AJHS
Asian Journal of Home Science
Volume **14** | Issue 1 | June, 2019 | 169-174

DOI: 10.15740/HAS/AJHS/14.1/169-174
ISSN : 0973-4732 ■ Visit us: *www.researchjournal.co.in*

Research Paper

Antibacterial and antifungal activity of toon tree bark's natural dye in aqueous medium

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Received: 27.09.2018; **Revised:** 06.05.2019; **Accepted:** 15.05.2019

■ **ABSTRACT :** Objectives: The present investigation was aimed to investigate the antibacterial and antifungal activity of natural dye from toon bark. Methods: Antibacterial and antifungal efficiency of natural dye were evaluated against gram positive bacteria *Staphylococcus aurous*, gram negative bacteria *Escherichia coli* and *Chrysosporium fungus*, *Fusarium oxysporium fungu*s using agar well diffusion method. Result: The dye was found to have potent antibacterial activity against all the test bacteria at all the tested concentrations. Highest antibacterial activity against all the test bacteria was recorded with 400 mg/ml treatment. The dye was found to have potent antifungal activity against all the test bacteria at all the tested concentration. Higher antifungal activity against all the test fungal 400mg/ml treatment. Conclusion: The study concluded that different selected barks can be a potential source of natural dye with remarkable antibacterial and antifungal potency which can applied in fabric for protective clothing.

■ **KEY WORDS:** Natural dye, Toon bark, Antibacterial activity, Antifungal activity

■ **HOW TO CITE THIS PAPER :** Tyagi, Deepa and Chhabra, Pankaj (2019). Antibacterial and antifungal activity of toon tree bark's natural dye in aqueous medium. *Asian J. Home Sci.,* **14** (1) : 169-174, **DOI: 10.15740/HAS/AJHS/14.1/169-174.**

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Colour is one of the elements of nature that made the human life more aesthetic and fascinating in the world. Dye is word derived from the English word Daeg or Daeh meaning colour. A dye can generally be described as a coloured substance that has an affinity to the substrate to which it is being applied (Upadhayay and Choudhary, 2014). Natural dyes are the gift of nature. Natural dyes are known for their use incolouring of food substrate, leather, wood as well as natural fibers like wool, silk, cotton and flax as major areas of application since ancient times. Natural dyes may have a wide range of shades and can be obtained from various part of plants including roots, barks, leaves, flowers and fruits (Saravanan *et al.*, 2013). Natural dye have better biodegradability and generally have higher compatibility with the environment. They are non-toxic, non-allergic to skin, non-carcinogenic, easily available and renewable (Saravanan and Chandramohan, 2011). The use of natural dyes for textile dyeing purposes, decreased to a large extent after the discovery of synthetic dyes in 1856. As a result, with a distinct lowering in synthetic dye stuff costs, the natural dyes were virtually unused at the beginning of 20th century (Saravanan *et al.*, 2014). Presently there is an excessive use of synthetic dyes, estimated at around 10,000,000 tons per annum, the production and application of which release vast amounts

of waste and unfixed colorants, causing serious health hazards and disturbing the eco-balance of nature. Currently, ecological considerations are becoming important factors in the selection of consumer's goods all over the world. Since the mid-1980s, more interest has been shown in the use of natural dyes and limited number of commercial dyes and small businesses have started to look at the possibility of using natural dye for coloration (Jothi, 2008). Natural dyes are deep and soft in color shades when compared with synthetic dyes and they are useful for human health because they have the antibacterial, insecticidal and healthy-properties that are due to the origin of them extracted from herb plants. Besides, with the increase of the worldwide concern for the environmental circumstances, many are anxious for the possibility of the natural dyes because they can overcome the defects of synthetic dyes such as harmfulness to human body, pollution and wastewater (Sivajiganesan, 2017).

Textile materials and clothing are known to be susceptible to microbial attack, as these provide large surface area and absorb moisture required for microbial growth. Nature fibres have protein {keratin} and cellulosic etc., which provide basic requirements such as moisture, oxygen, nutrients and temperature for bacterial growth and multiplication. This often leads to objectionable odor, dermal infection, product deterioration, allergic responses and other related diseases.This necessitates the development of clothing that could provide a desired antimicrobial effect (Singh *et al.,* 2004). textile materials are prone to microbial growth and multiplication resulting into discoloration, objectionable, allergic response and other related aliment (Prusty *et al.,* 2010). There is, therefore, an increased inclination across the world n functional textiles that can provide a higher level of hygiene, safety and health protection to people. In the last few decades, arrange of textile products based on synthetic antimicrobial agents such as triclosan, meta and their salts, organometallics, phenols and quaternary ammonium compounds, have been developed and quite a few are also available commercially (Joshi *et al.,* 2009). However, synthetic antimicrobial are a cause of concern due to the associated side effects, action on non-target micro-organisms and water pollution. Hence, there is a great demand for antimicrobial textiles based on ecofriendly agents which not only help to reduce effectively the ailing effects associated to microbial growth on textile material but also completely with the statutory requirements imposed by regulating agenices (Juneja and Pathriya, 2012). Althoughknown for a long time for dyeing as well as medicinal properties, the structures and protective properties of natural dyes have been recognized in the recent past. Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess remarkable antimicrobial activity (Chengaiah *et al.,* 2010). India is famed for its rich biodiversity and there are more than 450 plants which yields different dyes and pigments. Many of these plants and exhibit excellent antimicrobial activity (Patel and Desai, 2014). all of the naturally available dyes are eco-friendly (Siva, 2007). Therefore, antimicrobial agents extracted from plants have been more demanding for apparel technology. Effect of various antimicrobial plant extract on microorganisms have been studied by number of researchers (Liolious *et al.,* 2007). The present research was aimed to explore the different selected tree's barks and leaves as a source of antibacterial and antifungal natural dyes.

Toon has a straight cylindrical trunk and a spreading crown. Its bark is thin gray. It is found throughout the India subcontinent, especially in slightly elevated terrain of the tarai, the Shivaliks and the outer Himalayas from 400 to 50. Various parts of the plants, but especially the barks are used medicinally as an astringent and tonic to treat dysentery and to heal wounds. In South Asia the leaves as a vegetable. The bark is a powerful astringent febrifuge, tonic and antiperiodic. A resinous gum obtained from the bark is used to treat boils. The flowers are emmenagogue. Some extracts from the barks have insect-repellent properties (*www.ecoindia.com*).

The study this envisaged to investigate the antibacterial and antifungal activity of natural dye extracted from bark as well as cotton fabric dyed with the natural dye.

■ RESEARCH METHODS

Materials:

Source:

The barks of toon tree was collected from Tanshipur village, Haridwar district.

Experimental methods:

Dye extraction:

The collected bark was properly cleaned under

running tap water and then allowed for air drying in shade. The air dried bark was chopped into small pieces and coarsely ground using electric grinder. Aqueous medium was prepared in 300 mi of water without using chemicals and maintained the pH 6 separately 30g of dyestuff was added and the dye was extracted for 90 min. at 90°C the solution was filtered. The extract was directly used without any dilution.

Test solutions of natural dye:

Test solution of a series of concentrations *viz*., 20, 50, 100, 250, 400 mg/ml were prepared by dissolving natural dye obtained from bark in aqueous medium. All test solution were kept in refrigerator at 4°C for future use.

Antibacterial screening test:

Bacterial strains:

Antibacterial activity of natural dye obtained from different selected leaves and barks. The natural dye was tested against gram positive bacteria, *Staphylococcus* aureus and gram negative bacteria, *Escherichia coli*. The pure bacterial cultures were maintained on nutrient agent medium and each bacterial culture was further maintained by sub culturing on the same medium and was stored at 4°C before use in experiments. The bacterial strains obtained from IIT, Roorkee were used for evaluating antibacterial activity.

Preparation of bacterial inoculums:

Stock culture was maintained at 4°C on slopes of nutrient agar active culture for experiments was prepared by transferring bacteria in nutrient broth and that inoculated without agitation for 24 hrs. at 37°C. The bacteria spore suspension was adjusted to give a final concentration of 10×10^{10} PBS ml.

Preparation of media:

The medium was prepared by dissolving Muller Hinton Agar Medium (Himedia) in distilled water. The dissolved medium was auto calved at 15 Ibs pressure at 121°C for 15 minutes. The autoclaves medium was mixed well and poured into 100 mm petriplates (25ml plates) while still molten.

Assessment of antibacterial activity of natural dye:

The antibacterial activity of natural dye was tested against several bacterial isolated using agar well diffusion method. The culture plates were inoculated with 0.1 ml of standardized inoculums (1.0×10^{10} PBS ml) of each bacterium and spread with sterile swabs wells of 0.9 mm diameter were punched into MHA petriplates containing the bacterial inoculums with sterile core borer. The wells were filled with test solutions of natural dye. Commercially available antibiotics Ampicillin and Streptomycin discs (1.0 mg/disc each) were used. The plates thus, prepared were left at room temperature for 15 minutes allowing the diffusion of the extract into the medium. After incubation for 24 hrs. at 37°C, the plates were observed. Antibacterial activity was observed by an inhibition zone surrounding the well containing the natural dye. The zone of inhibition was measured and expressed in millimeters. Each experiment was repeated and diameter of inhibition zones were calculated. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism (Pal *et al.,* 2016).

Antifungal screening test:

Fungal strains:

The in vitro antifungal activities of the natural dye obtained from different selected leaves and barks. The natural dye was assessed against standard strains of two fungi namely *Chrysosporium* fungus and *Fusarium oxysporium* fungus. The pathogenesis grown in pure culture were maintained in potato dextrose agar (PDA). Culture slants at 4°C and used as stock culture throughout the study. The fungal strains obtained from IIT, Roorkee were used for evaluated antibacterial activity.

Preparation of fungal inoculums:

For the antifungal assay, cultivated slants were used for preparing spore suspension in 0.9 per cent saline water. The fungal spore suspension was adjusted to give a final concentration of 1×10^{5} Cfu/ml.

Preparation of media:

The medium was prepared by dissolving PDA media (Himedia) in distilled water and autoclaving at 121°C for 15 min. 20 ml of sterile PDA media was poured in sterilized pertridishes (9cm diameter) and allowed to solidify which were used for the antifungal assay.

Antifungal activity assay:

Antifungal activity of natural dye from different selected leaves and barks determined, using agar-well diffusion method. Spore suspensions (0.2 ml) were

applied on the surface of the presterilized and autoclaved PDA pertridishes and spread by using a sterile glass spreader. Wells of 0.6 mm diameter hole were made in the center of each of the PDA Pertri plates with help of sterilized cork border. The wells were filled with test solutions of natural dye. All the Pertri plates including treatment and controls were allowed to diffuse at room temperature for 2 hrs.And then incubated at room temperature for 72 hrs. after incubation, the antifungal activity of extracts was expressed in terms of diameter of zone of inhibition (Pal *et al.,* 2016).

■ RESEARCH FINDINGS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

In the present study, the inhibitory effect of natural dye extracted from different selected bark. The antibacterial and antifungal activity of natural dye was determined using agar well diffusion method and quantitatively assessed on the basis of inhibition zone (American Association Textile Chemists Colorists Test Method 100, 1999).

There has been an increasing consumer demand for natural dye coated traditional products like mat, coconut shell products, Palmyra leaf products and toys etc. because synthetic dyes coated material could be toxic to humans. Concomitantly, consumers have also demand for wholesome and safe products with long shelf life. These requirements are often contradictory and have put pressure on the traditional industry for progressive removal of chemical dyes and adoption of natural alternatives to obtain its goals concerning safe products with long shelf-life (Selvam *et al.*, 2012) in the present study the antibacterial efficacy of bark natural dye was evaluated according to their zone of inhibition against various bacteria and fungus.

Evaluation of antibacterial activity of natural dye:

In this study, two different bacterial pathogens were used to screen the possible antimicrobial activity of dye extract. Dye extract exhibited antibacterial activity against all tested micro-organisms.

Antibacterial activities of dye extract from toon bark:

It is evident from Table 1 that test solution of the natural dye at 250 ml concentration of showed highest antibacterial activity against other concentration. Treatment of the natural dye at 100 concentration showed minimum activity against *Escherichia coli* bacteria.

Table 1: Test against escherichia coli bacteria

Sr. No.	Natural dye Concentration(mg/ml)	Zone of inhibition (mm) Escherichia coli
1.	100	2
2.	200	4
3.	250	5
4.	400	4

It is evident from Table 2 that test solution of the natural dye at 250 ml concentration of showed highest antibacterial activity against other concentration. Treatment of the natural dye at 100 concentrations showed minimum activity against *Staphylococcus aureous* bacteria.

Table 2 : Test against *Staphylococcus aureous* bacteria

Sr. No.	Natural dye Concentration (mg/ml)	Zone of inhibition (mm) *Staphylococcus aureous*
1.	100	0
2.	200	4
3.	250	5
4.	400	3

Evaluation of antifungal activity of natural dye:

The antifungal efficiency of selected bark natural dye was evaluated according to their zone of inhibition against altogether two pathogenic fungi. The result as summarized in table that the natural dye exhibit antifungal activity against all the test fungi studied at all the experimented concentrations.

Antifungal activities of dye extract from toon barks:

It is evident from data presented in Table 3 that the natural dye from toon bark tested at different concentration exhibited varying the degree of antifungal activity against fungal species. Highest reduction in the growth of *Chrysosporium* test fungi is recorded with

Table 3 : Test against *Chrysosporium fungus*

Sr. No.	Natural dye Concentration (mg/ml)	Zone of inhibition (mm) *Chrysosporium fungus*
1.	20	-
2.	50	19

50 ml volume of natural dye. Treatment of the natural dye at 20 ml concentration however, showed no activity against the tested fungus.

It is evident from data presented in Table 4 that the natural dye from toon bark tested at different concentration exhibited varying the degree of antifungal activity against fungal species. Highest reduction in the growth of *Chrysosporium* test fungi is recorded with 50 ml volume of natural dye. Treatment of the natural dye at 20 ml concentration however, showed no activity against the tested fungus.

Table 4 : Test against *Fusarium oxysporium* fungus

Sr. No.	Natural dye Concentration (mg/ml)	Zone of inhibition (mm) *Fusarium oxysporium* fungus
1.	20	-
2.	50	7

Many natural dyes obtained from various plants are known to have antimicrobial properties. The flowers extract of acacia eburnean (l.f.) wild has a good natural dye source and also dye extract has shown good antimicrobial activity (Thiyagarajan *et al.*, 2016). Dyeing with extract of henna leaves is a very promising, simple and practical method for developing colour as well as antimicrobial effect on woolen yarn and can be proved an eco-friendly alternative source for expensive, synthetic toxic antimicrobial against available in the market today (Yusuf *et al.*, 2012).

Further, it is evident from results of antibacterial activity of toon bark natural dye had greater antibacterial activity toward the gram negative bacteria (*E.coli*) and gram positive bacteria (*S. aureus*) and results of antifungal activity of toon bark natural dye had greater antifungal activity toward the *Chrysosporium* fungus and *Fusarium oxysporium* fungus.

Conclusion:

With the rapid increase of world population natural lands have been converted to areas of residence and this leads to many problems in nature synthetically produced colouring agents have adverse impacts on human life and nature and pose a significant threat to human life (Colak, 2016).

Within the context of the current research, it was aimed to develop a natural dye from different selected barks and leaves to determine its antifungal and antibacterial activities. The study led to the conclusion that toon bark can be a potential source of eco-friendly natural dye with remarkable antifungal activity and the textile materials dyed with this natural dye can be useful for medical application users against common infections.

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