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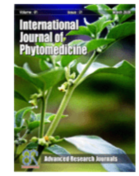

Original Research Article

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Studies on antimicrobial effect of natural dyes and pigments obtained from the leaf of *Artocarpus heterophyllus* Lam. and *Tectona grandis* L.f.

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Abstract

At present there is a renewed interest for value addition of textiles by using natural products. Many of the plant materials, from which natural dyes are obtained, also found to have some medicinal values. However, it is a matter of concern that the indigenous knowledge of extraction, processing and practice of using natural dyes has diminished to a great extent among the new generation of ethnic people due to easy availability of cheap synthetic dyes. In view of this, the present study was undertaken so as to revive and popularise the age-old art of dyeing with natural dyes.
During the investigation dyeing materials were prepared from leaves of *Artocarpus heterophyllus* Lam. (Jack fruit) and *Tectona grandis* L.f. (Teak). Silk fabrics were dyed with the extracted colouring materials and were estimated for their wash fastness to ensure the durability of the colour on the fabrics. Finally, the antimicrobial effect of the two different natural dyed fabrics was studied against *Klebsiella pneumoniae, Escherichia coli, Candida albicans* and *Aspergillus* sp. The dye prepared from leaves of *Tectona grandis* L.f. was found to be most effective by showing maximum zone of inhibition as compared to *Artocarpus heterophyllus* Lam. The textile material impregnated with two natural dyes resulted in the maximum zone of inhibition rate (65%) against *Escherichia coli* as recorded in the silk samples dyed with *Tectona grandis*.
It can be concluded that the natural dyes obtained from these two plants under study could provide special aesthetic values, which is not only environmentally friendly but gives added qualities to the textile production because of the antimicrobial potential of the dyes.
Keywords: Cisplatin Antimicrobial activity; *Artocarpus hetphyllus* Lam.; *Tectona grandis* L.f.; Silk fabric; Mordants; Disc diffusion.

Introduction

Natural dyes has gained importance due to the growing environmental awareness and due to the result of stringent environmental standards imposed by many countries in response to toxic and overall water quality issues due to effluents associated with synthetic dyes. Under such situation natural dyes derived from plants have gained economic advantage over synthetic dyes because of their non-toxic, non-carcinogenic and biodegradable nature.[1] Up to the end of 19th century natural dyes were the main colourants for textiles. Recently, interest in the use of natural dyes has been growing rapidly due to the result of stringent environmental standards imposed by many countries in response to toxic and allergic reactions associated with synthetic dyes.[2] Natural dyes may have a wide range of shades and can be obtained from various parts of plants including roots, bark, leaves, flowers, and fruit. [3]
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 amount of waste and unfixed colorants causing serious health hazards and disturbing the eco-balance of nature. [4] Now-a-days, fortunately, there is increasing awareness among people towards natural dyes. Natural dyes 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. [5]
Natural dyes are permanent as compared to synthetic colorant. In India, Rajasthan, Orissa (Kotapad, Nuapatana, and Berhampur) and Kutch still possess a rich tradition in the use of natural dyes for textile dyeing. The widely and commercially used synthetic dyes impart strong colours but causes carcinogenicity and inhibition of benthic photosynthesis. [6] Thus, India harbours a wealth of useful germplasm resources and there is no doubt that the plant kingdom is a treasure-house of diverse natural products. One such product from nature is the dye. Mordants are metal salts which produce an affinity between the fabric and the dye. [7]
Currently, both natural and synthetic fibres have inherent resistance to microorganisms. Textile materials provide an excellent environment for microorganisms to grow, because of their large surface area and ability to retain moisture. Microbial activity can be detrimental to textiles. It can cause unpleasant odour, lead to weakening of the substrate, discoloration, and even contribute to the spread of disease. For this reason, antimicrobials have been

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investigated as a finish for textiles. [8] Antibacterial finishes are applied to textiles for three major reasons: (a) to curtail the spread of disease and avoid the danger of injury-induced infection, (b) to curtail the development of odour from aspiration, stains and soil on textile materials, and (c) to curtail the deterioration of textiles caused by mildew, particularly fabrics made of natural fibres. [9] In order to obtain the greatest benefit, an ideal antimicrobial treatment of textile should satisfy a number of requirements; [10] of these, the antimicrobial agent should be effective against broad spectrum of bacterial and fungal species but at the same time exhibits low toxicity to consumers, e.g., not cause toxicity, allergy or irritation to the user. Natural dyes can produce special aesthetic qualities, which, combined with the ethical significance of the product that can evolve into an eco-friendly and value added textile fabrics.
The present study was undertaken with an objective to revive the age-old art of natural dyeing apart from the study of the antimicrobial potential present in the natural dyes obtained from the leaves of *Artocarpus heterophyllus* and *Tectona grandis*.

Materials and Methods

Collection of plant materials for the experiment

Artocarpus heterophyllus Lam. (Jack fruit) leaves and *Tectona grandis* L.f. (Teak) leaves collected from Balasore district of Odisha (India); 6.5 cm silk fabrics were used for dyeing.
Artocarpus heterophyllus Lam. (Moraceae) is a large tropical tree and is indigenous to India and popularly known as Jack tree (Figure 1 A, B). The leaves are simple, alternate, coriaceous, entire, dark, shiny green above, oblong, oval or eliptic in form, 4 to 6 inches in length, glabrous, hairless with smooth skinned surface. The leaves show the presence of sapogenin, cycloaratenone, cycloartenol, sitosterols and tannins. [11] The leaves are reported to be used in skin diseases and ash of leaves is useful in healing ulcer. The leaves are also useful in boils, wound, fever, skin diseases and vitiated condition of pitta and vata. [12]
Tectona grandis L.f., commonly known as teak, is a widespread woody plant with lot of biological properties. [13] Large deciduous tree, branchlets 4-angled. Leaves large, opposite, simple, obovate-elliptic, stellately (Figure 2 A, B); useful in skin diseases, leprosy, cooling, diabetes, and bronchitis. [14]

Figure 1 A: *Artocarpus heterophyllus* Lam.

Figure 1 B: Colour substances extracted from *A. heterophyllus* Lam.

Figure 2 A: *Tectona grandis* L.f.

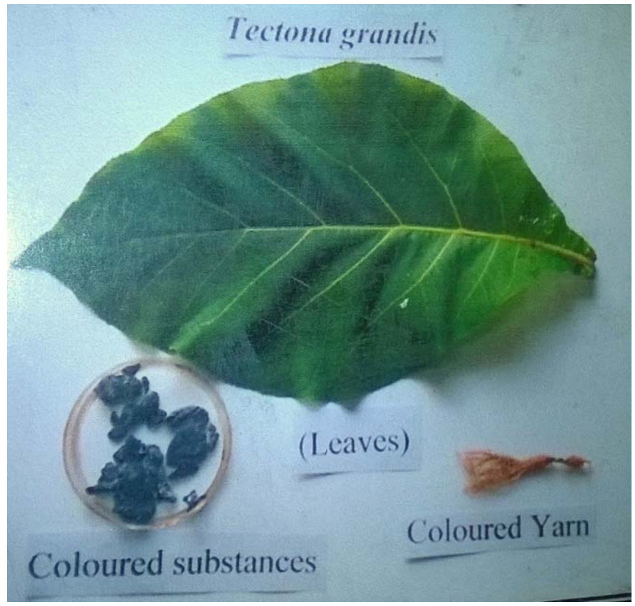

Figure 2 B: Colour substance extracted from *T. grandis* L.f.

Extraction of dye

Experiments were conducted for extraction of the natural dye with an effort to get the maximum amount from the leaves of *A. heterophyllus* and *T. grandis*. Samples collected were dried at room temperature (25%) completely before ground in the mixer grinder. 500 g of the leaf powder was taken in a soxhlet apparatus treated with 3500 ml of 10 % aqueous ethanol. On heating, the whole extracts underwent the process of distillation to obtain the crude dye as a pasty mass. The pasty extract was washed with a non-polar solvent such as petroleum ether followed by freeze drying to obtain the colouring materials in solved form. The final yield of material in case of *Artocarpus heterophyllus* and *Tectona grandis* was found to be 70 g (14%) and 74 g (14.80%) respectively, which was used as the experimental material.

Preparation of standard dye solution

The semi-solid dye materials were converted into different concentration of standard solution by 10% aqueous methanol solvent. Thus standard dye solutions were prepared at different concentrations such as 10%, 8%, 6%, 4%, and 1%. Dyeing procedure Dyeing was done with or without mordant as well as pre-mordanting and post-mordanting.

Artocarpus heterophyllus

Lam.For the purpose the fabric silk was dipped in the prepared mordant solution 20 ml (10 % Alum + turmeric powder) for 30-45 minutes at 50-60 ^{0}c. Then the mordanted silk was air dried for 15 minutes. The dye solution (20 ml) was prepared with 0.2% sodium hydroxide solution. The mordanted fabric was then dipped in the dye bath for 30-45 minutes at 60-70 ^{0}c for silk. The dyed fabric was left for 15 minute for air oxidation. The golden yellow colour was achieved.

Tectona grandis L.f.

The silk fabric was dipped in the prepared mordant solution of 20 ml (5% $CuSo_4$) for 30-45 minute at 50-60 ^{0}c. Then the mordanted silk was air dried for 15 minute. The dye solution (20 ml) was prepared with 0.2% sodium hydroxide solution. The mordanted fabric was then dipped in the dye bath for 30-45 minutes at 60-70 ^{0}c for silk. The dyed fabric was left for 15 minute for air oxidation. The brick red colour was achieved.

Test organism

Cultures of following microorganisms were used in the study: *Klebsiella pneumoniae* ATCC 1705, *Escherechia coli* ATCC 1053, *Candida albicans* ATCC 10231, *Aspergillus* sp. MTCC- 8790 obtained from Himedia were used in this study.

Determination of antimicrobial activity of natural dyes in solution

Disc diffusion method was used to screen the antimicrobial activities. The nutrient agar plates were prepared by pouring 15 ml of media into sterile Petri-plates and in the same way PDA plates were also prepared. The plates were allowed to solidify for 2 hrs and then prepared broth of 0.1% inoculums suspension was streaked uniformly. Sterile filter paper disc (diameter 5.0 mm) was placed on top of the seeded medium. 30 μl of different concentration of *Tectona grandis* and *Artocarpus heterophyllus* (1%, 4%, 6%, 8%, 10%) dye solution were loaded to diffuse for 2 minute and then the plates were kept for incubation at 37 ^{0}c for overnight (16 hrs) for bacteria. For fungi all procedure was similar but 50 μl dye solution was loaded on the filter disc and it was allowed to diffuse for 2 minute and the plates were kept for incubation at room temperature for 16 hrs. Inhibition zones formed around the disc were measured with ruler in centimetres. All the studies were performed in triplicates.

Determination of antimicrobial activity of dye fabrics

In this experiment the antimicrobial activity of dyed fabrics was tested. The 6.5cm fabric (dyed and undyed for control) was introduced in the 100 ml nutrient broth inoculated with the desired test pathogens and incubated at 37 ^{0}c over night for 24 hrs for bacteria and fungi for a room temperature for 16 hrs. The reduction in the growth of bacteria and fungi by the dye was expressed as follows.

R = B - A/B x 100

Where:

R = % Reduction in bacterial population

B = Absorbance (660 nm) of the media inoculated with microbe and undyed fabric.

A = Absorbance (660 nm) of the media inoculated with microbe and dyed fabrics.

Results and discussion

Screening of natural dyes for anti-microbial activity

Solution of different concentrations of the two natural dyes under study was screened for their activities against the test organisms. The results are reported in the Table 1 and Table 2. The data on the efficacy of the natural dyes upon the test organisms revealed that at 10%, 8%, 6% and 4% concentrations of the dye obtained from *Artocarpus heterophyllus* resulted in the significant inhibition zone. The results also indicated that there was a gradual increase in the zone of inhibition with the increase in the concentration of the leaf extract from 4% to 10% irrespective of the test organisms. However, at 1% concentrations, mild growth of microbes was observed along with zone of inhibition. *Artocarpus heterophyllus* was more effective against *Escherichia coli* as compared to other test organisms. However, *Artocarpus heterophyllus* was found to be less effective against *Candida albicans.*

Screening test for *Tectona grandis* showed that the dye was effective against all the four microbes irrespective of the concentration of the extract. Highest zone of inhibition could be seen against *Klebsiella pneumoniae* as a typical example as mentioned in Table 2. The results also indicated that there was a gradual increase in the zone of inhibition with the increase in the concentration of the leaf extract from 4% to 10% irrespective of the test organisms. However, a mild growth at 1% was observed thereby indicating that the dye was not effective to control the growth of the microbes at lower concentration.

The antimicrobial potential of both the dyes were studied by observing the zone of inhibition as presented in Table 1 and Table 2, which indicated that the increase in the concentration of dye could lead to increased inhibition as reflected by enhancement in diameter. It can be concluded that both the dyes could be used as effective antimicrobial agents. It is evident from Table 1 and 2 that with increasing concentrations of the dye, the zone of inhibition is increased almost linearly. The increase in zone width is relatively larger for *Artocarpus heterophyllus* than *Tectona grandis,* thus it is apparent that the dyes evaluated during the present study have bactericidal and fungicidal properties.

Table 1: Antimicrobial activity of natural dye (inhibition zone in cm) from leaves of *Artocarpus heterophyllus* Lam.

Dye	Sl.No	Test organisms	Measurement of inhibition zone (in cm)				
			Different conc. of plant extracts				
Artocarpus heterophyllus			1%	4%	6%	8%	10%
	1	*Klebsiella pneumoniae*	0.71±0.01	1.43±0.02	3.0±0.08	3.92±0.11	4.56±0.12
	2	*Esherichia coli*	0.69±0.00	1.2±0.01	2.8±0.04	3.57±0.08	4.6±0.14
	3	*Candida albicans*	0.56±0.01	0.97±0.01	1.56±0.02	2.33±0.03	2.53±0.03
	4	*Aspergillus* sp.	0.73±0.01	1.47±0.01	3.3±0.28	4.36±0.12	5.16±0.28

Table 2: Antimicrobial activity of natural dye (inhibition zone in cm) from leaves of *Tectona grandis* L.f.

Dye	Sl.No	Test organisms	Measurement of inhibition (in cm)				
			Different conc. of plant extracts				
Tectona grandis			1%	4%	6%	8%	10%
	1	*Klebsiella pneumoniae*	0.7±0.01	1.36±0.04	2.76±0.12	3.33±0.32	4.36±0.33
	2	*Esherichia coli*	0.69±0.00	1.16±0.04	1.93±0.20	2.76±0.24	4.06±0.16
	3	*Candida albicans*	0.56±0.01	1.2±0.08	2.3±0.21	3.43±0.41	3.86±0.12
	4	*Aspergillus* sp.	0.73±0.01	1.23±0.09	2.56±0.09	3.2±0.24	4.0±0.21

Antimicrobial activities of Fabrics treated with natural dyes

Since dyes showed good antimicrobial activities against selected microbes in solution, it was thought worthwhile to study their antimicrobial activities on dyed substrate (fabric). The silk samples dyed with these natural dyes were used as model system. It was revealed from Figure 3 that the dye obtained from *Tectona grandis* was 50% more efficient than the dye obtained from *Artocarpus heterophylus* with regard to the control of *Klebsiella pneumoniae, Escherichia coli* and *Candida albicans*, where as for *Aspergillus* sp. both the dyes were found to be more or less equally effective. It was evident from Table 3 that the dye from *Tectona grandis* reduced the growth in case of *Escherichia coli* up to 65% where as the dye obtained from *Artocarpus heterophyllus* reduced the growth of *Aspergillus* sp. up to (44%).

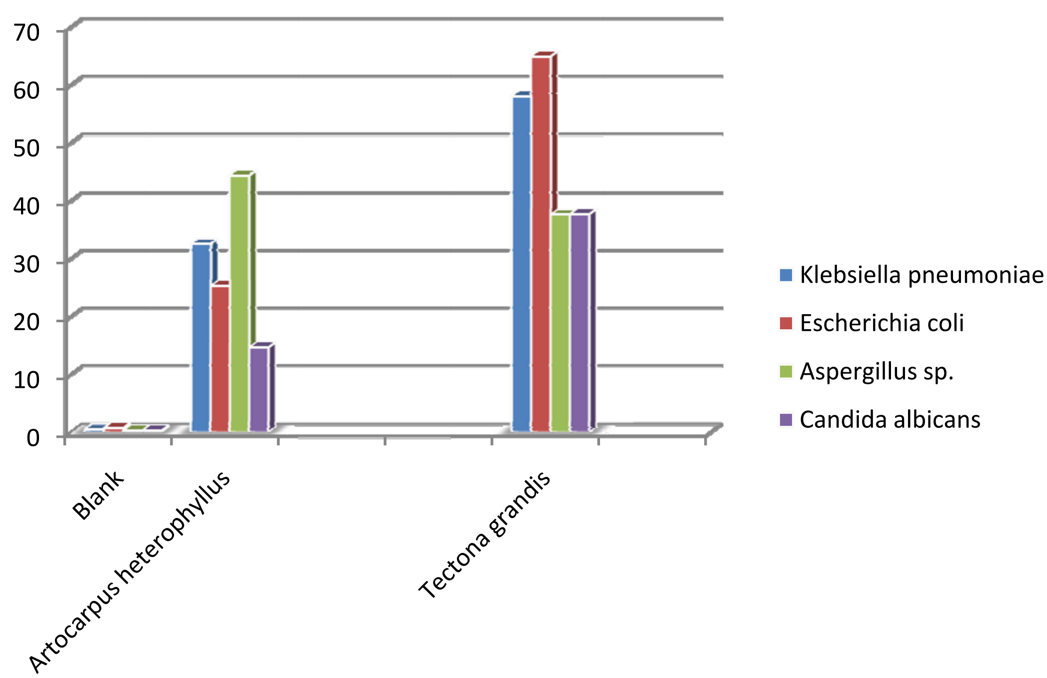

Figure 3: Antimicrobial activity of textile materials dyed with *Artocarpus heterophylus* and *Tectona grandis*

Table 3: Antimicrobial activity (reduction of growth in %) of the fabrics treated with natural dyes of the plants.

Test organism	Growth of test microbes				
	Initial Absorbance (O.D.)	Absorbance with dyed fabrics (O.D.)		Reduction in growth of test organisms (%)	
		Artocarpus heterophylus	*Tectona grandis*	*Artocarpus heterophylus*	*Tectona grandis*
Klebsiella pneumoniae	0.486	0.328	0.204	32.510	58.024
Escherichia coli	0.736	0.550	0.258	25.271	64.945
Candida albicans	0.300	0.256	0.187	14.66	37.66
Aspergillus sp.	0.282	0.157	0.176	44.33	37.58

Conclusion

The dyes obtained from the two plants (Artocarpus heterophyllus Lam. and Tectona grandis L.f.) used in the current study were found to have effective antimicrobial potential against the four microbial pathogens i.e. Klebsiella pneumonia, Escherichia coli, Candida albicans and Aspergillus sp. The dye obtained from Tectona grandis was 50% more effective to control the growth of Klebsiella pneumoniae, Escherichia coli and Candida albicans as compared to the dye obtained from Artocarpous heterophyllus. The efficacy of both the dyes in respect of the control of Aspergillus sp. was observed to be nearly similar. The effectiveness of the both the dyes were observed to increase linearly with the increase in concentration of the dye from 4% to 10%. It can be concluded that the natural dyes obtained from these two plants under study could provide special aesthetic values, which is not only environmentally friendly but gives added qualities to the textile production because of the antimicrobial potential of the dyes.

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