Phytochemical Profiling and Antifungal Efficacy of *Nigella sativa* and *Olea europaea* Extracts against Dermatophytes from School Children in Danko/Wasagu, Kebbi State, Nigeria

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Abstract*—The increasing prevalence of dermatophytoses and concerns over antifungal resistance have renewed interest in plant-derived therapeutics. This study evaluated the phytochemical constituents and antifungal potentials of leaf, root and seed extracts of *Olea europaea* (olive) and *Nigella sativa* (black seed) against clinically isolated dermatophytes from infected school children in. Plant materials were extracted using ethanol and phytochemical screening was performed using standard qualitative tests. Antifungal activity was assessed using the agar well diffusion method to determine zones of inhibition (ZOI) followed by broth microdilution to determine Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) against *Trichophyton tonsurans*, *T. rubrum, T. mentagrophytes* and *Microsporum canis.* Phytochemical analysis using standard method revealed the presence of alkaloids, saponins, steroids, glycosides, tannins and phenols in Olea *europaea* leaves and roots. *Nigella sativa* leaves contained similar compounds, but its seeds lacked phenols, tannins and flavonoids. *Olea europaea* leaf extract exhibited the strongest antifungal activity, with ZOIs up to 24.67 mm and the lowest MIC values (3.10-12.50 µg/mL). *Nigella sativa* leaf extract also showed significant potency. The root and seed extracts of both plants demonstrated comparatively weaker activity. The study concludes that leaf extracts of *Olea europaea* and *Nigella sativa* possess significant bioactive compounds with potent fungicidal activity against dermatophytes validating their traditional use and highlighting their potential as sources of novel antifungal agents.

***Keywords*— *Olea europaea*, *Nigella sativa*, Phytochemicals, Antifungal activity, Dermatophytes, MIC, MFC, Natural products**

I. INTRODUCTION

Dermatophytes are a group of keratinophilic fungi responsible for superficial infections of the skin, hair and nails, collectively termed dermatophytoses or tinea (Nweze and Eke, 2018). These infections are highly contagious and represent a major public health concern especially in tropical and subtropical regions with warm, humid climates conducive to fungal growth (Ameen, 2010). School-aged children are particularly vulnerable due to frequent close contact developing immune systems and often suboptimal hygiene practices (Teklebirhan and Bitew, 2015). In Nigeria, dermatophytosis is endemic, with studies reporting high prevalence rates among children; for instance, a 20% prevalence was reported in Osogbo (Adesiji*et al*., 2019) and 15.4% in Sagamu (Ayanlowo*et al.,* 2014). Rural communities like Danko/Wasagu in Kebbi State face compounded risks due to factors such as overcrowding, limited access to healthcare and low awareness of fungal infections.

The management of dermatophytosis typically relies on synthetic antifungal agents. However, issues of cost, accessibility, side effects and emerging antifungal resistance underscore the need to explore alternative therapeutic agents (Gnat *et al.,* 2020). Medicinal plants offer a vast reservoir of bioactive compounds with antimicrobial properties and form the basis of traditional treatment systems in many communities including Nigeria. *Nigella sativa* L. (black seed, Ranunculaceae) and *Olea europaea* L. (olive, Oleaceae) are two plants renowned in traditional medicine for their broad spectrum therapeutic applications. *Nigella sativa* seeds, rich in thymoquinone, have documented antibacterial, antifungal and anti-inflammatory properties (Ahmad

et al., 2013). Similarly, *Olea europaea* leaves contain potent phenolic compounds like oleuropein and hydroxytyrosol, known for their antioxidant and antimicrobial activities (Hashmi *et al.,* 2015).

Despite the ethnomedicinal reputation of these plants, there is a paucity of scientific data evaluating their specific efficacy against clinically relevant dermatophytes isolated from human infections in Nigeria, particularly from pediatric populations in rural settings. Localized epidemiological data on the circulating dermatophyte species in Kebbi State is limited. Therefore, this study aimed to: determine the prevalence and species distribution of dermatophytes among primary school children in Danko/Wasagu, Kebbi State, profile the phytochemical constituents of *N. sativa* and *O. europaea* extracts and evaluate the in vitro antifungal efficacy of these extracts against the isolated dermatophytes.

II. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in Danko/Wasagu Local Government in Kebbi State, Nigeria, covers approximately 4,208km^2 with an estimated population of about 265,271 (NPC, 2006). Located between latitude 11^{0}25'N and longitude 5^{0}40'E of the equator, it is bordered by Sakaba, Zuru and Bukkuyum LGAs, and comprises eleven administrative wards namely; Ayu, Bena, Dan-Umaru and Wasagu from the East zone, Kanya, Waje, Ribah/Machika, Kele/Gwanfi, Kyabu/Kandu, Shindi/Yalmo/Wari, and Danko/Maga from the West zone (Alhassan*et al.,* 2012).

2.2. Mycological Analysis

2.2.1. Direct Microscopy: Samples were cleared with 10% potassium hydroxide (KOH) and examined under a light microscope (Olympus CX23) for the presence of hyphae and arthroconidia.

2.2.2. Culture and Identification: Samples were inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05 g/L). Cultures were incubated at 28°C for up to 4 weeks. Dermatophyte isolates were identified based on macroscopic colony characteristics (texture, pigmentation) and microscopic morphology (macroconidia, microconidia) using lactophenol cotton blue mounts, following standard mycological keys (Weitzman and Summerbell, 2023).

2.3. Plant Material Collection and Extraction

Fresh leaves of *Olea europaea* and both seeds and leaves of *Nigella sativa* were collected from local sources in Kebbi State. The plants were authenticated at the Plant Science and Biotechnology Herbarium, Abdullahi Fodio University of Science and Technology, Aliero. Voucher specimens were deposited (*Olea europaea*: AFUSTA/PSB/H/Voucher No. 840 and *Nigella sativa*: AFUSTA/PSB/H/Voucher No. 839).Plant materials were washed, shade-dried and pulverized into fine powder. Approximately 100 g of each powder was macerated in 80% ethanol (1 L) for 72 hours with occasional shaking. The extracts were filtered using Whatman No. 1 filter paper and the filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator. The crude extracts were stored at 4°C until further use.

2.4. Phytochemical Screening

Standard qualitative phytochemical tests were performed on the crude extracts to detect the presence of secondary metabolites including alkaloids (Mayer's and Dragendorff's tests), flavonoids (alkaline reagent test), saponins (foam test), tannins (ferric chloride test), phenols (ferric chloride test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test) and glycosides (Keller-Kiliani test) as described by AOAC International. (2023).

2.4.1. Test for alkaloids

Plant extracts were dissolved in dilute hydrochloric acid (HCl) and filtered. The filtrates were then subjected to the following tests:

- Mayer's Test: Mayer's reagent (potassium mercuric iodide) was added to the filtrate. The appearance of a yellow precipitate indicated the presence of alkaloids.
- Dragendorff's Test: Dragendorff's reagent (potassium bismuth iodide) was added. Formation of a red precipitate confirmed the presence of alkaloids.

2.4.2. Test for saponins (foam test)

Approximately 2 mL of distilled water was added to the extract and shaken vigorously. Persistent frothing that lasted for at least 10 minutes indicated the presence of saponins.

2.4.3. Test for phenols

About 0.5 g of the crude extract was treated with a few drops of 2% ferric chloride ($FeCl_3$) solution. The appearance of a bluish-green or black coloration indicated the presence of phenolic compounds.

2.4.4. Test for tannins (ferric chloride test)
Each plant extract was stirred with 1 mL of distilled water and filtered. A few drops of ferric chloride reagent were added to the filtrate. A blue-black, green, or blue-green coloration indicated the presence of tannins.

2.4.5. Test for Glycosides
5mL of each plant extracts, 2mL of glacial acetic acid, and a few drops of ferric chloride solution was placed in a test tube. Alongside of the test tube, 2ml of concentrated H2SO4 was added. The presence of glycosides was shown by the formation of a brown ring at the contact.

2.4.6. Test for steroids
Approximately 10 mL of chloroform was added to 2 mL of the extract. To this, 1 mL of acetic anhydride was added followed by 2 mL of concentrated sulfuric acid. The formation of a blue-green color at the interface confirmed the presence of steroids.

2.4.7. Test for flavonoids (alkaline reagent test)
Extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow color, which became colorless upon the addition of dilute acid, indicated the presence of flavonoids.

2.5. Antifungal Susceptibility Testing

2.5.1. Preparation of Fungal Inoculum: Four clinically isolated dermatophytes, *Trichophyton tonsurans, Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum canis* were used. Inoculum suspensions were prepared from 14 day-old cultures in sterile saline with 0.1% Tween 80 and adjusted to a 0.5 McFarland standard (approximately $1–5 \times 10^6$ CFU/mL) (CLSI, 2017).

2.5.2. Agar Well Diffusion Assay: The antifungal activity was determined using the agar well diffusion method (Balouiri*et al.,* 2016). 100 µL of the standardized inoculum was spread evenly on SDA plates. Wells (6 mm diameter) were punched into the agar and filled with 100 µL of each plant extract at concentrations of 25, 50, 75 and 100 mg/mL (dissolved in 10% DMSO). Control wells contained 10% DMSO (negative control) and ketoconazole (20 µg/mL) (positive control). Plates were incubated at 28°C for 7 days. The diameter of the zones of inhibition (including well diameter) was measured in millimeters. Tests were performed in triplicate.

2.5.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC): The broth microdilution method was used in sterile 96-well plates. Serial two-fold dilutions of the extracts (100 mg/mL to 0.78 mg/mL) were prepared in Sabouraud Dextrose Broth. Each well was inoculated with 100 µL of the fungal suspension. The plates were incubated at 28°C for 96 hours. The MIC was defined as the lowest concentration that showed no visible fungal growth. For MFC determination, 100 µL from clear wells was subcultured on fresh SDA plates. The MFC was the lowest concentration yielding no growth on subculture after 7 days.

2.6. Data Analysis
Data were analyzed using SPSS software version 20.0. Descriptive statistics (frequencies, percentages, mean ± standard deviation) were used to summarize demographic data, prevalence and phytochemical results. The antifungal activity data (zone of inhibition) were analyzed using one-way ANOVA, followed by Duncan's multiple range test for post-hoc comparisons. A p-value of < 0.05 was considered statistically significant.

III. RESULTS

3.1. Phytochemical Constituents
The results of the qualitative phytochemical screening are summarized in Table 1. *Olea europaea* leaf and root extracts tested positive for alkaloids, saponins, steroids, glycosides, tannins, phenols and flavonoids. *Nigella sativa* leaf extract contained alkaloids, saponins, steroids, glycosides, tannins and phenols, but its seed extract lacked phenols, tannins and flavonoids.

Table 1: Phytochemical constituents of *Oleaeuropaea* and *Nigella sativa* extracts

S.N	Compound	*O. europaea*	*N. sativa*

		Leaves	Roots	Leaves	Seeds
1.	Glycosides	+	+	+	+
2.	Alkaloids	+	+	+	+
3.	Saponins	+	+	+	+
4.	Steroids	+	+	+	+
5.	Phenols	+	+	+	-
6.	Tannins	+	+	+	-
7.	Flavonoids	+	+	+	-

Key: + = Detected, – = Not detected

3.2. Antifungal Activity: Zones of Inhibition (ZOI)

All extracts showed concentration dependent antifungal activity. *Olea europaea* leaf extract demonstrated the strongest activity with the largest mean ZOI of 24.67 ± 0.47 mm against *T. tonsurans* at 100 mg/mL. *N. sativa* leaf extract also showed strong activity (ZOI: 21.00-22.67 mm). In contrast, *O. europaea* root and *N. sativa* seed extracts exhibited significantly lower activity ($p < 0.05$), with ZOIs generally below 20 mm at the highest concentration. The positive control (ketoconazole) showed a ZOI of 21.67 ± 0.94 mm. Results are detailed in Table 2.

Table 2: Mean zones of inhibition (mm) of plant extracts against dermatophytes.

Extract 100 Conc. (mg/mL)	*T. tonsurans*	*T. rubrum*	*T. mentagrophytes*	*M. canis*
O. europaea Leaves	24.67 ± 0.47^a	21.67 ± 1.25^a	20.33 ± 0.47^a	19.33 ± 0.94^b
O. europaea Roots	18.00 ± 4.97^b	19.33 ± 0.94^b	23.00 ± 2.16^a	20.33 ± 0.47^a
N. sativa Leaves	21.00 ± 0.82^a	21.67 ± 1.25^a	22.67 ± 1.70^a	20.33 ± 0.47^a
N. sativa Seeds	16.00 ± 1.63^b	15.33 ± 0.47^b	14.33 ± 0.47^b	14.00 ± 0.82^b
Ketoconazole (Control)	21.67 ± 0.94^a	21.67 ± 0.94^a	21.67 ± 0.94^a	21.67 ± 0.94^a

Values are presented as mean ± *SEM (n = 3), values in columns having same superscript are not significantly different with standard drug at (p>0.05) ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.*

3.3. Minimum Inhibitory and Fungicidal Concentrations (MIC and MFC)

The MIC and MFC results (Table 3) confirmed the superior potency of the leaf extracts. *Olea europaea* leaf extract had the lowest MIC value of 3.10 µg/mL against *T. rubrum*. Its MFC values were often equal to or one dilution higher than the MIC, indicating fungicidal action. *N. sativa* leaf extract showed MICs of 6.25-12.50 µg/mL. The root and seed extracts required much higher concentrations for inhibition (MICs 12.50-25.00 µg/mL) and fungicidal action (MFCs 25.00-100.00 µg/mL).

Table 3: MIC and MFC (µg/mL) of plant extracts against dermatophytes.

Test	Isolate	*O. europaea* leaves	*O. europaea* roots	*N. sativa* leaves	*N. sativa* seeds
MIC(µg/mL)	*T. Tonsurans*	6.25	12.50	6.25	25.0
	T. rubrum	3.10	12.50	6.25	12.50
	T. mentagrophytes	12.50	12.50	12.50	25.00
	M. canis	6.25	12.50	6.25	25.00
MFC(µg/mL)	*T. Tonsurans*	6.25	50.00	6.25	25.00
	T. rubrum	6.25	50.00	12.50	50.00
	T. mentagrophytes	12.50	25.00	50.00	50.00
	M. canis	12.50	50.00	25.00	100.00

Values (µg/mL) represent the lowest concentrations inhibiting visible growth (MIC) and preventing growth on subculture (MFC) after incubation. The lower the MIC/MFC value, the higher the effectiveness of the plant against isolated dermatophytes.

IV. DISCUSSION

The search for effective and affordable antifungal agents from medicinal plants is crucial, especially in resource limited settings. The phytochemical analysis confirmed that both plants are rich sources of diverse bioactive compounds with known antimicrobial properties. The potent activity of *Olea europaea* leaf extract can be attributed to its rich phenolic profile including oleuropein and hydroxytyrosol. These compounds are known to disrupt fungal cell membranes, inhibit ergosterol synthesis and induce oxidative stress (Almeida *et al*., 2022; Pereira *et al.,* 2020). The significant activity of *Nigella sativa* leaf extract which contained flavonoids andterpenoids absent in the seeds highlights the importance of selecting the correct plant part for medicinal use. The antifungal efficacy of *N. sativa* is often linked to thymoquinone; however, our findings suggest synergistic contributions from other compounds in the leaves (Akgü l*et al.,* 2023).

The superior performance of *O. europaea* leaf extract evidenced by the largest zones of inhibition and the lowest MIC values (as low as 3.10 µg/mL against *T. rubrum*) positions it as a highly promising candidate for further development. Its activity was comparable to, and in some cases exceeded the standard drug (ketoconazole). The fungicidal nature (MFC close to MIC) of this extract is particularly valuable for therapeutic applications as it suggests the ability to completely eradicate the pathogen, potentially reducing treatment duration and recurrence (Roukas, 2023). The weaker activity of *Niella sativa* seeds compared to its leaves and the variable activity of *Olea europaea* roots underscore a fundamental principle in pharmacognosy: bioactivity is not uniform across different plant organs and is dictated by the tissue-specific biosynthesis and accumulation of secondary metabolites (Bassam*et al.,* 2021).

V. CONCLUSION

This study confirms the antifungal potential of *Olea europaea* and *Nigella sativa* against clinically relevant dermatophytes. The leaf extracts of both plants particularly *Olea europaea* exhibited the strongest fungicidal activity supported by a rich array of bioactive phytochemicals. These findings provide a scientific basis for the traditional use of these plants in managing skin infections. Further research is recommended to isolate and characterize the specific active compounds, evaluate their safety and efficacy in animal models and develop standardized topical formulations for the treatment of dermatophytoses.

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