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Exploring dye extraction methods and preservatives for natural dyes

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Abstract
The use of natural dyes has been in India for thousands of years and is part of India's cultural identity. In the present scenario the craft of using natural dyes remained to smaller segment of craftsmen in the society, which may be due to difficulty in making and also due to lack of proper documentation about the process. As the synthetic dyes overtook the textile industry due to their ease of application and lower price, use of natural dyes retained to very small segment of textile wet processing industry. Hence, an attempt was made to identify the use of locally available natural dye sources and auxiliaries for preserving the natural dye extracts so as to use them as when required. For, the present study, simple, eco-friendly and easy method of extracting pigments from natural dye sources was explored. The process of extraction was validated by checking the quantity of pigment percent in the extracted solution. As, availability of the natural dye extracts was not there in the market, and craftsmen are not ready to extracts the pigments due to its lengthy process, their preservation was also studied. Three preservations *viz*., cow urine, cow ark and sodium benzoate were selected and their effect was assessed on natural dye extracts from sappan wood (*Caesalpinia sappan*), annatto seeds (*Bixa orellana L*.) and marigold flower (*Tagetes erecta*). The extracts were preserved for one year with and without preservatives and colour degeneration was evaluated through their absorbance values (*abs*) under visible light in spectrophotometer under transmittance mode. All the three extracts showed slight change in their light absorbance '*abs*' value after one year and cow urine found to be better preservative among the selected preservatives.

Keywords: Eco-friendly, natural dyes, extraction, spectrophotometer, *'abs'* value, cow urine, cow ark, sodium benzoate, sappan wood, annatto, marigold flower

1. Introduction
India has a rich biodiversity, it holds the world's twelveth position in mega diversity countries (Neha and Vidya, 2011). Natural dyes were used for coloring of textiles from ancient times till the nineteenth century when the synthetic dyes came to the textile industry (Kumar and Prabha 2018) [7]. Through the industrial revolution, textile industry was limited to use of natural dyes itself which quickly shifted to easy synthetic dyes. But now, use of natural dyes has become the need to textiles industry for their survival due to the pollution caused in use of synthetic dyes and harmful chemicals. The advantages of natural dyes over the synthetic dyes are harmless effluent, minimal pollution and good human health and eco system. Recently, a number of commercial dyers and small textile export houses have started looking at the possibilities of using natural dyes on regular basis for the dyeing and printing of textiles to overcome environmental pollution and health hazards caused by the synthetic dyes (Alemayechu and Teklemariam, 2014) [1]. Natural dyes produce very uncommon, soothing and soft shades when compared to synthetic dyes.
Now, the world is looking for use of eco friendly products in day to day life style like in textiles, interiors surface paints, food and beverages, holi colours, pharmaceutical etc. Market for natural dyes is still growing, but only its small part is applied to textile dyeing. Larger quantity consists of dyes for food colouring, because the concept on healthier foods is growing, and there is thus a need to replace synthetic colouring by healthier natural colours (Křížová, 2015) [6].
In textile industry, natural dyes are now been used in dyeing of yarns or fabric, which are used for apparel and home textiles; hand painting or printing using blocks, like in Kalamkari where the "Kalam" or pen is used to draw beautiful designs on cloth from south India, and bagru, ajrak from the western part of India. But, all the traditional methods of dyeing or printing with natural dyes is a laborious process and also need pre treatment of the fabric to be printed. Use of natural dyes in commercial textile dyeing and printing process is a challenge for young researchers starting from preserving the aqueous extract and to use them for various textile applications.

There is lot of research still going on the use of natural dyes for textile applications, based on the secondary data, the following extractions methods were explored and aqueous method was adopted for the study. Regarding preservation of natural dye extracts not much data was available; hence, in the present research three different natural and food based preservatives were studied. The spectrophotometer was used to analyze natural dye extracts.

2. Extraction of natural dyes

Use of natural dye always starts with the extraction of colouring pigment from a dry source. Extraction of colouring substance from dye stuff was considered to be an important step. There are many scientific methods of dye extraction procedures followed, which especially depends on the dye source, dye concentration, colour, end use of the extract, cost and equipment required in the process (Shrivastava and Dedhia, 2006) [9].

Environmentally safe and cheap extraction methods are therefore very important without affecting the extraction conditions and preserving the bio material without spoilage. Several extraction methodologies for natural dye that comply with both consumer preference and regulatory control are cost effective and becoming more popular (Chakrabarti and Vignesh, 2011) [2]. Following were the types of extraction methods used for natural dyes.

2.1 Aqueous extraction

It is one of the simple and oldest method of extracting dye from natural dye sources. It is still popular due to its eco friendly process, except the heat required in boiling process. Here, natural pigment yielding sources are boiled with required amount of water for optimum time, most likely for one hour. If required for hard woody materials, sodium carbonate is added to increase the pH of the extraction water. This is commonly termed as alkaline extraction. Once, the extract is cooled it is squeezed and then the pure extract is filtered for further use.

2.2 Solvent extraction

It is required for hard woody raw materials where, solvent is added to raw material in Soxhlet apparatus, which is a steam heated extractor. Different solvents such as acetone, chloroform, ether, n-hexane, alcohol, etc. are used for extraction. The process generally requires 4 hours, but use of solvents need to be focused for their eco friendly process.

2.3 Ultrasonic extraction

This method works on the Ultra-Sound Energy with lower temperature and time. Hence, improves product quality in dye extraction from natural dye sources and as well as in colouration of textiles.

2.4 Supercritical fluid extraction

In-order to avoid problems associated with solvents and other chemicals used along with high energy required boiling the raw materials, resulted in Supercritical Fluid Extraction Techniques. Here, clean, safe, inexpensive, nonflammable, nontoxic, environment-friendly, non-polluting solvent, such as carbon dioxide is used. But, the process is not available in large scale for local craftsmen working with natural dyes.

Hence, selection of required and safer extraction methods became an important step in natural pigment usages. Therefore, the traditional aqueous methods were opted for the study.

The other major issue with the use of natural dye sources was their storage for later use. As all the natural dye sources are seasonal and need proper preservation and later takes lot of time for preparation in production of dye extracts. It can be clearly said that once the natural dyes are available readily in dye liquor form there will be a huge market and many takers. Therefore, the major focus of the study was to preserve the natural dye extract in liquid form for further use.

3. Natural and food based preservatives: Preservatives perform an important and strategic part in food processing, pharmaceutical and other products that had a tendency to change when stored for certain useful duration i.e. addition of preservatives may enhance the product attributes and also help to improve the shelf life of final products.

According to Jandaik *et al*. 2015 [4], the cow urine has antifungal activities and the inhibitory activity was used in the control of fungi in menti and okra crop. The 15% concentration of fresh cow urine from local cow breed was found to be very effective against fungal pathogens (Fusarium oxysporum, Rhizoctonia solani, and Sclerotium rolfsii). *Gomutra* (Cow urine) is scientifically proven to act as an immunomodulatory along with its bacteriostatic action (Harshad *et al*. 2017) [4].

Another preservative selected for the study was Sodium benzoate, which is widely used in processed foods. Sodium benzoate helps to prevent bacteria and fungi from growing in organic and chemical products to extend shelf life, though it has several other uses. It was an odourless, crystalline powder made by combining benzoic acid and sodium hydroxide and it was commercially available in local market.

4. Spectrophotometer analysis: It was known that the colour was measured in various parameters i.e. light transmittance, reflectance and translucence, fluorescence, radiant energy and gloss. A spectrophotometer is a very powerful tool used in both the biological and chemical sciences yet operates by simply shining a beam of light, filtered to a specific wavelength (or very narrow range of wavelengths), through a sample and onto a light meter. An absorbance spectrophotometer is an instrument that measures the fraction of incident light transmitted through a solution. In other words, it is used to measure the amount of light that passes through a sample material and, by comparison to the initial intensity of light reaching the sample, they indirectly measure the amount of light absorbed by that sample.

The present study was focused on selection of extraction method from secondary data available and preserving natural dye extracts using various natural preservatives and it was planned with the following objectives.

5. Objectives of the study

- To select the feasible and eco-friendly natural dye extraction process
- To identify the natural and food based preservatives suitable for storing natural dye extracts in aqueous medium
- To assess the abs values of natural dye extracts stored for one year, with and without selected preservatives.

6. Materials and Methods

6.1 Materials

Following were the materials used for the study

Natural dye sources

Raw materials selected for the study were Marigold flowers (both yellow and orange colored flowers), Sappan heart wood chips and Annatto seeds were procured from local markets of Hyderabad and Chennai. Fresh Marigold flowers were procured and shade dried with proper ventilation in-order to avoid decomposition of flowers. Completely dried flowers, dried annatto seeds and sappan heartwood chips were used for the study.

Selection of preservatives

Two natural and one food based preservative was selected for the study. Cow urine collected from local cow breed and cow ark, which is distilled cow urine commercially available in Ayurvedic shops and sodium benzoate from food and confectionery shops were procured for the study.

6.2 Methods

Method of extracting pigments from natural dye sources

Aqueous extraction method was selected for the study, as it can be easily adopted by artisans at their own craft studios and also it requires minimal equipment for commercial production. According to Saxena and Raja, 2014, aqueous extraction can be used to extract the dye from natural dye sources, but alkali extraction method deepens the red color from sappan wood and reddish orange color from annatto seeds. Marigold flowers were directly boiled in aqueous medium. All the selected raw materials were soaked overnight in water in 1:7 ratios. Sappan wood and annatto seeds were extracted in alkaline water at pH 9, for which sodium carbonate was added to the measured quantity of water. The raw materials were boiled for one hour to extract the pigments from them. After the extract was cooled, the solution was then double filtered. Observing the pulp of the natural dye source which was boiled and filtered, there was lot of colour reaming in the pulp, hence, a second boiling of the pulp from first extraction process was done. This was studied to maximize the pigment yield from the selected natural dye source. The colour yield from first and second extractions was later analyzed using spectrophotometer through *abs* values.

Preservation percentages adopted for the study

Once, the dye extracts were ready, original extract and extractions with preservatives were stored in plastic bottles with tight lid and used for further analysis. Cow urine and cow ark were used in 5% on the volume of the extract to be preserved, similarly 2% of Sodium benzoate was used. All the extractions were stored for one year and their *abs* values were tested and compared with original extracts preserved for same the duration without any preservatives.

Spectrophotometer Analysis for natural pigment extracts

For this study, UV Spectrophotometer model– SS 5100A of Premier Colour scan was used. The instrument was used to measure the quantity of pigment present in extract from natural dye source and changes with the use of preservatives. The extracts were diluted with distilled water at 10% for better transmittance of the light rays in visible range. The colour analysis was given in the measurement as *'abs'* value-Absorbance value is the amount of light absorbed by a solution in transmittance mode. The colour values expresses color as three values: L* for the lightness from black (0) to white (100), a* from green (−) to red (+), and b* from blue (−) to yellow (+). Though c* not indicate colour, but represents whether the colour became (+) brighter or (-) dull.

7. Results & Discussion

Aqueous extraction methods with various pH range was a well known process. But the quantity of dye reaming in the pulp of extraction was needed to be analyzed for cost effectiveness of the dye quantity used as well as percent of dye source to be used for various applications. Hence, data related to first and second extractions from the same source was studied.

Effect of extraction methods for pigment yield from natural dye sources

All the three natural dye sources were boiled in water for two times separately and their pigment concentrations were studied from abs values. The details are given in the table 1.

Table 1: Concentration of pigment present in natural dye extractions

S. No	Dye Source	Abs value for first extraction	Abs value for second extraction
1	Annatto seed extract	36.554	27.947
2	Sappan heart wood extract	50.849	24.262
3	Marigold flower extract	23.799	17.643

The *abs* values of spectrophotometer data from the table 1, indicates that, there was more than half quantity of pigment present in the pulp of the natural dye boiled and squeezed after first extraction. Figure 1 revealed that sappan heart wood chips had high pigment concentration than annatto seeds and marigold flowers. Hence, darker shades were possible with sappan wood extract than annatto and marigold extracts at similar concentration of extracts used for dyeing or printing. Therefore, smaller quantity of sappan wood can be used to work. Comparing all the three natural dye sources, lowest pigment was present in marigold flowers, than annatto seeds and sappan wood chips respectively.

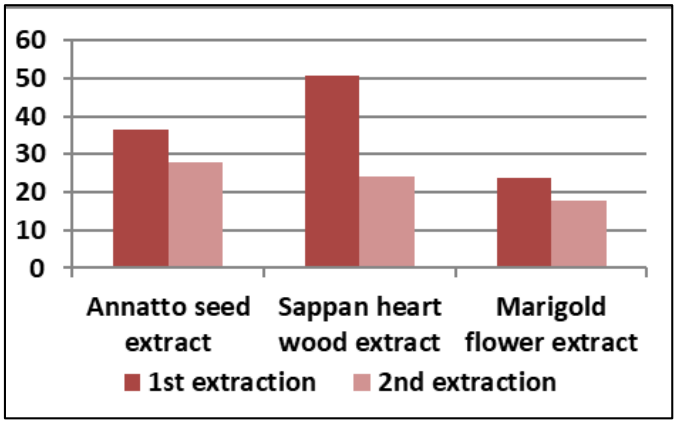

Fig.1 Pigment concentration of various dye sources in two different extractions

From fig 2 & 3 it was clearly evident that 40 per cent of pigment can be retrieved from annatto seed pulp after first extraction. Similarly, 35 per cent and 25 per cent of pigment was present in the pulp of sappan wood and marigold flowers respectively. Therefore, all the dye sources can to be boiled twice for better pigment yield or otherwise, improvised squeezing techniques like hydrolytic press can be implemented instead of manual squeezing of pulp after first extraction.

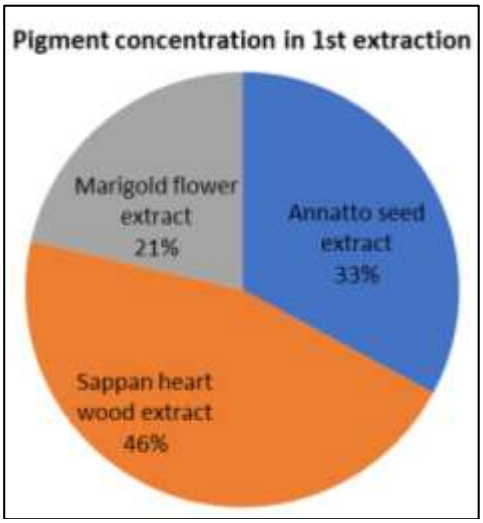

Fig 2: Pigment concentration from first extraction

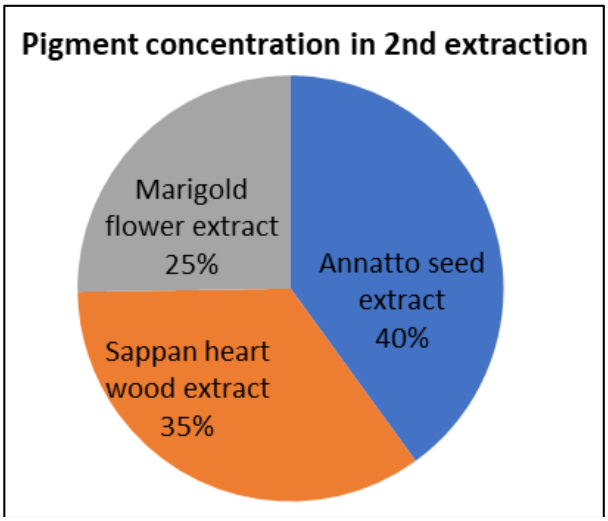

Fig 3: Pigment concentration from second extraction

Shelf life of the natural dye extracts with preservatives:

Here, the shelf life of pigments extracted over selected time period of one year was studied along with the effect of preservative used to store the aqueous extracted pigments. Here, abs values of original extract was compared with the stored extracts with three types of preservatives used for the study i.e Cow urine, cow ark and sodium benzoate. The abs values are given in the table 2.

Table 2: Colour absorbance values analyzed with UV Spectrophotometer for different preservatives

S.No	Source of Pigment extract	Abs value Original extract –year 2019	Abs value Original extract –year 2020	Abs value extract with preservative cow urine, year 2020	Abs value extract with preservative cow ark Year 2020	Abs value extract with preservative sodium benzoate year 2020
1	Annatto seed extract	36.554	28.723	36.523	35.431	23.565
2	Sappan heart wood extract	50.849	42.561	38.496	19.657	38.646
3	Marigold flower extract	23.799	23.617	22.521	17.780	20.510

From the above table it is evident that, the original extracts of sappan heart wood, Annatto seeds and Marigold flower when compared for shelf life of extractions after a year, it was found that, marigold extraction doesn't show any degeneration in quality compared to the sappan and annatto extractions. Subsequently, the same extractions were preserved using natural preservatives (Cow urine, Cow ark and Sodium benzoate) to enhance the shelf life of the extractions. It was observed that there was degeneration of colour in all the extractions but slightly low in marigold extractions.

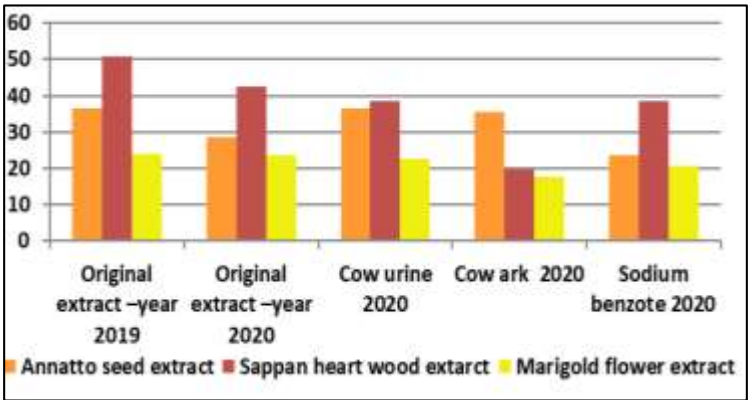

Fig 4: Colour absorbance values of natural dye extractions analyzed with UV Spectrophotometer for their shelf life

From figure 4 it was evident that out of three sources, marigold flower pigment showed good shelf life than the other two sources as there was little variation in the *abs* values of original extracts even after one year without any preservative followed by cow urine was found to be better preservative than other two preservatives *viz*., cow ark and sodium benzoate, as cow urine preserved extracts showed nearer values of that of original extract after one year. Sodium benzoate was the second best that can be used to preserve natural dye extract.

Effect of preservatives in colour values of natural dye extracts

L*a*b* and c* values of the each dye extract was analyzed both numerically and graphically. The details are as given below.

Table 3: Effect of preservative on annatto seed extract in one year duration

Natural Dye Extract	l* -lighter- black (0) to white (100)	a*- green (-) to red (+)	b*- Blue (-) yellow (+)	c*- dull to brighter
Original Extract	8.111	2.807	4.190	5.043
Original Extract + Cow urine	8.991	5.103	6.803	8.552
Original Extract + cow ark	8.102	0.253	4.546	4.553
Original Extract + sodium benzoate	12.090	13.894	21.314	20.394

From the above table 3 and figure 5 it was evident that sodium benzoate made annatto seed extract more yellower, brighter, redder and lighter than the original extract, when stored for one year. This may result in yellowish shade of annatto extract than orange shade. It should be noted that cow ark had more impact by turning orange shade of extract to greener shade.

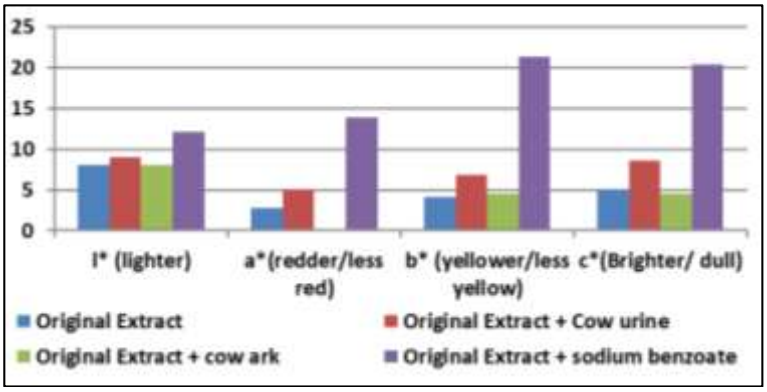

Fig 5: Effect of preservatives on annatto seed extract

Table 4: Effect of preservative on sappan heart wood extract in one year duration

Natural Dye Extract	l* -lighter- black (0) to white (100)	a*- green (-) to red (+)	b*- Blue (-) yellow (+)	c*- dull to brighter
Original Extract	8.525	28.743	22.687	36.628
Original Extract + Cow urine	2.529	5.092	12.286	13.299
Original Extract + cow ark	1.689	-12.432	7.750	14.654
Original Extract + sodium benzoate	1.924	-8.360	9.064	12.329

From table 4 and figure 6 it was observed that sappan wood extract was bright red pigment, which turned to be greener with cow ark and sodium benzoate. It was also noticed that all preservatives had reduced the colour values.

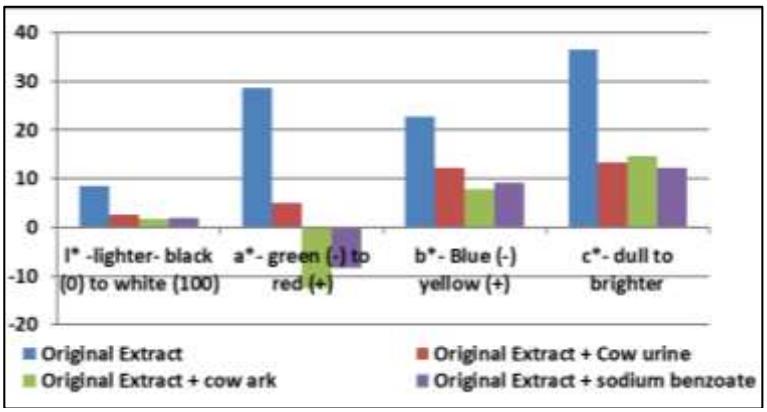

Fig 6: Effect of preservatives on sappan heart wood extract

Table 5: Effect of preservative on marigold flower extract in one year duration

Natural Dye Extract	l* -lighter- black (0) to white (100)	a*- green (-) to red (+)	b*- Blue (-) yellow (+)	c*- dull to brighter
Original Extract	31.770	8.742	27.551	28.905
Original Extract + Cow urine	40.666	10.772	46.691	46.944
Original Extract + cow ark	34.392	7.762	31.580	32.521
Original Extract + sodium benzoate	31.846	9.746	28.726	30.334

Table 5 and figure 7 indicate that cow urine had a positive impact on colour values of marigold flower extract. Cow ark and sodium benzoate also showed improved pigment colour values during preservation period.

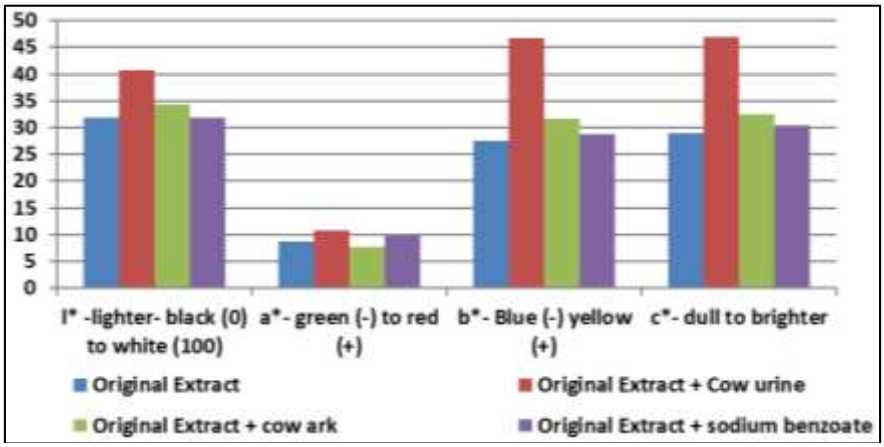

Fig 7: Effect of preservatives on marigold flower extract

From the above data, it is observed that cow urine is the best preservative when compared with cow ark and sodium benzoate for annatto and marigold extracts. Second best preservative was sodium benzoate followed by cow ark. Irrespective of preservatives used, sappan wood extraction decreased in its colour values during its storage.

8. Conclusion

It was evident that many of the textile wet processing units were been shut down due to poor or no effluent treatment plants (ETP). Many industries were not able to afford these ETP's or they were not educated to understand the need of ETP, which completely led to negative impact on textile wet processing industry. Presently, one solution to all the pollution problems was to replace synthetic dyes with natural dyes and auxiliaries. This study has made an attempt to extract pigment to the maximum level through proper extraction methods. It was also aimed to use natural preservatives to store natural pigments as well to avoid transportation of bulky dry dye sources. A community based extraction units can be developed to extract the pigment for each season and store till one year. It was observed that, no preservatives were required to store natural dye extractions until one year, if handled carefully. If required, cow urine found to be the best preservative among selected preservatives. To enhance the shelf life of natural dye extractions, one can study with more number of natural preservatives and process protocols to bring them as commercially viable dye liquors.

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