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Phytochemical analysis of *Babool* (*Acacia nilotica*) bark dye

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Abstract
Natural dyes derived from plant materials have gained prominence due to their safety, biodegradability, and environmental friendliness compared to synthetic dyes. In this study, *babool* bark (Acacia nilotica) was chosen as a natural dye source for cotton fabric. Phytochemical analysis was conducted using three different solvents (aqueous, ethanol, and methanol) to identify the bioactive compounds responsible for both coloration and potential health benefits. The results revealed the presence of alkaloids, tannins, flavonoids, and other phytochemicals, with anthraquinones, tannins, and flavonoids being significant contributors to coloration. The aqueous extraction method was selected for dye preparation, considering cost-effectiveness and simplicity. The findings suggest that *babool* bark extract could be a valuable natural dye source for textiles, offering both coloration and enhanced textile properties. This research aligns with the principles of green chemistry and highlights the potential of natural dyes in sustainable textile applications.

Keywords: Phytochemical, aqueous, ethanol, methanol and natural dye

Introduction
Natural dyes are colours or colouring agents that come from minerals, plants, animals, insects, and other living things. According to (Hossain *et al.* 2018) [5], the majority of natural dyes are made from dye-producing plant materials like leaves, stems, barks, seeds, flowers, and roots. Because they are non-carcinogenic and biodegradable, natural dyes are safer in handling and use than their synthetic cousins. (Haji, 2012) [4]. They have also gained importance due to the implementation of stringent regulations on production and use of synthetic dyes (Arun and Yogamoorthi, 2014) [2].

Natural dyes are biodegradable, contain medical qualities including antibacterial, antioxidant, and UV protection, and are generally more environmentally friendly. The process of using natural dyes for textile dyeing is already environmentally beneficial, but it becomes much more so when enzymes are used as pretreatments. The use of a set of principles that eliminates the use or generation of hazardous substances in the design, fabrication, and application is a component of green chemistry techniques in textiles, notably in the area of dyeing. The natural dyes discovered from diverse sources appear to offer an additional and prospective source for the creation of several lovely colours on all kinds of natural fabrics, including cotton, silk, wool, etc. (Anitha and Prasad, 2007; Samanta and Agarwal, 2009; Kumaresan *et al.*, 2012 and Mall *et al.*, 2014) [1, 12, 8, 10].

In Punjab, Haryana, Rajasthan, Bihar, and Uttar Pradesh, *babool (Acacia nilotica*), also known as kikar locally, is a common tree (Kumar *et al.*, 2015) [7]. The bark of *Babool* tree contains phenolic compounds, especially of quercetin. It generates a medium to deep brown hue by creating a substantial, insoluble colored complex involving cotton-gallotannins, catechin/epigallocatechin, and other pigments found in babool bark extract. This natural dye comprises a non-hydrolyzable tannin-rich color component, including minor quantities of catechin and epigallocatechin (Dhanania *et al.*, 2021) [3]. The *babool* bark also displayed significant antioxidant and antimicrobial properties (Saakshy *et al.*, 2013) [11]. In this current research, *babool* bark was selected as the dye source for colouring cotton fabric. Before dye application, an examination of the phytochemical composition of *babool* bark extract was carried out using three different solvents. This investigation sought to identify many bioactive substances with a variety of properties, including antibacterial activities and coloring properties.

Materials and Methods

Standard procedures were employed to perform phytochemical analysis tests on extracts from the babool bark plant. These tests aimed to identify the constituents of the extracts.

Material: *Babool* bark powder, ethanol, methanol, reagents

Plant collection (source of plant matter): After gathering the *babool* bark, it was cleansed to remove any impurities and dried in the shade. In order to protect it from changes in the environment until it was ready for use, it was first dried completely, then broken into tiny pieces, ground again into a coarse powder, and finally stored in airtight containers.

Selection of extraction method for *babool* bark dye: "Extraction involves the separation of the desired colour components through physical and chemical means, aided by a solvent. Three different extraction mediums were selected to detect the presence of phytochemical components in the dye extract, based on the simplicity of the process and cost considerations. The extraction of *babool* bark dye was carried out using the following methods:"

- **Aqueous extraction:** Traditionally, the preferred method for extracting dyes from plants and other materials has been through aqueous extraction. To enhance the efficiency of this process, the dye-containing material was initially broken down into smaller fragments, subsequently pulverized into a powder, and sifted. To facilitate the breakdown of cell structures, 10 grams of dye powder were soaked in 100 ml of distilled water within a stainless steel container overnight, resulting in the formation of an aqueous extract. To prepare the dye solution, the mixture was subsequently heated to a boiling point, maintaining a temperature range of 80 to 85 °C, for an hour. Afterward, the mixture was allowed to cool down to room temperature and filtered to eliminate any plant residues that were not part of the dye. (Lokesh and Kumara Swamy, 2013) [9].
- **Ethanolic extraction:** The dye powder was subjected to a soaking process in 100% ethanol, after which it was heated in a beaker placed in a water bath maintained at a temperature between 45-50 °C for one hour. This heating process was employed to aid in the extraction of the dye solution. Subsequently, the solution was left to cool down to room temperature and then filtered to eliminate any plant residues that were not part of the dye. (Lokesh and Kumara Swamy, 2013) [9]
- **Methanolic extraction: -** A quantity of 5 grams of dye powder was immersed in 100 ml of methanol and subjected to heating at a temperature range of 45-50 °C for a duration of 1 hour to extract the dye solution. Following this, the solution was permitted to naturally cool to room temperature. Subsequently, it underwent filtration to remove any plant residues not associated with the dye and was further refined by passing it through a fine mesh nylon cloth. (Lokesh and Kumara Swamy, 2013) [9].

All three extracts were filtered for phytochemical analysis through a fine nylon mesh screen.

Methods

- **Test for Alkaloids (Wagner's test):** The dye extract was heated in a water bath while being boiled with a 1% aqueous HCl (hydrochloric acid) solution, and it was then filtered. After obtaining the filtrate, it was treated with a solution made of 2 grams of iodine and 6 grams of KI (potassium iodide) dissolved in 100 ml of distilled water. Alkaloids were found in the dye extract when a dark or reddish-brown precipitate formed.
- **Test for Phenol (ferric chloride test)**: By mixing 2 ml of ferric chloride solution with 2 ml of dye extract, phenols were examined. The presence of phenols in the extract was indicated by the formation of a solution with a bluish-green color.
- **Test for Tannins (lead acetate test):** A small quantity of 1% lead acetate solution was introduced into 5 ml of the dye extract. The formation of a yellow precipitate served as an indicator of the presence of tannins within the extract.
- **Test for Saponins (froth test):** 5ml of the dye extract were boiled in a test tube with ten milliliters of distilled water, then the mixture was vigorously shaken for 30 seconds. The test tube was then let to stand still for 30 minutes. During this procedure, froth formed, which was a sign that saponins were present in the extract.
- **Test for Steroids:** For testing the presence of steroids, 1 ml of dye extract was dissolved in 10 ml of chloroform within a test tube. Carefully, an equal volume of concentrated sulfuric acid was added down the walls of the test tube. The emergence of a red colour in the upper layer, accompanied by yellow and green fluorescence, indicated the presence of steroids in the extract.
- **Test for Cardiac Glycosides:** In a test tube, 1 ml of the dye extract was mixed with glacial acetic acid, and a few drops of ferric chloride were added to assess the presence of cardiac glycosides. The test tube's inner walls were then carefully filled with strong sulfuric acid. Confirmation of the presence of cardiac glycosides in the extract was established by observing a reddish-brown color at the interface between the two layers and a bluish-green color in the upper layer.
- **Test for Anthraquinones:** 10ml of sulphuric acid and 5ml of dye extract were boiled together. While still hot, this mixture was filtered. In the preceding step's filterate, 5 ml of chloroform was added and shaken. Carefully transferring the chloroform layer into another test tube after carefully pipetting out the original one. 1 cc of diluted ammonia was put into the fresh test tube and added to the layer of chloroform. Any colour changes in the final solution were checked for. Anthraquinones might be detected in the dye extract by the appearance of colour changes.
- **Test for Flavonoids:** A vibrant yellow color promptly appeared upon the addition of a few drops of diluted sodium hydroxide (NaOH) to 1 ml of the dye extract. To this yellow solution, a few drops of dilute acid were added. The solution turned colourless upon the addition of dilute acid. This change from an intense yellow colour to colour less ness indicated the presence of flavonoids in the dye extract.
- **Test for Terpenoids:** The process involved mixing 1 ml of dye extract with an equal amount of chloroform. To this chloroform solution, 1 ml of acetic anhydride was introduced, followed by the addition of 2 ml of concentrated sulphuric acid. The mixture was observed for any colour changes. The development of a red hue serves as an indicator of the existence of terpenoids in the

dye extract.

- **Test for Reducing Sugar:** 1 ml of the dye extract was added 5 to 10 drops of Fehling solution. The mixture was then boiled for a total of 15 minutes. The identification of reducing sugars in the dye extract is established by observing the formation of a brick-red precipitate either during or after the boiling process.

Results and Discussions

Babool bark extracts were subjected to phytochemical examination using several solvents (aqueous, ethanol, and methanol), which disclosed the presence of numerous bioactive components. The results of this study's screening of phytochemicals in these three extraction mediums revealed the existence of a wide variety of phytochemical substances, as listed in Table 1.

Table 1 illustrates that alkaloids, reducing sugars, and tannins were detected in all three extraction mediums. Terpenoids were exclusively detected in the ethanol and methanol extraction solvents. Furthermore, flavonoids and anthraquinones were found in both the aqueous and the methanol extracts.

The aqueous extraction method was chosen for getting dye from plant material in order to dye cotton fabric in the subsequent study, taking into account elements like cost-effectiveness and the ease of the extraction procedure. Anthraquinones, tannins, and flavonoids were recognized as significant contributors to imparting and increasing color in the cotton fabric among the different phytochemicals found. Jame Rasha's (2018) [6] phytochemical analysis of different plant extract sections found that *babool* bark includes tannins, alkaloids, saponins, phenols, and glycosides, which supports the findings. Tepparin *et al*. (2012) [13] reported that tannins have the capacity to improve both the dye yield and the colour fastness characteristics of dyed fabrics. Additionally, flavonoids are known for their ability to provide colour to a variety of substrates. These phytochemicals also give off antibacterial and antioxidant characteristics, which raises the value of dyed materials overall.

Table 1: Phytochemical analysis of natural dye

Sr. No.	Phytochemicals	Extraction Mediums		
		Aqueous	Ethanol	Methanol
1.	Alkaloids	+	+	+
2.	Anthraquinone	+	+	-
3.	Cardiac glycosides	-	-	+
4.	Flavonoids	+	+	-
5.	Phenol	+	-	-
6.	Reducing Sugar	+	+	+
7.	Saponins	+	-	-
8.	Steroids	-	-	-
9.	Tannins	+	+	+
10.	Terpenoids	-	+	+

Conclusion

Babool bark extracts in aqueous, ethanol, and methanol revealed nearly identical phytochemical activity, which may justify its historical usage as a bacterial infection preventative. The presence of these common phytochemicals may also account for their historical use as textile dyes. By conferring UV protection properties to textile materials, they open up the potential for the development of even more innovative applications in textile fabric.

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