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In-vitro antimicrobial activity of *Achyrenthes aspera* L. with different extraction solvents

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Abstract
The present study was aimed to evaluate the effectiveness of antimicrobial activity with various extraction solvents against gram positive bacteria (*B. claussii*) and gram negative bacteria (*A. altamirensis and M. yunannensis*). Fresh root parts were extracted at room temperature using different solvents (Methanol, acetone, ethanol and distilled water). In the results, gram positive bacteria were found more susceptible as compared to gram negative bacteria. Solvents with methanol demonstrated better antimicrobial activity as compared to other solvents used. 100% extraction was observed as best inhibition zone. Statistical analysis demonstrated significant difference ($p<0.05$), where percentages of solvents as well as the type of microorganisms that have significant effect on the inhibition zone. Thus, this study revealed the importance with different extraction solvents used which showed satisfactory and reliable result on the antimicrobial activity of the three potent microorganisms.

Keywords: Antibacterial tests, antimicrobial activity, culture media, microorganism, solvents

Introduction
Achyranthes aspera is a species of plant in the Amaranthaceae family. It is distributed throughout the tropical world. It can be found in many places growing as an introduced species and a common weed. Textiles for medical and hygienic use have become important areas in the textile industry. Therefore, to reduce/prevent infections, various antibacterial compounds have been used for all types of textiles. The solutions of disinfectant used are generally active *in vitro*, but it is also necessary to know the effectiveness of disinfecting cloths in conditions of use (Kaur *et al*., 2005) [1] have studied the Antimicrobial Properties of *Achyranthes aspera* and found that Chloroform and methanol extracts of *A. aspera* showed good amount of antibacterial activity against *Klebsiella* sp. (Karim *et al*., 2009) [2]. Results also suggested that extract had significant antibacterial and antifungal activities against tested microorganisms. As the problem associated with microbial resistance continues to rise, yet there are still uncertainties in searching for the new antimicrobial drugs. Therefore, this study was aimed to assess the effectiveness of various solvents on *in-vitro* antimicrobial activity from root of *Achyranthes aspera* L. extract.

Methodology
Selection of fabric
Cotton material was chosen for the study because of low allergic reaction and good tolerance to skin.

Plant material
Root of *Achyrenthes aspera* L. was collected from different places of Jorhat District.

Preparation of plant material
Collected fresh samples of *Achyranthes aspera* L. roots were washed and cut into small portion, dried in drying oven at 50 ^{o}C until a constant weight was achieved. The dried roots were then made into powder.

Preparation of crude extracts
70 g of dry powdered plant material was extracted with 100 ml of different solvents (methanol, acetone, ethanol and distilled water) of 7:10 (w/v) for 48 hr.

The extraction was carried out at room temperature. After the respective time periods, the soaked powder-solvent mixtures were filtered through a Whatman No. 4 filter paper and then centrifuged at 10,000 rpm for separating the supernatant from the extract. Sterilized the extract and concentrated root extracts were then stored at -20 ºC until further use.

Culture media

Nutrient agar (NA) and nutrient broth (NB) were used during the study. NA and NB were used for the cultivation of bacteria. All bacterial cultures were incubated at 37 ºC for 24 hours.

Test of microorganisms

In-vitro antimicrobial studies were carried out on one Gram-positive bacteria (*Bacillus clausii)* and two Gram-negative bacteria (*Aureimonas altamirensis* and *Micrococcus yunnanensis*). All the microorganisms were obtained from the infected clothes.

Screening of antimicrobial activities

Disc diffusion method (Jothi, 2009) [3] was used for antimicrobial activity. From the prepared nutrient agar l5 ml of melted nutrient agar were poured in the Petri plates. After media were solidified, 250 µl of microbes cultures aged 48-72 hr were added to Petri plates and spread it by spreader evenly, dried it. After that holes were made by using 5mm cork borer. Each hole was filled with 150 µl of plant extract. Different concentrations were used i.e. 100%, 50%, 25%, 12.5%, 6.25% and 3.125%. The samples were slowly impregnated drop wise on the hole. Packed the Petri plates with paraffin. Plates were incubated at 28 ºC for 2-3 days. The zone of inhibition was recorded.

Durability of herbal extracts

Durability of the extracts was tested by observing the antimicrobial activities up to six months.

Preparation of cotton fabric for antimicrobial finish

Cotton fabric was desized before performing the treatment to remove the impurities. Acid steeping method was used for the purpose. Geometrical properties of the fabric are given in Table 1.

Table 1: Geometrical properties of the fabric

Types of fabric	Weave	Fabric count (Ne)		Thickness (mm)	Weight (g/m^2)
		Ends/in	Picks/in		
Cotton	Plain	94	66	0.21	129

Application of antimicrobial finishes on textiles

Direct application method (Sathianarayanan *et al.*, 2010) [4] was used for the study.

Antibacterial activity of treated fabrics

Antibacterial activities of the treated fabrics were evaluated by both qualitative (EN ISO 20645:2004) and quantitative (AATCC-100) methods. For agar diffusion plate test method (EN ISO 20645:2004), nutrient agar plates were prepared by pouring 15ml of media into sterile Petri dishes. The agar plates were allowed to solidify for 5 min and 0.1% inoculums was swabbed uniformly and allowed to dry for 5min. The finished fabric with the diameter of 2.0±0.1 cm was placed on the surface of medium and the plates were kept for incubation at 37 °C for 24 hr at the end of incubation, the zone of inhibition formed around the fabric was measured in mm and recorded. For quantitative analysis, (AATCC-100-2004) [5] test was used. Control swatches were inoculated with the test organism. After incubation the bacteria were eluted from the swatches by shaking a known amount of neutralizing solution. Apart from assessing antimicrobial effectiveness, wash durability as well as fitness for purpose, was considered as well as other physical tests which were relevant to the final intended use of the products.

Wash durability of finished fabrics

The antibacterial activity of the finished samples was evaluated after being subjected to several wash cycles (Erdem *et al.*, 2008) [6]. The finished fabrics were washed using standard detergent at 40 °C by hand wash.

Results and Discussion

Evaluation of herbal plant extracts:

To evaluate the herbal extract against microbes, disc diffusion method was used and zones of inhibition were measured. The herbal extract in 4 (four) solvents i.e. water, methanol, ethanol and acetone were screened against three bacteria i.e. *Aureimonas altamirensis*, *Bacillus clausii* and *Micrococcus yunnanensis* at six different conc. (100%, 50%, 25%, 12.5%, 6.25% and 3.125%). The results were presented below.

Table 2: Effect of water herbal extracts against isolated bacteria

Bacteria	Concentrations																	
	100%			50%			25%			12.5%			6.25%			3.125%		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Aureimonas altamirensis	15.0333	0.15275	0.08819	12.5167	0.12583	0.07265	8.0667	0.25166	0.1453	-	-	-	-	-	-	-	-	-
Bacillus clausii	12.5	0.1	0.05774	8.4333	0.15275	0.08819	6.6333	0.15275	0.08819	-	-	-	-	-	-	-	-	-
Micrococcus yunnanensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Data given are mean of five replicates

Table 3: Effect of methanol herbal extracts against isolated bacteria

Plant extracts	Concentrations																	
	100%			50%			25%			12.5%			6.25%			3.125%		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Aureimonas altamirensis	16.9333	0.15275	0.08819	15.25	0.13229	0.07638	12.95	0.22913	0.13229	-	-	-	-	-	-	-	-	-
Bacillus clausii	15.3333	0.15275	0.08819	12.8667	0.15275	0.08819	11.6167	0.20207	0.11667	-	-	-	-	-	-	-	-	-
Micrococcus yunnanensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Data given are mean of five replicates

Table 2 showed that in water extraction, *Achyranthes aspara* showed maximum inhibition (15.03 mm) at 100% than other conc. against *Aureimonas altamirensis*. It was observed that the water extract of *Achyranthes aspera* showed highest zone of inhibition (12.50 mm) against *Bacillus clausii* at 100% conc., whereas at 50% conc. *Achyranthes* showed zone of inhibition (8.43 mm) and at 25% conc. zone of inhibition found 6.33mm.

Table 3 showed that the maximum inhibition of *Aureimonas altamirensis* with methanol herbal extract of *Achyranthes aspera* was observed 16.93 mm at 100% conc. 15.25 mm at 50% conc. and 12.95 mm at 25% conc.

Achyranthes aspera with ethanol and acetone solvent were not effective against selected bacteria

Durability of _Achyranthes aspera_ extracts against isolated microbes

From the Fig. 1, it was revealed that in fresh condition, both water and methanol extracts of *Achyranthes aspera* showed approximately same inhibitory effects against *Aureimonas altamirensis* and *Bacillus clausii* at 100%, 50% and 25% conc. But methanol extracts of *Achyranthes aspera* remained effective (8.58 mm) and (5.60 mm) up to 4 months at 100% and 50% conc. against *Aureimonas altamirensis* whereas it was found effective (7.23 mm) and (4.30 mm) against *Bacillus clausii* up to 5 months at 100% and 50% conc. It was also observed from the result that neither fresh nor old methanol and water extracts of *Achyranthes aspera* were found effective against *Micrococcus yunnanensis* at any concentrations.

Evaluation of antimicrobial finished fabrics against bacteria

After application of extract (Sathianarayanan *et al*., 2010) [4] Treated and untreated (control) fabric samples were placed in intimate contact with AATCC bacteriostasis agar, which has been previously inoculated (Mat culture) with an innoculum of test organism. After incubation, a clear area of uninterrupted growth underneath and along the side of the test material indicated antibacterial effectiveness of the fabric.

Efficacy of antimicrobial activity of _Achyranthes aspera_ treated fabrics

Fig. 2 revealed a decreasing trend effective inhibition of *Aureimonas altamirensis* was observed when *Achyranthes aspera* treated cotton fabric tested as compared to herbal extract examined without fabric. Herbal extract of *Achyranthes aspera* alone showed effective inhibition of the tested bacterium (16.93 mm) at 100% conc. while treated fabric showed inhibition (16.77 mm) at 100% conc. It was followed by 50% conc. where inhibition was (15.25 mm) and (14.10 mm), and at 25% conc. (12.95 mm) and (12.40 mm), respectively. It was also observed from the Table that the effective inhibition of *Bacillus clausii* with the use of herbal extract without fabric and with fabric at 100% conc. (15.33 mm) and (15.26 mm), at 50% conc. (12.87 mm) and (12.63 mm) and at 25% conc. (11.62 mm) and (11.48 mm), respectively. This decreasing trend might be due to the pressure of the yarn and fabric structure. (Plate 1 and 2).

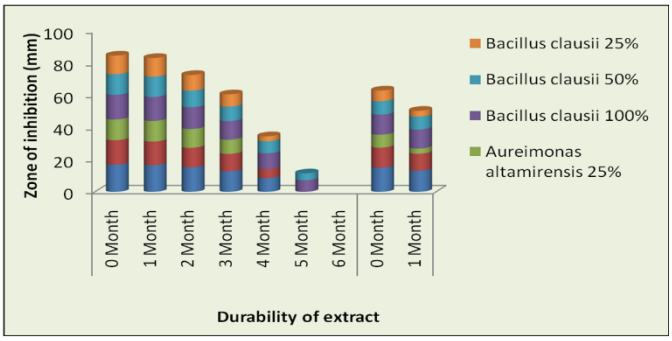


Fig 1: Efficacy of extracts against microbes

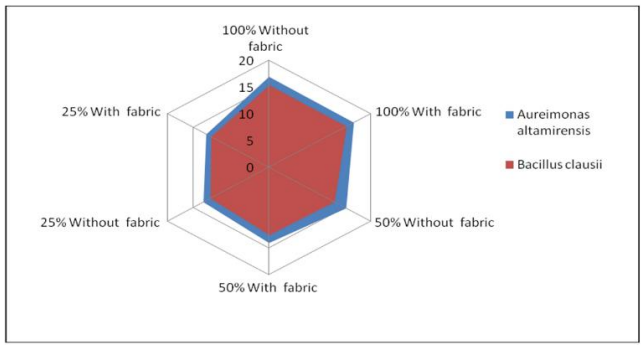
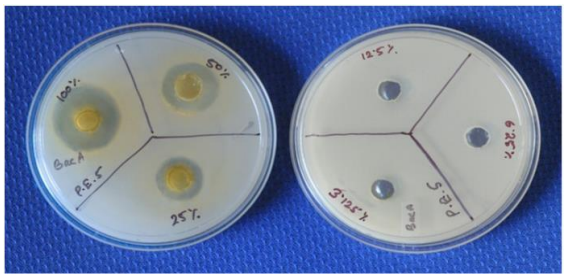
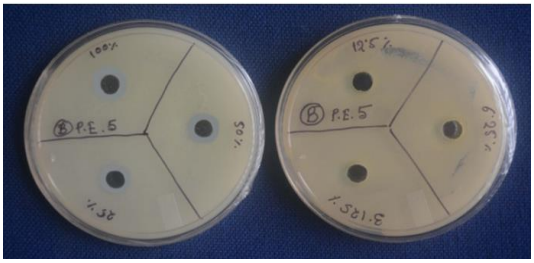


Fig 2: Efficacy of finish with control & treated fabrics

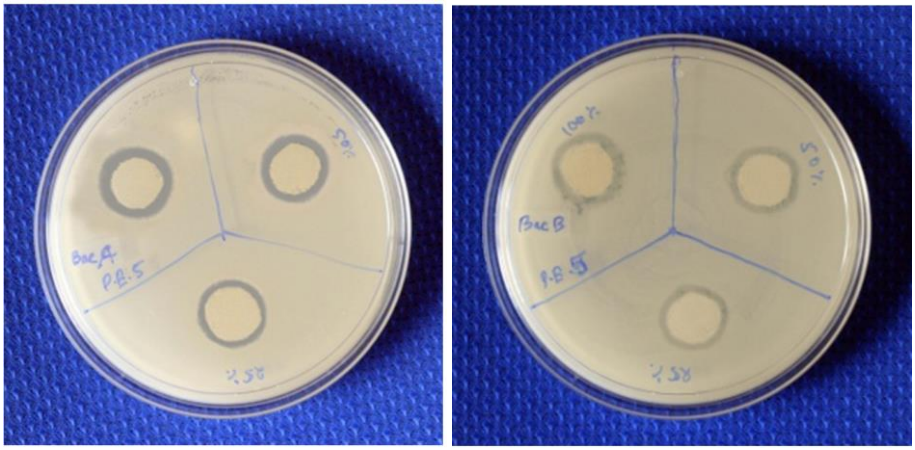


Aureimonas clausii



Bacillus clausii

Plate 1: Antimicrobial activity of *Achyranthes aspera* extract against bacteria



Aureimonas clausii *Bacillus clausii*

Plate 2: Antimicrobial efficacy of *Achyranthes aspera* treated sample against bacteria

Evaluation of antimicrobial finished fabrics against bacteria after washing

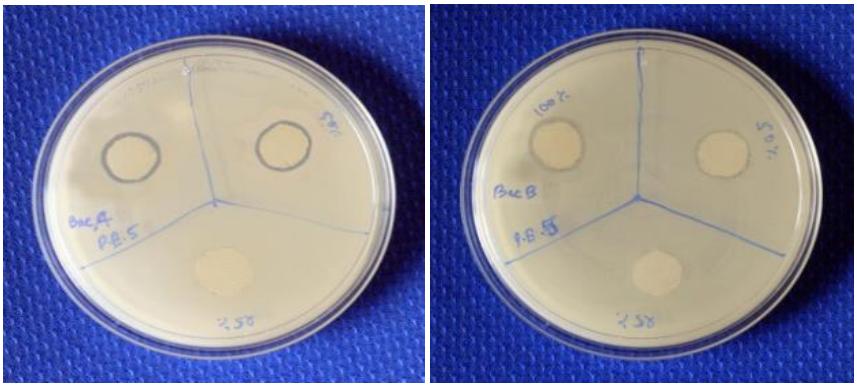
Tests were performed to evaluate the durability and effectiveness of the antimicrobial herbs used on cotton material. All the results were evaluated and compared their results whether the fabric was right for its proposed end use. To test the durability of the antimicrobial finish, treated samples were examined for antimicrobial efficacy from 1 to 10 home launderings. The procedure used for home laundering was the AATCC Test Method 61. Common detergent (2%) was used for washing the test samples. Home laundering was done with washing machine for 3min.

Table 6: Effect of laundering on antimicrobial finish of *Achyranthes aspera* extract

Bacteria	% Plant extracts	Wash cycle										
		Control	Wash 1	Wash 2	Wash 3	Wash 4	Wash 5	Wash 6	Wash 7	Wash 8	Wash 9	Wash 10
Aureimonas altamirensis	100%	16.77±0.03	15.20 ±0.06	13.38±0.07	11.85±0.05	8.42±0.06	5.68±0.04	3.27±0.07	1.08±0.06	0	0	0
	50%	14.10±0.06	13.53±0.09	12.53±0.10	9.73±0.09	7.83±0.06	4.83±0.06	2.57±0.06	0	0	0	0
	25%	12.40±0.06	11.78±0.06	10.82±0.04	8.50±0.09	5.22±0.07	0	0	0	0	0	0
Bacillus clausii	100%	15.27±0.09	14.63±0.09	11.83±0.07	9.68±0.07	7.08±0.09	4.93±0.07	2.77±0.09	0	0	0	0
	50%	12.63±0.09	11.77±0.09	9.63±0.09	6.82±0.07	3.28±0.04	0.87±0.07	0	0	0	0	0
	25%	11.48±0.12	10.43±0.03	8.52±0.04	5.78±0.06	2.62±0.06	0	0	0	0	0	0

*Data given are mean of five replicates ± Standard error

Table 6 revealed that *Achyranthes aspera* treated fabric showed effective inhibition of *Aureimonas altamirensis* up to 7 wash (1.08) as compared to control (16.77) at 100% conc. indicating long antimicrobial activity of the treated fabric. It was followed by (2.57) up to 6th wash at 50% conc. and (5.22) up to 4th wash at 25% conc., respectively. However, the efficacy of the finish fabric was reduced in each laundering gradually. It was also evident from the results that finish fabric was less effective against *Bacillus clausii* after laundering. Finish fabric showed effective inhibition of bacteria up to 6th wash at 100% conc. (2.77) as compared to control (15.27), followed by 5th wash (0.87) at 50% conc. and 4th wash (2.62) at 25% conc. (Plate3)



Aureimonas altamirensis *Bacillus clausii*

Plate 3: Antimicrobial activity of finish after 5 wash

Conclusion

The consumers are now increasingly aware of the hygienic life style and there is a necessity and expectation for a wide range of cotton fabric treated with anti-microbial finish. The antimicrobial finishes are applied to textile materials for two purposes: to protect the wearer and the fabric itself. The

antimicrobial finish is applied in such a way that appearance and feel of the fabric is not changed and no chemical odour remains. Clothing and textile materials are not only the carriers of micro-organisms such as pathogenic bacteria, odour generating bacteria and mould fungi, but also good media for the growth of the micro-organisms. These micro-organisms create problems in textile, including discoloration, stains and fiber damage, unpleasant odor and a slick, slimy feel. When fabric is worn next to skin infestation by microbes causes cross infection by pathogens and develop odors. The performance properties of cotton fabric are lost as a result of microbial attack. Antimicrobial agent destroys the growth of micro-organisms and their negative effects of odour, staining, and deterioration. Moreover anti-microbial agents are used on fabric to control bacteria, fungi, mould, mildew and algae. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, antimicrobial finished fabrics can be used to protect ourselves from infectious diseases caused by microbes.

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