



## Phytochemical analysis and cytotoxic activity of ant plant (*Myrmecodia tuberosa* Jack.)

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### Abstract

*Myrmecodia tuberosa* Jack (Rubiaceae) has been used as part of traditional Philippine remedies for a wide range of therapeutic usages. The results of this will prove the curability and effectiveness of this plant to some diseases and illnesses, and for the safety of aqueous and its ethanolic extracts. The present study was aimed to evaluate phytochemical constituents and to determine its toxicity. Qualitative phytochemical analysis was done by standard laboratory grade reagents and toxicity test was done using Brine Shrimp Lethality Analysis (BSLA). Results revealed that the phytochemical screening of tuber and leaf crude ethanolic extracts indicated the presence of saponins, tannins, flavonoids, alkaloids, and phenolic compounds. The brine shrimp lethality test of the ethanolic extracts of *M. tuberosa* tuber and leaves indicated LC<sub>50</sub> values of 38.68 and 126.62 µg/ml respectively. On the other hand, the decoction of tuber and leaves were toxic at LC<sub>50</sub> of 132.6 and 441.6 µg/ml respectively. From the results obtained, *M. tuberosa* extracts contained active compounds and that the ethanolic extracts were more potent for cytotoxic activity against brine shrimp as compared to the decoction.

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## Introduction

The importance of medicinal plants and traditional health systems in solving the healthcare problems of the world is gaining increased attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing tremendously. Historically, all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, and a lot more. Medicinal plants play a pivotal role in the health care of ancient and modern cultures. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Kadota, 2002). Plant contribution to the medicinal field is largely due to the activity of plant derived drugs. Ethnopharmacology is a highly diversified approach to drug discovery involving the observation, description, and experimental investigation of indigenous drugs and their biological activities. It is the scientific study which continues to provide new drugs and lead molecules for the pharmaceutical industry (Hamsar *et al.*, 2012).

In the Philippines, medicinal plants are considered to be one of the most popular ways of natural treatment of illnesses (Guevarra *et al.*, 2005). The Department of Health through the Philippine Institute of Traditional Alternative Health Care (PITAHC) under Republic Act No. 8423 endorsed the use of traditional medicines in the country. Merely 120 medicinal plants have been scientifically validated for safety and efficacy (Cayetano & Henry, 1984). Plant-based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost, and non-toxic nature when compared to modern medicine.

The *M. tuberosa* species is a member of Rubiaceae family and lives as epiphyte on other plants which also serve as habitat of ants that is why it is commonly called as ant plant. There are more than 13000 species which are distributed in 637 genera in Rubiaceae and have been used widely by various

indigenous people and particularly by traditional practitioners as remedies (Huxely, 1978). *Myrmecodia* spp. can be found in Malaysia, Philippines, south to the Cape York Peninsula in Queensland, southern Thailand, Cambodia, Vietnam and New Guinea. The ant-plants are saturated by ant colonies, and they are aggressive towards enemies of the host plant and are important for plant defense. Plant parts produce sweet secretions consumed by the ants, and the plant directly utilizes nutrients derived from animals. Local residents in Mindanao, specifically the Manobo tribe uses fresh tubers of *Myrmecodia tuberosa* to treat several diseases like goiter, stomach ache, and fever (Quisumbing, 1978). Meanwhile, there is still limited scientific evidence to prove the efficacy of *M. tuberosa* to cure diseases. The present study concentrates on phytochemical analysis and cytotoxic activity of ant plant (*M. tuberosa*) using brine shrimp (*Artemia salina*) lethality bioassay.

## Materials and Methods

### Plant collection and extraction

Ant plants were collected from mangrove areas of Hinatuan, Surigao Del Sur in December, 2014 (Fig. 1). The collected leaves and tubers were chopped separately for the preparation of extracts *viz* decoction, pure ethanolic extract, and hydro-ethanolic extract. Their preparations are summarized below.

Decoction – 100 g of each chopped tuber and leaves were with 200 ml of distilled water boiled for 5 minutes, was then freeze dried for three days.

Hydro-ethanolic Extract- 300 g of chopped tubers and leaves were air dried for two weeks, soaked in 1:1 ratio of distilled water and pure ethanol (100 mL: 100 mL) for three days, and concentrated through rotary evaporation *in vacuo* and freeze dried.

Ethanolic Extract- 300 g each of chopped tubers and leaves, air dried for two weeks, were soaked in pure ethyl alcohol for three days and concentrated in *vacuo*.

The extracts were used for the brine shrimp toxicity test with the following concentrations *viz.* 10 ppm, 100 ppm; 500 ppm, and 1000 ppm.

#### *Phytochemical screening of the plant extracts*

Phytochemical screening of the crude ethanolic extract of leaves and tubers of *M. tuberosa* was carried out using standard methods described by Harborne (1979).

#### *Screening for alkaloids*

Three grams of extract were stirred with ethanol containing 3% tartaric acid. The filtrate was shared into three beakers and tested for alkaloids as follows: into the first beaker, Hagar's reagent was added, into the second beaker, Mayer's reagent was added, and into the last beaker, Marquin's reagent was added. Precipitation in any of the three tests indicated the presence of alkaloids.

#### *Screening for saponin*

About 0.5 g of the plant extract was shaken with water in a test tube. Frothing, which persisted on warming was taken as a preliminary evidence for the presence of saponins. Few drops of olive oil were added to 0.5 g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicated the presence of saponin (Fansworth, 1984).

#### *Screening for tannins*

Water extract of the sample was treated with 15% ferric chloride test solution. The resultant color was noted. A blue color indicated the presence of hydrolyzable tannins. Into 10 mL of freshly prepared potassium hydroxide (KOH) in a beaker, 0.5 g of the extract was added and shaken to dissolve. A dirty precipitate observed indicated the presence of tannins (Sofowora, 1982).

#### *Screening for flavonoids*

About 2 g of the powdered leaves and tubers were completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while still hot. The filtrate was cooled and used.

Five millilitres of 20% NaOH were added to equal volume of the filtrate. A yellow solution indicated the presence of flavonoids.

#### *Cytotoxic bioassay*

##### *Brine shrimp lethality test*

Brine shrimp lethality test was carried out to investigate the potential cytotoxicity of extracts of *M. tuberosa*. This test costs little and utilizes small amount of test material. This provides a front line screen that can be backed up by more specific and expensive bioassays, once the active compound has been isolated. It is predictive of cytotoxicity and pesticidal activity (Meyer, 1982).

##### *Hatching of Artemia salina shrimps*

Brine shrimp eggs were obtained from the Drug Discovery Development Center at the Chemistry Department, MSU-IIT. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a vessel filled with sea water (brine solution) under constant aeration for 24 hours. The active shrimps were collected and used for the assay (Krisnaraju *et al.*, 2005).

About 4.5 mL of brine solution was taken in to each test tube. Suitable dilutions of the test substance (extract) were made as per the concentrations. About 0.5 mL diluted test solution was added to the test tubes.

Ten active shrimps were added into each test tube by drawing them with glass capillary tube. The solution was mixed thoroughly with the help of a cyclo-mixer. The number of surviving shrimps were counted and recorded after 24 hours.

#### *Statistical analysis*

Microsoft office excels 2010 and Probit analysis by Finney (1971) were used to determine the concentration at which lethality to brine shrimp represents 50% (LC<sub>50</sub>). LC<sub>50</sub> values less than 100 ppm were considered significant. As mentioned by Meyer, LC<sub>50</sub> value of less than 1000 µg/mL is toxic while LC<sub>50</sub> value of greater than 1000 µg/mL is non-toxic.

The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

**Results and discussion**

*Phytochemical screening*

Phytochemical analyses revealed the presence of flavonoids, alkaloids, tannins, phenolic compounds, and saponins in both tubers and leaves of the ant plant extracts. Moreover, tuber extracts contained more tannins and alkaloids compared to leaf extracts (Table 1).

**Table 1.** Phytochemical analyses of the crude extracts of tubers and leaves of *M. tuberosa*.

Phytochemicals	Tuber	Leaves
Tannins	++	+
Alkaloids	++	+
Saponins	+	+
Flavonoids	+	+
Phenolic compounds	+	+

+ present ++ abundant.

The present study agrees with the result of Efendi *et al.*(2010),that tuber ethanolic extracts of *M. tuberosa* have high concentration of phenolic compounds and have an antimicrobial potency against *C. albicans*, *E.coli*, and *S. aureus*.

The occurrence of the above phytochemicals is probably responsible for the use of these plants in the indigenous systems of medicine. Diverse uses of plants in treatment of wide variety of diseases are attributable to the presence of the phytochemicals.

**Table 2.** Brine Shrimp lethality test results of the various extracts of *M. tuberosa*.

Plant Parts	Extracts	Concentration (ppm)	Mortality, %	LC <sub>50</sub> (ppm)
Tuber	Decoction	10	3.33%	132.6
		100	36.66%	
		500	80%	
		1000	76.66%	
	Hydro-ethanolic	10	16.66%	23456.8
		100	13.33%	
		500	36.66%	
		1000	26.66%	
	Ethanolic	10	16.66%	38.68
		100	73.33%	
		500	80%	
		1000	90%	
Leaves	Decoction	10	30%	441.6
		100	36.66%	
		500	46.66%	
		1000	63.33%	
	Hydro-ethanolic	10	23.33%	14567.3
		100	26.66%	
		500	30%	
		1000	30%	
Ethanolic	10	46.66%	126.62	
	100	56.66%		
	500	86.66%		
	1000	83.33%		

The above-mentioned results showed that ant plant has rich sources of phytochemicals which can be isolated and further screened for different kinds of biological activities, depending on their reported ethno-botanical therapeutic uses.

A previous study from Abdul Wahab *et al.* (2011) reported *M. tuberosa* to contain alkaloids, phenolics, and terpenoids.

The plant extracts contained saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. (Itharat *et al.*, 2004). Some of the characteristics of saponins includes formation of foams in aqueous solutions and haemolytic activity. Saponins used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins.



**Fig. 1.** (A) Habit of *Myrmecodia tuberosa* and (B) cross section of tuber showing the holes and chambers made by ants.

In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss. It is also known to have anti-fungal properties.

Tannins are reported to exhibit antiviral, antibacterial, and anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and are also used as diuretic (Heslem, 1989). Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars.

Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergies, virus, and carcinogens (Itharat *et al.*, 2004).

Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity.

The medicinal properties of alkaloids are quite diverse. They can be used for the relief of pain, the discomfort of common colds, sinusitis, hay fever, and bronchial asthma. They also have anti-inflammatory and anti-bacterial properties and widely used as chemotherapeutic agents in the treatment of many types of cancer (Farnsworth, 1984).

Phenolic compounds are also known as nature's tender drugs, possessing numerous biological activities (Soeksmanto *et al.*, 2010). Recent reports indicated their antiviral, antifungal, antioxidant, and anti-inflammatory properties. Phenolics are employed in adaptive or defense mechanism (Kadota, 2002).

### Brine shrimp lethality assay

The results showed that among the extracts obtained, the tuber and ethanolic extract exhibited the highest percentage mortality in all doses having LC<sub>50</sub> of 38.68 µg/mL and 126.62 µg/mL respectively. There was a gradual increase in the percentage mortality with the increase in concentration of alcoholic extract. The results also showed that percent mortality has a direct relationship with concentration tested in all cases (Table 2). Maximum mortalities (90%) were observed at a concentration of 1000 ppm in tuber ethanolic extract. Decoction of tuber and leaves have LC<sub>50</sub> values of 132.6 µg/mL and 441.6 µg/mL, respectively. Hydro-ethanolic extracts of both leaves and tubers had no cytotoxic effect to brine shrimp.

Based on the results, the brine shrimp lethality of the plant extracts was found to be concentration-dependent. According to Meyer *et al.* (1998), crude plant extract is toxic (active) if it has an LC<sub>50</sub> value of less than 1000 µg/mL while non-toxic (inactive) if it is greater than 1000 µg/mL. The observed lethality of the plant extracts to brine shrimps indicated the presence of potent cytotoxic activities.

The present results confirmed the study of Soeksmanto *et al.* (2010) wherein the tuber and bark methanolic extracts exhibited the strongest cytotoxic effect against human colorectal cancer and human breast cancer with LC<sub>50</sub> of 27.61 µg/mL and 54.57 µg/mL, respectively.

### Conclusion

In the present investigation, varying degrees of lethality to brine shrimp were observed with exposure to different concentrations of the test samples. The degree of lethality was found to be directly proportional to the concentration of the extracts tested. Although the brine shrimp lethality bioassay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. However, the brine shrimp lethality assay actually has proven to be a convenient system for monitoring biological activities of several plant species that are used in the traditional medicine.

The mortality of brine shrimps due to extracts of *M. tuberosa* is an indication of the possible presence of potent cytotoxic components which warrants further investigation for the isolation, purification, and characterization of the active constituents responsible for the specific activity.

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