

ECO-FRIENDLY BIO-OVICIDAL ACTIVITY OF GREEN-SYNTHEZED COPPER OXIDE NANOPARTICLES FROM *Persicaria barbata* and *Crescentia cujete*: AGAINST GOLDEN APPLE SNAILS (*Pomacea canaliculata*)

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Abstract: This study addresses the destructive impact of golden apple snails on rice production by evaluating the bio-ovicidal activity of *Persicaria barbata* and *Crescentia cujete* extracts enhanced with green synthesized copper oxide nanoparticles (CuONPs). Ultrasound-assisted extraction and coconut sap vinegar were used as the optimal extraction methods. The synthesized CuONPs were characterized by UV-Vis spectrophotometer, showing surface plasmon resonance (SPR) bands at 301 nm for *C. cujete* and 307 nm for *P. barbata*. FTIR analysis indicated the presence of CuO bonds at 426.59 cm⁻¹ to 594.12 cm⁻¹, and SEM-EDX revealed unique morphological and elemental compositions, with strong signals for copper and oxygen, along with weaker signals for potassium, sulfur, and carbon, reflecting the phytochemical constituents in the extracts. Treated golden apple snail eggs showed no hatchability, and a changed color from bright pink to black were observed indicating mortality. Probit analysis revealed LC50 values of 67.55% for *P. barbata* CuONPs and 82.97% for *C. cujete* CuONPs, with *P. barbata* obtaining higher toxicity at lower concentrations. The study demonstrates that green synthesized CuONPs from *Persicaria barbata* and *Crescentia cujete* extracts effectively inhibit the hatchability of golden apple snail eggs, indicating the potential eco-friendly pesticide for pest control in rice production.

Keywords: *Persicaria barbata*, *Crescentia cujete*, Copper Oxide nanoparticles, golden apple snails

1. Introduction

Currently, the world population is approximately 8.05 billion, based on projections of the latest United Nations data (*World Population Review*, 2023). This increasing population over time requires more food and supportive compounds in the agricultural sector. As one of the rice producing country in Southeast Asia, the Philippines is being left behind in terms of productivity growth by its neighboring country. As rice is the staple food for about 80% Filipinos, there is a higher demand in the rice production as compare to other crops making it a major source of income for millions of Filipino farmers.

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One of the main causes of the shortage and high domestic pricing of rice is the presence of pests such as the golden apple snail (GAS). GAS also known as *Pomacea canaliculata* is a freshwater snail endemic to South America. It was introduced in Asia as a food (de Brito & Joshi, 2016) with high protein and in aquaculture. However, due to the ability of the GAS to adapt environmental conditions, they reproduce rapidly and became a major pest particularly in rice. GAS consume young, developing rice plants, cutting the stem at the base and destroying the entire plant and can completely destroy 1 m² of a field overnight if no control measures are taken (*IRRI Rice Knowledge Bank*, 2020). Moreover, they can colonize and invade a variety of habitats through different pathways, including irrigation canals, natural water distribution channels, sporadic flooding events, and human activity (Joshi & Parera, 2017).

Integrated Pest Management (IPM) introduced various strategies and techniques in controlling GAS which includes mechanical, chemical and biological methods. The use of chemical and synthetic molluscicides are still one of the most common strategies for controlling snails (Khay et al., 2018) such as the use of metaldehyde, niclosamide and fetin acetate due to their effectiveness, faster results and low cost (Mokhtar et al., 2019). Chemical methods, however, has a damaging impact to non-target organisms, human health and may pollute the environment. Hence, for addressing these harmful effects, the use of biological method has been a focus which are environmental-friendly and economically available specifically plants that have developed various secondary metabolites as a defense mechanism against pathogen attacks (Seimandi et al., 2021).

Persicaria barbata L. is a species of perennial herb in the family Polygonaceae growing in climates ranging from warm temperate to tropical. This family of plants has been associated with a range of medicinal applications specifically the species of *Persicaria* as an alternative medicine. This includes the treatment to colic pain, skin conditions such as scabies, boils, abscesses, ringworms, diuretic, inflammatory conditions like pain, knee pain, rheumatic pain, gout, menstrual pain and amenorrhoea, and etc. (Tonny et al., 2017) which may attributed to the different phytochemical group present (Shahid et al., 2023). Meanwhile, *Crescentia cujete* L. is also known as calabash tree or gourd tree and from the Bignoniaceae family having 110 genera and 800 species (Parente et al., 2016). Specifically, *cujete* genus is renowned for its numerous medicinal properties along with the others genus of this family (Islam et al., 2019). The study of Parvin et al. (2015) demonstrated that the leaves and bark of *C. cujete* exhibit anti-inflammatory and antibacterial properties, suggesting that the plant extract holds therapeutic properties for combating bacterial infections and influencing disease processes by disrupting biological membranes. Hence, *P. barbata* and *Crescentia cujete* plants have the potential source of natural therapeutic agents.

Moreover, copper(II) oxide is among the transition metal oxides of notable interest because of its exceptional attributes including its low toxicity at low concentrations, stability, affordability, abundance, and the capability for easy preparation in various sizes and shapes (Hussein et al., 2023). And green synthesis method for CuONPs is considered a viable and environmentally friendly substitute for the chemical approach, which typically relies on hazardous chemicals as stabilizing or reducing agents. Many researches have utilized plant extracts in synthesizing CuONPs. From the study of Narasaiah et al. (2017) used a green synthesis approach was used in synthesizing CuONPs from *Drypetes sepiaria* leaf extract and resulted a noteworthy catalytic activity. Whereas, Saif et al. (2016) in comparison to an engineered nanoparticle showed that the CuONPs synthesized using green methods exhibited greater stability and lower ion release when contrasted with the engineered CuONPs.

As golden apple snails (*P. Canaliculata*) have been continuously destroying the rice production in the Philippines, this study will be conducted to address this emerging concern to this invasive pest. The aforementioned literatures have proved the promising potential of *P. barbata* and *C. kujete* as medicinal and therapeutic agents. However, there are only limited studies that focused on the use of these particular plants as molluscicides. Further study is still in need for verification and development for these particular plants. Hence, this study will be conducted to further evaluate the bio-ovicidal activity *Persicaria barbata* and *Crescentia kujete* by incorporating green synthesized copper oxide nanoparticles (CuONPs) in their extracts.

2. Materials and Methods

2.1 Collection and Preparation of Extracts.

A selected portion of snail-infested rice fields in Munoz, Nueva Ecija, was monitored for the presence of golden apple snails (GAS). The snails were collected and placed in a designated area within the rice field, where they were allowed to lay eggs. The collected eggs were selected based on their bright pink color and the presence of slime, ensuring that they were freshly laid by the mother snails. The newly hatched egg clusters were utilized for different treatments in this study (Quitos & Catabona, 2023).

2.2 Collection and Preparation of Extracts

Leaves of *Crescentia kujete* L. and *Persicaria barbata* (L.) H. Hara were collected in Central Luzon State University (CLSU) and Bagong Sicat, Science City of Munoz, Nueva Ecija respectively. Both plant leaves undergone identification and authentication before utilization. The leaves were dried at room temperature for a couple of days and grounded using a food blender. The powdered materials were kept in an airtight vessel prior to use.

The extraction procedure of this study was adapted from the study of Zoubi et al. (2021) and Paragas et al. (2020) with few modifications for ultrasound-assisted extraction (UAE) and hot infusion extraction, respectively. Moreover, the extraction of *C. kujete* and *P. barbata* leaves was done using two solvents: Coconut sap vinegar and rice wash.

UAE was done by adding 100 g of plant material to 1 L of solvent and was subjected to ultrasound cleaning bath with a frequency of 37 kHz for 30 minutes at 50°C. Using filter cloth, the extracts that were obtained are filtered and evaporated under vacuum at 40°C on a rotary evaporator. Meanwhile, hot infusion was conducted by adding 100 g ground leaves into 1 L of boiling solvent. The mixture was stored for 15 minutes, filtered using filter cloth then the filtrate collected was subjected to rotary evaporator. The produced crude extracts from both extraction procedures were placed in a Petri dish, oven-dried and stored for further analysis.

2.3 Phytochemical Screening

Test tube method was applied from the study of Das et al. (2014) and Dubale et al. (2023) with few modifications to identify the presence of active chemical constituents such as tannins, saponins, flavonoids, glycosides, terpenoids, alkaloids, and coumarins.

Test for Tannins. 2 drops of 1% FeCl₃ was added to a 3 ml extract. The presence of tannins were confirmed when a greenish-black precipitate was observed.

Test for Saponins. In a test tube, 1 ml of plant extract was shaken vigorously. Saponins were identified from formation of stable foam in the solution.

Test for Flavonoids. 0.5 ml of 10% Ammonia was added to 1 ml of plant extract. Flavonoids were identified when a yellow precipitate was observed.

Test for Glycosides. 2 ml of extract was mixed with 0.5 ml chloroform. The mixture was added in the 0.5 ml concentrated H₂SO₄. The presence of glycoside is confirmed by the formation of a reddish brown ring at the interface of the two (2) solutions.

Test for Terpenoids. 0.5 ml of chloroform is mixed with a 2 ml plant extract. 1 ml of H₂SO₄ was slowly added to the mixture, and a reddish brown hue appears near the point of contact, indicating the presence of terpenoids.

Test for Alkaloids. Three drops of Mayer's reagent will be applied to 1 ml of plant extract. The presence of alkaloids is confirmed when there is turbidity or yellow precipitate observed.

Test for Coumarins. 1 mL of 10% NaOH solution and 1 mL of chloroform will be added to 3 mL of extract. The formation of yellow precipitates indicates the presence of coumarins.

2.4 Preparation of Positive and Negative Control Treatment

As a reference standard for comparison, a commercial pesticide powder was purchased and employed (Picardel et al., 2018 & Prabhakaran et al. 2017). The powder was dissolved in water and 10 ppm of the solution was prepared as a stock solution. The mass used were computed based on the mass/area that is given in the sachet. Then, it was further diluted to 0.5 ppm in distilled water that was used as positive control. Furthermore, distilled water was utilized as the negative control in this experiment.

2.5 Evaluation of the Bio-ovicidal Activity using the Extracts

The eggs were separated per cluster for each treatment. From the study of Ismail and Musa (2021), a spot spraying method was done. All egg surfaces must be in contact when sprayed. The length of time taken for the embryos to fully develop inside the eggs (hatching time, in days) and the number of hatchlings successfully emerged from the eggs (% hatching success) were the basis for assessing their mortality. Any eggs that failed to develop after 15 days were considered unsuccessful in terms of hatchability. The treatments that exhibited the least % hatchability for both extracts were used for the synthesis of CuONPs.

2.6 Green Synthesis and Optimization of Copper oxide Nanoparticles (CuONPs)

Copper oxide nanoparticles (CuONPs) were extracted following the procedure of Murthy et al. (2020) and Hussein et al. (2023) with some modification. A mixture was made by adding the previously prepared extracts and 0.05 M copper salt (anhydrous copper sulfate) at different volume ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 as adapted from the study of Thamer et al. (2018). For further studying the optimum condition for CuONPs, the resulting mixture was heated at different temperature at 50, 80 and 100degrees Celcius with constant stirring for 30 minutes. The effect of these parameters on the synthesis CuONPs were monitored by UV-visible spectrophotometer.

During heating, 0.1 M NaOH was slowly dropped and visual observation of the color transitioning from blue to light green and further changing to brown as precipitate was observed, indicating the formation of copper oxide nanoparticle. The mixture has undergone incubation at room temperature for 24 hours. Moreover, the solution was subjected to centrifugation at 10000 rpm for 10 minutes. Then the resulting g-CuONPs were thoroughly cleansed using deionized water to eliminate any impurities. Subsequently, the resultant powder was dried at 50 °C in an oven for 6 hours and will be stored for further analysis.

2.7 Characterization of Green synthesized Copper oxide Nanoparticles (g-CuONPs)

The optimal conditions, determined through UV-Vis spectrophotometer, including the volume ratio of extract to precursor and temperature, were selected for further characterization of CuONPs of both plants.

2.8 UV-Visible Spectroscopy Analysis

The g-CuO NPs was analyzed using UV-Vis spectrophotometer in the range of 290–600 nm to record the UV-visible absorbance of the samples against a blank extract solution.

2.9 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The g-CuONPs were further characterized with FTIR to determine its chemical characteristics. Potassium bromide (KBr) pellets were prepared, which will contain 1% of the finely ground sample g-CuONPs. The FTIR spectrum was recorded within the range of 400–4000 cm^{-1} .

2.10 Scanning Electron Microscopy – Energy Dispersive X-ray (SEM-EDX) Analysis

Scanning electron microscopy (SEM) was employed to determine the morphological, mean particle size and structural characteristics of g-CuO NPs. And further characterize with electron dispersive x-ray for its elemental composition.

2.11 Evaluation of the Bio-ovicidal Activity using Green Synthesized Copper oxide Nanoparticles

The collection of GAS eggs was similar on the aforementioned procedure above. The eggs were collected per cluster and distributed into 7 groups for each treatment. Treatment 1 was the positive control applying a 0.5 ppm commercial pesticide solution. Treatments 2, 3, 4, 5, and 6 were the prepared g-CuONPs at different concentrations wherein the pure g-CuONP (100%) was considered as Treatment 2. This was further diluted to prepare other treatments of 80%, 60%, 40% and 20% g-CuONP using distilled water for treatments 3, 4, 5 and 6 respectively. Moreover, distilled water was used as the negative control for Treatment 7.

For the assessment on the eggs of GAS, a spot spraying method was also used adapted from the study of Ismail & Musa (2021). The eggs were sprayed with up to 5 ml maximum of each of the treatments including the positive and negative control. Likewise, all egg surfaces must be in contact when sprayed. Their mortality was assessed from the length of time taken for the embryos to fully develop inside the eggs (hatching time, days) and the number of hatchlings successfully emerged from the eggs (% hatching success). Eggs that did not develop within a 15-day period will be considered as unsuccessful in hatchability. Moreover, the eggs treated with g-CuONPs were observed and calculated based on the color change happened, counting the number of discolorations from bright pink to black.

2.12 Statistical Analysis

During the experiment, the mortality of snails was recorded for the exposure period. Probit analysis was conducted to calculate the LC50 and LC90 values, along with their corresponding 95% confidence intervals (95% CI). The mortality of snails subjected to various concentrations was analyzed using a one-way analysis of variance (ANOVA), and mean comparisons will be conducted using Duncan's multiple comparison tests at a significance level of 5%.

3. Results and Discussion

Different extracts were successfully prepared from the plants using both hot infusion and ultrasound-assisted extraction (UAE) as extraction methods, and coconut sap vinegar and rice wash as extraction solvents.

P. barbata extracts were brown in color and has a rough surface after oven dried. Whereas, *C. cujete* extracts were also brown in color but shows a glass-like surface after drying. The extracts that used rice wash and hot infusion were somehow turbid as compared to those that used vinegar and UAE. These prepared extracts from *P. barbata* and *C. cujete* were utilized for further analysis.

3.1 Phytochemicals Present

Phytochemical screening of the plant extracts was conducted using the test tube method to identify the presence of phytochemicals of different methods and solvents used. As shown in Table 1 below, saponins, tannins, terpenoids, and cardiac glycosides were present. Coumarins were found at some of the extracts specifically at HRP and HVP extracts. However, the tests for flavonoids and alkaloids did not show positive results on other treatments indicating the absence of these phytochemicals on the extracts.

Table 1. Qualitative profiles of Phytochemicals in the leave extracts of different methods and solvents

	SAPONINS	TANNINS	COUMARINS	TERPENOIDS	ALKALOIDS	FLAVONOIDS	CARDIAC GLYCOSIDE
URP	+	+	-	+	-	-	+
UVP	+	+	-	+	-	-	+
HRP	+	+	+	+	-	+	+
HVP	+	+	+	+	+	+	+
URC	+	+	-	+	+	-	+
UVC	+	+	-	+	-	-	+
HRC	+	+	-	+	-	-	+
HVC	+	+	-	+	+	-	+

Method of Extraction- **U**: Ultrasound-assisted extraction, **H**: Hot Infusion;

Solvent used - **R**: Rice, **V**: Vinegar (coconut sap);

Plant - **P**: *P. barbata*, **C**: *C. cujete*

+: Positive, -: Negative

These results contradict the findings of Balogun and Sabiu (2021) and Maqbool et al. (2021), where alkaloids and flavonoids were reported in the leaves of *C. cujete* and *P. barbata*, respectively. The concentration of the extracts may have influenced the results, as the phytochemical composition of wild plant extracts varies with extract concentration (Bazamova & Ivanchenko, 2016). The extracts were turbid at high concentrations, necessitating dilution prior to phytochemical screening since the test tube method relies on visual observation, and such changes may not be detectable under these conditions.

Moreover, the detection of coumarins, flavonoids and alkaloids between hot infusion and ultrasound-assisted extraction (UAE) can be attributed to the differences in temperature, extraction efficiency and time associated with these methods. Hot infusion utilizes high temperature, which enhance the solubility and extraction of heat-stable compounds. Heat helps break down cell walls and facilitates the dissolution of the compounds into the water (Tzanova et al., 2020). On the other hand, UAE is often performed at lower temperatures which may not be as effective for heat-stable compounds. Thus, all UAE parameters, which may also apply to hot infusion, must be carefully optimized in order to avoid thermal degradation of phenolic compounds. The optimization parameters include temperature, extraction time, polarity and amount of solvent, amount and type of sample, ultrasound frequency and intensity, and number of pulses (Lu et al., 2015).

Further, vinegar and rice wash, being polar in nature due to their high water content, may not have effectively extracted certain phytochemical constituents, as non-polar groups cannot be dissolved in these solvents. Alkaloids exhibit non-polar and semi-polar properties (Widyawati et al., 2014), while coumarins are not highly soluble in water (Cysewski et al., 2022). Similarly, flavonoids possess both polar and non-polar counterparts, including isoflavones, flavanones, flavones, and flavonols as non-polars (De Luna et al., 2020). Considering this, the polarity of solvents affects the extraction efficiency of various phytochemicals and may contribute to obtaining negative results in test tube methods.

3.2 The Optimized Extraction Methods and Solvents

As presented in Figure 1, the extracts significantly affect the percent hatchability of the GAS eggs in comparison to the control treatments. These observations emphasize the potential bio-ovicidal activity of the plant extracts since the figures show a decrease in the percent hatchability as the extracts were applied.

Among the *P. barbata* extracts, the UVP treatment achieved a hatchability of 68.472%, while the UVC treatment from *C. kujete* extracts displayed an average hatchability of 67.499%. Both treatments had the lowest percent hatchability for each respective plant. However, the positive control still exhibits the lowest hatchability rate among those treated with extracts, with a rate of 26.580%. Therefore, there is a need to enhance the bio-ovicidal activity of the extracts by synthesizing nanoparticles using the aforementioned treatments with the lowest average percent hatchability on each of the plants.

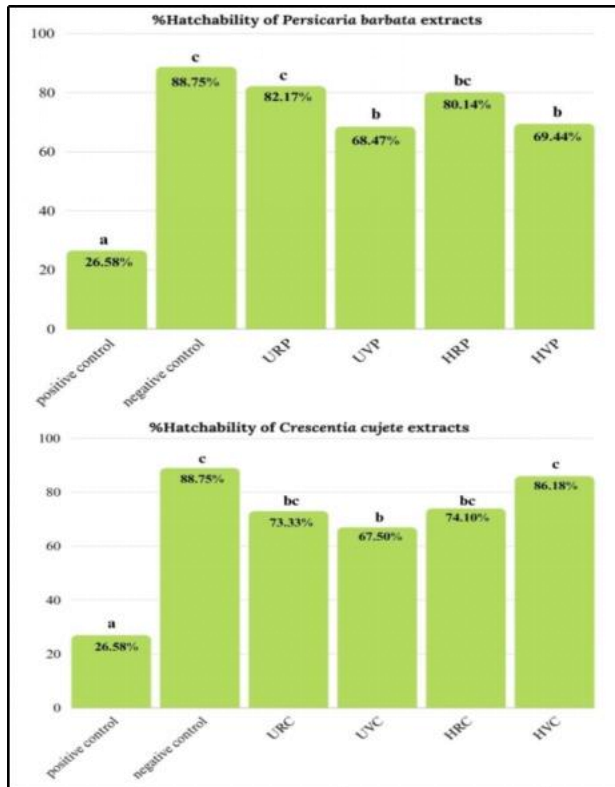


Figure 1: Percent hatchability of *C. cujete* and *P. barbata* leave extracts at different solvent and method of extraction

In this particular study, ultrasound-assisted extraction (UAE) and hot infusion were both used as the method of extraction, and rice wash and coconut sap vinegar as extraction solvents for different treatments to determine their bio-ovicidal effect on the plants. The results clearly demonstrate that both the extraction technique and the extraction solvent are crucial in determining the efficiency of phenolic compound extraction from plant matrices. This finding coincides with the results of the study of Cittan et al. (2018) that higher amounts (approximately 2-fold) of the phenolic compounds were extracted using UAE technique when compared to Infusion extraction (IE) method. However, the results from other extracts do not exhibit a direct relationship with the extraction method and solvent. It is evident that optimization is necessary across different parameters to achieve a more reliable comparison.

But several studies discussed the promising results of ultrasound-assisted extraction (UAE). UAE relies on acoustic cavitation, where collapsing cavitation bubbles and sound waves cause phenomena such as fragmentation, localized erosion, pore formation, and shear forces in the cellular matrix of the plants. This leads to increased absorption and swelling, reducing particle size and increasing surface area and mass transfer rates, thus enhancing solubilization of bioactive components (Roselló-Soto et al., 2015). With this, the localized damage (erosion) from cavitation bubbles further facilitates solvent contact, increasing extraction yield and giving a significantly the highest extract yield than infusion, micellar extraction, and microwave extraction (Somwongin et al., 2023). Given that the results from Figure 1 shows the lowest %hatchability of the extracts that had used UAE and coconut sap vinegar, these suggest that these treatments are the most effective for extracting the secondary metabolites needed for the bio-ovicidal properties of the plants.

3.3 Green Synthesis and Optimization of Copper oxide nanoparticles

Copper oxide nanoparticles (CuONPs) were successfully synthesized using the plants of UVP treatment for *P. barbata* and UVC treatment for *C. cujete* leave extracts as shown in Figure 2a and 2b, respectively.

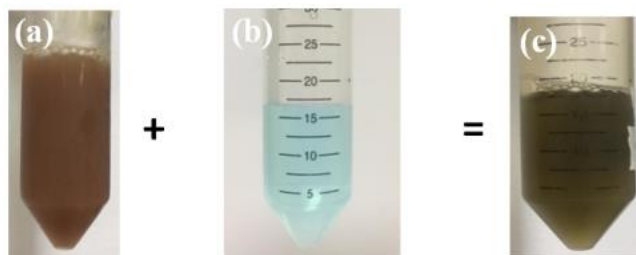


Figure 2a: Color reaction of the formation of green synthesized CuONPs (a) *P. barbata* leaves extract; (b) copper salt, (c) CuONPs from *P. barbata*



Figure 2b: Color reaction of the formation of green synthesized copper oxide nanoparticles, (a) *C. cujete* leaves extract; (b) copper salt, (c) CuONP from *C. cujete*

This was indicated by the change of the soft blue color to green after the addition of the extracts in the 0.05 M copper salt with 0.1 M NaOH. The plant extracts serve as the reducing and stabilizing agent from its various phytochemicals, donating electrons to Cu^{2+} ions, reducing them to metallic Cu^0 atoms and then further oxidize. Subsequently, these resulting complexes interact with similar complexes, facilitating the formation of small metal nanoparticles as presented in Figure 3.

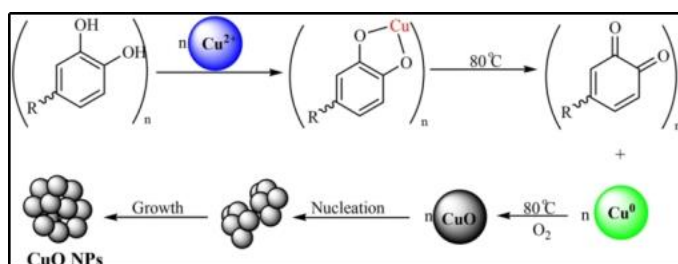


Figure 3: Plant-mediated synthesis of copper oxide nanoparticles (Veisi et al. (2021))

The presence of functional groups within the phytochemicals facilitates adsorption onto the surface of these nanoparticles, forming a protective layer that ensures their uniform size distribution which prevents agglomeration, and enhances colloidal stability (Irshad et al., 2024; Kazemi et al., 2023; Koteeswari et al., 2022).

Moreover, the synthesis of nanoparticles is influenced by various conditions, including extract-to-precursor volume ratio and temperature. Thus, optimization was done to achieve the optimal conditions

adjusting these parameters to improve the detectability and clarity of the absorbance peak associated with the synthesized nanoparticles.

Particularly, in this study, the optimization of the green synthesis of copper oxide nanoparticles (CuONPs) involves the adjustments of volume ratio between extract and copper salt, and the temperature. The optimal conditions for the synthesis of CuONPs using *P. barbata* were determined to be a 1:3 extract-to-copper salt ratio at 80°C, whereas for *C. cujete*, the optimal ratio was found to be 1:5 at the same temperature (Appendix II, Figure 7). These conditions were identified by showing better peaks among other solution mixture. Thus, these optimized conditions were used for further characterization and evaluation of their bio-ovicidal activities.

3.4 Characteristics of the Green Synthesized CuONPs

UV-Vis Spectroscopy. The formation of CuONPs was confirmed through the color change reaction of the resulting solution in Figures 2a and 2b. This was further identified using the UV-Vis spectrophotometer to absorb light and producing distinct spectra of chemical compounds.

Figure 4 reveals the maximum peaks for the synthesized CuONPs, with values of 229 nm and 230 nm for *C. cujete* and *P. barbata*, respectively similar to other studies on the green synthesis of CuONPs using plant extract (Gnanavel et al., 2017; Mali et al., 2019). This may be contributed by the copper salt used which influenced by a higher volume of precursor than of the extract in the synthesis. Small peaks were also observed at a lower absorbance level with 301 nm for *C. cujete* and 337 nm for *P. barbata* which shows the surface plasmon resonance (SPR) of CuONPs nearly coincides with the study of Balaji et al. (2023). The position of SPR band of CuONPs is highly sensitive to a variety of factors including the size, shape, capping agent, and temperature causing either red or blue shift in the spectrum (Thamer et al., 2018).

The increase of concentration of copper sulfate beyond 40 mM may decrease the SPR absorbance cause from a lower number of reducing agent molecules which converts all the Cu^{2+} ions into Cu atoms (Thamer et al., 2018).

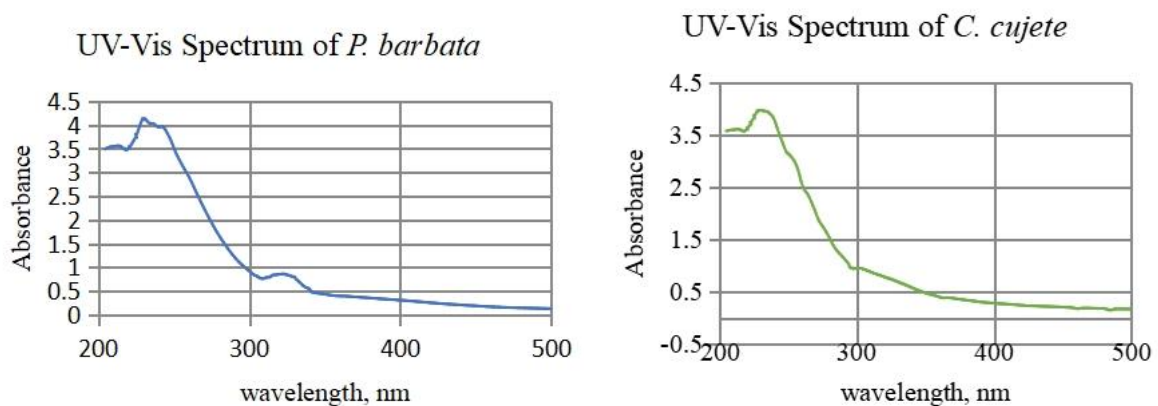


Figure 4: UV-Vis spectra of CuONPs from (a) *C. cujete*, (b) *P. barbata* leaves extract

This leads to the formation of fewer copper nuclei. The reduced number of nanoparticles results in lower SPR absorbance because there are fewer nanoparticles to interact with the light and exhibit plasmon resonance.

3.5 Fourier Transform Infrared Spectroscopy

Figure 5 presents the FTIR spectra of CuONPs derived from both *P. barbata* and *C. cujete*, revealing several characteristic absorption peaks. In the spectrum of *P. barbata* CuONP, the presence of an O-H stretch at 3252.10 cm⁻¹ indicates that the sample contains hydroxyl groups, likely from alcohols or phenols. Similarly, hydroxyl groups were also observed at 3274.32 cm⁻¹ in *C. cujete* CuONP. The C=C stretch observed at 1594.43 cm⁻¹ in *C. cujete* indicates unsaturation, such as in alkenes or aromatic compounds.

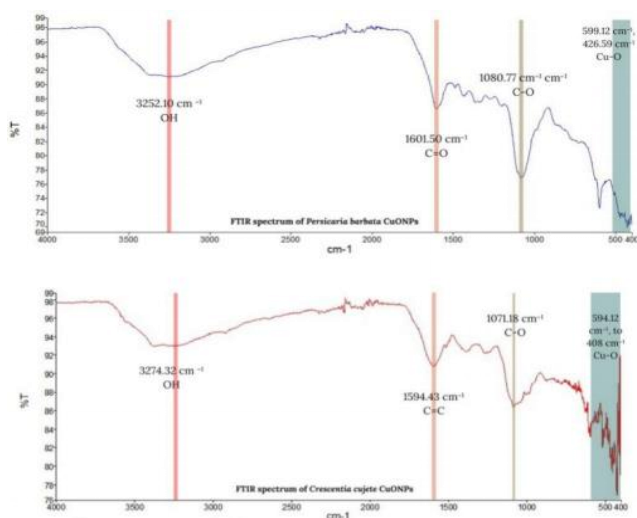


Figure 5: FT-IR spectra of (a) *P. barbata* and (b) *C. cujete* leaf extracts and CuONPs

For *P. barbata*, the presence of a C=O stretch at 1601.50 cm⁻¹ suggests conjugated alkenes. Moreover, the C-O stretch at 1071.18 cm⁻¹ in *C. cujete* and 1080.77 cm⁻¹ in *P. barbata* are characteristics of ether or ester functionalities, which may suggest the presence of such compounds.

Additionally, there are four major infrared absorption peaks exhibits in the vibrational modes of *C. cujete* CuONPs: 594.12cm⁻¹, 517.00cm⁻¹, 511.00 cm⁻¹, and 489.00 cm⁻¹ and two peaks for *P. barbata* CuONP: 599.12 cm⁻¹ and 426.59 cm⁻¹. These peaks correspond to the Cu-O bonds as also reported in the previous study of Thamer et al., (2018) and Nzilu et al. (2023). The small peak shift in the vibrational modes is associated with the corresponding change in the surface area of the prepared sample.

3.6 Scanning Electron Microscopy-Electron Dispersive X-ray (SEM-EDX) Analysis

The morphology and elemental composition of the green synthesized CuONPs was confirmed utilizing a scanning electron microscopy-electron dispersive x-ray. Figure 6 shows the SEM images of both CuONPs of the plants at different magnifications. It reveals the shape and size vary as different plant extract used in the synthesis process.

Significantly, the image differed from the standard spherical shape of CuONPs as described by the study of Aminuzzaman et al. (2017). Alternatively, CuO nanostructures vary due to the chemical composition

of the reducing agent, with the changes in surface morphology attributed to the phytochemicals in the extracts. This causes the irregular shape particles similar to the study of Rotti et al., (2022).

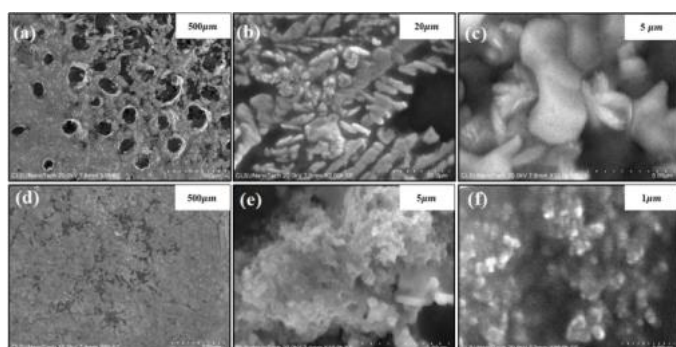


Figure 6: SEM Morphologies of *C. kujete* CuONPs at (a) 65X, (b) 2.00kX, (c) 10kX magnification, and *P. barbata* CuONPs at (d) 60X, (e) 10.0kX, (f) 30.0kX magnification

From the study of Mary et al. (2019), the shape of the synthesized CuONPs differed depending on the concentration of the plant extracts used in which increasing the concentration, the morphology changes from irregular to spherical, and the change in size of the NPs produced. These studies provide evidence that the morphology of the CuONPs changes as the plant extract used changed.

Moreover, since the UV-Vis peaks showed a high absorbance nearly 4, thus having a concentrated solution, it coincides the SEM morphology as it was also revealed that a high concentration of the precursor leads to the formation of larger NPs with faster agglomeration (Letchumanan et al., 2021). Whereas, similar study of Asemani and Anarjan (2019), Tran and Nguyen (2014) that a low concentration of the precursor results in the production of smaller-sized nanoparticles (NPs).

EDX analysis, as presented in Figure 7 shows strong signals of copper (Cu) and oxygen (O) for both green synthesized CuONPs in which similar study by Berra et al., (2018) also presents double signal peaks of Cu. The atomic and weight percentage represent the total weight of an element in the NPs observed. Cu and O atoms in *P. barbata* CuONPs are 9.49% and 61.32%, and 24.73% and 40.24%, respectively, whereas *C. kujete* CuONPs have an atomic and weight percentage of 14.85% and 61.28%, and 35.90% and 37.31%.

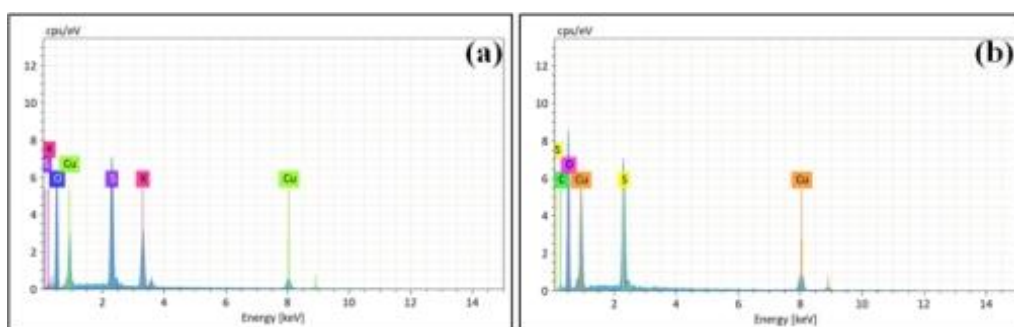


Figure 7: Electron Dispersive X-ray of graph of (a) *P. barbata* CuONPs and (b) *C. kujete* CuONPs

Additionally, other elements were detected with weaker signals: potassium (K), sulfur (S) and carbon (C) were present in the *P. barbata* CuONPs, while only C and S was detected in the *C. cuejete* CuONPs. The presence of other elements from both green synthesized CuONPs likely originates from the natural composition of the plant extracts (Aminuzaman et al., 2017), as plant contains various organic and inorganic bioactive compounds, which can be carried over into the nanoparticles during the synthesis process. Moreover, these additional elements can be attributed to the phytochemicals and minerals naturally present in the extracts, which may participate in the reduction and stabilization of the nanoparticles.

3.7 Evaluation of the Bio-ovicidal Activity using Green Synthesized CuONPs

The green synthesized copper oxide nanoparticles from *P. barbata* and *C. cuejete* were applied at different concentrations on the eggs of golden apple snails. The eggs were observed on a day-to-day basis, noting the changes of colors and hatched eggs. After the 15-day observation, only the negative control was observed for its hatchability.



Figure 8: Observed color change of golden apple snail (GAS) eggs (a) before application, (b) after 15 days after application

The treated eggs show no sign of hatchability along with the positive control. However, the treated eggs exhibit a color change from bright pink to black (Figure 8), concluding that the eggs were dead. The color change from the treated sample has been the basis in identifying their mortality as a bright pink color indicates that the eggs are alive and viable. The different log concentration of the green synthesized CuONP for both plants were compared as shown in the Figures 9a and 9b.

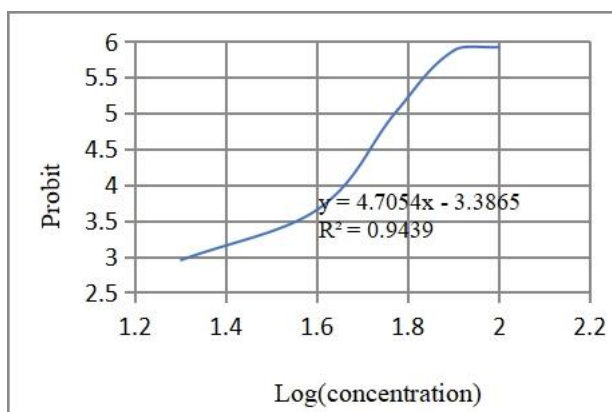


Figure 9a: Probit Analysis of *P. barbata* CuONPs

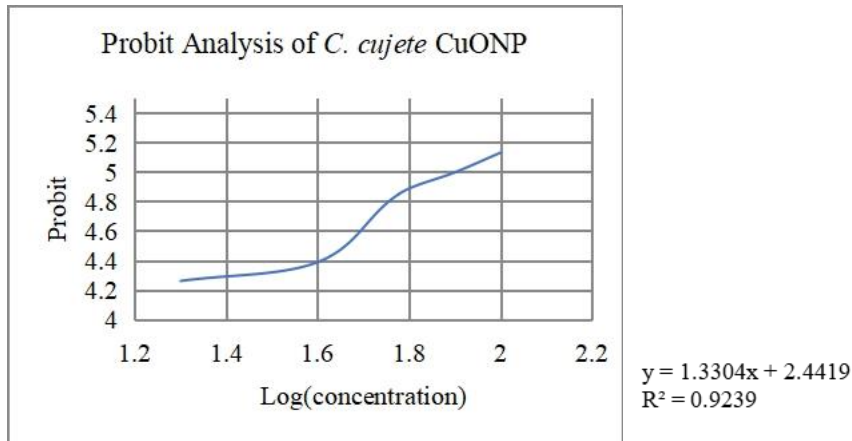


Figure 9b: Probit Analysis of *C. kujete* CuONPs

Figure 9a represents the probit analysis of *P. barbata* CuONP illustrating the toxicity of the synthesized NP against GAS eggs. Given the equation of the line, the LC50 was found at 67.55% as computed. On the other hand, in figure 9b, it was identified that the LC50 was found specifically at 82.97% concentration level of *C. kujete* CuONP. The LC90 of both CuONPs were calculated at more than a hundred percentage level, indicating that a higher concentration should be prepared to meet the 90% mortality on the eggs. Thus, it was further revealed that the *P. barbata* CuONPs demonstrate higher toxicity at a lower concentration, while *C. kujete* CuONPs require higher concentration to achieve similar mortality rates.

Table 2: Duncan’s Mutiple Comparison test of different treatment of Green synthesized CuONPs

Treatment	Mean %Mortality	
	<i>C. kujete</i> CuONP	<i>P. barbata</i> CuONP
postive control	85.38±1.27 ^a	85.38±1.27 ^a
100	62.40±0.81 ^b	85.26±0.90 ^a
80	58.35±0.66 ^c	83.94±0.74 ^b
60	53.25±1.44 ^d	60.29±2.25 ^c
40	38.80±1.30 ^e	23.89±0.55 ^d
20	35.57±1.05 ^f	18.18±1.42 ^d
negative control	16.56±0.72 ^g	16.56±0.72 ^d

Means not sharing subscripts differ significantly at 5% significance level.

Furthermore, Table 2 shows that treatments for *C. kujete* CuONPs were significantly different from each other along with the control. Meanwhile, *P. barbata* CuONP, the negative control and the 20% concentration level is not significantly different but significantly different with other treatment. The positive control, 100% and 80% concentration levels are not significantly different from each other but significantly different from the other treatments. 40% and 60% on the other hand, were significantly different from all the treatments of *P. barbata* CuONP.

The green synthesized CuONPs to GAS eggs have shown significant bio-ovicidal activity using plant extracts which enhances the efficacy of these nanoparticles due to their smaller size and increased surface area, which facilitates better interaction with the target organism (Haleem et al., 2023; Sajjad et al., 2023).

The mechanism by which CuO NPs affect golden apple snail eggs primarily involves the generation of reactive oxygen species (ROS). When CuO NPs was applied to the eggs, they induce oxidative stress by producing ROS, such as superoxide anions and hydroxyl radicals Chen et al. (2024) as OH bonds were seen in the characterization of the CuONPs of both plants. These reactive molecules cause cellular damage by (1) lipid peroxidation in which ROS attack the lipids in the egg membranes, leading to loss of membrane integrity and cell death (Chen et al., 2024), (2) protein oxidation wherein oxidative stress damages vital proteins within the egg cells, disrupting enzymatic functions and structural proteins necessary for cell viability and (3) DNA damage in which ROS can cause breaks in DNA strands and modify nucleotides, leading to genetic damage and preventing the development of viable embryos (Gupta et al., 2022; Sajjad et al., 2023).

Comparing the observation between the application of the extracts and CuONPs, high mortality was observed enhancing the extracts with the synthesized nanoparticles from the plant itself. Thus, this study successfully evaluates the bio-ovicidal activity of the CuONPs against GAS eggs.

4. Conclusion

Persicaria barbata and *Crescentia kujete* extracts itself shows an effect in decreasing the %hatchability of golden apple snail (GAS) eggs using a green solvents and methods of extraction. Phytochemical constituents were also identified including saponins tannins, coumarins, terpenoids, alkaloids, flavonoids and cardiac glycosides in both of the plants. Moreover, Copper oxide nanoparticles (CuONPs) were successfully synthesized using the extracts as reducing and stabilizing agent, and further used to enhance the extract to evaluate their bio-ovicidal activity. The green synthesized CuONPs showed a distinct discoloration on the GAS eggs at different percent concentration. Thus, the green synthesized CuONPs from *P. barbata* and *C. kujete* extracts effectively show bio-ovicidal activities which inhibit the hatchability of golden apple snail eggs, indicating the potential eco-friendly pesticides for pest control in rice production.

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Declaration of Interest Statement

The authors declare that they have no conflict of interests.

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