

Phytochemical Screening of Selected Ethnomedicinal Plants of Bolinao, Pangasinan, Northern Philippines

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Abstract — Ethnomedicinal plants are recognized as potential sources for the discovery and development of effective yet inexpensive pharmaceutical drugs. The study was conducted to detect the presence of different phytochemicals in the selected medicinal plants used by the Bolinao-Sambal *managtambal* for treating and curing various diseases. Healthy plant samples were collected from Bolinao, Pangasinan between December 2016 and January 2017. Phytochemical screening was done for the plants chosen from the list of plant used by herbalists of Bolinao, Pangasinan. The families of plants included Boraginaceae, Fabaceae, Poaceae, Astereaceae, Piperaceae, Compositaceae. The phytoactive constituents of the plants were screened. Results showed that there were detection of alkaloids, unsaturated fats, sterols and terpenes, flavonoids, steroid (cardio-active glycosides), saponins and tannins. Furthermore, it was observed that a strong culture retention was manifested on the use of plants in the folkloric illness pasma.

Keywords — *Phytochemical screening, secondary metabolites, ethnomedicinal plants,*

I. INTRODUCTION

Ethnobotany is an emerging science that documents our ancestors' use of plant resources which primarily aims to conserve biodiversity and preserve cultural heritage. Extensive use of herbs in medication by folks is a certain indication of their value and usefulness in the future (Ndam, 2014). In fact, an increase in the use of the medicinal plants in modern medicine is currently observed (Iordacescu and Dumitriu, 1988) as the demand for plant resources from rural and unpolluted areas by pharmaceutical and cosmetic industries proliferates (Ndam, 2014). Interestingly, traditional plant medicines are used by approximately 80% of individuals from developed countries (Yadav and Agarwala, 2011), implying that these are still preferred over synthetic drugs.

Although the significance of these plants used by indigenous peoples is clearly established, there has been an increasing erosion of culture on the plant utilization in all strata of the society due to change in lifestyles, habitat loss, land-use conversions and

exposure to modern technologies (Andrada, 2004). Thus, there is a need to document these practices and confirm their potentials to be sources of pharmaceutical drugs. The potential of these ethnomedicinal plants used for treating common diseases can be verified qualitatively using preliminary phytochemical analyses. Phytoactive substances such as tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids are some organic compounds that provide specific physiological action in the human body (Edeoga et al. 2005).

Plants produce primary and secondary metabolites, collectively known as phytochemicals, which are naturally occurring metabolites in leaves, flowers, roots, and stems. These are used for growth, maintenance, defense against herbivory, and protection from diseases. Primary metabolites are essential for the normal physiology which includes proteins, carbohydrates, lipids and chlorophyll (Mann, 1978). On the other hand, secondary metabolites are produced by the plant during development which are specific to tissues and organs which include tannins, flavonoids, phenolics,

saponins, and alkaloids (Wadood et al 2013). These phytoactive constituents are widely used in various areas of science such as human therapy, veterinary medicine, agriculture, and other related researches (Vasu et al 2009).

In the ethnobotanical study conducted in Bolinao, Pangasinan, 11 out of 32 barangays of the municipality were identified with traditional healers or “*managtambal*”. The research revealed that a total of 50 plants were used in the traditional healing, which are locally available in the community. These plants belong to families *Amaryllidaceae*, *Anacardiaceae*, *Anonaceae*, *Araceae*, *Arecaceae*, *Asteraceae*, *Bixaceae*, *Boraginaceae*, *Caricaceae*, *Compositae*, *Cucurbitaceae*, *Euphorbiaceae*, *Fabaceae*, *Labiateae*, *Lamiaceae*, *Leguminosae*, *Liliaceae*, *Lythraceae*, *Malvaceae*, *Menispermaceae*, *Musaceae*, *Myrtaceae*, *Oxalidaceae*, *Piperaceae*, *Poaceae*, *Rutaceae*, *Solanaceae*, *Verbenaceae*, and *Zingiberaceae* (Fajardo et al. n.d.).

The main objective of the study was to detect the presence of different phytochemicals in the selected medicinal plants from Bolinao-Sambal *managtambal* used for treating and curing various diseases. The obtained results are important in making recommendations concerning the cultivation of these plant species which may direct scientists in their search of promising plant species that contain effective bioactive constituents which can be helpful in the formulations of phytotherapeutical products, food supplements, cosmetics and agroecosystem chemical products (Ndam 2014). This study also aimed to find out the link between the secondary metabolites found in the plants vis-a-vis the ethnobotanical uses, and to increase public awareness of these medicinal plants so that residents will be

encouraged to do *in situ* and *ex situ* conservation of these plants.

II. MATERIALS AND METHODS

Study Area

The study area was in Bolinao, Pangasinan which included 11 barangays pre-identified with resident *managtambal*, or herbalists. These barangays visited were Arnedo, Binabalian, Cabuyao, Culang, Goyoden, Lucero, Pilar, Sampaloc, Samang Norte, Tara, and Victory. However, collection sites were mainly from Binabalian and Culang where most of the plants of interest were present.

Collection and Identification of Plant Specimens

Healthy plant samples were collected from Bolinao, Pangasinan between December 2016 and January 2017. The previous research on ethnobotanical study of traditional medicinal plants of Bolinao-Sambal was the basis for the collection (Fajardo et al. undated). Identification of plants was done on January 2017 at the PSU-Lingayen Biology Laboratory and were verified in the National Museum-Botanical Division, Manila.

Selection of Plants

Plants that were selected in the phytochemical screening was based on the least mentioned plants used by *managtambals* in treating identified illnesses and whether these plants were not widely known in folkloric medicine of Pangasinan and distinct only to Bolinao, Pangasinan. Such criteria were deemed to be significant since less study is conducted on the plants and the associated illness.

TABLE 1. SELECTED PLANTS USED IN PHYTOCHEMICAL SCREENING FROM SURVEY OF ETHNOMEDICINAL PLANTS OF BOLINAO, PANGASINAN USED FOR VARIOUS ILLNESSES AND THEIR METHODS OF PREPARATIONS.

	Local Name	Scientific Name	Family	Parts Used	Preparation/ Administration	Utilization/ Illness
1	Balikotkot	<i>Heliotropium indicum</i> Linn.	Boraginaceae	Leaves	Soak the washed leaves with warm water for few minutes. Then squeeze and mix with one teaspoon of honey to be drank by the patient. One teaspoon and 1 tablespoon for children and adults respectively.	Cough/ <i>Subi-subing</i> bata
2	Bani	<i>Pongamia pinnata</i> (L.) Pierre	Fabaceae	Leaves	Boil the leaves of tanubong, bani, inwad, kasoy, lagundi together with lazona. Decoction will be bathed by the patient	<i>Pasma</i>

Continuation...Table 1

	Local Name	Scientific Name	Family	Parts Used	Preparation/ Utilization/ Administration	Illness
3	Baranoy	<i>Cymbopogon citratus</i> L.	Poaceae	Leaves	Decoction of clean baranoy leaves. Then add calamansi juice into the decoction to be drank by the patient as a tea	Diabetes or for cleansing
4	Bugayong	<i>Abrus precatorius</i> Linn.	Fabaceae	Leaves	Wash ample amount of leaves then squeeze to extract the juice. Mix it with the extracted juice from dalayap juice to be drank by the adult patient. However, if the patient is a child, the juice of bugayong is added only with few drops of dalayap juice.	Cough and colds
5	Herbaca or Stamadia	<i>Parthenium hysterophorus</i> L.	Astereaceae	Leaves	Pound and grind the leaves then wrap them with banana leaves. Place ample amount of coconut oil then place it on the stomach for one hour.	<i>Sinisikmura/ Lamig sa tiyan/ Diarrhea with Vomiting</i>
6	Ikmo	<i>Piper betel</i> L	Piperaceae	Leaves	Clean 5 leaves of ikmo using clean cloth. Apply oil and a little amount of salt. Heat the leaf and then rub it on the body of the patient.	<i>Subi-subing bata</i>
7	Inwad	<i>Aegilops cylindrica</i> Host	Poaceae	Leaves	Boil the leaves of tanubong, bani, inwad, kasoy, lagundi together with lazona. Decoction will be bathed by the patient	<i>Pasma</i>
8	Sambong	<i>Blumea balsamifera</i> L. DC	Compositae	Leaves	Decoction of leaves then will be drank by the patient.	Cough
9	Silag	<i>Corypha utan</i> Lamelata	Arecaceae	Leaves	Decoction of leaves and will be bathed by the patient	<i>Pasma</i>
10	Tanobong	<i>Phragmites vulgaris</i> (Lam.) Trin.	Poaceae	Leaves	Boil the leaves of tanubong, bani, inwad, kasoy, lagundi together with lazona. Decoction will be bathed by the patient	<i>Pasma</i>

The phytoactive constituents of the plants were screened at the Pharmacy Laboratory of Virgen Milagrosa University, San Carlos City, Pangasinan. Certification was issued indicating the results were true and correct and that it was conducted in the same laboratory. Procedures and protocols were followed for the phytochemical screening (Guevarra *et al.* 2005). Results were analyzed based the chemical reactions manifested by the addition of various test solutions and with few methods applied (Guevarra, 2005).

Preparation of Extracts

About fifty grams (50) of the dried leaves were weighed and macerated in an Erlenmeyer flask with 300 ml of 95 % ethyl alcohol. The mixture was refluxed for an hour and filtered. The remaining residue was washed and added with ethyl alcohol to make a 500 ml the extract that was used in the photochemical screening.

Screening for Alkaloids

Seventy (70) ml of the ethanolic extract were evaporated to dryness on a steam bath. The residue was dissolved in 7 ml of 15 ml of hydrochloric acid,

aided by warming on the steam bath for 1 or 2 minutes. It was cooled, filtered and adjusted to a volume of only seven milliliters by washing the residue on the filter with sufficient quantity of 1% HCl. A few grains of the powdered sodium chloride were added to the filtrate, afterwards it was shaken, and filtered.

One milliliter of the filtrate was placed into each small test tube. To the first test tube, 3 drops of modified Mayer's Reagent was added; to the next 3 drops of Wagners's Reagent (Iodine and potassium iodine T.S); then 3 drops of Valser's and to the last test tube, 3 drops Bouchardat's reagent. Formation of white precipitate indicated positive result.

Screening for Unsaturated Sterol and Triterpenes

Thirty milliliters of the ethanolic extract were evaporated to dryness on water bath. The residue was allowed to cool at room temperature and fifteen milliliters of light petroleum ether were added, mixed well and filtered. More volumes of petroleum ether were added until the last volume of petroleum ether became colorless then ethereal filtrates were all collected. The defatted residue was set aside for screening for flavonoids and leucoanthocyanins.

The combined ethereal filtrates were evaporated to dryness and then the residue was dissolved in 15 ml of chloroform. The chloroformic solution was dried over anhydrous sodium sulfate, filtered, and the filtrate was divided equally into three test tubes. Two tests were performed which were essentially dehydration reaction and therefore moisture must be excluded in each of the experimental steps.

The first test was Lieberman-Burchard test wherein about five milliliters of the filtrate were placed in a suitable dry test tube and 0.3 ml of acetic anhydride was added and mixed gently. Then, one drop of concentrated sulfuric acid was added. Any color changes were observed immediately every 5 minutes thereafter over a sixty-minute period. Change of color into blue or green indicated positive result.

On the other hand, in Salkowski test about five milliliters of the filtrate were transferred to a dry test tube and a ring test was performed by adding 1 drop of concentrated sulfuric acid. The solution was shaken after 1-2 minutes and was observed for color change. Cherry red color indicated positive result.

Screening for Flavonoids

In the test for the presence of defatted plant extract, the residue was dissolved in 30 ml of 50% ethanol and was filtered 1-2 ml of the filtrate was placed in three separate test tubes.

For the Bate-Smith Metcalf Test, first test tube was treated with 0.5 concentrated hydrochloric acid and warmed in water bath for five minutes and observed for any color change within an hour. Red violet color indicated positive result.

For the Cyanidin test, the other tube was treated with 0.5 ml HCl and four pieces of magnesium turning were added on it. Any color change within 10 min had been indicated positive result.

Screening for Steroids (Cardioactive Glycosides)

Five ml of Kedde's reagent (2g-of 3-5-dinitro-benzoic acid in 100 ml of ethanol) were added to five ml of the ethanolic extract in an evaporating dish, and the solution was mixed well with glass stirring rod. Two ml of 1N sodium hydroxide were added to the mixture and was observed for color reaction. Purple ring indicated positive result.

Using Keller-killiani test (2-deoxysugars), about ten milliliters of the ethanolic extract was placed in an evaporating dish and dried on a steam bath. Three (3) ml of the FeCl₃ reagent (mix 0.3 ml of 10% FeCl₃ solution with 50 ml of Glacial acetic acid) were added, stirred to mix well, and then transferred to a small test tube held at 45 degree angle, 1 ml of concentrated H₂SO₄ was added by allowing it to run down the inside wall of the test tube.

In Kedde's Reaction test, 5 ml of kedde's reagent (2 g of 3,5-dinitro-benzoic acid in 100 ml of ethanol) were added to 5 ml ethanolic extract in an evaporating dish, and the solution was mixed well with a glass stirring rod. Two (2) ml of 1N sodium hydroxide was added to the mixture and was observed for color reaction. Purple ring color indicated positive result.

Screening for Saponins

In Froth Test, 10 ml distilled water were added in 2 separate test tubes, test tube 1 containing 2 ml of 10% *gugo* extract (control) and test tube 2 containing 2 ml of the ethanolic extract. Both tubes were shaken vigorously for 30 seconds. It was observed over a period of 30 minutes. Formation of honeycomb indicated positive result.

Test for Tannin and Phenolic Compound

About 100 ml of the plant extract was taken and evaporated to incipient dryness over a steam bath. It was cooled to room temperature; the residue was extracted with 25 ml of hot water. The cooled mixture was centrifuge for several minutes and the upper half from each tube used had been decanted. Three to four drops of 10% NaCl solution were added to salt out undesirable constituent through precipitation. Precipitates were filtered and was divided into three.

In Gelatin Test, three drops of 1% gelatin solution were added to test tube 1 and was observed if there was any formation of precipitate. Formation of precipitate indicated positive result. Another confirmatory test is gelatin black test wherein three drops of gelatin salt reagent (1% gelatin solution, 10% NaCl) were added to test tube 2 and was observed if there was any formation of precipitate. On the other hand, FeCl₃ test, several drops of FeCl₃ solution were added to the test tube. Blue-black or green-black color indicated positive result.

Test for Antraquinone Heterosides

Two tests were used which included Bortrager's test and Modified Bortrager's Test. In the first mentioned screening, 5 ml of the plant extract was taken and evaporated to dryness over a steam bath. About 5-10 ml of the petroleum ether was added to the residue and 50 ml of distilled water were added to the defatted residue, mixed well and then filtered into small separatory funnel. About 10 ml of benzene were added, mixed well as the two phases separate. The aqueous layer (bottom) was drained out and the benzene phase (upper layer) was transferred to a test tube, 5 ml of Ammonia TS was added and was observed for color in the benzene layer). Presence of cherry red or pink solution in the benzene layer indicated positive result. On the other hand, for modified Bortrager's Test, 0.3 g of the plant powder was heated with 10 ml of 0.5 N KOH and 1 ml of dilute H₂O₂ for 10 minutes. It was allowed to cool and the solution was filtered. Five (5) ml of the filtrate was acidified with approximately 10 drops of glacial acetic acid. The acidified solution was transferred to a small separatory funnel and partitioned with 10 ml benzene. The benzene phase was then filtered and 5 ml from it was transferred to the test tube containing 2.5 ml of Ammonia TS. It was observed for any color change. Presence of cherry red or pink solution indicated positive result.

Test for Cyanogenic Glycosides

Using Guignard's test, about 2-5 g of the crushed plant sample was placed in the test tube. It was moistened with water and few drops of chloroform

were added to enhance enzyme activity. The tube was covered with cork from which a piece of picrate paper was suspended. (Note: the paper strip must not touch the inner side of the test tube). The tube was warmed at 35-40 °C and kept at room temperature for 3 hours. Any change in color of the paper has been observed. Appearance of various shades of red within 15 mins indicated positive result.

III. RESULTS AND DISCUSSIONS

The study revealed the presence of phytochemicals considered to be of medical importance. In addition, plant extracts have their respective functions or uses and may provide what is needed by the body or as diet supplement for better body metabolism to uplift better body performance. Table 2 summarizes the results of phytochemical analysis showing that there was detection of alkaloids, unsaturated fats, sterols and terpenes, flavonoids, steroid (cardio-active glycosides), saponins and tannins. Results also showed that alkaloids and tannins were present in all of the ten plant samples. The biological role of alkaloids in plants is to serve as defenses against herbivores (Alfonso-Alejar and Dionisio-Sese 1999); however, pharmacological activities are associated with alkaloids which include antihypertensive effects (Dangi *et al.* 2002; Segura-Cobos and Vázquez-Cruz, 2006) antiarrhythmic effect (Jia-Qing 2002; Saxena *et al.* 2013; Loh, 2014) antimalarial activity (Johns *et al.* 2011; Frederich *et al.* 2008; Saxena *et al.* 2003) and anticancer actions (Liu *et al.* 2014; Koduru *et al.* 2007). Furthermore, tannins antioxidants, with anti-inflammatory, antidiarrhoeal, cytotoxic, antiparasitic, antibacterial, antifungal and antiviral activities (Wink, 2015).

Lipid molecules such as unsaturated fats, sterols and terpenes were found present in *H. indicum*, *C. citratus*, *A. precatorius*, *P. betel*, *A. cylindrical*, *C. utan*, *P. vulgaris*. Cellular membranes, suberin and cutin waxes are formed by fatty acids and serve as structural barriers to the environment (Beisson, 2007). Moreover, they contribute to inducible stress resistance through the remodeling of membrane fluidity (Iba, 2002). Their pharmacological functions include antimicrobial effect (McGaw *et al.* 2002) and antioxidant activity (Elagbar *et al.* 2016). On the other hand, sterols' biological roles in plants are involved in the control membrane fluidity and permeability, although some plant sterols have a specific function in signal transduction (Piironen *et al.* 2000). Interestingly, it was proven to decrease of total blood cholesterol (Bartnikowska, 2009; St. Jean, 2008; Marangoni and Poli, 2010; Ogbé *et al.* 2015)

and has anticancer activity (Bruce, 2013). Additionally, terpenes and terpene derivatives are involved in the synthesis of yellow carotenoids, chlorophyll and hormones such as abscisic acid and gibberellic acid (Alfonso-Alejar and Dionisio-Sese, 1999). Studies showed that terpenes have antiviral (Rezanka et al. 2009) and neuropharmacological effects such immunomodulatory, anticancer, anti-inflammatory, anti-anxiety, antidepressant, memory enhancer, antinociceptive, neuroprotective and other CNS actions (Parmar et al. 2013).

Furthermore, *C. citratus*, *P. hysterothorus*, *P. betel*, *A. cylindrical*, *B. balsamifera*, *C. utan* and *P. vulgaris* were found to contain flavonoids. These flavonoids are phenolic compounds that are produced by plants as an immune reaction to microbial

infection (Singh et al. 2007; Cowan, 1999). In addition, its pharmacological significance include gastroprotective activity (Mota et al. 2009), antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function and antiangiogenic property (Han et al. 2007) and antioxidant property (Brown and Rice-Evans, 1998; Krings and Berger, 2001).

Steroids were present in *C. citratus*, *A. precatarius*, *P. hysterothorus*, *A. cylindrical*, *B. balsamifera*, and *P. vulgaris*. Steroids have been shown to have antibacterial properties (Epanand et al. 2007), anti-inflammatory (Roddick and Melchers, 1985) and antitumor (Sun et al. 2005) activities.

Table 2. Phytochemical screening of secondary metabolites of the extracts selected plants of ethnomedicinal importance of Bolinao-Sambal

Local Name	Scientific Name	A				B		C		D		E	F			G		H	
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	
1	Balikotkot	<i>Heliotropium indicum</i> Linn.	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	
2	Bani	<i>Pongamia pinnata</i> (L.) Pierre	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	
3	Baranoy	<i>Cymbopogon citratus</i> L.	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	-	-
4	Bugayong	<i>Abrus precatorius</i> Linn.	+	+	+	+	+	-	-	-	+	-	-	+	+	+	-	-	-
5	Herbaca or Stamadia	<i>Parthenium hysterothorus</i> L.	+	+	+	+	-	-	-	+	-	+	-	+	+	+	-	-	-
6	Ikmo	<i>Piper betel</i> L.	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	-	-
7	Inwad	<i>Aegilops cylindrical</i> Host	+	+	+	+	+	+	-	+	-	+	-	+	+	+	-	-	-
8	Sambong	<i>Blumea balsamifera</i> (L.) DC	+	+	+	+	-	-	-	+	-	+	-	+	+	+	-	-	-
9	Silag	<i>Corypha utan</i> Lamelata.	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-
10	Tanobong	<i>Phragmites vulgaris</i> (Lam.) Trin.	+	+	+	+	+	-	-	+	-	+	-	+	+	+	-	-	-

Legend: A. Alkaloids; B. Unsaturated Fats, Sterols and Terpenes; C. Flavonoids; D. Steroid (cardio-active glycosides); E. Saponins; F. Tannin; G. Anthraquinone Heterosides; H. Cyanogenic Glycosides; a. Mayer's Reagent; b. Wagner's Reagent; c. Bouchardat's Reagent; d. Valsler's Reagent; e. Lieberman's Reagent; f. Salkowski Test; g. Bata-Smith Metcalf Test; h. Cyanidin Test; i. Kedde Reaction; j. Keller-Killiani Test; k. Froth Test; l. Gelatin Test; m. Gelatin Black Test; n. Ferric Chloride Test; o. Bortrager Test; p. Modified Bortrager Test; q. Gulnard Test; +presence; -absence

Claims in the ethnomedicinal study indicate that *H. indicum*, *C. citratus*, *A. precatarius* and *B. balsamifera* were used in the treatment of colds, cough, and cleansing wounds. It was suggested that since alkaloids and tannins were present in the mentioned plants, and these may be responsible for the treatment of the associated illnesses. *P. hysterothorus*' use in the treatment of diarrhea in Bolinao, Pangasinan may be explained because of the presence of flavonoids, alkaloids and tannins in their leaves which have gastroprotective, antidiarrheal, antimicrobial activities.

Most of the plants identified in the ethnobotanical study were used in the treatment of *pasma*. Such illness, with no English translation, is labeled as "medical fallacy" (Magat, 2011) which is perceived to occur when a condition to be "hot" is attacked by a "cold" element and vice versa (Tan, 2008). Several presentations of *pasma* were claimed by Bolinao herbalists which included hand tremors, sweaty palms, numbness and pain. In the study, *pasma* was associated with pain in joints of hands, arms, shoulders and feet. Such illness will appear if the patient was exposed to fluctuating hot and cold conditions for instance washing hands and feet or taking a bath immediately after long period of work

in the farm or any other exhaustive task. Plants such as *P. pinnata*, *A. cylindrica*, and *P. vulgaris* were used for the treatment of *pasma*. Decoction of the leaves of these three plants along with the leaves of *kasoy*, *lagundi* and *lasona* was done. Lukewarm decoction was bathed by the patient until the *pasma* disappears. The *Managtambal* then advise the patients to refrain from exposing themselves to “cold” again. They claimed that the same illness will be more serious if such recurred for a short period of time after the treatment. Thus, according to the *managtambal*, the simple way to avoid *pasma* is not to expose a person to water when he/she is too tired or has been exposed to heat. *Pasma* patients are often recommended to relax and take an hour of rest before getting wet.

The results have shown that the folkloric treatments of some of the selected plants for this study parallels the pharmacological action of the secondary metabolites they contain. Also, a strong culture retention was manifested on the use of plants in the folkloric illness *pasma*. In addition, it is recommended that isolation and characterization of specific type of present secondary metabolites is to be conducted accompanied by various assays that will confirm the effectiveness of such plant products.

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