SYNTHESIS OF SILVER NANOPARTICLES FROM MORINGA OLEIFERA: FORMULATION AND EVALUATION AGAINST CADIDIA ALBICANS

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ABSTRACT

The aim of present study was to investigate the effectiveness of the Moringa oleifera against Candida albicans. The work focused on to compare activity of plain aqueous leaves extract and silver nanoparticles (AgNPs) synthesized by using Moringa oleifera leaves. Silver nanoparticles were synthesized by green synthesis method and characterized by UV-visible Spectroscopy. Further study carried out to assess in-vitro diffusion and activity from two topical dosage forms viz. cream and ointment. Particle size and polydispersity index of topical ointment formulation were determined by using dynamic light scattering Malvern Zetasizer Nano-ZS90. Skin irritancy test on healthy albino rabbits proved safety of ointment containing AgNPs synthesized using Moringa oleifera leaves.

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INTRODUCTION

*Candida albicans* can affect areas such as mouth, throat, skin, genitals and can enter into blood circulation. It is the most common cause of mucosal candidiasis and is responsible for about half of all cases of candidemia in hospitalized patients [1]. Candidiasis is the third most common cause of nosocomial bold-stream infection (BSI) in critically ill patients, representing 11 percent and become more prevalent in recent years [2]. *Moringa oleifera* Lam (M o) family *Moringaceae* is one of the well known and most widely distributed species of a monogeneric and is popularly known as ‘Sahajan’ in Hindi and ‘Miracle tree’ in English [3] is reported to be medicinally important and almost all parts of *Moringa* tree are considered to possess medicinal properties [4]. Recently, an *in vitro* antimicrobial activity of *M. oleifera* L. seed extracts prepared in aqueous and organic solvents against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Aspergillus niger* and *Candida albicans* reported [5]. These properties of *M. oleifera* make it, as an effective alternative in many skin infections [6, 7]. Nanotechnology can play significant role in improved drug therapy. Nanoparticles synthesized by green method have applications in bactericidal, wound healing, medicine and electronics [8][9]. In the present study an attempt was made to observe the antimicrobial potential of silver nanoparticle with M.o. extract synthesize silver nanoparticles by green technology using *Moringa oleifera* leaves extract. Both silver nano particle and extract exhibits antimicrobial activity.

MATERIALS AND METHODS

Plant Material

*Moringa oleifera* leaves were provided as gift sample by PIRENS, Bbahaleshwar, Dist: Ahmednagar Maharashtra (India).

Chemicals

Materials used in present study were of AR grade and purchased from Research Lab Chemicals Pvt. Ltd, Mumbai (India), Himedia Mumbai, (India)

Fungal strain

The fungal strain *Candida albicans* (MTCC- 227) was procured from MTCC Chandigarh, Punjab (India).

Animal

Albino rabbits were obtained from animal house of Sanjivani College of Pharmaceutical Education and Research, Kopargaon

METHODOLOGY

Preparation of Extracts

The extraction of *Moringa oleifera* leaves was carried out by using solvents such as ethanol (95%), petroleum ether, chloroform, ethyl acetate using cold maceration method. The aqueous extraction of *Moringa oleifera* leaves was carried out using Soxhlet extraction method. The extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight container [9].

Preliminary anti-fungal activity

Anti-fungal activity of different extracts of *Moringa* leaves was carried out by agar well diffusion method against *Candida albicans*. The fungal isolates were allowed to grow on a sabouraud dextrose agar (SDA) at 25°C for 48 h. Thereafter, the fungal spores were harvested to the surface of the plate. The standardized fungal spore suspension (1000 μl) was evenly spread on the SDA. Wells were bored into the agar media using a sterile 6 mm cork borer and were filled with the extract, taking care not to allow spillage of the same onto the surface of the agar medium. Then plates were allowed to stand on the laboratory bench for 1h for proper diffusion of the extract into the medium. Thereafter plates were incubated at 25°C for 48 h and observed for zone of inhibition [10].

Biofabrication of silver nanoparticles using *Moringa oleifera* leaves

The reduction of silver ions (Ag⁺ to Ag⁰) done by green synthesis method [11]. 10 mL of leaf extract was mixed to 90 mL of 1 mM aqueous of AgNO₃, and was heated at 60 - 80 °C for 20 min. A change from brown to reddish color was observed. After the completion of reaction, the solution was incubated for 72 h and supernatant solution was again challenged with silver nitrate solution. The remaining solution was then centrifuged at 3000 rpm for 20 min. and then supernatant was discarded. The prepared silver nanoparticles were collected by drying the mass and supernatant solution [12].

Characterization of silver nanoparticles

The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer (Shimadzu 1650 PC) at 200-400 nm [13].

Formulation of topical preparations

Cream and ointment base are used to incorporate synthesized silver nano particles. Vanishing cream with composition [14] shown in Table 1.2 is selected for its non-oily nature. PEG (Macrogol) ointment is selected for its water soluble nature with composition [15] as shown in Table 3.

![Table 1: Formula of cream base.](www.iajpr.com)
Table 2: Composition of PEG ointment base.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients</th>
<th>Quantity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stearic acid</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Potassium hydroxide</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Glycerine</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Ag nanoparticles</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>q. s. 100</td>
</tr>
</tbody>
</table>

**Antifungal activity of topical preparations**

The *in-vitro* antifungal activity of both topical formulations was performed by agar well diffusion method against *Candida albicans* [11] [12] [14].

**Evaluation of topical preparations**

**pH**

The pH of cream and ointment were determined by pH meter (PICO+ Lab India); which was calibrated by using phosphate buffer solutions i.e. pH 4, 7 and 9 (triple point calibration). The electrode was inserted into the sample 10 min. prior to study at room temperature.

**Spreadability**

It is determined by applying 5g of sample between two glass slides and was compressed to uniform thickness by placing 1kg weight for 5 min. Then weight was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which upper glass slide moves the lower plate to cover a distance of 5 cm is noted. A shorter interval of the time indicates better spreadability. The spreadability S was calculated using the formula[14]

\[ S = \frac{m \cdot l}{T} \]

Where;

S: Spreadability, m: Weight tied to upper glass slide, l: Length moved on glass slide
T: Time to cover a distance of 5 cm by upper slide

**Tube extrudability**

The prepared formulations were filled in collapsible tube with a nasal tip of 5 mm opening and applied enough pressure on the tube. Tube extrudability was determined by measuring the amount of cream extruded through the tip when a pressure was applied on the tube [14].

**Viscosity**

The viscosity of topical preparations were determined using Brookfield Viscometer (LVII, Brookfield Inc., USA) equipped with the spindle no.4. The measurement was performed at ambient temperature in triplicate [14].

**Particle size and zeta potential analysis**

Particle size and polydispersity index of ointment formulation were determined by using dynamic light scattering Malvern Zetasizer Nano-ZS90. Sample preparation was done by dissolving 1g of ointment in 10 mL of dispersion medium i.e. distilled water in glass test tube. The sample was loaded into transparent cuvette having volume of 1cm³ in the thermostatic chamber at 25°C. Laser light scattering was monitored at fixed angle 90° [15].

**In-vitro diffusion study**

The diffusion cells were fitted with 0.45µ cellophane acetate membrane soaked in phosphate buffer pH 6.8 for 3 h at 37°C. The effective diffusion area was 3.14 cm², and the receptor compartment was filled with 14 mL of 20% phosphate buffer pH 6.8 which was constantly stirred by external driven magnetic stirrer and maintained at 37°C ± 0.5°C. Accurately weighed 1g of each semisolid preparation was placed on membrane in donor cell compartment. About 0.5 mL of each sample withdrawn from receptor compartment at predetermined time interval for 6 h, an equal volume of fresh phosphate buffer 6.8 was immediately replace after each sampling. The drug concentration in the receptor compartment was determined spectrophotometrically [15].

**Skin irritation study**
The Institutional Animals Ethics Committee approved the use of rabbit for the present study (1093/po/a/2007/CPCSEA). The skin irritation study was carried out as per the OECD 423 guidelines. Three healthy albino rabbits weighing 1.5-2.0 kg; maintained under controlled environmental conditions and exposed to a photoperiod of 12 h of daylight and 12 h of night, in animal house. The dorsal surface of rabbit was cleaned and hairs were removed by shaving of two different region of dorsal surface. The exposed skin was cleaned with rectified spirit. Blank formulation as control sample and ointment containing silver nanoparticles as test samples were applied topically on shaved surface. About 0.5 g sample was applied for each day and rabbits were secured. The selected animals were fed with standard feed and monitored on a regular basis for a period of 7 days [16].

Stability study
The formulations were exposed to 25°C ±2°C and 60 %± 5 %RH for three months in stability chambers [17]. The physical parameters were determined at the end of study and compared with initial results.

RESULT AND DISCUSSION

In-vitro antifungal activity of *Moringa oleifera* extracts
Aqueous extract of leaves shown maximum activity; whereas chloroform extract has not shown activity against *Candida albicans* (Table 3)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. mg/ml</th>
<th>Zone of Inhibition (mm) against C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>L1</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>L2</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>L3</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>L4</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>L5</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD (n=3)

Bio-fabrication and characterizations silver nanoparticles
As the *Moringa oleifera* extract was mixed with aqueous solution of AgNO₃ solution (1mM), it started to change the color from watery to yellowish brown and finally to reddish brown after 72 h.

![Absorption spectra of AgNPs](image)

In-vitro antifungal activity of topical preparations containing AgNPs
Both topical formulations were compared with marketed antifungal cream (Clotrimazole) as positive control. All formulations have shown significant activity against *Candida albicans*; where ointment containing silver nanoparticles provided better activity as compared to the cream formulation. Table 4

<p>| Table 4: Antifungal activity of topical formulations against <em>C. albicans</em>. |</p>
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cream with AgNPs</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Ointment with AgNPs</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>Marketed cream (Clotrimazole)</td>
<td>30</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD (n=3)

Evaluation of topical formulation

General Appearance

The cream and ointment were white and yellowish white in color respectively with glossy and smooth feel on application. Both formulations were with good consistency. Other parameters are shown in Table 5.

Table 5: Physical parameters of topical formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Viscosity (cps)</th>
<th>Spreadability (gcm/s)</th>
<th>Extrudability (%)</th>
<th>% Drug release after 6hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ointment</td>
<td>6.9± 0.1</td>
<td>8266± 2</td>
<td>23.37 ± 0.5</td>
<td>92 ± 2.0</td>
<td>86.955± 2.0</td>
</tr>
<tr>
<td>Cream</td>
<td>7.2 ± 0.1</td>
<td>11130± 2</td>
<td>18.06 ± 0.5</td>
<td>84.33 ± 1.5</td>
<td>71.263± 2.0</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD (n=3)

In-vitro diffusion study

From the diffusion study, it was found that the ointment and cream has shown (Figure 2) 87 % and 77 % of AgNPs release respectively after 360 min. Maximum drug release from PEG ointment base could be related to its water soluble nature.

![Release study of the synthesized AgNPs](image1)

Figure 2: Release study of the synthesized AgNPs

Particle size analysis of ointment

![Size distribution curve of silver nano particles in ointment](image2)

Figure 3: Size distribution curve of silver nano particles in ointment
The physical stability of vehicle dispersion depends upon particle size and particle size distribution. Dynamic light scattering measurement showed that peak particle size of ointment system in the range of 150-1000 nm. The peak particle size of AgNPs in ointment formulation was found to be 196.9 nm. The average particle size of all formulation was denoted by Z-Average (d. nm) which was found to be 343.4 nm. The droplet size distribution graph was found to be bell shaped with even distribution range (Figure 3). The droplet size of ointment containing M. oleifera aqueous extract was not significantly affected by incorporation of the silver nanoparticles when compared to the ointment prepared blank ointment. The polydispersity lowers the uniformity of particle size in the formulation. Therefore, the low polydispersity index indicates higher uniformity of particle size in the formulation. The polydispersity index of formulation was found to be 0.572 indicating higher uniformity of particle size in formulation [13].

Skin irritation test
In the present study effect of formulation was observed daily for sign of redness, irritation and inflammation by visual inspection for 7 days. There has been no sign of redness, erosion, puss, pruritis, irritation or inflammation observed (Figure 4). Thus, the ointment formulation containing silver nanoparticles is non-irritant to rabbit skin when compared with blank ointment results.

![Blank ointment](image1)
![Ointment with AgNPs](image2)

![Day 1](image3)

![Day 3](image4)

![Day 7](image5)

Figure 4: Results of the skin irritation study

Stability study
Both the cream and ointment formulations didn’t show any phase separation during six month storage in parameters and signs of instability during study period affirming the stability.

CONCLUSION
The silver nanoparticles synthesized using Moringa oleifera leaves extract showed significant topical antifungal activity related to decreased particle size and increased surface area. Both topical formulations viz. cream and ointment found to be stable after accelerated stability study. The skin irritation study on rabbits confirmed non-toxic nature of formulation containing silver nanoparticles. However, further structural study, especially in animal and human phase would be beneficial to assess its usefulness more exactly.

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Competing Interests
The authors declare no conflict of interest.
REFERENCES


