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Cytotoxicity and phytochemical screening of four species of plants from Pualas, Lanao del Sur, Philippines

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Abstract

Utilization of plants as traditional medicine is widely practiced by indigenous communities. This study aimed to test the cytotoxic activities and determine the phytochemicals present in the extracts of the four selected plants: *Cyathulageniculata, Peperomiapellucida, Marsdeniatinctoria,* and *Menthaarvensis* that are most commonly used as medicinal plants in Pualas, Lanaodel Sur. For cytotoxicity, the most potent was the ethanol extract of *M. tinctoria*with LC_{50} of 47.86 ppm against the test sample, *Artemiasalina*. Morever, the phytochemicals present in four plants indicate their potential to be source of drugs. This study illustrates the importance of traditional medicine in the treatment and management of ailments in Pualas, Lanaodel Sur and that confirming the safety and efficacy of the medicinal plants used should be given attention.

Key words : Drugs, ethanol extract, medicinal plants, traditional medicine.

INTRODUCTION

An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice [1]. Most of the medications of today are obtained from plants due to easy accessibility and affordability. The value of medicinal plants lies on their bioactive phytochemical constituents which show various physiological action on the human body [2,3]. Phytochemicals present in smaller quantities in higher plants include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many more. They have enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties [4].

The use of traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary healthcare delivery [5]. The World Health Organization (WHO) decided to begin cataloguing and evaluating the safety and efficacy of these remedies and discovered that 119 medicinally important chemical compounds are derived from higher plants [6]. Medicinal plants contain substances or chemical components that can be used for therapeutic purposes [3,7,8]. The presence of bioactive compounds such as flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, phlobatannins, and reducing sugars in plant extracts [3,8] further revealed their potential contribution to medicinal as well as physiological properties of the plants studied in the treatment of different ailments. Moreover, the plants screened for phytochemical constituents have the potential to act as a source of useful drugs and also to improve the presence of various compounds that are vital for good health [9].

This study aimed to evaluate the cytotoxicity of the extracts of selected medicinal plants using the brine shrimp lethality test (BSLT) and to determine the presence or absence of different compound classes in those medicinal plants through qualitative phytochemical analysis.

MATERIALS AND METHODS

Sample Preparation

C. geniculata, P. pellucida, M. tinctoria, and *M. arvensis* which are abundant in the study area and are commonly used as a remedy for cough, colds, fever, sprains, and UTI (Urinary Tract Infection) by the "Maranaos" in Pualas, Lanao del Sur were collected.

Plant parts used in this study were in accordance with the plant part/s used by the locals for medical purposes. About 1 kg of fresh plant materials was washed under running tap water and air-dried for 2-4 weeks, homogenized to fine powder using an electric blender, and stored in clean and air-tight sample containers prior to solvent extraction.

Plant Extraction

Fifty grams (50.0 g) of powdered air-dried plant samples were soaked separately in absolute ethanol (2.5 L) and 50:50 (1L:1L) ethanol-water for 48 hours and filtered. The solvent was filtered and then concentrated in *vacuo*using a rotary evaporator at a temperature below 40 °C as described by Peteros&Uy[4] with slight modifications. Extracts produced were stored in sample bottles in the refrigerator prior to use.

Another set of fresh plants was prepared for decoction. Five hundred grams (500.0.g) of thoroughly washed fresh plant parts were cut into pieces and then boiled under medium heat in 1000.0 ml distilled water corresponding to 1:2 ratio of water and sample for decoction. The mixture was filtered and freeze-dried to remove traces of water. Extracts produced were stored in sample bottles in the refrigerator prior to use.

Brine Shrimp Lethality Test

Brine shrimp hatching

The assay was carried out in accordance with the standard protocol described by Meyer et al.[10]. Eggs of *Artemiasalina*were obtained from the Chemistry Department of Mindanao State

University Iligan Institute of Technology (MSU-IIT), Philippines. The eggs were rehydrated with distilled water for 30 minutes then transferred to a glass container containing filtered sterile seawater. The hatching chamber had two partitions (dark and light areas). Shrimp eggs were added into the dark side of the chamber. The other side was lighted with lamp to attract the hatched nauplii. The nauplii were subjected to aeration until use for the toxicity test.

Brine shrimp lethality assay

Stock solutions of plant extracts with concentrations of 1000.0 ppm, 100.0 ppm, and 10.0 ppm were prepared by serial dilution. Aliquots of 500.0 µL, 50.0 µL, and 5.0 µL each of the stock solutions were transferred using micropipette to three test tubes to produce 1000.0 µg/mL, 100.0 µg/mL, and 10.0 µg/mL concentrations upon dilution to 5.0 mL of seawater. Three replicates were prepared for each concentration of the extract. Ten larvae of *A. salina*were placed in every test tube using a dropper. The test tubes were kept under 100 watt illumination and the number of survivors was recorded after 6 and 24 hours as described by Elias et al. [11].

Statistical analysis

Probit analysis was used to determine the lethal concentration (LC_{50}) with 95% confidence level. Chronic and acute LC_{50} represent the dose that rendered 50% mortality of the test animals after 24 hours and 6 hours exposure, respectively.

Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out using the standard procedure as described by Harborne[12] and Guevara [13]. Plant extracts were screened for the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, and phenols.

Phytochemical analysis

Alkaloids. Crude sample (0.25 g) was diluted to 10.0 ml with 2M HCl, warmed, and filtered. Diluted ammonia (2.0 mL) was added to 5.0 ml of the filtrate. An aliquot of 5.0 mL of chloroform was added to the mixture and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10.0 ml of acetic acid. Formation of a cream precipitate in Mayer's reagent indicated the presence of alkaloid.

Steroids. A 0.5 mg of each crude plant extract was mixed with 5.0 mL of chloroform and then added with 2.0 mL of concentrated H_2SO_4 draining along the sides of the test tube. Change in color in the upper layer in the test tube into red and the H_2SO_4 layer into yellow with green fluorescence indicated the presence of steroids.

Flavonoids. A 1.25 g of crude extract was heated with 10.0 mL of ethyl acetate over a steam bath for 3 min and then filtered. Filtrate (4.0 mL) was then mixed thoroughly with 1.0 mL of dilute ammonia solution. The presence of flavonoid compounds is represented by the yellow coloration.

Saponins. One millilitre (1.0 mL) of stock solution was mixed with 2.0 mL of distilled water and then shaken by hand for 15 minutes. Formation of a foam layer on top of the tube indicated the presence of saponin.

Tannins. Five milliliters of distilled water with 0.25 g of powdered plant sample were boiled in water bath and then filtered. Few drops of 0.1% $FeCl_3$ were added to the filtered samples. Brownish green or a blue black coloration indicated the presence of tannins.

Anthraquinones. A 10.0 mL distilled water was added to 1.0 g of powdered plant sample and then filtered. The filtrate was extracted twice with 5.0 mL portions of benzene, combining the benzene extracts. The solution was divided in two portions (1 portion as a control and 1 portion treated with 5.0 mL ammonia solution). Red coloration in the lower ammonia layer indicated the presence of anthraquinones.

Cyanogenic glycosides. A 1.0 g of powdered plant sample in a test tube was moistened with water and then added with a few drops of chloroform to enhance enzyme activity. Using a firm cork stopper, a strip of yellow picrate paper was suspended. The tube was warmed at 35-40 □C in a room temperature for 2 to 3 hours. Various shades of red indicated a positive result.

Fatty acids. 1.0 mL of 10% Na_2CO_3 was added in a powdered plant sample. Bubble formation indicated a positive result.

RESULTS

Brine shrimp lethality test

The level of toxicity of the four plants showed a directly proportional relationship with the concentration of the extracts (Table 1). The percentage of mortality of *A. salina*increased as the concentration and time increased. The highest lethality of *A. salina*was observed at 1000 ppm of ethanolic extract of *P. pellucida*and *Marsdeniatinctoria* wherein both had 100% mortality rate after the 12 h exposure.

Screening for phytochemical constituents

Qualitative analysis of phytochemicals found in the four medicinal plants is summarized in Table 2. The result showed the presence of alkaloids, steroids, flavonoids, saponins, tannins, and fatty acids in various degrees. Anthraquinone and cyanogenic glycosides were not detected during the test. The absence of anthraquinone and cyanogenic compounds might be because samples are way too little to be detected or the plants used for this test do not produce these compounds.

DISCUSSION

Brine shrimp lethality test

The lowest mortality rate was observed in *C. geniculata*(3.33%) at 1000 ppm after 12 h exposure. Results indicated that the ethanol extract from *M. tinctoria*with LC_{50} of 47.86 ppm was the most potent. Plant extracts with values of LC_{50} greater than 1000 ppm are considered inactive [14].

Although the trend for LC_{50} for each extract is not consistent (for example, Cg50>CgE>CgD and PpE>PpD>Pp_{50}), the result clearly showed that the LC_{50} for each extract was relatively not close to each other, thus showing that the mode of preparation can affect the cytotoxic activity of the plants. The difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the extracts could be the reason for the varied BSLT results [4]. Moreover, loss of some cytotoxic substances may be due to the different mode of extraction (aqueous, 50% and 95% ethanol extracts).

The results further revealed that the mode of extraction could affect the efficiency of the medicinal plants and on what extract could be used for further detection of the bioactivity present. Brine shrimp lethality bioassay is a general bioassay that has been

Table 1: Brine Shrimp Mortality Rate and LC50 of Four Medicinal Plants.

Plant	Percent Mortality						LC_{50} in ppm (after 12-h exposure)
	After 6-h exposure			After 12-h exposure			
	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	
CgD	0.00	0.00	6.67	3.33	13.33	50.00	1000.00
Cg50	0.00	0.00	0.00	0.00	0.00	3.33	5370.00*
CgE	0.00	3.33	13.33	3.33	10.00	36.67	2089.00*
PpD	0.00	0.00	10.00	13.33	0.00	0.00	1524.00*
Pp50	0.00	10.00	36.67	0.00	0.00	73.33	843.00
PpE	3.33	6.67	10.00	3.33	96.67	100	9772.00
MtD	0.00	0.00	0.00	0.00	6.67	13.33	-
Mt50	0.00	0.00	6.67	3.33	3.33	76.67	355.00
MtE	0.00	0.00	23.33	16.67	70.00	100.00	48.00
MaD	6.67	6.67	10.00	0.00	6.67	30.00	-
Ma50	0.00	3.33	3.33	0.00	43.33	50.00	-
MaE	0.00	0.00	16.67	10.00	100.00	53.00	912.00

CgD (*C. geniculata,* Decoction); Cg50 (*C. geniculata*, EtOH:H2O); CgE (*C. geniculata*, ethanol); PpD (*P. pellucida,* Decoction); Pp50 (*P. pellucida*, EtOH:H2O); PpE (*P. pellucida*, ethanol); MtD (*M. tinctoria,* Decoction); Mt50 (*M. tinctoria*, EtOH:H2O); MtE (*M. tinctoria*, ethanol); MaD (*M. arvensis,* Decoction); Ma50 (*M. arvensis*, EtOH:H2O); MaE (*M. arvensis*, ethanol); * extrapolated values.

Table 2: Phytochemical constituents of the four medicinal plants.

Phytochemicals	*C. geniculata*			*P. pellucida*			*M. tinctoria*			*M. arvensis*		
	Cg D	Cg 50	Cg E	Pp D	Pp 50	Pp E	Mt D	Mt 50	Mt E	Mt D	Mt 50	Mt E
Alkaloids	++	++	+	++	++	+	+	++	-	+	++	+
Steroids	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+	++	+++	++	+++	+++	+++	+++	+	+++	+++
Saponins	-	+	++	+	+++	+	-	+	+	-	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Cyanogenic glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Fatty acids	-	+++	++	++	++	++	++	+++	++	++	++	++

- absence; + poor or turbid only; ++ moderate amount; +++ heavy precipitate or dark color off solution; CgD (*C. geniculata,* Decoction); Cg50 (*C. geniculata*, EtOH:H_2O); CgE (*C. geniculata*, ethanol); PpD (*P. pellucida,* Decoction); Pp50 (*P. pellucida*, EtOH:H_2O); PpE (*P. pellucida*, ethanol); MtD (*M. tinctoria,* Decoction); Mt50 (*M. tinctoria*, EtOH:H_2O); MtE (*M. tinctoria*, ethanol); MaD (*M. arvensis,* Decoction); Ma50 (*M. arvensis*, EtOH:H_2O); MaE (*M. arvensis*, ethanol)

used for detection of a broad spectrum of bioactivity present in plant crude extracts [15], a good indicator for general toxicity, and also as a guide for the detection of antitumor and pesticidal compounds [10].

Screening for phytochemical constituents

Phytochemicals produced by plants possess physiological action on human body that could be of great value in the field of medicine. Alkaloids, tannins, and saponins exhibit toxic activities in treating common pathogenic strains [16,17,18]. Saponins contain antibiotic properties and protect against hypercholesterolemia. Steroids and triterpoenoids have analgesic activities and steroids and saponins are responsible for the activities in the central nervous system [9].

The phytochemical constituent flavonoids possess numerous pharmacological activities as anti-viral, anti-cancer, anti-inflammatory, and anti-allergic. Steroids possess anti-nociceptive actions while tannins have antimicrobial property [19,20]. These three groups are present in the four plants, all in good amount apart from the aqueous extract of *M. arvensis*and 50% ethanol extract of *C. geniculata*. This confirms the potential importance of the four plants as medicine.

Alkaloids exhibit antimicrobial activity that includes relieving the discomfort caused by common colds, sinusitis, hay fever, and bronchial asthma [21]. Hence, the presence of alkaloids in *M. tinctoria*and *M. arvensis*supports its folkloric use in treating fever, cough, and colds.

The medicinal value of any plant lies in its bioactive phytochemical constituents. Through phytochemical screening, one could detect the various important compounds which could be used as the basis of modern drugs for curing various diseases [2]. The phytochemical analysis of the four medicinal plants and their folkloric use showed nearly similar results due to the presence of the phytochemical constituents.

CONCLUSION

The highest lethality test of *A. salina*was observed at 1000 ppm of ethanolic extract of *P. pellucida*and *Marsdeniatinctoria*, both had 100% mortality rate after the 12 hours exposure. The lowest mortality rate was observed in *C. geniculata*(3.33%) at 1000 ppm after 12 hours exposure. Results indicated that themost potent plant for toxicity was the ethanol extract from *M. tinctoria*with LC_{50} of 47.86 ppm. Furthermore, positive bioactivity of the plant extracts might be due to the presence of various chemical compounds that are vital for good health. Hence, the presence of these phytochemical constituents supports the medical importance of these traditional plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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