Phytochemical Screening of Linlinna-aw (*Peperomia pellucida* Linn.) and its Analgesic, Diuretic and Anti-Hypertensive Properties

SOLITA EVANGELINE S. BANEZ, Ed.D.

Abstract

This study was conducted to perform phytochemical screening, analgesic, diuretic and anti-hypertensive testing on a local indigenous plant located in damp places commonly known as "Linlinna-aw" and scientifically known as Peperomia pellucida Linn. Specifically, this experimental investigation determined the chemical substances present in both the stems and leaves and tried to determine the pharmacological effects of the plant in terms of analgesic, diuretic and anti-hypertensive effects of the ethanol extracts (screened substances of "Linlinna-aw").

This study made use of the experimental research design in an actual laboratory set-up. There were three phases in the experimental process. Phase I included the Air Drying and Extraction process using the Rotavap apparatus, Phase II was the Phytochemical Screening using Mayer's Test for alkaloids, Fehling's test for glycosides, Gelatin Test for tannins, Froth Test for Saponins, Color Test for flavonoids and Liebermann-Burchard Tests for triterpenes and sterols. Phase III included the Pharmacological Testing in terms of Analgesic, Diuretic and Anti-hypertensive Properties.

In the pharmacological testing, 50 Swiss mice were used as test animals in Analgesia while six human beings were used in Diuretic and Antihypertensive properties of the plant.

Findings showed that "Linlinna-aw" contains the following chemical substances like alkaloids, glycosides, tannins, saponins, sterols and flavonoids in both the stems and leaves. Triterpenes were found to be absent in both the stems and leaves.

There were pharmacological effects of the ethanol extracts (screened substances) using male Swiss mice as test animals.

Under the conditions of this test, the ethanol extract of "Linlinna-aw" (Peperomia pellucida Linn) showed that it had an analgesic activity

when administered orally to male Swiss mice; it produced 23.81%, 42.86% and 71.43% protection against writhing at 500, 1,000 and 1,500 mg/kg respectively.

"Linlinna-aw" (Peperomia pellucida Linn.) had diuretic and antihypertensive properties. After the intake of ten "Linlinna-aw "plants, 3 times a day for 5-10 days, patients were relieved from urinary pain and their blood pressure was normalized.

Based on the conclusions, the researcher recommends a follow-up study to be conducted to quantify, isolate and identify the type of alkaloids, glycosides, tannins, saponins, sterols and flavonoids present in the stems and leaves of "Linlinna-aw" (Peperomia pellucida Linn). Further studies on its pharmacological tests such as anti-tumor, anti-septic, anti-hemorrhagic properties etc. are highly recommended.

Introduction

Herbal medicine has come a long way and is part of the health care system. Today, it is common to see modem and traditional medicine being practiced side by side not only in the remote areas but also in the urban centers. Ethnobotany has demonstrated its ability to generate important basic information and that this approach will continue to play an important role in the development of drugs from plants.

The primitive healer who uses herbs and *oracion* (prayer) to heal ailment is very popular in our midst. Science and technology want to validate this traditional doctor, and *is* giving support, in fact providing for the *herbolario's* research orientation.

The *herbolario* is as important as the scientific researcher because traditional healing can do much to save our people from the high cost of drugs.

Support for research and development (R&D) for traditional medicine is concretized by the integration of the traditional medicine program in the National Research and Development Plan; technical and financial grants for R&D activities; research information dissemination and technology transfer to concerned sectors. Among the different components of the traditional package, the Department of Science and Technology (DOST) has poured in a lot of resources in the development of herbal medicine. Access to drug, because of their high cost and inequitable distribution, is a major deterrent to health service delivery. Seventy percent of the population has not been provided with drug consumption of P360 per year, this is made bleaker with the fact that only 25% of the population accounts for 75% of the total sales. Sixty two percent of drug firms and 74% of pharmaceutical

laboratories are concentrated in Metro Manila. Presented with these facts and after a broad-based consultative process, the DOST- Philippine Council for Health Research and Development (PCHRD) ventured into a long-term program on the development of drugs from indigenous sources.

The National Integrated Program on Medicinal Plants **(NIRPROMP)** which was developed by a multisectoral-multidisciplinary group of researchers came up with the innovative approach in the investigation of medicinal plants. The new approach did a way with the phytochemistry studies prior to pharmacological testing. A more rapid, scientifically valid clinical screening process was developed to determine the efficacy and safety of the preparations. The integrated approach developed technology at two levels: low-middle technology and high level technology based on in-depth phyto-pharmacological studies.

Prioritization of medicinal plants was based on the health needs and problems of the population. Technologies for the development of drugs were developed from plants which alleviated symptoms such as fever, pain, cough, asthma, intestinal colic, high blood pressure, edema, skin ailment, and parasitism.

These technologies found their way into the production sector. Herba Pharma Inc., a primitive manufacturing firm bought the production technologies of four drugs from the medicinal plants namely: *Lagundi* for cough remedy, *Tsaang Gubat* as antispasmodic, *Sambong* as diuretic, and *Herba buena* as analgesic. *Lagundi* alone costs 30% lower than its commercial counterpart and is expected to yield savings for the government via its procurement of at least P3 million per year. The Department of Health (DOH), likewise adapted these technologies and established three production plants in the country - in Tacloban, Tuguegarao, and Cotabato to produce alternative drugs to meet the requirements of the regions (Infoscience: 1995).

In spite of the many hundreds of natural compounds that have been discovered, it is estimated that only about 20% of the plant species worldwide have been studied and the chances are good that with the richness of our natural resources many plant constituents with useful biological properties remain and are waiting to be discovered.

There is, thus, a need for an integrated natural products program, not only in the Philippines but within the region, which should discover new chemicals for medicine, agriculture and industry (Hernandez et al., 1998).

Linlinna-aw, (Peperomia pellucida linn.)" is a pantropic species of American origin and is found commonly in damp walls during the rainy seasons in different areas of the Philippines.

Linlinna-aw is a succulent, erect, branched herb, growing from 5 to 40 centimeters in height. The stems are round and often 5 millimeters thick. The leaves

are ovate, 1 to 3 centimeters long with pointed or blunt tip and heart shaped base, pale green, pellucid and shining. The spikes arc green, erect, very slender and 1 to 6 centimeters long. The fruit is found spherical less than one millimeter thick.

According to Eduardo Quisumbing in his book entitled "Medicinal Plants of the Philippines", the whole plant *(Peperomia pellucida* Linn.) is used as warm poultice for abscesses and boils. In the same book, Dalziel mentioned that in Tropical West Africa, they also use the plant as an ingredient in medicinal infusions for the treatment of convulsions (Folkloric remedies).

With the above statements, the researcher was greatly interested to look into the medicinal values of the indigenous plant *linlinna-aw (Peperomia pellucida* Linn.) particularly in its analgesic, diuretic and antihypertensive properties.

Objectives

This study aimed to perform phytochemical screening and pharmacological testing of *linlinna-aw Peperomia pellucida* Linn.).

Specifically, this study

1. identified the chemical substances present in the stems and leaves of *linlinna-aw (Peperomia pellucida* Linn); and
2. determined the pharmacological effects of *"linlinna-aw" (Peperomia pellucida* Linn) in terms of
   a. analgesia using male swiss mice as subjects of experimentation, and
   b. diuretic and antihypertensive properties using human beings as subjects of experimentation.

Scope and Delimitation

This study was limited to the phytochemical screening and pharmacological testing of *linlinna-aw (Peperomia pellucida* Linn.)

Only the stem and leaves were used in the experimental investigation. The plant samples were gathered mainly from Metro Vigan.

The determination of the chemical constituents was limited to qualitative rather than quantitative analysis. No attempt was made to determine the amount of specific chemical constituents but rather, only the presence or absence of general groups of organic compounds, glycosides, alkaloids, tannins, saponins, flavonoids, triterpenes, and sterols.

In the phannacological testing, it was limited to the testing of the analgesic, diuretic and antihypertensive effects of *linlinna-aw (Peperomia pellucida* Linn.)

Male Swiss mice were used as subjects for experimentation in analgesia while human beings were used for diuretic and antihypertensive properties.

The air-drying and extraction processes using "rotavap" were done at the UNP Chemistry Laboratory; the phytochemical screening was conducted at the ITDI, Department of Science and Technology (DOST), Bicutan, Taguig, Metro Manila and phannacological testing was done in Ilocos Sur.

Review of Related Literature

This section includes a summary of readings and studies both local and foreign researches which the researcher considered relevant to the study.

In a recent World Health Organization (WHO) Seminar on the Uses of Medicinal Plants in Health Care, experts urged the use of medicinal plants of proven effectiveness. They recommended greater awareness of the potential value of traditional remedies which can be used together with Western medicine.

Prof. G. Penson, World Health Organization (WHO) adviser on drug policies and management, noted that the increasing use of medicinal plants may be due to public awareness of the dangers of synthetic drugs. Medicinal plants, however, are usually mild forms of treatment with fever drawbacks than pure substances.

Guevara and Recio cited that there has been in more recent times an awakening towards the use of drugs and their preparation in a kind of "back-to-nature" movement, instead of the classical synthetic compounds manufactured in the advanced countries. While the use of synthetic drugs is of undoubted value, especially in advanced state of illness, it is believed that the use of herbal medicines of properly tested efficacy would be of great advantage in a developing country like the Philippines which is still blessed with bountiful plant resources. The idea is to keep people healthy by treating illness at an early stage instead of resorting to treatment when already at an advanced stage.

Researchers at the National Institute of Science and Technology (NIST), tho Philippine Council for Health Research and Development (PCHRD) and at the University of the Philippines (UP) have proven the effectiveness of medicinal plants.

In addition to this, one of the funded programs of the Department of Science and Technology is the intensified research on indigenous plant materials not only for drug manufacturers but also primarily for providing the rural areas with

adequate supply of medicines or drug preparation by the expanded utilization of the plants in their raw and semi-processed forms.

New drug originated from many different sources. Accidental observation on natural products, unexpected clinical findings on known compounds, basic physiological or biochemical investigation or even test tube experiments have provided leads to **great** therapeutic discoveries.

Today, most of the new drugs are known by systematic screening methods. The processes are so designed to distinguish useful drug materials from the non-useful ones as rapidly, comprehensively and inexpensively as possible.

Primary screening provides a general profile of the toxicity, pharmacological activities and pharmacokinetics of a new drug. The results obtained with the animal models are used to evaluate the safety of the material, its toxic effect and its intended therapeutic properties. Thus, it is essential that the phannacological and toxicological properties of the drug material be established before any clinical trials on man are conducted.

The University of Santo Tomas (UST) is also very much interested in the study of medicinal plants. It has come up with the publication of "Acta Manilana," a book on medicinal plants. A supplement of the "Acta Manilana" is a manual on the procedure of phytochemical, microbiological and pharmacological screening of medicinal plants.

Akilith (1984) conducted a study on the phytochemical and microbiological investigation of the leaves of *"Leucosyke hispidissima" (dael)*. Her findings on the photochemical assay revealed that the plant contains flavonoids, tannins, polyphenolic compounds as well as probable traces of saponins, triterpenoids and anthraquinones. Her microbiological assay done with the crude extract and the fraction from the chromatographic separation showed anti-microbial activity against gram-negative bacteria except those fractions that appeared to contain green components only.

The study of Akilith is different from this study because another plant species was used. Instead of pharmacological testing, she made use of microbiological assay.

As cited by Isleta, (1995), Estrada conducted a study on Pharmacological and Toxicologic Analyses of *lagundi, (Vitex negundo* Linn**)** in 1989. The phannacologic analysis showed that one hundred percent aqueous extract of *lagundi* has lethal dose 50 of 103 gm/ kg body weight in the adult albino mouse. Contractions of isolated tissue preparation of the rat duodenum, cat tracheal chain, and rat uterus were depressed. A bioassay method using the rat duodenum for the potency of batches of *lagundi* was established.

The present study is different from the study of Estrada because another plant species was used and Swiss mice were the subjects for experimentation and instead of toxicity, this study determined the analgesia, diuretic and anti-hypertension properties of *linlinna-aw (Peperomia pellucida* Linn.).

Bafiez (1993) conducted phytochcmical analysis and pharmacological Testing of *sanggumay* orchids *(Dendrobium superbum* Reichb) and found out that the plant has analgesic property. The present study is similar to her study because she also determined the analgesic property. They only differ in the species used and in the later study, she determined also the diuretic and anti-hypertension properties of the plant.

Banez et al. (2001) performed toxicologic analysis of *linlinna-aw (Peperomia pellucida* Linn.) and found out that the ethanol extract of *linlinna-aw* has toxic effect when administered orally to Swiss mice. The approximate lethal dose is 8 g/kg. From groups 1-4, after oral administration of the test drug five minutes after dosing, the mice manifested decreased motor activity and ptosis lasting for two hours. Five minutes after dosing, the mice in groups 8 and 9 manifested decreased motor activity and ptosis , writhing, hyperemia, abnormal gait, ataxia, diarrhea, convulsion and death of two mice within thirty minutes. The present study is different from the former study because photochemical, analgesic, diuretic and antihypertensive analyses were considered. They are similar because the same plant was utilized which is *linlinna-aw (Peperomia pellucida* Linn.).

Operational Definition of Terms

Certain terms are defined for clearer understanding of the reader.

Alkaloids. These are the medicinally important substances of plants from which they are derived. They constitute indispensable and most potent groups of substances and relief from suffering (Anderson: 1975). They are basic nitrogenated plant products usually possessing strong and dramatic physiological action. Alkaloids are anti-hypertensive, anti-neoplastic agents, and demonstrate encolytic (anti-tumor activity).

Analgesic. This is a medication that reduces or eliminates pain. In this study, it refers to the insensibility to pain without loss of consciousness of testing animals, the Swiss mice, using ethanol extract of *Linlinna-aw, (Peperomia pellucida* Linn).

Anti-hypertension. A medication that reduces excessive tension or high blood pressure.

Diuretic. An agent that increases the volume of urine.

Extraction. This is the process of obtaining the plant constituents by using ethyl alcohol as the solvent and by refluxing the system by using a sensitive apparatus, "the Rotavap".

Fehling's Test This refers to the test which is used for the determination of the presence or absence of glycoside in the ethanol extract, using anhydrous sodium carbonate and Fehling's solution. An increase in the amount of brick red precipitate in the hydrolyzed sample indicates the presence of glycosidcs.

Flavonoids. These are derivatives of glycosides usually occurring in plants wherein one or more of the phenolic hydroxyl groups are combined with sugar residue (Capal, 1992:6). Its presence in a sample is tested by tho formation of a red color with acidified magnesium.

Gelatin test. This refers to the test which is used for the determination of the presence or absence of tannins in the ethanol extract using gelatin solution. The formation of a very heavy precipitate suggests the presence of tannins.

Glycosides. These are the condensation products of sugar with various kinds of organic hydroxy compounds occurring in some plants (Santos, 1985:24).

Liebermann-Burchard Test. This refers to the test which is used for the determination of the presence or absence of triterpenes and sterols in the ethanol extract using acetic anhydride and sulfuric acid as reagents. A pink to red color is indicative of triterpenes, while a pink to blue color is indicative of sterols.

Mayer's Test. This refers to the test which is used to determine the presence or absence of alkaloids in the ethanol extract using mercuric chloride and potassium iodide in distilled water as reagents. The formation of precipitate upon the addition of Mayer's reagent in ethanol extract is suggestive of the presence of alkaloids.

Phytochemical. This refers to the chemical constituents or substances found in plants. The chemical constituents considered in this study are alkaloids, glycosides, tannins, flavonoids, triterpenes and saponins in the stems and leaves of *linlinna-aw (Peperomia pellucida* Linn.)

Saponins. These are glycosides which are characterized by their ability to froth when the aqueous solution is agitated.

Sterols. These are the derivatives of the so-called steroid nucleus (cyclopentanoperhydrophenanthrene). Members of these are cholesterol, dehydrocholesterol and ergosterol. They are also described as monohydric alcohols of higher fatty acids. The presence in a system is indicative of the fonnation of a blue color with the Liebermann-Burchard reagent.

Tannins. These are complex substances and usually occur as mixtures of polyphenol that are very difficult to separate since they do not crystallize. The formation of precipitate with the gelatin solution in a sample is an indication of the presence of tannins.

Triterpenes, These are found in essential oils of many plants and have carbon skeletons made up of isoprene units joined in a regular, head to tail way. These can be sources of Vitamin A. In the Liebermann-Burchard Test, a pink to red color was indicative of the presence of triterpenes.

Methodology

This section presents the design of the study, materials and experimental procedure and the statistical treatment of the data.

Design of the Study. This study made use of the experimental research design in actual laboratory set-up. Three phases were included in the pursuit of this study:

Phase I. The gathering of fresh stems and leaves of *linlinna-aw (Peperomia pellucida* Linn); the air drying process and the extraction process were included in this phase.

Phase II. This phase included the phytochemical screening to determine the presence of alkaloid, glycoside, tannins, saponins, flavonoids, triterpenes and sterols in the stems and leaves of *linlinna-aw (Peperomia pellucida* Linn.)

Phase III. This included the Pharmacological Testing using male Swiss mice and human beings as subjects of experimentation.

a. Analgesic Test (Writhing Method. PBQ)
b. Diuretic Test
c. Anti-hypertensive Test

Materials and Methods

Phytochemical Screening

linlinna-aw leaves
linlinna-aw stems
weighing balance
ethyl alcohol
petroleum ether
test tubes
distilled water
cheese cloth
glass funnel
water bath
filter paper
Erlenmeyer flask

test tube rack
graduated cylinder
test tube brush
spatula
evaporating dish
beakers
medicine dropper
glass rod
test tube holder
pipette

Pharmacological Testing

Ethanol extract of *linlinna-aw*
male Swiss mice
syringe and needle
mortar and pestle
pipette
laboratory counter
electronic balance
phenylbenzoquinone (PBQ)
aspirin
nonnal saline solution

This portion deals with the experimental procedures which were strictly followed during the conduct of this experimental study.

I. Preparation of Extract

Fresh stems and leaves of *linlinna-aw (Peperomia pellucida* Linn.) were gathered in Metro Vigan. They were washed thoroughly and air dried for four days.

The leaves were finely cut into small pieces. Five hundred grams of the finely cut materials were placed in an Erlenmeyer flask and were weighed in a balance. The material was completely submerged in a sufficient amount of ethyl alcohol. It was stoppered and soaked for twenty-four hours. Then it was filtered through a glass funnel.

The same procedure was followed for the stems. The flask and the plant material were rinsed with *95%* ethyl alcohol. Extraction was done in the Rotavap Apparatus.

The filtrates were concentrated under vacuo to about fifty milliliters. The exact volumes of the concentrated extracts were measured. Then, the extracts in tightly stoppered containers were stored inside a refrigerator. The extracts were ready for the phytochemical screening and pharmacological testing.

II. Phytochemical **Screening**

The following activities were done during the phytochemical screening to determine the presence of alkaloids, glycosides, tannins, saponins, flavonoids, triterpenes and sterols in the stems and leaves of *linlinna-aw (Peperomia pellucida* Linn). These methods and procedures were based on the methods available at the Chemistry and Pharmacological Division, Department of Science and Technology, Bicutan, Taguig, Metro Manila.

A. Screening for Alkaloids

Alkaloidal Test *(Leaves)*

Ten millimeters of the leaf extract was evaporated to a syup consistency on an evaporating dish over a water bath. To the concentrated extract, five milliliters of hydrochloric acid solution was added while heating. For about five minutes, the solution was stirred, and then cooled to room temperature. To this was added about 0.5 gram of sodium chloride powder. It was stirred and enough fresh hydrochloric acid solution was added to wash and bring the filtrate to a final volume of 3 milliliters.

To one milliliter of liquid, a few drops of Mayer's reagent were added. The formation of precipitate upon the addition of the Mayer's reagent was suggestive of the presence of alkaloids.

The same procedure was done using the stem extract. Precipitation that was formed upon the addition of Mayer's reagent indicated the presence of alkaloids.

B. Test for Glycosides (Fehling's test)

Ten milliliters of leaf extract was dissolved in hot water and filtered. The filtrate was used for the test. Two (2) ml each sample was placed in two test tubes. To sample I, 1 ml dil. HCL was added. To sample 2, nothing was added. Then the two test tubes were placed in a boiling water bath for 5 minutes. Then the test tubes were cooled. Both were neutralized with anhydrous sodium carbonate until no more effervescence was produced. Then Fehling's solution was added to the two test tubes which were heated over a water bath fur two minutes. An increase in the amount of brick red precipitate in the hydrolyzed sample as compared to the other sample indicated the presence of glycosides.

The same procedure was done using the stem extract.

Test for Tannins **(Gelatin** Test)

Ten milliliters of the ethanol extract of the leaves was evaporated to dryness over a water bath and then cooled. The residue was extracted with twenty milliliters of hot distilled water, cooled, then to it, five drops of IO % sodium chloride solution was added to salt out undesirable constituents and then the residue was filtered.

The filtrate was divided into two test tubes A and B. Test tube A was kept as the control. To test tube B, 3 drops of I% gelatin solution was added. The same procedure was done for the stem extracts. The formation of precipitation suggested the presence of tannins.

The same procedure was done using the stem extract.

C. Test for Saponins (Froth Test)

Ten milliliters of the leaf extract was dissolved in hot water. The aqueous extract was shaken vigorously for about thirty (30) seconds and was allowed to stand and was observed over a period of thirty (30) minutes. The formation of honeycomb froths at a height of three (3) cm indicated positive results.

The same procedure was done using the stem extract.

D. Test for Flavonoids (Color Test)

Two milliliters of the leaf extract was treated with two ml 10% hydrochloric acid and magnesium turnings. Formation of red color indicated a positive result.

The same procedure was done using the stem extract.

E. Test for Triterpenes and Sterols (Libermann-Burchard Test]

Two milliliters of leaf extract was dissolved in acetic anhydride. The soluble portion was decanted and to this, 1-2 drops of concentrated sulfuric acid were added. A pink to red color was indicative of triterpenes, while a pink to blue color was indicative of sterols.

The same procedure was done using the stem extracts.

III. Pharmacological Testing

Test Drug Material

The plant extract (ethanol extract) under study was absolutely free from extracting solvents, thus this was completely dry or syrupy in consistency.

The drug material was subjected to pharmacological testing together with a standard drug (aspirin) as positive control and the suspending vehicle (normal saline solution) as negative control.

Experimental Animals

Fifty (50) healthy male Swiss mice each weighing 17 to 30 gm at the start of the experiment were kept in individual observation cages. Female mice were not used because their emotional state is unstable due to menstrual syndrome. All animals were made to fast from food and water 16 hours before the test. Two hours after administration of the drug, the animals were given free access to food and water.

Analgesic Test by Writhing Method

Three increasing doses of the test material were given orally to the animals in groups of five (5) including the negative and positive controls. Thirty (30) minutes after dosing, the mice were injected intraperitoneally with phenylbenzoquinone (PBQ). The number of writhing and number of animals without writhing were closely observed for the first twenty (20) minutes after (PBQ) was administered. The percent protection was computed based on the control as follows:

$$\text{Percent Protection} = 100 - \frac{(\text{Experimental} \times 100)}{\text{Control}}$$

Diuretic and Anti-hypertensive Tests

Ten matured *linlinna-aw* plants were washed thoroughly and half boiled. These were eaten and the liquid was drunk by six patients for the duration of 5-15 days. For the anti-hypertensive test, blood pressure of the patients before and after the treatment was monitored.

Results and Discussion

Results on the phytochcmical screening of the stem and leaf extracts of *linlinna-aw (Peperomia pellucida* Linn) are exhibited in Table **I.**

Findings of this study show that the plant contains medicinal properties like alkaloids, glycosides, tannins, saponins, flavonoids, and sterols. The presence of these therapeutic substances confinn the purported use of *linlinna-aw (Peperomia pellucida* Linn.) as a medicinal plant.

Table 1. Results of the phytochemical screening of *linlinna-aw (Peperomia pellucida* Linn.).

SUBSTANCE DETERMINED	METHOD USED	RESULTS	
		Stems	Leaves
I. Alkaloids	Maver's Test	+	+
2. Glycosides	Fehling's Test	+	+
3. Tannins	Gelatin Test	+	+
4. Saponins	Froth Test	+	+
5. Flavonoids	Color Test	+	+
6. Tritemenes	Liebermann – Burchard Test	-	-
7. Sterols	Liebermann – Burchard Test	+	+

Legend:
+ = presence of substance detennined
- = absence of substance determined

Alkaloids. As shown in Table 1, the Mayer's test for alkaloids gave positive results on the stem and leaf extracts. This was due to the formation of precipitates upon the addition of Mayer's reagent to the solution.

Alkaloids are widely used in medicines like morphine, codeine, etc. Alkaloids are antihypertensive, antineoplastic agents and demonstrate encolytic property (anti-tumor activity). It is used to relieve nasal congestion, stop hemorrhage, combat malaria and dilate the pupil of the eye and also used as muscle stimulant (The US Educator Encyclopedia, 1984: 61). The stems and leaves of *linlinna-aw" (Peperomia pellucida* **Linn.)** can be a potential cure for illness related to the above-mentioned diseases.

Glycosides. In Fehling's test for glycosides, there was an increase of brick red precipitate in hydrolyzed sample as compared to the sample which indicated the positive results on the stems and leaves of the plant.

Glycoside is any of a group of compounds that yield sugar molecules in hydrolysis. All parts of a glycoside compound may be sugar molecules, so that sucrose, raffinosc, starch, and cellulose all of which hydrolyze into sugar molecules may all be considered glycosides. However, the name is usually applied to a compound in which part of the molecule is not a sugar. The non-sugar component is called the aglycon. Cardenolides and bufadienolides are cardiac glycosides as they act on muscles of the heart. Cardiac glycosides or simply digitalis is a group of chemically and pharmacologically related drugs. These cardiotonics increase the strength of the contraction of the heart muscle. The drugs are used mainly in the treatment of congestive heart failure and are unexcelled in this use. They may be used in the treatment of trial impulses from reaching the ventricle (Anderson, 1975). Thus the plant *linlinna-aw (Peperomia pellucida* Linn.) can be a source of medicine for the treatment of congestive heart failure.

Tannins. The Gelatin test for tannins gave positive results for both the stem and leaf extracts. A heavy precipitation upon the addition of gelatin solution was observed which indicated the presence of tannins.

Tannin is a yellowish-white to light brown amorphous powder, glistening scales and spongy masses, usually odorless with a strong astringent taste. Tannins could be a treatment for diarrhea and extensive bums and maybe used rectally for the relief of various rectal disorders (Santos, 1985:p.17). It is also used in the treatment of bed sore and weeping ulcers. It was also formerly used for sore throat and stomatitis. Therefore, the plant can be a potential source in the treatment of the above-mentioned diseases.

Saponins. The formation of honeycomb froths at 3.2 centimeters high in the froth test for saponins indicated a positive result. This means that the stem and leaf of *linlinna-aw* is an emulsifying agent and can be used as detergent to replace soap. Many of the saponins are markedly toxic. They usually exert a powerful hemolytic action on red corpuscles. They have been used as fish poison. Steroidal saponins are of great importance because of their relationship to such compounds as sex hormones, cortisone, Vitamin D and cardiac glycoside.

Flavonoids. The color test for flavonoids gave positive results for both the stems and leaf extracts. There was a fonnation of red color when the ethanol extract was treated with hydrochloric acid and magnesium turnings. This means that the plant has antiviral, antifungal, anti-inflammatory and cytotoxic activities (Capal, 1992).

Triterpenes. The Liebermann-Burchard test for triterpencs gave negative result on both the stems and leaves of *linlinna-aw (Peperomia pellucida* Linn.) The pink color did not change to red which indicated the absence of triterpenes. This means that the plant is not a good source of Vitamin A.

Sterols. Again, the Liebermann-Burchard test *was* used for sterols. There was a production of blue color which indicated the presence of sterols. Sterols are derivatives of the so-called steroid nucleus (cyclopentanoperhydrophenanthrene). Members of this are cholesterol, dehydrocholesterol and ergosterol.

In the blood, it plays an important role in transporting fatty acids in the form of cholesterol esters. Ergosterol is responsible for the low incidence of rickets because of the supply of Vitamin **D**; thus, the plant could be a good source of Vitamin **D.**

Pharmacological Screening

Analgesia

Results of the analgesic test by Writhing Method (PBQ) using ethanol extract from the stems and leaves of *inlinna-aw (Peperomia pellucida* Linn) using male Swiss Mice as test animals are exhibited in Table 2.

Under the condition of this test, the ethanol extract of *linlinna-aw (Peperomia pellucida* Linn.) showed that it has an analgesic activity when administered orally to male Swiss mice; it produced a 23.81%, 42.86% and 71.43% protection against writhing at 500, 1000, and 1,500 mg/kg, respectively.

Table 2. Results of analgesic test by writhing method.

Group #	Drug	Dose mg/kg	D	No. of Mice wlo Writhing/Total # of Test Animals	Number of Writhing	Percent Protection
I	Control	0	10	0/10	2	0
2	*Linlinna-aw*	500	10	5/10	16	23.81%
3	*Linlinna-aw*	1000	10	6/10	12	42.86%
4	*Linlirna-aw*	1500	10	7/10	6	71.43%
5	Asoirin	200	10	10/10	0	100%

Percent Protection= 100 - [(Experimental x 100)/Control]

The ethanol extract dose (mg/kg) of the experimental group is directly proportional to the percent protection of the male mice. (See Figure 2) This showed that as the dosage increased, the greater is the percent protection of the male Swiss mice. *As* long as *it* is below the toxidrome which is 8g/kg (Banez, 2001) the higher the dosage injected intraperitoneally to male Swiss mice, the more protection against writhing.

The positive control (Aspirin) showed the highest percent protection.

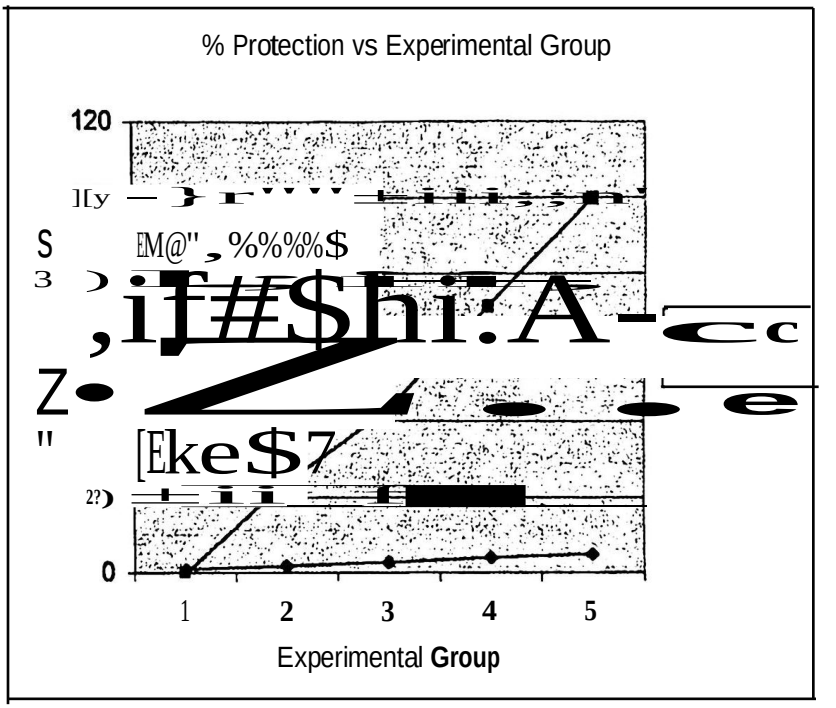

Figure 1. Graph showing the results of analgesic test by writhing method (PDQ) using male Swiss Mice as test animals.

Diuretic Properties

Result of the Diuretic Properties of *linlinna-aw (Peperomia pellucida* Linn.) is exhibited in Table 3.

It was found out that the *linlinna-aw (Peperomia pellucida* Linn.) plant can heal urinary problems as claimed by the four patients. Pain and difficulty of urinating were removed after 7-10 days of use and there was an increase in the volume of urine excreted. However, prolonged use of this caused dizziness, drowsiness and inactive mental alertness which are implications that this plant can lower blood pressure.

In the hypertensive test, it was found out that the two patients' blood pressure went down after the intake of *linlinna-aw (Peperomia pellucida* Linn) for 2-5 days from 180/120 mm/hg to 130/110mm/hg and later 110/100 mm/hg and from 140/110 mm/hg to 110/ 100 mm/hg.

Table 3. Results of diuretic properties of *linlinna-aw (Peperomia pellucida* Linn.)

Patient	Duration of Intake	Observation
I (Control)	no intake	Severe pain was felt for weeks and difficulty to urinate was experienced everyday
2	3x a day for IO days	For the first two days the flow of urine had become better and slight pain along the genital organ was experienced when urinating. After 8 days of using religiously, the pain vanished and the excretion of urine normalized. However, using continuously even without pain brought dizziness and decreased motor activity which lasted for 8 minutes.
3	3x a day for 10 days	After I ½ day using the herbal medicine, difficulty in urinating was removed and the pain had gone away. Pain was just felt along the inflamed testis but after IO days of continued drinking and eating *linlinna-aw* the infected area was totally healed.
4	3x a day for 15 days	After 2 days, the excretion of urine normalized and no pain was experienced after 7 days. Using until the 15 day brought drowsiness, dulling of mental alertness and even dizziness which lasted for 5 months and because of this the patient was subjected to medical treatment and found out that he was experiencing low blood pressure.
5	3x a day for 10 days	After 2 days using the medicine, difficulty of urinating and pain were completely removed. Using until the 10" day made the patient excrete more urine.

Conclusions

The ethanol extracts of the stems and leaves of *linlinna-aw (Peperomia pellucida* Linn.) contain therapeutic substances such as alkaloids, glycosides, tannins, saponins, sterols and flavonoids. This implies that the plant is a good

source of treatment for hypertension, tumor, congestive heart failure, wounds, sores, boils, stomach ache, diarrhea, sore throat, bums, ulcer, nasal congestion. hemorrhage, malaria, other rectal disorders, viral and fungal infections, inflammatory and cytotoxic activities. The plant is also an excellent emulsifying agent and can be used as detergent to replace soap. The Liebermann-Burchard Test for triterpenes showed negative results which implies then that *linlinna-aw (Peperomia pellucida* Linn) is not a good source of Vitamin A.

The ethanol extract of *linlinna-aw (Peperomia pellucida* Linn) had analgesic effect when administered orally to male Swiss Mice because of the percent protection the doses provided.

The *linlinna-aw* plant has diuretic and anti-hypertensive properties as claimed by the patients. Prolonged use of the plant, however, is not advised due to the observation that symptoms of ailments like low blood pressure and dizziness were felt by the patients. This implies that the plant contains only anti-hypertensive properties.

Recommendations

Based on the conclusions, the researcher presents the following recommendations:

1. A follow-up study should be conducted to quantify, isolate and identify the type of alkaloids, glycosides, tannins, saponins, sterols and flavonoids present in the leaves and stems.

2. The roots of the plant are recommended for other pharmacological testing such as anti-tumor, heart failure, etc.

3. The plant is recommended for Microbiological and Biological Assays.

4. Further studies on the plant's therapeutic properties are recommended to be undertaken by interested drug companies.

5. The result of this research is recommended to be listed in the compilation and documentation of Medicinal Plants in the Philippines through NRCP, DOST and UP and be indexed at PROSEA, Plant Resource of Southeast Asia.

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