



Antifungal Potentials of *Acacia nilotica*, *Ziziphus jujube* Linn and *Lawsonia inermis*

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Authors' contributions

This work was carried out in collaboration between all authors. Author ALA designed the study and wrote the protocol. Author UFM wrote the first draft of the manuscript. Authors AD and IHA managed the literature search, conducted the laboratory work and interpreted the results. Author MY carried out data analysis and took part in the supervision of the work. Author RSUW supervised the work and edited the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The increasing emergence of resistance to conventional antimicrobial drugs and the complicity of their usage is a serious challenge in Nigeria. In our previous report, it was demonstrated that methanol leaves extracts of *Acacia nilotica*, *Ziziphus jujube* Linn and *Lawsonia inermis* exhibited antibacterial activity against *Escherichia coli*, *Pseudomonas flourecense*, *Streptococcus* and *Staphylococcus aureus*.

Methodology: In this study, agar well diffusion method was employed to assess the antifungal potency of these plant extracts and were tested against *Aspergillus flavus*, *Trichophyton rubrum* and *Candida albicans*.

Results: Exclusive of *L. inermis* extract against *T. rubrum* at 100 mg/ml (zone of inhibition 34.33±1.89 mm). 100 mg/mL of all the extracts investigated have significantly lower (P<0.05) antifungal activity when compared to standard antifungal drug (Nystatin, 100 mg/ml). The activity of

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L. inermis against *A. flavus* was comparatively similar ($P>0.05$) to the control drug, but significantly higher ($P<0.05$) against both *T. rubrum* and *C. albicans* at 150 mg/ml. Conversely, the antifungal activity of *A. nilotica* extract against *T. rubrum* and *C. albicans* significantly surpass ($P<0.05$) that of the control drug, while *Z. jujube* Linn extract activity against *C. albicans* was comparatively similar ($P>0.05$) to it, but significantly higher ($P<0.05$) against *T. rubrum*. A dose dependent antifungal activity of the plants was observed, and *L. inermis* extract was the most potent antifungal agent with an MIC and MCF values of 5 mg/ml.

Conclusion: This study reveals that *L. inermis* leaves extract could be used as a sources of potential antifungal agents.

Keywords: Antifungal; *Acacia nilotica*; *Ziziphus jujube* Linn and *Lawsonia inermis*.

1. INTRODUCTION

Plants material provided a variety of compounds of known therapeutic properties, like analgesics, anti-inflammatories and others. Antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world [1,2]. Previous studies have demonstrated in laboratory trials that different plant tissues, such leaves, seeds and roots possess inhibitory properties against microorganisms (bacteria, fungi) and insects [2,3].

The leaves, roots and stem bark of *Lawsonia inermis* (henna), *Ziziphus jujube* Linn and *Acacia nilotica* are traditionally used for the management of bacterial and fungal infections [4]. Recent study on quantitative phytochemical analysis of crude methanol leave extracts of these plants revealed the presence of glycoside, tannins, phenols saponins and flavonoids [5]. The antibacterial potency of these plant extracts against *Escherichia coli*, *Pseudomonas flourecense*, *Streptococcus* and *Staphylococcus aureus* was efficient [5]. Presently, there is little evidence on the antifungal properties of the medicinal plants under investigation against phytogetic fungi.

Fungi are universal in the environment, and infection due to fungal pathogens is commonly among population. The aim of this study was to evaluate the potential antifungal activity of *Acacia nilotica*, *Ziziphus jujube* Linn and *Lawsonia inermis* extracts against *A. flavus*, *T. rubrum* and *C. albicans*, in order to verify possible inhibitory activity.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *A. nilotica*, *Z. jujube* Linn and *L. inermis* were collected from Achida, Wuruno Local

Government Area of Sokoto State. The samples were thoroughly washed with distilled water, then air-dried under shade.

2.2 Preparation of Plant Material

The plant leaf samples were pulverized to powder using pestle and mortar. 100 g pulverized plant materials were mixed with 1 litre of 95% methanol. The mixture was kept at room temperature for 72 hours and was then filtered with a Whatman filter paper No.1 of pore size 11 μ m. The filtrates were evaporated at 45°C using rotary evaporator. Then the methanol extracted material was dissolved in distilled water and the solution was used for antimicrobial studies.

2.3 Antifungal Screening Using Agar Well Diffusion Method

Clinical isolates of *A. flavus*, *T. rubrum* and *C. albicans* were collected from Microbiology Unit of Specialist Hospital Sokoto, Nigeria. The cultures were inoculated in Sabouraud dextrose agar medium for 10 days prior to the experiment. The isolates were subjected to antifungal studies by agar well diffusion method. Standard solution (50, 100 and 150 mg/ml) of the extracts were added onto test organism-seeded plates. The plate containing distilled water was used as negative control while Nystatin (a standard fungicide) (100 mg/ml) was used as positive control. Antifungal activity was determined at 28°C in 7 days incubation. The zones of inhibitions (mm) were defined by area of complete visible growth inhibition [6].

2.4 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for *A. nilotica*, *Z. jujube* Linn, and *L. inermis* against fungal species using Broth Dilution Method. A stock suspension of each organism was adjusted to 1.5×10^5 spores/ml in

sabouraud dextrose broth. Test tubes containing only the media were used as negative control, while those containing only sabouraud dextrose broth and fungi inoculums served as positive control. Visible turbidity were determined after incubation at room temperature for 72 hours and 37°C for 24 hours for moulds and yeast (*C. albicans*) respectively. The MIC values were extrapolated from the lowest concentration of extract that inhibited the visible growth of the tested organism [6].

2.5 Determination of Minimum Fungicidal Concentration (MFC)

In order to determine minimum fungicidal concentration (MFC), plates with no visible growth in the MIC assay were further subcultured in fresh sterile Sabouraud dextrose agar plates. The plates were incubated at room temperature until growth was detected in the growth control subculture. The MFC was then taken as the lowest concentration or highest dilution of the samples that did not show any visible growth [6].

2.6 Data Analyses

Results were expressed as mean \pm standard deviation and presented in tabular form. Data was analysed using In Stat Software package 3.0 version; San Diego USA. Differences between the means (n = 3) were established by One way ANOVA followed by Duncan's, multiple comparison test. Statistical significance was set at $P < 0.05$.

3. RESULTS

The antifungal activity of the methanol leaf extracts of *A. nilotica*, *L. inermis* and *Z. jujube*

Linn against *A. flavus*, *T. rubrum* and *C. albicans* are presented in Table 1. At 50 and 100 mg/ml, the antifungal patency of the extracts were significantly lower ($P < 0.05$) than that of standard antifungal agent, Nystatin 100 mg/ml, except for *L. inermis* against *T. rubrum* at 100 mg/ml with mean zone of inhibition value of 34.33 ± 1.89 mm. However, concentration of 150 mg/ml, the effect *L. inermis* extract against *A. flavus* was comparatively similar ($P > 0.05$) to that of the control drug. However, similar concentration of *L. inermis* extract exhibited significantly higher activity ($P < 0.05$) against both *T. rubrum* and *C. albicans* than Nystatin. On the other hand, the antifungal activity of *A. nilotica* extract against *T. rubrum* and *C. albicans* significantly surpass ($P < 0.05$) that of the control drug, while *Z. jujube* Linn extract activity against *C. albicans* was comparatively similar ($P > 0.05$) to that of Nystatin, but significantly higher ($P < 0.05$) against *T. rubrum*.

Table 2 shows the minimum inhibitory concentration (MIC) of methanol leaf extracts of *A. nilotica*, *L. inermis* and *Z. jujube* Linn against *A. flavus*, *T. rubrum* and *C. albicans*. The extract of *L. inermis* was most effective against the three fungi species with an MIC value of 5 mg/ml. The least antifungal potency was observed in *Z. jujube* Linn with visible *C. albicans* growth at MIC value 30 mg/ml.

The extract of *L. inermis* exhibited least MFC at 5 mg/ml on the three fungi species. The potency of *A. nilotica* was comparable to that of the standard antifungal agent, lower than that of *L. inermis* but higher than *Z. jujube* Linn extract (Table 3). Also, the least antifungal activity with MFC value of 35 mg/ml was observed in the activity of *Z. jujube* Linn against *C. albicans*.

Table 1. Antifungal activities of *A. nilotica*, *L. inermis* and *Z. jujube* Linn methanol leaf extracts (50, 100 and 150 mg/ml) and Nystatin (100 mg/ml)

Extract Conc.	Fungal spp.	Zone of Inhibition (mm)				
		<i>A. nilotica</i>	<i>L. inermis</i>	<i>Z. jujube</i> Linn	Nystatin	Distilled water
50 mg/ml	<i>A. flavus</i>	11.00 \pm 1.00 ^a	11.67 \pm 2.89 ^a	11.67 \pm 1.73 ^a	53.00 \pm 2.65 ^b	0.00
	<i>T. rubrum</i>	9.00 \pm 2.44 ^c	17.33 \pm 1.89 ^d	7.67 \pm 0.45 ^c	30.00 \pm 2.33 ^e	0.00
	<i>C. albicans</i>	11.45 \pm 0.89 ^a	11.00 \pm 0.45 ^a	14.33 \pm 0.67 ^f	31.00 \pm 2.89 ^e	0.00
100 mg/ml	<i>A. flavus</i>	22.33 \pm 2.50 ^a	41.67 \pm 2.90 ^b	11.00 \pm 1.73 ^c	53.00 \pm 2.65 ^d	0.00
	<i>T. rubrum</i>	28.00 \pm 0.44 ^e	34.33 \pm 1.89 ^f	27.67 \pm 0.33 ^e	30.00 \pm 2.33 ^g	0.00
	<i>C. albicans</i>	24.67 \pm 0.33 ^h	17.67 \pm 1.45 ⁱ	20.33 \pm 2.33 ^a	31.00 \pm 2.89 ^g	0.00
150 mg/ml	<i>A. flavus</i>	28.00 \pm 2.00 ^a	50.67 \pm 1.15 ^b	26.67 \pm 2.65 ^c	53.00 \pm 2.65 ^b	0.00
	<i>T. rubrum</i>	36.00 \pm 0.44 ^d	44.67 \pm 1.89 ^e	37.33 \pm 0.33 ^f	30.00 \pm 2.33 ^g	0.00
	<i>C. albicans</i>	35.30 \pm 0.33 ^d	38.33 \pm 1.45 ^f	30.33 \pm 0.33 ^g	30.33 \pm 2.89 ^g	0.00

Values are mean inhibition zones (mm) \pm S.D of three replicate experiment. Mean value having different superscript letters (abcdefg) along the rows are significantly different ($P < 0.05$) while values with the same superscripts letter in rows, are non significance ($P > 0.05$)

Table 2. Minimum inhibitory concentration (MIC) of methanol leaf extracts of *A. nilotica*, *L. inermis* and *Z. jujube* Linn

Samples	Fungal spp.	Concentration of extract (mg/ml)										
		50	45	40	35	30	25	20	15	10	5	MIC
A. nilotica	<i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-	10
	<i>T. rubrum</i>	-	-	-	-	-	-	-	-	-	+	10
	<i>C. albicans</i>	-	-	-	-	-	-	-	-	-	+	10
L. inermis	<i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-	5
	<i>T. rubrum</i>	-	-	-	-	-	-	-	-	-	-	5
	<i>C. albicans</i>	-	-	-	-	-	-	-	-	-	-	5
Z. jujube Linn	<i>A. flavus</i>	-	-	-	-	-	-	-	-	+	+	15
	<i>T. rubrum</i>	-	-	-	-	-	-	-	+	+	+	20
	<i>C. albicans</i>	-	-	-	-	+	+	+	+	+	+	35
Nystatin	<i>A. flavus</i>	-	-	-	-	-	-	-	-	+	+	15
	<i>T. rubrum</i>	-	-	-	-	-	-	-	-	+	+	15
	<i>C. albicans</i>	-	-	-	-	-	-	-	-	+	+	15

Key: (-) indicate no visible growth of the organisms and (+) indicate visible growth of the organisms

Table 3. Minimum fungicidal concentration (MFC) of methanol leaf extracts of *A. nilotica*, *L. inermis* and *Z. jujube* Linn

Samples	Fungal spp.	Concentration of Extract (mg/ml)										
		50	45	40	35	30	25	20	15	10	5	MFC
A. nilotica	<i>A. flavus</i>	-	-	-	-	-	-	-	-	+	+	15
	<i>T. rubrum</i>	-	-	-	-	-	-	-	+	+	+	20
	<i>C. albicans</i>	-	-	-	-	-	-	-	-	-	+	10
L. inermis	<i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-	5
	<i>T. rubrum</i>	-	-	-	-	-	-	-	-	-	-	5
	<i>C. albicans</i>	-	-	-	-	-	-	-	-	-	-	5
Z. jujube Linn	<i>A. flavus</i>	-	-	-	-	-	-	+	+	+	+	25
	<i>T. rubrum</i>	-	-	-	-	-	-	+	+	+	+	25
	<i>C. albicans</i>	-	-	-	-	+	+	+	+	+	+	35
Nystatin	<i>A. flavus</i>	-	-	-	-	-	-	-	-	+	+	15
	<i>T. rubrum</i>	-	-	-	-	-	-	-	-	+	+	15
	<i>C. albicans</i>	-	-	-	-	-	-	-	-	+	+	15

Key: (-) indicate no visible growth of the organisms and (+) indicate visible growth of the organisms

4. DISCUSSION

The increasing resistance of microorganisms to conventional antimicrobial drug, has lead to an increase/wider usage of plant, and other folk medicines as alternative ailment [7,8,9,10]. As previously reported, the methanol leaves extract of *A. nilotica*, *Z. jujube* Linn and *L. inermis* are rich in phytochemicals (glycoside, tannins, phenols saponins and flavonoids) and showed antibacterial potency [5]. In this study, the antifungal activities of the plant extracts were assessed. At 100 mg/mL the antifungal patency of the extracts was below that of standard antifungal agent (Nystatin), exclusive of *L. inermis* against *T. rubrum*. At a concentration of 150 mg/ml, the effect *L. inermis* extract was significantly increases against the fungi species beyond that of Nystatin. More so, the activity of *A. nilotica* extract against *T. rubrum* and *C. albicans* is higher than that of the control drug, while that *Z. jujube* Linn extract against *C.*

albicans was comparable to that of Nystatin, but higher against *T. rubrum*.

The outcome of this study conforms to earlier report suggesting a dose dependent antifungal activity of *A. nilotica* against *A. flavus* [11,12,13] and *C. albicans* [14,15]. The results of this study also conform to the findings of Yigit [16] which reported a strong antifungal activity of *L. inermis* against fungal isolates. This antifungal activity is attributed to the rich naphthoquinone content of it leaves extract [17,18,19]. The methanol leaves of *Z. jujube* Linn exhibited the lowest antifungal activity in comparison to the other samples investigated. These findings conform to the earlier reports of Manoj et al. [20] which revealed that the plant leaves extract had no effect against both *A. niger* and *C. albicans*. Whereas a report of Elaloui et al. [21] indicated promising antifungal effect of the plants leaves extract against *F. culmorum*, *F. solani* and *B. cinerea*. Similarly Naz et al. [22] reported a moderate

activity by methanol leave extracts of *Z. Jujuba mill* against *G. lucidum* but lower activity against *A. flavus*, *A. niger* and *A. alternate*. Abalaka et al. [23] reported the resistance of *A. niger* and *C. albicans* to ethanolic extracts of two *Ziziphus* species.

5. CONCLUSION

The findings of this study suggest that the leave extracts of *L. inermis*, *Z. jujube* Linn and *A. nilotica* showed antifungal activities against *A. flavus*, *T. rubrum* and *C. albicans*. Thus, these plants could served as potential sources of antifungal agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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