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RESEARCH ARTICLE

SCREENING OF ANTIMICROBIAL FLAX FIBRES TREATED WITH LEAF EXTRACT OF *Azadirachta indica*

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ABSTRACT

In many stuffed textile products, generally waste fibres from spinning mills are used to be filled. These natural fibres are susceptible of microbial infestation and can also infect human who comes into contact of this waste fibre. Thus, the study is aimed at the treatment of waste flax fibre with extract of green leaves of *Azadirachta indica* which can be extracted by indigenous method and screening for antimicrobial potentials of treated samples against the gram positive (Staphylococcus Aureus) and gram negative bacteria (*Escherichia coli*). Extract was applied on to the fibre samples in ten different concentrations from 100% to 10 % and evaluated by standard test methods of AATCC and ENISO, out of which each sample possess good antimicrobial activity against these microorganisms and hinder their growth.

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INTRODUCTION

Textile industry produces a large number of waste products. The waste fibre from spinning mills is generally used as filling in beddings or producing low grade fabric. Natural textile fibres such as cotton, linen, wool etc. are easily attacked by various harmful microorganisms present in the atmosphere. Therefore, the bacterial adherence on textile fabrics is an important field of study to prevent the growth and further proliferation of these harmful organisms (Bajpai *et al.,* 2011). There are a vast variety of natural and chemical based antimicrobial agents. Plant extracts are known to have an effective antimicrobial efficiency against a number of bacteria and fungi. There are also non toxic to human and are ecofriendly in nature. Neem (*Azadirachta indica*) is a tree of mahogany family *Meliaceae*. Neem is known for its medicinal and therapeutic properties (Bhuiyan *et al.,* 1997) for ancient times. Neem products are being used as a indigenous antimicrobial agent for very long time due to their antifungal and antibacterial property (Nagarajan, 2009) and are widely used for textile applications now days. *Azadirachtin* is the most important active component of Neem, is a tetranortriterpenoid abundant found in the seeds and present in a smaller concentration in its leaves also (Priscila *et al.,* 2009).

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MATERIALS AND METHODS

Extraction and Application

Green leaves of neem tree were washed with cold water and grinded with the help of grinder by adding some distilled water to get desired consistency. The paste was diluted again, adding distilled water in the ratio of 1:2. The material was then filtered to get clear filtrate devoid of residues and sediments. Acetone and copper sulphate were added to act as solvent and cross linking agent respectively. Ten different concentrations (100 to 10%) of extracted finish were applied on to the fibre samples. Samples were then dried in hot air oven at 80^0C.

Evaluation of Antimicrobial Activity

Two bacterial cultures of Staphylococcus Aureus and *Escherichia coli* were used to assess the antimicrobial activity of samples.

ENISO 20645: 2004 Agar Diffusion Test: Using a micro pipette, 200 µm of prepared culture was transferred to the agar plate and spreaded with the help of a spreader. Specimen of 25 mm. diameter was aseptically placed on to the inoculated agar plate. Plate was then closed, sealed and incubated for 18-24 hr at 37^0C. Test was replicated four times for each concentration. Plates were examined for bacterial growth and the presence of clear zone between the agar and the test specimen. Total inhibition zone across the specimen was measured using a ruler. Inhibition zone was calculated using the following expression:

Fig.1. Antibacterial activity of Neem extract treated sample against S. Aureus

Fig.2. Control Sample

Fig.3. Antibacterial activity of Neem extract treated sample against E. Coli

Fig.4. Control Sample

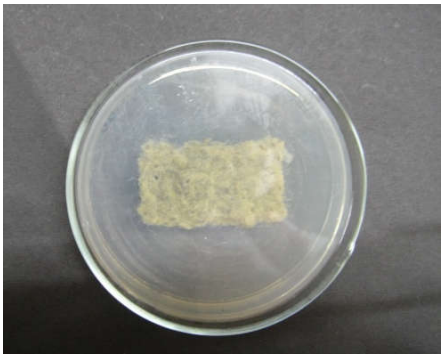

Fig.5. Antibacterial activity of Neem extract treated sample against S. Aureus

Fig.6.Control Sample

Fig.7. Antibacterial activity of Neem extract treated sample against E. Coli

Fig.8. Control Sample

Fig.9. Antifungal activity of Neem Extract Treated Sample

Fig.10. Control Sample

$$H=\left(D-d\ \right)\div 2,$$

where:

H- Inhibition zone in mm
D- Total diameter of specimen and inhibition zone in mm
d- Diameter of specimen in mm.

According to test method, >1-0 mm inhibition zones and no growth under specimen were accepted as effective, whereas 0 mm inhibition zone and slight growth were evaluated as limited effect.

AATCC 147: 2004-Parallel Streak Method: A loopful of the inoculum was taken to make five parallel streaks on the central area of a agar plate without refilling it again. Specimen 25×50 mm was placed onto inoculated plate transversely across streaks. Plates were then incubated for 18-24 hrs at 37^0C. After 18-24 hrs plates were examined for interruption of bacterial growth along the streaks and for a clean zone of inhibition beyond its edge. Zone diameter along a streak on either side of the test specimen was measured using a ruler.

The width of a zone of inhibition specimen was calculated using the following equation:

$$W = (T - D) \div 2$$

Where, W is width of clear zone of inhibition in mm
T is total diameter test specimen and clear zone in mm
D is diameter of the test specimen in mm.

Absence of bacterial colonies under the specimen in the contact area is considered as an acceptable antibacterial activity for parallel streak method.

AATCC 30-1993: Antifungal activity, Assessment of textile material: mildew and rot resistance of textile material (Humidity jar method)

500ml conical flask containing of Potato Dextrose Agar was prepared and sterilized at 121^0C for standard time. It was then allowed to cool. The samples were transferred aseptically into the flasks and kept at room temperature for 3 days. Then the growth of fungi in the flasks was observed after 3 days.

RESULTS AND DISCUSSION

Samples with different concentrations, when tested against S. Aureus according to ENISO 20645, the value of H found ranging from 21.50 to 2.89 (the highest and lowest values). 2.88 (10%) was the lowest value having effective antibacterial activity. Samples also showed effective antibacterial efficacy against *E. Coli* with 2.70 H value for lowest concentration (10%), while highest value was 15 for 100% concentration. Inhibition zone of the neem leaf treated samples (100%) against both the bacteria is shown in Fig. 1 and 3 and mentioned clearly in Table 1. Test control was found to have no antibacterial effect. The complete growth of bacterial colonies was found throughout the agar plate and below the samples as well Fig. 2 and 4. Samples when tested as per AATCC 147, showed sufficient antibacterial potential against S. Aureus with the W value of 20.50 to 2.63 for 100 to 10% concentrations. Samples were also found having antibacterial effect against *E. Coli* that is 14.63 to 02.50 (100 to 10%) as mentioned in Table 2 and Fig 5 and 7.

Table 1. Mean H values for samples tested with ENISO 20645: 2004

S. No.	%	Neem		Control
		S. A.	E. C.	S. A./E. C.
1.	100	21.50	15.00	
2.	90	21.25	12.50	
3.	80	17.25	09.75	
4.	70	10.00	08.75	
5.	60	07.00	08.50	00.00
6.	50	05.80	04.00	
7.	40	03.95	03.55	
8.	30	03.58	03.13	
9.	20	03.25	03.15	
10.	10	02.88	02.70	

S.A. = Staphylococcus AureusE.C. = *Escherichia coli*

Table 2. Mean W values for samples tested with AATCC 147: 2004

S. No.	%	Neem		Control
		S. A.	E. C.	S. A./E. C.
11.	100	20.50	14.63	
12.	90	20.20	12.25	
13.	80	16.38	09.38	
14.	70	09.25	08.75	
15.	60	06.50	08.00	00.00
16.	50	03.75	03.68	
17.	40	03.00	03.45	
18.	30	03.00	03.00	
19.	20	05.38	03.00	
20.	10	02.63	02.50	

The results against *E. Coli* were also found acceptable as the samples showed 14.625 and 2.5 value of W for 100 and 10% respectively. Results are clearly shown with the help of Table 2 and also illustrated through Fig 5 and 7. Test control did not show any antibacterial effect against both the test bacteria as there was no inhibition zone was found (Fig. 6 and 8). Samples were also assessed for its antifungal activity through humidity jar method. The results found, indicates that both the samples possess desired antifungal efficacy. There was no growth of fungi in the flask containing treated samples after 3 days (shown in Fig 9). Growth of fungi was clearly visible in the flask of test control (Fig 10).

Conclusion

Results found through both the tests methods, revealed that all the treated samples were found to have acceptable level of antimicrobial effect against gram positive and gram negative bacterium, when tested according to the test method EN ISO: 20645 and AATCC: 147. The results found during antifungal assessment using test method AATCC: 30 (Humidity Jar Method), indicate that all the samples possess desired level antifungal efficacy as there was no growth of fungi in the flask containing samples after 3 days.

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