



## Evaluating the Potential Cytotoxic Activity of *Ficus nota* Using Brine Shrimp Lethality Test

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### ABSTRACT

The plant species, *Ficus nota* locally known as "tbig" is traditionally used by the Manobo tribe in Talacogon, Agusan del Sur as an alternative medicine. However, there are no records or documentation on the phytochemical constituent or potential cytotoxicity of the stem of *Ficus nota*. This study was conducted to test for *in vivo* brine shrimp lethality of *Ficus nota*. Plant extracts were obtained through extraction of the stem samples with water and absolute ethanol. Three concentrations (100 ppm, 500 ppm, and 1000 ppm) of the extracts were tested and mortality of *Artemia salina* was noted after 24 h exposure. The results showed that both decoction and ethanolic extracts were active against the brine shrimp with  $LC_{50}$  values of 991.00 ppm and 852.22 ppm, respectively. Results indicated that both extracts may have substances that are cytotoxic and that active components of the plants are better extracted with absolute ethanol.

**Keywords:** alternative medicine, *Artemia salina*, decoction, ethanolic extract, stem.

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### INTRODUCTION

Medicinal plants are considered as rich sources of ingredients which can be used in drug development and synthesis. They play a critical role in the development of human cultures around the whole world [1] and have been used for centuries throughout the world by majority of the world's population which until today still rely on them as traditional and alternative medicine for primary health care needs [2]. Many plants used in traditional medicine contain chemical substances called phytochemicals [3] that produce a physiological action on the human body [4].

Moraceae is a family of flowering plants comprising about 40 genera and over 1,000 species that are mostly widespread in tropical and subtropical regions and a medicinally important family which comprises both wild and cultivated species [5]. Among the genera found in the family Moraceae is the genus *Ficus* which constitutes an important group of trees with immense medicinal value because it is a sacred tree of Hindus and Buddhists [6].

*Ficus nota*, locally known as "tbig" is a medicinal plant that belongs to family Moraceae [7, 8]. It is usually found in thickets and forest at low and medium altitudes [7, 8]. It is an erect, spreading, dioecious tree of about 8 meters (m) in height, with fruits around 2 to 3.5 cm in diameter, fleshy, green and becoming yellowish-white at the base [7, 8]. Among its traditional uses include drinking three times per day of the water extracted from the standing tree for fever, and muscle pain relief [7] and a decoction of the roots and bark for treatment of the urinary tract infection, hypertension, and diabetes [8]. In the Philippines, the Mansaka tribe in Compostela Valley used the decoction of the bark of this plant to treat asthma, cough, and other respiratory condition [9]. The Ayta people of Porac, Pampanga province in the Philippines used the stem of this plant species as repellent against hematophagous insects [10]. In this study, based from the interview of the Manobo tribe chieftain in the study area, stem of *F. nota* is traditionally used as an alternative medicine. It was found that *F. nota* occurs throughout the Philippines and found in Batan Island, Polilio, Mindoro, Palawan, Panay, Samar, and Leyte [7].

Scientific validation of traditional medicinal plant has been an important path of recent studies [11]. Some *Ficus* species like *F. racemosa* has long been used for various conditions as alternative medicine then scientific validation was made for its antidiabetic, antipyretic, anti-inflammatory, antitussive, hepatoprotective, and antimicrobial activities [12]. *Ficus septica* has also been found by certain studies to exhibit antifungal activity [13]. *Ficus nota* is among the *Ficus* species less explored on its bioactivity against certain conditions although a study on the chemical constituents of *F. nota* fruits has shown that the dichloromethane extract of the plant's unripe fruits has 4-(2-hydroxyethyl)- 2-methoxyphenol (1), a mixture of meso-2,3-butanediol (2a), (2R,3R)-2,3-butanediol (2b) and (2S,3S)-2,3- butanediol (2c) and  $\beta$ -sitosterol (3) [14]. However, no studies yet are reported on the phytochemical constituent of the stem of *F. nota* nor any documentation of its potential cytotoxicity. Hence, as an initial effort to scientifically support the utilization of *F. nota* stem as an alternative medicine, this study was done to determine the potential cytotoxicity of *F. nota* stem extract, as traditionally used by the Manobo tribe as an alternative medicine. *Artemia salina*, a primitive aquatic arthropod [15] is used as target organism to detect bioactive compounds in plant extracts in which the toxicity test against these animals has shown to have a good correlation with antitumor activity [16, 17]. Moreover, this study may also help in finding evidence in identifying important medicinal plants that may lead to possible development of new drugs.

## METHODOLOGY

### Plant Collection

The plant samples were collected in September 2014 from Bataran, Desamparados, Talacogon, Agusan del Sur, Philippines. Ethnopharmacological use was based on an interview with Lucrecio Durango, acting tribal chieftain of the Manobo Tribe in the area. The information gathered also included which part of the plant is traditionally used as medicine and its method of preparation. Approximately five kilograms of the stem of *Ficus nota* were collected. For the purpose of identification, small branches or twigs, healthy leaves and fruit were obtained and the plant was identified by Professor Edgrado Aranico, a botanist of the Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology, Philippines.

### Preparation of the Plant Decoction

Stem samples were washed with tap water followed by distilled water. Approximately 1 kg fresh and clean stem samples of the plant were cut into pieces and boiled in 1:2 ratio with distilled water for 5 minutes. The mixture was filtered, cooled, stored in glass containers and freeze-dried to give the concentrated decoction. It was then kept until required [18].

### Preparation of Crude Ethanolic Extract

An alternative procedure developed by other related studies was adopted with some modifications [19, 20]. Remaining samples were air-dried for about 2-3 weeks. Dried samples were pulverized using a sterile electric blender and soaked in pure absolute ethanol for at least three days. Solution was filtered and concentrated using rotary evaporator. Thirty milligrams from freeze-dried decoction were dissolved in 3 ml seawater to obtain the stock solution of the decoction. On the other hand, DMSO was used as emulsifying agent to dissolve the ethanolic crude extract and was added with 3 ml of seawater to obtain the stock solution of the crude ethanolic extract. From the stock solution, 100, 500, and 1000 ppm concentrations were prepared by the addition of 50  $\mu$ L, 250  $\mu$ L and 500  $\mu$ L of the solution, respectively to 20 mL test tubes all diluted to 5ml with seawater.

### Brine Shrimp Lethality Test and Statistical Analysis

*Artemia salina* eggs were obtained from Mindanao State University- Naawan Campus. Eggs were dehydrated in distilled water for 30 minutes. The tank filled with filtered sterile water was then divided into two compartments. The hatching chamber was covered with black cardboard before adding the dehydrated eggs of *A. salina*. The other half of the tank was illuminated to attract and separate the hatched shrimp from the unhatched eggs. The light is important to simulate the temperature of the natural habitat of the shrimp. The shrimps were then used for bioassay after 24 h.

Three replicates for each concentration were prepared. Ten *A. salina* nauplii larvae were transferred into each sample vial using glass dropper and filtered sterile seawater was added to make a total volume of 5 ml. Nauplii were counted macroscopically after 24 h and the deaths at control and each dose level were determined.

After 24 h, the lethal concentrations of *F. nota* extract resulting to 50% mortality of the brine shrimp ( $LC_{50}$ ) were determined. By means of a trendline fit linear regression analysis (MS Excel version 7) the dose-response data were transformed into a straight line. From the best-fit line obtained, the  $LC_{50}$  was derived.

Brine shrimps were exposed to different concentrations of both the decoction and crude ethanolic extracts to determine the relative toxicity. The relationship between the concentration of the extracts and mortality of the brine shrimp was done by plotting the concentration log (x-axis) versus mortality (y-axis). The LC<sub>50</sub> of *Ficus nota* stem extracts after 24 h were determined using trendline fit linear analysis in Microsoft Excel. Equation was obtained from the best-fit line and LC<sub>50</sub> was calculated.

## RESULTS AND DISCUSSION

The brine shrimp lethality of the two test samples both for decoction and ethanolic extract were found to be concentration-dependent as shown in Table 1. Both the decoction and ethanolic extracts showed direct relationship with the mortality rate of the brine shrimp because as the concentration of the *Ficus nota* extract increases, the value of brine shrimp mortality after 24 hrs. also increases. The study of Noraya *et al.* [21] also found that if the value of the concentration of the *Acmella grandiflora* extracts increases there is a direct relationship with the mortality rate of the brine shrimp. Morilla *et al.* [22] also found that the mortality rate of the brine shrimp exposed for 24 hours increased with increasing concentration of the *Kleinhovia hospita* extracts with the highest mortality observed at 1000 ppm specifically with ethanolic extract. In this study, LC<sub>50</sub> values for each extracts were 852.22 ppm (ethanolic), and 991.0 ppm (decoction). Crude plant extract with LC<sub>50</sub> value of less than 1000 ppm is considered toxic (active) while value higher than 1000 ppm is considered non-toxic (inactive) [23]. Even other *Ficus* species including *F. septica* through its ethanol extract from its leaves was already documented to exhibit cytotoxic properties and even antibacterial, antifungal, antiprotozoal, and cytotoxic properties [24]. Several plant species traditionally used as alternative medicine in the Philippines were also examined and showed active cytotoxicity against *A. salina* which included *Lantana camara*, *Chromolaena odorata*, and *Euphorbia hirta* as potent against brine shrimps through Brine Shrimp Lethality Assay with LC<sub>50</sub> values of 55 ppm, 10 ppm, and 100 ppm respectively [3]. Responde *et al.* [17] reported that the alcohol- based extracts (ethanol and water: ethanol) of *Eleusine indica* showed high mortality rate of *A. salina* with LC<sub>50</sub> values of 10 ppm, 100 ppm, 500 ppm and 1000 pm. The study of Jose *et al.* [25] also showed that the leaf extract of *Antidesma ghaesembilla* Gaertn (bugnai) in 100% percent, 50-50 ethanol extracts and decoction revealed cytotoxic activity against the brine shrimp *A. salina* indicating potential bioactive components of the plant extract.

**Table 1.** Chronic LC<sub>50</sub> of *Ficus nota* extracts on *A. salina* after 24-hrs Exposure.

Plant Extract	Concentration of the Extract (ppm)	Brine shrimp mortality (%) after 24 hrs.	Chronic LC <sub>50</sub> (after 24 hrs)
Decoction	100	38	991 ppm
	500	43	
	1000	50	
Ethanolic	100	20	852.22 ppm
	500	27	
	1000	38	

Comparing the two extracts from *F. nota*, ethanolic extract showed slightly higher lethality on *A. salina* after 24 hours. Several studies support the finding on better extraction of plant's cytotoxic component with ethanol extraction as shown in the study of Jose *et al.* [25], Olowa and Nuñez [3]; Lalisán *et al.* [18] and Morilla *et al.* [22]. In the cytotoxicity study of *Pseudelephantopus spicatus* leaves using *A. salina* lethality assay, plant extracts was obtained through decoction, ethanolic extraction, and extraction with ethanol-water. The results showed that the decoction and ethanol-water extract were inactive against brine shrimp. However, the ethanol extract showed a toxicity effect after 6h and 24h exposures with LC<sub>50</sub> values at 944.07 and 266.07 ppm, respectively [18]. The study on the brine shrimp lethality test of *Kleinhovia hospita* stem and bark showed that the bioactivity of *K. hospita* extract was more apparent in ethanol than in decoction extract which indicates that extraction with ethanol is effective in obtaining bioactive components of the plant species [22]. Other studies also reported the activity of ethanol extracts from certain plant species which included the study on examining the *in vitro* effect of *F. septica* ethanolic extract fractions on Balb/c mice macrophage phagocytosis and lymphocyte proliferation, ethyl acetate fraction has the highest immunomodulatory effects [26]. These support the finding of the present study on the greater cytotoxic activity of the ethanol extract of *F. nota* compared with the decoction. But a

report on the antibacterial activity of *Parkia clappertoniana* hot and cold water extract as well as ethanolic extract from its roots, stem, and leaves showed susceptibility of all the test organisms (*Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC10145, and *Staphylococcus aureus* ATCC 12600) to all extracts with zones of inhibition ranging between 14 mm – 27 mm for hot water extracts, 12 mm – 22 mm for cold water extracts and 12 mm – 25 mm for ethanolic extracts [27]. This could imply species-to-species difference among plants in terms of the cytotoxicity and bioactivities of their phytochemical constituents.

The findings on the active cytotoxicity of *F. nota* stem extracts may support the therapeutic use of the plant as an alternative medicine.

## CONCLUSION

The stem extracts of *Ficus nota* exhibited cytotoxic activity against the brine shrimp *Artemia salina*. After 24 h, ethanolic extract showed greater cytotoxic activity compared to the decoction extract. This further warrants phytochemical screening of the ethanolic extracts of *F. nota* for the determination of its active cytotoxic components.

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