A STUDY ON ANTIMICROBIAL EFFICIENCY OF MANGROVE LEAF EXTRACT ON COTTON FABRIC

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ABSTRACT

Leaf extract of mangrove plant *Exocoelearia agallocha* showed very promising antimicrobial properties against pathogenic strains on cotton fabrics. Methanol extract of leaves of the mangrove plant showed higher antimicrobial activity on all the Pathogenic strain as against aqueous extract of the same plant. The antimicrobial efficacy was found to be better when treated with combination of silicon softener and leave extracted antimicrobial finish. The treated samples sustained reasonable antimicrobial activity to repeated laundering.

KEYWORDS: Mangrove Plant, Bioactive Antimicrobial Finish, Value Addition, Silicon Softener, Zone of Inhibition, Washing Fastness

INTRODUCTION

Apparel sector, at present is one of the fast growing sectors in textiles. Various advanced marketing and consumers’ psychological tools are used by the garment manufacturing industries / fashion brands to lunch new products or to promote traditional products. Promoting traditional textiles by highlighting its value added aspects is a very common practice of Apparel Manufacturers/ prominent brands. Among the various value added tools employed presently with cotton fabrics, wrinkle free finish, antimicrobial finish and natural dyed colour are on the major front.

With growth in world population and the spread of diseases, the number of antibiotic resistant microorganisms is rising along with the occurrence of infections from these microorganisms. The need for antimicrobial textiles goes hand-in-hand with the rise in resistant strains of microorganisms. Consumers’ demands for hygienic clothing have created a substantial market for antimicrobial textile products. Many commercial antimicrobial agents are available but their toxicity to people and the environment are major issues. Over the past century, much focus has been placed on the sustainability of the Earth’s environment and healthy leaving of mankind. An innovative approach to make the cloth microbial resistant is to apply the extracts containing bio active substances from natural resources which are not only eco-friendly but also from renewable sources.

Antimicrobial finish is applied with a view to protect the wearer and the textile substrate itself. It is quite interesting to note that human sweat provides a suitable shelter for bacterial growth containing 1.4 million bacteria per gram which increases to 9000 million at 50 % moisture level. Antimicrobial textiles currently on the market are either disposable or used primarily for odour control, the availability of a reusable and durable antimicrobial textile effective against harmful pathogens will not only be beneficial to both medical industry workers and patients but also to the general public as well.

Studies shows that plant based bioactive agents have opened up new avenues in this area of research. Antimicrobial activity of cotton fabric treated with Quercus infectoria extract, using Neem (*Azadirachta indica*) extract, using Aloe vera extract, with Tea tree (*Melaleuca alternifolia*) , with Prickly chaff flower (*Achrysanthis aspera*) by
using Tulsi leaves oil extract have been studied by many researchers. Some of the other important natural products, such as Karanga oil, cashew shell oil, Henna or mehndi can also be explored on textile substrates for antimicrobial property which will have tremendous application in apparels and medical textile.

Very few literatures/ studies are available on the effect of mangrove plant extracts on different fabrics against pathogenic strains. In the present study an attempt has been made to evaluate the antimicrobial efficacy of plant extracts of mangrove species viz. *Excoecaria agallocha* so as to add one more step to the value addition aspects of cotton fabrics to be used in apparels.

Mangroves are salt tolerant plant found in intertribal regions of tropical and sub-tropical estuarine zones. Mangroves of India are confined to west court, east court and Andaman & Nicobar Islands. Mangrove of Bhitarkanika and Mahanadi delta are two mangrove ecosystems in Odisha. Mangrove plants have wide scale uses as medicines. Until now, more than 200 bioactive metabolic have been isolated from the true mangrove of tropical and sub-tropical populations. The antimicrobial efficacies of the leaf extract of mangrove plants on cotton hand woven fabrics have been studied during this experiment.

**MATERIALS AND METHODS**

**Materials**

100% cotton handloom fabric having following specifications have been used in the study.

- Warp count – 50\(^S\), Weft count - 49\(^S\), Ends/ inch- 64, Picks/ inch-61, Weave - 1 up 1 down.

Leave of mangrove plants (*EXOECARIA AGALLOCHA*) were collected from mangrove forests of Bhitarkanika, Wildlife Sanctuary of Kendrapara district of Odisha (Fig. 1&2). The leaves were air dried for 7 days, then grinded by a grinder to form leaf powders.

Fabric softener (silicone softener) was collected from Clarient Chemicals (Trade name and exact specifications were kept reserved due to company’s trade policy). Laboratory grade MgCl\(_2\) and acetic acid were used as catalyst and pH regulator respectively during the treatment.

**METHODS**

**Desizing of the Fabric**

Enzymatic desizing was carried out in order to remove the sizing materials (starch) by using Amylase-250, supplied by Chembond India, Pvt. Ltd.

**Preparation of Methanol Leaf Extract**

10 gms of leaf powder were taken in a 250ml conical flask (Fig-3). Then, 100 mL of methanol was added to it. The mouth of the conical flask was covered with aluminium foil and kept in an orbital shaker (SLM-OS-250D- machine).
for 48hrs in 100rpm speed. After 48hrs, it was removed from the orbital shaker and the solution was filtered using gauge cloth. Then the filtered solution was kept in a 250ml beaker and dried inside the oven at 30-50°Celsius to obtain the methanol extract of the leaf powder.

![Figure 3: Orbital Shaker](image)

The dried extract powder was scrapped and was filled inside the Apprendrof tube (fig-4 &5) so as to calculate the weight of the extract. The percentage of yield (for methanol extract) was calculated with respect to the amount of extract taken. The yield % (methanol extract) was found to be 45.34%.

![Figure 4: Scrap of Dried Methanol Extract](image) ![Figure 5: Filling of Extract into Apprendrof Tubes](image)

**Preparation of Aqueous Leaf Extract**

The residue in the conical flask was mixed with 100ml of distilled water. Then the mouth of the conical is covered with aluminium foil and kept on orbital shaker for 48hrs in 100rpm speed. After 48hrs, it is removed from the orbital shaker and the solution was filtered using gauge cloth (Fig: 6).

![Figure 6: Filtration](image)
Then, the filtered solution was kept in a 250ml beaker and dried inside the oven at 30-50°Celsius to obtain the aqueous extract of the leaf powder. The dried extract powder was scrapped and was filled inside the Appendix tube and the yield % was calculated in the similar way as done above for methanol extract. The percentage of yield (for aqueous extract) was noted to be 14.6%.

**Treatment of Fabric with Softener**

A solution was prepared by taking water 10 times the weight of the fabric and 40 grams per liter of silicon softener. The fabric was dipped in the softening solution for half an hour. Then it was passed through the padding mangle. After that the fabric was kept inside the oven at 130°C for few minutes and then taken out.

**Treatment of Fabric with Anti Microbial Agent**

Four types of fabric samples as mentioned below were taken for assessing antimicrobial efficacy;

Sample no.1- Control fabric (untreated fabric) – ‘F’

Sample no.2- Fabric treated with silicone softener- ‘S’

Sample no.3 - Control Fabric + Leaf extract - ‘F+E’

Sample no.4 - Softener treated fabric + Leaf extract – ‘S+E’

**Procedure and Evaluation for Bacterial Growth Methanol Extracted Leaf Powder**

Dilute solution of bioactive antimicrobial agent i.e. leave extract in methanol was prepared by mixing 50 mg of methanol extracted leave powder in 1 ml of water in a test tube. The solution was left for half an hour. Then the solution was poured in to two different beakers. Four numbers of fabric samples as mentioned above were then dipped in this methanol extract solution separately. Then the samples were taken out from the beaker and were dried in the hot air oven at 60 degree centigrade for 5 minutes. After 5 minutes samples are taken out from the hot air oven and kept ready for treatment with pathogenic stains.

- **Preparation of Flasks:**-100 ml of water was taken in conical flask then 3.1 gm. nutrient agar was added to the flask. It was slightly heated for complete dissolution of nutrient agar in the water (fig.-7).

- **Sterilization:**-Flask of culture medium, Petri plates, pipettes, beakers, forceps, cotton swabs, and test-tubes were wrapped accurately with paper, tied with thread so that no water can penetrate inside and then sterilized
A Study on Antimicrobial Efficiency of Mangrove Leaf Extract on Cotton Fabric

in autoclave at 15 lb. pressure and 120°C for 15 min. Four numbers of Petri plates were then kept in oven for drying. Then all the above mentioned equipment’s were kept in laminar air flow.

- **Inoculation**: Each Petri dish was filled up to half with nutrient agar. The agar medium was then allowed to solidify. Inoculation was done in totally sterile conditions in laminar flow.

**The Following Pathogenic Strains were Used during this Study**

- Staphylococcus aureus (MTCC-1144, gram +ve), Strain-1
- Shigella flexneri (Lab isolated, gram -ve), s- 2
- Bacillus licheniformis (MTCC-7425, gram +ve), s-9
- Escherchia coli (MTCC-1089, gram -ve) s-10

100microlitres of each type of the above pathogenic strains contained in test-tubes were added to each of the four Petri dishes and spread all over by means of the cotton swabs. Then by means of sterilized forceps, above prepared four numbers of specimens (cloth) were placed in each Petri plate and labelled (fig- 8).

![Figure 8: Inoculation](image)

- **Incubation**: The Petri dishes were kept in an incubator at 37degree Celsius for about 18-24 hours. After the stipulated time, the anti-microbial activity of the finish was evaluated by following Agar diffusion method i.e. measuring the developed ‘zone of inhibition’. The results so obtained are shown in Fig. 9 to fig.12 &Table-1.

**Procedure and Evaluation for Bacterial Growth (Aqueous Extracted Leaf Powder)**

Dilute solution of aqueous extract bioactive agent were prepared by mixing 50 mg of aqueous extract leaf powder with 1 ml of water in a test tube. The solution was kept for half an hour. The solution then shifted to two different beakers. In the similar way as done during methanol extract mentioned above, the four fabric samples as mentioned above were dipped in to the aqueous extract solution separately. The samples were taken out and dried in the hot air oven at 60 degree centigrade for 5 minutes. After 5 minutes, take the samples out from the hot air oven and put it in the Petri-plates. In the same manner as mentioned above, the sample fabrics which were treated with aqueous leave extract were placed into pathogen strained agar plates and in the similar way the anti-microbial activity of the finish was evaluated by measuring the developed ‘zone of inhibition’. The results so obtained are shown in Fig. 13 to fig.16 &Table-2.
Evaluation for Bacterial Growth of Fabrics Samples towards Washing Fastness Property

• Washing fastness to Methanol Extracted leave powder

The garments are subjected to repeated washes. If the treatment on the fabrics with bioactive antimicrobial finishes do not last for a good number of washes, then this type of finishes are not of much use for garments. Hence, study was conducted to measure the washing fastness property of the treated materials.

The samples (cotton fabrics) were treated with solution of methanol extract leave powder as mentioned above and were dried in hot air oven. Sixteen nos. of such samples of 1 cm. diameter each were are washed in a laundry-o-meter for 5mins, 10 mins, 15 mins and 20 mins wash cycle respectively. Samples are collected after wash, dried and then dipped in to the agar plates as prepared in the same manner as described above. Then anti-microbial activity of these fabrics was evaluated by the same ‘Agar diffusion test method’ i.e. by measuring the developed zone of inhibition. The results so obtained are shown in Fig. 17 to fig.20 & Table-3.

• Washing Fastness to Aqueous Extracted Leave Powder

The sample cotton fabrics were treated with solution of aqueous extract leave powder as mentioned above and were dried in hot air oven. Sixteen nos. of such samples of 1 cm. diameter each were are washed in a laundry-o-meter for 5mins, 10 mins, 15 mins and 20 mins respectively. Samples are collected after wash, dried and then dipped in to the agar plates as prepared in the same manner as described above. Then anti-microbial activity of these fabrics were evaluated by the same ‘Agar diffusion test method’ i.e. by measuring the developed zone of inhibition. The results so obtained are shown in Fig. 21 to fig.24 & Table-4.

RESULTS & DISCUSSIONS

Antimicrobial Efficiency of Leaf Extract

The purpose of this study was to evaluate the efficiency of application of bio active antimicrobial finishing prepared from mangrove plant leaf extracts against some gram +ve and gram –ve bacteria in cotton fabrics and also to study its durability to repeated washing of the treated fabrics.

Figures Showing ‘Zone of Inhibition’ on Different Strains (Methanol Extract Solution)

Figure 9: With ‘Staphylococcus aureus’  
Figure 10: With ‘Shigella flexneri’ Strains
A Study on Antimicrobial Efficiency of Mangrove Leaf Extract on Cotton Fabric

Table 1: Result Showing Zone of Inhibition of Different Strains Methanol Extracted Solution

<table>
<thead>
<tr>
<th>Pathogenic Strain</th>
<th>Control Fabric (Untreated) (in mm) ‘F’</th>
<th>Fabric with Softener Treated (in mm) ‘S’</th>
<th>Fabric with Leaf Extract Treated (in mm) ‘F+E’</th>
<th>Fabric with Softener and Extract Treated (in mm) ‘S+E’</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12</td>
<td>16</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>12</td>
<td>18</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>10</td>
<td>17</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td><em>Escherchia coli</em></td>
<td>10</td>
<td>15</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

Figures Showing ‘Zone of Inhibition’ on Different Strains (Aqueous Extracted Solution)

Figure 11: with ‘*Bacillus licheniformis*’ Strains

Figure 12: with ‘*Escherchia coli*’ Strains

Figure 13: With *Staphylococcus aureus*

Figure 14: With *Shigella flexneri*

Figure 15: With *Escherchia coli*

Figure 16: With *Bacillus licheniformis*
Table 2: Result Showing Zone of Inhibition for Different Strains Aqueous Extracted Solution

<table>
<thead>
<tr>
<th>Pathogenic Strain</th>
<th>Control Fabric (Untreated) (in mm) ‘F’</th>
<th>Fabric with Softener Treated (in mm) ‘S’</th>
<th>Fabric with Leaf Extract Treated (in mm) ‘F+E’</th>
<th>Fabric with Softener and Leaf Extract Treated (in mm) ‘S+E’</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>15</td>
<td>17</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>17</td>
<td>18</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>15</td>
<td>17</td>
<td>18</td>
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</table>

Fig. 9 to fig.12 &Table-1 shows the ‘zone of inhibition’ on different strains (methanol extract solution) and fig.13 to fig.16 & Table-2 shows the ‘zone of inhibition’ on different strains (aqueous extract solution) as assessed by standard agar diffusion test method. The result of the study revealed that leave extract of mangrove plant possess good antimicrobial resistance property against all the pathogenic micro organisms. Fabric treated with leaf extract (‘F+E’) showed better microbial resistance property against fabric treated with only silicone softener ( ‘S’). However Fabric treated with softener and leaf extract (‘S+E’) showed better antimicrobial activity as against fabric treated with only leaf extract (‘F+E’) or with only silicone softener ( ‘S’) . Most of the cotton fabrics used for garment purpose is generally given partial silicone softener treatment in order to impart better hand value/ handle property of the garments, which is a prime factor for consumers to decide before purchasing any garment. Handle property of any textile material refers to how smooth or how rough to touch; how stiff it is or conversely, how limp; how soft, or how harsh, etc.. The application of silicone softener in the finishing stage of fabrics improves the hand value of fabrics by making the fabric soft and flexible. Like synthetic dyes and other synthetic textile finishes , it is obvious that silicon would impart some antimicrobial property. The results above have also proved the same. But it is certainly not advisable to use more and more amount of silicone softener during garment finishing in order to improve the antimicrobial property of the garments, as because it is well known that the synthetic dyes and synthetic finishing chemicals cause health hazard by damaging skin. In some of the developed countries, the textiles with synthetic dyes and finish material are already banned. Hence, antimicrobial finishing treatment in garments by using bioactive agents like Mangrove leaf sources instead of any synthetic antimicrobial finish, certainly adds better value to the textile materials.

Wash Durability of Treated Samples

Fig- 17 to 20 & Table 3  and Fig- 21to 23 & Table 4  show the wash durability studies carried out on treated fabrics.

( Figures Showing Washing Fastness Property to Different Strains (Methanol Extracted Leave Powder )

![Figure 17: With Staphylococcus aureus](image1)

![Figure 18: With Shigella flexneri](image2)
Table 3: Result Showing Zone of Inhibition towards Washing Fastness Property Methanol Extracted Leave Powder

<table>
<thead>
<tr>
<th>Pathogenic</th>
<th>Control Fabric (Untreated) (in mm) ‘F’</th>
<th>Fabric with Softener Treated (in mm) ‘S’</th>
<th>Fabric with Leaf Extract Treated (in mm) ‘F+E’</th>
<th>Fabric with Softener and Extract Treated (in mm) ‘S+E’</th>
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<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>12</td>
<td>16</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>12</td>
<td>18</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>10</td>
<td>17</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Escherchia coli</td>
<td>10</td>
<td>15</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

Figures Showing Washing Fastness Property to Different Strains (Aqueous Extract )

Figure 19: With *Bacillus licheniformis*  
Figure 20: With *Escherchia coli*

Figure 21: *Staphylococcus aureus*  
Figure 22: *Shigella flexneri*  
Figure 23: *Bacillus licheniformis*  
Figure 24: *Escherchia coli*
Table 4: Result Showing Zone of Inhibition towards Washing Fastness Property 
(Aqueous Extracted Leave Powder)

<table>
<thead>
<tr>
<th>Pathogenic Strain</th>
<th>0 min Wash</th>
<th>5 min Wash</th>
<th>10 min Wash</th>
<th>15 min Wash</th>
<th>20 min Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30</td>
<td>21</td>
<td>21</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>22</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>31</td>
<td>24</td>
<td>24</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td><em>Escherchia coli</em></td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

It was noticed from the above, figures Fig- 17 to 20 & Table 3 and Fig- 21 to 23 & Table 4, that the antimicrobial treated fabrics could able to sustain a good number of washing cycles. This may be due to intensive binding of the finishing substrates in the internal structure of the fibre matrix. This is also supported by the Scanning Electron Microscope Test.

Figure 25: Untreated Sample

Figure 26: (05 Minutes Wash)

Figure 27: (10 minutes Wash)

Figure 28: (15 minutes Wash)

Figure 29: (20 minutes Wash)

The intensity of adherence of the bioactive antimicrobial treated finish intimately in the internal structure of the cloth after different washing cycles were very well noticed in the above SEM test photographs in figures 25 to 29. However, a slight reduction in the antimicrobial resistance capacity of the material with increase in number of washes was noticed.

CONCLUSIONS

From the above results, it was noticed that the leave extract of mangrove plant *Exocoecaria agallocha* showed very promising antimicrobial properties against cotton fabrics. Antimicrobial activity of fabrics showed a varied response with respect to different Pathogenic strains also. The results of the present study is very encouraging as it was found that these bio active antimicrobial treated fabrics could inhibit the growth of pathogenic microbes at very low concentration.

Very narrow zones of inhibition were observed in control (untreated) fabrics as because synthetic dyes were used in the yarns during dyeing process.
It was noticed that the treatment of fabrics with silicone softener also exhibited bacteria resistant properties. Hence, besides the prime advantages of using silicone finish for improving fabric hand value by imparting soft feel, silicon can also be used as antimicrobial finishing agent.

The methanol extract of leaves of the mangrove plant (Exocoecaria agallocha) showed higher antimicrobial activity on all the Pathogenic strain as against aqueous extract of the same plant.

The inhibition zone was found to be better in case of samples treated with a combination of silicon softener and leave extracted antimicrobial finish.

The treated samples rendered good washing fastness property as because it sustained antimicrobial activity even after 20 minutes wash cycles. Hence, may be strongly recommended for application with garments.

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