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Bio-efficacy of *Adansonia digitata L.* (Baobab) extract against aflatoxin produced by *Aspergillus* on *Sesamum indicum L.* (Sesame) seeds.

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Authors' Contribution	**Anddy, J.** developed the concept, designed the experiment, **U. Victory** collected samples from the village near Federal University Makurdi and performed statistical analysis.		
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ABSTRACT

The leaf and bark extracts of *Adansonia digitata* were used as inhibition agents to curtail the spread of aflatoxin produced by *Aspergillus niger on* sesame seeds. Susceptibility test was carried out using agar well diffusion method. The ethanoic and aqueous leaf and bark extracts of *Adansonia digitata* were subjected to phytochemical screening using standard methods of analysis and this investigation revealed the presence of tannins, flavonoids, saponins, glycosides, alkaloids, quinones and phenols. The test organism isolated from sesame seeds was identified as *Aspergillus niger*. The extracts at different concentrations 100mg/ml, 125 mg/ml, 200 mg/ml, 250 mg/ml and 500 mg/ml showed varied inhibitory activity against the fungus studied with ethanoic leaf extract having the highest inhibition at concentration of 500mg/ml with mean inhibition zone of 35.00±1.00. Ampicillin (250 mg) was used as a positive control. It is therefore recommended that bio-preservation of benniseed using *Adansonia digitata* be considered to avoid infestation by *Aspergillus niger* in humid and insect infested areas.

Keywords: *Adansonia digitata, extracts,* Aflatoxin, *Aspergillus, Sesamum indicum* and zone of inhibition.

INTRODUCTION: *Sesamum indicum L.* (sesame) belongs to the pedaliaceae family. It is an annual plant which grows either bush-like or upright, depending on the variety to a height of 1-2.0m (Ogbonna and Umar-Shaaba, 2011). *Sesamum indicum L.* (sesame) ranks eighth in the world production of edible oil seeds, with higher oil content than other oilseed crops. It is grown mainly for its seeds that contain approximately 50% oil and 25% protein. The presence of some antioxidants (sesamum, sesamolin, and sesamol) makes the oil one of the most stable vegetable oils in the world. In addition to having high oil content, sesame seed is known for its nutritional and medicinal qualities. The seed contains all essential amino acids and fatty acids and it is a good source of vitamins (pantothenic acid and vitamin E) and minerals such as calcium (1450 mg/100g) and phosphorous (570 mg/100g). The aflatoxin producing fungi *Aspergillus spp.,* are widely spread in nature and have severely contaminated food supplies of humans and animals, resulting in health hazards and even death (Nigeria Farmers Group and Cooperative Society, 2019). Aspergillus is commonly isolated from soil, plant debris, and indoor air environment. In particular, aflatoxins are produced by the soil-borne molds *Aspergillus* spp. that grows on the seeds and plants. (Microbial Secondary Metabolites, 2017). High concentrations of aflatoxins are most often found in plants with very nutritive seeds such as maize, nuts, cereal grains and *Sesamum indicum L.* (sesame) (Nigeria Farmers Group and Cooperative Society, 2019). Extracts from different plants, due to their fungicidal and fungistatic properties, have been found to affect the vegetative growth and associated aflatoxin production by *Aspergillus* spp (Soliman and Badeaa, 2002; El-Nagerabi *et al.*, 2013). This includes dry leaves and calyx extracts *of Hibiscus sabdariffa* (El-Nagerabi *et al.*, 2013), herbal compounds (Hashemi *et al.*, 2012), and fruit rinds of *Garcinia cowa* and *G. penduculata* (von Tippelskirch, 2018). *Adansonia digitata L.* (baobab) is a large iconic deciduous and stem-succulent tree that has been reported to be useful and this has attracted the interest of pharmaceutical companies and scientists. This is due to its various traditional uses as medicinal, nutritional and cosmetic plant (Buchmann *et al.*, 2010). This study intends to focus on bio-efficacy of *Adansonia digitata L.* (baobab) extract against Aflatoxin production in *Sesamum indicum L.* (sesame).

OBJECTIVES: The objectives of current study were (i) to screen/ determine the phytochemical constituent of *Adansonia digitata L.* (baobab) extract. To determine the bio-efficacy of Adansonia *digitata L.* (baobab extract) on *Aspergillus spp.*

MATERIALS AND METHODS: Sample collection: *Adansonia digitata* (baobab) leaves and barks were collected from a baobab tree in the Botany Garden of Federal University of Agriculture Makurdi. Seeds of stored sesame (benniseed) were obtained from North Bank Market, Makurdi, Nigeria

Preparation of baobab extracts: The leaves and bark were washed and shade dried at room temperature, pounded with a mortar and pestle and then sieved to get a fine powder. One hundred and ten grammes (110g) of baobab leaf and bark sample were separately weighed into two experimental bottles. 400ml and 700ml of ethanol and water were respectively added to the bottles. These were left for 48 hours to soak properly after which they were filtered using a filter cloth. The filtrate collected was then placed in a water bath to dry. The dried concentrated samples were then scraped and kept in sample bottles for various analyses. This process is known as maceration (Azwanida, 2015).

Phytochemical screening: Tannins: About three drops of 0.1% Iron Chloride ($FeCl_2$) was added to 1ml of baobab extract. A brownish green or a blue-black color indicated the presence of tannins (Ferric Chloride Test) (Herborne, 1973).

Flavonoids: Two to four drops of Iron chloride $FeCl_2$ were added to 2ml of baobab extract. A blackish red color showed the

presence of flavonoids (Ferric Chloride Test) (Herborne, 1973).

Saponins: 1ml solution of the baobab extract was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. Foamy lather formation indicated the presence of saponins (Foam test) (Herborne, 1973).

Glycosides: 5ml of Tetraoxosulphate (iv) acid (H_2SO_4) was added to 1ml of plant extract a 100ml flask. It was boiled for 15 minutes, cooled and neutralized with 10% Sodium hydroxide (NaOH). The Fehling solution A and B (1mL) were added to the neutralized solution and a brick red precipitate of reducing sugars indicated the presence of glycosides (Herborne, 1973).

Alkaloids: About 2ml of baobab extract was added to few drops of 2NHCl. An aqueous layer formed was decanted and one to two drops of Mayer's reagent was added. Formation of white turbidity or precipitate indicated the presence of alkaloids (Mayer's reagent test) (Herborne, 1973).

Quinones: To the extract (baobab), NaOH was added. Blue green or red color indicated the presence of quinones (Herborne, 1973).

Steroids: One gram of the plant extract was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated Tetraoxosulphate (iv) acid (H_2SO_4) was added along the sides of the test tube. The appearance of green color indicated the presence of steroids (Liebermann Burchard test) (Addy and Victory, 2021)

Fungal identification: A small portion of the fungal culture was picked carefully using a scalpel and pin, and then placed on a clean slide, stained with lactophenol cotton blue reagent and viewed under a low power (×10) and high power (×40) objectives. The hyphal structure, spore, shape, and arrangement were noted. Preliminary identification was done by macroscopic observation of the cultures, with regard to color and appearance (National Committee for Clinical Laboratory Standards (2000).

Preparation of concentration of baobab plant extract: Using double broth dilution method, one milligram (1g) of aqueous and ethanoic extracts was added to 2ml of distilled water and ethanol respectively to give a concentration of 500mg/ml. Other concentrations of 250mg/ml, 200mg/ml, 125mg/ml, and 100mg/ml were also prepared.

Antifungal susceptibility testing of the extract with the test organism: The inoculum was prepared by inoculating the nutrient broth and incubated at 37^{0}C for 24 hours. The cultures were diluted to 0.5 McFarland turbidity standard after the incubation. About 0.5ml each of the cultured organisms was pipetted onto the petri dish after which prepared Potato Dextrose Agar was pour plated and allowed to solidify. After the culture plates have gelled wells were bored on the surface of the agar plates using 4mm cork borer. About 0.2ml of the different concentrations of each extract was transferred into the well using Pastuer pipette. The wells were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. Ampicillin 250mg was used as positive control. The plates were incubated at 37^{0}C for 24 hours. The experiment was performed in triplicates and the resulting zones of inhibition was measured using a ruler in mm. Susceptibility testing was carried out using agar well diffusion method, according to the recommendation of the National Committee for Clinical Laboratory Standards (2000).

RESULTS: The phytochemicals present in *Adansonia digitata L.* (baobab) includes tannin, flavonoid, saponin, glycoside, alkaloid, quinone and phenol as shown in table 1.

Phytochemicals	Ethanoic Extracts	Aqueous Extracts
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Glycosides	+	+
Alkaloids	+	+
Quinones	+	+
Phenols	+	+

Table 1: Phytochemical screening of ethanoic and aqueous extract of *Adansonia digitata* (baobab).

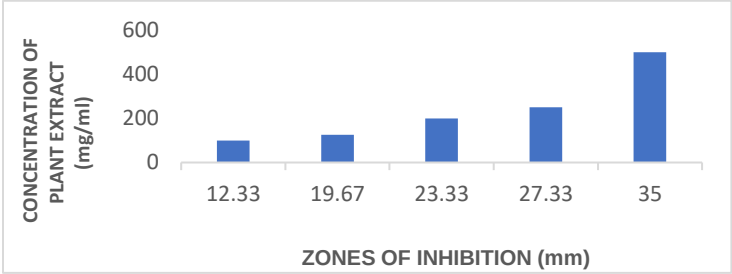

Figure 1: Graphical presentation of zones of inhibition of the ethanoic leaf extract of *A. digitata* (baobab) on *Aspergillus niger.*

These antifungal activities were analyzed at varying concentrations ranging from 100mg/ml to 500mg/ml. The ethanoic leaf extract elicited a diameter of inhibition ranging from 12.33±1.53 to 35.00±1.00 while the ethanoic bark had zones of inhibition ranging from 9.00±1.00 to 30.33±1.53 as shown in figure 2 and 3.

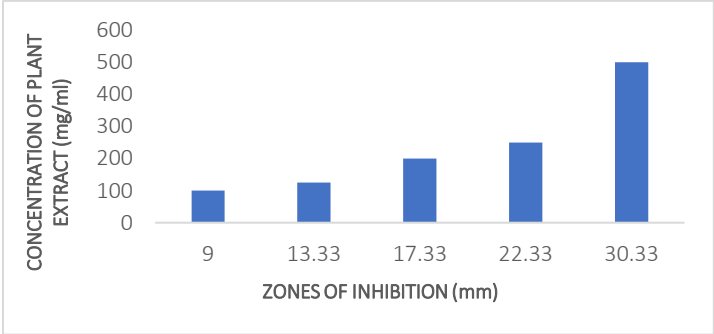

Figure 2: Graphical presentation of zones of inhibition of the ethanoic bark extract of *A. digitata* (baobab) on *A. niger.*

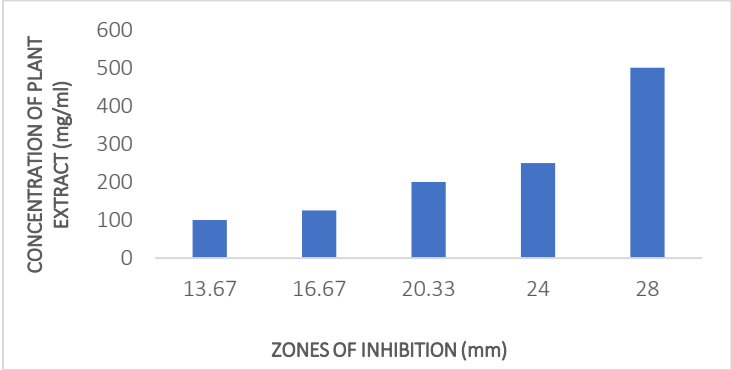

Figure 3: Graphical presentation of zones of inhibition of the aqueous leaf extract of *A. digitata* (baobab) on *A. niger.*

The aqueous leaf extract elicited zones of inhibition ranging from 13.67±1.53 to 28.00±1.00 while the bark extract had zones of inhibition ranging from 5.67±0.58 to 22.00±2.00 as shown in figure 4.

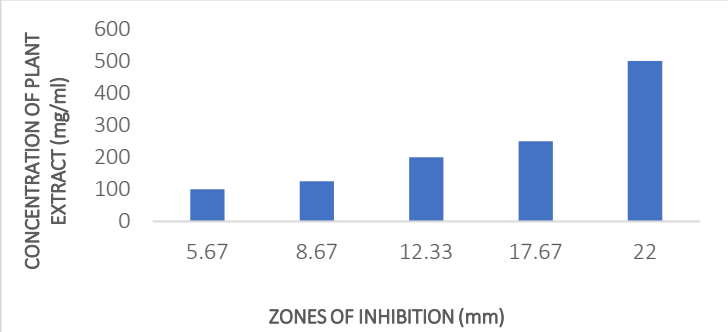

Figure 4: Graphical presentation of zones of inhibition of the aqueous bark extract of *A. digitata* (baobab) on *A. niger*.

The results in these figures revealed that the ethanoic leaf extract of baobab had the highest activity on the test organism with 35.00±1.00 at the highest concentration and 12.33±1.53 at the least concentration followed by the ethanoic bark extract which had 30.33 ± 1.53 at the highest concentration and 9.00 ± 1.00 at the least concentration as shown in figure 1 and 2. It was also observed that aqueous leaf extract of baobab had a higher activity on the test organism with 28.00±1.00 at the highest concentration and 13.67 ± 1.53 at the least concentration. The aqueous bark had the least activity on the test organism with 22±2 at the highest concentration and 5.67±0.58 at the least concentration as shown in figure 4 and 5. The effects of various concentrations of *Adansonia digitata* (baobab) leaf and bark extract against the production of *Aspergillus niger* in benniseed were recorded. figure 1, 2, 3 and 4 show the graphical presentation of antifungal activity of aqueous and ethanoic extracts of *Adansonia digitata L.* (baobab) on *Aspergillus niger*. The total production of *Aspergillus niger* was significantly reduced by the tested concentrations of baobab leaf and bark extracts.

Figure 5: showed the zones of inhibition of aqueous leaf extract of baobab on cultured *Aspergillus niger.*

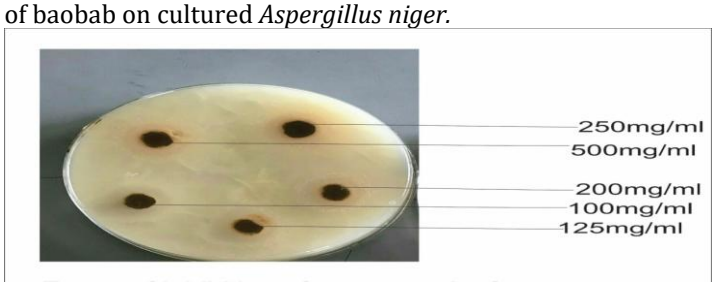

Figure 5: Zones of inhibition of aqueous leaf extract of *Adansonia digitata*.

Figure 6: showed the zones of inhibition of aqueous bark extract.

Figure 6: Zones of inhibition of Aqueous bark extract of *Adansonia digitata*.

Figure 7 and 8 depicts the zones of inhibition of prepared ethanoic leaf and bark baobab extracts respectively.

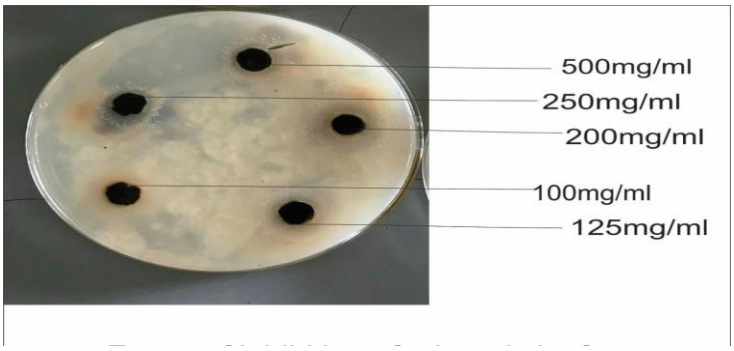

Figure 7: Zone of inhibition of ethanoic leaf extract of *A. digitata*

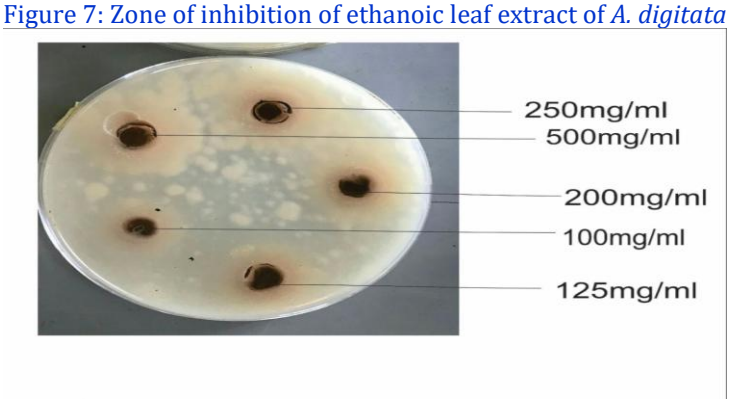

Figure 8: Zone of inhibition of ethanoic bark extract of *A. digitata*

DISCUSSION: The phytochemicals present in *Adansonia digitata L.* (baobab) includes tannin, flavonoid, saponin, glycoside, alkaloid, quinone and phenol as shown in table 1. These phytochemicals were present in both aqueous and ethanoic extracts. This finding corroborates with results were obtained by Zagga *et al.* (2018). The isolated fungus was identified as *Aspergillus niger*. This result agrees with Addy and Victory (2021) that also isolated *Aspergillus niger* from stale bread and onion. All the plant extracts showed various antifungal activities. The results from this study corroborates with the findings of El-Nagerabi *et al.* (2013).

with the research carried out by El-Nagerabi *et al.* (2013) on the fruit pulp extract of baobab to dictate its effect on fungal growth and aflatoxin production showed antifungal activities and inhibitory effect. This was carried out on two strains of Aspergillus; *A. flavus* and *A. parasiticus*. Different concentrations of essential oil of *A. digitata* on *Aspergillus flavus* and *A. parasiticus* indicated antifungal and inhibitory effects against the growth and aflatoxin production by the two strains (El-Nagerabi *et al.*, 2013).

CONCLUSION: This research work describes the effects of leaf and bark extracts on *Adansonia digitata* (baobab) on the growth of *Aspergillus spp* isolated from sesame. The phytochemicals present in the prepared baobab extracts were tannins, flavonoids, saponins, glycosides, alkaloids, quinones and phenols. The specie of Aspergillus isolated from the sesame sample was *Aspergillus niger.* The overall results demonstrate that both leaf and bark extracts inhibited the growth of *Aspergillus niger* with ethanoic leaf showing the greatest activity. Therefore, baobab leaf and bark can be suggested as plant additives and bio preservatives which enhance the nutritive value, quality and protection of benniseed against aflatoxin contamination as well as storage life.

CONFLICT OF INTERES: The authors declare no conflicts of interests.

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