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Original Article

ANTIFUNGAL ACTIVITY OF NATURAL COLOURANT FROM *MELIA COMPOSITA* BARK AND ITS APPLICATION IN FUNCTIONAL TEXTILE FINISHING

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ABSTRACT

Objective: The present study was aimed to evaluate the antifungal efficacy of natural dye obtained from *Melia composita* bark and its impact on the antimicrobial finishing of different types of textile fabrics.

Methods: Antifungal activity of *M. composita* bark dye was assessed against standard strains of five fungi namely *Aspergillus flavus, A. niger, A. parasiticus, Fusarium moniliforme* and *Penicillium canescens* using agar-well diffusion method. The minimum inhibitory concentration (MIC) was determined by broth dilution method. The antifungal potency of dyed fabrics (silk, wool, and cotton) against the five test fungi was quantitatively evaluated by reported method.

Results: Different treatment doses of natural dye exhibited a varying degree of antifungal activity against the five test fungi. The treatment dose of 100 µg/ml and above were considerably active. Growth inhibition impact of natural dye at 250 µg/ml dose was almost at par with positive control. Maximum growth inhibition in all the five test fungi was evident with 500 µg/ml dose followed by 250 and 100 µg/ml. MIC of the natural dye against test fungi were found in the range of 20.50-23.00 µg/ml. Dyed fabrics of silk, wool, and cotton also showed remarkable antifungal efficacy against all the test fungi. The highest growth reduction was observed in *A. parasiticus* whereas lowest in *P. canescens* with the three types of dyed fabrics. Dyed wool fabrics exhibited the maximum growth reduction followed by silk and cotton fabrics.

Conclusion: The study revealed the remarkable antifungal activity of natural dye from barks of *M. composita;* therefore, the plant can be considered as a good source of natural dye with functional properties and can be used in commercial dyeing and protective finishing of different kinds of textile fabrics.

Keywords: *Melia composita***,** Bark, Natural Dye, Dyed Fabrics, Antifungal activity

INTRODUCTION

Textile fabrics by virtue of their physicochemical characteristics and proximity to the human body are susceptible to microbial attack, as these provide large surface area and absorb moisture that aid microorganisms to grow, transfer and propagate infection [1]. This often results in objectionable odour dermal infection, product deterioration allergic responses and often related diseases [2]. With the growing awareness about cleaner surroundings and healthy lifestyle, the demand for protective clothing has increased among consumers. This has created significant challenges for textile researchers and industrialists to address the issue through innovative ways. Consequently, the competitive and textile market is globally witnessing a rapid growth in the development of technical textiles and their end-uses that have generated many opportunities for the application of innovative finishes [3]. Textiles with an antimicrobial finish and improved functionality find a variety of applications such as health, hygiene, and medical products, apart from healthy clothing.

Among all textile finishes, antimicrobial finishing has become a very promising, high-growth research area due to their potential to provide quality and safety benefits to different kinds of textile materials. Natural colorants from plant sources have been recently discovered as novel agents in imparting multifunctional properties to textiles such as antimicrobial, insect repellent, deodorizing, and UV protective besides imparting attractive shades. Application of natural colorants offers promise in developing antimicrobial textiles for aesthetic, hygienic, and medical applications owing to the presence of potent bioactive phytochemicals in their extracts. Substances and extracts isolated from different natural resources especially plants have always been a rich arsenal for controlling the fungal infections and spoilage. In the recent past, considerable research work has been undertaken on the application of natural dyes in coloration and antimicrobial finishing of textiles around the globe and use of natural dyes for antimicrobial finishing of textiles has been widely reported [4-7].

Melia composita Willd. syn *M. dubia* Cav. commonly known as Bakayan is a fast growing tree occurring in tropical forests and commercially valued for its multifarious uses. The plant belongs to the Mahogany (Meliaceae) family distributed in distributed in tropical and subtropical regions. The family is represented by 17 genera and 72 species of which 12 species and two varieties endemic to India [8]. Many species of this family were used in traditional medicine for treatment of various diseases and pest control. Chemically, the Meliaceae is characterized by synthesis of modified triterpenes known as limonoids derived from the precursor 4,4,8-trimethyl-17-furanylsteroid skeleton [9]. Over 300 limonoids have so far been isolated, and they are more diverse and abundant in this particular family than in any other family. In recent past, *M. composita* (= *dubia*), *M. toosendan* and *M. azedarach* have been characterized by the production of limonoids. It was also observed that all the three species having taxonomic similarities have many similarities in chemical profile as well, thus synonymous to each other [10-13]. The plant is well known as a rich source of bioactive limonoids [14] exhibiting a range of biological activities including insecticidal, antifeedant, growth inhibitor, antimicrobial, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans [15-17]. In the traditional system of medicine, the plant is regarded as a remedy for fevers, stomach worm, eczema, leprosy, asthma, malaria, venereal diseases, cholelithiasis, acariasis and pain [18-19]. The plant has been phytochemically investigated for various chemical constituents including alkaloids [20], limonoids [21], essential oil chiefly composed of monoterpenes and oxygenated monoterpenes with the small amount of alkanes, diterpenes sesquiterpenes and phenylpropanoids [22], unsaturated fatty acids, phenolic derivatives and lipophilic organic compounds [23, 24]. The plant is reported for wide range of biological properties including antimicrobial [25], analgesic [26], anti-inflammatory [27], hepatoprotective [28], anti-urolithiatic [29], ovicidal [30], antibacterial and cytotoxic [31], larvicidal [32], hypoglycemic and antidiabetic [33, 34] activities.

Plant-based antimicrobial natural colorants represent a vast untapped source of natural dyes by possessing enormous protective potential. They are effective in the antimicrobial textile finishing for various purposes while mitigating many of the adverse side effects often associated with their synthetic counterparts. In view of the fact, the study was aimed to investigate the antifungal activity of natural dye extracted from the stem bark of *M. composita* as well as of different textile fabrics dyed with the natural dye.

MATERIALS AND METHODS

Plant material and fabrics

Stem barks of *Melia composita* were collected from Dehradun, Uttarakhand (India) and authenticated by Botanical Survey of India, Northern Regional Centre, Dehradun (BSD) under accession No. 115503. A voucher specimen is preserved in the Chemistry, Division, Forest Research Institute, Dehradun, Uttarakhand, India.

Processing of plant material

The collected stem bark materials were surface sterilized with 0.1% $HgCl_2$ and washed two to three times with sterile distilled water. Properly cleaned barks were dried in the shade. Air dried barks ere cut into small pieces and then powdered using powdering mill to 50 mesh size. Powdered bark material was stored in sterile cellophane bags in a cool, dry place until further use.

Textile substrates

Silk, wool, and cotton textile fabrics were purchased from an authorized outlet of KVIC, Dehradun. The fabrics were washed for 30 min with nonionic detergent (1%) open width form (owf) for uniform washing and then rinsed and dried at room temperature. The scoured material was wetted in water for 30 min prior to dyeing.

Extraction of colourant

Natural colorants from *M. composita* bark was extracted under optimized conditions of material to liquor (water) ratio (MLR), pH and time. Bark powder was taken in a beaker and immersed in distilled water as per optimized MLR. The extraction was done for 70 min at the boiling temperature. Then, the solution temperature was allowed to cool at room temperature and was filtered through Whatman (No. 1) filter paper. The filtrate was distilled under reduced pressure and finally dried over a dehydrating agent in vacuo that resulted in natural dye powder.

Dyeing of fabrics

All the fabric samples were cleaned thoroughly off starch and other impurities and rinsed with cold water and dried. Silk, wool and cotton fabrics were dyed with a solution of natural dye extracted above. At the end, the dyed samples were removed, rinsed under running tap water in order to remove non-absorbed dyes and then dried at ambient conditions. It is worth mentioning that dyeing of all fabrics was performed under optimum conditions of MLR, pH, temperature and time acquired through experiments.

Preparation of test solutions

Test solutions of a series of concentrations viz, 25, 50, 100, 250 and 500 µg/ml were prepared from natural dye obtained from the stem bark of M. composita by dissolving the dye powder in Dimethyl sulfoxide (DMSO). All test solutions were kept in a refrigerator at 4 °C for future use.

Fungal strains

The in vitro antifungal activities of the natural dye obtained from M. composita stem bark and the dyed fabric were assessed against standard strains of five fungi namely Aspergillus flavus (AF), Aspergillus niger (AN), Aspergillus parasiticus (AP), Fusarium moniliforme (FM) and Penicillium canescens (PC) chosen based on their clinical and pharmacological importance [35]. The test fungi were isolated from fungal attacked textiles during monsoon season with high relative humidity and were identified based on their growth characteristic, mycelial morphology, spore morphology and other important characters using a standard protocol. The pathogens grown in pure culture were maintained in potato dextrose agar (PDA) culture slants at 4 °C and used as stock culture throughout the study.

Preparation of fungal inoculums

For the antifungal assay, cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of $1x10^5$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 petridishes (9 cm diameter) and allowed to solidify which were used for the antifungal assay.

Antifungal activity assay

Antifungal activity of natural dye from *M. composita* bark was determined, using agar-well diffusion method [36]. Spore suspensions (0.2 ml) were applied on the surface of the pre-sterilized and autoclaved PDA petridishes and spread by using a sterile glass spreader. Wells of 6 mm diameter were made in the centre of each of the PDA petriplates with the help of sterilized cork borer. The wells were filled with test solutions of natural dye as prepared above with three replications for each treatment. Control experiments were carried out under similar condition by using commercially available fungicides, Nystatin and Griseofulvin as a positive control and DMSO as a negative control. All the petriplates including treatments and controls were allowed to diffuse at room temperature for 2 h and then incubated at room temperature (28±2 °C) for 72 h. After incubation, the antifungal activity of extracts was expressed in terms of diameter of zone of inhibition and<9 mm inhibition zone was considered as less active, 9-12 mm as moderately active and>18 mm as very active [37].

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by the broth dilution method [38, 39]. Fungi were first grown in the potato dextrose broth for 24 h and then the inoculums were diluted for five times (10^{-5}dilution) to control its vigorous growth. Then each test tube was added with 1.8 ml of potato dextrose broth and different concentrations (5-25 µg/ml) of natural dye separately followed by inoculation of 0.2 ml of respective fungi and kept at 28 °C for 48 h. The tubes were examined for visual turbidity. Lowest concentrations of the extracts showing no turbidity (without microbial growth) were considered as the minimal inhibitory concentration.

Evaluation of the antifungal activity of dyed fabrics

The antifungal activity of the dyed fabrics (silk, wool and cotton) against the pathogenic fungi, *A. flavus, A. niger, A. parasiticus, F. moniliforme* and *P. canescens* was quantitatively evaluated by reported method [40]. Circular fabric swatches (5.00±0.1 cm) were placed in a container and sterilized for 15 min at 121 °C. Spore suspensions (1000 µl) of each fungus were added to the centre of fabric swatches and incubated for 24 hr at 37±1 °C. The fabric swatches were resuspended in dilution medium, vigorously shaken 1 min prior to the dilution. Ten-fold serial dilutions were made to all samples. A fixed volume of each dilution (100 µl) was inoculated on PDA plates, and the plates were incubated at 37±1 °C for 24 hr. Untreated circular fabric swatches of the same dimension were taken as control. Radial diameter (mm) of fungal growth on the agar plates (control & treatment) was measured, and the percentage of reduction in the fungal growth was calculated using following formula:

$$R\ (\%) = \frac{A - B}{A} \times 100$$

Where R = Reduction in fungal growth; A = Fungal growth on the control (untreated fabrics), and B = Fungal growth on the treated fabrics.

RESULTS AND DISCUSSION

In the present study, the inhibitory effect of natural dye extracted from *M. composita* bark as well as textile fabrics including silk, wool and cotton dyed with the natural dye were evaluated against altogether five fungal strains. The antifungal activity of natural dye was determined using agar well diffusion method and quantitatively assessed on the basis of inhibition zone. The minimum inhibitory

concentration (MIC) was determined by serial dilution method. Furthermore, the antifungal potency of silk, wool and cotton fabrics dyed with the *M. composita* stem bark dye was also assessed.

Evaluation of antifungal activity of natural dye

The antifungal efficacy of *M. composita* stem bark natural dye was evaluated according to their zone of inhibition against altogether five pathogenic fungi and the results (zone of inhibition) were compared with the activity of the standards, *viz*., Nystatin and Griseofulvin. The results as summarized in table 1 revealed that the natural dye exhibit antifungal activity against all the test fungi studied at all the experimented concentrations.

It is evident from data presented in table 1 that the natural dyes from *M. composita* stem bark tested at different concentrations exhibited varying the degree of antifungal activity against the five fungal species, *A. niger*, *A. flavus*, *A. parasiticus, F. moniliforme and P. canescens.* Highest reduction in the growth of all the test fungi is recorded with 500 μg/ml concentration of natural dye. The antifungal activity at this concentration is found even better than the positive control. Minimum growth inhibition in all the test fungi is recorded with 25 μg/ml concentration of natural dye. From the results, it is also evident that growth inhibition of all the test fungi increases with increase in test concentrations of natural dye. Of tested different concentrations of natural dye, inhibition of radial growth in all the test fungi is very low at the concentration of 25 μg/ml and moderate at 50 μg/ml whereas high growth inhibition is observed at 100 μg/ml. Natural dye at concentration of 100 μg/ml and above are very active in growth inhibition of all the tested fungi. Growth inhibition impact of natural dye at 250 μg/ml concentration is almost at par with positive control. Maximum growth inhibition in all the test fungi is evident at 500 μg/ml concentration of natural dye followed by 250 and 100 μg/ml concentrations

The mean radial growth inhibition of *A. flavus, A. niger*, *A. parasiticus, F. moniliforme* and *P. canescens* with various concentrations of *M. composita* natural dye ranged between 6.75-42.25, 6.23-40.26, 6.96-41.89, 6.16-40.53 and 5.89-41.43 mm respectively. Results indicated that all the treatments are effective as compared to negative control. Radial growth inhibition is maximum at 500 μg/ml and minimum at 25 μg/ml concentration of natural dye (table 1).

Table 1: Antifungal activity of *M. composita* stem bark dye against test fungi

Natural dye conc. (μg/ml)	Zone of inhibition (in mm)				
	AF	**AN**	**AP**	**EM**	**PC**
25	6.75±0.14	6.23±0.33	6.96±0.23	6.16±0.47	5.89±0.21
50	11.25±0.25	10.87±0.31	11.39±0.43	10.51±0.31	9.89±0.13
100	20.23±0.33	18.93±0.31	18.83±0.41	21.53±0.33	18.23±0.23
250	31.53±0.31	29.83±0.35	30.36±0.35	32.66±0.43	30.19±0.31
500	42.25±0.19	40.26±0.31	41.89±0.09	40.53±0.31	41.43±0.53
Nystatin	35.03±0.13	32.67±0.43	29.33±0.41	33.46±0.71	32.37±0.51
Griseofulvin	39.25±0.31	36.93±0.19	34.81±0.41	35.53±0.53	35.51±0.19
DMSO	–	–	–	–	–

Values are given in mean±SD for three replicates. AF-*Aspergillus flavus,* AN-*Aspergillus nigar,* AP-*Aspergillus parasiticus,*FM-*Fusarium moniliforme,* PC-*Penicillium canescens*

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is the lowest concentration able to inhibit any visible fungal population. The MICs of natural dye from *M. composita* bark against test fungi *A. flavus, A. niger, A. parasiticus, F. moniliforme* and *P. canescens* are recorded as 21.50, 21.00, 20.50, 22.50 and 23.00 μg/ml respectively (table 2).

Evaluation of antifungal activity of dyed fabrics

Silk, wool and cotton fabrics dyed with the natural dyes were also evaluated for their antifungal potency against the test fungi and was measured as a percentage reduction in fungal growth. Results of the study are presented in table 3.

Table 2: MIC of natural dye against tested bacteria

Fungal strains	MIC of natural dye (μg/ml)
AF	21.50
AN	21.00
AP	20.50
FM	22.50
PC	23.00

Values given are the average of three replications

MICs are important to confirm resistance of pathogenic fungi to an antifungal agent and regarded as the measurement of the activity of an antifungal agent against a fungus.

Table 3: Antifungal activity (reduction %) of dyed fabrics against test fungi

Dyed fabric substrates	Reduction in fungal growth (%)				
	AF	**AN**	**AP**	**FM**	**PC**
Silk	76.55	75.54	81.63	79.78	82.63
Wool	82.21	80.68	85.35	85.19	78.59
Cotton	70.33	72.76	75.56	75.33	80.23

Values given are the average of three replications. AF-*Aspergillus flavus,* AN-*Aspergillus nigar,* AP-*Aspergillus parasiticus,*FM-*Fusarium moniliforme,* PC-*Penicillium canescens*

It is evident from the results given in table 3 that all the three types of fabrics (silk, wool, and cotton) dyed with *M. composita* bark dye have considerable antifungal efficacy against all the test fungi. The highest growth reduction was observed in *A. parasiticus* whereas lowest in *P. canescens* for three types of dyed fabrics. Wool fabrics dyed with natural dye exhibited the maximum growth reduction in all the five test fungi followed by dyed silk and cotton fabrics as shown in table 3. Dyed cotton fabrics showed the minimum growth reduction in all the test fungi

Many natural dyes obtained from various plants are known to have antifungal properties. Natural dye from the bark of *Odina wodier* is reported to have antifungal activity [41]. Natural colorants derived from *Curcuma longa* (Turmeric), *Terminalia che*bula (Terminalli), *Psidium guajava* (Guava) and *Lawsonia inermis* (Henna) have been

shown to exhibit protective impact against fungi spoiling traditional craft made of natural fibres [42]. Also antibacterial and antifungal properties of natural dye *Lawsonia inermis* (Henna) and dyed wool fabrics have been reported [43]. The natural dye obtained from flowers of *Carthamus tinctorius*. L (Safflower) reported for antibacterial and antifungal activities [44]. In the present study, it was observed that the natural dye obtained from *M. composita* stem bark possessed activity against five pathogenic fungal strains, *A. flavus, A. niger*, *A. parasiticus F. moniliforme* and *P. canescens* (table 1). Different kinds of textile fabrics dyed with the natural dye also possessed a good antifungal activity against pathogenic fungi (table 3). The antifungal efficacy of the natural dye may be due the presence of bioactive chemical constituents, limonoids, terpenoids and phenolics which are known to possess antimicrobial properties.

CONCLUSION

Microorganisms which attack textile products not only have a destructive effect but also pose a significant danger to human health. Particularly dangerous are the pathogenic microorganisms present on fabrics which come into direct contact with the human body, such as on dressings and surgical masks; this may lead to skin infection, and even heart disorders and pneumonia. It is a significant challenge for manufacturers to produce fabrics with antimicrobial properties i.e. bioactive or functional fabrics, containing biocides to provide protection against pathogenic microorganisms. Plant-derived natural colorants have long been known to possess medicinal properties, and consequently, it seemed logical to explore their antibacterial activity considering the increasing interest and demand for functional textiles for protective clothing and clinical applications. In the current study, the antifungal potency of the natural dye obtained from *Melia composita* stem bark and different types of textile fabrics impregnated with the dye were evaluated. The study led to the conclusion that leaves of *Melia composita* can be a potential source of eco-friendly natural dye with remarkable antifungal efficacy and the textile materials dyed with this natural dye can be very useful in developing protective clothing to protect users against common infections. The study revealed the remarkable antifungal activity of natural dye from banks of *M. composite;* therefore, the plant can be considered as a good source of natural dye with functional properties and can be used in commercial dyeing and protective finishing of different kinds of textile fabrics.

ABBREVIATION

CFU (cfu)–Colony-forming unit; DMSO–Dimethul sulfoxide; MLR–Material to liquor ratio; PDA-Potato dextrose agar; OWF (owf)–Open width form

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest

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