Nanoherbal antiseptic medicine/ bandage for effective and safe treatment of external wounds

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ABSTRACT

The use of antibiotics has resulted in resistant microbes that hence need to be tackled by using safe green and effective treatments. Herbal formulations have reached extensive acceptability as a therapeutic agent for different diseases. Present research is designed to formulate a medicine that incorporate both the natural and traditional way of treating wounds. For this purpose ethanolic extracts of *Citrus limoniu, Azadirachta indica* and *Aloe barbadensis* leaves were used as reducing agent for the synthesis of silver and gold nanoparticles, thereby incorporating nanoparticles in the extract. The nanoparticle doped extract, was then checked for its efficacy against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Micrococcus Luteus*. Preliminary results suggest that the silver and gold nanoparticles have an inhibitory effect giving another hope for overcoming delayed healing and poor response to treatments of wounds.

Keywords: nanoherbal medicines, bacteria, skin wounds

1 INTRODUCTION

Medicinal plants are the backbone of several indigenous traditional systems of medicine and their pharmacological studies approved them as possible source of bioactive compounds [1]. Due to indiscriminate use of synthetic drugs, multi drug resistance in human and plant pathogen is developing, driving the need for screening of plants for novel bio active compounds. In many parts of world there is a rich tradition in the use of herbal medicine for treating many infectious diseases [2] with estimate of reliance of 80% population on traditional medicine in developing countries [3].

*Azadirachta indica* (Neem) an herbal plant is distributed in subcontinent during all seasons with each part having some medicinal property. Its leaves and bark extract and oil are commonly used for therapeutic purpose (Tewari, 1992). Biological activity of *Azadirachta indica* is reported for the crude extract of different fractions of leaf, bark, root, seed and oil [4].

*Aloe barbadensis* contain over 75 nutrients 200 compounds including enzymes, vitamins, minerals, lignin, saponions, amino acids [5]. Its gel has been used for tropical treatment of burns and wounds and studies indicated the glycoprotein fraction of Aloe vera which is involved in wound healing effect [6].

The application and development of nanotechnology has the potential to improve the quality of life. The understanding of nanoparticles and biological cell interaction has lead to the development of new and different capabilities [7, 8] like targeted drug delivery, magnetic resonance [9, 10].

2 MATERIALS AND METHODS

2.1 Plants material and extract preparation

Leaves of *Citrus limoniu* (LE 201), *Azadirachta indica* (NE 206) and *Aloe barbadensis* (AL 204) were collected from the botanical gardens of PCSIR, Lahore, Pakistan. They were further confirmed by Dr. Abdul Nisar (taxonomist), Department of Botany, University of the Punjab, Lahore, Pakistan and samples were deposited in the herbarium library of the University. Leaves were surface cleaned with running tap water, followed by distilled water and left for drying in shade for five days. 100g mildly crushed leaves were dipped in ethanol for 24 hours. After 24 hours the solution of ethanol and leaves was filtered.
using Whatman No. 1 filter paper [11] and the filtrate obtained was taken in two separate flasks (500 mL).

2.2 Preparation of Nanoparticles doped plant extract (Ag and Au)

The nanoparticles of silver and gold were prepared by using green synthesis route. 0.001M solutions of Ag and Au was prepared by adding 0.08g of AgNO₃ and 1.97g of 10% AuCl₂ solution in each of the three extracts separately. The flasks containing silver were wrapped with carbon paper to avoid silver decomposition. The formation of nanoparticles in each case was determined from the change in $\lambda_{\text{max}}$ value and absorbance of the extracts (scanned for 350-800nm) monitored with intervals for the span of 48h.

2.3 Determination of MIC

1000 mg/mL solution of dry plant extract (doped and undoped) was prepared in Dimethyl sulphoxide (DMSO) (MERCK, Germany) and further dilutions were made from this sample. 4 test tubes were taken, 9mL of sterilized nutrient broth was poured in the test tubes. 1mL of sample solution was added to first test tube and mixed well and then 1mL of this was transferred to second test tube and so on. 10 µL of diluted inoculums was added to all the test tubes as well. 1mL sample was added to first control test tube and mixed well and 10 µL of inoculums was added to second control test tube and allowed the growth of microorganism in the medium used. All the test tubes were incubated at 37ºC for 24 h for bacterial growth [12, 13].

2.4 Characterization

UV-vis spectrophotometer Analytikjena Specord200 was used to measure absorbance and $\lambda_{\text{max}}$ values. For characterization of nanoparticles AFM (scanning probe microscope VEECO, Model CPII run in non contact mode) and XRF (XRF-spectrometer by Spectro midex) are used.

3 RESULTS AND DISCUSSION

3.1 Characterization of nanoparticles

The three extracts belonging to three plants doped with silver and gold nanoparticles (Table 1) were characterized by AFM and XRF. The confirmation of nanoparticles formation was done by change in $\lambda_{\text{max}}$ values as the colour of plant extract is changed that can be clearly seen in Fig. 1.

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Metal</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus limonium</td>
<td>Silver</td>
<td>S-1</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>Silver</td>
<td>S-2</td>
</tr>
<tr>
<td>Aloe barbadensis</td>
<td>Silver</td>
<td>S-3</td>
</tr>
<tr>
<td>Citrus limonium</td>
<td>Gold</td>
<td>S-4</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>Gold</td>
<td>S-5</td>
</tr>
<tr>
<td>Aloe barbadensis</td>
<td>Gold</td>
<td>S-6</td>
</tr>
</tbody>
</table>

Table 1: Coding of plant extracts used in the study.

Fig. 2 presents the AFM images of the six samples (S-1 to S-6) which shows the particle size of silver nanoparticles are 7.14, 10.4 and 109 nm for samples S-1, S-2 and S-3 while gold samples S-4, S-5 and S-6 falls in range of 33.4, 55.4 and 97.6 nm.

Figure 2: AFM images of samples S-1 to S-6 showing nanosize of silver and gold particles.
For confirmation of doping and concentration of the silver and gold in samples, XRF analysis was carried out which shows that samples S-1, S-2 and S-3 has silver concentrations in the range of 3.33%, 3.09% and 3.32% while S-4, S-5 and S-6 has gold concentrations in the range of 2.26%, 2.84% and 4.31%.

### 3.2 MIC of S-1 to S-6

MIC (minimum inhibitory concentration) values of doped plant extracts (S-1 to S-6) are tabulated in Table 2 which shows that low concentrations of even gold can give activity against tested pathogens. Previous studies have showed that gold nanoparticles are not as such effective as antimicrobial agents [14] but in present case the prepared doped extract has shown great potential as bactericidal agent. Silver as reported in many other studies [15] has potential to be used in treatment of wound pathogens.

<table>
<thead>
<tr>
<th>Microbes</th>
<th><strong>Azadirachta indica</strong></th>
<th><strong>Citrus limoniu</strong></th>
<th><strong>Aloe barbadensis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Au</td>
<td>Ag</td>
<td>Au</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.0625</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>P. auregnosa</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. subtilius</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>M. luteus</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 2: MIC values (mg/ mL) of Gold and Silver nanoparticles against microbial pathogens

### 4 CONCLUSION

Ethanolic plant extracts of three plants *Citrus limonium*, *Azadirachta indica* and *Aloe barbadensis* were used to for green synthesis of silver and gold nanoparticles which are in the size range of 7.14 – 109 nm and 33.4 - 97.6 nm. All the samples either doped with silver and gold show good MIC values against 5 bacterial strains. So, it can be effectively stated that the prepared samples can be effectively used in treatment of wounds in a safe way.

### REFERENCES