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In vitro Anti Dermatophyte Activities of Crude Methanol and Aqueous Extracts of *Lawsonia inermis*

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ABSTRACT

The present study was carried out to investigate the *in vitro* anti dermatophyte activities of crude methanol and aqueous extracts of *Lawsonia inermis* leaves. The anti dermatophyte activities was tested against *Microsporum audouinii*, *Microsporum ferrugineum*, *Trichophyton megninii*, *Trichophyton tonsurans* and *Trichophyton rubrum*, recovered from rice farmers with fungal skin infections in Anambra State, Nigeria. From the result obtained, growth of dermatophytes was inhibited at the varied concentrations of crude methanol extract with the diameter zone of inhibition increasing with the increase in concentration. At the lowest concentration of 10 mg/disc, diameter zone of inhibition range of 8.0 mm-16.8 mm was recorded against the different dermatophytes. At 80 mg/disc, *T. rubrum* showed the highest diameter zone of inhibition (18.8 mm), followed by *M. audouinii* (18.0 mm) while *T. megninii* was the least inhibited (12.0 mm). Water extract of *L. inermis* also inhibited all the test dermatophyte. *M. audouinii* was the most susceptible with diameter inhibition zone of 14.5 mm. Minimum inhibitory concentration was recorded at 25 mg/ml for all dermatophytes while fungicidal action was recorded at concentrations of 50 mg/ml for *M. audouinii* and *T. rubrum*, 100 mg/ml for *M. ferrugineum* and 200 mg/ml for *T. megninii* and *T. tonsurans*. These results demonstrated that *L. inermis* has anti dermatophyte activities and could be a good source for the production of plant based antifungal drugs.

Keywords: Dermatophytosis; crude extracts; *Lawsonia inermis*; anti dermatophyte activity.

INTRODUCTION

Plants were the first source of treating infection even before the invention of antibiotics and synthetic drugs in the 20th century. These medicinal plants contain bioactive compounds that exhibit antimicrobial and other medicinal activities. Their use in treatment of various ailments is widely accepted and in great demands both in developing and developed countries. Today it is estimated that about 80% of people in developing countries rely on these plants for their primary health care. [1-2]

Fungal skin infections caused by dermatophytes had been extensively reported to be a public health problem in Nigeria and all over the world. [3-5] Most of these dermatophytes reside in soil from where they can infect the keratinized areas of a living host (man and animals). Farm workers that are exposed to various irritant agents like mud, cow dung or other types of fertilizers, herbicides, pesticides, dust and soil are often predisposed to this infection especially in developing countries where subsistence farming is still practiced. [3, 6-7] These farmers have low access to western health care and in most cases cannot afford the orthodox medication. [8-9] It is therefore necessary that plants with

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antifungal activity be identified, studied and probably used in production of plant based antifungal agents that will be affordable.

L. inermis (Henna) is one of such plants known for its medicinal use. It is a tall shrub or small tree, 2-6 M high. It is planted in home gardens as hedges and as ornamental. [10] In Nigeria, where it is locally called 'lali', the leaf of the plant is primarily used to dye skin, finger nail and hair, especially among the Northerners. This dying property has been attributed to a color agent, Lawson, contained in the plant, with its highest concentration detected in the petioles (0.5-1.5%). [11-12] Leaves, flowers, seeds, stem barks and roots of *L. inermis* have been found to exhibit antioxidant, anti-diabetic, hepatoprotective, anticancer, antimicrobial and wound healing properties; it also has cooling effect and is used to bring down fever. [10, 13]

The purpose of this research is to study the anti dermatophyte activities of crude methanol and aqueous extracts of *Lawsonia inermis* against five species of dermatophytes recovered from rice farmers in Anambra State, Nigeria.

MATERIALS AND METHODS

Source of dermatophytes

Two hundred and one samples were collected from rice farmers in Anambra State, Nigeria, with lesions suggestive of fungal infections. Dermatophytes were isolated and identified based on detailed study of their microscopic and macroscopic features. [7]

Plant material

Plant leaves of *L. inermis* were collected from their wild sources in Anambra State, Nigeria, and identified in the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria. The leaves were washed under running tap, dried overnight in an oven at 40°C, grinded into fine powder and stored in an airtight container for further use.

Extraction of crude extracts

Crude extract of *L. inermis* was extracted from thirty grams of powdered leaf by Soxhlet procedure using 300 ml Methanol (BDH) as solvent. [14] The Crude extracts were recovered using rotavapour apparatus. The extracts were dried and stored in a freezer for further use. Aqueous extract was also prepared by blending 20 g of chopped fresh leaf with 10 ml of water in a Moulinex blender for 5 min. The suspension was filtered and sterilized at 121°C for 15 min and used to impregnate discs (6 mm) for anti dermatophyte test. [15]

Inoculum preparation

Four day old dermatophyte culture grown on SDA plates was aseptically scraped and transferred into bijou bottle containing 10 ml of sterile water. The suspension was vigorously shaken, diluted Ten-fold and used for determining the anti dermatophyte activity. [16]

Determination of anti dermatophyte activity

Sterilized discs (6 mm) prepared from Whatman No 1 filter paper were impregnated with different concentrations (10 mg, 20 mg, 40 mg, 80 mg) of methanol extract dissolved in 2% DimethylSulphoxide (DMSO). The disc of the methanol and aqueous extracts were placed on SDA plates seeded with 0.1ml of 10^{-4} dilution of inoculum preparation. [17] The plates were prepared in duplicates, incubated at room temperature for 7 days and average diameter zone of inhibition recorded. Discs impregnated with 2% DMSO and 2 mg/disc ketoconazole served as negative and positive controls respectively.

Minimum Inhibitory Concentration (MIC) and Minimum fungicidal Concentration (MFC)

MIC: Two hundred milligrams/milliliter of the methanol crude extract was dissolved in 2% DMSO and serially diluted two fold in sterile water. Different tubes containing different concentrations (25mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the extracts were inoculated with 0.1ml of 10^{-4} dilution of the inoculum preparation (standardized suspension of the test dermatophyte) and incubated at room temperature for 7 days. These were done in duplicate and the broth medium containing no extract was used as control. [18] MIC was recorded as the tube with the lowest concentration of extract that failed to show any visible macroscopic growth.

MFC: Loopful from tubes of MIC and the preceding tubes were inoculated on sterile SDA plates without drug supplement. The plates prepared in duplicates, were incubated for 7 days at room temperature and observed for growth. The lowest concentration of the tube dilutions that showed no visible growth on SDA plates was considered as the MFC. [19]

RESULTS AND DISCUSSION

Based on the detailed study of the macroscopic and microscopic features of the isolated fungi, a total of 5 species of dermatophytes namely, *Microsporum audouinii, Microsporum ferrugineum*, *Trichophyton megninii*, *Trichophyton tonsurans* and *Trichophyton rubrum* were recovered from rice farmers in Anambra State, Nigeria. [7] The anti dermatophyte activities of crude methanol extract of *Lawsonia inermis* tested at four different concentrations with their zones of inhibition is represented in Figure 1. The crude methanol extracts of *L. inermis* at a concentration of 10 mg/disc inhibited all test dermatophytes. At that concentration, *M. ferrugineum* showed diameter inhibition zone of 8.0 mm, *T. megninii* (8.4 mm), *T. tonsurans* (12.2 mm), *M. audouinii* (14.8 mm) and *T. rubrum* (16.8 mm). However, the highest diameter zone of inhibition (18.8 mm) was observed with *T. rubrum* at a concentration of 80 mg/disc. This result agrees with the work of other researchers who also recorded total inhibition of test dermatophytes by methanol extracts of *L. inermis.* [10, 20] From the results obtained, the diameter zones of inhibition recorded by the different dermatophytes increased as the concentration of the crude extract increases. This observation was recorded

by Wagini *et al.*, [21] even though they used n-hexane extract of *L. inermis* against the dermatophytes.

Shown in Figure 2, is the average zone of inhibition of cold water extracts of *L. inermis*. The cold water extracts used in this work also showed inhibitory actions against the dermatophytes. *T. rubrum* and *M. audouinii* recorded millimeter zone of inhibition of 14.2 and 14.5 respectively. This agrees with the result of some researchers [10, 21], but differs from that of Abdelraouf *et al.*, [22] who recorded complete resistance of dermatophytes to methanol and water extracts of *L. inermis*. The results obtained in this work recorded increased bioactivity with methanol extract than the aqueous extract which is contrary to the reports of other workers. [10, 20]

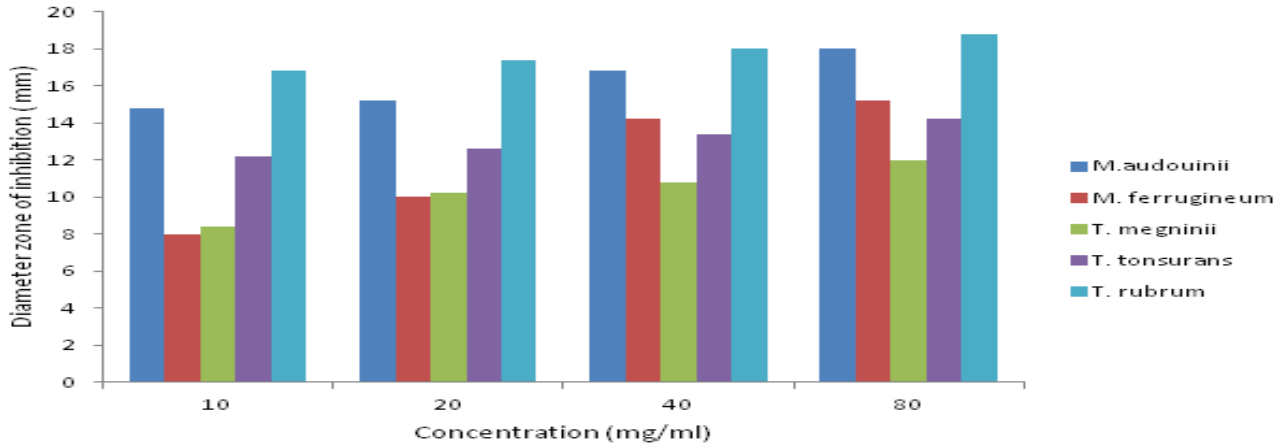


Fig. 1: Anti dermatophyte activity of crude methanol extract of *L. inermis*.

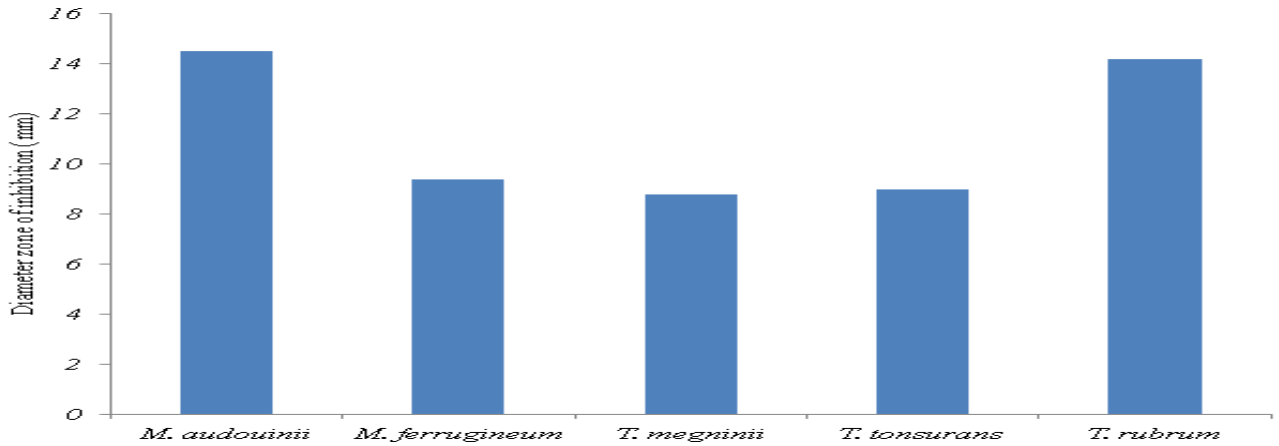
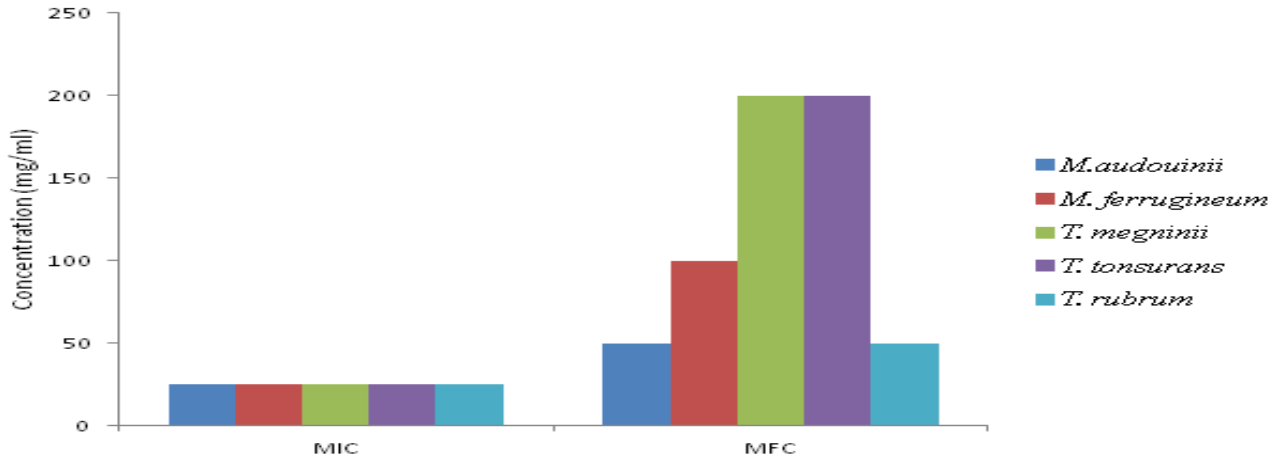


Fig. 2: Anti dermatophyte activity of cold water extract of *L. inermis*



MIC = Minimum inhibitory concentration; MFC= Minimum fungicidal concentration

Fig. 3: Minimum inhibitory concentration and minimum fungicidal concentration of crude methanol extract of *L. inermis*

As a general rule, plant extract is considered active against both fungi and bacteria when the zone of inhibition is greater than 6 mm. [23] The range of diameter zones of inhibition by methanol extract (8.0 mm - 18.8 mm) and aqueous extract (9.0 mm - 14.5 mm) of the leaf of *L. inermis* against the dermatophytes tested, confirms the anti dermatophyte activities of the extracts.

Represented in Figure 3, is the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the methanol crude extract of *L. inermis* against the dermatophytes. The methanol extract inhibited all test dermatophytes at the minimum concentration of 25 mg/ml while fungicidal actions were observed at a concentration of 50 mg/ml for *M. audouinii* and *T. rubrum*, 100 mg/ml for *M. ferrugineum* and 200 mg/ml for *T. megninii* and *T. tonsurans.*

The crude methanol extract of *L. inermis* showed total fungicidal action against the test dermatophytes, although at varied concentrations. This observation is supported by the work of many investigators [21, 24-25], who also reported absolute toxicity and fungicidal effects of *L. inermis* crude extracts against ringworm-causing fungi. The fungicidal effect of *L. inermis* leaves is attributed by researchers to the bioactive compound, 2-hydroxy-1:4naphthoquinone, contained in these leaves. [1, 10, 21, 26]

From the experimental observations in this study, the use of *L. inermis* is, therefore, highly recommended for the treatment of dermatophyte infections.

The results of anti dermatophyte activities of crude methanol and water extracts of *L. inermis* obtained in this work showed total fungicidal actions against the dermatophytes. The leaves could be used in the production of antifungal drugs that will be effective and affordable to the developing countries.

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