

Antifungal activity of silver nanoparticles loaded oregano oil nano-emulsion for topical treatment of mucocutaneous candidiasis

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World Journal of Advanced Research and Reviews, 2023, 19(03), 729–736

Publication history: Received on 20 May 2023; revised on 05 July 2023; accepted on 07 July 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.19.3.1281>

Abstract

Oregano essential oil (OEO) possesses antimicrobial, antioxidant, and preservative properties, making it a promising candidate for various biotechnological and biomedical applications. However, the inherent volatility, hydrophobicity, and susceptibility to external factors such as light, heat, and oxygen can compromise its stability and bioactivity. To overcome these limitations, silver nanoparticles (AgNPs) were incorporated into an oregano oil nanoemulsion, enhancing both its antimicrobial efficacy and mechanical properties. Chronic Mucocutaneous candidiasis, often associated with recurrent unexplained fungal infections, is characterized by inherited T-cell defects and typically requires prolonged antifungal treatment. The approved oral imidazole derivative, ketoconazole, is commonly used for the treatment of candidiasis, chronic Mucocutaneous candidiasis, and oral thrush. However, non-compliance, therapy failure, and systemic side effects have been reported with prolonged use of ketoconazole.

In this study, we developed a silver nanoparticle-loaded oregano oil nanoemulsion as a potential alternative. Our results demonstrated that the prepared nanoemulsion exhibited enhanced antimicrobial activity, as indicated by larger zones of inhibition compared to the standard ketoconazole and oregano oil combination, as confirmed by the disc diffusion method. This novel formulation holds great promise for the treatment of Mucocutaneous candidiasis, providing an effective and potentially safer alternative to current therapies.

Keywords: Oregano essential oil; Silver nanoparticles; Nanoemulsion; Antimicrobial activity; Chronic Mucocutaneous candidiasis; Ketoconazole

1. Introduction

Microbial infections pose a significant health problem worldwide, and the conventional approach to treating lethal infections involves the use of antibiotics (1). However, the long-term use of antibiotics has led to the emergence of drug-resistant microorganisms (2), resulting in the development of bacterial strains resistant to numerous antibiotics (3). This growing incidence of antibiotic resistance necessitates the development of effective and biocompatible antimicrobial agents with minimal or no microbial resistance. In recent years, the use of nanoparticles as alternative antibiotic agents has gained attention.

Silver nanoparticles (Ag NPs) have emerged as promising antimicrobial agents due to their ability to generate free radicals (4). Furthermore, Ag NPs disrupt ATP synthesis by blocking transcription and induce deformation in bacterial cell walls by binding to negatively charged components of proteins and nucleic acids (5-9). Silver ions have also been

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shown to interact with electron donor functional groups such as thiols, phosphates, hydroxyls, imidazoles, indoles, and amines (10).

Oregano essential oil (OEO) is derived from the *Origanum vulgare* plant and is rich in phenolic and terpenoid compounds (11,12,26). Oregano oil exhibits considerable antioxidant, antimicrobial, and anti-inflammatory activities (13). Combining the antimicrobial properties of silver nanoparticles and oregano essential oil may lead to a synergistic effect, enhancing the overall antibacterial and antifungal activity.

Nanoemulsions are charged, spherical, amorphous, and lipophilic dispersions of one liquid phase within a second continuous phase (14). They can exist in two forms: oil-in-water (o/w) or water-in-oil (w/o), and can also be formulated as double emulsions (o/w/o or w/o/w). Nanoemulsions have gained significant attention in the field of drug delivery systems due to their advantages, including enhanced drug loading capacity, improved drug targeting ability, protection against hydrolysis and enzymatic actions, and improved drug dissolution and bioavailability (15-18). Moreover, their small size imparts transparency and continuity, making them suitable for both laboratory studies and industrial scales (19).

Therefore, the aim of this investigation was to develop an optimized oregano oil nanoemulsion and incorporate it with silver nanoparticles to achieve improved antibacterial and antifungal activity. The combination of silver nanoparticles and oregano oil in a nanoemulsion form has the potential to offer a highly effective and biocompatible antimicrobial agent with enhanced efficacy against microbial infections. Bottom of Form

2. Materials and methods

Oregano oil was purchased from Misri Fumet Pvt. , Tween 80, Span 80 nutrient agar was purchased from Merck, India, silver nitrate was purchased Rankem, sodium citrate and sodium borohydride was purchased SRL , sodium chloride and peptone was purchased Loba Chmic Pvt. Ltd, beef extract was supplied by CDH, All other chemicals were of analytical grade or better.

2.1. Microorganism

Resistant bacterial strains *Candida albicans* were supplied from Sam Higginbottom Institute of Agriculture Technology and Sciences, Prayagraj.

2.2. Preparation of silver nano particles (AgNPs)

Silver nanoparticles were synthesized by adding 0.5 ml AgNO₃ 0.01 M into 20 ml stirring solution of sodium citrate 1 mM as a stabilizer. After 10 min, 0.5 ml sodium borohydride 0.01 mM which is a reducing agent was added to this solution after 10 minutes and the obtained mixture was stirred for 10 min. (19) Preparation of Ag NP-Loaded oregano oil (OO NE+Ag NPs) In order to prepare a clear nanoemulsion, aqueous phase was prepared by dissolving the surfactant mixture of Tween 80 and Span 80 (72:28) in ethanol and water under magnetic stirring. Then, using a low shear mixer the aqueous phase was mixed with the oil 15 min in order to obtain a transparent emulsion. consequently, Ag NPs were introduced to the system to obtain a oregano oil nano emulsion containing Ag NPs .

2.3. Characterization of Prepared Nanoemulsion (20)

2.3.1. UV-Vis Absorption Spectroscopy

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction was employed. Spectrum responses were recorded from 200 to 800 nm with 1-nm steps in a $4 \times 1 \times 1$ cm path quartz cuvette.

2.3.2. Field emission-scanning electron microscopy

It is used to estimate the roughness of the surface and visualization of the surface texture and morphology, prepared nanoparticles were examined by field-emission scanning electron microscopy operated at 30 kV acceleration. The photographs were recorded at different magnifications (12X- 1000KX).

2.3.3. Zeta Potential (ZP)

This is used to examine the surface charge of the prepared oregano oil loaded AgNPs. Suspend the 0.1g of the prepared particles in 50% v/v glycerol in isopropanol and then sonicated for 30 min. Transfer the suspension to a zeta-potential cell (21).

2.3.4. Particle size determination

The particle size analyzer was used to estimate the size of the nanoparticle. The instrument works on the principle of dynamic light scattering technique for size estimation. The smaller particles moved at higher speed than the larger.

2.3.5. Culture Media Nutrient Agar

Culture media Nutrient agar media was used for the culturing and Nutrient broth (Table 1) was used for incubation.

Table 1 Composition of nutrient agar

S.No.	Composition	Concentration (g/l)
1	Beef extract	1
2	Yeast extract	2
3	Gelatin extract	5
4	Sodium chloride	5
5	Agar	15
Composition of nutrient broth		
1	Gelatin peptone	5
2	Beef extract	1
3	Yeast extract	2
4	Sodium chloride	5

2.3.6. Media preparation

The 2.8g/ 100 ml of nutrient agar and 1.3g/100 ml nutrient broth were prepared in distilled water and poured into conical flasks test tubes plugged with the cotton wool and kept in an autoclave at 1.5 pounds pressure and 121°C for 15 min. The sterilized nutrient agar media was poured aseptically into sterilized Petri plates in a laminar flow hood. The media was allowed to be solidified in Petri plates for about an hour. Petri plates were kept in incubator at 37°C for 24 h in an inverted position in order to avoid evaporation of water from the media within the plates then these plates were used for culturing of bacteria and fungi (23-25).

2.3.7. Disc diffusion susceptibility method

Standardized inoculums of the test microorganism are inoculated in the agar plates. Then, the desired concentration of the test compound are placed on the agar surface with the filter paper discs (about 6 mm in diameter). Incubate the Petri dishes under suitable conditions. Usually, the growth of the test microorganism is inhibited by the diffusion of the antimicrobial agents into agar and then the diameters of the zone of inhibition is measured.

3. Characterization of Prepared Nano emulsion

3.1. UV-Visible Absorption Spectroscopy :-

Oregano oil's UV-visible absorption spectrum when combined with silver nanoparticles. Surface Plasmon reproduction absorbing, which would be caused by electronic excitation in conduction electrons produced by an electromagnetic field, is what gives silver nanoparticles their recognizable bright color. Silver colloids showed persistent spr groups with asymmetric shapes approximately 407 nanometers in the UV-visible spectrum (**Fig. 1**), as well as the broadband inside the Ultraviolet region was brought on by such an SNP electronic energy shift (245 nm).

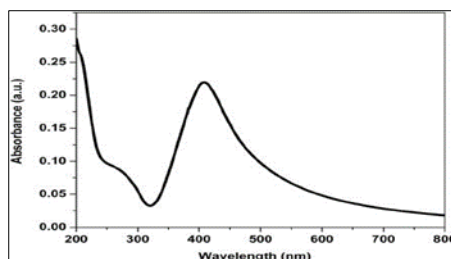


Figure 1 UV-Visible spectra of AgNPs Loaded Oregano Oil Nano Emulsion

3.2. Particle Size Determination and Zeta Potential Analysis:

the size of the nanoparticle and charge distribution of a chemically loaded oregano oil with AgNPs were assessed by particle size measurement and zeta size analysis by Zeta Size Nano ZS90, Malvern Instruments Ltd. Helps in determining the electrophoretic mobility. The zeta potential of the chemically AgNPs-loaded oregano oil was found to be -18.4 mV (**Fig. 2**), demonstrating its high stability. A nanoparticle is said to as extremely stable if its zeta potential is larger than +30 mV as well as lower than -30 mV. Additionally, a distinct peak was found at 91.28 nm in the plot in (**Fig 3**) that shows the relative percentage of light scattering for average particle size, indicating the presence of nanoscale aggregates in the samples.

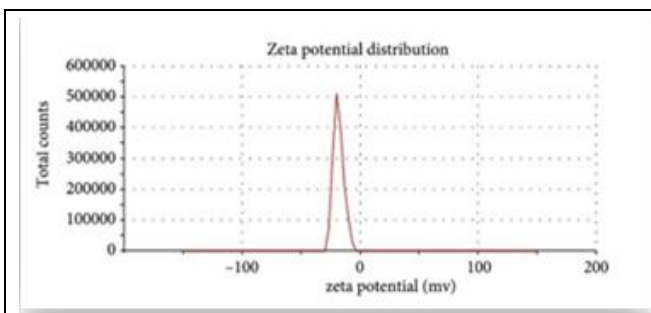


Figure 2 Zeta Potential of AgNPs Loaded Oregano Oil Nano Emulsion

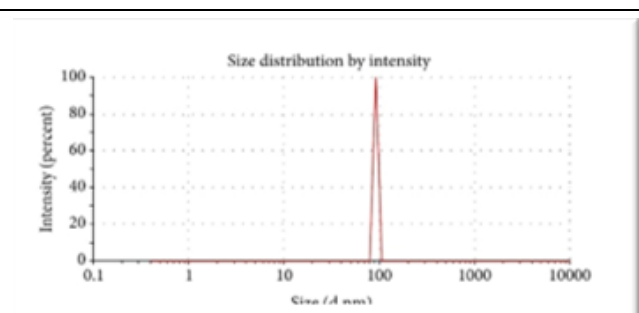


Figure 3 Particle Size Distribution of AgNPs Loaded Oregano Oil Nano Emulsion

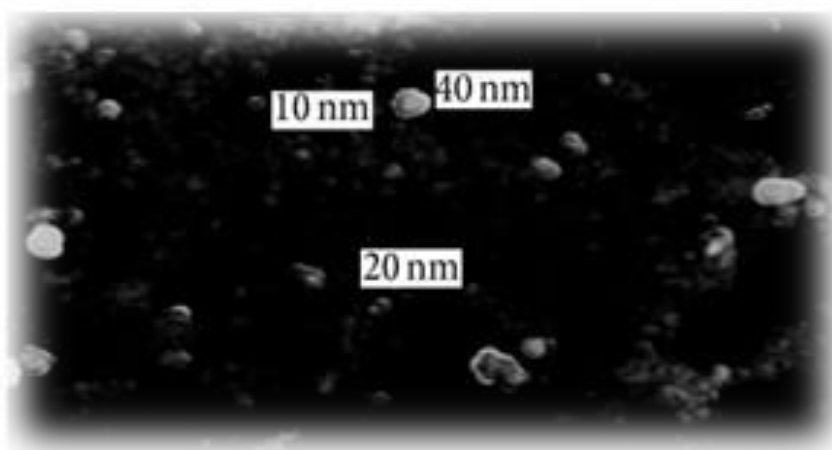


Figure 4 SEM image of AgNPs Loaded Oregano Oil Nano Emulsion

3.3. Scanning Electron Microscopy

SEM micrographs were spherical and the obtained particles are 10-40nm in size shown in fig. 4.

4. Results

4.1. Antifungal Activity of *C. Albicans* on the growth of Fungus Stains

The comparison of antifungal action (Fig.5) shows that the growth of fungal strains increased at different concentrations after 48 hours compared to 24 hours. When 0.3 mg/ml of oregano oil was added to Ag NPs in the culture conditions, it was found that treatment with ketoconazole at 0.1 mg/ml and loaded oregano oil nanoemulsion at 0.2 mg/ml completely inhibited the growth of *C. albicans*.

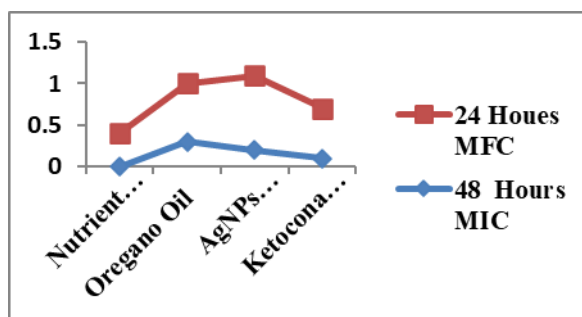


Figure 5 Graph Showing on Growth of Fungus Strain

4.2. Antifungal activity of *Candida albicans* Zone of inhibition (mm)

Figure 4.2 presents the antifungal activity of oregano oil, ketoconazole, and silver nanoparticles loaded oregano oil Nano emulsion at different concentrations. The data clearly demonstrate that as the concentration of oregano oil, ketoconazole, and silver nanoparticles loaded oregano oil Nano emulsion increases, the zone of inhibition also increases. Among the three, the silver nanoparticles loaded oregano oil Nano emulsion exhibits the highest zone of inhibition, surpassing both ketoconazole and oregano oil. The mean and standard deviation of oregano oil, silver nanoparticles loaded oregano oil Nano emulsion, and ketoconazole are presented in Table 1.

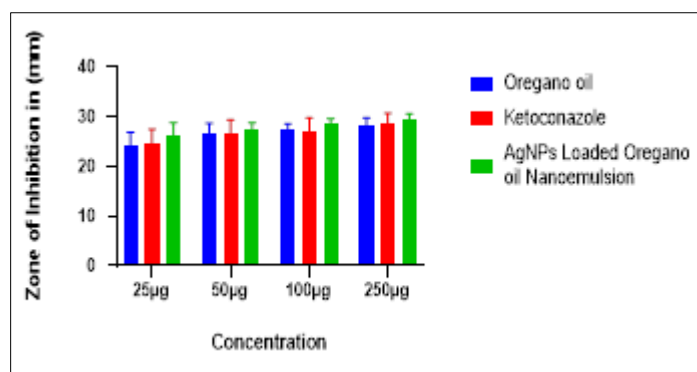


Figure 6 Graph Showing on Zone of inhibition in mm of Antifungal Activity

Table 1 Antifungal Activity Zone of Inhibition in (mm)

Antifungal Activity Zone of Inhibition in (mm)			
Mean ± SD			
Concentration	Oregano Oil	Ketoconazole	AgNPs Loaded Oregano Oil Nano emulsion
25µg	24.25 ± 2.58	24.05 ± 2.38	26.25 ± 2.58
50µg	26.75 ± 1.92	26.75 ± 2.58	27.25 ± 1.47
100µg	27.05 ± 1.11	27.00 ± 2.73	28.05 ± 1.11
250µg	28.25 ± 1.47	28.75 ± 1.92	29.05 ± 1.11

4.3. In Vitro Antifungal Activity of *Candida albicans*:

The in vitro antifungal activity was evaluated using the disc diffusion method in this study. The antifungal activities of oregano oil, AgNPs loaded oregano oil, and ketoconazole were compared, and the results obtained indicate that the antifungal activity is concentration-dependent. That is, as the concentration increases, the zone of inhibition also increases. Fig. 7 illustrates that the AgNPs loaded oregano oil exhibits a higher zone of inhibition at different concentrations compared to oregano oil alone. Based on these findings, it can be inferred that the AgNPs loaded oregano oil nanoemulsion demonstrates potential in vitro antifungal activity and could be topically administered for the treatment of Mucocutaneous candidiasis.

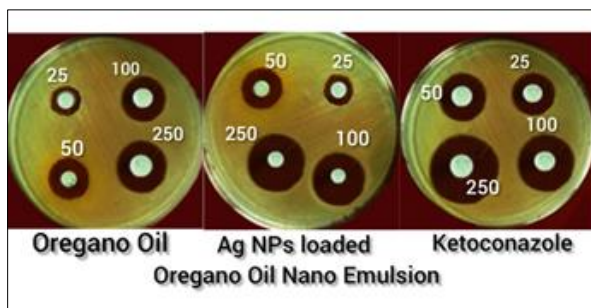


Figure 7 In vitro antifungal activity of the Mucocutaneous candidiasis effect of Oregano Oil Loaded on Silver Nanoparticles against *Candida albicans*. (a) Oregano Oil (b) AgNPs Loaded Oregano Oil Nano Emulsion (c) Ketoconazole

5. Discussion

The present study aimed to develop a silver nanoparticle-loaded oregano oil nanoemulsion as a potential alternative for the treatment of mucocutaneous candidiasis. The silver nanoparticles (AgNPs) into the oregano oil nanoemulsion to enhance its antimicrobial efficacy and mechanical properties. The study compared the antimicrobial activity of the prepared nanoemulsion with a standard combination of ketoconazole and oregano oil. The results demonstrated that the nanoemulsion exhibited enhanced antimicrobial activity, as indicated by larger zones of inhibition compared to the standard combination. This suggests that the combination of silver nanoparticles and oregano oil in a nanoemulsion form has a synergistic effect, leading to improved antibacterial and antifungal activity. The use of silver nanoparticles as antimicrobial agents has gained attention due to their ability to generate free radicals, disrupt ATP synthesis, and induce deformation in bacterial cell walls. Oregano essential oil, on the other hand, is known for its antimicrobial properties and is rich in phenolic and terpenoid compounds (13). Combining the antimicrobial properties of silver nanoparticles and oregano essential oil can potentially enhance the overall antibacterial and antifungal activity.

Nanoemulsions have been widely studied in the field of drug delivery systems due to their advantages, such as enhanced drug loading capacity, improved targeting ability, protection against degradation, and improved bioavailability. The small size of nanoemulsions also allows for transparency and continuity, making them suitable for various applications. The characterization of the prepared nanoemulsion revealed its stability and particle size. UV-Vis absorption spectroscopy showed the presence of silver nanoparticles, and field emission-scanning electron microscopy provided insights into the surface texture and morphology of the particles. Zeta potential analysis indicated the surface charge of the prepared oregano oil loaded with silver nanoparticles.

In vitro antifungal activity testing using the disc diffusion method demonstrated concentration-dependent inhibition of *Candida albicans* growth. The silver nanoparticle-loaded oregano oil nanoemulsion exhibited the highest zone of inhibition compared to oregano oil alone and ketoconazole. These findings suggest that the developed nanoemulsion has the potential to effectively treat mucocutaneous candidiasis, providing a safer alternative to current therapies. The study successfully developed a silver nanoparticle-loaded oregano oil nanoemulsion with enhanced antimicrobial activity against *Candida albicans*.

6. Conclusion

In conclusion, the development of a silver nanoparticle-loaded oregano oil nanoemulsion has shown significant promise as an alternative treatment for mucocutaneous candidiasis. The incorporation of silver nanoparticles into the oregano

oil nanoemulsion resulted in enhanced antimicrobial activity, surpassing the effectiveness of the standard combination therapy of ketoconazole and oregano oil.

The combination of silver nanoparticles and oregano oil in a nanoemulsion form exhibited a synergistic effect, leading to improved antibacterial and antifungal activity. This can be attributed to the antimicrobial properties of silver nanoparticles, which generate free radicals and disrupt bacterial cell walls, along with the natural antimicrobial compounds present in oregano oil.

The nanoemulsion formulation offers several advantages, including increased drug loading capacity, improved targeting ability, protection against degradation, and enhanced bioavailability. The stability and particle size of the prepared nanoemulsion were also confirmed through characterization techniques.

In vitro testing demonstrated concentration-dependent inhibition of *Candida albicans* growth, with the silver nanoparticle-loaded oregano oil nanoemulsion showing the highest zone of inhibition compared to oregano oil alone and ketoconazole. These findings suggest that the developed nanoemulsion holds great potential as an effective and potentially safer treatment option for mucocutaneous candidiasis.

Further research and studies are necessary to evaluate the efficacy and safety of this novel nanoemulsion formulation in clinical settings. If proven successful, it could provide a valuable alternative to existing therapies, addressing the growing concern of drug resistance and offering improved outcomes for individuals suffering from mucocutaneous candidiasis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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