

Antifungal Activity of Local Gin (Kai Kai) Extract of *Andrographis Paniculata* on Fungal Isolates

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Abstract:- *A. paniculata* (king of bitters) an herbaceous plant known for its bitter taste and medicinal properties has been used in treating different forms of infections and ailments. The antifungal activity of *A. paniculata* on fungi isolated from *Citrullus colocynthis* was investigated. *Colocynthiscitrullus* were bought from traders in a major market in Abia State, Nigeria. Fresh plant of *A. paniculata* was obtained from botanical garden of the Rivers State University and was identified in the botany department. The leaves of the plant were shade dried and blended into fine powder. Twenty grams (20g) of the powdered leaves was extracted using local gin 'kai kai'. The filtrate was evaporated and the resulting crude extract was used for antifungal sensitivity. The antifungal activity of the extracts was carried out using the well in agar diffusion method. In this method, 48 hours old fungal isolate was inoculated on dried Sabouraud Dextrose Agar plates in duplicates. five wells were bored using sterile 6mm cork borer on the dried seeded plates before 0.2ml of the different concentrations of 100, 50, 25, and 12.5mg/ml of the methanol extracts were transferred into the wells using sterile pipettes. *Aspergillus flavus*, *Rhizopus arrhizus*, *Aspergillus niger*, and *Mucor sp* were identified from the melon seeds. The zone diameter of the alcoholic extract of *A. paniculata* on *A. niger* was 16.50 ± 0.71 , 13.50 ± 0.71 , 10.50 ± 0.71 and 0.00 ± 0.00 mm at the concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml, respectively. While for *A. flavus*, the zone diameters were 33.50 ± 0.71 , 21.50 ± 0.71 , 19.50 ± 0.71 , and 0.00 ± 0.00 mm, respectively. The extract had no antifungal activity on *Rhizopus arrhizu* and *Mucor sp*. The antifungal activity of the extract was greatly influenced by the concentration of the extract, with higher concentrations of extract having high zone diameter.

Key words: Local gin 'kai kai' *A. paniculata*, antifungal activity, fungal isolates

I. INTRODUCTION

The use of plants and plant products for therapeutic purpose has been an ancient practice. Traditionally, plants or plant parts as well as other products are used to treat different types of ailment. Sanjoy and Yogeshwer[1] opined that the use of plant in traditional medical practice have remained an inestimable source of natural health products for humans, particularly in the last few decades, with more thorough researches having being carried out to explore natural therapies. The use of herbs in the treatment of diseases has become widespread and is increasingly achieving popularity worldwide not only due to their continuous usage in developing countries for primary health care of the poor, but also in societies where conventional medicine is prevalent in their health care system[2]. Approximately eighty percent

of the world's population practises herbal medicine, which may explain the constant rise in the annual global market value of these herbal remedies estimated at over US \$60 billion currently [3].

Contamination of food products by microorganisms could lead to deterioration of the food or food products. Fungi is one of the microorganisms that cause spoilage or deterioration of food or food products and such deterioration could be beneficial or dangerous especially when it affects the quality of the food or food products. Hocking *et al.* [4] posited that biodeterioration may lead to loss of nutritional value, organoleptic and color changes, and most importantly, safety of the food may become compromised. And the chemical changes in the organic substances of the food or plant product is caused by the action of specific enzymes produced by microorganisms, such as, moulds, bacteria, and yeasts. In order to satisfy the high demand of food or plant produce, proper storage of plant products would be of immense benefit. According to Hussaini[5], fungi are plant pathogens that are widely distributed in different environments and are the main cause of deterioration of foods and feed stuffs. The infections caused by these group of organisms go beyond the reduction in crop yield and quality despite the economic losses but also result in the production of poisonous mycotoxins which contaminate grains and ingestion of these contaminated products of plants by humans and animals is a serious public health concern since the secreted toxins could cause diseases.

Andrographis paniculata (king of bitters) is an erect yearly herb which tastes very bitter and it is native to India and Sri Lanka. Although the genus *Andrographis* is said to house twenty-eight species of small yearly shrubs that are very important and are found in tropical Asia, only few species are medicinal, of which *A. paniculata* is the most popular. Reports of the antifungal activity of the plant is well documented but there is paucity of information in respect to the chemical nature of the antifungal compounds inherent in the plant. The compound 3-O-β-D-glucosyl-14-deoxyandrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-didehydro and rographolide has been reported as an antifungal compound inherent in the plant[6]. Contamination of food by fungi as well as the resulting mycotoxicosis is caused by fungi are not very much considered as those resulting from bacterial infections. Thus, there is paucity of information in respect to fungi contaminants on crop products and the use of plant to control fungi contaminants. Previous studies have used different

solvents which include hexane, methanol, ethanol, etc. for extracting plant products [6, 7, 8] but studies on the use of local gin is scanty. The local gin is used for extraction of different herbs by locals. Herbs extracted with the local gin is consumed together with the gin by the locals. This current study is therefore aimed at evaluating the antifungal properties of *A. paniculata* extract extracted with local gin 'kai kai' on fungi isolated from melon seeds. The study no doubt would give insight in considering the use of local gin which is readily consumed as an extracting solvent for future studies.

II. MATERIALS AND METHOD

Collection of Materials

Fresh leaves of *A. paniculata* was collected from the botanical garden of the Rivers State University and was identified in the department of plant science and biotechnology. The leaves were shade dried in the laboratory before they were blended into fine particles. Alcohol (local gin) which is referred to as 'kai kai' was bought from a retail shop in Ghokana, Gokhana Local government, Rivers State Nigeria.

Extraction of *Andrographis paniculata*

The leaves of *A. paniculata* was extracted using local gin. In this method, 20g of the powdered leaves was immersed in 250ml beaker containing 200ml of alcohol (local gin) 'kai kai'. The set up was homogenized by swirling and was allowed to stand for forty-eight hours (48 hours). After forty-eight hours of extraction, the leave extracts were filtered with sterile filter paper (Whatman no1 filter paper) into sterile 250ml beakers. The filtrate was evaporated to dryness in the hot air oven at 45°C. The resulting oily residue was weighed and stored in sterile containers which were preserved in the refrigerator for further analysis. The percentage yield of the extract was calculated using the formula below;

$$\text{Yield (\%)} = \frac{\text{weight of Extract recovered}}{\text{weight of dry powder}} \times 100 \quad \text{Equation 1.}$$

Microbiological Analysis

Isolation and Identification of Fungi

Fungi isolates were isolated from the spoilt melon seeds using standard microbiological techniques. In this method, ten gram (10g) of the melon seeds was weighed and transferred into 250ml conical flask containing sterile 90ml normal saline. This was swirled gently to homogenization and to dislodge any attached particle. The stock was diluted serially using the 10-fold dilution as described by Amadi *et al.* [9]. Aliquots of appropriate dilution of 10^{-1} and 10^{-2} was inoculated in duplicates on tetracycline supplemented sabouraud dextrose agar plates. Plates were incubated at 25°C for 4 days (Douglas and Robinson, 2018). Identification of fungal isolates was done based on morphological appearance on plates and microscopy. Microscopic preparation of spores was made by transferring spores of 48 hours old fungal isolate on clean slide containing a drop of lactophenol blue. The slide was viewed under the light microscope using the $\times 10$ and $\times 40$

objectives (Douglas and Robinson, 2019). The identities of the fungal isolates were confirmed by comparing with those in the book of descriptions of medical fungi by Sarah *et al.* [10].

Preparation of Extracts Concentrations for antifungal Assay

Stock solution of the alcoholic extract was prepared by dissolving 100mg of the oily residue in 1ml of Dimethyl sulfoxide (DMSO) which gave rise to 100mg/ml stock of the ethanolic extract. Further two-fold serial dilution was carried out by diluting 1ml of the stock solution into 1ml of sterile distilled water to achieve the concentrations of 50mg/ml, 25mg/ml and 12.5mg/ml.

Antifungal Activity of Extracts

The antifungal activity of the extracts was carried out using the well in agar diffusion method as described by Robinson (2018). In this method, 48 hours old fungal isolates which were grown on 9ml sterile sabouraud dextrose broth was inoculated on well dried and labelled (according to the isolates) sabouraud dextrose agar (SDA) plates in duplicates. The plates were allowed to dry for 3 minutes before five wells were bored using sterile 6mm cork borer. About 0.2ml of different concentrations (100, 50, 25, and 12.5mg/ml) of the extracts were transferred into the bored wells using sterile pipettes. The positive control was 10mg/ml miconazole antifungal agent while the negative control was 10% DMSO. The plates were incubated at 25°C for forty-eight hours. Zone diameter were measured using graduated rule and the result was recorded. Sterility of the extract was confirmed by streaking the different extracts on nutrient and sabouraud dextrose agar plates which were later incubated for forty-eight hours. The absence of growth after incubation means that the extracts were sterile.

III. RESULT

The percentage yield of the alcoholic extract was 9.0g and the colour of the extract was dark brown. The fungal isolates characterized from the melon seeds belonged to three fungi genera and were identified as *Rhizopus arrhizus*, *Aspergillus niger*, *Aspergillus flavus*, and *Mucor* sp (Table 1). The result of the antifungal activity of alcoholic extract of *A. paniculata* as illustrated in Table 2 showed that both the miconazole antifungal agent and the alcoholic extracts of the leave had no antifungal effect on *R. arrhizus* where as high zones of inhibition were recorded in the isolates of *A. niger*, and *A. flavus*. *Mucor* sp on the other hand was only susceptible to the synthetic antifungal drug and was completely resistant to the extract.

Table 1. Description of fungal Isolates

Isolate code	Macroscopy	Microscopy	Identity
A1	Yellow-green spores, white periphery, brown reverse	Conidia is globose and rough walled head, with hyaline stipes of conidiophore	<i>Aspergillus flavus</i>
A2	White cottony, brownish grey to black-grey coloration, brown reverse	Smooth walled and non-septate branched sporangiophores, presence of rhizoids	<i>Rhizopus arrhizus</i>
A3	White periphery, densed black spores, dark brown reverse	Hyaline conidiophore phialides borne on vesicles, chains of conidia with septate hyphae	<i>Aspergillus niger</i>
A4	White loosed cotton wool-like aerial mycelium, white reverse	Non-septate mycelia bearing sporangiophore scattered all over the mycellium	<i>Mucor</i> sp

Table 2. Antifungal activity of Alcoholic extract of *Andrographis paniculata*

Isolate	c	100mg	50mg	25mg/	12.5mg/
		/ml	/ml	ml	ml
Zone Diameter (mm)					
<i>R. arrhizus</i>	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
<i>A. niger</i>	18.00±0.71 ^b	16.50±0.71 ^b	13.50±0.71 ^b	10.50±0.71 ^b	0.00±0.00 ^a
<i>A. flavus</i>	34.00±0.00 ^d	33.50±0.71 ^d	21.50±0.71 ^c	19.50±0.71 ^c	0.00±0.00 ^a
<i>Mucor</i> sp	19.00±0.71 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

*Means with similar superscript have no significant difference at $P \leq 0.05$

C: 10mg/ml Miconazole Nitrate.

IV. DISCUSSION

Fungi generally are ubiquitous and their mode of reproduction could be said to be a major factor in their ability to inhabit different environments since they reproduce by spores. Other factors which could predispose food to fungi include contaminations during harvest as well as post-harvest and pest infestations. *Aspergillus* as well as *Penicillium* sp has been classified as storage fungi and are known to contaminate grains in silos and other storage devices since they are able to tolerate and proliferate at lower water content [5]. The presence of fungi in agricultural products could be of public health significance since secondary metabolites (mycotoxins) secreted by most fungi are able to illicit different health challenges ranging from nephrotoxic, immunotoxic, teratogenic and mutagenic; and could also cause acute or chronic infections in both man and animals. Such infections could range from death to complications in the central nervous, cardiovascular, pulmonary systems and intestinal tract[13]. For instance, aflatoxin ingestion has been reported in Nigeria to be responsible for cases of hepatocellular carcinoma(HCC-liver cancer) [14].

The antifungal activity of the alcoholic extract of *A. paniculata* in this study depended on the concentration. The highest concentration of the extract had the highest zone of inhibition and was more effective on the fungal isolates. This observation could be that higher concentrations of the extracts has more phytochemicals or metabolites than in the smaller concentrations. The zone diameter of the alcoholic extract of *A. paniculata* on *A. niger* was 16.50±0.71, 13.50±0.71, 10.50±0.71 and 0.00±0.00mm at the concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml, respectively. While for *A. flavus*, the zone diameters were 33.50±0.71, 21.50±0.71, 19.50±0.71, and 0.00±0.00, respectively. The least concentration (MIC) of the extract to inhibit the growth of *A. niger* and *A. flavus* was 25mg/ml. it was also observed that the alcoholic extract of *A. paniculata* was more effective on *A. flavus* since higher zone diameter was recorded. As observed in Table 2, at 100mg/ml, the zone diameter of the extract on *A. niger* was 16.50±0.71mm while the zone diameter at same concentration on *A. flavus* was 33.50±0.71mm. At the 25mg/ml concentration, the zone diameter of the extract on *A. niger* was 10.50±0.71mm while 19.50±0.71mm was recorded at same concentration on *A. flavus*. The antifungal activity of the extracts of *A. paniculata* could be attributed to the phytochemicals inherent in the plant or leaves. This agreed with the control in which the DMSO which served as negative control exerted no antifungal activity against the fungal isolates. Abubakaret *al.* [8] in their study demonstrated the antifungal activity of extracts of *A. paniculata* on *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporiumcanis*, *Candida albicans*, *Candida krusei*, *Candida tropicalis*, and *Aspergillus niger* which are skin pathogenic fungi. They associated the antifungal activity of *A. paniculata* to the synergistic actions of the compounds extracted from the plants. Although, the results in our study showed no antifungal activity on *R. arrhizus* and *Mucor* sp, the antifungal activity exhibited on *A. niger* and *A. flavus* conforms the presence of antifungal compounds in the leaves of the plant. in another study of the antifungal activity of the extract of *A. paniculata* and andrographolide, it was reported that the methanol extract of the aerial parts of *A. paniculata* inhibited mycelial growth of *Fusarium solani* and spore germination of *Alternaria solani* and that the pure andrographolide compound inherent in the methanol extract inhibited spore germination of *A. solani*[6]. Aniland Sajad[15] in their study reported that *A. paniculata* exhibited antifungal activity on fungal isolates such as *A. paniculata* on *A. flavus* and *A. fumigatus*, *A. niger*, *Rhizoctoniasolani*, *Alternaria alternate* and *Fusarium oxysporum* but lacked antifungal activity against *Rhizopus stolonifer*. This agreed with our study in which *R. arrhizus* was resistant to the extract.

V. CONCLUSION

The alcoholic 'kai kai' extract of *A. paniculata* exhibited high levels of antifungal activity against *A. niger* and *A. flavus* but were very resistant to *Rhizopus* and *Mucor* sp. Although, no known antifungal compounds were isolated, *A. paniculata* extracts and compounds inherent in them could be used and

incorporated as antifungal agents both in the medical and agriculture in combating fungi pathogens. The use of local gin 'kai kai' should be adopted as extracting solvents in research since it is consumed by many in this part of the country. To our knowledge, this is the first study that have used local gin 'kai kai' as an extracting solvent for *A. paniculata*.

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