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Screening of functional activities of *Phyllanthus acidus*

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Abstract *Phyllanthus acidus* is commonly known as gooseberry tree belongs to family Phyllanthaceae which usually grow in tropical areas. In order to establish its functional activities, the antioxidant, phenolic content, antibacterial and molluscicidal activities of the plant were assessed. *P. acidus* aqueous extract showed 57.14 % radical scavenging activity and contained 29. 41 mg GAE/ g total phenolics. The bacterial inhibitory activity of *P. acidus* hot water extract against *E. coli* and *S. aureus* showed a mean diameter zone of inhibition of 0.58 mm and 5. 19 mm respectively. Whereas, the aqueous extract showed no bacterial inhibitory activity against the bacterial pathogens. Meanwhile, the molluscicidal activity of *P. acidus* against *Pomacea canaliculata* (golden apple snail) showed that the extract with a concentration of 1000 ppm had a mean of 2.38 which showed the highest mean mortality rate among the three concentration of aqueous extract tested. Results of the screening of functional activities of the plant extract showed a continuous effort to find a new pharmacological potentiality and plant molluscicides.

Keywords: Gooseberry tree, Molluscicide, Phenols, Radical scavenging activity, Saponin

Introduction

Phyllanthus acidus or most commonly known as gooseberry tree belongs to family Phyllanthaceae. According to Fern (2014), it is a deciduous tree, growing 20-30 ft. high, with an open sparingly branched, spreading crown. The leaves of *P. acidus* is completely green including the flowers. Leaves are simple, oblong acute or obtuse and slightly oblique measuring 14 mm long and six mm broad bearing inconspicuous flowers in pairs in their axils and each pair of flowers comprises one male and one female. In tropical areas, the tree is often grown as an ornamental plant (Jagessar *et al*., 2008).

At present, it had been observed that there is an increased appearance of numerous adverse reaction from drugs made from synthetic sources. Thus, various researches on the use of medicinal plants as alternative sources of medicines were being done. Therefore, it is necessary to do more scientific

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research which guarantees the rational and safe use of medicinal plant extracts (Rodriguez *et al.,* 2006). Compounds found in plants that can scavenge free radicals have a great potential in treating some diseases (Liu *et al.* 2008). These compounds are important because it act as antioxidant that stops the reaction of free radicals with other molecules in the body.

Moreover, plants that accumulate organic substances are more useful in pharmaceutical industry. Due to the increasing rate of drug resistant bacterial isolates, medicinal plants were used because of its bioactive molecules. According to Satish *et al.* (2008), drugs that are plant derived are safer compared to synthetic antibiotics. Plants were found to be natural resources of antimicrobials. Antibiotics are substances that have the ability to treat and effectively stop infection. As the use of antibiotics became widespread it led to increased occurrence of antibiotic resistant bacteria. Due to this, scientists were urged to explore other alternative or natural resources of antibiotics (Gerson 2002).

Furthermore, one of the most serious threats to the rice industry is *Pomacea canaliculata* or most commonly known as "golden kuhol," it is a single most serious invertebrate pest of rice. At present, there is a continuous search for possible sources of mollusicides that are cheaper and environmental friendly and are non-toxic to non-target animals and do not bring harmful effects to the aquatic environment (El-Sherbini *et al.,* 2009). In addition, plants have also been the subject of countless investigations since they are a rich source of medications. The usage of medicinal plants in industrialized civilizations has been expanding due to the extraction and creation of various medications (Rahman *et al*., 2011). This research developed a new molluscicide based on plant material that is effective against pests while still being safe for the environment and human health. Therefore, this study aimed to determine the functional activities of *P. acidus* to find new potential sources of natural antioxidants with antibacterial and molluscicidal activities.

Materials and methods

Collection and preparation of plant sample

Phyllanthus acidus leaves were collected at Bantug, Science City of Munoz, Nueva Ecija, Philippines. It was placed in clean brown paper bag and thoroughly washed to remove adhering debris using distilled water in

preparation for air-drying for about two weeks. Air dried plant sample were pulverized using a homogenizer.

Preparation of aqueous extract

In an Erlenmeyer flask, 30 grams of powdered leaves were mixed with 30 milliliters of distilled water. For 24 hours, the plant specimens were immersed. The plant sample was filtered via Whatman qualitative filter paper No. 1 to suspend all impurities. The filtered solution was placed in an amber container and kept refrigerated until it was needed.

Preparation of hot water extract

The functional components of the leaves were extracted using a hot-water extraction method, as described by Eguchi *et al.* (1999). Twenty grams of powdered plant sample were added in a 600 ml distilled water in a 1000 ml capacity Erlenmeyer flask. This was placed in a double boiler bath for 2 hours at $80^{O}C$ - $90^{O}C$. Filtration using Whatman qualitative filter paper no. 1 separated the powdered plant sample from the extract, which was then refrigerated until use.

DPPH radical scavenging activity assay

The antioxidant activity was determined using the radical scavenging activity following the procedure of Kolak *et al.* (2006) at Chemistry Laboratory of Center for Natural Sciences at St. Mary's University, Bayombong, Nueva Vizcaya. The plant aqueous extract was used to make a stock solution and aliquot was taken to make 1000ppm dilution. Catechin (1mg/ml) was used as control. One ml of prepared stock solution was mixed with 4 ml of 0.1 Mm DPPH solutions in separate plastic cuvette. Reactions were done in triplicates. The prepared mixtures were incubated in the dark at $37^{O}C$ for 30 minutes. Using the UV VIS spectrophotometer, absorbance readings were monitored at 517 nm. A lower absorbance of the reaction of the mixture indicated higher free radical scavenging activity. Computed radical scavenging activity of the plant aqueous extract was compared to the activity of the control. The DPPH radical was calculated using the following formula:

$$\% \text{ Radical Scavenging Effect} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where: *A control* - absorbance of the control (DPPH without the test sample); *A sample* - absorbance of the test sample (mixture of the DPPH and the sample)

Total phenolic content

The amount of total phenolic content in the extract was determined using Folin-Ciocalteu reagent as described hy Hodzic *et al*. (2009) at Chemistry Laboratory of Center for Natural Sciences at St. Mary's University, Bayombong, Nueva Vizcaya. The total phenolics were expressed as mg/g Gallic Acid Equivalents (GAE) using gallic acid as control. Concentrations of 31.25, 15.63, 7.81, 1.95, 0.98, 0.49 and 1 mg/ml of gallic acid were prepared in methanol. Concentration of 1 mg/ml *P. acidus* extract were also prepared in methanol and 0.5 ml of each sample were introduced into the test tubes and mixed with 2.5 ml of 10 fold diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The Folin-Ciocalteu reagent was sensitive to reducing compounds such as polyphenols. Then, tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature. Absorbance of the resulting blue color was measured at 760 nm. Experiment was performed in triplicates.

Antibacterial assay as eradicant

Previously plated plates with Mueller Hinton Agar (MHA) were inoculated with 0.1 ml of bacterial suspension using L-rod. The bacterial cell density was equivalent to $1.5x10^8$ bacterial cells/ml using the 0.5 McFarland Standard. The prepared discs soaked in hot water extract and aqueous extract of *P. acidus* as well as the discs soaked in streptomycin sulfate (positive control) and sterile distilled water (negative control) were seeded equidistantly in the previously inoculated plates. After which, plates were incubated within 48 hours at room temperature in an inverted position. After incubation, presence of inhibitions were measured using a vernier caliper indicating the potential antibacterial activity of the plant extracts.

Collection of golden apple snail

Matured golden apple snail (*P. canaliculata*) measuring 6-10 mm was collected from the rice field in Villa Pinili, Bantug, Science City of Munoz,

Nueva Ecija, Philippines, and placed in a plastic container. Collected snails served as an experimental animal in molluscicidal testing. Samples were transferred in improvised aquaria and acclimatized for three days with succulent leaves for their source of food before the application of the actual treatment.

Molluscicidal activity of Phyllanthus acidus

Following the method described by World Health Organization (1965), collected golden apple snails were tested side by side with the control and experimental treatments for 24 hours. Different concentrations were prepared to test against golden apple snail. Golden apple snails contained in a plastic containers were applied with 200 ml of each treatments. The snails were exposed in different treatments with three replicates each with 5 experimental snails; Treatment 1 (1000 ppm of *P. acidus* extract), Treatment 2 (500 ppm of *P. acidus* extract), Treatment 3 (100 ppm of *P. acidus* extract), Treatment 4 (Sure kill molluscicide solution; positive control) and Treatment 5 (water; negative control). Mortality rate was recorded every 4 hours within 24 hours. Snail mortality was determined when there is no observed contraction of the body within the shell, having a foul odor and discoloration of the shell (Ismail *et al.* 2016).

Results

Antioxidant property of *Phyllanthus acidus*

The aqueous extract of *P. acidus* leaves at 1000 ppm concentration had a significant radical scavenging activity (57.14%), which was somewhat lower than the catechin extract (60.50%). Furthermore, *P. acidus* leaves aqueous extract has 29. 42 mg GAE/g sample total phenolic content (Table 1).

Table 1. Radical scavenging activity and total phenolic content of *P. acidus*

Sample Description	Radical Scavenging (%)	Total Phenolic Content (Mg GAE/g)
P. acidus	57.14	29. 42
Catechin	60.50	

*Concentration 1000 ppm

Antibacterial activity of Phyllanthus acidus against Escherichia coli and Staphylococcus aureus

Eradicant method was used to screen the antibacterial activity of *P. acidus* aqueous and water extracts. Hot water extract inhibit the growth of *E. coli* with a mean diameter of 0.58 mm zone of inhibition but is not statistically comparable with streptomycin sulfate (positive control) with a mean diameter of 17. 38 mm zone of inhibition. Meanwhile, the aqueous extract did not inhibit the growth of *E. coli* and *S. aureus.* But the hot water extract inhibit the growth of *S. aureus* with a mean diameter of 5.19 mm which is significantly different to streptomycin sulfate (positive control) (Table 2; Figure 1).

Table 2. Antibacterial activity of *P. acidus* leaf extract against *E. coli* and *S. aureus* after 48 hours of incubation

Phyllanthus acidus	**Diameter Zone of Inhibition (mm)**	
	E. coli	*S. aureus*
Hot water extract	0.58 ± 0.77^{a}	5.19 ± 1.29^{a}
Aqueous extract	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Streptomycin (+)	17.38 ± 0.44^{b}	23.02 ± 1.29^{b}
Water (-)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

*Values are expressed as mean ± SD of three replicate test. Values with the same letters of superscript are not significantly different (P<0.05)

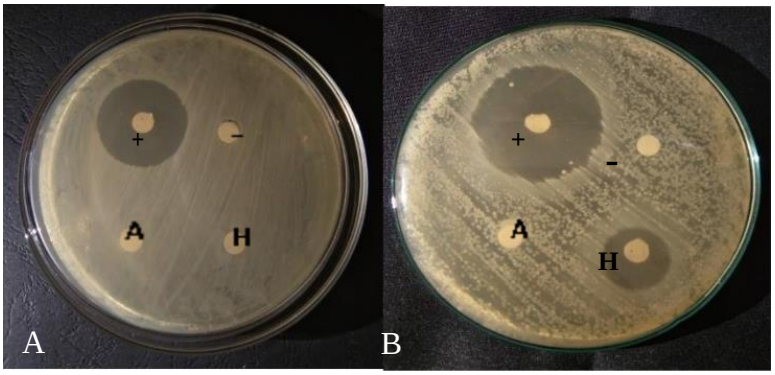

Figure 1. Antibacterial assay plates of *P. acidus* leaves aqueous extract against (A) *E. coli* and (B) *S. aureus* after 48 hour of incubation; (B) aqueous extract hot water extract (+) Streptomycin sulfate (positive control) and (-) water (negative control)

Molluscicidal activity of Phyllanthus acidus against Pomacea canaliculata

Aqueous extract of *P. acidus* was used as plant molluscicide against *P. canaliculata*. The mollusks reacted differently as they come in contact with the extract of *P. acidus* leaves. The snails were irritated as they increased their movement. Mucus secretion was observed in the foot. It has also partial retraction in the partially dead snails and no retraction in the dead snails. The mean mortality rates of golden apple snails treated with different treatments are shown on Table 3. Golden apple snails treated with commercial molluscicide (positive control) showed a significantly highest mean mortality which is 2.86 compared to aqueous treatments. However, 1000 ppm (Treatment 1) has the highest mean mortality with 2.38 which is significantly different to 500 ppm (Treatment 2) with 1.19 mean mortality and 100 ppm (Treatment 3) with zero mean mortality.

Table 3. Mean mortality of golden apple snails on the 24th hour of incubation

Treatment	Mean Mortality
1000 ppm	2.38 ± 0.44^{b}
500 ppm	1.19 ± 0.22^{c}
100 ppm	0.00 ± 0.00^{d}
Surekill (positive control)	2.86 ± 0.25^{a}
Distilled water (negative control)	0.00 ± 0.00^{d}

*Values are expressed as mean ± SD of three replicate test. Values with the same letters of superscript are not significantly different ($P<0.05$)

Discussion

Antioxidants found in plants are being studied because they aid in the prevention of oxidative cell damage by scavenging free radicals. The findings of this study imply that this plant has a lot of potential as an antioxidant that is biologically essential in avoiding and neutralizing free radical-related diseases, which medical industries can look into. Antioxidant compounds like polyphenols scavenge free radicals such peroxide, hydrogen peroxide, and lipid peroxyl, preventing oxidative mechanisms that could lead to degenerative illnesses (Badakshan *et al.,* 2012). The total phenolic content of *P. acidus* leaves aqueous extract was evaluated in this study, and it was found to be 29.42

mg GAE/g sample. Because of its hydroxyl-rich components like as phenols, flavonoids, and tannins, *Phyllanthus* species have a wide range of biological properties, including antioxidant activity utilizing polar solvent extracts (Mao *et al.,* 2016). According to Hadzri *et al.* (2014), different methods of extraction and solvents used play significant role in determining antioxidant activity.

In addition, plants are important resource of active ingredients that can be used in treating serious diseases. Since then, plants are been used in the treatment of various diseases which are bacterial in origin. Bacterial disease looks to be particularly manageable, led to strong hygiene and the availability of effective antibacterial treatments. However, the development of antibiotic resistance is an unavoidable consequence of their use (Dhale and Mogle, 2011). In this experiment, discs were used as reservoirs of the active compounds present in the plant. The plant extract was tested against two bacterial pathogens, *E. coli and S. aureus.* Results revealed that aqueous extract of the plant sample was ineffective in inhibiting the growth of *E. coli* and *S. aureus.* However, inhibitory activity was observed using the hot water extract. Similar to the study of Rahman *et al.* (2011) in which the pathogenic bacteria *E. coli* is susceptible to *P. acidus* extract but shows inhibitory effect in aqueous extract of *P. acidus* against *S. aureus.*

Moreover, molluscicidal screening methods are still regarded as an important means of controlling pest transmission in rice fields, such as the golden apple snail. The phytochemical components of *P. acidus* are responsible for its molluscicidal effect. Kumari *et al.* (2015) discovered that *P. acidus* contains saponins. Saponin is renowned for its structural diversity and numerous effects in animal cells, particularly on the permeability of cell membranes through generating pores (Francis *et al.,* 2002). It can reduce cholesterol levels in the blood and boost cholinesterase activity (Edoga *et al.,* 2005). Because of the complexity of their cellular responses, unambiguous functionality and structure surrounding the impacts of saponin in biological systems must be established (Francis *et al*., 2002).

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