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Research Article

Pharmaceutics for effective drug dosage

A Synergism of Eco-Friendly Dyeing of Cotton Fabric and Therapeutic Benefits of *Bixa Orellana* Seed

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Abstract: Background and objectives: The present research is aimed to study the eco-friendly nature and therapeutic application of the natural dye from the seed extract of *Bixa orellana* for maximizing the benefits of cotton fabric. Materials and methods: A natural dye was extracted from *Bixa orellana* seeds by hot water and ethanolic extraction method. This dye was examined for its ability with pre-mordanting and post-mordanting of cotton fabrics with ferrous sulphate, hydrated double sulphate and tamarind seed powder in order to improve the aesthetics and natural color shades on cotton fabrics. Furthermore, the dyed cotton fabric was used to analyze the antibacterial efficacy of cotton fabric against bacterial culture such as *Bacillus cereus, Escherichia coli, Proteus mirabilis, Salmonella typhi* and *Klebsiella pneumoniae*. Results: Our results suggest that pre-mordanting of cotton fabric with ferrous sulphate, alum and tamarind seed shows good dye fixation rate and stronger color than post-mordanting. Dyeing of cotton fabric with the ethanol extract of *Bixa orellana* seeds gave excellent and beautiful shades than the ones extracted with hot water. Furthermore, cotton fabric dyed with ethanol extracted was found significantly active against human pathogens compared to cloth dyed with hot water extract. The results evidenced that cotton knitted fabrics showed an increase in dye uptake with natural mordant and strength with a considerable reduction in antimicrobial activity. Conclusion: This research adumbrated developing a greener technology to cabalistic use of *Bixa orellana* seed extract applied on cotton cloths for coloring and in future find commercial use as a functional finishing agent (for newborns, clothing for burns, injured soldiers and hospitals) for health protection with semi-durable therapeutic properties.

Keywords: *Bixa orellana;* Antimicrobial activity; Natural dye; Cotton fabrics; Health protection.

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1. INTRODUCTION

Recent research has been focused on the development of natural bioactive clothing with therapeutic efficacy. [1] Modern and rapid growth in textile technology has generated eco-friendly and innovative finishes of textile materials used for healthy and hygienic lifestyle. Most of the textile fabrics composed of proteinaceous (silk, wool) and cellulosic materials (cotton, flax, jute, fibers) which have close contact with human body providing an optimum condition for multiplication of pathogenic microbiota resulting in dermal infection, allergies, objectionable odor, product deterioration and other skin related diseases.[2] These considerations include the establishment of technology for fabrics to be bacteria resistant with all the common desirable wearability features. These textile materials are used widely in various fields related to hygienic-lifestyle apart from traditional use of clothes.[3,4] Phenolic compounds, oxidizing agents, metal based complexes, halogens, and quaternary ammonium salts are well-known antimicrobial agents in textiles industries.[5] Although synthetic antibacterial agents are highly effective against a variety of microbiota and have a long lasting impact on textile fabrics, resulting in their use associated with complications, response on non-target microbes and expanding environmental impacts. [6] Many of the natural dyes are considered to be safe by their non-allergic, non-toxic, and biodegradable nature, [7] used in the coloration cosmetics,[8] paint, foodstuffs[9] and textile fabrics. [10, 11]Nowadays being trendy and fashionable makes it challenging to keep abreast with ecological concerns and environmental conservation, issues that have become priorities in the modern world. [12] Natural dyes extracted from plant flowers, bark, leaves, seeds, roots, insect secretions, fruits and minerals for eco-friendly and sustainable fabric dyeing decreases the uses of synthetic dyes in the international industrial markets. Awareness of sustainable ecosystems as well as risks of using synthetic dyes necessitates developing new strategies for eco-friendly products using natural resources. Greener technology is the strategy, development, and application of synthetic dyes and methods to diminish or eliminate the use and generation of hazardous substances that have a detrimental effect to the ecosystems and human-beings. [13]Natural coloration is considered as a substitute for textiles which guarantee aesthetic appeal without the use of toxic dyes, and therefore, *Bixa orellana* (annatto) seeds have been carefully chosen for knitted cotton fabrics as a natural plant source of dye and colorant. Worldwide annual production of dried *Bixa orellana* seed was at about 10,650 tons, 7000 of which were commercialized globally. [14] *Bixa orellana* seeds known as annatto, a condiment that gives an orange-red color is used in foodstuffs, cosmetics and paint industry. The orange-red pigment of annatto known as Bixin consists of carotenoid, a highly unsaturated compound that dissolves in vegetable oil and on heating, the bixin undergoes isomerization and degradation. *Bixa orellana* is reported to have numerous pharmacological properties such as wound healing, analgesic, hemostatic, antioxidant, and diuretic properties. [15, 16] Due to the wide-ranging application of *Bixa orellana* in the cosmetic industry, several studies about possible health risks have been analyzed. Researchers worldwide have been exploring applications of natural colorants for textiles and cosmetics, but most of the investigations have been focusing on standardizing the dyeing and mordanting methods, and fastness properties. [17, 18] Recent studies were concentrated on bioactive properties of natural colorants on textile materials,[19] However, there is a lack of systematic research on the durability of bioactive components of natural colorants after implementation on textile fabrics. Therefore, it must be determined whether *Bixa orellana* retains its antibacterial efficacy on textile fabrics. The goal of this study is to investigate the dyeing properties and antimicrobial efficacy of knitted cotton fabrics with *Bixa orellana* seed hot water and ethanol extract against commonly exposed microbes in hospital or household environments (*Bacillus cereus, Escherichia coli, Proteus mirabilis, Salmonella typhi* and *Klebsiella pneumoniae*). The experiment methodology was followed as Figure 1. These natural dyed cotton fabrics can be suitable in developing clothing for injured soldiers, infants, burning wounds, elderly and hospitalized people to guard them against bacterial infections. These textile fabrics may be used in carpets, curtains and bed linen, which are the major propagators of common infections.

2. MATERIALS AND METHODS

2.1 Fabric

Commercially bleached knitted cotton fabric (15x15 cm) was scored by using 1% sodium carbonate. The clothes were soaked in boiling water (80°C) for 30 minutes and left overnight, rinsed with distilled water and dried in shade for further analysis.

2.2 Extraction of Dyes

Dried seeds of *Bixa orellana* were purchased from local suppliers at Virudhunagar, Tamil Nadu, India. The collected plant was botanically authenticated by Dr. Rajarathinam Kanniappan, Professor, Department of Botany, VHNSN College, Virudhunagar. All the analytical grade reagents and chemicals were purchased from Sisco Research Laboratory Ltd (SRL, India) and Merck Ltd (USA). Dried *Bixa orellana* seeds were used for different dye extraction methods.[20] The hot water extraction was done by boiling 50 g of seeds in water (1:4) at 80°C for 45 minutes maintained in an induction cooktop (Prestige, India. Model - PIC 6.0 V3 2000 W). The filtering is repeated to remove as much dye as possible. The dye filtrate was evaporated to 15°C and the concentrated dye was dehydrated resulting in reddish-orange colorant powder (yield =21.50%). The ethanolic extraction was done by soaking 50 g of *Bixa orellana* seeds powder for two weeks in 100 ml of ethanol with continuous stirring. After two weeks, the first layer was removed and 50 ml of fresh ethanol was added as second layer and soaked for one week. Then the second layer was removed and 50 ml of ethanol was added as the third layer. All the reactions were carried out at room temperature, and the collected layers were mixed, and filtrated. The filtrate was placed at 70°C for 4 h in a water bath for evaporation resulting in a reddish-orange colorant powder (yield = 32%).

$$\text{Yield (\% w/w dry basis)} = \frac{\text{Weight of plant extracts (g)} \times 100}{\text{Weight of raw materials (g)}}$$

2.3 Phytochemical Screening

The *Bixa orellana* seeds hot extraction (water) and cold extraction (ethanol) were subjected to phytochemical analysis according to the standard methodology of Kumar *et al*. [21]

2.4 Mordanting and Dyeing

To enhance the colorant uptake, cotton fabrics were pretreated and post-treated with various positively charged metallic salts (ferrous sulphate, alum) and natural mordant (tamarind seed powder) at different concentrations under optimized extraction and dyeing conditions. Various concentrations (0.1%, 0.5%, 1% and 2%) of alum and ferrous sulphate were prepared to optimize the concentration of mordants in pre-mordanting and post-mordanting treatment. For pre-mordanting, the knitted cotton fabrics were soaked in boiling mordant solution (~80°C for 60 min.) and soaked for 12-18 hours, and then dried under shade. For post-mordanting, knitted cotton fabrics were dyed at optimized conditions and dried in under shade. Then, the cotton clothes were soaked in boiling mordant solution (~80°C for 60 min.) and dried under shade. For natural mordanting, the tamarind seed extract was prepared according to Prabhu and Teli. [22] The knitted cotton fabrics were soaked in a boiling mixture of 2% tamarind seed extract and 10% *Bixa orellana* hot water extract (~80°C for 30 min) and soaked for 18-24 hours, and then dried under shade.

2.5 Antibacterial activities

2.6 Test organisms

Bacillus cereus (MTCC 10403), *Escherichia coli* (MTCC 1687), *Proteus mirabilis* (MTCC 3310), *Salmonella typhi* (MTCC 98) and *Klebsiella pneumoniae* (MTCC 109) were obtained from V.H.N.S.N culture repository and confirmed biochemically according to Bergey's Manual and used for antimicrobial activity.

2.7 Agar diffusion method

The dyed and undyed cotton fabrics were sterilized to determine its antibacterial efficacy against wound isolates. The antimicrobial sensitivity tests of dyed and undyed cotton fabrics were performed using disc diffusion (Kirby-Bauer's) method. [23] Briefly, 1 inch of sterile cotton dyed and undyed samples were placed against the tested organism and incubated at 37°C for 24 h and the size of the clear zone of inhibition was measured around the sample.

2.8 Determination of antimicrobial activity

The antibacterial effectiveness of cotton dyed and undyed samples were investigated according to Leitner *et al*,[24] by measuring the OD values at 595 nm and the microbial growth reduction was calculated as follows:

$$R = \frac{(B - A)}{A} \times 100$$

where, R = percentage of reduction in microbial growth; B = absorbance at 595 nm of media inoculated with undyed cotton samples with microbes; A = absorbance at 595 nm of media inoculated with dyed cotton samples with microbes.

3. RESULTS

3.1 Extraction of dye from Bixa orellana seeds

The hot water extract collected and filtering was repeated to remove as much dye as possible. Dye filtrate was evaporated to 15°C and the concentrated dye was dehydrated resulting in a reddish-orange colorant powder (yield = 21.50%). The cold extract using ethanol was collected and filtered. The filtrate was placed at 70°C for 4 h in a water bath for evaporation resulting in a bright reddish colorant powder (yield = 32%). The extracted colour from *Bixa orellana* seeds using different solvents are depicted as Figure. 2.

3.2 Phytochemical analysis of Bixa orellana seeds

The present study revealed the presence of phytochemicals in the hot and cold extraction of *Bixa orellana* seeds as summarized in the table below (Table-1).

3.3 Impact of Various Mordanting Agents in Dye Fixation

The amount of dye uptake by cotton yarn samples was visually examined and different shades of orange to reddish brown color was developed depending on the types of mordant on pre and post treatment. The maximum strong color yield was observed in pre-mordanting with ethanol extracted compared to post-mordanting and hot water extracted dyed samples. In hot water, dyed fabric showed greenish orange in $FeSO_4$ and alum mordant whereas tamarind seed powder mordant gave reddish brown color with maximum strength. Ethanolic extract was used to dye the fabric with $FeSO_4$ mordant, it produced greenish red shade and a darker color than hot water treated fabric whereas alum treated dyed fabric showed reddish brown color. Tamarind seed produced excellent reddish brown color with maximum color strength in both pre and post-mordanting ethanolic extract dyed fabrics. Overall,

comparing the mordants, the natural mordant (tamarind seed) produced an excellent and strong reddish brown color compared to $FeSO_4$ and alum as shown in Figure. 3.

3.4 Antimicrobial activity of Bixa orellana dyed Cotton Fabrics

The eco-friendly dye extract showed a significant effect of antimicrobial activity against various organisms. Preliminary screening revealed that dyed cotton fabrics were effective against the microorganisms represented by a clear zone of inhibition as in Figure 4. The hot water and ethanol extracted dyed cotton fabrics without mordant were used in this study to assess the antimicrobial effects of *Bixa orellana* seeds. *Klebsiella pneumoniae* and *Salmonella typhi* showed higher sensitivity to hot water and ethanol extracted dyed cotton fabrics whereas, the undyed fabric (control) did not show any zone of inhibition. *Proteus mirabilis* was moderately sensitive to dyed cotton fabrics followed by *Escherichia coli,* whereas no differences were observed between hot water and ethanol extracted dyed fabrics. *Bacillus cereus* revealed lower sensitivity compared to all other organisms (Figure. 4). The hot water extracted dyed fabrics showed an effect against *Bacillus cereus* as compared to ethanol extracted dyed fabrics. Table 2 revealed that knitted cotton fabrics dyed with hot water and ethanol extracted *Bixa orellana* were found to be nearly equal in effect to standard ampicillin used in the experiment when correlating the antimicrobial activity of dyed fabrics. The antimicrobial efficacy of the hot water dyed fabrics against Bacillus *cereus* was 61.2% followed by *Escherichia coli* (70.1%), *Proteus mirabilis* (78.4%), *Salmonella typhi* (82.9%) and *Klebsiella pneumoniae* (86.8%). While ethanol extracted dyed fabrics decreased growth of Bacillus *cereus* (60.3%), *Escherichia coli* (72.5%), *Proteus mirabilis* (76.4%), *Salmonella typhi* (83.2%) and *Klebsiella pneumoniae* (88.6%).

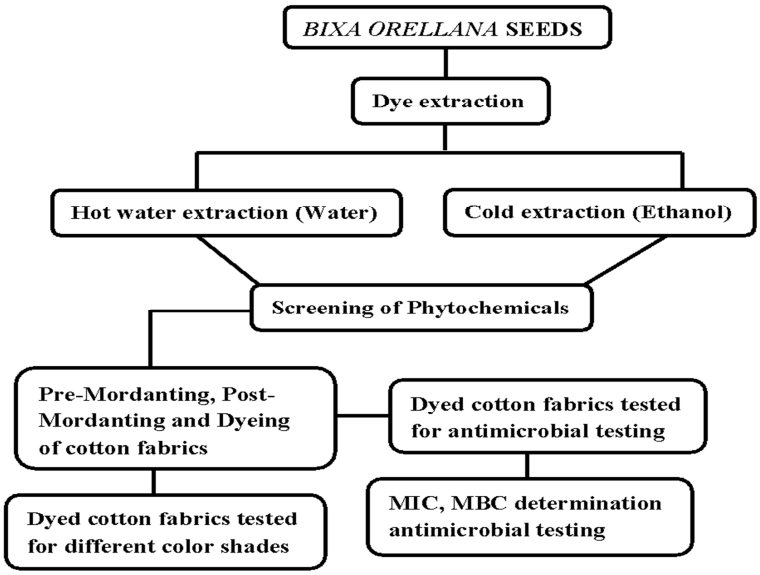

Figure 1: Schematic representation of the experimental layout.

Figure 2: Bixa orellana seeds and extraction of dye. (a) Annatto seeds (b) Grounded annatto seeds (c) Annatto seeds after extraction (d) 1. Hot water extraction of Bixa orellana seeds 2. Ethanolic extraction of Bixa orellana seeds.

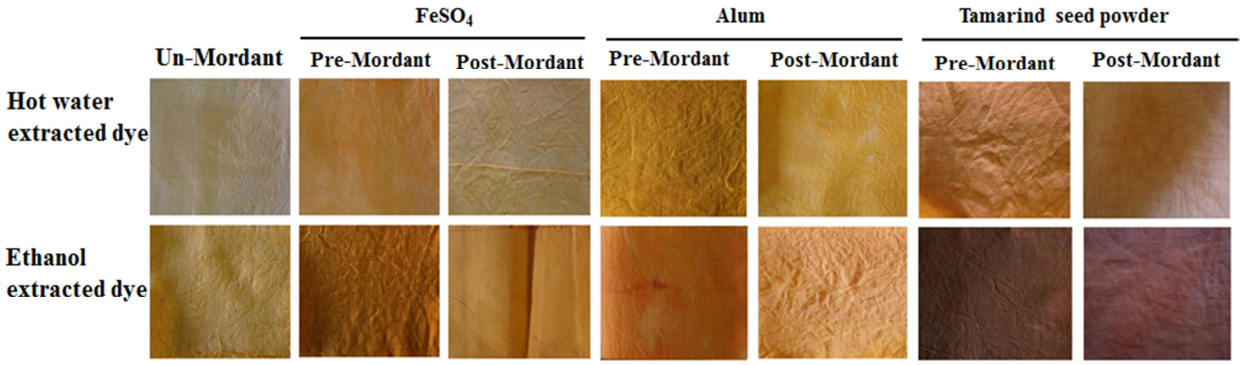

Figure 3: Colors of the samples dyed with Bixa orellana seeds hot water and ethanolic extract with pre and post-mordant samples treated with mordanting agents ($FeSO_4$, Alum and tamarind seed powder) in dye fixation.

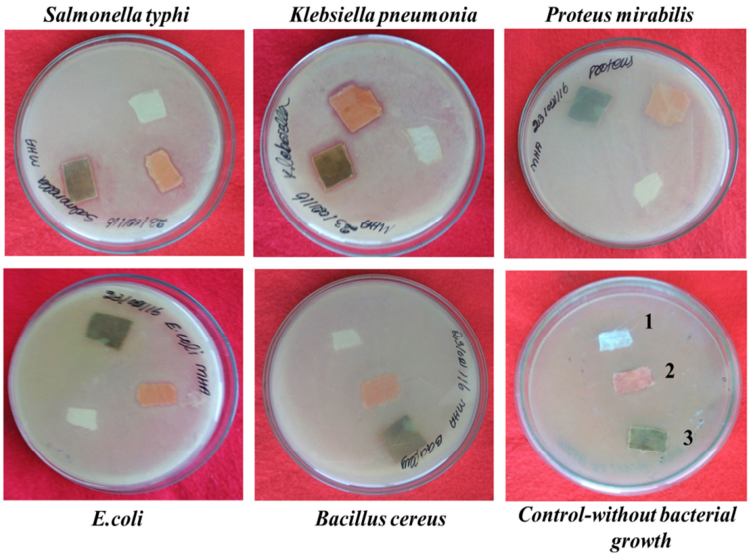

Figure 4: Disk diffusion assay of microorganism with samples dyed with and without Bixa orellana seeds. 1. Raw fabric, 2. Raw fabric dyed with hot water extract of Bixa orellana seeds (Greenish orange) and 3. 2. Raw fabric dyed with ethanolic extract of Bixa orellana seeds (reddish orange).

Table 1: Results of Phytochemical Screening of Bixa orellana Seeds.

Phytochemicals	Hot extraction (Water)	Cold extraction (Ethanol)
Alkaloids	+	+
Flavonoids	+	-
Steroids	+	+
Saponins	+	-
Terpenoids	+	-
Carbohydrates	+	+
Proteins	+	+

Table 2. Antimicrobial activity of *Bixa orellana* dyed Cotton Fabrics

	% Microbial Reduction				
Microorganisms	**Blank**	**Ampicillin**	**Control**	**Hot-water dye**	**Ethanol extracted dye**
Bacillus cereus	0	90.6	0.3	60.3	61.2
Escherichia coli	0	92.5	0.8	72.5	70.1
Proteus mirabilis	0	93.3	1.3	78.4	76.4
Salmonella typhi	0	96.7	1.8	82.9	83.2
Klebsiella pneumoniae	0	98.9	1.5	86.8	88.6

4. DISCUSSION

Fabrics have long been considered as a substrate for bacterial growth which provides ambient conditions for bacterial growth and multiplication. Antibacterial fabrics act as a protective shield against damage and safeguard the user against pathogenic and odor producing bacteria. It is therefore highly important for reducing the bacterial growth

in fabrics for long term storage and use. [25,26] The crude coloring dye obtained from *Bixa orellana* seeds extracted in hot water and ethanol (Figure. 2) was employed for dyeing cotton fabrics. Due to the harmful environmental effects from use of synthetic dyes and metal salt mordants, this study chose natural *Bixa orellana* seeds extracts and natural mordant (tamarind seed powder) for evaluating the dyeing potential (Figure. 2). Bixin and nor-bixin are color compounds present in *Bixa orellana* seed that consists of OH group, OH stretch for COOH group and NH group in primary amines are the functional groups responsible for physical bond formation between the compounds. [27-29] It is suggested that for the uniform diffusion and distribution of dye on the fabrics may be better or improved due to the smaller particle size of the colorants. [28] Mordant treated cotton fabrics showed stronger color than un-mordanted samples owing to the chelating effect between cotton fabrics, dye components and metal mordant. The ethanolic *Bixa orellana* seed extract with pre-mordant resulted in cotton fabrics with better shades than hot water *Bixa orellana* seed extract and post-mordant. In cotton fabrics for dyeing, pre-mordant and post-mordant the natural dye and mordant act as ecofriendly effective polyphenolic dye and bio mordant to produce a strong fabulous color on fabrics. For *Bixa orellana* seeds extract, the pre-mordant and post-mordant of natural mordant ranged from deep brown to burgundy color with bio mordant grenade giving attractive shades of altered tones. The highest color strength was observed with ethanolic *Bixa orellana* seeds extract than hot water extract and as estimated *Bixa orellana* seeds produced noteworthy dark shades (Figure. 3) It is for this reason, the *Bixa orellana* seeds can be used as a coloring agent in cosmetic, food and textile dyes due to its fabulous shades. This study has examined the dyeing potential and antibacterial activity of *Bixa orellana* and explores the feasibility of using this dye for large-scale applications. Augmented antibacterial efficacy of *Bixa orellana* seeds dyed cotton fabrics are based on functional groups and chemical structure presented in colorant. Antimicrobial activity of *Bixa orellana* seeds is due to the presence of flavonoids, phenolics acids (especially gallic acid) and carotenoids in the extract. [30] The phenolic ring presented in the colorant is poisonous to microorganisms and closely related to the presence of functional groups of pigment components.[31] The molecular mechanism underlying the phenolic ring toxicity for bacteria is the enzyme degradation by oxidized bioactive natural compound, nonspecific associations with proteins or reactions by sulfhydryl groups [32,33] found that *Bixa orellana* seeds extract possess antimicrobial effects against various Gram-positive bacteria. Rodrigues *et al*. [34] reported that *Bixa orellana* seeds extract contains terpenoids, flavonoids, tocotrienols and carotenoid derivatives such as bixin and norbixin that might be responsible for effective antibacterial action. The disc diffusion method revealed that *Klebsiella pneumonia* and *Salmonella typhi* are more sensitive as compared with other tested organisms. The un-dyed cotton fabrics (white) did not show any zone of clearance (Figure. 4) whereas the antibacterial function of the dyed cotton fabric is improved with both hot water and ethanolic extract. The chemical interaction between the pigments compounds and fiber can be modified by the functional groups effective for the antimicrobial activity. [35] The reduction in the rate of microbial growth by *Bixa orellana* seeds extract dyed knitted cotton fabrics was significant (Table. 2). The results from this study clearly demonstrate that the *Bixa orellana* seeds extract can be used for dyeing to develop garments that function as a protective shield against common pathogenic microorganisms has and possess potential for applications in home and hospital textiles.

5. CONCLUSION

This report focused primarily on the antibacterial activities of knitted cotton fabrics dyed with *Bixa orellana* seed extract. From this research, the influence of metallic salt mordants on fastness of coloring and antibacterial impact of cotton fabrics was assessed efficiently. The *Bixa orellana* seeds possess antibacterial effects, which is an eco-friendly natural color dye generating beautiful dark reddish to orange color and retain its biological activity on cotton fabrics. When cotton clothes were dyed with *Bixa orellana* seeds extracted without mordanting, colorant exhibited notable light fastness, fairly good to rub fastness and good wash fastness properties. Dyeing of cotton clothes with mordants increased noticeably the overall color fastness, with minor variabilities in shade and colour. Dyed cotton fabric samples exhibit reasonable durability with a good, relatively long life with effective color fastness. The antimicrobial effect is assumed to be lasting in practice and the eco-friendly nature might be a promising antibacterial colorant for clothing. *Bixa orellana* seed ethanolic extract has an effective scope for producing protective cotton fabrics clothing for use in newborns, clothing for burns, sportswear, injured soldiers and baby products for added value. Future work in this area should investigate more microbial types for the antibacterial properties of fabrics / dressing treated with *Bixa orellana* seeds extract.

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8. AUTHORS' CONTRIBUTIONS

AS, ND, KM, SD concept and design of the study, data acquisition, and supervision of the study. NAD, DV, AS, literature search, AS, ND, manuscript preparation, critical and SD, KM, DV, NAD final revision of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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