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Bioactivity of Textile Fabrics Impregnated with Crude Extract of *Biden Pilosa* Plant Species

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ABSTRACT

Aqueous extract of Biden pilosa plant species was obtained by boiling the dried and pulverized leaves in distilled water. The filtered aqueous extract was applied on scoured and bleached cotton and silk fabrics by adopting the procedure for post mordanting dyeing with natural dyes. The fixing agents (mordants) used were alum and ferrous sulphate. Nutrition agar (MHA) was used as culture media for the test microorganisms viz: Pseudomonas aeruginosa and Staphylococcus aureus and agar diffusion method was adopted for determination of antimicrobial activity. It was established that the impregnated cotton fabrics with the application of fixing agents/mordants inhibited microbial growth both underneath and around with poor growth rate towards the fabrics. However the cotton fabrics impregnated without the aid of mordants registered weak growth towards the fabric, no growth underneath but no growth inhibition towards the fabrics. All the impregnated silk fabrics registered strong growth inhibition underneath and around with weak growth rates towards the fabrics.

Keywords: Bioactivity, fabrics, medical textiles, mordant

1. INTRODUCTION

Bidens Pilosa found in Asteraceae family is an annual erect herbaceous plant about 60-90 cm. high. It a medicinal plant native to South America that nowadays is distributed all over the world, mainly in tropical and subtropical regions. [1] Bidens Pilosa has various classes of chemical constituents *viz;* polyacetylenes, polyacetylenic glycosides, aurons, auron glycosides, p-coumeric acid derivatives, caffeoylquinic acid derivatives, flavonoids and flavonoid glycosides, sesquiterpenes, acetylacetone, phenylheptadiynol, phenylpropanoidglucosides, pheophytins diterpenes. [2] These phytochemical components have been found to be responsible for the various medicinal activities of Bidenspilosa and these activities include antimalarial activity due to the presence of acetylene and flavonoid. [3] Bidenpilosa's roots, leaves, and seeds are reported to have antibacterial, anti-dysenteric, anti-inflammatory, and antimicrobial, antimalarial, antiallergic activity, hepatoprotective, and hypotensive properties. Biden pilosa is also reported as a vegetable or potherb among others in many cultures around the world.

Textiles play an important role in the daily lives of humans, and the demand for the various quality attributes are based on enhancing their properties through proper finishing. Textiles are excellent substrates for bacterial growth and microbial proliferation under appropriate moisture, nutrients and temperature conditions. Natural fibres are more liable to bacterial attack than synthetic fibres due to their porous and hydrophilic nature. On the other hand, direct contact with human body supplies warmth, humidity and nutrients, which provides a perfect environment and optimal conditions for microbial growth. [4] Bioactive textiles are those that induces specific biological activity on exposure to plant and animal tissues. Natural bioactive compounds with antimicrobial properties are gaining considerable attention as attractive eco-friendly alternative to synthetic antimicrobial agents for textile

applications, especially in medical and health care textiles, as they are safe, non-toxic and skin-friendly. [5] The purpose of imparting antimicrobial activity to textiles is to protect the material from microbial attack, prevent the transmission and spreading of pathogenic microorganisms, inhibit odour development resulting from microbial degradation, and creating a material that will act as preventive or curative treatment. [6]

The transfer of gram-positive bacteria, especially methicillin-resistant Staphylococcus aureus (MRSA), gram-negative bacteria (pseudomonas auregonosa) among patients is a growing concern. This results into increased risk of skin diseases like ring worms, small pox and scabies as a result of cross transfer among people from hospitals, homes, schools and camps through touch, sharing of beddings, tables and bed linens, towels, bathrooms and door handles. Antimicrobial finish causes a fabric to inhibit the growth of microbes in textile materials it also destroy or suppress the growth of microorganisms and their negative effects of odor, staining and deterioration. [7]

Above all, health issues are continuously challenging as pathogens evolve and the environment changes from time to time; a situation that calls for constant research in medical textiles and hence, the need to develop cheaper, safer and effective bioactive textiles fabrics through a research. In the present study, the bioactivity of extract of *Biden pilosa* plant species used as natural dye on cotton and silk fabrics is evaluated.

2. MATERIALS AND METHODS

2.1 Materials

Desized, scoured and bleached plain weave 100% cotton fabric was purchased from southern Range Nyanza Textiles Limited and 100% degummed and bleached knitted mulberry silk fabric was purchase from Uganda National Sericulture Centre, Kawanda, Wakiso district. The fresh leaves of *Biden pilosa* plants were collected from gardens around Busitema University campus, Busia-Uganda and brought to textile lab. Alum and iron sulphate were used as mordants. Nutrition agar (MHA) was used as culture media for the test microorganisms and an incubator for incubating the specimens.

2.2 Extraction of phytochemicals from plant material

Aqueous extraction method with a little heating was used. The fresh leaves (2000 g) were thoroughly washed with running tap water followed by distilled water to remove dirt. It was made to dry in the lab at room temperature for 4-5 days and pulverized. The pulverized leaf sample (150g) was transferred to a glass beaker and distilled water (1000 cm^3) added and heated to boiling and made to simmer at 90^0C for 30 minutes. The crude extract was made to cool at room temperature filtered and used and applied on fabrics as dye from plants.

2.3 Impregnation of fabrics with the crude extract

The fabrics were impregnated with the crude extract using the methods used in dyeing with natural dye for both cotton and silk fabrics.

Silk fabrics

All the samples were dyed using post mordanting method with Alum and iron sulphatemordants. Pieces of degummed and bleached silk fabrics (8x10 cm) were soaked in distilled water and transferred to dye bath liquor (700 cm^3) and the mixture heated gradually to 60^0C while stirring for 30 minutes. The fabrics were removed from the dye bath and immediately soaked in solution of a mordant. Different fabric samples were soaked separately in mordant solutions which were made to stand for 15 minutes. The dyed cloths were then rinsed repeatedly in clean water until there was no more color change observed in the cleaning water. The samples were thereafter dried at room temperature.

Cotton fabrics

The cotton samples was dyed according to the protocol described by. [8] The bleached cotton fabric samples was dipped in a dye

bath (150 cm^3) and after 10 minutes, 20% on weight of fabric (o.w.f) of sodium sulphate was added. The dyeing was carried out for one hour at 50°C with intermittent stirring. The dyed samples were removed from dye bath and squeezed to remove excess dye. The dyed fabrics were then soaked in a mordant solution (100 cm3) containing 10 % on weight of fabric (o.w.f) of a mordant at 60°C for 30 minutes with material to liquor ratio of 1:20. The dyed samples were washed with non-ionic soap solution (2 gpl) at 50°C for 10 minutes, rinsed with tap water and dried at room temperature.

2.4 Determination of bioactivity of the impregnated fabric samples

The quality of all media, reagents and samples used in the study were controlled to ensure accurate results. Sterility tests were conducted by incubating all prepared media at 37^0C for 24hrs and then inspected for microbial growth. The media were prepared according to the manufacturer's instructions, weighing balances were calibrated, and fridge temperatures were monitored using temperature charts. The temperature and pressure of the autoclave was checked and calibrated. The dyed fabric samples were sterilized in the autoclave.

In this step the procedures describe below were followed.

2.4.1. Preparation of plates

The study of antimicrobial activity was performed by the agar diffusion method. The nutrient agar was prepared according to standard procedure. [9]

Preparation of culture media (Mueller Hinton agar)

Mueller Hinton agar (MHA) used for direct sensitivity was prepared according manufacturer's (Laboratory Conda South Africa) instructions. The MHA media powder (30.4 g) was picked and suspended in distilled water (800 cm^3) in a conical flask, mixed thoroughly with frequent agitation to dissolve the powder completely (without heating). The media was sterilized by autoclaving at 121^0C for 15 min (at 15lb), after autoclaving, the media was cast in sterile petri dishes, made to set and then incubated at 37^0C for 24hrs to conform the sterility of the media.

2.4.2. Sub culturing the microorganisms

Isolates of pure strains of Pseudomonas aeruginosa and Staphylococcus aureus prepared and preserved were used in the experiment. Cultures of bacteria were grown in the nutrient agar at 37^0 C and maintained on the slopes of nutrient agar. The bacteria were transferred to separate petri dishes containing nutrient agarand kept overnight at 37^0C for reactivation. The bacteria suspension (inoculum) was made using normal saline water.

2.4.3. Inoculation of agar plates containing dyed fabrics

The petri dish containing media (MHA) from the incubator was used. Normal saline (50µl) was transferred in a test tube and two colonies of microbes were streaked using loop wire from each sub cultured microorganism *viz*; Pseudomonas aeruginosa and staphylococcus aureus, and then placed in each test tube containing normal saline water and stirred until the solution became turbid. The inoculum containing the test microorganisms was streaked across the petri dishes containing the media (MHA) that were previously incubated for 24hrs. A wire loop for streaking was used to transfer the inoculum from the test tubes onto the agar plates. The sterilized samples of dyed fabrics of (60 x60mm) were then laid firmly on the top of the inoculated plate and incubated for 24hrs at 37^0C. Incubated plates were examined for the interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge.

2.4.4. The evaluation in microbial population growth

After 24hrs, the inoculated plates were examined for bacterial growth along the streaks of inoculum directly beneath the fabric and around its edges using visible observation and a compound microscope. The microbial population growth rates

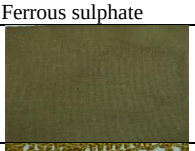
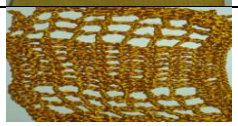
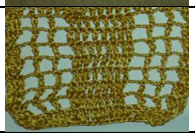
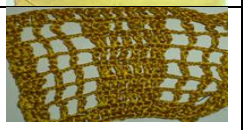
towards the fabrics and on fabrics surfaces were determined.

3. RESULTS AND DISCUSSIONS

3.1. Color shades developed on fabrics

The extract was applied on the fabric samples using the method applied for natural dyeing and in this case post mordanting method with alum and ferrous sulphate was adapted. The impregnated cotton and silk fabrics registered soft shades that varied with the mordant used. These shades developed on fabrics persisted as they were not washed away on through rinsing with water. Generally ferrous sulphate registered deeper shades as compared to alum. Table 1 below shows the shades produced on the fabric samples with the aid of ferrous sulphate and alum mordants.

Table 1. Shades produced on cotton and silk fabrics

Fabric sample	Alum	Ferrous sulphate	No mordant
Cotton			
Silk			

Shades produced on silk fabrics are more intense than those on cotton fabrics. The developments of persistent shades qualify *Biden pilosa* plant as a potential source of natural dye for textile application.

3.2. Bioactivity of the impregnated fabrics

Sterilized dyed fabric samples were placed in intimate contact with AATCC bacteriostasis agar, which was previously inoculated with an inoculum of the test microorganisms *viz;* staphylococcus aureus which is a gram positive bacteria and Pseudomonas aeruginosa which is a gram negative bacteria. After incubation, a clear area of interrupted growth underneath and along the side of the test material (fabrics) indicates its antibacterial effectiveness.

The fabric samples includes; impregnated fabrics with the plant extract using alum and ferrous sulphate, impregnated fabric without inorganic salts and fabrics which are not impregnated as control.

Cotton fabrics: Fig. 1 below shows the activity of cotton fabric samples against the selected types of bacteria. From left to right in the photograph, B_{1c} and $B_{2c}$are fabric impregnated with aid of alum and ferrous sulphate as mordants, $\underline{B}_C$ impregnated without mordant and P is not an impregnated fabric. As can be noted, the impregnated fabrics with the use of mordants registered interruption of microbial growth beneath and clear zones of inhibition towards the fabrics and those impregnated without the use of mordant registered interruption of growth beneath the fabric with no clear zone of inhibition towards them.

Figure 1.Growth of *staphylococcus aureus* and *Pseudomonas aeruginosa* on cotton fabrics in petri dishes

On the control fabric P, no activity was observed as there was uninterrupted microbial growth beneath the around the fabrics. This observation is true for both types of bacteria used. The summary of the result is tabulated in Table 1 below.

The microbial growth rates towards cotton fabrics registered for the fabrics impregnated with mordants were weak but a high growth rate was registered for impregnated unmordanted fabric as summarized in Table 3 below. This result reveals that the active components of the plant extract was able to diffuse through the agar gel thereby inhibiting growth towards the fabrics. The minimal activity exhibited by unmordanted cotton fabrics suggests that there was no or negligible diffusion of the active ingredients from the fabric to the gel. The light shades on the unmordanted cotton fabric as in Table 1 above could also suggest that the quantity of the bioactive components retained on the fabric is small as may be reflected on its bioactivity. Mordants are chemical substances that helps bind a dye compound to a fabric in this case the absence of a mordant could have resulted to reduced quantity of bioactive material attached to the fabrics.

Silk fabrics: The bioactivity of impregnated silk fabrics is shown in Fig. 2 below. All the impregnated fabrics mordanted and unmordanted registered interruption of microbial growth beneath the fabrics and clear zones of inhibition towards them. The non impregnated fabrics registered no activity on both type of the bacteria. The summary of the results is tabulated in Table 2 below. As can be noted from results in Table 3, the bacterial growth rates towards allthe impregnated silk fabrics were very weak.This is aimed at determining the ability of an antimicrobial agent impregnated in the fabric to diffuse through agar gel. [10]

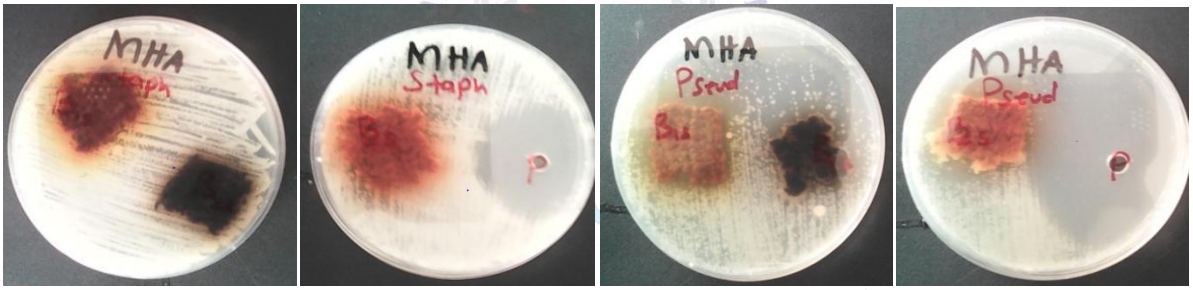


Figure 2. Growth *staphylococcus aureus* and *Pseudomonas aeruginosa* on cotton fabrics in petri dishes

Table 2: The antimicrobial activity of impregnated fabrics

Organism	Fabric					
	Cotton			Silk		
	A_a	A_f	A_n	B_a	B_f	B_n
S. aureus	++	++	+	++	++	++
P. auregenosa	++	++	+	++	++	++

++ = Maximum antimicrobial activity (interruption of the microbial growth beneath the fabric and a clear zone of inhibition around the fabric)

+ = Moderate antimicrobial activity (interruption of the microbial growth beneath the fabric but no clear zone of inhibition around the fabric)

The weak growth towards fabrics is a result of diffusion of the active components of the plant extract through the agar gel thereby inhibiting growth towards the fabrics. The immense bioactivity of impregnated silk fabrics is a function of the amount of bioactive constituent of extract that adhered to the fabrics. Assuming that the intensity of shades developed on the fabric is proportional to the quantity of bioactive constituents adhered on the silk fabrics, from Table 1, the intensity of color shades developed on silk fabrics can be visibly noted and more importantly on the unmordanted silk fabric. This observation reveals that the bioactive components from crude extracts of *Biden pilosa* plant binds to the protein based silk fibre better than the cellulosic cotton fibre.

Table 1: Microbial growth rate towards impregnated fabrics

Organism	Fabric					
	Cotton			Silk		
	A_a	A_f	A_n	B_a	B_f	B_n
S. aureus	W	W	H	W	W	W
P. auregenosa	W	W	H	W	W	W

W = Weak growth rate, H = High growth rate

Textile products are omnipresent in the field of human hygiene and medical practice. The bioactivity of the impregnated fabrics gives it a potential for use in medical and health care textiles. The bioactive fabrics can be developed for use as simple gauze, bandage material in wound dressing, barrier material (for infection control), wound care, hygiene and implantable material among others. Silk is a protein fibre which is used in the production of digestible satures for body tissue regeneration, a bioactive silk yarn makes it more suitable for this function.

CONCLUSION

The crude aqueous extract of the leaf of *Biden pilosa* plant species imparted persistent color shades and bioactivity characteristics on cotton and silk fabrics. The impregnated fabrics registered bioactivity for both gram-positive and gram-negative bacteria. However silk fabrics registered a strong bioactivity with both the mordanted and unmordanted samples. The persistence of color shades make *Biden pilosa* plant a potential source of natural dye from vegetables though its fastness on fabrics have to be established. Fabrics impregnated with extract from this plant can be further developed for application in medical and health care textiles in simple gauze, hospital bed and table linens, inpatient and cleaners garments to reduce cross infections from contagious diseases e.g. scabies, ringworms.

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