Antimicrobial Efficacy of *Terminalia chebula* Fruit Extract Treated Cotton Fabric for Healthcare Applications

R. Rathinamoorthy¹, S. Udayakumar² and G. Thilagavathi²

¹Department of Fashion Technology, and ²Department of Textile Technology, PSG College of Technology, Coimbatore - 641 004, Tamil Nadu, India.

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**ABSTRACT**

The study was focused on treatment of water and methanol extracts of *Terminalia chebula* fruits and citric acid as a cross linking agent on cotton plain-woven fabric. The active antimicrobial compounds in extracts were analyzed by high performance liquid chromatography. The fabric samples were tested for antibacterial activity against bacterial strains like *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Salmonella typhi* under agar diffusion test and quantitative analysis. The results indicated that the treated cotton fabric shows a clear microbial resistance with 27-38 mm zone of inhibition in the agar diffusion test against all the above mentioned strains. The treated samples showed 99% of reduction against *Staphylococcus aureus* and 86.25% reduction against *Escherichia coli* as per quantitative analysis. The Fourier Transform -Infra Red analysis confirmed the presence of active substances (saponin, ascorbic acid and gallic acid) in the treated samples. Process parameters were optimized using the response surface methodology adopted using Box-Behnken design and the correlation coefficient was found to be 0.932 in the case of *Staphylococcus aureus* and 0.66 in the case of *Escherichia coli*.

**KEY WORDS:** *Terminalia chebula*: agar diffusion method, HPLC; FT-IR; Box-Behnken method.

**Introduction**

In recent years, a large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases. Numerous plants have been screened for antimicrobial properties, for example Holetz et al., (2002) tested 13 plants used in Brazilian traditional medicine and they demonstrated activity against bacteria such as *Staphylococcus aureus* (*S. aureus*) and *E. coli*. Meléndez et al., (2006) tested 172 plant species used in Puerto Rico and they demonstrated that 14 of these showed activity against bacteria, including *S. aureus* and *E. coli*.

The traditional Indian systems of Ayurveda and Siddha medicines support the importance of medicinal plants to treat diseases (Beusher et al., 1994). According to World Health Organization (WHO) more than 80% of the world’s population relies on traditional medicine for their primary healthcare needs. *Terminalia chebula* is an important medicinal plant in Indian traditional medicine and it is most frequently used herb in Ayurveda. *Terminalia chebula* is a medium- to large-sized tree distributed throughout tropical and subtropical Asia, including China and Tibet. This tree is found in the forests of northern India, Uttar Pradesh, and Bengal and is common in Tamil Nadu, Karnataka and southern Maharashtra.

*Terminalia chebula* is routinely used as traditional medicine by tribal of Tamil Nadu in India to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection, and wound infections (Dash, 1991). Antibacterial activity of *Terminalia chebula* extracts against several bacterial strains have been reported (Bag et al., 2009). *Terminalia chebula* treated textile material and their efficacy analysis studies are meager. Hence, this present investigation aims at developing an eco-friendly natural antimicrobial finish for health care textiles for medical application. In the first phase of the work, methanol and water extracts of the *Terminalia chebula* powder were treated on cotton textile and the efficacy of microbial resistance of the treated fabrics were investigated. In the next phase, the herbal extract were treated on fabric in addition with cross linking agent to improve the durability of the treatment with different process parameter. Finally the process parameter, like extract concentration, curing temperature, and percentage of cross linking agent were optimized for better performance of the finished fabric. Here, to optimize the process parameter, the response surface methodology was adopted using Box-Behnken design.
Materials and Methods

Plant Material Extraction

*Terminalia chebula* fruits chosen for this study were purchased from the commercial outlets of the Coimbatore District, Tamilnadu, India. The collected quantities of *Terminalia chebula* fruits were shade dried and powdered. The methanol and water extract of the powders were obtained. 10 g of powder is soaked in water and methanol separately for 24 hours to obtain 10% concentrated solution, resulting in active substances being dissolved in methanol. The extract were filtered and used for antimicrobial finishing.

Microorganism

Bacterial cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The bacterial strains *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 6644), *Proteus vulgaris* (MTCC 742), *Salmonella typhi* (MTCC 733) were used.

Application Method

Plain woven Cotton fabric with 140 PPI and 78 EPI was desized, scoured, and bleached prior to the application of the antimicrobial finish. Both the water and methanol extracts were applied to the cotton fabric by dipping in the bath, with material to liquor ratio of 1:10 and then Pad-dry-Cured at 80°C for ten minutes. Finally, the fabric samples were tested for antimicrobial activity as per the AATCC test standards.

Agar diffusion Method (SN 195920)

The treated and untreated fabric samples were placed in the AATCC bacteriostasis agar (AATCC, 2005), which has been previously inoculated (Mat culture) with a test organism. After incubation, a clear area of uninterrupted growth underneath and along the side of the test material indicates the antibacterial effectiveness of the fabric. The area of the inhibition zone is a measure of antibacterial effectiveness of the material.

Quantitative Analysis (AATCC-100)

Specimens of the test material were shaken in a known concentration of bacterial suspension and the reduction in bacterial activity in standard time was measured. Both the water and methanol extracts were applied to the cotton fabric by dipping in the bath, with material to liquor ratio of 1:10 and then Pad-dry-Cured at 80°C for ten minutes. Finally, the fabric samples were tested for antimicrobial activity as per the AATCC test standards.

Characterization

**High Performance Liquid Chromatography (HPLC)**

HPLC fingerprints were prepared using waters HPLC equipped with UV-VIS detector. Solvents were pre-filtered and analysis was performed in Symmetry C18 column (4.6 x 250 mm). The methanol extracts of *Terminalia chebula* were injected in HPLC system. Injection volume was 20 µl. The flow rate was 0.7 ml/min and the active spots were identified.

**FT-IR Spectrometry**

FT-IR spectra for extract treated and untreated fabric were measured with a SHIMADZU spectrophotometer to identify the presence of active substance in the fabric. The spectra were obtained in the range of 400-4000 cm⁻¹.

**Durability Evaluation**

To test the durability of the antimicrobial finish, treated samples were examined for antimicrobial efficacy after 5, 10 home launderings (AATCC-61, 2003).

**Process Optimization by Response Surface Methodology**

Response surface methodology is an empirical modelization technique devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results (G. Annadurai et al., 1998).

In this study, the variable like extract concentration, Percentage of cross linking agent, and the curing temperature are the significant variables, designated as $X_1$, $X_2$, and $X_3$ respectively. The low, middle, and high level of the variables are designated as -1, 0, and +1 respectively and given in table 1. The calculation was carried out using multiple regression analysis using the least square method.

The quadratic polynomial equation approximates the mathematical relationship of 3 independent variable $X_1$, $X_2$ and $X_3$ on the response system. The equation was $Y = C_0 + C_1X_1 + C_2X_2 + C_3X_3 + C_1X_1X_2 + C_1X_1X_3 + C_2X_2X_3 + C_1X_1^2 + C_2X_2^2 + C_3X_3^2$.

Where $Y$ = Predicted yield, $C_0$ = Constant, $C_1$, $C_2$ and $C_3$ linear Coefficient, $C_{12}$, $C_{13}$ and $C_{23}$ cross product Coefficients, $C_{11}$, $C_{22}$ and $C_{33}$ Quadratic Coefficients.

The coefficients were obtained using multiple regression analysis to predict the response. The design of the experiment was for bone by Box-Behnken, three variable design. It is applicable once the critical value is identified (Kapat at al., 1998) The bacterial inhibition properties of the herbal extract treated fabric were prepared for optimization studies. The herbal treatment was done with variation in the critical variable like herbal extract concentration ($X_1$), cross linking agent percentage ($X_2$) and the curing temperature ($X_3$).

**Results and Discussions**

The antimicrobial properties of the material can be studied by qualitative as well as by quantitative test methods. However, it has been found that the quantitative test is good for testing the main agent or the treated fabric, provided the antibacterial agents used are capable of leaching out. On the other hand, quantitative test is the proper indicator of the degree of antimicrobial activity when the antimicrobial agent is fixed on the
textile material or unable to leach out. The test results were discussed in Table 1.

**TABLE 1**

Level of variables chosen for the trials.

<table>
<thead>
<tr>
<th>Condition</th>
<th>-1</th>
<th>0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of extract (in %)</td>
<td>15</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Cross linking agent conc (%)</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Curing Temp (in Degree C)</td>
<td>90</td>
<td>100</td>
<td>110</td>
</tr>
</tbody>
</table>

**Antimicrobial Efficacy**

**Agar Diffusion Test**

The results indicate the presence of clear zone of inhibition of 27-38 mm diameter for both water and methanol extract treated fabric against all the five selected microorganisms, namely *Staphylococcus aureus* (Gram-positive), *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Salmonella typhi* (Gram-negative). The untreated fabric (control) shows bacterial growth under the test specimen. Table 2 and Figure 1 show the antimicrobial efficacy of treated fabrics.

**TABLE 2**

Zone of inhibition of treated textile material for different strains.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Bacteria</th>
<th>Zone of inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Terminalia chebula fruit Water extract</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumonia</em></td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus vulgaris</em></td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella typhi</em></td>
<td>-</td>
</tr>
</tbody>
</table>

**Quantitative Analysis**

Table 3 indicates that the materials show high percentage of bacterial reduction against both *Staphylococcus aureus* and *Escherichia coli*. The methanol extract of *Terminalia chebula* shows 99.369% of reduction against *Staphylococcus aureus* and 98.11% reduction against *Escherichia coli*. The results show that, in agar diffusion and quantitative test, the methanol extract treated samples show high activity than the water extract against all the human pathogenic bacterial stains. This result supports the findings of Ahmed et al., (1998).

**HPLC Analysis**

Figure 3 shows the HPLC spectrum of crude extract of *Terminalia chebula*. It shows four prominent peaks with the retention time of 3.06, 3.32, 3.79, and 4.3. Out of four peaks the three peaks were compared with standards. The results show that the peaks at these retention times, 3.32, 3.79 and 4.3 minutes, were identified as saponin, ascorbic acid and gallic acid respectively were supported by Naik et al., (2004). Anjana Sharma et al., (2008) also documented the shigellocidal properties of Indian medicinal plants and identified the standard peak for saponin as 3.31 min which confirms the presence of saponin content in the *Terminalia chebula* extract. Figure 4 shows the standard peaks for active substance corresponding to gallic acid and saponin as per literature.
FTIR Analysis

Figure 5 shows the FTIR spectrum of the *Terminalia chebula* treated and untreated samples. From the spectrum, it is identified that the *Terminalia chebula* treated cotton fabric contains more carboxyl group [C(=O)-OH] due to the presence of active substances like gallic and ascorbic acid in their extract. The absorption in the region 3200 cm\(^{-1}\)-3600 cm\(^{-1}\) and 1200 cm\(^{-1}\)-1700 cm\(^{-1}\) of treated sample confirms the presence of -OH group stretching (William Kemp, 1987).

The presence of -(C=O)- group stretching is also confirmed by the absorption, in the region of 1600 cm\(^{-1}\)-1900 cm\(^{-1}\) in the treated sample. This stretching in 1760 cm\(^{-1}\)-1670 cm\(^{-1}\) confirms the presence of ester group. The Gallic acid could react with cellulose -OH group which resulted in the ester formation in the treated fabric. The presence of [C (=O)-OH] carboxyl group and –(OH) group confirms the presence of carboxylic acids (ascorbic and gallic acid) and the presence of ester group proves the deposits of glycosides (saponin) in the fabric (William Kemp, 1987).

Wash Durability Evaluation

The treated samples were evaluated for washing durability by AATCC Test Method 61. The antimicrobial activity of both water and methanol extract treated samples were assessed after 5 washes and 10 washes by using both agar diffusion and quantitative analysis. In both tests the microbe resistant activity of water extract treated samples at 10 washes and hence the methanol extract treated samples were analysed for activity. The results of wash durability of methanol extract treated fabrics are shown in Table 4 and 5.

**Agar Diffusion Test**

The results showed that, *Terminalia chebula* (methanol extract) treated samples showed high clear inhibition zone (above 24 mm) against all the bacterial strains even after 5 and 10 wash cycles, except *Salmonella typhi*.

**Quantitative Analysis**

Decreases in the percentage of bacterial reduction were obtained as the washing frequencies increased. Similarly, samples that had only been washed 5 times were shown very low percentage of bacterial reduction in the case of *Escherichia coli* (85.62% of reduction). But the sample that had been washed 10 cycles has no bacterial resistant property against *Escherichia coli*. However, while considering the *Staphylococcus aureus*, these above stated samples were noted 74.52% reduction after 5 wash, and no bacterial reduction percentage after 10 cycles of wash. It is noted that all the treated material have poor durability after commercial laundering. The poor durability of the antimicrobial agent in the textile material may be due to the weak Vander Val's or hydrogen bonds between antimicrobial agent and cellulose material (Thilagavathi et al., 2005).
TABLE 4
Durability evaluation of treated samples by agar diffusion method.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Bacterial Stains</th>
<th>Control (Un washed)</th>
<th>After 5 wash Inhibition zone (in mm)</th>
<th>After 10 wash Inhibition zone (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terminalia chebula</td>
<td>Staphylococcus aureus</td>
<td>34</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td>32</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Klebsiella pneumoniae</td>
<td>34</td>
<td>36</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteus vulgaris</td>
<td>34</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella typhi</td>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE 5
Durability test by quantitative measurement.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial Stains</th>
<th>Sample</th>
<th>Fabric</th>
<th>At &quot;0&quot; hour contact</th>
<th>At &quot;24&quot; hour contact</th>
<th>% of Bacterial reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S.aureus</td>
<td>Terminalia chebula</td>
<td>Control fabric</td>
<td>212X10^-4</td>
<td>540X10^-5</td>
<td>74.52%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After 5 Wash</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After 10 Wash</td>
<td>-</td>
<td>-</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>E.coli</td>
<td>Terminalia chebula</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After 5 Wash</td>
<td>160X10^-3</td>
<td>230X10^-2</td>
<td>85.62%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After 10 Wash</td>
<td>-</td>
<td>-</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Optimization Process Results

The durability results show that the herbal treated textile material possesses low durability after subsequent washing. Hence, in the second phase of this study, to improve the durability citric acid is used as crosslinking agent. Citric acid is one of the poly carboxylic acid which reacts readily with cotton material at an elevated temperature (Rowland Stanley et al., 1967). In the present investigation the process parameter for herbal finishing of is optimized by Box-Behnken method. The influence of three factors such as extract concentration, percentage of cross linking agent and curing temperature, and three levels investigated.

The regression equation obtained after analysis of variance gives the level of antimicrobial efficacy (Zone of inhibition) of herbal finished fabric against Gram-positive and Gram-negative as a function of different concentration, Cross linking agent percentage and the curing temperature. All the terms regardless of their significance are included in the equations (Table 6).

Where Y and Z are predicted response for S. areaus and E.coli respectively. For S. areaus, quadratic regression was significant at the level of 93.2%. Square regression was significant at the level of 86.9% and in the case of E. coli, the quadratic regression was significant at the level of 66%. The square regression was significant at the level of 44%. The microbial protection
value of the treated fabric at each experiment point is summarized in Table 6. The coefficients of equation are listed in Table 7.

### TABLE 6
Regression equation for the responses (S.Areaus and E.coli).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Equation</th>
<th>R</th>
<th>R²</th>
<th>F-Ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( Y=-26+0.9X_1+2X_2+0.650X_1X_2-0.035X_1X_3-0.015X_2+0.005X_2^2+0.001X_3^2 )</td>
<td>93.2%</td>
<td>86.9%</td>
<td>3.680</td>
<td>0.083</td>
</tr>
<tr>
<td>2</td>
<td>( Z=77+0.2X_1+0.85X_2-1.325X_1X_2-2X_1+0.01X_2+0.006X_2^2 )</td>
<td>66%</td>
<td>44%</td>
<td>0.431</td>
<td>0.871</td>
</tr>
</tbody>
</table>

### TABLE 7
Coefficient of the model.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient for Gram-positive bacteria ((S.Aureus))</th>
<th>Coefficient for Gram-negative bacteria ((E.Coli))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONSTANT</td>
<td>-26.000</td>
<td>77.000</td>
</tr>
<tr>
<td>Conc</td>
<td>0.900</td>
<td>0.200</td>
</tr>
<tr>
<td>Cl</td>
<td>2.000</td>
<td>0.850</td>
</tr>
<tr>
<td>Tem</td>
<td>0.650</td>
<td>-1.325</td>
</tr>
<tr>
<td>Conc*Conc</td>
<td>0.085</td>
<td>0.030</td>
</tr>
<tr>
<td>Cl*Cl</td>
<td>0.005</td>
<td>-0.090</td>
</tr>
<tr>
<td>Tem*Temp</td>
<td>0.001</td>
<td>0.008</td>
</tr>
<tr>
<td>Conc*Cl</td>
<td>-0.050</td>
<td>-0.040</td>
</tr>
<tr>
<td>Cl*Tem</td>
<td>-0.015</td>
<td>0.020</td>
</tr>
<tr>
<td>Conc*Tem</td>
<td>-0.035</td>
<td>-0.010</td>
</tr>
</tbody>
</table>

### Optimization of Extract Concentration with Curing Temperature

#### For Gram-Positive Organism

The contour plot in Figure 7a represents the maximum zone of inhibition versus extract concentration with curing temperature. Here the antimicrobial activity increases with the increase of extract concentration and with the increase in curing temperature. The activity reaching a maximum of 36 mm between the concentrations of 24%-25%. The optimum zone of inhibition (36 mm) can be achieved at the extract concentration of 25% and the curing temperature of 90°C.

#### For Gram-Negative Organism

In figure 7b with the Gram-negative microorganism, the inhibition zone increases with increase in concentration of extracts and the curing temperature increase. The maximum inhibition noted at a concentration of 24%-25% and at a temperature of 110°C.

### Optimization of Percentage of Cross Linking Agent with Curing Temperature

#### For Gram-positive organism

The plot in Figure 8a represents the maximum activity with cross linking agent percentage with curing temperature. It is noted that the increase in cross linking agent percentage reduces the antibacterial activity. Higher the amount of curing temperature cause maximum of activity 33 mm were observed at the 110°C. The increase in curing temperature causes improvement in extends of cross linking of cross linking agent. The optimum condition for the better activity was 4% of cross linking agent and 110°C of curing temperature.

#### For Gram-negative organism

In the case of Gram-negative, the increase in cross linking agent percentage increases the activity initially but after 10% it reduces step by step. While considering the curing temperature, the maximum of zone noticed only at high curing temperature. Hence the optimum point was noticed at 10% of cross linking agent with the curing temperature of 110°C.

The antimicrobial activity (both Gram-positive and Gram-negative) of the woven cotton fabric for different combination is given in Table 6. In the case of gram positive bacterial strain the maximum zone of inhibition (36 mm) is observed when the fabric is treated with high concentration of extract (25%) and minimum amount of citric acid (5%) as cross linking agent. The variation in curing temperature also shows significant influence in the antibacterial activity. The combination of high curing temperature and high concentration of cross linking agent shows a significant reduction in the zone of inhibition (27 mm).

In the case of Gram-negative bacterial strain, the maximum amount of inhibition zone observed (32 mm) was at extract concentration of 20% and high amount of citric acid (15%) as cross linking agent. The temperature
of 110°C identified as optimum for higher bacterial inhibition. The combination of lower cross linking agent and lower curing temperature reduces the inhibition area (21 mm). Because the Gram-negative bacterial strains have higher resistance towards the antibodies by their physical structure, the increase in temperature lead to better affinity of extract due to higher cross linking.

**Fig. 6.** Contour plot for Zone of inhibition versus Extract concentration and Cross linking agent percentage. a) Gram positive bacteria as surface response (SA) b) Gram negative as surface response (EC).

**Fig. 7.** Contour plot for Zone of inhibition versus Extract concentration and Curing Temperature. a) Gram-positive bacteria as surface response (SA) b) Gram-negative as surface response (EC).

**Fig. 8.** Contour plot for Zone of inhibition versus Cross linking agent percentage and Curing Temperature. a) Gram-positive bacteria as surface response (SA) b) Gram-negative as surface response (EC).
Conclusions

It is concluded from the results that the treated samples have 99% of bacterial reduction. Though the tested samples show poor wash durability, the unwashed samples show high and clear zone of inhibition against wide spectrum of human pathogenic bacterial stains. The Terminalia chebula treated materials are bactericidal as well as bacteriostatic.

The extract can be applied simply by the pad-dry-cure method, where the wash durability is the only problem. If the treated sample is for one-time usage like surgical mask, bandage gauze, wound healing bandages, wound management material, dressings etc, this antimicrobial treatment definitely has its potential. The process parameters were optimized using response surface methodology. The zone of inhibition of treated sample increases with the concentration of the herbal extract. The influence of curing temperature and crosslinking agent percentage on the zone of inhibition is significant for both the strains. The optimum zone of inhibition is observed at 15% concentration of crosslinking agent and 110°C curing temperature for Gram-negative (E.Coli) and 5% of crosslinking agent and 100°C curing temperature for Gram-positive (S.Areaus). Since Terminalia chebula is a natural resource, it is ecofriendly and because of its abundant availability, the scope of implementation and commercialization of herbal extract on cotton material is much possible.

References


Address correspondence to: R. Rathinamoorthy, Department of Fashion Technology PSG College of Technology, Coimbatore-641 004 Tamil Nadu, India. Email: r.rathinamoorthy@gmail.com