BIBLIOGRAPHY

YVONNE B. DEGAY-BOLAYO, November 2006. PHYTONUTRIENT ANALYSIS OF THE SHOOTS OF THE PASSION FRUIT VINE. (*Passiflora edulis Sims).*Benguet State University, La Trinidad, Benguet.

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ABSTRACT

Phytonutrient analyses of the shoots of the passion fruit revealed that this plant contain protein, carbohydrates, fat, minerals (potassium, calcium, phosphate, sodium, iron and zinc) and vitamins (A, C, and E). The presence of cyanogenic glycoside, alkaloids, flavonoids, steroids, anthraquinones, sugar and essential oils were determined in the phytochemical tests.

There is no risk associated with the consumption of the shoots of the passion fruit as vegetable due to presence of natural toxin particularly cyanogenic glycoside because it is easily eliminated through cooking. This is supported by the results of the brine shrimp assay which indicated that the toxicity of the different extracts of the shoots is insignificant.

Flavonoid, one of the compounds that impart bitter taste was partially isolated from the sticky brown acetate extract following a thin layer chromatography directed scheme. This fraction has a brown to yellow color and the flavonoid spot has an Rf value of 0.467 and is indicated by the blue spot upon spraying with potassium- ferricyanide-ferric chloride while it is a red color when sprayed with vanillin-sulfuric acid.

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INTRODUCTION

Background of Study

Foods of plant origin have long been regarded by folks for their nutritional, medicinal and protective values. Vegetables are known to be a good source of vitamins, minerals, proteins and certain hormone precursor (Shemilt, 1982). Many indigenous plants sold in the market nowadays were not used as vegetables before.

People avoid fruits and vegetables treated with commercial pesticides but often are unaware they are ingesting the natural toxins present in foods. There are some reports that claim the health risks from natural chemicals are more toxic than the risks from pesticide residues. Although this has not been conclusively proven, they assert that some constituents of commonly consumed vegetables like cabbage and broccoli are more toxic to human than chemical pesticides in foods (Pimentel et al, 1996).

Man utilizes for food the different parts of the plants like the seeds, leaves, roots and fruits. Plants are known to produce a wide array of chemicals. They contain natural chemicals that are needed for growth and health such as carbohydrates, sugars, proteins and vitamins. But some plants used for food contain undesirable natural products including some amino acids, proteins, antinutrients and glycosides (Mendoza et al, 1985). These chemicals are considered natural toxins in plants. New Zealand Food Safety Authority (1994)

cited some commonly consumed plants to contain toxins in the leaves, fruits, seeds and shoots.

The presence of the natural toxins in plants is to protect them from predators, as a natural pesticide to inhibit the action of insects or to protect them from spoilage by weather, handling, UV light and microbes (Randolph, 2005). Ingesting foods containing toxins may produce adverse effects such as allergic reactions or negative effects in the gastrointestinal, cardiac or nervous systems of animals and humans.

Edible plants, full of nutrients, vitamins and fiber as recommended by most professional health organizations are considered by people for their optimal diet. Even plants containing toxic compounds are considered staple food because people traditionally, developed effective processing methods to reduce or eliminate the toxins.

In the Cordillera particularly in Benguet and in Mountain Province, shoots of the passion fruit are utilized as vegetable. For the passion fruit, some species of this plant like giant granadilla have been reported to contain cyanogenic glycoside on the leaves, skin and immature seeds (Morton, 1987). The shoots of this plant have bitter taste. The popularity of this plant for food has motivated the researcher to study the suitability of the shoots of the passion fruit for vegetable use.

Objectives of the Study

The main objective of the study was to determine the suitability of the shoots of the passion fruit for vegetable use. Specifically, to:

1. Determine the phytonutrients of the shoots of the passion fruit.
2. Determine the toxicity of the shoots of the passion fruit.
3. Determine the compound responsible for the bitter taste of the shoots of the passion fruit.

Importance of the Study

The result of this study will provide the consumers adequate information regarding the health benefits and risks associated with the consumption of the plant as vegetable. It will also serve as baseline information for similar studies.

Time and Place of Study

The study was conducted from May 2006 to October 2006. The phytonutrient, phytochemical analysis, brine shrimp assay and partial isolation of the flavonoid were conducted at the Chemistry Department, Benguet State University and DOST Chemical Laboratories, La Trinidad, Benguet. Mineral analyses were done by the Philippine Institute of Pure and Applied Chemistry (PIPAC) at Ateneo de Manila, Quezon City.

REVIEW OF RELATED LITERATURE

Passion Fruit (*Passiflora edulis Sims*)

Botanical Description

Passion fruit *Passiflora edulis Sims* (Plate 1) is also known as masaplora or bantanas in Benguet. Morton (1987) described the plant as follows: vine of this plant is a shallow-rooted, woody, perennial, and climbing by means of tendrils.

A. Passion Fruit Vine B. Shoot of the Passion Fruit

C. Flower of Passion Fruit Plant D. Passion Fruit

Plate1. The Passion Fruit Plant (*Passiflora edulis Sims*)

The alternate, evergreen leaves, deeply 3-lobed when mature, are finely toothed, 3 to 8 in (7.5-20 cm) long, deep-green and glossy above, paler and dull beneath, and like the young stems and tendrils tinged with red or purple especially in the yellow form.

A single, fragrant flower (Plate 1c), 2 to 3 in (5-7.5 cm) wide, is borne at each node on the new growth. The bloom, clasped by 3 large, green, leaf like bracts, consists of 5 greenish-white sepals, 5 white petals, a fringelike corona of straight, white tipped rays, rich purple at the base, also 5 stamens with large anthers, the ovary, and triple-branched style forming a prominent central structure. The nearly round or ovoid fruit (Plate 1d) 1 1/2 to 3 inches wide has a tough rind, smooth waxy, ranging in hue from dark-purple with faint, fine white specks, to light -yellow. It is 1/8 inches thick, adhering to a 1/4 inches layer of white pith. Within a cavity is filled with a mass of double-walled, membranous sacs filled with orange-colored, pulpy juice and small, hard, dark-brown or black, pitted seeds.

The passion fruit (*Passiflora edulis Sims*) is a vine known for its edible fruit either green or ripe. This plant is also recorded for its pharmacological action. The plant is antiscorbutic, stomachic, and refreshing when drunk as lemonade. According to Holland and De Lanessan as mentioned by Quisumbing (1978), the roots possess narcotic properties. Also, it is used as diuretic and

emetic. Lirio et. al. (2006) mentioned that the juice from the fruit is also used to relieve hang-over, diarrhea, arthritis and hypertension.

Plant Nutrients

Water

Most fruits and vegetables contain up to 90 percent water. Strawberries, watermelon, lettuce, cabbage, celery, spinach and broccoli contain 90-99% water. Functions of the water in the body are: carries nutrients and waste products; maintains large molecules such as proteins and glycogen; participates in metabolic properties; aids in the regulation of normal body temperature and maintains blood volume (Whitney and Rolfes, 2005).

Energy Yielding Nutrients

The body uses carbohydrates, fats, and protein for energy to fuel all its activities. When these nutrients are used by the body, the bonds between each atom break, releasing energy. Some of the energy is released as heat, but some is used to send electrical impulses through the brain and nerves, to synthesize body compounds and to move muscles (Whitney and Rolfes, 2005).

Minerals

Minerals are inorganic elements (Whitney and Rolfes, 2005) required in physiological processes: they regulate fluid balance, muscle contractions and

nerve impulses (Encyclopedia, 2003). The major minerals which are needed in large amounts are calcium, phosphorus, potassium, sodium, chloride, magnesium and sulfur. Trace minerals, such as chromium, copper, fluoride, iodine, iron, selenium and zinc are needed in smaller quantities (Encyclopedia, 2003).

Vitamins

Whitney and Rolfes (2005) defined vitamins as organic, essential nutrients required in tiny amounts to perform specific functions that promote growth, reproduction, or the maintenance of health and life. Vitamins differ from carbohydrates, fats and proteins in the following ways: a) their structure- they are individual units and are not linked together as molecules of glucose or amino acids; b) function- they do not yield energy when broken down; they assist the enzymes that release energy from carbohydrates, fats and proteins; c) food contents- the amount of vitamins ingested from food are measured in terms of micrograms. Vitamins maybe water-soluble (like B vitamins and Vit. C) or fat-soluble (such as Vit. A, D, E, and K).

Phytochemicals

Scholbe (2001) defined phytochemicals as non-nutritive plant chemicals that contain protective, disease-preventing compounds. Guevara (2005) cited that these phytochemicals are some of the secondary plant metabolites like alkaloids,

saponins, steroids, flavonoids, tannins and polyphenols, anthraquinones, cyanogenic glycosides and terpenoids.

Alkaloids

These are small organic molecules, comprising several carbon rings with side chains in which one or more of the carbon atoms have been replaced by nitrogen. Examples of these bitter compounds are nicotine, quinine, srychnine, ergotamine atropine and caffeine. The action of alkaloids on the nervous system is to disrupt electrochemical transmission at nerve junctions, either preventing transmission or enhancing it inappropriately (Randolph, 2005).

Glycoalkaloids are found in all potatoes. Higher level of this toxin is found in the sprouts and the peel of the potatoes that taste bitter. These toxins are produced by the plant due to stress like presence of microorganisms, UV light and damage such as bruising (Delicious Organics Inc, 2004). Solanine is a cholinesterase inhibitor found in the surface of the potatoes exposed to light which turned green from chlorophyll production. Highest concentration is found beneath the peel. Peeling deeply to remove the green splotches and cooking in a steam or water reduces the toxin. Solanine can cause gastric upset and respiratory problems including the fatal depression of the activity of the central nervous system (Pimentel et. al., 1996). Spontaneous abortions in laboratory animals have been reported so pregnant women should avoid green potatoes even the cooked ones with bitter taste (Guilbert, 2001).

Flavonoids

These are phenolic plant pigments generally containing the γ-benzopyrone nucleus. Flavonoids include the anthocyanins, leucoanthocyanins, catechins, aurones and chalcones. Anthocyanins make up the most important coloring matter in plants (Guevarra, 2005). Drewnoski and Carneros (2005) stated that flavonoids that impart the bitter taste found in citrus fruits include flavanones (naringin), flavones (nobelitin) and flavonols (quercitin). In the report of Chang (1990) as mentioned by the same authors, genistin, a bitter and astringent isoflavone glucoside is thought to be responsible for the objectionable taste of soy protein. They also reported that catechin and epicatechin occur in tea. Epicatechin is more bitter than catechin. The bitter taste of chocolate may also be due to catechins, present in higher amounts than in milk chocolates.

Cyanogenic Glycosides

According to Food Standards Australia New Zealand (2004) glycosides are widely distributed in plants. Cassava and bamboo shoots contain cyanogenic glycosides, linamarin and taxiphillin, respectively. Cyanogenic glycosides are the glycosides of the alpha- hydroxynitriles. The toxicity of cyanogenic plant depends on the hydrogen cyanide that may be released upon consumption. If the cyanogenic plant is inadequately detoxified during processing or preparation of food, the potential hydrogen cyanide concentration, which may be released, is high. This compound is contained in kernels of almonds, lemons, limes, apples,

pears, cherries, apricots, prones and palms. Kidney, black-eyed peas and lima beans also contain bitter cyanogenic glycoside.

The other class of glycoside is the mustard oil glycoside or glucosinates, which are found exclusively in plants belonging to the family of Crucifereae. There is abundant evidence relating goiter as a result of excessive consumption of cruciferous plants. Other foods with antithyroid activity include plants in the genus Allium (onion group) and other vegetables. However, it has not been proven that these foods are goitrogenic unless they comprise an excessive high proportion of the diet (Pimentel, et. al. 1996).

Saponin is a glycoside, which occurs in many legumes. According to Mendoza et. al. (1985) these glycosides of hydroxylated steroids or tripenoid tend to depress the growth of experimental animals. The same author reports that cycasin, another toxic glycoside, when fed to rats, is a potent carcinogen producing tumors in the liver, kidney, intestine and lung.

Triterpenoids

According to Drewnoski and Carneros (2005) some dietary phytochemicals are bitter and toxic. They mentioned that some plants in the cucurbitaceae family like cucumbers and zucchini are bitter and inedible due to triterpenes, cucurbitacins or oxygenated tetracyclic triterpenes present in them.

Properties of Phytochemicals

Whitney and Rolfes (2005) mentioned that phytochemicals impart tastes, aromas and colors and other characteristics to foods. The burning sensation of hot peppers, the pungent flavor of garlics and onions, the bitter tang of chocolates and the dark red color of tomatoes are due to the phytochemicals present on them. The same authors further enumerated some phytochemicals found in fruits and vegetables: sulforaphane, cancer-fighting found in broccoli; flavonoid, protection against lung cancer found in apples; ellagic acid of strawberries may inhibit certain type of cancer; carotenoids, lutein and zeaxanthin found in spinach and other colorful vegetables which help to protect the eyes against mascular degeneration. The distinctive taste and smell of celery and its property of lowering high blood pressure and cholesterol levels are given by butyl phthalide (New Zealand Food Authority, 1994).

Natural Toxins

Plants utilized by man as food contain undesirable natural products called toxin. Harborne (1982) as cited by Mendoza et. al. (1985) lists some of the common natural products involved in the interaction of plants, animals and man. These includes proteins (protease inhibitors, amylase inhibitors, hemagglutinins/lectins, allergens); glycosides (cyanogenic glycosides, glucosinolates, saponins, vicine); amino acids (lathyrogens, hypoglycemic agents, mimosine) and others which include cycasin, pyrroligtine alkaloids, gossypol and

antivitamin. Different parts of the plant (stem, roots, leaves, and seeds) may contain varying concentrations of a toxin. The age of the plant also contributes to the difference in concentration. Young plants may contain lesser or more constituent toxins than mature ones (Klaassen, 2001). According to Trefry and Jameson-Jones (2004) plant diseases and environmental stress like drought, heat, cold and mineral deficiencies can affect toxin concentration in plants. Different varieties of the plant have also different concentrations of toxins. Liener (1983) as cited by Mendoza et. al. (1985) points out that evidence for a particular food constituent being toxic to man is only presumptive since most of the research in the toxicity of plant stuff is performed on animals. Such studies revealed the effects as inhibition of growth, decrease in food efficiency, goitrogenic response, pancreatic hypertrophy, hypoglycemia, and liver damage. Food poisoning due to tomatine from green tomatoes and dioscorine from yams have been observed in humans and domestic animals. The toxin in tomatoes causes their bitter taste. The leaves and stems of tomato also contain glycoalkaloids, making them inedible (Elpel, 2005). The toxicity of oxalic acid, which is present in rhubarb, depends on the age of the plant, the season, the climate and the type of the soil. The leaves of the plant contain the highest concentrations of oxalic acid. Poisoning of this type may cause muscle twitching, cramps, decreased breathing and heart action, pain headache, vomiting, convulsions and coma (New Zealand Food Safety Authority, 1994).

Detoxifying Processes

Shemilt (1982) reports cyanogenic glycosides can be detoxified by adequate processing techniques or traditional methods of preparation. A common processing method is heating or cooking of food. Mendoza, et. al. (1985) disclosed that proper cooking and processing should be done in plants containing protease inhibitors to make it safe for consumption.

Lectins can be readily denatured by sufficient heat treatment thus, beans classified as Phaseolus Vulgaris should be heated sufficiently at least 15 minutes in boiling water to make it safe for consumption (Shemilt, 1982).

Salda (1999) reported that many wild yam species possess sapogenins and alkaloids making them inedible. She also mentioned that the simplest method to detoxify tubers is by chipping the peeled tubers into uniformly thin slices, soaking these chips in brine solution and rasping them several times in running water.

Elpel (2005) mentioned that young milkweed shoots contain a toxic, bitter alkaloid that has to be properly cooked out to render the plants safe to eat. He also stated that the proper way of cooking is to plunge the herbs into boiling water for two minutes, drain the water, then repeat the process two more times until all bitterness is gone which makes it safe.

Processes employed to make plants containing cycasin safe for human consumption are fermentation, heating, water extraction or semi- drying (New Zealand Food Safety Authority, 1994).

Brine Shrimp Assay

The brine shrimp assay has been standardized for natural screening of bioactive substances in plant extracts. This uses the larvae (nauplii) of brine shrimp, Artemia Salina Leach. The lethal concentration for 50% mortality after 6 hours of exposure or after 24 hours of exposure is determined as the measure of the toxicity of the extract or compound. The solubility of the extract is of convenience to make the test simple and rapid. The use of the polar extracts would be of shorter exposure, whereas, lower concentrations is achieved in nonpolar extracts require longer time (Colegate & Molyneux, 1993).

Extraction Processes

Constituents of phytochemical and biological interests are soluble in 80% to 95% ethyl alcohol. Organic constituents from the plant can be extracted by alcohol and serial extraction with solvents of increasing polarity (Guevara, 2005). Nonpolar compounds can be extracted by nonpolar solvents like hexane and polar compounds can be extracted by ethyl acetate, a polar solvent (Pladio, 1999).

Extraction is a transfer of one solvent into another by means of distribution process. When a solution is shaken with a second solvent with which it is immiscible, the solute will distribute itself between the two liquid phases. When the two phases have separated again into distinct solvent layers, an

equilibrium situation will be achieved such that the ratio of the concentration of the solute in each layer will define a constant called distribution coefficient. The distribution coefficient has a constant value for each solute and is dependent on the nature of the solvents used in each case. The solute distributes itself between the two solvents so that its chemical activity (effective concentration) is the same in each phase.

Column Chromatography

Column chromatography is used to separate the mixture of two or more different compounds by distribution between two phases (one is stationary and the other is moving). In a solid-liquid phase partitioning technique, the solid are those materials that will not dissolve in the associated liquid phase such as silica gel and alumina. The more polar the functional group, the more strongly it will bind to alumina or silica gel. Elutants or the materials to be separated, move down the column at differing rates on their relative affinity for their adsorbent and the solvents or eluents .The versatility results of the chromatography can be varied due to the following: the adsorbent chosen, the polarity of the solvents chosen, the size of the column (both length and diameter) relative to the amount of the material to be chromatographed and the rate of elution or flow (Pavia et. al., 1976).

Thin Layer Chromatography

According to Guevarra (2005), thin layer chromatography can analyze most of the different classes of characteristic compounds present in the crude dry extract. This also provides semi-quantitative information on the active constituents of an extract enabling an assessment of plant quality. Thin layer chromatography (TLC) is also suitable for comparing different batches of sample plant and can indicate possible adulterations of material. Pavia et. al. (1976) stated that thin layer chromatography is related to column chromatography, however, rather than allowing the moving liquid phase to percolate down the adsorbent, it is allowed to ascend on a thin layer of adsorbent.

Rf value (ratio of front) is a constant value for any compound, corresponding to its physical property.

MATERIALS AND METHODS

Materials

Plant Materials

The plant material used in the study were the young shoots (edible part, about 3 to 4 leaves from the top) and mature leaves (inedible portion) of the passion fruit (*Passiflora edulis Sims.)* as shown in Plate 2.

Plate 2. Shoots of the *Passiflora edulis Sims*

Standard

Table 1 presents the materials used as standards or positive control in the phytonutrient analysis.

Table 1. Standard substances used in the phytonutrient analyses

ANALYSIS	STANDARD (Positive Control)
A. Vitamin	
A	Aflaxin (2500 IU)
E	Myra E 400
B. Sugar	(1 % aqueous solutions)
Molisch Test	Starch
Benedict's Test	Fructose
Barfoed's Test	Lactose
	Sucrose
	Ribose
C. Cyanogenic Glycoside	Cassava tuber peeling

Equipment

The following laboratory equipment were used in the analyses: analytical balance, blender (Plate 3A), separatory funnel (Plate 3B), rotary evaporator (Plate 3C), water bath, refractometer, oven and column chromatography (Plate 3D).

A. Blender (sample preparation)

B. Separatory Funnel (partitioning of crude extract)

Plate 3. Equipment used in the extraction of bitter constituent of the passion fruit

Plate 3 continued…

C. Rotary Evaporator (concentration of solutions under vacuo)

D. Column Chromatography (separation and isolation)

Phytonutrient Analyses of the Shoots of *Passiflora edulis Sims.*

Proximate Analysis

Sample Preparation. Fresh shoots of the passion fruit plant were washed and finely chopped prior to analysis.

Classical methods of analyses (Table 2) were used to determine the moisture, ash, crude fat, and crude protein contents of the shoots of the passion fruit. All analyses were replicated three times.

Table 2. Proximate analysis of the shoots of the passion fruit vine

PROXIMATE ANALYSIS (Wet Basis)	METHOD OF ANALYSIS
% Moisture	Oven- drying
% Ash	Gravimetric
% Crude Protein	Kjeldahl
% Crude Fats	Soxhlet
% Nitrogen free-extract	By difference

*Source: Official Methods of Analysis of AOAC International, Lees, (1975)

Mineral Content Analysis

About 1 kg of the fresh shoots of the passion fruit was sent to the Philippine Institute of Pure and Applied Chemistry (PIPAC), Ateneo de Manila for mineral content analysis. The analytical methods used were Ashing-Acid Digestion/Atomic Absorption Spectrophotometry for Potassium, Calcium, Sodium, Iron and Zinc and Ashing-Acid Digestion/ Spectrophotometry for Phosphate.

Vitamin Analysis

The presence of Vitamin A and E in the sample was determined qualitatively while Vitamin C was quantitatively analyzed. Table 3 presents the different methods used.

Table 3. Vitamin analysis

VITAMINS	METHOD
Vitamin A	Carr-Price Reaction
Vitamin E	Modified Furter- Meyer's Test
Vitamin C	Titrimetric Method/ Oxidation-Reduction

*Source: Biochemistry Laboratory Manual in Benguet State University (2006)

Carr-Price Reaction. The content of Vitamin A capsule, Aflaxin 2500 IU, was transferred in a test tube. This served as first positive control. Another positive control was prepared by mixing two ml of fresh extract with another capsule of Aflaxin. One ml of chloroform was added to each control, afterwards were allowed to cool in an ice bath. Two ml of cold saturated chloroform solution of antimony (III) chloride was then added. Formation of a blue violet precipitate indicates a positive result. The same procedure was applied to two ml of the fresh extract of the shoots.

Modified Furter-Meyer Test. Two positive controls were prepared in separate test tubes, a capsule of Vitamin E (Myra E) and two mL of fresh extract mixed with Myra E. To each test tube, one ml of each chloroform, n-butyl alcohol and concentrated HNO_3 were added, and then was heated in a water bath for ten minutes. A positive result was indicated by the formation of a yellow solution. The same procedure was followed using only the fresh extract sample.

Oxidation-Reduction Titration. Five grams of fresh shoots of the passion fruit vine was homogenized in a blender with 10 mL 1% oxalic acid. The resulting solution was filtered and the filtrate was collected in a 50 ml volumetric flask. A volume of 25 ml filtrate was transferred to a burette. Two ml of 2,6-dichlorophenolindophenol dye was titrated with the solution until the end point, from red to light pink color was attained. The volume of the titrant (filtrate) was

noted. This procedure was repeated for the second and third trial. The vitamin C content was computed using the formula;

$$\text{mg Vitamin C/ g sample} = \frac{(0.3 \text{ mg of Vit. C})(50 \text{ mL})}{(\text{Average vol. of sol'n needed to Consume the dye})(5 \text{ g sample})}$$

Sugar Analysis

The tests used for the determination of sugar presence in the sample are presented in Table 4.

Table 4. Sugar analysis

SUGAR	METHOD
General	Molisch test
Reducing/non reducing	Benedict's test
Monosaccharide	Barfoed's test
% Sugar	Refractometer

Sample preparation. Fresh shoots were homogenized using a blender and the juice was squeezed using cheese cloth. The extract was filtered using an ordinary filter paper.

Molisch test. This was used to determine the presence of all types of carbohydrates. To each of separate ten test tubes ten drops of 1 % of a standard sugar sample (fructose, lactose, ribose, sucrose, and starch) was added. A drop of

Molisch reagent was mixed followed by gradual addition of one ml of concentrated sulfuric acid. The color at the junction of the two liquids was noted. A positive result was indicated by the formation of a violet purple color. The same procedure was followed for the fresh extract and the water soluble fraction of the alcohol extract from the plant sample.

Benedict's Test. This was used to determine the presence of reducing sugars in the sample before and after hydrolysis. Five ml of the fresh shoot extract, water soluble fraction, and starch were first subjected to hydrolysis by adding 5 drops of concentrated hydrochloric acid to each sample and then boiled for thirty minutes. To each ten test tubes containing sugar samples (fructose, lactose, ribose, sucrose, and hydrolysate from starch), two mL of Benedict's reagent was added and mixed. The mixture was boiled in a water bath for three minutes. Formation of precipitate after three minutes indicated the positive result. The same procedure was also followed for the hydrolyzed and unhydrolyzed filtrates from the fresh shoots extract and water soluble extracts.

Barfoed's Test. This was used to distinguish between monosaccharide and reducing disaccharide sugars. To one ml of each standard sugar sample, hydrolysate of the starch, fresh extract and water soluble fraction from the shoots, 2 ml of Barfoed's reagent was added and boiled for three minutes. A positive result is indicated by the formation of the precipitate after three minutes. The

same procedure was employed for the unhydrolyzed fresh extract and water-soluble fraction of the shoots.

Phytochemical Screening for Secondary Metabolites

Sample Preparation

The fresh shoots of the passion fruit were air- dried at room temperature and then ground using a blender. A volume of 450 mL of 80% ethyl alcohol was used to completely submerge about 100 g of the ground plant material. The plant materials were soaked for 48 hours and then filtered using a Buchner funnel. The filtrates collected were concentrated using the rotary evaporator. The filtrates were used in the phytochemical analysis.

Phytochemical Analysis

Phytochemical tests (Table 5) were done to determine the possible class of compounds present in the extracts of the shoots of the *Passiflora edulis Sims*. Procedure used was based from the methods of Guevara (2005).

Table 5. Phytochemical tests using test tuber reactions

TEST	COMPOUND
Mayer's, Dragendorff's and Wagner's*	Alkaloids
Keller-Kiliani	Cardenolides and Bufadienolides
Kedde	Cardenolides and Bufadienolides
Bate-Smith and Metcalf	Flavonoids
Willstatter	Flavonoids
Froth	Saponins
Gelatin	Tannins and Polyphenolic compounds
Ferric Chloride	Tannins and Polyphenolic compounds
Guignard	Cyanogenic glycoside

*Mojab et al, (2003)

Thin- layer Chromatography (TLC) Screening

To confirm the presence of the phytochemicals, thin-layer chromatography was performed on the crude extract using procedures as shown in Table 6 (Guevarra, 2005).

Table 6. Phytochemical tests using Thin- Layer Chromatography (confirmatory test)

COMPOUND TESTED	SPRAY REAGENT	OBSERVABLE RESULT FOR A POSITIVE TEST
Flavonoids Steroids	Antimony(III) Chloride	Intense yellow to orange visible zones appear on spraying for glycosidic flavonoids
Phenols, Tannins Flavonoids	Potassium ferrricyanide-ferric chloride	Blue Spots
Alkaloids	Dragendorff's reagent	Brown-orange visible spots immediately on spraying
Coumarins Anthraquinones, Anthrones, Phenols	Methanolic potassium hydroxide (Borntrager's reagent)	Anthraquinones- orange color Anthrones- yellow Coumarins- blue
Higher alcohols Phenols Steroids Essential Oils	Vanillin- sulfuric Acid	Triterpenes and sterols appear as blue- violet spots Essential oils- zones with wide range of colors
Sugars	α-naphthol-sulfuric acid	Blue spots

Effect of Heat on Cyanogenic Glycoside

To determine the effect of heat on the cyanogenic glycoside content of the sample, the shoots were blanched for 2-3 minutes. The cyanogenic glycoside content of the blanched and the uncooked shoots were tested using the Guignard test. The same test was also performed on samples of uncooked and blanched

mature leaves. The colors of the picrate papers used in the samples and standards were observed and compared. The standard used for positive test was cassava tuber peelings.

Toxicity Test

Brine Shrimp Assay

The larvae (nauplii) of Brine Shrimps were used in the bioassay. The eggs of the Brine shrimps were obtained in a pet shop.

Hatching of Brine Shrimp. The plastic dish and the artificial sea water (3.8 g of rock salt per 100 ml distilled water) were prepared for hatching the shrimp's eggs. Incubation lasted for 36 hours after sowing the cysts in the dish containing artificial sea water.

Dilution Series. Different concentrations (parts per million) of the plant extract were prepared by dilution. Three replications were done for every concentration. Table 7 shows the dilution series.

Table 7. Dilution series

TEST TUBE NUMBER	DILUTION SERIES (ppm)
1	10,000
2	1:10
3	1:100
4	1:1000
5	1:10,000

Assay. The assay began 36 hours after sowing the cysts. Twenty nauplii were collected using a pipette from the hatching dish and transferred using the minimum amount of artificial seawater to a petri dish containing the sample extract. A control containing artificial sea water was used.

The numbers of dead nauplii after 24 hours were counted in every dish. The nauplii were considered dead if they were immobile at the bottom of the dish. A hand lens was used to check the inactivity. To confirm the number of shrimps, nauplii that were alive were also counted.

Series of tests were conducted on the different concentrations of fresh, ethanol, hexane, ethyl acetate and the water-soluble extracts.

Analysis of Results

Extract concentrations were converted to log concentrations to make the wide range of values easier to deal with. The numbers of dead nauplii were counted and the percent mortality (death) was computed. The % mortality versus log extract concentration was plotted and from the equation of the line obtained, the LD_{50} for each sample extract were determined. The LD_{50} is the dose that is lethal to half of the animals tested. Activities or plant toxicity were considered significant if the LD_{50} is less than 30 µg/ ml. or 0.03 mg/ ml (Saupe, 2006).

Extraction and Partial Isolation of Bitter Constituent of the Shoots of the Passion Fruit

The overall process for the extraction and partial isolation of bitter constituent of the shoots of the passion fruit is shown in Figure 1.

Extraction

The plant materials were prepared prior to extraction. Batch of passion fruit shoots were gently washed and air-dried. About 1.8 kg of the air-dried materials was homogenized with 2.845 L of distilled technical grade of ethyl alcohol. This was allowed to soak overnight. The liquid was filtered using buchner funnel and ordinary filter paper.

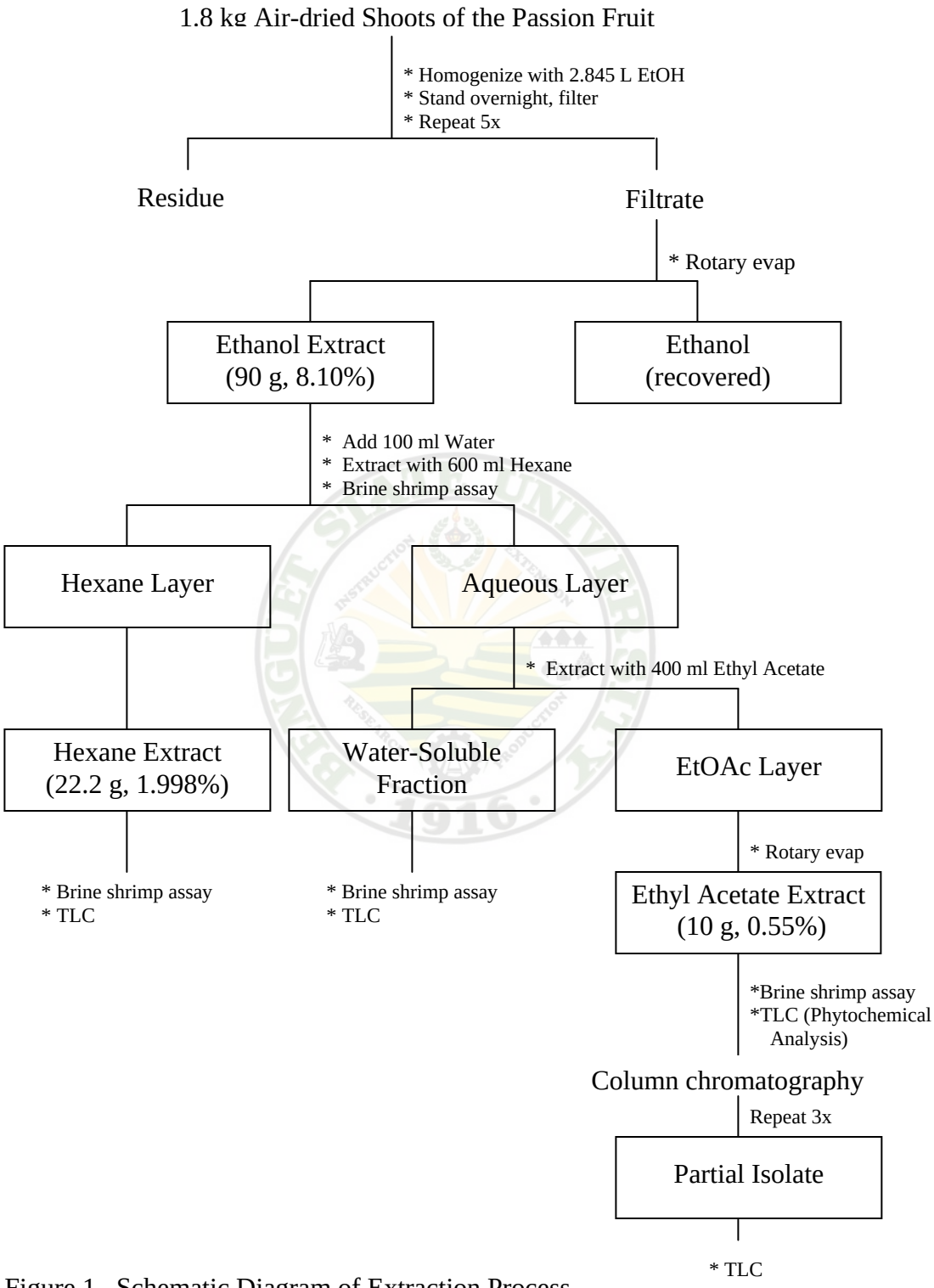


Figure 1. Schematic Diagram of Extraction Process

The filtrate was concentrated using rotary evaporator with the water bath maintained at 40°C. The crude ethanol extract obtained was partitioned using solvents of increasing polarity, hexane (Absolute Reagent) followed by distilled technical grade ethyl acetate. An ordinary separatory funnel was used in partitioning the crude ethanol extract. The hexane and ethyl acetate extracts were concentrated under rota-evaporator. To maximize the yield, the soaking of the plant residue was repeated five times using ethanol recovered from the rota-evaporator.

Monitoring of Bitter Constituent.

To determine the presence of the bitter constituents of the plant, the hexane and ethyl acetate extract were subjected to phytochemical analysis using thin layer chromatography (TLC). The extract that gave the distinct positive result to the flavonoid test was subjected to chromatographic separation.

Isolation (Partial)

To separate the bitter constituent, the extract was subjected to repeated column chromatography using vacuum elution. Two different column sizes, 4.5 and 2.0 cm diameters were used. The column was packed with silica gel 60 G (Merck) to a height of 8.0-cm. Starting with pure hexane and increasing at 5-10% gradient ethyl acetate- hexane mixture up to 100% ethyl acetate, eluates were collected in about 20 ml fraction using test tubes. The collected eluates were

spotted on a TLC and were developed using different concentrations of the ethyl acetate –hexane mixture based on the polarity of the fraction. The spots were first visualized with potassium ferricyanide-ferric chloride and then with vanillin-sulfuric acid. Fractions that showed positive result with the flavonoid test were pooled together and were again subjected to column chromatography using isocratic elution (40% ethyl- acetate hexane mixture).

The Rf value of the spot positive for the flavonoid test was determined by dividing the distance traveled by the component by the distance traveled by the solvent.

$$Rf = \frac{\text{Distance traveled by component}}{\text{Distance traveled by solvent}}$$

RESULTS AND DISCUSSIONS

Phytonutrient Analyses

Proximate Analysis

The proximate compositions of the shoots of *the Passiflora edulis Sims* as shown in Table 8 were determined on a wet basis.

Table 8. Proximate Composition of the Shoots of the Passion Fruit

PARAMETER	COMPOSITION (%) (wet basis)
Moisture	81.03
Ash	1.5
Crude Protein	7.73
Crude Fats	0.9 3
Nitrogen free-extract	8.81

Moisture content. The moisture or water content of the shoots is very high (81.03%), which suggests that it is very susceptible to spoilage. Bennion and Scheule (2000) stated that the perishability of the food is related to its water content. The food with higher water content is more perishable. This relationship occurs because microorganisms require water for their growth. Fresh vegetables

have usual water activity values of 0.95 to 0.99 causing them to be more perishable.

Ash content. The ash content of the shoots is 1.5 %. According to the Official Methods of Analysis of (AOAC) International, the ash content shows the inorganic constituent of the shoots. James (1995) states that the ash content is the residue that remains after the moisture has been removed and the organic materials (fats, protein, carbohydrates, vitamins, organic acids) have been oxidized to volatile materials through ignition at very high temperature. This represents the mineral content of the plant.

Crude protein. The percent crude protein content of the shoots was 7.73%. This value was computed by multiplying the total nitrogen by the protein factor suitable to the food (Portugal et. al., 1997). Total nitrogen was determined by the Kjeldahl method and the protein factor used is 6.25. Aurand and Woods, (1973) mentioned that proteins are essential components of every living cell and are utilized in the formation and regeneration of tissue. They also pointed out that certain proteins serve as enzymes, antibodies and provide functions in metabolic regulation and contractile processes. The percent crude protein value of the passion fruit shoots is low compared to plant foods with relatively high proteins (20% to 30%) such as legumes (Bennion and Scheule, 2000). As reported by Wills .et al, (1989) protein is mostly functional, for example as enzymes rather than storage pool as in grains and nuts. The low value for percent crude protein

implies that the shoot of passion fruit is not an important contributor of protein in the diet.

Crude fat. The amount of the crude fat extracted from the shoots was found to be 0.93%. This value does not represent the true fat content, but rather the lipid fraction of the food which are the constituents soluble in non-polar organic solvents. The crude fat includes fats, phospholipids, sphingolipids, waxes, steroids, terpenes and fat soluble vitamins (James, 1995). As reported by Brody (1994), dietary fats and oils are excellent sources of energy and they contribute to the palatability of the diet. In the study, the value obtained showed that the shoots contain low amount of fats compared to 46% in groundnut as reported by Ezeagu et al, (2006). This means that the shoots are not a good source of energy. Bennion and Scheule (2000) explained that the leaf is actively working or metabolizing part of a plant and does not generally store energy.

Nitrogen free extract or carbohydrates. The nitrogen free extract or carbohydrates according to James,(1995), was calculated "by difference" using the formula suggested by Lees, R. (1975):

% Nitrogen free extract = 100-(%water + %protein + % ash +% fat)

This calculation included the indigestible carbohydrate component as well as other components of the food item that are not measured as water, protein, fat and ash (Portugal et. al, 1997). The computed value of the nitrogen free extract of the shoots was 8.81%. This value is higher than what was reported by Portugal et. al,

(1997) on the carbohydrate content of some green leafy vegetables such as chayote leaves (2.3%), pechay leaves (3.2%) and saluyot leaves (7.5%). Compared to cereals with high carbohydrate contents, the shoots of the passion fruit is not a good source of supplemental carbohydrates.

Mineral Content

The mineral contents of the shoots *of Passiflora edulis Sims* is presented in Table 9. These were determined from the ash content. Differences in composition may occur as a result of the loss of some volatile inorganic constituents such as chlorides during the ashing process and the presence in the ash residue of constituents, such as sulfur in protein (James, 1995). He also classified the minerals as major elements if there values are greater than 100 ppm or 0.01% and trace elements if the values are lesser than 100 ppm or 0.01%.

Table 9. Mineral content of the shoots of the passion fruit

MINERAL	COMPOSITION (ppm)
Potassium	5100
Calcium	980
Sodium	15
Phosphate as P	1070
Iron	22
Zinc	10

Major elements. Based from the results obtained (Table 9), potassium (5100 ppm), calcium (980 ppm) and phosphate (1070 ppm) are considered major elements since their values were higher than 100 ppm. Wills et. al. (1989) stated that potassium is the major mineral found in fruits and vegetables, especially the green leafy vegetables with parsley containing the highest amount. They further reported that health authorities in many countries are urging increased consumption of potassium from fruits and vegetables, to counter the effects of sodium in the diet.

Encyclopedia (2003) gives the importance of the different dietary minerals. Potassium is important for the heart and other muscles. It helps muscle contraction and maintains fluid and electrolyte balances in the body. It also helps transmit nerve impulses and release energy from fat, protein and carbohydrates during metabolism. Aurand and Woods (1973) stated that calcium is most needed during growth periods, pregnancy and lactation. Calcium is also essential for building strong bones and teeth, as well as maintaining bone strength and density. It also plays a role in muscle contraction, blood clotting and maintenance of cell membranes (Encyclopedia, 2003). The same source mentioned that another key element for building strong bones and teeth is phosphorus. It also helps the body release energy from fat, protein, and carbohydrate during metabolism. It also plays a role in the formation of genetic material, cell membranes and certain enzymes.

Trace elements. Sodium (15 ppm), Iron (22 ppm), and zinc (10 ppm) are considered trace elements since they have values lower than 100 ppm. Whitney and Rolfes (2005) stated that sodium is the main cation outside the cells. Sodium is used by the body to produce muscle contractions, maintain fluid balance, conduct nerve impulses, and carry nutrients to cells (Encyclopedia, 2003). Aurand and Woods (1973) pointed out that iron is an indispensable element for the normal function of hemoglobin of red blood cells, myoglobin of heart muscle and the cytochromes of respiration. Finally, the role of zinc, an elemental form of protein is to promote wound healing (Encyclopedia, 2003).

Vitamin Analysis

Shown in Table 10, Vitamin A, E and C were found present in the extracts of fresh shoots of the passion fruit vine.

Table 10. Vitamin content of fresh shoot extract

VITAMIN	TREATMENT		
	Standard	Standard	Fresh extract
VITAMIN A	(Aflaxin)	(Aflaxin +fresh extracts)	
	+	+	+
VITAMIN E	(Myra E)	(Myra E + fresh extract)	
	+	+	+
VITAMIN C			1.43mg/g

Vitamin A. In the Carr Price reaction (Plate 4), it was observed that the shoots gave yellow green and violet precipitate, the same as that of the two standards solutions. The results indicate the presence of Vitamin A in the fresh extract shoot. Aurand and Woods (1973) reported the importance of Vitamin A; plays an important part in vision, essential for the integrity of the epithelial tissues of the body and necessary for normal growth and development. Wills et. al, (1989) reported that prolonged deficiency to Vitamin A can lead to blindness. He also mentioned that Retinol, a vitamin A compound, is not present in vegetables but some carotenoids found in dark green leafy vegetables can be converted to retinol by man.

Plate 4. Vitamin. A analysis

Vitamin E. Result of the Modified Furter –Meyer test for Vitamin E (Plate 5) showed that the fresh shoot extract gave a yellow green to dark yellow solution, which is similar to the results obtained in the two standards used. The yellow colors of the solutions indicate a positive result. Thus, the shoots of the passion fruit contains Vitamin E. This Vitamin as cited by Whitney and Rolfes (2005) is a fat soluble oxidant and one of the body's primary defenses against the adverse effects of free radicals. He also mentioned that it could reduce the risk of heart disease by protecting low density lipoproteins against oxidation. Brody (1994) stated that good sources of Vitamin E are vegetable oils. Dark green leaves and nuts are also rich in this vitamin (Aurand and Woods, 1973).

Plate 5. Vitamin E analysis

Vitamin C. The amount of Vitamin C in the passion shoots is 1.43 mg/g. This value is very low compared to Amti leaves (58mg/g), kangkote leaves (35 mg/g) and chayote leaves (20mg/g) as reported by Portugal (1997). Although Vitamin C is only a minor constituent of vegetables, it has a major importance to man by preventing diseases (Wills et.al, 1989). Vitamin C is important for the gums, arteries and other soft tissues, bone, for nerve and brain function, for nutrient metabolism and for antioxidant defense against free radicals (Kohlmeier, 2003).

Morton (1987) cited that the pulp and seeds of passion fruit are rich in Vitamins A and C. This means edible portions of the passion fruit vine are rich in these vitamins.

Sugar Analysis

Table 11 shows the results of the qualitative sugar analyses performed on the shoots of the passion fruit (*Passiflora edulis Sims*).

General test for all types of carbohydrates. In the Molisch test (Plate 9) for determining the presence of carbohydrates, glycosidic bonds are hydrolyzed by concentrated sulfuric acid to monosaccharides which are dehydrated to furfural, hydroxymethylfurfural and other decomposition products. These products react with alpha-naphthol forming violet color. Although this is not a specific test for carbohydrate because furfural forming substances give positive

result, a negative result is an indication of the absence of carbohydrates (Biochemistry Manual for use at Benguet State University, 2006).

Table 11. Sugar Analysis of the shoots of the *Passiflora edulis Sims*

SAMPLE	QUALITATIVE TEST			QUANTITATIVE TEST
	General Test	Test for Reducing Sugar	Test for Monosaccharide	% Sugar
	(Molisch's)	(Benedict's)	(Barfoed's)	
Standard				
Starch	+	No ppt (-)	Blue, no ppt (-)	
Fructose	+	Red (+)	Red ppt (+)	
Lactose	+	Red (+)	Blue, no ppt (-)	
Sucrose	+	No ppt (-)	Blue, no ppt (-)	
Ribose	+	Red (+)	Red ppt (+)	
Fresh Extract				
Hydrolyzed	+	Yellow-green ppt (+)	Yellow-green ppt (+)	
Unhydrolyzed	+	Yellow-green ppt (+)	Yellow-green ppt (+)	10 ˚Brix
Water-soluble extract				
Hydrolyzed	+	Yellow-green ppt (+)	Yellow-green ppt (+)	
Unhydrolyzed	+	Yellow-green ppt (+)	Yellow-green ppt (+)	15 ˚Brix

Khadem (1988) states that the carbohydrates were first thought to be hydrated carbons but today, it applies to a large number of organic compounds, monomeric, oligomeric and polymeric in nature which do not necessarily have their hydrogen and oxygen atoms in the molecular ratio of 2:1 but which can be either synthesized from or hydrolyzed to monosaccharides. The formation of the violet ring at the junction of the solutions in the Molisch test reveals the presence of carbohydrates in the shoot extracts as shown in Plate 6.

Plate 6. General test for carbohydrate (Molisch Test)

Benedict's test. Reducing sugars are carbohydrates that react with mild oxidizing agents under basic conditions to give an aldonic acid (Bettelheim et.al. 2004). In this test for reducing sugars, the cupric ion was reduced to cuprous ion. The cuprous ion was less soluble thus it precipitated out of the alkaline

(Biochemistry Manual for use at Benguet State University, 2006)). Khadem (1988) revealed that all monosaccharides are reducing but in disaccharides there are reducing and nonreducing. The results of the test reveal the presence of reducing sugar in the shoots. In the test, yellow -green and brown precipitate was formed in the sample since the extracts were colored. The basis for the positive result was the formation of the precipitate after heating in the water bath. These results are shown in Plate 7.

Plate 7. Test for Reducing Sugar (Benedict's Test)

Barfoed's test. Barfoed's test was done to distinguish between the monosaccharides and reducing disaccharides. Results of the test showed that the extracts formed precipitates after three minutes of heating which implied that the sample contain monosaccharide carbohydrates. In contrast, the disaccharide standards gave a blue solution indicating a negative result (Plate 8). In acidic

medium, monosaccharides are more easily oxidized than disaccharides (Biochemistry Manual for use at Benguet State University, 2006).

Plate 8. Test for Reducing Sugars (Barfoed's Test)

Refractometry. The total soluble solid or % sugar content of the fresh crude extract determined was 10° Brix at 20°C and 15.2° Brix at 20°C for the water soluble extract. The Brix value refers to % used as the soluble solid content in a solution such as sugar, salt, protein acid, etc. which dissolve in water. James, (1995) explains that as the light passes from one medium to the aqueous solution, the light rays are refracted. The refractive index of the solution is dependent on the concentration of materials in a solution. Hence, refractive index is used as a measure of the % sugar in a sample or solution.

Phytochemical Screening for Secondary Metabolites.

Literature search reveals that the bitter taste and toxins of many food sources are due to the presence of phytochemicals like alkaloids, flavonoids, cyanogenic glycosides and triterpenes. Phytochemicals which are considered nonnutrient compounds found in plants impart their tastes (Cataldo et. al, 2003). Drewnoski and Carneros (2005) reported some dietary phytochemicals that are bitter, toxic and lethal; flavonoids, cyanogenic glycosides and alkaloids are bitter compounds.

The phytochemical analysis of air-dried shoots extract using test tube reactions are presented in Table 12. The results revealed that only alkaloid, flavonoid, steroids and cyanogenic glycoside are found in the crude extract of the shoots.

Alkaloid

Observation of heavy precipitation (+++) in the Mayer's and Wagner's tests, indicates presence of alkaloids in the sample. The negative precipitation on the Dragendorff's test may be due to interfering factors like the sensitivity of the reagent.

Table 12. Phytochemical analysis (air- dried shoots extract)

COMPOUND	TEST	OBSERVATION	RESULT
Alkaloids	Mayer's	yellow precipitate	+++
	Wagner's test	brown precipitate	+++
	Draggendorrf's	no orange ppt	-
Steroids	Keller-Kiliani	reddish brown color at the interface	+
Flavonoids	Bate-Smith and Metcalf	strong red-violet color	+
	Willstatter	dark brown color	+
Saponins	Froth	froth is less than 1 cm	-
Tannins and Polyphenolic compounds	Gelatin	no precipitate	-
	Ferric Chloride	no black solution	-
Cyanogenic glycoside	Guignard	orange color of picrate paper	+

Flavonoid

The ethanolic exctract of the shoots of the passion fruit gave a positive result for the Bate-Smith and the Metcalf test, which indicates that the one type of flavonoid present in the shoots is leucoanthocyanins. Also, positive result in the Wilstatter test signifies that another flavonoid with an γ- benzopyrone nucleus is found in the shoots.

Cyanogenic Glycoside.

Result of the Guignard test shows that cyanogenic glycoside is found in the shoots and mature leaves of the passion fruit. Color comparison on the change of color in the picrate paper was done to determine the presence of cyanogenic glycosides in the sample. A change in color from yellow to orange of the picrate paper implies a positive result.

The mature leaves have higher amount than the shoots as indicated by the dark orange color of the picrate paper compared to the lighter orange shade obtained from the shoots (Plate 9). This result is confirmed by Klaasen (2001) who reported that the age of the plant contributes to the variability of chemical constituents. He further mentioned that in spring pokeweed, sprouts and young leaves are safer to eat than mature leaves because of lesser toxins.

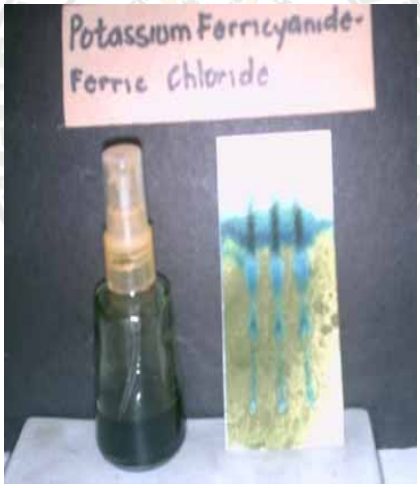
Plate 9. Guignard test for cyanogenic glycoside

Using thin-layer chromatography (TLC), the presence of the same metabolites was confirmed. These results are shown in Table 13. The color of the chromatograms shown in Plate 10 indicates the presence of alkaloids and flavonoids. The intense blue color is for the flavonoids (Plate 10A and B) while the brown orange color (Plate 10C) refers to the alkaloid. Other compounds which tested positive are steroids (Plate 10A), phenols, anthraquinones (Plate 10D), anthrones (Plate 10D), triterpenes, essential oils (Plate 10E) and sugars (Plate 10F).

Table 13 Phytochemical analysis using Thin Layer Chromatography method

COMPOUND TESTED	SPRAY REAGENT	OBSERVATION	RESULT
Flavonoids Steroids	Antimony(III) Chloride	Intense yellow upon spraying	(+)
Phenols, Flavonoids Flavonoids	Potassium ferrricyanide-ferric chloride	Blue Spots	(+)
Alkaloids	Dragendorff's reagent	Brown-orange visible spots immediately on spraying	(+)
Coumarins, Anthraquinones, Anthrones Phenols	Methanolic potassium hydroxide (Borntrager's reagent)	Anthraquinones-orange color Anthrones-yellow Coumarins- no blue color	(+) (+) (-)
Higher alcohols, Phenols Steroids, Essential Oils	Vanillin- sulfuric Acid	Triterpenes and sterols appear as blue- violet spots Essential oils-zones with wide range of colors	(+) (+)
Sugars	alpha-naphthol-sulfuric acid	Blue spots	(+)

A. Test for flavonoids and steroids



B. Test for phenols and flavonoids

Plate 10. Chromatograms for Phytochemical Analysis using Thin Layer Chromatography

Plate 10. Continued…

C. Test for alkaloids

D. Test for anthraquinones, anthrones and phenols

Plate 10. Continued…

E. Test for phenols, steroids, essential oils

F. Test for sugars

Effect of Heat on Cyanogenic Glycoside

Table 14 presents the results of the Guignard test performed to determine the effect of the heat on the cyanogenic glycoside content of the shoots of the passion fruit vine. Plate 11 shows the color change in the picrate paper used. In the uncooked shoots and cassava tuber peeling, the picrate paper changed to orange (+) while there was no change in the cooked sample (-). This shows that the cyanogenic glycoside can be lost during heating or cooking. This finding corroborates the report of Shemilt (1982) that cooking process either by boiling, roasting or sun-drying inactivates the enzyme linamarase found in cassava which liberates hydrogen cyanide.

Table 14. Effect of Heat on the Cyanogenic Glycoside Content

TREATMENT	OBSERVATION (color of picrate paper)
Control (+) cassava tuber peeling	Brick red (+)
Shoots	
a. Uncooked	Orange (+)
b. Cooked	No change (-)
Mature Leaves	
a. Uncooked	dark orange (+)
b. Cooked	No change (-)

Plate 11. Guignard Test for Cooked and Uncooked Shoots

According to Food Standards Australia New Zealand, (2004) all parts of the cassava are cyanogenic and the starch –rich tuber must be properly processed before it can be eaten. Sweet varieties of cassava with low cyanide content can be processed adequately by peeling and cooking.

In cassava when its cellular integrity is disrupted as what occurs in grinding and maceration, a highly specified β- glucosidase catalyzes the hydrolysis of the glycosidic bond; to produce acetone cyanohydrin and glucose are formed. Hydroxynitrilyase or oxynitrilase are the second enzyme catalyzing in the dissociation of cyanohydrin to form HCN and acetone. The acetone and HCN are resembled and volatiles can be leached away with water from the starchy

residue. Drying the residue after sufficient washings or boiling can be used to remove the volatile (Food Standards Australia New Zealand, 2004).

Toxicity Test

Brine Shrimp Assay

LD_{50} (Lethal Dose). Using the brine shrimp assay, the toxicity of the different extracts (fresh, ethanol, hexane, and water- soluble) were determined at different concentrations. Toxicity was measured in terms of $LD_{50.}$ Calculation of LD_{50} is presented in appendix C.

Table 15 presents the percent mortality and LD_{50} obtained from the brine shrimp assay. All the extracts have LD_{50} values higher than 30 ppm, which according to Saupe means that the toxicity of the plant extracts were not significant. This means that the shoots of the passion fruit vine is not toxic. From the table, the water soluble extract has the lowest LD_{50} or the most toxic fraction, but still the value is insignificant. This finding supports the result of the Guignard test which revealed the presence of cyanogenic glycoside, a toxic substance. This substance is maybe responsible for the toxicity revealed in the assay, but the amount present is low, which explains the low toxicity observed.

Table 15. Brine shrimp assay (after 24 hours)

EXTRACTS	% MORTALITY (after 24 hours)					LD_{50} (ppm)
	Concentration (ppm)					
	1	10	100	1000	10,000	
Fresh	10	23.35	43.35	81.65	93.35	1.985
Ethanol	16.65	33.35	45	71.65	96.65	73.45
Hexane	8.35	16.65	38.35	66.65	95	173.38
Ethyl Acetate	15	23.35	48.35	85	98.35	1.824
Water Soluble Extract	16.5	30	55	78.35	98.35	54.203

Extraction of Bitter Constituents of Passion Fruit Vine

Extraction of the Flavonoids

The yield obtained in hexane extract (22.2g or 1.998%) and ethyl acetate extract (10 g or 0.55%) from 1.8 kg of air-dried shoots of the passion fruit vine are very low. This caused the difficulty in isolation of the bitter constituents. The presence of the plant constituents; alkaloids, flavonoids, cyanogenic glycosides and triterpenes in the shoots of the *Passiflora edulis Sims* was revealed by phytochemical analysis and was confirmed using thin layer chromatography except for the cyanogenic glycoside. Phytochemical tests showed that the alkaloid, triterpenes and flavonoids are present in the ethyl acetate extract. From the observable results in the thin layer chromatography, the flavonoid showed a very distinct color spot on the different spray reagents used. This may imply higher amount of flavonoid is present in the extract.

Table 16 shows that the ethyl acetate extract is positive for alkaloid and flavonoid while the hexane extract is negative to both tests.

The sticky brown acetate extract was subjected to chromatographic separation monitored by thin layer chromatography. Fractions that were positive to the flavonoid test were pooled and further subjected to repeated column chromatography to yield the Bb2 fraction. This fraction has a brown to yellow color. Table 17 shows there are several components of the Bb2 fraction. The

flavonoid is indicated by the blue-colored spot upon spraying with potassium-ferricyanide-ferric chloride and red color when sprayed with vanillin -sulfuric acid.

Table 16. Phytochemical Analysis Using Thin Layer Chromatography Method

COMPOUND TESTED	RESULT		
	Hexane extract		Ethyl Acetate Extract
Flavonoids, Steroids	No Intense yellow appear	(-)	Intense yellow appear on sprayi
Phenols, Flavonoids	No blue Spots	(-)	Blue Spots
Alkaloids	No brown-orange spots upon spraying	(-)	Brown-orange spots upon spra
Coumarins	Anthraquinones- orange color	(+)	Anthraquinones- orange color
Anthraquinones,	Anthrones- yellow	(+)	Anthrones- yellow
Anthrones,Phenols	Coumarins- no blue color	(-)	Coumarins- no blue color
Higher alcohols,Phenols	Triterpenes and sterols appear as blue- violet		Triterpenes and sterols appea
Steroids, Essential Oils	spots	(+)	violet spots
	Essential oils- wide range of colors	(+)	Essential oils- wide range of col
Sugars	Blue spots	(+)	Blue spots

Table 17. Characteristics of the Bb2 Fraction

FRACTION	SOLVENT SYSTEM (Ethyl Acetate – Hexane)	RF	COLOR		
			Vanillin-Sulfuric Acid	Iodine Vapor	Potassium Ferricyanide – Ferric Chloride
Bb2	65%	0.883	Violet	Yellow	
		0.567	yellow -green	Yellow	brown
		0.467	Red		blue
		0.367	Green	Yellow	Yellow

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

This research aimed to determine the suitability of the shoots of the passion fruit for vegetable use. The specific objectives were to determine the phytonutrients present in the shoots, determine the toxicity of the shoots and to determine the compound responsible for the bitter taste of the shoots. This study was conducted from April to October 2006 at the Benguet State University, D.O.S.T .and P.I.P.A.C. laboratories.

Varied sample preparations were followed for every analysis performed. Proximate analysis showed that for every 100 g fresh sample, there were 81.03 %, moisture, 1.5 % ash, 7.73 % crude protein, 0.93 % crude fat and 8.81 % nitrogen free- extract.

The mineral content of the shoots were: potassium (5,100) ppm, calcium (980 ppm), sodium (15 ppm), phosphate as phosphorus (1070 ppm), iron (22 ppm) and zin (10 ppm). Ashing-acid digestion and Atomic Absorption Spectrophotometry were used in the analysis.

Qualitative vitamin analysis for A and E were employed to reveal their presence. Vitamin C in the shoots was quantitatively determined and was found to contain 1.43 mg/g sample.

The presence of sugar was also determined using general and specific test for reducing sugars and monosaccharides. The tests showed the presence of

reducing sugar and a monosaccharide. Refractive index was also measured. The presence of sugar was also confirmed in the thin-layer chromatography using the alpha-naphthol as the spray reagent.

Phytochemical analysis revealed that flavonoids, steroids, phenols, alkaloids, anthraquinones, anthrones, triterpenes, essential oils, sugar and cyanogenic glycoside are present in the shoot samples. The bitter taste of the shoots of passiflora maybe attributed to the presence of alkaloids, flavonoids and cyanogenic glycosides. Through cooking, cyanogenic glycoside were volatilized or volatilized.

The toxicity of the shoots was determined by the brine shrimp assay. The assay revealed that the LD_{50} obtained from the different extracts (fresh, ethanol, hexane, ethyl acetate and water soluble extracts) were not significant, since they are higher than 30 ppm. Activity or toxicity is significant if obtained values of LD_{50} is lower than 30 ppm.

The flavonoid, one of the bitter constituent of the shoots was extracted and partially isolated by repeated vacuum column chromatography, monitored by thin-layer chromatography using potassium ferricyanide-ferric chloride as spray reagent.

Conclusion

Based on the results obtained, the following conclusions were drawn:

1. The shoots of the passion fruit is a rich source of phytonutrients such as proteins, carbohydrates, vitamins (A, E and C), minerals and the phytochemicals cyanogenic glycoside, alkaloids, flavonoids, steroids, anthraquinones, sugar and essential oils.
2. There is no risk due to the presence of the natural toxin particularly cyanogenic glycoside because it is easily eliminated through heating or cooking.
3. Brine shrimp assay showed that toxicity of the shoot extracts are insignificant.
4. Phytochemical analysis determined the presence of flavonoid, one of several compounds that impart bitter taste to food. Repeated column chromatography yielded the yellow brown fraction Bb2, whose TLC showed the flavonoid spot to have an Rf value of 0.467. The chromatogram was developed using 65% ethyl acetate-hexane solvent system and spray reagent vanillin sulfuric acid.

Recommendations

Based on the findings and conclusions, the following are recommended

1. The shoots of the passion fruit is highly recommended for vegetable consumption.
2. Studies on the bioactivity of the plant should be done.
3. Further study should be done on the isolation and characterization of the bitter constituents of the passion fruit.
4. Other similar studies should be done on the shoots of other indigeneous plants used as vegetables.

APPENDICES

Appendix A. Preparation Of Test Reagents

Reagents for Proximate Analysis:

- Bromocresol green solution: Dissolve 100 mg bromocresol green in 100 mL ethanol
- Methyl red indicator: Dissolve 0.1 g methyl red in 100 mL methanol
- Receiver solution: Dissolve 40 g of boric acid in about 500-600 ml very hot de-ionized water. Mix and add more de-ionized water to volume of 900 mL. Cool the solution and add 10 mL of bromocresol green solution and 7 mL of methyl red solution. Dilute to 1 L with de-ionized water and mix carefully.
- Sodium hydroxide, 40%: Dissolve 400 g NaOH in water and make up to 1 liter.

Reagents for Phytochemical Analysis

- Ammoniacal Chloroform: To 1 L chloroform add 3.6 mL of 28% ammonia. Add sufficient anhydrous sodium sulphate to take up the water. Filter or decant.
- 1M sulfuric acid: Add 2.8 mL of the concentrated sulfuric acid to enough distilled water to make up 100 mL solution.

- Mayer's reagent: Dissolve 1.4 g mercuric iodide in 60 mL water. Pour the resulting mixture into a solution of 5.0 g potassium iodide dissolved in 10 mL water. Add enough water to make 100 mL .
- Dragendorff's reagent:

  Solution A- dissolve 0.85 g bismuth(III) nitrate in a mixture of 10 ml acetic acid and 40 mL water

  Solution B- dissolve 8 g potassium iodide in 20 mL water

  Stock solution- mix equal parts of Solution A and Solution B. The resulting mixture can be stored in dark bottle for long time at room temperature.

  To prepare the reagent mix 1 mL stock solution with 2 mL of acetic acid and 10 mL water.
- 2M Hydrochloric acid: Add 17 mL of concentrated HCl to enough distilled water to make 100 mL solution
- 0.3 M Hydrochloric Acid: Add 2.5 mL of concentrated HCl to enough distilled water to make 100 mL solution.
- Iron(III) chloride reagent: Dissolve 3 mL of 1% Ferric chloride in 50 mL glacial acetic acid
- Iron(III) chloride solution, 1%: Dissolve 1.0 g Ferric chloride in 100 mL distilled water.

- Sodium Chloride, 10%: Dissolve 10 g NaCl in enough distilled water to make 100 mL solution
- Gelatin-salt reagent: Mix an equal amount of 1% gelatin solution with 10% sodium chloride
- Sodium picrate solution: Dissolve 5 g sodium carbonate and 0.5 g picric acid to enough water to make 100 mL solution
- Potassium-ferricyanide-ferric chloride- Mix equal volume of 1% potassium ferricyanide and 1% ferric chloride solution
- Antimony(III) chloride: Prepare solution of 10% antimony chloride in chloroform(dissolve 10 g of antimony(III) chloride in enough chloroform to make 100 mL solution)
- Methanolic Potassium Hydroxide,: Dissolve 5 g potassium hydroxide in enough methanol to make 100 mL solution
- Vanillin-sulfuric acid: Add 50 mL of concentrated sulfuric acid to 10 mL of 2.5% vanillin in ethyl alcohol. Mix with cooling
- alpha-naphthol-sulfuric acid: Mix 10.5 mL of 15% ethanolic A-naphthol with 6.5 mL concentrated sulfuric acid. Dilute with 40.5 mL ethanol and 4 mL water.

Appendix B. Proximate Analysis

Oven- drying for moisture content. Moisture content was determined on the basis of weight loss of water from the samples after oven-drying at specified temperature. Ten gram of fresh sample was accurately weighed using an analytical balance. The sample was placed in an oven maintained at 105°C and was dried for four hours. The sample was then removed from the oven and cooled in a desiccator and weighed to constant weight.

Gravimetric method for ash determination. Five gram of the fresh sample was weighed in a crucible and then placed in a temperature controlled furnace preheated to 600°C. Heating was maintained for 8 hours. The crucible was removed and was directly transferred to cool in a desiccator. The crucible was weighed until constant weight was obtained.

Kjeldahl method for crude protein. The weighed sample was placed in a digestion tube. Two Kjeltabs Cu 3.5 was added. Twelve ml of sulfuric acid was gently added. A blank was also prepared in the same manner. The sample and the blank were digested until a clear solution was obtained. After digestion, the tubes were removed from the digester, cooled and diluted with water. The tubes were then placed in a Kjeltec (distillation unit). A conical flask containing 25 ml of boric acid (containing bromocresol green and methyl red indicator) was placed under the condenser outlet. Twenty five ml of 40% NaOH was dispensed to the

tubes and distilled for 4 minutes. The ammonium borate solutions formed were titrated with 0.1 M HCl to purplish-gray end-point (James, 1995).

Soxhlet method for crude fat. About 2 grams of dry sample from the moisture analysis was extracted with anhydrous ether in a Soxhlet apparatus for four hours. After extraction, the ether was recovered and the flask with fat was dried in an oven (110°C) for 30 minutes. The weight of the crude fat was obtained.

Thin-Layer Chromatography: Phytochemical Analysis

Preparation of plant extract. Two grams of the dried powdered shoots was defatted with 10 mL hexane and was heated over a water bath for 5 minutes. The solvent was decanted. The defatted plant residue was treated with 10 mL mixture of chloroform and acetic acid (99:1 v/v) and was heated for 5 to 10 minutes over a water bath. This was filtered and labeled as solution A. The resulting residue from solution A was treated with 10 mL mixture of chloroform, methanol and acetic acid (49.5:49.5:1) and was heated for 5 to 10 min. over a water bath. This was filtered and labeled as solution B. The residue from solution B was extracted with 10 ml of a mixture of methanol and water (1:1) and was heated for 5 to 10 minutes. This was filtered and labeled finally as solution C. Solutions A, B, and C were spotted on a TLC plate and visualized with the spray reagents.

Appendix C Toxicity test using Brine ShrimpAssay

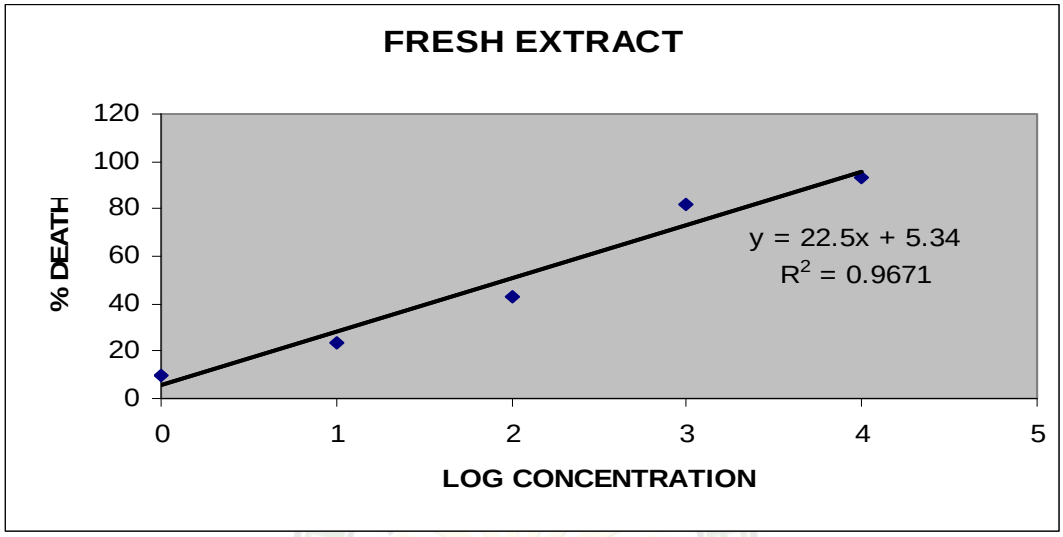


Figure 2. Fresh Extract

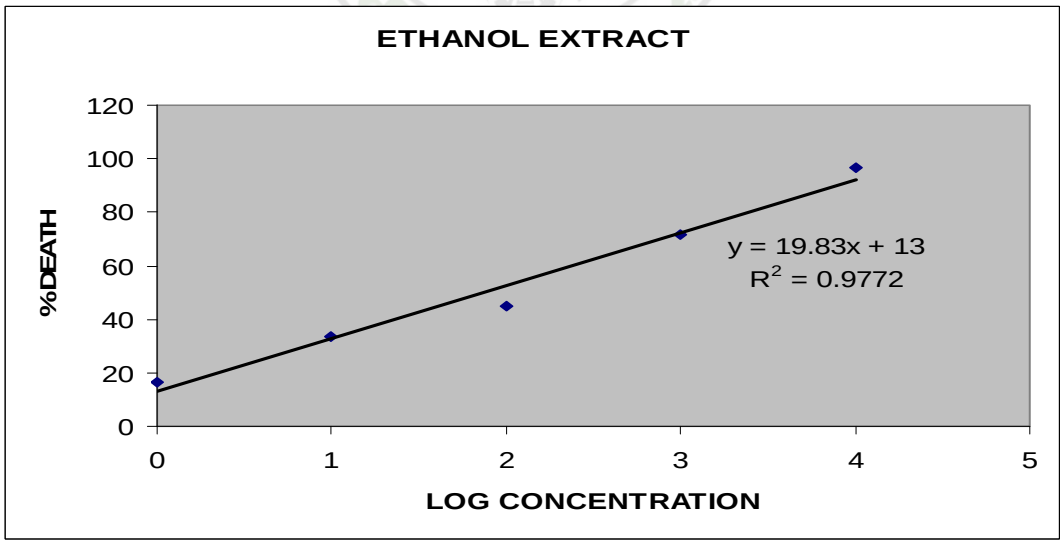


Figure 3. Ethanol Extract

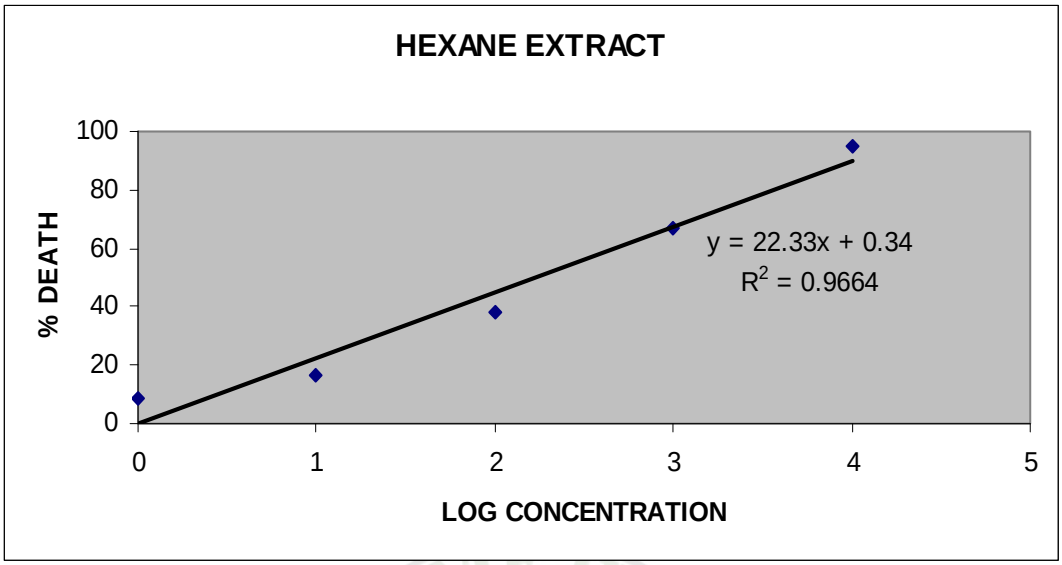


Figure 4. Hexane Extract

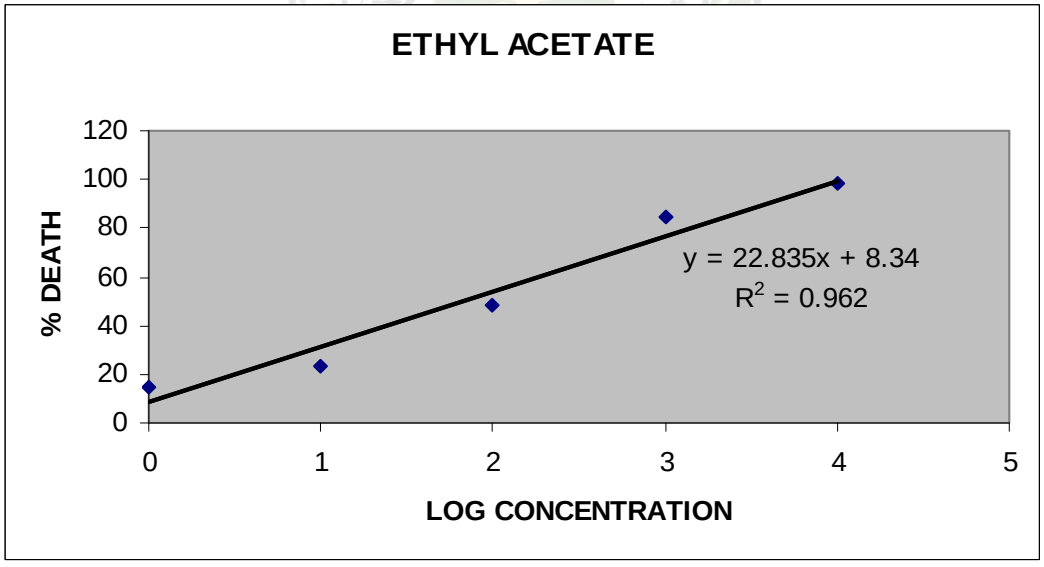


Figure 5. Ethyl acetate Extract

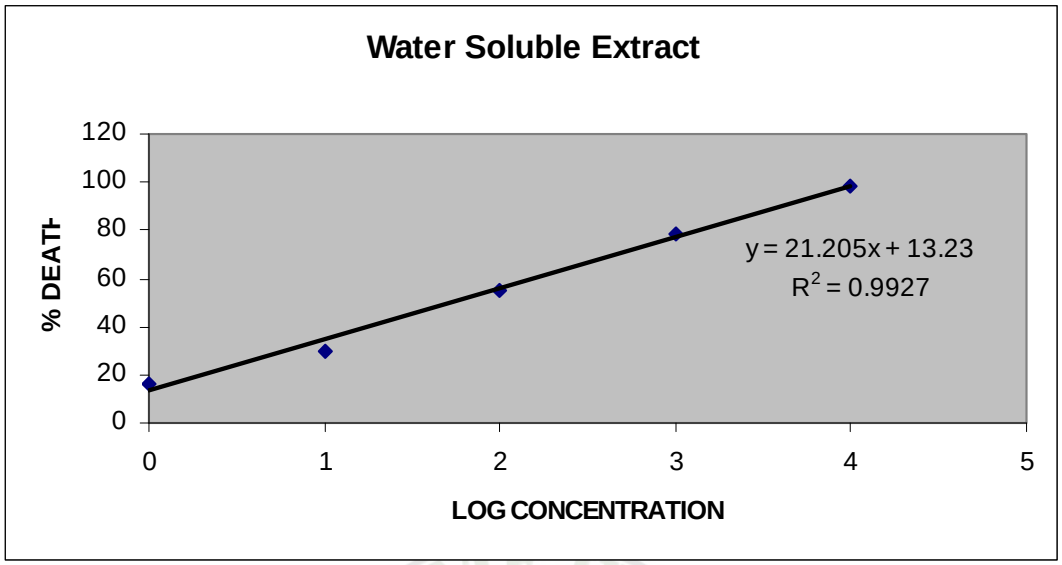


Figure 6. Water Soluble Extract

BIOGRAPHICAL SKETCH

The author was born on November 3, 1977 at Sagada, Mountain Province She is the second child among the four children of Mr. Manuel C. Degay and Mrs. Stella B. Degay .

She finished her elementary education at Bangaan Elementary School. Her secondary education was obtained at MPGCHS- Bangaan Annex (now Bangaan National High School), Sagada, Mountain Province. She was consistently an honor student. In March 1999, she received her degree of Bachelor of Science in Chemistry at Saint Louis University, Baguio City.

On June 2004, she was given the opportunity to teach chemistry subject at Benguet State University for two months as a substitute. One year later, she was again hired for the same position of the University for one semester. She is currently an instructor in chemistry at the College of Arts and Sciences .

She is married to Crispolo N. Bolayo of Tublay, Benguet. They are blessed with three daughters: Craishel, Zai and Chelsea.