Antibacterial Treatment of Guava Leaves on Cotton

Zeba Jamal* and Vivek Singh**

Consumers of 21st century are aware of the importance of hygienic and safe clothing for them. They not only want a clothing safe for them but also for the environment as well. The demand from these consumers have lead to the development of eco friendly treatments. The antibacterial treatment from Guava leaves is one such attempt to treat cotton fabric for safety of skin and environment. The extract of Guava leaves was prepared by cold maceration and applied through exhaust method. The treated cotton sample showed a considerable reduction in bacterial colony counts of Bacillus spp. as compared to controlled sample.

[Keywords: Consumers, Guava leaves, Maceration, Exhaust, Cotton]

1. Introduction

Microbes are the tiniest creatures which cannot be seen with the naked eye and can be found almost everywhere in the environment. Micro-organisms include a variety of organisms like bacteria, fungi, algae and viruses. Bacteria grow very rapidly under warmth and moisture. Some specific types of bacteria are pathogenic and cause cross infection as these are capable of invading the body of a human, where they replicate and cause tissue damage. Pathogenic micro-organisms like Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus aureus, Bacillus spp. and Candida albicans have been found on textiles. They damage the textile substrate and may also promote skin contamination and inflammation in sensitive people (Haug, et al. 2006).

* Ph. D Scholar, Department of Textile & Apparel Designing, COHS, CCS Haryana Agricultural University, Hisar, Haryana (India) E-mail: <zebajamal@hau.ernet.in>

** Associate Professor, Department of Textile & Apparel Designing, COHS, CCS Haryana Agricultural University, Hisar, Haryana (India) E-mail: <vivek_hau@rediffmail.com>
Bacterial growth on the textiles can be inhibited by applying chemical and natural botanical anti-microbial agents. In recent years, anti-microbial agents like triclosan, quaternary ammonium compounds, metal salts solutions, antibiotics and nanosilver are available for use on textiles (Mahesh, et al., 2011). Synthetic anti-microbial agents leads to fabric strength loss, change the colour of the fabric and also harm the environment (Hussain, 2006). Due to these problems natural products in textile substrate are gaining significance momentum. Among them, plants and plant products are more reliable for its renewability and therefore considered as catalyst for human welfare (Mahesh, et al. 2011). Plants have their own self defense mechanism and protect themselves from microbes due to the presence of substances known as phytochemicals. Guava, the most popular fruit is a powerhouse of nutrients.

Health benefits of Guava fruit are known to most of us but we are unaware of the fact that even Guava leaves have several medicinal properties and offer an array of health benefits. The phenolic compounds such as \( \alpha \)-pinene, \( \beta \)-pinene, limonene, menthol, \( \beta \)-copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, \( \beta \)-sitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4- arabinofuranoside (aviclarin) and 3-L-4 pyranoside (Essential oil), resin, tannin, eugenol, caryophyllene, azulene, Guajivolide and guavenoic acid, triterpene oleanolic acid, triterpenoids, found in Guava leaves possess antioxidant and antimicrobial properties (Kumar, 2012). Aqueous extract of Guava leaves is described to be effective against a number of microbial strains.

Considering the health problems faced by the consumers due to environmental hazards like microbes as well as understanding the importance of cotton in our life, the present study was conducted for the application of Guava leaves extract treatment on cotton fabric for microbial resistance with following objectives:

1. To prepare the extract from leaves of Guava tree for application on cotton fabric.
2. To determine microbial resistance of the treated fabric

2. Materials and Methods

Two types of materials namely cotton woven fabric and leaves of Guava plant were procured. A survey was conducted in local market of Hisar city of Haryana state to procure cotton woven fabric. On the basis of visual inspection, pure grey cotton woven fabrics suitable for apparel during summer season were collected. The collected fabrics were subjected to burning, physical and chemical tests for conformity of pure cotton fabric. The cotton fabric in grey state exhibiting light to medium weight was selected for the study.

Fresh, fleshy, mature and green leaves of Guava plant (Hisar Surkha) were collected from Agriculture Farm of CCS Haryana Agricultural University Hisar, because of easy accessibility.
To ensure complete wetting and uniform absorbency of the extract, cotton woven fabric was subjected to preparatory processes. Cotton woven fabric was desized and scoured. After that the fabric was rinsed thoroughly and dried on a flat surface (Saini, 2014). Samples taken from scoured cotton fabric was kept as controlled sample.

Collected leaves were washed and allowed to dry in shade to avoid breakdown of important phytochemicals. These were crushed and ground by grinder mixer to make into a fine powder. After that, the powder was sieved to remove the dirt and unkind particles. The dry powder was weighed and subjected to aqueous extraction by maceration. 100 gm dry powder of Guava leaves was placed in two closed vessels containing 800 ml distilled water (Yimer et al., 2014) for 12 and 24 hours separately at room temperature. During this period shaking was done occasionally.

The extract obtained was weighed and percentage yield was calculated in terms of air dried powder weight of the plant material using the following formula.

\[
\text{Yield percentage} = \frac{\text{Yield obtained (G)}}{\text{Weight of the dry plant material (g)}} \times 100
\]

The time period having greater yield percentage was selected for extraction.

Agar well diffusion method of Dey et al., 2010 with slight modification, was employed to study the antibacterial property of plant extracts. The Nutrient Agar medium (28 g nutrient agar in 1000 ml distilled water) was prepared. After autoclaving, the media was poured into sterilized petri plates with a uniform thickness of approximately 4 mm. The agar plates were allowed to solidify at ambient temperature and were used after 24 hours. Four uniform wells were prepared with the help of sterile cork borer of 5 mm diameter in agar plates. The entire agar surface was then inoculated with bacterial culture by spreader and left for 3 to 4 minutes. Different concentrations 1mg/ml, 3mg/ml and 5mg/ml of Guava leaves extract were added to the grooves and one control (sterilized distilled aqueous) was added to groove on agar plate (Plate 3). The plates were incubated for 24 hours at 300 C. Plates were examined and zone of inhibition of the bacteria growth was measured in mm by using diameter measurement scale. The assays were performed in triplicate and expressed as average values. Antibacterial
activity of Guava leaves extract and control was interpreted as no activity (- mm), weak (<6 mm), moderate (7-12 mm) and strong activity (>12 mm).

**Plate 3 : Zone of inhibition of Guava leaves extract at different concentrations**

Extract was applied in one concentration (the one exhibiting the best zone of inhibition) on scoured cotton fabric by using standard exhaust method (Hooda, 2012).

Cellulose degrading bacterium i.e. Bacillus spp. was selected for the present study as cotton is susceptible to its growth and culture was also readily available in the Department of Microbiology, college of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar for use in research. Culture of procured bacterium was maintained on Carboxy Methyle Cellulose petri plates and stored at 4°C.

The inoculated broth was incubated at 30°C for microbial counts on 0 day (soon after 24 hours of inoculation), 7th, 14th, 21st and 28th days. The plates were placed in an incubator for bacterium at 30°C. After 24 hours the colonies of bacterium were counted manually (Saini, 2014). The results were enumerated as percent reduction in the bacterium count of the treated samples in comparison to the controlled and treated samples and were calculated as under:

Formula used for CFU calculation :

\[
CFU = \frac{\text{No. of colonies}}{\text{Amount plated (ml)}} \times \text{dilution factor}
\]

(Malpani, 2013)

Where,

CFU-Colony Forming Unit

\[
\% \text{ Reduction} = \frac{\text{Initial cell no.} \times \text{Final cell no.}}{\text{Initial cell no.}} \times 100
\]

(Karolia, 2007)

Data for Guava leaves treated fabric samples were coded, tabulated and analyzed by the application of statistical tools.

3. **Results and Discussion**

3.1 **Determination of Yield Percentage of Guava leaves extract**

Data presented in Fig 1 showed the yield obtained by cold maceration extraction process of Guava leaves at different time periods. Results revealed that the yield obtained for 12 hours was 6 g and for 24 hours was 15 g. Thus, the yield
percentage of Guava leaves was found to be higher for 24 hours (15%) as compared to yield percentage for 12 hours (6%). The reason behind increased yield percentage with an increase in time period may be because more phytochemicals are extracted due to increase in keeping time. The results are in alignment with the findings of Gupta, (2016) who reported increase in mass yield percent of Syzygium cumini (L.) extract with increased length of extraction period.

3.2 Antibacterial Property of Guava Leaves Extract at different Concentrations

The data in Table-1 highlighted that sterilized distilled aqueous kept as control had no inhibition. The concentrations 1 mg/ml and 3mg/ml exhibited moderate zones of inhibition i.e. 10mm and 12 mm, respectively while 5 mg/ml concentration had a strong zone of inhibition i.e. 19 mm against Bacillus spp. The increase in antibacterial property with increase in concentration may be because of presence of more phytochemicals and more extract gets dissolved in higher concentration as compared to lower concentration. The results coincide with the results of Saini (2014) who reported that as the concentration of all treatments of karanja extracts increased percentage reduction of Pseudomonas and Aspergillus growth in all treated samples increased. Gupta (2016) also revealed that the activity of the treated sample increased with the increase in extract concentration.

Table-1 : Antibacterial property of Guava leaves extract at different concentrations

<table>
<thead>
<tr>
<th>Guava leaves extract Concentrations</th>
<th>1 mg/ml</th>
<th>3 mg/ml</th>
<th>5 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone of inhibition (mm)*</td>
<td>10</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Control (sterilized distilled aqueous)</td>
<td>No Inhibition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3 Efficacy of Guava leaves Extract Treatment against Growth of Bacillus Spp.

The efficacy of Guava leaves extract treatment against growth of cellulose degrading bacteria i.e. Bacillus spp. on treated samples, was counted quantitatively by AATCC-I00 test method. The antibacterial activity of treated cotton woven fabric inoculated with Bacillus spp. was compared to its controlled sample by calculating the percent reduction in bacterial count. The observations
were recorded soon after 24 hours of inoculation of *Bacillus spp.* on the same day and also on 7th, 14th, 21st and 28th days of inoculation with dilution factors 107 and 108. Mean values of 107 and 108 dilution factors were calculated.

There was 100 percent reduction on 0 day soon after 24 hours of inoculation i.e. no bacterial growth was observed in treated fabric with mean CFUs in $10^7$ and $10^8$ dilution factors. The percent reduction on 7th day was 93 percent with $7 \times 10^8$ mean CFUs in $10^7$ and $10^8$ dilution factors. The percent reduction on 14th, 21st and 28th day of inoculation was found to be 86, 77 and 67 with $1.4 \times 10^9$, $2.3 \times 10^9$ and $3.3 \times 10^9$ mean CFUs in $10^7$ and $10^8$ dilution factors respectively.

**Table 2: Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.***

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>Bacillus spp.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day (soon after 24 hours)</td>
</tr>
<tr>
<td>Treated fabric</td>
<td>0</td>
</tr>
<tr>
<td>Percent reduction (%)</td>
<td>100</td>
</tr>
<tr>
<td>Controlled fabric</td>
<td>Confluent growth</td>
</tr>
</tbody>
</table>

Plate 4: Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.* at different incubation periods

The antimicrobial activity of Guava leaves extract may be due to the presence of several phytochemicals as tannins, flavonoids etc. The attached herbs’ extract disturb the cell membrane of the microbes through the physical and ionic phenomena (Sarkar and Munshi, 2003). The antibacterial assessment of the treated fabrics confirmed presence of active compounds such as tannin, eugenol and mallic acid etc. The results are very much in confirmation with the results obtained by Saini (2014) who reported that with an increase in the incubation period there was continuous increase in the bacterial colonies. Rajendran (2011) highlighted that there was percent reduction of 95.7 for *S.aureus* and 89.4 for *E. coli* in treated fabric against its respective control samples.

### 4. Conclusion

Extract of Guava leaves was observed as potential natural source for antibacterial activity. Understanding the importance and significance of cotton in
day to day lives of Indian people and this being a comfortable habitat for most of the microbes, was used as a substrate for the application of Guava leaves extract. Guava leaves extract treatment applied with exhaust method with 5 gm/l concentration exhibited remarkable improvement in antibacterial properties of the treated fabric against cellulose degrading bacteria i.e. *Bacillus spp.*

**References**


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