsamifera* L. HWE), T_7 (positive control) and T_8 (Distilled water

Table 3: Measured zone of inhibition against *E. coli* after 12, 18, and 24 hours of incubation using crude and hot water extract

TREATMENTS	Diameter of zone of inhibition (mm)		
	12 hours	18 hours	24 hours
T_1 (*C. alata* L. CE)	2.83mm ± 2.56	4.17 mm ± 3.82	2.83 mm ± 2.47
T_2(*C. alata* L. HWE)	0	0	0
T_3(*P. pellucida* L. CE)	0	0	0
T_4(*P. pellucida* L. HWE)	0	0	0
T_5 (*B. balsamifera* L. CE)	0	0	0
T_6 (*B. balsamifera* L. HWE)	0	0	0
T_7(Positive control)	17.00 mm ± 2.60	18.83 mm ± 3.05	17.00 mm ± 3.28
T_8 (Distilled water)	0	0	0

***Means in each column followed by a common letter are not significantly different at 5% level by DMRT**
***0 – No inhibition**

CONCLUSION

Alkaloids, tannins, saponins, and terpenoids were the phytochemicals present in the three plants namely *C. alata* L., *P. pellucida* L., and *B. balsamifera* L. On the other hand, all plants are reported to have potential antioxidant activity which can scavenge free radicals. In terms of antibacterial testing, the *C. alata* L. inhibited the growth of *E. coli*.

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