
Synthesis of zinc nanoparticles from extracts of the medicinal plant *Kaempferia galanga* L. and their antifungal activity

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This study aims to investigate the biogenic synthesis of zinc oxide nanoparticles (ZnO NPs) using the rhizome oil and acetone extract of *Kaempferia galanga* and to evaluate their efficacy against a wide range of fungal phytopathogens. In this work, the biogenic synthesized nanoparticles were characterized using UV-Vis spectroscopy and Scanning Electron Microscopy (SEM). After antifungal screening, the results showed that while the control or abiotic ZnO (0.2%) provided the highest inhibition against *Colletotrichum* (75.71%) and *Fusarium* (47.86%), the biogenic nanoparticles notably the acetone extract-mediated ZnO achieved the highest inhibition against *Epicoccum* (73.33%), while the oil-mediated ZnO nanoparticles was the most effective against *Alternaria* (71.13%), *Cercospora* (62.07%), and *Rhizoctonia* (100%). These findings suggest that the use of *K. galanga*—particularly from its essential oil—enhances the antifungal profile of ZnO particles, supporting the potential of biogenic nanofungicides as sustainable alternatives to conventional treatments.

Keywords : Biogenic synthesis, essential oil, fungal pathogens, *Kaempferia galanga*, nanoparticles, plant extract, Zinc oxide nanoparticles

INTRODUCTION

Plant extracts and essential oil have been used for a long time as natural medicines to treat various ailments. These practices dates back to hundreds of years and has been perfected and concentrated over the years. (Petrovska, 2012). They have been known to have antimicrobial properties and, in some cases, found to be more effective in inhibiting pathogenic microbes than other important commercial antibiotics (Kavanaugh and Ribbeck, 2012). Analysis from the oil and extracts of various plants were found to have important antifungal and antibacterial compounds like cinnamic acid, cinnamaldehyde, tannin, phenol and thymol, including plants which are rich in phytochemicals like saponin, steroids, triterpenoids, glycosides, flavonoids, anthraquinone, amino acids and proteins (Burt, 2004; Bhalodia and Shukla, 2011).

Their high level of antioxidant makes them good candidates as food preservatives especially during post-harvest (Anjum and Akhtar, 2012; Dubey *et al.* 2017). Solvents also play an important part in extraction of important compounds from plant parts as certain compounds of importance are dissolvable in certain solvents (Bacon *et al.* 2016). Since plant extracts and essential oil are able to degrade in soil and water quickly, have low toxicity to mammals and are relatively easy to obtain, they are thus considered to be good alternatives to conventional fungicides and pesticides due to their environmentally friendly properties (Isman, 2000). While biogenic synthesis of nanoparticles has been explored with other Zingiberaceae members (Varghese *et al.* 2021); and silver nanoparticles synthesized from *Kaempferia galanga* have also been reported (Renjusha and Kumar, 2023), the specific interaction between the *K. galanga* and zinc ions remains uninvestigated. This research fills that gap by providing a detailed analysis of how oil and acetone extracts from the plant facilitates

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nanoparticle formation and contributes to antifungal activity.

MATERIALS AND METHODS

Extraction of essential oil

The rhizomes collected were thoroughly washed in running water to remove debris and then cut into fine pieces. 500 gm of the rhizome was subjected to hydro-distillation using a Clevenger apparatus at 40 °C for 4 hours. The oil collected was then stored at - 20 °C until required for bioassay.

Preparation of plant extract

According to the methods as described by Mahlo *et al.* (2016) and Abirami *et al.* (2021), the rhizomes were collected, washed, air dried at room temperature and grounded into fine powder. 20 gm of the powdered rhizome material was then soaked in 200 ml of desired solvent for 72 hours in a thermal shaker with constant rocking at 26 °C. Different solvents with increasing polarity were chosen namely hexane, ethyl acetate, chloroform, acetone, methanol and water. The samples were then filtered in a conical flask using Whatman No. 44 filter paper till a clear solution with no debris was obtained. It was then left to dry in an oven at 16 - 18 °C. The solid residue from the extract was then scraped from the bottom of the conical flask and dissolve at 0.1 g/ml in Tween20. It was then stored at 4 °C until required.

Synthesis of Zinc Oxide particles using Kaempferia galanga Oil and Acetone Plant Extract

This was done following the method of Raj and Jayalakshmy (2015), where 0.01 M of Zinc acetate dihydrate or $(\text{CH}_3\text{COO})_2\text{Zn}\cdot 2\text{H}_2\text{O}$ was prepared using sterile distilled water. To 50 ml of the solution, 500 μl of the rhizome extract or oil was added slowly by continuous stirring in a magnetic stirrer and slight modification was done by maintaining a temperature of 30- 40°C. Then 1.0 M of sodium hydroxide (NaOH) was added to get a desired pH of 12. It was then allowed to stir for 4 hours until the formation of yellow or light-yellow

precipitate was observed. The mixture was then centrifuged at 10,000 rpm for 15 minutes. The supernatant was discarded and the pellet which has settled at the bottom of the centrifuge tubes was washed using distilled water and then dried overnight in a hot air oven. The resulting dried yellow powder was then put in an oven at 70 °C for 2 hours, collected carefully and stored at room temperature for further use. The ZnO nanoparticles synthesized from only NaOH was taken as control. The control ZnO were prepared the same without the addition of the plant extracts.

Isolation of fungal phytopathogens

Pathogens infecting the plants parts of *Solanum tuberosum*, *Phaseolus vulgaris* and *Tetragonia tetragonioides* inducing characteristic visible symptoms like spots, blights, etc., were collected. For the isolation of the pathogenic fungi, agar plate method (Muskett and Malone, 1941) was used; infected and diseased parts of the plants were cut into small pieces and inoculated on sterilized petriplates containing Potato Dextrose Agar (PDA) media. The inoculated plates were then incubated at 28 °C and left for 7 days. Pure culture of the fungal colonies were done on Czapek Dox Agar media and incubated again. Identification of the fungal colonies was done based on the microscopic observations of the spores and fruiting bodies using standard manuals and from the colony characteristics (Sutton, 1980; Barnett and Hunter, 1998).

Antifungal Activity

The ZnO powder synthesized from the essential oil, plant extract and control were measured according to their desired concentration and suspended in distilled water. It was then subjected to sonication using an ultrasonic bath at 40,000 Hz to break down agglomeration of the particles. It was then mixed with Czapek Dox Agar media for testing of antifungal activity. A 5 mm disc of mycelial material was taken from the edge of pure fungal cultures and placed in the centre of each petriplates. Fungal mycelial disc inoculated on petriplates free from the ZnO particles were also taken. It was then incubated at 28 °C. The efficacy of the treatment was evaluated by measuring the diameter of treated

fungal colonies with the untreated fungal colonies (Arciniegas-Grijalba *et al.* 2017). The antifungal assay was conducted using three replicates with three independent experimental repeats and inhibition percentage was calculated as follows :

Inhibition Percentage =

$$\frac{\text{Growth of untreated fungi} - \text{Growth of treated fungi}}{\text{Growth of untreated fungi}} \times 100$$

Statistical analysis was done accordingly using SPSS software where a one-way analysis of variance (ANOVA) was conducted to evaluate the inhibitory activity of ZnO nanoparticles at a concentration of 0.2%, with the significance level set at $p \leq 0.05$.

Characterization of the Prepared ZnO Particles

● UV-Vis Absorption Spectrum

The optical absorption spectra of ZnO dispersed in water were recorded using a Lambda 35 UV/VIS spectrophotometer (PerkinElmer). The peak absorption spectrum was observed and measured from the range of 230nm to 600 nm.

● Scanning Electron Microscopy

Scanning Electron Micrographs of the synthesized ZnO particles and their antagonistic activity against phytopathogens were observed under the JEOL JSM-6360 SEM model at 20k, performed at SAIF- NEHU, Shillong (The Sophisticated Analytical Instrument Facility - North-Eastern Hill University).

RESULTS AND DISCUSSION

From the absorbance taken at different wavelengths from the range 230 nm to 600 nm, the control ZnO shows highest absorbance at 327 nm and 332 nm, oil synthesized ZnO from 282 nm-312 nm, plant extract synthesized ZnO at 328 nm. This is illustrated in Fig. 1.

The acetone plant extract shows the highest antifungal activity as compared to other solvents, therefore only acetone plant extract was further

proceeded for synthesis of nanoparticles. The conventionally synthesized ZnO particles at 0.2% concentration shows highest inhibition percentage against *Colletotrichum* and *Fusarium* at 75.71% and 47.86% respectively than the oil synthesized and plant extract ZnO, while for *Epicoccum* highest inhibition was seen from the acetone plant extract synthesized ZnO at 73.33%. The highest inhibition against the three remaining phytopathogens i.e., *Alternaria*, *Cercospora* and *Rhizoctonia* at 71.13%, 62.07% and 100% respectively was by the oil synthesized ZnO particles. Table 1 shows the antagonistic activity of the above ZnO particles, while Fig. 2 shows the Scanning Electron Micrographs on the effect of the ZnO particles on the hyphal morphology of the phytopathogens.

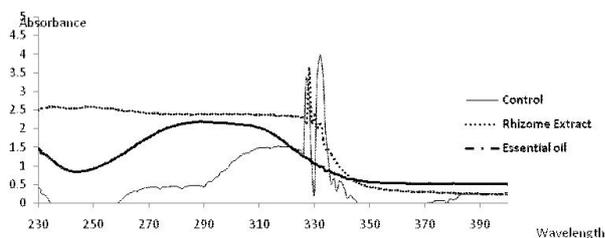
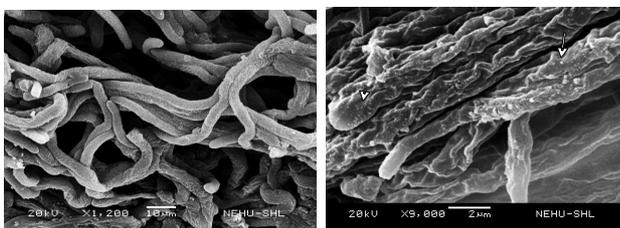
All the ZnO particles synthesized from different sources show high absorbance peak in the range of 251 to 332 nm. These are in accordance with the works of others which reported ZnO nanoparticles having peak absorbance from the range 200-400 nm (Verma *et al.* 2020; Mekky *et al.* 2021).

The antifungal activity of the biogenic ZnO nanoparticles (b-ZnO) showed higher antifungal activity than that of the conventionally synthesized nanoparticles (c-ZnO) against *Epicoccum*, *Cercospora*, *Alternaria*. The phytopathogen *Rhizoctonia* in particular showed 100% inhibition at a concentration as low as 0.05% from the oil synthesized nanoparticles. While the c-ZnO required a concentration of 0.5% to demonstrate inhibitory effects, the b-ZnO particles exhibited significant antifungal activity at a much lower concentration that is at 0.05% and 0.1%. These results are similar with previous findings by Gunalan *et al.* (2012), which reported the superior antimicrobial potency of biosynthesized ZnO compared to their abiotic counterparts. The morphological analysis of the treated fungal specimens using Scanning Electron Microscopy (SEM) revealed distinct distortions in the hyphae of the treated fungal specimens which include surface indentations and flattened hyphae characterized by dense nanoparticle deposition. In contrast, untreated control hyphae maintained a healthy, smooth, and undeformed structure. These observations suggest a direct physical and

Table 1: Inhibitory action of ZnO particles synthesized from oil, acetone plant extract and NaOH against the phytopathogens

Phytopathogens	Oil			Plant Extract			Control		
	0.05%	0.1%	0.2%	0.05%	0.1%	0.2%	0.05%	0.1%	0.2%
<i>Alternaria</i>	46.39±8.18	48.45±0.67	71.13±1.26 ^a	8.04±1.26	42.86±0	65.00±5.0 ^b	0.00	0.00	50.89±1.26 ^{ba}
<i>Cercospora</i>	31.03±3.37	37.93±3.03	62.07±3.03 ^a	15.00±7.07	45.00±7.07	52.50±3.53	11.00±1.41	20.00±0	41.00±4.24 ^a
<i>Colletotrichum</i>	24.07±2.62	25.93±0	56.48±9.17 ^a	21.05±3.72	61.84±1.86	69.74±1.86	0.00	12.86±2.02	75.71±6.06 ^a
<i>Epicoccum</i>	34.89±2.21	36.56±4.57	70.78±1.25 ^a	21.33±1.89	66.67±0	73.33±0 ^b	0.00	5.00±3.03	54.29±0 ^{ab}
<i>Fusarium</i>	11.59±2.25	14.93±2.26	44.95±2.42	17.50±3.54	26.88±0.88	31.88±0.88 ^a	0.00	0.00	47.86±3.03 ^a
<i>Rhizoctonia</i>	100.00±0	100.00±0	100.00±0	55.00±7.07	79.38±2.65	89.38±2.65	0.00	0.00	87.50±0

ANOVA of the Inhibitory Activity at 0.2% of ZnO particles synthesized from different sources, where letters in the same row indicates significant difference at $p \leq 0.05$. \pm represents standard deviation from three replicate

**Fig. 1:** Wavelength (nm) of the synthesized ZnO particles from different sources**Fig 2 :** Scanning Electron Micrographs showing antagonistic activity of biogenic ZnO particles on fungal hyphae-Left - Untreated fungi and Right- Treated Fungi.

Arrow marks indicate deposition of ZnO nanoparticles on the fungal hyphae

oxidative interaction between the nanoparticles and the fungal cell wall.

Furthermore, the utilization of *Kaempferia galanga* provides an eco-friendly alternative to traditional fungicides. Zinc-based nanoparticles are particularly advantageous due to their biocompatibility where ZnO is classified by the FDA as 'Generally Recognized as Safe' (GRAS) for topical applications, whereas other metal nanoparticles like silver nanoparticles face stricter regulatory constraints and environmental runoff

concerns. This foundational investigation addresses a significant gap in the literature, as the synthesis of ZnO nanoparticles specifically using *K. galanga* remains largely unreported. The primary scientific contribution of this work lies in the optimization of a novel biogenic synthesis protocol and the demonstration of the enhanced antifungal efficacy of the resulting hybrid particles. While the current results are promising, future research on evaluating the phytotoxicity of these particles to ensure their safety for broad-scale agricultural application is important and this in vitro study served as a critical first step.

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CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

REFERENCES

- Abirami, S., Edwin Raj, B., Soundarya, T., Kannan, M., Sugapriya, D., Al-Dayyan, N., Ahmed Mohammed, A. 2021. Exploring antifungal activities of acetone extract of selected Indian medicinal plants against human dermal fungal pathogens. *Saudi J. Biol. Sci.* **28**:2180-2187.
- Anjum, T., Akhtar, N. 2012. Antifungal activity of essential oils extracted from clove, cumin and cinnamon against blue

- mold disease on citrus fruit. *International Conference on Applied Life Sciences*. pp: 321-326. IntechOpen. Doi: 10.5772/intechopen.84099.
- Arciniegas-Grijalba, P.A., Patiño-Portela, M.C., Mosquera-Sánchez, L.P., Guerrero-Vargas, J. A., Rodríguez-Páez, J. E. 2017. ZnO nanoparticles (ZnO-NPs) and their antifungal activity against coffee fungus *Erythriciumsalmonicolor*. *Appl. Nanosci.* **7**: 225–241.
- Bacon, K., Boyer, R., Denbow, C., O'Keefe, S., Neilson, A., Williams, R. 2016. Evaluation of different solvents to extract antibacterial compounds from jalapeño peppers. *Food Sci. Nutr.* **5**(3):497-503. Doi: 10.1002/fsn3.423.
- Barnett, H.L., Hunter, B.B. 1998. *Illustrated genera of imperfect fungi* (4th edition) APS Press.
- Bhalodia, N. R., Shukla, V. J. 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L.: An ethnomedicinal plant. *J. Adv. Pharm. Technol. Res.* **2**(2): 104–109.
- Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* **94**(3): 223-253. Doi: 10.1016/j.ijfoodmicro.2004.03.022.
- Dubey, N.K., Kumar, A., Kumar, A., Das, S. 2017. Efficacy of *Luvunga scandens* Roxb. essential oil as antifungal, aflatoxin suppressor and antioxidant. *J. Food Technol. Preserv.* **1**: 37-41.
- Gunalan, S., Sivaraj, R., Rajendran, V. 2012. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Prog. Nat. Sci.-Mater.* **22**(6):693–700. Doi: 10.1016/j.pnsc.2012.11.015.
- Isman, M.B. 2000. Plant essential oils for pest and disease management. *Crop Prot.* **19**:603–608.
- Kavanaugh, N.L., Ribbeck, K. 2012. Selected antimicrobial essential oils eradicate *Pseudomonas* spp. and *Staphylococcus aureus* biofilms. *Appl. Environ. Microbiol.* **78**(11): 4057–4061. Doi: 10.1128/AEM.07499-11.
- Mahlo, S.M., Chauke, H.R., McGaw, L., Eloff, J. 2016. Antioxidant and antifungal activity of selected medicinal plant extracts against phytopathogenic fungi. *Afr. J. Tradit., Complementary Altern. Med.* **13**(4): 216-222.
- Mekky, A.E., Farrag, A.A., Hmed, A.A., Sofy, A.R. 2021. Preparation of zinc oxide nanoparticles using *Aspergillus niger* as antimicrobial and anticancer agents. *J. Pure Appl. Microbiol.* **15**(3):1547-1566.
- Muskett, A.E., Malone, J.P. 1941. The Ulster method for the examination of flax seed for the presence of seed-borne parasites. *Ann. Appl. Biol.* **28**: 8-13.
- Petrovska, B. B. 2012. Historical review of medicinal plants' usage. *Phcog. Rev.* **6**(11): 1–5. Doi: 10.4103/0973-7847.95849.
- Raj, L. F. A., Jayalakshmy, E. 2015. Biosynthesis and characterization of zinc oxide nanoparticles using root extract of *Zingiber officinale*. *Orient. J. Chem.* **31**(1): 51-56.
- Renjusha, S., Kumar, G. S. 2023. Green synthesis of silver nanoparticles using *Kaempferia galanga* extract and study of its antibacterial effect. *Int. J. Sci. Res.* **12**: 1812-1815.
- Sutton, B.C. 1980. *The Coelomycetes: Fungi imperfecti with pycnidia, acervuli and stromata*. Commonwealth Mycological Institute, Kew.
- Varghese, B. A., Nair, R. V. R., Jude, S., Varma, K., Amalraj, A., Kuttappan, S. 2021. Green synthesis of gold nanoparticles using *Kaempferia parviflora* rhizome extract and their characterization and application as an antimicrobial, antioxidant and catalytic degradation agent. *J. Taiwan Inst. Chem. Eng.* **126**: 166-172.
- Verma, P., Khan, F., Banerjee, S. 2020. *Salvadora persica* root extract-mediated fabrication of ZnO nanoparticles and characterization. *Inorg. Nano-metal Chem.* **51**(3): 427-433. Doi: 10.1080/24701556.2020.1793355.