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Effect of *Phyllanthus niruri* and *Passiflora foetida* Extracts on the Mortality and Survival rate of the Brine Shrimp *Artemia salina*

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Abstract

Phyllanthus niruri *and* Passiflora foetida *are known to have pharmacological properties. This study tested the lethality effects of the different extract concentrations of P. niruri and P. foetida on brine shrimps in order to assess the bioactivity of the plants. The sampled plants were taken from selected areas in Mindanao, Philippines. Plant extractions were done using three solvents: water, absolute ethanol and 50:50 ethanol-water. LC50 values were calculated to evaluate the toxicity of the plant extracts. The LC50 values of* P. niruri extracts *were >1000 µg/ml for plant extract with water as solvent, 251.19 µg/ml for plant extract with absolute ethanol as solvent, and 281.84 µg/m for plant extract with 50:50 ethanol-water solvent. The LC50 values of* P. foetida *with the same solvents were >1000 µg/ml, 749.89 µg/ml and >1000 µg/ml, respectively. These results suggest a low toxicity level of the plant extracts. This implies that the use of these medicinal plants especially in the traditional approach which is through the decoction method has the least risk of danger. The results also suggest that* P. niruri *and* P. foetida *may contain bioactive compounds needing further elucidation and tests.*

Keywords: Bioactivity, concentrations, lethality, pharmacological, toxicity.

Introduction

Interest on medicinal plants to ameliorate health care problems is increasing locally and internationally[1]. Over the years, advances in drugs and other pharmaceutical products originating from plants are revealed and are introduced[2]. Many developing countries address health concerns by depending on phytomedicine. Most of the people in Africa make use of traditional herbal medicine to meet their health requirements[3]. In addition, the use of traditional remedies has already been engaged since the Ayurvedic treatment in India ranging from Asia and to Europe[4].

Passiflora foetida (Linn.) belonging to Passifloraceae family is an herbaceous climber with the auxiliary tendrils and yellowish white hair stems. It is frequently found growing in thin soil. It is native in tropical America and is wild in several parts of India[5]. *Passiflora* species are significant because of their high therapeutic values. Tribes with traditional medicinal knowledge recommended *P. foetida* L. as a good source of high value pharmaceutical plant[6].

P. foetida is a common weed found in farmlands where it could invade monocrops[7] and could affect the yield of the farmers. Nevertheless, its invasive ability in agricultural aspects is paralleled with its benefit as this weed is tested to have useful features. Because of its substantial effect, it may be necessary to yield additional medicines to cure or ease illnesses. *P. foetida* leaf extract is reported to possess anti-inflammatory effects and analgesic activity[8]; anti-histamine[9]; anti-depressant effects[10] and estrogenic activity[11]. The fruit extract is reported to have anti-inflammatory effects[12] and the whole plant possesses immune-modulatory effects[13]. Traditional medicinal practitioners of Chittor District of India use it against asthma and various neurological disorders[14]. In addition to its curative effect in nervous ailments, it is also used to treat problems on arthritis[15]. Other species of *Passiflora* is used as sedative, antidote for anxiety and hypertension[16].

Phyllanthus had been widely used as ethnomedicinal agent around the world[17]. Recently, there is a rising consciousness of reviewing and developing the pharmacological and biochemical assets of this plant because of its active mechanism. This invites further research for health care applications18. Six different species of *Phyllanthus*, dominated by *Phyllanthus amarus*, are sold for their homeopathic causes in the market of southern India[19]. Many are already reported about the positive effects of *Phyllanthus*, used as a therapeutic treatment against hepatic issues[20,21], renal complications[22] and can also be antioxidant and reactive oxygen species scavenger[23,24].

Phyllanthus niruri is one of the species of *Phyllanthus*. It is an herbaceous plant with small, green, short-petiole and bitter-taste leaves; has thin and glaucous under surface; unisexual, monoecious and minute flowers in the axil of the leaves[25]. Some *Phyllanthus* species in some countries like Thailand propagate

together in open places, wastelands and secondary forest[26]. In a study conducted using RAPD polymorphism, *Phyllanthus* species were distinguished from each other. *Phyllanthus urinaria*, *P. debilis* and *Phyllanthus embilica* were found grouped into one major group in dendrogram based on the RAPD polymorphism yet the *P. urinaria*, *P. debilis* and *P. amarus* have more resemblances in vegetative morphology[27].

Phyllanthus belongs to the family Euphorbiaceae- family of flowering plants. In the Philippines,there are more than 40 *Phyllanthus* species. The common names for the genus used in this study are "sampasampalukan, surusampalok, talikod, or taltalikod, San Pedro, malakirum-kirum, turutalikod" and other tags depending on the local or cultural terms. These were found as weeds growing throughout the Philippines at low and medium altitudes[28].

Passiflora foetida, like *P. niruri*, is also a weed with agricultural significance. It is usually grown to reduce soil erosion or to impede the growth of *Imperata cylindrica* grass ("kogon") in a coconut plantation[29].

Phyllanthus, tagged as a "wonder plant" because of its numerous beneficial effects[30], has been widely studied for its species classification, phylogeny and morphology of its flower[31]; phytochemical observation[32]; phytochemical screening, anti-inflammatory and cytotoxic activities[33,34], efficacy of its extracts in induced hepatitis[35], and phytochemical and pharmacological properties[36]. There are limited studies on the toxicity of *P. niruri*[37]. Further investigation of this medicinal plant is needed in the Philippines to supplement the existing lethality evaluation done on medicinal plants in the country[38]. Toxicity test is essential to evaluate the lethal implications of the medicinal plant to be used[3], and may suggest the plant's supplementary bioactivity[39]. This further draws attention to any biochemically significant compounds found in the plant[40]. The application of brine shrimp lethality bioassay is mostly general because it is functional, low-cost and fast assessment requiring only ample sample size and requirement[41].

This study used brine shrimp lethality test to investigate the toxicity level of *P. niruri* and *P. foetida,* to know if these plant species have possible hazard when employed as therapeutic agents and to test the bioactivity of the plant extracts which could be of high importance to further pharmacological studies on the plants.

Material and Methods

Plant Collection and Identification: The plant samples of *P. niruri* and *P. foetida* were collected from Mt. Matutum of South Cotabato; Kalubihon, Tibanga and Brgy. Abuno of Iligan City, Philippines in the month of August 2013. The collected samples were properly labelled and identified. Plant identification was based on taxonomic keys and published works[42].

Crude extracts preparation: One to two kilograms (kg) of fresh samples of leaves and stems was properly washed with tap water and rinsed with distilled water. The rinsed samples were air- dried for one week or until when the samples were already crispy enough upon crumpling. The dried samples of each plant were pulverized using a sterile electric blender. The powder was weighed, divided into two equal parts and stored in glass containers. One part was saturated with enough absolute ethanol and the other part was soaked in 50:50 ethanol - water for three days (72 hours). Each solution was filtered using Whatman filter paper and collected in a glass container. Adequate amount of the filtered solution was then concentrated in a rotary evaporator to achieve the crude plant extract. After extracting, the 50:50 ethanol-water extract was freeze dried to eliminate the excess water.

Plant decoction preparation: About 450grams (g) of fresh and clean samples of the plants were cut into pieces and boiled in about 900 ml distilled water corresponding to a 1:2 ratio of water and sample for decoction for five minutes. The mixture was then filtered. The solvent was then freeze dried to remove traces of water, then cooled and stored in glass containers until needed for the lethality testing.

Toxicity Assay: Brine shrimps hatching: Brine shrimp eggs were hatched in sterile seawater. A rectangular glass aquarium was divided into two compartments with holes on the partition. The eggs were placed into the first compartment (covered) where the brine shrimp eggs hatched while the second compartment was illuminated. Mature nauplii (larvae) swam towards this illuminated compartment. Eggs hatched after 24 hours incubation at room temperature (25-29^{0}C) with constant exposure to light. They were then collected by glass capillary from the lighted side and added into each test tube.

Brine Shrimp Lethality Test: Thirty milligrams (ml) of the extracts from each fraction were dissolved with a sufficient amount of solvent to obtain 10,000 ppm stock solution. In triplicate, serial dilutions (1000 µg/ml, 500 µg/ml, 100 µg/ml, and 10 µg/ml) were made in each test tube and dissolved in dimethyl sulfoxide (DMSO). Ten nauplii and seawater were added to each well obtaining a 5ml volume of each tube. The test tubes were examined and the number of dead (non-motile) nauplii in each test tube was counted after 24 hours.

Statistical Analysis: Brine shrimps were exposed to four different extract concentrations to determine the relative toxicity of the extracts51. The relationship between the concentration of the extracts and mortality of the brine shrimps was shown by plotting the concentration log (x-axis) *versus* mortality (y-axis). LC50 is the dose that resulted to 50% mortality of the brine shrimps.

Results and Discussion

Results showed that there is an increasing mortality rate of the brine shrimps with increasing concentration of the extracts. This suggests a direct proportional relation between concentration

and mortality. Table-1 shows that the absolute ethanol extract of *P. niruri* has the most potent activity with LC50 value of 251.19 µg/ml. The *P. foetida* extracts had the lowest toxicity. Extracts with LC50 values of 1.0 - 10µg/ml are treated as toxic while values > 100µg/ml are considered non-toxic43. *P.niruri* and *P. foetida* results failed to show highly toxic levels of extracts.

Table-1
Chronic LC50 values of *Phyllanthus niruri* and *Passiflora foetida* extracts against the Brine Shrimp *Artemia salina*

Plant	Extract	Chronic LC50 (24 hours), µg/ml
Phyllanthus niruri	Decoction/water	>1000
	Absolute ethanol	251.19
	50:50 ethanol-water	281.84
Passiflora foetida	Decoction/ water	>1000
	Absolute ethanol	749.89
	50:50 ethanol-water	>1000

The results of this study showed that at increased concentrations, *P. niruri* and *P. foetida* have variable levels of toxicity. The mortality of the brine shrimps administered with different concentrations of the *P. niruri* and *P. foetida* extracts may be attributed to the possible constituents or compounds present in them which have beneficial effects as therapeutic agents parallel to an investigation evaluating medicinal plants[43].

The leaves and stems of the sampled plant extracts used here presented low toxicity or non- toxicity as shown in the LC50 values which are higher than 100µg/ml. This means that the plant could not convey harm upon critical exposure because of its minor or low toxicity, particularly the water extracts (decoction). This suggests that the decoction, which is commonly practiced in traditional medicine, has no distinct toxic effect in acute administration[43]. The low toxicity effect using the brine shrimp lethality test was also reported on four Indonesian plants[24]. The study may have resulted into twice the value of LC_{50} compared to the present study but both outcomes denote a low cytotoxic properties present in the *P. niruri* extract. This is because most toxic

extracts showed an LC_{50} of less than 1µg/ml but not exceeding 100µg/ml[43]. This minute level of harmful effect is parallel with another study, wherein the whole plant extract was found to be non-toxic and not genotoxic with the acute administration at 300mg/kg in rats[3].

Greater shrimp death indicates high noxiousness at 50% or less concentration and, for the extract to be considered slightly toxic, it needs to cause 50-70% cell death in higher dilutions. In toxicology, pharmacological compounds found even at higher concentrations (non-toxic dose) draw possibilities of useful and functional toxic compounds[44].

The LC50 values of *Phyllanthus acidus* were 3.12 µg/ml, 12.5 µg/ml and 70% mortality above 50 µg/ml and considered highly toxic 2. High lethality indicated by the brine shrimp lethality test suggests the presence of cytotoxic elements and high bioactivity of the plant extract. The extract of *P. amarus* (synonymous to *P. niruri*45) is found to have tannins and phenols having healing significance32. Aside from the limited literatures regarding the toxicity of *P. niruri*, change in environmental factors could also affect the chemical properties of the plant. Thus, more tests are recommended about its chemical properties after some time, to confirm prevailing data[37].

This study showed the lethality effect of 749.89 µg/ml of *P. foetida* leaf and stem extracts implying its low toxicity. The necessity of elevated concentration value may be the same with the 200mg/kg and 300mg/kg doses of its leaf extracts as a painkiller to the ache caused by Eddys hot plate[46,47] and almost the same effect with the study on the activities of *P. foetida* L8. This trivial level of lethality relates to its chronic toxicity ineffectiveness when tested in mice[48]. The report revealed that 20g/kg dose administered exhibited neither any abnormality to the tested group, nor significant changes in the body weight, food intake and relative organ weight compared to the control group. Alteration in haematological parameters and biochemical parameters both range at doses 800 to 1600mg/kg which are still covered in the normal range. In a study, the ethanol leaf extract of the plant did not bring about any death or abnormality to tested mice even at the highest dose of 200mg/kg after three days[8]. A study also showed that acetone and ethanol extracts of *P. foetida* leaves have anti-microbial property[49]. It validated the plant's curative activity in higher concentrations and traditional treatment of infections affected by microbes or bacteria.

The threshold of toxicity could be considered to have significant biological activity, proposing strong injurious compounds which warrant further investigation[50]. Brine shrimp assay has been considered as an examination for antimicrobial, antitumor, anti-malarial, antifungal and insecticidal activities[51]. The present results merit further investigation of *P. niruri* and *P. foetida* plants to verify their medicinal potentials.

Conclusion

P.niruri and *P. foetida* extracts showed no toxicity as indicated by the need to have a higher concentration of the extracts to trigger mortality on the brine shrimps. This points out that usage of the plant extracts as a therapeutic agent poses no high risk of harm/ toxicity upon acute administration especially when prepared in a traditional way which is the decoction method. However, the death of some of the nauplii also suggests that *P.*

niruri and *P. foetida* may have compounds that need further research given that some literatures have shown some of their antioxidant activities and other biochemical properties.

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