Study of the Synergistic Effect of Antibiotics and Plant Extracts Against Clinical Staphylococcus aureus Strains.

Ibrahim TA*, Fagbohun ED², and Olalumade BB¹.

¹Department of Food Science and Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.
²Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria.

ABSTRACT

The synergistic anti-bacterial action of two plants (Eucalyptus and Thuja) extracts on plasma coagulation by staphylococcus aureus and the effect of conventional chemotherapy combined with plant extracts below the MIC. Coagulation was inhibited in plasma containing (10, 20, 50,70 and 100mg\L) for both extracts. The MICs of ampicillin and ciprofloxacin for S.aureus markedly reduced from (0.25, 0.5 and 1mg\L) of the four resistant strains to 0.06mg\L in Muller Hinton agar (MHA) plates with 20mg\L Eucalyptus extract and 50mg\L Thuja extract. The results suggest that T. catappa and E. camaldulensis extracts can be used in treating diseases caused by the test organisms.

INTRODUCTION

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last years, resistance to these drugs by bacteria has increased and it now becomes a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents [1].

Staphylococcus aureus is recognized as one of the major causes of infections in humans occurring in both the community and the hospital. Multidrug resistant staphylococci have become a major nosocomial pathogen [2]. Therefore the importance of identifying new effective antimicrobial agents cannot be overemphasized. Among the potential sources of new agents, medicinal plants have long been investigated. In rational drug therapy, the concurrent administration of two or more drugs is often essential and sometimes mandatory in order to achieve the desired therapeutic goal or to treat co-existing diseases. However, the drug interaction may have different effects on the host as well as the infecting microorganism. The potential benefits of using combined antimicrobial therapy can be treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity, reducing the time needed for long-term antimicrobial therapy and prevention of the emergence of resistant microorganisms [3,4].

Drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept and has been recently reported by [1,5]. Many studies reported the antibacterial activity of the Eucalyptus leaf extract [6,7,8] and Thuja seed extract [9,10] against many microorganisms. Therefore, this present study aims to evaluate the synergistic effect between Ampicillin and Ciprofloxacin and the extracts of Eucalyptus and Thuja plant against RSA.

MATERIALS AND METHODS

Test Bacterial strains

Eighteen Staphylococcus aureus strains were isolated from patients’ clinical specimens who attending the general Federal Medical Center, Owo. These isolates were examined for plasma coagulation and antibacterial activities of Ampicillin and Ciprofloxacin and the they were grown in tryptic soy broth at 37°C overnight incubation, the
bacterial cells were harvested by centrifugation at 6000 r.p.m. for about 10 min, then resuspended in a sterile normal saline solution and centrifuged again, the process was repeated three times and then the washing bacteria resuspended [11] .

Collection of Plant Materials and Preparation of extracts

The method of [12] was used to obtain the plant extract, 50gm of Eucalyptus leaves and 50gm of Thuja fruits were washed by water and dried for about 48h at room temperature, then crushed and extracted 3 times with 800 of 70% acetone (1h with continuous stirring). All extracts were rotary evaporated under vacuum to remove acetone, and stored into clean and dried airtight vials at room temperature and store at 4°C prior to use.

Plasma Coagulation Assay

Cell suspensions of S.aureus (100 /cell per ml) were used for the inoculation . About 0.5ml of rabbit plasma alone (control) and the ones supplemented with Eucalyptus extract (10, 20, 50, 70 and 100mg/L) or Thuja extract (10, 20, 50, 70 and 100 mg/L), aclot (plasma coagulation) were checked for coagulation after incubation for about 24hours at 37°C.

Determination of Minimum Inhibitory Concentration (MIC) of extracts and antibiotics

The MIC of Eucalyptus extract and Thuja extract against the four resistant strains of S.aureus was determined in Muller-Hinton agar (MHA) using the agar plate method and the MIC of Ampicillin and Ciprotab was also examined in MHA and MHA with 20mg/L Eucalyptus extract and MHA with 50 mg/L Thuja extract using the method of [13].

RESULTS AND DISCUSSION

Plasma coagulation assay

A total of 18 S.aureus strains coagulate plasma alone after 24h incubation at 37°C, while there were 4(22.22%), 10 (55.55%), 14 (77.77%) and 18 (100%) negative strains to plasma coagulation in plasma containing Eucalyptus extract with concentration 10, 20, 50 and 70, 100 mg/L respectively and 4(22.22%), 8 (44.44%), 10 (55.55%) and 18 (100%) negative strains to plasma coagulation in plasma containing Thuja extract with concentration 20, 50, 70 and 100 mg/L respectively but there were no one reported in concentration 10 mg/L, as shown in table 1.

Table 1: The percentage of S.aureus strains that have a negative result to plasma coagulation at different concentration of Eucalyptus and Thuja extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Extracts Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus</td>
<td>10 20 50 70 100</td>
</tr>
<tr>
<td>Thuja</td>
<td>0% 22.22% 44.44% 55.55% 100%</td>
</tr>
</tbody>
</table>

These findings indicate that there were only four resistant isolates of S. aureus reported with 10mg/L of Eucalyptus extract and 20mg/L of Thuja extract; thus it has been selected to evaluate the effect of both extracts on their growth and detect the synergistic effect between Ampicillin and Ciprotab and the extracts of Eucalyptus and Thuja plant against RSA.

MIC of Extracts

The results of the minimum inhibition concentration(MIC) of Eucalyptus extract and Thuja extract against the four resistant strain of S. aureus which are S3, S8, S14 and S17 were presented in table 2 which explained that the MIC of S. aureus isolates was (20 and 50mg/L) for Eucalyptus extract inhibited two isolates for every concentration and (50 and 70mg/L) for Thuja extract with same trend in respect to the antibacterial activity.

Table 2: The MICs (mg/L) of Eucalyptus and Thuja extract for S.aureus strains on MHA plates

<table>
<thead>
<tr>
<th>Extracts</th>
<th>S3</th>
<th>S8</th>
<th>S14</th>
<th>S17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus</td>
<td>20mg/L</td>
<td>50mg/L</td>
<td>50mg/L</td>
<td>20mg/L</td>
</tr>
<tr>
<td>Thuja</td>
<td>50mg/L</td>
<td>70mg/L</td>
<td>70mg/L</td>
<td>50mg/L</td>
</tr>
</tbody>
</table>
Both *Eucalyptus* and *Thuja* extract have an astringent effects refer to the present of tannins which known as an active antimicrobial agent against many microorganism \[^{14}\]\ These findings may be due to that source, concentration, and chemical properties which are important factors that influence antimicrobial activity of tannin extracts \[^{15}\]\ where the antimicrobial mechanisms of tannins can be summarized as follows:(1) The astringent property of the tannin may induce complexation with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when mixed with tannins.(2) A tannin's toxicity may be related to its action on the membranes of the microorganisms.(3) Complexation of metal ions by tannins may account for tannin toxicity \[^{11}\]\.

**MIC of antibiotics and extracts**

Figure 1 showed the MICs of Ampicillin for the four resistant strains of *S.aureus* in MHA with or without *Eucalyptus* extract (20mg\(\text{L}\)) and *Thuja* extract (50 mg\(\text{L}\)). As shown the MICs of Ampicillin decreased to 0.06mg\(\text{L}\) in MHA with the 20mg\(\text{L}\) *Eucalyptus* extract and 50mg\(\text{L}\) *Thuja* extract.

\[
\begin{array}{c}
0 & 0.2 & 0.4 & 0.6 & 0.8 & 1 \\
S3 & S8 & S14 & S17 \\
\end{array}
\]

**Figure 1:** The minimal inhibition concentration(mg\(\text{L}\))of Ampicillin with and without *Eucalyptus* and *Thuja* extract for *S.aureus* strains on MHA plates.

*Am= Ampicillin,*E= *Eucalyptus,*T= *Thuja*

Figure 2 shows the MICs of Ciprofloxacin for the four resistant strains of *S.aureus* in MHA with or without *Eucalyptus* extract (20mg\(\text{L}\)) and *Thuja* extract (50 mg\(\text{L}\)). The MICs of Ciprofloxacin also decreased to 0.06mg\(\text{L}\) in MHA with the 20mg\(\text{L}\) *Eucalyptus* extract and 50mg\(\text{L}\) *Thuja* extract.

\[
\begin{array}{c}
0 & 0.2 & 0.4 & 0.6 & 0.8 & 1 \\
S3 & S8 & S14 & S17 \\
\end{array}
\]

**Figure 2:** The minimal inhibition concentration(mg\(\text{L}\)) of ciprofloxacin with and without *Eucalyptus* and *Thuja* extract for *S.aureus* strains on MHA plates.

*Ac= ciprofloxacin,*E= *Eucalyptus,*T= *Thuja*

Since both Ampicillin and Ciprofloxacin possess antibacterial activity against RSA, it is necessary to assess whether the anti-RSA effect observed in the presence of the two antibacterial agents (two antibiotics and two plant extracts) is an additional one or a synergistic one. Therefore, the MICs of Ampicillin and ciprofloxacin were determined
against RSA strains in the absence or presence of (20mg/L) Eucalyptus extract and (50 mg/L) Thuja extract respectively and it has been observed that the effect was a synergistic one. These findings may be due to the damage occurring in the cell wall and in the cell membrane caused by epigallocatechin gallate and an increase in the permeability would be responsible for the potent synergy as reported by [16].

The present study indicated that both extracts of the studied plants showed an increase in the antimicrobial activity of certain drugs that can be used against S. aureus, and synergistic interaction of plant extracts is possible with antimicrobial drugs and these results are consistent with previous reports which showed that some plant extracts can increase the activity of antimicrobial drugs in vitro against bacteria [1,17,18,19] and also consistent with study of [16] who found that an extract of Arctostaphylos uva-ursi markedly reduced the MICs of β-lactam antibiotics such as Oxacillin and Cetmetazole against methicillin resistant S.aureus (MRSA). This high synergism rate shows the need for more studies concerning the molecular basis of these interactions to understand the synergistic mechanism which is fundamental to development of pharmacological agents to treat bacterial infections using medicinal plants.

CONCLUSION

The results from this study showed that there exist synergistic effect in the plant extracts used and the antibiotics used against the multidrug resistant Staphylococcus aureus which implies that the extracts and the antibiotics could be combined to treat diseases caused by the MRSA

REFERENCES