Antioxidant activity and phytochemical screening of the methanol, ethyl acetate, and hexane extracts of Lansium domesticum seeds

Adriel Noel Andonaque[1], Novie Dorado[1], Kyle Jeremiah Ledesma[1] and Laureen Manalo[1]

[1] *Philippine Science High School Western Visayas Campus - Bito-on, Jaro, Iloilo City 5000, Department of Science and Technology, Philippines*

Abstract – This study intended to conduct a qualitative phytochemical test and an antioxidant assay on *Lansium domesticum* seeds grown in the Philippines. The seeds were extracted through maceration in methanol, ethyl acetate, and hexane. The antioxidant activity of the seeds were tested by measuring the absorbance of the free radical, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) or ABTS through the use of a UV/Vis spectrophotometer. The results were measured in % free radical scavenging activity, using 2% [g/ml] Vitamin C in distilled water as the positive control.

Introduction. – Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. These can be found in various foods including fruits and vegetables. These certain types of cell damages are caused by free radicals and is also thought to be a factor in some diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration amongst others. [1]

Generally, there are two types of antioxidants: synthetic antioxidants and natural antioxidants. Most antioxidants in commercial use are synthetic, for example tert-butyl hydroxyquinone (TBHQ) butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These have been prohibited by governmental policy because they are carcinogenic when given in vivo. [2]

Plants are potential sources of naturally occurring antioxidants and have garnered interest as possible sources of antioxidants and other biological active substances. These include ascorbic acid, benzoic acids, carotenoids, cinnamic acids, flavonoids, folic acid, tocotrienols and tocopherols [3].These antioxidants prevent free radical damage and reduce the risk of chronic diseases. The search for newer natural antioxidants has been increasing, namely those belonging to plant origin. [4]

Lansium domesticum, otherwise locally known as lanzones is a tropical fruit indigenous to throughout the entire Southeast Asia region. According to several studies, lanzones have been known to exhibit many medical properties but it is said to be underutilized here in the Philippines [5]. Its skin and leaf extracts are antimalarial [6], anti-melanogenic which is for the regulation of skin pigmentation [7], and antipyretic which is used in the prevention of fever [8].

A study by Klungsupya et al (2015) made use of *Lansium domesticum* grown in Thailand which underwent ethyl acetate fractionation of the peel and maceration extraction using 50% aqueous ethanol. The total phenolic content and total flavonoid content was then solved for. The results showed a high potential for antioxidants. This study aims to determine the antioxidant activity and the phytochemicals found in the methanol, ethyl acetate and hexane extracts from the seeds of *Lansium domesticum* found in the Philippines.

Methods. – The Methods is composed of two main parts; During the phytochemical testing a change in color to brownish- black indicated the presence of phenols [10], a colorless sample indicate the presence of flavonoids, and a foam layer indicates the presence of saponins [10, 11].

The subjects of the study are limited to extracts from the seeds of a variety of *Lansium domesticum* found in the Philippines. The seeds will be used instead of the fruit itself because these fruits are edible while these seeds are considered as wastes. Additionally, the samples used for the 10% concentration was prepared separately from the other concentrations using a different batch of samples. This was done due to time constraints. The different batches of lanzones were assumed to be of similar species and grown at similar conditions.

Chemicals. Ferric chloride(5%), sodium hydroxide(10%), hydrochloric acid (5N), Wagner's reagent, manganese dioxide (70%), and phosphate buffer (pH 7; 01.M) were obtained from Philippine Science High School- Western Visayas chemistry stockroom. Ascorbic acid was purchased from a local drug store and 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) diammonium salt (ABTS) was purchased from iChemical. Methanol, hexane, and ethyl acetate were purchased from Patagonian Enterprises. All chemicals were stored at room temperature away from direct sunlight.

Sample Preparation. Two batches of lanzones weighed eight kilograms in total. *Lansium domesticum* seeds were dried at 40-45C for 12-16 hours in an oven. The dried samples were then ground, crushed and then sifted. 38g of the sample was added to three identical amber reagent bottles with 190mL of solvent in each bottle- one with hexane, another with methanol and the last with ethyl acetate- making the solvent to dry weight ratio 5:1.

Evaluation of phytochemical screening. A phytochemical analysis was conducted to test for the presence of selected phytochemicals that are related to this activity. The samples were allowed to soak for approximately 24 hours while being frequently agitated before being subjected to the phytochemical analysis.

The phytochemical screening was done in accordance to the methods indicated by Tiwari et al (2011) with slight modifications. Phenols were tested by preparing a stock solution (1 ml) which was pipetted into a test tube and 3-4 drops of 5% ferric chloride solution was added. A change in color to brownish- black will indicate the presence of phenols.

Flavonoids was tested using a stock solution (1 mL) which was pipetted into a test tube. Using a dropper, 10% sodium hydroxide solution was added drop by drop until an intense yellow colour appears in the test tube. 5N hydrochloric acid was then added into the mixture drop by drop until the liquid turns colorless to indicate the presence of flavonoids.

Saponin test was done with a stock solution (1 mL) that was placed in a graduated cylinder and diluted with 20 mL of distilled water. It was then shaken by hand for 15 min. If a foam layer of roughly 1 cm appeared on the top of the test tube, which indicates the presence of saponins.

Alkaloid test was done with a stock solution (1 mL) which was placed in a test tube and 5-15 drops of Wagner's reagent was added. The presence of a brown-colored precipitate indicated the presence of alkaloids

Evaluation of antioxidant capacity. The various extracts were tested on their ability to scavenge the 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic-acid),or ABTS+ radical cation. The assay will be conducted as stated in studies by Bayoumi et al. and El-Sherbeni et al. Prior to the the ABTS+, a 7.0 pH 0.1 M phosphate buffer was made. The ABTS+ was prepared by mixing of ABTS solution (0.1g/100mL) with of manganese dioxide (25mg/mL) in a 2:3 ratio both prepared in the phosphate buffer (pH 7; 0.1M) and mixed in an amber reagent bottle. The mixture was then centrifuged at a little above 2000 RCF for 10 mins. The supernatant was collected and it was allowed to stand in the dark inside an amber reagent bottle wrapped in tin foil in until the absorbance was stable.

Prior to the assay, the remaining mixture of solvent and sample after the phytochemical analysis was filtered out and then allowed to evaporate under a fume hood until each solvent was at the same volume. The 120 mL mixture was reduced by 83.33% to give a 20mL solution. Different concentrations (50%, 25%, and 10%) of the reduced mixture was prepared by making 1:1 extract to buffer mixture and served as the stock solution. The extracts were diluted with methanol instead of the mother solvent, this is to allow the more nonpolar solvents, hexane and ethyl acetate, to mix with the buffer.

A 0.2 mL stock solution was pipetted into 1.8 mL of the ABTS+ solution in a the cuvette. Measurements were be taken as soon as the extract is placed, after 1min, 5min, 15min, and 30min. A blank sample was run without ABTS, using only the phosphate buffer. 0.2 mL of 1:1 solvent to buffer ratio was used instead of the stock solution for the negative control. All measurements were done in triplicate and the means of the measurements were used in the final equation.

The antioxidative activity of the tested samples will be calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$E = ((Ac - At)/Ac)100 \quad (1)$$

where: At and Ac are the respective absorbance of tested samples and control. ABTS+ radical scavenging activity will be expressed in percent scavenged.

Results. – This study aims to determine the antioxidant activity and the phytochemicals found in the methanol, ethyl acetate, and hexane extracts from the discarded seeds of *Lansium domesticum*.

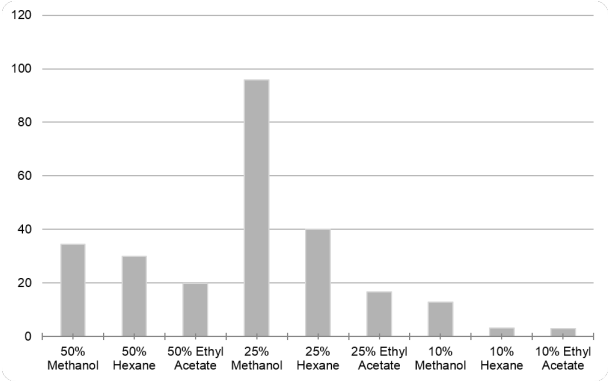

Figure 1: Summary of antioxidant activity in every concentrations and solvents

		Phenols	Flavonoids	Saponins	Alkaloids
First Batch Samples	Methanol	-	+	+	-
	Ethyl Acetate	-	+	-	-
	Hexane	-	-	+	-
Second Batch Samples	Methanol	-	-	+	-
	Ethyl Acetate	-	-	-	-
	Hexane	-	-	+	+

Figure 2: Results of the phytochemical tests performed on the seed samples collected in September-October (First Batch Samples) and March/April (Second Batch Samples). + = positive; - = negative

Discussion. – *Phytochemical tests.* The results are in contrast to the study of Solidum which conducted a phytochemical screening on lanzones found in Manila. The study showed that lanzones was positive for alkaloids for all the samples which was only present in the hexane crude extracts in this study. Saponins were also not present in the samples of the study.

There was no color change to brownish-black in any of the samples which indicates an insignificant amount of phenols found in the sample. This may have been due to the prolonged soaking period of the first batch and the short soaking period of the second bath.

The concentrations used in this study are not based on the concentration of the sample in the solvent. Rather, the remaining mixture of solvent and sample after the phytochemical analysis was filtered out and then allowed to evaporate to 16.67% of the original amount of solvent. The concentrations use this mixture as the basis of the 100% concentration.

ABTS assay. The radical scavenging activity of ethyl acetate yielded the smallest percentages out of all 3 extracts reaching 20% free radical scavenging activity on both 25% and 50% concentrations and 3% free radical scavenging activity at 10% concentration after 30 minutes of reacting with ABTS+. Hexane on the other hand, had the second largest yield with 30% free radical scavenging activity at 25%, and 50% concentrations and 3% free radical scavenging activity at 10% after 30 minutes. The methanol extracts had the strongest free radical scavenging activity in all three concentrations. For the 25% concentration, free radical scavenging activity reached 90% at 15 minutes and stabilizing there until the 30 minute mark. Free radical scavenging activity reached 34% for the 50% concentration and 13% for the 10% concentration by the 30 minute mark (refer to Figures 1, 2, and 3).

Throughout the conducting of this study, there has been much room for error. The solvents ethyl acetate and hexane are very volatile and this study was conducted in small amounts so some of these results may have been affected by constant error especially the results from the 10% concentrations.

Dead End. – More comprehensive testing is required in order to verify the potency of lanzones as potential source of antioxidants. Based on the results of the study, the following courses of action are recommended:

- To measure the strength of each phytochemical in each extract.
- Focus on either measuring different concentrations at one time or one concentration at different times.
- Investigate the difference of saponins found in the methanol and hexane extracts or why saponins were not found in the ethyl acetate extract.
- Investigate the difference of phytochemical content and antioxidant activity based on the different seasons of lanzones.
- Produce a crude extract before conducting the assays.
- Try smaller concentrations to check for optimal absorbance.

Conclusion. – More compounds with antioxidant properties can be found in the methanol extracts of *Lansium domesticum* seeds than in the hexane and ethyl acetate extracts and that Lanzones can be a viable source of antioxidants. More comprehensive testing is required in order to verify the effectiveness of lanzones as potential source of antioxidants.

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