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Evaluation of Anthocyanin in *Amaranthus Gangeticus* L. (Nurawsuraw) Leaf Extract as Hair Colorant

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ABSTRACT

This study was conducted to Evaluate Anthocyanin in *Amaranthus gangeticus* (Nurawsuraw) leaf extract as hair colorant. Spectrophotometric analysis screening was done to measure the absorbance of anthocyanin and the amount of light that passes through a sample solution at different wavelengths in percentage solutions.

Spectrophotometric analysis showed that the detected highest absorption of anthocyanin was indicated by the highest peak in less percentage solutions at maximum wavelength from which anthocyanin absorption can be found, while the lowest peak in higher percentage solutions was at the minimum wavelength with least absorbance.

The hair colorant effect of the *Amaranthus gangeticus* (Nurawsuraw) leaf extract being mixed with the developer was viewed on the microscope and was observed by a desired coloration occurred on hair sample, which appeared to be red in color. In comparison to the hair colorant effect of the commercial hair color, it produces thick violet coloration to hair strand. Therefore, it is concluded that *Amaranthus gangeticus* (Nurawsuraw) could be used as natural hair colorant.

Keywords: *Amaranthus gangeticus (Nurawsuraw), anthocyanin, hair colorant*

INTRODUCTION

Hair coloring is the practice of changing the color of the hair. The main purposes of this practice are cosmetic to cover gray hair, to change to a color regarded as more fashionable or desirable, or to restore the original hair color after it has been discolored due to old age or aging and by hair dressing process, or sun bleaching.

Hair dyeing which is an ancient art involves treatments of the hair with various chemical compounds. Hair coloring involves the use of chemicals capable of removing, replacing and/or covering up pigments naturally found inside the hair shaft. Hair dyeing is now a multibillion dollar industry that involves the use of both plant-derived and synthetic dyes.

A dye can generally be described as a colored substance that has an affinity to the fiber, fur or hair. Synthetic hair coloring products are widely used and are known to cause problems such as breakage of hair stands, loss of hair, dry scalp, and risk of cancer (Zheng et al., 2002). Currently, there is a popular trend in using pigments and colorants from plant extracts as substitutes for synthetic colorants, because natural pigments and colorants are non-toxic and non-mutagenic, have desirable pharmacological properties (Lazzé et al., 2003). Amaranthus gangeticus L. (Nurawsuraw) may be a good source of natural hair colorant.

It has long been known that plants containing colors are usually those that are of great benefit to human health. One suggestion for this is the inclusion of colored plants, which contain compounds having a number of beneficial effects to human health. Such types of compounds include the anthocyanin.

Anthocyanins, the natural colorants that belong to a group of plant compounds called flavonoids, are responsible for the most of the red, orange, purple, violet, magenta and blue colors in fruits, vegetables, legumes, flowers, and other plants, which explains their name, in Greek: anthos , means flower; and kyanos, means blue (Markaris, 1982).

METHODOLOGY

For effective interpretation of data gathered the researcher used experimental design under laboratory condition, determining the physical properties as well as the secondary metabolites and evaluating the Anthocyanin present in Nurawsuraw leaves.

The leaves were washed, strained, and then weighed. The samples were pounded using a mortar and pestle, for every 100 grams of the sample, the extracted juice were filtered through a fine cloth.

Physical Properties Determination

The color of the Nurawsuraw leaf extract was identified using the sense of sight of a total of five respondents. About five (5) mL of Nurawsuraw extract was contained into a clear transparent test tube. Then, using the sense of sight, the respondents described the color of the extracts. The obtained perception of the color from the respondents was gathered and analyzed for proper color determination.

Odor determination was tested on the extracts of Nurawsuraw using the olfactory sense of a total of five respondents. About five (5) mL of extracts was contained into a clear transparent test tube. Then, using the sense of smell, the respondents described the odor of the extracts. The obtained odor from the respondents was gathered and analyzed for proper odor determination.

The density was determined by weighing a known volume of the extract. It was computed using the formula:

$$density = \frac{mass}{volume}$$

pH was determined by dipping the pH meter into a 50 mL beaker containing 5 mL of the extract. The reading was recorded in three replicates.

The solubility of the prepared extract of the Nurawsuraw was determined by using 2 mL of extract was placed in three separate test tubes and each test tube was added with 2 mL of different solvents. The solvents used were hexane, distilled water, and ethanol. After a given hour the researcher recorded the results whether it is miscible, slightly miscible or immiscible.

Determination of Secondary Metabolites

The presence of secondary metabolites such as Alkaloid, Anthocyanin Flavonoid, Leucoanthocyanin, Tannin, and Terpenoidwere determined from the leaf extract of Nurawsuraw separately using the procedures of Guevara (2005) as follows:

Test for the Presence of Alkaloid

In this test, the Dragendorff's reagent and Mayer's reagent was used to determine the presence of Alkaloid. A positive result indicates the presence of orange precipitate in Dragendorff's reagent and a white precipitate in Mayer's reagent. An equivalent of 20 grams of Nurawsuraw leaf extract was placed in an evaporating dish. Then, it was evaporated to a syrupy consistency over a steam bath. Five (5) mL of 2M hydrochloric acid (HCl) was added. Next, it was heated with stirring for about 5 minutes and cooled. Then, about 0.5 g of sodium chloride (NaCl) was added. Then, it was stirred and filtered. The residues were washed with enough 2M HCl to bring the filtrate to a volume of 5 mL, and then the filtrate was separated into two parts. To the first part, 2-3 drops of Dragendorff`s reagent was added and to the other part, 2-3 drops of Mayer`s reagent was added

Confirmatory Test for Alkaloid

To the remaining 3 mL of the filtrate, enough 28% ammonia was added until the solution is alkaline in litmus. The alkaline solution was extracted three times in small portion of less than 10 mL chloroform. The chloroform extracts were combined and the upper aqueous layer was reserved. The chloroform extract was evaporated to dryness under the fume hood and over the water bath. The five (5) mL of 2M HCl was added to the residue and stirred over a steam bath for about two minutes and cooled and filtered. Then the filtrate was divided into 2 portions. One portion was tested with Dragendorff's reagent and the other portion was tested with the Mayer's reagent. The results obtained were recorded.

Test for the Presence of Anthocyanin

In this test two(2) mL of extract was added to 2 mL of 2 N HCl and NH_3. The appearance of pink red or blue violet indicates the presence of anthocyanin.

Confirmatory Test for Anthocyanin

The presence of anthocyanin in the extract was confirmed by performing the following test (Harbore, 1973).

One mL of the extract was mixed with 2 N HCl and heated for 5 minutes at 100°C. When the extract remained the stable purple (Magenta) color confirmed the presence of anthocyanin.

Test for the Presence of Flavonoids

In this test, about 2 mL of the extract was added for a few drops of dilute NaOH solution. A yellow precipitate appeared in the test tube of dilute acid that indicated the presence of Flavonoid.

Test for the Presence of Leucoanthocyanin: Bate-Smith and Metcalf method

A two (2) mL of Nurawsuraw extract was evaporated into incipient dryness over a steam bath then cooled at room temperature, the residue was defatted by adding 9 mL hexane and water. The hexane was discarded. Then the defatted aqueous layer was diluted with 10 mL of 80% ethyl alcohol. It was filtered and divided into two test tubes.

One portion of the above alcohol filtrate was treated with 0.5 mL conc. hydrochloric acid (12M), and was observed for any color change. It was warmed up for 15 minutes in a water bath. It was observed for further color change within an hour and was compared with the control. The results were recorded. A strong red or violet color indicated the presence of leucoanthocyanins.

Test for the Presence of Tannin

The gelatin test was used to determine the presence of tannin. The formation of the jelly precipitate indicated the presence of tannin. Ten (10) grams of Nurawsuraw Leaf extract was formulated as stock extract and was evaporated to incipient dryness over a steam bath. Then residue was extracted with 20 mL of hot distilled water and it was added with 5 drops of 10 % sodium chloride solution. Then it was filtered and the filtrate was divided into three test tubes. One portion was the control. Then the aqueous solution tannic acid serves as a reference standard. Then one portion of the filtrate of the plant extract was treated with three drops of gelatin salt reagent. Do likewise to the tannic acid solution, and the reference standard. Then it was compared with the control and the reference standard.

Confirmatory Test for Tannin

Gelatin test was used for the confirmatory test of tannin. Formation of white precipitate indicated the presence of tannin.

Preparation of the Gelatin salt reagent

Equal amount of 1 % gelatin solution 10 % NaCl solution were combined to prepare the gelatin salt reagent. A portion of the extract was treated with three drops of gelatin-salt reagent. The same procedure was done to tannic solution which is the reference standard.

Test for the Presence of Terpenoid

Terpenoid was determined by treating the leaf extract with acetic anhydride and concentrated H_2SO_4. Formation of blue, green ring indicates the presence of Terpenoid. About 2 mL of the extract was added to 2 mL of acetic anhydride and 2 drops of concentrated sulfuric acid. The results were recorded.

Absorbance Determination

Extract Preparation

The researcher used spectrophotometric analysis to determine the Anthocyanin content in Amaranthus gangeticus (Nurawsuraw) using UV-Vis spectroscopy. A solution of 10% solution was formulated as stock solution. This sample preparation was repeated in two other trials of plant extract. To check whether Beer's Law was obeyed, five more solutions of lower concentration (1%, 2%, 3%, 4%, and 5%) were prepared from the stock solution.

The Spectrophotometer was warmed up of about 15 minutes. The wavelength settings were noted on the point where the sample absorbed the moderate amount of incident light. With the sample compartment closed, the adjustment knob was turned to zero until the display register indicated 0% transmission. The sample chamber where the sample tube was inserted was opened and the reference solution (or blank) of about ¾ full was inserted. The sample chamber was shut off and the knob was turned to 100% T adjustment until the needle read 100% transmission (zero on the absorbance scale). The adjustment was repeated until no further changes were necessary. The sample tube filled with sample solution of about ¾ full was inserted in the chamber, and then the chamber was closed. The absorbance was recorded. Anthocyanin can be measured with different readings in spectrophotometry, which is why different wavelengths were used. Measuring the absorbance at different wavelengths provides an absorption spectrum for the pigments and colorants in the extracts. Because different pigments and colorants have different absorption spectra, pigments and colorants can be tentatively identified by their absorption spectra (Ross, 1974).

Preparation for Natural Hair Color

In preparing natural hair color, 25g of henna was used. Henna serves as a bleaching agent. Henna will not lighten the color of the hair, but it may tent or highlight. It was mixed with the developer by 1:1 ratio. The developer is the oxidizing agent that allows the hair color to do its job. Then it was applied to hair sample within one (1) hour until the hair turned to brown in color from which it was covered with aluminum foil. Then after an hour it was rinsed with water and it was dried for another 30 minutes letting the hair very dry. Then 10 ml of extract was used and were mixed with 10mL developer and it was subjected directly to unpigmented hair, and it was sun dried. The sun rays worked with the hair strand giving the hair beautiful low lights/ undertones in shades of color. (Kyley Zimmerman et al), then it was observed in a compound microscope. It was compared with the commercial hair color. The results were recorded. (Madhusudan Rao et al).

RESULTS AND DISCUSSION

Physical Properties

After conducting, the following results were obtained from the evaluation of anthocyanin in *Amaranthus gangeticus* L. (Nurawsuraw) leaf extract as hair colorant. Based on the study the following physical properties were observed on the extracts of Nurawsuraw leaf.

It was observed by most of the respondents that the color of the Nurawsuraw leaf extract was red-violet and its odor was unpleasant.

It can be claimed from the data above that the Nurawsuraw leaf extract has a density of 0.9 g/ml. Average density was computed by adding all three trials and then dividing it by the used trials which was three.

It was observed that the pH of the Nurawsuraw leaf extract was neutral based from the used pH meter which resulted from an all 7.2 readings in the three trials. This means that Nurawsuraw leaf extract is neutral and is neither acidic nor basic.

Using three different solvents, it was determined that the Nurawsuraw leaf extract has a polar constituents which was observed by its miscibility in water and ethanol solvents which are extremely polar compounds and its immiscibility in hexane which is non- polar compound.

Table1. Summary of Physical Properties of Nurawsuraw Leaf Extract

Physical Properties	Observed Results
Color	Red-violet
Odor	Unpleasant Odor
Density	0.9 g/mL
pH	7.2 (Neutral)
Solubility	Immiscible (in Hexane)
	Miscible (in Ethanol)
	Miscible (in Water)

Secondary Metabolites Characterization

The tests for the presence of secondary metabolites were done under laboratory conditions. After extraction, the extracts of the Nurawsuraw leaf were then subjected to different tests. The results were validated and interpreted below by the following tables.

The extracts were found to be positive in alkaloid which was observed by the formation of orange and white precipitates both on Dragendorff's and Mayer's reagents, respectively. This result implies that the Nurawsuraw leaf extract can be a source of nitrogen bound alkaloid compounds. Once the presence of alkaloid is established other tests are used to identify if the alkaloid is really present, hence confirmatory test for Alkaloid was done.

Nurawsuraw leaf extract was tested for the presence of anthocyanin by observing pigment color which resulted from the formation of pink and violet coloration when it was treated with HCl and NH3 which was the indication of the presence of anthocyanin.

The results showed that the mixture was stable in color and did not lose color when boiled. A purple color was observed

when adding HCl to the extract, which indicated the confirmation of anthocyanin in Nurawsuraw extracts.

The flavonoid screening test for the leaf extract of Amaranthus gangeticus (Nurawsuraw) was done in three trials. All the three trials showed the same result, which resulted to the formation of yellow color appeared in a test tube when it was treated with dilute NaOH solution. This clearly indicates that Nurawsuraw is positive from the presence of flavonoid.

It was observed that Nurawsuraw extract was subjected to Leucoanthocyanin test. All the three trials showed the same results, which resulted to violet coloration after it was treated with 0.5 HCl and was evaporated to incipient dryness over a steam bath. This clearly indicates that Nurawsuraw is positive on the presence of leucoanthocyanin.

The extract of Nurawsuraw was subjected to tannin screening tests. The extracts were found to be positive in tannin which was observed by the formation of white precipitates in gelatin salt solution.

It was observed from the test for tannin in the leaf extract of Amaranthus gangeticus in resulted to the formation of white precipitate when it was treated with gelatin-salt solution. This clearly indicates that Nurawsuraw is positive from the presence of tannin.

It was observed from the test for terpenoid that the extract did not exhibit a blue or green coloration once it was treated with acetic anhydride and conc. sulfuric acid at the end of the procedure which indicated that terpenoid was not present in the leaf extract of Nurawsuraw.

Table2. Summary of Secondary Metabolites Characterization

Secondary Metabolites	Results	Interpretation
Alkaloid	White ppt. formed when treated with Mayer's reagent and Orange ppt. formed when treated with Dragendorff's reagent	Positive
Flavonoid	Yellow ppt. formed when treated with dilute NaOH	Positive
Anthocyanin	Formation of purple coloration when treated with HCl and was heated	Positive
Leucoanthocyanin	Violet coloration when treated with HCl	Positive
Tannin	Jelly ppt. formed in gelatin salt solution	Positive
Terpenoid	No formation of blue or green rings	Negative

As such, these five secondary metabolites were the most important because alkaloid when characterized can be a source of antimicrobial and antiviral compounds which can be further improved to make medicines. Anthocyanins have become increasingly important to the food industry as alternatives to artificial colors have become widespread and knowledge of their health-promoting properties has become more evident. Flavonoid acts as flavorants, colorants and antioxidants. Also goes the same with Tannin which acts as an astringent in making ink, and in dyeing, same with Leucoanthocyanin which is classified as flavonoids.

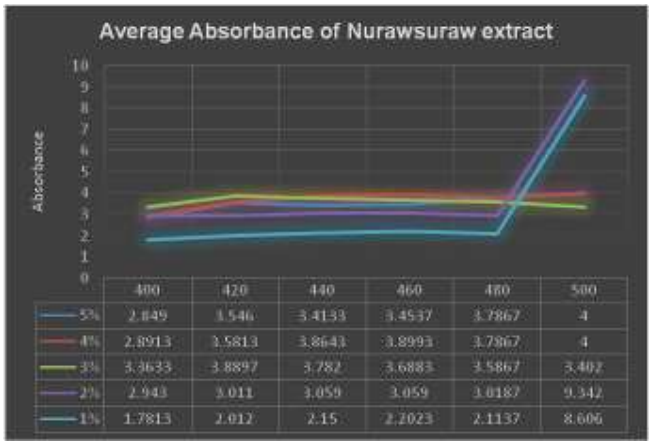
Absorbance Determination
Spectrophotometric Analysis

Table 3 presents the absorbance determination of anthocyanin in the leaf extract of Amaranthus gangeticus (Nurawsuraw) detected at certain wavelength at light energy absorbeb and transmitted.

Table3. Average Absorbance of Nurawsuraw extract in UV light in three trials

% Nurawsuraw extract solution	Wavelength (nm)					
	Average Absorbance for three trials					
	400nm	420nm	440nm	460nm	480nm	500nm
5%	2.849	3.546	3.4133	3.4537	3.7867	4
4%	2.8913	3.5813	3.8643	3.8993	3.7867	4
3%	3.3633	3.8897	3.782	3.6883	3.5867	3.402
2%	2.943	3.011	3.059	3.059	3.0187	9.342
1%	1.7813	2.012	2.15	2.2023	2.1137	8.606

In this table, it showed that in a 1% to 2% solution in turn held higher absorbance compared to 3%, 4% and 5% solution in 500 nm which was the absorption spectrum for anthocyanin. It only showed that among the percentage solution, the lower concentration which is 1% solution, the higher the light it absorbs and the absorbance value is greatercompared to higher concentration which is 5% solution by which it absorbs less light and the absorbance value is lesser.



	400	420	440	460	480	500
5%	2.849	3.546	3.4133	3.4537	3.7867	4
4%	2.8913	3.5813	3.8643	3.8993	3.7867	4
3%	3.3633	3.8897	3.782	3.6883	3.5867	3.407
2%	2.943	3.011	3.059	3.059	3.0187	9.342
1%	1.7813	2.012	2.15	2.2023	2.1137	8.606

Figure1. Average Absorbance of Nurawsuraw extract

In this graph it can be viewed that the detected highest absorption of light was indicated by the highest peak at maximum wavelength in less percentage solutions, while the lowest peak was at the minimum wavelength in higher percentage solutions with least absorbance. It can also be viewed on the table that there is a uniform peak from 400 nm to 480 nm. In less percentage solutions which is 1% and 2% solutions it deflects from a wavelength of 400 to 500 nm. It can be further observed that the absorption peak in a wavelength 500 nm tends to be shifted towardsthe long wavelength region and the absorption peaks tend to be larger (Masayoshi Nakahara).The larger its value, the greater the absorption. Because according to Beer's law, absorbance is directly proportional to the concentration. .

The values of the wavelength of maximum absorbance and minimum absorbance show that the capability of absorbing visible light is strongly affected by anthocyanin and by solvent composition.

Sample Application to Hair Color

The prepared extract of Nurawsuraw was subjected to the unpigmented hair. About 10 ml of Nurawsuraw extract was used and it was mixed with 10 mL developer.

Study on the dyeing effect

The formulated dye was applied over black hair sample, the hair that was being bleached and dried. It was observed for thirthy (30) minutes, and it was set outside for the sunlight. Then it was dried and it was viewed in a compound microscope and also the commercial hair color was applied to other portion of the hair to compare its difference. The plant extract produces the desired coloration to the hair which appeared to be red in color when it was viewed on the microscope. In comparison to the hair colorant effect of the commercial hair color, it produces thick violet coloration to hair strand when it is viewed on the microscope. This clearly indicates that *Amaranthus gangeticus* (Nurawsuraw) could be used as a natural hair color since the produced hair color from plant extract gives pigments to hair strand sample.

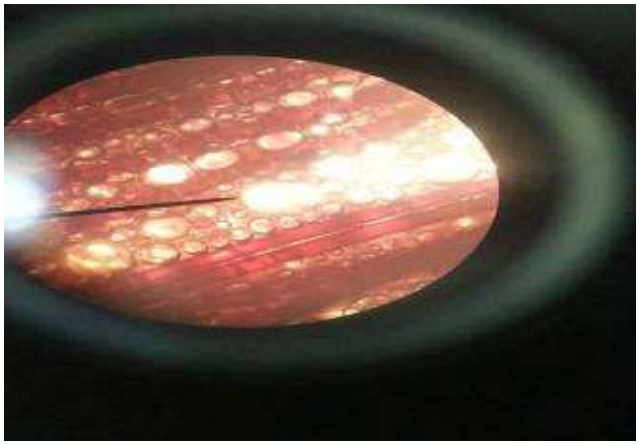


Figure2. Microscopical Observation of the Nurawsuraw Extract as Hair Colorant

CONCLUSIONS

The Physical Properties of *Amaranthus gangeticus* (Nurawsuraw) leaf extract is violet in color, has unpleasant odor, has a density of 0.9 g/mL, a neutral pH of 7.2 and is polar in its chemical constituents. The Secondary Metabolites that were present in Nurawsuraw are the Alkaloid, Anthocyanin, Flavonoid, Leucoanthocyanin, and Tannin. Only Terpenoid is negative on the leaf extract. In absorbance reading using spectrophotometric analysis, the detected highest absorption of anthocyanin is indicated by the highest peak in 500 nm in less percentage solution indicated strong anthocyanin absorption. *Amaranthus gangeticus* (Nurawsuraw) leaves can be extracted and can be used as hair colorant.

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