Recently the prevalence of dermatophyte infection increased dramatically to 20–25% of the world population, which coincided with fungal resistance to known antifungals. This has led to more only a small proportion of species may be considered pathogenic.

While people are exposed to fungal microorganisms regularly, only a small proportion of species may be considered pathogenic. Most fungal infections afflict the epidermis, comprising the coetaneous and subcutaneous tissues. The coetaneous fungi are keratinophilic and so generally infect skin keratins, afflicting millions of people and animals globally [1]. A prominent source of keratinophilic pathogen is the genus *Trichophyton*, which is one of the most widely distributed of the human and animal dermatophytes [2,3], most commonly leading to *Tinea*. The genera most commonly associated with *Tinea* include *Trichophyton*, *Epidermophyton*, and *Microsporium* [4]. Many studies show that such dermatophytes are transmitted directly and indirectly through contact with dogs, as they are part of the normal flora in canines but lead to infection in humans [3].

Recently the prevalence of dermatophyte infection increased dramatically to 20–25% of the world population, which coincided with fungal resistance to known antifungals. This has led to more studies being conducted to find new antifungals that can replace modern treatments that fail in the plight of growing resistance [5,6].

The nanotechnique has achieved popularity in medicine and pharmacy research and is now considered to have enormous potential for diagnosis and treatment of diseases [11]. In the current study the nanotechnique was used to prepare a nanohybrid antifungal from griseovulvin (Gri) and zinc oxide. Gri-ZnO was confirmed by changes in the FT-IR and x-ray spectrums, compared with zinc oxide. New nanodimensions were observed in atomic force microscopy. The antifungal activity of free griseovulvin and Gri-ZnO was studied against 12 patient dermatophytes belonging to three species, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis*. While some of the isolates showed resistance to both Gri and Gri-ZnO others were susceptible, with average inhibition zone sup to 15 mm.

## 1. INTRODUCTION

While people are exposed to fungal microorganisms regularly, only a small proportion of species may be considered pathogenic. Most fungal infections afflict the epidermis, comprising the coetaneous and subcutaneous tissues. The coetaneous fungi are keratinophilic and so generally infect skin keratins, afflicting millions of people and animals globally [1]. A prominent source of keratinophilic pathogen is the genus *Trichophyton*, which is one of the most widely distributed of the human and animal dermatophytes [2,3], most commonly leading to *Tinea*. The genera most commonly associated with *Tinea* include *Trichophyton*, *Epidermophyton*, and *Microsporium* [4]. Many studies show that such dermatophytes are transmitted directly and indirectly through contact with dogs, as they are part of the normal flora in canines but lead to infection in humans [3].

Recently the prevalence of dermatophyte infection increased dramatically to 20–25% of the world population, which coincided with fungal resistance to known antifungals. This has led to more studies being conducted to find new antifungals that can replace modern treatments that fail in the plight of growing resistance [5,6].

The antifungal griseovulvin belongs to the heterocyclicbenzofuran group and was discovered in 1939 after it was isolated from *Penicillium griseofulvum*. Griseovulvin came to be considered an important antifungal, particularly since it has low toxicity and can therefore be taken orally or as an active in skin ointments [7,8]. Griseovulvinis a crystalline white-yellow powder with formula \(\text{C}_{17}\text{H}_{18}\text{C}\text{O}_{6}\) and 352.767g/mol molecular weight. It is soluble in ethanol, methanol, dimethyl- and formamide, while partially soluble in water [9,10].

Recently the nanotechnique has achieved popularity in medicine and pharmacy research and is now considered to have enormous potential for diagnosis and treatment of diseases [11]. In the current study the nanotechnique was used to prepare a nanohybrid antifungal from griseovulvin (also spelled griseofulvin) and restore its efficacy in the face of growing resistance.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of antifungal nanoparticles from zinc oxide and griseovulvin by Sol gel ion exchange

The antifungal ZnO nanoparticles were prepared from griseovulvin and zinc oxide layers by the sol-gel ion exchange method which was modified from Kolekar et al., [12]. Briefly, 50 ml of griseovulvin (10 mg/ml) was added drop by drop to a zinc oxide solution (1 g of zinc oxide in 50% ethanol (20 mg/ml). Then, the solution was stirred at room temperature for 2 hr. After that, the mixture was incubated with shaking at 37°C for 18 hr then put in an incubator at 40°C for 24 hr. The solution was then centrifuged at 5000 rpm for 20 minutes and the precipitant was washed with deionized water several times then dried at 40°C. Finally, the dried product (precipitate) was ground and stored in refrigerator.

### 2.2 Nanohybrid antifungal diagnosis (screening)

Several methods were used to confirm the success of the nanohybrid antifungal (Gri-ZnO) formation, which included spectral methods and atomic force microscope (AFM).

#### 2.2.1 Nanohybrid antifungal diagnosis using infrared spectrum (FT-IR)

A tablet was prepared from the nanohybrid and potassium bromide (KBr) then finely ground (crushed). The infrared spectrum was measured at wavelengths ranging from 400–4000 cm\(^{-1}\).

#### 2.2.2 Nanohybrid antifungal diagnosis using x-ray diffraction (XRD)

The nanohybrid antifungal Gri-ZnO was characterized by XRD to clarify the layer thickness’ differences (d) before and after antifungal insertion using Bruker n. \(\lambda = 2d\text{Sine} = \text{equation to calculate layer’s thickness (d)}.

#### 2.2.3 Nanoantifungal using atomic force microscope (AFM)

The AFM was used to test the nanohybrid antifungal Gri-ZnO and measured the diameter, size, and nanoparticles’ grouping. The crystallinity index was calculated using the equation: Crystalinity Index = \(\text{Dp}/L\) [13].

#### 2.2.4 Antifungal activity of free Griseovulvin and nanohybridGri-ZnO using well diffusion assay against dermatophytes

The antifungal inhibition activity of free griseovulvin and Gri-ZnO against 12 strains of dermatophytes were measured in a well diffusion assay as described byEgorovc [14]. Also the minimum inhibition average of the free griseovulvin and nanohybrid against the isolated dermatophytes was determined using several concentrations (5, 1, 0.5, 0.25, 0.1 and 0.01 mg/ml).

## 3. RESULTS AND DISCUSSION

### 3.1 Nanohybrid antifungal screening

The free Griseovulvin showed many significant peaks (bands) with specific frequencies as shown in Figure 1. The appearance of bands at the frequency 3392.90 cm\(^{-1}\) referred to the stretch vibration of the aromatic C-H bonds. In addition, the C-H
stretched at frequencies 2847.03 and 2941.54 cm⁻¹ is from the alkene bond on the aliphatic side of the molecule. While the bands 1660.77 and 1708.99 cm⁻¹ are indicative of the vibration of the carbonyl groups (C=O). The appearance of the band at 1460.16 cm⁻¹ referred to the vibration at the bending C-H backbone.

3.1.2 Nanoantifungal of zinc oxide layers screening using infrared spectrum
The zinc oxide layers showed a band at 400-500 cm⁻¹ as a result of vibration at the metal ZnO bond (Figure 2).

3.1.3 Nanohybrid antifungal (Gri-ZnO) screening using infrared spectrum
Figure 3 shows that the Gri-ZnO complex has formed which is indicated by a substantial reduction of the bands at the frequencies 3396.76 cm⁻¹ and 2943.47 cm⁻¹. This indicates proton substitution in the formation of the Gri-ZnO complex. The two bands at 1618.33 and 1707.06 cm⁻¹ are retained since the carbonyl groups are unchanged in the reaction. The bands at 428.21 and 439.78 cm⁻¹ are also retained since the stretch on the metal bond in Zn-O is still there [15].

3.2 Diagnosis using X-ray diffraction spectrum
The x-ray diffraction spectrum for nanohybrid Gri-ZnO and ZnO layers was studied to know the difference in the layer thickness before and after the hybridisation of griseovulvin and ZnO. Figure 4 shows the ZnO x-ray diffraction spectrum of level 100 at 31.29° angle for crystal distance (d) equal 0.281 nm and the level 002 at the 34.82° angel for distance equal to 0.259 nm. While the level 101 at 36.29° for crystal distance equal to 0.247 nm [16]. Also the ion exchange between the ZnO and the antifungal griseovulvin were done and the results shown five diffractions at the levels 204, 212, 111,102and 101 of Griseovulvin in addition to ZnO diffraction levels. These results confirmed that ZnO still kept its natural state and the antifungal griseovulvin is encapsulated between the ZnO layers as shown in Figure 5.
3.3 Atomic force microscopy (AFM) screening

The surface morphology of the nanohybrid Gri-ZnO was studied. Figure 6a shows two dimensional photos of the molecular clusters of semi-spherical shapes of antifungal Gri-ZnO. While Figure 6b shows three dimensional photos of a section of surface of the nanohybrid Gri-ZnO. This shows molecular grouping increases with limits of 1.47 nm. This result confirmed the presence of a new product, the nanohybrid antifungal from the free griseovulvin and ZnO layers.

The molecular size range of nanohybrid antifungal Gri-ZnO is almost 81.85 nm. Preparation of the nanohybrid antifungal gave molecules with diameter ranging from 20 – 100nm (20, 25, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100nm) with corresponding percentages (1.72, 5.75, 2.87, 4.02, 1.15, 4.02, 2.30, 2.87, 1.15, 5.75, 5.75, 4.60, 6.32, 4.02, 4.02% respectively). The crystallinity index of the nanohybrid antifungal was found to be 1.75 according to data in Table 1 and XRD spectrum (Figure 5). The current study agreed with another study [15], where the prepared spherical shape of the nanohybrid molecules size (50-76nm) is similar.

<table>
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<tr>
<th>Isolate No</th>
<th>Disease</th>
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<th>7</th>
<th>6</th>
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<td></td>
<td></td>
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<tr>
<td></td>
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<td></td>
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</table>

| Isolates average | 14.47 | 11.08 | 14.58 | 15.02 | 10.44 | 15.00 | 12.08 | 0.00 | 8.88 | 9.61 | 9.52 | 5.76 | Isolates average |

* The vertically different capitals indicate significant differences (p <0.05) between the compounds.
* The different small letters horizontally indicate significant differences (p <0.05) between fungal isolates.

<table>
<thead>
<tr>
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</table>

* The different capitals clearly indicate significant differences (p <0.05) between the concentrations used.
4.3 Inhibition activity of nanohybrid antifungal Gri-ZnO against dermatophyte isolates

The inhibition activity against 12 dermatophyte isolates (numerated from 1 to 12) was studied in vitro. The dermatophyte isolates included six strains of Trichophyton mentagrophytes, which were isolated from three patients suffering from Tinea corporis (isolates No. 1, 5 and 6) and three patients suffering from Tinea faciei, Tinea manuum and Tinea capitis (isolates No. 2, 3 and 4 respectively). Also three strains of Microsporum canis were isolated from patients suffering from Tinea capitis (isolate No. 7) and Tinea corporis (isolates No. 8 and 9). Three strains of Epidermophyton floccosum were isolated from patients suffering from Tinea cruris, Tinea unguium and Tinea pedis (isolates No. 10, 11 and 12 respectively) as shown in Table 2.

The statistical analyses of antimicrobial activity demonstrated that there was a significant differences ($p < 0.05$) between the fungal isolates. The isolates numbered 6, 10 and 12 were the most susceptible to the studied compound, with significant differences between the isolates. Zones of inhibition were 15.00, 14.47 and 14.58 mm respectively. While fungal isolates numbered 1, 2, 3, 8, 9 and 7 were less susceptible to the treatment, no significant differences between the isolates ($p>0.05$) was measured. However, isolate number 4 showed high resistance toward both the free griseovulvin and the hybrid Gri-ZnO as shown in table 3.

References

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