Lafia Journal of Scientific & Industrial Research (LJSIR), Vol. 4(2), 2026
p-ISSN: 3026 – 9288 e-ISSN: 3027 – 1800 pages: 1-8
https://lafiascijournals.org.ng/index.php/ljsir/index ⇒ Published by the Faculty of Science,
Federal University of Lafia, Nasarawa State, Nigeria

Crude and Nano-Enhanced Antifungal Properties of *Acalypha wilkesiana* Leaf Extract against *Trichophyton mentagrophytes*

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Abstract

Trichophyton mentagrophytes is a dermatophyte fungus responsible for infections of keratinized tissues such as the skin, hair, and nails. This disease can be caused by poor hygiene, a warm environment, and an individual's weakened immune system. In this study, we examined the antifungal properties of the crude and nano-enhanced green synthesis of *Acalypha wilkesiana* against *T. mentagrophytes*. Leaves of *A. wilkesiana* were extracted with ethanol by maceration. Phytochemical analysis was conducted using conventional methods, and zinc oxide nanoparticles were prepared using green synthesis technique. Antifungal potential of *A. wilkesiana* was evaluated by agar well diffusion test and microdilution method. Data were analyzed using two-way ANOVA, with a significant value of $P \leq 0.001$. The presence of alkaloids (5.82 ± 0.12 mg/g), flavonoids (8.35 ± 0.15 mg/g), and phenols (7.96 ± 0.21 mg/g) was noted during phytochemical screening. The gas chromatography-mass spectrometry (GC–MS) analysis confirmed ten compounds in the extract. Among the compounds, palmitic acid had the highest percentage peak (18.2 %) with a molecular weight of 256 and retention time of 5.43 minutes. Nano-enhanced formulation demonstrated greater zones of inhibition than the crude formulation. Particularly, nano-formulated *A. wilkesiana* demonstrated significant improvement in antifungal properties against *T. mentagrophytes* ($F = 1{,}261.50$; $P \leq 0.001$), with a zone of inhibition of 32.00 ± 1.00 mm compared to 23.00 ± 1.00 mm for the crude extract. Minimal Inhibitory Concentration (MIC) values showed a dose-response effect on the fungi.

Keywords: Antifungal resistance, *Acalypha wilkesiana*, Dermatophytes, ZnO nanoparticles

Article History

Submitted
February 04, 2026

Revised
May 13, 2026

First Published Online
May 15, 2026

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doi.org/10.62050/ljsir2026.v4n2.813

Introduction

Fungal infections are major health problems affecting millions of people globally, especially those caused by dermatophytes such as *Trichophyton mentagrophytes*, which have the ability to infect keratinized tissues such as hair, nail, and skin [1]. Although, it is a superficial infection, it has the potential to cause discomfort, stigmatization, and economic losses, especially in cases where the infection is not adequately controlled by the available drugs due to the emergence of resistance [2]. *Trichophyton mentagrophytes* infection has shown resistance to antifungal drugs such as terbinafine, thereby affecting the control of dermatophytosis [3]. The rising cases of antifungal resistance have made it challenging to control fungal infections effectively [4]. The most commonly used drugs in the control of fungal infections are azoles and allylamines. However, these drugs have shown inefficacy due to drug resistance, toxicity, and high cost of drugs [5]. According to a research carried out in Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa state, dermatophytes are the major cause of skin diseases [6].

Acalypha wilkesiana has traditionally been used in different places for its medicinal benefits. In Trinidad, a poultice of leaves is applied to treat headache, cold, and swelling [7]. Various compounds have been identified by research on *A. wilkesiana*. The leaves contain saponins, tannins, anthraquinones, and glycosides [8]. Various studies have been carried out on the antimicrobial activity of *A. wilkesiana* [9]. Various crude and ethanol extracts of different plants, including *A. wilkesiana*, were tested for activity against methicillin-resistant Staphylococcus aureus. Moderate bactericidal and inhibitory activities were found. Onocha [10] confirmed the antihelmintic activity of *A. wilkesiana*. The antimicrobial activity of the plant has also been confirmed by [11]. The plant extracts were found to have activity against gastrointestinal and skin infections, mainly in neonates. Aboaba and Omotoso [12] found that the secondary metabolite of the plant has antioxidant activity. The compound has significant antioxidant activity. However, there are some drawbacks of the use of crude extracts, such as poor solubility, instability, and poor bioavailability, which may confine the use of the plant extracts.

Nanotechnology has shown promise in improving the efficacy of natural products. The green synthesis of metal oxide nanoparticles by the use of plant extracts has shown promise in improving the antimicrobial activity of plant extracts. The use of nanoparticles has shown promise in improving the antifungal activity of plant extracts. The use of nanoparticles has shown promise in improving the activity of plant extracts compared to the crude extracts. Even though there is sufficient evidence for the use of nanoparticles in improving the antimicrobial activity of plant extracts, there is limited information on the comparison of the crude and nano-enhanced activity of *A. wilkesiana* plant extracts in treating fungal infections in Nigeria [13].

This study investigated the nano enhanced antifungal properties of *Acalypha wilkesiana* leaf extract.

Materials and Methods

Study area

The study was carried out in Nasarawa State; Established in 1996 from the neighboring Plateau State, Nasarawa State lies in Nigeria's NorthCentral region. It shares borders with the Federal Capital Territory to the West, Kaduna to the North, Benue and Kogi to the South, and Plateau and Taraba States to the East. It is located on coordinates 8.490° N, 8.199° E and made up of 13 Local Government Areas (Fig. 1). Its major occupation is non-mechanized farming, which predisposes them to pathogenic fungi.

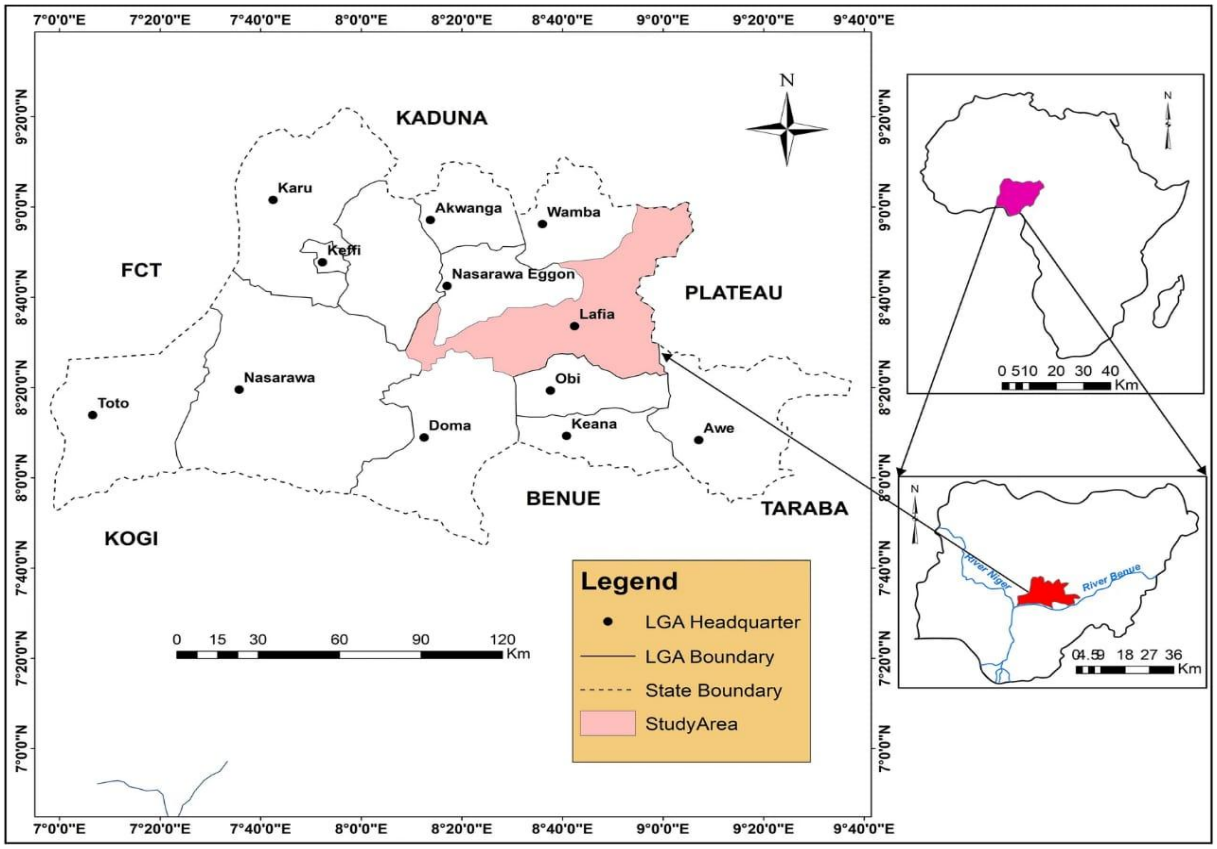

Figure 1: Map of Nasarawa State showing all the 13 Local Government Areas

Collection of fungi specie

A previously identified clinical isolate of *Trichophyton mentagrophyte* was obtained from Jos University Teaching Hospital, Plateau State. The isolate was then subcultured on Sabouraud dextrose agar in the laboratory at Federal University of Lafia, Nasarawa State.

Collection of plant and authentication

The plant species, *A. wilkesiana*, were collected from the Federal University of Lafia, take off site, and Federal University Teaching Hospital Nasarawa state. The plant species were authenticated at the Department of Plant Science and Biotechnology, Federal University of Lafia, and FUL/PSB/H.LAB/0072 was assigned.

Preparation of *Acalypha wilkesian* extracts

The *A. wilkesiana* plant species were thoroughly washed with distilled water to avoid contamination. The plant species were then air-dried at room temperature, away from sunlight, for two weeks. After two weeks, the plant species were ground using an electric blender. The powder was then kept in tightly sealed, labeled plastic containers [14].

For the preparation of extracts, 100 g of the powder was soaked in 500 ml of 80 % methanol solution for 72 h with intermittent shaking. The solution was then filtered using Whatman No. 1 filter paper, and the solution was

concentrated using a rotary evaporator under reduced pressure at 40 degrees Centigrade. The solution was then kept in a refrigerator at 4 degrees Centigrade until further use [15].

Phytochemical screening of *Acalypha wilkesiana* leaf

The phytochemical screening of the plant species was carried out to identify key phytochemicals using a standard qualitative test, as described by Dubale [16]. The screening identified the presence of Tanins, which were identified using a change in color with Ferric Chloride solution, Saponins, which were identified using constant frothing, Alkaloids, which were identified using Mayer`s and Dragendorff`s reagents, Carbohydrates, which were identified using a purple interface with Molisch`s solution, Cardiac Glycosides, which were identified using a change in layer, and Flavonoids, which were identified using a change in Shibita`s reaction, and Steroids, which were identified using a change in Liebermann-Burchard`s solution.

Green synthesis of ZnO nanoparticles

The leaf extract was utilized to synthesize ZnO nanoparticles using a green synthesis approach, where the leaf extract was utilized as a reducing and capping agent. Zinc nitrate hexahydrate was utilized as a precursor, and sodium hydroxide was utilized to adjust pH. Approximately 30 mL of *A. wilkesiana* leaf extract was utilized, where it was heated to 60 °C, followed by the gradual addition of zinc nitrate hexahydrate solution (0.2 M) with continuous stirring. The mixture was then kept at 60 °C until a paste was formed, after which it was calcined at 400 °C for 2 h to form ZnO nanoparticles. The synthesized nanoparticles were kept in a sterile container for further analysis [17].

Gas chromatography – mass spectrometry analysis (GC-MS)

GC-MS analysis was carried out at the Analysis Research Laboratory, Afe Babalola University Ado Ekiti, Nigeria. Ten microlitres (10 µL) of each sample was dissolved and diluted in 0.5 mL hexane and dried in anhydrous sodium prior to GC-MS analysis. The analyses were performed on Varian 3800/4000 gas chromatograph mass spectrometer; nonpolar fused silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The oven temperature was set at 70 °C for 4 min (min) and ramped at 8 °C /min to final temp 240 °C and held for 20 min. The flow rate of the carrier gas, Helium, was 1mL/min. The injection volume was 1 µL of diluted oil in hexane. The mass spectrometer detector was used in electron ionization mode and all spectra were acquired using a mass range of 40 to 800 m/z and Automatic Gain Control (AGC). The identification of compounds was based on the retention time match and mass spectra match against standards and NIST mass spectra library. Using computer searches on a NIST Ver. 08 MS data library and comparing the spectrum obtained through GC-MS, compounds present in the plant samples were identified. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Characterization of zinc oxide nanoparticles

A*calypha wilkisian* ZnO nanoparticles were characterized at the Analysis Research Laboratory, Afe Babalola University Ado-Ekiti, Nigeria, using a combination of analytical and spectroscopictechniques, including UV-vis (UV-visible spectroscopy) and FTIR (Fourier Transform Infrared Spectroscopy).

The Biochrom Libra PCB 1500 UV –Visible spectroscopy was the specific instrument used to accomplish this determination using UV-Visible spectroscopy to identify the wavelength with the maximum absorption .The absorbance of Zinc nanoparticles dispersed in a quartz cuvette with a 1cm optical path was measured using a small aliquot taken from the reaction liquid, the wavelength was varied from 200 to 800 nm and 300 to 800 nm in every 60 or, 90 min until a steady absorbance was established at a maximum wavelength.

Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out in the Analysis Research Laboratory, Afe Babalola University Ado-Ekiti, Nigeria which used Agilent Technology to identify the functional group in *A. wilkesiana* extracts that are responsible for the creation of ZnO (Zinc oxide) nanoparticles.

Preparation of fungal inoculum

The isolated microbe was inoculated on Sabouraud dextrose agar medium and cultured at 35 °C for 48 h. The fungi obtained from the culture were then inoculated in distilled water to prepare a suspension of 1.5×10^8 colony forming units per milliliter, equivalent to 0.5 McFarland Standard [18].

Determination of antifungal activity of extract and ZN nanoparticles extract

The effectiveness of the extracts and nanoparticles against the fungus *T. mentagrophytes* was determined using the agar well diffusion assay in vitro [18]. About 20 ml of Sabouraud dextrose agar (molten and sterile) was dispensed into sterile Petri dishes under aseptic conditions and then allowed to solidify. The plates were kept overnight in order to ensure their sterility.

After being inoculated with the fungal strain using a sterile cotton swab, the plates were allowed to air dry. Seven wells (6 mm diameter) equidistant from each other were aseptically created using a sterile cork borer. Different concentrations of the extract (500, 250, 125, 62.5, 31.25, 15.63, and 7.82 mg/mL) and the nanoparticle suspensions (500, 250, 125, 62.5, 31.25, 15.63, and 7.82 mg/mL) were placed in these wells.

A positive control was prepared using 0.1 mL of fluconazole (50 mg/mL). This was done by dispensing the drug into one of the wells. Afterward, the plates were allowed to stay undisturbed for 40 min to allow

adequate diffusion. Subsequently, the plates were incubated at 25 °C for 48-72 h and observed for the development of growth inhibition zones.

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was obtained by using the broth dilution technique in 96-wells, as recommended by the CLSI M100 guideline [19]. A sterile inoculum of 10 µL was sub-cultured into SDA plates from the non-growing wells obtained in MIC assays and cultured for 5-7 days at 28-30 °C. The Minimum Fungicidal Concentration (MFC) was taken as the lowest concentration of the extract/fraction that totally inhibited fungal growth, suggesting a total fungicidal effect on *T. mentagrophytes* [20].

Statistical analysis

The results obtained from antifungal susceptibility testing were subjected to analysis using IBM SPSS 27. The results were summarized using descriptive statistics, including mean values and standard deviation, especially for zone of inhibition values. The results were considered normally distributed, and homogeneity was observed, thus satisfying the requirements. The two factors, extraction method and concentration, including their interaction, were subjected to two-way ANOVA. The results obtained were further subjected to post hoc analysis, especially when there was a significant difference, using Tukey's HSD. The concentrations that did not show inhibition in one method were excluded to ensure balance. All calculations were done using two-tailed values, with $P < 0.05$ considered statistically significant.

Results and Discussion

Phytochemical composition

The phytochemical screening result revealed that the *A. wilkesiana* leaves contained a high level of absorbance of bioactive phytochemical compounds such as alkaloids, flavonoids, and phenolic compounds, as corroborated by the findings of Akinkunmi and Ibibia [21]. The result also revealed the moderate level of tannins, saponins, and terpenoids as compared to the previous study. The level of glycosides and steroids was also found to be low as compared to the previous study, as indicated in Table 1. The present study is in line with the findings of some researchers [21, 22], who found that the level of the secondary metabolite is high in the leaves of *the A. wilkesiana* plant. The present study is not in line with the findings of Dada *et al.* [22], who found the presence of many phytochemical compounds in the *A. wilkesiana* leaves' extract. Apart from alkaloids, saponins, and anthraquinones, the variation in the type of phytochemical compounds present in the leaves of the *A. wilkesiana* plant may be attributed to the climate and season, as also found by Dada *et al.* [22].

The phytochemical compounds present in the *A. wilkesiana* leaves provide the biochemical rationale for the enhanced antifungal activity of the zinc oxide nanoparticle-based antifungal formulation.

Table 1: Phytochemical composition of the extracts

Phytochemicals	*A. wilkesiana* (Qualitative)	Quantitative (mg/g) ±SD
Alkaloids	+++	23.4 ± 1.2
Flavonoid	+++	11.5 ± 0.8
Phenols	+++	10.3 ± 0.7
Tannins	++	8.6 ± 0.5
Saponins	++	19.2 ± 1.0
Terpenoids	++	4.5.± 0.3
Glycosides	+	7.2 ± 0.4
Steroids	+	6.0 ± 0.4

+++ = Highly present; ++ = moderately present; + = present in small amount; **ND** = Not detected

From the analysis of GC-MS as presented in Table 1 and Fig. 2, there was an indication of the existence of various phytochemicals such as monoterpenes, phenolics, esters, terpenoids, and fatty acids. The presence of such phytochemicals in *A. wilkesiana* leaf extract may be attributed to antifungal properties, as earlier documented [24].

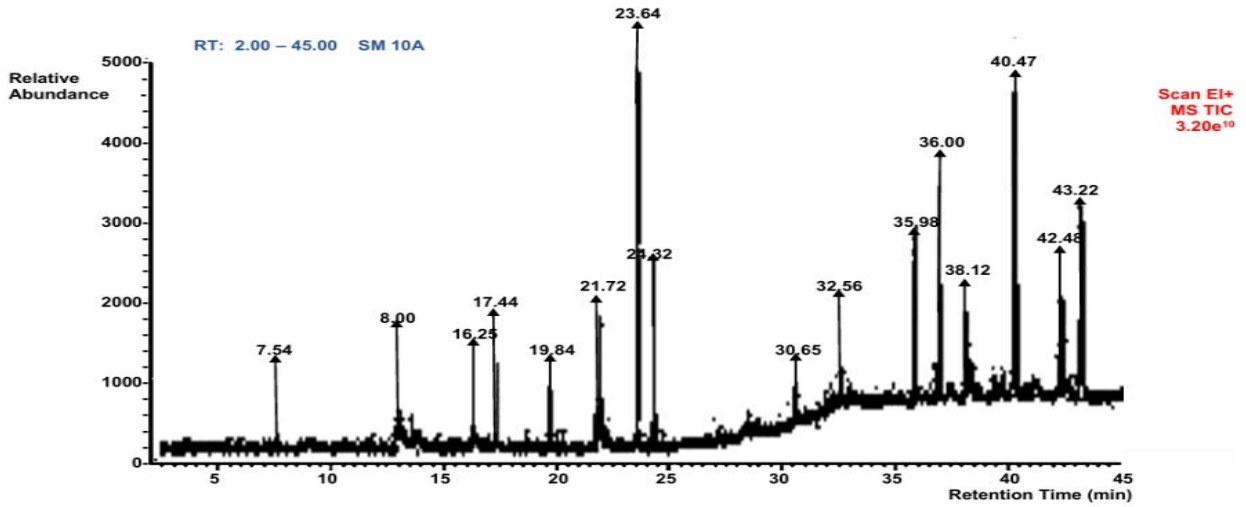

Figure 2: Chromatography-mass spectrometry (GC-MS) analysis for *A. wilkesiana*

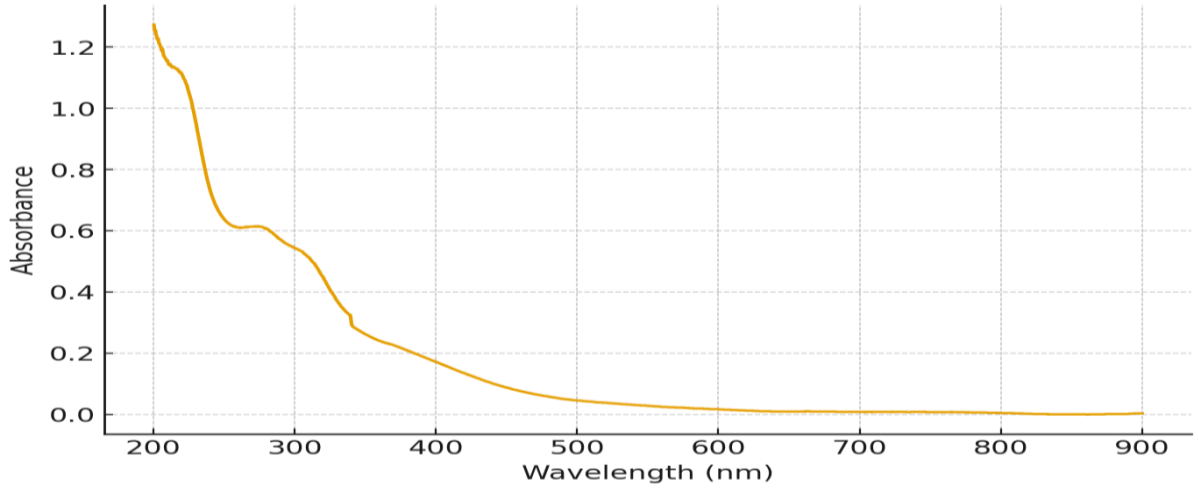

Figure 3: Ultra violet-visible spectra analysis for *A. wilkesiana*

The Ultraviolet–visible spectroscopy analysis

The Ultraviolet–Visible spectroscopy analysis (Fig. 3) showed characteristic absorption peaks in the range typically associated with ZnONPs, confirming nanoparticle formation. The distinct absorbance bands observed between 320 and 380 nm are consistent with earlier reports by Shinde [25], who demonstrated that plant-derived phenolics act as natural reducing agents during nanoparticle synthesis. This observation corroborates the research by Idowu [22], that phytochemical and biomolecular constituents of *A. wilkesiana* act as bioreducing and capping agents ,mediating the reduction of Zn^{2+} ions and stabilizing the nucleation and growth of the synthesized zinc oxide nanoparticles synthesis.

Fourier-transform infrared (FTIR) spectroscopy analysis

Fourier-transform infrared (FTIR) spectroscopy analysis (Fig. 4) confirmed the involvement of hydroxyl, amide, and carbonyl groups, which act as reducing and capping agents during ZnONP formation. These functional groups likely originate from polyphenols, proteins, and polysaccharides present in the plant and fungal extracts. The presence of metal–oxygen stretching bands below 700 cm^{-1} further corroborates nanoparticle synthesis [26]. Similar functional group interactions were reported by AlDhabi *et al.* [27] who found that hydroxyl and carbonyl moieties from plant extracts significantly influence nanoparticle morphology and bioactivity.

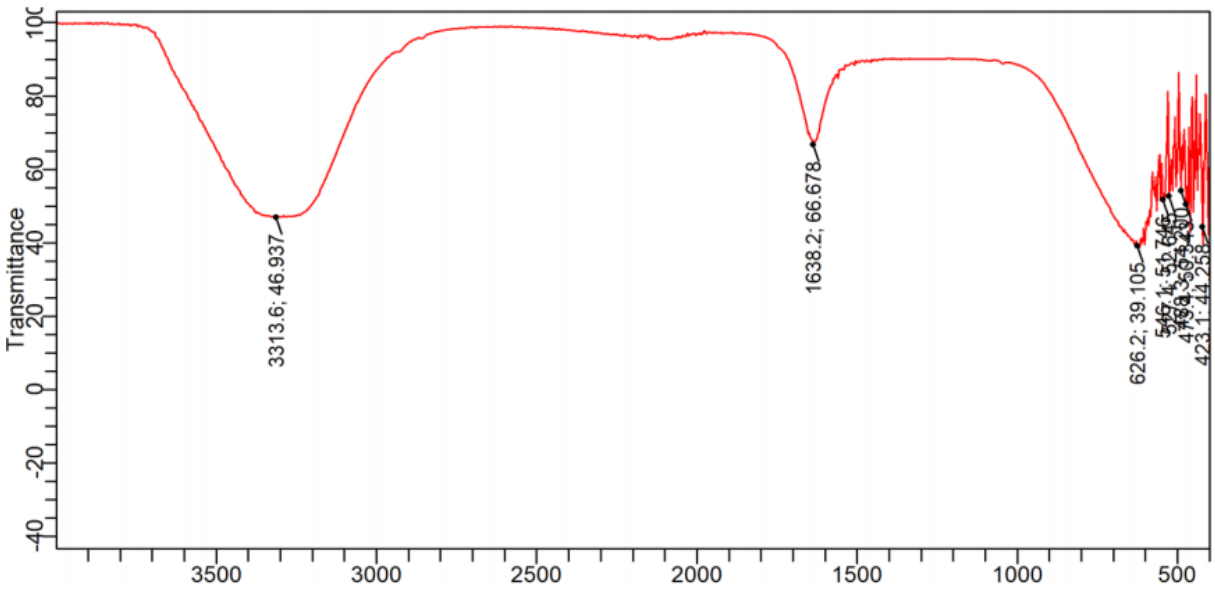

Figure 4: FTIR Spectrum ZnONPs of aqueous extract of *A. wilkesiana*

Antifungal activity of crude and nano extract of *A. wilkesiana* against *T. mentagrophyte*

The agar well diffusion assay indicated that all the extracts exhibited clear zones of inhibition, thus showing antifungal potency against *T. mentagrophyte*. The anti-fungal assay activity of the extracts was concentration-dependent, showing that the highest zone of inhibition was recorded at 500 mg/ml, while the

smallest zones of inhibition were recorded at 782 mg/ml. Thus, it can be concluded that the nano-formulated extracts recorded larger zones of inhibition compared to the crude extracts at the same concentrations. This is because the formulation of the extracts in the nano-form enhanced the diffusibility of the extracts, thus improving the interaction of the drug with the cell membrane of the test organisms. The results of this experiment are in agreement with the reports of some workers [28, 29] who recorded enhanced antifungal efficiency of nano-formulated herbal extracts compared to crude extracts. The enhanced size of the zones recorded for the nano-formulated extracts further confirms that the use of nanotechnology can enhance the efficiency of natural products.

The minimum inhibitory concentration of *A. wilkesiana*

Antifungal activity from the above agar well diffusion assay showed that nano-enhanced extracts possess a high inhibitory effect compared to their crude forms. The inhibitory zone of *A. wilkesiana* (nano) is significantly larger compared to crude and fluconazole as a standard control. This shows that ZnONP synthesis increases the antifungal potential of biological sources. The minimum inhibitory concentration (MIC) shown in Table 2 and minimum fungicidal concentration (MFC) shown in Table 3 below prove that nano-formulated samples completely inhibit fungi at 7.82 mg/ml compared to 15.63 mg/ml in crude form. These results are in line with earlier research proving that ZnONPs synthesized from a biological source possess enhanced antifungal activity due to their nano-scale dimensions and increased surface activity [30, 31]. The nano-scale dimensions enable ZnONPs to bind more effectively to microbial membranes and disrupt membrane structure by inducing oxidative stress and disrupting membrane fluidity. The synergistic effect of ZnONPs and bioactive compounds in plants increases membrane binding and disrupts ergosterol synthesis in fungi [32]. The results obtained in this research show the potential of biogenic ZnONPs as a source of eco-friendly fungicides compared to conventional chemical fungicides, particularly against dermatophytes resistant to conventional drugs like fluconazole [33].

Table 2 MIC for crude and nano enhanced *A. wilkesiana* against *T. mentagrophyte* using (96-well micro-dilution Assay)

Test sample	Concentration (mg/ml)						
	500	250	125	62.5	31.25	15.63	7.82
A. wilkesiana (crude)	–	–	–	+	+	+	+
A. wilkesiana (Nano)	–	–	–	–	–	–	+
Fluconazole (control)	–	–	–	–	–	–	+

Control: fluconazole 500 mg/ml, + = visible growth, – = no visible growth (complete inhibition)

Table 3 Minimum fungicidal concentration of crude and zinc nano enhanced A. *wilkesiana* against *T. mentagrophyte*

Test sample	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC Ratio	Interpretation
A. wilkesiana (crude)	15.63	31.25	2.0	Fungicidal
A. wilkesiana (Nano)	7.82	7.82	1.0	Fungicidal
Fluconazole (control)	7.82	7.82	1.0	Fungicidal

Table 4: Comparison of zone of inhibition between crude and nano extracts of *A. wilkesiana* across different concentrations

Conc. (mm)	N	Crude extract Mean ± SD	Nano extract Mean ± SD	F	P
Control	3	23.00 ± 1.00	32.00 ± 1.00	1,261.50[a]	<0.001
62.5	3	15.67 ± 0.58*	28.00 ± 1.00*	187.13[b]	<0.001
125	3	19.67 ± 0.58*	32.00 ± 1.00*	4.00[c]	0.015
250	3	24.67 ± 0.58*	37.00 ± 1.00*		
500	3	29.00 ± 1.00*	41.00 ± 1.00*		

a = indicates F and corresponding P-value for comparison of extracts (crude vs nano); **b** = indicates F and corresponding P-value for concentrations; **c** = indicates F and corresponding P-value for interaction between extracts vs concentrations; * = Indicates statistically significant difference in pair wise comparison for zone of inhibition between treatment against the control

Minimum fungicidal concentration of crude and zinc nano enhanced A. *wilkesiana* against *T. mentagrophyte*

The minimum fungicidal concentration (MFC) values of the crude and zinc nano synthesized ethanol extracts of *A. wilkesiana* against *T. mentagrophyte*. The MFC values were 31.25 mg/ml for crude extracts and 7.82 mg/ml for Zinc nano enhanced extracts. The MFC/MIC value for all the samples was ≤ 4, indicating that they were fungicidal, not fungistatic [34]. Table 3 indicates that all the samples were fungicidal, and those that were formulated with nano particles were more potent than those that were not.

The ANOVA result shows a significant difference in the inhibition zone mean compared to the agar well diffusion indicating that the nano extract produced significantly greater zones of inhibition than agar well diffusion across the tested concentrations (Table 4). Therefore, the two groups differ significantly in their antifungal activity.

Conclusion

Acalypha wilkesiana possess antifungal properties, which are significantly enhanced upon zinc nanoparticle synthesis. The nano-enhanced extracts showed MIC and MFC values, confirming stronger fungicidal action against *T. mentagrophytes. A. wilkesiana* ZnONPs exhibited the highest inhibitory zones, suggesting that it`s phytochemical composition—rich in terpenoids and phenolics—plays a central role in enhancing antifungal potency. The combined effects of surface modification, high surface-to-volume ratio, and synergistic phytochemical activity likely account for the improved performance of nano-formulations.

Conflict of interest: None; neither was there any funding received for this work.

Acknowledgements: The authors are grateful to Department of Microbiology Federal University of Lafia for the opportunity and conducive environment to carry out this research work also Jos University Teaching Hospital for providing the organism used in this research work.

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Citing this Article

Ogbonnaya, N. A., Owoseni, M. C., Chuku, A., & Nworie, C. R. (2026). Crude and nano-enhanced antifungal properties of *Acalypha wilkesiana* leaf extract against *Trichophyton mentagrophytes*. *Lafia Journal of Scientific and Industrial Research*, *4*(2), 1–8. https://doi.org/10.62050/ljsir2026.v4n2.813