Antifungal Activity of Natural Dye from Aerial Biomass of *Barleria prionitis* L. and Dyed Fabrics

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ABSTRACT: *Natural dye extracted from aerial parts of Barleria prionitis and different kinds of textile fabrics dyed with the natural dye were investigated for their antifungal activity. Antifungal activity of natural dye and dyed fabrics 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 MIC was determined by the broth dilution method. Further, the antifungal potency of dyed fabrics (silk, wool, and cotton) against the test fungi was quantitatively evaluated by the reported method. Different treatment doses of natural dye exhibited a varying degree of antifungal activity against the five test fungi. The highest growth reduction in all the test fungi, however, was recorded with 500 μg/ml concentration of natural dye. The antifungal activity at this concentration is found almost at par with the positive control. The Minimum Inhibitory Concentrations (MICs) of natural dye against test fungi were ranged within, 22.50-23.50 μg/mL. Dyed silk, wool, and cotton fabrics also showed remarkable antifungal efficacy against all the test fungi. Dyed silk fabrics exhibited the maximum growth reduction followed by wool and cotton. The study revealed the remarkable antifungal activity of natural dye from B. prionitis aerial biomass and dyed fabrics. Therefore, B. prionitis can be considered as a potential source of natural dye with functional properties and can be used in the protective finishing of different kinds of textile fabrics.*

KEYWORDS: *Barleria prionitis; Aerial biomass; Natural Dye; Dyed Fabrics; Antifungal activity.*

INTRODUCTION

Natural dyes are known for their use in textile colouring since pre-historic times. However, with the advent of cheaper synthetic dyes with better fastness properties and low cost in 1856, the use of natural dyes has declined to a great extent [1]. Due to worldwide awareness over the adverse health and environmental consequence

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1021-9986/2018/1/213-221 9/$/5.09
DOI:

of production and use of synthetic dyes, there has been a revival of interest in natural dyes in recent years [2]. The use of non-allergic, non-toxic and eco-friendly natural dyes on textiles has become a matter of significant importance to avoid hazard from synthetic dyes [3]. Further, a number of plants used for dye extraction are considered as medicinal and some of these have recently been shown to possess remarkable antimicrobial activity [4-11]. However, plant-derived colorants are scantily screened for use as antimicrobial agents for protective textile finishing.

Barleria prionitis L (Acanthaceae), commonly known as Vajradanti or Kundan is an erect, prickly shrub distributed throughout India, Srilanka, Burma, Malaya and extends westwards to tropical and South Africa [12]. It is also found in many other parts of the world, like USA, Australia, Indonesia, Malaysia, and the Philippines. The plant is acclaimed for numerous medicinal properties and healthcare applications. In Indian system of medicine, the aerial parts are used in fever, toothache, inflammation and gastrointestinal disorders; bark as an expectorant; whole plant and especially the roots as tonic and diuretic [13]. Phytochemical investigation of the plant led to the isolation and characterization of a number of compounds including 6-hydroxyflavones [14], irridoids named as acetyl barlerin and barlerin [15,16], iridoid glycosides 6-O-Trans-pcoumaroyl-8-O-acetylshanzhiside methyl ester and its Cis-isomer [17]; acbarlerin, barlerin, β-sitosterol, flavanol glycoside, iridoids and scutellarein-7-neohesperidoside [18], balarenone, pipataline, lupeol, prioniside A,B & C [19], phenylethanoid glycoside, barlerinoside along with other iridoid glycosides, namely 7-methoxydiderroside and lupulinoside [12]. Phytochemical screening has recorded the presence different types of chemicals in different parts of the plant [20,21]. Different parts of the plant have been pharmacologically investigated for diuretic[22]; antidiarrhoeal[23], anti-inflammatory [24,25], antidiabetic [26], anti-fertility [27,28], antioxidant [12, 29, 30], hepatoprotective [31], antimicrobial [32, 33], anthelmintic [34]; anti dental decay [35, 36], anxiolytics [37] and mast cell stabilization and membrane protection [38] activities. No work has so far been done to explore the potential of *B. prionitis* as a source of functional natural dye. The present paper reports the studies carried out to assess the antifungal activity of natural dye derived from aerial parts of *Barleria pronitis* L., against some common pathogenic fungi which infest textiles materials causing deterioration of fabric itself resulting in unpleasant odor, dermal infection, allergic responses and often related diseases to the user.

EXPERIMENTAL SECTION

Plant materials

Aerial parts of *Barleria prionitis* was collected from suburbs of Dehradun, Uttarakhand (India) and authenticated by Systematic Botany Section of Botany Division, Forest Research Institute (FRI), Dehradun under accession No. 164282. A voucher specimen is preserved in the Chemistry, Division, Forest Research Institute, Dehradun, Uttarakhand, India for future reference.

Processing of plant material

The collected plant material was surface sterilized with 0.1% $HgCl_2$ and washed with sterile distilled water. Properly cleaned plant material was dried in shade. Air dried plant material was cut into small pieces and then powdered (50 mesh) using an electric grinder. Powdered material was stored in sterile cellophane bags in a cool dry place till further use.

Textile substrates

Silk, wool, and cotton textile fabrics were purchased from an authorized outlet of Khadi and Village Industries Commission (KVIC), Dehradun. The fabrics were washed with non-ionic detergent (1% owf) for 30 min to remove starch and other impurities, then rinsed and dried at room temperature. The scoured material was wetted in water for 30 min prior to dying.

Extraction of natural dye

Natural dye from dried and powdered aerial parts of *B. prionitis* was extracted under optimized conditions of Material to Liquor (water) Ratio (MLR), alkali content and time. Optimum values of MLR, alkali content and time were 12 g/100ml, 0.2 %, 4.0 and 60 min for respectively for dye extraction determined through process optimization experiments [39]. Powdered plant material was taken in a beaker and immersed in distilled water made alkaline with Sodium carbonate (0.2%) as per optimized MLR and alkali content. The extraction was done for 60 min at the boiling temperature. The extract so obtained was allowed

to cool at room temperature and then filtered with 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.

Phytochemical screening of natural dye

The natural dye derived from *B. prionitis* aerial biomass was successively extracted with ethylacetate, acetone, methanol and distilled water and extracts so obtained were subjected to qualitative phytochemical screening to detect the presence of different types of phytochemicals by standard methods [40-42]. All the qualitative tests were replicated thrice for confirmation.

Dyeing of fabrics

Silk, wool, and cotton fabrics were dyed with a solution of the extracted natural dye. Dyeing of all fabrics was performed with the optimum value of dye concentration, pH and dyeing time for different fabrics determined through experiments. Optimum values of dye concentration, pH and time were 1.2%, 4.0 and 60 min for silk; 0.2%, 2.0 and 60 min for wool and 0.2%, 3.0 and 90 min for cotton respectively. Finally, the dyed fabrics were washed with 5g/l non-ionic detergent and then rinsed with water and dried in air at room temperature.

Evaluation of antifungal activity

Preparation of test solutions

Test solutions of a series of concentrations *viz*, 50, 100, 200, 300, 400, 500 and 1000 μg/ml were prepared from the natural dye by dissolving the dye powder in Dimethyl sulfoxide (DMSO). All test solutions were kept in a refrigerator at 4°C till further use.

Fungal Strains

Antifungal activity of the natural dye and different dyed fabrics were assessed against standard strains of five fungi namely *Aspergillus niger, A. flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium canescens* chosen based on their clinical and pharmacological importance [43]. The pathogens grown in pure culture were maintained in PDA culture slants at 4°C and used as stock culture throughout the study.

Preparation of fungal inoculums

Spore suspensions were prepared in 0.9% saline water using cultured slant. 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 Potato Dextrose Agar (PDA) (HiMedia) in distilled water and autoclaving at 121^0 C for 15 minutes. For the antifungal assay, 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm diameter) with an equal thickness and allowed to solidify.

Antifungal activity assay

Antifungal activity of the natural dye was determined by agar-well diffusion method [44]. Spore suspensions (0.2mL) were applied and uniformly spread on the surface of the pre-sterilized and autoclaved PDA petriplates using a sterile glass spreader. Wells of 6 mm diameter were made in the centre of each PDA petriplates with the help of sterilized cork borer. The wells were filled with test solutions of natural dye with three replications for each treatment. Control experiments were carried out under similar condition by using nystatin and griseofulvin as positive and DMSO as a negative control. All the petriplates including treatments and controls were allowed to diffuse for 2 hours and then incubated at room temperature (28 ± 2^0C) for 72 hours. After incubation, the antifungal activity of dye solutions was measured and expressed in terms of diameter (mm) of the zone of inhibition.

Determination of minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was determined by broth dilution method [45, 46]. Fungi were first grown in the potato dextrose broth for 24 hrs and then inoculums were diluted 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 (10-50 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. The MIC was determined based on lowest concentrations of the extracts showing no turbidity (without microbial growth).

Evaluation of the antifungal activity of dyed fabrics

The antifungal activity of the dyed fabrics (silk, wool, and cotton) against pathogenic fungi, *A. flavus, A. niger, A. parasiticus, F. moniliforme* and *P. canescens* was

quantitatively evaluated by the reported method [47]. Circular discs (5.00 ±0.1 cm dia.) of dyed fabrics were placed in PDA plates and sterilized for 15 min at 121°C. Spore suspensions (1000 µL) of each fungus were added to the center of fabric discs and incubated for 24 hr at 37±1^{0}C. Test solutions of natural dye were made through ten-fold serial dilutions. A fixed volume of each dilution (100 µl) was inoculated on PDA plates and the plates were incubated at 37±1^{0}C for 24 h. Untreated circular fabric discs 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 the following formula:

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

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

Phytochemical screening of natural dye

The results of qualitative phytochemical screening of the different extracts of natural dye from *B. prionitis* aerial biomass are presented in Table 1.

The qualitative phytochemical screening recorded the presence of a various group of phytochemicals in the different extracts of natural dye. The results presented in table 1 indicate the presence of alkaloids, steroids, terpenoids, flavonoids, phenolics in ethylacetate extract; terpenoids, flavonoids, phenolics, and anthraquinones in acetone extract; alkaloids, steroids, terpenoids, flavonoids, phenolics, anthocyanins, saponin and glycosides in methanol extract and flavonoids, phenolics, anthocyanins, saponin and glycosides in aqueous extract. These compounds are responsible for several biological functions in the human body, therefore have great pharmaceutical applications. Among these compounds tannins and flavonoids, anthocyanins are the substances which can give the colour. Tannins are the most important ingredients which are necessary for dyeing.

Evaluation of antifungal activity of natural dye

The antifungal efficacy of *B. prionitis* 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 2 revealed that the natural dye exhibit antifungal activity against all the test fungi studied at all the experimented concentrations.

It is evident from the data presented in Table 2 that the natural dyes from *B. prionitia* aerial parts tested at different concentrations exhibited a varying degree of antifungal activity against the five fungal species. Highest reduction in the growth of all the test fungi was recorded with 500 µg/ml concentration of natural dye. The antifungal activity at this concentration is found almost at par with the positive control. Minimum growth inhibition in all the test fungi is recorded with a concentration of 25 µg/mL. From the result, it is also evident that growth inhibition of all the test fungi increases with increase in concentrations. Of different tested concentrations of natural dye, inhibition of radial growth in all the test fungi was low at a concentration of 25, 50 and 100 µg/mL, moderate at 200 and 300 µg/mL, and high at 400 and 500 µg/mL (Fig. 1).

The mean radial growth inhibition of *A. niger, A. flavus A. parasiticus, F. moniliforme* and *P. canescens* with various concentrations of *B. prionitis* natural dye ranged between 2.55-35.25, 1.86-33.53, 3.16-34.75, 2.75-35.19 and 2.35- 35.25 mm respectively. Results indicated that all the treatments are effective as compared to the negative control. Radial growth inhibition is maximum at 500 µg/mL and a minimum at 25 µg/mL concentration of natural dye (Fig. 1).

Determination of MIC

MIC is the lowest concentration able to inhibit any visible fungal population. The MICs of the natural dye against test fungi *A. niger, A. flavus, A. parasiticus, F. moniliforme* and *P. canescens* are recorded as 23.25, 22.75, 23.50, 22.50 and 23.00 µg/ml respectively. MIC is regarded as a measurement of the activity of an antifungal agent against a fungus that confirms resistance of pathogenic fungi to an antifungal agent.

Evaluation of antifungal activity of dyed fabrics

Antifungal property of silk, wool and cotton fabrics dyed with the natural dyes were also evaluated against the five test fungi and was measured as the percentage reduction in fungal growth. Results of the study are presented in Table 3.

Table 1: Qualitative phytochemical analysis of B. prionitis natural dye.

Phytochemicals	Extracts			
	Ethylacetate	Acetone	Methanol	Water
Alkaloids	+	–	+	–
Steroids	+	–	+	–
Terpenoids	+	+	+	–
Flavonoids	+	+	+	+
Phenolics	+	+	+	+
Tannins	–	–	+	+
Anthocyanins	–	+	+	+
Saponins	–	–	+	+
Glycosides	–	–	+	+

(+) Present, *(–) Absent*

Table 2: Antifungal activity of natural dye from B. prionitis against test fungi

Natural Dye Conc. (µg/ml)	Zone of Inhibition (in mm)				
	A. niger	*A. flavus*	*A. parasiticus*	*F. moniliforme*	*P. canescens*
25	2.55±0.13	1.86±0.25	3.16±0. 26	2.75±0.19	2.35±0.23
50	5.43±0.16	3.29±0.13	5.21±0.19	4.63±0.15	5.21±0.25
100	12.15±0.23	10.25±0.26	11.35±0.16	10.85±0.26	12.09±0.13
200	20. 59±0.21	17. 19±0.19	20. 16±0.23	19.39±0.23	20. 15±0.15
300	28.69±0.33	24.35±0.23	26.19±0.13	27.36±0.15	27.75±0.06
400	33.53±0.31	32.26±0.15	32.86±0.21	32.25±0.21	33.63±0.25
500	35.25±0.19	33.53±0.33	34.75±0.16	35.19±0.23	35.25±0.23
Nystatin	36.16±0.15	35.59±0.26	35.86±0.26	37.25±0.15	36.35±0.15
Griseofulvin	35.85±0.31	34.66±0.25	36.13±0.15	36.25±0.21	35.35±0.33
DMSO	–	–	–	–	–

Results presented in Table 3 showed that all the three types of dyed fabrics have considerable antifungal efficacy against all the test fungi. Dyed silk exhibited highest fungal growth reduction in *A. flavus* whereas maximum growth reduction was exhibited by dyed wool and cotton fabrics in *A. niger*. Dyed silk fabric showed highest growth reduction in all the five test fungi followed by dyed wool and cotton fabrics as envisaged in Fig 2. Dyed cotton fabrics however showed the minimum growth reduction in all the test fungi.

In recent years, studies have been conducted on the antifungal activity of phenolic compounds including

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

Dyed Fabric Substrates	Reduction in fungal growth (%)				
	A. niger	*A. flavus*	*A. parasiticus*	*F. moniliforme*	*P. canescens*
Silk	80.63	82.55	81.49	79.78	81.25
Wool	75.45	72.83	73.33	71.36	72.59
Cotton	66.39	65.76	65.49	63.69	65.23

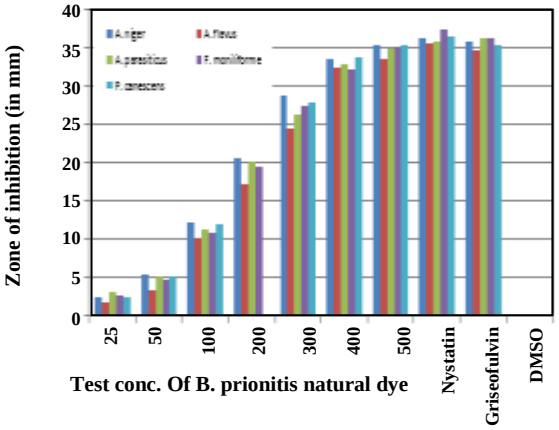

Fig. 1: Antifungal activity of B. prionitis natural dye against test fungi.

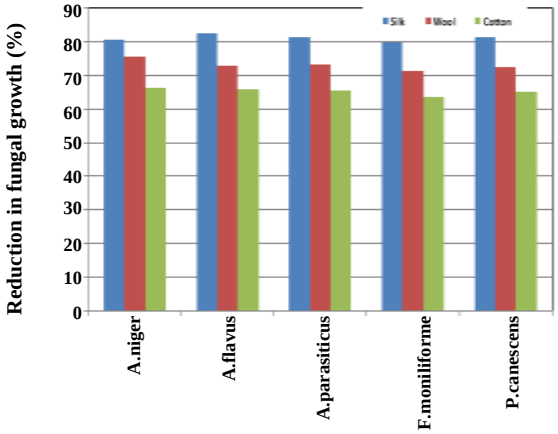

Fig. 2: Antifungal activity (reduction %) of dyed fabrics against test fungi.

flavones and related flavonoid glycosides, coumarins and derivatives, and anthraquinones [48, 53]. Presence for a large number of tannins in some of the common plant-derived nature dyes and naphthoquinones such as lawsone in henna, juglone in walnut [54] and lapachol in alkannet are reported to exhibit antibacterial and antifungal activity [55]. The antifungal potency of natural dye derived from *B. prionitis* aerial parts may be due to the presence of phenolic chemical constituents including flavonoids, tannins, anthocyanins, etc, of complex molecular structure vis-a-vis diverse action mechanisms. However, further studies are needed to characterize the natural dye in term of its color imparting constituents.

CONCLUSIONS

Several medicinal properties have been attributed to plant-derived natural dyes [56]. In view of the growing interest and resultant need for developing functional textiles for protective clothing and clinical applications, assessment of natural dyes for their antimicrobial efficacy has been thought imperative. Results of the present study showed that the natural dye derived from aerial parts of *B. prionitis* as well as fabrics dyed with it have significant antifungal activity. With its remarkable antifungal activity as evident from the studies, the textile materials dyed with natural dye can be very useful in developing protective clothing to protect users against common infections. The study led to the conclusion that the medicinally acclaimed plant *B. prionitis* 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.

Conflict of Interests

The authors declare no conflicts of interest.

Acknowledgments

The authors are grateful to the Director, Forest Research Institute, Dehradun for providing necessary facilities for carrying out this work.

Received: Mar. 6, 2017; Accepted: Jun. 19, 2017

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