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Cytotoxicity and Phytochemical Screening of *Heliotropium indicum* L. Extract from Malita, Davao Occidental Philippines

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Abstract

This study aimed to determine the cytotoxicity properties and phytochemical profile of the different extracts of *Heliotropium indicum L.* The cytotoxicity properties of Heliotropium indicum L. were evaluated using the Brine Shrimp Lethality Assay (BSLA), while qualitative phytochemical screening was also tested on its leaf extracts. Cytotoxicity was evaluated in terms of LC50 (lethality concentration), which measures the ability of the extract to kill 50% of test animals in a specific observation period. Brine shrimps were tested in three different extraction solvents with different concentrations: 1, 10, 100, and 1000 µg/mL. Their toxicity values (LC50) were computed and described using Clarkson's toxicity. Probit analysis and LC50 were employed to measure the degree of toxicity of the brine shrimps after 6 and 24 hours of treatment. Results of the cytotoxicity assay showed that the decoction and aqueous extracts have the highest toxicity values. Phytochemical screening was assayed using a standard protocol. The qualitative phytochemical assessment of *Heliotropium indicum L.* Leaf extracts showed the presence of alkaloids, flavonoids, and saponins from decoction and aqueous extract. The phytochemical components detected in *Heliotropium indicum L.* could account for their rich pharmacological potential. Thus, the results show tentative support for its pharmacological claims and its utilization as an alternative to traditional medicine.

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Introduction

The species of *Heliotropium indicum L.* from the family of Boraginaceae locally known as Trompang elepante is known by various names in the Philippines. It is characterized by its erected, thicked, fetid, annual, or perennial herb with bushy ascending branches that reach approximately 20 and 60 cm in height (Dash KG, Abdullah SM. 2013). The leaf architecture ranges from opposite or sub-opposite, alternate or sub-alternate, straightforward, sheet-shaped from ovate to elliptical, hairy, sharp, and usually 5–10 cm long. The leaf margins are oscillate; the nerves are conspicuous on two pair sides and are serrulate or cordate and observable under the leaves (Kandemir *et al.* 2020); the petiole is approximately 1–7 cm long, while the flowers began apically within the cymose; at maturity; nutlets are located at the base of the inflorescence. Flowers are white or whitish-violet; regular, permanently attached, axillary, and has an estimated 5 mm in diameter. Sepals are distributed with hairs outside, deep green in color, linear to lanceolate, serrated, and approximately 5–3 mm long. The fruits are dry with 2–4 lobed, with or without united nutlets, and 3–6 mm long. This species thrived in tropical places that favored elevated topography at around 800 meters (Ghosh *et al.* (2018).

The utilization of traditional medicine is cheaper and more affordable, and according to the patient's ideology, it is generally perceived to have an undesirable effect on the patient. It also satisfies someone's desire for more personalized health care, and it liberates broader public access to health information. When individuals in underdeveloped countries, like the Philippines, experience sickness, they tend to turn into traditional healers, particularly those who reside in rural areas. The paradigm focuses on the individual's overall health rather than the specific affliction or disease that the patient is experiencing. The utilization of herbs is an essential aspect of every traditional practice of medicine (Mancao LS, Lumogdang LP, 2023). *Heliotropium Indicum L.* has been tested to treat a variety of diseases. The plant exhibited antioxidant, analgesic, antimicrobial, anticancer, antituberculosis, antiplasmodial, anticataract, antifertility, wound healing, anti-inflammatory, antinociceptive, antihyperglycemic, anthelmintic, diuretic, antitussive, antiglaucoma, antiallergic, and larvicidal properties. (Sarkar *et al*, 2021).

Currently, no studies have been conducted on the cytotoxicity and phytochemical screening of *Heliotropium indicum Linn* harvested locally in Malita, Davao Occidental Philippines.The study's findings provide significant information about the herb's indigenous therapeutic capabilities, as it has been used as an alternative medicine for a various of ailments. This study aimed to evaluate the cytotoxicity properties using brine shrimp lethality assay (BSLA) and determine the potential phytochemicals active in the plant extract on *Heliotropium indicum Linn*. It specifically sought to assess the cytotoxicity of trompang Heliotropium indicum Linn leaves using various extraction solvents, including decoction, aqueous, and ethanolic extract. Moreover, the plants were screened for the following phytochemicals, namely alkaloids, anthocyanins, flavonoids, quinones, saponins, and tannins. The findings of the study will contribute to the database of the pharmacological properties of *Heliotropium indicum L.*

Methodology

Sample collection

Heliotropium indicum L. was collected from Barangay Felis, Malita, Davao Occidental. The sample preparation, such as preparation of extract and brine shrimp lethality assay and phytochemical screening, was conducted at the Research and Laboratory Services Center of Southern Philippines Agribusiness and Marine and Aquatic School of Technology (SPAMAST).

Preparation of extract

To prepare the aqueous extract, 50 grams of the powdered leaves were weighed and decanted into a 500-mL conical flask and will be added with 400 mL of distilled water. The mixture was kept for 12 hours

with constant agitation at 30-minute intervals. In the extraction using ethanol, 25 grams of powdered leaves were used and mixed with 250 mL of pure ethanol in a flask and left to macerate for 30 minutes. Lastly, to prepare a decoction, 20 grams of fresh leaves were boiled in 400 mL of distilled water in a 1000 mL beaker and then filtered. Lastly, 1-3 drops of Dimethyl Sulfoxide (DMSO) were added to the extracts.

Brine shrimp lethality assay

Brine shrimp eggs were procured in a local supplier in San Roque St., Barangay Poblacion, Malita, Davao Occidental, and hatched in the SPAMAST hatchery. Brine shrimps were hatched using a transparent plastic container filled with brine solution under constant aeration and left to submerge for 48 hours. After two days, ten nauplii were separated in plastic containers for each concentration (1, 10, 100, and 1000 µg/mL) of each extraction solvent (decoction, aqueous extract, and ethanolic extract). The stock solutions were made using identical quantities of decoction, aqueous, and ethanol. Ten nauplii were added to each concentration of the produced extract, while another ten were added to the control set-up (sterile seawater). Each container had 10 mL of seawater. After 6 hours and 24 hours, the containers were checked and the number of dead (non-motile) nauplii in each was recorded. The brine shrimp lethality assay was conducted using the modified protocol of Lumogdang *et al*., 2021.

Phytochemical screening

The leaves of *Heliotropium indicum Linn* went through different tests to know whether there are active phytochemicals—alkaloids, anthocyanins, flavonoids, quinones, saponins, and tannins—present in the leaves of the plant. The tests were done by following standard procedures by Shaikh JR and Patil MK (2020).

Results

Table 1 shows the cytotoxicity of brine shrimp lethality of decoction, aqueous extract, and ethanolic extract from the leaves of *Heliotropium indicum Linn* on brine shrimps in the initial reading of 6 hours. In a decoction, one (1) µg/mL has a mortality rate of 20%, 10 µg/mL, and 100 µg/mL, 0%, which was the same as 1000 µg/mL, 0%. Therefore, the decoction was described to be highly toxic and has a toxicity value of 31.5949 µg/mL. In the aqueous extract, 1 µg/mL has a 0% mortality rate, 20% at 10 µg/mL, 0% at 100, and 10% at 1000 µg/mL. Its toxicity value is 28. 0149 µg/mL and described as highly toxic. The ethanolic extract has a 30% mortality rate at 1 µg/mL, 50% at 10 µg/mL, 30% at 100 µg/mL, and 0% at 1000 µg/mL. It has a toxicity value of 128.5434 µg/mL and was described as medium toxic.

Table 1. Cytotoxicity assessment on *Heliotropium indicum L.* after 6 Hours.

	CYTOTOXICITY					
SOLVENT	1 µg/mL (%)	10 µg/mL (%)	100 µg/mL (%)	1000 µg/mL (%)	LC50 (µg/mL)	CLARKSON'S TOXICITY INDEX
Decoction	20	20	0	0	31.5949	Highly toxic
Aqueous	0	20	0	10	28.0149	Highly toxic
Ethanolic	30	50	30	0	128.5434	Medium toxic

Table 2 presents the cytotoxicity of brine shrimp lethality of decoction, aqueous extract, and ethanolic extract from the leaves of *Heliotropium indicum Linn* after 24 hours of reading. The final reading of the brine shrimp was held after 24 hours. In decoction, there was an increase in brine shrimps' mortality, resulting in a 70% mortality rate at 1 µg/mL, 70% at 10 µg/mL, 50% at 100 µg/mL, and 40% at 1000 µg/mL. Its toxicity value (LC50) heightened at 83.7091 µg/mL and was described as highly toxic. In the aqueous extract, 1µg/mL has a 50% mortality rate, 70% at 10µg/mL, 50% at 100µg/mL, and 90% at 1000µg/mL. It has a toxicity value of 465. 8054 µg/mL and was described as moderately toxic. In the

ethanolic extract, 1µg/mL has a mortality rate of 100%, 90% at 10µg/mL, 80% at 100µg/mL, and 40% at 1000µg/mL. It has a toxicity value of 3046.6763 µg/mL and was described as non-toxic. The cytotoxicity values shown by the three (3) solvents, which range from 28.0149 to 128.5434, which is considered highly toxic, are promising results indicative of the abundant bioactive compounds present in the extracts.

Table 3 presents the data gathered from the phytochemical screening of *Heliotropium indicum Linn* using three different solvents (aqueous, decoction, and ethanol). In the decoction, the extraction of flavonoids and saponin appeared affirmative, while alkaloids, anthocyanin, quinones, and tannins appeared negative in qualitative tests.

The phytochemical screening of plant decoctions exhibited the presence of glycosides, flavonoids, phenols, alkaloids, carbohydrates, tannin, and terpenoids, while phlobatannin, volatile oil, vitamin C, protein and amino acids, cardiac glycosides, saponins, and hydrolyzable tannin could not be detected. In the aqueous extract, alkaloids, flavonoids, and saponin were present, while tannins, anthocyanin, and quinones were not detected in the phytochemical tests.

Table 2. Results of Cytotoxicity evaluation after 24 Hours.

	CYTOTOXICITY					
SOLVENT	1 µg/mL (%)	10 µg/mL (%)	100 µg/mL (%)	1000 µg/mL (%)	LC50 (µg/mL)	CLARKSON'S TOXICITY INDEX
Decoction	70	70	50	40	83.7091	Highly toxic
Aqueous	50	70	50	90	465.8054	Medium toxic
Ethanolic	100	90	80	40	3046.6763	Non-toxic

Discussion

Brine Shrimp Lethality Assay (BSLA) is a rapid, cheaper, and simple assay to assess the bioactive potential of plant extracts, which frequently corresponds with cytotoxic and anti-tumor activities (Stepanie *et al.* 2018). Brine Shrimp Lethality Assay (BSLA) is a simple, high-throughput assay for bioactive compounds' cytotoxicity. It is based on the principle of testing chemicals' capacity to kill brine shrimp, a straightforward zoological creature (*Artemia salina*). This study agrees that BSLA is an accessible and economical way to conduct assays (Lumogdang *et al.*2021).

The hatching of brine shrimps didn't take so long to utilize, and the brine shrimps themselves were effective in extraction solvents or reagents that had been diluted in different concentrations, and the objectives were met. In this study, the LC50 after 6 hours of exposure to Artemia salina was known to be 31.5949, 28.0149, and 128.59 for decoction, aqueous, and ethanolic extracts, which could be interpreted as highly toxic. Moreover, after 24 hours, the LC50 is now 83.7091 for decoction, which is still considered highly toxic, 465.8054 for aqueous, which is considered medium toxicity, and 3,046.6766 for ethanolic extracts, which could be interpreted as non-active. The previous results of the aqueous extracts of the leaves of H. indicum also showed low toxicity (Chunthorng-Orn *et al.* (2016). The indicators of the toxicity among the brine shrimps are based on Clarkson's toxicity index: an LC50 of <100 µg/mL is highly toxic, an LC50 of 100-500 µg/mL is medium toxic, an LC50 of 500-1000 µg/mL is low toxic, and an LC50 > 1000 µg/mL is nontoxic (Hamidi *et al.* 2014).

The qualitative phytochemical screening of the ethanolic extracts of leaves reveals the presence of various bioactive components, which include steroids, saponins and tannins, alkaloids, and flavonoids (Pauline Fm. 2016), but in contradiction, none of any

phytochemicals were detected in the qualitative test using the ethanolic extract. As shown in Table 4, leaf extracts using decoction and aqueous extract demonstrated the presence of alkaloids, flavonoids, and saponin qualitative tests. Based on the results, it could be inferred that extraction solvent plays a significant role in the cytotoxicity property of H. indicum extracts. Aqueous extract performs better than ethanolic and decoction extracts, which demonstrates the versatility of water as an extraction solvent (Raven *et al*. 2014).

The result of this study was also observed in the study of Chunthorng-Orn *et al*.2016 where the ethanolic extract showed cytotoxic activity against lung cancer cell lines. Moreover, the methanolic extracts of the stem and leaf of *Heliotropium indicum* reported to have excellent anticancer properties and cytotoxic activity (Sivajothi *et al.* (2015). Furthermore, the chloroform fraction of Heliotropium indicum was found to be most efficient in inhibiting cell growth and inducing apoptosis (Paul *et al*. 2015). The cytotoxicity results of *H. indicum* support the traditional claim of the plans. The phytochemicals found in H. indicum namely Saponins (Adetuyi *et al*.2020 and Idowu ID, Tolulope O. (2023) Alkaloids and Flavonoids (Mohammad *et al.* 2014) were previously reported.

Table 3. Results of Phytochemical Screening of *Heliotropium indicum Linn*.

Extracts	Phytochemicals					
	Alkaloids	Anthocyanin	Flavonoids	Quinones	Saponin	Tannins
Decoction	-	-	+	-	+	-
Aqueous	+	-	+	-	+	-
Ethanolic	-	-	-	-	-	-

Legend: (+) indicates positive or presence and (-) negative or absence of the phytochemical constituents in the extraction solvent.

The findings reported in this study which utilize only brine shrimp lethality assay which is only considered as a preliminary assessment of cytotoxicity properties. To further, substantiate the cytotoxic properties, a quantitative method with a high detection limit is highly suggested. In terms of phytochemical properties, there are only six (6) parameters that were tested in this study namely alkaloids, anthocyanins, flavonoids, quinones, saponins, and tannins. An inclusion of the other phytochemicals is highly suggested.

This study reported the highly cytotoxic properties of Heliotropium indicum and the detection of selected phytochemicals, namely alkaloids, saponins, tannins, and flavonoids. The findings of the study highlighted the potential medicinal properties of Heliotropium indicum, and its conservation and propagation are highly desirable, especially in remote places where hospitals and healthcare facilities are difficult to access. Moreover, this study also promotes the valuable added resource of Heliotropium indicum as an alternative medicine.

Conclusion

The cytotoxicity of *Heliotropium indicum L.* was assessed successfully using the brine shrimp lethality assay. Decoction and aqueous extract of *Heliotropium indicum Linn* exhibited remarkable cytotoxicity activity, which could potentially be attributed to its rich bioactive compounds. The bioactive components of *H. indicum L.* could be attributed to its active phytochemicals such as alkaloids, flavonoids, and saponins. The results of the study are highly promising for further screening of its specific pharmacological activities. This study highlights the important medicinal properties and value-added resources of *Heliotropium indicum*. Propagation and its conservation are highly suggested.

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Abbreviation

BSLA Brine Shrimp Lethality Assay.

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