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"Formulation and Evaluation of Nanogel on Pumpkin Peel Extract –Silver Nanoparticles Congugates against Candida Albicans"

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ABSTRACT

The present study focuses on the formulation and evaluation of pumpkin peel extract–silver nanoparticle (AgNP) conjugates for their antifungal efficacy against Candida albicans. Pumpkin peel, an agro-waste rich in phenolic and flavonoid compounds, was utilized as a natural reducing and stabilizing agent for the green synthesis of silver nanoparticles. The synthesized AgNPs were characterized for their particle size, zeta potential, morphology, and stability, confirming the formation of stable, spherical nanoparticles within the nanometres range. The nanoparticles were subsequently incorporated into Nano gel formulations (N1–N9) using suitable polymers to enhance bioavailability and topical application potential. The antifungal activity of the formulations was evaluated using the agar well diffusion method against Candida albicans. All formulations exhibited concentration-dependent antifungal activity, with inhibition zones increasing as the concentration increased from 250 µg/mL to 1000 µg/mL. Among all, the optimized formulation (N5) demonstrated the maximum antifungal activity, showing a zone of inhibition of 21.2 ± 1.0 mm at 1000 µg/mL, which was comparable to the standard antifungal drug Fluconazole (25 mm).The enhanced antifungal effect of the optimized nanogel may be attributed to the synergistic action of bioactive phytochemicals in pumpkin peel extract and the nanosized silver particles that facilitate efficient fungal cell membrane disruption. The findings suggest that pumpkin peel extract–silver nanoparticle conjugates represent a promising, eco-friendly, and cost-effective alternative for the management of Candida infections, supporting the potential application of agricultural waste in advanced nanomedicine.

Keywords: Pumpkin peel extract, Silver nanoparticles, Candida albicans, Antifungal activity, Nanogel, Green synthesis

I. INTRODUCTION

Fungal infections are a major global health problem, with Candida albicans representing one of the most prevalent opportunistic pathogens affecting skin, mucous membranes, and internal organs. Conventional antifungal drugs, such as azoles and polyenes, often show limited efficacy, resistance development, and systemic toxicity [11].Therefore, there is increasing interest in developing alternative, plant-based nanotechnological formulations that can enhance therapeutic effectiveness while minimizing side effects.

Nanotechnology has revolutionized pharmaceutical science by offering novel systems for drug delivery, particularly through nanoparticles, nanogels, and nanoemulsions[2]. Among these, metallic nanoparticles—especially silver nanoparticles (AgNPs)—exhibit strong antimicrobial and antifungal activity because of their small size, high surface area, and ability to interact with microbial membranes [4]. However, their biomedical use requires safe, eco-friendly, and biocompatible synthesis routes.

Green synthesis employs biological materials such as plant extracts, bacteria, fungi, and algae as reducing and stabilizing agents, avoiding toxic chemicals [1]. Plant-mediated synthesis is particularly attractive due to simplicity, low cost, and environmental compatibility [5]. In this context,

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agricultural by-products such as pumpkin peel (Cucurbitapepo) offer valuable phytochemicals that can act as natural reducers and stabilizers for nanoparticle formation [10].

Pumpkin peel is rich in phenolics, flavonoids, carotenoids, and vitamins, which impart antioxidant, antimicrobial, and antifungal properties [8]. Its utilization aligns with green chemistry principles and supports waste valorization. When combined with AgNPs, pumpkin peel extract may enhance antifungal efficacy through synergistic mechanisms.

Nanogels—cross-linked polymeric networks in the nanoscale range—serve as excellent carriers for sustained and targeted drug release [2]. Incorporating pumpkin-peel-mediated AgNPs into a nanogel provides a controlled topical delivery system that can penetrate the skin barrier and deliver active compounds directly to infection sites.

II. METHODOLOGY

2.1 Overview

The study involved the green synthesis of silver nanoparticles (AgNPs) using Cucurbitapepo (pumpkin) peel extract, formulation of AgNP-loaded nanogel, and evaluation of antifungal efficacy against Candida albicans. Each step followed standard laboratory protocols with slight modifications from published methods [10].

2.2 Preparation of Pumpkin Peel Extract

Fresh pumpkin peels were washed, shade-dried, and powdered. Ten grams of the powder were extracted with 100 mL of distilled water by heating at 60 °C for 30 minutes. The extract was filtered through Whatman No. 1 paper and stored at 4 °C for further use (Sharma et al. 2023).The aqueous extract served as both the reducing and stabilizing agent in AgNP synthesis due to its phytochemical richness, including phenolics, flavonoids, and carotenoids.

2.3 Green Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$, 1 mM) was used as a precursor. Equal volumes of $AgNO_3$ solution and pumpkin peel extract were mixed and incubated under continuous stirring at room temperature until a color change from pale yellow to brown indicated nanoparticle formation (Rautela et al. 2017).The reduction process was monitored by UV–Vis spectroscopy, observing the surface plasmon resonance (SPR) peak between 420–440 nm [11]. The synthesized nanoparticles were centrifuged at 10 000 rpm for 15 minutes, washed with distilled water, and dried at 40 °C.

2.4 Characterization of Silver Nanoparticles

1. UV–Visible Spectroscopy:
Confirmed nanoparticle formation by detecting characteristic SPR peaks.

2. Fourier Transform Infrared (FTIR) Analysis:
Identified functional groups involved in reduction and stabilization.

3. Scanning Electron Microscopy (SEM):
Determined morphology and size distribution.

2.5 Formulation of Nanogel

The optimized AgNP dispersion was incorporated into a topical nanogel base using Carbopol 940 as the gelling polymer [2].
Procedure:
1. Carbopol 940 (1 % w/w) was soaked in distilled water overnight.
2. The AgNP dispersion and glycerinwere added under slow stirring.
3. Triethanolamine was used to adjust the pH (6.5–7.0) and form a clear gel.
4. Preservatives and penetration enhancers were added to stabilize the formulation.

The resulting nanogel was labeled F1–F9 based on varying polymer concentrations and AgNP ratios for optimization.

2.6 Evaluation of Nanogel

a. Physical Properties

The formulated gels were visually inspected for color, homogeneity, and phase separation.

b. pH Measurement

Determined using a calibrated digital pH meter to ensure skin compatibility.

c. Viscosity and Spreadability

Measured using a Brookfield viscometer. Optimal viscosity ensures uniform application and drug release.

d. Swelling Index

Assessed by immersing gels in phosphate buffer (pH 7.4) and recording weight change.

e. Drug Content and Entrapment Efficiency

Calculated by dissolving a known amount of gel in solvent, measuring absorbance atλmax =

420 nm [4]. Entrapment efficiency (%) = (Entrapped drug / Total drug) × 100.

f. Zeta Potential and Particle Size

Determined by dynamic light scattering (DLS) to evaluate stability.

7 Antifungal Activity Evaluation

Antifungal efficacy was evaluated against Candida albicans using the agar well diffusion method [10].Wells were filled with nanogel samples at concentrations of 250 µg/mL, 500 µg/mL, and 1000 µg/mL.Fluconazole (25 µg) was used as the standard.Zone of inhibition was measured after 48 hours incubation at 37 °C.Formulations showing higher inhibition zones were selected for further analysis.

III. MATERIALS

3.1 Plant Material

The pumpkin peel (Cucurbitapepo L.), belonging to the family Cucurbitaceae, was chosen for this study due to its high content of phytochemicals with antioxidant and antifungal properties [10]. Fresh pumpkins were collected from local markets, authenticated by a qualified botanist, and the peel was separated manually.After washing, the peels were shade-dried for seven days, ground into fine powder, and stored in airtight containers until extraction [8]. This method ensured the preservation of thermolabile bioactive compounds such as flavonoids and phenolic acids.

3.2 Chemicals and Reagents

All chemicals used were of analytical grade.

Reagent Purpose/Use

Silver nitrate ($AgNO_3$)-Precursor for silver nanoparticle synthesis

Carbopol 940-Gelling polymer for nanogel formation

Glycerin -Humectant and emollient

Triethanolamine -pH adjustment and gel consistency

Ethanol -Extraction solvent and dispersant

Phosphate buffer (pH 7.4) - Dissolution and swelling medium

Distilled water Solvent for all formulation

3.4 Composition of Nanogel Formulations

Nine formulations (N1–N9) were prepared by varying the polymer (Carbopol 940) concentration and AgNP content to optimize consistency and release behavior.

FORMULATION CODE	PUMPKIN PEEL EXTRACT (ML)	CARBOPOLIN(%)	AgNP 03 (mm)
N1	3ml	1	3
N2	3ml	1.5	3
N3	3ml	2	3
N4	3ml	1	5
N5	3ml	1.5	5
N6	3ml	2	5
N7	3ml	1	7
N8	3ml	1.5	7
N9	3ml	2	7

Formulations N1–N9 were tested for physical properties, drug content, spreadability, viscosity, and antifungal activity.

3.5 Microorganism Used

Candida albicans was selected as the test organism because it is the most common fungal pathogen associated with mucosal and cutaneous infections [11].

The strain was obtained from a microbiology research laboratory culture collection and maintained on Sabouraud Dextrose Agar (SDA) at 4 °C. For each experiment, fresh subcultures were prepared.

3.6 Culture Media and Growth Conditions

Media: Sabouraud Dextrose Agar (SDA) for solid cultures; Sabouraud Dextrose Broth (SDB) for liquid inoculation.

Incubation: Plates were incubated at 37 °C for 48 hours.

Sterilization: All glassware and media were sterilized by autoclaving at 121 °C for 15 minutes.All microbiological procedures were performed aseptically to avoid contamination [6].

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3.7 Safety and Waste Management

All procedures involving silver nitrate and microbial cultures were conducted under controlled laboratory conditions following biosafety guidelines. Waste containing nanoparticles was neutralized using sodium thiosulfate before disposal [3].

IV. RESULTS AND DISCUSSION

4.1 Green Synthesis of Silver Nanoparticles

The aqueous extract of Cucurbitapepo peel successfully reduced Ag^+ ions into silver nanoparticles (AgNPs), as observed by a distinct color change from pale yellow to brown, confirming nanoparticle formation [1] [10].

UV–Visible spectroscopy showed a characteristic surface plasmon resonance (SPR) peak at 430 nm, consistent with typical AgNP spectra [5]. The stability of the nanoparticles was evidenced by the absence of peak shifts over time, indicating effective capping by phytochemicals.

FTIR analysis revealed strong absorption bands at 3420 cm^{-1} (O–H stretching), 1630 cm^{-1} (C=O stretching), and 1380 cm^{-1} (C–N vibration), confirming the role of hydroxyl, carbonyl, and amide groups in reduction and stabilization [4].

SEM imaging displayed nearly spherical nanoparticles ranging from 30 – 80 nm in diameter, while XRD patterns showed distinct peaks at 2θ = 38°, 44°, 64°, and 77°, verifying their crystalline silver nature [7].

4.2 Nanogel Formulation

The AgNPs were successfully incorporated into Carbopol-based nanogels that were smooth, homogeneous, and free from grittiness. pH values ranged from 6.5 – 7.0, suitable for topical application on human skin.

Viscosity measurements confirmed that the gels exhibited pseudoplastic flow behavior — a desirable property ensuring easy spreadability and retention on the skin surface.

Formulations N4 and N5 showed optimal consistency and clarity with no phase separation.

4.3 Drug Content and Entrapment Efficiency

The drug content among the formulations varied between 88 % and 97 %, confirming uniform distribution of AgNPs in the polymeric matrix [2].

Entrapment efficiency increased with polymer concentration up to 1.5 % Carbopol (N5), after which aggregation occurred, slightly lowering efficiency. This trend aligns with previous reports indicating that moderate cross-linking improves encapsulation while maintaining release balance [3].

4.4 Swelling Index and Spreadability

The swelling index of the gels increased proportionally with polymer concentration, affecting drug diffusion and release rate.

Spreadabilitytests showed that formulations N4 and N5 had optimal texture and surface coverage, confirming good patient acceptability for topical use [6].

4.5 Particle Size and Zeta Potential

Dynamic Light Scattering (DLS) analysis indicated that the average particle size in nanogels was 80–120 nm, confirming nanoscaledispersion.The zeta potential value of –32 mV demonstrated strong electrostatic stability, preventing aggregation during storage[11].

4.6 In-Vitro Antifungal Activity

Antifungal activity was evaluated by the agar well diffusion method against Candida albicans.

FORMULATION CODE	PATHOGEN	250µg	500µg	1000µg
N1	Candida albicans	7.5±0.4	9.2±0.3	12.0±0.5
N2	Candida albicans	8.0±0.5	10.1±0.4	13.2± 0.6
N3	Candida albicans	8.5±0.5	10.8±0.4	14.5±0.4
N4	Candida albicans	9.0±0.4	11.5±0.3	14.0±0.5
N5	Candida albicans	11.0±0.6	14.0±0.5	21.2±1.0
N6	Candida albicans	8.2±0.4	10.3±0.3	13.0±0.6
N7	Candida albicans	7.8±0.3	9.5±0.4	14.7±0.5
N8	Candida albicans	8.1±0.5	10.6±0.5	15.0±0.4
N9	Candida albicans	7.9±0.4	10.0±0.3	16.4±0.6
STANDARD(FLUCONAZOLE 25µg)	-	-	-	22.4±0.6
NEGATIVE CONTROL (GEL BASE)	-	-	-	No inhibition

The inhibition zone increased with concentration, showing a dose-dependent antifungal effect. Formulation N5 exhibited the maximum inhibition (21.2 ± 1.0 mm) at 1000 µg/mL, comparable to the standard antifungal drug fluconazole.

This enhanced effect is attributed to the synergistic interaction of bioactive phytochemicals and silver nanoparticles, which disrupt fungal cell walls, generate reactive oxygen species (ROS), and inhibit biofilm formation .

Mechanism of Antifungal Action

Silver nanoparticles penetrate the fungal cell wall, bind to sulfur- and phosphorus-containing biomolecules, and cause structural deformation [9].

The phytochemicals in pumpkin peel extract, such as flavonoids and phenolic acids, enhance oxidative stress, leading to cell apoptosis and inhibition of hyphal growth in C. albicans[8].

Thus, the pumpkin-peel-mediated AgNPnanogel exhibits a dual mechanism — direct cell-wall disruption by AgNPs and biochemical inhibition by phytochemicals, ensuring higher efficacy with reduced resistance risk.

4.8 Stability Studies

The optimized formulation (N5) was stored for 60 days at 25 ± 2 °C and 40 ± 2 °C / 75 % RH.

No significant changes in color, pH, or viscosity were observed, confirming good physical stability. Antifungal activity remained within 95 % of the initial value, indicating long-term retention of efficacy [2].

4.9 Comparative Discussion

The findings corroborate previous research where plant-mediated AgNPs exhibited potent antimicrobial activity [4].

The pumpkin-peel nanogel demonstrated superior antifungal performance due to synergistic phytochemical–silver interaction and sustained drug release through the gel matrix.

Compared to chemically synthesized nanoparticles, the green-synthesized system is safer, eco-friendly, and more biocompatible[6].

V. CONCLUSION

This study successfully demonstrated the green synthesis, formulation, and evaluation of a nanogel containing silver nanoparticles (AgNPs) synthesized using Cucurbitapepo (pumpkin) peel extract. The pumpkin peel, an agro-waste material, proved to be an effective natural reducing and stabilizing agent, aligning with the principles of green chemistry and environmental sustainability.

The UV–Vis, FTIR, SEM, and XRD analyses confirmed the formation of spherical, stable, and crystalline silver nanoparticles capped with phytochemicals. The AgNPs were effectively incorporated into a Carbopol-based nanogel, producing a homogeneous and skin-compatible formulation with excellent viscosity, spreadability, and pH stability.

Among all formulations, N5 exhibited the highest antifungal efficacy, producing a zone of inhibition of 21.2 ± 1.0 mm against Candida albicans, which was comparable to that of the standard drug Fluconazole (25 mm). The enhanced antifungal activity resulted from a synergistic effect between silver nanoparticles and the bioactive compounds present in pumpkin peel extract.

The nanogel demonstrated good stability over 60 days, with minimal changes in physical parameters and sustained antifungal potency. This indicates that green-synthesized silver nanoparticles, when integrated into nanogels, can provide an effective, safe, and eco-friendly alternative for the treatment of fungal infections.
Future work can focus on in-vivo evaluations, toxicological studies, and scale-up production to develop this formulation for clinical and pharmaceutical applications.

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