Atsuete (*Bixa orellana* L.) Seeds and Alugbati (*Basella alba* L.) Stem Ethanolic Extract as Potential Hair Dye Tested via Spectrophotometry

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Abstract

Coloring agents were obtained from natural sources such as plants and animals. The biosphere is gifted as more than 500 plant species which yield natural dyes. With the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has undoubtedly once again gained interest and momentum. This study aimed to analyse the level of absorbance and (%) transmission of Atsuete seeds and Alugbati stem dye extract through spectrophotometer as potential hair dye substitute synthetic hair dye. Parallel group design was used in this study with three treatments groups and one control group and was subjected to spectrophotometric analysis at 200nm, 250m, and 400-550nm. Results showed that Treatment3 is the most potential hair dye coloring agent because it absorbs less light from ultraviolet to visible light and this may protect the hair strands from being brittle due to light absorption of color applied in the hair stands. Further, Treatment3 has the highest transmission among the treatment and control groups with transmission of 6.028%-6.300% at 200-250nm respectively; and 99.46% at 400-550nm. High Transmission property of Treatment3 can protect the hair from phototendering due to light when applied by this dye. Generally, this study found that combination of dyes from plant can enhance light transmission to help protect hair structure and color from sunlight exposure and that atsuete and alugbati have a potential as hair dye. Recommendation for home-made use for the said extract is recommended.

Keywords: Absorbance level, Alugbati stem, Atusete seeds, Percent Transmssion, Spectrophotometric analysis
INTRODUCTION

Coloring agents were obtained from natural sources such as plants and animals. The biosphere is gifted as more than 500 plant species which yield natural dyes. Coloring agents of these plants are derived from leaves, roots, trunks, barks, or fruits. Natural dyes are environmental friendly, nontoxic, non-carcinogenic, non-allergic and biodegradable substance, which have high regenerating, compatibility and renewable source [1]. However, the replacement of natural dyes could happen until the introduction of synthetic dyes. [2]. With the advent of widely available and cheaper synthetic dyes in 1856, the use of natural dyes with poor to moderate wash and light fastness declined drastically and was replaced by more moderate to excellent color fastness properties of synthetic materials [3].

In the research conducted in 1970, it reported the use of eighty indophenol – which are inorganic coloring matters [4], and indamine synthetic dyes evaluated for their substantivity to human hair, their fastness to light, and the stability to storage of the dye solution. Research of Tucker published in 1971 is one of the research evidences that synthetic dyes are being promoted in color/dye industry and over-throwing natural dyes.

Despite the market competition between natural dyes and synthetic hair dyes, natural dyes made a way to be in demand again due to environmental and health issues pose about by the use of synthetic dyes. A published article in 2012 reports that the demand for natural dyes in the modern world is increasing day by day and back-to-nature trend is being practiced by the developed world [5]. In fact, in 1995 Food and Agriculture Organization (FAO) reported that the food sector is now experiencing a trend back towards natural colorants. This change has not been driven by the food industry but by consumers in developed countries who are concerned over possible health risks associated with synthetic food additives [6].

Natural dyes are now a days in demand not only in textile industry but in cosmetics, leather, pharmaceuticals [7] food [7] [3], handicrafts articles, drawings [3], and cotton fabrics [1] [2]. Natural dyes from plants have been given much interest in recent years due to the threat and harmful effects raised by synthetic dyes and environmental awareness created by researchers [2]. With the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has undoubtedly once again gained interest and momentum. [7]. Today, an undeniable fact is that there are increasing demands for natural dyes in textile industries all over the world [3]. The aspect of producing products without negatively altering ecological balance, affecting both human and environmental health, is an important focal point to be pursued [9].

Extracted dyes from plants undergo several testing for its effectiveness to color materials and retain its color even for a long period of exposure to sunlight. This property of dyes is called as color fastness to light. Colorfastness testing is used to determine the loss and change of color [10]. Some dyes can exhibit a reversible colour loss (change) on exposure to light [11]. Colorfastness to light is an essential quality for any dye/fabric combination intended for sunlight exposure for a significant length of time. One need only look at garments or fabric that has been in a shop display window to see that fading due to sunlight is a problem for most fabric dyes [12].

With the interest of producing natural dyes that has low fading, machine has been used to study dye from plant extracts. Nearly all natural dyes will fade badly during an exposure to articial light, or to a much smaller dose of daylight [13]. It is of great importance to manufacturers of all types of materials and
colorants that the effects of radiation, particularly visible and ultraviolet, can be measured in some quantitative way [Pugh].

Nowadays, dyes are being subjected to spectrophotometric analysis. Spectrophotometric analysis was introduced in 1941 [14] and spectrophotometry is mainly used for the measurement of specific substances and aggregate parameters in water and wastewater. However, other applications for physical parameters like measurement or characterisation of color had also been reported [15]. Some other uses of spectrophotometric analysis in research field are in biostain [16] [17], anti-oxidant [18], spray dye [19], dye for solar cell [20], and bixa dye for food that is tested for absorbance level; and optical property test of biosynthesized silver nanoparticles [21]. This study aimed to conduct spectrophotometric analysis on the different ratio mixture of Atsuete (Bixa orellana L.) seeds and Alugbati (Basella alba L.) stem ethanolic extract as potential source of hair dye.

Atsuete (Bixa orellana L.). Bixa orellana L., a representative of Bixaceae is rich in bixin and nor-bixin pigments. These pigments were proved to be non-toxic, non-carcinogenic and non-mutagenic. The pigment was found to be a potent antioxidant as well as bactericidal against opportunistic bacteria [Nathan]. Further, Atsuete has been documented that it contains important amounts of tocotrienols, tocopherols, terpenes and flavonoids both in the seed level and in the leaves. Vitamin E is the generic name given to all compounds that exerts the biological functions of a-tocoferol. Atsuete is widely cultivated in all tropical regions around the world [22], thus, availability is not a problem. Two thirds of the production is commercialized as dried seeds and the rest as colorant. Latin America produces 60% of the total world production, followed by Africa (27%) and Asia (12%) [22].

On the other hand, Alugbati (Basella alba L.) a plant that thrives in tropical Asia, Africa, Malaya and is found cultivated throughout the Philippines. Basella alba fruit with dark blue skin and deep red violet flesh is a potential source of natural colorant. [sk reshmi]. Different studies have proved that the plant is rich in vitamin A and vitamin C along with flavonoids, saponins, carotenoids, many amino acids and organic acids. Various in vivo and in vitro studies revealed that the plants is enriched with active substances/principles having medicinal potential. Physical and biological properties of Basella alba stem mucilage has studied and reported that mucilage is mainly a polysaccharide with pH ranging between 5.3 to 5.4, contains Dgalactose as a major monosaccharide and exhibits slow swelling capacity, mild antioxidant activity and no tyrosinase inhibiting activity [24]. These two plants will be utilized for the production and formulation of potential hair dye that can replace synthetic hair dye in the market.

OBJECTIVES OF THE STUDY

This study aimed to analyse the level of absorbance and (%) transmission of Atsuete seeds and Alugbati stem extract through spectrophotometer.

Specifically, this study aimed to:

1. determine the absorbance and transmission of treatment groups and control group at 200-250nm;
2. determine the absorbance and transmission of treatment groups and control group at 400-550nm; and
3. determine if there is a significant difference exist between treatment groups and control groups in terms of absorbance and transmission.
MATERIALS AND METHODS

Research Materials

The research materials were Atsuete (Bixa orellana L.) seeds and Alugbati (Basella alba L.) stem collected at the locale of San Jacinto and brought at National Museum Botany Division for authentication. Plate 1 shows the certification of the plant used in this study. One kilogram (1kg) of each plant was set aside for the extraction process.

Other research materials such as the Bremod synthetic hair dye was bought in the cosmetics market. Spectrophotometer was used to determine the level of absorbance and transmission at 200-250nm and at 400-550nm.

Research Design

Table 1. The Parallel Group Design

Table 1 shows the research design used in this study. This research design is known as Parallel Group Design which composed of three treatment groups and one positive control group. Each treatment groups and control group have their three replicates.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Atsuete</td>
</tr>
<tr>
<td>Treatment 1 (1:1)</td>
<td>10mL</td>
</tr>
<tr>
<td>Treatment 2 (1:2)</td>
<td>10mL</td>
</tr>
<tr>
<td>Treatment 3 (2:1)</td>
<td>20mL</td>
</tr>
</tbody>
</table>

General Procedures

a. Ethanolic extraction of Dye stuff

One kilogram of Atsuete (seeds were macerated with 1200mL of ethanol for three days, afterwards it was extracted and evaporated until the pure extract is acquired. The same process was done to Alugbati (Basella alba L.) stem. This was performed at Virgen Milagrosa University Foundation San Carlos City, Pangasinan.

Plate 1. Certificate of Plant Authentication

Plate 2. One kilogram of Atsuete and Alugbati and laboratory materials
b. Collection and Stocking of dye for treatment groups and control group

Table 2. Mixture Ratio of Atsuete and Alugbati dye extract

Obtained dye from Atsuete and Alugbati was prepared by dividing into three formulation ratio shown in Table 2.
- T1 = 10mL of Atsuete dye extract + 10mL of Alugbati dye extract (1:1 ratio);
- T2 = 10mL of Atsuete dye extract + 20mL of Alugbati dye extract (1:2 ratio);
- T3 = 20mL Atsuete dye extract + 10mL Alugbati dye (2:1 ratio).

Stocking of the dyes was carried out at San Jacinto National High School, San Jacinto, Pangasinan (See Plate).

c. Spectrophotometric Analysis

Using Spectrophotometer, the researchers tested the level of absorbance and transmission of Atsuete-alugbati dye and Bremod synthetic dye at 200nm, 250nm, and at 400-550nm [12][25][27][28]. Effect of light to colorfastness of dye has been tested through light exposure that can reach up to 10 hours before the dye is rated as ‘satisfactorily’ [4].

Plate 1. Collecting and Stock formulation of dye per Treatment

Plate 4. Researcher with their consultant conducting Spectrophotometric Analysis

d. Statistical Analysis

Average mean was employed to raw data from spectrophotometer. Average mean data was then subjected to One way Analysis of Variance (ANOVA) to determine if there is a significant difference exist between treatment groups and control group in their absorbance and transmission at 200 and 250nm and spectrum at 400-550nm.
RESULTS AND DISCUSSIONS

Spectrophotometric Analysis of Atsuete-alugbati dye and Bremod synthetic dye

Table 3. Mean Level of Absorbance of Atsuete and Alugbati dye and Bremod synthetic dye

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Treatment groups</th>
<th>+Co group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>200nm</td>
<td>2.7336</td>
<td>1.9913</td>
</tr>
<tr>
<td>250nm</td>
<td>2.063</td>
<td>1.986</td>
</tr>
<tr>
<td>400-550nm</td>
<td>3.448</td>
<td>1.776</td>
</tr>
</tbody>
</table>

Table 3 shows that at 200nm wavelength, Treatment 3 has the lowest absorbance level followed by Treatment 2 and Bremod dye respectively. Treatment 1 has the highest absorbance level at 200nm. At 250nm, Treatment 3 has the lowest absorbance level still followed by Treatment 2 and Treatment 1 respectively. Bremod dye has the highest absorbance level at 250nm.

Further, the table shows that in visible light wavelength at 400-500nm, Treatment 3 still has the lowest absorbance level and again followed by Treatment 2 and Bremod dye respectively. Treatment 1 has the highest absorbance level of visible light at 400-550nm.

The higher the absorbance level, the faster the color fading and higher risk of photodegradation [25] and phototendering [11]. Thus, the researcher implied that Treatment 3 – combination of 20mL of Atsuete and 10mL of Alugbati –, is the most potential dye to be used as hair coloring agent because it absorbs less light and will not be too sensitive to light that can cause fading [25]. Further, the table shows that Treatment 3 has decreasing absorbance level in increasing wavelength. Therefore, the researcher can say that exposure to sunlight will not fade the color produced by Treatment 3 fast. Furthermore, Treatment 3 can be used as coloring agent compared to Bremod synthetic dye.

Analysis of Variance showed there is a significant difference exist between the treatment groups and control group in terms of absorbance level at 200nm (F(0.05; 3,8) Fc 22.67>Ft 4.07); 250nm (F(0.05; 3,8) Fc 13.04>Ft 4.07); spectrum at 400-550nm (F(0.05; 3,8) Fc 4.95>Ft 4.07). Generally, Scheffe’s test revealed that Treatment 3 gives the significance.

Table 4. Mean Percent Transmission of Atsuete and Alugbati dye and Bremod synthetic dye

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Treatment groups(%)</th>
<th>+Co group</th>
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<tbody>
<tr>
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</table>

Table 4 shows that Treatment 3 has the highest light transmission at all wavelength used in this study. In the ultraviolet wavelength, approximately 6% of light was transmitted. This is highest among the experimental treatments and most importantly higher than the synthetic dye with transmission percentage ranging only from 0 to 0.748% at 200-250nm. Further, in the visible light wavelength, Treatment 3 was able to transmit 99.46% of the light compared to synthetic dye which only transmitted 0.732% of light at 400-550nm.

The results in the table 4 further justify that Treatment 3 is the most ideal dye as coloring agent presented in this study that can be used rather than bremod synthetic dye. High Transmission property of Treatment 3 can protect the hair from phototendering [13] when applied by this dye.
CONCLUSIONS AND RECOMMENDATIONS

The researchers conclude the following:

1. Treatment3 is the most potential hair dye coloring agent because it absorbs less light from ultraviolet to visible light.
2. Treatment3 has the highest transmission among the treatment and control groups with transmission of 6.028%-6.300% at 200-250nm respectively; and 99.46% at 400-550nm. High Transmission property of Treatment3 can protect the hair from phototendering when applied by this dye.

The researchers recommend the following:

1. Atsuete and Alugbati dye extract should be used as alternative hair dye coloring agent.
2. Higher amount of Atsuete dye than Alugbati dye extract should be formulated to decrease light absorbance and increase light transmission than can last for long period of time in sunlight exposure.

ACKNOWLEDGMENTS

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REFERENCES


