

INDIGENOUS USE, PHYTOCHEMICAL AND ANTIMICROBIAL TESTING OF THE LOCAL *Phaseolus lunatus* Linn LEAVES

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Abstract

The plant materials were grown and gathered in Don Lorenzo Querubin, Caoayan, Ilocos Sur. Normally, the plants are cultivated for production since it's used for the local popular dish called "pinakbet" and dinengdeng. However, the plant is also a threat to the goat raisers since it is believed to be toxic to the rudiment animals. This study is the first step in the conduct of the toxicologic testing of the leaves of the local patani (*Phaseolus lunatus* Linn) grown in the province of Ilocos Sur.

Phytochemical analysis on the leaves was conducted at Saint Louis University, Baguio City. Strongly positive constituents were noted in gums, mucilage, glycosides (Lead acetate test), carbohydrates, reducing sugars (Fehling's test), tannin and derivatives in Millon's test. Weak positive results were found in Fehling's test (tannin and tannin derivatives, dragendorff's and Mayer's test (alkaloids). Keller-Killiani (for deoxy sugars), Wilstatter "cyaniding" (for benzopyrene) and Ferric chloride test (for polyphenolic test).

The Kirby-Bauer Disk Diffusion Method showed that the leaves extract had a moderate antimicrobial activity against staphylococcus aureus and negative against E. coli and Candida albicans.

Based from the results, the focus group participants narrated the indigenous use of the patani leaves as a major material in bagoong making from "Ipon" in the earlier times but discontinued due to the availability of insecticides to prevent the flies from hovering the produce and the unavailability for its immediate.

The study recommends an in depth investigation on the indigenous values of patani leaves in larger scope of study. Other substances should be analyzed in both of the agricultural waste of the plant such as the leaves and fruits. Other pharmacological effects such as anti-inflammatory and diuretic effects of the leaves and root extract must also be tested.

Background of the Study

Patani (*Phaseolus lunatus* Linn) is very extensively cultivated in the Philippines for its edible seeds. It is usually eaten as a green bean or before it has become hard and dry. There are several varieties, including some with white seeds and other with colored or variegated seeds. The white forms are the best. In cooking, the colored and variegated varieties should be boiled in several changes of water to render them wholesome. The lima beans or patani is a popular vegetable in both tropical and temperate zones. A form of ordinary patani with dark-colored seeds is common in thickets in some parts of the Philippines. The seeds are edible, but sometimes maybe poisonous, deaths having occurred from eating them. According to Steyn, the wild lima bean contains dangerous amounts of phaseolunatin, while the cultivated bean is free or contains very small quantities of this glucoside. Analyses of lima beans show that they are very high in carbohydrates and fairly high in protein, but only pair in iron, and deficient in calcium. (www.wikipedia.com)

Wehmer reports that the phaseolunatin is also present in the leaves (0.026-0.063 percent hydrocyanic acid) and stems but not in the root. An emulsion like enzyme and linamarase are present in the beans. Serrano examined the hydrocyanic acid content of several varieties of patani grown in the Philippines. (www.wikipedia.com)

Patani (*Phaseolus lunatus* Linn) is a major agricultural crop in the province of Ilocos Sur primarily used as an ingredient in the famous Ilocano dish "Pinakbet"

Lima beans are an excellent source of the trace mineral, molybdenum, an integral component of the enzyme sulfite oxidase, which is responsible for detoxifying sulfites. Sulfites are a type of preservative commonly added to prepared foods like delicatessen salads and salad bars. Persons who are sensitive to sulfites in these foods may experience rapid heartbeat, headache or disorientation if sulfites are unwittingly consumed. If you have ever reacted to sulfites, it may be because your molybdenum stores are insufficient to detoxify them. A cup of lima beans will give you 86.5% of the daily value for this helpful trace mineral. (www.wikipedia)

Fishing and farming were the two major means of live hood in the place. Ipon, a small seasonal fish is one of the major produce of the locality. In terms of farming, patani is one of the major crops being cultivated. In the municipality of Caoayan, the leaves of patani was originally used to prevent formation of larvae on top of the fermented fish called "Ipon" These had been the practice in the previous generations but no longer practiced as to the present times. The leaves therefore were left to rot or considered as agricultural wastes.

Objectives

This study was conducted to determine the indigenous use, phytochemical and antimicrobial analysis of patani (*Phaseolus lunatus* Linn) Saint Louis University, College of Natural Sciences, Natural Sciences Research Unit (NSRU), Baguio City.

Scope and Delimitation

This study was delimited to the determination of the indigenous use of patani leaves, as well as phytochemical and antimicrobial screening.

Review of Related Literature

Lima beans, also called butter beans for their rich texture and flavor, are the seeds of the lima bean plant pod. Canned and dried lima beans are available all over the country, while fresh beans are found during the summer and fall. Lima beans aren't just green and boring; there are lots of different varieties with different flavors for most palates. The lima bean, according to the Oklahoma State University Extension, originates from Peru. Lima beans, in one form or another, have been grown since 6000 B.C. The name of the bean comes from Peru's capital city, Lima. Lima beans also go by the name chad bean, as well as the aforementioned butter bean. Lima beans are not eaten raw as they contain a small portion of a cyanide compound, produced by linamarin, which is highly poisonous. In the United States, regulations are in place to limit the amount of linamarin in a bean variety. According to foodreference.com, beans from Java and Burma contain 20 to 30 times more concentrated linamarin than most western countries allow. According to the Whole Foods website, lima beans are a good source of dietary protein. A cup of lima beans contains 14.7 grams of protein, 29 percent of your daily protein values. This also provides 52.6 percent of a day's dietary fiber needs, with only a small amount of fat intake. Lima beans are also a prime source of iron, blood sugar stabilizers and antioxidants. Lima beans come in three main bean varieties, says Oklahoma State University: large, small and dwarf beans. Large lima beans are usually green and speckled and have a stronger flavor than light green bean varieties, which usually fall into the small bean category. Dwarf beans are also called butter peas, and are white and speckled and contain less starch. Fresh lima beans are difficult to find in the U.S. due to small crops and regulations. Most lima beans are found canned, frozen or dried, but fresh beans are available to grow, or purchase from small farmers markets. Limas are tropical beans named after Lima, the capital city of Peru. In the U.S. we grow large, white lima beans selected especially because they contain very small amounts of the poison cyanide. Lima beans are not eaten raw as they contain a small portion of a cyanide compound, produced by linamarin, which is highly poisonous. In the United States, regulations are in place to limit the amount of linamarin in a bean variety. According to foodreference.com, beans from Java and Burma contain 20 to 30 times more concentrated linamarin than most western countries allow. According to the Whole Foods website, lima beans are a good source of dietary protein. A cup of lima beans contains 14.7 grams of protein, 29 percent of your daily protein values. This also provides 52.6 percent of a day's dietary fiber needs, with only a small amount of fat intake. Lima beans are also a prime source of iron, blood sugar stabilizers and antioxidants. Lima beans come in three main bean varieties, says Oklahoma State University: large, small and dwarf beans. Large lima beans are usually green and speckled and have a stronger flavor than light green bean varieties, which usually fall into the small bean category. Dwarf beans are also called butter peas, and are white and speckled and contain less starch. Patani is very extensively cultivated in the Philippines for its edible seeds. It is usually eaten as a green bean or before it has become hard and dry. There are several varieties, including some with white seeds and other with colored or variegated seeds. The white forms are the best. In cooking, the colored and variegated varieties should be boiled in several changes of water to render them wholesome. The lima beans or patani is a popular vegetable in both tropical and temperate zones. A form of ordinary patani with dark-colored seeds is common in thickets in some parts of

the Philippines. The seeds are edible, but sometimes maybe poisonous, deaths having occurred from eating them. According to Steyn, the wild lima bean contains dangerous amounts of phaseolunatin, while the cultivated bean is free or contains very small quantities of this glucoside. Analyses of lima beans show that they are very high in carbohydrates and fairly high in protein, but only pair in iron, and deficient in calcium. (www.gardenguides.com/114680-lima-bean.html)

Florendo , Bañez and Queddeng (2013) explored the possibility of the Buri (*Corypha elata* Roxb.) leaf extract as an alternative source of herbal medicine. Extraction through infusion of the pulverized leaves was used for the phytochemistry and antimicrobial activity determination. Results of the analyses of the leaf extract showed the presence of sterols, saponins, glycosides, and tannins, and complete antimicrobial activity, 10mm total mean zone of inhibition with a reactivity of 2 to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Based on the results, the researchers concluded that the buri leaf extract has a promising contribution to the field of herbal medicine and can be used as an alternative herbal medicine for the treatment of diseases caused by aforementioned microorganisms.

A similar study was conducted by Bañez and Castor (2013) on phytochemical screening on Mother in Law's Tongue (*Sansevieria trifasciata*) their results showed that the ethanol extract of the leaves and rhizomes of the plant contain alkaloids, saponins, flavonoids, glycosides, tannins, sterols and triterpenes. Their study and the current study differ because they looked into the insecticidal properties of the leaves and rhizomes of *Sansevieria trifasciata* and found out to be more effective than carbamate which the current study did not consider.

On Toxicity, a similar study was conducted by Bañez (2016) entitled, "Insecticidal Effects of Arrowhead (*Syngonium podophyllum*) & Jicama (*Pachyrhizus erosus*) against Odorous House Ants (*Tapinoma sessile*)". The researcher compared the toxicity efficacy of aqueous and alcoholic extracts prepared from the mature leaves of two species arrowhead (*Syngonium podophyllum*) and jicama (*Pachyrhizus erosus*) against odorous house ants (*Tapinoma sessile*). Her results showed that there is a significant interaction effects between the treatments (aqueous and alcoholic) and the kind of plant used. Arrowhead alcoholic leaf extract when compared with aqueous extract; positive and negative controls have significant differences between and among all pairs, same is true with jicama. Jicama is more effective than arrowhead in both the treatments alcoholic and aqueous, the mean difference is highly significant at 0.05 level. The alcoholic extract is more effective than the aqueous. The jicama leaf extract is more effective than arrowhead.

Another study on Analgesic and Toxicologic Analyses of Purple Rain(*Dendrobium superbum*) confirms the result of the present study . The researcher concluded that The following chemical substances like alkaloids, glycosides, tannins, saponins and sterols were found to be present in both the stems and leaves of purple rain (*Dendrobium superbum* Reichb). There are pharmacological effects of the ethanol extract screened) substances using male Swiss mice as test animals. Under the conditions of this test, the ethanol extract showed that it has an analgesic activity when administered orally to male Swiss mice; it produced a 6.25%, 31.25% and 56.25% protection against writhing at 500, 1000 and 1,500 mg/kg, respectively. The approximate Lethal Dose (LD) of the sample ethanol extract of purple rain administered orally in male Swiss mice is 2,500mg/kg. Toxidrome ranged from ptosis, decreased motor activity and convulsion leading to the deaths of male Swiss mice. In the autopsy findings, all mice that died immediately and those sacrificed and autopsied after fourteen (14) days had grossly normal findings (Banez,2017).

Queddeng, (2009) studied both the leaves and roots of the Kalunay (*Amaranthus spinosus* L.) using various solvents of ethyl acetate, ethanol, water and reagents. The qualitative tests of both the leaves and roots manifested the presence of alkaloids, and saponins. Alkaloids indicated yellowish precipitates while saponins showed a formation of more than 1.2 cm of “honeycomb froth”. It was noted, however, that only fresh concentrated root extract manifested the presence of glycosides. All the other tests on fats and oils, flavanoids, triterpenes, sterols, leucoanthocyanins as well as resins showed negative results. In the concentrated root extract of Kalunay, a formation of brick-red precipitate in the hydrolyzed sample was noted; thus, it showed the presence of glycosides.

Methodology

Fresh plant materials were grown and gathered in Barangay Don Lorenzo, Caoayan, Ilocos Sur. Plant samples were sent to Saint Louis University, College of Natural Sciences, Natural Sciences Research Unit (NSRU), Baguio City for phytochemical analysis and antimicrobial susceptibility test.

Focus group discussion using proper protocol was also conducted to determine the indigenous use of patani leaves as an indigenous material in the local livelihood of the local community, the bagoong making from “Ipon”, a small edible fish locally found in Caoayan, Ilocos Sur. It’s a newly zygote fish, with the scientific name of *sycyopterus lachrymosus*. This small fish that measures about 1 inch in length and ¼ inch in diameter is only endemic to the provinces of Ilocos Norte, Ilocos Sur and Cagayan Valley.

It is also called a lunar fish because they only appear after counting ten days from full moon. They can catch this fish from 3-5 days once in a month. Fishermen make one harvest in the morning and one in the afternoon.

Results and Discussion

Table 1. Result of the Qualitative Test of *Patani*(*Phaseolus lunatus*Linn)Leaves

Preliminary Test	Result	constituents
Lead Acetate test	++	Gums, mucilages, glycosides
Fehling's test	+	Carbohydrates, reducing sugars
Ferric chloride test	+	tannin, and tannin derivatives
Millon's test	+++	proteins and its derivatives
Tests for Physiologically Active Constituents		
1. Alkaloids Dragendorff's test Mayer's test	-- +	Alkaloids alkaloids
2. Sterols Keller-Killiani Lieberman-Buchard Kedde test	+ + --	Deoxysugar Unsaturated sterols Unsaturated lactones
3. Anthraquinones Borntrager's test	--	anthraquinones
4. Flavonoids Bate & Metcalf Wilstätter "cyanidin"	+ +	Leucoanthocyanins & cyanidin γ-benzopyrene
5. Saponins Froth test Lieberman-Buchard	-- +	Saponins Unsaturated sterols & triterpenes
6. Cyanogenic glycosides Guinard test	--	Cyanogenic glycosides

Qualitative test showed positive results on lead acetate test with the presence of gums, mucilages, carbohydrates, glycosides, Fehling's test (Carbohydrates, reducing sugars), and Millon's test (proteins and its derivatives). In the physiologic active constituents, alkaloids, deoxysugars, unsaturated lactones, leucoanthocyanins and cyaniding, γ-benzopyrene, unsaturated sterols and triterpenes were noted to be positive. The presence of cyanidin in the Bate & Metcalf test confirms the presence of small amounts of the poison cyanide in the plant as stated in the reference at www.gardenguides. This also indicates that effectiveness on the indigenous use of patani (*Phaseolus lunatus*Linn) leaves on the fermented fish of the local community in Caoayan, Ilocos Sur prevented the larval formation on the products.

Antimicrobial Activity of Patani (*Phaseolus lunatus* Linn.) Leaf Extract Against the Test Organisms

Zone of inhibition of *S. aureus* as affected by Lima bean’s leaves extract using agar disc diffusion method

Sample	Replication (measured in mm)			Mean
	I	II	III	
100%	12	11	10	11

Zone of inhibition of *C. albicans* affected by Lima bean’s leaves extract using agar disc diffusion method

Sample	Replication (measured in mm)			Mean
	I	II	III	
100%	0	0	0	0

Zone of inhibition of *E. coli* affected by Lima bean’s leaves extract using agar disc diffusion method

Sample	Replication (measured in mm)			Mean
	I	II	III	
100%	0	0	0	0

LEGEND:

- + =WEAK
- _ =NEGATIVE
- C=CONTROL

The table showed a negative antimicrobial activity of the two test organisms namely *C. albicans* and *E. coli* a moderate antimicrobial activity against *staphylococcus aureus* with a grand mean of 11 This indicates a positive antimicrobial activity of the plant extract.

Indigenous Use of Patani (*Phaseolus lunatus* Linn) Leaves Focus Group

Moderator:

Dr. Mercita Q. Queddeng

Co-Moderator:

Conrado Director, RM

There were eight participants with who participated in the focus group with the indigenous use of patani leaves conducted from 9:00 AM to 12:00 NN in the local community of Caoayan. The group included three males and five females whose ages ranges from 45-65 years old. . Seven of them were married and speaks their Ilocano dialect; they also speak Tagalog and English language. Two of the participants finished vocational course while 4 finished secondary education while the three only finished elementary education. Six of the participants are full time fisher folks while two are homemakers.

Focus Group Protocol

The focus group covered the activities involving the indigenous use and its effectiveness of patani leaves in their livelihood of the seasonal bagoong making from “Ipon” during the earlier times and the reasons of discontinuance on its use.

Indigenous Use and its Effectiveness of Patani Leaves in Bagoong making from “Ipon”

The focus group participants unanimously agreed that during the earlier times, the patani leaves play a major important role in the bagoong making from “Ipon”. They discussed in simultaneous manner and in narration and expressed that the usage of the plant material started only after opening the lid of the locally made earthen container the “burnay” (earthen jar) to prevent larval formation of the produce. The supply of the patani leaves was in abundance since it is a major crop of the place according to the participants from the focus.

In terms of effectiveness, the patani leaves were very efficient in the prevention of larval formation on the top of previously opened lid of the “bagoong nga Ipon” (locally fermented fish).

Discontinuance on the Use of Patani Leaves on “Bagoong nga Ipon”

The focus group participants expressed their opinions in the discontinuance on the use of patani leaves in “Bagoong nga Ipon”. One of the reasons was the decrease of the produce bagoong that no longer require the use of the leaves. The amount produced is just enough for small scale only. The availability of insecticides to prevent the flies from hovering the produce is also one of the reasons.

Conclusions

1. Patani (*Phaseolus lunatus* Linn) leaves extract contain traces of alkaloids, glycosides, flavonoid and sterols.
2. The leaf extract showed a moderate antimicrobial activity against *Staphylococcus aureus* and negative against *E. coli* and *Candida albicans*.
3. Focus group participants narrated the indigenous use of the patani leaves as a major material in bagoong making from “Ipon” in the earlier times but discontinued due to the availability of insecticides to prevent the flies from hovering the produce and the unavailability for its immediate.

Recommendations

1. An in depth investigation and don the indigenous values of patani (*Phaseolus lunatus* Linn) leaves should be conducted in larger scope of study.
2. Detailed Data Documentation of the techniques must be made as part of cultural preservation of the local indigenous practice.
3. Dissemination and utilization of the research output be made to the stakeholders focusing on the use on maximizing potentials of agricultural waste.
4. Other substances should be analyzed in both the leaves and fruits of the plant.

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