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Development of Antibacterial Biomaterial: Optimization method of *Bauhinia varigata* L. Extract on Cotton Bamboo Blended Fabric

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Abstract:
Aim: The present study reports the development of antibacterial biomaterial by optimization method of *Bauhinia varigata* Linn. extract. *Bauhinia Variegata* is an edible plant belongs to family (Fabaceae/Leguminosae). *Bauhinia varigata* Linn., commonly referred to as Kanchna. Ayurvedic medical practice has long utilized Bauhinia's leaves, blossoms, and stem bark as a treatment for a variety of illnesses.
Methods: Cotton Bamboo fabric (50+50 cotton-bamboo blend) were finished using optimization parameters such as herbal concentrations (1, 2 and 3%), binder concentrations (1, 2 and 3%) and wet pick-up percentages (50, 100 and 200%). The EN ISO 20645 test technique for antibacterial activity was employed as the functional test to examine all the samples under various optimum settings. The completed samples were examined using textile standards for their physical and comfort characteristics after the most optimal condition was determined.
Result: In bamboo cotton, RUN-16 finished with 3% herbal concentrates showed maximum inhibitory zones. The fabric's count, thickness, air permeability, and wicking qualities did not significantly change following the application of selected herbal concentrations. It demonstrates that ideal finishing conditions have no effect on the intrinsic characteristics of fabric samples. Statistical information from the analysis further confirmed it. It was found that higher herbal concentrations of 3% in all selected RUNs expressed stronger antibacterial inhibitory zones against the two test bacteria.
Conclusion: The obtained results emphasized that the developed antibacterial biomaterial of *B. varigata* finished fabrics shall be used for technical textile applications.

Keywords: *Bauhinia varigata*, Biomaterial, *Escherichia coli*, *Staphylococcus aureus*, Optimization study.

1. Introduction

One of the areas of the global textile industry that is expanding the quickest is biomaterial textiles. Antimicrobial fabrics are particularly useful in hospitals, especially in areas where bacteria are undesirable. Numerous germs that are easily spread from one person to another may be present on the clothing that physicians, nurses, and patients wear [1]. Certain chemicals substances are referred to be antimicrobials because they may be used to combat a variety of microorganisms [2]. Numerous prospects for significant research and development exist, transforming the spectrum into high-value items such gowns, medical linens, wound dressings, sanitary products, functional bed linens [3]. Antimicrobial properties can be provided by adding biocides to synthetic fibers before extrusion or by finishing with antimicrobial agents. Triclosan and other chemicals have the potential to cause skin irritation, behave as bio accumulators, and be nonbiodegradable [4]. Some research is being done on natural antimicrobial substances as a potential replacement to stop the growth of germs. Thus, research on environmentally friendly antimicrobial textile treatments made of essential oils, plant extracts, and other natural materials is now needed. [5]. The orchid tree Bauhinia variegata L., commonly referred to as Kachnar, is a member

of the Fabaceae family of legumes. Lavender or purple flowers are grouped in the axils of leaves. The leaves resemble the anatomy of cow hooves in the wild [6]. The plant's aerial parts include lutine, dimeric flavonoids, flavone glycosides, and β-sitosterol. stems and roots are utilized as anti-inflammatory treatments for skin conditions, dyspepsia, and snake poisoning. This plant has historically been used in traditional medicine to treat a wide range of conditions, such as inflammatory disorders. It is also used as a tonic and treatment for, ulcers, tumors, and neck pain. [7-10]. Although the literature study indicates that B. variegata plant parts have numerous therapeutic advantages, the current work examines the phytochemical composition and pharmacological assessment of B. variegata leaf extracts with regard to their antibacterial and antioxidant capabilities.

2. Materials and methods

Bauhinia varigata plant utilized in this study was gathered from several locations within the Salem district of the South Indian state of Tamil Nadu, specifically the Yercaud hill station. The Department of Agricultural and Farmer's Welfare, Karur, Tamil Nadu, verified and authenticated the plant specimens. Based on its pharmacological and therapeutic qualities, Bauhinia variegata L. was chosen from the literature. Table 1 shows the plant's taxonomy, and Fig. 1. Cotton bamboo blended (50:50) fabric are selected for the present research was presented in Fig. 2. The fabric was manufacture from Looms industry in the Tamil Nadu region of Erode.

Table 1: Taxonomy of Plant source

Order	Fabales
Family	Fabaceae
Genus	Bauhinia
Species	*Bauhinia variegata* L.

Fig 1: *Bauhinia varigata* L. plant

Fig 2: Selected Fabric sample

2.1 Extraction of powder

The plant leaves were carefully cleaned with distilled water and then allowed to dry in the shade at a temperature of 40°C until the water completely evaporated. They were then fully dried for the grinding machine and processed into a fine powder. For future usage, the powdered leaves were evaluated and stored in airtight, sealed containers. The plant component was over-dried for 24 hours at 50°C in separate, well-organized aluminum trays in order to achieve water vaporization below 10%. Before being utilized with the Soxhlet Extractor device, the dried plant material was kept at room temperature and sieved using a metal sieve to produce an incredibly fine powder. The Soxhlet idea is a method of extracting chemicals from plants using infusions. In this extraction procedure, finely ground plant powder was stored in a porous bag made of cellulose, a long-lasting filter paper. The bag was placed into the device. For this experiment, the Soxhlet Extraction infusion technique was used. After being heated, the extraction solvent evaporated into a thimble-shaped compartment, condensed, and finally drained into an upper condenser. This procedure is repeated when the liquid content is discharged into the bottom flask and the siphon arm has been reached. Plant powder was put in a thimble and extracted for six hours at 60 °C using a Soxhlet apparatus. The extraction temperature was gradually increased up to a maximum of 100 °C. An

extraction occurs through a side arm tube into a flask with a round bottom and in the heating mantle underneath. Figure 3 shows the plant after it was removed independently using a similar procedure.

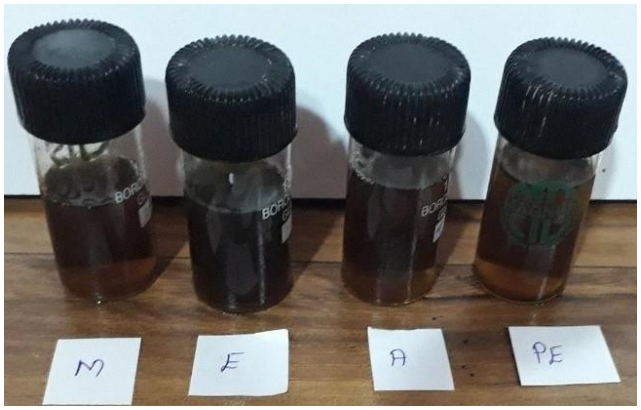

Fig 3: *Bauhinia varigata* L. extraction solvents
(M-Methanol, E-Ethanol, A-Acetate, PE- Petroleum ether)

2.2 Preparation of fabric

Each piece of scaled cloth has its dimensions cut to 10 cm × 10 cm. First, the material was soaked in ordinary fresh water at 70°C for 20 minutes. Using running water, the cleaned fabrics were carefully washed to remove any remaining dirt and other impurities. Each and every item was given a gentle hand squeeze before being let to air dry. Non-ionic detergent was used to enhance the excess starchy-like components from the fabric's surface and interstices. For 10 minutes, a typical washing machine was operated at a low speed. After washing, fabrics were left to unwind in the shade for one hour.

2.3 Finishing of fabric

2.3.1Synthesis of reactive plant dyes

This process was utilized to change the reactive and finishing plant extracts onto a few chosen cloth samples. In a water bath heated at 37°C, 2% of each plant extract was suspended in 20 milliliters of deionized water to make reactive dye. This solution was mixed with 0.04M cyanuric chloride to make the extracts reactive. Drop by drop, 0.04M NaOH was added while the suspension was maintained at 37°C. Thus, produced reactive extracts were stored at room temperature in order to finish the fabric materials.

First, a recipe solution for fabric finishing was developed. Wetting agent 0.5%, flexible acrylic binder 1%, and specific reactive plant extracts 1% (v/v) are all included in the solution. The aforementioned recipe was completed using a conventional M:L ratio of 1:20 on the selected fabric. Padding mangles were used to finish each type of reactive extract with fabric under conventional finishing settings (pH 7.0, 28^0C to 30^0C, 80% wet pick up). The next stage involved drying all of the completed cloth with extract at room temperature first, then in a standard oven at 80^0C for 10 minutes. The curing procedure was completed as the fourth phase. For the purpose of effectively attaching reactive plant dyes onto the surface and interstices of the fabric materials, all of the textiles were subjected to a high temperature for a brief period of time (120^0C/5min). After being UV-sanitized for 30 minutes in a laminar air flow cell, these samples were stored for use in future studies in a sterile environment. The images of finished fabrics were presented (Figure 4).

Fig 4: Reactive herbal dye finished fabric sample

2.4 Optimization of different factors for effective antibacterial finishing

Three different parameters were chosen to optimize the finishing conditions in order to impart efficient antibacterial qualities into fabric materials through the use of herbal extracts. Table 2 displays the optimization parameters that were used for the present study. Herbal concentration (1%, 2%, and 3%), binder concentration (1%, 2%, and 3%) and wet pick up (50%, 100%, and 200%) are the chosen parameters and their corresponding concentrations. Based on functional testing (antibacterial activity), the optimal finishing conditions for each kind of cloth were chosen from the optimization studies previously examined. About 21 trials were carried out with a fabric sample. Every experiment was given the designation "RUN" and a sequential number between 1 and 21.

Table 2: Optimization of different factors for effective antibacterial and aroma finishing

S. No.	Optimization Parameter	Concentration (%)		
1	Herbal composite concentration	1%	2%	3%
2	Binder Concentration	1%	2%	3%
3	Aroma Oil Concentration	1%	2%	3%

2.5 Optimization study - Functional tests

After finishing all the test swatches as per optimization factors, Antibacterial activity functional test was performed to identify the best optimized parameters for each type of fabric finished separately with each type of reactive herbal dyes.

2.6 Antibacterial screening of plant

Bacterial strains (Escherichia coli and Staphlococcus aureus) from the Gram Positives Research and Development Laboratory in Coimbatore were utilized in the investigation. The range of temperatures that are maintained for bacteria is 4°C. The crude extract's antibacterial activity against test organisms was assessed using the well diffusion method. For a duration of one to two days, test cultures of Staphylococcus aureus and Escherichia coli were maintained in a sterile nutritional broth. One liter of distilled water, 0.5 grams of beef extract, 1 gram of yeast extract, 2.5 grams of peptone, 2.5 grams of sodium chloride, and a final pH of 7.0 ± 0.320 make up the nutritious broth medium. Hinton and Mueller 17.0 g/L of agar, 2 g/L of beef extract, 1.5 g/L of starch, and 17.5 g/L of casein acid hydrolysate are all contained in agar plates. Final pH should be adjusted to 7.3 ± 0.321. Distinct layers of suspensions of Staphylococcus aureus and Escherichia coli inoculum were released throughout the agar surface, with a degree of around 0.1%. Surface-mounted agar plates with 6 mm wells cut out of them were kept in a sterile environment. The well was then filled with twenty microliters of each plant extract fraction. Each plate had an incubator.

For a whole day, each plate was incubated at 37ºC. The antibacterial activity was assessed on the inoculated NA plates, in the zone of inhibition, and surrounding the well. Millimeters should be used to measure and record the inhibitory clear zones.

2.7 Physical properties of finished fabrics

The physical qualities of each type of fabric were assessed after selecting an optimal condition, and they were contrasted with those of control unfinished materials. The physical characteristics include air-permeability, wicking, weight, thickness, and count of the fabric. The finished fabrics differences as well as those between the unfinished and finished fabrics were statistically determined.

2.7.1. Fabric Count

The fabric count is the number of threads in a square inch or square centimeter of cloth. To determine the fabric count, use a pick glass to count the weft and warp per centimeter in line with ASTM D 3887-96. The fabric was counted at ten different sites within the chosen textiles, and the average number of weft and warp per centimeter was calculated.

2.7.2. Fabric Thickness

The distance between a material's top and bottom surfaces under a specific pressure is what the American Society for Testing and Materials refers to as the thickness of textile materials. Fabric thickness is measured with a thickness gauge. To make sure there are no wrinkles in the fabric, a portion of it is placed on the instrument's reference plate. The gauge reading is obtained when the pressure foot has been lowered gently and has rested on the cloth for thirty seconds. The sample's average measured thickness is determined by taking the mean of the 10 measurements made at different locations on the cloth.

2.7.3. Absorbency test - Wicking properties (AATCC TM 197)

The samples were conditioned in a standard atmosphere with 65% relative humidity and a temperature of 22°C for a whole day. Every test, which was set out on a glass slide, was precisely measured to be 1.5 cm by 5 cm and was kept entirely submerged in distilled water in a reservoir. The wicking height of the flowing liquid front was visually assessed as a function of time after five minutes. A standard ruler scale was used to assess each sample's water absorption color on the fiber surface, and the findings were noted.

2.7.4. Air-Permeability test (ASTM D 737-96 test method)

The air volume in cubic centimeters that is passed through one square centimeter of fabric per second under a pressure of one centimeter head of water is known as the fabric's air permeability. The Shirley Air Permeability Tester is a device that may be used to assess air permeability. As per ASTM D-737-96 Test Method, air permeability was measured. Unless otherwise indicated in a material specification or contract order, the specimens were evaluated in the standard environment for testing textiles, which is 21 ± 1°C and 65 ± 2% relative humidity. To prevent changing the material's inherent condition, every kind of fabric specimen was treated with extreme care. The test was conducted in accordance with the manufacturer's operating instructions after each test specimen was put onto the test head of the test apparatus. Every test result was documented in terms of SI units, namely cm3/s/cm2.

3. Results and Discussion

3.1 Optimization of different factors for effective antibacterial finishing

Optimization of effective antibacterial finishing of reactive plant extracts on to cotton fabrics were studied using selected factors. The functional parameter of antibacterial activity for each sample was measured in terms of inhibitory zones and the values and its respective images were presented in Table-3, Fig. 5 for Cotton+Bamboo.

3.1.1 Cotton+Bamboo

In Table-3, antibacterial activity of cotton+bamboo finished with *Bauhinia varigata* extracts for each RUN was presented. During the analysis, all the RUNs showed inhibition zones against both test bacteria. Among all the experiments, Maximum inhibitory zones of around 32 mm and 33 mm, respectively, were shown by RUN-16 against Escherichia coli and Staphylococcus aureus. RUN-11 showed almost similar inhibitory zones of 33mm and 31mm for the respective test bacteria. RUN-17 expressed inhibitory zones of 32mm and 32mm against *Escherichia coli* and *Staphylococcus aureus.* In RUN-5 and in RUN-21 inhibitory zones of about 31mm, 32mm; and 31mm, 31mm was found evident against the respective test organisms. Cotton+bamboo blended samples, RUN-16 showed more antibacterial activity which was finished with 3% *Bauhinia varigata* extracts. This indicated that lower concentrations of herbal extracts (1%) and binder (1%) and lower wet pick-up percentage 50% showed lesser inhibitory zones compared to the higher concentrates and higher wet pick-up percentage.

Table 3: Experimental setup for optimization studies

Run	Factor-1: Herbal concentration (%)	Factor-2: Binder concentration (%)	Factor-3: Wet pick up (%)	Response: Antibacterial activity (ISO 20743)	
				E. coli	*S. aureus*
1	1	1	50	29	27
2	1	2	100	29	28
3	1	3	200	28	29
4	1	1	100	29	30
5	**1**	**2**	**200**	**31**	**32**
6	1	3	50	29	28
7	2	1	50	28	30
8	2	2	100	30	30
9	2	3	200	30	30
10	2	1	100	30	29
11	**2**	**2**	**200**	**33**	**31**
12	2	3	50	29	28
13	3	1	50	30	29
14	3	2	100	30	30
15	3	3	200	31	29
16	**3**	**1**	**100**	**32**	**33**
17	**3**	**2**	**200**	**32**	**32**
18	3	3	50	29	30
19	1	1	200	28	30
20	2	1	200	30	31
21	**3**	**1**	**200**	**31**	**31**

From the current research, the cause of the herbal extract's potent antibacterial action was eagerly sought after. Interestingly, throughout our search, we came across a number of research publications discussing the biological and phytochemical substances of B. variegata that have been linked to antibacterial, antioxidant, and anticancer effects, among other qualities. The study emphasizes how the plant's strong and promising antibacterial activity is directly caused by secondary metabolites such anthraquinones, alkaloids, flavonoids, saponins, phenolics, terpenoids, and tannins that are present in the solvent extracts of Bauhinia variegata. Numerous studies demonstrating the importance of the various phytochemical components of the chosen plant were also discovered to support this concept. The function of flavonoids in B. variegata was previously discussed by Dixon et al. (1983). In these states, plants produce flavonoids—hydroxylated phenolic molecules—in response to microbial infection.

According to Aboaba & B. M. Efuwape (2001), these plants contain non-toxic glycosides that, following hydrolysis, give phenolics that are detrimental to microbial pathogens. The significance of terpenoids and their antimicrobial efficacy against various microbes were documented by Sikkema et al. (1994). The researchers also noted that antibacterial activity was demonstrated by membrane destabilization and proton motive force loss in the bacterial membrane structures, which resulted in cell disintegration and death. The antibacterial qualities of higher plants are ascribed to secondary metabolites such alkaloids, flavonoids, tannins, and other phenolic compounds, according to Pandey (2007) and Mahomoodally et al. (2005). The function of flavonoids in B. varigata was previously discussed by Dixon et al. (1983); they said that flavonoids are hydroxylated phenolic compounds that plants produce in reaction to microbial infection. Thus, the antibacterial activity of finished fabric samples containing B. varigata extract revealed the presence of several phytochemical components, which were proven to be responsible for the outcomes seen in this study.

3.2 Physical and comfort properties

In this present study, many physical properties that contribute to its comfort qualities were examined. The finished and unfinished samples were nomenclature in Table-E for better understanding. The same nomenclature was used in the following analysis.

Table 4: Nomenclature of the finished and unfinished fabric samples

S.No.	Types of fabric samples	Nomenclature
1	Cotton+Bamboo	CB3
2	Cotton+Bamboo finished	CBF3

3.2.1 Fabric count - Wales per centimetre (WPC)

Wales per centimeter (WPC) fabric count is regarded as one of the most important comfort characteristics of fabric materials that are finished with herbal extracts. All of the fabric samples that were analyzed in terms of fabric count (WPC) revealed the following observations. When compared to unfinished fabric samples, CBF3 to their corresponding unfinished CB2 samples, they showed increases of +4.54%, respectively. The fabric samples that were treated with herbal extracts had just a little increase in count in terms of wales per centimeter (Fig. 5).

Table 5: Fabric count of CB3 and CBF3 Fabric samples (WPC)

S. No.	Samples	Fabric count (WPC)	% loss or gain	Statistical analysis Between the fabrics and between unfinished and finished fabrics	
				F-Value	Significance
1	CB3	21	-	F = 1.0	P = 1.0
	CBF3	22	+4.54		

The fabric count of the CB3 and CBF3 fabric samples was determined statistically using the F value. The previously cited Table 5 unequivocally demonstrated that there was no statistically significant difference (P = 1) between CB3 and CBF3 in terms of fabric count in relation to Wales per centimeter (WPC).

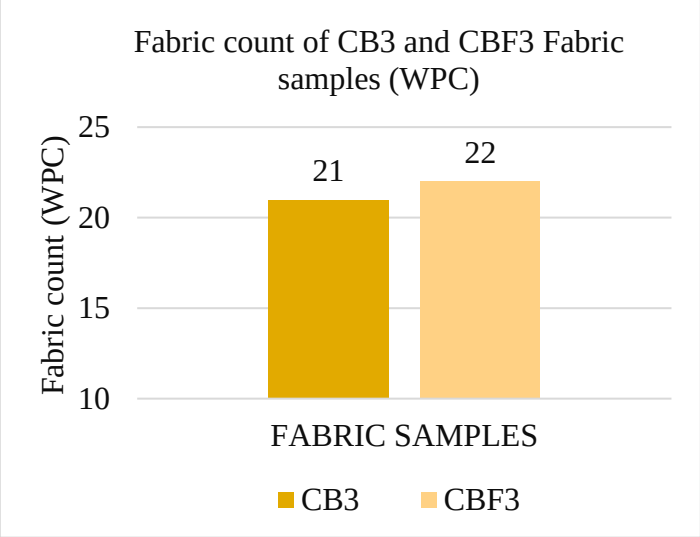

Fig. 5: Fabric count of CB3 and CBF3 Fabric samples (WPC)

3.2.2 Fabric count - Course per centimetre (CPC)

The fabric count measured in terms of courses per centimeter (CPC) is regarded as one of the most important comfort characteristics of fabric materials finished with herbal extracts. In terms of fabric count (CPC), the following findings were observed in every fabric sample evaluated. When comparing finished fabric samples made from herbal extract to unfinished fabric samples, CBF3 to their corresponding unfinished CB3 sample, they showed increases in fabric count of +5.26%, respectively. In fabric samples, the use of herbal extracts resulted in a marginal increase in the count per centimeter of course in the final samples (Fig. 6).

Table 6: Fabric count of CB3 and CBF3 Fabric samples (CPC)

S. No.	Samples	Fabric count (CPC)	% loss or gain	Statistical analysis Between the fabrics and between unfinished and finished fabrics	
				F-Value	Significance
1	CB3	18	-	F = 1.0	P = 1.0
	CBF3	19	+5.26		

The fabric count of the CB3 and CBF3 fabric samples has been determined statistically using the F value. The previous Table 6 demonstrated that there was no statistically significant difference (P = 1) between CB3 and CBF3 in terms of fabric count in relation to Course per centimeter (CPC).

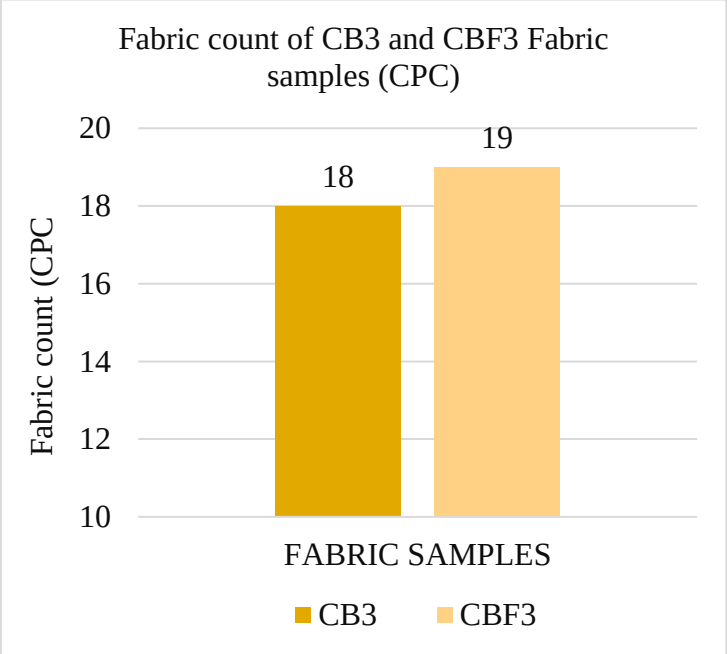

Fig. 6: Fabric count of CB3 and CBF3 Fabric samples (CPC)

The variation in fabric counts before and after ending with herbal extracts was shown in Tables 4 and 5 as a percentage increase or loss among the samples. When comparing finished fabric to unfinished textiles, there was no discernible change in the fabric count in terms of Wales per centimeter and Course per centimeter, according to the table values and statistical analysis (F = 1 and P = 1).

3.2.3 Fabric thickness

Fabric thickness of the CB3 and CBF3 fabric samples was measured in millimeter. Among the herbal extract finished fabrics, CBF3 exhibited +2.77% increase in fabric thickness compared to its respective unfinished CB2 samples (Fig. 7).

Table 7: Fabric thickness of CB3 and CBF3 Fabric samples

S. No.	Samples	Fabric thickness (mm)	% loss or gain	Statistical analysis Between the fabrics and between unfinished and finished fabrics	
				F-Value	Significance
1	CB3	0.35	-	F = 1.0	P = 1.0
	CBF3	0.36	+2.77		

The fabric thickness of the CB3 and CBF3 fabric samples was statistically determined using the F value. The previous Table 7 made it evident that the CB3 and CBF3 fabric samples thicknesses did not differ statistically significantly (P = 1).

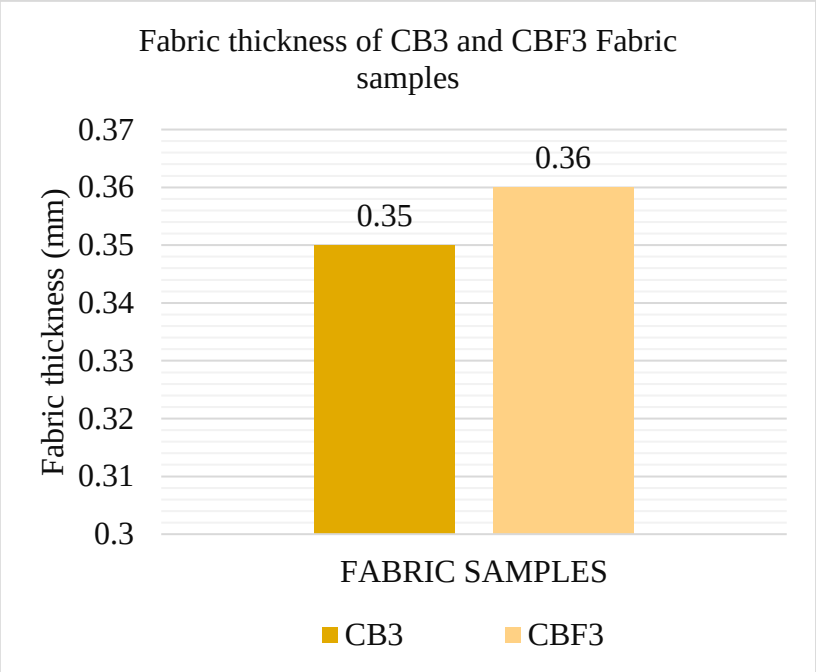

Fig. 7: Fabric thickness of CB3 and CBF3 Fabric samples

3.2.4 Air permeability Test

In Table-8 and Fig. 8, air permeability in terms of unit (m3/cm2/sec) was evaluated for all unfinished and finished fabric samples.

Table 8: Air permeability values of CB3 and CBF3 Fabric samples

Samples	Air-permeability ($m^3/cm^2/sec$)	% loss or gain	Statistical analysis Between the fabrics and between unfinished and finished fabrics	
			F-Value	Significance
CB3	20.7	-	F = 1.0	P = 1.0
CBF3	20.3	-1.93		

The F value was utilized to statistically evaluate the air-permeability of both CB3 and CBF3 fabric samples. The previous Table 8 made it evident that the air-permeability of the CB3 and CBF3 fabric samples did not differ statistically significantly (P = 1).

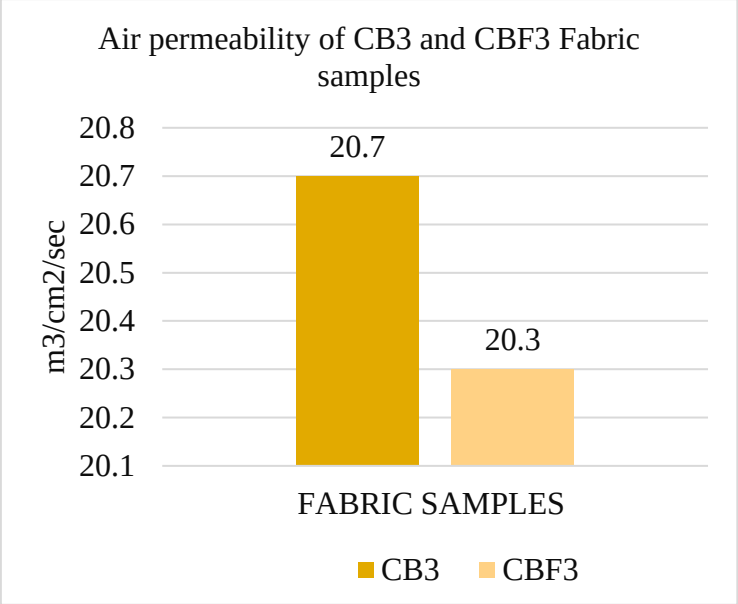

Fig. 8: Air permeability of CB3 and CBF3 Fabric samples

The air-permeability between the unfinished fabrics was analysed first and found no significant difference among the samples. Among the finished fabric sample the air permeability was found to be CBF3, about 20.3m3/cm2/sec of air permeability value was exhibited. From this analysis in terms of air-permeability, *Bauhinia varigata* extract finished samples showed almost similar air-permeability values of unfinished fabric samples. This was evident from a marginal percentage loss of about -1.93% between the finished and unfinished samples (Table 8).

3.2.5 Wicking properties

Table 9: Wicking properties of CB3 and CBF3 Fabric samples

Samples	**Wicking absorbency (cm)**	**% loss or gain**	**Statistical analysis Between the fabrics and between unfinished and finished fabrics**	
			F-Value	**Significance**
CB3	4.6	-	F = 1.0	P = 1.0
CBF3	4.5	-2.23		

The F value was utilized to statistically evaluate the wicking properties of both CB3 and CBF3 fabric samples. The previous Table 9 made it evident that the wicking properties of the CB3 and CBF3 Fabric did not differ statistically significantly (P = 1).

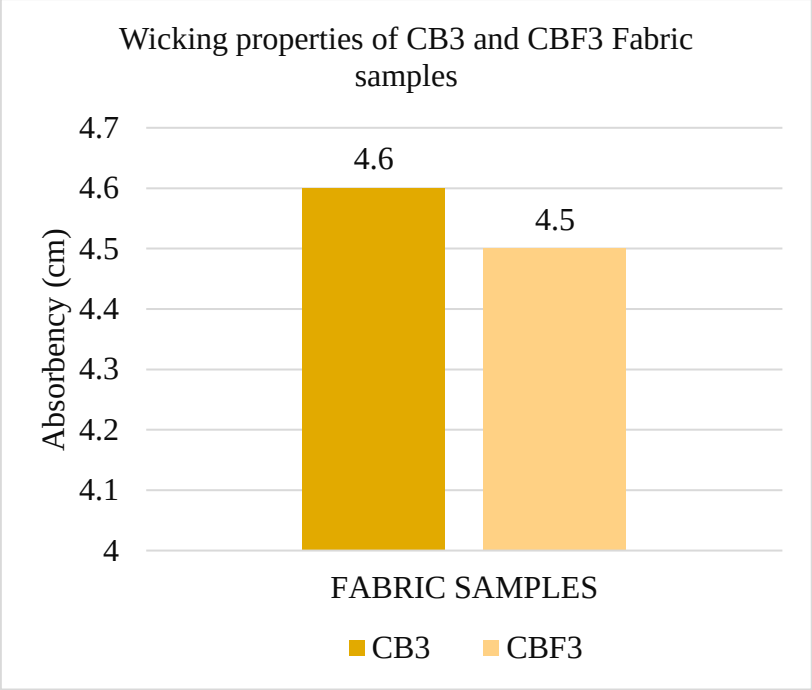

Fig. 9: Wicking properties of CB3 and CBF3 Fabric samples

In Table-9 and Fig. 9, wicking or absorbency property of CB3 and CBF3 Fabric samples were evaluated. The results were interpreted based on the ability of fabrics absorb more water vertically; the height of water absorbed along the fabric surface was noted and measured in centimetre. CBF3 samples also showed negligible decrease in absorbency -2.23% respectively; which was confirmed from percentage loss values presented in Table-9.

4. Conclusion

With the aim of finding development of antibacterial biomaterial by optimization method of *bauhinia varigata* l. Extract on cotton bamboo blended fabric**,** the present study was studied. Higher herbal concentrations of 3% in all optimally selected RUNs were found to exhibit greater antibacterial inhibitory zones against both test microorganisms during the study. The literature review revealed that many kinds of important phytochemical components are present in the herbal extracts. The obtained results underscored the need for using B. variegata finished fabrics' antibacterial activity in biomedical and biotextile applications for a range of human ailments that are spread through textiles and fabrics used in hospitals and healthcare facilities.

5. Conflict of interest

Authors declare no conflict of interest in the present research

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