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Evaluation of three plant extracts in the control of fungal pathogens isolated from Garri (fried mashed fermented cassava) in Makurdi, Nigeria

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ABSTRACT

An evaluation of three plant extracts, Parkia biglobosa, Moringa oleifera and Daniellia oliveri in the control of fungal pathogens isolated from garri (fried mashed fermented cassava) in Makurdi was conducted. The fungal pathogens isolated from the garri samples were Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae and Aspergillus fumigatus. Water and ethanol extracts of the plant species at concentrations of 20%, 40%, 60%, 80% and 100% were tested against the fungal isolates in vitro. The inhibitory effects of water extracts of Moringa oleifera, Parkia biglobosa and Daniellia oliveri at 100% on all test organisms ranged from 1.15 to 4.95cm and at 20% it ranged from 3.50 to 8.00cm. The inhibitory effect of ethanol extract of Moringa oleifera, Parkia biglobosa and Daniellia oliveri at 100% on all test organisms ranged from 0.70 to 4.00cm and at 20% it ranged from 2.50 to 9.50cm. The antifungal activity of the extracts (water and ethanol) increased with increase in concentration. Analysis of Variance revealed higher significant inhibitory effects of both water and ethanol extracts at 100% (2.02cm) and (2.50cm) respectively and least inhibitory effect at 20% (6.06cm) and (7.08cm) respectively for most fungal isolates. There was no significant difference in the grand mean inhibitory effect of both water and ethanol extract on the test fungi at P = 0.05. The extracts of Parkia biglobosa, Moringa oleifera and Daniellia oliveri are potentially useful antifungal agents, inhibiting fungal growth at all concentrations.

Keywords: Evaluation, Garri, Plant extracts, fungal pathogens, Makurdi.

INTRODUCTION

Garri is the most popular cassava product in Africa [1] (Thoha *et al.*, 2007). It is widely accepted in both rural and urban areas [2] (Frazier, 2000). Traditional preparation techniques vary by region and by ethnic group. The variation in the preparation techniques practiced among different localities has resulted in a non – uniform product with respect to quality, shelf life and safety practices associated with the production and storage of the product.

Microbial proliferation in garri is a major economic concern. The major agents that contaminate or render it unfit for consumption are moulds, insects and mites [3] (Ogiehor and Ikenebomeh, 2005). The growth of mold in garri raises the risk of mycotoxin related illnesses to consumers. Plant extracts have been recorded to have significant activities against fungal organisms in plant products. Also the use of extracts from higher plants in the control of fungal organisms that contaminate food products have been reported [4] (Tiwari, 1997).

This study therefore evaluates the extracts of *Parkia biglobosa*, *Moringa oleifera* and *Daniellia oliveri* in the control of fungal pathogens isolated from Garri in Makurdi.

MATERIALS AND METHODS

Collection of Garri Samples
A total of 20 samples of Garri were collected in polyethylene bags from the major markets within Makurdi and taken to the Botany laboratory of the Benue state university for isolation of fungal pathogens.

Media Preparation
The medium used for isolation of fungi was Potato Dextrose Agar (PDA). This was prepared according to manufacturer's instruction. About 36.9g of powdered PDA was dissolved in 1 litre of sterile distilled water and sterilized at 121^0C for 15mins. After cooling, it was poured into sterile Petri dishes.

Isolation of Fungal pathogens
The garri which were tied in muslin bags were surfaced sterilized by dipping in 5% Sodium hypochloride (NaOCl) solution for 3 minutes. These were then rinsed in several changes of sterile distilled water. The surface sterilized garri was then loosened from the muslin bags and 10 grams of it was dispersed into 90mls of sterile distilled water in a beaker and allowed to stand with occasional stirring for 5 minutes. One millilitre of the solution was transferred to a test tube contain ning 9mls of sterile distilled water. The process was repeated to yield a serial dilution of 10^4. 1 millilitre portion of 10^4 test tube was pipette into a separate Petri dish after which 15 – 20mls of molten PDA was added. The Petri dish was swirled gently on the laboratory bench to effect proper dispersion. The Petri dish was incubated at 25 – 27^{o}C for 5 – 7days. After 5 – 7days of fungal growth, subcultures were made on sterile Petri dishes and incubated at 25 – 27^{o}C for 7days.

Identification of fungal pathogens
Two techniques; visual observation of fungal growth in Petri dishes and microscopic identification in slide culture using lactophenol cotton blue dye were used for identification of fungal isolates. The isolates were then matched with standards from [5] (Barnett & Hunter 1972) and other electronic documentations on the genera isolated.

Collection of plant material: Fresh leaves of three selected plant species namely *Parkia biglobosa* (locust bean tree), *Moringa oleifera* (Drumstick) and *Daniellia oliveri* (African Copaiba Balsam) were collected and taken to the Botany laboratory of the Benue state University for preparation of crude extractions.

Preparation of Crude Extractions
About 50grams each of fresh leaves of the selected trees were weighed for both water and ethanol extractions respectively. The leaves were washed with tap water and rinsed with distilled water after which they were pounded separately using a mortar and pestle. The macerates each were transferred into different beakers each containing 100ml of sterile distilled water and 90% ethanol respectively for water and ethanol extracts. The set up was left to stand for 6 hours after which the macerates were sieved using a muslin cloth into separate beakers for water and ethanol respectively of each plant species.

Extract Concentrations
Serial dilutions of the crude extracts (water and ethanol) of the selected plant species were prepared to give 20%, 40%, 60%, 80% and 100% respectively. Extract concentration of 20% (w/v) was obtained by adding 80mls of sterile distilled water to 20g of each botanical paste in a beaker for water extraction and 90% ethanol for ethanol extraction respectively. Extract concentration of 40% (w/v) was obtained by adding 60mls of sterile distilled water to 40g of each botanical paste in a beaker for water extraction and 90% ethanol for ethanol extraction respectively. The same principle applied to all the other extract concentrations.

Anti-fungal activity of plant extracts on fungal isolates in vitro
The pour plate method was used to investigate the efficacy of the extracts on test fungi.1milliliter of 20%, 40%, 60%, 80% and 100% of the crude extracts (water and ethanol) for each of the plant species was dispensed in sterile Petri dishes after which 15 – 20mls of molten PDA was added. The mixture was swirled gently on the work bench and allowed to set. The medium was then inoculated centrally with 4 mm discs obtained from 5 - 7 days old cultures of the test fungi. Three replications were set for each experiment. Controls were Petri dishes containing PDA with no botanical extract, inoculated with the test fungi. The plates were arranged on laboratory desks following complete randomized design. The Petri plates were incubated at 25 – 27^{o}C for 5 – 10 days during which measurement of growth of the fungal colony was carried out using a meter rule at intervals of twenty four hours. Growth inhibition of the fungi was calculated using the formula;

$$\text{Growth inhibition of fungi} = \frac{R_1 - R_2}{R_1} \times 100$$

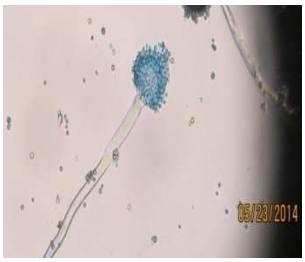
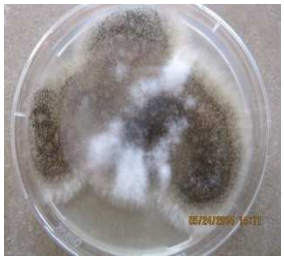
Where R_1= growth of the fungi in control
R_2= growth of fungi in treatment

Data Analysis
Data generated from this study was analysed using Analysis of Variance (ANOVA) and the Fishers Least Significant difference was used to separate the means at 5% level of significance.

RESULTS

Fungi Isolated From Garri Samples
A total of four fungi were isolated from the garri samples. They are *Botryodiplodia theobromae, Aspergillus flavus, Aspergillus niger* and *Aspergillus fumigatus* as shown in Table I.

Table I: Characterization of fungal isolates from Garri on PDA.

Macro/Microscopic characteristics	Appearance on PDA	Photomicrograph	Probable Organism
Colony is deep green in colour. Conidia are grey green to pale blue and are spherical to sub spherical, about 3-6µm in diameter. Conidiophores are heavy walled and hyaline.			***Aspergillus flavus***
Colony is fast growing and bears abundant erect conidial structures, typically deep brown to black in colour covering the entire colony. Conidiophores are hyaline and faintly brown near the apex. Conidia are typically spherical.			***Aspergillus niger***
Colony colour is grayish. Conidia colour is dark green to dark blue and is globose. Conidiophores are uncoloured to grayish and are smooth to finely roughen.			***Aspergillus fumigatus***
The colony has white aerial mycelia that turned grey to black with age and formed black pycnidia. Pycnidium is oval, brownish with one septum in the middle			***Botryodiplodia theobromae***

The inhibitory Effects of water extract of *M. oleifera* on Test Fungi.
This showed that there was significant inhibitory effect of *Moringa oleifera* on *B. theobromae* and *A. fumigatus*. On *B. theobromae*, the highest significant inhibitory effect was observed at 100% (1.15cm) and the least significant

inhibitory effect at 20% (7.75cm). On *A. fumigatus*, the highest significant inhibitory effect was observed at 100% (1.10cm) while the least significant inhibitory effect was at 20% (4.10cm). On *A. flavus*, highest inhibitory effect was observed at 80% (1.45cm) and the least inhibitory effect at 100% (4.95cm). On *A. niger*, highest inhibitory effect was at 100% (3.60cm) and the lowest inhibitory effect was observed at 20% (6.05cm) as shown in table II.

Table II: Inhibitory effects of water extract of *M. oleifera* on test fungi.

Conc./ml	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	3.50	6.05	7.75^{a}	4.10^{a}
40%	2.90	4.10	5.65^{ae}	2.95^{ae}
60%	2.40	5.90	4.95^{be}	2.10^{bef}
80%	1.45	5.25	4.45^{ce}	2.60^{ce}
100%	4.95	3.60	1.15^{d}	1.10^{df}
LSD(0.05)	**NS**	**NS**	**2.15**	**1.41**

Footnote: Means not tagged with same alphabets are significant at P=0.05
NS - No significant difference.

The Inhibitory Effects of Ethanol Extract of *M. oleifera* on Test Fungi

The inhibitory effects of ethanol extract of *M. oleifera* on test fungi showed that there was a significant inhibitory effect on *A. niger* and *B. theobromae*. On *A. niger*, the highest significant inhibitory effect was at 40% (2.25cm) and 100% (2.25cm) and the least significant effect was at 20% (5.35cm). On *B. theobromae*, the highest significant inhibitory effect was observed at 100% (1.70cm) while the least was at 20% (6.00cm). On *A. flavus*, highest inhibitory effect was at 100% (0.70cm) and the least inhibitory effect was at 20% (2.90cm). On *A. fumigatus*, highest inhibitory effect was at 100% (1.60cm) and the lowest inhibitory effect was at 20% (6.00cm) as shown on table III.

Table III: Inhibitory effects of Ethanol extract of *M. oleifera* on Test Fungi

Conc./ml	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	2.90	5.35^{a}	6.00^{a}	6.00
40%	1.90	2.25^{b}	3.55^{b}	3.90
60%	2.15	2.35^{b}	2.55^{c}	3.00
80%	2.65	2.50^{b}	2.25^{d}	4.50
100%	0.70	2.25^{b}	1.70^{e}	1.60
LSD(0.05)	**NS**	**0.41**	**0.14**	**NS**

Footnote: Means not tagged with same alphabets are significant at P=0.05
NS - No significant difference.

The Inhibitory Effects of water Extracts of *Daniellia oliveri* on Test Fungi

The inhibitory effects of water extracts of *D. oliveri* on the test fungi showed that *A. flavus* was most significantly susceptible at 100% (2.00cm) and least significantly susceptible at 20% (6.95cm). Also *B. theobromae* was most significantly susceptible at 100% (2.95cm) and least significantly susceptible at 20% (5.00cm). However, *A. niger* showed highest inhibitory effect at 100% (3.50cm) and least inhibitory effect at 20% (8.00cm). *A. fumigatus* showed highest inhibitory effect at 100% (3.15cm) and lowest inhibitory effect at 20% (7.75cm) as shown in table IV.

Table IV: Inhibitory effects of water extract of *D. oliveri* on Test Fungi

Conc./ml	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	6.95^{a}	8.00	5.00^{a}	7.75
40%	4.15^{bf}	6.05	4.25^{ad}	7.50
60%	5.65^{cf}	4.50	8.00^{b}	6.50
80%	4.60^{df}	4.25	3.90^{ae}	3.40
100%	2.00^{e}	3.50	2.95^{cde}	3.15
LSD(0.05)	**1.82**	**NS**	**1.64**	**NS**

Footnote: Means not tagged with same alphabets are significant at P = 0.05
NS - No significant difference.

The Inhibitory Effects of Ethanol Extracts of *Daniellia oliveri* on test Fungi

The inhibitory effects of ethanol extract of *D. oliveri* on the test fungi showed that *A. flavus* was most significantly susceptible at 100% (4.00cm) and least significantly susceptible at 20% (8.85cm) while *B. theobromae* was most significantly susceptible at 100% (3.10cm) and least significantly susceptible at 60% (7.00cm). On *A. niger*, highest inhibitory effect was observed at 100% (2.00cm) and least inhibitory effect was observed at 20% (9.50cm). On *A. fumigatus*, highest inhibitory effect was observed at 100% (2.15cm) while the least inhibitory effect was observed at 20% (7.75cm) as shown in table V.

Table V: Inhibitory effects of Ethanol Extract of *D. oliveri* on Test Fungi

Conc./ml	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	8.85[a]	9.50	6.00[a]	7.75
40%	7.25[a]	7.05	5.36[b]	6.50
60%	6.65[b]	5.50	7.00[c]	5.60
80%	6.70[b]	3.25	4.90[d]	6.40
100%	4.00[c]	2.00	3.90[e]	2.15
LSD(0.05)	**1.8**	**NS**	**2.05**	**NS**

Footnote: Means not tagged with same alphabets are significant at P = 0.05
NS - No significant difference.

The Inhibitory Effects of Water Extract of *Parkia biglobosa* on Test Fungi

The inhibitory effects of water extract of *P. biglobosa* on the test fungi showed that there was significant inhibitory effect on *A. flavus.* On *A. flavus*, the highest significant inhibitory effect was observed at 100% (1.65cm) and the least significant inhibitory effect at 20% (6.35cm). On *A. niger, B. theobromae* and *A. fumigatus*, the highest inhibitory effect was observed at 100% (2.65cm), (2.25cm) and (1.80cm) respectively while the lowest inhibitory effect was observed at 20% (5.75cm) and (5.75cm) for *A. niger and B. theobromae*. *A. fumigatus* showed least inhibitory effect at 40% (3.90cm) and 60% (3.90cm) respectively as shown in table VI.

Table VI: Inhibitory effects of water extract of *Parkia biglobosa* on Test Fungi

Conc./ml	*A. flavus*	*A .niger*	*B. theobromae*	*A. fumigatus*
20%	6.35[a]	5.75	5.75	2.55
40%	4.35[be]	5.60	5.80	3.90
60%	4.70[ae]	4.35	4.05	3.50
80%	3.55[ce]	4.75	4.80	3.90
100%	1.65[d]	2.65	2.25	1.80
LSD(0.05)	**1.76**	**NS**	**NS**	**NS**

Footnote: Means not tagged with same alphabets are significant at P = 0.05
NS -No significant difference.

The Inhibitory Effects of Ethanol Extract of *Parkia biglobosa* on Test Fungi

The inhibitory effects of ethanol extract of *P. biglobosa* on the test fungi showed highest inhibitory effect at 100% and 80% (1.25cm) on *A. flavus* and least inhibitory effect at 40% (4.30cm). On *A. fumigatus*, *A. niger and B. theobromae*, the highest inhibitory effect was observed at 100% (2.75cm), (3.45cm) and (2.75cm) respectively and their least inhibitory effect at 20% (7.50cm), (7.75cm) and (6.90cm) respectively as shown in table VII.

Table VII: Inhibitory effects of Ethanol extract of *P. biglobosa* on Test fungi

Conc./ml	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	4.15	7.75	6.90	7.50
40%	4.30	6.00	5.35	6.30
60%	3.35	4.80	3.85	5.20
80%	1.25	4.00	3.90	3.50
100%	1.25	3.45	3.45	2.75
LSD(0.05)	**NS**	**NS**	**NS**	**NS**

Footnote: No significant difference at P= 0.05
NS – No Significant difference.

Table VIII: Comparative inhibitory effects of water extract of the three plant species on the test fungi.

Conc./ml	*A .flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	5.60	6.60[a]	6.17[a]	4.80
40%	3.80	5.25[ae]	5.23[a]	4.78
60%	4.25	4.92[be]	5.67[a]	4.03
80%	3.20	4.75[cef]	4.38[ac]	3.30
100%	2.87	3.25[df]	2.12[bc]	2.02
LSD(0.05)	**NS**	**1.58**	**2.31**	**NS**

Footnote: Means not tagged with same alphabets are significant at P = 0.05
NS -No significant difference.

Comparative Inhibitory Effects of Water Extract of the Three Plant Species on the Test Fungi

The comparative inhibitory effect of water extracts of the three plants species on the test fungi showed that there was significant inhibitory effect on *A. niger* and *B. theobromae.* On *A. niger*, the highest significant inhibitory effect was observed at 100% (3.25cm) and the least significant inhibitory effect at 20% (6.60cm). On *B. theobromae*, the highest significant inhibitory effect was at 100% (2.12cm) and the least significant inhibitory effect was at 20% (6.17cm). On *A. fumigatus*, the highest inhibitory effect was observed at 100% (2.02cm) and the least inhibitory

effect at 20% (4.80cm) and on *A. flavus*, the highest inhibitory effect was observed at 100% (2.87cm) and the least inhibitory effect at 20% (5.60cm) as shown in table VIII.

The Comparative Inhibitory effects of ethanol extract of the three plant species on the test fungi

The comparative inhibitory effect of ethanol extracts of the three plant species on the test fungi showed that, highest significant inhibitory effect was observed at 100% (3.07cm) and the least significant effect at 20% (7.03cm) on *A. niger*. On *A. fumigatus*, the highest significant inhibitory effect was observed at 100% (2.50cm) and the least significant effect at 20% (7.08cm). *A. flavus* had highest inhibitory effect at 80% (2.83cm) and lowest inhibitory effect at 20% (4.07cm). On *B. theobromae*, highest inhibitory effect was observed at 100% (2.70cm) and the least inhibitory effect at 20% (5.97cm) as shown in table IX.

Table IX: Comparative inhibitory effects of ethanol extract of the three plant species on the test fungi

Conc./ml	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
20%	4.67	7.03^{a}	5.97	7.08^{a}
40%	3.45	4.77^{ae}	4.38	5.90^{ad}
60%	3.72	3.88^{be}	4.80	4.90^{ae}
80%	2.83	3.58^{ce}	3.35	3.80^{bdef}
100%	3.20	3.07^{de}	2.70	2.50^{cf}
LSD(0.05)	**NS**	**2.58**	**NS**	**2.36**

Footnote: Means not tagged with same alphabets are significant at P = 0.05
NS -No significant difference.

Comparative Inhibitory Effect of water and ethanol extracts of the three plant species on test fungi

The comparative inhibitory effect of water and ethanol extract of the three plant species on the test fungi showed that there was no significant inhibitory effect between water and ethanol extracts of the plant species on the test organisms as shown in table X.

Table X: **Comparative inhibitory effects of water and Ethanol extracts on the test fungi**

Extract	*A. flavus*	*A. niger*	*B. theobromae*	*A. fumigatus*
Water	3.94	4.95	4.71	3.79
Ethanol	3.57	4.67	4.24	4.84
LSD 0.05	**NS**	**NS**	**NS**	**NS**

Footnote: No significant difference at P=0.05
NS- No Significant difference

DISCUSSION

The results of this study clearly show that garri sold in Makurdi habour different fungi such as *A. flavus, A.niger, B. theobromae* and *A. fumigatus.* These fungi have also been reported in various stored foods [6] (Brauman, 1995). Their presence in the garri samples indicate the poor handling techniques during processing, transport and storage. The presence of the organisms in garri suggests an imminent public health danger since the metabolites (mycotoxins) produced by them may lead to devastating clinical conditions in the consumers.

In another set of experiments, the aqueous and ethanol extracts of three plant species namely; *P. biglobosa, D. oliveri* and *M. oleifera* inhibited the growth of *A. flavus, A. niger, B. theobromae and A. fumigatus* in vitro. In general, it was observed that susceptibility of the fungal isolates increased with increasing concentration of the plant extracts. These results agree with the reports of other workers on the inhibitory action of plant products employed on the mycelia growth and spore germination of other pathogenic fungi [7](Bankole and Adebanjo,1995), [4] (Tiwari,1997),[8] (Ajayi and Olufolaji,2008).

All the plants had antifungal potentials at various concentrations. The antifungal potentials of these plant extracts can be explained by the phytochemicals present in them [9] (Emmanuel *et al*., 2013). [10] (Ahmad *et al.,* 1998) reported that phytochemical screening of these plants revealed the presence of alkaloids, saponins, tannins, flavonoids which have been found to form reversible complexes with fungal organisms.

The variation in the inhibitory effects of the plants extracts may be due to the qualitative and quantitative actions of the bioactive components present in the plants.

CONCLUSION

The extracts of *P. biglobosa*, *Daniellia oliveri* and *M. oleifera* are potentially useful antifungal agents, inhibiting their growth at all concentrations. The bioactive compounds from these plants can therefore be employed in the formulation of antifungal drugs for ailments in which these fungal isolates have been implicated as etiologic agents.

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