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ANTIMICROBIAL ACTIVITY OF NATURAL DYES AND PIGMENTS OBTAINED FROM *MORINDA CITRIFOLIA* L. AND *TERMINALIA CATAPPA* L. LEAVES

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ABSTRACT

The global consciousness reveals about the use of eco-friendly natural dyes in textiles and food industries instead of hazardous and carcinogenic synthetic dyes. Many of the plant materials, from which natural dyes are obtained, found to have some medicinal values. During the present study, dying materials were prepared from leaves of *Morinda citrifolia* L. and *Terminalia catappa* L. The antimicrobial potential of the two different dyed fabrics was studied against *Klebsiella pneumoniae, Escherichia coli, Candida albicans* and *Aspergillus* sp. The dye obtained from leaves of *Morinda citrifolia* was found to be more effective by exhibiting maximum zone of inhibition as compared to *Terminalia catappa*. The fabrics impregnated with two natural dyes resulted in the maximum inhibition rate (67 %) against *Candida albicans* as observed in the silk samples dyed with *Morinda citrifolia*. The dyed fabrics also showed reasonably good wash fastness; hence have practical potential for adding antibacterial properties along with vibrant colours to textiles of medical and other delicate uses.

KEYWARDS: Antimicrobial effectiveness; Natural dye; *Morinda citrifolia* L.; *Terminalia catappa* L.; Colour fastness; Silk fabric

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INTRODUCTION

In the human civilization plants were used not only for the basic needs of life such as food, fiber, fuel, cloths and shelter but also as sources of natural dyes for dying cloths, designing and painting. Dyes are the natural / synthetic compounds used to add a colour to or change the colour of materials. Dyes are capable of being fixed to materials and do not wash out with detergents and water or fade easily on exposure to light. The majority of natural dyes are made from plant parts such as leaves, flowers, berries, roots, bark, rhizomes, tubers, shoots, sap and wood. Natural dyes can provide the essential alternative to the complex world of chemical dyes. These dyes are environmentally sound and can be grown by organic methods. They are inherently carbon neutral. Vibrant colours can be produced from natural dyes.[1, 2] Textiles find immense applications in day to day life and there has been a growing need to develop finishes for textile materials that can offer improved protection to the users from microbes. Many of the natural dyes derived from plant sources were classified as medicinal and possess remarkable antimicrobial activity.[3] Now-a-days, the demand for antibacterial fabric and health care has increased; so, finding a method to get antibacterial textile is a challenge. Fibers, especially natural fibers (wool and cotton), provide basic requirements such as moisture and nutrients for bacterial growth and multiplication. Using synthetic non-biodegradable chemical compounds for these approaches cause environmental and health concerns. Although natural dyes are known for a long time for dyeing as well as medicinal applications, the structures and protective properties have been recognized only in the recent past.[4] 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.[5] The dyes extracted from natural sources were less competitive and were therefore gradually replaced by synthetic dyes. But now-a-days, extraction and application of natural dyes are becoming more popular owing to the growing awareness of environmental problems coupled with the toxicity associated with synthetic dyes.[6] A variety of antimicrobial textile agents are reported such as organometalics, phenols, quaternary ammonium salts and organo-silicones.[7] Synthetic compounds are more complex and it will take a long time for them to complete their natural cycles and return to nature; thus causing a lot of environmental pollution. Due to the fact that natural dyes can often inhibit the growth of microorganisms traditionally, different plants have been used as natural dyes in textile and carpet industries and it is believed that these dyes are less allergic and more stable than the chemical ones.[8]

Natural dyes can produce special aesthetic qualities, which, combined with the ethical significance of a product that is environmentally friendly, gives added value to textile production as craftwork and as an industry.[9] Much has been written about the healing power of colour and their effect on human health and well being through the selection of the wrong or right colours that we surround and cloth ourselves with.[10]

In the world today, the position of natural dyes and colorants, ‘natures colours’ are at a crossroad: They will either vanish and with them the ancient knowledge and skill that has advanced with their use over centuries and across civilisations or they will evolve to create a new form of colouration and with this be applied to new environmental/sustainable applications and possibly be recognised for the health/healing aspects they can provide.[11, 12] Currently there was increasing interest into natural dyes and colorants, as interest grows within the Industrialised Nations in natural (green) products and sustainable ways of living. But this could also be in response to the negative impact synthetic dye manufacturing is having on the environment and human health. This is caused during dye synthesis, the release of undesirable/hazardous and toxic chemicals in nature and the effect synthetic dyes can have upon human health, as irritants/sensitizers, synthetic dyes are often the cause of allergic reactions.[11, 13]

In the current investigation, the antimicrobial activity of two natural dyeing agents available in Odisha has been examined. The dyed fabrics have been tested for their wash fastness also to ensure the durability of the antimicrobial effect. The purpose of this research is to study the antimicrobial effect of two dye powders, obtained from *Morinda*

citrifolia L. and *Terminalia catappa* L. leaves against 4 microbes. The antimicrobial activity of silk fabric treated with these natural dyes has also been evaluated.

MATERIALS AND METHODS

Natural dyes were prepared from leaves of two plant species (*Morinda citrifolia* L. and *Terminalia catappa* L.) collected from Bhubaneswar, Odisha (India).

Morinda citrifolia L.

Morinda citrifolia L. belongs to family Rubiaceae, is commonly known as 'achhu' (Fig.1). It is a small tree with straight trunk, distributed throughout the greater part of India. Leaves broadly elliptic, bright green, glabrous; flowers white indent; fruits are ovoid, glossy white when ripe. Leaves are used as a healing application for wounds and ulcers. The juice of the leaves is externally applied in gout. The skin health benefits of the 'achhu' fruit and leaf have been alluded to by many traditional healers, especially those in the Islands of Tonga, in the South Pacific.[14] It was a common practice for traditional healers of Odisha to treat various forms of skin ailments.

Terminalia catappa L.

Terminalia catappa L. is a tree, 5-7 m tall, with blackish-brown bark; leaves obovate, coriaceous, glabrous, cordate at base; flowers pale-yellow, fragrant in 6-20 cm long spikes; fruit 3-5.5 x 1.6-2.5 cm, ellipsoidal, brown when ripe (Fig. 2). It belongs to the family Combretaceae. Frequently planted in gardens and avenues. The juice of the leaves is ingested for cough. An infusion of the leaves is used to treat jaundice. Leaves sudorafic, also the paste is applied to rheumatic joints. Bark diuretic and cardiotonic also used in dysentery. The juice of the young leaves is used in South India to prepare an ointment for scabies, leprosy and other cutaneous diseases.

Extraction of dye

The leaves were collected and completely dried at room temperature (25 ^{0}C) shade drying and then ground in mixer grinder. The processed leaf powder (500 g) was taken in a soxhlet apparatus and treated with 10% aqueous ethanol (3500 ml) for the extraction of colouring materials by the solvent extraction methods on heating. The whole extract undergoes the process of distillation to obtain the crude dye as a pasty mass. The pasty extract was washed with non-polar solvent such as pet-ether followed by freeze drying to obtain the colouring materials in solid form. The yield of *Morinda citrifolia* L. and *Terminalia catappa* L. gives 60 g (12 %) and 50 g (10 %) respectively.

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.

Morinda citrifolia L.

Silk fabric was dipped in prepared mordant solution (20 ml) (10 % alum) at 50^0 to 60 ^{0}C for 30-45 minutes. Then the mordanted fabric was air dried for 15 minutes. The dye solution was prepared and the mordanted silk fabric was then dipped in dye bath for 30-45 minutes at 60-70 ^{0}C. The dyed fabric was left for 15 minute for air oxidation. Finally red colour was achieved.

Terminalia catappa L.

In the same way silk fabric was dipped in the prepared mordant solution (20 ml) (5% $CuSo_4$) for 30-45 minute at 50-60 ^{0}C. Then the mordanted fabric was air-dried for 15 minutes. The dye solution was prepared. The mordanted fabric was then dipped in the dye bath for 30-45 minutes at 60-70 ^{0}C. The dyed fabric was left for 15 minute for oxidation. The brown shade colour was achieved as final result.

Test organisms

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

Antimicrobial screening test

Susceptibility of the bacterial and fungal strains to the natural dyes was investigated using the disc diffusion method. Nutrient Agar and Potato Dextrose Agar medium (15 ml) was poured into each sterile petri dish. The plates were allowed to solidify for 10 minutes 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. The experiment was carried by using 5 different concentration dye solution (10, 8, 6, 4, and 1%) of *Morinda citrifolia* L. and *Terminalia catappa* L. All the experiments were done in triplicates and a control set was run along with each test. 30 µl of different concentration of dye solution were loaded on the sterilized filter disc and it allow for 2 minutes for better diffusion, then the plates were kept for incubation at 37 ^{0}C for overnight (24 hrs.) for bacteria. For fungi all procedure was similar but 50µl dye solution was loaded on the filter disc and it was allowed to diffuse for 2 minutes 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. After overnight incubation at 37 ^{0}C, the zone of inhibition was measured. In the second set of experiment the antimicrobial activity of dyed fabrics was tested. The 6.5 cm 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 overnight for (24 hrs.) for bacteria and fungi in room temperature for 16 hrs. The reduction in the growth of bacteria and fungi by the dye was expressed as follows:

$R=B-A/B\times100$

Where;

R=% of reduction of microbial 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 fabric

RESULTS AND DISCUSSION

Antimicrobial activity of natural dyes in solution

Solution of different concentrations of the two natural dyes under study was screened for their activities against the test organisms. The results are presented in the Table 1 and Table 2. It was observed that increase in dye concentration led to increase inhibition reflected by enhancement in diameter of the inhibition zone. *Morinda citrifolia* L. resulted that there was a gradual increase in the zone of inhibition with the increase in the concentration of the leaf extract from 1 to 10% irrespective of the test organisms. But in case of *Klebsiella pneumoniae* at 1% concentration, mild inhibition zone was observed. In case of *Aspergillus* sp. maximum zone of inhibition was observed. However, *M. citrifolia* L. was found to be less effective against *Klebsiella pneumoniae* as compared to other test pathogens. Screening test for *Terminalia catappa* showed that the dye was effective against all the four microbes irrespective of the concentration of the extract. 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 1 to 10% irrespective of the test organisms. It was observed to be highly effective against *Escherichia coli, Klebsiella pneumoniae, Candida albicans* and *Aspergillus* sp. as mentioned in Table 2. The antimicrobial potential of both the dyes were studied by observing the zone of inhibition as presented in Table 1 and Table 2. It can be concluded that both the dyes could be used as effective antimicrobial agents.

Antimicrobial activity of natural dyes on substrate

As both the experimental natural dyes exhibited good antimicrobial activities against selected microbes in solution, it was thought to study their antimicrobial activities on dyed substrate (fabric). The silk samples dyed with these natural dyes were used as model system. Quantitative evaluation of anti-microbial activity was possible on the fiber by spectrophotometric assessment. The percentage of effectiveness of dye against the mentioned microorganism is listed in the Table 3. It can also be visualized from Fig. 3 that *M. citrifolia* has more effective antimicrobial properties as compared to *T. catappa*. The highest reduction (67%) in growth of *Candida albicans* was seen in *M. citrifolia* treatment, whereas the reduction in growth of *Aspergillus* sp. was least affected (2.33%) in *T. catappa* treatment.

Table 1
Antimicrobial activity of natural dye (inhibition zone in cm) from leaves of Morinda citrofolia L.

Dye	Sl.No	Test organisms	Measurement of inhibition zone (in cm)				
			Different conc. of plant extracts				
			1%	4%	6%	8%	10%
Morinda citrifolia	1	*Klebsiella pneumoniae*	0.70±0.01	1.97±0.01	2.23±0.01	2.66±0.02	2.86±0.04
	2	*Esherichia coli*	2.4±0.08	2.83±0.12	3.26±0.02	3.54±0.04	3.83±0.01
	3	*Candida albicans*	2.45±0.03	2.88±0.01	3.43±0.04	3.77±0.01	4.0±0.09
	4	*Aspergillus* sp.	2.52±0.02	3.23±0.02	3.5±0.01	3.99±0.08	4.4±0.08

Table 2
Antimicrobial activity of natural dye (inhibition zone in cm) from leaves of Terminalia catappa L.

Dye	Sl.No	Test organisms	Measurement of inhibition zone (in cm)				
			Different conc. of plant extracts				
			1%	4%	6%	8%	10%
Terminalia catappa	1	*Klebsiella pneumoniae*	2.45±0.01	2.93±0.02	3.53±0.01	3.97±0.02	4.38±0.02
	2	*Esherichia coli*	2.48±0.16	2.88±0.01	3.38±0.01	3.88±0.01	4.2±0.16
	3	*Candida albicans*	2.5±0.01	2.9±0.01	3.47±0.02	3.94±0.05	4.3±0.02
	4	*Aspergillus* sp.	2.52±0.01	2.88±0.06	3.36±0.04	3.8±0.8	4.33±0.04

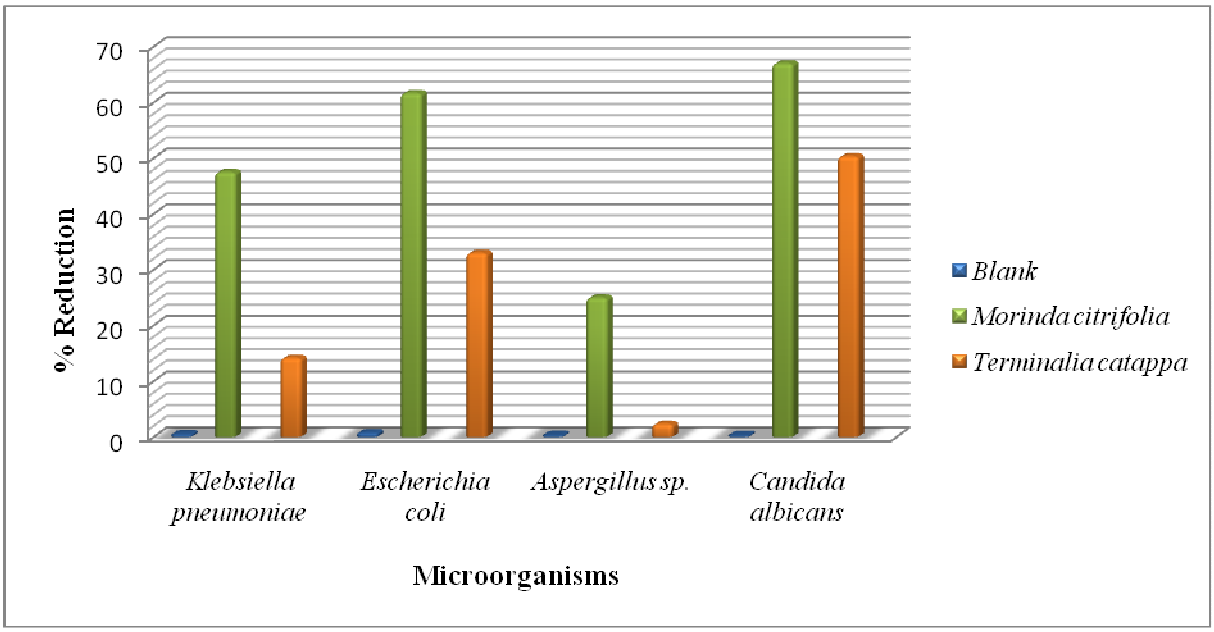
Table 3
Antimicrobial activity (reduction of growth in %) of the fabrics treated with natural dyes of the plants

Test organism	Growth of test microbes				
	Initial Absorbance (O.D.)	Absorbance with dyed fabrics (O.D.)		Reduction in growth of test organisms (%)	
		Morinda citrifolia	*Terminalia catappa*	*Morinda citrifolia*	*Terminalia catappa*
Klebsiella pneumoniae	0.486	0.255	0.417	47.53	14.19
Escherichia coli	0.736	0.282	0.493	61.68	33.01
Candida albicans	0.282	0.093	0.140	67.02	50.35
Aspergillus sp.	0.300	0.255	0.293	25	2.33

Figure 1
***Morinda citrifolia* L. (Indian mulberry)**

Figure 2
***Terminalia catappa* L. (Almond)**

Figure 3
Antimicrobial activity of textile materials dyed with *Morinda citrifolia* L. and *Terminalia catappa* L.



CONCLUSION

The dyes obtained from *Morinda citrifolia* and *Terminalia catappa* used in the present investigation were found to possess effective antimicrobial potential against four pathogenic strains i.e. *Klebsiella pneumoniae, Escherichia coli, Candida albicans* and *Aspergillus* sp. The colouring material extracted from *Morinda citrifolia* was more effective in controlling the growth of all the tested microbial pathogen as compared to the dye obtained from *Terminalia catappa*. The effectiveness of both dyes was also observed to increase with the increase in concentration of the dye up to 10% irrespective of the organisms tested. Besides, the dyed fabrics also showed good wash fastness; hence have practical potential for adding antibacterial properties along with vibrant colours to textiles of medical and other delicate uses.

REFERENCES

1. Chengaiah B., Rao K. M., Kumar K. M., Alagusundaram M. and Chetty C. M., Medicinal importance of natural dyes a review: International Journal of Pharma Tech Research, 2 (1): 144-154, (2010)
2. Srivastava S. K., Singh S. K., Rajkumar S. D. and Gautam R. P., Inventorisation and documentation of dye yielding plant diversity of eastern Uttar Pradesh: International Journal of Research in Engineering and Bioscience, 2 (5): 70-75, (2014)
3. Giridev V. R., Venugopal J. R., Sudha S., Deepika G. and Ramakrishna S., Dyeing and antimicrobial characteristics of chitosan treated wool fabrics with henna dye: Carbohydrate Polymers, 75 (4): 646-650, (2009)
4. Mohammad M. and Mina A., Comparison between antibacterial activity of some natural dyes and silver nanoparticles: Journal of Nanostructure in Chemistry, 37: 1-3, (2013)
5. Gerson H., Fungi toxicity of 1, 4-napthoquinones to *Candida albicans* and Trichophyton mentagrophytes: Can. J. Microbiol, 21: 197–205, (1975)
6. Narayanaswamy K. N., Gowda N and Sudhakar R., Natural dye from the bark of *Casuarina equisetifolia* for silk: Int J Pharm Bio Sci, 4(3): 94 -104, (2013)
7. Yang Y., Corcoran L. and Vorlicek K., Durability of some antibacterial treatments to repeated laundering: Text Chem Color Am Dyestuff Rep, 32(4): 48-54, (2000)

8. Mehrabian S., Majd A. and Majd I., Antimicrobial effects of three plants (*Rubia tinctorum, Carthamus tinctorius* and *Juglans regia*) on some airborne microorganisms: Aerobiologia, 16: 455-458, (2000)
9. Kanchana R., Fernandes A., Bhat B., Budkule S., Dessai S. and Mohan R., Dyeing of Textiles With Natural Dyes - An Eco-Friendly Approach: International Journal of Chem Tech Research, 5(5): 2102-2109, (2013)
10. Quinn B., Textile Futures: Fashion, Design and Technology, Berg, Oxford, 102-107, (2010)
11. Kumar Samanta A. and Konar A., Dyeing of textiles with natural dyes: Natural Dyes, Perrin Akcakoca Kumbasar E (ed.), In Tech, 29-56, (2011)
12. Bhandari K., Natural compounds and its medical activity: Proceedings of the Vegetable Dye and its Application on Textiles National Workshop and Seminar, India, 59-63, (2011)
13. Fletcher K., Sustainable Fashion and Textiles: Design Journeys, Earthscan, London, 51-55, (2008).
14. Afa K. Palu, Shixin Deng and Brett J. West, Sunburn (*fohia*) Healing Effects of Noni: Is it a Mechanism Involving its Inhibitory Effects on MMP, COX-2 and Cat-G Enzymes?: Journal of Applied Pharmaceutical Science, 02 (08): 40-50, (2012)