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Research Article

Evaluation of Natural Color from Annatto Seeds for Pharmaceutical Use

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ABSTRACT

The aim of the present investigation was to study the suitability of Annatto seed extract as coloring agent for pharmaceutical use and to evaluate its stability of color intensity when exposed to different light sources like Ultraviolet, tungsten and sunlight. The Annatto seed extract was extracted from the Annatto seeds by solvent extraction method using ethyl acetate as a solvent. Annatto seed extract was subjected for color intensity stability study in dry form and in solution of 5% potassium hydroxide, the tablets and simple syrup IP was prepared using Annatto seed extract as coloring agent were subjected to color intensity stability studies by placing them at different light sources like Ultraviolet, tungsten and sunlight. In all cases color intensity was decreased with time but rate of decrease was more with sun light. This suggested that annatto seed extract was light sensitive. To establish this fact same study was conducted with tablets packed in aluminum foil and results suggested that loss of color intensity was very negligible or no loss of color intensity. Annatto seed extract comprised with many coloring principles among all bixin an oil soluble and nor bixin water soluble attributed more for its coloring characteristics. To prove which principle was responsible for light sensitivity, bixin and norbixin were isolated from the extract by conducting Thin Layer Chromatography and Preparative Thin Layer Chromatography. The isolated pure bixin and norbixin were subjected for color intensity stability. The results proved that color intensity of bixin decreased whereas norbixin not with sunlight. So bixin was attributed light sensitivity and study was concluded that Annatto seed extract could be useful as coloring agent for pharmaceutical dosage form packed in light resistance containers.

Key words: Annatto seed extract, solvent extraction method, color intensity stability study, bixin, norbixin.

1. INTRODUCTION

The application of natural colorants to the food and beverages gained increased importance in recent times since natural colorants are less harmful than synthetic dyes and consumers acceptability also more compared with synthetic dyes as they cause harmful.[1] The colorants play major role in acceptability of food and beverages product, most of the time the quality of these products is first adjudged on the basis of its color. The suitable color in food and beverages product has impact on perception of flavor, attraction, quality and consumption. In pharmaceutical dosage forms the colorants play a major role in formulation development. Colorants are used mainly to impart a distinctive appearance to a all pharmaceutical dosage and cosmetics preparation, enhances the acceptability by the patients by improving the attractiveness. Colorants attributed to maintain uniformity between batches to batch. [2] The natural colorant extracted from Annatto seeds obtained from *Bixa orellana* L. fruits *Bixa orellana* is a large, rapidly growing tree native to tropical America and is now grown in many tropical countries in south and Central America, Africa and Asia. It bears clusters of brown or crimson capsular fruits, containing 10 to 50 seeds covered with thin, highly colored resinous coatings. The major producers of annatto seeds are Peru, Brazil and Kenya.[3] The Annatto seed extract contains many color principles among all bixin, oil soluble and norbixin, water soluble principles are responsible for its dye characteristics. Bixin responsible for imparting reddishness and norbixin for yellow. [4] Annatto has been used for over two centuries as a food color especially in dairy products and various forms are now used in a wide range of food products. Annatto used in food dye, body paint, treatment for heart burn and stomach distress, sunscreen and insect repellant. The main actions of annatto color are it kills bacteria, parasites, germs, increases urination, stimulates digestion, lowers blood pressure, mildly laxative, and protects liver. The other actions of annatto color includes it reduces inflammation, cough, cleanses blood, soothes membrane, reduces fever, blood sugar, heals wounds.[5]

2. MATERIALS AND METHODS

2.1 Materials

Annatto seeds were obtained from the tree of *Bixa orellana* locally available in the Shimoga city. Ethyl acetate was purchased from Himedia laboratories Pvt. Ltd. (Mumbai,India). Benzene, Methanol, Potassium hydroxide was purchased from S.D.Fine chemicals, Mumbai, India. Silica gel G was purchased from Himedia laboratories Pvt. Ltd. (Mumbai, India).Ethanol was purchased from Merck Ltd. India. Concentrated hydrochloric acid was purchased from S.D. Fine chemicals Mumbai, India. All the other chemicals were of analytical grade.

2.2 Extraction of Color from Annatto Seeds

2.2.1 Annatto Seed Extract (Solvent extraction method)

Seeds of the annatto tree (*Bixa orellana* L.) are abraded to remove the coloring matter bixin, norbixin. Seeds are treated with the organic solvent like ethyl acetate followed by solvent removal, crystallization and drying. Then heated on water bath oily part obtained is bixin. It contains several colored components, major is cis-bixin and a minor colouring principle is trans-bixin. In the next step of the process aqueous alkali (5% w/v potassium hydroxide) is added to the resultant powder which is then heated on water bath to hydrolyse the coloring matter and cooled. The aqueous solution is filtered and acidified with concentrated hydrochloric acid a few drops slowly from sides to precipitate norbixin. The precipitate is filtered, washed, dried, and milled to give a granular powder

Solvent extracted norbixin contains several colore components the major part is cis-norbixin and minor part is trans-norbixin. Thermal degradation products of norbixin may also be present as a result of processing. The obtained dried powder is passed through sieve. No # 44 to get fine powder.

2.2.2 Color Stability Study of Annatto Seed Color Dry Powder and Solution at Different Light Sources

The extracted annatto seed extract in dry powder form and dissolved in 50ml 5% Potassium hydroxide solution were subjected color stability study. Solubility of extract in 5% potassium hydroxide was achieved by mixing the components in rotary shaker for 24hrs. Then the dry form and solution form were placed in three different light sources like UV light source, tungsten lamp and sunlight. Initial and final color content was determined after 6hrs, 12hrs, and 24hrs.

2.3 Preparation of Tablets using Annatto Seed Color

oven for one hr at 110 ^{0}C. Prepare 5% solution of colour sample in 95% ethanol and apply 10µL to plate. Allow

To study the physical and chemical color stability and integrity of annatto seed color. Dummy tablets were prepared using lactose as diluent, starch and starch paste as a disintegrating agent and binding agent respectively. Annatto color dissolved in potassium hydroxide to make 1% solution was then added to the starch paste and was used in wet granulation with other excipients and then passed through sieve no #10 and granules were dried in hot air oven. The dried granules were passed through sieve no # 22, these granules were punched in single station tablet punching machine using 9mm punch and die set, Magnesium stearate used as lubricant. The final weight of tablet was 250mg.

2.4 Estimation of Initial Color Content in Tablets

The initial color content present in the prepared tablet was determined by crushing the tablet in mortar and pestle dissolved in 50ml 5% Potassium hydroxide solution placed in rotary shaker for 24hours. After 24hours final volume made up to 100ml and filtered. Absorbance is measured using UV-Visible spectrophotometer at 453nm using Potassium hydroxide as blank.

2.5 Color stability study in tablets at different light sources

The prepared tablets were directly exposed to different light source like UV light, Tungsten lamp and sunlight to determine the stability of Annatto seed color. After incubation at 6hrs, 12hrs, 24hrs and 48 hrs tablets were recovered, crushed, dissolved in 50ml 5% Potassium hydroxide solution placed in rotary shaker for 24hrs and the color content of tablet was determined using UV-Visible spectrophotometer at 453nm. Initial and final color content was compared. Tablets were packed in aluminum foil and repeat the same procedure for color stability study.

2.6 Color Stability Study in Oral Liquid Dosage form at Different Light Sources

Annatto color in5% Potassium hydroxide solution prepared to make 0.1% concentration solution of color and then added to the simple syrup IP. The color simple syrup was divided into three equal parts and placed at the three different light sources like UV light source, tungsten lamp, sunlight. Initial color content was noted & after incubation for 6hrs, 12hrs & 24hrs the final color content was determined. Stability of color in simple syrup was measured using UV-Visible spectrophotometer at 453nm.

2.7 Separation of Bixin, Norbixin and their Color Stability Study at Different Light Sources

A thin layer chromatography (TLC) was employed to determine the R_f value both bixin and norbixin. The procedure as follows, Activate a thin layer chromatography plate (silica gel G 250µm thickness,size 5X20) kept in hot air drying and developing using a mixture of benzene, ethyl acetate, and methanol(35:35:30). Until solvent front has

ascended about 10cm. Allow to dry, bixin and norbixin appear as yellow spots.

2.8 Preparative Thin Layer Chromatography

Activate a Thin layer chromatography plate with very thick film to 500 µm with silica gel G , kept in hot air oven for one hour at 110 ^{0}C. Prepare 10% solution of color sample in 95% ethanol and apply to the plate and allow it to dry and develop using mixture of benzene, ethyl acetate, methanol, until solvent front has ascended about 10cm.

Then separate the bixin and norbixin portion containing silica gel G by mixing with ethanol or ethyl acetate, filter it. Silica gel G gets separated in filter paper, and then the filtrate obtained is evaporated in water bath. Bixin and norbixin obtained separately. The isolated bixin and norbixin were subjected to color stability study by placed at different light source like UV light source, tungsten lamp and sunlight. Initial absorbance was noted. Then after 6hrs, 12hrs, 24hrs the stability of bixin and norbixin was determined.

3. RESULTS AND DISCUSSION

3.1 Color Stability Study of Annatto Seed Color Dry Powder and Solution at Different Light Sources

The color stability of dry extract powder and extract in 5% potassium hydroxide solution were studied and results were shown in Table No 1, the color intensity was decreased with increase in time and for both dry powder and solution form the degradation of color was much significant in sun light compared to UV and tungsten light. This study suggested that annatto seed color was sun light sensitive.

Table 1: Color intensity stability study of annatto seed colour in dry powder,in solution, in tablets and in simple syrup IP at different light sources

Stability	Light Source	Initial Colour Content (mg/ml)	After 6 hrs (mg/ml)	After 12 hrs(mg/ml)	After 24 hrs (mg/ml)
Dry extract powder	UV Light	10.55	8.85	6.5	5.5
	Tungsten Lamp	10.55	8.58	7.45	5.1
	Sunlight	10.55	6.05	5.3	4.5
In 5% Potassium Hydroxide	UV Light	10.55	8.80	6.45	5.5
	Tungsten Lamp	10.55	8.50	7.30	5.1
	Sunlight	10.55	6.03	5.25	4.5
In Tablets	UV Light	6.25	6.02	5.44	5.04
	Tungsten Lamp	6.25	5.9	5.6	5.1
	Sunlight	6.25	5.05	4.14	3.05
In Simple Syrup IP	UV Light	4.67	4.17	3.88	2.08
	Tungsten Lamp	4.67	3.83	2.55	2.23
	Sunlight	4.67	3.67	2.08	1.50

3.2 Color Stability Study in Tablets at Different Light Sources

The tablets consists annatto seed color as a colorant were subjected for color stability study by placing them at different light sources like UV, tungsten lamp and sunlight. Initial color content in tablet was noted & after 6hrs,12hrs and 24hrs the color intensity in same tablet was determined, were shown in Table No 1, the color loss from the tablet placed in sunlight is more than the tablets placed in other two light sources. This suggested that the annatto

color is sensitive to the sunlight. Color loss from the tablets placed in U.V. & tungsten lamp was also noticed. This showed that the annatto color is light sensitive when it is subjected to direct light source. The color tablets were packed in the aluminum foil and then were exposed to sunlight; color loss from the tablets was significantly reduced or no color loss.

3.3 Color Stability Study in Oral Liquid Dosage form at Different Light Sources

The simple syrup IP prepared and placed at different light sources like U.V., tungsten lamp and sunlight. Its initial color was noted and after 6 hours,12 hours and 24 hours again color intensity was determined there was no color change but only color intensity was found to be decreasing ,this was more in the case of syrup placed in direct sunlight.There was not much difference in annatto color placed at UV & tungsten lamp.

3.4 Separation of Bixin, Norbixin and their Color Stability Study at Different Light Sources

After performing thin layer chromatography the two distinct yellow points were appeared for bixin and norbixin with R_f value of about 0.50 to 0.45 respectively. The preparative thin layer chromatography was performed to isolate bixin and norbixin. The isolated bixin and norbixin were subjected to color stability study at different light sources and it was observed that bixin the oil soluble form of annatto color intensity was found to be less stable when placed at sunlight than norbixin water soluble form of annatto color.

4. CONCLUSION

The present investigation was carried out to study the suitability of Annatto seeds extract for pharmaceutical use as coloring agent for tablets and liquid orals. The dry extract powder and solution in 5% potassium hydroxide were subjected for color stability study by exposing at three different light sources like UV, Tungsten and sunlight. The color intensity was decreased with increase in time and rate of decrease was more with sun light than UV and Tungsten light source. The same phenomenon was observed with compressed tablets having annatto seed extract as coloring agent when the tablets were directly exposed to different light sources, but when tablets were packed in an aluminum foil then exposed to different light sources it was observed that loss of color intensity was much decreased or no loss. This observation concluded that Annatto seed extract was light sensitive. The same observations were observed with liquid orals. The annatto seed extract comprised many components among all chemical constituents bixin and norbixin contributes for its coloring properties. To find out which component was responsible for light sensitivity, the two components were isolated by preparative TLC method and isolated bixin and norbixin were subjected color stability study by placed them at different light sources like UV, Tungsten and sunlight separately. After measuring color intensity it was observed the color intensity the bixin, oil soluble component was degraded with time in all light sources but the rate of degradation was high with sunlight. The norbixin, a water soluble component was stable in all light sources. From the above observation study was concluded that the annatto seed extract could be a suitable choice for pharmaceutical use as a coloring agent dosage forms were packed in light resistance containers.

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