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Anti-dermatophytic activities and time-kill kinetics of the methanol extracts of *Napoleona imperialis*

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Abstract:

Background: Dermatophytosis is one of the most common cutaneous infections in the world. This is a superficial fungal infection that pose public health challenges to man and animals. The objective of this study is to evaluate the anti-dermatophytic activities of the different parts of *Napoleona imperialis* extract against selected clinical dermatophytes isolated from school children in Anambra State, Nigeria.
Methodology: The pulverized materials of the authenticated plant parts were extracted using cold maceration method for 48 hours in methanol. Stock solutions of the extracts were prepared by dissolving 1000mg of the extract in 2ml of dimethylsulphoxide (DMSO) to obtain a final concentration of 500mg/ml, that was used to primarily screen the plant extracts for their anti-dermatophytic activities on the selected dermatophytes by the agar well diffusion method. For determining the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), stock solution of the plant extracts was prepared by dissolving 10000mg of the extract in 2 ml of DMSO to attain a final concentration of 5,000 mg/ml, which was used to prepare different concentrations of the extract by 2-fold serial dilution. The extracts were then tested on selected dermatophytes consisting of two isolates of *Trichophyton mentagrophytes*, one isolate of *Microsporum audouinii,* one isolate of *Microsporum canis*, and two isolates of *Microsporum ferrugineum*. The time kill kinetics was also performed using standard method.
Results: The mean (±SD) inhibition zone diameter produced by the stem bark extract against the different dermatophytes at concentration of 500mg/ml was significantly higher than all other plant extracts used ($p<0.05$) with *M. ferrugineum* as the most susceptible. The MICs and MFCs were the same for the leaf, root, stem bark and seed extracts of the plant, which ranged from 31.25-250 mg/ml for *M. audouinii*; 62.5-250 mg/ml for *M. canis,* and 0.978-62.5 mg/ml for *M. ferrugineum* 2. Similarly, the MICs and MFCs were the same for the root extract of the plant against *T. mentagrophyte* 2 (62.5 mg/ml), stem bark extract against *T. mentagrophyte* 1 (3.912 mg/ml) and against *T. mentagrophyte* 2 (1.956 mg/ml). The seed extract against *T. mentagrophyte* 1 and against *M. ferrugineum* 1 also had the same MIC and MFC (15.625 mg/ml). In the time -kill assay, there was drastic reduction in the number of viable cells count within 0-1 hour, and at 8-hour time period, there were no viable cells.
Conclusion: Crude methanol extracts of *N. imperialis* stem bark exhibited higher fungicidal effect when compared to the other plant parts. This extract can be a complementary source of novel antifungal agents. The study provides evidence for the use of this plant in traditional settings for the treatment of dermatophytosis

Keywords: *Napoleona imperialis;* anti-dermatophytic; minimum inhibitory concentration; minimum fungicidal concentration; time-kill kinetics

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Activités anti-dermatophytiques et cinétique de destruction temporelle des extraits méthanoliques de *Napoleona imperialis*

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Résumé:

Contexte: La dermatophytose est l'une des infections cutanées les plus courantes au monde. Il s'agit d'une infection fongique superficielle qui pose des problèmes de santé publique pour l'homme et les animaux. L'objectif de cette étude est d'évaluer les activités anti-dermatophytiques des différentes parties de l'extrait de *Napoleona*

imperialis contre des dermatophytes cliniques sélectionnés isolés chez des écoliers de l'État d'Anambra, au Nigéria.
Méthodologie: Les matières pulvérisées des parties de plantes authentifiées ont été extraites à l'aide de la méthode de macération à froid pendant 48 heures dans du méthanol. Des solutions mères d'extraits ont été préparées en dissolvant 1000mg de l'extrait dans 2ml de diméthylsulfoxyde (DMSO) pour obtenir une concentration finale de 500mg/ml, qui a été utilisée principalement pour tester les extraits de plantes pour leurs activités anti-dermatophytiques sur les dermatophytes sélectionnés par la méthode de diffusion en puits d'agar. Pour déterminer la concentration minimale inhibitrice (CMI) et la concentration minimale fongicide (CMF), une solution mère d'extraits de plantes a été préparée en dissolvant 10000 mg de l'extrait dans 2ml de DMSO pour atteindre une concentration finale de 5000mg/ml, qui a été utilisée pour préparer différentes concentrations de l'extrait par dilution en série de 2 fois. Français Les extraits ont ensuite été testés sur des dermatophytes sélectionnés constitués de deux isolats de *Trichophyton mentagrophytes*, d'un isolat de *Microsporum audouinii*, d'un isolat de *Microsporum canis* et de deux isolats de *Microsporum ferrugineum*. La cinétique de la destruction temporelle a également été réalisée à l'aide d'une méthode standard.
Résultats: Le diamètre moyen (± ET) de la zone d'inhibition produite par l'extrait d'écorce de tige contre les différents dermatophytes à une concentration de 500mg/ml était significativement plus élevé que tous les autres extraits de plantes utilisés (p<0,05) avec *M. ferrugineum* comme le plus sensible. Les CMI et les CMF étaient les mêmes pour les extraits de feuilles, de racines, d'écorce de tige et de graines de la plante, qui variaient de 31,25 à 250mg/ml pour *M. audouinii*; 62,5-250 mg/ml pour *M. canis* et 0,978-62,5mg/ml pour *M. ferrugineum* 2. De même, les CMI et les CFM étaient les mêmes pour l'extrait de racine de la plante contre *T. mentagrophyte* 2 (62,5 mg/ml), l'extrait d'écorce de tige contre *T. mentagrophyte* 1 (3,912mg/ml) et contre *T. mentagrophyte* 2 (1,956 mg/ml). L'extrait de graine contre *T. mentagrophyte* 1 et contre *M. ferrugineum* 1 avait également les mêmes CMI et CFM (15,625mg/ml). Dans le test de temps de destruction, il y a eu une réduction drastique du nombre de cellules viables dans un délai de 0 à 1 heure, et après 8 heures, il n'y avait plus de cellules viables.
Conclusion: Les extraits bruts au méthanol de l'écorce de la tige de N. imperialis ont montré un effet fongicide supérieur à celui des autres parties de la plante. Cet extrait peut être une source complémentaire de nouveaux agents antifongiques. L'étude fournit des preuves de l'utilisation de cette plante dans des contextes traditionnels pour le traitement de la dermatophytose.

Mots clés: *Napoleona imperialis*; anti-dermatophytique; concentration minimale inhibitrice; concentration fongicide minimale; cinétique de temps de destruction

Introduction:

Dermatophytes are the fungal pathogens of humans and animals infecting the keratinized tissues which are most likely found in hot humid areas (1). They belong to the genera *Microsporum, Trichophyton, Epidermophyton* (anamorphic state) and the genus Arthroderma (telemorphic state). The main aetiological agents in man are *T. rubrum, T. tonsurans, T. mentagrophytes* complex, *M. canis, M. gypseum* and *E. floccosum* (2). Fungal infections are included in the most difficult diseases to manage in humans (3). Fungal pathogens have been mostly neglected by the public and public health officials (4). Children are particularly susceptible to dermatophyte infections because of their poor personal hygiene habits and poor environmental sanitation (5).

Dermatophyte was the second most frequent encountered aetiological agent in the nine-year study conducted by Ayanbimpe et al., (6) and the predominant dermatophyte specie isolated was *T. mentagrophyte*. The occurrence of dermatophytosis caused by *Trichophyton, Epidermophyton* or *Microsporum* specie (7) has increased significantly especially among immunocompromised patients (8, 9). Dermatophyte infection affects up to 25% of people globally with higher rates observed in Africa and Asia (10). Antifungal resistant dermatophyte has recently emerged as a global public health concern (11).

The antifungal potential of medicinal plants and their secondary metabolites against different types of fungal pathogens have been widely studied (12). The World Health Organization (WHO) has stated that medicinal plants would be the best source of a variety of novel drugs (13).

*Napoleona imperialis (*P. beauv) is the common *Napoleona* in Nigeria. They are trees or shrubs rarely more than 6mm high, branching low down and with very dense crown (14). According to Odeyemi et al., (15) the phytochemical composition of *N. imperialis* showed that the stembark contains alkaloid, steroid, terpenoid, flavonoid, saponin, tannin, cardiac glycoside, phenol, phytate and hydrogen cyanide. The methanol extract of *N. imperialis* has shown antibacterial and wound healing properties (16). The leaves contain pharmacologically active compounds with antidiarrhoeal activities which may be the reason for its anti-diarrhoeal application in traditional medicine (17). The methanolic extract of the leaves possess anti-inflammatory effect (18). The plant also contains nutritive ingredients, minerals, vitamins and antioxidants. (19).

The objective of this study is to evaluate the anti-dermatophytic activities of the different parts of *N. imperialis* extracts against selected clinical dermatophytes isolated from school children in Anambra State, Nigeria.

Materials and method:

Study design:

This was an evaluation study of the anti-dermatophyte activity and time kill kine-

tics of the methanol extracts of the different plant parts (leaves, stem barks, roots and seeds) of *N. imperialis* against selected dermatophytes; two isolates of *Trichophyton mentagrophytes*, one isolate of *Microsporum audouinii,* one isolate of *Microsporum canis*, and two isolates of *Microsporum ferrugineum* collected from Primary School pupils (consent obtained from their parents) in Anambra State, Nigeria.

Ethical approval:

Ethical approval to conduct the study was obtained from Nnamdi Azikiwe University Teaching Hospital (NAUTH) Ethical Committee.

Plant collection, identification and preparation:

The plant was identified and authenticated as *N. imperialis* by a botanist at the Botany Department of Nnamdi Azikiwe University, Awka, Nigeria. Fresh mature leaves, stem barks, roots and seeds of *N. imperialis* were collected and dried under shade separately. They were pulverized separately and stored in brown bottles in dry environment until ready for extraction.

Extraction:

Different quantities; 60g, 55g, 90g and 120g of the pulverized materials of *N. imperialis* leaves, roots, stem barks and seeds respectively were extracted using cold maceration method for 48 hours in methanol. The methanol extracts were collected and concentrated almost to dryness (MeOH fraction) under vacuum at 45 ± 5^0C using rotary evaporator. The fraction obtained were stored at 4^0C.

Preparation of stock solutions:

Stock solutions of the extracts were prepared by dissolving 1,000mg of the extract in 2mL of DMSO to obtain a final concentration of 500mg/ml for preliminary screening of the anti-dermatophytic activities of the extracts. For determining the MICs and MFCs of the extracts, stock solution of the plant extract was prepared by dissolving 10,000mg of the extract in 2ml of DMSO to attain a final concentration of 5000mg/ml. These were transferred to a screw capped bottle and stored at 4°C.

Test organisms:

Six previously identified clinical dermatophytes from primary school pupils (consent obtained from parents) in Anambra State, Nigeria were used for the study. These consist of two isolates of *T. mentagrophytes*, one isolate of *M. audouinii*, one isolate of *M. canis*, and two isolates of *M. ferrugineum*. These organisms were maintained by monthly subculturing on Sabouraud dextrose agar (SDA) media at 25-27°C.

Inoculum standardization of test organisms:

All test isolates were inoculated onto SDA plates and incubated at 25°C for 7–10 days to obtain young, actively growing cultures consisting of mycelia and conidia. A mycelial disc, 5mm in diameter, cut from 7–10-day-old cultures, was aseptically inoculated into tubes containing Sabouraud dextrose broth. The tubes were incubated at 25°C for 2-3 days. After incubation, the tubes were placed on a vortexing machine and vortexed for about 15-20 min to properly disperse the cells in the broth.

The concentration of organisms in the tubes was standardized by adjusting to a concentration of about 10^4 CFU/ml from 10^8 CFU/ml already produced from standardizing to 0.5 MacFarland standard. Dimethylsulphoxide was used as control in time kill kinetics, and does not have any dermatophyte in it.

Primary screening of extracts for anti-dermatophyte activity:

The anti-dermatophyte activities of the extracts were determined by the agar well diffusion method as described by Onyegbule et al., (20) with little modifications. Concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, and 15.125mg/ml were prepared from the 500mg/ml stock solution of the extracts by doubling dilution of the extracts. Twenty millimetres of molten SDA was poured into sterile Petri dishes (90 mm) and allowed to set. Standardized inoculum (10^4 CFU/ml) of the test isolates grown in Sabouraud dextrose broth were swabbed aseptically on the agar plates and holes (6mm) were made in the agar plates using a sterile metal cork-borer.

A 20µl volume of the various concentrations of each extract and the controls were put in each hole under aseptic condition, kept at room temperature for about 30 minutes to allow the extracts to diffuse into the agar medium and incubated accordingly. Ketoconazole (50µg/ml) was used as the positive control, while dimethylsulphoxide (DMSO) was used as the negative control. The plates were then incubated at room temperature for 24-28 hours and the inhibition zones diameters (IZDs) were measured and recorded. This procedure was conducted in triplicate and the mean IZDs calculated and recorded.

Determination of minimum inhibitory concentration of the extracts on test isolates:

The MIC of the plant extract on the test isolates was determined by agar dilution method using the method described by Ali-Shtayeh and Abu Ghdeib (21). The stock solution (5000 mg/mL) was further diluted in a 2-fold serial dilution to obtain the following concentrations; 2500, 1250, 625, 312.5, 156.25, 78.26, 39.12, 19.56 and 9.78mg/ml. Agar plates were prepared by pouring 9ml of molten double strength SDA into sterile Petri plates containing 1ml of the various dilutions of the

extract making the final plate concentrations of 500, 250, 125, 62.5, 31.25, 15.625, 7.826, 3.912, 1.956 and 0.978mg/ml respectively.

The test isolates (grown overnight in broth) were adjusted to 0.5 McFarland standard and streaked onto the surface of the agar plates containing the extracts. The SDA plates were incubated at room temperature (25-27^{o}C) for 5-7 days, after which all plates were observed for growth. Control culture plates, which contained no plant extract, were also made with the test. The minimum concentration (highest dilution) of the extracts completely inhibiting the growth of each organism was taken as the MIC.

Determination of minimum fungicidal concentrations of the extracts on test isolates:

The MFC of the extracts was derived by sub-culturing portions of the agar from plates that showed no growth in the tests for determination of MICs as described by Ali-Shtayeh and Abu Ghdeib (21). These agar portions were transferred into plates containing freshly prepared SDA and incubated at 25-27^{o}C for 5-7 days and then observed daily for mycelial growth. The absence of growth at the end of incubation period signifies total cell death. The minimum concentration of the extracts that produces total cell death is taken as the MFC.

Determination of fungicidal activities of the extracts on test isolates by time-kill kinetics:

Determination of the fungicidal activities of the extracts on test isolates by time-kill kinetics was achieved using the method described by Adeniyi et al., (22) with little modifications. Standardized inoculum (10^4CFU/ml) of the logarithmic phase culture of test isolates were prepared. An appropriate quantity of the extract was added to a sterile test tube containing Sabouraud Dextrose Broth, and 1ml of the standardized test culture was added to 9ml of the extract-broth mixture to give a microbial concentration 10^3 CFU/ml and a concentration equal to the MFC of the extract.

Sterile molten SDA was poured into sterile Petri plates and allowed to set. 0.1mL of the extract-broth-culture mixture was put onto the agar and spread with a sterile spreader. This is to give control time 0 minutes count. Samples were taken after 1, 4, 8, and 24 hours intervals for subcultures on SDA agar. The procedure was carried out in triplicate to ensure accuracy. Plates were incubated at 25-27^{o}C for 5-7days and visible colonies were counted. For controls, one strain each of *M. ferrugineum, M. audouinii*, and *M. canis* was grown in tubes containing broth with no added plant extract and samples taken at the indicated time intervals. Control culture plates were also incubated. The numbers of colony forming units (CFU) were counted after the period of incubation.

Data analysis:

Analysis of variance (ANOVA) was used to compare the mean inhibition zone diameter produced by the different plant part extracts against the selected dermatophytes. This was done using Statistical Packages for the Social Sciences (SPSS) version 26.0. The level of significance was set at $p<0.05$.

Results:

Anti-dermatophytic activities of the extracts and control antifungal:

The positive control, ketoconazole (50 µg/ml), produced mean inhibition zone diameter of 36mm to *M. ferrugineum* strain 1, 32mm to *M. ferrugineum* strain 2, 28mm to *T. mentagrophyte* strain 1, 30mm to *T. mentagrophyte* strain 2, 10mm to *M. audouinii*, and 8mm to *M. canis.* The negative control used (DMSO) produced no mean inhibition zone diameter against all the selected dermatophytes. The mean inhibition zone diameters produced to all the extracts and the ketoconazole control was highest for *M. ferrugineum*, followed by *T. mentagrophyte*, *M. audouinii* and *M. canis* (Figs 1-4).

The mean (±SD) inhibition zone diameter among different plant part extracts (*N. imperialis*) at 500mg/ml concentration against the selected dermatophytes is shown in Table 1. At this concentration, the extracts from the stem barks exhibited significantly higher anti-dermatophytic activity against all the selected dermatophytes (*T. mentagrophytes* 1 and 2, *M. ferrugineum* 1 and 2, *M. audouinii* and *M. canis*) compared to the extracts from the leaf, root, and seed ($p<0.001$).

Comparing the antimicrobial activities of the different plant extracts against each other on *T. mentagrophyte* (1 and 2) and *M. ferrugineum* (1 and 2), there were no significant differences in the mean (± SD) inhibition zone diameters between the leaf and the seed extracts but there were significant differences between other plant parts against each other showing that leaf and seed extracts produced significantly higher inhibition zone diameters (higher activity) than the root extract, and the stem bark extract produced significantly higher activity than the leaf (A v C), root (B v C) and seed (C v D) extracts ($p<0.05$). Invariably, the stem bark extract produced the highest inhibition zone diameters (highest anti-dermatophytic activity) among the plant extracts (Table 1).

For *M. audouinii* there was no significant difference in the mean inhibition zone diameter between the leaf and root extracts (A v B), and between the root and seed extracts

(B v D). However, there were significant differences in anti-dermatophytic activity across the other plant parts against the dermatophytes; leaf extract was significantly higher than seed extract (A v D) and stembark extract was significantly higher than leaf (A v C), root (B v C) and seed (C v D) extracts ($p<0.05$).

For *M. canis*, there were no significant difference in the anti-dermatophytic activity between the leaf and root extracts (A v B), leaf and seed extracts (A v D), root and seed extracts (B v D), but the stembark extract had significantly higher activity ($p<0.05$) than the leaf (A v C), root (B v C) and seed (C v D) extracts (Table 1).

Minimum inhibitory and fungicidal concentrations of the plant extracts:

The MICs and MFCs of the leaf, root, stem bark and seed extracts of *N. imperialis* were the same for *M. audouinii*; 125mg/ml, 250mg/ml, 31.25mg/ml and 250mg/ml respetively: for *M. canis*; 250 mg/ml, 250 mg/ml, 62.5 mg/ml, and 250 mg/ml respectively and for *M. ferrugineum* strain 2; 15.625 mg/ml, 62.5 mg/ml, 0.978 mg/ml and 15.625 mg/ml respectively.

The MICs and MFCs were the same for the root extracts of *N. imperialis* against *T. mentagrophyte* 2 (62.5 mg/ml), stem bark extracts against *T. mentagrophyte* 1 (3.906 mg/ml) and *T. mentagrophyte* 2 (1.956 mg/ml) (Table 2). The MICs and MFCs were also the same for the seed extracts against *T. mentagrophyte* 1 (15.625 mg/ml) and *M. ferrugineum* 1 (15.625 mg/ml).

However, for the other dermatophytes, the MICs and MFCs of the extracts were not the same. From Table 2, the leaf extract had MIC of 15.625 mg/ml and MFC of 31.25 mg/ml against *T. mentagrophytes* 1 and 2. The root extract had MIC of 62.5 mg/ml and MFC of 125 mg/ml against *T. mentagrophytes* 1, and MIC of 31.25 mg/ml and MFC of 62.5 mg/ml against *M. ferrugineum* 1. The seed extract had MIC of 15.625 mg/ml and MFC of 31.25 mg/ml against *T. mentagrophytes* 2.

The fungicidal concentrations of the extracts against *M. audouinii* and *M. canis* were higher when compared to *Trichophyton* species. *M. ferrugineum* was highly inhibited by the stem bark extract. Extract of *N. imperialis* stem bark exhibited a higher fungicidal effect when compared with other plant part extracts.

Results of the time-kill assay:

The results of the time-kill assay are presented in Tables 3-6 and control in Table 7. Changes in the $\log_{10}$ CFU/ml of the test isolates indicated that the extracts exhibited significant fungicidal activities evident in the rate of reduction in the viable count of isolates in relation to time. At 1-hour contact time with the extract, the viable count of the fungi isolates drastically reduced, and at 4-8 hours, the reduction was minimal but clearly evident while at 24 hours contact time, there was total (100%) kill of the test isolates. With the control, the isolates were still multiplying at 8th hour (Table 7).

Discussion:

Dermatophytes were reported earlier to respond well to antifungal agents, but due to an upsurge in resistance with high cost of the antifungal agents, there was increased use of medicinal plants for the treatment of dermatophytes (23). The extracts of leaves, roots, stem barks and seeds of *N. imperialis* and the control antifungal agent (ketoconazole) inhibited the growth of *M. ferrugineum* the most with the screening method on agar well diffusion, followed by *T. mentagrophyte* while *M. audouinii* and *M. canis* were not inhibited at the concentration of 15.625 mg/l. However, all the dermatophyte species were inhibited at a higher concentration of 500mg/l. The stem bark extracts of *N. imperialis* showed higher antimicrobial effect on the selected dermatophytes when compared with the other plant part extracts ($p<0.05$). In the study by Fowora et al., (23), aqueous extracts of *Anilotica* leaves showed good anti-dermatophyte effects against all dermatophytes tested which included *A. otae, A. verperitilii*, *A. quadritidum A. multifidum*, *T. interdigitale, T. mentagrophyte,* and *M. ferrugineum.*

In our study, methanol extract of *N. imperialis* demonstrated fungicidal effect against all the fungal isolates tested. The stem bark extract of *N. imperialis* showed higher inhibitory effect on the selected dermatophytes when compared with the other plant part extracts. Extracts of *N. imperialis* leaves, roots, stem barks and seeds have the same inhibitory concentrations with fungicidal concentrations against *M. audouinii* and *M. canis.* Where minimum inhibitory concentration is the same with minimum fungicidal concentration, it indicates that the plant has a great therapeutic potential.

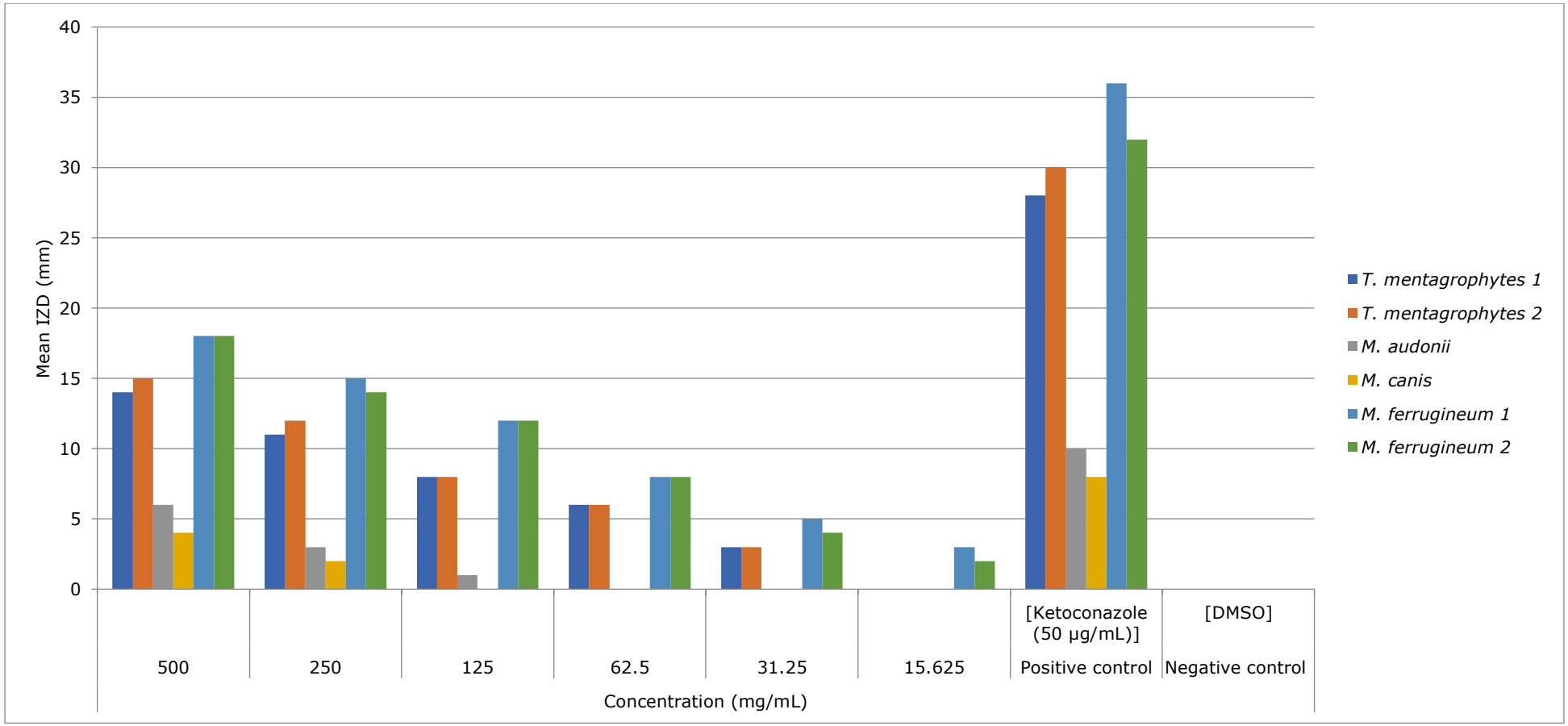

Fig 1: Mean inhibition zone diameters in millimeters (mm) produced by methanol extract of *Napoleona imperialis* leaf and the controls against various dermatophytes

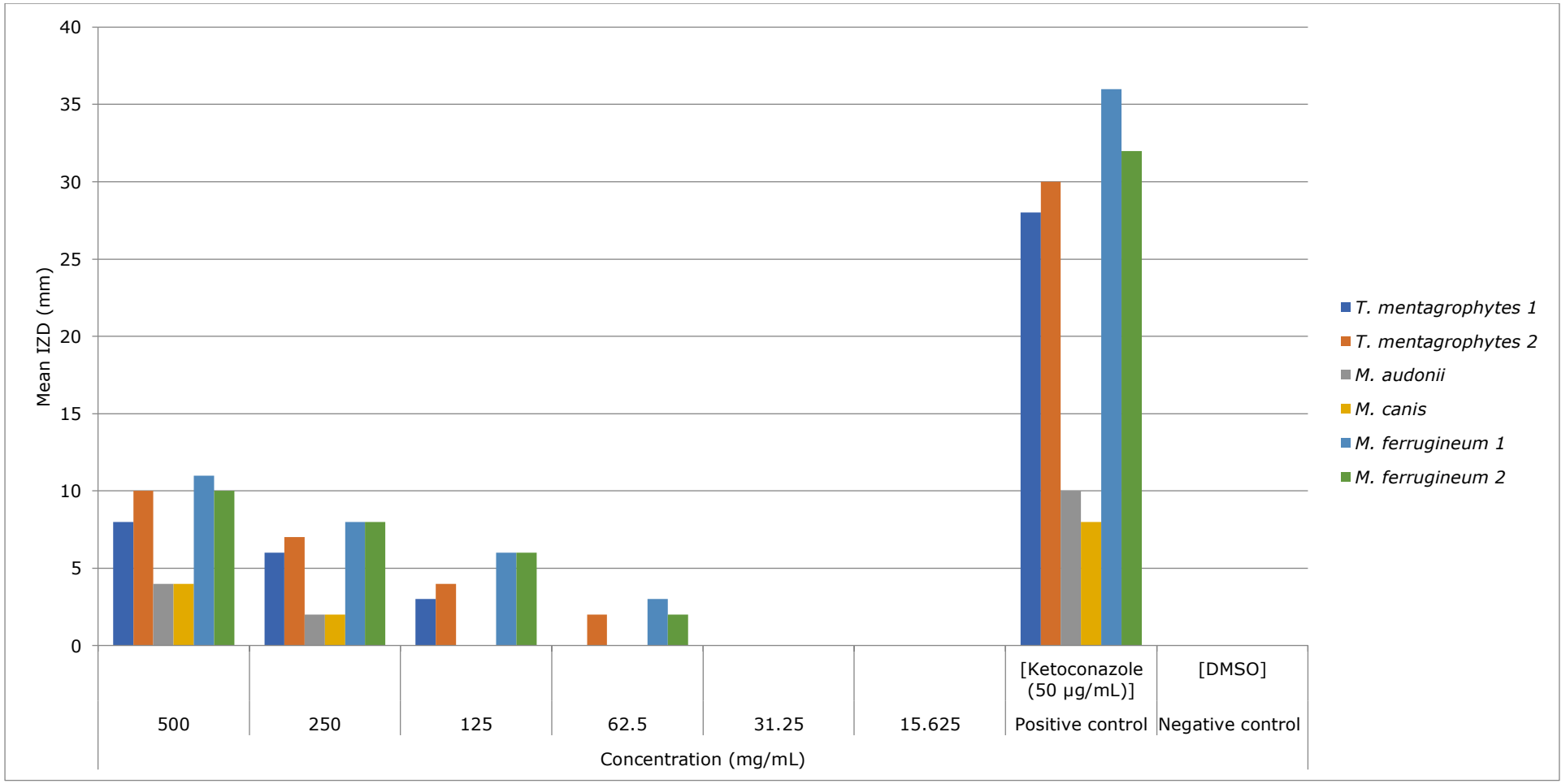

Fig 2: Mean inhibition zone diameters in millimeters (mm) produced by methanol extract of *Napoleona imperialis* root and the controls against various dermatophytes

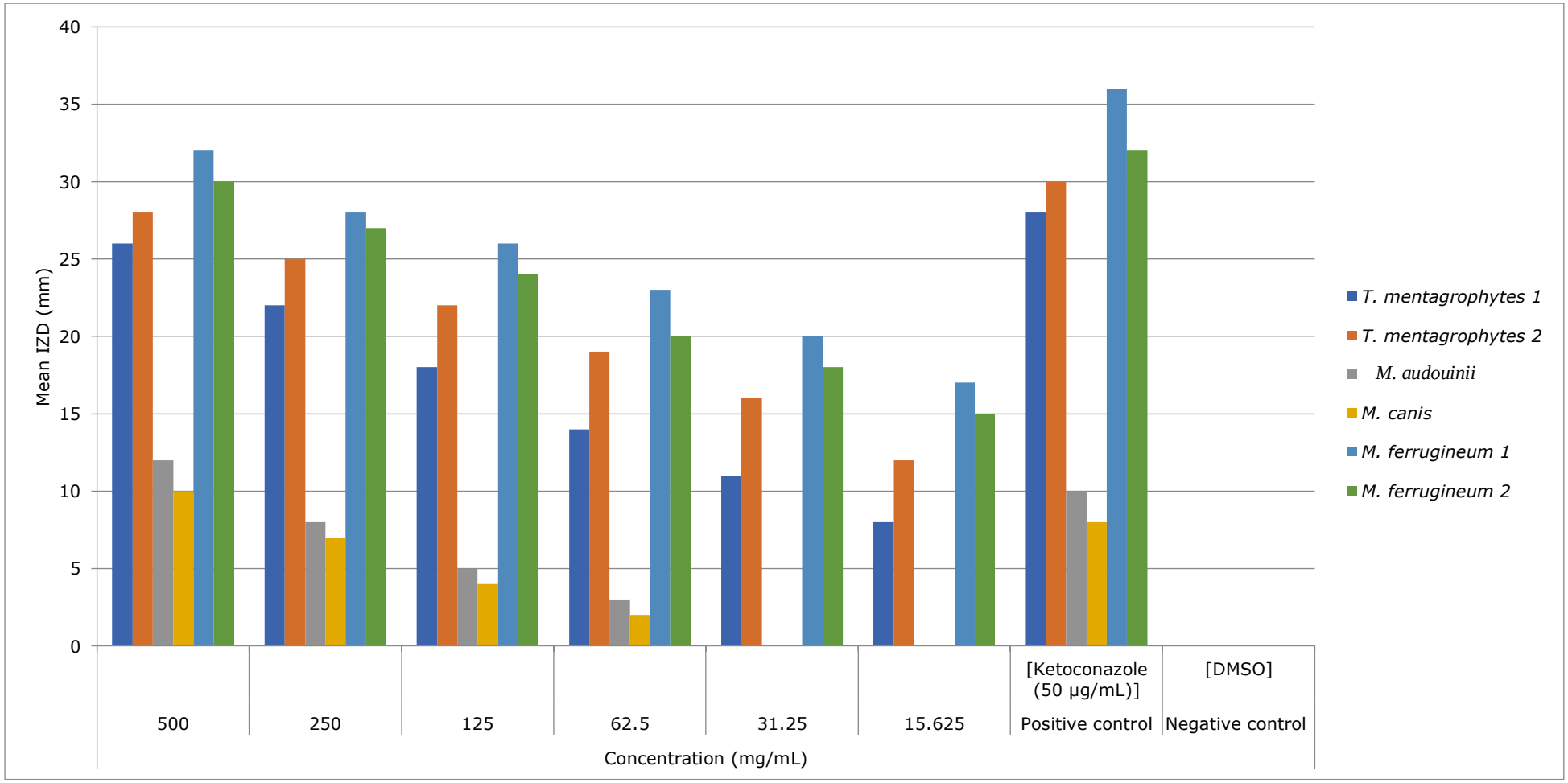

Fig 3: Mean inhibition zone diameters in millimeters (mm) produced by methanol extract of *Napoleona imperialis* stem bark and the controls against various dermatophytes

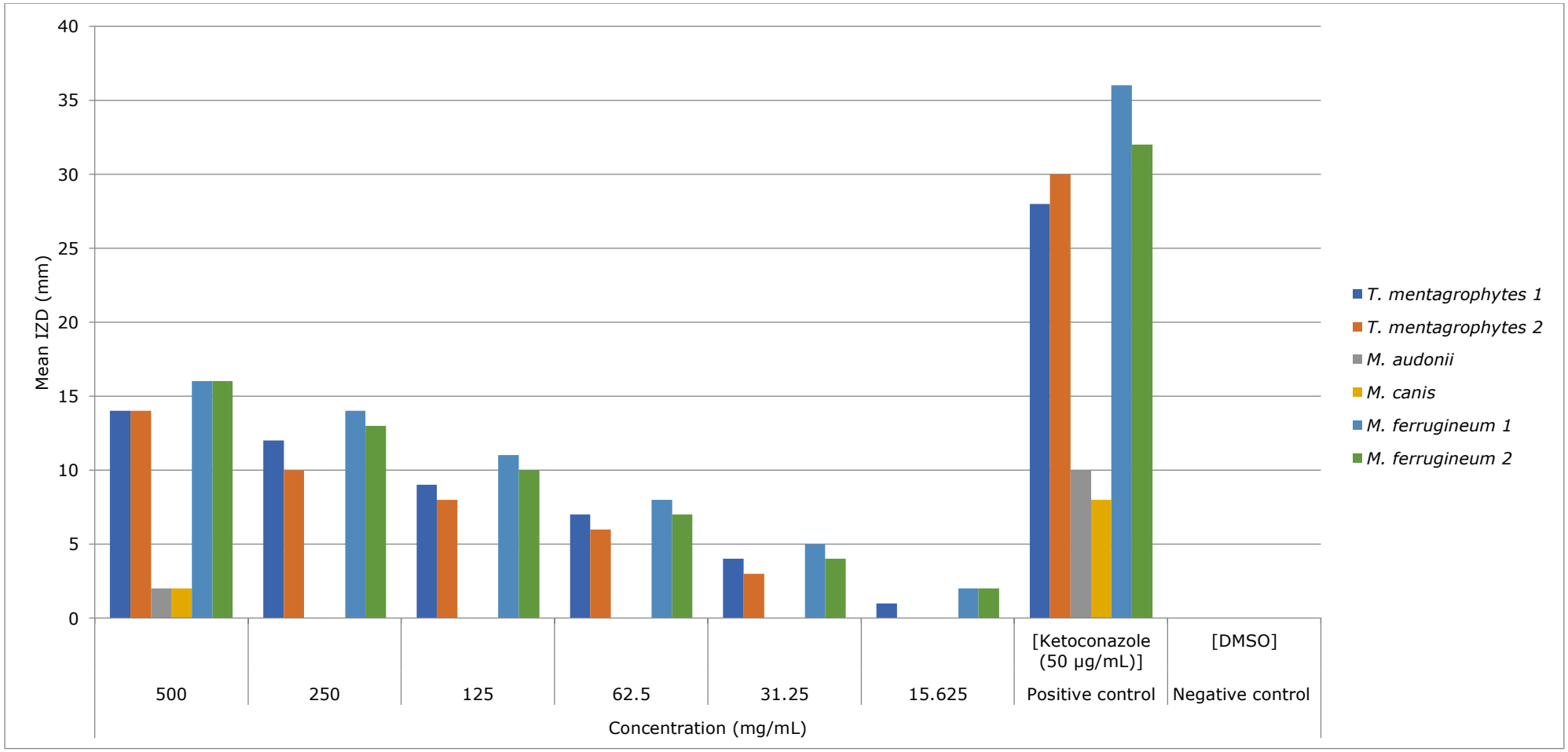

Fig 4: Mean inhibition zone diameters in millimeters (mm) produced by methanol extract of *Napoleona imperialis* seed and the controls against various dermatophytes

Table 1: Comparison of mean inhibition zone diameter among different plant extract at 500mg/ml

Category	*T. mentagrophytes 1*	*T. mentagrophytes 2*	*M. audouinii*	*M. canis*	*M. ferrugineum 1*	*M. ferrugineum 2*
Leaf extract (A)	14.00±1.00	15.00±1.00	6.33±0.57	4.00±1.00	18.33±0.57	18.00±1.00
Root extract (B)	8.00±1.00	10.33±0.57	4.33±0.57	4.33±0.57	11.00±1.73	10.00±1.00
Stem bark extract (C)	26.00±2.00	27.67±0.57	12.00±2.00	10.00±2.00	32.67±1.15	30.00±1.00
Seed extract (D)	13.67±0.57	13.67±0.57	2.33±0.57	2.33±0.57	16.33±0.57	16.00±1.00
F-value	108.684	345.778	41.667	23.608	205.578	211.000
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A vs B	0.002	<0.001	0.359	1.000	<0.001	<0.001
A vs C	<0.001	<0.001	0.002	0.002	<0.001	<0.001
A vs D	1.000	0.298	0.014	0.748	0.359	0.240
B vs C	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
B vs D	0.003	0.003	0.359	0.442	0.002	<0.001
C vs D	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Statistically significant at $p<0.05$

Table 2: Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of *Napoleona imperialis* extracts on test isolates

Test isolates	MICs of the various plant extracts (mg/ml)				MFCs of the various plant extracts (mg/ml)			
	Leaf	Root	Stem bark	Seed	Leaf	Root	Stem bark	Seed
T. mentagrophytes strain 1	15.625	62.5	3.912	15.625	31.25	125	3.912	15.625
T. mentagrophytes strain 2	15.625	62.5	1.956	15.625	31.25	62.5	1.856	31.25
M. audounii	125	250	31.25	250	125	250	31.25	250
M. canis	250	250	62.5	250	250	250	62.5	250
M. ferrugineum strain 1	15.625	31.24	0.978	15.625	15.625	62.5	0.978	15.625
M. ferrugineum strain 2	15.625	62.5	0.978	15.625	15.625	62.5	0.978	15.625

Table 3: Time-kill assay of the fungicidal activity of methanol extract of *Napoleona imperialis* leaf against the dermatophytes

Test isolates	**Concentration (MFC) mg/ml**	**Incubation period (hours)**	**0**	**1**	**4**	**8**	**24**
T. mentagrophytes strain 1	31.25	Viable Cell Count ($x10^4$CFU/mL)	292	91	12	0	0
T. mentagrophytes strain 2	31.25		328	121	25	0	0
M. audouinii	125		243	68	8	0	0
M. canis	250		321	92	18	0	0
M. ferrugineum strain 1	15.625		333	101	23	0	0
M. ferrugineum strain 2	15.625		214	84	14	0	0

Table 4: Time-kill assay of the fungicidal activity of methanol extract of *Napoleona imperialis* root against the dermatophytes

Test isolates	**Concentration (MFC) mg/ml**	**Incubation period (hours)**	**0**	**1**	**4**	**8**	**24**
T. mentagrophytes strain 1	125	Viable Cell Count ($x10^4$CFU/mL)	201	88	18	0	0
T. mentagrophytes strain 2	62.5		322	119	26	0	0
M. audouinii	250		188	66	12	0	0
M. canis	250		224	105	20	0	0
M. ferrugineum strain 1	62.5		328	134	35	0	0
M. ferrugineum strain 2	62.5		295	92	23	0	0

Table 5: Time-kill assay of the fungicidal activity of methanol extract of *Napoleona imperialis* stem bark against the dermatophytes

Test isolates	**Concentration (MFC) mg/ml**	**Incubation period (hours)**	**0**	**1**	**4**	**8**	**24**
T. mentagrophytes strain 1	3.912	Viable Cell Count ($x10^4$CFU/mL)	240	121	29	0	0
T. mentagrophytes strain 2	1.956		322	99	38	0	0
M. audouinii	31.25		202	84	26	0	0
M. canis	62.5		188	75	2	0	0
M. ferrugineum strain 1	0.978		154	60	8	0	0
M. ferrugineum strain 2	0.978		168	65	13	0	0

Table 6: Time-kill assay of the fungicidal activity of methanol extract of *Napoleona imperialis* seed against the dermatophytes

Test isolates	**Concentration (MFC) mg/ml**	**Incubation period (hours)**	**0**	**1**	**4**	**8**	**24**
T. mentagrophytes strain 1	15.625	Viable Cell Count ($x10^4$CFU/mL)	315	122	38	0	0
T. mentagrophytes strain 2	31.25		282	82	11	0	0
M. audouinii	250		257	87	8	0	0
M. canis	250		256	88	10	0	0
M. ferrugineum strain 1	15.625		372	156	24	0	0
M. ferrugineum strain 2	15.625		266	91	18	0	0

Table 7: Time-kill assay of the control (SDA agar with no extract) against the dermatophytes

Test isolates	**Incubation period (hours)**	**0**	**1**	**4**	**8**	**24**
T. mentagrophytes strain1	Viable Cell Count (x104CFU/mL)	353	728	1222	1748	2386
T. mentagrophytes strain 2		421	879	1325	1932	2658
M. audouinii		242	644	1080	1620	2324
M. canis		286	682	1040	1688	2232
M. ferrugineum strain 1		341	779	1125	1871	2654
M. ferrugineum strain 2		222	642	1082	1620	2128

The time-kill kinetic study exhibited a significant reduction in the cell count at 0-1 hour, and within 1-4 hours and 4-8 hours, the reduction in viable count was minimal, but at 8 hours, there were no viable cells, indicating that the plant has fungicidal properties.

Conclusion:

Treatment of dermatophyte infections which are among the commonly diagnosed skin diseases in Africa with medicinal plants can be a complementary source of antifungal treatment. This study showed that stem bark of *N. imperialis* exhibited higher fungicidal activity against *M. ferrugineum* and *T. mentagrophyte* than against *M. audouinii* and *M. canis.* With no viable cells at 8hours in the time-kill kinetic assay for all the tested dermatophytes, our study showed that extracts of the different parts of *N. imperialis* have great potential in the treatment of dermatophytosis, the property confirms the usage of the plant in traditional setting in the treatment of dermatophytosis.

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Contribution of authors:

OO and EIB contributed to the conception and design of the study. Material preparation and analysis were performed by OO. The whole work was supervised by EIB. The first draft of the manuscript was written by OO and proofread by EIB. Both authors commented on previous versions of the manuscript, read and approved the final manuscript.

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The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References:

1. Nweze, E. I., and Okafor, J. I. Dermatophytosis in Western Africa. A review. Pakistan J Biol Sci. 2010; 13: 649-659. doi:10.3923/pjbs.2010.649.656
2. Arenas, R., del Rocío Reyes-Montes, M., Duarte-Escalante, E., Frías-De-León, M. G., and Martínez-Herrera, E. Dermatophytes and Dermatophytosis. In: Mora-Montes, H., and Lopes-Bezerra, L. (eds) Current Progress in Medical Mycology. Springer, Cham. 2017. https://doi.org/10.1007/978-3-319-64113-3_13
3. Savarirajan, D., Ramesh, V. M., and Muthaiyan, A. Invitro anti-dermatophytic activity of bioactive compounds from selected medicinal plants. J Anal Sci Technol. 2021; 12:53. https://doi.org/10.1186/s40543-021-00304-3
4. Rodrigues, M. L., and Nosanchuk, J. D. Fungal diseases as neglected pathogen: a wake-up call to public health officials. PLoS Negl Trop Dis. 2020; 14 (2): e 0007964. https://doi.org/10.1371/journal.pntd.0007964
5. Enweani, I. B., Ozan, C. C., Agbonlahor, E. E., and Ndip, R. N. Dermatophytosis in school children in Ekpoma, Nigeria. Mycoses. 1996; 39: 303-305. https://doi.org/10.1111/j.1439-0507.1996.Tb00143x
6. Ayanbimpe, G. M., Enweani, I. B. and Solomon, E. G. Fungal infections in Jos: A 9-year-Study. Afr J Clin Exper Microbiol. 2003; 4 (2): doi:10.4314/ajcem.v4i2.7302
7. Weitzman, I., and Summerbell, R. C. The dermatophytes. Clin Microbiol Rev. 1995; 8: 240-259. doi:10.1128/CMR.8.2.240
8. Bergg, J. C, Hamacher, K. L., and Roberts, G. D. Pseudomycetoma caused by *Microsporum canis* in an immunocompromised patient: a case report and review of the literature. J Cutaneous Pathol. 2007; 34: 431-434. doi:10.1111/j.1600-0560.2006.00628.x.
9. Nir-Paz, R., Helinaav, H., Pierard, G. E., et al. Deep Infection by *Trichophyton rubrum* in an immunocompromised patient. J Clin Microbiol. 2003; 41: 5298-5301. doi:10.1128/jcm.41.11.5298-5301.2003
10. Ortiz, B., Ballesteros-Monrreal, M. G, Rosales-Tamashiro, J., Bush, M., Salmanton-García, J., and Fontecha, G. Global Insights and Trends in Research on Dermatophytes and Dermatophytosis: A Bibliometric Analysis. Mycoses. 2024; 67 (10): e13803, doi:10.1111/myc.13803
11. Gold, J., Benedict, K., Lockhart, S. R., et al. Recognition of Antifungal-Resistant Dermatophytosis by Infectious Diseases Specialists, United States. Emerg Infect Dis. 2024; 30 (9): 1978-1980 doi:10.3201/eid3009.240118
12. Chahal, R., Nanda, A., Akkol, E. K, et al. *Ageratum conyzoides* L and it's secondary metabolites in the management of different fungal pathogens. Molecules. 2021; 26 (10): 2933.doi:10.3390/molecules26102933
13. Ezeonu, C. S., Tatah, V. S., Imo, C., et al. Inhibitory effect of aqueous and ethanol extracts of neem parts on fungal rot disease of Solanum tuberosum. Pakistani J Biolog Sci. 2019; 22 (5): 206-213.doi:10.3923/pjbs.2019.206.213
14. Ajuru, M. G. Anatomical and Polynological Studies on *Napoleona imperialis* P. Beauv. (Lecythidaceae). Sci Rev. 2018; 4 (2): 16-21.
15. Odeyemi, O., Onochie, A. U., Odeyemi, S. O., and Oshim, I. O. Evaluation of qualitative and quantitative phytochemical constituents of *Napoleona imperialis* stem bark. GSC Biol Pharmaceut Sci. 2023; 24 (01): 202-208 doi:10.30574/gscbps.2023.24.1.0246
16. Esimone, C. O., Ibezim, E. C., and Chah, K. F. The wound healing effect of herbal ointments formulated with *Napoleona imperialis*. J Pharma ceut Allied Sci. 2005; 3: 294-299 doi:10.4314/jophas.v3i1.34994
17. Kelechi, C. O., Omeh, Y. N., Emmanuel, U., and Ofoezie, R. C. Phytochemical Composition and Anti-Motility Effects of *Napoleona imperialis* Methanol Crude Leaf Extract and Fractions in Rats. J Complem Altern Med Res. 2023; 22 (3): 8-16.doi:10.9734/jocamr/2023/v22i3458
18. Ben, I. O., Etim, O. E., and Udo, N. M. Anti-inflammatory effects of *Napoleona imperialis* P. Beauv. (Lecythidaceae) on rat model of inflammation. Indian J Health Sci Biomed Res. 2016; 9(1):89-95.doi:10.4103/2349-5006.183686
19. Odeyemi, O., Onochie A. U., Ifeanyi, O. O., Odeyemi, S. O., Nwaka, A. C. and Chioma, M. O. Evaluation of Proximate compositions, Vitamins, Micro and Macro elements of *Napoleona imperialis* stem bark. World J Biol Pharm Health Sci. 2023;14 (02): 028-036. doi:10.30574/wjbphs.2023.14.2.0192
20. Onyegbule, A. F., Anowi, C. F., Gugu, T. H., and Uto-Nedosa, A. U. Evaluation of antimicrobial properties of ethyl acetate extract of the leaves of *Napoleona imperialis* Family Lecythiaceae. Int J Drug Res Technol. 2011; 1 (1): 45-51.
21. Ali-Shtayeh, M. A., and Abu Ghdeib, S. I. Antifungal activity of plant extracts against dermatophytes. Mycoses. 1999; 42(11-12): 665-672.doi:10.1046/j.1439-0507.1999.00499.x
22. Adeniyi, B. O., Obasi, O.J., and Lawal, T. O. *Invitro* antifungal activity of *Distemonanthus benthamianus* stem. Int J Pharm Pharmaceut Sci. 2011; 3 (4): 52-56
23. Fowora, M., Onyeaghasiri, F., Olanlege, A., Edu-Muyideen, I., and Adebesin, O. *In vitro* Susceptibility of Dermatophytes to Anti-Fungal Drugs and Aqueous *Acacia nilotica* Leaf Extract in Lagos Nigeria. J Biomed Sci Eng. 2021; 14: 74-82. doi:10.4236/jbise.2021.142008