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Comparative Anti-fungal Activities of Leaf Extracts of Pawpaw and Neem against Tinea capitis: An in vitro comparative study of aqueous and ethanolic extracts against Trichophyton spp.

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Abstract- ***Tinea capitis is a contagious dermatophyte infection of the scalp and hair, commonly associated with Trichophyton and Microsporum species. Interest in plant-derived antifungals has increased due to conventional treatment failure, tolerability, access, and emerging antifungal resistance complicate management. This study compares the in vitro antifungal activities of aqueous and ethanolic leaf extracts of pawpaw (Carica papaya) and neem (Azadirachta indica) against Trichophyton spp. Collected leaves of both neem and pawpaw plants were dried, pulverized and extracted using water or ethanol. The extracts were reconstituted to 25, 50, 75, 100, and 150 mg/mL and were tested using an agar-based susceptibility assay. Inhibition-zone diameters, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) were determined. Fluconazole at 5 mg/mL was used as a control. The results showed no inhibition was recorded at 25 or 50 mg/mL. At 75, 100, and 150 mg/mL, pawpaw aqueous extract produced zones of 11, 13, and 25 mm; pawpaw ethanolic extract produced 17, 21, and 30 mm; neem aqueous extract produced 8, 15, and 19 mm; and neem ethanolic extract produced 9, 18, and 21 mm, respectively. Pawpaw ethanolic extract had the lowest MIC (100 mg/mL) and the only reported MFC (150 mg/mL). Pawpaw aqueous and neem ethanolic extracts had MICs of 150 mg/mL. Activity increased with concentration and was enhanced by ethanolic extraction. Pawpaw ethanolic extract showed the greatest in vitro activity.***

Keywords: Azadirachta indica; Carica papaya; dermatophytes; leaf extract; minimum fungicidal concentration; minimum inhibitory concentration; Trichophyton; tinea capitis

I. INTRODUCTION

Tinea capitis (scalp ringworm) is an infection of scalp skin and hair shafts caused by dermatophyte moulds. *Trichophyton* and *Microsporum* species are the principal aetiological genera, and disease is especially common among children, although adults may also be affected (Hill et al., 2024). Clinical manifestations range from non-inflammatory scaling and alopecia to inflammatory kerion, and transmission may occur through infected people, animals, fomites, or contaminated environments.

Because infection involves the hair shaft, effective management generally requires systemic antifungal therapy; topical agents alone are usually inadequate (Al Aboud & Crane, 2023; Hill et al., 2024). Nevertheless, prolonged treatment, cost, adverse effects, reinfection, diagnostic delay, and changing susceptibility patterns can undermine successful therapy. These constraints support continued investigation of antifungal candidates, while recognising that an in vitro plant extract is not a substitute for clinically validated treatment.

Pawpaw (*Carica papaya L.*) contains phenolics, alkaloids, flavonoids, enzymes, and other secondary metabolites with reported antimicrobial potential. Ethanolic pawpaw leaf extract has inhibited *Candida albicans* and has demonstrated synergy with fluconazole in vitro (Noorie & Chenthamarai, 2021). Related work with papaya fruit extract has also shown

inhibition of *Microsporum canis* and measurable minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) endpoints (Aljuhani et al., 2024). Neem (*Azadirachta indica* A. Juss.) similarly contains limonoids, terpenoids, flavonoids, tannins, and other compounds. Neem leaf extracts have shown antidermatophytic activity against species including *Trichophyton rubrum* and *T. mentagrophytes* (Mahmoud et al., 2011; Ospina Salazar et al., 2015).

Solvent polarity affects the composition and yield of recovered phytochemicals. Comparing aqueous and ethanolic preparations of both plants at identical concentrations therefore provides a useful preliminary indication of extraction efficiency and relative antifungal potential. This study compares the concentration-dependent activities of pawpaw and neem leaf extracts against Trichophyton spp. using inhibition zones and dilution endpoints.

II. AIM AND OBJECTIVES

Aim of the Study

The aim of the study is to compare the in-vitro antifungal activities of aqueous and ethanolic leaf extracts of pawpaw and neem against a *Trichophyton specie* associated with tinea capitis, and the objective with which the above is to be achieved are as listed below:

Specific Objectives of the Study

- To prepare aqueous and ethanolic crude extracts from the collected pawpaw and neem leaves.
- To prepare varying concentrations of the aqueous and ethanolic extracts of both plant extracts.
- To prepare 5mg/ml fluconazole concentration.
- To collect and subculture the *Trichophyton species.*
- To determine the inhibition zone of the extracts at 25, 50, 75, 100, and 150 mg/ml against the test isolate, using 5mg/ml fluconazole concentration as control.
- To compare the effects of plant species, extraction solvent, and varying concentrations.
- To determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of each extract within the tested concentration range.
- To identify the most active extract and define limitations requiring confirmatory investigation.

III. MATERIALS AND METHODS

Plants Sample Collection

Leaf samples of pawpaw and neem plants were collected at the botanical garden of the Department of Science Laboratory Technology, Igbesa, Ado Odo Ota Local Government Area of Ogun State. The collected samples were rinsed and dried in the shade. The dried leaf samples were pulverized and kept in an airtight container until they are required for further required.

Plants Extracts Preparation

The stored pulverised leaves of pawpaw and neem were weighed separately (50 g) and soaked in 500 mL of both aqueous and ethanol solvents for a period of 48 hours and 72 hours, respectively. The mixtures were extensively shaken together periodically and reconcentrated using a rotary evaporator.

150 mg/ml, 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml of the extracts were prepared by dissolving 1g of the each extracts in 6.67 ml, 10 ml, 13.3 ml, 20 ml, and 40 ml of dimethyl sulphoxide (DMSO) respectively.

Collection of Test Isolate

A *Trichophyton* isolate was collected from the Microbiology teaching laboratory at the Ogun State Institute of Technology, Igbesa, Ado Odo Ota Local Government Area, and was subcultured for 24 hours. The subcultured isolate was later adjusted 0.5 McFarland standard.

Susceptibility Testing (Agar Well Diffusion Method)

The adjusted test isolate was streaked onto 24 different plates containing Potato Dextrose agar and a sterile cork-borer with 7mm size. 0.4 mL of the extracts were dispensed into the bored holes of the appropriately labeled plates in duplicate for each of the extracts concentrations while the remaining 4 were used for the controls in duplicate (DMSO and 5 mg fluconazole. The plates were incubated for 5 days at a regulated temperature of 30^{o}C.

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Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungal Concentration (MFC)

The minimum inhibitory concentration (MIC) was carried out on the various extract concentrations of neem and pawpaw against *Trichophyton spp*. using a potato dextrose broth. Nutrient broth (5 mL) was dispensed into each of the 24 test tubes and sterilized at 121°C for 15 minutes, and allowed to cool to 40–45°C. 0.5 mL of 0.5 McFarland standard of the test isolate was introduced into the 24 sterile different test tubes, while 5 mL of each extract concentration (150 mg/mL, 100 mg/mL, 75 mg/mL, 50 mg/mL, and 25 mg/mL of aqueous and ethanolic extracts of neem and pawpaw) were introduced into each tube in duplicate. 5 mL of fluconazole was equally dispensed into two sterile tubes (in duplicate), while 5 mL of DMSO was added to the other two tubes containing the isolate, labeled accordingly. The tube with the least concentrations showing no fungal growth after 4 days of incubation at 35ºC (using modified CLSI standard) were recorded as the lowest tested concentrations reported to prevent visible fungal growth. Material from inhibited cultures was subsequently assessed for regrowth on a potato dextrose agar incubated at 28ºC for 5days; the MFC was the lowest concentration reported to prevent regrowth. Antifungal susceptibility was assessed with reconstituted extracts, and activity were recorded as the diameter of the growth-inhibition zone in millimetres (Clinical and Laboratory Standards Institute [CLSI], 2017).

Data analysis

The reported mean values were entered into a concentration-by-extract matrix and analysed descriptively. Comparisons included ranking at each active concentration, changes between 75 and 150 mg/mL, mean inhibition across the three active concentrations (75–150 mg/mL), and MIC/MFC ordering. Zero values at 25 and 50 mg/mL are treated as no recorded inhibition, consistent with the supplied graph.

IV. RESULTS

All four extracts showed no recorded inhibition at 25 and 50 mg/mL. Activity appeared at 75 mg/mL and increased with concentration. Pawpaw ethanolic extract produced the largest zone at every active concentration, reaching 30 mm at 150 mg/mL

Table 1. Mean Zone of inhibition of pawpaw and neem leaf extracts against *Trichophyton spp.*

Concentration (mg/ml)	Pawpaw aqueous extract (mm)	Pawpaw ethanolic extract (mm)	Neem aqueous (mm)	Neem ethanolic extract (mm)
25	0	0	0	0
50	0	0	0	0
75	11	17	8	9
100	13	21	15	18
150	25	30	19	21

Note. Values are mean measurements. A value of 0 indicates no inhibition recorded in the source graph.

Table 1a. Mean Zone of inhibition of Fluconazole 5mg/ml against Trichophyton spp.

Fluconazole (mg/ml)	Test Isolate (mm)
5	10

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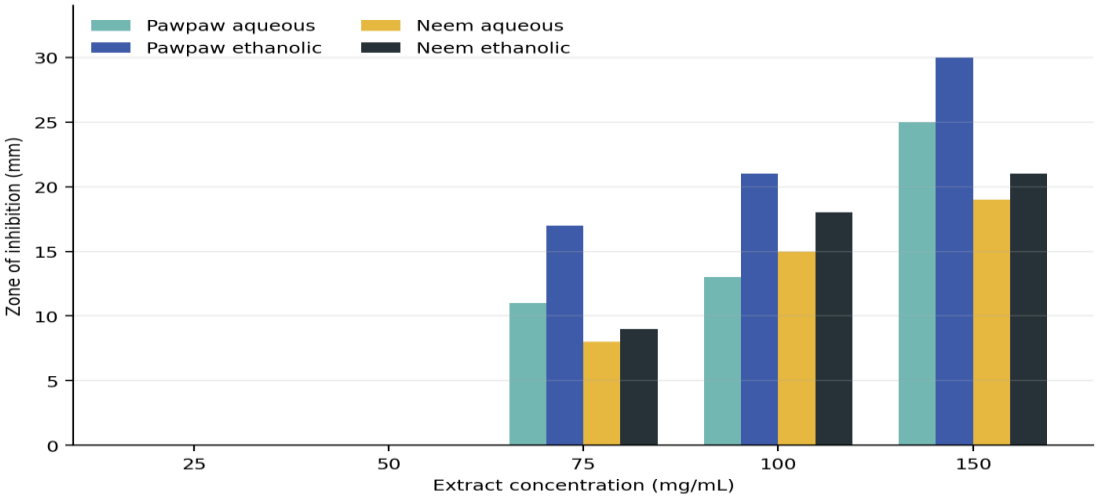

Figure 1. Concentration-dependent inhibition zones of the four leaf-extract preparations.

Minimum inhibitory and fungicidal concentrations

Pawpaw ethanolic extract inhibited visible growth at 100 mg/mL and was fungicidal at 150 mg/mL. Pawpaw aqueous and neem ethanolic extracts reached inhibitory endpoints only at 150 mg/mL. Neem aqueous extract did not reach an MIC within the tested range, and no MFC was reported for either neem preparation or pawpaw aqueous extract.

Table 2. MIC and MFC endpoints

Plant	Solvent	MIC (mg/mL)	MFC (mg/mL)
Pawpaw	Aqueous	150	>150
Pawpaw	Ethanolic	100	150
Neem	Aqueous	>150	>150
Neem	Ethanolic	150	>150

Note. “>150” means the endpoint was not attained at the highest concentration tested; it is not an estimated numeric endpoint.

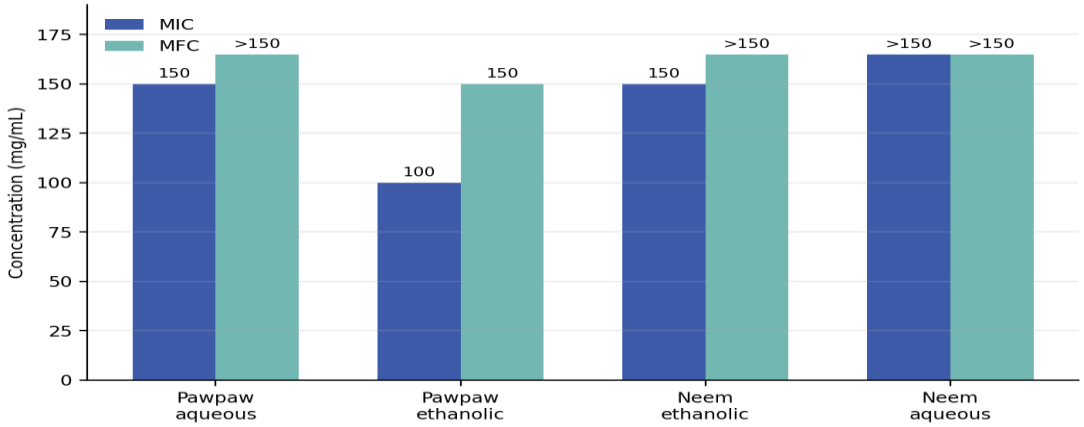

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Figure 2. MIC and MFC endpoints. Bars labelled >150 indicate that the endpoint was not reached within the tested range.

V. DATA ANALYSIS

Table 3. Descriptive comparison across active concentrations (75–150 mg/mL)

Extract	Mean zone (mm)	Maximum zone (mm)	Increase 75→150 (mm)	Rank by mean
Pawpaw ethanolic	22.7	30	13	1
Pawpaw aqueous	16.3	25	14	2
Neem ethanolic	16.0	21	12	3
Neem aqueous	14.0	19	11	4

Pawpaw ethanolic extract had the highest mean zone across active concentrations (22.7 mm), followed by pawpaw aqueous (16.3 mm), neem ethanolic (16.0 mm), and neem aqueous (14.0 mm). From 75 to 150 mg/mL, inhibition increased by 13 mm for pawpaw ethanolic, 14 mm for pawpaw aqueous, 12 mm for neem ethanolic, and 11 mm for neem aqueous. At 150 mg/mL, the pawpaw ethanolic zone was 20% larger than pawpaw aqueous (30 versus 25 mm) and approximately 43% larger than neem ethanolic (30 versus 21 mm).

The solvent effect was plant-dependent but generally favoured ethanol. For pawpaw, ethanol exceeded water by 6, 8, and 5 mm at 75, 100, and 150 mg/mL, respectively. For neem, ethanol exceeded water by 1 and 3 mm at 75 and 100 mg/mL, but neem ethanolic remained 2 mm greater at 150 mg/mL. The MIC ordering supports the same conclusion: pawpaw ethanolic required the lowest concentration for inhibition and was the only preparation with a fungicidal endpoint.

VI. DISCUSSION

The principal finding is a clear concentration-response pattern among the four crude extracts from 75 to 150 mg/mL. Such a pattern is biologically plausible because higher extract concentrations deliver larger quantities of antifungal phytochemicals to the diffusion interface. Pawpaw ethanolic extract consistently produced the greatest inhibition and achieved both inhibitory and fungicidal endpoints, suggesting that ethanol recovered a more active or more diffusible mixture from pawpaw leaves than water.

This interpretation is consistent with prior reports of antifungal activity in C. papaya preparations. Noorie and Chenthamarai (2021) observed greater activity of ethanolic pawpaw leaf extract at higher concentration against C. albicans, while Aljuhani et al. (2024) reported measurable inhibition, MIC, and MFC for methanolic papaya fruit extract against M. canis. Although the plant part, extraction solvent, organism, and test method differ, these studies support the broader proposition that papaya contains compounds capable of impairing fungal growth. Phenolics, flavonoids, alkaloids, and other constituents may act individually or synergistically.

Neem also demonstrated concentration-dependent inhibition. Previous work has found antidermatophytic effects of neem leaf extracts and associated activity with terpenoid-rich fractions (Mahmoud et al., 2011; Ospina Salazar et al., 2015). In the current dataset, however, neem preparations were less active than pawpaw ethanolic extract, and neither produced a fungicidal endpoint at or below 150 mg/mL. The small aqueous–ethanolic differences for neem suggest that solvent advantage may vary with plant chemistry, extraction yield, and agar diffusion behaviour.

The reported fluconazole control produced only a 10mm inhibition at 5 mg/mL. The findings should not be interpreted as evidence that topical pawpaw or neem preparations cure human tinea capitis, but as an indication that the two plants possess an adequate

metabolite to inhibit the biological activities of Trichophyton spp.. The hair-shaft location of infection usually necessitates systemic therapy (Al Aboud & Crane, 2023; Hill et al., 2024). Before clinical translation, active compounds must be standardised; cytotoxicity, irritation, stability, pharmacokinetics, formulation, and in vivo efficacy must be evaluated; and randomised clinical trials must demonstrate safety and benefit.

Summary
Aqueous and ethanolic leaf extracts of pawpaw and neem were screened against Trichophyton spp. at 25–150 mg/mL. Activity was absent at the two lowest concentrations and increased from 75 to 150 mg/mL. Pawpaw ethanolic extract was the strongest preparation, producing zones of 17, 21, and 30 mm, an MIC of 100 mg/mL, and an MFC of 150 mg/mL. Pawpaw aqueous and neem ethanolic extracts reached MIC at 150 mg/mL, while neem aqueous extract did not attain MIC within the tested range. These results support further laboratory investigation but remain preliminary.

VII. CONCLUSION AND RECOMMENDATIONS

Under the reported in vitro conditions, pawpaw and neem leaf extracts inhibited Trichophyton growth in a concentration-dependent manner. Ethanolic pawpaw leaf extract had the best overall performance and was the only preparation to demonstrate fungicidal activity at 150 mg/mL. It is therefore the leading candidate among the tested extracts for fractionation and confirmatory study.

Future work should authenticate the fungal species; deposit plant voucher specimens; quantify extraction yield; standardise inoculum and media; test biological replicates; include valid positive, negative, vehicle, growth, and sterility controls; apply CLSI-aligned broth dilution; determine confidence intervals; chemically characterise active fractions; and undertake cytotoxicity and formulation studies before any animal or human investigation.

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