Research Article

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ANTIBACTERIAL PROPERTIES OF *Ichnocarpus frutescens* (BLACK CREEPER) FINISHED COTTON MATERIALS

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Received on: 15/09/17 Accepted on: 20/10/17

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DOI: 10.7897/2277-4343.09123

ABSTRACT

The interest in the study of a medicinal plant, *Ichnocarpus frutescens* containing significant pharmacological compounds has triggered a concept of developing a medical textile. The pharmacological compounds present in the extracts were investigated for its antibacterial properties in the present research. The cotton fabrics were finished with three different concentrations of herbal extracts (1X, 2X and 3X) using a standard pad-dry cure process. Herbal extract finished fabrics were subjected to qualitative and quantitative antibacterial assays using AATCC test methods (AATCC 147 and AATCC 100). Among the three different concentrations, cotton finished with 3X herbal extracts produced maximum inhibitory zones of 34.6 mm and 32.3mm when tested against *Escherichia coli* and *Staphylococcus aureus*. The herbal extract finished fabrics showed increased bacterial reduction percentage ($p<0.05$). Maximum bacterial reduction percentage was observed for cotton fabrics against *Escherichia coli* (90%) and *Staphylococcus aureus* (95%). The effective antibacterial properties of the herbal extract finished cotton fabrics revealed the presence of significant pharmacological compounds in *lchnocarpus frutescens.* The extracts shall be analyzed for its specific antibacterial and other pharmacological compounds in future for obtaining more promising results in the field of medical textiles and pharmacology.

Keywords: *Ichnocarpus frutescens*, Medical textiles, Pharmacology, Antibacterial activity, Herbal extracts.

INTRODUCTION

Ichnocarpus frutescens is also termed commonly as Black Creeper used in traditional Indian medicine for centuries to treat different microbial infections. Some common names of this plant are Dudhi, Shyamalata in Bengali and 'Udarkoti' in Tamil[1]. Roots, flowers and leaves of *Ichnocarpus frutescens* are used to treat syphilis, diaphoretic, hemiplegia, dyspepsia, demulcent and diuretic[2]. Different phytochemical compounds like polyphenols, terpenoids, alkaloids, phytosterols, carbohydrates, coumarins, glycosides, flavonoids were attributed to the pharmaceutical and antibacterial properties of the plants[3].

The antibacterial and antifungal activity of different solvent extracts of *Ichnocarpus frutescens* was reported earlier. A study on chloroform and aqueous extracts of *Ichnocarpus* roots were carried out to evaluate their antimicrobial activity. According to Pandurangan et al[4], the chloroform extract revealed maximum antifungal and antibacterial properties against the test bacteria *Eschericia coli* and test fungi *Aspergillus flavus*. The researchers also highlighted that when the concentration of chloroform extract was increased, the diameter of inhibition zone was also increased. The ethyl acetate (EtOAc) extract of *I. frutescens* study revealed that the root extract possessed potential antibacterial activity against pathogenic bacteria, *Shigella flexneri*, *Shigella dysenteriae*, *Vibrio cholerae* non.0139 (L4), *Vibrio cholerae* non.0139 (CSK6669), *Streptococcus pneumonae* and *Escherichia coli*[5]. Raju and Subin (2013) studied the antibacterial activity of methanol and aqueous extracts of *lchnocarpus frutescens* against two pathogenic bacteria namely *Escherichia coli* and *Bacillus subtilis* under *in vitro* conditions[6].

The interest in the study of medicinal plant, *lchnocarpus frutescens*, which has a source of pharmacologically active compounds, and the increase in the Multiple DrugResistant pathogens havetriggered a concept of developing medical textile finished with the solvent extracts of *lchnocarpus frutescens*.

MATERIALS AND METHODS

Fabrics used in the study

100% cotton fabrics were selected based on their eco-friendly properties. The specification of the fabric materials tested using standard textile standards was presented in Table 1.

Pre-Treatment of Cotton Fabric

To ensure complete wetting and uniform absorbency of the extract and other finishing agents during finishing process, cotton fabric was subjected to different pre-treatments like enzymatic desizing and scouring.

Enzymatic desizing

The woven cotton fabric was treated with 3% concentration of alpha amylase (Amylase-543). Ratio of 1:20 material to liquor at 60°C for 45 min reaction time at pH 6-7 level was used for enzymatic desizing process. The fabric was thoroughly rinsed with hot water followed by cold water and then dried at 80°C using hot air oven[7].

Enzymatic Scouring[8]

The desized woven cotton fabric was treated with 2% pectinase with 1:20 material to liquor ratio for 45 min at 40ºC. After enzyme treatment, the fabric was thoroughly rinsed with hot

water followed by cold water and then dried at 80°C using hot air oven.

Herbs and shell materials

Ichnocarpus frutescens (Udarkoti) herbal powders and shell material, gum acacia (Hi Media) was commercially procured from a local supplier at Coimbatore, Tamil Nadu, India.

Bacterial cultures used

Two bacterial cultures, *Escherichia coli* and *Staphylococcus aureus* which have the ability to cause hospital-acquired infections were commercially procured from a diagnostic laboratory at Coimbatore, Tamil Nadu, India.

Methanol extraction of *Ichnocarpus frutescens*[9]

Ichnocarpus frutescens (Udarkoti) herbal powders were extracted using methanol. Methanol extraction of the leaf powder was performed with aid of soxhlet extraction apparatus. About 100g leaf powder of the selected herb was extracted with 500ml of methanol at 60 – 80°C for two continuous cycles. The solvent containing active constituents was transferred to rota vapour to evaporate the solvent and to get solid extract. The extract was stored in cool and dry place for further analysis.

Finishing cotton fabrics with herbal extracts[10]

Cotton fabric was finished with methanol extracts of *Ichnocarpus frutescens* leaves using pad-dry-cure process. The *Ichnocarpus frutescens* extracts were prepared in three different concentrations (10%, 20% and 30%) and noted as 1X, 2X and 3X throughout the study. Cotton fabric was immersed in the finishing solution containing 10%, 20% and 30% *Ichnocarpus frutescens* extracts separately. Ratio of 1:20 material to liquor was used to finish the fabrics. Gum acacia as shell material, 50g/l cross linking agent and 10g/l magnesium chloride was added to the reaction mixture and maintained at pH 5.5 for 30 to 40min. Each test fabric was then passed in the padding mangle between the rollers at 2kg/cm^{2}pressure in such a way to maintain 100% moisture content in the fabric. The fabric was then dried at 80°C for 10min and cured at 120°C for 5min in a curing chamber.

Assay for qualitative antibacterial properties of finished cotton (AATCC 147-2012)[11]

Cotton fabrics finished with herbal extracts were subjected to antibacterial assay. The assay used for measuring antibacterial properties was based on the AATCC Test Method 147-2012. Briefly, finished cotton fabrics were cut into pieces (25mm x 50mm) and hygienically transferred to testing conditions. The 50mm length of the cotton swatches permits the specimen to lay across 5 parallel inoculums streaks each of diminishing width from both 8mm to 4mm wide. Sterile AATCC bacteriostasis agar plates were prepared. Using sterile 4mm inoculating loop, one loop full of culture was loaded and transferred to the surface of the agar plate by making five parallel inoculum streaks spaced 10mm covering the central area of the petridish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with agar surface. The plates were incubated at 37°C for 18-24 hours.

The inoculated plates were examined for the interruption of growth along the streaks of inoculum beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen calculated in mm.

Assay for quantitative antibacterial properties of finished cotton (AATCC 100-2012)[12]

The antibacterial properties of materials can be studied by quantitative (AATCC-100) test methods. Quantitative test is the proper indicator of degree of antibacterial activity when the antibacterial agents are fixed on to the textile material or are unable to leach out. The different tests carried out in this study were based on such consideration.

Cotton fabrics finished with herbal extracts were subjected to antibacterial assay. The assay used for measuring antibacterial properties was based on the AATCC Test Method 100-2012. Briefly, 1.0 ml of 12 hours challenge bacterial inoculum was dispersed as droplets over the test swatches (fabrics) using a micropipette. The swatches were inoculated in pre-sterilized 250 ml Erlenmeyer flasks. After all the samples were inoculated, the flasks were incubated at 37 ± 2 °C for 18 hour before being assayed for bacterial population density. The bacterial population density was determined by extracting the bacteria from the fabric by adding 100 ml of distilled water to each flask and shaken using an orbital shaker for 1 min. Then aliquots were serially diluted and pour plated to determine the bacterial density. The difference in number of viable bacteria was evaluated on the basis of the percentage reduction. Percentage reduction was calculated using the following formula.

$$R = (A-B) / A \times 100$$

Where, *R* is percentage reduction; *A* is the number of bacteria in the broth inoculated with finished test fabric sample immediately after inoculation i.e., at zero contact time and *B* is the number of bacteria recovered from the broth inoculated with finished test fabric sample after the desired contact period of 18 hours.

Statistical analysis of total viable bacteria on herbal finished fabrics

Chi-square non parametric test using SPSS-9 for Windows 7 was used as a statistical tool to determine the effect of herbal extracts on bacterial reduction. The hypothesis selected (H_0) was that there is significant quantitative antibacterial activity of herbal extracts on the test organisms. The difference in the bacterial reduction percentage between the extract finished and unfinished fabrics were statistically calculated with $p< 0.05$ considered significant.

RESULTS

Qualitative antibacterial activity of the herbal extract finished fabrics

Qualitative antibacterial activity was determined based on the zone of inhibition around the finished fabrics against the test organisms. Maximum zone of inhibition was influenced by the greater action of finished herbal particles. Cotton fabrics finished with three herbal concentrations (1X, 2X and 3X) showed antibacterial activity under *in vitro* conditions. Among the three different concentrations, cotton finished with 3X herbal extracts produced maximum inhibitory zones when tested against *Escherichia coli* and *Staphylococcus aureus* (Fig-1 and Fig-2). Cotton finished with similar herbal extract concentration produced inhibitory zones of 34.6 mm and 32.3mm against their respective test cultures (Table-2). The zones around the test

fabrics clearly indicate the leaching capacity of the herbal extract thus inhibiting the growth of the bacteria.

Quantitative antibacterial activity of the herbal extract finished fabrics

Cotton fabric finished with herbal extracts (3X herbal concentration) was quantitatively assessed by AATCC-100 (bacterial reduction) test method. The anti-adherent activity of finished fabrics against the test organisms, *Escherichia coli* and *Staphylococcus aureus* was concentration dependent as the reductive effect of herbal particles was in the range of 90% to 95% (Table-3). Bacterial reduction percentage was calculated from the CFU of the herbal extract finished fabrics after exposing 0^{th} hour and 18^{th} hour. Maximum bacterial reduction percentage was observed for cotton fabrics against *Escherichia coli* (90%) and *Staphylococcus aureus* (95%). In Fig-3 and Fig-4, the difference in the number of bacterial colonies between 0^{th} hour and 18^{th} hour plates were presented. Bacterial reduction percentage up to 100% shall be achieved if the finishing conditions are optimized.

Statistical analysis of total viable bacteria on herbal extract finished fabrics

Using chi-square statistical analysis, the effect of herbal extracts on bacterial adherence was determined. The difference in bacterial reduction percentage of finished and unfinished fabrics were taken as the experimental design. The hypothesis selected (H_0) was that there is significant quantitative antibacterial activity of herbal extracts on the test organisms. The difference in the bacterial reduction percentage between the extract finished and unfinished fabrics were statistically calculated with $p<0.05$ considered significant. For all the data, the calculated value was less than the table value. In Table-3, the calculated value of each finished fabrics tested against the test organisms was presented. Since the calculated value was less than the table value, the assigned hypothesis is accepted. The statistical survey of the research proved the quantitative antibacterial activity of the herbal extracts finished fabrics.

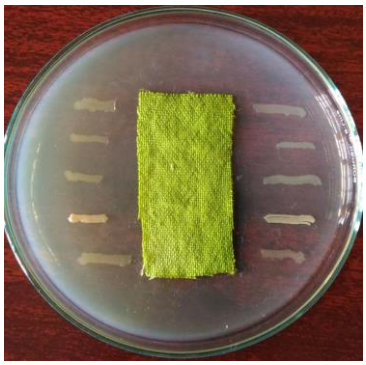

Figure 1: Qualitative antibacterial activity of *Ichnocarpus frutescens* extracts finished cotton fabrics (*Escherichia coli*)

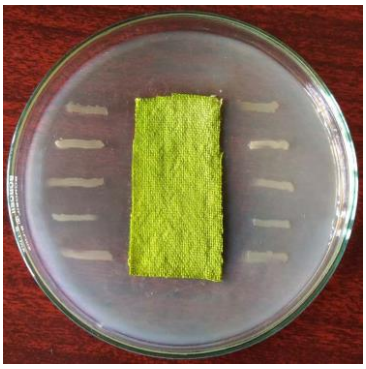

Figure 2: Qualitative antibacterial activity of *Ichnocarpus frutescens* extracts finished cotton fabrics (*Staphylococcus aureus*)

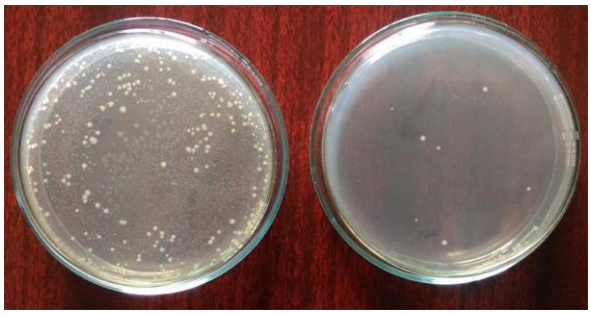

Figure 3: Quantitative antibacterial activity of *Ichnocarpus frutescens* extracts finished cotton fabrics (*Escherichia coli*)

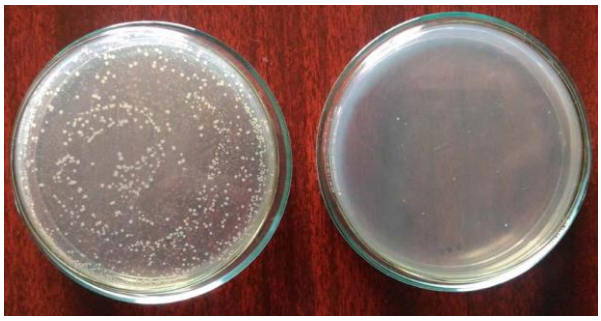

Figure 4: Quantitative antibacterial activity of *Ichnocarpus frutescens* extracts finished cotton fabrics (*Staphylococcus aureus*)

Table 1: Test fabrics and its parameters

Fabric Parameter	100% Cotton
Courses per cm	20.06
Wales per cm	16.50
GSM	120.00
Tightness factor	15.20
Loop shape	1.50

All parameters were selected based on the textile standards

Table 2: Qualitative antibacterial activity of herbal extract finished fabrics

Fabric sample	Herbal concentration	Zone of inhibition (mm)	
		Escherichia coli	***Staphylococcus aureus***
Cotton	Unfinished	0	0
	1X	28.3	27.6
	2X	30.6	29.3
	3X	**34.6***	**32.3***

*Maximum inhibitory zones

Table 3: Quantitative antibacterial activity of the optimized herbal extract finished fabrics

Fabric samples	Test Bacteria	Bacterial reduction (%)		Statistical significance (Chi square test)
		Unfinished	finished	
Cotton	***Escherichia coli***	0	**90**	p<0.05
	Staphylococcus aureus	0	**95**	p<0.05

* Calculated value is less than the statistical table value, so the assigned hypothesis is accepted

DISCUSSION

A wide range of textile products for medical, technical, industrial, home furnishing and apparel sectors with the application of antimicrobial properties have gained special interests to protect human being against harmful microbes. Commercially available antimicrobial agents and their compliance to the regulations imposed by international bodies like EPU is still unclear. Recent developments on herbal and its oil extracts for functional finishing onto textile fabrics raised a novel avenue in the biomedical textile research.

In the present study, cotton fabrics finished with three different herbal concentrations (1X, 2X and 3X) individually showed good qualitative antibacterial activity. Qualitative antibacterial activity revealed the antimicrobial effectiveness against standard test cultures *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) organisms. The zone of bacterial inhibition was indicated by a halo around the fabric samples. It was apparent that the activity of herbal extract finished fabrics was excellent for 3X herbal concentration. The qualitative antibacterial analysis clearly reveals the potential of *Ichnocarpus frutescens* extract gets increased with increase in concentration of extract. It emphasizes that, the plant comprising the natural ingredients have potential to reduce bacterial growth; and its mechanism of action is effective, safe to human and environment. The effective mechanism of the herbal finished fabrics was reported earlier. According to Ghannam[13] bacterial inhibition was due to the slow release of active substances from the fabric surface. Amino groups of the herbal extract were responsible for its excellent antimicrobial activity. Hecht[14] reported that in presence of slight acidity the amino groups will be converted to positive amino group ions. Thus converted ions will react with the negatively charged protoplasm of microorganisms and breaks the cell wall to destroy the microorganisms.

After selecting the herbal concentration from qualitative assay (3X) the finished fabrics also showed good bacterial reduction percentage during the quantitative antibacterial assay. Quantitative antibacterial activity tested by AATCC 100 test method revealed that antibacterial agent gets attached to the substrate through bond formation on the surface. According to Sarkar *et al*[15]the finished antibacterial compounds in the fabric samples disrupts the cell membrane of the microbes through the physical and ionic phenomenon. The finishing agent inhibited growth of test organisms by using an electrochemical mode of action to penetrate and disrupt their cell walls. When the cell walls are penetrated, leakage of metabolites occurs and other cell functions are disabled, thereby preventing the organism from duplication. Bacterial reduction percentage obtained during the study also attributed that the compounds such as tannin, saponin, flavonoids and phenols in *Ichnocarpus frutescens* leaves extract are the responsible bioactive agents (the phytochemical analysis was not performed in this study). The antibacterial activity of such phytochemcials was reported by earlier studies and revealed that they inhibited the growth of microbes in many ways such as by inhibiting protein synthesis, inferring with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting metabolic pathway, inferring with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms[16].

CONCLUSION

All the fabric materials finished with *Ichnocarpus frutescens* herbal extracts showed excellent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* due to the active biological compounds in the prepared concentrations. Mode of action of these compounds on the cell membrane and peptidoglycan layer of bacteria thus proved its antibacterial activity. This indicated that the *Ichnocarpus frutescens* extracts could act as bactericidal and bacteriostatic. The fabrics with these finishing conditions shall be used for garments like bed linen sheet in hospital, surgical gowns, patient uniforms and other healthcare dress materials. Since these herbs and shell materials are abundantly available in tropical and sub-tropical countries, the scopes of imparting antibacterial finish in different fabrics are high. Since the raw materials used in this study are 100% natural, the antibacterial finished fabrics are considered to be eco-friendly possessing economic, social and health benefits to the human beings.

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Cite this article as:

Christie Jennifer K and Sangeetha K. Antibacterial properties of *Ichnocarpus frutescens* (black creeper) finished cotton materials. Int. J. Res. Ayurveda Pharm. 2018;9(1):118-122

Source of support: Nil, Conflict of interest: None Declared