Synthesis of ZnO-H\textsubscript{NPS} for Functional Fabric Finishing using Zinc-Herbal Nano Complex

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Abstract: Nano technology is one of the key technologies which plays a major role in medical textiles nowadays. It is also used in all the other textile industries like Geo textiles, hygienic textiles, and technical textiles. Zinc oxide has unique physical and chemical properties. The Zinc Oxide nano particle is synthesized with natural plant extracts by sol–gel technique with Citric Acid s a cross-linking medium. The Developed nanoparticles were applied on to the selected fabric by Pad-Dry-Cure Method. The functional testing of the treated fabric samples was subject for the functional properties Viz: Scanning Electron microscopy (SEM), the antibacterial testing, Antifungal activity, Vitro Wound Scratch Assay, Cytotoxicity Analysis of Zinc Oxide-Herbal Nano particles (Zno-H\textsubscript{Nps}) – MTT Assay.

Keywords: Nanoparticle, SEM, Antibacterial, Antifungal, Vitro Wound Scratch Assay, MTT Assay.

1. Objectives

- To study the suitable chemical for synthesis of nano particles.
- To carry out the synthesis of Zinc Oxide nano partical with extracts of Kedrostis Foetidissima leaf’s by sol–gel technique with ethanol as medium.
- To prepare the fabric samples for treatments with nano particles.
- To apply nano particles on to the fabric samples by PAD-DRY-CURE method.
- To characterize the treated fabric samples for nano particles using Scanning Electron Microscope (SEM).
- To Study the effects of application of nano – particles on the fabric by subjecting the fabrics to Antibacterial activity’s and Antifungal activity.
- To analyze the Wound Healing and toxicity.

2. Introduction

In this study the Zinc Oxide nano particle is synthesized with extracts of Kedrostis Foetidissima leaf’s by sol–gel technique with citric acid as medium. In this study the Kedrostis Foetidissima plant leaf’s had taken and dried in a room temperature for 48hr’s. Then the dried leaves are crushed and added into the soxhlet extractor for the extraction. Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures.

For the present study, soxhlet method which follows the principle of infusion method was chosen to extract the content from the selected herbs, Appakovaithalai (Kedrostisfoetidissima). In the Soxhlet extraction method, the leaves, stems and fruits of Kedrostis foetidissima were collected near udumalpet area, Tirupur district, Tamil Nadu, India. The leaves were washed and shad dried for 48hr’s. Then the dried leaves are crushed and added into the soxhlet extractor for the extraction. Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures.

3. Methodology

![Methodology Diagram]

4. Materials and Methods

The leaves, stems and fruits of Kedrostis foetidissima were collected near udumalpet area, Tirupur district, Tamil Nadu, India. The leaves were washed and shad dried for 48hr’s. Then the dried leaves are crushed and added into the soxhlet extractor for the extraction. Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures.

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finely ground sample—Kedrostisfoetidissima herbal leaf powder was placed in a porous bag or “thimble” made from a strong filter paper or cellulose, which is placed, is in thimble chamber of the Soxhlet apparatus.

Extraction solvent is heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents is emptied into the bottom flask again and the process is continued. For the study, infusion method of Soxhlet Extraction had been adopted. The powdered herbs of Kedrostisfoetidissima were filled in the thimble and placed in the soxhlet extractor. The extractor had been filled with solvent solution of acetone and the temperature of 60°C was set and left for 6 hours.

In this project, the sol-gel method was used for preparation of zinc oxide nanoparticles (ZnO-NPs). In a typical procedure 10.5g of zinc sulphate and 5% of Magnesium sulphate was used with 0.1N NaOH. The mixture was stirred at 800rpm in a magnetic stirrer to form Zinc oxide nanoparticles (ZnONPs). To the developed zinc oxide nanoparticles, about 50ml of herbal extracts was added to with continuous stirring to dissolve zinc completely. Then the solution was heated to 50°C and 600 ml of absolute alcohol was added slowly with stirring. After this, 6ml of hydrogen peroxide H₂O₂ (% 47) was added drop wise to the vessel and mixed it using a magnetic stirrer to get clear Zinc oxide-herbal nanoparticles (ZnO-HNPs) solution.

The fabric samples were finished with zinc oxide-herbal nanoparticles using citric acid as cross-linking agent. Developed nanoparticles were applied to the selected fabric with material-to-liquor ratio of 1:20 at 40°C using 8% citric acid concentration. Nanoparticles were padded onto the fabric materials for 30min. All the samples were taken and dried at 100-120°C for 5min and cured at 180°C for 3min.

SEM analysis of the nanoparticles revealed two different shapes, spherical shape and cube shape. Spherical shaped nanoparticles showed the average size of 650.0 nm. Cube shaped particles measured in micron size and hence these particles were not considered for the analysis.
B. Antibacterial activity (AATCC-30 test method) of zinc oxide-herbal nanoparticles (ZnO-H\textsubscript{NPs}) finished fabrics against (Staphylococcus aureus and Escherichia coli)

The fabrics finished with Zinc oxide-herbal nanoparticles (ZnO-H\textsubscript{NPs}) were analyzed for their antibacterial testing using the standard AATCC – 147 test methods (Parallel streak method).

Briefly, test specimens (fabrics) were cut into pieces (25mm x 50mm). A 50mm length permits the specimen to lay across 5 parallel inoculums streaks each of diminishing width from both 8mm to 4mm wide. Sterile AATCC bacteriostasis agar plates were prepared. Using sterile 4mm inoculating loop, one loop full of culture (Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 6538) was loaded and transferred to the surface of the agar plate by making five parallel inoculum streaks spaced 10mm covering the central area of the petridish without refilling the loop.

The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with agar surface. The plates were incubated at 37°C for 18-24 hours. The inoculated plates were examined for the interruption of growth along the streaks of inoculum beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen calculated in mm.

Results:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test Bacteria</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>33</td>
</tr>
</tbody>
</table>

C. Antifungal activity (AATCC-30 test method) of zinc oxide-herbal nanoparticles (ZnO-H\textsubscript{NPs}) finished fabrics against fungus (Candida albicans and Candida tropicalis)

The fabrics finished with Zinc oxide-herbal nanoparticles (ZnO-H\textsubscript{NPs}) were analyzed for their antifungal testing using the standard AATCC-30 test method. Briefly, test specimens were cut into pieces (50mm in diameter). Sterile Potato Dextrose agar plates were prepared. Using sterile cotton swab the test fungal cultures (Candida albicans and Candida tropicalis) was transferred by swabbing all around the surface of the agar plate and also covering the central area of the petridish. The plates were incubated at 30°C for 72 hours. The inoculated plates were examined for the interruption of growth along the swabs of inoculum beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen calculated in mm.

Results:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test Fungi</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida albicans</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>Candida tropicalis</td>
<td>64</td>
</tr>
</tbody>
</table>

Fabric samples finished with Zinc oxide-herbal nanoparticles (ZnO-H\textsubscript{NPs}) exhibited excellent antibacterial activity and antifungal activity. Good inhibitory zones against the bacterial species (E. coli and S. aureus) and fungal species (Candida spp) were observed for the finished fabrics in the present study. The antibacterial and antifungal inhibitory zone obtained for the finished fabrics against the test organisms indicated the
presence of different antibacterial compounds in the Zinc oxide-herbal nano composites. The finished fabric confirmed the presence of different phytochemical compounds like phenol, alkaloids, flavonoids and terpenoids in the herbal extracts which are proved to be attributing for enhanced antibacterial and antifungal activity. The activity obtained in the present research was attributed due to several biological functions of Zinc oxide-herbal nanoparticles. The functions are as follows,

More adsorbed compounds of Zinc oxide-herbal nanoparticles would evidently result in greater changes in the structure and in the permeability of the cell membrane of bacteria. This would suggest that the antibacterial mode of action of Zinc-oxide is dependent upon the host microorganism. Another proposed mechanism is the binding of biological compounds of Zinc oxide and herbal mixtures with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via the penetration of biological compounds into the nuclei of the microorganisms.

D. In vitro wound scratch assay

L929 mouse fibroblast cells were grown in 24 well plates at a density of 1X105 cells/ml and cultured until ~ 80% confluency. A small linear scratch was created in the confluent monolayer by gently scraping with sterile cell scrapper as per the method described by Yanling Chen (2011). Cells were thoroughly rinsed with 1 X PBS to remove cellular debris and treated with Zinc oxide-herbal nanoparticles (ZnO-HNPs) at a ratio of 1:1 (25µl). Cell proliferation was monitored at different time points: 0th hour, 18th hour and 24th hour images of the migrated cells were taken at all different time points using inverted phase contrast microscope. Extent of wound healing was determined by the distance traversed by cells migrating into the denuded area.

Results:

The cell migration, cell proliferation of L929 mouse fibroblast cell lines and wound closure was measured for a known concentration (100µg) of developed Zinc oxide-herbal nanoparticles (ZnO-HNPs) at three different time periods (0th hour, 18th hour and 24th hour). The self-wound healing ability of ZnO-HNPs showed that, at 0th hour, no cell migration and proliferation was observed including control (Distilled water). At 12th hour, the samples showed positive cell migration and cell proliferation. And after 24hours, more cell proliferation was evident and thus indicating the wound healing ability of the developed ZnO-HNPs.

E. Cytotoxicity analysis of zinc oxide-herbal nanoparticles (ZNO-HNPS) - MTT assay

1) Cell line

The mouse fibroblast cell lines (L929) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2) Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x105 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution (Zinc oxide-herbal nanoparticles - ZnO-HNPs) was diluted to twice the desired final maximum test concentration with serum free medium.

Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

3) MTT assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.
**Results:**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Samples</th>
<th>Concentrations (µg/ml)</th>
<th>% cell inhibition (L929)</th>
<th>% viable cells (L929)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>10</td>
<td>11.3</td>
<td>88.2</td>
</tr>
<tr>
<td>2</td>
<td>Zinc oxide-herbal nanoparticles - ZnO-HNPs</td>
<td>60</td>
<td>12.6</td>
<td>87.3</td>
</tr>
</tbody>
</table>

Zinc oxide-herbal nanoparticles (ZnO-HNPs) samples used in the study were subjected for toxicity test after exposing the required concentrations on the mouse fibroblast cell lines (L929). During the analysis, the concentration of nanoparticles (60µg/ml) did not exhibit any toxicity for the mouse fibroblast cell lines (L929). This was evident from the percentage of viable cells (80% of viable cells); and the percentage of cell inhibition was recorded as only 12.6% which when compared to control samples showed close to 11.3%. And hence the developed nanoparticles were proved to be highly biocompatible and non-toxic.

**6. Conclusion**

The developed nano samples have good antibacterial, antifungal activities. The nano finished fabrics shows excellent wound healing ability. Hence this nano finished fabrics can be further developed using many other herbals and can be used in many medical textile products.

**References**


