Original Research Article

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

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 leave 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 *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 were 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.

Key words: Antifungal, *Acacia Nilotica*, *Ziziphus Jujube* Linn and *Lawsonia Inermis.*

Comment [P1]: 100 mg/ml mic as a control is too high, what is recommended mic of Nystatin in vitro and stick to that.

Comment [P2]: I wonder why you are silent on your mic results, rather than loud on 100 and 150 mg/ml results?

1.0 INTRODUCTION

The leaves, roots and stem back of *Lawsonia inermis* (henna), *Ziziphus jujube* Linn and *Acacia Nilotica* are traditionally used for the management of bacterial and fungal infections [1]. Recent study on quantitative phytochemical analysis of crude methanol leave extracts of these plants revealed the presence of glycoside, tannins, phenols saponins and flavonoids

[2]. The antibacterial potency of these plant extracts against *Escherichia coli*, *Pseudomonas flourecense*, *Streptococcus and Staphylococcus aureus* was efficient [2]. In the present study, the antifungal property of the individual plant extracts was tested against *Aspergillus flavus*, *Trichophyton rubrum* and *Candida albicans*.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *A. nilotica*, *Z. jujube* Linn and *L. inermis* were collected from Achida, Wurno 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. The pulverized plant materials were mixed with 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 µm. 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 isolates were subjected to antifungal studies by agar well diffusion method. Sabouraud dextrose agar plates were inoculated with fungal culture (10 days old) by point inoculation. Standard solution (50, 100 and 150 mg/ml) of the extracts were added onto test organism-seeded plates. The plate containing distilled water (100 ml) was used as positive control while Nystatin (a standard fungicide) (100 mg/ml) was used as Negative 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 [3].

Comment [P3]: How possible

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 x 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^{0}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 [3].

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 [3].

2.6 DATA ANALYSES

Results were expressed as mean ± 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 were established by One way ANOVA followed by Duncan's, multiple comparison test. Statistical significance was set at $p< 0.05$

3.0 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 100mg/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*.

Comment [P4]: What does this mean

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)				
		A. nilotica	*L. inermis*	*Z. jujube* Linn	Nystatin	Distilled Water
50mg/ml	A. flavus	11.00±1.00[a]	11.67±2.89[a]	11.67±1.73[a]	53.00±2.65[b]	0.00
	T. rubrum	9.00±2.44[c]	17.33±1.89[d]	7.67±0.45[c]	30.00±2.33[e]	0.00
	C. albicans	11.45±0.89[a]	11.00±0.45[a]	14.33±0.67[f]	31.00±2.89[e]	0.00
100mg/ml	A. flavus	22.33+2.50[a]	41.67±2.90[b]	11.00±1.73[c]	53.00±2.65[d]	0.00
	T. rubrum	28.00±0.44[e]	34.33±1.89[f]	27.67±0.33[e]	30.00±2.33[g]	0.00
	C. albicans	24.67± 0.33[h]	17.67±1.45[i]	20.33±2.33[a]	31.00±2.89[g]	0.00
150mg/ml	A. flavus	28.00±2.00[a]	50.67±1.15[b]	26.67±2.65[c]	53.00±2.65[b]	0.00
	T. rubrum	36.00±0.44[d]	44.67±1.89[e]	37.33±0.33[f]	30.00±2.33[g]	0.00
	C. albicans	35.30±0.33[d]	38.33±1.45[f]	30.33±0.33[g]	30.33±2.89[g]	0.00

Values are mean inhibition zones (mm) ± S.D of three replicate experiment. Mean value having different superscript letters along the rows are significantly different ($P<0.05$)

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. ablicans* growth at MIC value 30mg/ml.

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	*A. flavus*	-	-	-	-	-	-	-	-	-	+	10
	T. rubrum	-	-	-	-	-	-	-	-	-	+	10
	C. ablicans	-	-	-	-	-	-	-	-	-	+	10
L. inermis	*A. flavus*	-	-	-	-	-	-	-	-	-	-	5
	T. rubrum	-	-	-	-	-	-	-	-	-	-	5
	C.ablicans	-	-	-	-	-	-	-	-	-	-	5
	A. flavus	-	-	-	-	-	-	-	-	+	+	15

Z. jujube Linn	*T. rubrum*	-	-	-	-	-	-	-	+	+	+	20
	C. ablicans	-	-	-	-	+	+	+	+	+	+	35
	A. flavus	-	-	-	-	-	-	-	-	+	+	15
Nystatin	*T. rubrum*	-	-	-	-	-	-	-	-	+	+	15
	C. ablicans	-	-	-	-	-	-	-	-	+	+	15

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

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. ablicans*.

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	*A. flavus*	-	-	-	-	-	-	-	-	+	+	15
	T. rubrum	-	-	-	-	-	-	-	+	+	+	20
	C. ablicans	-	-	-	-	-	-	-	-	-	+	10
L.inermis	*A. flavus*	-	-	-	-	-	-	-	-	-	-	5
	T. rubrum	-	-	-	-	-	-	-	-	-	-	5
	C. ablicans	-	-	-	-	-	-	-	-	-	-	5
Z.jujube Linn	*A. flavus*	-	-	-	-	-	-	+	+	+	+	25
	T. rubrum	-	-	-	-	-	-	+	+	+	+	25
	C. ablicans	-	-	-	-	+	+	+	+	+	+	35
Nystatin	*A. flavus*	-	-	-	-	-	-	-	-	+	+	15
	T. rubrum	-	-	-	-	-	-	-	-	+	+	15
	C. ablicans	-	-	-	-	-	-	-	-	+	+	15

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

4.0 DISCUSSION

The increasing resistance of microorganisms to conventional antimicrobial drug, has lead to a increase/wider usage of plant, and other folk medicines as alternative ailment [4, 5, 6, 7]. As previously reported, the methanol leaves extract of *A. nilotica*, *Z. jujube* Linn and *L. inermis* are rich in phytochemicals and showed antibacterial potency [2]. 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* [8, 9, 10] and *C. albicans* [11, 12]. The results of this study also conform to the findings of Yigit [13] which reported a strong antifungal activity of *L. inermis* against fungal isolates. This antifugal activity is attributed to the rich naphtoquinone content of it leaves extract [14, 15, 16]. 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*., [17] which revealed that the plant leaves extract had no effect against both *A. niger and C. albicans.* Whereas a report of Elaloui *et al.* [18] indicated promising antifungal effect of the plants leaves extract against *F. culmorum*, *F. solani* and *B. cinerea*. Similarly Naz *et al.* [19] 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*. [20] reported the resistance of *A. niger* and *C. albicans* to ethanolic extracts of two *Ziziphus* species.

5.0 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. ablicans*. Thus, these plants could served as potential sources of antifungal agents.

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