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EXTRACTION OF NATURAL DYE FROM ARECA PRECIPITATE USING DIVERSE TECHNIQUES AND INVESTIGATION OF ITS INDUSTRIAL USES

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Abstract

Natural dye was extracted using areca precipitate. Varied color intensities were obtained by the dyes recovered from areca precipitate at varied concentrations (10%, 5%, and 1%), using methanol and water as a solvent. When extracting colors, water produced a more vibrant hue than when methanol was used as the solvent. The UV-Vis absorption spectra of the water and methanol extracts were obtained, and it was discovered that the absorption maxima of both extracts were in the same range: 241 nm for the water extract and 246 nm for the methanol extract. The color intensity of extracted dyes increased with increasing pH under various alkaline circumstances (pH 8–12). There was a qualitative phytochemical analysis carried out. The components that were present were tannins, amino acids, and saponins; all other substances were lacking. Retardation factor (Rf) for the most resolved component was found to be closer to tannic acid, gallic acid, and catechin. The extracted phytochemicals' Rf values matched those of the real samples. Different dye concentrations resulted in fabric that was colored in various colors of brown with differing intensities. On fabric treated with the same mordant, 1% aqueous and alcoholic dye created a very light shade of brown, whereas 5% dye generated muddy brown and bright brown colors. By employing different mordants, different shades of brown were produced. For example, practically black color was produced when FeSO4 was used as a mordant; varied shades of brown were produced using alum as a mordant; and reddish-brown color was produced with lodhra as a mordant. In the Textiles Committee lab, dyed fabric's light fastness and wash fastness were evaluated. The colored fabric's fastness qualities, such as its wash and light fastness, were rated 3 and 4 on the grey scale, respectively, meaning that it possessed fair to good fastness qualities. Against every examined bacterium, the extracted color exhibited antibacterial action. Staphylococcus aureus, Sarcina, and E. coli were all susceptible to the areca dye's commendable antibacterial action. The appearance of pale pink and light brown color served as the endpoints for calculating the normality of strong acid, HCl, utilizing phenolphthalein and areca precipitate extract as indicators.

***Keywords*:** Areca precipitate, natural dye, antimicrobial activity, histological stain, indicator

1. Introduction

Natural dyeing is receiving a lot of attention in the textile industry right now because of the increased awareness of the problems associated with aquatic pollution and its environmentally beneficial qualities, like raw material sustainability and biodegradability.[4, 2]

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Areca catechu is a palm species commonly known as the betel tree. The chewed areca nut, sometimes known as fruit, is consumed with the betel leaf. Betel trees are grown all over the world in tropical climates. Areca nut is utilized as a medication worldwide because it contains components including sugars, lipids, tannins, polyphenols, and alkaloids. It has antioxidant, anti-inflammatory, antifungal, antibacterial, and anthelmintic properties. Another characteristic of Areca nuts is their tannin content, specifically the forms of condensed tannins known as proanthocyanidins. Catechins are the primary class of tannins[11].

The residue left over after processing areca nut is called areca precipitate. During harvest season, ripened fruits are picked, and uncooked fruits are peeled to extract kernels. Once the kernel is removed, it must be cooked at a high temperature in large metal pots for at least 12 hours. It is necessary to fill the container with the areca-nut kernel and mix it with enough water. The liquid that remains after the kernels are removed from the boiling liquid is called areca precipitate. To achieve a decent color, the areca-nut precipitate from the previous batch must be blended in with the water while boiling.

Mordants, which have an affinity for both the dyeing material and the fabric, are often salts of metallic compounds and are used to apply natural dyes to textiles5. Mordants are substances that promote textile fibers' absorption of natural dyes. Even a single natural dye can generate several hues when combined with various types of mordants. Thus, the dye determines the final pigment's brightness and color fastness, which are achieved by varying the dye's concentration and using the mordants deftly [3]

Commonly used metallic mordants include metal salts of copper (cupric sulfate), iron (ferrous sulphate), chromium (potassium dichromate), aluminum (alum), and tin (stannous chloride). Metallic mordants have been replaced by bio-mordants because some of the mordants contain heavy metals, which are extremely hazardous and can cause health and environmental problems. As a bio-mordant, pomegranate peels, myrobalan, and the herbal plant Lodhra (Symplocusracemosa) can be substituted for other harmful mordants.

In an article published in 13 summarized the research conducted over a 15-year period (1998–2013) in a variety of extracted dye applications, with particular reference to technological advancements in natural textile dyeing and the use of natural dyes in food coloring, dye-sensitized solar cells, and functional textile finishing. Among the recently found uses for natural dyes are histology staining, fabric deodorizing, insect repellant and UV protection.

The current study's goals were to determine the effectiveness of extraction at various concentrations, the pH and solvent conditions under various extraction techniques, the ability to dye cotton fabric using extracted dyes, the assessment of areca precipitate extract as a pH indicator in an acid-base titration, and the ability to test the sample extract's antibacterial activity against specific microbes and examination of the extract's effectiveness as a histological stain as well.

2. Materials and methods

2.1 Materials required

Areca precipitate, Cora cotton fabric (washed), mordants (alum, FeSO4 and lodhra), NaOH and acetic acid. All chemicals used in the work were analytical grade.

2.2 Modes of extraction

Three modes of extraction were used:

1. Extraction by boiling
2. Extraction using sonicator
3. Extraction using microwave

2.2.1 Extraction by boiling

The areca precipitate that had solidified was crushed into a powder. After precisely weighing 5g of powder, the dye was extracted by boiling it in 50ml of water. After boiling the powder for ten to fifteen minutes, the extract was filtered through muslin fabric. The filtrate was then centrifuged for 10mins at 5,000 rpm. Pellet was discarded and the supernatant was used for dyeing.

2.2.2 Extraction using sonicator

5g of powdered sample was weighed accurately and placed along with 50ml of water, in an ultrasonic bath for 30 mins. After which the extracted dye was filtered and centrifuged at 5,000 rpm for 10mins. The same procedure was repeated using methanol as solvent.

2.2.3 Extraction using microwave

5g of powdered sample was dissolved in 50ml of water and heated using microwave oven for 30-60 seconds, and then the dye extract was filtered and centrifuged. Microwave was also used to extract dye with methanol as solvent instead of water.

2.3 Extraction at different concentrations

Varied solvent systems, such as methanol and water, were used to extract color at varied quantities. After precisely weighing 5g, 2.5g, and 0.5g of samples, 50ml of water was added to obtain 10%, 5%, and 1% of the dye concentration. To extract dye, a sample combined with water was heated for ten to fifteen minutes. After being removed, the dye was filtered, centrifuged, and used to dye fabric. Similarly, using methanol as the solvent, the extraction was done at three different concentrations: 10%, 5%, and 1%.

2.4 Extraction at changed pH conditions

The dye was extracted at different pH conditions. Briefly, 10g of sample was dissolved in warmed water. Then the initial pH of the solution was noted using pH meter. Then the pH was adjusted using dilute NaOH and dye was extracted at pH 8-12. Intensities were measured using UV-Vis spectrophotometer.

2.5 Phytochemical analysis

The bioactive components were analyzed using the solvent extracts. Qualitative analysis of phytochemicals using standard procedures, including

alkaloids (Dragendorff test, Hager's test, and Mayer's test), tannins (Ferric chloride test), flavonoids (Alkaline test and Lead acetate test), glycosides (Fehling's test and Benedict's test), terpenoids (Salkowski test), phytosterols (Salkowski's test), saponins (Foam test), amino acids and proteins (Ninhydrin test and Millon's test), and phenolic compounds (Ferric chloride test and Lead Acetate test).

2.6 Chromatography

TLC analysis was performed on the 10% extract using the boiling, sonicator, and microwave methods. The developing solvent, 6% aqueous acetic acid, was added to a saturated chamber in which the plate was put. The sample components are carried up the plate by the solvent through capillary action. Before the solvent front touched the stationary phase's top, the chromatogram was taken out of the chromatography chamber, dried, and examined.

2.7 Mordanting

The fabric was treated using the pre-mordanting technique prior to being dyed with extracted natural dye. The textile substrate is initially treated for 60 minutes at 70°–80°C with a liquor ratio of 1:30 in an aqueous solution containing mordants such as alum, FeSO4, and lodhra (natural mordant). After that, it is rinsed with water. Different mordants were applied to the fabrics for the same amount of time at the same temperature. After that, the fabric that had been mordanted was dyed under ideal dyeing circumstances.

2.8 Dyeing

Mordanted fabric material was dyed using a variety of dye samples that were acquired using a variety of extraction procedures, including the use of solvents (water and methanol), methods such as boiling, sonication, and microwave, and dye samples with varying concentrations (10%, 5%, and 1%). Fabric materials that had been mordanted were colored with a 1:15 liquor ratio. The fabric was dipped in dye, heated to 70°–80°C for 30 minutes on a water bath, and then let to soak in the dye for 12 hours. Following the dyeing process, the cloth was squeezed, stretched, and allowed to air dry before being rinsed three times with 500 ml of distilled water.

2.8.1 Fastness test

2.8.1a. Light fastness

At the Textiles Committee in Bangalore, the light fastness characteristics of dyed cloth were assessed using a xenon arc lamp in accordance with AATCC16.3:2012.

2.8.1b. Washing fastness

At the Textiles Committee in Bangalore, color fastness to washing was assessed using IS/ISO 105 C-10 A (1)-2006.

2.9 Antibacterial property of dye

The dye extract's antimicrobial activity was evaluated in the following ways: The nutrient agar medium (g/L: Yeast extract 1.5; Peptone 5.09; Beef extract 1.5; NaCl 5.0; Agar 20.0; pH 7.5) was made and sterilized for 20 minutes at 121°C. The sterilized petriplates were poured with equal thickness of nutrient agar. The microorganisms used for antibacterial activity were; Staphylococcus aureus, Sarcina (gram positive) and E.coli, (gram negative) were grown overnight in nutrient broth. This broth culture was used for inoculating the nutrient agar plates. Antimicrobial activity was carried out by agar well diffusion method. Every examined bacterium's zone of inhibition was determined after it had been incubated for 24 hours at 37°C.

2.10 Alternate applications

2.10.1 pH indicator

For strong acid-strong base titrations, 10 ml of 0.1 N NaOH in a burette and 10 ml of 0.1 N HCL in a conical flask were used. Three titrations were conducted to ensure accuracy and dependability using dye extract as an indicator.

2.10.2 Histological staining

It is possible to color tissue histologically using natural dyes. Onion peel was dyed using the sample's 10% methanol extract in the manner shown below: On a dry, spotless glass slide, a drop of distilled water was placed. After using forceps to carefully remove a translucent onion peel (epidermis), the epidermis was placed on a glass slide, 2-3 drops of sample dye were applied, and the skin was left to get stained for 30 seconds. The skin is dyed and then placed onto a slide that has a drop of glycerin on it. After covering the dyed cells with a cover slip, the cells were examined under a microscope.

3. Results and discussion

3.1 Dye extracted at different concentrations

The dyes extracted with methanol and water at several concentrations (10%, 5%, and 1%), yielding varying color intensities (Fig. 1). When pigments were extracted using water instead of methanol as a solvent, the colors were more vibrant. As dye concentration rose, so did color intensity. At lesser concentrations (5% and 1%), alcoholic dyes exhibited an orange-red tinge, while aqueous dyes had deeper brown hues. Both the water and methanol extracts' absorption spectra were obtained in the UV-Visible region, and it was discovered that their absorption maxima—241 nm for the water extract and 246 nm for the methanol extract—were in the same range (Fig.2).

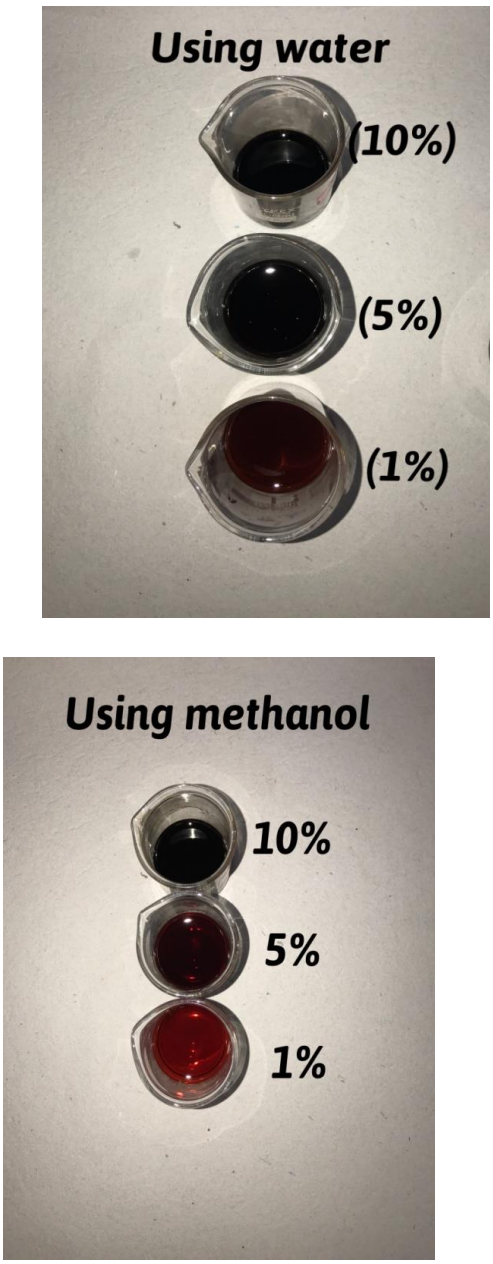


Fig.1.Extracted dyes at different concentrations using water and methanol as solvents.

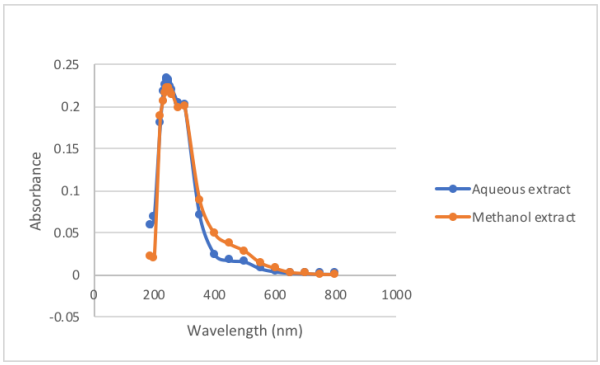


Fig. 2. Absorbance spectra of aqueous and methanol extract in UV-Visible range.

3.2 Effect of pH on dye extraction

Increased color intensity was observed in extracted dyes at various alkaline settings (pH 8–12) (Table 1). The color was brown at slightly alkaline pH levels, but at greater alkaline pH levels, blackish brown was seen (Fig.3).

Diode was extracted at various alkaline pH settings, yielding varying color intensities. It was discovered that extraction peaked at pH.11, after which there was little apparent change in hue. The dye extracted at an alkaline pH and the dye removed without changing pH showed a noticeable color variation. Both before and after pH adjustments, UV-Visible spectra were obtained (Fig.4).

Table.1. Absorbance maxima of dye extract at different pH conditions.

pH	Absorbance maxima (nm)
8	241
9	242
10	244
11	245
12	245

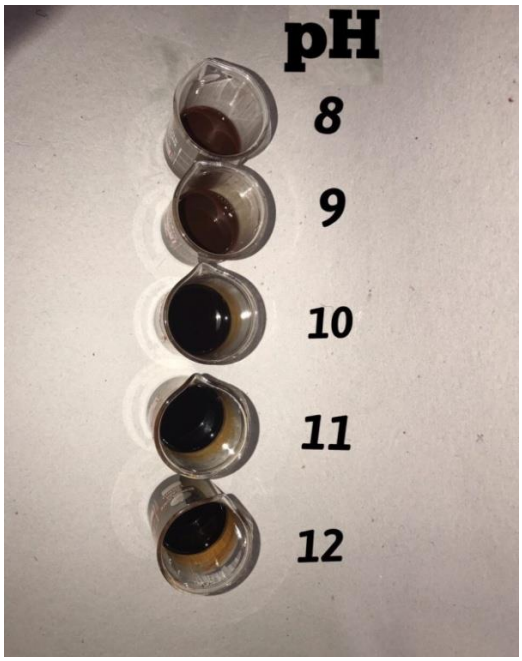


Fig.3. Dye Extracted at different pH.

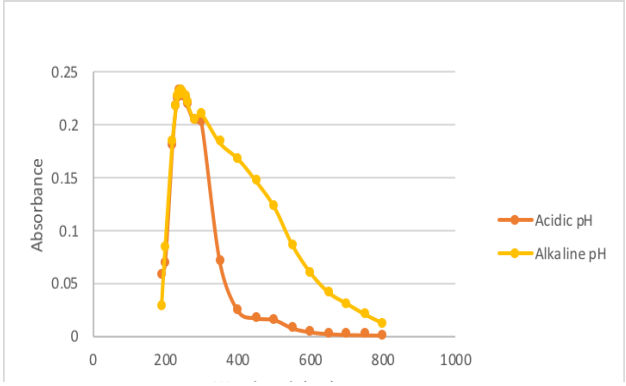


Fig.4. Absorption spectra of acidic and alkaline extract in UV-Visible range.

3.3 Analysis of phytochemicals

The standard methodology was followed while performing the phytochemical analysis, and the findings are presented in Table 2. The components that were present were only tannins, amino acids, and saponins (Fig. 5). All other components were lacking. When ingested, areca nut affects most human organs, including the heart, brain, lungs, gastrointestinal tract, and reproductive organs. Heart arrhythmias, neuronal damage, hepatotoxicity, asthma, central obesity, myocardial infarction, type II diabetes, hyperlipidemia, metabolic syndrome, and other pre-existing illnesses are all made worse by it. Areca nut has an endocrine system effect that can result in infertility, hypertrophy of the prostate, and hypothyroidism. Additionally impacted is the immunological system, which results in decreased cytokine production and T-cell activity reduction. Using areca nut during pregnancy can have harmful effects on the developing foetus. All of this is mostly brought on by the alkaloids, particularly arecoline, that are present in areca nuts. Because areca precipitate extract has no alkaloids, it is safe to use[7].

Table.2. Phytochemical status of aqueous areca precipitate extract.

Phytochemical	Status
Alkaloids	Absent
Flavonoids	Absent
Saponins	Present
Tannins	Present
Phenolic compounds	Absent
Amino acids	Present
Triterpenoids	Absent
Phytosterols	Absent
Reducing sugars	Absent

Using areca nut, the primary phytochemicals in Swietenia macrophylla's alcohol extract of the leaf, seed, and central-fruit axis were compared qualitatively and quantitatively. Common bioactive substances, such as alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, and volatile oils, were shown to exist in the phytochemical tests and were subsequently measured [6].

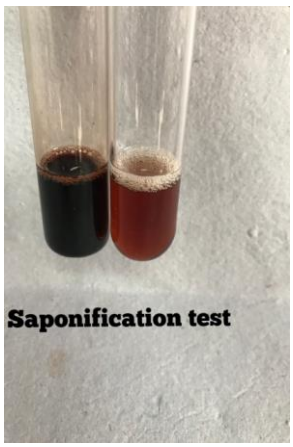


Fig.5. Qualitative tests showing presence of saponins, tannins and amino acids.

3.4 Chromatography (Thin Layer Chromatography)

The crude aqueous sample extract, obtained through various extraction methods, was subjected to thin layer chromatography. The results showed that different components were resolved (Fig. 6). The retardation factor (Rf) of the most resolved component (Table 3) was compared with the Rf values of authentic samples of tannins and related phenolics (Table 4)10. Ratios of these phytochemicals were nearly the same, and the most resolved component had a retardation factor closer to tannic acid, gallic acid, and catechin.

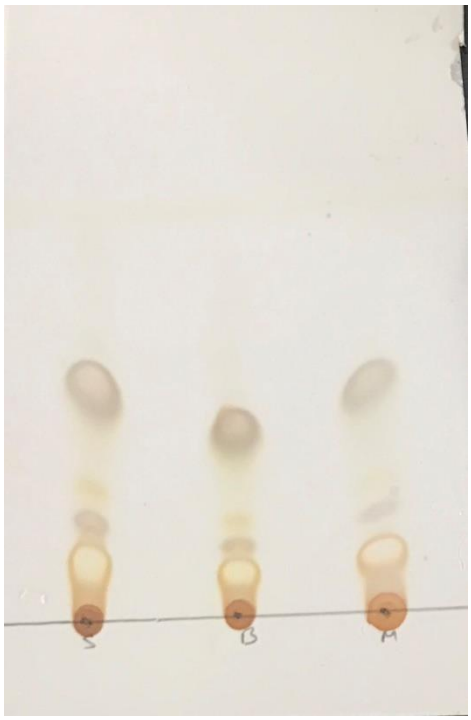


Fig.6. Thin layer chromatogram of extracts obtained from sonication, boiling and microwave.

Samples	R_f in 6% acetic acid as developing solvent
Sample 1 (Sonicated)	0.55
Sample 2 (Boiled)	0.43
Sample 3 (Microwaved)	0.55

Table.3. Rf values of crude sample extracts.

Tannins and Related Phenolics	R_f in 6% acetic acid as developing solvent
Gallic acid	0.47
Tannic acid	0.47
Catechin	0.47
Catechol	0.71

Table.4. Rf values of some authentic sample of tannin and related phenolics.

3.5 Effects of different parameters on dyeing

The effects of several mordants on dye uptake is demonstrated in a clear manner (Fig. 7). When 10% aqueous extract was used as a mordant for dyeing, the fabric took on a blackish hue. On the other hand, fabric dyed a medium brown color when alum was used as a mordant. Fabric dyed with Lodhra alone turned out to be reddish-brown; fabric dyed with alum and Lodhra combined yielded a dark brown color**.**

Different dye concentrations resulted in fabric that was colored in various colors of brown with differing intensities as shown in Figure 7. On fabric treated with the same mordant, 1% aqueous and alcoholic dye created a very light shade of brown, whereas 5% dye generated muddy brown and bright brown colors..

Research was done to revive the age-old practice of dying using natural dye derived from Odinawodier L bark; the tree belongs to Anacardiaceae family. Cotton fabrics were dyed and bleached using different mordants for the investigation. Different mordanting techniques were used to carry out the dyeing process12. The dyed fabrics demonstrated good light, rubbing, and perspiration fastness characteristics. The different color shifts were measured using computer color matching software. The dye's antimicrobial properties was also investigated 15. The dye extract lacked heavy metals, as demonstrated by investigations conducted using inductively coupled plasma mass spectrometry (ICP-MS).

Based on the data obtained it's concluded that dyeing process is influenced by various factors such as concentration, pH, extraction method, and mordants used. The dye that was extracted from the sample solution varied depending on the extraction method used. When the dye was recovered from the sonicator as opposed to boiling and microwaving, the intensity of the dye increased dramatically. Even the extraction solvents had an impact on the process.

Using different mordants produced varied hues of brown. For example, using FeSO4 as a mordant nearly produced a black colored fabric; using alum as a mordant produced various shades of brown; and using lodhra as a mordant produced a reddish brown color. This demonstrates how a variety of shades achieved and tints of a same color by merely utilizing different mordants.
Natural mordants like lodhra can be used instead of poisonous metallic ones that are bad for the environment and human health8. More natural mordants were used in place of poisonous ones to reduce the production of toxic wastes with little to no negative effects on humans or the environment.

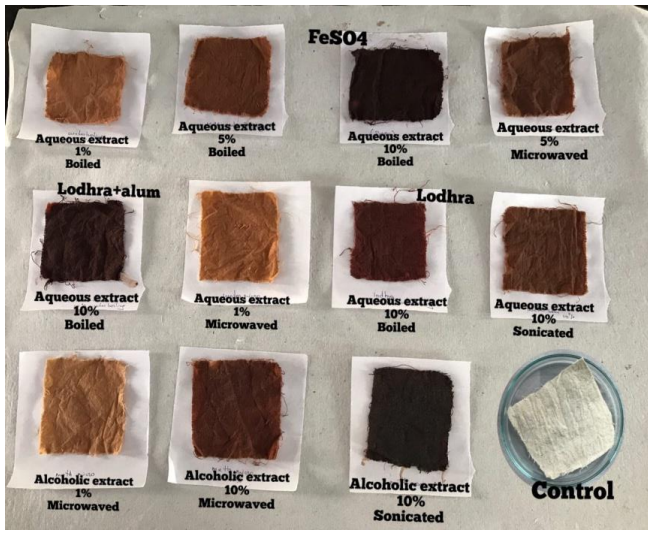


Fig.7. Dyed fabric showing different shades of color.

3.6 Fastness properties

The Textiles Committee laboratory tested the light fastness and wash fastness of dyed fabric; the results are tabulated in Table 5.5. The dyed fabric's light fastness and wash fastness received ratings of 3 and 4 on the Grey scale,

respectively, meaning that it had fair-good fastness properties.

The resistance of color to weaken or drain from a dyed or printed textile fabric to various sorts of impacts, such as perspiration, water, rubbing, washing, light, etc., which they are routinely exposed to in the textile industry and in daily usage, is known as the color fastness of a dyeing material.

A material receiving a 3 on the grey scale is considered fair, indicating a mild color shift in the sample material following testing. A score of four suggests that, even after receiving the proper care, the material's color changed very little.

TESTS	GREY SCALE RATING	COLOR CHANGE
Color Fastness To Light	3	Fair
Color Fastness To Washing Change in color Staining on cotton Staining on wool	 3-4 3 4	 Fair-Good Fair Good

Table.5. Color fastness test results

3.7 Antibacterial activity

This study investigated the potential antibacterial properties of dye extract using a variety of bacterial infections. All verified microbes were resistant to the antibacterial effects of areca dye (Fig. 8a, b, and c). The dye shown good antibacterial efficacy against E. Coli, Sarcina, and Staphylococcus aureus (Table 6)

Given that the dye employed demonstrated antibacterial action against both Staphylococcus aureus and Escherichia coli, two common microorganisms known to degrade textile fibers, it may be helpful in preventing microbial growth on textiles.

NAME OF THE MICROBE	ZONE OF INHIBITION (in cm)
Staphylococcus aureus	1.55
Sarcina species	1.025
E.coli	1

Table.6. Zone of inhibition of *Staphylococcus aureus, Sarcina* and *E.coli.*

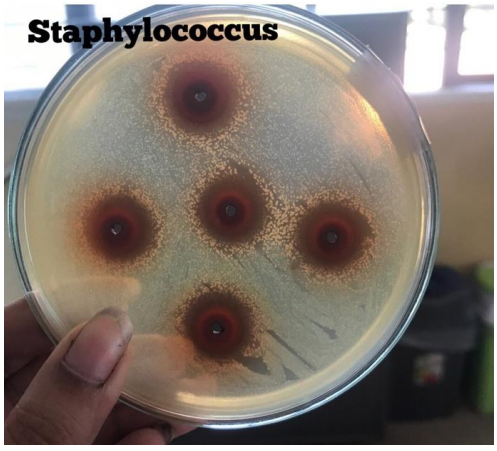


Fig.8a. Plate showing zone of inhibition for Staphylococcus aureus. 8b. Plate showing zone of inhibition for Sarcina

Fig.8b. Plate showing zone of inhibition for Sarcina

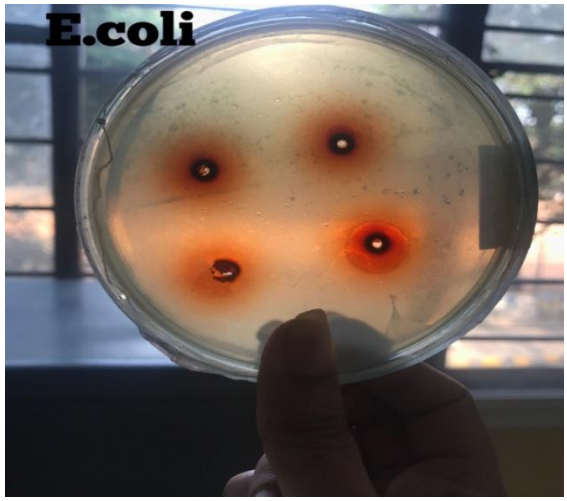


Fig.8c.Plate showing zone of inhibition for *E.coli*

Using isolated organisms14, the dye's in vitro antibacterial, fungal, and viral activity was assessed. Using a spectrophotometric approach to measure the growth of a range of human and veterinary isolates, it was discovered that numerous gram positive and negative organisms were susceptible to the areca dye extract.

3.8. pH indicator

Using phenolphthalein and areca precipitate extract as indicators, the normality of strong acid, HCl, was determined. The appearance of pale pink and light brown hue served as the end points (Fig. 9). The outcomes were tabulated. Table 7.

An effective substitute for phenolphthalein indicator in pH indicators is natural color derived from areca precipitate. Given the high cost of the phenolphthalein indicator and the extremely little variation in the two indicators' calculated normality, sample extract can be utilized as a low-cost substitute.

In analytical chemistry, pH indicators are just as significant as plant material or plant extracts. For instance, it appears that a red cabbage aqueous dye changed color depending on the pH: red (pH=2), purple (pH=3), violet (pH=5), blue (pH=7), blue green (pH=9), and green

(pH=12). The concepts pertaining to the pH indicator mechanism in the systematic quantifications that connect volumetric analysis were designated as [3].

INDICATOR	NORMALITY OF ACID CALCULATED
Phenolphthalein	0.97 N
Areca precipitate extract	0.96 N

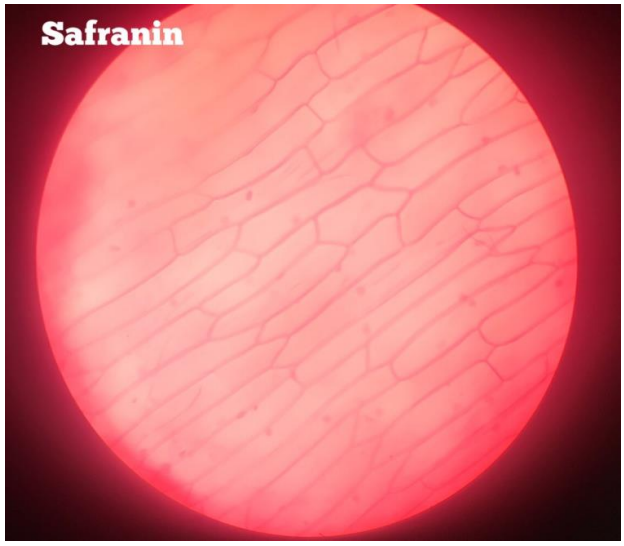
Table.7. Comparison of normality calculated using phenolphthalein and areca dye as indicators.

Fig.9. End point of acid-base titration using phenolphthalein and areca precipitate as indicator.

3.9 Areca dye as histological stain

Onion peels treated with an alcoholic sample extract effectively absorbed the stain. The following findings were made: under a microscope, stained onion cells revealed a huge number of consistently shaped cells laying side by side, with each cell having its own unique cell wall. There was a unique nucleus at the edge of every cell. Every cell had cytoplasm that was lightly pigmented. For comparison, safranin-stained cells were employed (Fig.10). Plant cells, such as onion epidermis, were stained with the dye's alcoholic extract. The stain was efficiently absorbed by the cells, allowing for the clear visualization of the cells under a microscope.

Using onion bulb epidermal cells, the dye characteristics of the *Lawsonia inermis* plant were investigated as a basis for dye for cytological investigations. Using onion epidermal cells, the study compares the ethanolic leaf extract of *Lawsonia inermis*, stain with safranin[9] to explain cellular architecture. According to this work, alcoholic leaf extract from *L. inermis* could be a cheap local supply of dye for investigations into cells and structures[1].



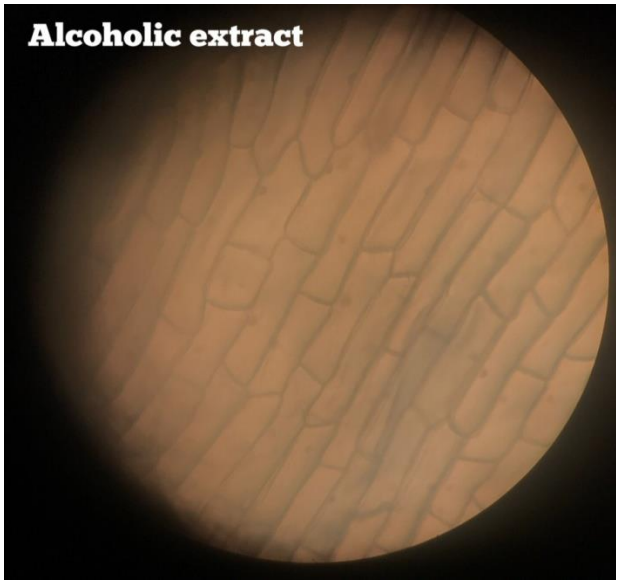


Fig.10. Onion epidermis stained with alcoholic sample extract and with safranin under 40x

4. Conclusion

The study's findings demonstrated the significance of concentration, pH, mordants, and extraction method in the dying of textiles. The color intensity of extracted dyes at varying alkaline conditions (pH 8–12) increased as pH rose. The color was brown in lower alkaline pH levels and blackish brown in higher alkaline pH levels. All other phytochemicals, such as alkaloids, were lacking, leaving just tannins, amino acids, and saponins. The most resolved component was found to have a retardation factor that was more similar to that of tannic, gallic, and catechin. These phytochemicals' Rf values were essentially within the same range.

The colored fabric's wash and light fastness ratings on the Grey scale came in at 3 and 4 on the same scale, respectively, meaning that its fastness was rated as fair to good. When used against E. coli, Sarcina, and Staphylococcus aureus, the dye demonstrated good antibacterial efficacy. Given that the dye used demonstrated antibacterial action against both Staphylococcus aureus and Escherichia coli, two common microorganisms known to infect textile fibers, it may be helpful in preventing microbial growth on textiles.

Given the high cost of phenolphthalein indicator and the extremely small variation in normality calculation between the two indications, dye extract can serve as a low-cost substitute indicator. Plant cells, such as onion epidermis, were stained with the dye's alcoholic extract. The stain was efficiently absorbed by the cells, allowing for the clear visualization of the cells under a microscope.

Plant dyes have been used in a variety of outdated and recently discovered application fields by the scientific community, despite their widespread perception as being low-cost, non-toxic, reusable, and ecological resources with no ecological impact. Due to the wide variety of natural dye sources available, enlightening procedures of extraction and use as well as creating cost-effective methods are currently confusing. Given their many benefits and potential uses, natural dyes lend themselves to further study.

5. Conflict of Interests

The authors declare no conflict of interest.

6. References

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