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Stability Study of the Effect of Light on the Ethanol-Water Natural Colour Extract of Bixa Orellana L. Plant Seeds

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ABSTRACT

The stability of ethanol-water natural colour extract from Bixaorellana L. plant seed under darkness and light conditions were studied. The changes of the colour intensity due to darkness and light were monitored for two weeks with a UV/Visible spectrophotometer at 400 nm and 510 nm. The effect of storage condition on colour loss was more predominant on distilled water extract followed by distilled water-ethanol extract and lastly ethanol extract. At the end of the study, the colour intensity ofsamples kept under direct sunlight reduced by 2.7-47.2%, while those kept in darkness reduced by 0.4-3.4.0%. Kinetic study indicated that the colour degradation followed first order kinetics for all conditions. The rate, however, varied with solvent type and exposure. Extract solutions kept under direct sunlight degrade fastest (k = 0.0653, 0.00316 and 0.00213), than those kept in darkness (k = 0.00113, 0.00103 and 0.0005).

Keywords: Bixa orellana, degradation, ethanol, kinetics, stability, UV/Vis spectrophotometer, water.

INTRODUCTION

Natural dyes comprise of colorants that are obtained from animal or vegetable matter without any chemical processing [1]. The major areas for the use of natural dyes since pre-historic times is in colouring of food substrates, leather, as well as natural protein fibres like wool, silk and cotton [2].

Some of the well-known ancient dyes include Madder, a red dye made from the root of the *Rubiatinctorum* plant, blue indigo from the leaves of *Indigoferatinctorum* plant, yellow from the stigma of the *Saffron* plant and from *Turmaric* plant [3].

Bixa orellana is a tropical tree whose seed extracts are generally formulated to impart colour shades in the range of red, orange and yellow in different foods especially dairy products for over 200 years [4].

However, most of the natural colourants are highly unstable because they strongly absorb light and are highly unsaturated [5], which thus limited their applications, owing to some processing procedures. Heat treatment, pH changes, light exposure and storage conditions cause colour loss and therefore require the addition of synthetic colourants to recover the lost colour and enhance appearance [6]. There are however large differences in stability. For instance, colour from butterfly pea [7] and Red dragon fruit [6] were shown to be susceptible to pH, light, temperature and storage conditions. Bixa orellana L. plant colour extract subjected to high temperature heat treatment was found to be sensitive and therefore not suitable for heat processed food [8]. Knowing the limitation of each colour means that a specific colour can be avoided for certain applications in which the conditions are unfavourable for the colour [5].

Therefore, the objective of this study is to report on the stability of the ethanol-water *Bixa orellana* L. plant seed extract in response to light.

MATERIALS AND METHODS

Colour extraction

Colour was extracted from *Bixa orellana* plant seeds at three different conditions: 100% distilled water, 100% ethanol and a mixture of distilled water and ethanol at a ratio 1:4. About 16 g each of the seeds was mixed with 200 ml each of solvent in a 250 ml conical flask and heated in a water bath for 2h at a temperature of 60℃. The mixture was filtered and the extract was used for stability study.

Spectrophotometric analysis

The maximum wavelength (λ_{max}) and absorbancies of the extracted colour were measured at the visible wavelength (200-800 nm) using a spectrophotometer.

Effect of light on the stability of colour extract

In analyzing the effect of light on the stability of colour, 60 ml each of the filtered extract was stored in the dark, kept in the room and exposed to the sun. The changes in absorbance at the maximum wavelength (λ_{max}) were measured for 2 weeks. Samples were taken right after extraction and after day 2, 4, 7, 9, and 11, and analyzed using a Shimadzu UV spectrophotometer. Kinetic study of degradation of *Bixa orellana* seed colour was carried out by

monitoring the changes in colour with time. The data was fitted to n^{th} order kinetic model using integral method to evaluate order of reaction n and the rate constant k.

RESULTS AND DISCUSSION

Absorbance measurement

Freshly extracted colour was scanned through with a spectrophotometer between the wavelengths of 200 to 800 nm. The absorption spectrum showed major absorption peaks at 400 and 510 nm. Absorption peaks at 400 and 510 nm is a characteristic absorption for yellow-orange-red colours of *Bixa orellana* seed colour extract [8, 9],

Stability study

The absorbancies of the colour extract before and after degradation were measured after extraction and at day 2, 4, 7, 9 and 11. Measurements were carried out at wavelengths of 400 and 510nm and the percentage of degradation was calculated using Equation 1.0 [10]

$$\text{Degradation} \quad \% \quad = \quad [1-\frac{At}{Ao}]\times 100 \qquad (1.0)$$

Where A_t is the absorbance after time t and Ao is the initial absorbance before degradation.

The changes in intensity of *Bixa orellana* seed colour extract in the presence and absence of light was monitored for 2 weeks. Results (Figures 1.0-3.0) revealed sunlight as the major factor of *Bixa orellana* seed colour extract degradation.

Figures 1.0-3.0 present the effect of time and storage condition on the degradation of natural colour extract from *Bixa orellana* L. plant seed.

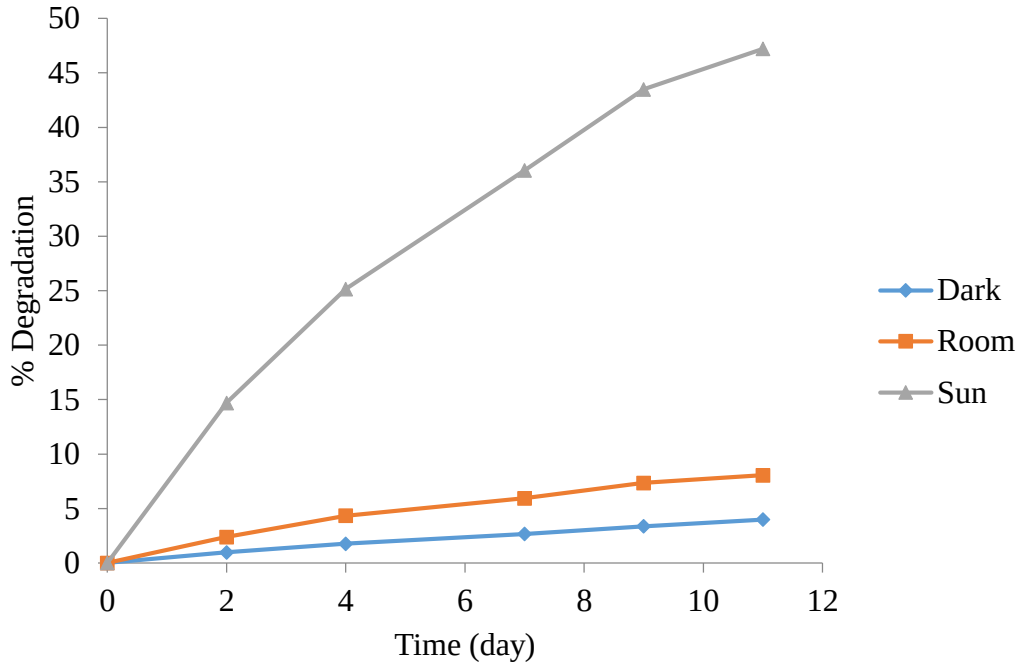

Figure 1.0: Percentage degradation with time for distilled water colour extract

From the Figures, it can be seen that the colour loss increased with time at all conditions. About 3.99 %, 8.06 % and 47.21 % colour was lost in 2 weeks for solutions kept in darkness, room and sunlight.

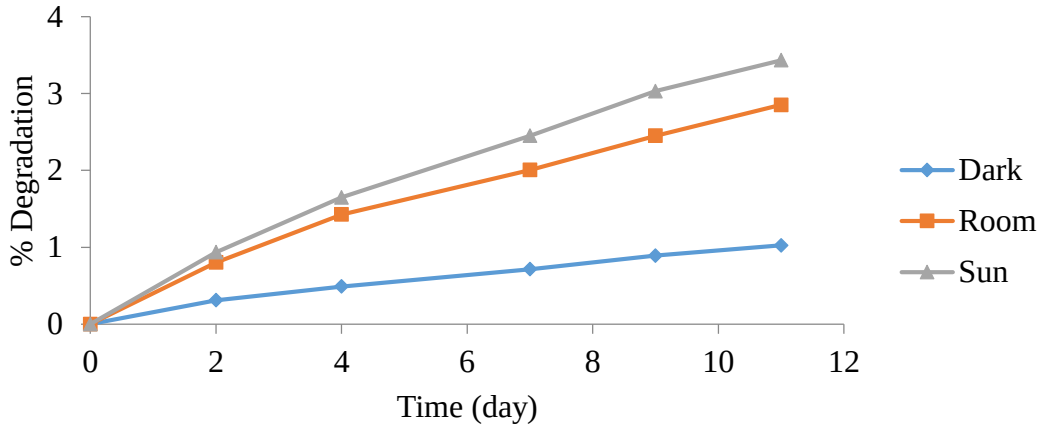

Figure 2.0: Percentage degradation with time for distilled water- ethanol colour extract

Bixa orellana colour extract consists of a system of conjugated double bonds which when exposed to high temperature, light, oxygen and low pH, undergoes oxidation reaction [11], with a resultant loss of colour.

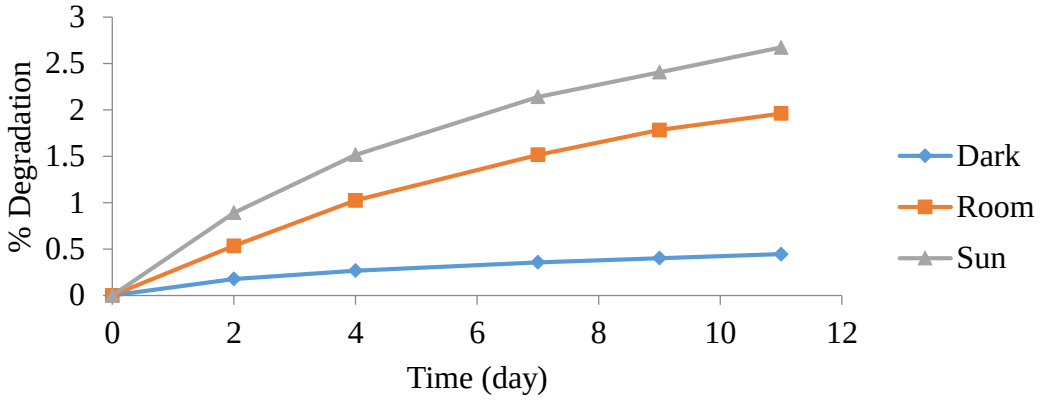

Figure 3.0: Percentage degradation with time for ethanol colour extract

From the present study, 0.89-47.21 % colour was lost for solutions kept in sunlight as against 0.18-3.99 % colour lost for solutions kept in darkness.

Degradation kinetics

The stability of *Bixa orellana* colour extract solutions in response to darkness and light were evaluated. Kinetics studies over a period of 2 weeks indicated that the colour extract degradation followed first order kinetics for all conditions (Table 1.0). The rate of degradation, however (Table 1.0), varied with solvent type and exposure. Extract solutions kept under direct sunlight degrade faster (highest k values), while solutions kept in darkness degrade slowly (lowest k values). Ethanol extract solution is the least affected by exposure conditions and is therefore the most stable, while distilled water extract solution is the most affected by exposure and therefore least stable.

Table 1.0: Kinetic parameters for degradation of natural colour extract from *Bixa orellana* plant seed

	Kinetic parameters					
	Darkness		Room		Sunlight	
Extract	K (d^{-1})	n	K (d^{-1})	n	K (d^{-1})	n
Distilled water	0.00113	1.04	0.00795	1.22	0.0653	1.28
Distilled water-ethanol	0.00103	0.96	0.002	1.13	0.00316	1.23
Ethanol	0.0005	0.94	0.00198	1.00	0.00213	1.17

CONCLUSION

The stability of *Bixa orellana* colour extract solutions in response to darkness and light was evaluated. The following conclusions were drawn from the results of the experiments:

1. Degradation of *Bixa orellana* colour extract solution followed first order kinetics in the presence and absence of light.
2. Extract solutions kept under direct sunlight degrade faster than those kept in darkness.
3. Ethanol extract solution is the least affected by exposure conditions and is therefore the most stable, while distilled water extract solution is the most affected by exposure and therefore least stable.
4. Extract solutions exposed to direct sunlight lost 0.9-47.2% colour, while samples protected from light retained 96.0-99.6% colour.

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