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Antifungal activity of different natural dyes against traditional products affected fungal pathogens

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ABSTRACT

Objective: In the present study to evaluate the anti fungal activity of natural dyes against traditional products affected fungal pathogens. **Methods:** Many traditional craft products affected fungal pathogens were isolated using potato dextrose agar medium. The isolated fungus were identified by morphological and microscopically characterization using Alexopolus manual. 50 μl of Turmeric, Terminalli, Guava and Henna natural dyes were poured into the wells of the culture plates. If antifungal activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the natural dye. **Result:** At a dose level of 50 μl of terminalli dye was able to inhibit the growth of all the fungi tested. The absorbance rate of natural dyes analyzed by UV Spectrophotometer. The absorbance rate is high in terminalli (2.266) and turmeric (2.255). **Conclusions:** Natural dyes were bound with traditional products to give good colour and good antimicrobial activity against isolated fungal pathogens

1. Introduction

The various types of traditional craft work being organic origin attack microorganisms due to the carbohydrate content in it. Biological degradation affect the usage, strength utility and value of the products leading to decay and disintegration, splits or cracks, unsightly strains and blotches. So it is essential to treat the raw material and finished products to extend durability and prolong of life. The traditional craft products made from Grass reed leaf and fiber; Bamboo; Areca nut; Palmyra leaf and coconut shell etc. These products are often damaged by fungal biofilm. So they were rejected by exporters and local consumers. So the microbiological intervention is to be applied to prevent the microbial damage in craft products. Many of the fiber products get fungal attack during monsoon seasons with high relative humidity. This fungal attack from a layer over the craft products and consumers reject these products as the fungal infection makes an ugly and dark colour biofilm over the products. This is a major problem the traditional crafts worker endures to address this imperative nee, a microbiological preventive for fungal inhibition is a need of the day.

In the last few decades there has been considerable interest in the active compounds present in natural dyes. In example the major curcuminoid is called curcumin (diferuloyl methane), which makes up approximately 90% of the curcuminoid content in turmeric, followed by demethoxycurcumin and bidemethoxycurcumin [20]. Hundreds of in vitro and animal studies have been published describing the antioxidant, anti-inflammatory, antiviral, and antifungal [4, 19] properties of curcuminoids [3, 5, 11, 29, 30]. The curcuminoid give turmeric its bright yellow color. Turmeric oil was tested for its antifungal activity against *Aspergillus flavus, Aspergillus parasiticus, Fusarium moniliforme and Penicillium digitatum* [8]. The natural dye was tested for antimicrobial activity against *E.coli, Bacillus subitillus, Psedomons aeruginosa and Styphallococcus areus* [2]. The extract of henna leaves showed obvious antibacterial activity against *E.coli* [14, 27,16]. Antibacterial activity of natural dye coated wool fabrics reported [13, 12]. When combined with amphotericin B or Fluconozole, curcumin provided a greater fungicidal effect in the treatment of systemic fungal infections such as candidiasis and candidemia [25, 26]. The natural dyes are using many

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purposes in example hair dyeing, food preparation and etc [24]. The incorporation of curcumin dye with natural fibers that are used to make handicraft products will add value to the traditional crafts and help to store perishable materials in baskets made of natural fibers. Considerable research work has been undertaken on the application of natural dyes in the coloration of textiles around the globe in the recent past [2, 9, 10, 15, 28].

2. Material and methods

2.1. Preparation of different natural dyes

The four different dyes were prepared using Continuous Stream Distillation Process.

2.1.1. Turmeric dye

1. Turmeric roots were collected from agricultural field at Alwarkurichi region.
2. The roots were dried and powdered. Hundred grams of powdered turmeric roots were boiled in a vessel with 2 liters of water.
3. After 45 min heating the turmeric emitted a strong smell and the water turned a dark orange color.
4. Finally 5ml of lemon juice used as a mordant.

2.1.2. Terminalli dye

1.The Terminalli seeds were dried and powdered and hundred grams of powdered terminalli seeds were boiled in a vessel with 2 liters of water.
2. After 45 min heating the terminalli emitted a strong smell and the water turned a dark brown color.
3. Finally alum used as a mordant.

2.1.3. Guava dye

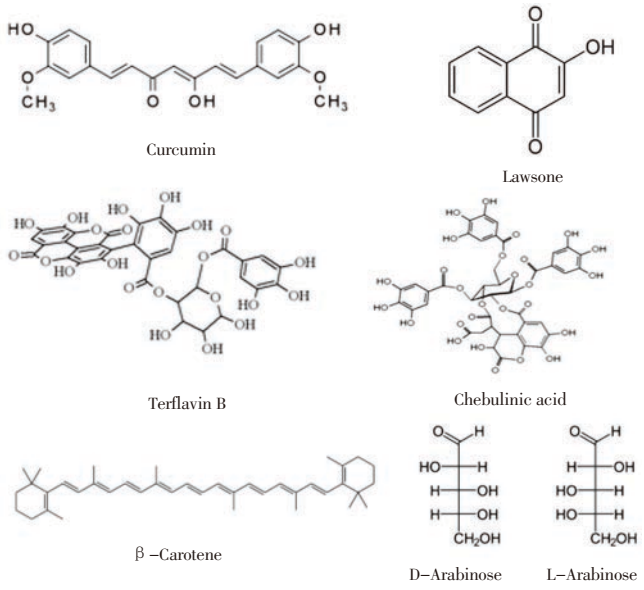
1. The guava stem outer cuticle collected from the garden and powdered.
2. 100gms of powdered guava cuticle poured in 2 liters of water and boiled with low temperature at 45 minutes.
3. After 45 min, the guava emitted colour. The water turned a gray colour.
4. Finally alum used as a mordant.

2.1.4. Henna dye

1. Henna leaves were collected from agricultural field in our college campus and it to form a paste.
2. 100gms of henna leaf paste were boiled in a vessel with 2 liters of water.
3. After 45 min, the paste emitted a strong smell and the water turned a greenish yellow colour.
4. Finally 5ml of lemon juice used as a mordant.
After prepared dyes were used for further testing.

2.2. Major groups of antifungal compounds from natural dyes

Plants have an almost limitless ability to synthesize aromatic substances of different functional groups, most of which are phenols or their oxygen substituted derivatives [6]. Most are secondary metabolites, of which at least 13,000 have been isolated that is less than 10% of the total [21]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some plants used for their odors (terpenoids), pigment, (quinones and tannins) and flavor (terpenoid capsaicin from chili peppers) were found to be endowed with medicinal properties. Some of the herbs and spices used by humans as season food yield useful medicinal compounds.



The major chemical compound in turmeric is Curcumin, Terflavin B and Chebulinic acid are present in terminallia, Lawsone is active compound of henna and β –Carotene and D & L– Arabinose are present in guava plants. These compounds were good inhibitory effects against fungal pathogens [23, 18].

2.3. Isolation of fungal pathogens

Many of the fiber products (Traditional craft products) are got fungal attacks during monsoon seasons with high relative humidity. The fungal attacked traditional products collected from local traditional craft workers. The collected traditional products (Areca nut plates) were entering to laboratory condition. The fungal pathogens were isolated from collected traditional products using potato dextrose agar medium.

2.4. Fungal identification

The isolated fungus were identified by morphological and microscopically characterization using Alexopolus manual.

2.4.1. Morphological identification

The morphological identification of fungal pathogens using Potato dextrose agar medium and Rose Bengal agar medium. The fungus growth were different from one medium to

another medium.

2.4.2. Microscopical identification

Morphologically differentiated the four fungal hypae, sporangium and spore structure seen through the Light Microscopy (450 X) using Lacto phenol cotton blue as a dye. The four funguses were different from one to other. These four fungal species identified using Alexopoulas manual.

2.5. Antifungal activity

The antifungal activities of natural dyes were tested against fungi isolated from areca nut plates (traditional product) using agar well diffusion method [15]. The culture plates were inoculated with 100 μl of standardized inoculums of each fungi and spread with sterile swabs. Wells are 6 mm sizes were made with sterile borer into agar plates containing the fungal inoculums. 50 μl of the natural dyes were poured into the wells of the culture plates. After inoculation the plates were incubated for 24 hrs at 37°C, the plates were observed. If antifungal activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the natural dye. The zone of inhibition was measured and expressed in millimeters. Antifungal activity was recorded if the zone of inhibition was greater than 6 mm. The antifungal activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active [1].

2.6. UV spectrophotometer analysis of natural dyes

The natural dye absorption and transmittance was analyzed using UV-visible spectrophotometer in wave length of 250nm. The colour strength is analyzed by spectrophotometer [7, 17].

2.7. Microscopically view of natural dye coated plant materials

Natural dyes coated plat materials seen through the Light microscopy (450 X). The dyes to give colour from the (traditional products) plant materials.

3. Result

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. The terminalli natural dye possessed activity against traditional graft products affected fungal pathogens (Table 1). In the present study it was observed that natural dyes were good antifungal activity against pathogenic microbes. At a dose level of 50 μl the terminalli dye was able to inhibit the growth of all the fungi tested (Table 1). This indicates that the traditional craft products can be colored with these natural dyes at the time of making. Many different fungus affected in the traditional products at monsoon time (Figure 1 & 2). In example our result shows four different fungi isolated from areca nut plates (Figure 13, 14, 15 &16) (traditional products). Then these funguses were identified by microscopically and morphologically (Figure 3, 4, 5, 6, 11 & 12) using Alexopoulas manual. The different natural dyes are prepared and analyzed absorbance and transmittance in using UV -Visible Spectrophotometer (Table 2&3). The absorbance rate is high in terminalli (2.266) and turmeric (2.255) natural dyes are to give good colour in the traditional products (Figure 7&8) and good antimicrobial activity against isolated fungal pathogens (Table 1). Natural dyes were tested against isolated fungus, the terminally dye is high activity against all isolated fungal pathogens (Table 1). The natural dye coated plant material seen through the microscopy (Figure 7, 8, 9&10), terminally and turmeric dye coated plant materials high concentrated colour and inhibit the fungal growth (Table 1), (Figure 7&8).

Figure 1: Normal Plate

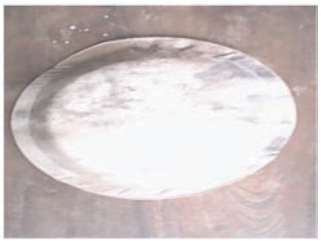


Figure 2: Fungal attacked Plate

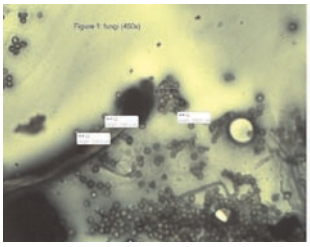


Figure 3: fungi - Spore size is 0.0011- 0.0012μm 0.0012μm

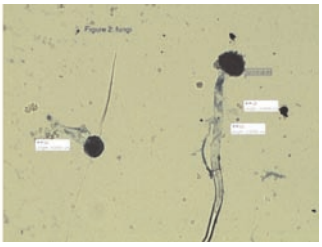


Figure 4: fungi - Spore size is 0.0006 -0.0007μm

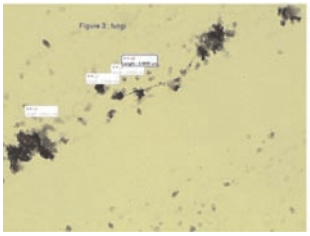


Figure 5: fungi - Spore size is 0.0009 -0.0011μm

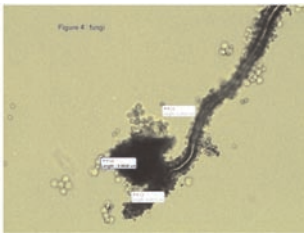


Figure 6:fungi - Spore size is 0.0016μm

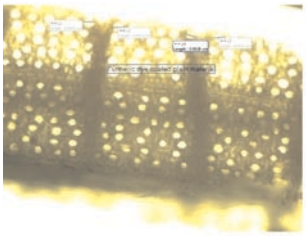


Figure 7: Turmeric dye coated plant material

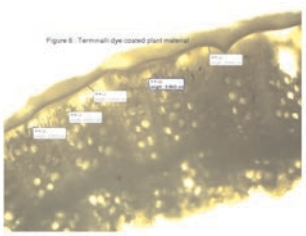


Figure 8: Terminalli dye coated plant material

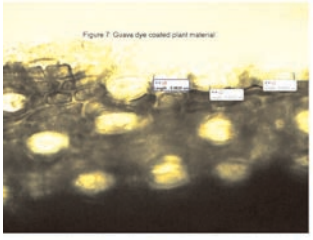


Figure 9: Guava dye coated plant material

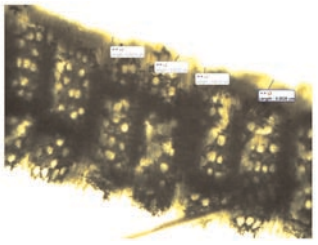


Figure 10: Henna dye coated plant material

Figure 11: The isolated fungus are grow on Potato Dextrose agar medium

Figure 12: The isolated fungus are grow on Rose Bengal agar medium

Figure 13: fungi 1

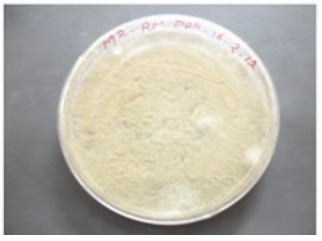


Figure 14: fungi 2

Table 1

Antifungal activity of different Natural dyes.

S.No	Fungal Samples	Zone inhibition of Natural Dyes (mm)			
		Turmeric	Terminalli	Guava	Henna
1	*Cladosporium spp*	15	12	11	–
2	*Emerisella spp*	13	17	–	12
3	*Candida spp*	14	16	10	11
4	*Rhizopus spp*	–	18	–	–

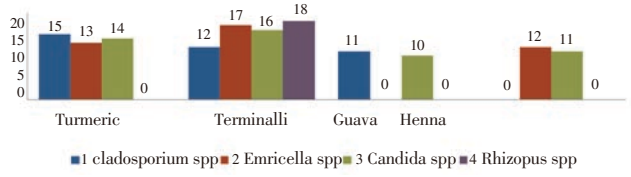


Figure 15: fungi 3

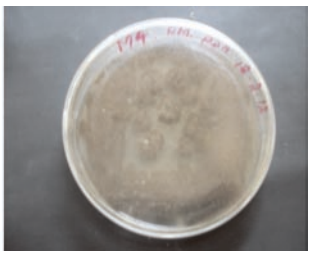


Figure 16: fungi 4

Table 2

UV Spectrophotometer analyses of Natural dyes.

S.No	Natural Dyes	Absorbance (Wave Length 250 nm)
1	*Curcuma longa* (Turmeric)	2.255
2	*Terminalia chebula* (Terminalli)	2.266
3	*Psidium guajava* (Guava)	2.249
4	*Lawsonia inermis* (Henna)	2.251

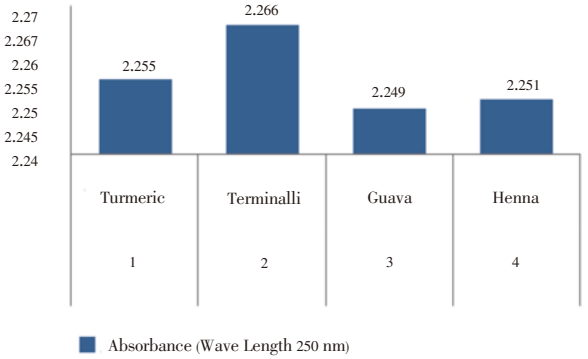
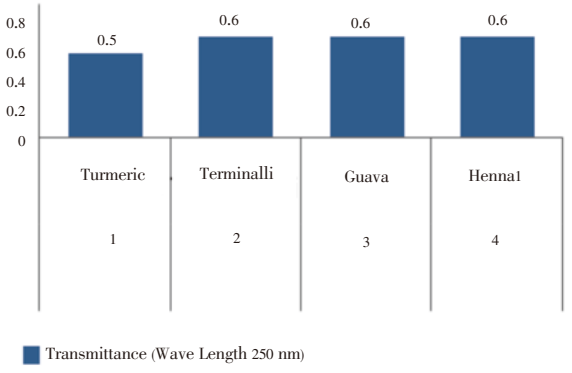


Table: 3

UV Spectrophotometer analyses of Natural dyes.

S.No	Natural Dyes	Transmittance (Wave Length 250nm)
1	*Curcuma longa* (Turmeric)	0.5
2	*Terminalia chebula* (Terminalli)	0.6
3	*Psidium guajava* (Guava)	0.6
4	*Lawsonia inermis* (Henna)	0.6



4. Discussion

In the present study to evaluate anti fungal activity of natural dyes against traditional products affected fungal

pathogens. There has been an increasing consumer demand for natural dye coated traditional products. In India being one of the countries, which possesses the natural wealth in the form of plantation of plenty. This has provided relatives better opportunity for the development of industries in the country. The raw materials for production of natural dyes were plentifully available in India. Natural dyes do not cause any harm to human skin and no hazards are anticipated in their manufacturing, rather some of the dyes act as health care. The chemical reaction is almost absent in the manufacture of natural dyes and no pollution problem. All these dyes are harmonized with nature. These requirements have put pressure on the traditional industry for progressive removal of chemical dyes [22] and adsorption of natural alternatives to obtain goals concerning safe products with long shelf life.

Conflict of interest statement

We declare that we have no conflict of interest.

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