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Research Article

Antimicrobial Activity of Perspiration Pads and Cotton Cloth Fabricated with the Ethyl Acetate Extract of *Eichhornia crassipes* (Mart.) Solms

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Waterhyacinth is one of the most dangerous aquatic weeds causing various ecological and environmental issues. The plant has several pharmaceutical properties in spite of its harmful nature. In the present study, perspiration pads and cotton cloth fabricated with the ethyl acetate extract of waterhyacinth were evaluated for its activity against *Corynebacterium*. Perspiration pads and samples of cotton cloth were fabricated with ethyl acetate extract of *Eichhornia crassipes* by three methods, namely, dipping, sonication, and homogenization. The antimicrobial activity of the fabricated textile materials was tested against *Corynebacterium*, a microorganism commonly seen in human sweat. Disc diffusion method (AATCC 147) was used for evaluating the antimicrobial nature of the fabricated samples. Sonication was found to be efficient for coating of the extract on the cotton cloth, whereas dipping method was found to be efficient in case of perspiration pads. No bacterial growth was observed under and on the fabricated cloth and perspiration pads indicating that the fabrics possess bacteriostatic property and not bactericidal property and the absence of leaching of the extract. The results showed significant antimicrobial activity of the ethyl acetate extract of *Eichhornia crassipes* coated onto fabrics against *Corynebacterium* with no growth under and on the test sample.

1. Introduction

Human body represents a multitude of microenvironments, each with its own normal flora. In general, intact skin serves as a natural protective barrier against invasion by most infectious disease growth. Species found repeatedly on the skin of groups of individuals are referred to as resident flora or microbiota of the skin. The conditions of the skin favour the growth of *Streptococcus*, *Micrococcus*, *Staphylococcus*, fungi, diphtheroid bacilli, and yeast [1]. Human axillary odour is formed by the action of *Corynebacteria* on odourless axilla secretions. The skin in human armpits contains a dense arrangement of sweat glands. Volatile substances evaporating from these areas make a key contribution to human body odour. However, sweat secreted from apocrine glands in these skin areas is initially odourless, and skin bacteria release the odoriferous principles from nonsmelling substrates present in these secretions. The axilla is a skin region colonized by an unusually dense bacterial population, with a species composition dominated by the two genera *Staphylococcus* and *Corynebacterium*. Most individuals carry a flora that is dominated by either one of these two genera, and there is a strong correlation between a high population of *Corynebacteria* and strong axillary odour formation. Subjects whose axillary skin is mainly colonized by *Staphylococci* emit only low levels of odour [2].

The three major components contributing to odour production are sweat secretion, bacterial population, and a moist environment. There are many ways to inhibit sweat odour and one among them is the use of antimicrobial

fabrics. Many products are available in the market for the consumers but most of them are synthetic based and may not be environmentally benign. Their compliance to the international bodies such as EPU is essential [3]. Plant extracts have long been used as antimicrobial textile finishes owing to its ecocompatible nature. Natural antimicrobial agents are nontoxic and nonallergenic and do not cause the problems of microbial resistance [4] Perspiration pads are widely used by men and women to keep clothes clean and dry. Several commercial pads are in the market. Utilization of waterhyacinth, a throw away weed in the fabrication of sweat pads, will help in the switch over of a waste to wealth.

Eichhornia crassipes, popularly called waterhyacinth [5], is considered as a menace to the environment due to its rapid growth. In spite of the many problems that it causes, the solvent extracts of the plant possesses good antimicrobial activity, antioxidant activity, wound healing activity, and larvicidal activity [5] and is not toxic up to a tested dosage of 2000 mg/kg body weight [6].

Hence in the current study, the ethyl acetate extract of fresh *Eichhornia crassipes* was coated on to the cotton cloth and perspiration pad by three different methods, namely, sonication, dipping, and homogenization and was tested for its antimicrobial activity against *Corynebacterium* adopting standard procedures.

2. Materials and Methods

2.1. Collection of Plant. Eichhornia crassipes was collected from the lakes near Kurichi at Coimbatore between January 2012 and March 2012. The plant sample was identified by Dr. G. V. S. Murthy, Scientist F & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore, with the number BSI/SRC/5/23/2011-12/Tech.

2.2. Extraction of Plant Material. Eichhornia crassipes (80 g) was extracted with ethyl acetate (100 mL) for 30 minutes and desolvetised yielding ethyl acetate extract.

2.3. Phytochemical Tests. Phytochemical screening was carried out for the chloroform, ethanol and methanol extracts of waterhyacinth [7].

2.4. Preparation of Fabrics

2.4.1. Fabrication of Cotton Cloth with Ethyl Acetate Extract of Eichhornia crassipes. The white cotton cloth material was purchased with respect to the expected quality requirements. Cotton cloth (8 × 8 cm size) and perspiration pads (commercially available ones) were washed with Millipore water. The gel absorbent inside the perspiration pads was removed. The cotton cloth and the perspiration pad were air-dried. The samples were cut into small pieces; the diameter and size of the samples were measured. The samples were fabricated with the ethyl acetate extract of *Eichhornia crassipes* by sonication, dipping, and ultrasonic homogenizer methods. Similar procedure was adopted for coating standard sample ciprofloxacin.

2.4.2. Fabrication of Perspiration Pads with Ethyl Acetate Extract of Eichhornia crassipes. Ethyl acetate extract of *Eichhornia crassipes* was taken in a 500 mL beaker and then dissolved in Millipore water. The samples were subjected to sonication, dipping, and homogenization for 1 hour separately. After 1 hour, the samples were air-dried and used for the antibacterial assessment against the *Corynebacterium* strain.

2.5. Determination of Antimicrobial Effect of Fabrics

2.5.1. Test Organism. The inoculums for the experiment were prepared in fresh nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards by the addition of sterile saline.

2.5.2. Disc Diffusion Method (AATTC 147). A sterile swab was dipped in the standardized inoculums and inoculated onto the plates prepared earlier (aseptically). The excess of inoculum was removed by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60°C after each application. Finally the swab was passed round the edge of the agar surface. The inoculums were left to dry at room temperature with the lid closed. Each Petri dish was divided into 2 quadrants. In one quadrant, discs coated with the extract were placed and in the other quadrant standard Ciprofloxacin 10 μg were placed with the help of sterile forceps. Then Petri dishes were placed in the refrigerator at 4°C for 1 hour for diffusion and incubated at 37°C for 24 hours. The zone of inhibition produced by leached substance was measured. Unleached samples do not give zone of inhibition but no growth should be observed above and below the sample cloth and perspiration pads.

3. Results and Discussion

3.1. Phytochemical Screening. The phytochemical screening tests carried out for the ethyl acetate extract of fresh waterhyacinth show that the plant possesses phytochemicals of commercial importance. The results of the phytochemical screening tests for the ethyl acetate extract of fresh waterhyacinth are given in Table 1. The extract tested positive for alkaloids, flavonoids, sterols, terpenoids, anthraquinones, anthocyanins, phenolics, quinones, and carbohydrates.

3.2. Fabrication of Cotton Cloth and Perspiration Pads with Ethyl Acetate Extract of Eichhornia crassipes. The amount of ethyl acetate extract coated onto the cotton cloth and perspiration pad by different methods is given in Table 2. Sonication method resulted in better coating of the extract (35 mg) onto the cotton cloth, whereas dipping was found to be better method of coating for perspiration pads (13.3 mg).

Growth of moulds, yeasts, and bacteria causes discolouration, quality deterioration, and formation of odour. This necessitates protection of textile fabrics from microbial attack

Table 1: Phytochemical screening of the ethyl acetate extract of fresh waterhyacinth.

S. number	Phytochemicals	Ethyl acetate extract
1	Alkaloids	
a	Meyers test	+
b	Wagner's test	+
c	Hager's test	+
2	Flavonoids	
a	Lead acetate test	−
b	H_2SO_4 test	−
3	Sterols	
a	H_2SO_4 test	−
b	Liebermann-Burchard test	+
4	Terpenoids	
a	Chloroform test	+
b	Liebermann-Burchard test	−
5	Anthraquinone	
a	Borntrager's test	+
6	Anthocyanins	
a	NaOH test	−
7	Proteins	
a	Ninhydrin test	−
8	Phenolics	
a	Ferric chloride test	−
b	Liebermann's test	+
9	Quinones	
a	HCl test	+
10	Carbohydrates	
a	Molisch's test	+
b	Fehling's test	+
11	Saponin	
a	Foam test	−
12	Tannin	
a	Braymer's test	−

and this warrants antimicrobial treatment for textile materials to avoid cross infection by pathogenic microorganisms. The antimicrobial agents can be applied to the textile substrates by techniques like exhaust, pad-dry-cure, coating, spray, and foam techniques. Various test procedures have been used to test the effectiveness of the antimicrobial activity, to evaluate antimicrobial fabrics, and to protect users and textile against action mechanisms of antimicrobial compounds [8].

The cotton cloth was fabricated with the ethyl acetate extract of fresh plant of *Eichhornia crassipes* by three methods, namely, sonication, dipping, and using ultrasonic homogenizer. It is reasonably apparent from the results that sonication method yields good coating of the extract onto the fabric. Nearly three times the extract was coated onto the cloth material even after a wash, compared to dipping method and use of ultrasonic homogenizer. The coated cloth was washed by dipping in water after drying it for about 30 minutes.

Sonication in a sonic bath disperses the extract making it fine for efficient coating onto the sample cloth. Dipping involves slack adherence of the extract onto samples that it is removed in washing. The results attest the efficacy of sonication method in coating of extracts onto cotton cloth.

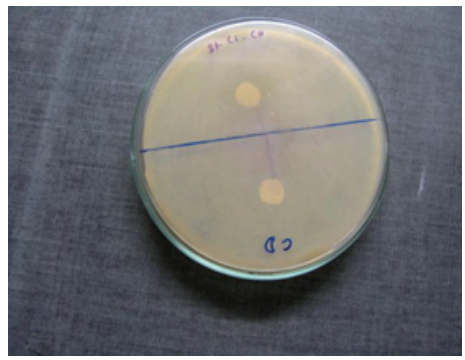

Figure 1: Antibacterial activity of cotton cloth fabricated by dipping method against *Corynebacterium*.

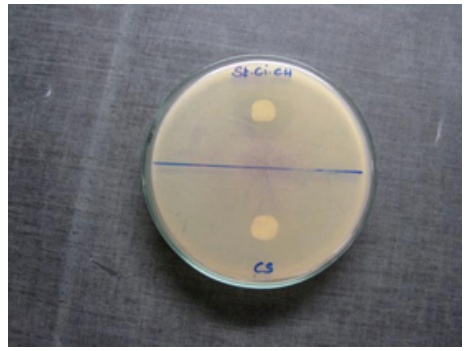

Figure 2: Antibacterial activity of cotton cloth fabricated by sonication method against *Corynebacterium*.

3.3. Determination of Antimicrobial Effect of Fabrics Coated with Ethyl Acetate Extract of Eichhornia crassipes. The antimicrobial activity of the cotton cloth (8 × 8 cm) and perspiration pads coated with ethyl acetate extract of fresh leaves and shoot portion of *Eichhornia crassipes* against *Corynebacterium* adopting AATCC 147 method are shown in Figures 1, 2, 3, 4, 5, and 6. No bacterial growth was observed under and on the fabricated cloth and perspiration pads indicating that the fabrics possess bacteriostatic property and not bactericidal property and the absence of leaching of the extract.

The perspiration pads were fabricated with the ethyl acetate extract of fresh plant of *Eichhornia crassipes* by sonication, dipping, ultrasonic homogenizer methods as in the case of cotton cloth. The commercial samples of perspiration pads were purchased and used as blank samples. The perspiration pads were washed with water to remove the existing gel coating in the commercial samples and were coated with the extract to test the efficacy of the extract. Similar procedure was adopted to quantify the amount of sample coated onto the pads.

Table 2: Various methods of finishing cotton cloth and perspiration pads.

Sample	Methods of coating of extract onto the sample	Weight of the cloth/perspiration pads (g)		Difference of weight (mg) coating
Cotton cloth [8 × 8 cm]	Sonication	137	172	35
	Dipping	199	207	8
	Ultrasonic homogenizing	262	276	14
Perspiration pads [8 × 8 cm]	Sonication	180	191	11
	Dipping	206	219	13
	Ultrasonic homogenizing	154	162	8

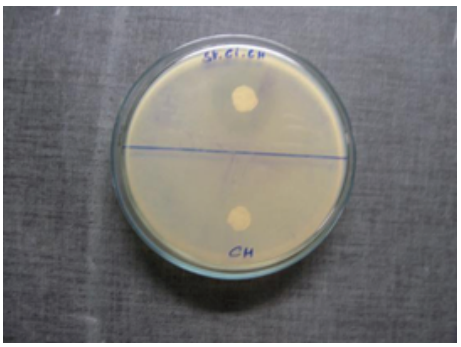

Figure 3: Antibacterial activity of cotton cloth fabricated by homogenizer method against *Corynebacterium*.

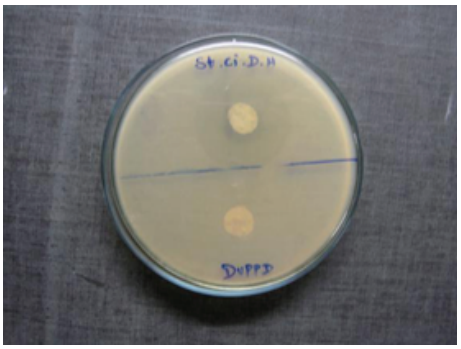

Figure 4: Antibacterial activity of perspiration pads fabricated by dipping method against *Corynebacterium*.

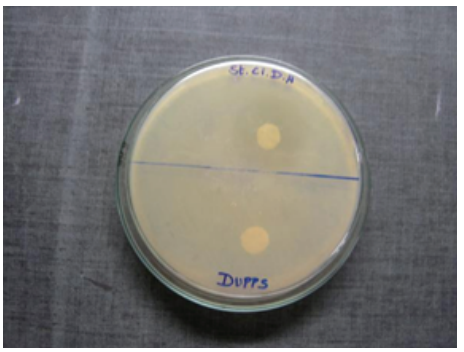

Figure 5: Antibacterial activity of perspiration pads fabricated by sonication method against *Corynebacterium*.

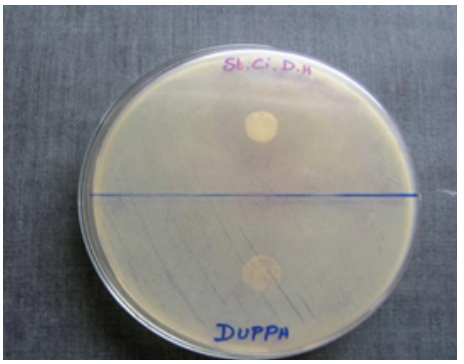

Figure 6: Antibacterial activity of perspiration pads fabricated by homogenizer method against *Corynebacterium*.

Ciprofloxacin was used as a standard and was also coated onto the cotton cloth and perspiration pads for one hour using homogenizer method. After washing it was found that 11.4 mg of the standard was coated onto the cloth and 11.5 mg of the standard was coated onto the perspiration pads.

In the case of coating of perspiration pads, dipping proved to be more efficient than the other three methods. This may be attributed to the nature of the pad materials which, being more of absorbing tendency, may have taken up the extracts immediately after dipping and retained it. In the case of sonication, desorption would have taken place since sonication would have dispersed off the extracts from the fabric in the one-hour period. This can be minimized by reducing the time of coating using sonic waves.

All the fabricated cotton cloth and perspiration pads were tested for antimicrobial activity against *Corynebacterium*. The fabricated cotton cloth and perspiration pads were placed on the surface of the Petri dish that has been inoculated with *Corynebacterium* and incubated for 24 hours. No bacterial growth was observed under and on the fabricated cloth and perspiration pads. According to qualitative AATCC 147 method for a successful result, there should be no bacterial growth observed under and on the test sample. This test is very easy to assess with antimicrobials that leach, as a zone of inhibition. With antimicrobials that are fixed to the fabric, there is no zone of inhibition and no bacterial growth under the test sample indicating that the fabrics possess bacteriostatic property and not bactericidal property.

In the present study, there was no leaching of the extracts as was seen from the absence of zone of inhibition. Good antimicrobial activity was noted since there was no growth in all the fabric samples, suggesting the success of the study.

The antimicrobial activity of the cotton cloth and perspiration pads may be attributed to the presence of phytochemicals like alkaloids, flavonoids, sterols, terpenoids, anthraquinone and anthocyanins, phenolics, quinones, and carbohydrates in the ethyl acetate extract of fresh waterhyacinth. Duangsri et al. [9] have reported the antimicrobial activity of the fabric coated with tobacco leaf containing higher polyphenol content. Steroids and phenols have been attributed to the antimicrobial activity of the cotton fabrics coated with peony extract and clove extract, respectively [10]. Flavonoids have also been demonstrated to possess bacteriocidal property [11].

Thymus vulgaris and *Citrus paradise* have shown significant antimicrobial activity when tested against the sweat bacteria including *Corynebacterium xerosis* [12]. In case of standard ciprofloxacin, the zone of inhibition around the cloth samples indicate the leaching of ciprofloxacin.

4. Conclusion

The results of the present study indicate that the ethyl acetate extract possesses activity against *Corynebacterium*. Further studies may be carried out to prove the effectiveness of the treatment. Hence, the plant may be successfully employed in the pharmaceutical industry as antibacterial finishes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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