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Characterization and Antifungal Activity of Zinc Oxide Nanoparticles of Edible Mushroom against Selected Dermatophytic Fungi

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Abstract

Dermatophytic infections continue to contribute to the burden of superficial fungal infections, hence the need for alternative antifungal agents from natural sources. This study comparatively evaluated the efficacy of the crude extract of *Cremini kigali* mushrooms and its ZnO nanoparticles against selected dermatophytic fungi. Crude extracts were prepared by maceration, while ZnO nanoparticles were synthesized using the green synthesis technique and characterized. Antifungal activity against selected fungi isolates was assessed using agar well diffusion and broth microdilution methods. The proximate composition of the mushrooms revealed the presence of protein (28.00 %), moisture (26.85 %), carbohydrates (20.65 %), crude fiber (11.50 %), ash (9.00 %), and crude fat (4.00 %). The GC–MS spectral profile of the ethanol extract revealed the presence of diverse bioactive metabolites. Synthesis of ZnO nanoparticles was confirmed through UV spectroscopy with absorption band in the ultraviolet region at 350–380 nm. The FTIR spectrum revealed the presence of functional groups such as O-H, C-H, C=O, and C-N. TEM micrographs revealed the nanoparticles to be spherical in shape with a size, ranging from 15 to 45 nm. Zinc oxide Nanoparticles showed the highest antifungal activity against *Trichophyton tonsurans* at a concentration of 62.5 mg/ml, with zone of inhibition measuring 26.67 mm ± 1.53, compared to crude extract, which measured 7.33 mm ± 1.53 with $P<0.001$. Results obtained for the minimum inhibitory concentration and minimum fungicidal concentration revealed the antifungal efficacy of the crude extract on test fungi at concentrations of 31.25-6.25 and 31.25 mg/ml respectively for the ZnO nanoparticles, indicating the enhanced antifungal efficacy of the nano-synthesized extract compared with the crude extract.

Keywords: Antifungal resistance, ZnO nanoparticles, Dermatophytes, edible mushroom

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Introduction

Dermatophytic fungi are a type of keratinophilic pathogen that causes skin, hair, and nail infections, collectively known as dermatophytoses. These infections are still highly prevalent worldwide, especially in tropical and subtropical regions because of favorable environmental conditions such as heat and humidity, which favor the growth and transmission of these pathogens [1]. Despite the fact that antifungal compounds such as azoles and allylamines are employed in the management of these infections, an increase in recurrence and treatment failure has led to a concern over their potential effectiveness over a long period of time [2].

Dermatophytic infections are a public health concern in Nigeria, especially among children and young people. Studies carried out on infections in North Central Nigeria reported *Trichophyton rubrum* and *T. mentagrophytes* as major infecting pathogens among school children diagnosed with *Tinea capitis*, while variable antifungal susceptibility patterns were observed among these pathogens *in vitro* [3]. Other studies carried out on epidemiological investigations on school children in Calabar reported high rates of dermatophyte infections, indicating a high rate of transmission in Nigeria [4]. Other studies carried out on antifungal susceptibility tests on pathogens isolated from patients in Anambra State reported variable responses of dermatophytic pathogens to commonly used antifungal compounds such as terbinafine and fluconazole [5]. Despite these studies providing an overview of dermatophyte infections and antifungal responses in Nigeria, there is a lack of information on these infections and responses in Nasarawa State, indicating a need to investigate alternative antifungal compounds.

Edible mushrooms are a potential source of bioactive compounds such as phenolics, terpenoids, fatty acids, and polysaccharides with reported antifungal and antibacterial properties [6, 7]. Some species of mushrooms, such as *Pleurotus ostreatus*, have been reported to exhibit inhibitory activity against a range of

fungal pathogens in vitro. However, these crude extracts are limited by factors such as reduced solubility and bioavailability.

Nanotechnology has the potential for the enhancement of the biological properties of natural products. The use of the green synthesis of metal oxide nanoparticles using biological extracts offers an environmentally friendly approach for the enhancement of the antimicrobial properties of natural products [8]. Nano-formulations have the advantage of increasing surface area interaction, cell penetration, and potentially increasing the antifungal properties of the product compared to the conventional extracts [9]. Despite the accumulation of scientific evidence on the antimicrobial properties of nano-enhanced antimicrobial products, the comparative antifungal properties of crude and nano-formulated extracts of edible mushrooms against clinically relevant dermatophytes have not been adequately investigated, particularly in Nigeria.

The objective of this study was therefore to investigate the comparative antifungal properties of crude and nano-formulated extracts of *Cremini kigali* against clinically relevant dermatophytic fungi. By combining the bioactive properties of *Cremini kigali* with the technology of nanoparticles, this study sought to evaluate whether the antifungal properties of the product could be significantly enhanced using the nano-technology formulation

Materials and Methods

Collection and identification of mushroom samples

Edible mushroom was used for this study, namely *Cremini kigali*, purchased from Shoprite, Jabi, Abuja. Sample was identified at the Department of Plant Science and Biotechnology, Federal University of Lafia.

Sample identification number

Taxonomic confirmation was carried out at the Department of Plant Science and Biotechnology, Federal University of Lafia. Voucher specimen was assigned identification number FUL/SC/PSB/HBL/LAB 0056 (*C. kigali*)

Fungal isolates

Clinical isolates of *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Epidermophyton floccosum* were obtained from the Microbiology Unit of the Federal University Teaching Hospital, Nasarawa State.

Determination of proximate composition

Proximate composition of the edible mushroom sample was determined according to standard methods of the Association of Official Analytical Chemists [10].

Moisture content

Moisture content was determined by oven drying. Three grams of each pulverized sample were weighed into pre-weighed crucibles and dried at 105 °C for 24 h until constant weight was achieved. The crucibles were cooled in a desiccator and reweighed. Moisture content was calculated as:

$$\frac{W_2-W_3}{W_2-W_1}\times 100$$

Where: W_1= weight of crucible; W_2=weight of crucible + sample before drying; W_3= weight of crucible+ sample after drying

Crude protein

Crude protein content was determined using the Kjeldahl method. Three grams of dried sample were digested in a Kjeldahl flask containing 10 mL concentrated H_2SO_4 and 0.2 g catalyst mixture (Na_2SO_4, $CuSO_4$, and SeO_2 in ratio 98:1:1) until a clear solution was obtained. A blank was prepared similarly. The digest was distilled, and liberated ammonia was collected in boric acid solution containing mixed indicator and titrated against 0.05 M H_2SO_4. Nitrogen content was calculated as:

(% N) = V_S-V_B ×N_{acid}× 0.014/W×D×100

Where: V_S= Volume (ml) of acid used; V_B= Volume (ml) of acid used in the titration; N_{acid}= Normality of the acid; W=Weight of sample in gram; D=Dilution factor; % Crude protein= %N ×6.25 (Conversion factor)

$$Titre\ value = \frac{0.2\ x\ 1.4\ x\ 6.25}{weight\ of\ \ sample\ used}$$

Ash content

Five grams of moisture-free sample were weighed into crucibles and incinerated in a muffle furnace cat 550 °C for 6 hours until white or light grey ash was obtained. After cooling in a desiccator, samples were reweighed. Ash content was calculated as:

%Ash content= W_3-W_1/W_2-W_1×100

Where: W_1= Weight of empty crucible; W_2=Weight of crucible + weight of sample before ashing; W_3= Weight of crucible + weight of sample after ashing

Crude fibre

Four grams of moisture-free sample was sequentially digested with 1.25 % H_2SO_4 and 1.25 % NaOH under boiling conditions for 30 min each. The residue was washed, treated with ethanol and acetone, dried at 105 °C for 24 h, weighed, and subsequently ignited at 600 °C for 6 h. Crude fibre was calculated as:

% crude fibre= (W_1/W_2) ×100; W_1= Initial weight of sample; W_2=Weight of sample after ashing

Crude fat content

Crude fat content was determined by solvent extraction using diethyl ether. The solvent was evaporated using a rotary evaporator, and fat content was calculated gravimetrically as percentage of initial sample weight. The crude fat was calculated as:

Crude Fat (%) =Weight of ether extract/ weight of dried sample

Carbohydrate content

Carbohydrate content was determined by difference method, by taking a total of the all the other parameter, and subtracting it from 100. The carbohydrate content of the sample was calculated using the formula:

Total carbohydrate (%) =100- (% moisture + %protein + % ash + % crude fibre +% fat)

Gas chromatography–mass spectrometry (GC–MS) analysis of the mushroom sample

The GC–MS analysis of the mushroom sample was carried out according to the standard practice outlined in ASTM E1618, which employs EPA and ISO-based methods for identification of organic compounds. The GC–MS analysis was carried out using a Varian 3800/4000 GC–MS (Agilent Technologies, USA), equipped with an HP-5 capillary column (30 m × 0.25 mm ID). The carrier gas used was high-purity nitrogen at a constant flow rate of 1 mL/min. The GC–MS analysis was carried out with helium as the supporting gas.

The injector and detector were maintained at 300 °C during analysis. The temperature was initially set at 50 °C for 2 min and then raised to 100 °C at 10 °C/min and held for 2 min. The temperature was then raised to 250 °C at 5 °C/min and then to 300 °C at 3 °C/min and held for 15 min. A 1 µL aliquot each of all the extracts was injected in splitless mode.

The MS was scanned in scan mode in the range 30–400 amu. The ion source and quadrupole were maintained at 150 and 230 °C respectively.

The identification of compounds was carried out by comparing with library spectra in NIST and Wiley databases with a similarity value of 90 % or higher.

Preparation of fungal inocula

For fungi inocula preparation, 48-hour-old cultures of dermatophytes grown on Sabouraud Dextrose Agar medium were suspended in peptone water, and the turbidity of the suspension was adjusted to a 0.5 McFarland standard for uniform inoculum density. The inoculum was then used for agar well diffusion and broth minimum inhibitory concentration testing, following the procedures for antimicrobial susceptibility testing [11].

Green synthesis of ZnO nanoparticles

Zinc oxide nanoparticles were synthesized by a green synthesis technique, where mushroom extract was used as a reducing agent and stabilizing agent. Zinc nitrate hexahydrate was used as a precursor, and sodium hydroxide was used to maintain pH levels. Approximately 30 mL of mushroom extract was heated to 60 °C, and then zinc nitrate hexahydrate solution (0.2 M) was added slowly to the solution while continuously stirring (Fig. 1). The solution was maintained at 60 °C until a paste is formed, and then it was calcined at 400 °C for 2 h to produce ZnO nanoparticles. The nanoparticles were stored in sterile containers for further analysis [8].

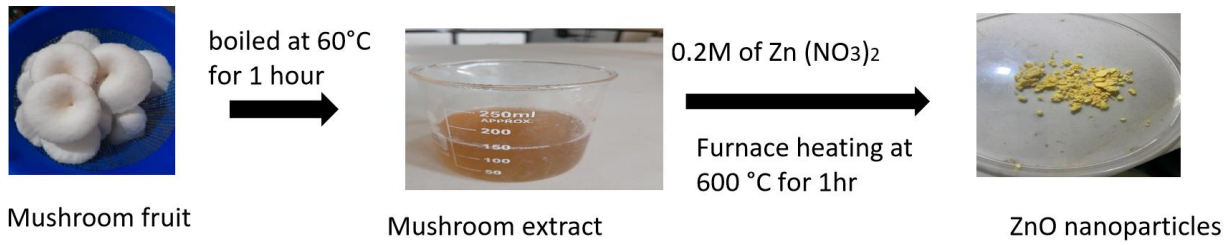

Figure 1: Schematic diagram of the green synthesis of ZnO nanoparticles using mushroom

Characterization of the green-synthesised ZnO nanoparticles

The ZnO nanoparticles synthesized using the green synthesis technique were characterized using UV-Visible spectroscopy, Fourier Transform InfraRed spectroscopy, and Transmission Electron Microscopy techniques. The UV-Visible spectroscopy was carried out using a UV-Visible spectrophotometer (e.g., Shimadzu UV-1800, Shimadzu Corporation, Japan) with the range of 200-800 nm using distilled water as the blank solution. The characteristic absorption peak of the ZnO nanoparticles was determined using the UV-Visible spectroscopy technique. The surface functional groups of the ZnO nanoparticles which interact with the phytochemicals for reduction stability were characterized using an FTIR spectrometer (e.g., Perkin Elmer Spectrum Two, Perkin Elmer Inc., USA). The range of the instrument was set from 4000-400 cm^{-1} using the KBr pellet or ATR technique. The morphology of the ZnO nanoparticles was characterized using Transmission Electron Microscopy (TEM) (e.g., JEOL JEM-2100, JEOL Ltd., Japan). The accelerating voltage was set at 200 kV. A drop of the ZnO nanoparticle solution was placed on the copper grid coated with carbons while solution was then allowed to dry.

Preparation of crude ethanolic extract

Dried mushroom samples were pulverized into fine powder using a clean mortar and pestle to increase surface area for efficient extraction. The powdered mushroom samples were stored in airtight containers before use and afterwards subjected to the maceration method for extraction. The powdered mushroom sample was soaked separately in absolute ethanol at a solvent-sample ratio of 1:10 w/v to ensure immersion of the powdered mushroom sample. Absolute ethanol was

used for extraction because it can extract a wide range of moderately polar bioactive compounds with antimicrobial properties. The mixture was maintained at room temperature (25 to 28 °C) for 72 with intermittent agitation to enhance solvent penetration and phytochemical diffusion. After 72 h, the mixture was filtered using Whatman filter paper to obtain clear filtrate. The filtrate obtained was concentrated using a water bath at 40 °C to enable solvent evaporation until a semi-solid crude extract was obtained. The obtained extract was stored in a sterile airtight container at 4 °C until further use [11].

Preparation of extract concentrations

Crude extract solution was prepared by adding 1 g of dried crude extract to 2 mL of sterile distilled water to obtain a concentration of 500 mg/mL. The solution obtained was thoroughly mixed before use. Two-fold serial dilutions were made from the crude extract solution to obtain 250, 125, 62.5, 31.25, 15.625, and 7.8125 mg/mL extract concentrations. The obtained extract concentrations were used for antifungal susceptibility tests with sterile distilled water used as a negative control [11].

Antifungal activity assay

Antifungal activity of crude and zinc nanoparticles extracts was evaluated using the agar well diffusion method. Sterile molten SDA was poured into Petri dishes and allowed to solidify. Plates were inoculated by evenly swabbing fungal suspensions. Wells of 6 mm diameter were created in the medium using cork bearer. Each well was filled with 0.1 mL of plant extract at concentrations of 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 mg/ml. Fluconazole was used as a positive control at a concentration of 500 mg/ml, while ethanol was used as a negative control. Plates were left for 40 min before incubation to allow diffusion and then incubated for 48–72 h at 25 °C. The diameter of the zone of inhibition was measured in millimeters using a ruler. All tests were done in triplicate [12].

Microbroth dilution assay for MIC determination

The minimum inhibitory concentration of both crude and nano-enhanced plant extracts was determined using the microbroth dilution method in a 96-well microtitre plate. Peptone broth was aseptically added to each well in a microtitre plate. Diluted plant extract and zinc oxide nanoparticles were then added to each well in peptone broth. Three microlitres (3 µl) of standardized fungal spore suspension was added to each well. Plates were mixed gently to mix all contents. Fluconazole was used as a positive control, while sterility controls were broth alone. Plates were then incubated for 48 h in a thermostat incubator at 28°C. Minimum inhibitory concentration was determined as the lowest concentration of *Cremini kigali* extract that completely inhibited fungal growth with changes in colour using resazurin indicator [6].

Determination of minimum fungicidal concentration (MFC)

MFC was determined by subculturing aliquots of the contents of wells that showed no signs of growth onto SDA agar plates, which were incubated at 35 °C for 24–48 h. The least concentration of the extract that showed no fungal growth was recorded as the MFC [8].

Statistical analysis

Data collected from the antifungal susceptibility assays were encoded and analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 27.0; IBM Corp., Armonk, NY, USA). Zones of inhibition were recorded in millimeters and expressed as mean values ± SD. Before proceeding to inferential statistics, the assumptions underlying the parametric test were checked. Normality of distribution of each type of extract-concentration combination was tested using the Shapiro-Wilk test, whereas homogeneity of variance was tested by Levene's test. Only those datasets that met the assumptions of parametric statistics were subjected to inferential statistical analysis. A two-way factorial analysis of variance (ANOVA) was carried out to determine the effects of the type of extract (nano vs. crude) and the concentration of the extract (15.63, 31.25, 62.5, 125, 250, 500 mg/ml, including the control where applicable) on the antifungal activity of the respective extract, as well as the interaction of the two factors. If significant differences were found, post hoc comparisons were carried out by Tukey's Honestly Significant Difference (HSD) test to determine the differences among the groups. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Results and Discussion

UV-vis analysis

The UV–Vis absorption spectrum of the green synthesized ZnO nanoparticles exhibited a strong and well-defined absorption band in the ultraviolet region, typically centered around 350–380 nm, which corresponds to the intrinsic band-gap absorption of ZnO arising from electron transitions from the valence band to the conduction band (Fig. 2). The sharp absorption edge indicated the formation of nanosized ZnO with good crystallinity, while any slight blue shift relative to bulk ZnO ($\lambda \approx 368$ nm; $Eg \approx 3.37$ eV) can be attributed to quantum confinement effects associated with reduced particle size [13]. The absence of significant absorption in the visible region suggests high phase purity and minimal defect-induced mid-gap states, although minor tailing may arise from surface defects or phytochemical residues from the plant extract used in the green synthesis. The spectrum therefore confirms successful nanoparticle formation and supports the nanoscale dimensions observed in TEM analysis, while also implying potential suitability for photocatalytic and antimicrobial applications due to efficient UV light absorption.

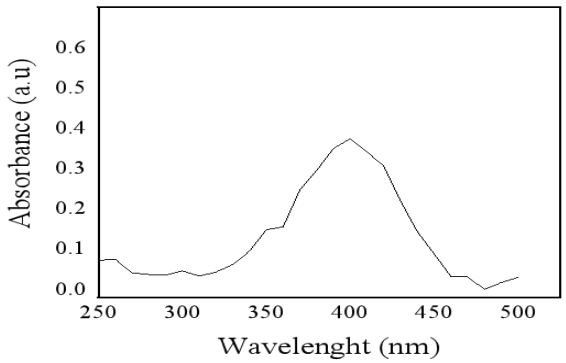

Figure 2: UV-Vis plot of the green synthesized ZnO nanoparticles

The UV–Vis absorption spectrum of the ZnO nanoparticles showed a strong absorption band in the ultraviolet region between 350 and 380 nm, which is characteristic of the intrinsic band gap absorption of ZnO, related to the transition between the valence and conduction bands in ZnO nanoparticles. The presence of this absorption band in the UV region confirmed the formation of ZnO nanoparticles, and the strong absorption band is related to the optical properties of ZnO nanoparticles [14].

FTIR analysis

The FTIR spectrum of the green synthesized Zinc oxide nanoparticles confirmed the successful formation of ZnO and the presence of phytochemical residues acting as stabilizing agents. The broad absorption band centered around ~3330 cm^{-1} is attributed to O–H stretching vibrations of hydroxyl groups from adsorbed water molecules and plant-derived phenolic compounds, indicating surface-bound biomolecules (Fig. 3). The peaks observed near 2924 and 2853 cm^{-1} corresponded to asymmetric and symmetric C–H stretching of aliphatic groups, suggesting the presence of organic capping agents. A distinct band around 1638 cm^{-1} is assignable to C=O stretching or amide I vibrations, while the peak at ~1742 cm^{-1} may have indicated carbonyl functionalities from esters or carboxylic acids present in the extract. The bands at ~1377 and 1161 cm^{-1} were associated with C–N stretching and C–O or C–O–C vibrations, further supporting the involvement of biomolecules in reduction and stabilization processes [15]. Additionally, the strong absorption in the low-wavenumber region between ~620 and 450 cm^{-1} corresponded to Zn–O stretching vibrations, which is the characteristic fingerprint region confirming the formation of crystalline ZnO nanoparticles. Overall, the spectrum demonstrated that phytochemicals from the plant extract not only facilitated the reduction of zinc precursors but also remained partially adsorbed on the nanoparticle surface, providing stabilization and preventing excessive agglomeration.

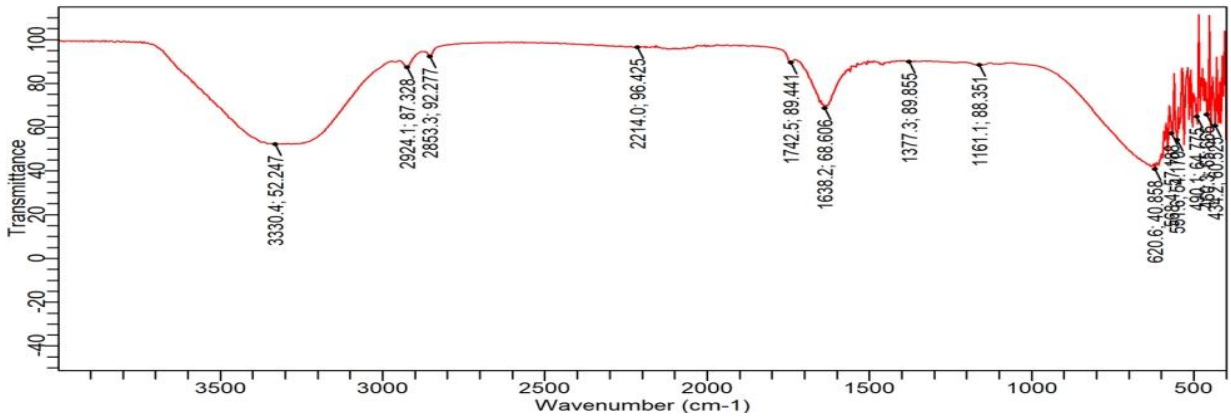

Figure 3: FTIR spectrum of the green synthesized ZnO nanoparticles

FTIR analysis confirmed the formation and stabilization of the ZnO nanoparticles. The absorption band observed in the range around ~3330 cm^{-1} was related to the stretching vibration of the O–H bond in hydroxyl groups, phenolic compounds, or water molecules. The absorption bands observed in the range around 2924 and 2853 cm^{-1} were related to the stretching vibration of the C–H bond in aliphatic groups, while the absorption band observed in the range around 1638 cm^{-1} was related to the stretching vibration of the C=O bond in amide groups or proteins and biomolecules in the plant extract. The absorption bands observed in the range around 1377 and 1161 cm^{-1} were related to the stretching vibration of the C–N and C–O bonds, respectively, indicating the presence of organic compounds in the plant extract, acting as a reducing agent in the formation of ZnO nanoparticles. The absorption band observed in the range around 450–620 cm^{-1} was related to the Zn–O stretching vibration, indicating the formation of ZnO nanoparticles [14].

TEM analysis

TEM analysis revealed that the green synthesized ZnO nanoparticles were predominantly spherical (Fig. 4) with particle sizes ranging from approximately 15 to 45 nm, average particle size of 35 nm. The particles exhibited moderate agglomeration, which may be attributed to high surface energy and interparticle van

der Waals interactions despite phytochemical stabilization during synthesis. The relatively uniform contrast suggests consistent composition and crystallinity. The nanoscale dimensions confirmed successful synthesis within the nanoregime and imply enhanced surface area, which is advantageous for photocatalytic and antimicrobial applications [16].
Similar morphological characteristics have been reported for biologically synthesized ZnO nanoparticles using plant-derived metabolites, where phytochemicals act as both reducing and capping agents to control particle size and morphology [14, 17].

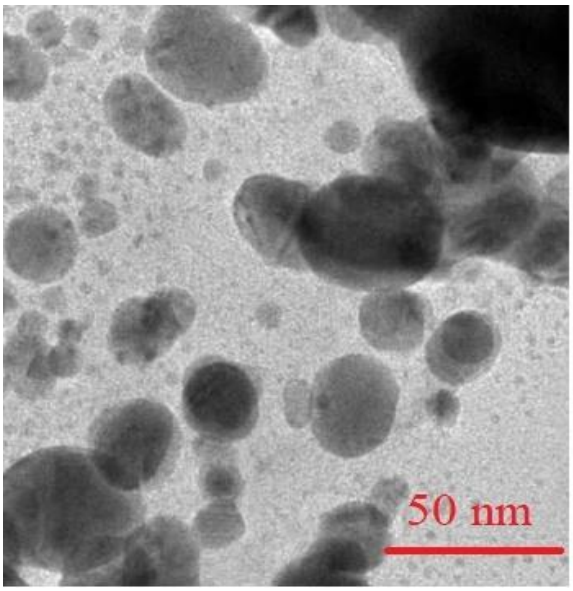

Figure 4: TEM image of the synthesized ZnO nanoparticles

Table 1: Proximate composition of *Cremini kigali* mushrooms

Parameter	*Cremini Kigali* (%)
Moisture	26.85
Ash	9.00
Crude fibre	11.50
Crude fat	4.00
Protein	28.00
Carbohydrate	20.65

Proximate composition of mushroom samples
The proximate composition of *Cremini kigali* (Table 1) showed a nutritionally important composition, characterized by high protein content (28.00 %), moderate levels of carbohydrates (20.65 %), appreciable amounts of crude fibre (11.50 %), ash content (9.00 %), lower crude fat content (4.00 %) and moisture content (26.85 %). This study has supported the already established classification of edible mushrooms as nutrient-rich and important functional foods, rich in proteins, fibres, and minerals, and bioactive substances [18].
The high protein content of the *Cremini kigali*, established in this study has been supported by other studies on cultivated mushrooms, which have shown their potential to be important alternative sources of dietary protein in the diet of humans [19]. The moisture content of 26.85 % established in this study was lower than the moisture content of 80-90 % established for fresh edible mushrooms. This could be attributed to the edible mushroom being subjected to the process of drying, which has been reported to have the potential to reduce water activity and increase the stability of edible mushroom sample, thus minimizing microbial and enzymatic degradation and increasing the nutrient content of the edible mushroom sample [18]. Edible mushrooms have been reported to be important and rich in essential minerals, including potassium, phosphorus, magnesium, and iron, which have been shown to make important contributions to metabolic and physiological functions in the body [18]. The level of crude fibre (11.50 %) is attributed to structural polysaccharides, chitin, and beta-glucans, which are well documented for their immunomodulatory, antioxidant, and antimicrobial activities, contributing to the functional properties of edible mushrooms [19]. The level of crude fat in this study (4.00 %) is relatively low, and this is in agreement with the general characterization of mushrooms, which are generally recognized as low-fat foods. Although the total fat content is generally low in mushrooms, there are nutritionally significant levels of unsaturated fatty acids, such as linoleic acid, that contribute to the functional properties of mushrooms [18].The level of carbohydrates (20.65 %) could be attributed to storage and structural polysaccharides, beta-glucans, and other non-digestive carbohydrates, which are well documented for their immunomodulatory, antioxidant, and antimicrobial activities, contributing to the functional properties of mushroom extracts [19].

Gas Chromatography mass spectrometry (GC–MS) profiling of the ethanol extracts of *Cremini kigali*
GC–MS chromatographic profiling of the ethanolic extracts of and *Cremini kigali* revealed complex but chemically related metabolite patterns, as illustrated in Fig. 4. The retention times across all species ranged from 2 to 45 min, indicating the presence of both low and high molecular weight volatile constituents.
The gas chromatography–mass spectrometry profiling of the ethanolic extract of *Cremini kigali* showed the presence of chemically diverse volatile and semi-volatile compounds (Fig. 5). The wide range of the distribution of the chromatographic peaks over the retention time indicated the presence of both low and high molecular weight compounds. This diversity of the chemical nature of the compounds is generally associated with the biological/therapeutic potentials of the macrofungal. Long-chain hydrocarbons identified in the present GC-MS analysis of the edible mushrooms have been found to exhibit antimicrobial as well as antifungal properties. These hydrocarbons may have interacted with the lipid membranes of the microbes, which may alter the integrity of the membranes, thus inhibiting the growth of fungi [20].

In addition to the long-chain hydrocarbons, some of the other hydrocarbons identified in the GC-MS analysis of the edible mushroom *Cremini kigali* were found to be in the form of branched hydrocarbons, including 2,2,4,9,11,11-hexamethyldodecane and 2,6,10,14-tetramethylpentadecane derivatives. Branched hydrocarbons have been identified as products of the secondary metabolism of fungi, which may be involved in the ecological defense mechanisms of the fungi to protect them from the effects of other microorganisms in the natural habitat of the mushrooms [21]. The identification of the presence of hydrocarbons such as heptadecane, octadecane, and tricosane in the GC-MS analysis of the edible mushroom *Cremini kigali* is similar with the GC-MS studies of *Agaricus bisporus*, as well as other edible mushrooms, that were conducted in the past [22, 23].

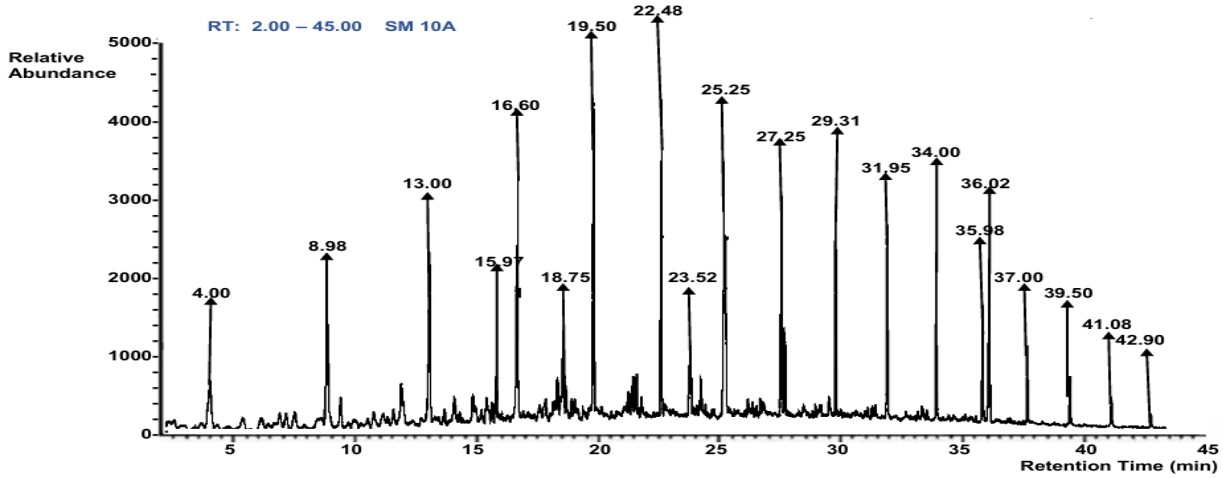

Figure 5: GC-MS spectrum representing potential bands in *Cremini kigali*

Table 2: Effect of extract type and concentration on the zone of inhibition for *Cremini mushroom kigali*

Concentration (Mg/ml)	n	Nano (Mean ± SD)	Crude (Mean ± SD)	F	*P*
Trichophyton mentagrophytes					
Control	3	27.67 ± 10.50	16.50 ± 7.78	10.24*	**<0.005**
15.63	3	9.00 ± 2.65	-	8.50**	**<0.001**
31.25	3	8.00 ± 2.00	-	0.74***	0.575
62.5	3	13.00 ± 3.00*	7.67 ± 2.08*		
125	3	16.00 ± 3.61	13.67 ± 2.52		
250	3	19.00 ± 2.00	16.00 ± 3.00		
500	3	27.00 ± 2.00	21.33 ± 3.21		
T. rubrum					
Control	3	31.67 ± 4.51	36.00 ± 1.73	0.03*	0.856
15.63	3	8.00 ± 2.00	-	16.86**	**<0.001**
31.25	3	9.67 ± 3.79	-	0.70***	0.600
62.5	3	13.00 ± 2.00[a]	15.67 ± 3.06[a]		
125	3	13.33 ± 3.51[a]	13.67 ± 4.73[a]		
250	3	19.00 ± 6.25[a]	17.67 ± 5.69[a]		
500	3	25.33 ± 5.51[a]	21.00 ± 8.54[a]		
T. tonsurans					
Control	3	24.00 ± 6.56	31.67 ± 5.51	40.80*	**<0.001**
15.63	3	14.33 ± 4.16	-	5.69**	**0.003**
31.25	3	16.33 ± 1.15	-	7.41***	**<0.001**
62.5	3	24.67 ± 1.53[a]	7.33 ± 1.53[a]		
125	3	26.00 ± 4.00	13.00 ± 2.00		
250	3	34.00 ± 6.00	16.67 ± 1.15		
500	3	34.00 ± 6.00	15.50 ± 0.71		
Epidermophyton floccosum					
Control	3	31.67 ± 6.11	19.00 ± 9.00	12.39*	**0.002**
15.63	3	9.67 ± 2.08[a]	-	7.20**	**<0.001**
31.25	3	10.67 ± 1.53[a]	-	1.26***	0.320
62.5	3	15.67 ± 3.06[a]	8.67 ± 2.52		
125	3	16.33 ± 3.06[a]	800 ± 2.00		
250	3	18.67 ± 3.79	16.00 ± 5.57		
500	3	19.33 ± 6.81	18.00 ± 3.00		

Means with superscripts are significantly different from the control at P <0.05.
P-values in boldface are statistically significant, lightface, not statistically significant; *F_1: indicates F and corresponding *P*-value for extract type (crude vs. nano extracts); **F_2: indicates F and corresponding *P*-value for concentrations
***F_3: indicates F and corresponding *P*-value for interaction between extract type vs concentrations
NB: all F and corresponding *P*-values were computed only for concentrations and crude extracts with zone of inhibition

Antifungal activity of the crude extracts and nano extracts of *Cremini kigali* against the test fungi

Table 2 presents the effect of extract type and concentration on the zone of inhibition of *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans* and *Epidermophyton floccosum*. Statistically, with P value set at $P<0.005$, Zinc oxide nanoparticles showed the highest antifungal activity against *Trichophyton tonsurans* at a concentration of 62.5 mg/ml, with zone of inhibition measuring 26.67 mm ± 1.53, compared to crude extract, which measured 7.33 mm ± 1.53. Conversely, the least antifungal activityof ZnO nanoparticles, 9.67 mm ± 2.08 was recorded for *Epidermophyton floccosum* with the crude extract showing no activity at a concentration of 15.63 mg/ml.

The antifungal activity of the zinc oxide nanoparticles was relatively higher in terms of zone size when compared to the crude extract. Such result can only indicate that ZnO nanoparticles have improved antifungal activity against pathogenic fungi when compared to the crude extract of the mushroom. This has been reported previouslyfor ZnO nanoparticles biosynthesized using plant metabolites [24].

A reason for the high activity observed by the zinc oxide nanoparticles in this study may be production of reactive oxygen species, including hydroxyl radicals, superoxide anions, and hydrogen peroxide, which induce oxidative stress in the target cell [25]. This oxidative stress disrupts cellular macromolecules such as proteins, lipids, and nucleic acids, ultimately inhibiting fungal growth and leading to cell death [25]. ZnO nanoparticles can also directly interact with the cell membrane of target fungi, leading to increased permeability and cell membrane destabilization. This disrupts intracellular components and metabolic processes essential for fungal growth and survival [25].

The antifungal activity of the crude mushroom extract can also be attributed to naturally occurring bioactive compounds found in *Cremini kigali*. Edible mushrooms such as *Cremini kigali* are known to possess a wide range of bioactive compounds such as phenolic compounds, polysaccharides, sterols, and fatty acids, which possess antifungal and antioxidant properties. During green synthesis, bioactive compounds found in mushroom extract can act as reducing and stabilizing agents, which can enhance antifungal activity and nanoparticle synthesis. The nanosize of ZnO nanoparticles can also enhance antifungal activity by increasing surface area, thus enabling better penetration into target cells and enhancing antifungal activity [24].

Minimum Inhibitory Concentration of the crude extract and zinc oxide nanoparticles of *Cremini kigali* against the test fungi

The minimum inhibitory concentration (MIC) for crude extract was at 62.5 mg/ml (Table 3), while that for the zinc oxide nanoparticles was at 15.63 mg/ml (Table 4).

Tables 3: Minimum inhibitory concentration of the crude extract of *Cremini Kigali* against test fungi

Concentration (mg/ml)	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*	*E. floccosum*
500	-	-	-	-
250	-	-	-	-
125	-	-	-	-
62.5	-	-	-	-
31.25	-	+	+	+
15.63	+	+	+	+
7.83	+	+	+	+
Control	-	-	-	-

Control: fluconazole 500 mg (≥ 19 mm susceptible ≤14 mm resistances) CLSI; += Growth; - = No Growth

Tables 4: Minimum inhibitory concentration of zinc oxide nanoparticles of *Cremini kigali* against test fungi

Concentration (mg/ml)	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*	*E. floccosum*
500	-	-	-	-
250	-	-	-	-
125	-	-	-	-
62.5	-	-	-	-
31.25	-	-	-	-
15.63	-	-	-	-
7.83	+	+	+	+
Control	-	-	-	-

Control: fluconazole 500 mg (≥ 19 mm susceptible ≤14 mm resistances); += Growth (no Inhibition, organisms utilize dye, alter pH and color change)- = no Growth (Inhibition, no color change)

The crude extract inhibited the growth of *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Epidermophyton floccosum* at a concentration of 31.25 mg/mL, which represents the MIC for the crude mushroom extract (Table 3). At lower concentrations (15.63 and 7.83 mg/mL), fungal growth was observed, indicating that these concentrations were insufficient to suppress dermatophyte proliferation. These findings suggest that although the crude extract possesses antifungal properties, relatively higher concentrations are required to achieve fungi inhibition.

In contrast, the ZnO nanoparticle-enhanced mushroom extract exhibited a lower MIC of 15.63 mg/mL against all tested dermatophytic fungi (Table 4). This reduction in MIC indicates that the incorporation of ZnO nanoparticles may have significantly improved the antifungal potency of the mushroom extract. The enhanced activity observed in the nano-formulation may also be attributed to the increased surface area and improved dispersion of bioactive compounds when integrated with nanoparticles, which enhances interaction with fungal cells. Zinc oxide nanoparticles have also been reported to possess intrinsic antifungal activity which can enhance the antimicrobial performance of natural extracts by increasing their bioavailability and facilitating penetration into microbial cell walls [27].

One of the major mechanisms underlying the antifungal activity of ZnO nanoparticles is the generation of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide. These reactive molecules induce oxidative stress, which damages

cellular components such as lipids, proteins, and nucleic acids within fungal cells [28, 29]. ROS-mediated oxidative damage disrupts cellular metabolism, leading to inhibition of fungal growth and eventual cell death. Additionally, ZnO nanoparticles may release zinc ions (Zn^{2+}), which further interfere with enzymatic systems essential for fungal survival as observed in this present study.

The MIC values obtained in the present study are consistent with previous research demonstrating enhanced antifungal activity when antimicrobial agents are combined with ZnO nanoparticles. For example, [30] reported that ZnO-based nanoparticle formulations significantly improved the inhibitory activity of antifungal compounds against dermatophytic fungi such as *Trichophyton mentagrophytes*. Similar studies have shown that nanoparticle formulations reduce the concentration required to inhibit fungal growth due to improved delivery and stronger interaction with microbial cells.

Minimum fungicidal concentration of the crude and zinc oxide nanoparticles of *Cremini kigali* against test fungi

The Minimum fungicidal concentration (MFC) results for the crude extract showed that at 250 mg/ml, fungi death was recorded (Table 5) while at a concentration of 31.25 mg/ml, no fungi growth was recorded for zinc oxide nanoparticles (Table 6)

Tables 5: Minimum fungicidal concentration of the crude extract of *Cremini kigali* against the test fungi

Concentration (mg/ml)	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*	*E. floccosum*
500	-	-	-	-
250	-	-	-	-
125	-	+	-	-
62.5	-	+	+	+
31.25	+	+	+	+
15.63	+	+	+	+
7.83	+	+	+	+
Control	+	+	+	+

Control: fluconazole 500 mg/ml (≥19 mm susceptible ≤14 mm resistance) CLSI, += Growth (no cidal effect); - = no Growth (cidal effect)

Tables 6: Minimum fungicidal concentration of the nano synthesized of *Cremini kigali* (C)

Concentration (mg/ml)	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*	*E. floccosum*
500	-	-	-	-
250	-	-	-	-
125	-	-	-	-
62.5	-	-	-	-
31.25	-	-	-	-
15.63	+	+	+	+
7.83	+	+	+	+
Control	+	+	+	+

+= Growth, - = No growth

The minimum fungicidal concentration (MFC) assay evaluates the lowest concentration of an antimicrobial agent required to kill fungal cells rather than merely inhibit their growth. In the present study, both *Cremini kigali* extract and ZnO nanoparticle-enhanced mushroom extract demonstrated fungicidal activity against selected dermatophytic fungi including *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Epidermophyton floccosum*. However, the nanoparticle-enhanced extract exhibited stronger fungicidal activity compared with the crude mushroom extract.

From the results presented in Table 6, the ZnO nanoparticle-enhanced mushroom extract showed fungicidal activity at concentrations as low as 31.25 mg/mL, whereas fungal growth was observed at lower concentrations of 15.63 mg/mL and below. In contrast, the crude mushroom extract required higher concentrations to exert comparable antifungal effects, indicating lower fungicidal potency. This pattern suggests that nanoparticle incorporation significantly enhanced the antifungal efficiency of the mushroom extract.

The observed antifungal activity of the crude *Cremini kigali* extract may be attributed to the presence of bioactive compounds such as phenolics, flavonoids, terpenoids, and polysaccharides that are known to exhibit antimicrobial properties. These compounds can interfere with fungal metabolism, disrupt cell membrane integrity, and inhibit spore germination. Previous studies have reported that phenolic extracts from *Cremini kigali* possess antifungal activity against pathogenic fungi, supporting the antifungal potential of crude mushroom extracts [31]. The antifungal activity of mushroom extracts is largely linked to their rich content of secondary metabolites capable of inhibiting microbial growth and interfering with cellular processes in fungal pathogens [32].

Several mechanisms may explain the enhanced antifungal activity of nanoparticle-based formulations. Zinc oxide nanoparticles possess intrinsic antimicrobial properties and can damage fungal cells through multiple pathways. One key mechanism involves the generation of reactive oxygen species (ROS), which induce oxidative stress and lead to damage of essential biomolecules such as proteins, lipids, and DNA within fungal cells [32].

In addition, ZnO nanoparticles can also directly interact with the fungal cell membranes, thus causing an increase in the permeability of the membranes and the leakage of the cellular components, which also contributes to the fungicidal activity [27].

Conclusion

This study demonstrated that zinc oxide nanoparticles of *Cremini kigali* exhibited significantly greater antifungal activity against *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum* compared to their crude extracts. The nano-formulated extracts consistently produced larger zones of inhibition and lower MIC and MFC values, indicating improved antifungal potency. These findings confirm that nano-formulation enhances the bioactivity of edible mushroom extracts and suggests that delivery strategy plays a critical role in antifungal effectiveness.

Conflict of interest: The authors whose names are mentioned hereby declare that they have no conflict of interest in this research article and that in case any of such comes up, it will be resolved hitch-free. The authors also declare that this research is solely sponsored by Tertiary Education Trust Fund (TETFund).

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