Formulation and Evaluation of Hair dye from Atsuete *(Bixa orellana L.)* Seeds and Alugbati *(Basella alba L.)* Stem

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Abstract

The quest for alternative and organic products has always been common in the community. Parallel group design was used in this study with three treatments groups and one control group and was subjected to spectrophometric analysis, substantivity, and acceptability evaluation. Using Atsuete seed and Alugbati stem, the researchers formulated and evaluated a semi-permanent hair dye from these plants that could be used as alternative hair dye. The researchers evaluated the aborbance level of the Atsuete-alugbati hair dye via spectrophotometer and found that the Treatment 3 (20mL Atsuete seeds dye with 10mL Alugbati stem dye) formulation yield for the least absorbance of light (therefore can protect the hair strands from photodegradation and phototendering). Compared to Bremod synthetic hair dye in the market, Treatment3 has generally decreasing absorbance level of light in increasing wavelength while Bremod synthetic hair dye has generally increasing absorbance level of light in increasing wavelength. Further, the researcher evaluated the substantivity level (a property of hair dye to retain its color after eight washes) of the hair dye and Treatment3 was rated as 'Moderately Substantive' while Bremod synthetic hair dye was rated as 'Not Substantive'. Furthermore, Atsuete-Alugbati hair dye was found to be generally 'Highly Substantive'. With this, the use of Atsuete-Alugbati hair dye for home-made use is recommended.

Keywords: Acceptability Evaluation, Alugbati stem, Atsuete seeds, Spectrophotometric Analysis, Substantivity Evaluation

INTRODUCTION

Natural dyes are derived from natural resources; these are broadly classified as plant, animal, mineral, and microbialdyes. Natural dyes can be used for dyeing almost all types of natural fibers [1]. Among the Egyptians, there were hairdressers and the art of dyeing hair with vegetable dyes was known already at that time. The first artificial dye was synthesized in the laboratory in 1856, and permanent hair colorants have been in commercial use for over 100 years [2]. The replacement of natural dyes could happen until the introduction of synthetic dyes. [3]. 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 [1]. But, natural dyes from plants have been given much interest in recent years due to the threat and harmful effects arised by synthetic dyes and environmental awareness created by researchers [3].

Natural dyes are now a days in demand in cosmetics, [4] food [4] [1], handicrafts articles, drawings [1], and cotton fabrics [5] [3]. 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 [3]. 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. [4]. The aspect of producing products without negatively altering ecological balance, affecting both human and environmental health, is an important focal point to be pursued [6].

Today most of the human beings are very careful about their beauty and hairs play an important role in this. Nearly 70% of human beings above 50 years struggle with the problem of graying of hair [7]. There has been a significant occurrence of premature graying specially in women, attributable probably to stress and use of synthetic shampoos. Loss of color in hair is due to varied reasons like genetic influence, effect of environmental factors, use of alcoholic preparation and etc. [2]. Though the permanent and semi-permanent synthetic hair dyes are available in varied colors and ranges retain natural luster, they have the chief disadvantage of producing hypersensitive reactions in some individuals. Some hair dyes marketed as natural dyes contains 1% to 3% Phenylenediamine which is a synthetic hair dye (carcinogenic) and stain skin and clothes during use [8].

To provide an alternative hair dye agent, the researchers used Atsuete (*Bixa orellana* L.) and Alugbati (*Bixa orellana* L.) stem dye extract that has been reported be potential hair dye [20]. *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 [9]. Further, Atsuete has been documented that it contains important amounts of tocotrienols, tocopherols, terpenes and flavonoids both in the seeds 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 [10], 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%) [10].

Alugbati (*Basella alba* L.) a plant that thrives in tropical Asia, Africa, Malaya and is found cultivated throughout the Philippines. It has been found to be a good source of calcium, iron, vitamin A and vitamin C. In the Indian system of medicine, the plant has immense potential in androgenic activity, antiulcer activity, antioxidant, cytotoxic and antibacterial activity, anti-inflammatory activity, central nervous system (CNS) depressant activity, nephroprotective and wound healing properties etc. [11]. *Basella alba* plnt has been studied and

results showed that it has phenolic and flavonoids content. Further, Ferric ion reducing antioxidant power (FRAP) and antimicrobial action was reported to be Basella alba plant activity [12]. With this, the researcher will be using Atsuete and Alugbati plants to formulate hair dye.

OBJECTIVES OF THE STUDY

Generally, the objective of this study is to formulate a hair dye out of Atsuete *(Bixa orellana* L.) seeds and Alugbati (*Basella alba* L.) stem evaluate its effectiveness.

Specific objectives are listed below:

1. Determine the absorbance level of the hair dye at 200nm, 250nm, and 400-550nm;
2. Perform substantivity testing of the hair dye in hair stands;
3. Determine the level of acceptability of the treatment and control groups in terms of odor, texture, and color; and
4. Determine if there is a significant difference between the treatment groups and control group in terms of absorbance.

MATERIALS AND METHODS

A. 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 available Virgen Milagrosa University Foundation (VMUF), San Carlos City, Pangasinan was used to determine the level of absorbance level of the hair dye and Bremod synthetic dye at 200nm, 250nm, and 400-550nm.

B. 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 three replicates.

Replicates	*Treatments*			*+Control Group*
	T_1	T_2	T_3	Bremod hair dye
R_1				
R_2				
R_3				

C. General Procedures

a. Ethanolic extraction of Dye stuff

One kilogram of Atsuete (*Bixa* orellana) seeds were prepared and (Plate 1) macerated with 1200mL of ethanol (Plate 2) for three days, afterwards, it was extracted until the pure extract is acquired (Plate 3). The same process was done to Alugbati (*Basella alba* L.) stem.

Plate 1. One kilogram of Atsuete and Alugbati and laboratory materials

Plate 2: Plant Maceration

Plate 3: Extraction of Dye from Atsuete seed (left) and Algubati stem (right)

b. Collection and Stocking of hair dye per treatment and control

Table 2. Mixture Ratio of Atsuete and Alugbati dye extract

Treatments	*Ratio*	
	Atsuete dye	Alugbati dye
Treatment 1 (1:1)	10mL	10mL
Treatment 2 (1:2)	10mL	20mL
Treatment 3 (2:1)	20mL	10mL

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 Atsuetedye extract + 10mL Alugbati dye (2:1 ratio).

Stocking of the dyes was carried out at San Jacinto National High School, San Jacinto, Pangasinan (Plate 4).

Plate 4. Collecting and Stock formulation of dye per Treatment

c. Spectrophotometric Analysis

Using Spectrophotometer, the researchers tested the level of absorbance of Atsuete-alugbati dye and Bremod synthetic dye at 200nm, 250nm, and at 400-550nm [13][14][15][16]. 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' [17].

d. Application of Hair dye

The hair strands treatment groups and control group were washed with concentrated shampoo and wiped with cloth and blow dried. Afterwards it was subjected to application of Atsuete-alugbati hair dye and Bremod synthetic hair dye separately.

Plate 5. Application of Hair dye to a sample replicate

e. Substantivity Evaluation

This was carried out by washing the hair strands of the treatment and control groups for eight times and pictured every wash for substantivity evaluation. The researchers compiled the pictures per treatment and certified by the Adviser, Special Science Class head teacher, and OIC principal to prove that the pictures were not edited. The pictures were brought to the panelists to rate the level substantivity.

Plate 6. Substantivity Evaluation of a Sample replicate

f. Acceptability Evaluation

The hair dye formulations and the synthetic hair dye were evaluated by beauty care experts and people of the community that uses hair coloring products.

Plate 9. Acceptability Evaluation

g. Statistical treatment

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 level at 200 and 250nm and spectrum at 400-550nm.

RESULTS AND DISCUSSIONS

Spectrophotometric Analysis of Atsuete-alugbati hair dyes fromulations and Bremod synthetic hair dye

Table 3. Mean Absorbance of the Treatment groups and Control group

Wavelength	***Treatment groups***			***+Co group***
	T_1	T_2	T_3	Bremod
200nm	3.7336	2.9913	2.2253	3.1316
250nm	3.063	2.986	2.168	3.112
400-550nm	4.448	2.776	1.002	3.973

Table 3 shows that at 200nm wavelength, Treatment3 has the lowest absorbance level followed by Treatment2 and Bremod dye respectively. Treatment1 has the highest absorbance level at 200nm. At 250nm, Treatment3 has the lowest absorbance level still followed by Treatment2 and Treatment1 respectively. Bremod dye has the highest absorbance level at 250nm.

Further, the table shows that in visible light wavelength at 400-500nm, Treatment3 still has the lowest absorbance level and again followed by Treatment2 and Bremod dye respectively. Treatment1 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 [14] and phototendering [18] in hair strands [19]. Thus, the researcher implied that Treamtent3 – combination of 20mL of Atsuete and 10mL of Alugbati –, is the most effective hair dye agent because it absorbs less light and will not be too sensitive to light that can cause fading [14]. Further, the table shows that Treatment3 has decreasing absorbance level in increasing wavelength. Therefore, the researcher says that that exposure to sunlight will not fade the color produced by Treatment 3 fast. Furthermore, Treatment3 can be used as coloring agent compared to Bremod synthetic dye. The researcher suggests that Treatment3

should be used as hair dye to reduce sunlight damage to the hair due to absorption of sunlight.

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 Treatment3 gave the significance.

Substantivity Evaluation of the Atsuete-alugbati hair dye and Bremod synthetic hair dye

Table 4. Mean Level of Substantivity of the Treatment groups and Control group

Treatments	*Mean*	***Interpretation***
T_1	2.25	*MS*
T_2	3.00	*S*
T_3	2.50	*MS*
Bremod dye	1.25	*NS*

1.00-1.75 – Not Substantive (NS)
1.76-2.50 – Moderately Substantive (MS)
2.60-3.25 – Substantive (S)
3.26-4.00 – Very Substantive (VS)

Table 2 reveals that Treatment2 (M=3.000 substantive; Treatment1 (M=2.25) and Treatment3 (M=2.50) were moderately substantive, while +Control (M=1.25) not substantive. This means that Treatment2 is the most effective among the treatment groups and the control groups in terms of substantivity level. In table 3, we can see that Treatment2 has decreasing absorbance values in increasing wavelength and it is the second among the treatment groups and control group that has low absorbance level. The researcher can say that Treatment2 has enough property on transmitting light to limit hair damage or photodegradation [14] and phototendering [18] in hair strands [19] and at the same time substantive to washing. However, Treatment3 formulation, having the least absorbance of light, is recommended to have minimized damage of sunlight to hair because Treatment3 transmits more light and is rated as Moderately Substantive (M = 2.50) close to Treatment2 formulation.

Acceptability Level of the Atsuete-alugbati hair dye and Bremod synthetic hair dye

Table 3. Mean Level of Acceptability of the Atsuete-alugbati hair dye and Bremod synthetic hair dye

Treatments	Mean	Interpretation
T_1	3.78	HA
T_2	3.94	HA
T_3	3.94	HA
+Co	3.41	A

4.30-5.00– Very Highly Acceptable
3.50-4.20– Highly Acceptable
2.70-3.40– Acceptable
1.90-2.60– Moderately Acceptable
1.00-1.80– Not Acceptable

Table 3 reveals Treatments 1, 2, 3 were found to be 'Highly Acceptable' while the synthetic hair dye is found to be 'Acceptable'. This means that the Atsuete-alugbati hair dye is generally highly acceptable in terms of texture, odor, and color as to Bremod synthetic hair dye, it is acceptable. The researcher suggest that when coloring the hair semi-permanently, Atsuete-alugbati dye can be used alternatively rather than synthetic products.

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings, the researchers came up with the following conclusions:

1. Treatment 3 is the most effective in terms of absorbance level. In addition, Treatment3 has decreasing absorbance values in increasing wavelength. This means that Treatment3 hair dye can best

limit the absorption of light that cause damage or photodegradation [14] and phototendering [18] in hair strands [19]. Lastly, Treatment3 is rated as Moderately Substantive (M = 2.50)

2. The Atsuete-alugbati hair dye is generally highly acceptable while the Bremod synthetic hair dye is acceptable in terms of texture, odor and color.
3. There is a significant difference exist among the treatment and control groups in terms of absorbance level at 200nm, 250nm, and at 400-550nm. Scheffe's test revealed that Treatment3 gave the significance

Based on the conclusions, the researchers recommend the following:

1. The Hair Dye out of Atsuete (*Bixa orellana* L.) seeds and Alugbati (*Basella alba* L.) stems should be used as alternative hair dye with the formulation ratio of 2:1 (Table 2)
2. Further studies about production of hair dye from Atsuete (*Bixa orellana* L.) seeds and Alugbati(*Basella alba* L.) stems using different extraction process and test for shelf-life should be conducted.

ACKNOWLEDGMENTS

Special thanks to the Laboratory Staff of Virgen Milagros University Foundation for the technical assistance given to the researcher during the laboratory part of the study.

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