Original Research Article

EVALUATION OFTHE COMBINATION EFFECTS OF EXTRACTS OF *JATROPHA TANJORENSIS* AND *ADANSONIA DIGITATA* ON CLINICAL FUNGAL ISOLATES.

ABSTRACT

Introduction: The proliferation of fake drugs and the multiple resistance seen in conventional fungicidal drugs has led to the use of medicinal plants to treat fungal infections.There are claims that *Jatrophatanjorensis*and *Adansonia digitata* have fungicidal potentials, hence this study. Aim: To evaluate the combination effects of the extracts of *Jatropha tanjorensis* and *Adansonia digitata* on clinical fungal isolates. **Study design**: Randomized sampling of the plants was done by harvesting the plants from different farm in Nsukka Enugu State.

Place and duration of study: This study was conducted in Enugu State, South East of Nigeria**,** from November 2023 to March 2024.

Methodology: Phytochemical screening was done on the two plants.The methanolic and aqueous (cold and hot) extracts of these plants bark and stem were assessed for their antifungal activities against 6 fungal isolates using agar diffusion method. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were done according to standard methods.

Result: The plants contain alkaloids, terpernoids, saponins, flavonoids, tannin, glycoside and phenol. The hot water extracts of *J. tanjorensis stem* gave higher zones of inhibition diameter (IZD) than the methanolic extracts and the methanolic extracts of *A. digitata* bark gave higher IZD than the aqueous extracts. The combination of these two potent extracts showed greatest inhibition on *Aspergillus niger*, *Candida albican* and then least inhibition *on Trichophyton shoenleinii*, this was found to be statistically significant at(P<.05),the P value was(P=0.001). The lowest (MIC) and (MFC) were recorded in *A. niger* and *Candida albican* at (1.5625mg/ml) while the highest (MIC) and (MFC) were recorded in *T. shoenleinii*, at (25mg/ml*)*.

Conclusively, this study revealed that the combined plant extracts were more effective against opportunistic fungi than the dermatophytes.

Keywords: *Jatropha tanjorensis, Adansonia digitata, Fungi organisms, Antifungal susceptibility, Minimum inhibitory concentration.*

INTRODUCTION

Jatropha tanjorensis isa common weed of field crops and a gregarious shrub of about 1.8 m in height and it belongs to the family Euphorbiaceae;. It is usually grown in rainfall forest zones of West Africa. *Jatropha* is known for its purgative and laxativepotentials, it has other medicinal values. All parts of the plant including seeds, leaves and bark; fresh or as a decoction, are used as treatment regime in traditional and folk medicine [1]. While *Adansonia digitata* is a big,long-lived, deciduous tree that can be found in the sub-Saharan Africa [2]. It grows to a height of approximately 15 m and has a hugely swollen, unevenly folded trunk [3].

Fig 1; *J. tanjorensis*(uguoyibo) or (hospital too far). [4].

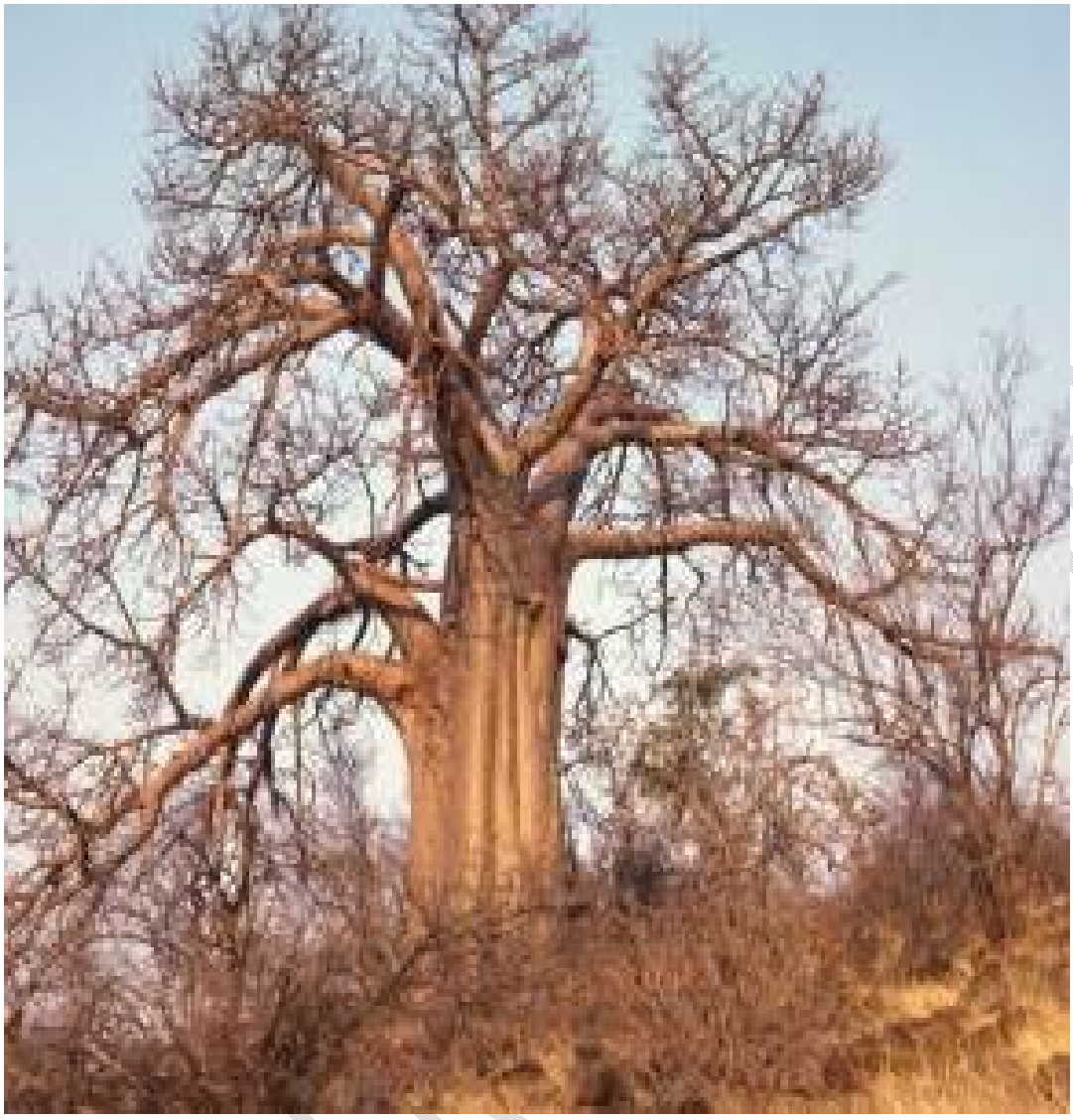

Fig 2; *A. digitata* (oyili-akpu) tree. [5]

These plants have been reported by [6] to be used for medicinal purposes in the treatment of various diseases. [7]observed *in vitro* and *in vivo* investigation of the antifungal properties of *Jatropha curcas* and *Ricinus communis* seed extracts against the mycelia growth and rot development of yam caused by *Fusarium verticillioides* and *Aspergillus flavus*.

The high cost of conventional drugs, adverse drug reactions and the proliferation of fake drugs has brought about resistance of some organisms to the conventional drugs[8],and that has led to the use of medicinal plants so as to curb this menace. *Jatropha tanjorensis* and *Adansonia digitata* have been used by many traditional healers to treat various non-communicable and communicable diseases, such asEczema, Candidiasis, Aspergilosis,Tenia

capitis, Tenia corporis, malaria, diarrhea and anaemia, hence the aim of this study;To evaluate the combination effects of the extracts of *Jatropha tanjorensis* and *Adansonia digitata* on clinical fungal isolates.

MATERIALS AND METHODS

This study was conducted in Enugu State, South East of Nigeria**,** from November 2023 to March2024.

Collection of Plants materials and Phytochemical screening: The bark and stem of the plants were obtained from a farmland in Nsukka, Enugu State and the Phytochemical screening and authentication were done in Herbarium unit of the Department of Pharmacognosy, University of Nigeria, Nsukka. Enugu State.

Preparationof plant extract: The various plant parts were rinsed in running tap water and sun-dried and thengrounded to fine powder using a mechanical grinder.The powdered plant materials were extracted using methanol, cold and hot water by method of [9-10]with little modification.

Test microorganism: Clinical isolates used in the study were obtained from Mycology Unit of the University of Nigeria Teaching Hospital, Ituku-ozalla, Enugu State. They include C.*albican*, *A.niger*, *A. fumigatus, Penicillum*spp*, T.schoenleinii*and *T. tonsurans*. These isolates were confirmed by standard mycological methods according to [11].

Susceptibility testing: The Antifungal susceptibility testing of the extracts were done usingagar well diffusion method by [11-12]. Freshly prepared Sabouraud dextrose agar (containing chloramphenicol) was allowed to solidify and using a swab stick an overnight broth of fungal culture was swabbed all over the surface of the plate, 4 holes of 6mm diameter were bored in the plate and the different concentration (100mg/ml, 50mg/ml, 25mg/ml and 12.5 mg/ml) of the different extracts were seeded in 40ul amount into the holes, Fluconazole and DMSO were used as positive and negative control respectively, they were incubated at 26^{o}Cfor 48hours and the zones of inhibition diameter were measured and compared withthe positive and negative controls.

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) value of the combined extracts of *J. tanjorensis* and *A. digitata* against fungal isolates were determined using Modified dilution method of [13]. The initial concentration of plant extracts (100mg/ml) was serially diluted by

transferring 2ml from (stock) solution into 2ml of sterile SDA broth and mixing the content in a test tube and then serially diluting in five other test tubes, giving concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.563.Then 0.2ml of the standardized broth culture of fungi was inoculated into the tubes and were incubated accordingly. The lowest concentration that inhibited growth of test organisms was noted as the minimum inhibitory concentration. The content of the tubes weresubcultured into freshly prepared Sabouraud dextrose agar and the concentration that showed no growth is the minimum fungicidal concentration.

Data analysis: The data collected was analyzed and graphed using Minitab Version 20.4 and Microsoft Excel 2016. Kruskal Wallis test was performed on the data, followed by a post-hoc analysis (Dunn's test) using a level of significance of 0.05. When (*P<0.05), the difference was significant.

RESULTS

The data obtained from this study revealed that Phytochemical analysis of both plant extracts had secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, saponins, steroids, reducing agents, phenols and glycosides.

Table 1: Anti-fungal Susceptibility Pattern of Hot Water Extract of *J. tanjorensis*Stem (JTS).

Fungal Isolate	**Extract (mg/ml)**			
	100mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**
Aspergillus niger	*42*	38	31	27
Candida albican	38	34	29	21
Aspergillus fumigatus	40	36	30	23
Penicillium spp	36	29	23	17
T. schoenleinii	22	23	19	13
T. tonsurans	34	27	23	19
P-value	**0.007**			

The table 1 presents the inhibition zone diameter (IZD) of the fungal isolates at different level of concentrations, 100mg concentration had the highest mean IZD among the four levels of concentrations. The differences in IZD across the concentrations compared using Kruskal Wallis test which gave a P-value of 0.007at (P <.05) indicating a significant difference of IZD across the different levels of concentrations.

Table 2: Anti-fungal Susceptibility Pattern of Methanolic Extract of *A.digita* bark.

Fungal Isolate	**Extract (mg/ml)**			
	100mg/ml	**50mg/ml**	**25mg/ml**	**12.5mg/ml**
Aspergillus niger	42	40	37	28
Candida albicans	42	37	33	27
Aspergillus fumigatus	44	40	37	30
Penicillium spp	36	31	25	22
T. schoenleinii	27	23	20	13
T. tonsurans	31	28	22	17
P-value	**0.032**			

The table 2, shows the inhibition zone diameter (IZD) of the fungal isolates at different level of concentrations for the methanolic extract of *A. digita* bark. Here, 100mg/ml concentration had the highest mean IZD among the four levels of concentrations. The differences in IZD across the concentrations compared using Kruskal Wallis test which gave a P-value of 0.032 at ($P < .05$) indicating a significant difference of IZD across the different levels of concentrations.

Table 3: The combined Hot water Extract of *J. tanjorensis*Stem and Methanolic Extract of *A. digitata* bark.

Fungal Isolate	**Combined Extracts (mg/ml)**			
	100mg/ml	**50mg/ml**	**25mg/ml**	**12.5mg/ml**
Aspergillus niger	61	55	52	50
Candida albicans	58	54	48	43
Aspergillus fumigatus	56	50	47	37
Penicillum spp.	54	51	46	39
T. shoenleinii	53	47	42	36
T. tonsurans	56	51	45	40
P-value	**0.001**			

The table 3 shows the inhibition zone diameter (IZD) of fungal isolates at different level of concentrations for combined hot water extract *J. tanjorensis* stem and methanolic extract of *A. digitata* bark. The IZD was highest at 100mg/ml concentration of the extracts. The differences in IZD across the concentrations compared using Kruskal Wallis test which gave a P-value of 0.001 at (P<.05) indicating a significant difference of IZD across the different levels of concentrations.

Table 4: Minimum Inhibititory Concentration (MIC) of the Combined Extracts of Hot water Extract of *J. tanjorensis*Stem and Methanolic Extract of *A. digitata* bark. (JTS + ADB).

Organisms	Different concentrations of the combined extract (Jts+Adb). 100mg	50mg	25mg	12.5mg	6.25mg	3.125mg	1.5625mg
A. niger	-	-	-	-	-	-	+
C. albican	-	-	-	-	-	-	+
A.fumigatus	-	-	-	-	-	+	+
Penicillium spp	-	-	-	-	+	+	+
T. shoenleinii	-	-	+	+	+	+	+
T.tonsurans	-	-	-	+	+	+	+

Key:- = No growth; **+** = Growth.

From table 4, the combined extract was able to inhibit the growth of all the opportunistic fungi even at very low concentration, while the dermatophytes were not inhibited at very low concentrations,

DISCUSSION

It was observed that the hot water extracts of *J. tanjorensis*and the methanol extracts of *A. digitata* gave the highest activity than cold water extracts of the plants, this shows that the proportion of water insoluble compound was greater than that of water soluble compound hence hot water and methanol aided solubility, this is in line with the results of [14].

The antifungal activity of the hot water extract of *J. tanjorensis* (JTS) demonstrated in table 1, showed that at 100mg/ml, both *A.niger*and *A. fumigatus* were most susceptible followed by *C. albicans* and *Penicillium*spp while the least susceptible fungi was*T. shoenleinii*, this is in agreement with the work of [7], who also isolated A. fumigatus in his work. The differences in IZD across the concentrations were compared and P-value of

0.007,which indicatedthat there is a significant difference in their IZD, hence the J.tanjurensis significantly inhibited the growth of these fungi.

The antifungal activity of the methanol extract of *A. digitata* bark (ADB) against the test isolates were shown in table 2, and it revealed that *A. fumigatus* was the most susceptible at 100mg/ml, followed by *A.niger*and then other fungi,this is in agreement with the work of[15], where the methanol extract of *A. digitata* stem- bark inhibited mycelial development of *Aspergillus* spp.

Tables 3, shows that the fungi isolates used in this study were highly susceptible to the combination of hot water extract of *J. tanjorensis and* the methanol extract of *A.digitata*,especially on *A. niger, andC. albican*, this agrees with the work of [4].

Table 4, showed the minimum inhibition concentration (MIC) of the combined extracts on *A. niger* and *C. albican*to be low (1.5625), is not in agreement with the findings of [16], where the MIC for *A. niger* and *C.albicans* was greater than 25mg/ml. The minimium fungicidal concentration (MFC) in this study ranges from 1.5625mg/ml to 6.25mg/mlfor the opportunistic fungi and 12.5mg/ml - 25mg/ml fordermatophytes, this is not in agreement with the workperformed by [14] where the MFC was 500ug/ml against *C. albican* and 1000ug/ml against *A. niger.*

CONCLUSION

The result of the analysis revealed that *J. tanjorensisextract has significant inhibitory action on Aspergillus species and some other fungal organisms more the A. digitata*, but the combined extracts of *J.tanjorensis*and *A. digitata* is a better inhibitor of the fungal organisms therefore they can be used in combination as a potential source of antifungal agent for the treatment of opportunistic fungaldiseases such as Eczema, Aspergillosis and Candidiasis, and the extracts were not very inhibitory to the dermatophytes, hence they cannot be used to treat infections caused by dermatophytes.

LIMITATIONS

The limitation experienced in the course of this was insufficient fund, if there was enough fund, further studies like the molecular studies of the extracts would have been done.

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