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Application of Enriched Fraction of Seabuckthorn Leaf Extract as Antimicrobial Finish on Technical Textile

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ABSTRACT

Flavonoid-rich fraction (FRF) from Seabuckthorn leaves extract was prepared by acid hydrolysis process. Total flavonoid content of Seabuckthorn leaves extract and FRF estimated as rutin equivalent was found to be 116.98±3.06 and 277.14 ± 6.78 mg/g of extract/FRF respectively. Its major constituents myrcetin, quercetin, kaempferol and isorhamnetin, were determined by reverse phase high performance liquid chromatography (RP-HPLC). Aramid (NomexIIIA) fabric was treated with triethylene tetramine to increase the wicking height of the fabric for better uptake of FRF. Then, FRF was coated using citric acid as cross linking agent on to aramid fabric by pad-dry-cure method for improved wash durability. FRF coated fabric was characterised using universal attenuated total internal reflection Fourier Transform Infrared spectroscopy. Effect of FRF coating on flammability property of coated fabric was estimated using flammability tester. There was no significant difference in the char length of the FRF coated fabric and control samples. Antimicrobial activity of the FRF coated fabric was assessed by both qualitative (agar diffusion method; AATCC 147-2001) and quantitative (percentage reduction test; (AATCC 100-2001) methods using test organisms. The zone of inhibition by agar diffusion method for *E. coli* and *S. aureus* was found to be 12.4 mm and 16.7 mm, respectively. Quantitative assessment by percentage reduction test showed a reduction percentage of 96.00 per cent and 93.00 per cent for *S. aureus* and *E. coli*, respectively. The results of the above study indicate FRF as a valuable ingredient for the development of antimicrobial textiles.

Keywords: Antimicrobial activity; Flavonoids; Seabuckthorn; Nomex IIIA

1. INTRODUCTION

Hippophae rhamnoides commonly known as seabuckthorn (SBT), a wild shrub of family Elaeagnaceae, is being used in different parts of the world for its medicinal and nutritional properties[1]. It is a dwarf to tall (3 m – 4 m in height), branched and thorny nitrogen fixing deciduous plant, native to Europe and Asia. A wide spectrum of pharmacological effects of SBT have been reported recently such as antioxidant, immunomodulatory, antiatherogenic, anti-stress, hepatoprotective and tissue repair properties[2,3]. Every part of the plant is considered to be a rich source of biologically active substances like flavonoids, carotenoids, vitamins (C, E and K), tannins, glycerides of palmitic, stearic and oleic acids and some essential amino acids[4] which contribute to its wide usage as a natural antioxidant and antimicrobial agent[5-7].

Textiles provide an excellent environment for the growth of the microorganisms because of their large surface area and their ability to retain the moisture. This growth leads to various undesired effects on the fabric such as unpleasant odour, degradation of the fabric and other aesthetic changes such as stain formation and discolouration. Such infected fabrics causes allergy, irritation, infection of the skin of the wearer. Functional aspects such as antibacterial and UV protection thus play an important role in the development of fabrics [8-11].

Antimicrobial finishing of textile materials aims to protect the users from the harmful microorganisms and to ensure a proper functionality of textiles[12]. An ideal antimicrobial treatment should provide protection against a wide range of bacterial and fungal species. The treatment should be durable to washing, dry cleaning, hot pressing and compatible with textile chemical finishing processes such as dyeing and also it has to be cost effective[13]. A number of chemicals such as, benzophenone, organometallics, iodophors (substances that slowly release iodine), phenols and thiophenols, metallic salts, antibiotics, heterocyclics with anionic groups, nitro compounds, ureas and related compounds, formaldehyde derivatives and amines[14-16] have been employed to impart antimicrobial activity to textiles. Many of these chemicals, however, are toxic to humans and do not easily degrade in the environment. In recent years, the textile industry looks for non toxic, non allergic and eco-friendly natural antimicrobial agents that do not adversely affect the quality of the textile material[17] as substitute for synthetic toxic chemicals. The herbal antimicrobial finishes overcome the disadvantages of the chemical finishes because they are eco-friendly, non toxic and also non allergic[18,19]. Some studies

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have been reported on herbs that contain a wide variety of compounds having beneficial antimicrobial activity and hence used for antimicrobial coatings on the fabrics[20-22]. The earlier studies have looked into the broad spectrum of antimicrobial agents of SBT that are responsible for Anti-microbial properties, while there is no published literature on the application of the enriched fraction of SBT on the technical textiles.

The present investigation primarily aims at developing an eco-friendly natural antimicrobial finish from SBT leaf for textile application. Enriched fraction of SBT leaf extract was applied to Nomex IIIA fabrics and screened for their antimicrobial activity. The coated fabric was characterised by Fourier Transform Infrared Spectroscopy (FTIR) for the confirmation of the loading of the active agent on the fabric surface. An extensive study was conducted to assess the antimicrobial effectiveness of Enriched fraction of SBT leaf extract by employing standard test methods.

2. MATERIALS AND METHODS

2.1 Fabric sample

Nomex IIIA (NIIIA) fabric was used in the present study. Specifications of the fabric are (Blend of 93 per cent Nomex, 5 per cent kevlar, 2 per cent nylon; Count: Warp – 2/40s, weft-2/40s).

2.2 Collection of plant material

The SBT leaves were collected from the hilly regions of the North-West Himalayas (the region lies between latitude 32-36^0 North and longitude 76-79^0) in the month of September, where the plant grows widely under natural conditions. Plant material was characterised by an ethnobotanist at the Defence Institute of High Altitude Research (DIHAR), Leh, India.

2.3 Preparation of Seabuckthorn leaves crude extract

Fresh leaves were cleaned thoroughly with ultrapure water, dried under shade in a clean and dust-free environment. Maceration method was used to prepare the 70 per cent ethanol extract of SBT leaves. Here the powdered dry leaves were soaked with 70 per cent ethanol (1:5 w/v) at room temperature (25 ± 1^{o}C). After 24 h, the supernatant was decanted and the residue was re-soaked in fresh solvent. The process was repeated three times for complete extraction. The supernatant was pooled, filtered through muslin cloth, and centrifuged at 5000 *g* (Remi PR 24) for 10 min at 4 ºC. Ethanol content of the hydroalcoholic extract was evaporated using rotary evaporator (Buchi R-124 Labortechnik AG, Postfach, CH-9230, Flawil, Switzerland) at 40 ºC. Finally, the supernatant solution was lyophilised (Lyophilizer, HITOSICC, Heto-Holten A/S, Denmark) and the dried extract was stored in an air tight dark bottle at 4 ºC.

2.4 Preparation of Flavonoid Rich Fraction

0.5 g of obtained crude extract was dissolved in 500 mL of 2 N HCl : Methanol (1:1) solution in a 1000 mL round bottom flask and refluxed at 80 °C for 1 h and 30 min. It is further cooled to the room temperature and the methanol content was removed in a rotary evaporator. The aglycone part was extracted several times with ethyl acetate. Finally the flavonoid rich fraction (FRF) was obtained by the removal of ethyl acetate content in a rotary evaporator[23].

2.5 Determination of Total Flavonoid Content

One mL aliquot of appropriately diluted sample solution was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a 5 per cent $NaNO_2$ solution. After 6 min, 0.15 mL of 10 per cent $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 mL of 4 per cent NaOH solution was added to the mixture. Then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was measured at 510 nm against a prepared blank. Rutin was used as the standard compound for the quantification of the total flavonoid content. The flavanoid content was expressed as milligram of rutin equivalents per gram of sample[24].

2.6 Pre-treatment of Fabrics

Fabrics (size: 30 cm x 30 cm) were pre-treated at 80˚C for 60 min with 70 per cent triethylenetetramine(15 mL solution for every gram of fabric). After treatment, the samples were washed in water at 100 ˚C for 30 min, dried in an oven at 80˚C for 30 min and conditioned at 25˚C at 65 per cent RH for 60 min in an environment chamber[25].

2.7 Assessement of Hydrophilicity

The hydrophilic behaviour of the treated and untreated fabrics was analysed using wicking test (BS 4554). Fabric strip of 0.5 cm x 8 cm dimension was suspended vertically with its lower edge in contact with distilled water. A spontaneous rise in the water level in the fabrics due to capillary force was observed. The rise in the water level for a given period of time is taken as the direct indication of wicking height of the fabrics. The rise of water level in the fabric was measured at 20 s, 40 s, 60 s, 120 s, 180 s, and 240 s. The wicking height measured at 240 s was considered for the assessment of hydrophilicity of fabrics [26].

2.8 Coating onto Fabrics

SBT extract was applied on the fabric up to a wet pick up of 80 per cent by the ‘pad-dry-cure’ method. The NIIIA fabric (size: 30 cm x 30 cm) was immersed in the solution containing FRF (3 per cent W/V) for 5 min and then it was passed through a laboratory padding mangle (RGE make), which was running at a speed of 20 rpm at an applied pressure of 1.5 kgf cm^{-2}. After padding, the fabric was air-dried and then cured for 5 min at 70˚C. The treated samples were then evaluated for antimicrobial activity.

2.9 Bacterial Strains

Escherichia coli MTCC 25922 and *Staphylococcus aureus* MTCC 25293 as representative of Gram-negative bacteria and Gram-positive bacteria respectively were used for testing antimicrobial susceptibility according to AATCC standard method. The strains were cultured on nutrient agar (Hi-Media, Mumbai, India) and incubated aerobically at 37 ºC overnight.

2.10 FTIR Characterisation

The functional group analysis of the FRF-coated fabric was determined using attenuated total internal reflection Fourier Transform Infrared (ATR-FTIR) Spectrophotometer.

The spectra of the NIIIA and FRF-coated NIIIA fabric samples were recorded using Perkin Elmer (Spectrum 100) spectrophotometer in the range of 4000 cm^{-1}- 650 cm^{-1}. ATR consists of Zn-Se single crystal that collects the spectra from the fabric of dimensions 10 mm x 10 mm placed on to it. Pressure was applied to the samples to ensure good contact between the sample and the crystal to prevent the loss of the incident IR radiation. The spectra were obtained for each sample at a resolution of $4cm^{-1}$ with 32 scans.

2.11 Flammability test at an angle of 45° (ASTM D1230)

Coated and control NIIIA fabric sample of 5 cm x 15 cm of fabric surface was inserted in a Flammability tester at an angle of 45°. A standardised flame (16-18mm) as per ASTM standard[27] is applied to the surface near the lower end of the fabric for 12 s, and the char length generated after 12 s exposure was recorded.

2.12 Qualitative and Quantitative Antimicrobial Activity Assessment

Antibacterial activity was evaluated by both qualitative and quantitative test methods. The following are the descriptions of test methods employed for this study. Qualitative assessment was carried out by agar diffusion method (AATCC 147-2001). Treated and untreated control fabric samples were placed in intimate contact with AATCC bacteriostasis agar which has been previously inoculated (Mat culture) with inoculums of test organisms. After incubation, a clear area of interrupted growth underneath and along the side of the test material indicates the antibacterial effectiveness of the fabric.

Quantitative assessment was done by percentage reduction test (AATCC 100). Specimens of the test material were shaken in a known concentration of bacterial suspension and the reduction in bacterial activity at standard time duration was measured. The efficiency of the antimicrobial treatment is determined by comparing the reduction in bacterial concentration of the treated sample with that of control sample expressed as a percentage reduction in the standard time duration.

per cent Reduction = $A\text{-}B/A \times 100$

Where A and B are the number of surviving cells (CFU/mL) in the flasks containing the control (untreated NIIIA fabric) and test samples (SBT-treated NIIIA fabric), respectively, after 18 hr of contact time duration.

3. RESULTS AND DISCUSSION

3.1 Extraction of SBT, Determination of Total Flavonoid Content and Application on to NIIIA Fabrics

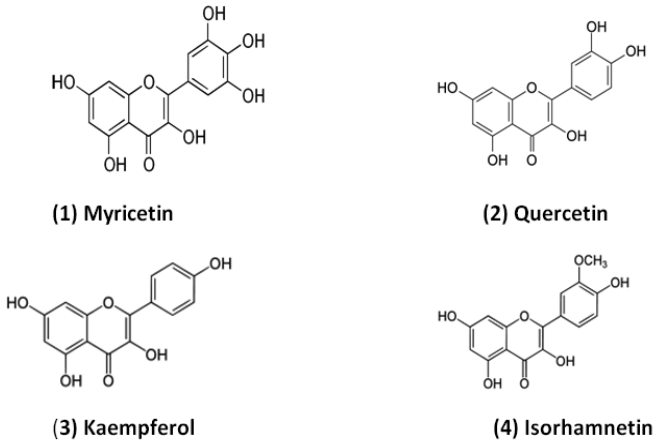
In our previous study, SBT leaves crude extract was applied on the fabric and studied the antimicrobial activity of the fabric[22]. Therefore in the present study, Flavonoid-rich fraction (FRF) was prepared from this crude extract (SLE) by acid hydrolysis process with a yield of 19.80 per cent. Total flavonoid content estimated as rutin equivalent was found to be 116.98 mg/g for SLE and 277.14 mg /g for FRF, indicating that total flavonoid content is higher in FRF. Dry add-on percentage of the NIIIA fabric coated with 3 per cent FRF solution with wet pick up of 80 per cent using the 'pad-dry-cure' method was found to be 3.9 per cent.

A simple and gradient elution-based reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the qualitative analysis of SLE and FRF for various major constituent myricetin, quercetin, kaempferol and isorhamnetin. Various solvent systems, including different combinations of acetonitrile, methanol and water with ortho phosphoric acid were tried in order to develop an effective mobile phase. Finally, a solvent system of acetonitrile:methanol (75:25) containing 0.3 per cent ortho phosphoric acid in water was proved to be successful. This allows the separation of maximum number compounds with better resolution. Myricetin, quercetin, kaempferol and isorhamnetin (Fig.1(a)) that might contribute to the antimicrobial behaviour were identified in SLE and FRF by HPLC (Figs. 1(b) and 1(c)). Identification of compounds was performed by coinjections and spectral matching of standards. The results shown in Figs. 1(b) and 1(c) indicate the presence of higher myricetin, quercetin, kaempferol and isorhamnetin contents in FRF in comparison to SLE.

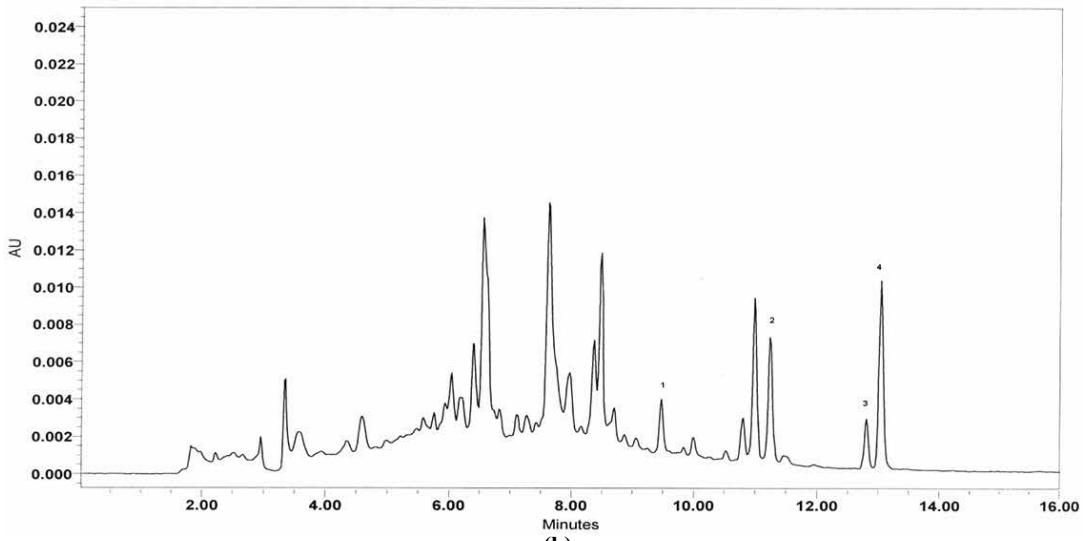
Flavonoids form a class of benzo-γ-pyrone derivatives includes flavones, flavanes, flavonols, anthocyanidines, and catechins. They possess a wide spectrum of biological activities such as anticancer, antibacterial, antifungal, antiviral, spasmolytic, hypoglycaemic, antihistaminic and radioprotective potential[23,28]. Some of these activities derive from the free radical-scavenging properties of flavonoids. There are many reports relating to the reactivity of flavonoids with active oxygen species. Recent interest in these substances has been stimulated by the potential health benefits arising from their antioxidant and antimicrobial activities.

3.2 Effect of Amine Treatment on the Hydrophilicity of the Fabric

The effect of amine treatment on the hydrophilicity of the fabrics was determined by measuring the wicking height at different interval of time (20 s to 240 s). Graph was plotted using wicking height (mm) against time (s) shown in the Fig. 2. Wicking height of the treated fabric was found to be more than two times that of the untreated fabric[22]. This indicates that the amine treatment enhanced the formation of polar species on the surface of the fabric due to etching making it hydrophilic. Similar



(a)



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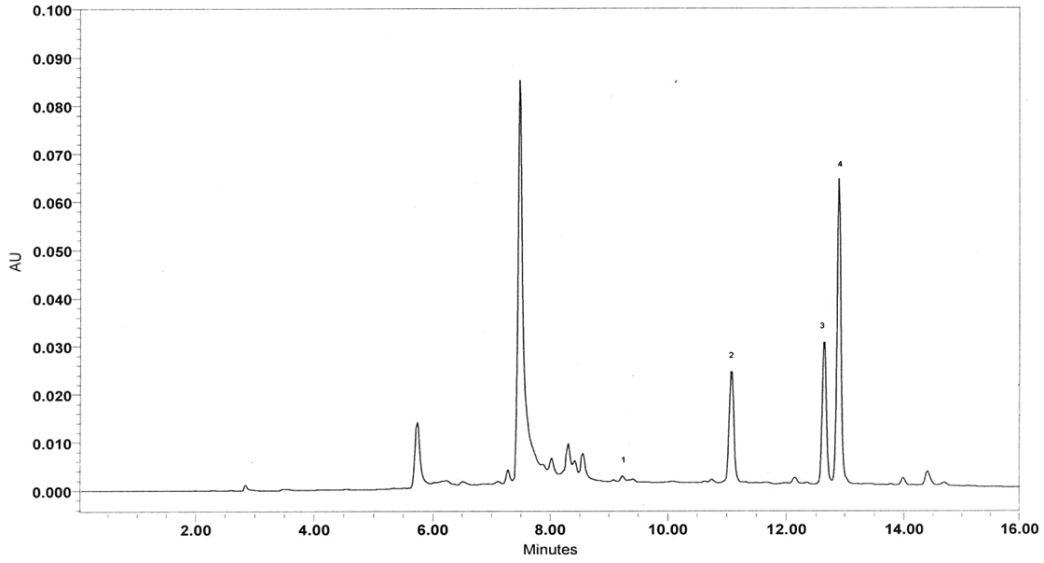


(c)

Figure 1. Structure of the quantified flavonoid compounds (a), HPLC chromatogram of SLE (b) and HPLC chromatogram of FRF of Seabuckthorn leaves(c).

results were reported for the effect of amine treatment on Nomex yarns leading to enhanced wicking height and dye uptake[25].

3.3 FTIR Characterisation

ATR FTIR, a surface sensitive technique was used to study the chemical modification on the surface of the treated fabrics. The IR spectrum of the control and coated fabric samples are shown in Figs. 3(a) and 3(b). The FRF-treated NIIIA fabric has characteristic peak at wavelength of 3337 cm^{-1} for hydroxyl and at 1728 cm^{-1} for carbonyl group. These characteristic peaks confirmed the coating of the flavonoids onto the NIIIA fabric as the control NIIIA fabric does not show such characteristic peak. Further, there is much increased intensity of the peak at 1728 cm^{-1} for the FRF treated NIIIA fabric. The prominence of this feature may be ascribed to the relatively high content of carboxylic acid and hydroxyl containing compounds in FRF.

Similar results on the characteristic hydroxyl and carbonyl groups at 3307 cm^{-1} and 1641 cm^{-1} respectively have been reported on the plant extract containing

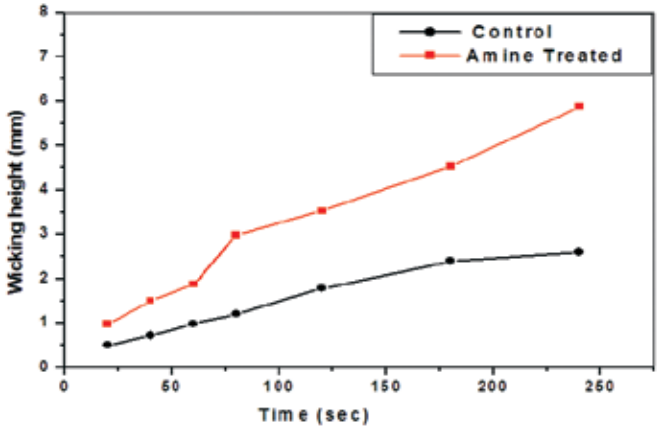
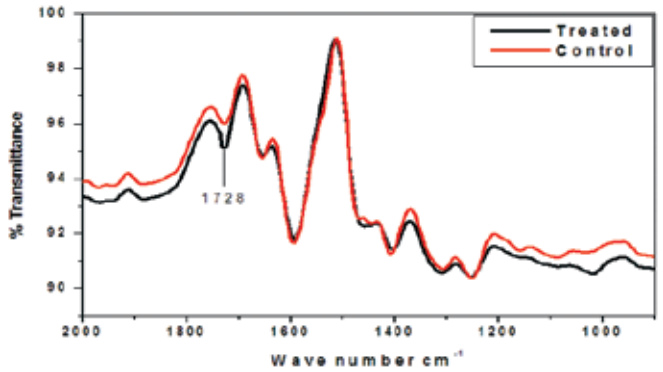
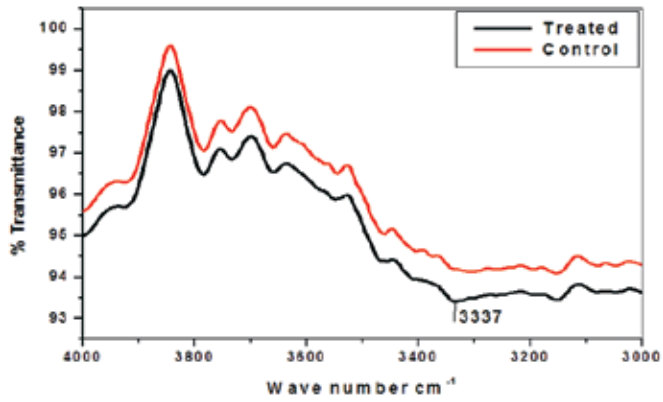


Figure 2. Wicking rate of control and amine treated fabrics.

flavonoids[22,29]. Another study also reported the bands at 3351 cm^{-1} and 1714 cm^{-1} for the plant extract containing rich flavonoid content[22,30]. The fabric without FRF coating does



(a)



(b)

Figure 3. (a) FTIR Spectra of treated and untreated fabrics (2000-900 cm^{-1}). (b) FTIR Spectra of treated and untreated fabrics (4000-3000 cm^{-1}).

not have these characteristic peaks. In yet another study it was confirmed that the peaks at ~3500 cm^{-1}, correspond to –OH stretching and peaks at 1740 cm^{-1}, correspond to C=O groups[31].

3.4 Flammability Test

This test method used to measure and describe the properties of fabrics in response to heat and flame under controlled laboratory conditions. Char length of treated and control samples was estimated using flammability tester. No significant difference was found in the area of char produced by both the samples as shown in Table 1. It indicates that the treatment has no effect on the flammability parameters of the fabric.

Table 1. Flammability characteristics of fabric samples

Sample	*Char length (mm) (±SD)	After glow
Control	21.83(±0.75)	—
Treated	21.98(±0.79)	—

*Values expressed as mean ± Standard deviation of three determinations.

3.5 Qualitative and Quantitative Antimicrobial Activity Assessment

The results of agar diffusion method against the standard test organisms *S. aureus* (Gram positive) and *E. coli* (Gram negative) are given in Figure 4 (a) and 4 (b) There was a clear zone of inhibition around the fabric treated with FRF against the above two test organisims in contrast to the control fabric which allowed the growth of these organisms. The zone of inhibition exhibited FRF-treated sample was found to be 16.7 ±1.9 mm for S aureus and 12.4±1.3 mm for *E. coli* which indicated the antimicrobial activity of FRF-treated sample.

The results of percentage reduction tests are shown in Table 2. The reduction percentage for E. *coli* and *S. aureus* correspond to the reduction in bacterial numbers on the test samples compared to the respective control. The reduction percentage was found to be 97.3 per cent for *S. aureus* and 94.2 per cent for E.*coli.*

Textiles provide an excellent environment for the growth of the microorganisms because of their large surface area and their ability to retain the moisture. A number of antimicrobial chemicals have been developed and quite a few are also available commercially[32]. Although the synthetic

Table 2. Percentage reductions of both the test organisms

Test Organism	Survival cells (CFU/mL)		per cent Reduction
	Control fabric	Treated fabric	
S aureus	5.52 x 10^7	5.37 x 10^5	97.30
E coli	5.59 x 10^7	5.27 x 10^5	94.20

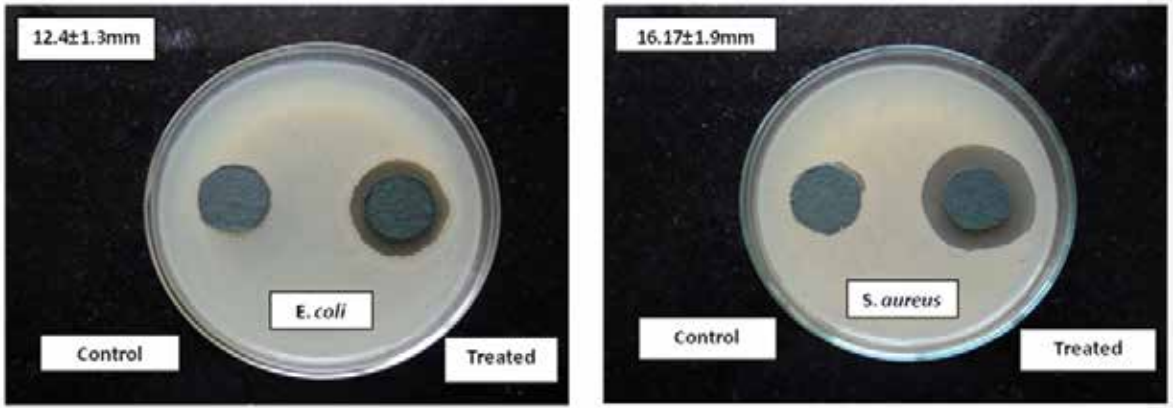


Figure 4. Photograph showing zone of inhibition- by disc diffusion method. (a) *E. coli* and (b) *S. aureus.*

antimicrobial agents are very effective against a range of microbes and give a durable effect on textiles, they are cause of concern due to the associated side effects, action on non-target microorganisms and water pollution[17]. Hence, there is a great demand for antimicrobial textiles based on ecofriendly natural antimicrobial agents. Similar studies have been reported on natural products that contain a wide variety of compounds having beneficial antimicrobial activity and hence used for antimicrobial coatings on the fabrics[20-22].

4. CONCLUSIONS

In this study, bioactive flavonoid rich fraction (FRF) was prepared from SBT leaves by acid hydrolysis process and coated on the aramid fabric by pad-dry-cure method. FRF coated fabric was characterised using Fourier Transform Infrared spectroscopy. The FRF-coated fabric showed a high degree of bactericidal activity against test organisms *E.coli* and *S.aereus*. In future, we are interested in increasing the durability of the FRF coated NIIIA fabric using various cross-linking agents, so that the antimicrobial property of FRF is retained in the fabric even after few washes and thus enhancing durability. Fungicidal activity of FRF also will be studied in the future. By this way, it is desired to develop an eco-friendly antimicrobial aramid textile with a novel natural antimicrobial agent.

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