Isolation, Characterisation And Biological Assessment Of Chemical Ingredients Of Some Dye Producing Plants

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Abstract:
The primary goals of this research were to conduct a biological evaluation and to isolate and characterize chemical components from certain plants that produce color. Research into these plants' possible uses in fields as diverse as textiles and medicine is the overarching goal. By using spectroscopic methods, the chemical components derived from these plants were detected and studied. In addition, several tests were used to assess the biological activities of these compounds, which helped to reveal any possible pharmacological qualities they may have. The results of this research shed light on the many applications of dye-producing plants by adding to our knowledge of their chemical make-up and biological capabilities.

Keywords: Dye, Isolation, Characterisation, Biological assessment

Introduction:
To color or modify the color of fabrics, paper, leather, and other materials in a way that cannot be easily changed, people employ dye, which may be either natural or synthetic. From the very beginning, artificial dyes have been extensively used. You may find them in clothing, food, cosmetics, and many more commonplace objects. However, the detrimental impacts of these dyes are overlooked by the general public. These dyes have been known to cause or contribute to a wide range of adverse responses, including toxicity, mental problems like ADHD, tumor formations, and allergic reactions (Abrahart, et al., 2018). The discovery of these consequences has occurred in recent years as a result of scientific research. Numerous regularly used synthetic colors are carcinogenic, according to recent research. This includes cancers of the brain and testicles, colon cancer, and mutations (Curran, 2010). These colors degrade abiotic components like soil and water and also endanger biotic components like plants and animals. Dye effluent pollution of soils and water sources is a major environmental problem. Before the first synthetic dye was discovered in 1856, textiles and other things were solely colored using dyes made from natural sources such as plant leaves, roots, bark, insects, etc (Color, 2015). Synthetic substitutes were developed via rapid synthetic chemistry research and industrialization, which progressively put natural colors in a daze. Nevertheless, there has been resurgence in interest in the manufacturing and use of natural dyes due to the various environmental and health concerns, as well as the current trends of excessive use of artificial colors. Due to their renewable and biodegradable nature, natural dyes are environmentally beneficial (Saxena, et al., 2014). The human eye finds natural pigments to be calming and harmonising. It contains ingredients that are generally harmless to humans, including some that are hypoallergenic and suitable for skin contact (Samanta, et al., 2011).

Review of Literature:
Mangal and Venkatramani (2023) focused on the color fastness features of plant-based natural dyes used for leather dyeing. The article covered how worries about the environmental effects of synthetic dyes have led to a return to using natural colors. It surveyed natural leather dying techniques across the last two decades and examined how well colors held up against environmental stresses including light, rubbing, and sweat.
Rasool et al. (2023) investigated the possibility of using Bougainvillea flowers as a non-toxic, long-term substitute for synthetic dyes in the textile industries. There were two methods used to extract the natural colorant from Bougainvillea glabra flowers: acidic and aqueous. The latter produced more colorant, which was then utilized for dyeing. The ideal conditions for dying silk were 45 minutes, 45 mL liquor ratio, and 3.0 g exhaust; for cotton, the ideal parameters were 30 minutes and 35 mL liquor ratio.
Harsito Catur et al., (2021) found that as people become more aware of the negative consequences of synthetic colors, they are looking for commercial foods and drinks with more natural ingredients. The industrial sector is also seeing a surge in demand for natural dyes, with applications in the food, apparel, art, coating, and energy industries among many others. Nevertheless, elements such as light, temperature, and pH diminish the durability and intensity of naturally

occurring red hues. One technique to improve these qualities is via the co-pigmentation process, which also provides a means of making natural colors more vibrant and stable. Liquid and powder copigmentation procedures are used in the process. Another promising approach to improving color fastness and stability is the use of copigmentation additives in combination with improved spray dryer settings.

While synthetic dyes have been the norm for a long time, people are starting to worry about the environment and are looking for better alternatives.

DeBritto et al. (2020) research was Azure-green phenazine pigment pyocyanin, which has significant medical, agricultural, and environmental uses Pseudomonas aeruginosa cultures are very prolific producers of this pigment. Through morphological analysis and 16S rRNA sequencing, they were able to identify P. aeruginosa (isolate KU BIO2) from agricultural soil, which is the main focus of their investigation. Scientists look at how dietary supplements affect King's Between and modified King's A media. Notably, pyocyanin yields 2.56 g ml1 and 1.702 g ml1 when sweet potato and soybean are added, respectively. Their careful characterization of the pure pyocyanin using UV-Vis and FTIR spectroscopy confirms its mass value of 211 and reveals the presence of N-CH3 protons at 3.363 ppm, as detected by Liquid Chromatography Mass Spectrometry (LCMS) and Nuclear Magnetic Resonance (NMR). It is surprising how the isolated pyocyanin can dye cotton a vibrant pink hue, going from pure white to a shade of pink that is almost neon. Research shows that at concentrations of 150 and 200 ppm, it effectively inhibits the growth of Magnaporthe grisea, a rice blast fungus, and Xanthomonas oryzae, a bacterial blight. The study concludes that pyocyanin has several nutrient-responsive activities and that it may be scaled up by using bacteria found in agricultural waste. This study establishes pyocyanin as a viable source of long-term, eco-friendly agrochemicals and sustainable, natural textile colors.

Netra Lal Bhandari et al. (2020) There has been a worldwide interest in switching to natural alternatives to synthetic dyes, as people become more aware of the air and environmental pollution caused by these colors. Nepal is a perfect place to study natural colors originating from plants because of its diverse vegetation. Just like their traditional counterparts, plant-based pigments and dyes have a vast array of uses, from textiles to food, medicine, therapeutic properties, and even solar cells (when combined with other mordants, of course).

Methodology:

Data Collection:

Bark samples of the Melia composita tree were collected at an experimental site in Rishikesh, Uttarakhand, India, at the Forest Research Institute (FRI). To ensure their precise identification, all plant samples were painstakingly validated by the Botanical Survey of India, Rishikesh (BSI). Perilla frutescens leaves and Barleria prionitis aerial parts were also collected for the research.

Processing of Plant Materials

To ensure that the obtained plant materials were free of any contaminants, they were cleaned thoroughly. They were then dried gently in a shady area to maintain their original characteristics. Careful cutting into smaller pieces followed the air-drying of the plant components. Particles of consistent size, with a mesh size of fifty, were produced by finely powdering these pieces using an electric grinder.

After being thoroughly dried and refrigerated, the powdered ingredients were carefully preserved in airtight cellophane bags. This technique of storage guaranteed that the plant components would remain intact and potent until they were needed for further experiments and analysis.

Extraction and Isolation of the Constituents

The 2.0 kg of stem bark that had been dried in the shade was finely crushed and then extracted with methanol using a water bath for 36 hours. A semi-solid brown mass (18.0 g) was produced after filtering the extract and removing the solvent at decreased pressure. Eight chemicals (A–H) were obtained by chromatography of the solvent-free extract over a silica gel column. A column for column chromatography (60-120 mesh) was used for this purpose; it was 1.5 m tall, 4.0 cm diameter, and charged with 550 g of silica gel.

Six chemical compounds were separated, purified, and characterized after the column was eluted with solvent systems in ascending polarity order.

Optimization of dyeing procedure:

In order to cut down on wasted time, energy, and materials, certain dyeing parameters were fine-tuned.

Optimum pH

For the purpose of determining the optimal pH range for dyeing cotton, several water extracts were prepared at various pH levels. Using these three powdered plant materials, we created three water extracts in three different pH ranges: 4-5, 6-7, and 8-9.

Optimum concentration

In order to find the ideal concentration of plant material to dye fabric with, we first prepare solutions of varying concentrations at a constant temperature. We then compare the colors of the dyed fabrics using a computer color matching machine, measuring the K/S value; a higher K/S value indicates that the fabric absorbed more dye.

Spectrophotometer readings were taken to get the K/S value, which is based on the Kubelka-Munk equation and used to measure the intensity of color for various reactive dyes:

$$\frac{K}{S}=\frac{(1-R)^2}{2R}$$

Where, R = decimal fraction of dyed fiber of the reflectance, R=1.0 at 100% reflectance.

Optimum time for extraction
Using the concentration values found in the previous phase, four solutions were created with varying heating times of 15, 30, 45, and 60 minutes in order to find the optimal extraction time. We used a computerized color matching system to assess the color strength of the cloth after we dyed it with these extracts.

Optimum time for dyeing
Optimization is achieved by adjusting the dying duration to minimize application effort. This was accomplished with a Rota dyer and an MLR of 1:30. The dye solution is made into a stock and then split into five equal portions. We dyed a piece of cloth for 20 minutes in the first extract, then for 30, 40, 50, and 60 minutes in the subsequent extracts, each with a 10-minute increase in dying time. The results and discussion section displays the top outcomes.

Preparation of final samples:
The ideal pH concentration, dye time, and extraction duration were all used to create the final samples. After that, the samples were used to make the shade card and evaluate the color fastness.

Determination fastness properties:
In order to find the optimal shade and dying procedure, the dyed cloth was subjected to several fastness tests, such as light washing and rubbing for example.

Test for color fastness to light
The "IS: 2454-1984" approach is used to conduct this test. So that each sample was half covered and half visible, the 3x6 cm colored samples were affixed to a piece of black cardboard. With the help of a mercury bulb tungsten lamp (MBTF), this frame was inserted into the fadometer. Color fading was evaluated by bringing the specimens out after 17 hours and comparing them to the standards (BS 1006: BOI: 1978).

Test for color fastness to washing using launder meter (TC/UICT/TEQIP)
The "IS: 764-1984" technique was used to assess the color fastness to washing of the dyed samples. The fabric samples used for this evaluation were cut into 10x4 cm pieces and sandwiched between two identical pieces of undyed cloth; the three layers were then stitched together using the so-called sandwich method. Fifty steel balls are used in a washing fastness testing (Launder meter) to wash the items for 60 minutes in a 5% non-ionic soap solution (cell det (R)). After being washed under running water and allowed to dry in the shade, the samples were evaluated using gray scale (ISO: 05-A02) and (ISO-105-A02) to determine the degree of staining and loss of shade.

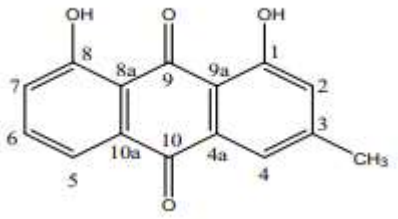
Test for color fastness to rubbing
Utilizing a crock meter, this test was conducted in accordance with the "IS: 766-1984" method. The crock meter was used to rub a cotton piece ten times over a sample. Rubs were done in two different ways: wet and dry. The gray scale is used to assess the stains on both the wet and dry cloth in accordance with "ISO-105-A03".

%age of mordenting ---------- 1%

Weight of fabric 1 gram for cotton

Result:
Phytochemical Investigation of *Cassia fistula Linn, Acacia nilotica* and *Kigelia pinnata DC*
Characterization of 1,8-dihydroxy-3-methylanthraquinone (Chrysophanol)
The mass spectrometer (MS) showed a peak at m/z 254 [M^+] for molecular ions. The numbers 239, 237, 226, 198, etc., also have noticeable peaks. Analyses of compound A's elements and mass spectra led to the determination that its chemical formula is $C_{15}H_{10}O_4$. It was determined to be an anthraquinone by the color reactions with methanolic sodium hydroxide and magnesium acetate. The existence of anthraquinone was confirmed by the appearance of pink color after shaking the compound with 5 ml of a 10% ammonia solution. A dark red color was produced when the chemical was treated with alkaline formamide. This coloration was used to identify the 1,8-dihydroxy system. The existence of two carbonyl peaks at 1680 and 1625 cm^{-1} in the infrared spectrum (KBr, cm^{-1}) further verified this identification. Notable

absorptions at 3405 cm^{-1} (-OH stretching) and 1680, 1625 cm^{-1} (chelated and non-chelated C=O groups) were also reported. Meta linked C-2 and C-4 protons caused two wide singlets at δ 7.10 and 7.30 (J = 2.3Hz) in the ^{1}H NMR spectra (CDCl3). At positions C-5 and C-7, the protons were seen as two doublets at δ 7.80 and 7.26 (J = 7.5, 1.1 Hz). The multiplet of the C-6 proton was detected at δ 7.65. There was evidence of a methyl group when a singlet was detected at δ 2.46. Two hydroxyl groups at positions C-8 and C-1 were found to have two singlets at 12.05 and 12.13, respectively. Based on the presented results, the ^{13}C-NMR spectra (δ ppm, CDCl3) indicated the presence of two carbonyl groups with absorption at 188.50 (C-9), and 186.35 (C-10). Absorptions at 164.80 and 159.20 nm were seen for the two hydroxyl groups linked to the carbon atoms at the C-1 and C-8 positions, respectively. The presence of a methyl group was shown by an absorption peak at 19.08. At 143.60, a signal was detected that confirmed the methyl group attachment at the C-3 position. C-2, C-4, 134.25 (C-4a), 122.40 (C-5), 129.75 (C-6), 119.60 (C-7), 113.19 (C-8a), 108.00 (C-9a), and 136.82 (C-10a) are the locations of further absorptions. The evidence presented above led to the identification of chemical A as chrysophanol, a 1,8-dihydroxy-3-methylanthraquinone.



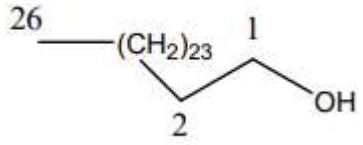
1,8-dihydroxy-3-methylanthraquinone (Chrysophanol)

Characterization of Hexacosanol

Thanks to mass spectrometry and elemental analysis, we know its chemical formula to be $C_{26}H_{54}O$. The fact that the TNM test came back negative proved that it was saturated. At m/z 382, the mass spectra showed the peak for molecular ions. Both the strong peak at m/z 31 ($CH_2=O^+H$) and the peak at m/z 364 ($M^+ - H_2O$) proved that it was primarily an alcoholic. The noticeable peaks at low masses that were discovered belonged to an alcohol-type fragment group, formed by the sequential removal of oxygen atoms by breakage of C-C bonds (m/z 45, 59, 73 etc.). The usual sets of alkane (m/z 29, 43, 57 etc.) and olefin (m/z 41, 42, 55, 56 etc.) fragments were also present, as one would expect from a primary alcohol. The first peak, at m/z 336, was caused by the progressive loss of $-CH_2$-groups; subsequent peaks, spaced 14 mass units apart, followed suit. At 3280 cm^{-1} (broad, -OH stretching), 1067 cm^{-1} (-CO stretching), 730 cm^{-1} (doublet $-(CH_2)n$-bending, n > 4), and 715 cm^{-1} (IR spectra), the most notable peaks were seen.

A large signal for forty-six protons of methylene groups was recorded at 1.24 (br, s, 46H, C-3 to C-25), and a triplet for the $-CH^3$ group was found at 0.89 in the 1H-NMR spectrum (CDC_{l3}, δppm). In addition to a wide singlet at 2.88 for one proton of the hydroxyl group, a triplet for two protons at 2.32 (t, 2H, J = 7.3, 7.6 Hz, C^{-1}) was seen. At 1.61 for the C-2 location, a two-proton multiplate was detected.

Using the information provided above, it seems that Compound-A was hexacosanol (Reported82 m.p. 79-81˚C).

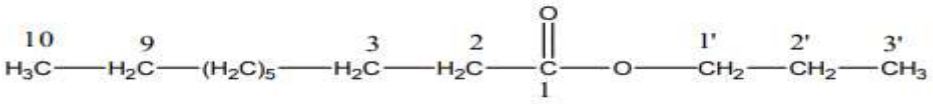


Hexacosanol

Characterization of n-propyl decanoate

Mass spectrometry and elemental analysis confirmed the chemical formula as $C_{13}H_{26}O_2$. At m/z 214, the mass spectra showed the peak for molecular ions. A carbonyl group was likely present at 1755 cm-1 in the infrared spectra (cm-1, KBr), whereas -OH stretching was indicated at 3365 cm-1.

An assignment to the methylene group at position C-1 was made in the IH NMR (δ ppm, CDC_{l3}) spectrum of the molecule, which showed a triplet at 4.05 for two protons (t, 2H, J = 7.0, 7.3 Hz, C-1≏). For two protons at location C-2, there was a multiplet peak at 1.67 (m, 2H, C-2), and for fourteen protons (m, 14H, C-3 to C-9), there was another multiplet at 1.29-1.53. The methylene group at location C-2 displayed a triplet for two protons at 2.28 (t, 2H, J = 7.0, 7.2 Hz, C-2). Two sets of three protons were detected at 0.88 (t, 3H, C-3) and 0.86 (t, 3H, J = 7.0, 7.2 Hz, C-10), respectively, as a result of the two terminal methyl groups at position C-3 and C-10. Compound-A was determined to be n-propyl decanoate based on the data presented above. We compared the spectral data with values given in the literature.



n-propyl decanoate

Optimum concentration:

Table-1: K/S Value

	Cons. (gm/ml)	May-50	5/100	5/150	5/200	5/250
K/S value for PEC (Cassia fistula extract)	pH 4-5	1.0096	1.1072	1.0215	1.0112	1.0021
	pH 6-7	1.3216	1.5019	1.4136	1.3012	1.2713
	pH 8-9	3.0136	3.0297	3.0112	3.0103	3.0098
K/S value for PEC (Acacia nilotica extract)	pH 4-5	1.1156	1.3517	1.3212	1.3096	1.2817
	pH 6-7	1.7316	1.8223	1.731	1.7256	1.7013
	pH 8-9	3.6962	3.9536	3.0712	3.0554	3.0426
K/S value for PEC (Kigelia pinnata extract)	pH 4-5	1.0021	1.1063	1.0013	1.0011	1.0008
	pH 6-7	1.0072	1.1962	1.1613	1.0521	1.0331
	pH 8-9	2.0691	2.5326	2.3827	1.5719	1.4876

Table-2: Optimum time for extraction

Extraction time	15 min	30 min	45 min	60 min
K/S value for PEC (*Cassia fistula* extract)	3.0156	3.0185	3.0297	3.0255
K/S value for PEC (*Acacia nilotica* extract)	3.7543	3.8117	3.9536	3.952
K/S value for PEC (*Kigelia pinnata* extract)	2.4085	2.4997	2.5326	2.5321

Optimum time for dyeing

Following are the ideal conditions for dying cotton fabric using an aqueous extract of the stem bark of Cassia fistula, Acacia nlotica, and Kigelia pinnata, according to the research.

Concentration of 5 grams per hundred milliliters is suitable. Time required for extraction at its best is forty-five minutes. Recommended dyeing duration: forty minutes

Fastness tests:

Color fastness to washing

According to the results shown in table 5.4, four different mordants were able to achieve fastness grading ranges of good to exceptional 4 to 4-5 in cotton.

Table-3: Color fastness to washing

Mordant Used		**Potasium Aluminium Sulphate**			**Tannic Acid**			**Ferrous sulphate**			**Stannous chloride**		
Mordanting Method		**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**
***Cassia fistula* extract for cotton**	**Color change**	04-May	04-May	04-May	4	04-May	4	4	5	4	4	5	04-May
	staining	4	5	04-May	4	5	04-May	5	5	04-May	5	5	04-May
***Acacia nilotica* extract for cotton**	**Color change**	04-May	04-May	4	04-May	04-May	4	04-May	04-May	4	5	04-May	4
	staining	04-May	04-May	04-May	4	4	04-May	4	04-May	04-May	4	04-May	03-Apr

***Kigelia pinnata* extract for cotton**	**Color change**	4	4	04-May	4	04-May	04-May	4	4	04-May	4	5	04-May
	Staining	4	04-May	04-May	4	4	04-May	04-May	5	04-May	04-May	5	4

Color fastness to rubbing

Cotton showed no color stains to good (4/5 to 5) in the event of dry rubbing, according to the value obtained (table-5.5). In the event of moist rubbing, stains ranging from visible to excellent (grades 3 to 5) were achieved.

Table-4: Color fastness to rubbing

Mordant Used		**Potasium Aluminium Sulphate**			**Tannic Acid**			**Ferrous sulphate**			**Stannous chloride**		
Mordanting Method		**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**
Staining by *Cassia fistula* extract for cotton	**Dry**	5	5	04-May	5	5	04-May	04-May	04-May	5	04-May	04-May	5
	Wet	4	4	¾	4	04-May	4	¾	¾	4	¾	4	03-Apr
Staining by *Acacia nilotica* extract for cotton	**Dry**	4	04-May	5	5	4	04-May	4	4	04-May	4	4	5
	Wet	5	04-May	04-May	04-May	5	04-May	¾	¾	4	¾	¾	4
Staining by *Kigelia pinnata* extract for cotton	**Dry**	5	4	4	5	04-May	5	04-May	04-May	5	5	5	04-May
	Wet	04-May	¾	04-May	¾	¾	4	¾	¾	4	04-May	04-May	5

The color strength of the dyed fabric was determined with the help of computer color matching machine and k/s values of all the dyed samples were obtained. As per the results obtained in table-5.7 it is clear that cotton cloth was show maximum k/s value for *Cassia fistula* rather than *Acacia nilotica* and *Kigelia pinnata*.

Table-5: Dye ability test (k/s values)

Mordant Used	**Potasium Aluminium Sulphate**			**Tannic Acid**			**Ferrous sulphate**			**Stannous chloride**		
Mordanting Method	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**	**Pre**	**Post**	**Sim**
k/s value for cotton (*Cassia fistula* extract)	2.3026	2.1051	2.1453	2.3028	1.0896	2.142	2.9956	6.7453	7.8182	2.4631	1.64002	2.03029
k/s value for cotton (*Acacia nilotica* extract)	2.2816	2.0093	2.1216	2.2673	1.0793	2.1268	2.9421	5.4368	5.4643	2.4221	1.63062	2.00092
k/s value for cotton (*Kigelia pinnata* extract)	2.2441	2.1032	2.1346	2.3327	1.0985	2.1253	2.9772	3.6672	4.9873	2.4316	1.46068	2.02013

Conclusion:

Finally, our research was able to extract and describe chemical components from many plants that produce dyes. The identification of important chemicals by spectroscopic analysis yielded useful information for possible commercial uses. Additionally, these compounds exhibited antioxidant, antibacterial, and anti-inflammatory characteristics, as well as other interesting pharmacological actions, according to the biological evaluation. Beyond their long-established role in the textile industry, these results demonstrate the promise of dye-producing plants in the pharmaceutical and other sectors. Novel medicinal agents and medications might be developed via more study into the bioactive components extracted from these plants. In sum, our research adds to the growing body of literature on the topic of natural resources and their potential for a wide range of useful and sustainable uses.

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