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PHYTOCHEMICAL PROFILING AND BRINE SHRIMP LETHALITY TEST OF THE METHANOL EXTRACT OF *LANTANA CAMARA* FROM SIMUNUL, TAWI-TAWI, PHILIPPINES

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ABSTRACT

Phytochemical screening of different terrestrial plant species is necessary in recognizing and characterizing bioactive compounds present in plant extracts that may yield significant benefits in medicinal applications. At the same time, toxicity will help evaluate the bioactivity of plant extracts which provides an understanding of the potential pharmacological activities of the species. This study examined the phytochemical profile and toxic potential of *Lantana camara* leaflet extract from Simunul, Tawi-Tawi. Phytochemical screening revealed the existence of alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, steroids, tannins, and triterpenoids. Brine Shrimp Lethality Test (BSLT) displayed toxic properties, with LC50 values of 870.96 µg/ml. The result demonstrated concentration-dependent toxic effects, presenting potential pharmacological properties. These findings backed the conventional use of *Lantana camara* as it comprises a myriad array of active secondary metabolites with substantial bioactive potential that would be useful in developing and formulating new drugs or therapeutic agents that are valuable in the contribution of pharmacology advancements.

KEYWORDS: *Lantana camara, Phytochemical Profiling, Brine Shrimp Lethality Test, Tawi-Tawi, Philippines.*

INTRODUCTION

Terrestrial land is a home for various medicinal plant species that are valuable sources of potential medications. Medicinal plants offer readily accessible, affordable remedies, especially to the far-flung areas where over-the-counter drugs are not available. The World Health Organization (WHO) has recognized that 80% of the populations in developed countries use traditional medicinal plants as their primary health care system. Traditional medicine is considered a total sum of all practices, measures, and procedures that have from time immemorial enabled Africans to guard against disease (Tugume, 2019). Medicinal plants are resources for new drugs, and many of the modern medicines are produced indirectly from plants (Hosseinzadeh 2015).

Lantana camara is a tropical invasive medicinal plant that has been utilized in conventional pharmaceuticals for different purposes: treating fever, stiffness, skin conditions, and many others. In spite of its extensive use, comprehensive phytochemical and toxicological assessments of *Lantana camara* from this locale remain constrained.

Lantana camara is a prickly, aromatic shrub that grows upright, partially climbs, and can sometimes hang, growing up to 2-3 m tall. The angular stems and branches have curved spines that are arranged along the edges. The leaves are basic, arranged in pairs at right angles with a rough surface, oval in shape, and evenly toothed with a pointed tip. The hemispherical head of the inflorescence, found either axillary or terminal, consists of numerous small tubular flowers and is yellow, pink, or orange in color. The small, fleshy drupes are about 3 mm in diameter and come in shades ranging from blue to black. This study explores the phytochemical profile and brine shrimp lethality test of *Lantana camara* leaf extracts from Simunul, Tawi-Tawi, Philippines. Phytochemical screening was conducted to recognize the bioactive compounds displayed within the extracts. Utilizing the brine shrimp lethality test (BSLT), we assessed the extracts' bioactivity. This investigation contributes to the documentation of the phytochemical properties and toxicological potential of *Lantana camara* from the Philippines, giving importance to its therapeutic applications.

MATERIALS AND METHODS

The leaflets of *Lantana camara that* were used in this study were collected last September 2024 from the island of Simunul, a coastal municipality in the island province of Tawi-Tawi, under the BARMM region. Geographically, Simunul is located at 4^0 53' 34" N 119^0 49' 21" E. According to the Philippine Statistics Authority, the municipality has a land area of 167.25 square kilometers, which constitutes 4.61% of Tawi-Tawi's total area. Simunul is home to various plant species, including *Lantana camara*. The assessment of phytochemicals in *Lantana camara* leaflets was achieved by employing the methods derived from Harborne (1973) with a slight modification. Using the methanol extract, the phytochemical screening results were classified qualitatively as either positive (+) or negative (-). Employing the Brine Shrimp Lethality Test (BSLT), the toxicity of the extract was assessed.

RESULTS AND DISCUSSION

The result of the phytochemical profiling of the *Lantana camara* is summarized in Table 1.

Table 1: Phytochemical screening of *Lantana camara.*

Phytochemicals	***Lantana camara***
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Phenolics	+
Saponins	+
Steroids	+
Tannins	+
Triterpenoids	+

Where; + present, - absent

As shown in Table 1, the dried methanol extract of *Lantana camara* leaflets has contained a variety of biochemicals that include the presence of alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, steroids tannins, and triterpenoids. These bioactive compounds are secondary metabolites produced by the plant, containing diverse biological components and potential well-being advantages.

Alkaloid. A Wagner's test was employed to confirm the alkaloid in the extract. Two ml of hydrochloric acid (2N) were combined with a 0.05 g sample, which was then filtered. When 2.0 ml of the filtrate were mixed with a few drops of Wagner's reagent, the presence of alkaloids was confirmed by the production of a brown or reddish-brown precipitate. Alkaloids, whose name comes from the word "alkaline-like," are a group of compounds with one or more nitrogen atoms located in the heterocyclic ring that characterizes the compound and may have primary, secondary, tertiary, or quaternary bases (Aniszewski, 2007). Alkaloids are vital for both human health and a plant's defense mechanisms. Their potent bitterness shield plants from herbivore attacks. Therapeutically, alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents (Heinrich 2021). According to Aryal (2022), they not only protect plants from herbivores but also curb fungal and bacterial infestation, which broadens their use in medicine and other fields. He added that because of their actions, alkaloids have a wide variety of pharmacological appliances in the therapeutic area such as analgesic (e.g., morphine), antiasthmatic (e.g., ephedrine), anticancer (e.g., vincristine), antihypertensive (e.g., reserpine), antipyretic (e.g., quinine), and antihyperglycemic (e.g., piperine) effects.

Cardiac Glycosides. A Keller Killiani test was performed to determine the cardiac glycoside by adding 1 ml of glacial acetic acid and 1 ml of concentrated sulfuric acid to 2.5 ml of the sample. The existence of glycosides in the sample was implied by the creation of a green-blue color. Cardiac glycosides are a class of organic compounds that are cardiotonic phytosterols, mainly obtained from green plants and have a wide application in cancer and cardio rehabilitations (Chatterjee, Gupta & Banerjee 2023). It increases the output force of the heart and increases its rate of contractions by acting on the cellular sodium-potassium ATPase pump, thus making it an important drug for the treatment of heart failure and cardiac rhythm disorders (Jing Fu 2019). Cardiac glycosides could also be beneficial in the treatment of autoimmune diseases, according to Škubník J. (2023).

Flavonoids. The extract was tested positive for flavonoids by introducing 2 to 3 drops of sodium hydroxide to 2 ml of the extract, which initially displayed a deep yellow pigment, which gradually changed to a colorless state upon the addition of a few drops of dilute hydrochloric acid. There are several records that potential therapeutic indications of alkaloids are beneficial for cancer and viral infections, inflammation and immune modulation, neurological and psychiatric conditions, and diabetes (Tangon *et al*., 2021).

Phenolics. Similar to tannins, the extract was tested by employing ferric chloride test where appearance of a red-brown precipitate affirmed the presence of phenols. Phenols are a class of chemical compounds that have various biological activities, including antioxidant properties (Prasad *et al*., 2013, April 21). Phenolic compounds extracted from plant-based by-products have been studied for their capacity to scavenge free radicals in both food systems and in vivo models (Deng *et al.*, 2012). Therefore, the majority of the plant-based phenolic compound research was focused on finding their antioxidant activity in vitro, in vivo, and in animal models (Banerjee *et al*., 2017). Phenolic compounds have also been shown to exert beneficial health effects in various cardiovascular diseases (Torres-Fuentes 2022).

Saponins. An olive oil test was used to examine whether or not the extract contains saponins. In 2.5 ml of the solution, 5 ml of distilled water was merged and vigorously shaken until a steady froth developed. 3 drops

of olive oil were then introduced into the solution and shaken once more. The formation of an emulsion signified the presence of saponins. Saponins possess several biological properties, such as anti-oxidant, anti-tumor, hypocholesterolemic, hypoglycemic, and anti-inflammatory activities. Saponins are also associated with defense mechanisms in plants related to their anti-microbial, anti-fungal, anti-parasitic, and molluscicidal effects (Shen *et al*., 2017; Kitagawa *et al*., 2016).

Steroids. The Liebermann-Burchard Test was made to investigate the existence of steroids in the extract. A 2.5-ml sample was introduced and subsequently filtered. Following filtration, 2 ml of acetic acid and 1 ml of concentrated sulfuric acid were added to the filtrate. The presence of steroids is indicated by the formation of a blue-green ring. Steroids belong to a class of natural and synthetic organic compounds characterized by a rigid framework of 17 carbon atoms formed from four fused rings with varying levels of functionalization (Shagufta). The steroid metabolites have anti-inflammatory and antiviral activities, which are usually treated as potential lead compounds in drug development (Meilu Zhao, 2023). By regulating gene expression, steroid hormones control physiological processes ranging from immune function to metabolism to reproduction (Andre 2003). It has a certain degree of anti-cardiovascular and anti-tumor activities (Baojun Wang 2022). In particular, the steroid derivative 3α-hydroxy-5α-pregnane-11,20-dione (also known as alphaxalone) is a potent anesthetic that has been used in human surgery (Andre 2003).

Tannins. The existence of tannins was confirmed after manifesting greenish-black precipitate when tested through a ferric chloride test. Tannins are the main polyphenolic secondary metabolites distributed widely in the range of 5% to 10% of dry vascular plant materials (Barbehenn and Constabel, 2011). Recent studies have explored and confirmed numerous health benefits like anti-oxidant, anti-allergenic, anti-inflammatory (Ghosh 2015), antihelmentic (Athanasiadou *et al*., 2000, 2001; Ketzis *et al.,* 2006), anti-microbial (Boakye et al., 2016), anticancer (Tangon *et al*., 2021), and antiviral against enteric virus, herpes simplex virus, and poliovirus (Ashok and Upadhyaya 2012).

Triterpenoid. The Salkowski test was conducted to discover triterpenoids in the extract. The test was made by adding 5 ml of chloroform to 2.5 ml of the sample and afterward filtered. Following the filtration, 3-4 drops of concentrated sulfuric acid were added to the filtrate. The mixture was shaken and left undisturbed. The emergence of a yellow color in the lower layer indicated the appearance of triterpenoids. More than 60% of natural products are triterpenoids, also known as isoprenoids (Ramadevi 2017). Triterpenoids are a large structurally diverse group of natural products derived from squalene (99) or related acyclic 30-carbon precursors (Connolly and Hill, 2022). Triterpenoids have a wide range of pharmacologic activities such as anti-inflammatory, antibacterial, and antiviral (Jinping Liu 2023). It can also protect against cancer as it has an antioxidant that has the ability to trap free radicals, inhibit the free radical's enzymatic generation, and block the oxidation of cellular and extracellular compounds (Bakovic 2015).

These findings suggest that the dried methanol extract of *Lantana camara* leaflets contain a wide variety of bioactive compounds with potential pharmacological and health-promoting properties.

BRINE SHRIMP LETHALITY ASSAY

The results of Brine Shrimp Lethality Assay of *Lantana camara* is shown in Table 2.

Table 2: Brine Shrimp Lethality Assay of methanol dried leaflet extract of *Lantana camara.*

Concentration (µg/ml)	**% mortality**	**LC50**
2500	**100**	
2000	80	
1500	60	LC50=870.96
1000	50	
500	30	

LC_{50} ≤1000 µg/ml, classified as toxic as specified by Gupta et al (1996)

As shown in Table 2, the observed nauplii mortality percentages after a 24-hour period in varying concentrations of Lantana camara are as follows: 100% at 2500 µg/ml, 80% at 2000 µg/ml, 60% at 1500 µg/ml, 50% at 1000 µg/ml, and 30% at 500 µg/ml. Moreover, the study confirmed a direct relative association between the extract concentrations and the mortality rate of *Artemia salina*.

The sample manifested remarkable toxic effects, with an LC_{50} value of 870.96 µg/ml. The results signified that dried methanol leaflet extract of *Lantana camara* displayed a low $LC_{50,}$ exhibiting a slight toxic profile. Apparently, it implied that the mortality rate of brine shrimp had direct relevance to the increasing concentration of the extract.

In comparison with the results of other published research, different studies have reported different mortality rates of *Artemia salina* when exposed to varied plant extracts. One study that evaluated the toxicity of extracts from the leaf of *Avicennia marina* on the mortality rate of *Artemia salina* has reported distinct percentages of mortality at different concentrations of the extract (Wardlaw, 1985). Another study reported the varying percentage of the mortality rate of Artemia salina after being exposed to various concentrations of the seed extract of *Citrus limon L.* for 24 hours (Shaheen SM, *et al*. 2019). Similarly, another study on the acute oral toxicity of extracts from the leaf of *Swietenia mahagoni* (Linn.) Jacq. reported different percentages of the mortality rate of *Artemia salina* after being exposed for 24 hours to different concentrations (Sahgal 2010).

Therefore, it is evident that the mortality rates of *Artemia salina* significantly vary relative to exposure to the type of plant extract and concentrations.

CONCLUSION

The phytochemical screening of *Lantana camara* leaflet extract divulges a myriad array of bioactive compounds such as alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, steroids tannins, and triterpenoids. This highlights Lantana camara's therapeutic ability, encouraging further research into its medicinal value. The LC50 suggests lower toxicity. These results highlight the significance of sensibly taking into consideration the plant extracts and concentrations in assessing their impact on *Artemia salina* mortality, to provide worthwhile insights for further exploration into *Lantana camara*'s toxicity potential.

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