



In vitro* Efficacies of Some Nigerian Medicinal Plant Extracts against Toxigenic *Aspergillus flavus*, *A. parasiticus* and *A. ochraceus

Yemisi Adefunke Jeff-Agboola^{1*} and Anthony Kayode Onifade²

¹Department of Biological Sciences, Ondo State University of Science and Technology, P.M.B 353 Okitipupa, Nigeria.

²Department of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author YAJA designed the study, wrote the protocol, analyze the study and wrote the first draft of the manuscript. Author AKO managed the literature searches, managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/20390

Editor(s):

(1) Dongdong Wang, Department of Pharmacogonosy, West China College of Pharmacy, Sichuan University, China.

Reviewers:

(1) Norbert De Kimpe, Ghent University, Belgium.

(2) Antonello Santini, University of Napoli Federico II, Italy.

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(4) Daniela Hanganu, Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania.

Complete Peer review History: <http://sciencedomain.org/review-history/11768>

Original Research Article

Received 25th July 2015
Accepted 20th September 2015
Published 16th October 2015

ABSTRACT

The aim of this work was to find an alternative to chemical fungicides currently used in the control and prevention of activities of toxigenic *Aspergillus flavus*, *A. parasiticus* and *A. ochraceus* in food and feed. The antifungal activity of extracts of five medicinal plants used in native medicine in Nigeria is reported. All plant extracts were screened for their minimum inhibitory concentration (MIC) against toxigenic *Aspergillus flavus*, *A. parasiticus* and *A. ochraceus*. Antifungi efficacies of the medicinal Plant extracts showed that maximum antifungal activity was demonstrated by ethanolic, hot and cold water extracts of *Borreria verticillata*, *Lactuca capensis*, *Colocasia esculenta*, *Jatropha gossy* and *Ageratum conyzoides*. Leaves part of the five medicinal plants were investigated for antifungal activities against toxigenic *Aspergillus flavus* using radial growth technique. The plant extracts of *Borreria verticillata* inhibited the growth of the test organisms. *Borreria verticillata*

*Corresponding author: Email: yjeffagboola@yahoo.com;

appears to be more effective as an antifungal agent than other plant extracts. The ethanolic extracts were more effective than the cold and hot water extracts against the tested organism.

Keywords: Antifungi; toxigenic Aspergillus spp; medicinal plant.

1. INTRODUCTION

Fungal deterioration of stored seeds and grains is a chronic problem in the Nigeria storage system because of the tropical hot and humid climate. Harvested grains are colonized by various species of *Aspergillus*, under such conditions leading to deterioration and mycotoxin production. *Aspergilli* are the most common fungal species that can produce mycotoxin in food and feedstuffs. Aflatoxins are primarily produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which contaminate a wide variety of food and feed commodities including maize, oilseeds, spices, groundnuts, tree nuts, milk and dried fruits [1]. Presence of aflatoxins in food chain is associated with decrease in quality and quantity of food and feed materials. In addition, consumption of aflatoxin-contaminated products can pose a risk of development of various diseases in human and animals. *Aspergillus* can attack crops at different times, in the field, during harvest, transport and storage. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market [2].

The known success of traditional medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotic [3,4].

Medicinal Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones [5-7]. Which have been used worldwide in traditional medicine to treat several diseases and infection [8-10]. Many studies all over the world have shown that these plants and their extract have multi-antimicrobial properties [11-13].

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine [14].

Over Sixty percent of Nigeria's rural population depends on traditional medicine for their

healthcare need [15]. Medicinal plants contain pharmacologically active principles which over the years have been exploited in traditional medical practice for the treatment of various ailments [14]. Medicinal plants play a paramount role in the management of various ailments in rural communities considering the vast potentiality of medicinal plant as sources for antimicrobial agents.

Although restrictions are being imposed to protect food quality and the environment, chemicals are still our only recourse at present to prevent diseases of food crops. In recent years, the need to develop disease control measures as alternative to chemicals has become a priority of scientists worldwide. Therefore, it is important to find a practical, cost effective and non-toxic method to prevent fungal deterioration of field crop. Use of natural plant extracts and biocontrol agents provides an opportunity to avoid chemical preservatives. Therefore, the present study was designed to evaluate the *in vitro* antifungal activity of some plant extracts against toxigenic strains of *A. flavus*, *A. parasiticus* and *A. ochraceus*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves of five medicinal plants (Plates 1-5) of *Borreria verticillata*, *Lactuca capensis*, *Colocasia esculenta*, *Jatropha gossy* and *Ageratum conyzoides* were collected from Akure metropolis in Nigeria. The leaves were collected in sterile resealable bags. The leaves were washed under tap water, dried in hot air oven at 60°C for 4 days and grounded to powder to pass through 20 mesh sieves and stored in sterile resealable bags.

2.2 Preparation of Aqueous Extracts from the Plant Leaves

Fifty gram (50 g) sample of each powder was weighed separately into 200 mL of hot and cold distilled water respectively and allowed to stand for 24 hrs. The extracts were then filtered through a millipore filter into different conical

flasks. The extracts obtained were evaporated to dryness using a rotary evaporator. The extracts were assayed against the test organisms to determine the antifungal properties as described by [16]

2.3 Preparation of Ethanolic Extracts from the Plant Leaves

For the preparation of ethanolic extract, a modified method [17] was used. Fifty grams (50 g) sample of each powder was weighed separately into 200 mL ethanol (SDH Chemical Ltd), water and allowed to stand for 24 hrs. The extracts were then filtered through a millipore filter into different conical flasks. The extracts obtained from ethanolic extract were evaporated to dryness using a rotary evaporator. The extracts were assayed against the test organisms to determine the antifungal properties.



Plate 1. Photograph showing fresh leaf of *Borreria verticillata* on the ground



Plate 2. Photograph showing fresh leaf of *Lactuca capensis* on the ground



Plate 3. Photograph showing fresh leaves of *Colocasia esculenta*



Plate 4. Photograph showing fresh of *Jatropha agossy* on the ground



Plate 5. Photograph showing fresh leaves of *Ageratum conyzoides*

2.4 Collection of Toxicogenic Aspergillus flavus, A. parasiticus and A. ochraceus Strain and Culture Conditions

Mycotoxin producing *A. flavus*, *A. parasiticus* and *A. ochraceus* were previously isolated from poultry feed samples.

All the test organisms were collected from the Food Microbiology Lab of Food Science and Technology Department, Federal University of Technology, Akure, Nigeria.

Spore suspension was prepared by growing the fungi on Petri dishes for 7 days with potato dextrose agar (PDA) containing 50 mg/L of streptomycin. After incubation at 25°C, spores were harvested by adding mixture of sterilized distilled (10 mL for each plate) water and 10µl of

tween twenty on each plate. The spore suspension thus obtained was filtered using cheesecloth, and spores were counted using a haemocytometer and brought to a final concentration of 10^6 conidia/ml.

2.5 Determination of Minimum Inhibitory Concentrations (MIC)

The MIC was determined as the concentration above which the fungal growth was totally suppressed and below which the fungus resumed growth. The MIC at which no mycelia growth of the test fungus was seen or 100 percent inhibition of the fungus growth was determined. Study was carried out by poisoned food technique using different concentrations of extracts against all the test fungi.

2.6 Procedure of MIC

A culture of the test fungi was grown on Potato Dextrose Agar (PDA) medium for certain period (generally 7 days) at the optimum temperature ($25\pm 2^\circ\text{C}$) for growth. PDA supplemented with different plant extracts at four concentrations (0.5, 1.5, 2.5 and 3.5%) was poured in the Petri plates under aseptic conditions. After solidification, small dish (0.5 cm dia.) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down at the center of petri dish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA without extract. Solvent checks were maintained to check out the inhibitory effect of solvent on fungi in which PDA was mixed with solvent (a solvent, which is used for dissolving extracts). Petri plates were incubated at $25\pm 2^\circ\text{C}$. The radial five fungi were selected for bioassay viz. *Borreria verticillata*, *Lactuca capensis*, *Colocasia esculenta*, *Jatropha gossy* and *Ageratum conyzoides* leaves. Growth of fungus colony was measured after every 24h till the fungus in the control plates completely occupied it. Three replications were maintained for each treatment. The antifungal activity was evaluated by measuring the relative growth of fungus in treatment vis-à-vis control. To maintain aseptic condition (the word aseptic means without microorganisms) throughout the experiment is very important. The percent growth inhibition over control was worked out using the formula. $I = \frac{C-T}{C} \times 100$ Where, I is inhibition percent, C is colony diameter in control (cm) and T is colony diameter in treatment (cm) [17,18].

2.7 Data Analysis

The experiment was conducted using a completely randomized design. Standard errors of means of three replicates were computed using computer software Microsoft Excel.

3. RESULTS AND DISCUSSION

The results of antifungal screening of extracts of *Borreria verticillata*, *Lactuca capensis*, *Colocasia esculenta*, *Jatropha gossy* and *Ageratum conyzoides* are given in Tables 1-5.

The results in Table 1 show that ethanolic, hot and cold water extract were effective against *A. ochraceus* but have no effect on *A. flavus* and *A. parasiticus* with maximum mean value concentration from hot water extract (49.23%) compared with ethanolic extract (42.84%) and cold water extract (30.22%). The result in Table 2 shows that ethanolic extract of *Jatropha gossy* has the highest mean concentration (40.85%) than hot water extract (30.12%) and cold water extract (23.61%) against *A. flavus* but have no inhibitory concentration against the other fungi used.

Ethanolic extract of *Colocasia esculenta* was more effective against *A. flavus* and *A. parasiticus* (Table 3). The highest mean concentration of the extract that inhibited the growth of *A. flavus* was observed with ethanolic extract (45.37%) compared with the hot water extract (24.27%) and cold water extract (26.36). Also the ethanolic extract was able to inhibit the growth of *A. parasiticus* at mean concentration of 29.77% while the hot and cold water extract had no inhibitory effect on *A. parasiticus*. It was also observed that the three extract had no inhibitory effect on *A. ochraceus*.

The result of the effect of different concentrations of ethanolic, hot and cold water extracts of *Lactuca capensis* on the radial growth of fungi (Table 4) shows that the maximum inhibitory effect was observed from ethanolic extract against *A. flavus* (56.20%) and *A. parasiticus* (62.76%) compare to hot water extract (44.83, 57.13)% and cold water extract (41.30, 42.4)% respectively. All the extracts of *Lactuca capensis* had no inhibitory effect on *A. ochraceus*.

Table 1. Effect of different concentrations of ethanolic, hot and cold water extracts of *Ageratum conyzoides* on the radial growth of fungi

Fungus species	Concentration/Growth inhibition (%) ethanolic extract						Concentration/Growth inhibition (%) hot water extract						Concentration/Growth inhibition (%) cold extract						
	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	
<i>Aspergillus flavus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>A. parasiticus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>A. ocheracus</i>	0.00	22.35	35.25	45.00	68.75	42.84	0.00	25.50	39.25	55.00	77.15	49.23	0.00	10.15	28.26	35.23	47.25	30.22	

Table 2. Effect of different concentrations of ethanolic, hot and cold water extracts of *Jatropha gossypifolia* on the radial growth of fungi

Fungus species	Concentration/Growth inhibition (%) ethanolic extract						Concentration/Growth inhibition (%) hot water extract						Concentration/Growth inhibition (%) cold extract						
	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	
<i>Aspergillus flavus</i>	0.00	23.65	37.00	44.50	58.25	40.85	0.00	15.00	24.65	35.66	45.15	30.12	0.00	10.00	18.55	28.15	37.75	23.61	
<i>A. parasiticus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>A. ocheracus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3. Effect of different concentrations of ethanolic, hot and cold water extracts of *Colocasia esculenta* on the radial growth of fungi

Fungus species	Concentration/Growth inhibition (%) ethanolic extract						Concentration/Growth inhibition (%) hot water extract						Concentration/Growth inhibition (%) cold extract						
	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	
<i>Aspergillus flavus</i>	0.00	20.15	35.66	55.50	75.15	45.37	0.00	13.25	20.00	28.68	35.15	24.27	0.00	14.65	22.15	30.00	38.62	26.36	
<i>A. parasiticus</i>	0.00	10.00	20.15	33.25	55.67	29.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>A. ocheracus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Effect of different concentrations of ethanolic, hot and cold water extracts of *Lactuca capensis* on the radial growth of fungi

Fungus species	Concentration/Growth inhibition (%) ethanolic extract						Concentration/Growth inhibition (%) hot water extract						Concentration/Growth inhibition (%) cold extract					
	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean
<i>Aspergillus flavus</i>	0.00	33.25	45.00	58.22	88.36	56.20	0.00	28.65	38.00	47.65	65.00	44.83	0.00	25.00	35.65	45.77	58.76	41.30
<i>A. parasiticus</i>	0.00	35.00	50.15	69.17	96.70	62.76	0.00	30.10	48.15	65.00	85.25	57.13	0.00	22.35	37.35	49.25	60.65	42.4
<i>A. ocheracus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5. Effect of different concentrations of ethanolic, hot and cold water extracts of *Borreria verticillata* on the radial growth of fungi

Fungus species	Concentration/Growth inhibition (%) ethanolic extract						Concentration/Growth inhibition (%) hot water extract						Concentration/Growth inhibition (%) cold extract					
	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean	Control	0.5	1.5	2.5	3.5	Mean
<i>Aspergillus flavus</i>	0.00	45.15	68.55	88.35	105.25	76.83	0.00	40.00	65.15	79.78	97.35	70.57	0.00	32.45	55.36	70.15	88.95	61.73
<i>A. parasiticus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>A. ocheracus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

It was revealed in this study, that increase in the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. This finding agrees with the report of [19,20] that higher concentration of antimicrobial substance showed appreciation in growth inhibition. All the plant extracts tested exhibited different degrees of antifungal activity against toxigenic *A. flavus*, *A. parasiticus* and *A. ochraceus*. The maximum antimycotic activity was shown by ethanolic extract of *Borreria verticillata* against toxigenic *A. flavus* followed by hot water and cold water extract of *Borreria verticillata* against toxigenic *A. flavus*. The extract of *Borreria verticillata* was effective against fungal pathogens causing diseases in plants, human beings and animals [21] The fact that the results of this study showed that extract of the plants were effective against the test organisms may be due to the presence of active principles in the plant materials. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs [22] Plant products still remain the principal source of pharmaceutical agents used in orthodox medicine [22]. The MIC values of the plant extracts against the test organism showed that fungi vary widely in the degree of their susceptibility to antifungal agents. This observation therefore suggests that the antifungal substances contained in the extracts were fungistatic at lower concentrations while becoming fungicidal at higher concentrations of the extracts. Similar observations have been reported by [22]. When comparing data obtained in different studies, most publications provide generalizations about whether or not plant possesses activity against fungi. However, not all provide details about the extent or spectrum of this activity. Some publications also show the relative activity of plant and extracts by comparing results on different extracts tested against the same organism(s) [23]. It has been found that essential oils with anti-mould effectiveness is able to consistently cause morphological changes in *Aspergillus* species including lack of sporulation, loss of pigmentation, aberrant development of conidiophores (flattened and squashed) and distortion of hyphae (budding, lack of cytoplasm, swelling anomalous apex bifurcation) [24]. These findings suggested that the mode of antifungal activity of plants could include an attack on the cell wall and retraction of the cytoplasm in the hyphae ultimately resulting in death of the mycelium and spore germination. The results found in this study showed fungicidal

activities of *Borreria verticillata*, *Lactuca capensis*, *Colocasia esculenta*, *Jatropha gossy* and *Ageratum conyzoides* ethanolic, hot and cold water extracts providing interesting information about rapidity and stability of its anti-*Aspergillus* property. It also showed that the growth and survival of food and feed -related *Aspergillus* species could be controlled by the use of the extracts, particularly, *Borreria verticillata* Several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi [25-30]. The inhibitory effect of tested extracts might be due to natural bioactive materials present in these extracts [24]. This may be because the bioactive compounds of these plants may differ in quantity and quality compared to other tested plants.

It was observed that the effect of different concentrations of ethanolic, hot and cold water extracts of *Borreria verticillata* on the radial growth of fungi shows that the three extracts were able to inhibit the growth of *A. flavus* with the highest mean concentration observed from ethanolic extract (76.83%) followed by hot water extract (70.57%) and cold water extract (61.73%). The plant extract has no inhibitory effect on *A. parasiticus* and *A. ochraceus*.

Several workers have made similar observations by using essential oils or complex mixture from higher plants [31]. For example some water-soluble compounds may have a higher diffusion power and lower antimicrobial activity [32-35].

The inhibitory effect of some ethanolic plants extracts under in vitro condition might be due to the presence of phenolic compounds (polyphenols, phenolic acids, coumarins) in the extracts. Earlier work of [33] supports the finding where the polyphenols, phenolic acids, coumarins of plants were found to inhibit the growth and enzyme production (ligninolytic and pectinolytic enzymes) of *Botryosphaeria* spp. The Inhibitory activity of some other plants by the flower extracts of *Daturainnoxia* under in vitro condition was reported [33]. This also supports the presence of phenolic compounds (polyphenols, phenolic acids, coumarins) in the plant extract which cause the inhibition of enzymes activity.

The plant extracts of *Borreria verticillata* inhibited the growth of the test organisms except *Aspergillus parasiticus* and *A. ochraceus*, *Borreria verticillata* appears to be more effective as an antifungal agent than other plant extracts.

Ethanol extracts were more effective than the cold and hot water extracts against all the test fungi.

4. CONCLUSION

The results obtained from this study show that the extracts of *Borreria verticillata*, *Lactuca capensis*, *Colocasia esculenta* and *Jatropha gossy* used exhibit antifungal activity against *A. flavus* and *A. parasiticus*. While extracts of *Ageratum conyzoides* alone had inhibitory effect on *A. ochraceus*, Extracts of the plant used in this study could be useful in the treatment of fungal infections caused by *A. flavus*, *A. parasiticus* and *A. ochraceus*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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