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Research Article

Phytochemical screening, antibacterial and cytotoxic activities of *Aristolochia philippinensis* Warb. leaf extracts

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Abstract: The *Aristolochia philippinensis* Warb. (Aristolochiaceae) is commonly used in traditional medicine among indigenous people in the Philippines. The leaves of *A. philippinensis* are used to treat illnesses such as stomach aches, bodily aches, and physical relapse. This study was conducted to identify the phytochemical compounds and evaluate the antibacterial and cytotoxic activity of *A. philippinensis.* The leaf extracts were subjected to qualitative phytochemical screening, in vitro testing for antibacterial activity using agar well-diffusion assay against selected gram-positive and gram-negative bacteria and cytotoxic activity using 3-(4,5-dimethylthiazol-2-y-l)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay against human colorectal carcinoma cells (HCT 116). The results of the phytochemical screening of the leaf extracts showed to contain flavonoids, tannins, alkaloids, and steroids; however, there is an absence of saponins, anthraquinones, and cyanogenic glycosides. The agar well-diffusion assay also revealed that leaf extracts have inhibitory effects on all test organisms but are relatively lower compared to the control (Chloramphenicol). In contrast, aqueous leaf extract showed no inhibitory effects. Furthermore, the MTT cell viability assay demonstrated that leaf extracts inhibited the growth of HCT116 effectively with a half-maximal inhibitory concentration (IC_{50}) of 141.57 ppm than the aqueous extract with an IC_{50} of 506.66 ppm. This study demonstrated that the use of *A. philippinensis* has a pharmacological basis as an ethnomedicine and could be vital in drug discovery.

1. INTRODUCTION

Due to the growth of multi-resistant bacteria that cause infections resistant to currently available antibiotics, antimicrobial resistance poses a danger to global health and development (WHO, 2023). Excessive use of incorrect prescriptions, frequent agricultural use of antibiotics, and a shortage of novel antibiotics all contribute to the development of drug resistance in bacteria (Ventola, 2015; Bhuyan *et al.*, 2017). Naturally occurring chemicals produced by a wide variety

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of higher plants are valuable sources of natural antibacterial agents (Naik *et al.*, 2014). Plant-based medicines continue to be a significant source of therapeutic agents due to their availability, reduced costs, and nontoxic when compared to modern medicine (Demetillo *et al.*, 2017). The active plant chemical constituents are often composed of a combination of secondary metabolites including alkaloids, saponins, tannins, glycosides, phenols and flavonoids. Such phytochemicals must be investigated, extracted, isolated, detected, and identified in order to establish quality control, the biological mechanisms of their action on the body (Borah *et al.*, 2021; Ogunmefu, 2018; Saxena, 2013). Many chemotherapy medications are utilized for the treatment of cancer however, the issue of selective toxicities and severe side effects remains (Ogbole *et al.*, 2017). Furthermore, recent studies indicated that the majority of the secondary metabolites that were separated from a wide range of plant families had the potential to be developed into anticancer drugs (Amna *et al.*, 2019). The most prevalent anticancer technique in the treatment of cancer cells in the body is the induction of apoptosis in tumor or cancer cells. A number of natural plant extracts and phytochemicals have been shown to induce apoptosis in cancer cell lines (Dubey *et al.*, 2022).

Aristolochia species have been utilized for various illnesses and diseases. For instance, the root of *Aristolochia indica* is known to heal wounds, enhance fertility in males, treat inflammations, dry coughs, stomach pains, constipation, and excessive flatulence (Taid *et al.*, 2014; Mathew *et al.*, 2020). The tubers of *Aristolochia acuminata* are used to treat diarrhea and dysentery (Lerma-Herrera *et al.*, 2022). In traditional Chinese medicine, the dried ripe fruits of *Aristolochia debilis* and *Aristolochia contorta* have been used for haemorrhoids, coughs, and asthma and the dried stems or leaves are used for epigastric pain, joint pain, and oedema. The dried roots from *Aristolochia debilis* are used for the treatment of dizziness, headaches, abdominal pain, carbuncles, boils, and snake and insect bites (IARC, 2002). In addition, *Aristolochia clematitis* has been reported to have antibacterial and antifungal properties (Ozen *et al.*, 2020). Other species of *Aristolochia* also has been utilized to treat snake bite and headache (Alonso-Castro *et al.*, 2017).

Despite the Philippines being a hotspot in biodiversity and known to be rich in traditional medicinal plants (Garcia *et al.*, 2018) and with various indigenous tribes having their own ethnobotanical practices for utilizing these plants, there have been few studies and no clinical reports concerning the use of *Aristolochia philippinensis* Warb. This plant is locally known as 'Bicaca', and is a member of the family Aristolochiaceae, and it is endemic to the Philippines. *A. philippinensis* is a low, shrubby, smooth plant that can grow up to 1.5 meters tall. The leaves are simple, exstipulate, and spiral, and the blade is oblong-lanceolate and serrate, measuring 5 to 18 cm in length and 2 to 5 cm in width. Flowers are arranged in terminal inflorescences. Fruits are capsular and dehiscent, measuring up to 1 cm in length (Wiart, 2006). A decoction of *A. philippinensis* leaves is often used to treat stomach aches (Carag and Buot, 2017), body aches and physical relapse (Madjos and Ramos, 2021).

The scarcity of scientific data on *A. philippinensis* traditional knowledge necessitates further exploration of commonly used traditional medicine among Indigenous people to prioritize species that have implications for health and find new lead plants in drug discovery. Thus, this study was conducted to identify the bioactive compounds of *A. philippinensis*, determine the antibacterial activity through the agar well-diffusion method against selected gram-positive and gram-negative bacteria, and evaluate the cytotoxic activity using the 3-(4,5-dimethylthiazol-2-y-l)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay against human colorectal carcinoma cells (HCT 116).

2. MATERIAL and METHODS

2.1. Sample Collection

The leaf samples of *A. philippinensis* were collected in the coastal areas of General Luna, Siargao Island, province of Surigao del Norte, Philippines in October 2022. Plant identification

was verified by Dr. Florence Roy Salvaña, a botanist from the University of Southern Mindanao, Philippines. A voucher specimen was deposited in the Natural Science Museum, MSU-Iligan Institute of Technology (NSM-4582). The collected leaves were washed and rinsed with distilled water, air-dried for 30 days, pulverized into a fine powder using a blender, and stored in a glass container for further use.

2.2. Preparation of Ethanol Extract

A total of 250 g of pulverized leaves of *A. philippinensis* were soaked in 1500 mL of 95% ethanol for 7-days. Whatman filter paper No. 1 was used to filter the supernatant, and the filtrates were concentrated through rotary evaporation at 45 °C and reduced pressure. A total of 44 g of dry residue was recovered from the 250 g powdered leaves with a yield of 17.6%. The residue retained in rotary evaporation was collected and stored in a glass vial for further analysis.

2.3. Preparation of Aqueous Extract

Aqueous extraction of leaves followed the method of Bhuyan *et al.,* 2017, with modifications. Fifty (50) g of powdered leaf samples were added to 1000 mL of distilled water, stirred, and heated at 85 °C for 15 minutes in a shaking water bath. Subsequently, the mixture was allowed to cool to room temperature and filtered using Whatman filter paper No. 1. The supernatant was concentrated using rotary evaporation at 55 °C at reduced pressure. The 4.8 g residue retained in rotary evaporation from the 50g of powdered leaves (9.6% yield) was kept in a glass vial.

2.4. Phytochemical Screening

A qualitative screening was performed to identify active components of ethanol and aqueous leaf extracts of *A. philippinensis* and the result was recorded using a 3-point scale (+ turbid, ++ moderate, and +++ heavy) based on the “Handbook of Philippine Medicinal Plants” (De Padua *et al.,* 1977).

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2.7.1. *Detection of alkaloids*

In a test tube, 2 g of crude extracts were added with 5-10 mL of 2M HCl and 0.5g of sodium chloride in a test tube. It was immersed in a boiling water bath for 5 minutes then cooled, filtered and subsequently, the residue was rinsed with 2M HCl. After rinsing, the combined filtrates

were split into three equal pieces. 3-5 drops of Wagner's reagent were added to the first portion, Mayer's reagent was added to the second part, and the third component acted as the control. The production of dark precipitate with Wagner's reagent and white precipitate with Mayer's reagent indicates the presence of alkaloids (Aguinaldo *et al.*, 2004).

2.7.2. *Detection of flavonoids*

Crude extracts were diluted in 10 mL of 80% alcohol, filtered, and the filtrate was separated into two equal amounts. The first component was added with 0.5 mL of 12M HCl, while the second part acted as the control. Two test tubes were placed in the hot water bath. The observation of red coloration in the solution is indicative of the presence of flavonoids (Aguinaldo *et al.*, 2004).

2.7.3. *Detection of saponins*

Saponins were detected using the froth's test procedure. In a test tube, 2 mL of leaf extract was added to an equal volume of water. For around 30 seconds, the plant extract was shaken. The production of foam of 2 cm in height indicates a favorable result for saponins (Aguinaldo *et al.*, 2004).

2.7.4. *Detection of tannins*

Crude extracts were mixed with 20 mL of boiling water, followed by 2-3 drops of 10% NaCl solution. The resulting solution was filtered, and the residue was rinsed with water. The mixed filtrates were then collected, rinsed, and separated into three equal portions. 3-5 drops of 1% ferric chloride were added to the first portion, followed by 3-5 drops of Gelatin-Salt reagent in the second portion, and the third part acted as the control. The experiment yielded positive results in two respects: firstly, the presence of a black or blue-black precipitate when ferric chloride was utilized; secondly, the formation of a white precipitate when gelatin and sodium chloride were employed (Aguinaldo *et al.*, 2004).

2.7.5. *Detection of anthraquinones*

Two grams of crude extract were diluted with 10 mL of distilled water, then the resultant solution was filtered and stirred. 5 mL of benzene was used to extract the filtrate twice then, the extracts were allowed to sit for a few minutes to allow separation of the aqueous and benzene layer. Then, the benzene layer was transferred using a pipet in a test tube containing 1 mL ammonia reagent and it was shaken for a few seconds. The formation of a reddish-pink color in the solution indicates the presence of anthraquinones (Aguinaldo *et al.*, 2004).

2.7.6. *Detection of steroids*

In a test tube, 2 mg of the plant extract was dissolved with 2 mL of chloroform. The resulting solution was separated equally into two parts. The first component was mixed with 1 mL of concentrated sulfuric acid passing through the test tube's wall. A positive result will reveal the production of violet to blue colored rings at the aqueous extract's border area (Aguinaldo *et al.*, 2004).

2.7.7. *Detection of cyanogenic glycosides*

In a 20 mL test tube, 1 mL of crude extract was poured and was added with 5 drops of chloroform. A picrate paper was then suspended above the solution. The test tube was placed in the hot water bath, and covered with an inverted dropper. The positive result for cyanogenic glycosides was revealed by the quick production of red color on the surface of the picrate paper (Aguinaldo *et al.*, 2004).

2.8. *In vitro* Antibacterial Activity

The *in vitro* antimicrobial activity of *A. philippinensis* leaf extracts was screened using the agar well-diffusion method against selected microorganisms. The ethanol and aqueous leaf extracts were tested against two gram-positive bacteria, *Bacillus subtilis* (UPCC 1295), *Staphylococcus*

aureus (UPCC 1143), and two gram-negative bacteria, *Salmonella typhimurium* (UPCC 1368), and *Escherichia coli* (UPCC 1195), for which microbial suspensions approximately 1.5 x 10^8 cfu/mL were prepared from each of the 18-24-hour old cultures, and 0.1% peptone water was used as a suspending medium. Agar plates were pre-poured with Muller Hinton Agar about 3 mm thick and inoculated with the respective microbial suspension by swabbing the agar surface. The sterile cotton swab applicator was dipped into the microbial suspension, rotated several times, and then firmly pressed against the tubes' interior wall above the fluid level to remove excess inoculum. To ensure uniform inoculum coverage, the swab was streaked over to the entire agar surface twice, with the plate rotated by 60 degrees in between each time. A 10 mm diameter cork borer was used to drill three equidistant wells, and 200 µL of ethanol and aqueous extracts were inserted in each of the wells. Plates were incubated at 35°C and observed after 24 hours; 30 µg of a 6 mm diameter disc of chloramphenicol was used as the positive control.

All tests were performed in triplicate, and the diameter of the inhibition zone (in millimeters) was measured and the mean value was calculated to determine the antibacterial activity of the tested sample.

2.9. *In vitro* Cytotoxic Activity

2.9.1 *Treatment preparation*

Before the assay, 10,000 ppm sample stock solution was prepared by dissolving 3 mg of leaf extract with 300µL of Dimethyl Sulfoxide (DMSO). Concentrations of 12.5, 25, 50, 100, 200, and 400 parts per million (ppm) were obtained via serial dilution with cell culture medium as a diluent.

2.9.2 *Cell culture*

Human colorectal carcinoma cells (HCT116) were procured from the American Type Culture Collection (CCL-247; Manassas, VA, USA). Cancer cells were grown and cultured following the instructions recommended by the manufacturer (Promega Technical Bulletin, 2012). HCT 116 were subcultured using Ham's F12 and Dulbecco Modified Eagle Medium (DMEM) containing 1.2 g/L sodium bicarbonate, 15 mM HEPES (pH 7.35), and 10% horse serum in a tissue culture flask and incubated for 24 hours in a 5% CO_2 atmosphere at 37°C humidified temperature. The harvesting of cells was performed using trypsin-EDTA to determine cell number, and the trypan blue dye exclusion method was employed to assess cell viability.

2.9.3 *Measurement of MTT cell viability*

The CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) was used to determine the cell viability based on the manufacturer's procedure (Promega Technical Bulletin, 2012) with modifications. A 90µL cell suspension containing 200,000 cells was seeded in a 96-well plate, excluding the blank wells, and incubated overnight at 37°C in a 5% CO_2 atmosphere. The 10µL of prepared samples were added to their respective wells on a 96-well plate. The culture medium in the negative control was changed to 100µL culture medium + 1% sterile DMSO, and 100µL culture medium + 1% sterile DMSO was also added to the blank wells. Subsequently, the 96-well microplate was incubated overnight at 37°C in a 5% CO_2 atmosphere. Then, a 15µL dye was added to each well and was incubated for 4 hours at 37°C with 5% CO_2. Following the addition of 100µL of solubilization solution to each well, the plate was left to sit at room temperature in the dark for one hour. The absorbance at 570nm wavelength was measured using Victor X3 Spectrophotometer with a Plate Reader. All experiments were performed in two trials with three replicates. The percentage in the treated cultures relative to the untreated cells was given as means ± SD in the results. Digitonin, a positive control, was employed. Using the following formula, the percentage of cell viability was determined:

Cell Viability (%) = [(AS-AB)/AC-AB)]x100

AS: Average Sample Absorbance

AB: Average Blank Absorbance

AC: Average Control Absorbance

Furthermore, the half maximal inhibitory concentration (IC_{50}) value indicates the concentration at which 50% of the cells are killed. This was calculated from linear regression plots of the percent cell viability vs. the log concentration.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

The results of the phytochemical screening indicated the presence of secondary metabolites in ethanol and aqueous leaf extracts of *A. philippinensis.* These extracts showed a heavy presence of flavonoids, tannins, steroids, and a turbid presence of alkaloids; anthraquinones, cyanogenic glycosides, and saponins were absent (Table 1).

Table 1. Phytochemical screening of the *Aristolochia philippinensis* ethanolic and leaf extract.

Secondary Metabolite	Ethanol	Aqueous
Alkaloids	+	+
Flavonoids	+++	+++
Saponins	-	-
Tannins	+++	+++
Anthraquionones	-	-
Steroids	+++	+++
Cyanogenic Glycosides	-	-

(+) indicates present: + turbid, ++ moderate, and +++ heavy, (-) indicates absent

3.2. Antibacterial Activity

The antibacterial activity of the ethanol and aqueous leaf extracts of *A. philippinensis* were assessed against selected gram-positive and gram-negative bacteria and were expressed in terms of mean inhibition zone (Table 2). The results showed that ethanol leaf extracts displayed inhibitory effects on all test organisms ranging from 11.6mm to 13.3mm. However, the aqueous extract showed no inhibitory effect. Positive control (Chloramphenicol) showed the highest inhibition zone, ranging from 27 mm to 30 mm.

Table 2. Antibacterial activity of the *Aristolochia philippinensis* ethanolic and leaf extract.

Type of Bacteria	Test Organism	Sample	Mean Inhibition Zone (mm)
Gram-positive	*Bacillus subtilis* (UPCC 1295)	Ethanol	12.66 ± 0.33
		Aqueous	nd
		Chloramphenicol	28.00 ± 0.57
	Staphylococcus aureus (UPCC 1143)	Ethanol	11.66 ± 0.33
		Aqueous	nd
		Chloramphenicol	27.33 ± 0.33
Gram-negative	*Salmonella typhimurium* (UPCC 1368)	Ethanol	12.16 ± 0.16
		Aqueous	nd
		Chloramphenicol	27.46 ± 0.29
	Escherichia coli (UPCC 1195)	Ethanol	13.43 ± 0.43
		Aqueous	nd
		Chloramphenicol	30.06 ± 0.58

nd= not detected; Each value is a mean of three biological replicates

3.3. Cytotoxic Activity

The effect of the different concentrations of *A. philippinensis* ethanolic and aqueous leaf extract are recorded in terms of percent cell viability (Figure 1) and the concentration dose required to

inhibit 50% of cell growth and proliferation (Table 3). The inhibitory effect of the cell growth of human colorectal carcinoma cells was observed to be concentration dependent in both extracts, the viability of cell growth decreases as the concentration of the extract increases. Moreover, the highest cytotoxic activity was observed in the ethanolic extracts of *A. philippinensis* with an IC_{50} value of 141.57 ppm compared to the aqueous extract with an IC_{50} value of 506.66 ppm.

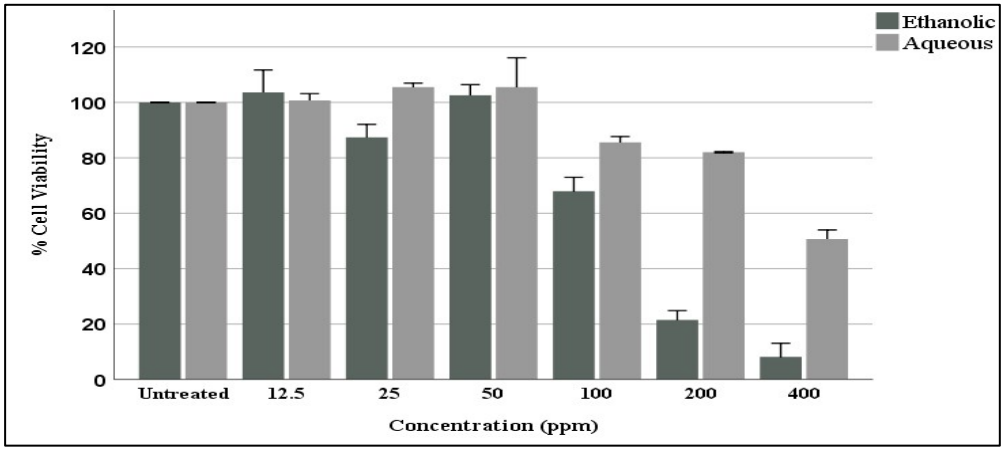


Figure 1. Percent cell viability of *Aristolochia philippinensis* leaf extracts against Human colorectal carcinoma cells (HCT 116). Data are represented as means ± SD of the percentage in the treated cultures compared with those of the untreated cells.

Table 3. IC_{50} values of cell proliferation inhibition of *Aristolochia philippinensis* leaf extracts.

Cell	IC_{50} (ppm)		
Human colorectal carcinoma cells (HCT 116)	Ethanol	Aqueous	Digitonin
	147.57*	506.66	13.83**

**Strong: IC50<100ppm, *Moderate: 101-200 ppm, Weak: IC50>200ppm

4. DISCUSSION and CONCLUSION

The phytochemical screening of *A. philippinensis* showed both ethanolic and aqueous leaf extracts have the presence of flavonoids, tannins, steroids, and alkaloids. These bioactive secondary metabolites from plants are known for their pharmacological, food products, and industrial applications.

Flavonoids are bioactive polyphenol compounds with antibacterial action (Gutiérrez-Venegas *et al.,* 2019). Studies on the antibacterial activity of plant flavonoids have shown to influence bacterial growth, such as in *Staphylococcus aureus* (Adamczak *et al.*, 2020) and in *Staphylococcus epidermidis*, which causes acne vulgaris (Nishino *et al.*, 1987). Flavonoids are regarded as constitutive antibacterial agents due to their potential to exert antibacterial activity through cytoplasmic membrane damage, inhibition of energy metabolism, and inhibition of nucleic acid production (Tan *et al.*, 2022).

Tannins are naturally occurring bioactive substances and are known for their various applications, including dietary supplements and medications (Das *et al.,* 2020). Tannins are notable for their antibacterial, antioxidant, antibacterial, antiparasitic, antiviral and anti-inflammatory activities. (Tong *et al.,* 2022) Like tannins, plant steroids also possess antioxidant, anti-inflammatory, antibacterial, and antifungal properties among tested organisms (Marjoni and Zulfisa, 2017; Raju *et al.,* 2021; Miya *et al.,* 2023).

Alkaloids are naturally occurring organic molecules with extremely divergent chemical properties that encompass more than 20,000 different molecules in organisms. Alkaloids show several pharmacological activities on human health, such as local anaesthetics and stimulants, analgesics, anti-asthma therapeutics, vasodilators, anti-cancer, anti-inflammatory, anti-

malarial, antimicrobial, anti-hypertensive, anti-diabetic, and antioxidants (Kuete, 2014; Rajput *et al.*, 2022). The presence of alkaloids explains the use of *A. philippinensis* leaves as a treatment for bodily aches, physical relapses, and stomach aches.

From the antibacterial study conducted, the ethanolic extracts inhibited all test organisms but were lower than the standard antibiotic chloramphenicol. Various factors may influence the level of inhibition of the plant extract. The duration of storage of the plant extract has been reported to result in a sharp decline in antibacterial activity (Eloff, 1999; Lawal and Tshikalange, 2020), and the solvent used to extract compounds (Borges *et al.,* 2020). However, these factors may not be associated with the lower inhibitory effect on test organisms since the plant extract was used immediately and ethanol was used as a solvent of extraction, which is considered the best solvent for extraction, and it extracts active compounds that exhibit maximum antimicrobial activities (Al Farraj *et al.,* 2020). Likewise, utilizing ethanol as a solvent result in a substantial increase in the solubility of plants' bioactive compounds in comparison to water alone (Abubakar *et al.,* 2015), since ethanol can cleave diverse types of compounds including polar and non-polar, simple and complex chemical structures compared with water (Ezemokwe *et al.,* 2020). Furthermore, the difference between ethanol and aqueous extract suggests that the bioactive compounds that greatly contribute to the antibacterial activity could be identical in structures in both extracts, only that they are more soluble in ethanol as it is more lipophilic than water due to the following reasons: compounds extracted by ethanol might have greater potency as there maybe impurities that are soluble with water than ethanol which could lead to decrease in activity or there may be varying impurities that exhibit different antagonisms against the active components, with the impurities dissolved in the ethanol extract being less antagonistic than those extracted by water (Thakur *et al.,* 2011; Ezemokwe *et al.,* 2020).

Despite this, compounds with lesser antibacterial activity may continue to play a crucial role in the synthesis of new antibacterial drugs since plant extracts and their active substances can be employed in place of chemical additives (Kaci *et al.,* 2021).

Moreover, the antiproliferative bioactivity of *A. philippinensis* against human colorectal carcinoma (HCT116) cells displayed a concentration-dependent inhibition of the proliferation of HCT116, as seen in the study the cell viability rates of HCT 116 decreased as the concentration increased in both extracts. Furthermore, the study by Kosasih *et al.,* (2019) reported that the potency of a substance in inhibiting a biological process by half, IC_{50}, could be divided into strong (<100 ppm), moderate (101-200 ppm), and weak (>200 ppm) activities. In this study, the ethanol leaf extract of *A. philippinensis* displayed moderate cytotoxic activity with an IC_{50} value of 141.57 ppm. In contrast, the aqueous leaf extracts showed weak cytotoxic activity with an IC_{50} value of 506.66 ppm. This suggests that the ethanol extract has inhibited the growth of HCT116 more effectively than the aqueous extract. However, it is relatively low compared to the control Digitonin, with an IC_{50} value of 13.83 ppm.

In conclusion, *Aristolochia philippinensis* leaves contain bioactive compounds that exhibit antibacterial and cytotoxic activity. Specifically, the ethanolic extract demonstrated antibacterial activity and moderate toxicity against HCT 116. Consequently, these preliminary investigations on *A. philippinensis* provide scientific evidence for the folkloric use of the plant as an alternative medicine for various diseases. Further research is necessary to fully establish the biological activity of the plant. This research should include chemical profiling, characterization, and *in vitro* studies using other extraction solvents.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Vanessa Grace V. Narvasa: Investigation and experimentation, literature review, preparation of draft-final manuscript. **Eddie P. Mondejar**: Literature review, manuscript proofreeading, supervision, and validation.

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REFERENCES

Abubakar, M.A., Zulkifli, R.M., Hassan, W.N.A.W., Shariff, A.H.M., Malek, N.A.N.N., Zakaria, Z., & Ahmad, F. (2015). Antibacterial properties of *Persicaria minor* (Huds.) ethanolic and aqueous-ethanolic leaf extracts. *Journal of Applied Pharmaceutical Science*, *5*(Suppl 2), 50–56. https://doi.org/10.7324/JAPS.2015.58.S8

Aguinaldo, A.M., Espeso, E.I., Guevara, B.Q. & M.G. Nonato. (2005). Phytochemistry. In: Guevara B.Q. [ed.] A Guidebook to Plant Screening: Phytochemical and Biological. University of Santo Tomas, Manila, Philippines.

Al Farraj, D.A., Ragab Abdel Gawwad, M., Mehmood, A., Alsalme, A., Darwish, N.M., Al-Zaqri, N., & Warad, I. (2020). In-vitro antimicrobial activities of organic solvent extracts obtained from *Dipcadi viride* (L.) Moench. *Journal of King Saud University - Science*, *32*(3), 1965–1968. https://doi.org/10.1016/j.jksus.2020.01.007

Alonso-Castro, A.J., Domínguez, F., Ruiz-Padilla, A.J., Campos-Xolalpa, N., Zapata-Morales, J.R., Carranza-Alvarez, C., & Maldonado-Miranda, J.J. (2017). Medicinal plants from north and central america and the caribbean considered toxic for humans: The other side of the coin. *Evidence-Based Complementary and Alternative Medicine*, 2017, 9439868. https://doi.org/10.1155/2017/9439868

Amna, U., Halimatussakdiah, Wahyuningsih, P., Saidi, N., & Nasution, R. (2019). Evaluation of cytotoxic activity from Temurui (*Murraya koenigii* [Linn.] Spreng) leaf extracts against HeLa cell line using MTT assay. *Journal of Advanced Pharmaceutical Technology and Research*, *10*(2), 51–55. https://doi.org/10.4103/japtr.JAPTR_373_18

Bhuyan, D.J., Vuong, Q.V., Chalmers, A.C., van Altena, I.A., Bowyer, M.C., & Scarlett, C.J. (2017). Phytochemical, antibacterial and antifungal properties of an aqueous extract of *Eucalyptus microcorys* leaves. *South African Journal of Botany*, *112*, 180–185. https://doi.org/10.1016/j.sajb.2017.05.030

Borah, P.J., Borah, D., Das, U., Das, T.J., & Sarma, R. (2021). A review on ethnopharmacological utility, traditional knowledge and phytochemistry of *Aristolochia species* in assam, india. *Notulae Scientia Biologicae*, *13*(3), 1-18. https://doi.org/10.15835/nsb13311027

Carag, H., and Buot Jr, I.E. (2017). A checklist of the orders and families of medicinal plants in the Philippines. *Sylvatrop, The Technical Journal of Philippine Ecosystems and Natural Resources*, *27*(1 & 2), 40–83.

Das, A.K., Islam, M.N., Faruk, M.O., Ashaduzzaman, M., & Dungani, R. (2020). Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany*, *135*, 58–70. https://doi.org/10.1016/j.sajb.2020.08.008

Demetillo, M.T., Baguio, M.L., Uy, M.M., & Nuñeza, O.M. (2017). Phytochemical analysis and cytotoxic activity of ant plant (*Myrmecodia tuberosa* Jack.). *J Bio Env Sci*, *2017*(6), 85–91.

De Padua, L.S., Lugod, G.C., & Pancho, J.V. (1981). *Handbook of Philippine Medicinal Plants.* University of the Philippines-Los Baños.

Dubey, A., Yadav, P., Peeyush, Verma, P., & Kumar, R. (2022). Investigation of proapoptotic potential of *Ipomoea carnea* leaf extract on breast cancer cell line. *Journal of Drug Delivery and Therapeutics*, *12*(1), 51–55. https://doi.org/10.22270/jddt.v12i1.5172

Eloff, J.N. (1999). The antibacterial activity of 27 southern African members of the Combretaceae. *South African Journal of Science*, *95*(3), 148–152.

Ezemokwe, G.C., Aguiyi, J.C., & Chollom, F.P. (2020). The antibacterial activity of aqueous and ethanolic leaf extracts of *Balanites aegyptiaca* (l.) del plant on some selected clinical human pathogens. *Journal of Advances in Microbiology*, *20*(10), 51-66. https://doi.org/10.9734/jamb/2020/v20i1030290

Lawal, F., & Tshikalange T.E. (2020). Chapter 43: Terminalia prunioides. Elsevier.

Garcia, J.S., Borja, N., Sevilla, J.B., Villanueva, J.D., & Peyraube, N. (2018). An ethnobotanical study of medicinal plants and perceptions on plant biodiversity conservation in Leyte, Philippines. *Journal of Human Ecology*, *7*(1), 26–42.

Gutiérrez-Venegas, G., Gómez-Mora, J.A., Meraz-Rodríguez, M.A., Flores-Sánchez, M.A., & Ortiz-Miranda, L.F. (2019). Effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque. *Heliyon*, *5*(12), e03013-e03018. https://doi.org/10.1016/j.heliyon.2019.e03013

International Agency for Reseach on Cancer. (2002). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 82, Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene.* IARC Press, Lyon, France.

Kaci, F.N., Rüzgar, D., Görmez, A., & Efe, D. (2021). The Evaluation of cytotoxic and antibacterial activity of the ethanol extract of *Punica granatum* L. peels. *Journal of the Institute of Science and Technology*, *11*(3), 2319–2327. https://doi.org/10.21597/jist.875449

Kosasih, K., Sumaryono, W., & Supriono, A. (2019). Cytotoxicity of ethyl acetate extract of Cantigi (*Vaccinium varingiaefolium* (Blume) Miq. young leaves on *Artemia salina* L. larvae, MCF-7, T47D, and vero cell lines. *Journal of Pharmacognosy and Phytochemistry*, *8*(4), 24–33.

Kuete, V. (2014). *Health effects of alkaloids from African medicinal plants: Toxicological survey of African medicinal plants.* Elsevier

Lerma-Herrera, M.A., Beiza-Granados, L., Ochoa-Zarzosa, A., López-Meza, J.E., Navarro-Santos, P., Herrera-Bucio, R., Aviña-Verduzco, J., & García-Gutiérrez, H.A. (2022). Biological activities of organic extracts of the Genus *Aristolochia:* A review from 2005 to 2021. *Molecules*, *27*(12), 1–19. https://doi.org/10.3390/molecules27123937

Madjos, G., & Ramos, K. (2021). Ethnobotany, systematic review and field mapping on folkloric medicinal plants in the Zamboanga Peninsula, Mindanao, Philippines. *Journal of Complementary Medicine Research*, *12*(1), 21. https://doi.org/10.5455/jcmr.2021.12.01.05

Marjoni, M.R., Zulfisa, A. (2017). Antioxidant activity of methanol extract / fractions of senggani leaves (*Melastoma candidum* D. Don). *Pharmaceutica Analytica Acta*. *8*(8), 1–6. https://doi.org/10.4172/2153-2435.1000557

Mathew, L.S., Mtewa, A.G., Ajayi, C.O., Deyno, S., Weisheit, A., Tolo, C.U., Denge, A.L., & Ogwang, P.E. (2020). Ethnopharmacology, pharmacology and phytochemistry of. *East Africa Science Journal*, *2*(1), 23–28.

Miya, G.M., Oriola, A.O., Payne, B., Cuyler, M., Lall, N., & Oyedeji, A.O. (2023). Steroids and fatty acid esters from *Cyperus sexangularis* leaf and their antioxidant, anti-inflammatory and anti-elastase properties. *Molecules*, *28*(8), 1-14. https://doi.org/10.3390/molecules28083434

Naik, S.K., Mohanty, S., Padhi, A., Pati, R., & Sonawane, A. (2014). Evaluation of antibacterial and cytotoxic activity of *Artemisia nilagirica* and *Murraya koenigii* leaf extracts against mycobacteria and macrophages. *BMC Complementary and Alternative Medicine*, *14*, 1–10. https://doi.org/10.1186/1472-6882-14-87

Nishino, C., Enoki, N., Mori, A., Kobayashi, K., Fukushima, M., & Tawata, S. (1987). Antibacterial Activity of Flavonoids against *Staphylococcus epidermidis*, a Skin Bacterium. *Agricultural and Biological Chemistry*, *51*(1), 139-143. https://doi.org/10.1271/bbb1961.51.139

Ogunmefun, O.T. (2018). Phytochemicals-God's Endowment of Curative Power in Plants. https://doi.org/10.5772/intechopen.77423

Ogbole, O.O., Segun, P.A., & Adeniji, A.J. (2017). In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. *BMC Complementary and Alternative Medicine*, *17*(1), 1–10. https://doi.org/10.1186/s12906-017-2005-8

Ozen, T., Bora, N., Yenigun, S., & Korkmaz, H. (2020). An investigation of chemical content, enzyme inhibitory propert, antioxidant and antibacterial activity of *Aristolochia bodamae* Dingler (develiotu) (Aristolochiaceae) root extracts from Samsun, Turkey. *Flavour and Fragrance Journal*, *35*(3), 270–283. https://doi.org/10.1002/ffj.3559

Promega Corporation. (2012). *CellTiter 96® Non-Radioactive Cell Proliferation Assay Instructions for Use of Products G4000 AND G4100.* Promega Corporation Technical Bulletin.

Rajput, A., Sharma, R., Bharti, R. (2022). Pharmacological activities and toxicities of alkaloids on human health. *Materials Today: Proceedings*, *48*(5), 1407-1415. https://doi.org/10.1016/j.matpr.2021.09.189

Raju, Renjith, Prakash, T., Rahul, R., Poonangadu, S.S., Senthil Kumar, S., Sonaimuthu, P.T. Chua, J.M., & T. Capili, J. (2021). Phytochemical analysis of three common medicinal plants *(Gliricidia sepium, Melothria pendula*, and *Pithecellobium dulce*) in the Philippines. *Scholars Academic Journal of Biosciences*, *9*(3), 84-88. https://doi.org/10.36347/sajb.2021.v09i03.004

Saxena, M., Saxena, J., Nema, R., Singh, D., Gupta, A. (2013). Phytochemistry of medicinal plants. J*ournal of Phamacognosy and Phytochemistry*, *1*(6), 13-14. https://doi.org/10.1007/978-1-4614-3912-7_4

Taid, T.C., Rajkhowa, R.C., & Kalita, J.C. (2014). A study on the medicinal plants used by the local traditional healers of Dhemaji district, Assam, India for curing reproductive health related disorders. *Pelagia Research Library Advances in Applied Science Research*, *5*(1), 296–301.

Tan, Z., Deng, J., Ye, Q., Zhang, Z. (2022). The antibacterial activity of natural-derived flavonoids. *Current Topics in Medicinal Chemistry*, *22*(12),1009-1019.

Thakur, L., Ghodasra, U., Patel, N., & Dabhi, M. (2011). Novel approaches for stability improvement in natural medicines. *Pharmacognosy Reviews*, *5*(9), 48-54. https://doi.org/10.4103/0973-7847.79099

Tong, Z., He, W., Fan, X., & Guo, A. (2022). Biological function of plant tannin and its application in animal health. *Frontiers in Veterinary Science*, *8*(January), 1-7. https://doi.org/10.3389/fvets.2021.803657

Ventola, C.L., (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharm Therapeutics*, *40*(4), 277–283.

Wiart, C. (2006). *Medicinal plant of Asia and the Pacific.* Taylor and Francis Group.

World Health Organization. (2023, March 24). https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance