Odeyemi & Enweani-Nwokelo. *Afr. J. Clin. Exper. Microbiol.* 2025; 26 (4): xxxx https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Oct 2025; Vol.26 No.4
AJCEM/2437. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Phytochemical screening, *in vitro* anti-fungal activities, and time-kill kinetics of methanol extracts of *Nauclea latifolia* against selected dermatophytes

*[1]Odeyemi, O., and [2]Enweani-Nwokelo, I. B.

[1]Faculty of Medical Laboratory Science, Achievers University, Owo, Nigeria
[2]Faculty of Medical Laboratory Sciences, Nnamdi Azikiwe University, Awka, Nigeria
*Correspondence to: yemiode@yahoo.com; Tel: +2347032971869

Abstract:

Background: Dermatophytosis is a highly contagious superficial mycosis of the genera *Trichophyton, Epidermophyton* and *Microsporum* that affects both man and animal. The different plant parts of *Nauclea latifolia* are known to have ethnomedicinal uses. The objective of this study is to access the phytochemical components of *N. latifolia* plant parts and to investigate the anti-fungal activities and time-kill kinetics of the plant parts against isolated dermatophyte species.
Methodology: The pulverized parts (root, stem, leaf, fruit) of authenticated *N. latifolia* plant were extracted using cold maceration method for 48 hours in methanol. The extracts were then concentrated almost to dryness and the phytochemical constituents assessed. The preliminary screening of the anti-dermatophytic activities of the extracts was determined by the agar disc diffusion technique on selected dermatophytes, consisting of two isolates of *Microsporum ferrugineum* (strains 1 and 2), two isolates of *Trichophyton mentagrophytes* (strains 1 and 2), one isolate of *Microsporum audouinii* and one isolate of *Microsporum canis.* The minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and time-kill kinetics of the extracts were determined using standard methods on the selected dermatophytes.
Results: The pulverized stem bark of the plant had higher yield of 12.73% when compared to other plant parts (leaf; 4.44%, root; 1.53%, and fruit; 4.16%). All extracts of the plant parts contain flavonoids and tannins, with the stem bark and fruit extracts containing tannins in higher concentrations. The leaf and root extracts of the plant contained saponin, but this is absent in the stem bark and fruit extracts. There were no zone diameters of inhibition produced by different concentrations of the root and leaf extracts against the selected dermatophytes while the mean zone diameters of inhibition produced by the stem bark and fruit extracts was appreciably low (range 2.0±00-10±2.00mm) at 500mg/ml of the extracts. The MICs and MFCs were the same for stem bark and fruit extracts at 62.5mg/ml for *T. mentagrophyte* strain 2 and *M. ferrugineum* strain 2, and at 250mg/ml for *M. audouinii*. Similarly, the MICs and MFCs were the same for the stem bark extracts at 125mg/ml for *T. mentagrophyte* strain 1. In the time-kill assay, reduction in the count of the viable cells occurred within 0-1 hour, and at 8 hours, there were no viable cells.
Conclusion: Crude methanol extracts of *N. latifolia* stem bark and fruit exhibited fungicidal activity against the selected dermatophytes, albeit at high concentration above what is considered to indicate potential efficacy of an extract for further therapeutic exploration. Nevertheless, the time-kill assay result holds promise that the stem bark and fruit extracts of the plant potentially possess anti-dermatophytic activity that could justify the plant use for treating dermatophyte infections.

Keywords: Dermatophyte, phytochemicals, methanol extraction, time-kill kinetics, *Nauclea latifolia*

Received Jul 6, 2025; Revised Sep 9, 2025; Accepted Sep 10, 2025

Criblage phytochimique, activités antifongique *in vitro* et cinétique de destruction temporelle des extraits méthanoliques de *Nauclea latifolia* contre certains dermatophytes

*[1]Odeyemi, O., et [2]Enweani-Nwokelo, I. B.

[1]Faculté des Sciences de Laboratoire Médical, Université Achievers, Owo, Nigéria
[2]Faculté des Sciences de Laboratoire Médical, Université Nnamdi Azikiwe, Awka, Nigéria
*Correspondance a: yemiode@yahoo.com; Tél.: +2347032971869

Résumé:

Contexte: La dermatophytose est une mycose superficielle très contagieuse des genres *Trichophyton*, *Epidermophyton* et *Microsporum* qui touche aussi bien l'homme que l'animal. Les différentes parties de la plante *Nauclea latifolia* sont connues pour leurs usages ethnomédicaux. L'objectif de cette étude est d'identifier les composants phytochimiques de parties de *N. latifolia* et d'étudier leurs activités antifongique et leur cinétique d'élimination contre des espèces de dermatophytes isolées.
Méthodologie: Les parties pulvérisées (racine, tige, feuille, fruit) de *N. latifolia* authentifiée ont été extraites par macération à froid pendant 48 heures dans du méthanol. Les extraits ont ensuite été concentrés presque jusqu'à siccité et leurs constituants phytochimiques ont été évalués. Le criblage préliminaire des activités anti-dermatophytiques des extraits a été déterminé par la technique de diffusion sur disque d'agar sur des dermatophytes sélectionnés, constitués de deux isolats de *Microsporum ferrugineum* (souches 1 et 2), de deux isolats de *Trichophyton mentagrophytes* (souches 1 et 2), d'un isolat de *Microsporum audouinii* et d'un isolat de *Microsporum canis*. La concentration minimale inhibitrice (CMI), la concentration minimale fongicide (CMF) et la cinétique de tuer le temps des extraits ont été déterminées à l'aide de méthodes standard sur les dermatophytes sélectionnés.
Résultats: L'écorce de tige pulvérisée de la plante présentait un rendement supérieur de 12,73% à celui des autres parties de la plante (feuilles; 4,44%, racines; 1,53% et fruits; 4,16%). Tous les extraits de parties de la plante contiennent des flavonoïdes et des tanins, les extraits d'écorce de tige et de fruits contenant des concentrations plus élevées de tanins. Les extraits de feuilles et de racines de la plante contenaient de la saponine, mais celle-ci est absente dans les extraits d'écorce de tige et de fruits. Français Il n'y avait pas de diamètres de zone d'inhibition produits par les différentes concentrations des extraits de racines et de feuilles contre les dermatophytes sélectionnés tandis que les diamètres moyens de zone d'inhibition produits par les extraits d'écorce de tige et de fruit étaient sensiblement faibles (plage de 2,0±00 à 10±2,00 mm) à 500mg/ml des extraits. Les CMI et les CMF étaient les mêmes pour les extraits d'écorce de tige et de fruit à 62,5mg/ml pour la souche 2 de *T. mentagrophyte* et la souche 2 de *M. ferrugineum*, et à 250mg/ml pour *M. audouinii*. De même, les CMI et les CMF étaient les mêmes pour les extraits d'écorce de tige à 125mg/ml pour la souche 1 de *T. mentagrophyte*. Dans le test de tuer le temps, la réduction du nombre de cellules viables s'est produite dans un délai de 0 à 1 heure, et à 8 heures, il n'y avait plus de cellules viables.
Conclusion: Des extraits méthanoliques bruts d'écorce de tige et de fruit de *N. latifolia* ont montré une activité fongicide contre les dermatophytes sélectionnés, bien qu'à une concentration supérieure à celle considérée comme indiquant l'efficacité potentielle d'un extrait pour une exploration thérapeutique ultérieure. Néanmoins, les résultats du test de temps de destruction laissent entrevoir une activité anti-dermatophytique potentielle des extraits d'écorce de tige et de fruit de la plante, justifiant ainsi son utilisation pour le traitement des infections dermatophytiques.

Mots-clés: Dermatophyte, composés phytochimiques, extraction au méthanol, cinétique de temps de destruction, *Nauclea latifolia*

Introduction:

Dermatophyte fungal diseases are part of the frequently occurring infectious diseases in the universe and amongst the most commonly diagnosed skin infections in Africa in which the dermatophyte spectrum and the clinical symptoms are completely different from the ones seen in other parts of the world, probably because African environment is hot and humid giving it the main reason for the high prevalence (1). They are contagious and are caused by group related fungi in the genera of *Trichophyton, Microsporum* and *Epidermophyton* (2). Infection with any of the disease-causing dermatophyte species result in dermatophytosis and affects both man and animal with grave economic impacts (3,4).

Resistance and the after effects from the usage of common topical and systemic antifungal medications for dermatophytosis emphasizes the necessity for novel therapies (5). *Nauclea latifolia* is a multi-stemmed evergreen woody plant that can reach a height of 200m, commonly found in the Savannah forests and wet tropical rainforest zones of West and Central Africa (6). The phytochemical components displayed positive results in healing many diseases through different processes such as antioxidative, anti-secretory, anti-inflammatory, anti-ulcerogenic and anti-hypertensive effects (7).

Phytochemicals are substances in plants that occur naturally and play an important role in defense mechanisms to protect them from diseases (8). *Nauclea latifolia* has served as herbal medicine for treating different ailments and diseases (9). Bekoe et al., (10) reported that the usage of *N. latifolia* in treating viral diseases, hypertension, stomach pain, helminthiasis, back pain, diabetes, fever, cancer, gastric ulcer, malaria, measles, diarrhoea and conjunctivitis have been well established. It is therefore, imperative to assess the anti-fungal properties of *N. latifolia* extracts on dermatophytes, with the aim of providing alternative therapeutics of dermatophytosis.

Materials and method:

Study area and design:

The study was conducted at Nnamdi Azikiwe University, Awka, Anambra State, Nigeria as an evaluation of the qualitative phytochemical constituents of methanol extracts of *N. latifolia* plant parts (leaf, stem bark, root and fruit), and determination of *in vitro* anti-dermatophyte activity and time kill kinetics of the extracts against selected dermatophytes obtained from dermatophytic lesions of primary school pupils (with consent from their parents).

Ethical approval:

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

Plant collection, identification, preparation and extraction:

Nauclea latifolia plant was identified and authenticated by a Botanist at Botany Department of Nnamdi Azikiwe University, Awka, Nigeria. Fresh mature leaves, roots, stem barks and fruits of the plant were collected and separately dried under shade. They were pulverized separately and stored in brown bottles in dry environment until ready for extraction. Pulverized materials of *N. latifolia* leaves (170 g), roots (130g), stem barks (150g), fruits (143g), were extracted using cold maceration method for 48 hours in methanol.

Methanol extracts of the plant parts were collected, and then concentrated almost to dryness (MeOH fraction) under vacuum at 45 ± 5^0C using rotary evaporator. The resultant fractions were separately stored at 4^0C. The crude methanol extracts were screened for phytochemical constituents. The percentage yield was calculated using the formula as shown below; percentage yield = weight of extract/weight of pulverized materials of *N. latifolia* x 100 (11).

Phytochemical analysis:

Qualitative phytochemical analysis was carried out using the methods of Trease and Evans (12) to determine the presence of the different phytochemicals in the leaves, roots, stem barks and fruits of the tested plant.

Preparation of stock solution:

The stock solutions were prepared by dissolving 1000mg of the plant part in 2 ml of DMSO for a final concentration of 500 mg/ml to be obtained and used for the preliminary screening of the anti-dermatophyte activities of the extracts. This was done for each of the plant part extract. For determining the MICs, stock solution of the extract of plant was prepared by dissolving 10,000 mg of the extract in 2 ml of DMSO to obtain a final concentration of 5000mg/ml. These were transferred to screw capped bottles and kept at 4°C until ready for use.

Test dermatophyte organisms:

These were dermatophyte organisms (six isolates) previously identified from clinical samples collected from primary school pupils whom their parents consented, and consist of two isolates of *Microsporum ferrugineum*, two isolates of *Trichophyton mentagrophytes*, one isolate of *Microsporum audouinii* and one isolate of *Microsporum canis.* These organisms were maintained by monthly sub-culturing on Sabouraud Dextrose Agar (SDA) at 25-27°C.

Inoculum standardization of test organisms:

All the test isolates were inoculated onto SDA plates and incubated for 7–10 days at a temperature of 25°C to obtain young, actively growing cultures consisting of mycelia and conidia. Mycelial disc of 5 mm in diameter, cut from the periphery of the 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. Following 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 organism concentration in the tubes was standardized by adjusting to concentration which was about 10^4 CFU/ml from 10^8 CFU/ml already produced from standardizing to 0.5 MacFarland standard. The control used in time kill kinetics was dimethylsulphoxide which did not contain any dermatophyte.

Preliminary testing of the extracts for anti-dermatophyte action:

The preliminary testing of the extracts for anti-dermatophyte activities was carried out by the agar well diffusion procedure using Onyegbule et al., (13) with slight modifications. Dilutions of 250, 125, 62.5, 31.25, and 15.125mg/ml were prepared from the 500mg/ml stock solution of the extracts by doubling dilution of the extracts. Molten SDA (20ml) was poured into sterile petri dishes (90 mm) and left to gel. The aseptically prepared agar plates with holes (6 mm) made in them using a sterile metal cork-borer were swabbed with standardized concentrations (10^4 CFU/ml) of cultures of test isolates grown in Sabouraud dextrose broth.

A 20µl volume of the different dilutions of each extract and the controls were put in each hole under aseptic condition and kept at room temperature for about 30 minutes to allow the extracts and controls to diffuse into the agar medium and incubated accordingly. Ketoconazole (50µg/ml) was utilized as positive control and dimethylsulphoxide (DMSO) as the negative control. The plates were incu-

bated for 24-28 hours at room temperature for fungal isolates and the inhibition zones diameters (IZDs) were measured and recorded. The cork borer (6mm) size was subtracted from the figures recorded for the IZDs to get the actual figure. The process was conducted for three times and the mean IZDs calculated and recorded.

Determination of MIC of extracts on isolates:

The MIC of the extract of the plant parts on test isolates was carried out by agar dilution method using the method described by Ali-Shtayeh and Suheil (14). The extract stock solution (5000 mg/mL) was further diluted in a 2-fold serial dilution to obtain the following extract concentrations; 2500, 1250, 625, 312.5, 156.25, 78.26, 39.12, 19.56 and 9.78mg/ml. The agar plates were made by pouring 9ml of the molten double strength SDA into sterile Petri dishes that contained 1 ml of the different dilutions of the extract to make the final extract concentrations of 500, 250, 125, 62.5, 31.25, and 15.625, 7.826, 3.912, 1.956 and 0.978 mg/ml respectively.

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

Determination of MFCs of extracts on isolates:

The MFC of the extracts was carried out by subculturing portions of the agar from the plates that showed no growth in the MIC tests as described by Ali-Shtayeh and Suheil (14). The agar parts were plated into plates containing freshly prepared plain SDA which were incubated at room temperature (25-27^{o}C) for 5-7 days and checked every day for fungal growth. The absence of growth at the conclusion of incubation period implies total cell death. The minimum concentration of the extracts that produced total cell death was considered as the MFC.

Determination of fungicidal action of extracts on isolates by time-kill kinetics:

Determination of fungicidal action of the extracts on test isolates by time-kill kinetics was performed using the procedure of Adeniyi et al*.,* (15) with slight modifications**.** Standardized concentrations (10^4 CFU/mL) of 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 9 ml 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. The procedure was done in triplicate to ensure accuracy. The agar plates were incubated at 25-27^{O}C for 5-7days and observable colonies were counted. Each of the organisms was also grown in tubes containing broth with no added plant extract (control) and samples taken at the indicated time intervals and treated. Control plates were similarly incubated. The numbers of colony forming units (CFU) were counted after the period of incubation.

Data analysis:

The Student's *t*-test was used to compare the mean inhibition zone diameter produced by the plant part extracts against the dermatophytes, with the Statistical Packages for the Social Sciences (SPSS) version 26.0. The level of significance was set at $p<0.05$.

Results:

Extract yield of pulverized *N. latifolia* plant:

The percentage extract yield of the pulverized *N. latifolia* stembark was highest at 12.73%, followed by leaf (4.44%), fruit (4.16%) and roots (1.53%) (Table 1).

Phytochemical constituents of *N. latifolia* plant extracts:

Tannin was observed to be in high quantities in stem bark and fruit but moderate in root and leaf. Flavonoid was present in high quantities in all the plant parts (leaf, root, stem bark and fruit). Alkaloid, steroid and terpenoid were absent in all the parts of the plant. Saponin was absent in stem bark and fruit but present in moderate quantities in root and leaf (Table 2).

Table 1: Percentage extract yield of the pulverized *Nauclea latifolia* plant parts

Extract	Pulverized materials (g)	Yield (g)	% yield
Leaf	170	7.55	4.44
Root	150	2.29	1.53
Stem bark	130	16.55	12.73
Fruit	143	5.95	4.16

Table 2: Phytochemical constituents of *Nauclea latifolia* plant Extracts.

Phytochemical constituents	Extracts			
	Leaf	Root	Stem bark	Fruit
Alkaloid	-	-	-	-
Saponin	++	++	-	-
Steroid	-	-	-	-
Terpenoid	-	-	-	-
Flavonoid	+++	+++	+++	+++
Tannin	+++	+++	++++	++++

+ = low, ++ = moderate, +++ = high, ++++ = very high

Anti-dermatophyte activities of *N. latifolia* extracts:

The mean (±SD) inhibition zone diameters at different concentrations of the extracts of the plant parts against the selected dermatophytes are shown in Fig 1 for the stem bark and Fig 2 for the fruit. In Fig 1, the crude methanol extract of *N. latifolia* stem bark against the test dermatophytes at the least concentration of 62.5mg/ml, produced mean zone diameter of inhibition of 1 mm to *T. mentagrophyte* strain 2, 3 mm to *M. ferrugineum* strain 1 and 2 mm to *M. ferrugineum* strain 2*.* At 125 mg/ml, it was 2 mm to *T. mentagrophyte* strain 1, 3 mm to *T. mentagrophyte* strain 2*,* 6 mm to *M. ferrugineum* strain 1 and 5 mm to *M. ferrugineum* strain 2.

At concentration of 250 mg/ml, it was 4mm to *T. mentagrophyte* strain 1*,* 6mm to *T. mentagrophyte* strain 2, 9mm to *M. ferrugineum* strain 1 and 8 mm to *M. ferrugineum* strain 2. At the highest concentration of (500 mg/ml,) the extract produced mean zone diameter of 7 mm to *T. mentagrophyte* strain 1, 9 mm to *T. mentagrophyte* strain 2, 3 mm to *M. audouinii*, 2 mm to *M. canis*, 12 mm to *M. ferrugineum* strain 1 and 10 mm to *M. ferrugineum* strain 2.

In Fig 2, the mean inhibitory zone diameters (mm) produced by the crude methanol extract of *N. latifolia* fruits against the test dermatophytes at the least concentration of 62.5 mg/ml, was 2 mm to *T. mentagrophyte* strain 2, 3 mm to *T. ferrugineum* strain 1 and 2 mm to *M. ferrugineum* strain 2. At the concentration of 125 mg/ml, it was 3mm to *T. mentagrophyte* strain 1, 4 mm to *T. mentagrophyte* strain 2, 6 mm to *M. ferrugineum* strain 1 and 5 mm to *T. ferrugineum* strain 2. At the concentration of 250 mg/ml the extracts produced 5 mm to *T. mentagrophyte* strain 1, 7 mm to *T. mentagrophyte* strain 2, 9 mm to *M. ferrugineum* strain 1 and 8 mm to *M. ferrugineum* strain 2.

At the highest concentration of 500 mg/ml, it produced 8 mm to *T. mentagrophyte* strain 1, 10 mm to *T. mentagrophyte* strain 2, 2 mm to *M. audouinii*, 2 mm to *M. canis*, 12 mm to *M. ferrugineum* 1 and 10 mm to *M. ferrugineum* strain 2. The positive control (ketoconazole at 50 µg/ml) produced mean inhibition zone diameter of 28 mm to *T. mentagrophyte* strain 1, 30 mm to *T. mentagrophyte* strain 2*,* 10 mm to *M. audouinii,* 8 mm to *M. canis,* 36 mm to *M*. *ferrugineum* strain 1 and 32 mm to *M*. *ferrugineum* strain 2. The negative control (DMSO) produced no inhibition zone diameter to the selected dermatophytes.

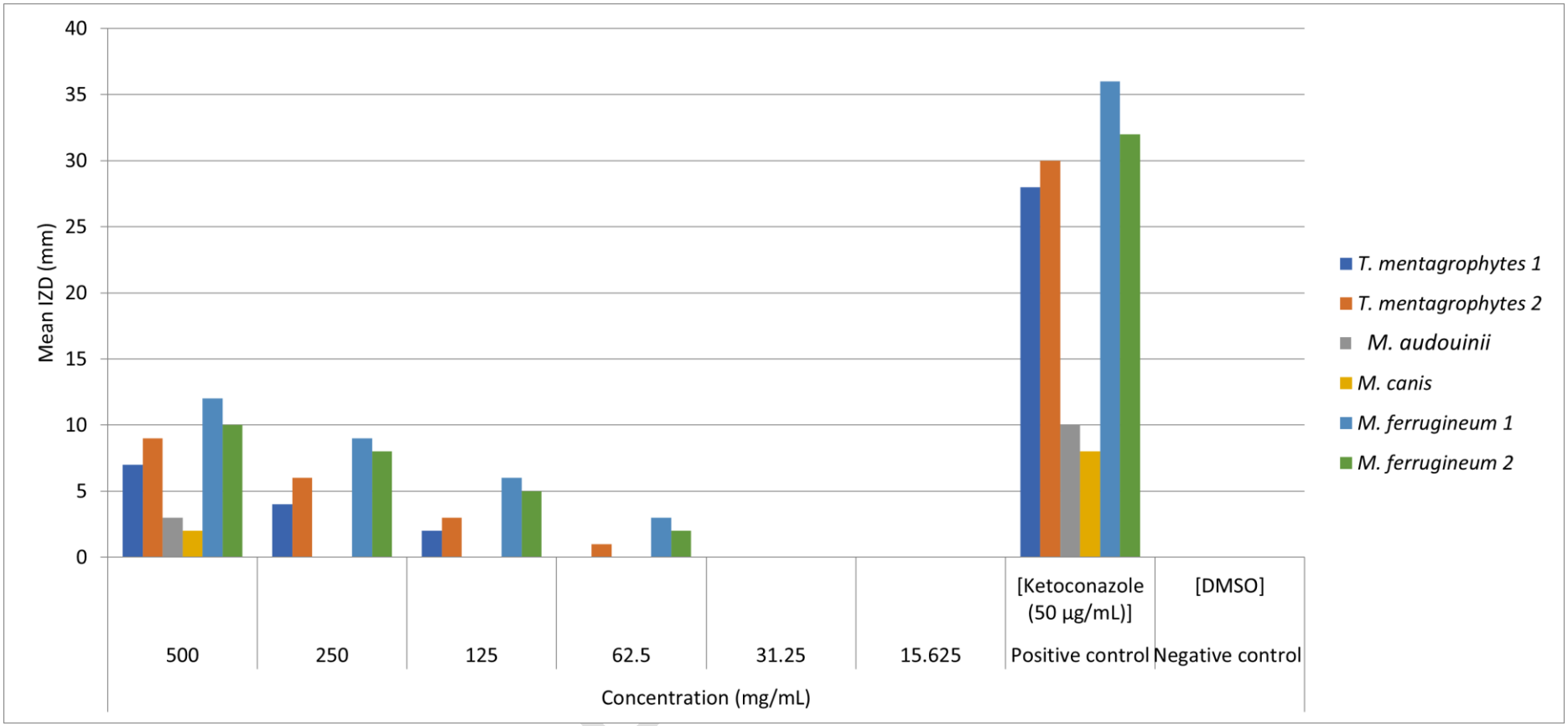

Fig 1: Mean inhibition zone diameters (IZDs) in millimeters (mm) produced by crude methanol extract of *Nauclea latifolia* stem barks and the control against selected dermatophytes

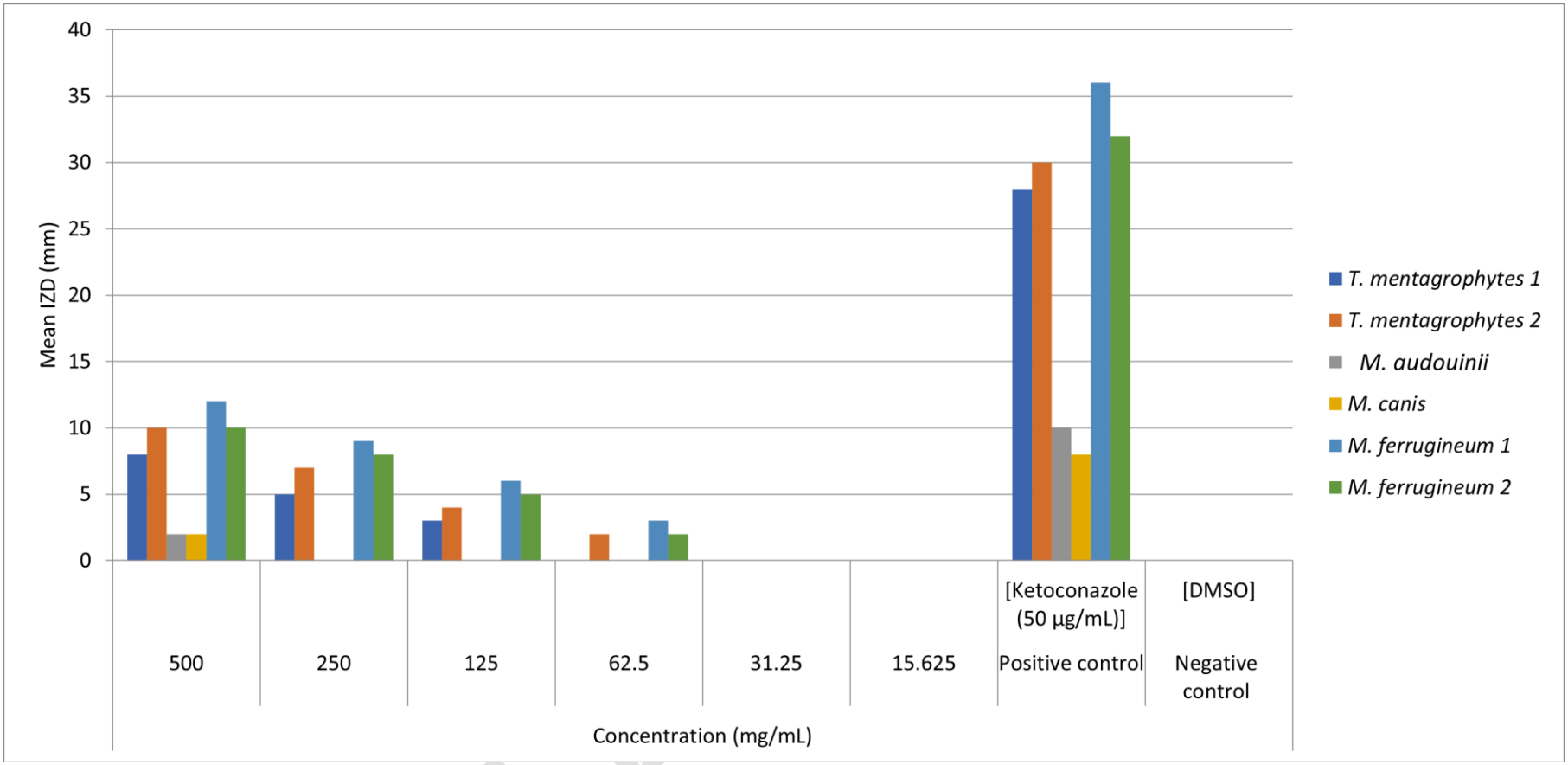

Fig 2: Mean inhibition zone diameters (IZDs) in millimeters (mm) produced by crude methanol extract of *Nauclea latifolia* fruits and the controls against selected dermatophytes

Table 3 shows the comparative mean (±SD) inhibition zone diameter of the stem bark and fruit extracts of the plant at 500 mg/ml concentration (the concentration at which the extracts exhibited some antimicrobial activities against the selected dermatophytes). The results showed that there were no significant differences in the antimicrobial activities of the two plant parts at 500 mg/ml concentration against the selected dermatophytes (*T. mentagrophytes* 1and 2; *M. ferrugineum* 1 and 2; *M. audouinii* and *M. canis*) (p>0.05). The leaf and root extracts were not considered because even at this high concentration, the extracts did not give any zone of inhibition against the dermatophytes.

Minimum inhibitory and fungicidal concentrations of the plant extracts:

The leaf and the root extracts of *N. latifolia* had no antimicrobial activity on all the selected dermatophytes. The minimum inhibitory concentration (MIC) of the stem bark and fruit extracts of *N. latifolia* against *M. audouinii* and *M. canis* was 250 mg/ml while against *T. mentagrophyte* strain 2, the MIC was 62.5 mg/ml. For *M. ferrugineum* strain 1; the MIC was 31.25 mg/ml, and *M. ferrugineum* strain 2 it was 62.5 mg/ml.

The minimum fungicidal concentration (MFC) was the same as the minimum inhibitory concentration (MIC) of the fruit and stem barks extracts against *T. mentagrophyte* strain 2 (62.5mg/ml) and *T. ferrugineum* strain 2 (62.5mg/ml), *T. mentagrophyte* strain 1 stem bark extracts (125mg/ml) and *M. audouinii* stem bark extract (250 mg/ml). The MFC of the stem bark extract against *M. canis* was 500 mg/ml and for *M. ferrugineum* strain 1 was 62.5 mg/ml. The MFC of the fruit extract against *T. mentagrophyte* strain 1 was 125 mg/ml, *M. audouinii* 500 mg/ml, *M. canis* 500 mg/ml and *M. ferrugineum* strain 1 62.5 mg/ml (Table 4).

Table 3: Comparison of mean inhibition zone diameter of the stem bark and fruit extracts at 500mg/ml concentration

Organisms	**Means (±SD) zone diameter (mm) of inhibition**		***t*-test**	***p*-value**
	Stem bark	**Fruit**		
Trichophyton mentagrophytes 1	7.00±1.00	8.00±1.00	-1.225	0.288
Trichophyton mentagrophytes 2	9.00±1.00	10.00±2.00	-0.775	0.482
Microsporum audouinii	3.00±0.00	3.00±1.00	<0.001	1.000
Microsporum canis	2.00±0.00	2.00±0.00		
Microsporum ferrugineum 1	8.67±4.16	12.00±2.00	-1.250	0.279
Microsporum ferrugineum 2	10.00±2.00	10.00±2.00	<0.001	1.000

Table 4: Minimum inhibitory and fungicidal concentrations of the extracts on selected dermatophytes

Test dermatophyte isolates	**MICs of extracts (mg/ml)**		**MFCs of extracts (mg/ml)**	
	Stem bark	**Fruit**	**Stem bark**	**Fruit**
Trichophyton mentagrophytes 1	125	62.5	125	125
Trichophyton mentagrophytes 2	62.5	62.5	62.5	62.5
Microsporum audouinii	250	250	250	500
Microsporum canis	250	250	500	500
Microsporum ferrugineum 1	31.25	31.25	62.5	62.5
Microsporum ferrugineum 2	62.5	62.5	62.5	62.5

MIC = Minimum inhibitory concentration; MFC = Minimum fungicidal concentration

Time kill assay:

Results of the time kill assay shown in Tables 5 and 6 indicated that within 0-1 hour, there was drastic reduction in the number of viable cell count. Within the succeeding 1–4 hours, there was also reduction but at the 8th hour, all the dermatophyte isolates were dead. The control showed an increase in the viable cell count of each of the selected dermatophyte even after the 8th hour (Table 7).

Table 5: Fungicidal action of crude methanol extract of *Nauclea latifolia* stem barks incorporated into Sabouraud dextrose broth against selected dermatophytes

Test dermatophyte isolates	Concentration (mg/ml)	Incubation period (hours)	0	1	4	8	24
Trichophyton mentagrophytes 1	125	Viable Cell Count ($x10^4$CFU/mL)	301	102	32	0	0
Trichophyton mentagrophytes 2	62.5		356	141	20	0	0
Microsporum audouinii	250		246	72	22	0	0
Microsporum canis	500		296	88	16	0	0
Microsporum ferrugineum 1	62.5		322	112	34	0	0
Microsporum ferrugineum 2	62.5		282	198	21	0	0

Table 6: Fungicidal action of crude methanol extract of *Nauclea latifolia* fruits incorporated into Sabouraud dextrose broth against selected dermatophytes

Test dermatophytes isolates	Concentration (mg/ml)	Incubation period (hrs)	0	1	4	8	24
Trichophyton mentagrophytes 1	125	Viable Cell Count ($x10^4$CFU/mL)	264	82	11	0	0
Trichophyton mentagrophytes 2	62.5		291	69	8	0	0
Microsporum audouinii	500		272	91	19	0	0
Microsporum canis	500		265	96	13	0	0
Microsporum ferrugineum 1	62.5		332	121	25	0	0
Microsporum ferrugineum 2	62.5		278	83	16	0	0

Table 7: Viable cell counts of selected dermatophytes in Sabouraud dextrose broth without incorporation of extracts

Test dermatophyte isolates	Incubation period (hrs)	0	1	4	8	24
Trichophyton mentagrophytes 1	Viable Cell Count ($x10^4$CFU/mL)	353	728	1222	1748	2386
Trichophyton mentagrophytes 2		421	879	1325	1932	2658
Microsporum audouinii		242	644	1080	1620	2324
Microsporum canis		286	682	1040	1688	2232
Microsporum ferrugineum 1		341	779	1125	1871	2654
Microsporum ferrugineum 2		222	642	1082	1620	2128

Discussion:

Medicinal plants have since been recognized as notable source for treating variety of diseases and might be regarded as precursor of contemporary medicine (16,17,18). In this study, pulverized *N. latifolia* stem bark yielded the highest extract percentage, followed by the leaf, fruit and root, and contained tannin, flavonoids, and saponin. This result agrees with that of Malami et al., (19) on the leaves extract of *N. latifolia* containing flavonoids, tannins and saponins, and also with the study of Adepoju et al., (20) on the stem bark of *N. latifolia* containing tannin. However, the methanol extracts of the leaf, root, stem bark, and fruit in this study did not contain alkaloids, steroids and terpenoids that were present in the study of Malami et al., (19) and Adepoju et al., (20). If these were present in the extracts in our study, they might have been so minimal such that the crude methanol extraction method we used could not qualitatively detect them. In the study of ethanol and methanol leaf extracts of *N. latifolia* and phytochemical testing of metabolites by Olusola (21), the author did not detect tannin but detected saponin, flavonoid and alkaloid. It could be possible that *N. latifolia* plant part contains alkaloid, flavonoid, saponin, terpenoids, tannins and steroid as reported by Odeyemi et al., (22) in the qualitative phytochemical testing of the methanol stem bark extract of *Napoleona imperialis*.

Although, *N. latifolia* leaves and roots have been acclaimed to possess medicinal and antimicrobial properties, the methanol extracts in our study did not exhibit any antifungal activity or inhibitory properties at all the extract concentrations against the selected dermatophytes in the preliminary agar well diffusion assay. However, extracts of the stem bark and fruit of the plant inhibited the growth of *M. ferrugineum* strains 1 and 2, and *T. mentagrophyte* strain 2 at high extract concentration of 62.5mg/ml (62,500µg/ml), which is 1250 times higher than the control ketoconazole concentration of 50µg/ml, producing zone diameter of inhibition of 1-10mm, compared to the zone diameter produced by ketoconazole of 10-36mm against the selected dermatophytes. At a further higher extract concentration of 500mg/ml (500,000µg/ml), all the selected dermatophytes were inhibited by the extracts of the two plant parts, with mean zone diameter of inhibition that ranged from 2.0±00 to 10.0±2.0mm. At this extract concentration there was no significant difference in the inhibition activity of the methanol extracts of the two plant parts ($p>0.05$) on the selected dermatophytes. The anti-dermatophytic activities of these extracts may therefore appeared to be insignificant for further therapeutic exploration.

The minimum inhibitory concentrations (MICs) of the methanolic extracts of *N. latifolia* stem barks and fruits against the selected dermatophytes ranged from 31.25 mg/ml for *M. ferrugineum* strain 1 to 250 mg/ml for *M. audouinii* and *M. canis*. The MICs of the methanolic extracts of *N. latifolia* stem barks against *T. mentagrophyte* strain 1 and 2 were different, and although they are the same dermatophyte species, their genetic composition might be different, to account for this disparity. This similarly applied to the methanolic extracts of the stem barks and fruit of *N. latifolia* against *M. ferrugineum* strain 1 and 2. It was also observed that the MICs of the methanolic extracts of both the stem barks and fruits of *N. latifolia* were the same against each of the selected dermatophytes except for *T. mentagrophyte* strain 1. Higher concentrations of the methanolic extract of the stem barks and fruits (250-500 mg/ml) were required to completely kill (fungicidal) *M. canis* and *M. audouinii*.

The time-kill kinetic study revealed a progressive reduction in the viable cell count at 0-1 hour, 1-4 hours, and at the 8th hour, there was no viable cell, while with the control, the dermatophytes were still multiplying at the 8th hour. This was a similar observation in the previous study of methanol extracts of various plant parts (stem bark, leaves, seed and root) of *N. imperialis* by Odeyemi et al., (23), which reported anti-fungal activity, with the time-kill kinetics showing no viable dermatophyte at the 8th hour.

Conclusion:

Our study showed that crude methanol extracts of the stem bark and fruits of *N. latifolia* exhibited anti-fungal property, but at a much higher extract MIC values than what is generally considered to indicate potential efficacy of an extract or isolated compound from an extract for further therapeutic exploration (24). Nevertheless, with no viable cells at 8th hour in the time-kill kinetic assay for all the selected dermatophytes, the stem bark and fruit parts of the plant may potentially possess anti-dermatophytic activities that justifies the usage in traditional medicine. In this study however, the methanol extract of the leaf and root parts of the plant do not have any inhibitory activity against the selected dermatophytes.

Acknowledgments:

The authors wish to acknowledge Dr. Peter Eze of the Department of Pharmaceutical Microbiology, Nnamdi Azikiwe University, Awka for his assistance during the experimen-

tal procedures in the laboratory. The parents who obliged us with written informed consent to collect samples from their children are also appreciated.

Contribution of authors:

Both authors contributed to the conception and design of the study. Preparation of the materials and the 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.

Source of funds:

Authors received no external funding

Conflict of interest:

Authors declare no conflict of interest.

Declaration of authors:

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. Delis, J. S., Kazeem, H. M., Chah, K. F., et al. Molecular identification of dermatophytes isolated from sheep and goats in Zaria, Nigeria. Sokoto J Vet Sci. 2024; 22 (1): 20-26. doi:10.4314/sokjvs.v22i1.3
3. Hubka, V., Peano, P., Cmokova, A., and Guillt, J. Common and Emerging Dermatophytoses in Animals: Well-Known and New Threats. In: Seyedmousavi, S., de Hoog, G. S., Guillot, J., and Verweiji, P. E (eds). Emerging and Epizootic fungal infections in Animals. Springer International Publishing AG. 31-79. doi: 10.1007/978-3-319-72093-7_3
4. Fawzi, E. M., Abd-Elmergeed, M. M., El-Mekkawi, M. F., El-Diasty, E. M., Morsi, A. M., and Abd-Elfatah, E. B. Prevalence and some risk factors with therapeutic trial of sheep dermatophytosis in Egypt. Iraqi J Vet Sci. 2023; 37 (1): 31-38. doi: 10.33899/ijvs.2022.133376.2214
5. Massiha, A., and Muradov, P. Z. Comparison of antifungal activity of extracts of ten plant species and griseofulvin against human pathogenic dermatophytes. Zahedan J Res Med Sci. 2015; 17 (10): e2096. https://doiorg/10.17795/zjrms-2096
6. Balogun, M., Oji, J., Besong, E., Ajah, A., and Micheal, E. Antiulcer activity of aqueous leaf extract of *Nauclea latifolia* (Rubiaceae) on Indomethacin-induced gastric ulcer in rats. Afri J Biotechnol. 2013; 12 (32): 5080-5086. doi: 10.5897/ajb2013.12788
7. Balogun, M. E., Beson, E. E., Obu, D. C., Obu, M. S. U., and Dijobissie, S. F. E. *Nauclea latifolia:* A Medicinal economic and pharmacological review. Int J Plant Res. 2016; 6: 34-52 doi: 10.5923/j.plant.20160602.03
8. Bansal, A., and Priyadarsini, C. Medicinal properties of phytochemicals and their production. In Intech Open eBooks. 2021
9. Ezeagba, C. C., Duru, C. P., Agbasi, I. S., et al. Nutritional properties of Nauclea latifolia root and leaves. GSC Biol Pharm Sci. 2024; 26 (01): 130-138. https://doi.org/10.30574/gscbps.2024.26.1.0006
10. Bekoe, E. O., Lartey, M., Gordon, A., and Asante, B. Medicinal uses, Pharmacological activities, and bioactive compounds of N. latifolia and implications in the treatment of tropical disease. Health Sci Invest J. 2024; 5 (1): 670-685. https://doi.org/10.46829/hsijournal.2024.6.5.1.670-678
11. Vogel, A. I. Vogel's Textbook of Practical Organic Chemistry (5th ed). Prentice Hall. 1996.
12. Trease, G. E., and Evans, M. C. Textbook of Pharmacognosy, Bailliere Tindall London 12th edition. 1983: 343-383.
13. 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. http://dx.doi.org/10.13040/IJPSR.0975-8232. 3(7).2018-23
14. Ali-Shtayeh, M. S., and Suheil, I. A. Antifungal activity of plant extracts against dermatophytes. Mycoses. 1999; 42: 665-672. doi:10.1046/j.1439-0507.1999.00499.x
15. Adeniyi, B. O., Obas, I. O. J., and Lawal, T. O. *In vitro* anti-fungal activity of *Distemonanthus benthamianus* stem. Int J Pharm Pharmaceut Sci. 2011; 3 (suppl 4): 52-56
16. Mintach, S. O., Asafo-Agyei, T., Archer, M., et al. Medicinal plants for reatment of prevalent diseases. In IntechOpen eBooks, 2019.
17. Salmoin-Manzano, E., Garrido-Cardenas, J. A., and Manzano-Agugliaro, F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Publ Health. 2020; 17 (10): 3376 doi: 10.3390/ijerph17103376
18. Theodoris, S., Drakou, E. G., Hickeler., T., Thines M., and Nogues-Bravo, D. Evaluating natural medicinal resources and their exposure to global change. The Lancet Planetary Health. 2023; 7 (2): e155-e163 doi: 10.1016/s2542-5196(22)00317-5
19. Malami, Y. G., Lawal, F. B., and Garba, S. T. Phytochemical analysis and antibacterial efficacy of leaf extracts of *Nauclea latifolia*. Int J Med Sci Appl Biosci. 2019; 4 (3): 65-69
20. Adepoju, A. J., Esan, A. O., Olawoore, I. T., Ibikunle, G. J., and Adepoju, V. O. *Nauclea latifolia* stem back extracts: Potentially effective source of Antibacterial, Antioxidant, Antidiabetic and Anti-Inflammatory Compounds. J Appl Sci Environ Manag. 2024 ; 28 (1) 49-59. https://dx.doi.org/10.4314/jasem.v28i1.6
21. Olusola, S. E. Phytochemical screening and anti-bacterial potential of African peach Nauclea latifolia leaves extracts against some clinical isolates of pathogenic bacteria from *Clarias gariepinus.* COAST. J Fac Sci. 2020;1 (2): 164-171
22. 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
23. Odeyemi, O., and Enweani-Nwokelo, I. B. Anti-dermatophytic activities and time-kill kinetics of the methanol extracts of *Napoleona imperialis*. Afr J Clin Exper Microbiol. 2025; 26 (1): 81-93 doi:10.4314/ajcem.v26i1.10
24. Bubonja-Šonje, M., Knežević, S., and Abram, M. Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds. Arh Hig Rada Toksikol. 2020; 71: 300-311. doi: 10.2478/aiht-2020-71-3396,