*International Journal of Multidisciplinary Research and Modern Education (IJMRME)*
*ISSN (Online): 2454 - 6119*
*(www.rdmodernresearch.com) Volume II, Issue II, 2016*

# EFFECT OF TEMPERATURE, LIGHT, PH ON THE STABILITY OF ANTHOCYANIN PIGMENTS IN COCCULUS HIRSUTUS FRUITS

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**Abstract:**

*The fruit of Cocculus hirsutus (L) Diels is often considered as waste so the study was aimed at exploring the feasibility of using the fruit as natural colorant using acidified methanol extraction. The extracted anthocyanin pigments then were exposed to number of environmental conditions, which could destabilize the anthocyanin molecules. These environmental conditions were included different pH, various temperatures and presence or absence of light. The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the anthocyanin molecule. Cocculus hirsutus fruit antocyanin extract was more stable at pH 1.0 and 3.0, temperature at 4° C and 37° C both in the presence and absence of light. The results show that anthocyanin obtained from Cocculus hirsutus fruit has a high potential to be used as a natural food colorant.*

**Key Words**: *Cocculus Hirsutus* Fruits, Anthocyanins, pH, Temperature & Light

**Introduction:**

*Cocculus hirsutus* (L) Diels (Synonym-*Cocculus villosus*) locally called Jaljamini, belonging to the family Menispermaceae is a climbing scandent shrub with hairy sepals. The plant grows all over India, especially in dry regions. It is widely distributed in tropical and subtropical countries. The fruit is a drupe which is size of small pea with red purple endocarp. The plant is used in treatment of gonorrhea, spermatonorrhea, Urinary troubles, diarrhea and hyperglycemia (Kirtikar and Basu, 2002). The leaves of the plant have been evaluated for anti hyperglycemic (Badole *et al*., 2006); antibacterial (Panda *et al*., 2007); diuretic and laxative (Ganapaty *et al*., 2002).

Anthocyanins have a high potential for use as natural colorants due to their attractive orange, red, purple, and blue colours; however, they have stability problems (Markakis, 1982; Francis, 1989). The colour and stability of anthocyanin pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity and quality, presence of copigments, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products and sulfur dioxide, among others (Mazza & Miniati, 1993; Francis, 1989). They have antioxidant, antiinflammatory and anticancerous property. That it can be better studied as the natural source of food colorant. However, the lower stability of natural plant pigments against environmental factors could pose restriction to their utilization as food colorant in industry (Tang and Norziah, 2007). In this research was to investigate the stability of *Cocculus hirsutus* fruit anthocyanins at different pH, various temperatures and presence of light.

**Materials and Methods:**

**Sample Collection:**

*Cocculus hirsutus* fruits were collected from in and around Pollachi district, Tamilnadu, India and stored at -20°C. The plant was identified by the botanical survey of India Coimbatore.

**Extraction of Anthocyanin:**

The anthocyanin from *Cocculus hirsutus* fruit was extracted by incubating the fruit with 1% HCl in methanol overnight at room temperature, followed by a filtration through whatman paper no.4. Methanol was removed by a rotary evaporation under 35°C and the extracts were stored for further study (Lachman *et al.,* 2003).

**Analytical Procedure:**

**Flavanoid Confirmatory Test (Harbone 1998)**

**A. $FeCl_3$**: 1ml of sample extract was added with a small amount of $FeCl_3$, and results were observed.

**B. $AlCl_3$**: 1ml of sample extract was added with 5% of $AlCl_3$ solution, and results were observed.

**Confirmative Test for Anthocyanin:**

**A. 2M HCl**: 1ml of sample extract was added with 2ml of HCl and heated for 5min at 100°C, and the results were observed.

**B. 2M NaOH**: 1ml of sample extraction was added with 2ml of NaOH, and the results were observed.

**Stability of Anthocyanin:**

The influence of key factors, including pH, temperature and light, on the stability of anthocyanin were studied (Wang *et al*., 2010).

**Stability of Anthocyanin at Different pH**:

In the pH study, buffers at five pH values (1.0, 3.0, 5.0, 7.0 and 9.0) were used for analysis and prepared as follows: The extract of anthocyanin was dissolved in different pH buffers (5mg / 5mL) in separate volumetric flasks and placed for 10 days at room temperature under the same conditions. The absorbance of each extract was detected every day at the same time using a visible spectrophotometer.

In order to study the effect of temperature and light on the stability of anthocyanin, extracts were prepared as follows: A mass of 25mg of anthocyanin was dissolved in buffer at pH 3.0. The samples were diluted to appropriate concentration and the initial absorbance measured in 527nm.

**Stability Studies on Temperature:**

The effect of temperature on colorant stability was done with samples inside capped glass vials covered with aluminium foil sealed with parafilm and kept at different temperatures ranging from 4°C, 37°C, 50°C and 100°C The UV/Vis spectra were recorded for freshly made samples (‘‘0’’), after 2 day, and then after 4, 6 and 8 days at 527nm.

**Stability Studies on Light:**

Light effect on colorant stability was performed with samples inside capped glass vials sealed with parafilm and kept at different temperatures ranging from 4°C, 37°C, 50°C and 100°C The UV/Vis spectra were recorded for freshly made samples (‘‘0’’), after 2 day, and then after 4, 6, and 8 days at 527 nm .

**Result and Discussion:**

**Flavonoid Confirmation Test:**

In the presence of $FeCl_3$, acidified methanol extract showed brown color (Figure 1A) and blue color was observed in the presence of $AlCl_3$ (Figure 1B). These confirm the presence of flavonoid in the extract.

**Anthocyanin Confirmations Test: (Horbone, 1998)**

**A. 2M HCl**

The presence of anthocyanin was confirmed in the presence of 2M HCl. The red color was found to be stable when allowed to heat at 100°C (Figure 2A).

**B. 2M NaOH**

The extract was again confirmed for the presence of anthocyanin with 2M NaOH. On addition of NaOH, the initial red color changed blue to green and gradually faded (Figure 2B).

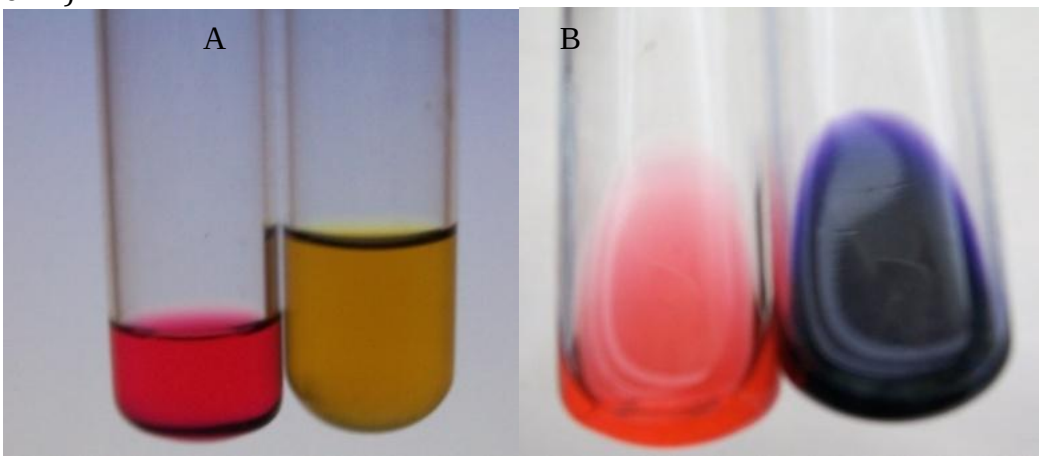


Figure 1: Flavonoid confirmation test A-$FeCl_3$; B- $AlCl_3$

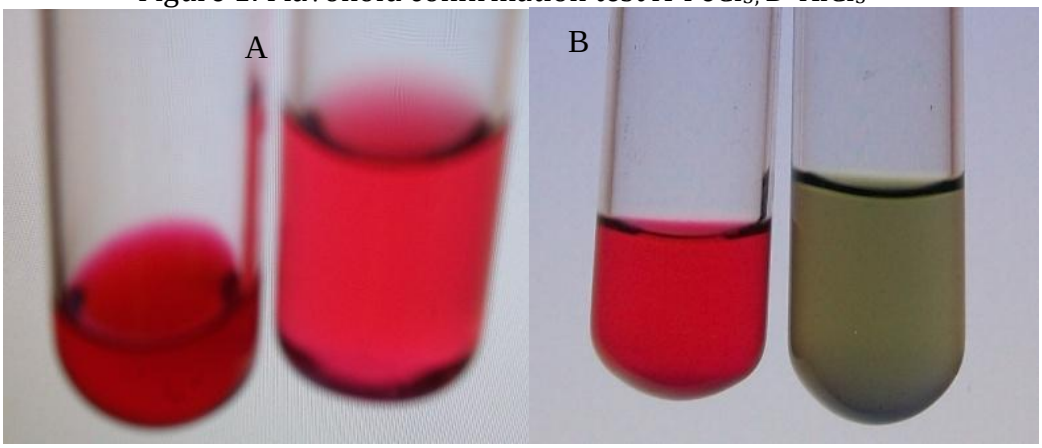


Figure 2: Anthocyanin confirmation test: A – HCl; B – NaOH

**Stability of Anthocyanins:**

**The Effect of pH on the Stability of Anthocyanins:**

Based on our results, pH had a great influence on the stability of anthocyanin pigment. The anthocyanin was more stable at pH 1 and 3, in the extracts of *C.hirsutus* fruit. The absorbance of each buffer solution of anthocyanin determined every day is presented (Figure 3). The absorbance decreases with anthocyanin degradation and in the current study decrease in absorbance was not significant at pH≤5.0, but it decreased significantly at pH≥7.0, indicating the hasty degradation of anthocyanin. The results demonstrate evidently that increasing in pH causes greater destruction to anthocyanin in samples. This evidenced that the anthocyanin were stable under low pH values (< 5.0) and is unstable under alkaline conditions.

Wang *et al*, (2010) reported similar results in blueberry extract, where in the anthocyanins were stable at pH below 5.0 and indicated the possibility of using the extract as food colorants in acidic food products. Similar results were reported by Kirca *et al,* (2007) in black carrot, where the stability of anthocyanins decreased significantly at pH above 5.0. The flavylium cation form presents anthocyanins to be stable under low pH conditions (Francis, 1992).

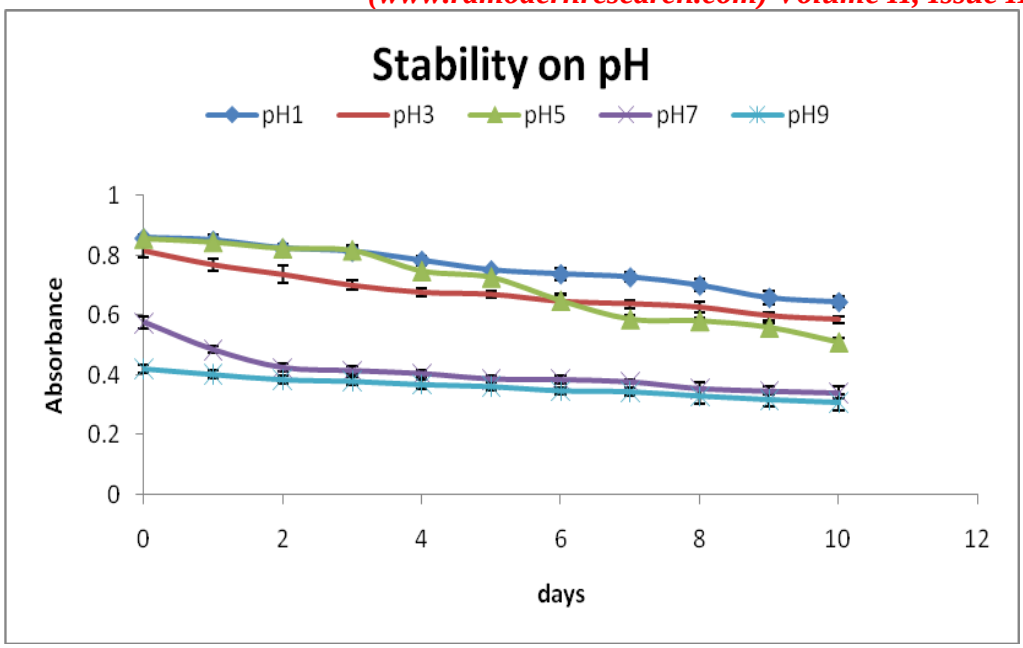


Figure 3: Effect of the pH on the stability of anthocyanins (mean±S.D.) N=3

**Stability Studies on Temperature:**

Temperature is a one factor that affects the stability of anthocyanin. The speedy destruction of anthocyanin at higher temperatures (dark) is proved in the present study. Anthocyanins degrade at high temperature (100°C), which is evident by the decrease in absorbance (Figure 4). However, the effect of low temperatures (≤50°C) on the stability of anthocyanin is significant. Analogous results have been reported by Jenshi roobha *et al*., (2011) in *Musa acuminata* bract, wherein the effects of temperature on the destruction of anthocyanin extract under dark. Hence the data reveals that that anthocyanin are sensitive to high temperature (100°C) and the present study suggest that high temperature and long heating should be avoided in the processing, storage and usage of anthocyanin extracted from *C. hirsutus.*

**Stability Studies on Light:**

The effect of temperature (under light) on anthocyanin extract of *C. hirsutus* fruit was studied at four different temperatures (4, 37, 50 and 100°C). The results showed higher level of degradation of anthocyanin at 50 and 100°C, indicating that higher temperature (50 to 100°C) should be avoided in the processing, storage and usage of anthocyanin extract from *C. hirsutus*. However, the anthocyanin extract is stable at temperatures 4°C and 37°C (Figure 5) and hence can be finely employed for storage. Similar consequences were reported in *Musa acuminata* bract for the effect of temperature on the destruction of anthocyanin under light (Jenshi roobha *et al*., 2011). A temperature raise has been shown to shift anthocyanin equilibrium towards the chalaone form, which has negligible effect in the visible range and a large decrease in absorbance with increasing temperature. The quantitative recovery of colour on cooling of copigment solution is due to changes the copigment complex (Brouillard *et al.,* 1989).

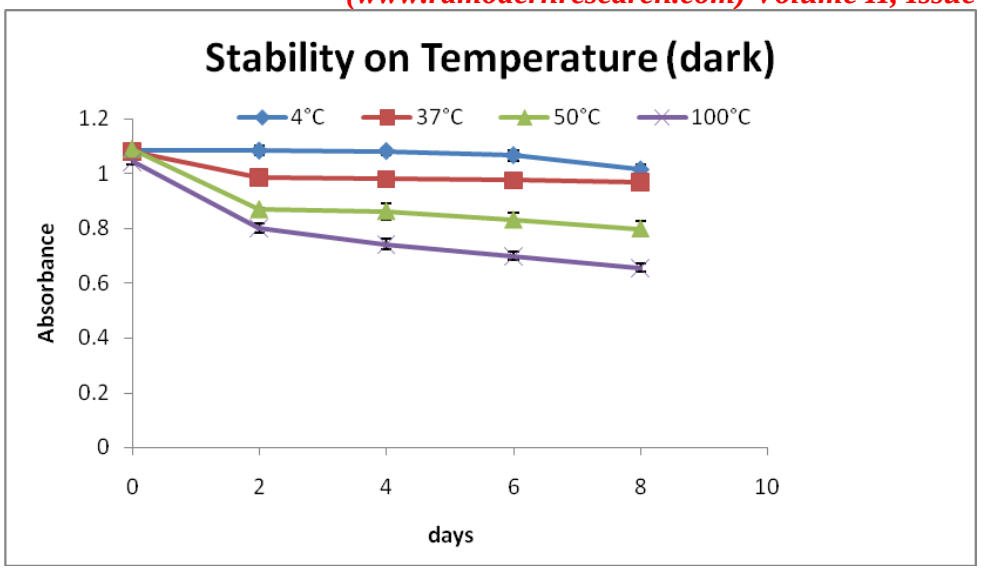


Figure 4: Effect of temperature (dark) on the stability of anthocyanin (mean±S.D) N=3

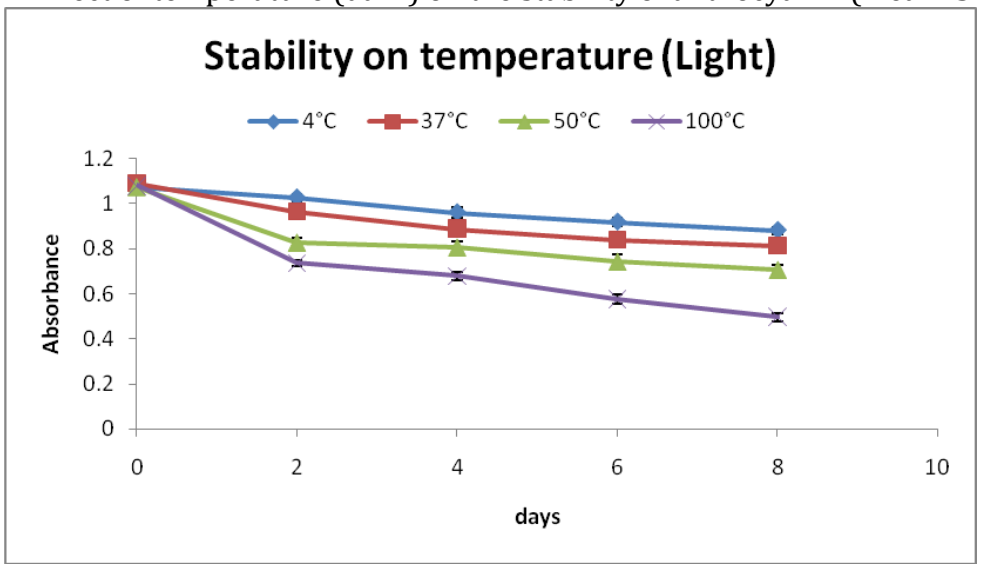


Figure 5: Effect of temperature (dark) on the stability of anthocyanin (mean±S.D) N=3

Comparitively, anthocyanin degradation under dark condition is very low than degradation under light. Also anthocyanin is slowly degraded in dark and highly degraded under light. At high temperatures, the equilibrium shifts to chalcones, which means loss of color. The return of chalcones to flavylium is slow (Centurion *et al*., 2008). Storage temperature is the main cause for the loss of anthocyanins. During storage, anthocyanin content decreased in both customary and low-calorie jams. The loss of anthocyanin in strawberry jams was higher when the samples were stored at room temperature than at 4°C (Kopjar *et al*., 2009).

**Conclusion:**

From the results it can be concluded that anthocyanin extracts of *Cocculus hirsutus* fruit were highly or moderately resistant to the pH, temperature and light factors tested. *Cocculus hirsutus* anthocyanin extract was more stable at pH 1 and 3, temperature at 4° C and 37° C both in the presence and absence of light. Increase in environmental factors like pH, temperature and light accelerates destruction of anthocyanin. There is a need for replacement of the artificial dyes used in the food industry with natural dyes because of the general toxicity presented by artificial dyes, making them undesirable for human consumption. Thus these results suggest that

anthocyanin extract from *Cocculus hirsutus* fruit represent inexpensive crops with high pigment yield that could be sources of anthocyanins for the food colorant market.

**Acknowledgement:**

The authors are grateful thanks to the president Dr. B. K. Krishnaraj Vanavarayar, principal Dr. P. Badri Sreeman Narayanan, Head of the Department R.Kavitha Krishna, NGM College, Pollachi for providing necessary facilities to do this research work.

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