Biological Activities of *Cyperus imbricatus* Retz (Shingle Flat Sedge) Leaf Crude Extract

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ABSTRACT

Cyperus imbricatus Retz. (Shingle Flat Sedge) is traditionally used in Ifugao to treat urinary tract infections. This study determined the biological activities of *C. imbricatus* Retz (shingle flat sedge) leaf crude extract using in vitro assays. Using an experimental design, ethanol-extracted leaf crude was screened for secondary metabolites via Thin Layer Chromatography (TLC) and tested for antimicrobial, antioxidant, anti-inflammatory, and cytotoxic properties through in vitro assays. Phytochemical screening revealed that *C. imbricatus* contains phenols, flavonoids, coumarins, and triterpenes that support the pharmacological potential of *C. imbricatus*. Antimicrobial activity was assessed against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, in which no inhibition was observed. Strong antioxidant activity was observed in the DPPH assay, and strong anti-inflammatory activity in the egg albumin denaturation test. Cytotoxicity, determined via brine shrimp lethality assay, showed high toxicity with an LC_{50} at a very low concentration. An infographic poster summarizing the findings was shared with local communities to enhance awareness and promote informed use of traditional medicinal plants.

Keywords: Anti-inflammatory activity, antimicrobial activity, antioxidant activity, cytotoxicity, phytochemical screening

INTRODUCTION

Background of the Study

Cyperus imbricatus, commonly known as Shingle flat sedge, is one of the herbal plants prepared by decoction to treat Urinary Tract Infections (Tamayo, 2018). A number of community members of Lamut, Ifugao, have claims on the detoxifying activity of the plant. In a review of the phytochemical composition, biological activity, and health-promoting effects of *Cyperus* sp., it was concluded that these species are employed for their medical potential with their plethora of pharmacological attributes such as antioxidant, anti-inflammatory, antimicrobial, and anticancer (Taheri et al., 2021a). These biological activities offer significant potential to address a range of health conditions and enhance overall well-being (Dar et al., 2023).

While existing studies have extensively explored the biological activities of various species within the *Cyperus* genus, *C. imbricatus* Retz. remained notably understudied. This lack of scientific investigation was particularly evident regarding its potential antimicrobial, antioxidant, anti-inflammatory, and cytotoxic properties. Recognizing this gap, the study addressed the scarcity of research on *C. imbricatus* by evaluating its biological activities, thereby contributing to a more comprehensive understanding of the species and its potential applications in natural product research and drug development.

Statement of the Objectives

The study sought to determine the biological activities of *C. imbricatus* Retz (shingle flat sedge) leaf crude extract using in vitro assays. It was done from January 2024 to May 2025.

Specifically, this study answered the following questions:

1. What are the secondary metabolites from the ethanolic extract of *C. imbricatus* leaves?

2. What is the zone of inhibition of the ethanolic extract of *C. imbricatus* leaves against *K. pneumoniae* and *P. aeruginosa*?
3. What is the antioxidant capacity of the crude extract of *C. imbricatus* leaves as measured by its ability to reduce DPPH radical activity?
4. What is the efficacy of *C. imbricatus* leaf crude extract in preventing egg albumin protein denaturation, and what does this suggest about anti-inflammatory activity?
5. What is the effect of *C. imbricatus* leaf crude extract on the viability and metabolic activity of Brine shrimps as determined by Brine shrimp lethality assay?
6. What Information Education and Communication (IEC) strategy will be employed to inform the residents of Lamut, Ifugao, about the biological activities of *C. imbricatus*?

METHODOLOGY

Research Design

This study employed an experimental research design to evaluate the biological activities of the crude extract from *Cyperus imbricatus* Retz. leaves. The extract was obtained through standard procedures involving maceration, filtration, and solvent evaporation. It was subsequently subjected to a series of in vitro bioassays to determine its antimicrobial, antioxidant, anti-inflammatory, and cytotoxic properties.

Study Site and Sample Collection

This study was conducted at the Center for Natural Sciences (CNS) research laboratory at Saint Mary's University (SMU) in Bayombong, Nueva Vizcaya. The plant species selected for this study is *C. imbricatus* Retz, which is commonly found in wetland environments, including swamps and marshes. Samples were collected from Lamut, Ifugao. The collection site has been confirmed to be free from known pollution sources, including vehicle emissions, toxic substances, heavy metals, and other contaminants. The samples were collected prior to air drying, which facilitated subsequent extraction.

Specimen Identification

The plant specimen was collected from Lamut, Ifugao, and was submitted to the Research Extension and Training (RET) Division of Nueva Vizcaya State University for authentication and certification of its botanical or taxonomic identity. The test organisms, *P. aeruginosa*, *K. pneumoniae*, Brine shrimp eggs were obtained from CNS-SMU.

Data Gathering Procedure

Plant Material Collection and Preparation

Leaves of *C. imbricatus* were collected, washed, and drip-dried. Samples were chopped, air-dried, and pulverized using an electric mill.

Crude Extraction of Plant Material

Following Toklo et al. (2023), 400 g of pulverized material was macerated in ethanol for 24 hours. The extract was filtered (Whatman No. 1), evaporated with a rotary evaporator, and refrigerated at 4°C for storage.

Phytochemical Screening via Thin Layer Chromatography

Phytoconstituents were analyzed via TLC (Kalemba et al., 2024) using a 7:3 ethyl acetate chloroform solvent. Extracts were visualized under UV light (254 nm and 365 nm) and treated with reagents as described by Guevarra et al. (2005).

Antimicrobial Activity Testing

The Kirby-Bauer Method was employed using Mueller-Hinton Broth standardized to 0.5 McFarland (1.5×10^8 CFU/mL). Inoculated agar plates were treated with extract discs and incubated at 35°C for 16–18 hours. Zones of inhibition were measured in mm.

Antioxidant Activity Testing

Using Kalemba et al. (2024), serial dilutions (1.95–1000 µg/mL) of extracts were mixed with 0.02% DPPH solution and incubated for 30 mins at 37°C. Absorbance at 517 nm was recorded, and radical scavenging activity was calculated using the formula:

$$\text{DPPH Scavenged (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Anti-inflammatory Activity Assay

Based on Daram et al. (2021), reaction mixtures containing egg albumin, PBS, and varying concentrations of the extract were incubated and heat-treated. Absorbance at 660 nm was used to compute % inhibition of protein denaturation:

$$\text{\% Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Cytotoxicity Assay

Brine shrimp eggs were hatched in saline for 48 hrs. Extracts (1.95–1000 µg/mL) were tested, and % mortality was assessed after 24 hrs. LC_{50} values were calculated via Probit analysis in Excel and classified according to the standards of Meyer et al. (1982) and Clarkson et al. (2004).

Treatment of Data

All quantitative data obtained from the bioassays were recorded, tabulated, and statistically analyzed to determine the biological activity of the *Cyperus imbricatus* Retz. Leaf crude extract. Results from the five replicates per treatment in the bioassays, except for the cytotoxicity assay, were expressed as mean } standard deviation (SD).

For the Brine Shrimp Lethality Assay (BSLA), mortality data were used to calculate the median lethal concentration (LC_{50}) using probit analysis. A dose-response curve was plotted, and linear regression was performed to determine the relationship between concentration and mortality rate. Higher LC_{50} values were interpreted as indicative of lower cytotoxicity and vice versa.

Ethical Consideration

The study was approved by the Saint Mary's University Research Ethics Board (SMUREB) under reference code 2025 0852. Laboratory procedures were conducted under the supervision of the CNS laboratory assistants. The waste materials generated from the study were discarded after the post-in vitro assay.

RESULTS AND DISCUSSIONS

Section 1. Secondary metabolites detected from *C. imbricatus* leaf extract

This section encapsulates the results for the determination of secondary metabolites present in *C. imbricatus* leaf crude extract. The secondary metabolites were detected using the corresponding reagents listed in the first column of the table.

Table 1

Secondary Metabolites from C. imbricatus Extract

Reagent	Compound Tested	Result
Preliminary test (H_2SO_4 + heat)	Essential oils	+
Vanillin sulfuric acid	Triterpenes and sterols	+
	Essential oils	-
	Phenols	+
	Fatty acids	+
Naphthol-sulfuric acid + heat	All carbohydrates (except tetroses and trioses)	+
Methanolic potassium hydroxide (KOH–MeOH)	Anthraquinones	+
	Coumarins	+
	Anthrones	-
Potassium ferricyanide + ferric chloride	Tannins	+
	Flavonoids	+
	Phenols	+
Dragendorff's reagent	Alkaloids	-
Magnesium acetate + heat	Anthraquinones	+
Ninhydrin	Amino acids	-

Table 1 presents that the ethanolic extract of *C. imbricatus* leaves was revealed to contain essential oils, triterpenes, sterols, phenols, fatty acids, all carbohydrates except tetroses and trioses, anthraquinones, coumarins, tannins, and flavonoids.

The presence of essential oils, phenolic acids, and flavonoids align with the bioactive compounds found by Peerzada et al. (2015) and Bezerra et al. (2023) from *Cyperus spp*. Additionally, similar results were found in the study of Kumar et al. (2017) on *C. rotundus*, a species in the same genus, which confirmed the presence of carbohydrates, quinones, terpenoids, and coumarins while the study of Zhang et al. (2022) confirmed the presence of tannins and sterols from *C. esculentus*, another species of Cyperus genus. These compounds are known to essentially contribute to the bioactivities exhibited by the *C. imbricatus* (Udari, 2018).

Based on the obtained results, *C. imbricatus* is abundant with secondary metabolites that qualify for various biological activities. In light of this, this plant shows potency for pharmacological studies and product formulation.

Section 2. Antimicrobial activity of *C. imbricatus* leaf extract against *K. pneumoniae* and *P. aeruginosa*

This section summarizes the values obtained from Kirby-Bauer disk diffusion. The plant extract and the controls were tested against *K. pneumoniae* and *P. aeruginosa*. In the determination of the ZOI of the plant extract, the Mean } SD of the five replicates was recorded in a table.

Table 2

Zone of Inhibition of C. imbricatus against K. pneumoniae and P. aeruginosa

Test Substances	***Replicates (mm)***								
	1	***2***	***3***	***4***	***5***	***Mean***	***SD***	***Mean±SD***	***Result***
					K. pneumoniae				
C. imbricatus (1000 μg/mL)	*7.5*	*6.8*	*6*	*6.2*	*14.9*	*8.28*	*3.74*	*8.28± 3.75*	*Inactive*
Streptomycin (+)	*15.3*	*12.9*	*12.3*	*14.3*	*12.3*	*13.42*	*1.33*	*13.42±1.33*	
24% DMSO (-)	*6*	*6*	*6*	*6*	*6*	*6*	*0*	*6*	
					P. aeruginosa				
C. imbricatus (1000 μg/mL)	*6*	*6*	*6*	*6*	*6*	*6*	*0*	*6*	*Inactive*
Streptomycin (+)	*10.4*	*10.8*	*9.3*	*10.1*	*10.3*	*10.18*	*0.55*	*10.18±0.55*	
24% DMSO (-)	*6*	*6*	*6*	*6*	*6*	*6*	*0*	*6*	

Table 2 presents the zone of inhibition of the replicates after the extract was introduced to *K. pneumoniae* and *P. aeruginosa*. On *K. pneumoniae*, 1000 μg/mL of *C imbricatus* has a mean inhibiting activity of 8.28mm with a standard deviation of about 3.75mm, which demonstrates inactivity against *K. pneumoniae*. On the other hand, it showed no inhibition on *P. aeruginosa*, thus absolutely inactive.

The *C. Imbricatus* extract contained essential oils, fatty acids, triterpenes, sterols, coumarins, and anthraquinones. These non-polar compounds were found to exhibit antimicrobial activity (Burt, 2004, as cited by Falleh et al., 2020; Sonkoue et al., 2023; Arellano et al., 2023; Smyth et al., 2009, as cited by Hassanein et al., 2020; Qun et al., 2023). The little to no inhibition of the extract in the disk diffusion method may have been attributed to the poor diffusion of non-polar compounds into the MHA (Bubonja-Ssonje et al., 2020). Moreover, the poor solubility of essential oils found in this plant poses a major limitation, particularly when incorporating the extract into the media. This drawback significantly reduced their antimicrobial efficacy in practical applications (Gyawali & Ibrahim, 2014).

Based on the mean ZOI against the bacteria, *C. imbricatus* demonstrated no inhibitory activity on *K. pneumoniae* and *P. aeruginosa*. This implies that the plant extract has no potential as an antimicrobial agent for UTI and thus suggests that its effect on UTI patients may not be attributable to this biological activity.

Section 3. Scavenging activity of the *C. imbricatus* extract to neutralize DPPH radicals.

This section presents the scavenging activity of *C. imbricatus* to neutralize DPPH radicals.

The result was presented through a bar graph, highlighting the mean % inhibition of the different concentrations of the plant against DPPH radicals. The error bars represent the standard error of the mean.

Figure 1

Inhibitory Activity of C. imbricatus against DPPH Radicals

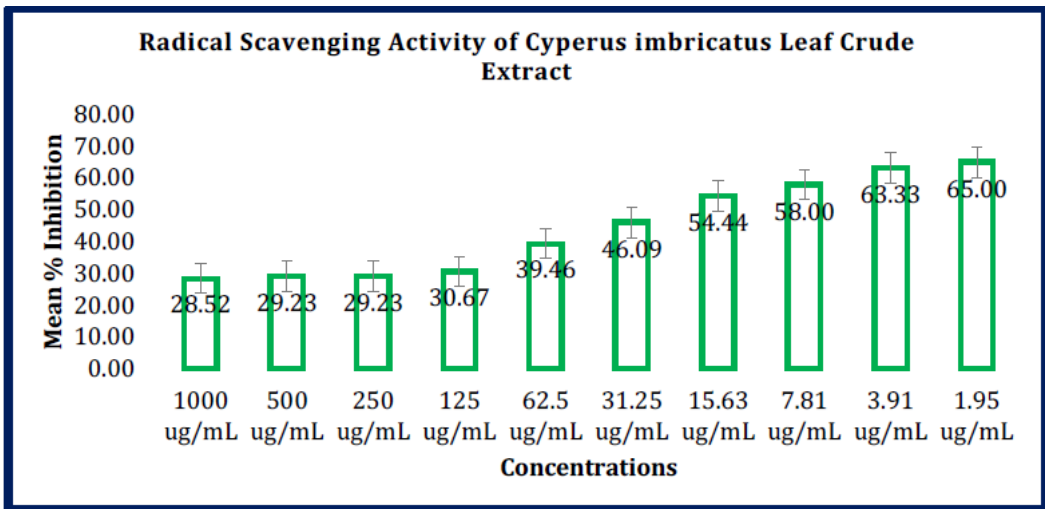


Figure 1 shows a chart of the scavenging activity of *C. imbricatus* against DPPH radicals. The graph illustrates the antioxidant activity of the plant at concentrations ranging from 1000-1.95 µg/mL. The highest concentration has the lowest antioxidant activity, reaching a mean inhibition of 28.52%. In contrast, the lowest concentration has the highest antioxidant activity, reaching a mean inhibition of 65%, approaching the inhibitory activity of Ascorbic, which is 75% at 1.95 µg/mL. The negative control shows minimal to no activity, confirming that the observed results are attributable to the extract. The chart clearly shows an increasing trend of scavenging activity as the concentration decreases (from 1000 ug/mL down to 1.95 ug/mL). This indicates a dose-dependent effect, where lower concentrations of the extract exhibit stronger radical scavenging activity within the tested range.

This strong antioxidant activity of *C. imbricatus* is comparable to the antioxidant activity of the *Cyperus spp*. studied by Bezerra et al. (2023) and Taheri et al. (2021), showing high antioxidant properties. However, the relationship between concentration and activity is observably inversely proportional in this study, in which the lower the concentration of *C. imbricatus* extract, the higher the antioxidant activity is and vice versa. As cited by Chaves et al. (2020) from the study of Li et al. (2014), the antioxidant capacity of phenols in plant extracts is generally effective at low concentrations.

Therefore, the ability of *C. imbricatus* extract to scavenge DPPH radicals is most effective at low concentrations due to its phenolic content, as shown in Section 1. This implies that the plant is high in antioxidant properties, making the plant essential in neutralizing free radicals that cause various diseases by cell damage, thus improving immune function. Moreover, it can be utilized in the formulation of pharmacological products, food supplements, cosmetics, and preservatives.

Section 4. Anti-inflammatory activity of *C. imbricatus* against protein denaturation

This section presents the inhibitory activity of *C. imbricatus* against the denaturation of egg albumin caused by heat. The mean percentage of the activity per concentration of the extract is represented in Figure 5. The error bars describe the significance of the statistical differences of the mean.

Figure *2*

Inhibitory activity of C. imbricatus against egg albumin denaturation

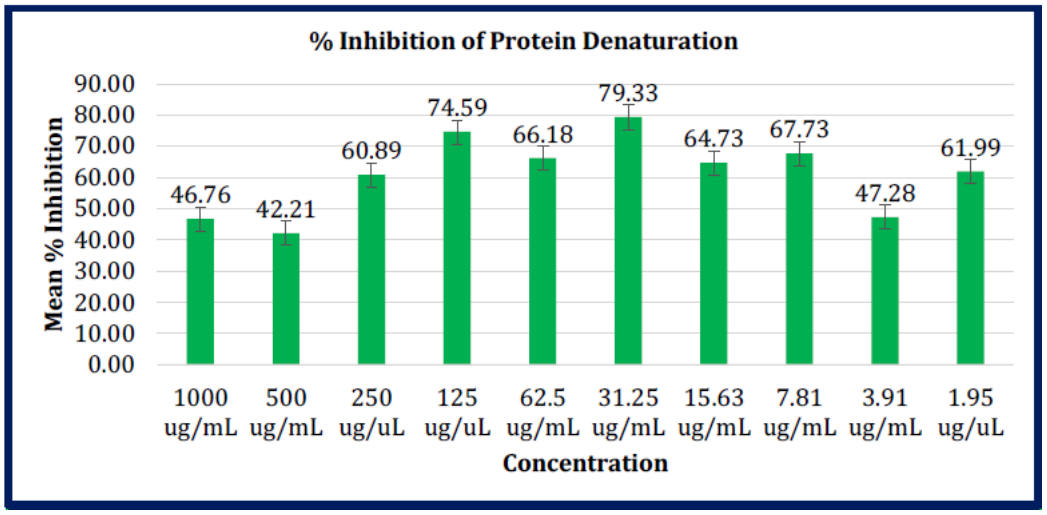


Figure 2 illustrates the ability of *C imbricatus* extract to inhibit protein denaturation after the egg albumin has been exposed to heat. The chart demonstrates that the crude extract possesses the ability to inhibit protein denaturation in a concentration-dependent manner. The optimal concentration for this activity appears to be around 31.25 ug/mL, achieving 79.33% inhibition. In comparison, its MIC is observed at 1.95 ug/mL. While the inhibition generally increases as the concentration decreases from 1000 ug/mL to this optimal point, concentrations lower than 31.25 ug/mL showed reduced efficacy. The generally short error bars indicate good precision in the experimental measurements, allowing for confidence in the observed trends and the identification of concentrations with significantly different inhibitory effects.

Previous studies on *Cyperus* presented bioactivities of plant species belonging to the genus, including anti-inflammatory activity (Bezerra et al., 2022). This biological activity is concluded by Pratiwi et al. (2024) to be attributable to the detected secondary metabolites, such as flavonoids, alkaloids, tannins, sterols, and triterpenes, which are detected in the plant as shown in Section 1. The synergism of these compounds, which are generally found in medicinal plants such as *C. imbricatus*, exhibit pharmacological activities like anti-inflammatory activity (Gonfa et al., 2023). Moreover, polysaccharides are carbohydrates that play a significant role in immunomodulatory and anti-inflammatory activity (Elbandy, 2022).

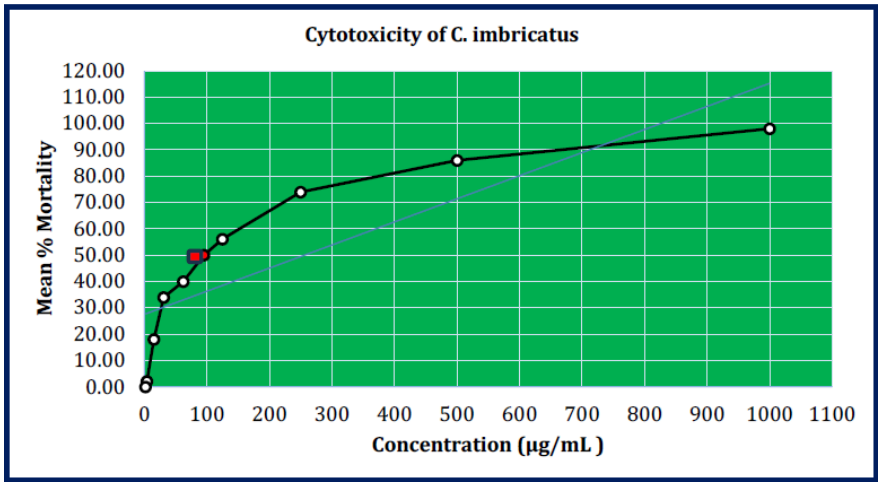
Based on the data and related studies, *C. imbricatus* exhibits promising anti-inflammatory activity, especially at the identified optimal concentration, as determined by its ability to reduce the denaturation of egg albumin. While the immune system functions to protect the body from
infection by inflammation, uncontrolled inflammation leads to cell and tissue damage, which can be fatal. The plant's anti-inflammatory activity is crucial to reducing uncontrolled inflammation, thus its crucial role in immune function.

Section 5. Cytotoxic activity of *C. imbricatus* on brine shrimps

This section shows a dose-response curve showing the cytotoxicity of *C. imbricatus*. The X-axis indicates varying concentrations of the *C. imbricatus* extract. In contrast, the Y-axis indicates the mean percentage of cell mortality at each concentration. The data was analyzed using probit analysis, and a linear regression chart was used to present the LC50.

Figure 3

Cytotoxicity of C. imbricatus to Nauplii Brine Shrimps



Note. Data points show the mean % mortality at each concentration. The red mark indicates the LC_{50} and the regression line illustrates the dose-response relationship between extract concentration and mortality.

Figure 3 shows the mean mortality rate of nauplii brine shrimp after it has been exposedto the different concentrations of *C. imbricatus* extract. The lethal concentration that caused 50% mortality (LC50) to the nauplii brine shrimps was 95.66 µg/mL as determined using Probit analysis and regression formula. Using the classification of toxicity in Table 1, the plant is considered highly toxic.

Based on the results obtained from the phytochemical screening, as presented in Section 1, *C. imbricatus* extract was positive for Tannins. In the study of Younus et al. (2021), it was suggested that Tannins may have cytotoxic activity. It was further proven that this secondary metabolite exhibits a substantial cytotoxic effect against tumor cells due to a specific Tannin called Corilagin (Jit et al., 2024). In the study of Chudzik et al. (2015), as cited by David et al.(2024), triterpenes were proven to have anticancer and chemo preventive properties and toinduce cancer cell apoptosis without damaging the normal cells. Abotaleb et al. (2020) also studied polyphenols, such as tannins and flavonoids, which exhibit cell proliferation reduction and apoptosis. In addition, coumarins exhibit similar activity (Rawat & Reddy, 2022).

Therefore, *C. imbricatus* extract is proven to exhibit cytotoxic effects on nauplii brine shrimps due to the presence of cytotoxic compounds like triterpenes, phenols, and coumarins. This bioactivity sets its qualification as a cytotoxic agent that combat cancer cell proliferation. However, this study also suggests caution for oral intake due to its highly toxic nature. This also supports the caution advised by the users on the dosage and frequency of consumption due to its possible side effects.

Section 6. Information Education and Communication

This section presents the IEC material used to disseminate the results of this study. Scientific data were summarized and translated for better understanding among the community. It was submitted to the barangay officials with the attached transmittal letter for approval.

Figure 4

Infographics on The Biological Activities of C. imbricatus

Figure 4 shows an infographic poster containing the biological activities of *C. imbricatus*.. It presents the results obtained and interpreted in the previous sections, such as its secondary metabolites, antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activity of the plant. This IEC material serves as a means to spread awareness about the bioactivities of this plant, especially the need for caution on its cytotoxic tendency.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study determined the biological activities of *C. imbricatus* leaf crude extract, namely, its antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activity. The result of the

phytochemical screening revealed that *C. imbricatus* leaf ethanolic extract contained essential oils, triterpenes and sterols, phenols, fatty acids, all carbohydrates except tetroses and trioses, anthraquinones, coumarins, tannins, and flavonoids. It was also tested for its antimicrobial activity, and it showed no zones of inhibition against *K. pneumoniae* and *P. aeruginosa*. While the antimicrobial assay obtained negative results, it showed high antioxidant and anti-inflammatory activity at low concentrations. It was found to be highly toxic as its LC50 against nauplii brine shrimps was determined at a low concentration. However, its level of toxicity suggests caution before consumption. The results were disseminated in the community in order to spread awareness on the biological activities, as well as caution on its consumption.

Recommendations

Based on the obtained results of this study, the following are recommended:

1. Determine the RF value of the secondary metabolites to identify organic compounds and chemical reactions and purifications.

2. Further exploration of the bioactivities, in vitro and in vivo, of C. imbricatus extract is needed to provide a stronger scientific foundation.

3. Determine the *C. imbricatus* extract's cytotoxicity through molecular diagnostic tests to determine the extent of its cytotoxicity.

4. Conduct an ethnopharmacological and translational toxicology study aimed at developing an evidence-based health policy for the safe use of a traditionally utilized medicinal plant.

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