ANTIMICROBIAL TEXTILE DYEING BY APPLYING NATURAL COLORANTS OF BROWN SEAWEED (*CYSTOSEIRA INDICA*)

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ABSTRACT

The current investigation was carried out to develop a sustainable dyeing method by utilization of a marine brown seaweed Cystoseira indica as natural dye. Moreover, the dyeing process was optimized by varying fixing agent percentage and dyeing time. The influence of different variables on color uptake (K/S), fastness rating and color measurement were examined. The bioactive efficiency of seaweed extract and dyed fabric were also investigated against human pathogens such as Escherichia coli, Staphylococcus aureus and Candida albicans and were compared with commercial antibacterial and antifungal drugs. It was found at the optimized dyeing condition has 1 percent formic acid as fixing agent with 120 min dyeing period. At this condition, the dye molecule shows 70 percent exhaustion and 59 percent fixation along with acceptable fastness and antimicrobial activities Furthermore, the dyed fabric shows effective inhibition against all tested organisms. Fabric samples showed greater antimicrobial efficiency for Candida albicans and poorer than for Escherichia coli.

Keywords: Brown seaweed, fixing agent, optimization of dyeing process, antimicrobial activity, fastness properties.

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INTRODUCTION

Synthetic dyes are known for their wide range of producing bright shade, low price and considerably improved fastness properties (Mirjalili *et al.,* 2011). Despite having these socio-economics benefits, these dyes release toxic chemicals which effects the worker, consumers and environment (Khandare and Govindwar, 2015; Venil *et al.,* 2017). The substitute of these carcinogenic chemicals the use of natural pigment from seaweed as it is safer and has less toxic to environment (Adeel *et al.,* 2018). The lack of information about dye yielding plants and their accessibility might be a problem for these colorants. Only few species have been identified for the utilization of the plants for colorant extraction (Ajmal *et al.,* 2014; Khan *et al.,* 2014; Bhuiyan *et al.,* 2018). Besides the overutilization of these colorant threatens the survival of those species and hence the eco-balance disturb. To prevent the specie loss and ecosystem balance, seaweeds or marine algae used as source of natural colorant extraction (Khan *et al.,* 2014; Doty *et al.,* 2016). Because of the fast growing rate, the utilization of seaweed colorant does not disturb the eco-balance and also the environment.

Seaweed or Algae are the extensively available photosynthetic aquatic organisms with variety of different coloring pigments. These abundantly available seaweed floras may be a good source for harnessing natural colorants and has no detrimental effect on the worker, consumer and environment. (Kadir *et al.* 2014). Fabric dyeing with seaweed colorant gaining popularity due to their bioactive properties like antimicrobial, antioxidant properties and also it does not cause irritation on skin, biodegradable and biocompatible (Wang *et al.,* 2005). Natural colorant has been extracted from green algae and applied on wool fabric (El-Khatib *et al.,* 2016). Utilization of seaweeds in textile dyeing process has also been reported (Janarthanan and Senthil, 2017). Similarly, Sustainable fabric dyeing has been reported as a potential benefit of brown seaweed, *Stoechospermum marginatum* (Rani *et al.*, 2020).

The brown seaweed *Cystoseira indica* is a member of class Phaeophyceae. Distributed around the Atlantic-Mediterranean coasts, and comprises of about 40 species (García-Fernández and Bárbara, 2016). *Cystoseira* comprises of a wide variety of secondary metabolites like lipids, terpenoids, phenolic compounds, carbohydrates, phlorotannins, steroids, pigments and vitamins. (Carolina *et al.,* 2017). Extracts of different species shows the bioactive properties like antimyobacterial, antiprotozoal (Spavieri *et al.,* 2017), antiviral (Pujol *et al.,* 2012) antifungal (Calvo, Cabafies and Abarca, 1986), cytotoxic (Spavieri *et al.,* 2017), antioxidant (Vizetto-Duarte, *et al.,* 2016) and antitumor activities (Vizetto-Duarte, *et al.,* 2016).

In this study, a novel approach to extend the knowledge of sustainable fabric dyeing with brown seaweed colorants has been evaluated. Optimization of colorants was performed in alkaline medium to yield highest extract by weight. To enhance the fixation efficacy tannic acid was used as bio-mordanting agent. Furthermore, dyeing process was optimized by varying dyeing time and fixing agent percentage. Dyed cotton fabric were also assessed for color coordinates in term of lightness (L*), redness-yellowness (a*), blueness-greenness (b*), Chroma (C*), h° (hue angle), color strength (K/S) and color fastness properties in term of wash, light and crocking. Moreover, antimicrobial assessment of the dye extract and dyed fabric were evaluated towards human pathogens *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*

MATERIAL AND METHODS

Materials

The scoured and bleached S/J 100% cotton fabrics were provided from Masood Textile Mills, Faisalabad for dyeing and mordanting process. Sodium hydroxide (99%) from Sigma-Aldrich used for extraction of natural colorants, Tannic acid from E. Merck Darmstadt were used as bio-mordanting agent, sodium chloride (99.95), sodium carbonate (99%) form Riedel-de Haën AG and formic acid (89.5-91%) from BDH laboratory were used as exhausting agent, Nutrient agar and potato dextrose agar from Merck KGaA were used for the growth of micro-organisms, antimicrobial drugs such as ampicillin form GlaxoSmith Kline and fluconazole from Bryon were considered as standard and laboratory grade distilled water were used for stock solutions preparation.

Seaweed Collection and Processing

The samples of brown seaweed *Cystoseira indica* were collected from the Buleji coastal area of Karachi, Pakistan during a period of January to March, 2018. The collected specimen were washed with tap water to remove extraneous foreign particles and epiphytes attached on them. Finally, rinse the collected sample with distilled water and shade dried at room temperature. The dried specimens were grind to powder form and used for further experimental work. The entire thalli of samples were identified in the Department of Botany, University of Karachi and voucher specimen has been deposited in the Herbarium Department of Dr. Muhammad Afzal Hussain Qadri, Biological Research Centre. The experiment was carried out at Leather Research Centre – Pakistan Council of Scientific and Industrial Research, Karachi. Fig. 1 shows growing form of *Cystoseira indica.*

<**Fig. 1.** Growing form of *Cystoseira indica*.>

Harnessing Natural Colorants form *C. indica*

Harnessing of colorants was performed in Memmert 854 Schwabach water bath. The extraction was carried out in alkaline medium using 5g/L sodium hydroxide at material to liquor ratio of 1:20. The processing temperature was set at 80°C for three hour. After completing three hour extraction time, cool and filtrate the extract using a piece of cotton. The filtrate extract was set for drying at water bath until a gummy extract was obtained, then utilized for utilized for further dyeing application.

Dyeing and Mordanting Process

Dyeing and mordanting were carried out in Memmert 854 Schwabach water bath, keeping the material to liquor ratio of 1:20 at 80 °C. The 3g piece of S/J 100% cotton was soaked in clean water for 30 min prior to dyeing and mordanting to remove the dirt and swelling of fabric. For mordanting, 12 % on the weight of fabric (o.w.f.) tannic acid were dissolved in pre-heated distilled water. Sodium chloride (12% o.w.f.) and sodium carbonate (6% o.w.f.) solution were prepared at room temperature, added to tannic acid solution as exhausting agent. Complete the mordanting cycle for 60 min with occasional stirring. After completion of mordanting process the bath dropped, squeezed the fabric than air dried at room temperature. In order to remove the superfluous molecule, rinse the fabric with cold water prior to dyeing.

Prepare a dye bath having material to liquor ratio of 1:20. Dyeing processes were optimized by varying fixing agent percentage form 1, 2, 3 and 4 and dyeing time were changed to 60, 90, 120 and 150 min. Add amount of fixing agent and allowed the mordanted fabric to sit in the dye bath for completing respective dyeing time at 80°C. Upon completion of the dyeing process, the fabric were squeezed and air dried at room temperature for 10 min. Finally, rinse the dyed fabric with cold water and air dried. Fig. 2 illustrate the shades developed by varying fixing agent percentage with 120 min dyeing cycle.

< **Fig. 2.** Shade developed by varying fixing agent percentage and dyed under optimized dyeing time (120 min).>

Dye Exhaustion and Fixation Percentage

The dye exhaustion percentage (E %) is defined as percentage of dye molecules that has moved from dye bath to the fabric during dyeing process. Dye exhaustion percentage was recorded by Beckman Coulter DU-730 Life Science UV/Vis Spectrophotometer at maximum wavelength ($\lambda_{max.}$). The dye absorbance were recorded before and after dyeing process and calculated with the help of following formula (Vankar *et al.,* 2011),

$$\textbf{Dye Exhaustion}\ \% = \frac{\mathbf{A2 - A1}}{\mathbf{A2}} \times \mathbf{100}$$

Where,

A2 and A1 are the absorbance values of dyes offered and spent liquor, respectively.

The percentage of exhausted dye chemically bind with the surface of fabric is called as dye fixation percentage (F %). It is evaluated by measuring color strength values (K/S) of dyed samples before and after hot washing. Dye Fixation percentage were calculated by the following empirical formula (Burkinshaw and Katsarelias, 1995),

$$\textbf{Dye Fixation}\ \% = \frac{(\mathbf{K/S})\mathbf{2}}{(\mathbf{K/S})\mathbf{1}} \times \mathbf{E}\ \%$$

Where,

(K/S)1 and (K/S)2 are the color uptake before and after hot washing, respectively.

Color Co-ordinates and Fastness Property of Dyed Fabric

Color strength (K/S) and the CIE Lab co-ordinates (L*, C*, a*, b* and h°) of the dyed, mordanted and pre-mordanted samples were tested by Data Color SF-650 spectrophotometer having software version 2.0.4 and calibrated on setting SCI UV 400.

Fastness studies like wash, light and crock fastness of the dyed samples were also tested. Wash fastness were evaluated through AATCC Test Method 61-2 on ATLAS Laundry-o-meter at 49 degrees for 30 min and 40 RPM. Light fastness were determine according to AATCC 16 E-2014 Test Method on SUNTEST+, SDL-ATLAS brand, Black penal temperature 63 + 1 °C, Chamber air 43 + 2 °C and Humidity 30 + 5%. Crock fastness were measured through AATCC Test Method 8 on ATLAS Crock meter CM-5. The change in color was assigned using the AATCC Chromatic Transference Scale. Gray scale rating of 1 (poor) – 5 (excellent) was assigned to each treated fabric sample.

Antimicrobial Assessment by Disc Diffusion Method

For assessment of antibacterial activity test organisms *Staphylococcus aureus* and *Escherichia coli* were used. Nutrient agar medium were prepared and autoclaved at 121°C for 15 min. Sterilized petri dish were prepared with equal thickness of nutrient agar. *Candida albicans* were used as test organisms for antifungal activity. Potato dextrose agar used as medium for antifungal assessment. Susceptibility of test organisms against natural dyes was investigated using disc diffusion method (NCCLS, 1997). The surface of petri dish was inoculated with desired test organisms. 12 mm whatman-3 filter paper disc were sterilized and impregnated with 1, 2 and 3 g dye extract solution for 30 min and dry. Place the dye extract treated disc on the top of seeded medium than incubate plates for 18-24 h for antibacterial activity and 72 h of antifungal activity (Gao and Cranston, 2008).

In the second set of experiment, qualitative antimicrobial assessment of dyed fabrics were tested by disc diffusion method (AATCC Test Method 147-2001). Approximately 3 by 3 cm piece of dyed fabric having 1, 2, 3 and 4 percent formic acid as fixing agent were placed on the top of seeded medium. Standard antimicrobial drug ampicillin was used as positive (+ve) control for bacteria and fluconazole for fungi while un-dyed fabric were considered as negative (−ve) control. Incubate the plates for respective time, a clear zone of interrupted growth indicated the effectiveness of the dye extract. The average width of the zone of inhibition was calculated by the following formula (Murray *et al.,* 2009),

$$\textbf{Zone of inhibition (mm)} = \frac{\mathbf{T - I}}{\mathbf{2}}$$

Where,

T is the width of clear zone along with the specimen and I is the width of test specimen.

Statistical Analysis

The mean and standard error of mean values of the triplicates for each treatment were calculated. SPSS (Statistical package for social sciences) version 20 was used to examine the sample statistics. The parametric one-way analysis of variance (ANOVA) was used to evaluated the effect of antimicrobial agent of dye molecules on the growth of micro-organisms. ANOVA - Duncan’s multiple range test was used to differenciate the difference among the treatments at P <0.01.

RESULTS AND DISCUSSION

Effect of Fixing Agent Percentage on Dye Exhaustion and Fixation Percentage

Fig. 3 shows that formic acid percentage has considerable influence on the dye exhaustion and fixation percentage property of dyed fabric. The dye exhaustion and fixation percentage of the pre-mordanted sample were significantly higher than the dyed fabric. It was clear that the pre-treatment with tannic acid were improved the color characteristic and fixation property of the pre-mordanted fabric by forming the covalent bond between the fiber and dye molecules than only dyed with *C. indica* extract (Agarwal and Patel, 2002). The maximum dye exhaustion and fixation percentage were observed in 1 % formic acid exhausted sample followed by 2 % and 3 % that of 4 %. The pre-mordanted samples having 1 % formic acid as fixing agent changes the pH of dye bath to alkaline that was 9 while further increase in exhausting agent results in shifting of dye bath pH from alkaline to acidic (pH = 5) which decline the fixation efficacy of the colorants. Moreover, 4 percent fixing agent gives over exhaustion which results in the aggregation of colorant on the surface of fabric and results in poor fixation (Adeel *et al.,* 2018).

<**Fig. 3.** Dye exhaustion and fixation percentage of dyed fabric by varying fixing agent percent with 120 min dyeing time.>

Effect of Color Strength (K/S) on Dyeing Time

The color strength (K/S) values of the pre-mordanted fabric higher than the dyed fabric. The mordant treatment improved the interactive forces between the dye and pre-modanted fabric. Therefore, the color strength has direct relation with mordant (Khan *et al.,* 2011; Prusty *et al.,* 2010; Yusuf *et al.,* 2012). Fig. 4 illustrate the influence of color uptake on dyeing time. The color uptake of the dyed fabric was increases as the dyeing time were increase to 60, 90 and 120 min. The maximum absorbance was obtained at 120 min, then a plateau attained after 120 up to 150 min. The dye molecules reached their saturation level at 120 min, a further increase will result in shifting of dye molecules from fabric to dye bath (Kamel *et al.,* 2007). Thus, the optimized dyeing time have been fixed 120 minutes.

<**Fig. 4.** Effect of color strength (K/S) on dyeing time.>

Color Measurements

Bio-mordanting with tannic acid significantly enhance the dye ability and durability of textile substrate by forming complex with fabric and dye molecule. The results of colorimetric properties of shades obtained are represented in table-1 in terms of CIE Lab color co-ordinates lightness (L*), chroma (C*), redness-yellowness (a*), blueness-greenness (b*), hue angle (h°) and color uptake (K/S). Mordanting has little effect on the colorimetric properties in comparison to un-mordanted fabric. Furthermore, fixing agent and dyeing time has slight effect on the color coordinates. From the table-1, it can be shown that the darkest shades were perceived with 1% fixing agent while further increase results in lighting of shade. L* values of pre-mordanted fabric gradually decrease results in deepening of shade as compared to only dyed and mordanted fabric. a*-b* values of all dyed samples were positive which reveals that shade was on reddish-yellow axis and there is slight effect on these values by varying dyeing time (Fig. 5). Chroma (C*) was the saturation of coloring compounds and its values ranges from 18.34-33.14. H° of dyed, mordanted and pre-mordanted fabric varies form 81-87° which indicates that samples were on red-yellow axis.

<**Fig. 5.** a*-b* plot of pre-mordanted fabric using 1 percent fixing agent and varying dyeing time form 30, 90, 120 and 150 min.>

<**Table-1.** Colorimetric properties of dyed, mordanted and pre-mordanted (Pre-M.) samples.>

Fastness property

The color fastness of sample dyed with brown seaweed extract *C. indica* are given in table-2. Fastness property of treated fabric always depend on the different factors, like the mode of dyeing as well as the nature of colorant, mordant and fabric used (Adeel, Zuber and Rehman, 2018). The wash fastness property of all dyed sample varies from good to excellent and no staining on adjacent multifibre strip were observed. In all cases, dry crock fastness of all dyed fabrics were found excellent except the mordanted sample which has good dry crocking rating. The wet crocking rating of dyed and pre-mordanted samples were found good to very good except mordanted and dyed sample have 3 and 4 grading. The results of fastness property to light varies from moderate to good that is graded from 3-4. The chromophores of the natural dyes are sensitive to photochemical degradation which shows poor to moderate light fastness rating (Zarkogianni, 2011). This property could be improve by further mordanting treatments hence taken for further research.

<**Table-2.** Color fastness property of dyed fabric.>

Preliminary Antimicrobial Screening

Susceptibility of the test organisms viz., *C. albicans, E. coli* and *S. aureus* to natural extract of *C. indica* was investigated by disc diffusion assay technique. The antimicrobial screening results were express as clear zone of inhibition as depicted in fig. 6. The dye extract has shown the effective inhibition as the amount of the dye increases form 1, 2 and 3 g. The compound shows active inhibition in *C. albicans* followed by *S. aureus* than *E. coli*. The control shows no zone of inhibition. Distilled water was used as solvent in our study which shows no inhibition against all test organisms. Therefore, it is effectively concluded that the whole activity is due to the dye molecules.

<**Fig. 6.** Preliminary assessment of seaweed extract by disc diffusion method.>

Antimicrobial Assessment of *C. indica* Dyed Cotton Fabric

The extract of seaweed showed good bioactivity against all tested organisms, it is worthwhile to study the effect of seaweed colorants after application on cotton fabric. The results of untreated (-ve control), dyed, tannic acid mordanted, pre-mordanted fabric having different percentage of fixing agent and commercial antimicrobial drug treated fabric (+ve control) are summarized in table-3. From the given results it is clear that the dyed fabric with *C. indica* extract without mordant treatment exhibited higher activity than mordanted sample while pre-treatment observed significant improvement in antimicrobial assessment. The samples dyed and pre-mordanted with *C. indica* extract were observed significant inhibitory effect which is comparatively similar to standard antimicrobial drugs (+ve control). The zones of inhibitions were increases as the fixing agent percentage were increases from 1-4 percent. The maximum zone of inhibition were observed in 4 percent fixing agent and has better antimicrobial activity against *C. albicans, E. coli* than *S. aureus* (Fig. 7).

The undyed fabric (–ve control) shows no zone of inhibition against all test organisms. The zones of inhibition of all treated samples are represented in table in the form of mean ± standard error of means. There is significant difference among means and are statistically approved by ANOVA-Duncan's multiple range test.

<**Table-3.** Qualitative antimicrobial assessment of dyed fabric.>

<**Fig. 7.** Qualitative antimicrobial assessment of pre-mordanted fabric having 4 % fixing agent against *E. coli* (Panel-A) and *C. albicans* (Panel-B).>

CONCLUSION

The feasibility of brown seaweed *C. indica* extract as a natural dye for dyeing cotton fabric via color measurements and color fastness testing was examined in this study. The optimization of dyeing process by varying formic acid percentage and dyeing time were also studied in the course of investigation. Furthermore, the dye exhaustion, fixation percentage, color strength and antimicrobial assessment of dye and dyed fabric were also investigated. The experimental results shows that by varying fixing agent percentage from 1-4 percent the development of the shade or color strength decreases with increasing fixing agent. The dye molecule exhausted better at alkaline pH than the acidic. Hence, the optimized dyeing condition is

1 percent formic acid as fixing agent with 120 min dyeing cycle. At this optimized condition, the dyed fabric yielded higher exhaustion and fixation percentage along with acceptable fastness and antimicrobial activity. Therefore, it is concluded that *C. indica* extract can be effectively use as natural dye along with novel antimicrobial properties.

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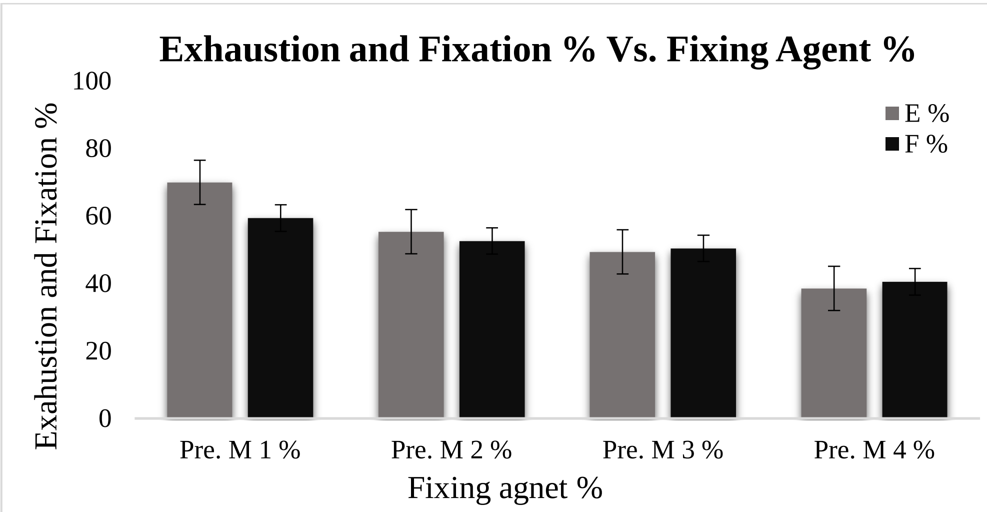
Fig. 1. Growing form of *Cystoseira indica*.

Fig. 2. Shade developed by varying fixing agent percentage and dyed under optimized dyeing time (120 min).



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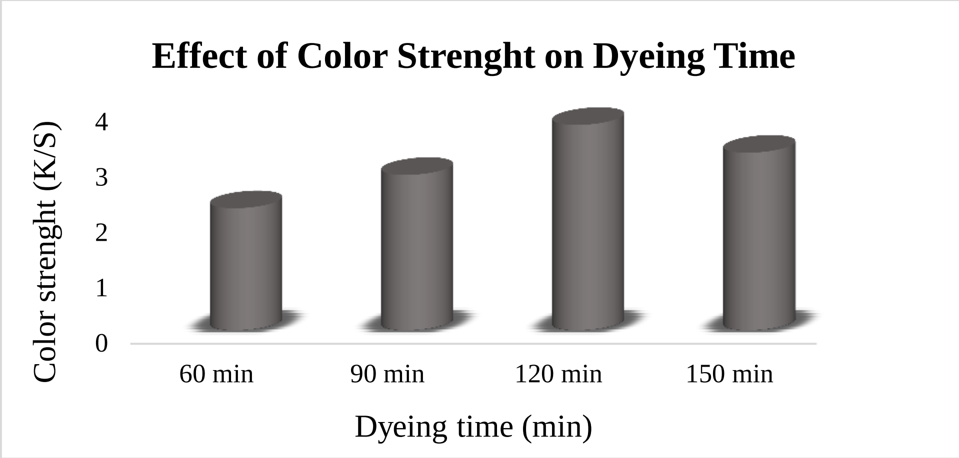

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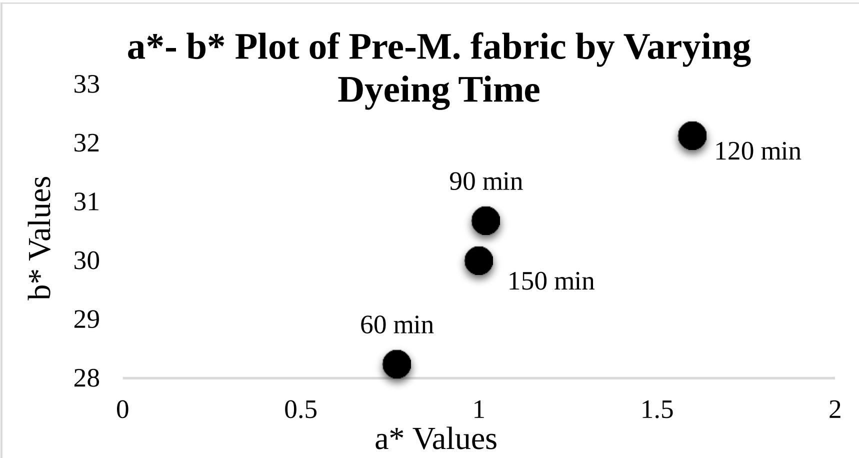

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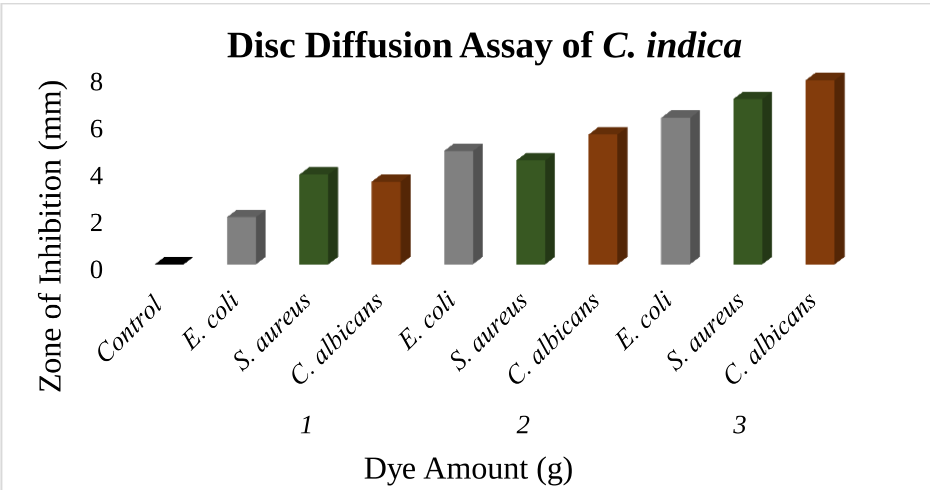

Fig. 6. Preliminary assessment of seaweed extract by disc diffusion method.

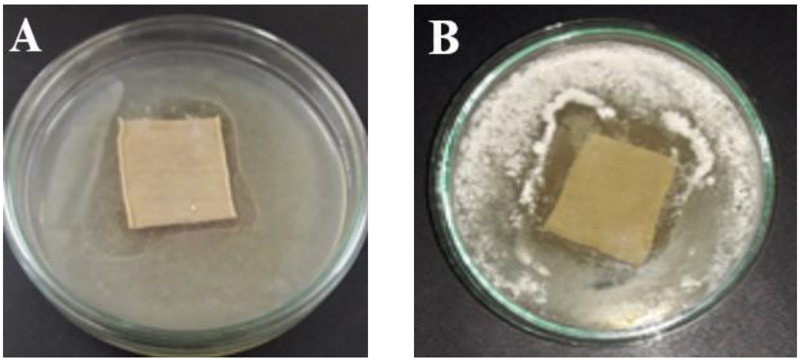

Fig. 7. Qualitative antimicrobial assessment of pre-mordanted fabric having 4 percent fixing agent against *E. coli* (Panel-A) and *C. albicans* (Panel-B).

Table-1. Colorimetric properties of dyed, mordanted and pre-mordanted (Pre-M.) samples.

Samples	L*	a*	b*	C*	h°	K/S	E %	F %
Dyed	84.24	2.62	18.34	18.52	81.86	1.5	21.98	10.45
Mordanted	76.66	2.43	28.94	29.04	85.20	7.9	00	00
Pre. M. 1 %	72.32	2.87	29.44	29.57	84.43	3.7	69.94	59.34
Pre. M. 2 %	75.25	1.57	30.78	30.82	87.08	3.4	55.26	52.55
Pre. M. 3 %	77.45	1.60	33.14	33.17	87.23	3.0	49.35	50.34
Pre. M. 4 %	78.91	1.56	32.67	32.70	87.26	2.6	38.48	40.45

Table-2. Color fastness property of dyed fabric.

Samples	Acetate	Cotton	Naylon	Poly-Ester	Acrylic	Wool	Dry	Wet	Light Fastness
	Before Curing - 2A Wash Fastness – Staining						Crocking		
Dyed	4-5	4-5	4-5	4-5	4-5	4-5	5.0	4.0	3-4
Mordanted	4-5	4-5	4-5	4-5	4-5	4-5	4.0	3.0	3.0
Pre. M 1 %	4-5	4-5	4-5	4-5	4-5	4-5	5.0	4-5	4.0
Pre. M 2 %	4-5	4-5	4-5	4-5	4-5	4-5	5.0	4-5	4.0
Pre. M 3 %	4-5	4-5	4-5	4-5	4-5	4-5	5.0	4.0	3.0
Pre. M 4 %	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4.0	3.0

Table-3. Qualitative antimicrobial assessment of dyed fabric.

Microbes	Dyeing Parameters	Zone of Inhibition (Mean ± SEM)			
E. coli	Dyed and control	**Dyed**	**Mordanted**	**+ve Control**	**-ve Control**
		22.96 ± 0.317^{d}	21.73 ± 0.145^{c}	18.96 ± 0.296^{b}	0 ± 0^{a}
	Pre-M / fixing agent	**1%**	**2%**	**3%**	**4%**
		33.86 ± 0.240^{e}	34.96 ± 0.202^{f}	37.96 ± 0.338^{g}	38.83 ± 0.371^{h}
S. aureus	Dyed and control	**Dyed**	**Mordanted**	**+ve Control**	**-ve Control**
		19.60 ± 0.208^{c}	18.30 ± 0.152^{b}	20.70 ± 0.435^{d}	0 ± 0^{a}
	Pre-M / fixing agent	**1%**	**2%**	**3%**	**4%**
		30.86 ± 0.49^{e}	33.20 ± 0.529^{f}	34.00 ± 0.230^{f}	35.03 ± 0.317^{g}
C. albicans	Dyed and control	**Dyed**	**Mordanted**	**+ve Control**	**-ve Control**
		25.96 ± 0.317^{c}	20.80 ± 0.152^{b}	40.76 ± 0.120^{g}	0 ± 0^{a}
	Pre-M / fixing agent	**1%**	**2%**	**3%**	**4%**
		35.00 ± 0.230^{d}	37.06 ± 0.233^{e}	39.00 ± 0.230^{f}	41.13 ± 0.240^{g}