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NUTRACEUTICAL PROFILING OF LUPLUPIIT (Parameria laevigata A. JussMoldenke) BARK AND STEM ETHANOLIC CRUDE EXTRACTS

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Abstract

This research was conducted to discover the secondary metabolites present in Luplupiit (Parameria laevigata) bark and stem ethyl crude extract. Many people are using traditional plants which have compounds derived from medicinal plants. However, these plants should be investigated to better understand their properties, safety, and efficacy. Hence, nutraceutical profiling of the plant is conducted to determine its nutraceutical potential. The tests included in the nutraceutical profiling are phytochemical screening, antioxidant screening, and amino acid characterization.

Keywords: Ethanolic crude extract, Luplupiit, Nutraceutical profiling, Parameria laevigata A. JussMoldenke.

1. Introduction

Medicinal plants have long been utilized in traditional times although there were only none or little information is known about the plant's composition that makes them medically useful. In the beginning, medical plants were used instinctively, more on trial and error, and based on experience. People of the past used them without any knowledge of how and why they are effective in curing diseases and illnesses. With the use of advanced technology as well as the advancement of the way of thinking of humans, thorough observation and analysis of medicinal plants have become possible. From the study of Arunkumar & Muthuselvam (2009), approximately 80% of people from developing countries use traditional medicines which have compounds derived from medicinal plants. Nonetheless, these plants should be investigated to better understand their properties, safety, and efficacy.

According to Dr. Tomislav Mestrovic (2022), the nutraceutical is a term used to describe any product derived from food sources with extra health benefits in addition to

the basic nutritional value found in foods. The term "nutraceutical" combines the two words "nutrient", which is a nourishing food component, and "pharmaceutical", which is associated with a medical drug. Nutraceutical products can be considered non-specific biological therapies used to promote general well-being, control symptoms, and prevent malignant processes.

Parameria laevigata A. JussMoldenke is commonly known as "Dugtong-ahas", "Lupiit", or "Luplupiit" in the Philippines and it is found in low to medium-altitude thickets and open forests. It is a member of the Apocynaceae family - a family of flowering plants that includes trees, shrubs, herbs, stem succulents, and vines. *Parameria*leavigata is an evergreen climbing shrub producing stems 10 meters long. The plant is valued mainly for its medicinal virtues and is often harvested from the wild for local use. It also supplies a useful fiber and latex. The bark and branches are sometimes sold for medicinal use in local markets.

Luplupiit is considered to be medically useful, but there are only minimal studies and literature about this plant. With this, the scientific interest of the researchers has been piqued, and thus wants to focus on nutraceutical profiling of plant extracts. The nutraceutical profiling of the Luplupiit bark and stem extract includes phytochemical screening, antioxidant screening, and amino acid characterization.

Materials and Methods

Design

Collection of Test-plant Sample. The plant samples, Luplupiit bark and stem, are bought from Nueva Vizcaya.

Preparation of the Bark and Stem. After gathering the plant samples, the bark and stem of Luplupiit are air-dried before pounding until pulverized.

Extraction of the Ethyl Crude Extract. The pulverized Luplupiit bark and stem are weighed separately on a Mettler balance and then transferred to a beaker. Ethanol is then poured onto the samples. The beakers are covered with gasket paper, and allowed to sit on a fume hood for one to three days before being collected with a funnel, cotton, and triad. The collected extracts are then subjected to a water bath for the alcohol to evaporate and the beaker to contain crude extracts.

Phytochemical Screening. First is the preparation of the developing solvents and reagents: Dragendorff's Reagents and Potassium ferricyanide-ferric chloride. The bark and stem ethyl crude extracts that are spotted on TLC plates are developed in a 5:1 ratio of chloroform (CHCl3) and methanol (MeOH) solvent systems. UV light was

used to visualize the UV active spots corresponding to a certain metabolite. Then, spray reagents were used on the TLC plates to determine the different classes of compounds present in the extract. After that, a preliminary test was conducted using 0.5 % sulfuric acid (H2SO4) dried in hot plate and underwent to Vanillin-sulfuric test (50 ml H2SO4; 2.5 mL Vanilin) to test the presence of sterols, steroids and triterpenes. For the detection of alkaloids, Dragendorff's was used. Potassium ferricyanide-ferric chloride reagent was used for detecting the presence of phenolics. Then, potassium hydroxide (KOH-MeOH) was used to detect the presence of anthrones, coumarins, and anthraquinones.

Antioxidant Screening. First is the preparation of standard by 2-fold serial dilution by preparing a stock solution of 1ppm ascorbic acid then adding 3mL of distilled water. Dilute it into a 20 fold serial dilution by preparing the following concentrations, 0.5ppm, 0.25ppm, 0.125pmm, and 0.0625ppm of ascorbic acid. Take 0.5mL from the 1mL solution place in another container then add water until it reaches 1mL, take again 0.5 mL from the newly prepared solution then add water until it reaches 1mL. Do the same until you reach the last serial dilution concentraion. Next is UV-VIS Spectroscopic Analysis of the Control Solution is conducted by placing in separate cuvettes 200µL each of the concentraions of ascorbic acid. Then add 200µL distilled water, 200µL of Folin-Ciocalteu and 400µL of Na2CO2 solution and placed in another plastic cuvette. Then UV-VIS Spectroscopic Analysis of the Sample Solution was conducted after making the calibration curve, 200µL of 1ppm of the sample is mixed with 200µL of Folin-Ciocalteu and 400µL of Na2CO2 solution in a cuvette. Measure the absorbance of the sample in the spectrophotometer at 680nm. It should be carried out in triplicates.

Amino Acid Characterization. The first step of this test is putting the droplets of the acquired bark and stem ethyl crude extracts on a TLC plate. Then preparation of Locating Reagent was conducted through weighing 0.003g of ninhydrin then putting it in a flask. 100mL of distilled water is then added to it. 3mL of acetic acid is measured and put in a flask, 97mL of distilled water is then added to make it a 100mL solution. Next is preparation of developing solvent by mixing 5mL ninhydrin solution, 5mL acetic acid solution, and 10mL butyl alcohol in an Erlenmeyer flask. The droplets of the samples on the TLC plate are subjected to chromatography. After that, these TLC plates are exposed to heat using the hot plate.

Results and Discussion

Table 1. Secondary Metabolites Tested in Luplupiit Bark and Stem Extracts

Metabolites Tested	Bark Ethanolic Crude Extract	Stem Ethanolic Crude Extract
Essential Oil	+++	++
Anthraquinone	+++	+++
Alkaloids	+++	++
Flavonoids, tannins, and phenols	++++	+++
Phenolics	++	+++
Fatty Acid	+++	++

+: fair amount; ++: good amount; +++: frequent amount; ++++: very frequent amount

Table 1 shows the results of the phytochemical screening of Luplupiit bark and stem ethanolic crude extracts. Both Luplupiit bark and stem ethanolic extracts contain the different metabolites tested, such as essential oil; anthraquinone; alkaloids; flavonoids, tannins, and phenols; phenolics; and fatty acids. Although both contain the tested metabolites, the bark ethanolic crude extract has higher content of metabolites compared to the stem ethanolic crude extracts.

Luplupiit bark has a high content of flavonoids, tannins, and phenols. Containing these phytochemicals means that the Luplupiit bark has antioxidants that can help prevent the development of diabetes, cancer, and cognitive diseases such as Alzheimer's and dementia. From the conduct of the study, the presence of frequent amounts of anthraquinone and alkaloids was detected in the Luplupiit bark which means that it also contains antiviral, antifungal, antibacterial, laxative, insecticidal, and antioxidant properties, which makes it suitable to be used in the treatment of various ailments, such as antimetastatic effects on various types of cancers both in vitro and in vivo (Natural Alkaloids: Basic Aspects, Biological Roles, and Future Perspectives - PubMed, 2014). The study also shows the presence of good amounts of phenolics, which means that Luplupiit's bark is beneficial to human health due to its potential antioxidants, which avert the damage to cells resulting from free-radical oxidation reactions (Kumar & Goel, 2019). Luplupiit also carries fatty acids that play an important role in various bodily functions, including heart health, cancer prevention, cognitive function, skin health, and obesity prevention.

Luplupiit stem ethyl crude extract also carried high content of flavonoids, tannins, and phenols. These phytochemicals mean that the Luplupiit stem has antioxidants that can help in preventing the development of diabetes, cancer, and cognitive diseases. From the result of the study conducted by the researchers, frequent amounts of anthraquinone and a good amount of alkaloids are present in the Luplupiit stem which means that it also contains antiviral, antifungal, antibacterial, laxative, insecticidal, and antioxidants properties that are helpful in the treatment of various ailments such as antimetastatic effects on various type of cancer. The study also shows that the stem of the luplupiit contains frequent amounts of phenolics which are beneficial to human

health due to their antioxidants that avert the damaged cells resulting in free-radical oxidation reactions. The study also detected a good amount of fatty acids that play an important role in various body functions as well as heart health, cancer prevention, skin health, and obesity prevention.

The metabolites present in the stem and bark are essential oil, anthraquinone, alkaloids, flavonoids, tannins, phenols, and phenolics. Based on the study conducted by Binay-an & Chan (2016), phytochemical screening revealed that 'dugtong-ahas' also known as 'lupiit' (*Paramerialeavigata*A. JussMoldenke) methanolic bark has the presence of secondary metabolites such as flavonoids, saponins, tannins, and polyphenols. Thus, comparing the results to the study of Binay-an & Chan (2016), both the bark methanolic and ethyl crude extracts contain flavonoids and tannins.

Table 2. Result of the Antioxidant Screening of Luplupiit Bark and Stem Extracts

Sample	**Absorbance Reading**	**Ascorbic Acid Equivalent (AAE)**
Bark	0.124	16.96 mg in every 1g of sample
Stem	0.140	19.96 mg in every 1g of sample

Table 2 shows the results of the antioxidant screening of Luplupiit bark and stem ethanolic crude extracts. The ascorbic acid equivalent (AAE) or the total phenolics content is 16.96 mg in every 1g of the bark sample and 19.96 mg in every 1g of stem sample. Based on the data, it could be inferred that the stem sample has higher phenolics content compared to the bark sample.

According to Johnston (2013), ascorbic acid or vitamin C plays a role as a redox cofactor and catalyst in a broad array of biochemical reactions and processes. The result from the foregoing table implies that the bark and stem of Luplupiit have ascorbic acid content, albeit in minimal amounts.

Table 3. Result of the Amino Acid Characterization of Luplupiit Bark and Stem Ethanolic Crude Extracts

Amino Acids	Approximate *Rf* value (developing solvent)	*Rf* value of screened AA Bark	*Rf* value of screened AA Stem
Alanine	0.29		
Arginine monohydrochloride	0.15		0.15
Asparagine	0.20		
Cystine	0.12		
Glutamic acid	0.33		0.34-0.36
Glycine	0.11		
Leucine	0.57		0.58
Lysine	0.10	0.08-0.09	
Methionine	0.47		
Serine	0.25		0.25
Tryptophan	0.55		
Tyrosine	0.52		
Valine	0.40	0.40-0.43	

**the Rf values of the screened AA bark and stem are compared to the approximate Rf value using the developing solvent*

Table 3 shows the amino acids and their corresponding approximate *Rf* values which are compared to the *Rf* values of the screened AA (amino acid) bark and stem. The results imply that Luplupiit bark has lysine and valine, while the stem has the following: arginine monohydrochloride, glutamic acid, leucine, and serine. The Luplupiit stem sample has more amino acids screened as compared to the bark sample.

Arginine monohydrochloride is an amino acid that people use for chest pain and various blow flow issues, erectile dysfunction, high blood pressure during pregnancy, and a serious disease in premature infants called necrotizing enterocolitis (NEC). Glutamic acid, on the other hand, is an amino acid used to form proteins and in the body, it turns into glutamate which is a chemical that helps nerve cells in the brain send and receive information from other cells. Leucine can be used by skeletal muscles to give energy during exercise. According to Kobayashi (2015), valine is a white crystalline powder in appearance and is easily soluble in water, but insoluble in ethanol. Valine is an essential amino acid, which means that the body cannot produce it on its own and must be supplemented through dietary sources. It can promote the normal growth of the body, repair tissues, regulate blood sugar, and provide the required energy. Lysine is an essential amino acid, like valine. According to Chen et al (2021), serine or threonine kinases (STKs) play important roles in prokaryotic cellular functions such as growth, differentiation, and secondary metabolism. Its main function is signal transduction.

Luplupiit bark contains lysine and serine, which play an important role in growth, differentiation, and secondary metabolism. The stem of Luplupiit has arginine monohydrochloride, glutamic acid, leucine, and valine, all of which can be used to treat chest pain, give energy to the body, help in sending and receiving signals from the brain, promote normal growth of the body, repair tissues, regulate blood sugar, and provide the required energy for the body.

4. Conclusion

The following conclusions can be drawn from the results of the three tests: phytochemical screening, antioxidant screening, and amino acid characterization. Luplupiit bark and stem can be used to prevent the development of diabetes, cancer, and cognitive diseases. Both the stem and bark of Luplupiit also contain antiviral, antifungal, antibacterial, laxative, insecticidal, and antioxidant properties which can be helpful in treating various ailments such as cancer. Since phytochemical screening is a series of different tests to determine the presence of specific secondary metabolites, various extracts may result in different conclusions, thus using more extracts can have more accurate results. After conducting antioxidant screening of the plant sample, Luplupiit is discovered to have ascorbic acid in each of the extract, bark, and stem, wherein for every 1g of the bark sample, there are 16.96mg of phenolics content and for every 1g of the stem sample, there is 19.96mg of phenolics content. Therefore,

approximately 1.7% of the bark extracts and approximately 2% of the stem extract has an equivalent amount of ascorbic acid. The amino acids detected from the Luplupiit bark extract are lysine and serine while the stem extract are arginine monohydrochloride, glutamic acid, leucine, and valine.

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