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STUDY ON NATURAL FOOD COLORANTS EXTRACTED FROM PHYLLANTHUS RETICULUM FRUITSAS A HEALTHIER ALTERNATIVE TO SYNTHETIC FOOD COLOR

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Abstract:

Natural dyes find use in coloring of textiles, drugs, cosmetics and food products due to their nontoxic effect and the research has led to revive the production of natural food dyes on commercial scale in innumerable sectors considering health sensitive applications. The *Phyllanthus reticulatus* fruit has concentrated source of valuable nutrients, as well as bioactive constituents of therapeutic interest highlighting its importance as a biocolorant. In present study, the aqueous fruit extract of *Phyllanthus reticulatus* (Euphorbiaceae) commonly known as a roast potato plant, is used as a food colorant. The aqueous fruit extract was used for phyto-chemical evaluation, pH stability, thermostability, photostability, storage stability, Sun fastness test and TLC analysis. The obtained results suggest that very aesthetically pleasing colorants can be derived from *Phyllanthus reticulatus* fruit extractis a suitable organic replacements for the chemical colorants used in the food industry.

Keywords: Natural dyes, Phyllanthus reticulatus, phytochemical evaluation, pH stability, thermostability, photostability, storage stability, Sun fastness test, TLC analysis.

Introduction

The use of various types of synthetic and natural colorants in food and drinks is a significant factor to food manufacturer and consumer in determining the acceptability of processed food[1]. Biocolorants are prepared from renewable sources and majority are of plant origin which contains usually a mixture of natural dyes enlisted in Fig.No.01.[2]There is a demand for food colorants from natural sources that can serve as alternatives to the use of

synthetic dyes due to health benefits of biocolorant. Replacing synthetic dyes with natural colorants poses a challenge due to the higher stability of synthetic colorants with respect to light, temperature, and pH, among other factors.[3] Synthetic dyes which are commonly used in food items are carcinogenic, neurotoxic, and genotoxic also cause allergenic and intolerance reactions etc. As a resulting, there has been a worldwide interest in the development of food colorants from natural sources[4] .

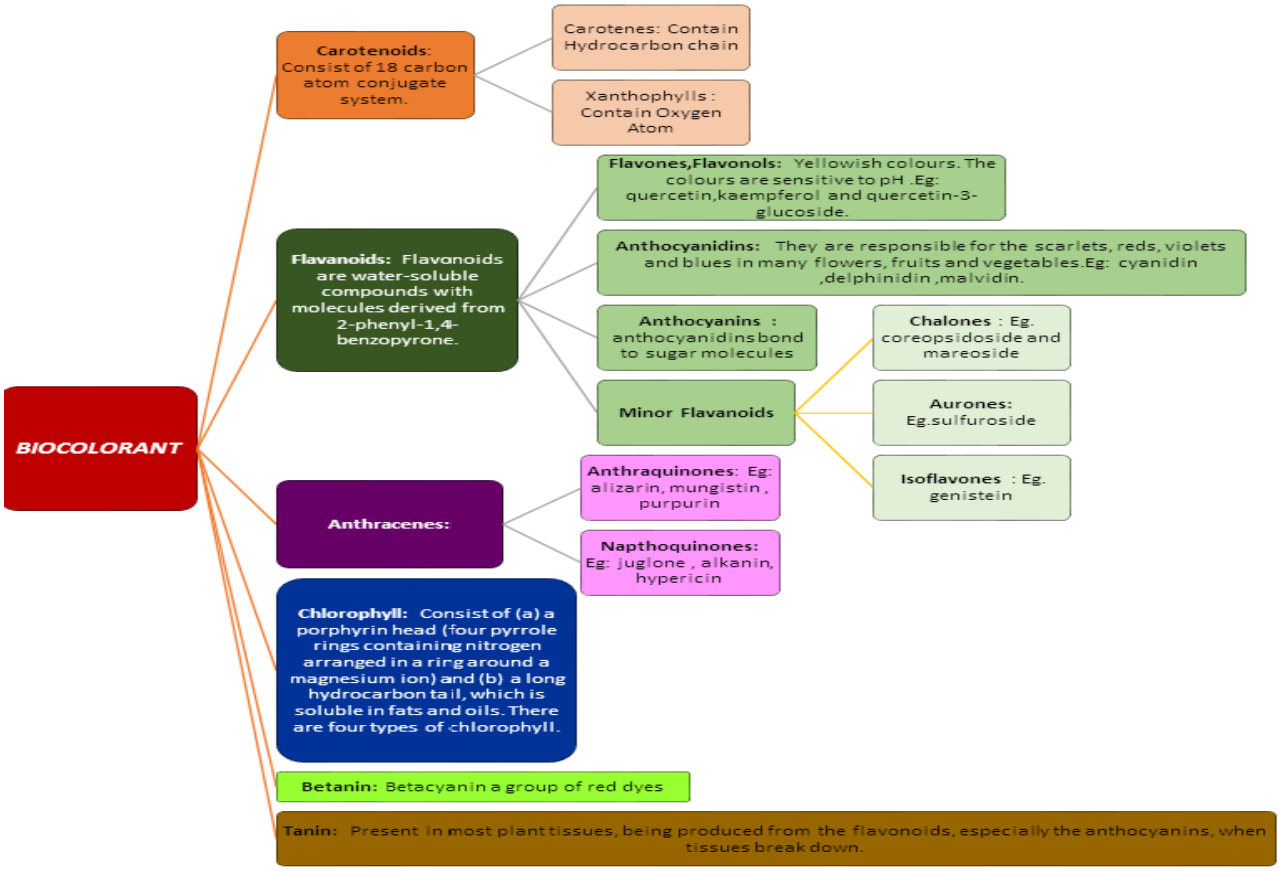


Fig.No.01: Classification of Natural Dyes present in plants

Advantages of biocolorants in food substances:

- Maintain the original food appearance
- Color uniformity for avoiding seasonal variations in color tone
- Intensify normal color of food and thus to maintain its quality
- Act as a light-screen support
- Increase acceptability of food as an appetizing item
- Health-promoting properties: Biocolorants may also play an important role in human health as they contain some biologically active compounds, which possess a number of pharmacological properties like strong

antioxidant (Carotenoids, Lycopene, zeaxanthin, Betacyanin, xanthophylls), antimutagenic, anti-inflammatory and antiartheritic, anti-tumor (Carotenoids, Allomelanins, chlorophylls), vasotonic, vasoprotective, chemo- and hepatoprotective activities. health benefits of biocolorants include enhancement of immune system function, protection from sunburn; improve visual acuity properties.[5,6,7]

- Low-fat content
- Environment friendly and can be recycled after use

The aim of this study was to extract pigment from and to evaluate the major constituents of pigments and their other properties

Material and Method:

The blue fruits of *Phyllanthus reticulatus* from family Euphorbiaceae were collected from trees near to the campus. Figure.No02 enlists the medicinal uses of *Phyllanthus reticulates*fruit [8].The fruit pulp was manually separated from seeds and immediately transferred to solvent (water)for pigment extraction. Aqueous extract used for evaluation study.

Fig.No.02: Medicinal uses of *Phyllanthus reticulatus* plant[8].

Extraction of dye:

Collected fruits 10 g were digested with distilled water and magnetically stirred for 1hr at room temperature. The procedure was repeated until the solvent become colorless. Reddish colour extract was collected and concentrated by rotary evaporator.

Qualitative analysis[9]:

Collected extract used for determination of various phyto-constituents in it by chemical testing.

Sun fastness test:

Degree of sunfastness was determined by 120 min exposure to sunlight at 25-28 ^{0}C

Stability Assays: Stability assays in terms of temperature/ thermal, pH and storage were carried out.

a) Thermal stability: In the present work a study was conducted to determine the heat tolerance of extracted colour from the fruits in the range of 25– 120^{0} C for 10 min in each. Samples were cooled immediately in an ice bath, followed by measuring the absorption spectra of the solution at λmax and then percentage of color loss was calculated

b) pH stability: A preliminary study was conducted to test the stability at different pH values 2,4,6,7,8,10,12 for 30 min. After the treatment, color loss was calculated by spectroscopic measurements at λmax.

c) Storage stability: Amount of degradation in content during storage was studied in two different time periods. Amount of dye was quantified at the time of extraction and a part of the extract was kept in a closed container at room temperature for 3 months. After completion of three months extract was again quantified and compared with the previous value.

Thin layer chromatography:

TLC is an important method for the isolation, purification and confirmation of natural products. Compared with other chromatographic methods, TLC is often considered to be deficient in reproducibility and accuracy, but some

distinctive attributes of this tool should be considered: low cost analysis, high-throughput screening of samples, minimal sample preparation, whole sample integrity, disposable stationary phase. Thin Layer Chromatography (TLC) is a solid-liquid type in which the two phases are a solid (stationary phase) and a liquid (moving phase). Solids most commonly used in chromatography are silica gel (SiO2 x H2O) and alumina (AL2O3xH2O). In our experiments thin layer chromatography (usually 5 µl of a 10 mg extract/ml solution) is loaded on silica gel plates using chloroform: methanol (5:5) mixture as eluents.

Application of dye extract as food colorant:

Water Extract was added to food items to find out the concentration of pigment needed to colour the food items and their stability also. Different food items like lemon juice (at pH 3,5), beaten rice (pH 7), Carbonated drink (pH above 7) were prepared according to standard recipe. Extract used for application study was taken after 3 months storage in room temperature to get a better understanding about the practical applications of this extract as a food colorant at different pH.

Results and Discussion:

Qualitative analysis: Qualitative analysis determination of fruit extract shown in Table 1entitled as Phyto-chemical screening of aqueous extract

Table No.01: Phytochemical screening of aqueous extract

Sr.No	Name of the Test	Result
1	Carbohydrates	+
2	Tannins	+
3	Saponins	-
4	Flavonoids	+
5	Alkaloids	+
6	Quinones	+
7	Glycoside	-
8	Cardiac glycosides	-
9	Terpenoids	-
10	Triterpenoids	-
11	Phenols	+
12	Steroids &Phytosteroids	+
13	Phlobatannins	-
14	Anthraquinones	-

* Presence + ;- absence

Degree of sun fastness: The result of sunfatness test shows that the dye solution has no change in color upto 28^{o}C with good stability for 120 min. The absorbance of extract was carried out at λmax which found to be 620nm having absorbance 0.92 as per shown in Table 2 UV absorbance and Color stability of aqueous extract and Figure 3 Effect of physical interpretation no degree of colour change after keeping (a) With sun exposure (b) Without sun exposure.

Table No.02: UV absorbance and Color stability of aqueous extract

UV absorbance	Before sun fastness test	After sun fastness
	0.92	0.93
Color stability	Stable	Stable

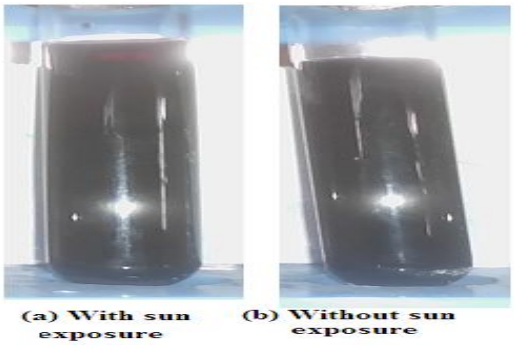


Fig.No.03: Effect of physical interpretation no degree of colour change after keeping (a) With sun exposure (b) Without sun exposure.

Stability Assays:

a) **Thermal stability:**

Aqueous extract were subjected to various temperature for 10 min, their colour absorbance were detected at 620nm at 25^{o}C, 60^{o}C, 100^{o}C, 120^{o}C. Results shown that up to 60^{o}C colour of dye was stable as shown in bellow Figure 4Thermal stability at 25oC,60 0 C, 1000 C, 1200 C. Which concluded that, above 100 ^{0}C ; absorbance decreases and colour found to be unstable. Table 3 shows the results regarding UV absorbance and Color stability of aqueous extract for Thermal stability study at 25^{o}C, 60^{o}C, 100^{o}C, 120^{o}C**.**

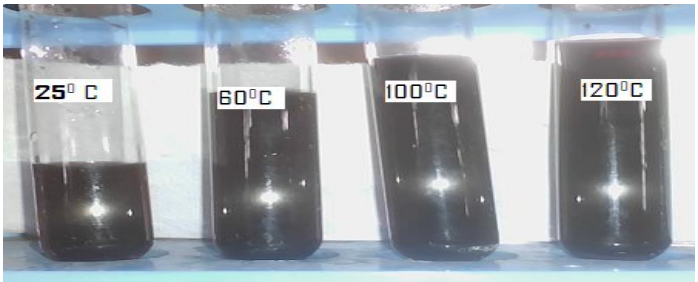


Fig.No.04: Thermal stability at 25^{o}C, 60^{0} C, 100^{0} C, 120^{0}C.

Above 100^0 C absorbance decreases and colour found to be unstable.

Table No.03: UV absorbance and Color stability of aqueous extract for Thermal stability study at 25°C, 60°C, 100°C, 120°C.

Temperature	25°C	60°C	100°C	120°C
UV absorbance a 620nm	0.80	0.83	0.95	0.89
Color stability	Stable	Stable	Stable	Unstable

b) pH stability: A preliminary study was conducted to test the stability at different pH values 2,4,6,7,8,10,12 for initial period and after 30 min incubation. From observation extract showed development of red to yellow color at various pH as shown in following Figure 5 and Table 4

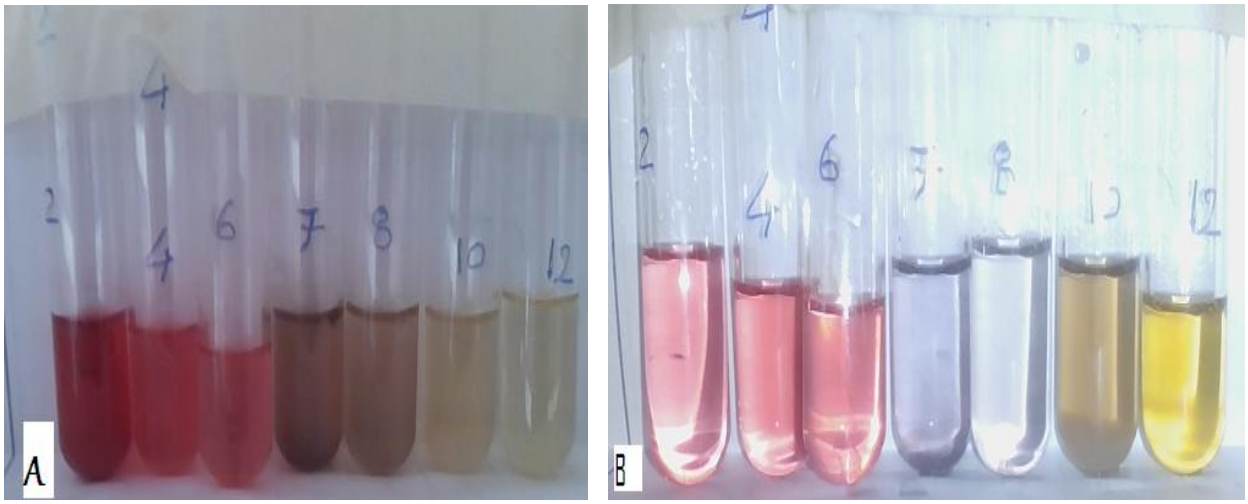
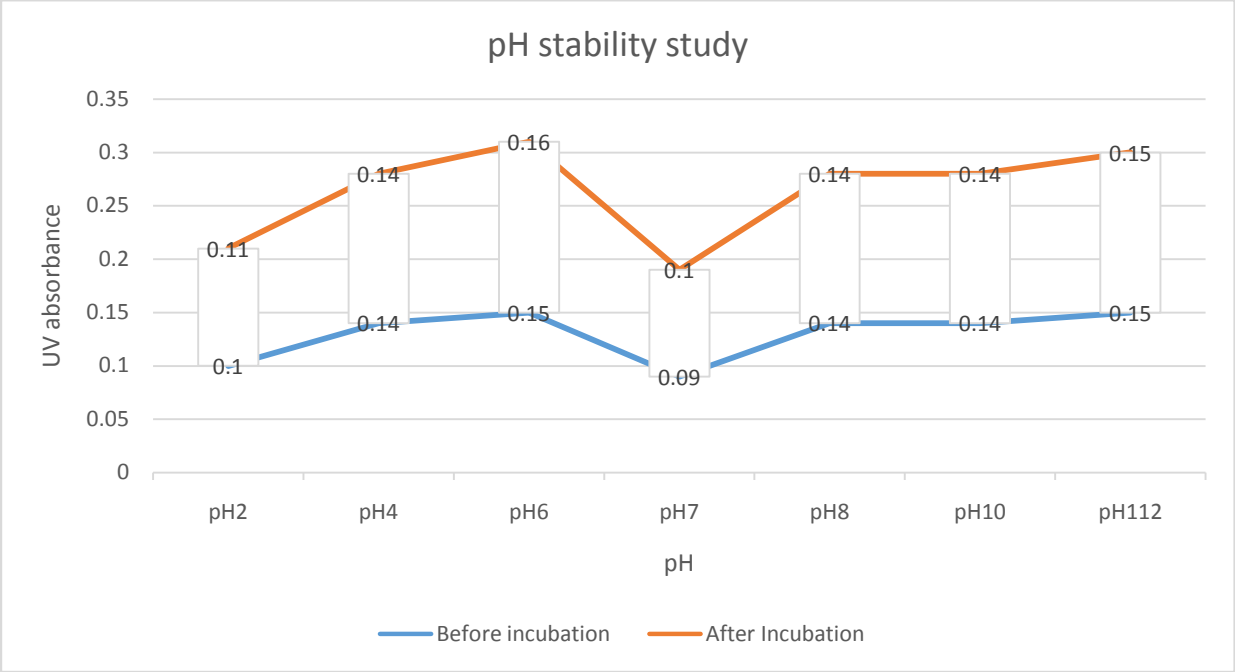


Fig.No.05: Aqueous extract develop red to yellow colour at various pH range.

Table No.04: pH stability study

pH	2		4		6		7		8		10		12	
	Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation
UV absorbance at 620nm	0.10	0.11	0.14	0.14	0.15	0.16	0.09	0.10	0.14	0.14	0.14	0.14	0.15	0.15



c) Storage stability: Storage stability assay proved that there was degradation in dye content by time. Further studies are needed in this direction to increase the storage stability of the anthocyanin extract but from application studies it became clear that the reduction in stability will not affect the potential of aqueous extract to use as a food colorant. As shown in Fig.6

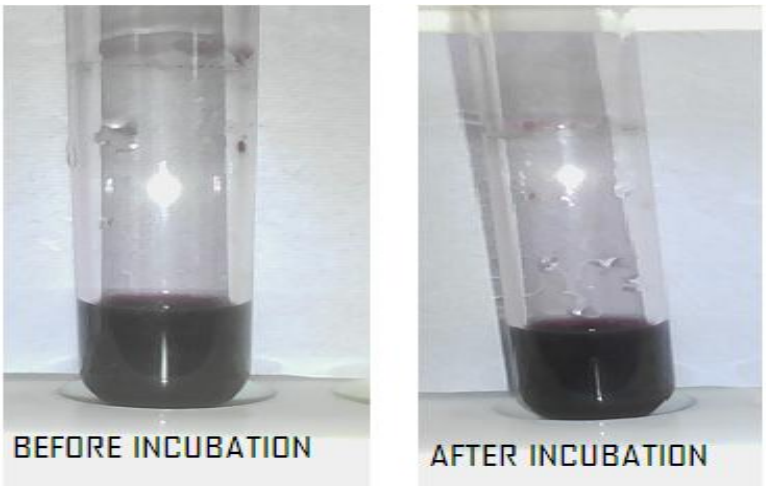


Fig.No.06: Storage stability study

Table No.05: Storage stability study

UV absorbance	Before Incubation	After incubation
	0.92	**0.49**
Color stability	**Stable**	**Stable**

Thin layer chromatography:

In our experiments thin layer chromatography (usually 5 µl of a 10 mg extract/ml solution) is loaded on Merck TLC F254 silica gel plates using chloroform: methanol (5:5) mixture as eluents in which the dye separated as distinct point.

Application of dye extract as food colorant:

Different food items like lemon juice (at pH 3,5), beaten rice (pH 7), Carbonated drink (pH above 7) were selected for study. It indicates good stability of color at different ph. Figure 7showsApplication of dye extract to different food item like rice and lemon juice at different pH.

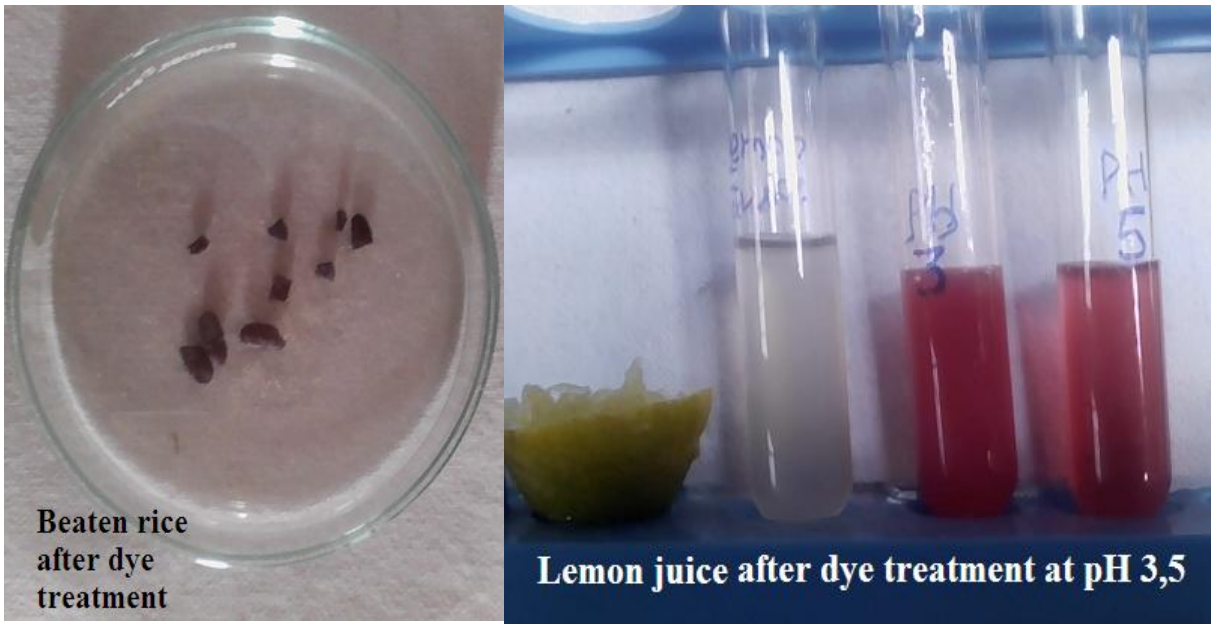


Fig.No.07: Application of dye extract to different food item.

Conclusion:

Natural dyes are not only having dyeing property but also having the wide range of medicinal properties. 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. The purpose of this research work has been to explore the natural reserves, identify plants from which dyes can be extracted and applied to food .This task was accomplished by means of assisted extraction of natural dye as a food colorant which shows good acceptability, pH stability, Thermal stability and satisfactory storage stability. The present work concluded that this fruit extract can be used as a potential alternative to the synthetic dye used in food coloration process. Also due to its health benefits can increases the status of natural food colorant in food industries.

Future Prospects:

Comparative genotoxic studies between natural food colorant as fruit extract and synthetic food colorant by onion root tip culture, cell line studies and sensory evaluation will be carried out for extracted dye.

Conflict of Interest: We declared that this review does not have any conflict of interest.

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