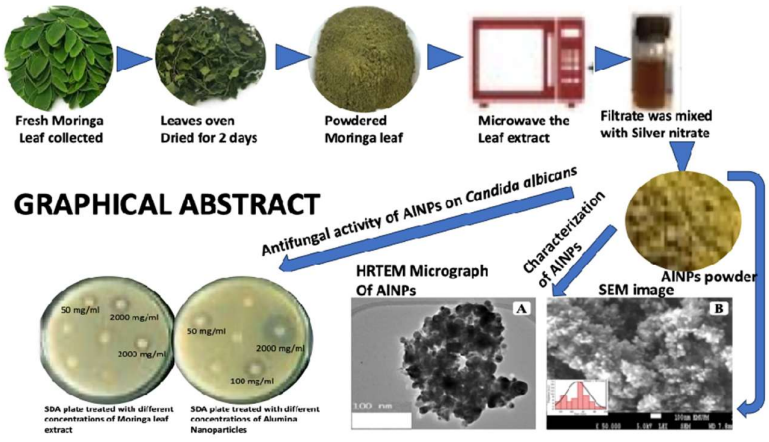
International Academic Journal of Advanced Pure & Applied Science Research
ISSN: 5280-5269 | Volume 10, Issue 1 | January, 2025 | pages 1 – 8
DOI: 2229442831425272771011
Double Blind Peer Reviewed International Research Journal
https://arcnjournals.org
arcnjournalsa@gmail.com

Microwave-assisted Green Synthesis and Characterization of Alumina Nanoparticles using *Moringa oleifera* for Management of Oral Candidiasis

Aisha Ahmad Tijani, Zainab Hassan Isa, Alhaji Modu Bukar*, Modu Z. Kyari, Hassan Grema, Hadiza Bunu Goni, Aisha Muhammed Ngubdo, Aisha Musa Bulama & Fatima Y. usman and Yakubu Baban Kanada
Department of Science Laboratory Technology, School of Applied Sciences & Technology, Ramat Polytechnic, Maiduguri P.M.B. 1070 Maiduguri Borno state – Nigeria

Abstract: *In the recent days, research in medicine is targeting towards protecting communicable diseases that are caused by microorganisms. Unfortunately, proper management and treatment of the diseases remain the major challenges to both human and animals. In addition, the existing commercially produced antibiotics are not effective and can cause serious antibiotic resistance problems. Nanotechnology in the biomedical science sector aimed at enhancement of the antimicrobial products by providing innovative novel delivery platform towards successful management of fungal disease. The research work involved the green synthesis of alumina nanoparticles (AlNPs) using aqueous Moringa oleifera leaf extract by microwave-assisted method for the management of Candida albicans. The morphologies and particle sizes of green synthesized nanoparticles from leaf extract were characterized by using TESCAN Vega LMU- scanning electron microscope (SEM) and JEOL and transmission electron microscope (TEM). TEM exhibits spherical shaped nanoparticles with an average particle size range from 12 to 46 nm. The synthesized alumina nanoparticles were screened for antifungal activity by agar-well diffusion method. Effective zones of inhibitions against the test organisms the causal agent of oral candidiasis, was achieved after exposure to green synthesized AlNPs. Significant dicreases in zones of inhibition was recorded when the Candida albicans was exposed to different concentrations of the nanoparticles. The current investigation suggested that green-snthesized nanoparticles can revolutionize the field of candidiasis management by utilizing an effective approach for disease management and playing a potential part in biomedical industries. This area of research is in need of adoption and exploration for the management of oral candidiasis.*

Keywords: Candida *infections,* Candida*, antifungals, resistance, alternative antifungal drugs.*



Introduction

In the recent days, the term phytonanotechnology less commonly known as green synthesis has received great attention own to it several advantages such as inexpensively, simplicity, scalability, applicability and most importantly biocompatibility, by using aqueous solution which acts as a reduction medium. Green synthesis of nanoparticles serve as relatively the most effective approach that is relatively advantageous compare to chemicals and physical methods. The green synthesis Biological processes implicate fungal, bacterial, and plant enzymes that involve convoluted procedures for sustaining cell cultures under aseptic conditions while significant production of NPs was achieved by employing plant extracts, encompassing simplicity and applicability with low energy consumption; however, chemically synthesized NPs comprise of toxic reagents that remain as residues along with particles and ultimately nurture toxicity problems within human system (Bukar et al., 2024; Oberdorster et al, 2005).

In recent times, simple, inexpensive and most importantly eco-friendly synthesis of nanoparticles has been consciously employed to overcome the cytotoxic effects associated with nanoparticle synthesized from chemical approach. Being an environmentally friendly and a multifactorial phytogenic material, application of NPs is receiving great attention due to their specific physicochemical and physiochemical properties, serves as an approach for assembling of innovative functional materials used almost in every area of science and technology like medicine, engineering, environment, and agriculture (Bukar et al., 2022; Ismail et al., 2021; Sharma and Sharma, 2019). Due to the interdynamic properties of NPs, there are high opportunities to explore the potential of NPs while the nano-elicitive behavior of these minute particles may rely on their nature and methods of synthesis *(Allen et al 2015).*

Alumina (Al)NPs serves as potential antifungal agents by demonstrating stout bacterial and fungal activities which highlighted their importance in medical applications (Sharma and Sharma, 2019; Jalal et al., 2016). Synthesis of novel antimicrobial agents for the management of disease caused by fungal in human (Sharma and Sharma, 2019). Some recent research reported that antifungal activity of nanoparticle on conidial development and fungal hyphae as well as presenting strong inhibitory effects of nanoparticles. In addition, the existing literature, nanoparticles have been applied to inhibit microbial growth. Among the different types of metals, AlNPs have been used extensively against different microbial pathogens. AlNPs from *Carica papaya* leaf extracts have shown high effect against bacterial pathogens from the genus Staphylococcus spp and Escherichia coli. Its single applications of 10 and 100 µg/ml of alumina nanoparticles resulted in inhibition of microbial cells (Bukar et al., 2024)

Candidiasis is an opportunistic mycosis with high annual incidence worldwide. Candidiasis is caused by different Candida species, with C. albicans being the main cause of the disease worldwide. *Candida albicans* as the result of overgrowth of a type of yeast that lives on human body. A candidiasis infection often appears on your skin, vagina or mouth, where Candida naturally lives in small amounts (Wainwright, 2008; *Barchiesi et al, 1997)*. The *Candida* genus comprises opportunistic fungi that can become pathogenic when the immune system of the host fails. *Candida albicans* is the pathogenic species most frequently isolated. However, other species such as *C. glabrata, C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. famata, C. guilliermondii*, and *C. lusitaniae* have been increasingly isolated, mainly in human immunodeficiency virus (HIV)-infected individuals *Candida albicans* is the most important and prevalent species *(Allen et al 2015)*. Polyenes, fluoropyrimidines, echinocandins, and azoles are used as commercial antifungal agents to treat candidiasis. However, the presence of intrinsic and developed resistance against azole antifungals has been extensively documented among several *Candida* species *(Barchiesi et al, 1997)*. The advent of original and re-emergence of classical fungal diseases have occurred as a consequence of the development of the antifungal resistance

phenomenon. In this way, the development of new satisfactory therapy for fungal diseases persists as a major challenge of present-day medicine *(Allen et al 2015)*.

Among the available antifungal agents, azoles are the preferred and most frequently used drugs for treatment of *Candida* infections. Depending on the type of infection, the anatomical site in which it occurs and the sensitivity profile of species, other antifungals can also be used. Among these, there are polyenes, echinocandins, nucleoside analogs and allylamines *(Allen et al 2015)*. Fluconazole (FLZ), a type of azole, is often preferred in treatments of *Candida* infections because of its low cost and toxicity, in addition to availability in varied formulations. However, there are many reports in the literature on the development of resistance among *Candida* species, especially in relation to azoles, which is essential for the determination of resistance mechanisms presented by fungi with the objective of developing new classes of antifungal for treatment of *Candida* infections *(Barchiesi et al, 1997)*. The need of the hour includes the development of a more effective therapy, since the phenomenon of resistance caused the appearance of new fungal infections, in addition to facilitating the resurgence of the existing ones *(Calderone and Fonzi, 2001).* In this way, the control of *Candida* infections is a challenge in the modern clinic. The design of new drugs from the traditional ones used in the clinic and the identification of new molecules with antifungal potential for the manufacture of new drugs, more effective and less toxic, are fundamental to face the challenge (Ismail et al., 2021;

Allen et al 2015).

The design of original drugs from traditional medicines provides new promises in the modern clinic. The urgent need includes the development of alternative drugs that are more efficient and tolerant than those traditional already in use. The identification of new substances with potential antifungal effect at low concentrations or in combination is also a possibility. The present study examines antifungal activity of AlNPs against *Candida* species and focuses on the mechanisms of action associated with the traditional agents used to treat those infections.

Materials and Methods

Collection of Plant Material and Procurement of Pathogen Inoculum Fresh leaves of *Moringa oleifera* were collected from the tree planted in the vicinity of Ramat Polytechnic Maiduguri. Alumina nitrate ($AlNO_3$, 99.0%) and Saboroud dextrose agar (SDA) (Merck, Darmstadt, Germany) was purchased from Sigma-Aldrich. Pure culture of *Candida albicans* was kindly provided by Abubakar Mursal of Microbiology Laboratory, University of Maiduguri Teaching Hospital – Nigeria. The strain was maintained, subcultured fortnightly, and preserved on PDA medium in glass culture tubes at 4°C. All the solutions were prepared with distilled water. All glasswares were rinsed and sterilized before use to avoid possible contamination.

Preparation of *Moringa oleifera* Leaf Extract

The healthy and fresh leaves of *Moringa oleifera* were collected from Agro-farm Ramat polytechnic Maiduguri – Nigeria and transported to the chemistry laboratory, Department of Science Laboratory Technology, Ramat Polytechnic Maiduguri – Nigeria. The samples were rinsed thoroughly with double distilled water to remove dust particles. Leaves were oven-dried at 55°C temperature for 24 hours. 20g of dried samples was dissolved in 100 ml of double distilled water and boiled at 90°C for 20 min in a temperature-controlled water bath. The leaves extract was allowed to cooled down at room temperature

and filtered through Whatman filter paper. The extract was stored at 4°C and used within 2 weeks. This extract was used as a stabilizing and reducing agent.

Synthesis of *Moringa* Leaf Extract-Capped Alumina Nanoparticles

The reaction conditions for the green synthesis of Moringa-AlNPs were optimized. Typical reaction contained 5 ml of Moringa, mixed with 45 ml of 2.5 mM AlNO3, pH 8.0 in a 100-ml flask. For rapid microwave synthesis, the synthesis product was subjected to a domestic microwave oven (Panasonic NN-CT651M) operating at the power of 1,100 W for a short pulse of 30 s. The mixture was then allowed to stand at room temperature for further use. The reaction conditions were optimized by performing the experiment for different conditions, wherein the reactions of Moringa-AlNO3 were performed as the function of the concentrations of alumina nitrate (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 mM), amount of extract-Moringa (1, 2, 3, 4, 5, 8, and 10 ml), pH (2, 4, 6, 8, and 10), and microwave irradiation (5, 10, 15, 30, 45, 60, and 75 s). All the reactions were performed at ambient temperature at room temperature. A radical change in color from pale yellow to deep brown was observed. The greenynthesis of Moringa-AlNPs was observed by color change from yellow to deep brown.

Characterization of Moringa Leaf Extract-Capped Alumina Nanoparticles

The greensynthesis of Moringa -AlNPs was characterized by using TESCAN Vega LMU- scanning electron microscope (SEM) and JEOL and transmission electron microscope (TEM). and Fourier-transform infrared (FTIR) spectra for Moringa -AlNPs were obtained in the range of 4,000–400 cm–1 with an FTIR (Thermo Scientific Nicolet 6700).

Antifungal Activity of Moringa Leaf Extract-Alumina Nanoparticles

The synthesized alumina nanoparticles were screened for antifungal activity by agar-well diffusion method. The sabouraud dextrose agar (SDA) was prepared, autoclaved (121°C for 15 min) and allowed to cool 25°C and the media was aseptically in 25 mL amount into sterile petri dishes. Antifungal activities of the alumina nanoparticles were evaluated using varying concentrations of the AlNPs. After 24 h of incubation, agar plugs of uniform size (4 mm in diameter) at the center of the petri plates of 3-day-old cultures of *Candida albican* and the AlNPs were aseptically poured to the center of each medium plate amended with different concentrations of NPs. Control plates were also prepared by using double distilled water only. All the plates were incubated at 28 ± 2 °C for 10 days. All the assays were performed in triplicate (Jalal et al., 2016).

Results

The morphological and size wise characterization of Microwave-mediated greens synthesized Moringa Leaf Extract-Alumina Nanoparticles. The representative SEM images are shown in Figures 1. The Moringa-AlNPs observed were all spherical in shape in nature. TEM images confirmed the synthesis of AlNPs by depicting variable and predominantly spherical and crystalline Moringa-AlNPs with dark edges (graphical abstract). Graphical abstract SEM and HRTEM shows the histogram pattern of green synthesized AlNPs. The particle size distribution ranges from 12 to 46 nm with an average diameter of 28.04 nm.

Antifungal Activity of Moringa Leaf Extract-Alumina Nanoparticles

The Moring-AlNPs expressively inhibited the growth of *Candida albicans* which clearly indicated that these NPs have the potential to be used as an active antifungal agent. Observations were recorded after

10 days for different concentrations. It is evident from the results that higher concentrations (50–200 µg/ml) of AlNPs repressed fungal activity with 79–98% inhibition rate as compared to the control ($p < 0.05$). However, less than 50% inhibition was noted at lower concentrations, i.e., 50–100 mg/ml after the required incubation period in contrast to higher ones. The highest inhibition rate (98.2 ± 0.15) was observed at 140 µg/ml of alumina nanoparticles while the lowest rate (10.6 ± 0.379) was observed at 50 mg/ml.

Table 1: Antifungal activity of green synthesized nanoparticles against *Candida albicans*

Categories	**Concentration (mg/mL)**	***C. albicans***
Alumina nanoparticles	200	18.5 ± 0.8^{ab}
Alumina nanoparticles	100	16 ± 1.00^{a}
Alumina nanoparticles	50	15.3 ± 0.70^{a}
Moringa extract	200	7.0 ± 0.0
Moringa extract	100	7.0 ± 0.0
Moringa extract	50	7.0 ± 0.0

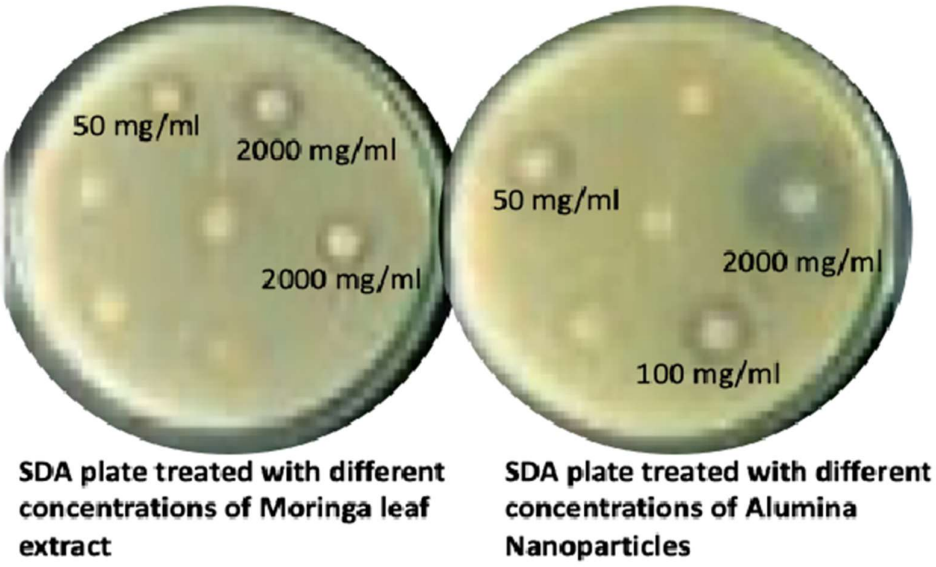


Figure 1: Zones of inhibition exhibited by Alumina nanoparticles and Moringa leaf extract against *Candida albicans*.

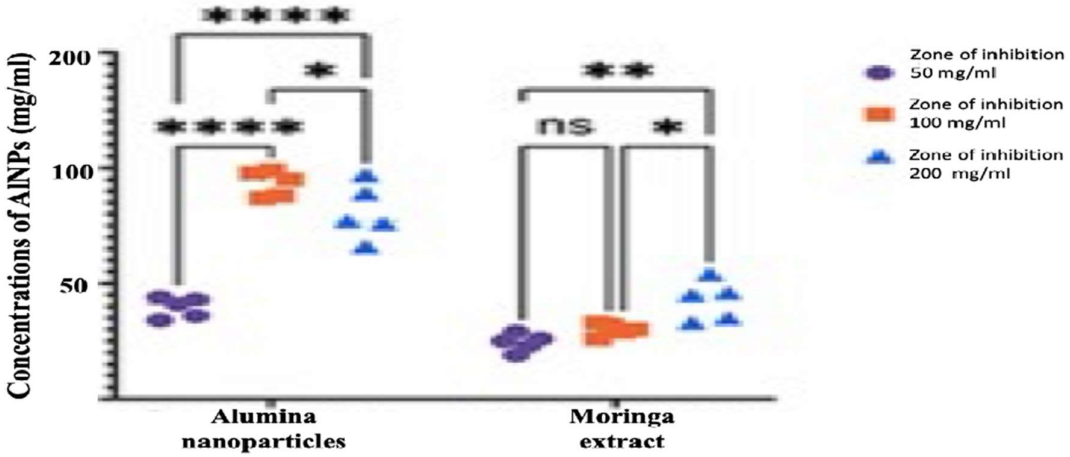


Figure 2. Showing antifungal activity of Moringa leaf extract and alumina nanoparticles

Table 1 and figure 2 Plates showing antifungal activity of Moringa leaf extract and alumina nanoparticles (AlNPs) at different concentrations (50 to 200 mg/ml) after 7 days of incubation at 28°C.

Discussion

In the recent days, research in medicine is targeting towards protecting communicable diseases that are caused by microorganisms. Unfortunately, proper management and treatment of the diseases remain the major challenges to both human and animals. In addition, the existing commercially produced antibiotics are not effective and can cause serious antibiotic resistance problems. Nanotechnology in the biomedical science sector aimed at enhancement of the antimicrobial products by providing innovative novel delivery platform towards successful management of fungal disease. In recent years, the green synthesis of NPs has recorded remarkable success in producing environment-friendly nanoparticles with huge range of application in biomedical field. The reduction of alumina ions A+ into AlNPs is as the result of presence of active biomolecules in the moringa leaf extract (Bukar et al., 2024; Oberdorster et al, 2005). Interestingly, the fast rate of reaction observed in the present study is as the result of constant and intensive heating of the medium, which provide uniform nucleation and synthesis of nanoparticle (Jalal et al., 2016). The findings of this study agreed with the findings of Sharma and Sharma (2018), who clearly reported on the synthesis of AlNPs using *Moringa olifera* as reducing and capping agent.

In addition, the findings of this study are in agreement with that of Jalal et al. (2016) who synthesized AlNPs after 30 s of microwave irradiation. It is therefore suggested that microwave-assisted green synthesis by using Moringa serves as both reducing and capping agent. The results of the present study clearly shows how different concentrations of synthesized alumina nanoparticles has inhibited the *Candida albicans* as compared to the leave extract of moringa which shows no significant activity against the fungi. The existing studies have favorably reported antifungal activities of the AlNPs that shows similar zones of inhibitions. Bukar et al. (2024) reported antifungal activity of AlNPs synthesized from Papaya against some medically important fungi, and their findings showed that plate treated with nanoparticles have wider zones of inhibition as compared to leaf extract alone.

Conclusion

Nanotechnology plays a dynamic role in introducing multiple approaches for suppressing disease, enhancing disease diagnostics, and developing new measures for management of oral candidiasis. Thus, microwave-assisted green synthesized moringa-AlNPs is an inexpensive, simple and less toxic approach to control *Candida albicans*. The synthesized alumina nanoparticles Thus, this study provides a basis that green synthesized NPs can be used as an alternative to conventional oral *Candida albicans*. Hence, they have the ability to overcome the current challenges of synthetic antifungal drugs.

Reference

1. *Allen D., Wilson D., Drew R., Perfect J. (2015). Azole antifungals: 35 years of invasive fungal infection management. Expert Rev. Anti Infect. Ther. 13 787–798. 10.1586/14787210.2015.1032939* [DOI] [PubMed] [Google Scholar]

2. *Álvares C. A., Svidzinski T. I. E., Consolaro M. E. L. (2007). Candidíase vulvovaginal: fatores predisponentes do hospedeiro e virulência das leveduras. J. Bras. Patol. Med. Lab. 43 319–327. 10.1590/S1676-24442007000500004* [DOI] [Google Scholar]

3. *Barchiesi F., Falconi Di Francesco L., Scalise G. (1997). In vitro activities of terbinafine in combination with fluconazole and itraconazole against isolates of Candida albicans with reduced susceptibility to azoles. Antimicrob. Agents Chemother. 41 1812–1814.* [DOI] [PMC free article] [PubMed] [Google Scholar]

4. Bukar, A.M., Abdullah Jesse, F.F., Che Abdullah, C.A. *et al.* In vitro cytotoxicity evaluation of green synthesized alumina nanoscales on different mammalian cell lines. *Sci Rep* **14**, 22826 (2024). https://doi.org/10.1038/s41598-024-53204-y

5. Bukar AM, Jesse FFA, Abdullah CAC, Noordin MM, Lawan Z, Mangga HK, Balakrishnan KN, Azmi MM. Immunomodulatory Strategies for Parapoxvirus: Current Status and Future Approaches for the Development of Vaccines against Orf Virus Infection. Vaccines (Basel). 2021 Nov 17;9(11):1341. doi: 10.3390/vaccines9111341. PMID: 34835272; PMCID: PMC8624149.

6. *Calderone R. A., Fonzi W. A. (2001). Virulence factors of Candida albicans. Trends Microbiol. 9 327–335. 10.1016/S0966-842X(01)02094-7*

7. *Canuto M., Rodero F. G. (2002). Antifungal drug resistance to azoles and polyenes. Lancet Infect. Dis. 2 550–563. 10.1016/S1473-3099(02)00371-7* [DOI] [PubMed] [Google Scholar]

8. Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Particle Fibre Toxicology. 2005; 2: 1–60.

9. Giammar DE, Maus CJ, Xie LY. Effects of particle size and crystalline phase on lead adsorption to titanium dioxide nanoparticles. Environmental Engineering Science. 2007; 24: 85–95.

10. Hristovski K, Baumgardner A, Westerhoff P. Selecting metal oxide nanomaterials for arsenic removal in fixed bed columns: from nanopowders to aggregated nanoparticle media. Journal of Hazardous Materials. 2007; 147: 265-274.

11. Hiemstra T, Venema P, Van Riemsdijk WH. Intrinsic proton affinity of reactive surface groups of metal (hydr) oxides: the bond valence principle. Journal of Colloid and Interface Science. 1996; 184: 680-692.

12. Sharma P., Sharma N., (2019), Optical Properties of Aluminium Oxide Nanoparticles Synthesized by Leaf Extract of Ocimum Sanctum, J. Nanosci. Tech., Vol. 5 (4), (pp 817 – 819).

13. Jalal, M., Ansari, M. A., Shukla, A. K., Ali, S. G., Khan, H. M., Pal, R., ... & Cameotra, S. S. (2016). Green synthesis and antifungal activity of Al2O3 NPs against uconazole-resistant Candida spp isolated from a tertiary care hospital. RSC advances, 6(109), 107577-107590

14. Ismail, A.; A., Kabary and Samy, A. (2021). Synthesized of α-Al2O3 Nanoparticles from Pepsi Cans Wastes and Its Fungicidal effect on Some Mycotoxins Producing Fungal Isolates. Journal of inorganic and organometallic polymers. Inpress

15. Wainwright M. Some highlights in the history of fungi in medicine – A personal journey. Fungal Biology Reviews. 2008; 22: 97– 102.