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Synthesis and Application of Natural Dye from Azadirachta Indica and its Biological Activity

[1]Dr. B. Jini kumari, [2] R. Reesha & [3]R. Blessy Praise

[1]Assistant professor in chemistry, Department of Chemistry, St.Alphonsa College of Arts and Science, Soosaipuram, Karinkal-629157, Tamilnadu, India.

[2] III B.Sc Chemistry, Department of Chemistry, St.Alphonsa College of Arts and Science, Soosaipuram, Karinkal-629157, Tamilnadu, India.

[3] III B.Sc Chemistry, Department of Chemistry, St.Alphonsa College of Arts and Science, Soosaipuram, Karinkal-629157, Tamilnadu, India..

[1]Email: [1]drjinikumari@stalphonsa.edu.in

Abstract: *The present study focuses on the extraction of natural dye from the neem bark of Azadirachta Indica. The chemical compounds present in neem bark extract were investigated by phytochemical tests. Natural dye was extracted from the bark powder with water at 90◦ for one hour. In this research, three dyeing methods such as pre-mordanting, post mordanting, one pot dyeing and mordanting methods and also three different mordants such as alum, copper sulphate and cobalt nitrate were used. The effects of dyeing methods and mordants on properties of dyed cotton fabrics were also investigated. Microbes Esherichia Coli and Staphylococcus Aureus were isolated from distilled water using Agar well diffusion method.*

Key Words: *Natural dye, mordant, cotton fabric, phytochemicals, Antimicrobial.*

1. INTRODUCTION:

Dyes are organic chemical compounds which have property of producing colour by light absorption. Dyes are used to colouring various materials like textiles, fabrics, paper, wood, medicine, varnishes, leather, cosmetics, ink, and food stuff etc. Natural dyes which was derived from all renewable natural sources such as plants, vegetables and insects[1]. Most natural dyes are adjective types which need the use of chemicals called mordant to promote the absorption, fixing and to prevent bleaching and fading of the colours. Natural dyes are safer than synthetic dye because it does not contain harmful chemicals. There is a very low possibility for causing skin allergies, but plant dyes are safer to human health such as it helps to prevent skin allergies caused by other synthetic dyed fabrics and its does not produce any unpleasant odour and better to inhalation than synthetic dyed materials. Colour obtained from multitudes of plants, animals and fungi has been used to colour skin, hair, food we eat, clothes and other different kinds of natural fibres that have been collected, examined and analysed as sources of raw materials for textile industries.

Nowadays, the use of synthetic dyes in textile industry is increasing rapidly. All parts of the neem tree leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin disease and dental disorders[2]. Neem juice shows many activities against diseases like diabetes, liver problems and microbial infections, herbal medicine for diarrhea, menorrhagia, stomach aches, skin infections, inflammations, and rashes. Neem juice may also show wound healing and anti-inflammatory and antioxidant properties.

Fig 1 Azadirachta Indica (Neem)

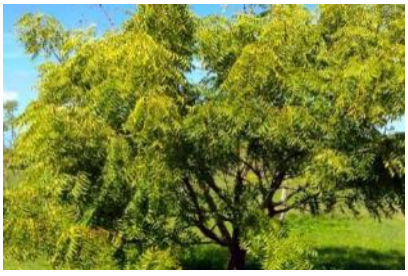
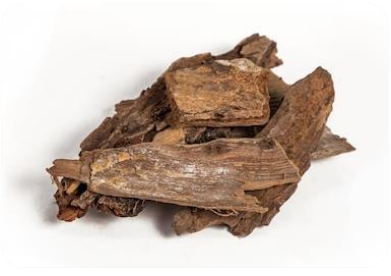


Fig 2 Azadirachta Indica Bark (Neem Bark)



Neem dyes are completely harmless and less hazardous than their synthetic equivalents in terms of health and safety. Many natural colours are allowed as food additives[3]. They have pharmacological effects and may be beneficial. Neem dyes have the advantage that, while having low wash fastness ratings, they do not stain nearby materials during washing since the dye is not strongly attached to the cloth.

Phytochemicals are generally used to describe plant compounds that are under research with established effects on health, and are not scientifically defined as essential nutrients. They help to resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. Some phytochemicals have been used as poisons and others as traditional medicine[4]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive neem bark substances include alkaloids, flavonoid, saponins, tannins, phenol, Coumarins, proteins, carbohydrate, glycoside, terpenoids, and triterpenes[5]. Plant products have been part of phytomedicines since time. This can be derived from barks, leaves, flowers, roots, fruits, seeds. This study proved to protect the environment from the pollution caused by synthetic dyes. It is less toxic, cost effective and alternative method using natural renewable plant material[6].

2. MATERIALS:

Stem bark of neem, cotton fabric, beaker, test tube, stirrer, funnel, distilled water, copper sulphate, cobalt nitrate, and potash alum were used in dying process.

METHOD:

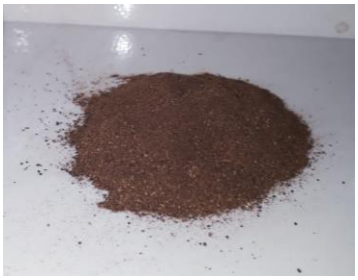
2.1. PREPERATION OF FABRIC MATERIALS:

One of the best pure cotton fabric materials were used as the dying substrate. The pure white material were cut into desired size and used.

2.2. EXTRACTION OF NATURAL DYE

Neem bark was collected in the morning time and cleaned it with distilled water and dried at room temperature. After drying the samples were powdered by grinding machine. The powder was collected and stored for further works.

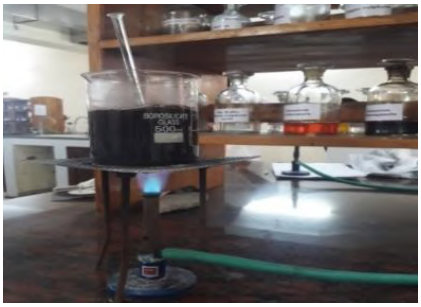
Fig 3 Neem Bark Powder



2.3. EXTRACTION OF DYE SOLUTION

About 3 g of bark powder and 400 ml water were boiled in a stainless-steel vessel at 90^0 C for 1 h to obtain the dye solution of 200ml. This solution is cooled and filtered using filter paper. It was used for dying of cotton fabrics.

Fig 4 Preparation of Bark extract of Azadirachta Indica

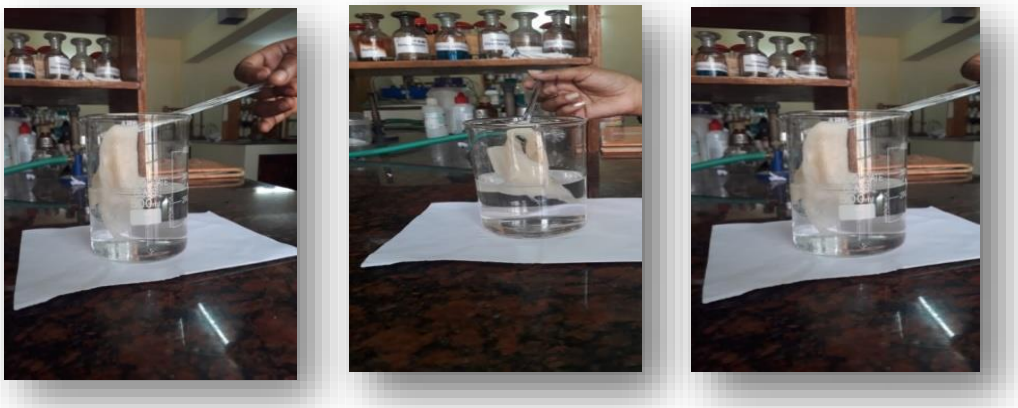


2.4. DYING PROCESS

MERCERIZATION OF FABRIC

Sodium hydroxide was added to the cotton fabrics. It was soaked in that solution for 1 h and rinsed with water, then the cotton fabrics were soaked into the solution of acetic acid and water for half an hour to neutralize[7]. Then the cotton fabrics were washed with water and dried at room temperature and tested with few drops of iodine.

Fig 5 Mercerization of Different Variety of cotton Fabrics

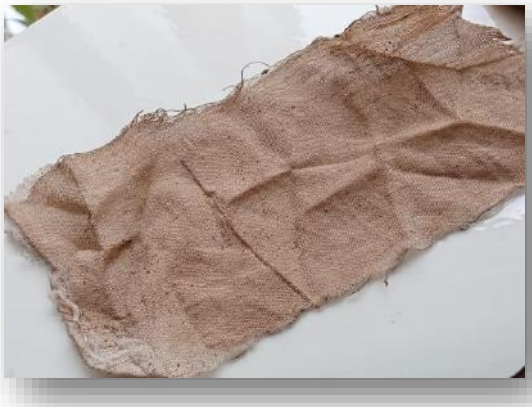


PRE MORDANTING-METHOD

In this method the solution was prepared by heating 1.25 ml alum with 200 ml water. It was heated to 80^0 C. A small piece of mercerized fabric was boiled in mordant solution for about 45 minutes. During mordanting the fabric was frequently stirred to obtain good penetration of mordants and then it was rinsed with water.

After mordanting the dye liquid was added to a beaker and heated at 80^o C. Then the fabric was boiled in this solution for about half an hour. Make sure the dye covers evenly. Boil and simmered until the desired colour was obtained. Then the fabric was rinsed with water. The wet fabric are dropped in the tile and dried in shades.

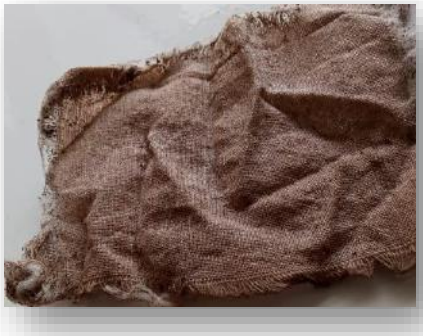
Fig 6 Potash alum mordant



POST MORDANTING METHOD

Mordanting bath was prepared by heating 1.25 g copper sulphate, with 200 ml water. This solution was heated to 80^0 C and small piece of mercerized fabric was simmered in it for 45 minutes. The fabric was frequently stirred to obtain good penetration of mordant into the material then fabric was rinsed in water[8]. After mordanting the dye liquid was added to the beaker and heated to 80^0 C. Then the mordant fabric was simmered in this solution for about 30 minutes and rinsed with water and allowed to dried in shades.

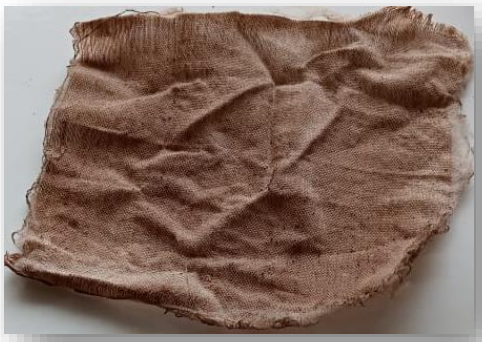
Fig 7 Copper sulphate mordant



ONE POT MORDANTING METHOD

Dye liquid was added to a beaker containing 1.25 g of cobalt nitrate with 200 ml water then the dye bath was heated at 80^0 C and mercerized fabric was simmered in that solution for about 30 minutes. The fabric was frequently stirred to obtain good penetration of mordant into the materials. After that fabric was rinsed with water. After mordanting the dye liquid prepared was added to the beaker and heated at 80^0 C. Then the mercerized fabric was simmered in the solution for about 30 minutes. Then the fabric was rinsed with water and allowed to dried in shades

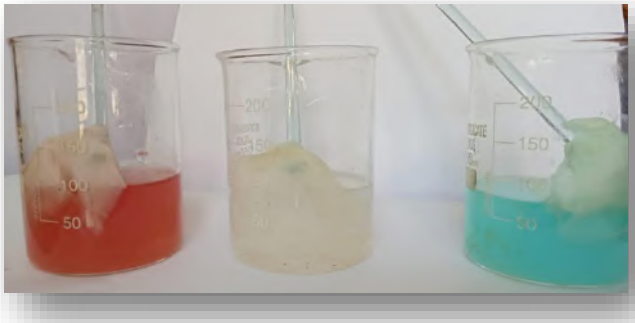
Fig 8 Cobalt nitrate mordant



2.5. TESTING FOR COLOUR FASTNESS

After dyeing, testing for colour fastness of dyed fabric was carried out. The fabric was tested by soap solution. After washing the sample was rinsed with water for 3-4 times rubbing with a finger. The specimen was placed on the cabinet was covered. Then the specimen was exposed on sunlight for 7 days.

Fig 9 Mercerized Fabric Dipped in Three Different Mordant



3. ANTIBACTERIAL ACTIVITY

The newly synthesized Neem bark extract have been screened for antibacterial activity against gram-negative *Escherichia coli and* gram-positive *staphylococcus.* As a reference, Levofloxacin is used and a comparison of the data obtained from the study shows that the new compound now screened appeared to have remarkable antibacterial activity. These are classified into highly active (inhibition zone > 15mm), fairly active (inhibition zone >10mm), moderately active (inhibition active > 8mm) and minimum active (inhibition zone = 6mm)

Table 1 Antibacterial activity of *Azadirachta Indica* bark extract

Concentration	Bacteria strain name and zone size in diameter	
	Escherichia coli	Staphylococcus aureus
25 µl	7	6
50 µl	6	7
75 µl	6	7
100 µl	7	6
Positive control (Levofloxacin)	**32**	**27**

4. PHYTOCHEMICAL INVESTIGATION OF NEEM BARK :

The preliminary phytochemical tests were carried out to study the main compounds which are present or absent in neem bark. Tests for alkaloids, flavonoid, saponins, tannins, phenol, Coumarins, proteins, carbohydrate, glycoside, terpenoids, and triterpenes were performed according to the methods and procedures prescribed in the book of ‘‘A Modern Techniques of Plant Analysis.’’

Table -2 Preliminary phytochemical screening of bark extract of *Azadirachta Indica*

SI NO	CONSTITUTION	TEST	RESULT
1	Flavonoids	Lead Acetate Alkaline reagent Ferric chloride	+
2	Alkaloids	Wagner’s test	+
3	Proteins	Millan’s test Ninhydrin test	+
4	Carbohydrates	Fehling’s test Benedict test Molisch test Iodine test	+
5	Phenol	Ferric chloride test	+
6	coumaris	Sodium hydroxide test	_
7	Tannins	Ferric chloride test	+
8	Saponins	Froth test	+
9	Triterpenes	Salkowski test	+
10	Terpenoids	Chloroform test	+
11	Glycoside	Glycoside test	+

5. RESULT:

The extraction of natural dyes from the bark of, *Azadirachta Indicia* can be used as a potential source for cotton fabric. There is a need for an alternative solution, this follows a less toxic approach by using only natural ingredients to synthesis a dye and using it in cotton fabrics. This study proved to protect the environment from the pollution caused by synthetic dyes. It is less toxic, cost effective and alternative method using natural renewable plant material. The Phytochemical test was done to find out the presence of active chemical constituents such as alkaloids, flavonoid, saponins, tannins, phenol, Coumarins, proteins, carbohydrate, glycoside, terpenoids, and triterpenes reveals that bark extract of *Azadirachta Indicia* exhibits medicinal as well as pharmacological activities. The antibacterial activity of

Azadirachta Indicia bark extract determined by Agar well diffusion method showed in Table 1, the new compounds now screened have remarkable or minimum activity since it is eco-friendly, cost effective, nontoxic, natural biodegradable and pollution free.

6. CONCLUSION:

Brown dye was extracted from freshly collected neem bark of Azadirachta Indica. The extracted brown dye was applied on cotton material using the fixing agent alum, copper sulphate and cobalt nitrate. The dye was fixed to the material intensely, in the cotton material did not faded during the time of rinsing with soapy reagent. The bark extract of *Azadirachta Indicia* is a good source of phytochemical activity, the study contains higher number of chemical components such alkaloids, flavonoid, saponins, tannins, phenol, Coumarins, proteins, carbohydrate, glycoside, terpenoids, and triterpenes. Many evidences gathered in previous studies have confirmed that the presented phytochemicals are bioactive. These phytochemical functions which accounts for the use of bark of neem plant in traditional medicine and pest control. The *Azadirachta Indica* bark extract was then investigated to evaluate its antimicrobial activity against two bacteria (Esherichia Coli and Staphylococcus Aureus) using Agar well diffution method. Evaluation of antimicrobial activity of the *Azadirachta Indica* bark extract shows minimum activity against *Esherichia Coli* and Staphylococcus Aureus.

The bark extract from neem plant can be a good source of drugs for pharmaceutical industries. Thus, it also claims that the traditionally medicin can be recommended and suggest for further research to identify the active constituents of Azadirachta Indica bark extract. So, this can be one of the alternatives to utilize the phytochemicals of the medicinal plants and replace the hazardous chemicals in the environment. These positive results encourage us the continuation of the present research study.

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