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In vitro antibacterial and antioxidant activities of cotton fabrics treated with bael fruit shell extract

Sukumar Roy[1], Sourav Banerjee[2], Mangala Joshi[1], Satya Narayan Naik[2,3], Subbiyan Rajendran[4] & Wazed Ali[1,2,a]

[1]Department of Textile and Fiber Engineering, [2]School of Interdisciplinary Research (SIRe), [3]Centre for Rural Development and Technology, Indian Institute of Technology Delhi, New Delhi 110 016, India

[4] School of Engineering, University of Bolton, Bolton BL3 5AB, United Kingdom

The present study is aimed at evaluating the antibacterial and antioxidant properties of bael fruit shell (BFS) extract. Hot water extraction (HWE) and ultrasonic assisted extraction (UAE) techniques have been used to understand the effectiveness of the extraction process and its relation to impart enhanced functional property of cotton fabric. The cotton fabric has been treated with BFS extract by using padding mangle. The effectiveness of antibacterial activity against both the Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and antioxidant property has been evaluated both qualitatively and quantitatively. The results reveal that UAE-BFS treated fabric shows 93% reduction for *E coli* and 82% for *S aureus*, which is higher as compared to HWE-BFS treated fabric (91% for *E coli* and 61 % for *S aureus*). This trend has also been observed in qualitative zone of inhibition method. Antioxidant efficacy of UAE treated fabric is 86%, whereas HWE treated fabric registers 80% activity.

Keywords: Antibacterial, Antioxidant, Bael fruit shell, Biomedical application, Cotton

1 Introduction

The current pandemic situation demands the increasing use of antimicrobial textiles, that are prone to pathogenic microbes, such as hospitals and other healthcare settings. It is highly likely that textiles and garments used by the doctors, healthcare workers, patients and other frontline teams who are managing the infectious patients may carry a broad range of disease causing and infectious pathogens and transmit them from one person to another[1].

It should be mentioned that antimicrobial textiles refer to any fabrics or garments that protect against the growth of pathogenic microorganisms, such as bacteria, virus and so on. Synthetic and natural polymers and chemicals that impart antimicrobial property to textile can target a broad range of microbes and they are called as antimicrobial agent[2]. Unfortunately, there is growing evidence that textiles act as disease vectors, transmitting infectious diseases from person to person especially in healthcare settings. In order to prevent or reduce the chances of transmission of microbes from one person to another, textiles are incorporated with antimicrobial functionality[3,4]. In addition to imparting antimicrobial property, textiles can also be capable in delivering antioxidant property. The antioxidant textiles can serve as a storage system which can gradually provide the required antioxidants to our skin. Basically, when antioxidant textiles are in contact with skin, it would help to scavenge the free radicals produced by skin degeneration and protect tissues from oxidative damage[5]. Antioxidant dressings regulate the correct balance between low or high levels of Reactive Oxygen Species (ROS), which are essential for effective wound healing process[6]. A case study demonstrates that antioxidant wound dressing enhances the healing of hard-to-heal wounds[7]. It should be noted that antioxidant and antibacterial properties can also be imparted to face masks, patient outfits and other special garments for persons who have various skin diseases[8].

Nanoparticle based antimicrobial agents are increasingly used mainly because of their high surface area to volume, that enhances the antimicrobial effectiveness. It should be mentioned that among different methods, mostly coating is used to imbue nanoparticle antimicrobial agents into both natural as well as synthetic textiles. Silver nanoparticles have the potential to destroy a broad range of microbes along with lower toxicity towards human cell[9]. In addition to silver nanoparticles, there are other metal and metal oxide nanoparticles, like zinc, copper, tin, and titanium, which have also been used to impart antimicrobial property on natural and synthetic textiles.

[a]Corresponding author.
E-mail: wazed@textile.iitd.ac.in

There are mainly three mechanisms which are responsible for antimicrobial activity of functionalized copper oxide nanoparticles (CuONP) coated textiles against both the Gram-positive and Gram-negative bacteria. The mechanisms involved are: generation of reactive oxygen species; direct interaction of nanoparticles with bacteria; and the release of copper ions[10]. Currently, application of inorganic nanostructured materials prepared by biosynthesis gain popularity in developing antimicrobial textiles[11]. In green agriculture research, several ecofriendly nanohybrid polymers such as oligochitosan (obtained from crab shells) and nano-silica (obtained from rice husk), are used to produce antimicrobial fabrics and they are resistant against *Phytophthora infestans* fungus[12]. Besides, several antimicrobial agents have been biosynthesized for use in fabrics. Animal products, essential oils and plant extract like neem[13], aloe vera[14], honey,[15] etc. have been used to impart antimicrobial property in fabrics, wound dressings and other medical related applications. It is reported elsewhere that different plant and animal based bioactive agents are gaining popularity to produce green based eco-friendly antimicrobial textiles[16]. Similarly, sustainable technology is also gaining attention of the industries. Natural antimicrobial compounds like cyclodextrin have been used in sustainable textile finishing. The cyclic oligosaccharides possess hydrophilic outer surface and a lipophilic central cavity. The cyclodextrin and its derivatives (α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin) are widely used in producing antimicrobial textiles by sustainable finishing technology[17]. Lignin, which is extracted from the sugarcane bagasse, is also used for antimicrobial coating of fabrics against *Staphylococcus epidermidis*[18]. Chitosan is also used for producing antimicrobial and anti-odor fabrics[19]. It should be mentioned that chitosan is a cationic polysaccharide, which is obtained by alkaline deacetylation of chitin[20].

Bael or *Aegle marmelos* (fruit, leaf, shell) is widely used in traditional ayurvedic medicine because of its excellent dietary and nutritive values. It is interesting to mention that bael fruit, leaf, root extract showed significant antimicrobial activity against pathogens[21,22]. Recent studies have demonstrated that phytochemical present in bael plant has the potential to prevent COVID-19 spread and acts as an anti-coronavirus agent[23]. It is worth to mention that the textile application of bael fruit and its associated parts has not been studied but a few researchers have already used it for effluent treatment, as a bio adsorbent and as a source of activated carbon[24-26]. Bael fruit is edible and has more nutritive values and is used to produce various products, such as jam, jelly, juice, candy, squash, powder, capsules, toffee, wine, etc.[27]. The bael fruit contains bioactive compounds, namely pectin, tannins, flavonoids, alkaloids, carotenoids, phenolic acid, terpenoids, and coumarins[28]. A study has reported that bael fruit pulp contains 0.42 % of tannic acid, whereas the fruit shell possesses 1.03 % tannic acid[29]. Results showed that bael fruit contains 1755-3000 mg per 100 g of polyphenols combined with tannic acid[30]. Total phenols and tannins are lower, when compared to green bael shell, in ripe fruit shell perhaps they degrade due to sugar synthesis in peel and fruit over time. Also, the original bitter taste of unripe fruit diminishes with tannin reduction[29].

The antibacterial effect of bael against *Shigella dysenteriae* is reported elsewhere[31]. The inhibitory effect is due to the present of coumarin compounds present in the extract. Pandey[28] studied the antibacterial properties of bael fruits, leaves and peels against various pathogens. He reported that highest antibacterial efficacy against pathogens is found in fruits of the bael as compared to those in peels and leaves. Several studies have reported the antioxidant property of the aqueous extract of bael fruit pulp[32]. In addition, the bael fruit beverage contains high amounts of total phenolic compounds (83.89/37.6 mg gallic acid equivalents/100 mL), which are responsible components for good antioxidant property and show high value in DPPH (2,2-diphenyl-1-picrylhydrazyl) assay[33].

It is recognised that nowadays consumers are conscious of health, environment and sustainability issues and they are keen in using biobased hygiene textiles. This encourages researchers for developing novel eco-friendly and sustainable textile based medical products. The present study focuses on developing novel antibacterial and antioxidant cotton fabrics utilizing unripe bael fruit shell (as it contains maximum tannin) extracts for healthcare and other biomedical applications.

2 Materials and Methods

2.1 Materials

Bael fruit (*Aegle marmelos*) was collected from Indian Institute of Technology (IIT), Delhi campus

(India). It was cleaned thoroughly in tap water, rinsed in distilled water, dried for one week at room temperature (~30°C) followed by separating the shell from the fruit. Then fruit shell was converted into fine particles by crushing and grinding in a mixer grinder for 15-20 min and stored in an airtight container. 100% plain woven bleached cotton fabric (80 g/m^2) was used for bael fruit shell (BFS) extract treatment.

2.2 Methods

Extraction of Bael Fruit Shell

Two types of extraction methods, namely hot water extraction (HWE) and ultrasonic assisted extraction (UAE), have been used.

Hot Water Extraction (HWE)

BFS powder (25 g) was immersed in 250 mL water at 100 °C for 60 min using a hot plate (Lab Companion TS 14SG) (Fig. 1). The crude extract was filtered repeatedly several times using cloth sieve followed by Whatman filter paper 40 (pore size, 8 µm). BFS powder was isolated from the extract by using rotary evaporator.

Ultrasonic Assisted Extraction (UAE)

In the case of ultrasonic-assisted extraction, an ultrasonic bath was utilized as an ultrasonic supply. BFS powder (25 g) was immersed in 250 mL water and kept in a laboratory sonication bath at room temperature (~30°C) for 30 min. The sonication water bath has a rectangular container (24 cm × 13 cm × 10 cm) in which 50 kHz transducers were galvanized at the bottom side. The bath power rating (250 W) varies from 40% to 100%. Cavity that forms due to ultrasonic wave in ultrasonic bath results in shear disruption of cell as well as contraction of cell membranes, which leads the solvent to penetrate into BFS powder efficiently (Fig. 1). This helps in causing mass transportation of BFS powder extracted molecules to get diffused into the water. Mechanical and cavitation impacts are main factors for the ultrasonic extraction purpose[34,35], The crude UAE based extract was filtered using the procedure as mentioned above (hot water extraction process).

2.3 Characterization of BFS Extract

Phytochemical analysis was carried out to identify various secondary metabolites in BFS extract. The ferric chloride test was performed to analyse the presence of tannin in BFS extract.

UV-Vis absorbance of the BFS extract was studied using UV-Vis spectroscopy (Shimadzu, Japan) in the wavelength range of 200-400 nm.

2.4 Fabric Finishing

The bleached cotton fabric (10 cm × 10 cm) was treated with both HWE and UAE solutions (100g/L). The fabrics were dipped in the solution for 10 min and padded through laboratory padding mangle for 2-dip-2- nip operation. Finally, the padded fabrics were dried at 100°C for 5 min. The add-on percentage was maintained at 5% in both the cases.

2.5 Characterization of the Finished Fabric

FTIR Analysis

FTIR analysis was carried out using a Thermo-fisher Scientific Nicolet Is50 FTIR instrument. The samples were scanned in the range of 4000-400 cm^{-1} in transmittance mode. The fabric samples were studied in ATR (Attenuated Total Reflection) mode, whereas the analysis for the extracted powder sample was performed in DRIFT (Diffuse Reflectance Infrared Fourier Transform) mode.

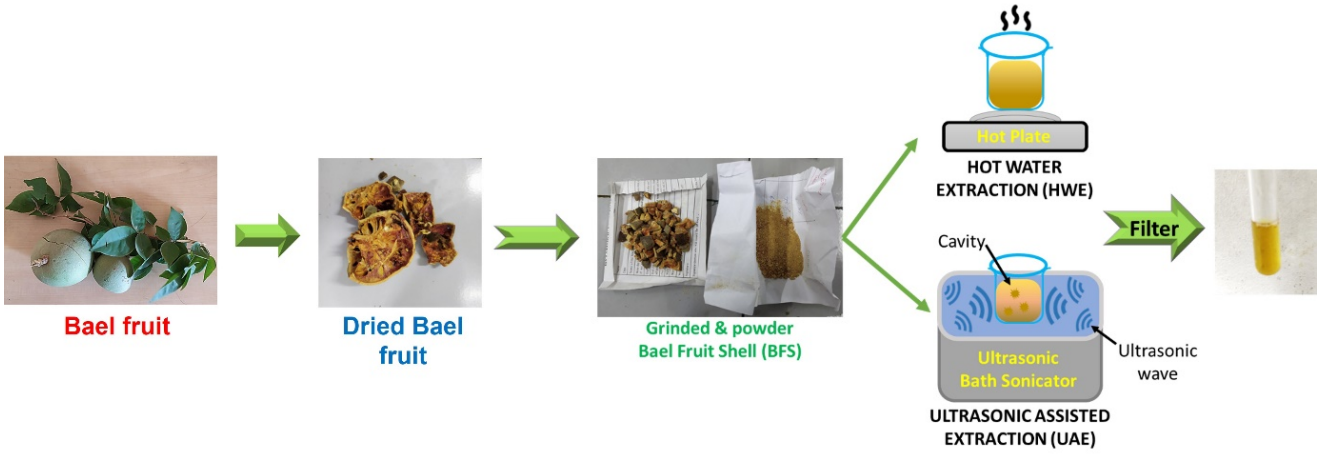

Fig. 1 — BFS extraction process

Antibacterial Test

The antibacterial activity of the untreated and the finished cotton fabrics was tested qualitatively and quantitatively against both the Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria by disc diffusion (AATCC 90) and reduction percentage (AATCC 100-2004) methods. For AATCC 100, circular swatches of 4.8 ± 0.1 cm in diameter were cut from the test fabric. The cut pieces were stacked in 250 mL conical flux followed by sterilization at 121 °C for 15 min. Then 0.5 mL of the bacterial suspension was added to the swatches so that whole of it is absorbed by one swatch. Just after inoculation ("0" contact time), 50 mL of sterilized saline water was added to the conical flux followed by 15 min shaking in a laboratory shaker. After that 100 µL of this diluted bacterial suspension was spread into the prepared nutrient-agar plate and left for 24 h in an incubator at 37 °C. The additional conical flux containing inoculated treated swatch was kept for 24 h in the incubator at 37 °C ("24" h contact time). After 24 h, 50 mL of sterilized saline water was added to this conical flux followed by 15 min shaking in the shaker. As mentioned above, 100 µL of this diluted bacterial suspension was spread uniformly into the nutrient-agar plate and left for 24 h in the incubator at 37 °C. After 24 h, the number of bacterial CFU on the Agar plate was counted. Reduction percentage was calculated using the following equation:

$$R\ (\%) = \frac{(A-B)}{A} \times 100 \qquad \ldots (1)$$

where R is the % reduction; A, the number of bacteria recovered from the inoculated treated test specimen swatches in the conical flux immediately after inoculation (at "0" contact time); and B, the number of bacteria recovered from the inoculated treated test specimen swatches in the conical flux incubated over the desired contact period.

For disc diffusion method, the nutrient-agar solution was prepared by adding 2 g each of Luria broth and agar-agar to 100 mL of deionized and distilled water. The concentrations of the bacteria suspension were approximately 1.6×10^6 CFU/ mL. 30 mL of the nutrient-agar solution was added to the Petri dishes and allowed to solidify. 100 µL of the bacterial suspension was then spread evenly onto the Petri dish. Then 20 mg of each sample was sterilized and placed on the Petri dish. The plates were then incubated at 37°C for 24 h and the zone of inhibition was measured after 24 h.

Analysis of Antioxidant Property

In this method, the control and treated fabric samples were added to 3.5 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl) methanol solution, which was shaken into a shaking bath for 25 min at 25°C in dark environment. The absorbance of the treated and untreated sample containing DPPH was measured at 517 nm. The following equation was used to calculate the antioxidant % or radical scavenging efficiency:

$$\text{Radical scavenging efficiency (\%)} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of blind control sample}} \times 100 \qquad \ldots (2)$$

2.6 Physical Properties of Finished Fabrics

Both the treated and control samples were conditioned at 25°C, 65 % RH for 24 h. The tensile strength of fabrics along warp and weft directions was carried out as per ASTM D 5035 (2006) using universal testing machine (UTM, Tinius Olsen, England, H5KS). Ten samples were analyzed for each test, and the average was calculated. Bending length measurement was performed for both the finished and control samples using Eureka Bending Length tester as per ASTM D 1388-08. The colour strength of the fabric samples was measured utilizing Gretag Macbeth, Color Eye-7000-A.

3 Results and Discussion

3.1 Characterization of BFS Extract Treated Fabrics

3.1.1 FTIR Analysis

FTIR spectra of HWE powder, UAE powder and BFS extract treated cotton fabric are shown in Figure 2. The peak at 3126 cm^{-1}, in the case of UAE powder, is due to the stretching of -OH bond of polyphenolic groups. It indicates the presence of hydroxyl groups of tannins and alkaloids[36]. But there are no such groups observed in the case of HWE powder. Also, the peak at 1566 cm^{-1} is due to the presence of C=C stretching of cyclic alkenes. The UAE powder peak at 1414 cm^{-1} again indicates the -OH bending of phenol or tertiary alcohol. On the other hand, HWE treated cotton fabric shows the peak at 3321 cm^{-1} and for the UAE treated cotton fabric, the peak is shifted to 3276 cm^{-1} from 3334 cm^{-1}. This shift is due to the interaction of hydroxyl groups (polyphenols in cotton structure) present in UAE powder. However, the absence

of -OH group in HWE powder does not show any major shifting in HWE treated fabric. The UAE treated fabric is showing a peak at 1597 cm^{-1} for C=C stretch which is also a shifted peak at 1566 cm^{-1} of the powder sample. The presence of intense hydroxyl groups in unripe fruit shell of UAE is due to higher tannin contents which are increasingly responsible for altering various functional properties. Also, ultrasonic action enhances the penetration of water into the matrix of fruit shell powder. As the cavity formed in ultrasonication disrupts cell of fruit shell, it proceeds to wash out more phytochemical components from the UAE-BFS powder[37-39].

3.1.2 Phytochemical Analysis

The presence of hydrolysable or condensed tannins in the extract is determined by adding a few drops of 5% ferric chloride into HWE and UAE powder solution. The colour change indicates their presence. A bluish black colour precipitation indicates that the extract contains hydrolysable tannins (gallo tannins and ellagitannins), whereas the condensed tannin shows brownish green. It is observed that the UAE solution produces prominent black color, which indicates the presence of hydrolysable tannin in BFS extract (Figure 3). It is reported that bael fruit shell contains hydrolysable gallotannin[38]. The major responsible groups for colour changes in BFS are polyphenols, tannins like gallic acid and gallotannin.[26,27,40]

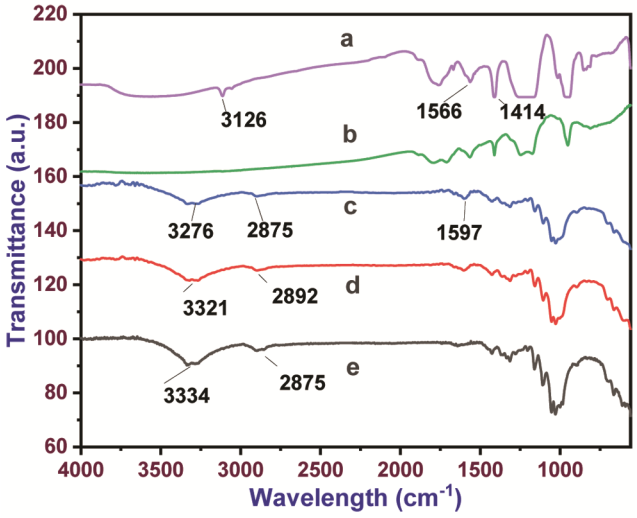

Fig. 2 — FTIR spectra of (a) UAE powder, (b) HWE powder, (c) UAE treated cotton fabric, (d) HWE treated cotton fabric, and (e) control cotton fabric

3.1.3 UV-Vis Absorbance of Extracts

Figure 4 shows the UV-Vis absorbance characteristics of BFS extract. The analysis was carried out at a wavelength range between 200 nm and 400 nm. Due to the presence of tannins in the extract, the UV spectrum shows absorption at 220-350 nm. It is evidenced that about 9% and up to 20% tannins are present in fruit pulp and shell of bael respectively. It should be noted that tannin is also present in leaves as skimmianine[41]. Peak absorbance for HWE and UEA at 312 nm and 315 nm indicates the presence of gallic acid and gallotannin.

3.2 Functional Properties

3.2.1 Antioxidant Efficacy

The phenolic and tannin components of the BFS treated fabric are seen as useful scavengers to the free radicals of DPPH and their remarkable scavenging efficacy is shown in Fig. 5 and Table 1. It is reported elsewhere that antioxidant activity of bael fruit is caused due to presence of tannin, flavonoids and other polyphenols[21,42,43]. HWE BFS

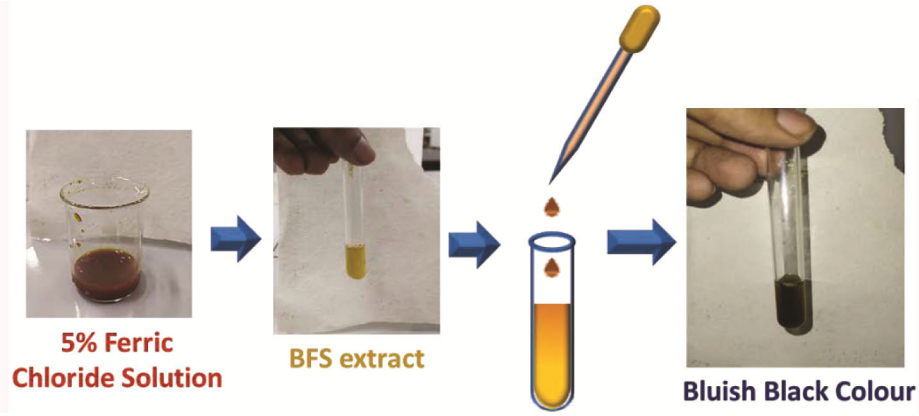

Fig. 3 — Ferric chloride colour changing test

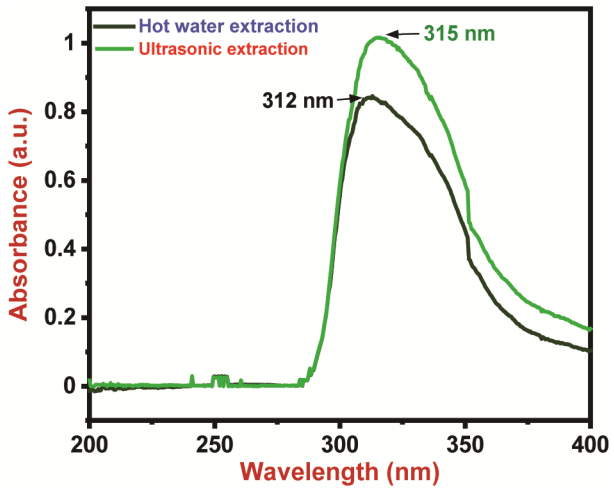

Fig. 4 — UV-Vis absorbance of BFS extracts

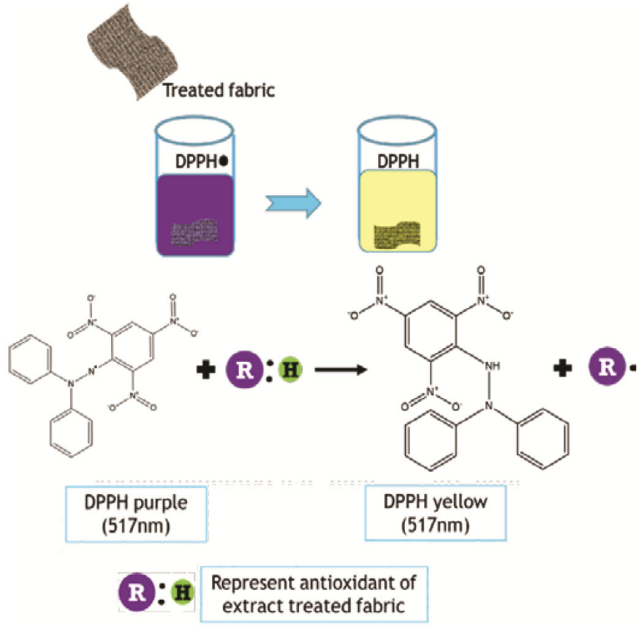

Fig. 5 — Mechanism of DPPH assay

treated fabric shows 80.5% scavenging activity and UAE treated fabric shows the maximum efficacy of 86.5%. The increased value for UAE extract treated fabric may be due to more biomolecules (polyphenols) extracted through the cavity in ultrasonic technique than hot water extraction. It should be noted that the presence of polyphenols is reaffirmed in FTIR analysis (Fig. 2). Further, it could be possible that tannin breaks into smaller fragments due to higher temperature in hot water extraction, which results in lower activity in HWE than UAE[21].

Table 1 — Antioxidant activity of BFS treated fabrics

Sample	Absorbance (at λ_{max} = 517 nm)	Antioxidant efficiency, %
Blank	2.4214	-
Control cotton fabric	2.4214	0
HWE-BFS treated fabric	0.4720	80.5
UAE-BFS treated fabric	0.3247	86.5

Table 2 — Antibacterial activity of BFS treated fabrics

Sample	Zone of inhibition mm		Reduction %	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
Control	0	0	-	-
HWE-BFS treated fabric	1.2	0.7	91	61
UAE-BFS treated fabric	2.0	1	93	82

3.2.2 Antibacterial Effect

Qualitative assessment of antibacterial activity of the treated fabric is done by disk diffusion method as shown in Fig. 6. It can be seen from Table 2 that the sample treated with UAE shows higher inhibition zone (2 mm for *E coli* and 1 mm for *S aureus*) as compared to HWE treated sample (1.2 mm for *E. coli* and 0.7 mm for *S. aureus*). This trend agrees with the trend published elsewhere[26], in which the hot water extraction registers lower antibacterial activity than the ultrasonic extraction. It should be mentioned that the chemical compounds, responsible for antibacterial effect of BFS, are tannins, phlobatannins, saponins, terpenoids, alkaloids and various other polyphenols[24]. The presence of these chemical compounds in BFS is evidenced in FTIR and phytochemical analyses.

Quantitative evaluation of antibacterial activity of the treated fabric is carried out by calculating the reduction percentage. Antibacterial activity of all the samples is shown in Figure 7. As shown in Table 2, the UAE-BFS treated fabrics show 93% reduction for *E. coli* and 82 % for *S. aureus*, which are higher as compared to those of HWE-BFS treated fabrics (91% for *E. coli* and 61 % for *S. aureus*). This substantiates the higher antimicrobial activity of ultrasonic extracted sample as compared to hot water extraction. The absence of hydroxyl groups in HWE-BFS powder, as observed in FTIR spectra (Fig. 2) indicates that the absence of polyphenolic compounds results in lower antibacterial activity for HWE-BFS treated fabric.

3.3 Physical Properties

The tensile strength, bending length and colour strength of BFS treated fabrics are presented in Table 3. It is observed that the tensile strength retention of HWE treated fabrics in both warp and

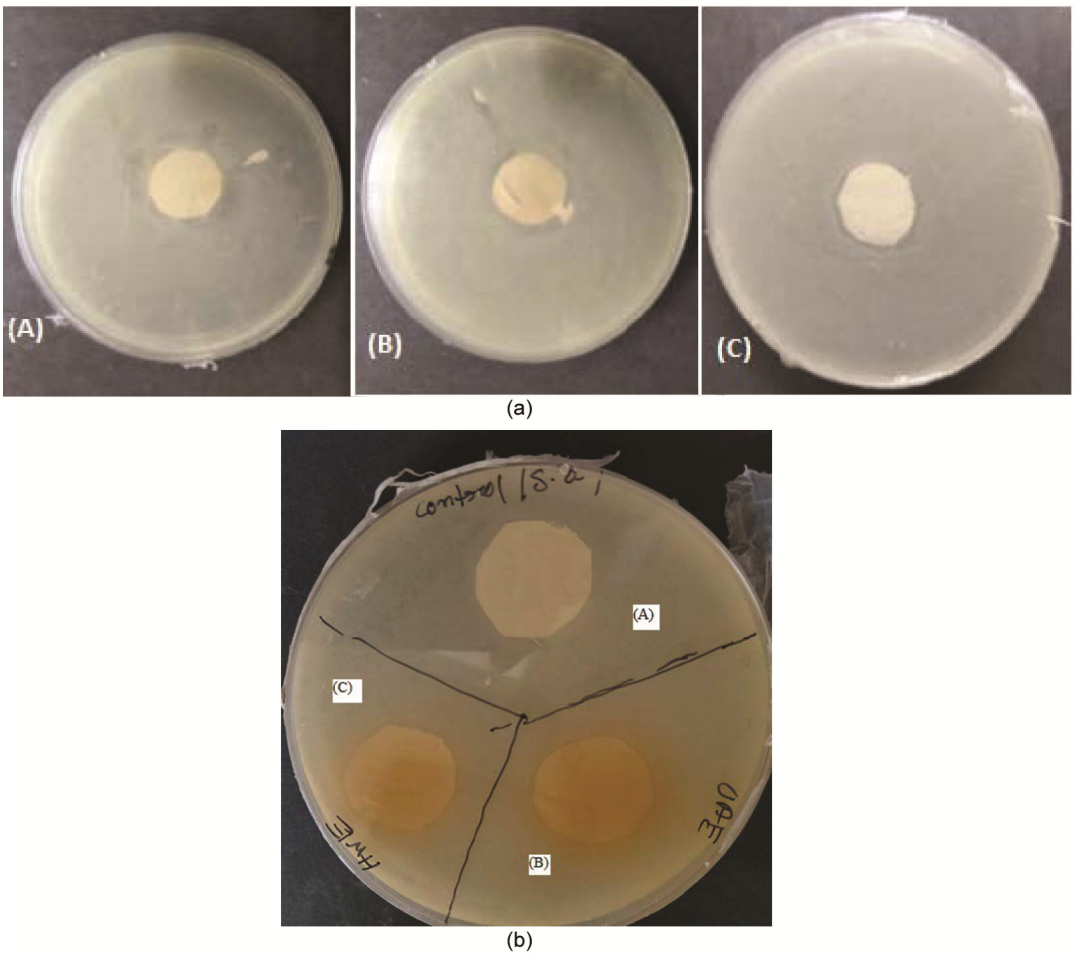

Fig. 6 — Zone of inhibition of the sample (A) control, (B) UAE, (C) HWE treated fabric against (a) *E coli* and (b) *S aureus* bacteria

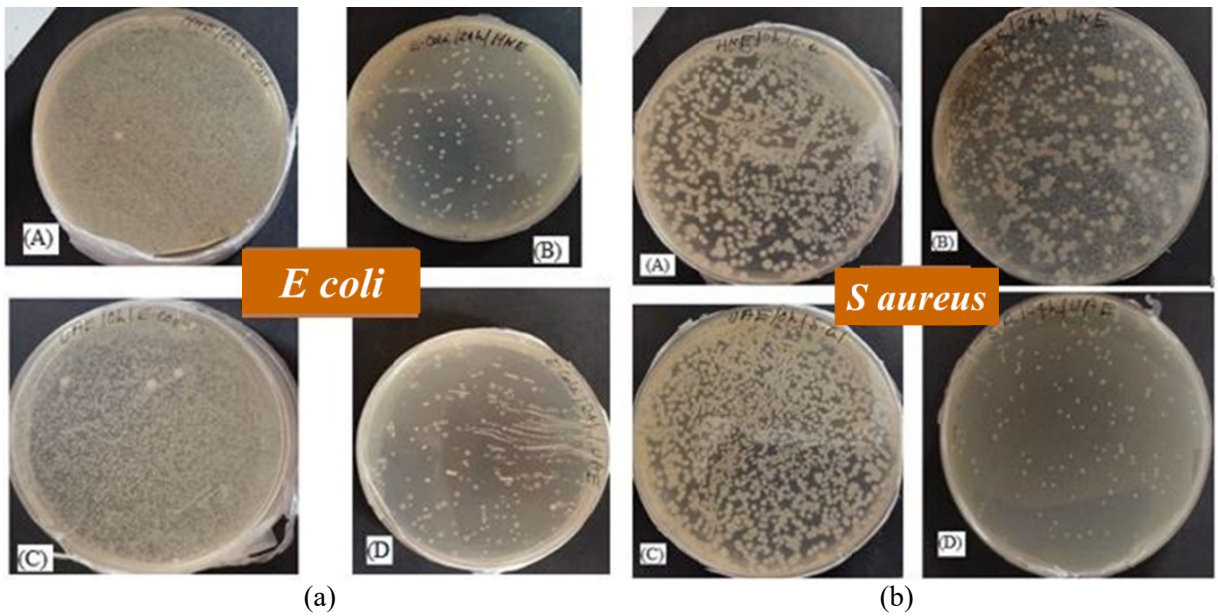

Fig. 7 — (A) CFU of HWE-BFS treated fabric at "0" contact time, (B) CFU of HWE-BFS treated fabric at "24" h contact time, (C) CFU of UAE-BFS treated fabric at "0" contact time, (D) CFU of UAE-BFS treated fabric at "24" h contact time against (a) *E. coli* and (b) *S. aureus* bacteria

Table 3 — Physical properties of BFS treated fabrics

Sample	Strength retention %		Avg. bending length, cm		Avg. colour strength (*K/S*)
	Warp	Weft	Warp	Weft	
Control	-	-	3.1	2.6	1.922
HWE-BFS treated fabric	94.5	95.1	3.6	3.4	4.684
UAE-BFS treated fabric	98.5	98.9	3.3	2.9	4.959

weft is similar (95%). However, the UAE treated fabrics (warp and weft) exhibit 99% strength retention. The results ascertain that BFS treatment (HWE and UAE) does not influence the tensile strength of fabrics. In addition, it can also be observed in Table 3 that both the treated fabrics show a limited variation in increase in bending length (warp and weft) than the untreated; perhaps due to the attachment of biomolecules in the fabric structure which makes the fabric slightly stiffer. The attachment of biomolecules in the treated fabric structure is reaffirmed, as evidenced (Table 3) by the fact that the colour strength of treated fabrics is noticeably high than that of untreated sample. It is also evidenced from Table 3 that the colour strength of UAE-BFS treated fabric is higher (4.96) than that of HWE-BFS treated ones (4.69), justifying the fact that the number of active biomolecules attached in UAE-BFS is high.

4 Conclusion

In this study, it is observed that UAE-BFS treated fabric exhibits higher antibacterial activity (93% reduction for *E. coli* and 82% for *S. aureus*) than HWE-BFS treated fabric (91% reduction for *E. coli* and 61% for *S. aureus*). A similar trend is also observed in zone of inhibition method. Samples treated with UAE show higher antimicrobial activity (inhibition zone 2 mm for *E coli* and 1 mm for *S aureus*) than HWE treated samples (inhibition zone 1.2 mm for *E coli* and 0.7 for *S aureus*). The efficacy of UAE technique is further substantiated by higher antioxidant activity (86.5%) of the BFS extract treated samples than HWE-BFS treated (80.5%) ones.

The active ingredients of tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols, which deliver antibacterial activity, in BFS is confirmed by FTIR analysis and phytochemical testing. The strength retention of BFS treated fabrics shows a marginal increase for UAE treated fabrics (98% for both warp and weft direction) than HWE treated ones (95% for both warp and weft).

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