CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1. Silks

Untreated *Samia ricini* silk (*Eri*), *Antheraea assamensis* silk (*Muga*), and *Bombyx mori* silk (*Pat*) were collected from sericulture-practicing local weavers of Dhakuakhana, Lakhimpur, Assam, India, in January 2021. The silks were prepared by the weavers in the year 2020, which makes them fresh samples. The silks were wrapped separately in white paper and stored in a polythene bag in the laboratory at room temperature.

3.1.2. Washing agents

For commercially available washing agents, detergent powder (SUR); laundry detergent (TID); detergent bar (RIN); indigenous soap ball (LOCAL); liquid fabric stiffener (REVI); fabric conditioner (COMF); gentle liquid detergents (EZY); detergent booster and stain remover (VOX); and hair shampoo (SHAMPOO) were used purchased from the local market. Fruits of *Musa balbisiana* (for preparation of *Kolakhar*), were collected from a banana plantation near Tezpur University campus. Activated charcoal (ACT. CHARCOAL) was purchased from Merck, India. *Citrus Limon* (Citrus) was purchased from Tezpur University's local vegetable vendors.

3.1.3. Dyeing materials

Curcumin (Himedia) has been used as a dye. Tannic acid (loba) was used as a mordant. For bleaching 35% Hydrogen peroxide was used.

3.2. Methods

3.2.1. Washing Formulation preparation, washing methods, and analysis methods

3.2.1.1 Preparation of *Kolakhar* Suspension

Kolakhar suspension was prepared following the method of Deka and Talukdar (2007) with some minor changes [1]. The peels of *M. balbisiana* fruits were sun-dried until constant weight was achieved. From the sun-dried peels, 500g were burned to charcoal and ashes, which is termed as *Kolakhar*, and stored in an airtight container. To

make the suspension, 3.00 g of *Kolakhar* was mixed in 60 ml of sterile distilled water using a magnetic stirrer (Tarsons Motorless Magnetic stirrer, Model 4050, India) for 45 minutes at 500 rpm. It was filtered using a muslin cloth to obtain a light opaque suspension as can be seen in Fig 3.1. The collected filtrate was used freshly.

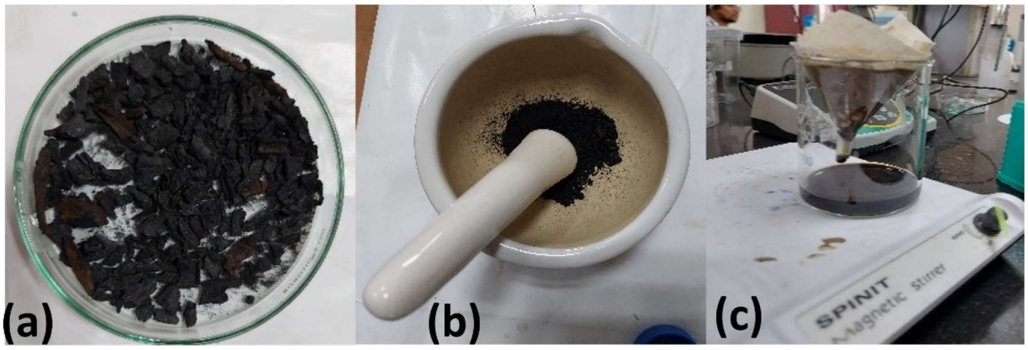

Fig 3.1: a) *Kolakhar* b) grinding of *Kolakhar* c) Filteration of *Kolakhar*

3.2.1.2 Preparation of activated Charcoal suspension

The activated charcoal was prepared using 3.00 g of activated charcoal in 60 ml of sterile distilled water mixed for 45 minutes in a magnetic stirrer at 500 rpm. It was further filtered using a muslin cloth that gave a semi-transparent suspension, as can be seen in Fig 3.2. The method of Deka and Talukdar (2007) for *Kolakhar* described in section 3.2.1.1 has opted for the preparation of activated charcoal suspension with some changes [1, 2]. The final concentration of the charcoal solution after filtration was found to be approximately 12.37 ± 1.08 mg/ml.

The activated Charcoal suspension was prepared after the completion of the first objective, where results of some treatment suggested some possible chances of activated charcoal. This has also been added in the first objective to show its comparison.

Fig 3.2: Filtration of activated charcoal

3.2.1.3. Preparation of *Citrus Limon* juice:

The *C.limons* were washed before cutting. After cutting, the pieces were squeezed to obtain 30 ml of juice without seeds for direct use for washing. The idea was taken from the degumming process described by Choudhury and Devi (2018) with some modifications [3].

3.2.1.4. Preparation of commercial washing agent solution

The preparation of commercial washing agents was similar to *Kolakhar* preparation with some changes [1]. Each washing agent, detergent powder (SUR), laundry detergent (TID), laundry bar (RIN), commercial indigenous laundry soap ball (LOCAL), liquid fabric stiffener (REVI), fabric conditioner (COMF), gentle liquid detergent (EZY), fabric whitener (VOX) and hair washing shampoo (SMP) was weighed one gram (1.00g) and mixed in 30 ml of distilled water at room temperature. The solution was mixed until all the agents were dissolved completely. To compensate for the filtration loss of *Kolakhar* and activated charcoal, in the case of commercial washing agents, instead of 1.50 g per 30 ml (0.05g/ml), the concentration used was 1.00g per 30 ml (0.03g/ml) done in approximation [4]. The setup can be seen in Fig 3.3.

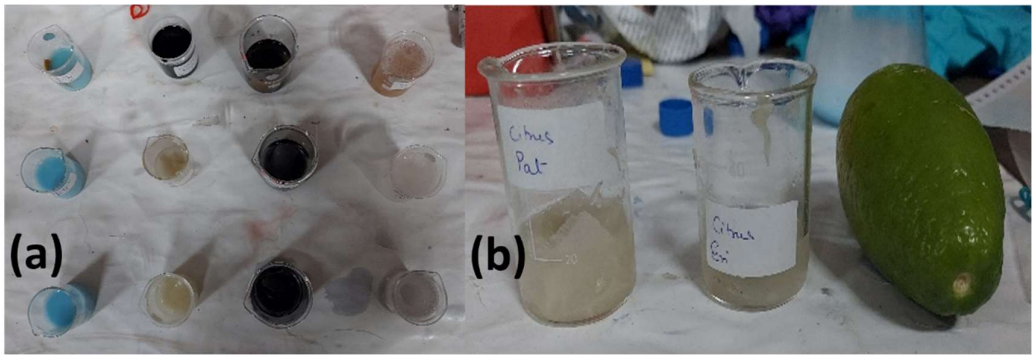

Fig 3.3: a) Washing solution of commercial agents and *Kolakhar* b) Citrus juice wash

3.2.1.5. Washing of *Eri, Muga, and Pat* silk fabric

S. ricini, *A. assamensis,* and *B. mori* silks were cut into equal weights of 60.00 mg of similar sizes of a particular silk and dipped into the commercial as well as traditional washing solutions prepared. The material-to-liquid ratio (MLR) was kept at 0.002 (g/ml) which was reduced compared to the degumming of the cocoon described by Choudhury et. al (2016) as the silks already had been degummed [5]. The solutions with the silk fabrics in them were gently shaken and allowed to rest for 30 minutes which was also adapted for degumming. It was followed as described by Choudhury and Devi (2018), with temperature taken at room temperature instead of high temperature [3-5]. The rinsing and drying set-up of washed samples is shown in Fig 3.4. The silks were taken out of the washing solution and rinsed gently in distilled water until complete removal of any remains of the washing solution. A distilled water wash control was also carried out. The samples were air-dried at room temperature for 12 hours and subsequently stored in labeled polypropylene zipped bags for analysis. All the experiments were carried out in triplicates.

Fig 3.4: a) Rinsing of washed samples of *Eri, Muga and Pat* fabric b) drying procedure

3.2.1.6 Optical microscope imaging of the surface of washed *S. ricini*, *A. assamensis*, *B. mori* silks, and their control

The changes in the fabric caused by different washing agents were checked under the microscope (Labomed, vision 2000) at 100X magnification and 40X. The sample silk was sandwiched between two glass slides and the eyepiece of the microscope was attached to a digital eye (Abron, 5 MP) connected to a laptop (Lenovo ideapad 330) for image capturing.

3.2.1.7 Scanning electron microscope (SEM)

The analysis of the surface morphology of the silk fibers pre and post-washing treatments was done in scanning electron microscope (JSM6390 LV, Jeol, Japan) visualized in JEOL software (windows-based). The accelerating voltage of 20 kV was used for the evaluation of the placed samples. The magnification was done at 100X.

3.2.1.8 Hue, Saturation, and Luminosity (HSL)

The Red-Green-Blue (RGB) values of the pre and post-washing treatments were done as per the methodologies described by Pan et al. (2009) and Saravanan et al. (2016) with some modifications [6, 7]. To maintain the constant conditions, the silk fabric samples were scanned at 600 dpi using a Canon scanner (Lide 220). The scanned samples were subjected to the determination of RGB values using ImageJ software [8]. ImageJ is an image processing software (Java-based) developed jointly by the National Institutes of

Health, Bethesda, Maryland, United States, and the Laboratory of Optical and Computational Instrumentation, University of Wisconsin–Madison, Madison, Wisconsin, United States. The software produced the RGB values and the mean of each sample. The RGB data were made in the range of 0-1. Hence, the values were divided by 255 (the highest value of RGB).

$$R' = \frac{R}{255} \qquad \text{(i)}$$

$$G' = \frac{G}{255} \qquad \text{(ii)}$$

$$B' = \frac{B}{255} \qquad \text{(iii)}$$

Using the RGB values, HSL values were calculated using the following formulas,

$$C_{max} = Max\ (R', G', B')$$

$$C_{min} = Min(R', G', B')$$

$$\Delta = C_{max} - C_{min}$$

Luminosity was calculated with the formula,

$$L = \frac{C_{max} - C_{min}}{2} \qquad \text{-----3.1}$$

Saturation was calculated using formula,

$$S = 0, \qquad \Delta = 0$$

$$S = \frac{\Delta}{1 - |2L - 1|}, \Delta <> 0 \qquad \text{-----3.2}$$

Hue was calculated using the formula,

$$H = 60^0\ x\ \left(\frac{G' - B'}{\Delta} mod\ 6\right), \text{ } C_{max} = R'$$

$$60^0\ x\ \left(\frac{B' - R'}{\Delta} + 2\right), \text{ } C_{max} = G'$$

$$60^0\ x\ \left(\frac{R' - G'}{\Delta} + 4\right), \text{ } C_{max} = B' \qquad \text{-----3.3}$$

3.2.1.9 Color difference (ΔE)

The deviation of color of different washes is calculated by the color difference from the control sample denoted by ΔE has been determined. The calculation was carried out using the RGB data in the ΔE calculator available online (http://colourmine.org/deltae-calculator / cie2000) [9].

3.2.1.10 Fourier Transform-Infrared Spectroscopy (FTIR)

The washed samples of *S. ricini*, *A. assamensis*, and *B. mori* silks with different agents were cut into finer parts (20mg) and placed in 0.5µl Eppendorf tubes separately and labeled. The samples were then subjected to FTIR analysis in Chemical Sciences under SAIC, Tezpur University. The FTIR instrument used was an FTIR spectrophotometer (IMPACT 410, NICOLET, USA), and the measure was done between 4000 to 400 cm^{-1} with step size 1.0 cm^{-1}. The analysis of the results was carried out in ORIGINPro 8.5.

3.2.2 Dyeing and its Analysis of *Eri*, *Muga*, and *Pat*

For dying of *Eri*, *Muga*, and *Pat* fabrics, the samples were cut into pieces of 250 mg.

3.2.2.1 Bleaching

All the silk pieces were subjected to bleaching separately. Bleaching was carried out using Uddin's (2014) method with some modifications. Hydrogen peroxide (35%) was mixed in distilled water at a concentration of 3ml/L. The material-to-liquid ratio (MLR) was kept at 1:50 at pH 9. The temperature was kept at 60^0C in a water bath for 60 minutes [10]. The setup is shown in Fig 3.5. After bleaching, the samples were rinsed in distilled water to remove any remaining bleaching agents. The samples were allowed to dry at room temperature indoors. The bleached samples were tagged as 'B'= Bleached.

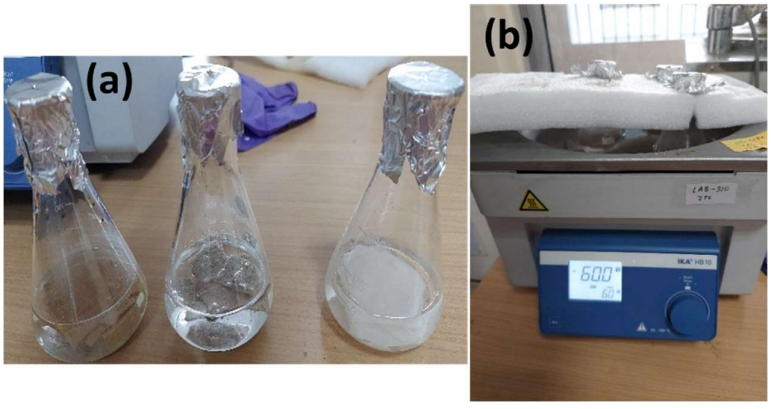

Fig 3.5: a) Samples in conical flask for bleaching b) set-up for bleaching procedure

3.2.2.2 Application of mordant

The bleached samples were subjected to the application of tannic acid as a mordant. The samples were weighed, and 5% of the obtained weight was the amount of tannic acid to be used. The MLR was 1:50 in distilled water [10]. The closed setup was suspended in the water bath at 60^0C for 60 minutes. The setup is shown in Fig 3.6. The samples were then rinsed with distilled water and allowed to air dry at room temperature indoor. The dried samples were stored in zipped polypropylene bags for further experiments. The bleached and mordant samples were marked as 'BM'= Bleached-Mordant.

Fig 3.6: Mordant application on *Eri, Muga* and *Pat* fabric in water bath

3.2.2.3 Pre-Mordant dyeing

The bleached and mordant samples were subjected to dyeing with 0.03% Curcumin solution in 50 % ethanol, as described by Han & Yang (2015) with some minor changes [11-14]. The samples were placed at 80^0C water bath for 60 minutes [10]. The samples were rinsed until complete removal of unbound dye and air dried indoors at room temperature for 12 hours. These samples are tagged as 'BMD'= Bleached-Mordant-Dye.

3.2.2.4 Post-Mordant dyeing

After carrying out the bleaching described in section 3.2.2.1, the samples were dyed with 0.03% Curcumin solution in 50% ethanol at 80^0C in a water bath for 60 minutes [10]. The silk samples were rinsed for complete removal of unbound Curcumin. The samples were air-dried in room temperature indoor. The dried samples were subjected to mordant as described in section 3.2.2.2. The samples after re-drying were stored in zipped polypropylene bags and marked as 'BDM' = Bleached-Dye-Mordant.

3.2.2.5 Simultaneous dyeing

The bleached samples were allowed to be in the solution that contained 0.03% Curcumin and 5% tannic acid (of fabric weight) and the simultaneous dyeing and mordanting were done at 70^0C for 60 minutes at water bath. After rinsing the unbound mordant and dye, the samples were air dried at room temperature and stored marked as 'SIM'= Simultaneous Dyeing.

3.2.2.6 Pre and Post dyeing

The Pre-mordant dyed samples were subjected to post-mordant, which was done as described in section 3.2.2.3. The dried samples are stored and marked as 'BMDM' = Bleached-Mordant-Dye-Mordant.

3.2.2.7 Only dye

The bleached samples were directly dyed with 0.03% Curcumin at 80^0C for 60 minutes, air dried at room temperature, and stored as 'BD'= Bleached-Dye.
All the treated samples were air-dried indoors, as shown in Fig 3.7.

Fig 3.7: Drying method after bleaching, mordanting, and dyeing *Eri, Muga,* and *Pat* fabrics

3.2.2.8 Light Fastness testing of *Eri*, *Muga,* and *Pat* silk fabrics

The samples, after treatments with mordant sequence dyeing, were tested for color fastness following methods described by Shiekh *et al.* (2006) with some modifications. Since a lower concentration of dye was used; For light fastness, the exposure to sunlight time has been reduced [15]. The treated fabric samples (25 cm^2) were exposed to sunlight for 1 hour (sides altered at 30 minutes) at lux 83860-100884 value ranging and temperature from 29^0C -31^0C and humidity ranging from 35-40%. The same exposure has been carried out for 5 cycles. In each cycle, the samples were scanned before starting the next cycle. The scanned samples were analyzed for color change as described in sections 3.2.1.8 and 3.2.1.9. The setup is shown in Fig 3.8.

Fig 3.8: Set-up for light fastness with monitor of humidity and temperature

3.2.2.9 Wash-fastness testing of *Eri, Muga*, and *Pat* silk fabrics

The treated samples with different sequences of mordant were tested for color fastness against washing. For washing colorfastness, the *Eri, Muga*, and *Pat* silk fabrics were washed at 42°C for 30 minutes and were subjected to five times of repeated wash tests in a standard soap solution according to ISO 105CO1 [16] as can be seen in fig 3.9. The dry samples, after each wash, were subjected to scanning where analysis for color change was carried out as described in sections 3.2.1.8 and 3.2.1.9.

Fig 3.9: Washing of samples to check wash-fastness at 42°C

3.2.2.10 Hue, Saturation, and Luminosity (HSL)

The Hue, Saturation, and Luminosity (HSL) of the samples were carried out as described in section 3.2.1.8. Samples of each cycle have been checked for the change in HSL.

3.2.2.11 Color difference (ΔE)

The color difference (deviation) of each cycle has been carried out as mentioned in section 3.2.1.9

3.2.2.12 FTIR of dyed samples

The treated samples were checked for their bonds due to mordant applications with dye. The FTIR test has been carried out as mentioned in section 3.2.1.10.

3.2.3 Biocompatibility of treated and untreated *Eri, Muga,* and *Pat* silk fabric

To check the biocompatibility of *Eri, Muga,* and *Pat* fabric, antioxidant assay, hemolysis assay, and antimicrobial activity have been checked.

3.2.3.1 2, 2-Diphenyl-1-picrylhydrazyl mediated antioxidant assay (DPPH)

The DPPH radical scavenging assay was carried out to evaluate the antioxidant activity of the treated and untreated samples. The method described by Jha and Matsuoka (2000) was used to carry out the experiments with some modifications [17]. Here, instead of different dye concentrations, fabric concentration was considered. A volume of 1.5 ml of DPPH solution (0.1 mM) in methanol was mixed with 10 mg, 20 mg, 40 mg, 80 mg, and 160 mg, respectively, and 3.5 ml of methanol was added to make the total volume 5 ml as seen in fig 3.10. The set-up was incubated for 30 minutes in the dark at room temperature. The samples were subjected to spectroscopic analysis at 517 nm. The equation that was used to determine the DPPH scavenging activity:

$$Antioxidant\ activity\ (\%) = 100\ x\ \frac{A_{control} - A_{sample}}{A_{control}} \qquad \text{---------- 3.4}$$

Here, $A_{control}$ is the initial absorbance of the DPPH, A_{sample} is the absorbance of DPPH after 30mins of addition of fabric.

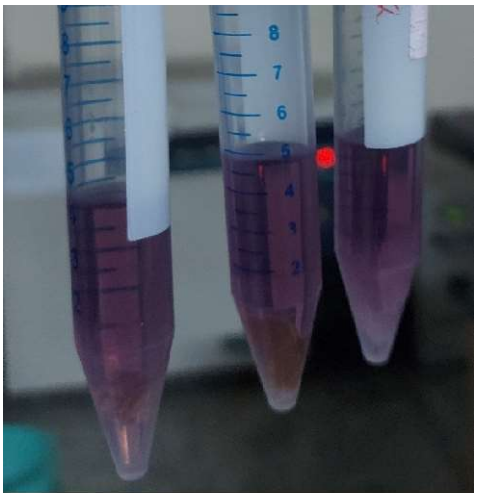

Fig 3.10: Antioxidant assay set-up of *Eri*, *Muga* and *Pat* fabrics

3.2.3.2 Hemolysis

The blood compatibility was assessed using the red blood cell (RBC) hemolytic assay. Goat blood has been collected from a Tezpur government-licensed butcher shop. Blood has been collected from the goat in an amber reagent bottle containing 1/10th volume of sodium citrate (3.8% (w/v)) of the total volume. The blood is then centrifuged at 3000 g for 10 minutes in a 50 ml centrifuge tube. The pellet containing RBC was suspended in 10 volumes of pH 7.4 phosphate buffer saline (PBS), discarding the platelet-poor plasma-containing supernatant. For the buffy layer of RBC to be entirely removed, the washing process was repeated two to three times. To obtain a homogenous suspension of cells, the cells were ultimately suspended in 10 volumes of PBS [18].

A random weight of 25 mg of each treated and non-treated sample was cut and placed in tubes to which 3 ml of blood was poured and incubated for 90 min at 37 ◦C. After the incubation period was over the samples were centrifuged at 3000 × g for 5 min to pellet out RBC cells. A positive control has been used where 0.5 ml of distilled water has been used in the tubes and for negative control, PBS has been used. The supernatants were carefully separated using a 1 ml micropette and used for absorption recording at 540 nm. The increasing absorption means more hemolysis [18, 19].

Hemolysis rate (%) = $x = \frac{A-A0}{A0-A}$ x 100 ----3.5

where A: optical density (OD) of the sample; A0: OD of PBS (negative control); and A1: 100% OD of hemolysis (water) as positive control. All values used in the formula are the average values of groups of three samples tested in parallel.

3.2.3.3 Antimicrobial activity of *Eri, Muga,* and *Pat* silk fabrics

3.2.3.3.1 Disc diffusion

The protocol of the Clinical and Laboratory Standards Institute (2013) for disc diffusion tests was conducted with slight modifications [20]. Curcumin and Tannic acid were dissolved in 100% DMSO at concentrations of 50 mg/ml and 100 mg/ml. Both were sterilized using a Nylon-66 syringe-driven filter of pore size 0.22 µm and 25mm Diameter. To perform the antimicrobial tests, fresh cultures of *Staphylococcus aureus* (MTCC 3160), and *Klebsiella pneumoniae* (MTCC 618) were made in autoclaved Mueller Hinton Broth (50 ml) [21]. Both the bacteria were received as a gift from the microbiology laboratory of Prof. Manabendra Mandal, Professor, MBBT, Tezpur University, who purchased the same from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The optical density of the media has been tested for determination of the Colony-forming Unit (CFU) using McFarland standards and diluted accordingly to obtain the desired suspension containing $1x10^8$ CFU/ml bacteria. Petri plates were poured with 20ml of Mueller Hinton Agar. The plates were allowed to solidify and left undisturbed in the LAF for evaporation of excess water. After the plates were solidified, 100 µL of the serially diluted culture containing $1x10^8$ CFU/ml bacteria were evenly spread on it. Discs of diameter 5mm were prepared and sterilized in an autoclave for disc diffusion. The disc was impregnated with 500 µg of Curcumin for Curcumin only, 250 µg of tannic acid for Tannic acid only, and 250 µg Tannic acid followed by 500 µg Curcumin for the combination type. Discs impregnated by DMSO were considered as negative control. Gentamicin sulphate was tested as a positive reference standard (10 µg) to check the sensitivity of each strain used. Another combination of Gentamicin and Curcumin was also checked for any synergistic effect on the antibiotic. Each Petri plate was allotted with 4 numbers of discs equidistant from each other. The inoculated Petri dishes were kept at 4°C in a sterile autoclaved bag for nearly 1 hour to allow the diffusion of the loaded compounds in agar. The plates were then incubated at 37°C for 16 hours in

an incubator without upside down to avoid any fall of the discs. Plates were taken out of the incubator and were checked for inhibition zones from the downside. For each plate inhibition zone was measured using a millimeter scale to check the antibacterial activity against the test organisms post 16-hour incubation. Each test compound was tested in triplicates.

3.2.3.3.2 Antibacterial Screening of Fabric

To determine the efficacy of the antibacterial properties of raw silk fabrics as well as treated silk fabrics, the AATCC 100-2004 test method, also known as the antimicrobial fabric test, has been carried out. *S. aureus* (MTCC 3160), a gram-positive bacterium, is well known for its cross-infection in hospitals which also makes it one of the most frequently evaluated species. In the case of gram-negative, *K. pneumoniae* (MTCC 618) has been opted for. These two strains were already in use as antibacterial test organisms for fabric being another reason for their selection. The untreated silk fabrics were considered as negative control and Gentamicin treated samples were considered as positive control. The fabrics were cut into 2.4 x 2.4 cm^2 and each sample was placed in test tubes and autoclaved at 121^oC for 15 minutes properly sealed. The fabrics were allowed to come to room temperature, followed by the addition of 500 µL of the 24-hour incubated culture, which was poured directly into the fabric as inoculums using a micropipette, as can be seen in Fig 3.11 (a). The media was allowed to be completely absorbed by the fabric without any free-flowing media. The fabrics were submerged in a way that no drying occurred during incubation. The fabrics were incubated at 37^oC for 24 hours in the same sterile test tubes in the incubator. After 24 hours, 10ml of sterile PBS solution was added to the fabric containing test tubes in Laminar Air Flow to maintain sterile condition. The test tubes have been vortexed vigorously to elute out all the bacterial colonies formed during incubation in the fabric as can be seen in Fig 3.11 (b). From the test tubes, 1 ml of the solution was added to 9 ml of nutrient broth with 1N sodium hydroxide as a neutralizer in another test tube. The test tubes were incubated at 37^oC for four hours after which the Optical density of the broth at 600 nm in a Split beam UV-visible Spectrophotometer (Thermo Scientific, Vantaa, Finland) was done using UV quartz cuvette (Sigma-Aldrich, Wilmington, Delaware, US) in triplicates. The entire set-up can be seen in Fig 3.11(c). To evaluate the antimicrobial activity, reduction in optical

density between the treated and untreated fabric samples after incubation. The results are expressed as a percent reduction of bacteria (R) by Eq. (1).

$$R\% = \frac{A-B}{A} \times 100 \qquad \text{------ 3.6}$$

Here, A stands for OD of untreated fabric culture, and B stands for treated fabric culture after inoculation and incubation. The experiment was done in triplicates.

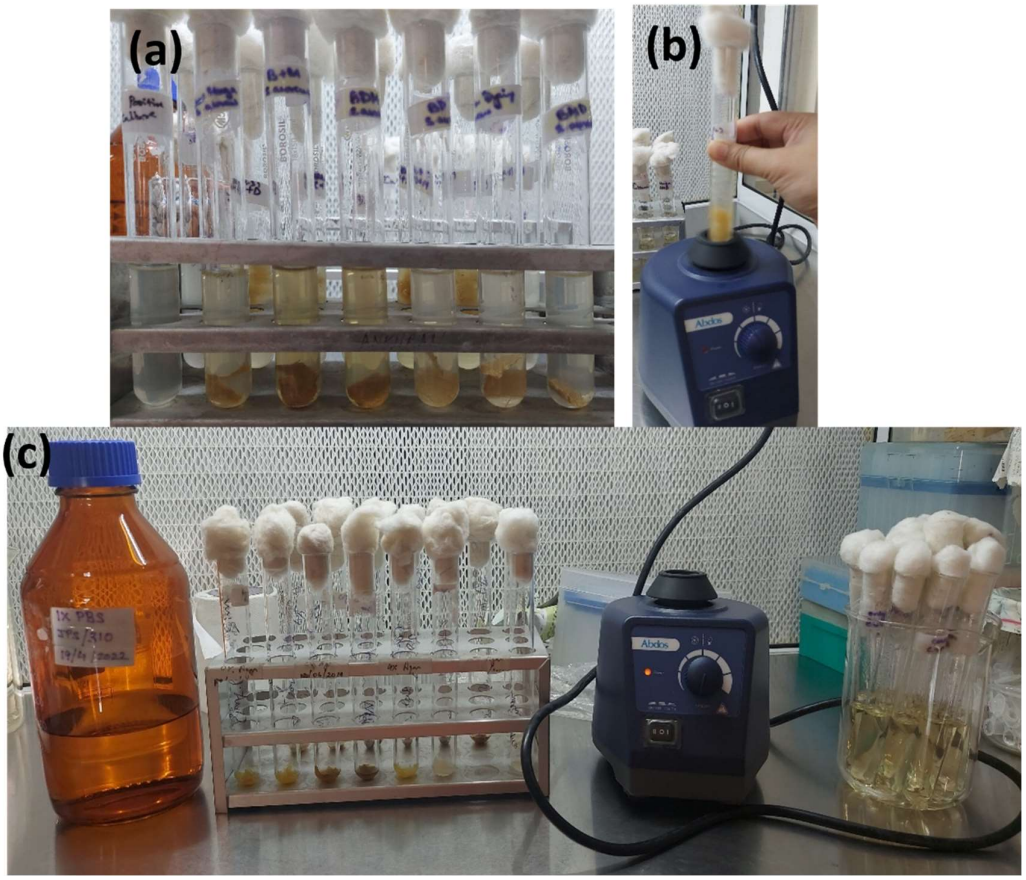

Fig 3.11: a) Inoculating test tubes with fabrics, b) Vortex of the test tubes to detach all the bacteria, c) Complete set-up of antibacterial test

3.2.4 Biodegradation

3.2.4.1 Water holding capacity (WHC) of soil

For the determination of water-holding capacity, Wilke's (2005) method was followed; a standardized method for knowing the water-holding capacity of soil against

gravity [22]. Few necessary changes were made. A perforated base cylinder was covered with filter paper in its base. The testing soil was weighed 50 g and filled in the cylinder in small portions, and homogeneous spreading was confirmed by taping the cylinder. The cylinder was capped and submerged into the water bath at room temperature, keeping the water level lower than the soil surface until the soil moistened to the surface. The cylinder was lowered after that to the soil surface and was kept in that position for 12 hours. The cylinder was removed from the water bath and kept in a tray of sand to allow the draining of excess water. Capping is important to avoid evaporation of water. The cylinder was weighed every 3 hours until a constant weight was achieved. The soil was dried at 105^0C in an oven for 24 hours and then cooled in a desiccator and re-weighed. The maximum water holding capacity was calculated following the equation,

$$WHC_{max} = \frac{m_s - m_t}{m_t - m_b} \; x \; 100 \qquad \text{-----3.7}$$

Here, m_s is the mass (g) of the beaker containing water-saturated soil. m_t is the mass of the beaker containing oven-dried soil and m_b is the mass of the beaker. For soil burial, 60% of the WHC_{max} was used.

3.2.4.3 pH of the soil

The pH of the soil was measured using pH meter. A 1:5 ratio suspension was prepared of soil and water [23]. The pH was calculated in triplicates.

3.2.4.4 Soil burial method

The samples of objectives 1 and 2 were selected to check the effect on the biodegradation of treated samples. The soil burial method was carried out using Arshad et. al (2014) and Park et. al (2004) described method with some modifications [24, 25]. Here, instead of a 1000 ml beaker, a 500 ml phyta jar was used, and hence, the sample size was also reduced to half, and so is the position height, as seen in Fig 3.12 (a). The soil was transferred to the 500ml phyta-jars. The weighed 2.5 x 2.5 cm^2 sized samples were buried in the filled jars horizontally at a depth of 4.25cm. Long term (120 days) burial period was considered. Flags were used to tag the set-ups for differentiation. The temperature and humidity were kept constant during the burial period at 25^0C and 95% by placing the setup in an incubator. After 120 days, samples were removed cautiously

and rinsed in ethanol/water (70%/30% volume fraction) solution serially for approximately 10 min, as seen in Fig 3.12 (b) before drying at room temperature as in Fig 3.12 (c). The samples were stored for analysis.

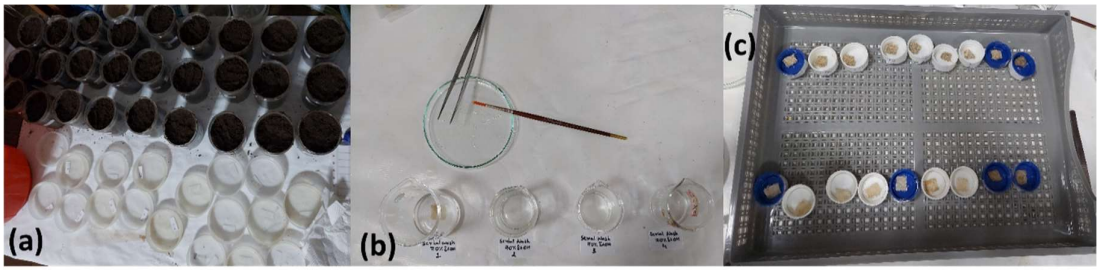

Fig 3.12: a) Phyta-jars used for soil burial b) serial washing of degraded samples c) drying of washed degraded samples

3.2.4.5 Determination of weight loss post-burial

The rinsed samples were conditioned at standard atmosphere conditions for 24 hours and then weighed. A comparison of the buried and control fabrics was done in terms of mass. The percent of mass loss is calculated as per the formula,

$$Weight\ loss\ (\%) = \frac{W_i - W_f}{W_i} x100 \qquad \text{----- 3.8}$$

Where W_i is the initial weight, and W_f is the final weight after being buried in the soil[26].

3.2.4.6 FTIR

The FTIR of the samples was carried out as mentioned in section 3.2.1.10.

3.2.4.7 Visual and Morphological Characteristics

The biodegraded samples were compared in terms of visual and morphological characteristics. For visual analysis, the samples were scanned in a scanner (Canoscan lide 220). Visual analysis shows the attack reasons in the silk samples. For morphological analysis, optical microscopy and SEM images were studied, as mentioned in sections 3.2.1.6, 3.2.1.7, and 3.2.1.8.

3.2.5 To develop a silk-based water filtration setup and a formulation for silk washing

3.2.5.1 Antimicrobial filter from the best combination

Inspired by the silk paper filter by Zheng et. al, a similar method has been applied with some modifications [27]. Here, the treated silk and the untreated silk were folded two times, to have a silk book. The weight of the silk used was 500 mg for *Pat*, 1 g for *Muga*, and 2 g for *Eri* samples. 100 ml of *Escherichia coli* contaminated water was used to check the filtration. The O.D. of the water before and after filtration has been used as the percent reduction.

$$Reduction\ \% = 100 - (\frac{\mathrm{Ab}-\mathrm{A}}{\mathrm{Ab}} x\ 100) \qquad \text{-----3.9}$$

Where Ab= O.D before filtration and Aa= O.D after filtration.

3.2.5.2 Optimization of the best washing formulation

As *Kolakhar* was found to be a better washing agent for *Muga*, through the literature survey, it was found that the main component of *Kolakhar* is charcoal and a trial has been made to check if activated charcoal can be used as an alternative for *Muga* washing. Hence, the same procedure for *Kolakhar* preparation has been used, which is also described in objective 1 for comparison. Apart from the regular comparison (SEM, colour analysis), glossiness has also been analyzed as the surface appeared to be shiny after charcoal wash. Glossiness value at 20°, 60°, 80° has been analyzed. The HSL and SEM images of *Kolakhar*, Act. Charcoal, detergent, and water had also been used for comparison. Glossiness value at 20°, 60°, and 80° of treated and raw *Muga*.

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