Volume 2, Issue 7 July 2016 ISSN NO: 2454 - 7514

North Asian International Research Journal Consortium

North Asian International Research Journal

Of

Science, Engineering and Information Technology

IRJIF IMPACT FACTOR: 3.821

Welcome to NAIRJC

ISSN NO: 2454 -7514

North Asian International Research Journal of Science, Engineering & Information Technology is a research journal, published monthly in English, Hindi. All research papers submitted to the journal will be double-blind peer reviewed referred by members of the editorial board. Readers will include investigator in Universities, Research Institutes Government and Industry with research interest in the general subjects

Editorial Board

Address: -North Asian International Research Journal Consortium (NAIRJC) 221 Gangoo, Pulwama, Jammu and Kashmir, India - 192301, Cell: 09086405302, 09906662570, Ph. No: 01933-212815,
Email: nairjc5@gmail.com, nairjc@nairjc.com, info@nairjc.com Website: www.nairjc.com

IRJIF IMPACT FACTOR: 3.821

PHYTOCHEMICAL COMPONENTS AND CYTOTOXIC PROPERTIES OF LEAVES AND STEMS OF VIOLET PHILIPPINE EGGPLANT (*SOLANUM MELONGENA LINN.)*

ANGELICA ORDANZA-CORTEZ*

*Math and Science Professor, College of Education, Nueva Ecija University of Science and Technology, Cabanatuan City

ABSTRACT

The study was on a determination of phytochemical components and cytotoxic properties of violet Philippine eggplant scientifically known as Solanum Melongena Linn. Phytochemical screening includes Test for Alkaloids, Test for Flavonoids through Bate/Smith and Metcalfe Test, Test for Saponins by "Froth Test" and Liebermann-Burchard Test, and Test for Tannins by Protein Binding Test. The cytotoxic property of Solanum Melongena Linn. was detected using the Brine Shrimp Assay.

The stems and leaves of Solanum Melangena Linn. containalkaloids and saponins. The presence of bioactive parts and components such as alkaloids, flavonoids, saponins and tannins and its cytotoxic property is a way leading to a source of much cheaper but more effective synthetic drugs.

Keywords: ***Solanum Melongena Linn., Phytochemical, Cytotoxic, Alkaloids, Flavonoids, Saponins, Tannins.***

INTRODUCTION

Nature has endowed Philippine forest with almost 3,500 arborescent species. [6] Since time immemorial, people have depended on plants for their source of food, shelter, and most especially medicine. Plants have been used for medicinal purposes as old as time even before man started the art of writing. Ancient Chinese and Egyptian writings describe medicinal uses for plants as early as 3,000 BC while indigenous cultures in African and Native American used herbs in their healing rituals and herbal therapies. [4] Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes. One of the races who are fanatic in the use of herbal medicine is the Filipino people. [5]

The evolution of Philippine traditional medicine is an interesting subject that is partly influenced by religion, mysticism, magic, superstition, folkloric herbalism and western medicine. When the importance of the traditional medicine was appreciated by former President Fidel V. Ramos a law is known as Republic Act 8423 (R.A. 8423), or the Traditional and Alternative Medicine Act (TAMA) of 1997 was signed. According to the Department of Health, the "Top 10 Medicinal Plants in the Philippines" were Akapulko (*Cassia alata),*Ampalaya (*Momordicacharantia*), Bawang (*Allium sativum*), Lagundi (*Vitexnegundo*), Bayabas (*Psidiumguajava),*Niyog-Niyogan (*Quisqualisindica L.*), Sambong (*Blumeabalsamifera*), TsaangGubat (*Ehretiamicrophylla Lam.*), Yerba Buena (*Clinopodiumdouglasii*), and Ulasimang Bato or Pansit-Pansitan (*Peperomiapellucida*).[9] It is noticeable that eggplant (*Solanummelongena Linn.)* is not in the 10 top medicinal plants in the country but is present in the long list of Philippine Herbal Medicinal Plants update.[8]

Eggplant or *Solanummelongena Linn*. is a vegetable long prized for its beauty as well as its unique taste and texture. Also commonly known as nightshades, and are kin to the tomato, bell pepper, and potato. Eggplants grow in a manner much like tomatoes, hanging from the vines of a plant that grows several feet in height.[1] [2] [3] This vegetable is produced by almost all provinces in Luzon and abundant in many months of the year, but only its fruit is consumable to human. Since eggplant is a known vegetable and its consumption has been part of the everyday meal of Filipino, its helpful benefits were already established, but there was the limited textual form on the phytochemical components and cytotoxic properties of its stem and leaves which were the focus of the present study.

THE PROBLEM

This study determined the bioactive components of the stems and leaves of *Solanum Melongena Linn.*
More specifically, the study answered the following questions:

1. What phytochemical components are present in the leaves and stems of the violet eggplant?
2. What percent of cytotoxic substance is present in the leaves and stems of the violet eggplant?

SCOPE AND DELIMITATION

The study delimited itself in the extraction of sample leaves and stems of *Solanummelongena Linn.* for phytochemical and cytotoxic tests. The tests for the detection of the biochemical components of the extracts of the

plant parts and the test for cytotoxic were done in the Physico-Chem Laboratory at the Philippine Rice Research Institute.

SIGNIFICANCE OF THE STUDY

Results of the study may lead to the utilization of waste eggplant parts such as leaves and stems in the manufacture of future drugs. This study will also provide a benchmark for the students and teachers to consider other waste products in the farm as the source of prospective research problems. Results, when extended to the community, may serve as a guide in the realization that other products can be produced out of the leaves and stems of violet eggplant which are commonly treated as waste after the period of harvesting.

THE RESEARCH PARADIGM

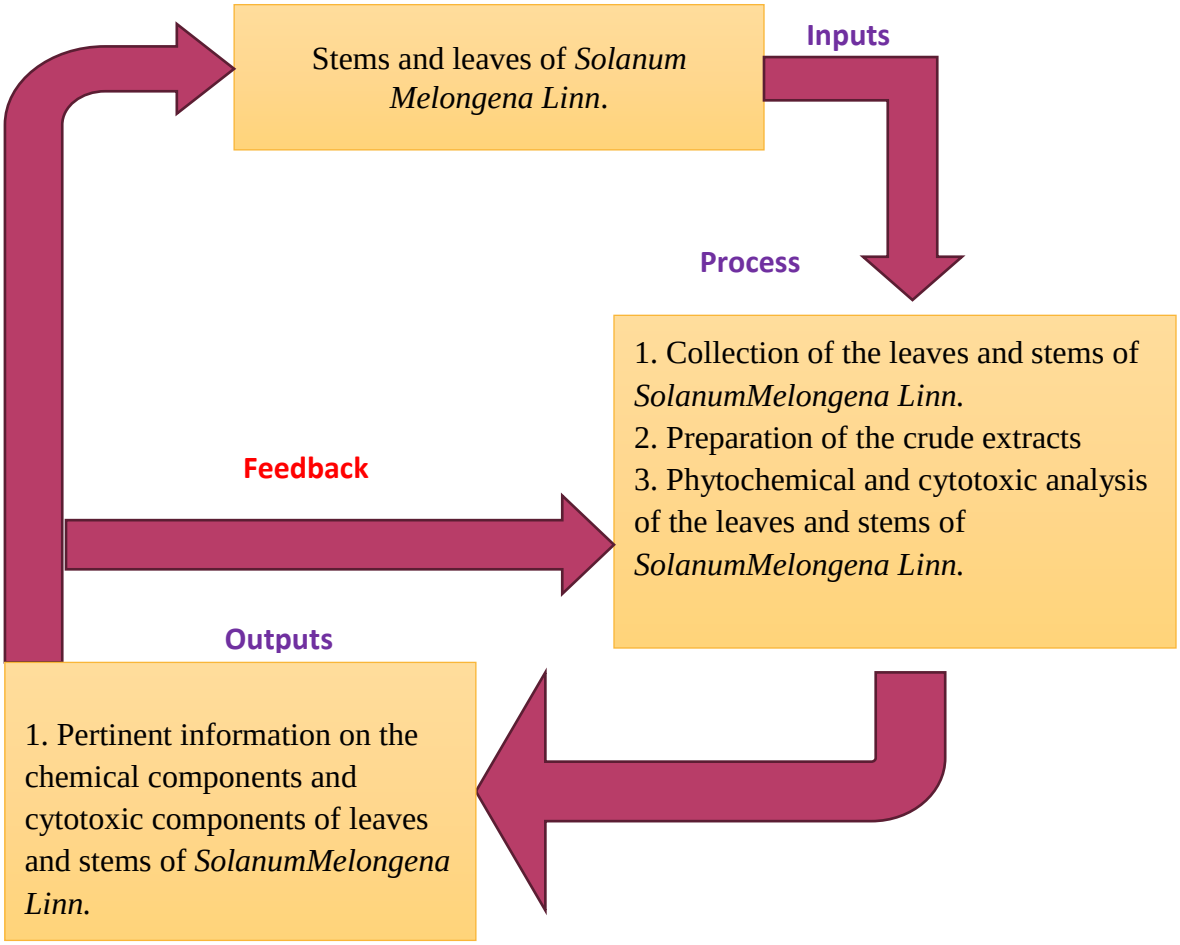

Figure 1: Diagrammatic Representation of the Phytochemical and Cytotoxic Analysis of *Solanum Melongena Linn.*

METHOD AND PROCEDURES

RESEARCH METHOD

The after-only experimental method of research was utilized in the study. Palispis [7] said that this type of research method describes "what will be" when certain variables are carefully controlled or manipulated. The focus is on a variable relationship.

MATERIALS AND PROCEDURE

Materials: Erlenmeyer Flasks, Test Tubes, Knife, Paper bags, masking tape, Ethanol, Bulb, Socket and Cord, Steam bath, Yeast, Paraffin, Ferric Chloride, 2M HCl, Mayer reagents, Anhydrous sodium sulfate, Chloroform, Hexane, Acetic anhydride, Concentrated sulfuric acid, Sodium Chloride

Procedure:

Collection of plant samples. The sample leaves and stems of *Solanum Melongena Linn*. were collected from the farm of the researcher in Laur, Nueva Ecija.

Air drying of samples. Plant materials were cut into pieces. Each of the parts was suspended from each other. It was placed in an empty room and was air dried for a week and then ground. Samples were stored in preparation for extraction.

Preparation of plant extracts. Two hundred fifty grams (250g) of air-dried ground plant was loaded in a 2.5Liter capacity bottle. The bottle was stoppered to avoid the evaporation of the solvent. The mixture was set aside for two days at room temperature then filtered through a funnel with gentle suction. The residue was discarded while the filtrate was concentrated under vacuum to about 75mL using rotary evaporator. The concentration of the plant material per mL of the plant extract was computed using the formula:

$$\text{Plant Extract Concentration} = \frac{\text{Mass in grams of plant materials}}{\text{Volume in mL of the plant extract}}$$

The extracts were placed in a lightly stoppered amber bottle to prevent the decomposition of the components and kept refrigerated until analysis. The extracts were subjected to phytochemical screening and cytotoxic testing.

Phytochemical Screening:

a. **Test for Alkaloids.** Ten (10mL) of the plant extract was evaporated to a syrupy consistency over a steam bath, about 5mL of 2M HCl was added, and the mixture was heated with stirring for about 5 minutes. After cooling, about 0.5 grams of NaCl was added. The mixture was stirred and filtered with enough 2M HCl to wash the residue and to bring the filtrate to 5mL. One (1) mL of the filtrate was tested with two to three drops of Mayer's reagent.

A positive result was indicated by the presence of orange precipitate over the white precipitate with Mayer's reagent.

+ Slight turbidity
++ Define turbidity
+++ Heavy precipitation
- No precipitate

A +, ++, or +++ is indicative of the presence of primary, secondary and tertiary alkaloids respectively, while negative (-) indicates their absence.

Confirmatory Test for Alkaloids. Twenty-eight percent ammonia was added to the remaining 3.0mL aliquot until the solution is alkaline to the litmus paper. The solution was extracted three times with 10mL chloroform ($CHCl_3$). The $CHCl_3$ extracts was combined and evaporated over a steam bath. The upper alkaline aqueous layer was reserved for quarter nary and or amine oxide test.

The residue chloroform was added with a 5mL of 2m HCl and stirred over a steam bath for about two minutes. It was cooled and filtered and the filtrate was divided into two equal portions.

Mayer's reagent was added. The presence of alkaloids was indicated as +, ++, +++, respectively, based on the amount of precipitate formed.

b. **Test for Flavonoids**. An equivalent of 10mL plant extract was taken and evaporated to incipient dryness over a water bath. It was cooled to room temperature. The residue was deflated by treating with hexane or petroleum ether until the extract is almost colorless. The hexane or petroleum ether was discarded.

Ten (10mL) of 80% ethyl alcohol was added to the residue and the mixture was filtered. The filtrate was divided into two test tubes one portion was used as control.

Bale/Smith and Metcalf Test for Leucoanthocyanins. One part of the filtrate was treated by adding 0.5mL concentrated HCl and was observed for any color change. It was warmed for 15 minutes in a water bath. Further change in color was observed within an hour. A strong red or violet color would indicate the presence of leuconthocyanins.

c. Test for Saponins.

Froth Test. A "gogo" extract was used as a control about one gram of the bark of Entadaphaseolides, locally known as "gogo" was extracted with 10mL of ethyl alcohol. A volume of alcohol extract equivalent to 2g plant material was placed in a test tube and 2mL of the "gogo" extract in another test tube. Ten 910mL) of distilled water was added to each test tube. The tubes was stoppered, shake vigorously for 30 seconds and allowed to stand for 30 minutes. A honeycomb froth above the surface eof the liquid measures in minutes as the indicator of the results when compared to that of the "gogo' extract.

Liebermann-Burchard Test. The extract equivalent to 10mL plant material was evaporated to dryness over a water bath. It was allowed to cool at room temperature. The material was deflated by treating the residue with 10mL hexane extract. The treatment was repeated once until most of the materials were removed. The resulting residue was treated with 10mL chloroform and the mixture was stirred for about 5 minutes. It was allowed to stand, and the chloroform extract was pipetted off. The chloroform extracts were dried by filtering the mixture with 100mg anhydrous sodium sulfate over a dry filter paper. The filtrate was divided into two portions. One portion was set aside as the control. The second portion was treated with three drops of acetic anhydride and one drop of concentrated sulfuric acid. Any immediate change was observed. It was allowed to stand for an hour to observe further any color change. Results were compared with the control.

d. Test for Tannins. One (1mL) of ferric chloride ($FeCl_3$) was allocated. It was then put on the watch glass. A drop of the crude extract of the samples was then dropped on the watch glass after the $FeCl_3$. The brown or black color that appeared after the sample was dropped indicated the presence of tannins.

Confirmatory Test by Protein Binding Test. About 0.25 grams of Bouvine Serum Albumin (BSA) was placed in 10mL of distilled water in the test tube and was shake vigorously until the BSA was completely dissolved. Three drops of each of the crude extracts were dropped in the solution. The precipitation showed the presence of tannins.

Cytotoxic Screening:

Shrimp Hatching. About one gram of brine shrimp eggs was hatched in a jar half-filled with 35 ppt (parts per thousand) saline solution. It was covered with a plastic screen to avoid the entry of undesirable insects. The eggs were allowed to hatch and mature for 48 hours (shrimp can be used 48-72 hours after initiation of hatching, after 24 hours they should be discarded). A pinch of yeast was added to serve as food for nauplii.

Sample Preparation. Solution A was prepared by dissolving enough volume of the isolates in enough volume of ethanol to make 10mg/mL solutions. Solution B was prepared by diluting an enough volume of solution A and with enough volume of ethanol to make 0.5mg solutions. An appropriate amount of solution (100uI B, 1.0uI B, and 500uI A for 10, 100, 1000ug/mL respectively) was transferred into separate test tubes. These were dried in vacuous for one hour. The control was prepared using ethanol only.

Brine Shrimp Bio-Assay. Ten (10) shrimps were transferred into each sample test tube using a disposable pipette. Saline solution was added to make 5mL. Against a lighted background, the nauplii were counted macroscopically in the stem of the pipette. A drop of dry yeast suspension (3 mg in 5mL saline solution) was added to each test tube. After 24 hours, the survivors were counted with the aid of the magnifying glass. The computation involved the actual counting of living shrimp in both the controls and the treatment test tubes.

$$\% \text{ Mortality due to treatment} = \frac{x - y}{X} \times 100$$

Where: x represents the number of living in the control

y represent the number of living in the treatments test tubes

x-y represent the number of dead shrimps by the treatment

RESULTS AND DISCUSSION

Description of the Crude Extracts

Approximately, 300mL crude extracts were recovered in each replication made. They were concentrated in the rotary evaporator, and the volume of the concentrate ranges from 75-80mL. The stem extracts or each replicate is yellowish with some brownish particulate. The leaf extract is a dark green solution.

Table 1: Results of Phytochemical Screening

Phyto-chemical	Plant Part	Test/Reagent Used	Results	Observations
Alkaloid	Leaves	Mayer's Reagent	+++	Heavy white precipitate. It changes its color from dark brown to orange.
		Mayer's Reagent	+++	Same result.
	Stems	Mayer's Reagent	++	Slightly produced white precipitate and changes its color from brown to orange.
		Mayer's Reagent	++	Same result.
Flavonoids	Leaves	Bate/smith and Metcalfe Test	_	The crude extract changes its color from dark brown to black.
	Stems	Bate/smith and Metcalfe Test	_	The crude extract changes its color from brown to black.
Saponins	Leaves	Liebermann Burchard Test	++	The crude extract changes its color from dark brown to dark green.
		Froth Test	++	The bubbles of the control are smaller that of the experimental set-

				up.
	Stems	Liebermann Burchard Test	++	The crude extract changes its color from brown to yellow green.
		Froth Test	++	The bubbles of the control are smaller than the experimental set-up.
Tannins	Leaves	Ferric Chloride Test	-	The crude extract changes its color from dark brown to black after it was dropped to ferric chloride solution.
		BSA Test	-	No precipitate.
	Stems	Ferric Chloride Test	-	The crude extract changes its color from brown to light brown after it was dropped to ferric chloride solution.
		BSA Test	-	No precipitate.

The data shown in Table 1 were the results of the phytochemical screening test and all the treatments and medium used in the process. The test used for alkaloid was by dropping a Mayer's Reagent to 10mL of crude extract. The amount of precipitation shows the presence of alkaloid. The positive result was indicated by a white precipitate. The crude extract of the leaves produced heavy precipitation while the crude extract of the stems produced slight white precipitation. The results showed that both extracts contain alkaloids.

The test used for the detection of flavonoids was the Bate/Smith and Metcalfe procedure. The positive result was indicated a red or violet solution. The results showed no violet solution and that the crude extracts do

not contain flavonoids. Saponins have distinctive property of forming honeycomb froth when agitated with water. The saponins in the crude extracts were easily determined by the froth test. The crude extract of the leaves increased to 0.8cm compared to the control which is only 0.65 cm. The crude extract of the stems increased by 0.05 cm from its original height of 0.45cm to 0.50cm, which persisted for 30 minutes. Following the Liebermann-Burchard procedure, results were confirmed. Saponins were glycosides which are characterized by their ability to change color upon addition of sulfuric acid. An immediate change in color of the crude extract revealed the presence of saponins. Tannins were likewise screened from the crude extracts of leaves and stems of plant sample. The ferric chloride test was used. The crude extract of the leaves changes its color from dark brown to dark green. The yellowish with brown articulate crude extract of the stem changes its color to yellow-green. To confirm the results which indicated the presence of tannins and not of polyphenols, BSA test was conducted and showed that no precipitate occurred. The results show that the crude extract of the stems and leaves of the eggplant contain polyphenols instead of tannins.

The leaves and stems of *Solanummelongena Linn*. contained alkaloids and saponins but not flavonoids and tannins and instead of tannins polyphenols are present.

Table 2: Cytotoxic Test of Leaves and Stems of *Solanummelongena Linn.*

Plant Part	Replicates	Percent Mortality	
		12 hour-exposure	24 hour exposure
Leaves	1	83%	100%
	2	100%	100%
	3	33%	100%
	Mean	**72%**	**100%**
Stems	1	100%	100%
	2	100%	100%
	3	100%	1005
	Mean	**100%**	**100%**

The percentage mortality of brine shrimp after 12 and 24 hours was shown in Table 2.

The brine shrimp were hatched for two days. Ten brine shrimp were placed in each of the test tubes. The control and experimental set-ups were divided into three replicates. It was observed after its 12th and 24th hour.

After 12 hours, six of the brine shrimp in the test tubes of the control set-up were left in the three replicates. On the experimental set-up which contains the crude extract of the leaves, the first was left with one brine shrimp. In the second test tube, all brine shrimps died and on the last replicate, four were left. On the experimental set-up which contains the crude extracts of the stems, all the brine shrimp in the three replicates died.

FINDINGS AND CONCLUSIONS

Alkaloid and saponins were all present in the crude extracts of leaves and stems of *Solanummelongena Linn.*thus these parts have the significant amount of alkaloids and saponins but not tannins and flavonoids. Also, The crude extracts of the stems of Solanummelongena Linn. hasgreater cytotoxic component than that of the crude extract of the leaves and that the cytotoxic property was evident as a result of the mortality rates of the brine shrimp after 12 and 24 hours.

RECOMMENDATIONS

Other organic solvent with different polarities can be used to extract the bioactive components of other varieties of eggplant. Also, other bioactive components of other parts of the sample plant can also be subjected to test such as its roots, flowers, and sepals of eggplant. Antimicrobial assays and pharmacological screening method can also be done to completely establish the phytochemical components of the plant sample in this study.

REFERENCES

[1] Bliss RM, Elstein D. (2004). Scientists get under eggplant's skin. ARS Magazine, 2004 January; 52 (1): http://www.ars.usda.gov/is/AR/archive/jan04/skin0104.htm.

[2] Ensminger AH, Ensminger, ME, Kondale JE, Robson JRK. (1983). Foods & Nutrition Encyclopedia. Pegus Press, Clovis, California.

[3] Ensminger AH, Esminger M. K. J. e. al. (1986). Food for Health: A Nutrition Encyclopedia. Clovis, California: Pegus Press; PMID: 15210.

[4] Izzo AA, Ernst E. (2009). Interactions between herbal medicines and prescribed drugs: an updated systematic review. Drugs; 69(13):1777-98.

[5] Kraft, K.(2009). Complementary/Alternative Medicine in the context of prevention of disease and maintenance of health. Prev. Med.

[6] Lesley, Tierra. (2003). Healing with the Herbs of Life Paperback.

[7] Palispis, Epitacio (1991). Educational Research. National Bookstore Inc.

[8] Stuart Xchange. (2015). List of Philippine Herbal Medicinal Plants. Update.

[9] Copyright © 2011 www.medicalhealthguide.com, USA, UK, Canada, Australia, India, Philippines, Singapore

Publish Research Article

Dear Sir/Mam,

We invite unpublished Research Paper,Summary of Research Project,Theses,Books and Book Review for publication.

Address:- North Asian International Research Journal Consortium (NAIRJC)
221, Gangoo Pulwama - 192301
Jammu & Kashmir, India
Cell: 09086405302, 09906662570,
Ph No: 01933212815
Email:- nairjc5@gmail.com, nairjc@nairjc.com , info@nairjc.com
Website: www.nairjc.com