Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Extraction of Pigment from *Rhodotorula Mucilaginosa* and its Application in Textile

Hemashenpagam.N[1*], Karuppusamy.R[2] and Pratheeba .M[3]

[1*]Associate Professor, PG and Research Department of Microbiology
[2,3]II M. Sc Microbiology, Hindusthan college of Arts and Science Coimbatore-641028

ABSTRACT

Yeasts are more convenient than algae or molds for large scale production in fermenters, due to their unicellular nature and high growth rate.The present investigation was undertaken with an aim to identify and characterize pigment from yeast. Yeast were isolated from kitchen waste mixed soil sample collected from Avinashi, Tirupur, Tamil Nadu, India. The potent strain (KAR1*) Rhodotorula mucilaginosa*. The extraction of pigment was done by solvent extraction method using acetone and purified by column chromatography. Mango bark used as a mordant. Antibacterial activity of pigment, mordant and pigment with mordantinhibitory action against *E. coli, Pseudomonas, Staphylococcus aureus, Klebsiella pneumonia, Proteus, Bacillus cereus* were checked. Antifungal activity against fungal pathogens like *Candida albicans* and*Trichoderma*were tested against microbial pigment, mordant and dye + mordant to evaluate its antifungal activity. As a source of natural pigment these pigments were used for textile dyeing.

KEYWORDS: Natural pigment, microbial pigment, yeast, extraction of pigment, antimicrobial activity, Textile application

***Corresponding author:**

Hemashenpagam. N

Associate Professor,

PG and Research Department of Microbiology

Coimbatore-641028

INTRODUCTION

The toxicity problems caused by synthetic dyes to the environment have created amounting interest towards natural pigments. Pigments from microbial sources as natural dyes are potentially good alternative ones to synthetic pigments. There are several organisms which produce many varieties of intracellular and extracellular pigments including melanin with different biological functions. Pigments were primarily used as a colouring agent in various industriesfrom the past decade. Researchers have focused the usage of natural pigments for various industries including pharmaceutical for antitoxic and antioxidant agents[1]

Most of the natural pigments are extracted from plants like annatto, beet root, marigold, grapes, carrot, paprika, etc. and microorganisms like yeast of the genera*Phaffia*, *Cryptococcus* and *Sporobolomyces*, fungi like *Blakesleatrispora,Monascus sp.*,and algae such as *Dunaliella* and *Haematococcus* and bacteria such as *Flavobacterium* and Micrococcus are reported to produce carotenoids [2] Pigmented yeasts like *Rhodotorula* and *Rhodosporidium* produce the major carotenoid pigments viz. carotene, torulene, torularhodin.

Yeasts can synthesize pigment when cultivated in commercial medium, containing various carbon sources, such as glucose, xylose, cellobiose, sucrose, glycerol and sorbitol nevertheless these type of medium represents high costs. Therefore there have been growing interest in the use of natural substrates as carbon sources[3] By-products from industrial processes are pollutants to the environmental and their treatment represents high costs. In recent years raw materials and agro-industrial wastes origin have been proposed as low-cost alternative carbohydrate sources.

The textile industry discharges large proportion of effluent that mainly consists of synthetic dyes. Synthetic dyes been extensively used in the textile industries due to their ease and cost effectiveness, high stability towards light, temperature and technically advanced colours covering the whole colour spectrum. However, these synthetic dyes are often toxic, mutagenic and carcinogenic leading to several human health problems such as skin cancer and allergic reactions [4,5]. Thus, the worldwide demand for the dyes of natural origin is increasing rapidly in the textile industry.

MATERIALS AND METHODS

Sample collection:

Soil samples were collected from different areas of Avinashi,Tirupur, Tamil Nadu, India.Kitchen waste mixed withsoil and fruits and vegetable waste composted soil were used

Isolation of yeast cells:

Isolation of yeast cells was performed by serial dilution and spread Plate technique using Yeast malt agar(YMA) and Potato dextrose agar(PDA). Plates were incubated at 30°c temperatures at 3 to 4 days.

Screening of pigment producing yeasts:

Yeast strains were isolated in this study and screen the efficient isolates. Potential isolates were chosen from primary screening and subjected through staining technique (Lacto phenol cotton blue stain). Morphological characteristics were detected.

Fermentation process of pigment producing yeasts:

The pigmented yeast isolate was incubated into production media YMB(Yeast malt broth) at 30°c for 7 Days on a rotatory shaking incubator at 100 rpm.YMB –Yeast Malt Broth: Dextrose-10g, Peptone-5g, Malt extract-3g, Yeast extract-3g, Distilled water-1000ml.

Extraction of pigment:

The yeast culture was inoculated on fermentation broth and incubated at 25 ºC for 5 days. A known amount (500mg) of freeze-dried red yeast was hydrolyzed with 1 ml of 1N hydrochloric acid in water bath at 70 °C for one and half hour. After removal of excess acid by washing with water, the cells were soaked overnight in acetone: methanol (1:1) solution. The pigment was extracted with acetone until the entire colour was leached out from the cells.Acetone extracts were transferred to light petroleum (20ml) at (40-60 °C) in a separating funnel and washed thrice with distilled water.The pigment was collected and stored at 4°c.

Purification of pigment:

Using a silica gel chromatography column, the extracted solution was separated, and the pigments were eluted using hexane. It consists of glass tube with bottom portion of the column-packed with glass wool/cotton. Above which absorbent is packed. Stationary phase (absorbent) – Silica gel was packed in the column. Sand was loaded in the top of the cotton and then silica gel was then packed the column. The crude extract was loaded at the top of the column and eluted using ethyl acetate as solution system. Fraction was collected at 20 minutes intervals. The fraction is further check qualitative and quantitative analysis.

Mordant preparation:

5gm of Mango bark powder is dissolved in 100ml of distilled water and boiled for 1hr. Then the extract was filtered using Whatmann no. 1 filter paper.

Antimicrobial activity:

Antibacterial activityof the pigment, mordant and pigment + mordant was tested by well diffusion method. Some pathogenic bacterialike (*E. coli*, *Pseudomonas, Staphylococcus aureus, Klebsiella pneumonia, Proteus, Bacillus cereus.)* were used against extracted Pigment, mordent and dye + mordent to evaluate its antibacterial activity. Then the wells were filled with appropriate amount of samples (50 µl) and it was incubated at 37°c for 24 hours and the result was observed by measuring zone of inhibition.

Antifungal activity of the pigment, mordant and pigment + mordant was tested by well diffusion method. Some fungal pathogenic like *Candida albicans* and *Trichoderma* were used against microbial pigment, mordant and dye + mordant to evaluate its antifungal activity. Then the wells were filled with appropriate amount of samples (50 µl) and it was incubated at room temperature for 3 days and the result was observed by measuring zone of inhibition.

Dyeing experiment:

A textile material (cotton) which is commercially available was selected for the experiment. Material was cut into equal size of 5 cm. Pigment in acetone was used as the stock solution. From this stock solution 5 ml solution was applied to the cloth material in a warm surface. The cloth material was allowed to dry at room temperature for about 1 hour. A white cloth material was taken as a control.

Washing performance:

The textile material dyed by pigment was tested for wash performance at room temperature.The textile material was washed with soap solution for 30 minutes at room temperature. Thetextile materials were washed with running tap water and allowdrying. The result was observed physically with other dyed unwashed textile material[6].

Molecular identification of yeast:

Genomic organization of the 18srRNA and ITSI sequencing

RESULT AND DISCUSSION

Sample collection and isolation of Pigmented yeast:

Soil samples were collected from different areas of Avinashi, Tirupur, Tamil Nadu, India. Kitchen waste mixed with soil samples and fruits and vegetable waste composted soil samples were collected and processed. The Samples were processed in YMA, PDA and SDA media's. Six different yeast strains were identified. The potent strain was found to be pigmented producing yeast(***Rhodotorulamucilaginosa***). (Shows figure 1).

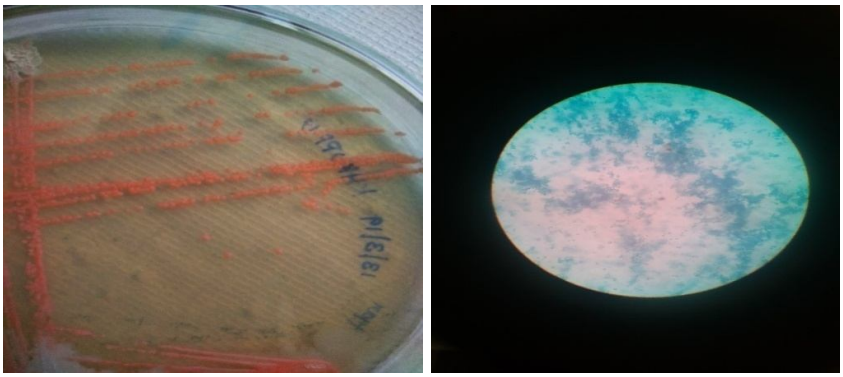

Figure 1 ***Rhodotorulamucilaginosa*****inplate YMA and LCB Staining**

Fermentation Process for Pigment Production

The isolated pigmented yeast was incubated in production media consists of Yeast Extract -3g, Malt Extract -3g, Dextrose -10g, Peptone -5 g,Distilled Water -1000ml. (pH 6) at 30°c for 4 to 8 Days on a rotatory shaking incubator at 100 rpm for fermentation process.

Extraction of pigment:

The yeast culture was inoculated on fermentation broth and incubated at 8 to 10 days. A known amount (500mg) of freeze-dried red yeast was hydrolyzed with 1 ml of 1N hydrochloric acid in water bath at 70 ℃ for one and half hour was shown in figure 2.After removal of excess acid by washing with water, the cells were soaked overnight in acetone: methanol (1:1) solution.The pigment was extracted with acetone until the entire colour was leached out from the cells was shown in the figure 3Acetone extracts were transferred to light petroleum (20ml) at (40-60 °C) in a separating funnel and washed thrice with distilled water.The pigment was collected and stored at 4°c. (Figure 4 shows the extracted pigment).

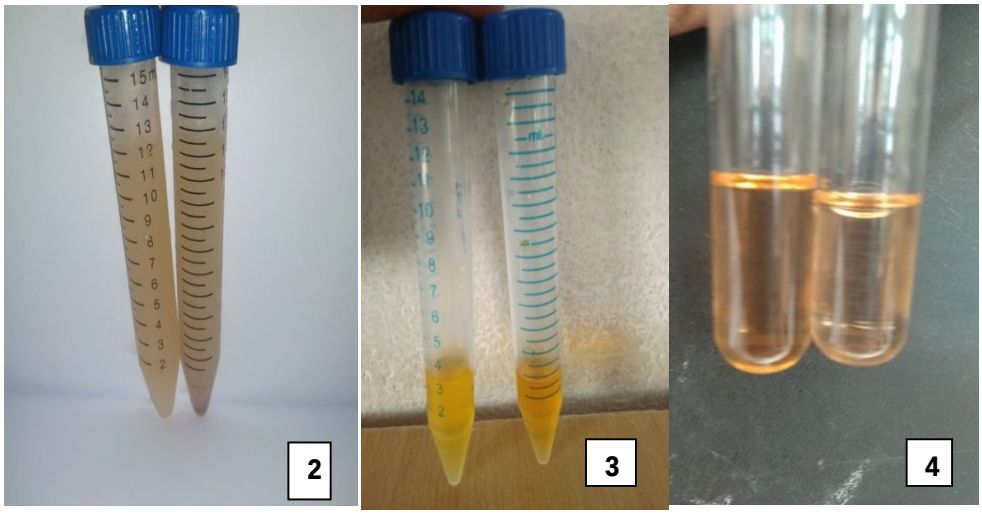

Figure 2, 3and 4 Pigment extraction process

Purification of Pigment

Using a silica gel chromatography column, the extracted solution was separated, and the pigments were eluted using hexane.It consists of glass tube with bottom portion of the column-packed with glass wool/cotton. Above which absorbent is packed.Stationary phase (absorbent) – Silica gel was packed in the column. Sand was loaded in the top of the cotton and then silica gel was then packed the column was shown in the figure 5.4.The crude extract was loaded at the top of the column and eluted using ethyl acetate as solution system. (Figure5 shows the purification dye).Fraction was collected at 20 minutes intervals were shown in figure 5The fraction is further check qualitative and quantitative analysis.

Figure 5Column chromatography

Mordant Preparation

5gm of Mango bark powder is dissolved in 100ml of distilled water and boiled for 1hr. Then the extract was filtered using Whatman no. 1 filter paper.

Antimicrobial Activity

Antibacterial activityof the pigment, mordant and pigment + mordant was tested by well diffusion method. Some pathogenic like (*E. coli, Pseudomonas, Staphylococcus aureus, Klebsiella pneumoniae, Proteus, Bacillus cereus.)* were used against extracted Pigment, mordent and dye + mordent to evaluate its antibacterial activity. Then the wells were filled with appropriate number of samples (50 µl) and it was incubated at 37°c for 24 hours and the result was observed by measuring zone of inhibition in Figure 6

Klebsiella pneumonia is found to be more resistant for microbial pigment than compare to *E. coli, Pseudomonas, Staphylococcus aureus, Proteus* and *Bacillus cereus.E.coli* is found to be more resistant for mordant than compare to *Pseudomonas, Staphylococcus aureus, Klebsiella pneumonia, Proteus, Bacillus cereus* in Figure 6..

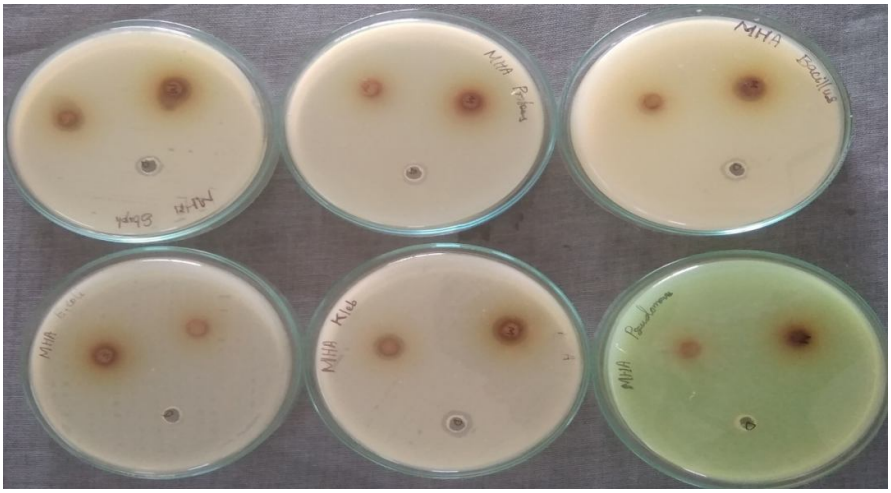

Figure 6 Antibacterial activity of Dye, mordant and Dye+mordant

Table 1 Antimicrobial activity of pigment extract with and without mordant

ORGANISMS	PIGMENT(DYE)	MORDANT	DYE+MORDANT
S. aureus	**8mm**	**12mm**	**13mm**
Klebsiella pneumonia	**10mm**	**13mm**	**11mm**
Proteus sp.,	**6mm**	**12mm**	**8mm**
E.coli	**8mm**	**25mm**	**18mm**
Bacillus sp.,	**8mm**	**11mm**	**11mm**
Pseudomonas sp,.	**4mm**	**11mm**	**9mm**

Antifungal Activity

Antifungal activity of the pigment, mordant and pigment + mordant was tested by well diffusion method. Some fungal pathogenic like *Candida albicans and Trichoderma*were used against microbial pigment, mordant and dye + mordant to evaluate its antifungal activity. Then the wells were filled with appropriate amount of samples (50 µl) and it was incubated at room temperature for 3 days and the result was observed by measuring zone of inhibition. (TheAntifungal activity of dye**,** Mordant, Dye + Mordant was shown in the figure7).

Candida albicans is found to be more sensitive for microbial pigment, microbial pigment + mordant and mordant than compare to *Trichoderma*.(figure shows 7).

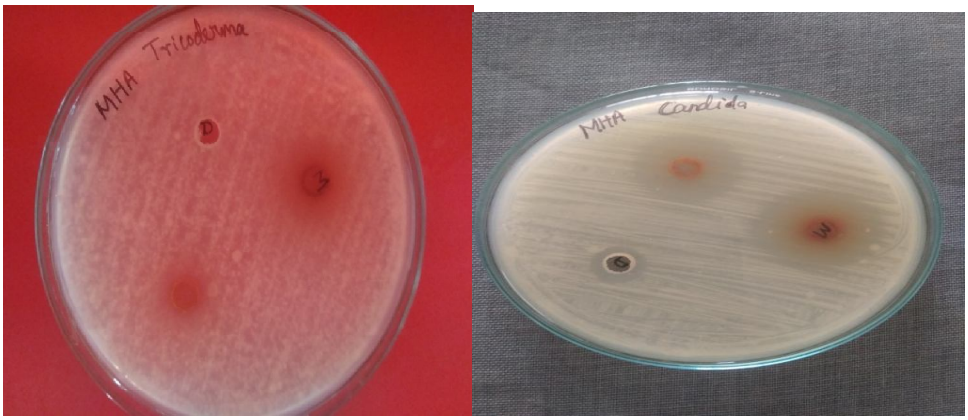

Figure 7 Antifungal Activity of Dye, Mordant and Dye+Mordant

Table 2:Antifungal activity in dye with and without mordant

ORGANISMS	PIGMENT(DYE)	MORDANT	DYE+MORDANT
Candida	12mm	25mm	20mm
Trichoderma	0mm	17mm	11mm

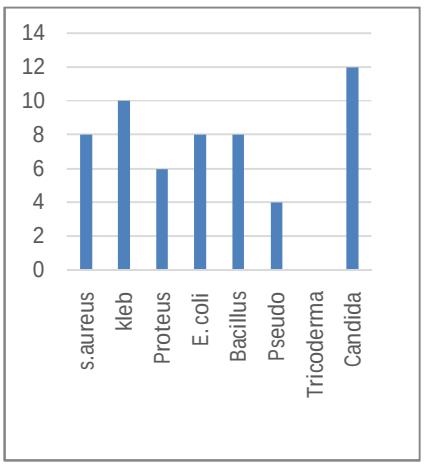

Figure 8 Zone of inhibition in microbial Pigment

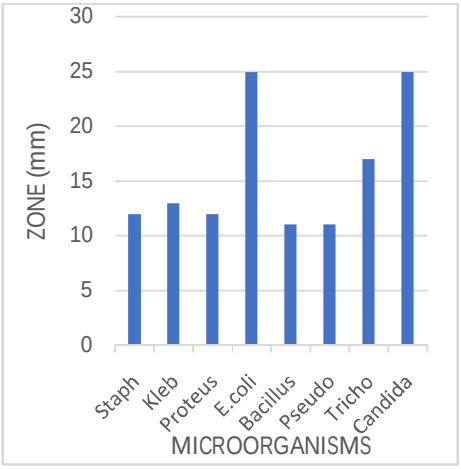

Figure 9 Zone of inhibition in Mordant

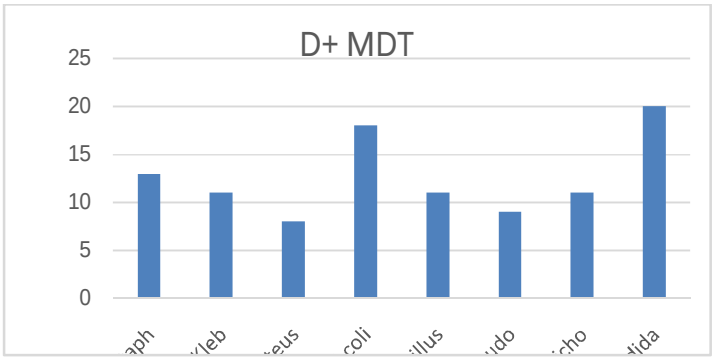

Figure 10 Zone of inhibition of dye and mordant

Figure 8,9,10Antimicrobial activity of Microbial dye, Mordant and Microbial dye+ Mordant against test organisms

Dyeing Experiment

A textile material (cotton) which is commercially available was selected for the experiment. Material was cut into equal size of 5 cm. Pigment in acetone was used as the stock solution. From this stock solution 5 ml solution was applied to the cloth material in a warm surface. The cloth material was allowed to dry at room temperature for about 1 hour. A white cloth material was taken as a control. Figure11 shows Pigmented cloth materials before washing.

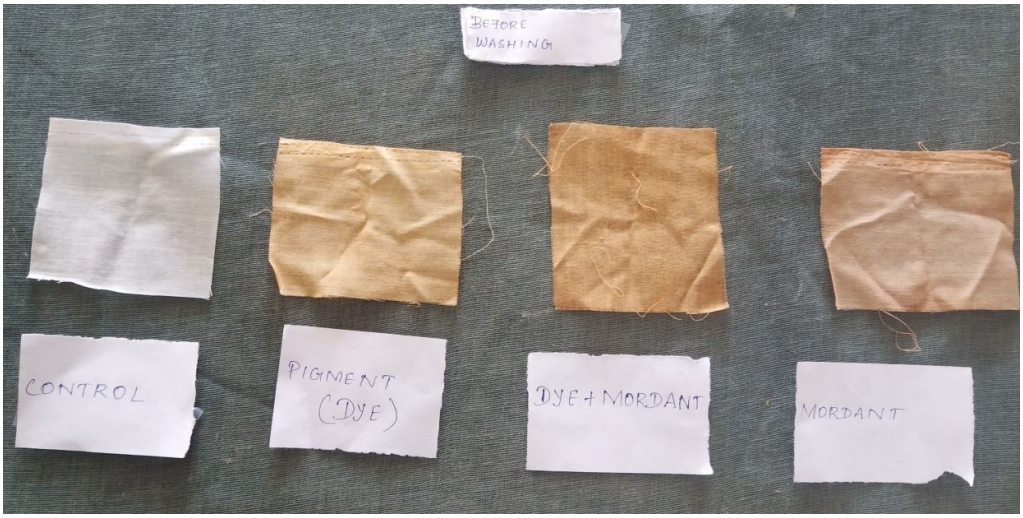

Figure11 Pigmented cloth material before washing

Washing performance:

The textile material dyed by pigment was tested for wash performance at room temperature.The textile material was washed with soap solution for 30 minutes at room temperature. Thetextile material was washed with running tap water and allow to dry. The result was observed physically with other dyed unwashed textile material.

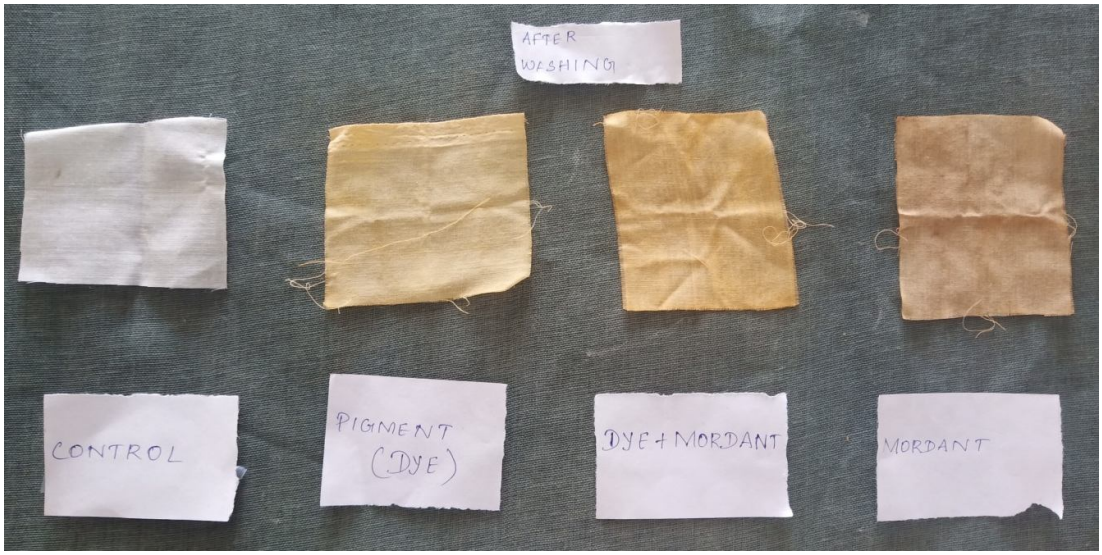

Figure 12 Pigmented cloth material after washing

Dye + Mordant was found to effective then mordant and dye alone. It retain the orange colour after washing with soap solution. Then (Dye) orange colour decolorized to sandal colour was found to be retentive for the textile materials shown in figure 12.

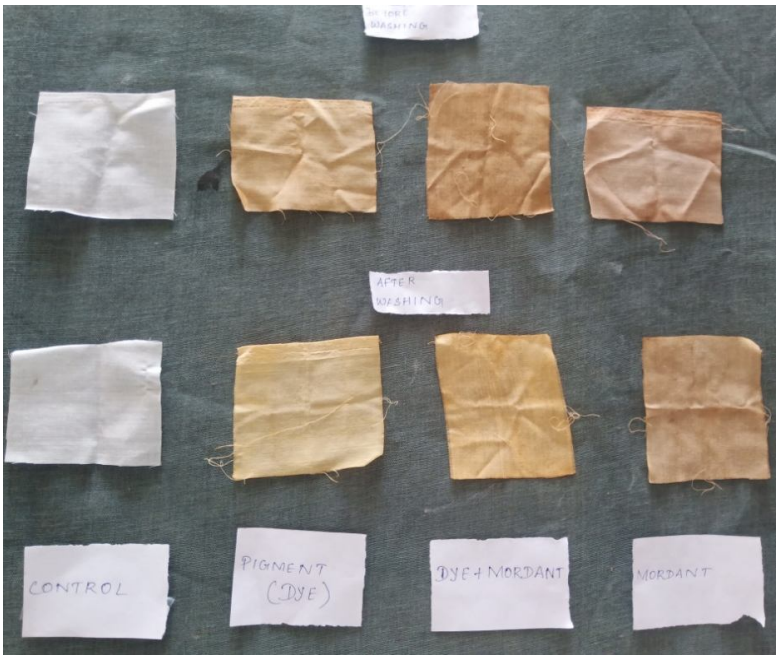

Figure 13 Comparison (Before Washing and After Washing) Of Microbial Dye In Cloth And After Washing

The textile material was to check the dyeing property of the extracted pigment. The textile material after dyeing was subjected to three consecutive normal water wash treatments and detergent water wash treatment. Then orange colour decolorized to sandal colour was found to be retentive for the textile materials. (Figure 13). In textile industries, these pigments extracted from biological

source can be used as an alternative to the synthetic colorants and also which are safe and cost effective[7,8].

Molecular Identification of Yeast

Genomic organization of the 18srRNA and ITSI sequencing were performed. DNA is isolated and further subjected to PCR Amplified. Then read the primers and then submitted in BLAST.The organism is confirmed by above using sequencing method as *Rhodotorulamuciaginosa*[9](Figure 14)

Figure 14 Molecular Identification of yeast

BLASTN 2.6.0+

Database: Nucleotide collection (nt)

43,634,103 sequences; 150,987,822,336 total letters

Query= 190327-R04_I05_KAR_1_ITS1 1 1200

Length=1200

Sequences producing significant alignments:	Score (Bits)	E Value
KC113304.1Rhodotorulamucilaginosa isolate PKU Y1 18S ribosomal...	1044	0.0
LT548978.1Rhodotorulamucilaginosa genomic DNA sequence contain...	1042	0.0
LT548977.1Rhodotorulamucilaginosa genomic DNA sequence contain...	1042	0.0
KF726105.1Rhodotorulamucilaginosa strain R-4685 18S ribosomal ...	1042	0.0
KC113312.1 Fungal sp. PKU Y9 18S ribosomal RNA gene, internal tr...	1042	0.0
KR912272.1Rhodotorulamucilaginosa strain YY39 internal transcr...	1040	0.0
KT876700.1Rhodotorulamucilaginosa isolate A5-7 internal transc...	1040	0.0
KC113310.1Rhodotorulamucilaginosa isolate PKU Y7 18S ribosomal...	1040	0.0
LT548979.1Rhodotorulamucilaginosa genomic DNA sequence contain...	1038	0.0
LC229714.1 Rhodotorula sp. EY12114 genes for ITS1, 5.8S rRNA, IT...	1038	0.0

KY104794.1Rhodotorulamucilaginosa culture-collection CBS:10946... 1038 0.0

KT876501.1Rhodotorulamucilaginosa isolate X5-3 internal transc... 1038 0.0

KF411538.1Rhodotorulamucilaginosa strain DY115-21-1-Y46 intern... 1038 0.0

KF411535.1Rhodotorulamucilaginosa strain DY115-21-1-Y38 intern... 1038 0.0

DQ186608.1 Rhodotorula sp. Y11 18S ribosomal RNA gene, partial s... 1038 0.0

LC277143.1Rhodotorulamucilaginosa genes for SSU rRNA, ITS1, 5.... 1037 0.0

CONCLUSION

In the near future, the product with natural colors may have an increased demand, not only for the safety of health and environment but also for their beauty and novelty. Increased awareness for eco-friendly products in the developed countries has opened up a new channel for the export of hand printed fabrics printed with natural dyes. Natural colors should not be taken as a threat to synthetic colors. It may take decades to manufacture natural colors in a ready to use form if all it is possible. A very long and consistent effort is required, since we have just begun our search for natural color source. It is estimated that worldwide up to 70% of all plants have not been investigated fully and that only 0.5% has been exhaustively studied.

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