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The Antimicrobial Activities of Crude Extracts of Fruits of *Andasonia digitata.*

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Abstract

Adansonia digitata plant is a tree with a wide range of medicinal properties that can be used to treat a variety of infectious diseases locally. The main objective of this study is to determine the antimicrobial activity of *Adansonia digitata* methanol, ethanol, and distilled water extract against pathogenic microorganisms. The antimicrobial activity of methanol, ethanol, and aqueous extracts of *Adansonia digitata* fruit pulp extracts was thoroughly examined against pathogenic microorganisms such as *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae, Staphylococcus aureus, Fusarium oxysporium, Trichoderma harzianum*, *Aspergillus flavus*, and *Pyricularia oryzae*. The antimicrobial activity of these extracts against both bacteria and fungi strains was investigated using the paper disk diffusion method, Agar well diffusion technique and the minimum inhibitory concentration (MIC) method. It was observed that the distilled water, methanol, and ethanol extract of *Adansonia digitata* were effective against all the test organisms used in this study. The *Adansonia digitata* plant also demonstrated antimicrobial qualities as it showed zone of inhibition against some fungi which include *Aspergillus flavus*, *Fusarium oxysporum*, *Pyricularia oryzae* and *Trichoderma harzianum*. The MIC values obtained from this study showed that distilled water, methanol, and ethanol extract of *Adansonia digitata* were effective against all the test organisms used in this study at the concentrations of 50µg/ml and 25µg/ml. The range of zone of inhibition against fungi (13mm – 17mm) was significantly higher than the range of zone of inhibition of the bacteria.

Keywords: *Andasonia Digitata*; Zone of Inhibition; Antimicrobial; MIC.

1.0 Introduction

Since ancient times, people have been searching for drugs in nature. Humans have always been using the medicinal plants for their ailments. The origin of the use of these medicinal plants can be traced back to the lack of information about their usage. As a result, people became more focused on explicatory facts instead of the proper use of these natural medicines. Petrovska, (2012). The oldest known evidence of the use of medicinal plants for the preparation of drugs dates back to 5000 years ago. It was found on a clay slab from Nagpur. The evidence contains 12 drug preparation recipes that refer to over 250 different plants, some of which are alkaloid such as the henbane and the poppy. The diversity of plants and their potential as a bio-based drug source has

led to the development of numerous new drugs. Kelly, (2009). The importance of plants as raw materials and their role in traditional medicine cannot be overemphasized. Since they are used to treat diseases and maintain physical and mental health, they have a significant role in the development of the pharmaceutical industry. Akinyemi *et al*., (2018). It has been found that many countries in the Asia-Pacific, Africa, and Latin America have adapted to the use of traditional medicine to improve their primary health care. In most cases, while about 80 percent of the African population uses traditional medicine for primary health care.

Adansonia digitata is a member of Bombacaceae family and belongs to the genus Adansonia. It has eight species, known by many names these include African baobab, Ethiopian sour gourd, monkey tamarind, Senegal calabash, monkey bread tree, and upside down tree. *Adansonia digitata* is a medium sized, deciduous tree. It is native to tropical Africa. This tree has a strange shape and has grotesquely swollen trunks Kamatou (2011). Baobab can grow up to a height of 25 meters. It has a diameter of about nine meters. These trees have white flowers with large white centers and the fruit of the baobab tree is also edible. One of the longest lived trees in the world. It can tolerate temperatures of up to 40 degrees Celsius. The tender roots, stems, and flowers are all edible and are used as ingredients in African dishes Sundarambal *et al*., (2015). Baobabs have been valued as sources of food and water for thousands of years. They are associated with superstition and legend. European explorers were known to carve their names on the trees. Phytochemical analysis revealed the presence of various phytosterols, amino acids, flavonoids, fatty acids, vitamins, and minerals. It is used for scurvy related diseases Sundarambal *et al*., (2015).

Fig 1: A baobab tree in Ekiti state, western region of Nigeria.

Fig 2: A baobab fruit pulp found in Ekiti state, Nigeria

2.0 Materials and Methods

2.1 Collection and Processing of Sample

The fruit of the plant *Adansonia digitata* was harvested from a local farm in Owo town, Ondo State. The bark of the fruit was peeled and the whitish edible fruit was removed and air-dried for five days before it was crushed to powder using an industrial grinding machine. The powdered fruit was then stored in a clean sterilized beaker covered with aluminum foil and taped with masking tape and stored until further use.

2.2 Extract of Plant Materials

25g of the powdered fruit of *Adansonia digitata* was weighed accurately using an electronic weighing balance. 25g of the powdered fruit *Adansonia digitata* was weighed in three different conical flasks. Three different solvents were used for the extraction of the samples. The solvent used were methanol, ethanol, and distilled water. Each conical flask was labelled according to the solvent used. The solutions were allowed to stay for 24hours before filtering using a Whatman filter paper No.1.The sample was allowed to dry at room temperature. The extract was scraped from the glass plates and transferred to a clean sterilized beaker with appropriate labels to prevent mix up and covered with aluminum foil and masking tape and kept at cool temperature until further use.

2.3 Reconstitution of the Extract

Dimethyl sulfoxide (DMSO) was used for the reconstitution of the extract to increase the volume and improve the efficiency of the extract during the antimicrobial susceptibility test. 10 per cent of DMSO was prepared for the reconstitution of the standard concentration.

2.4 Disk Diffusion Method

The method used by Ponmurugan and Shyamkumar (2012) was adopted for this procedure in this work. The paper disk embedded with the extract was made by using a perforator to make disk shape from a filter paper and these paper disks were soaked in the crude extract for few hours to absorb about 0.015ml of the extract before using the paper disk for antimicrobial susceptibility test. The standard media used for Kirby-Bauer test was used. Mueller-Hinton agar which is the standard agar used for the Kirby-Bauer test was prepared and aseptically, a swab stick was dipped in a test tube broth was used to streak the surface of the plate containing the Mueller-Hinton agar. Thereafter, the already soaked paper disks that have absorbed the extract were transferred on the streaked agar plate and pressed on the agar using a sterile forceps. The plates were covered and placed in an incubator at 37^{o}C for 24 hours for all test bacteria.

2.5 Agar Well Diffusion Test

Mueller-Hinton agar was prepared and poured into plates and allowed to gel. Aseptically, a swab stick was used to transfer the test fungi unto the Agar plate by streaking. A sterile cork borer was also used to make a hole of 4mm in diameter on the plate containing the gel Mueller-Hinton agar. The extract was transferred into the holes made by the use of a sterile syringe. Each hole was able to contain 0.1ml of the extract. Dimethylsufoxide was used as control. The plate was covered and store in an incubator for 48 hours at 37^{0}C for all test fungi.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of a plant extract is the lowest concentration that is capable of killing or completely inhibiting the growth of a certain type of organism. This concentration is determined by analyzing the sensitivity of the extract against varied concentrations. The control test tube contained only broth and the solvent. The test tubes were incubated for 24 hours at 37^{o}C for the bacteria and 48 hours for fungi at 35^{o}C. The lowest concentration which gave no visible growth was recorded as the Minimum Inhibitory Concentration (MIC).

3.0 Results

The antimicrobial activity of distilled water, methanol, and ethanol extract of fruit content of *Adansonia digitata* was examined in this study to find out their activity against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Staphylococcus aureus, Fusarium oxysporium, Pyricularia oryzae, Aspergillus flavus, and Trichoderma harzianium*. The result obtained from this investigation was recorded.

3.1 Paper Disk Method

The prepared paper disk was able to absorb about 0.015 mL of the extract and different zone of inhibition was observed as shown on plates 1-3 and recorded on table 3.1 below.

Table 3.1: Zone of Inhibition in Paper Disk Diffusion Method Against *E. coli, S. aureus, P. aeroginosa,* and *K. pneumoniae*

Organisms	**Zone of Inhibition (mm)**			
	Distilled water	**Methanol**	**Ethanol**	**Control**
Escherichia coli	13	12	11	-
Staphylococcus aureus	-	-	18	-
Pseudomonas aeroginosa	-	-	9	-
Klebsiella pnemoniae	7	-	15	-

Plate 1: Distilled water extract of *A. digitata* against *E. coli*

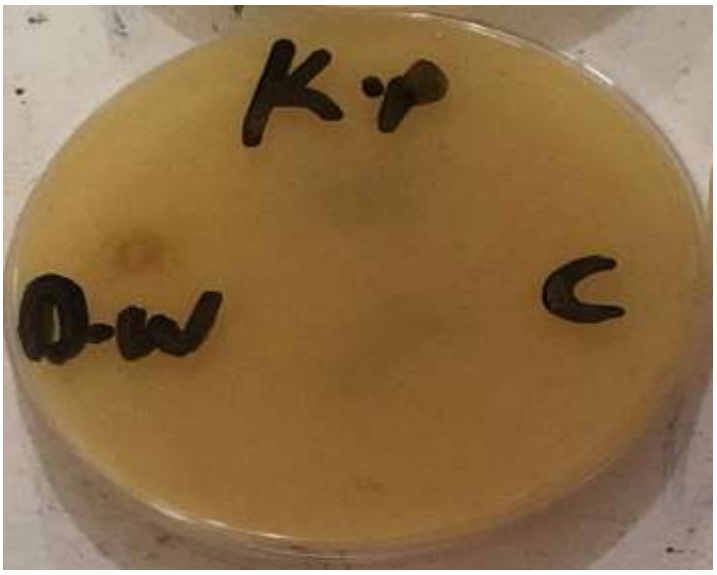

Plate 2: Distilled water extract of *A. digitata* against *K. pneumonia*

Plate 3: Ethanol extract of *A. digitata* against *E. coli*

3.2 Agar Well Diffusion Method

Each well made with a sterile cork borer was able to contain 0.10 mL of the extract and different diameter of zone of inhibition was observed against the fungi used as shown in table 3.2.

3.3 Minimum Inhibitory Concentration (Mic)

Minimum Inhibitory Concentration in µg/ml for Distilled water, Methanol, and Ethanol extract of *A. digitata* against *F. oxysporum, T. harzianum, P. oryzae, A. flavus E. coli, S. aureus, P. aeroginosa,* and *K. Pneumonia.* The result is as shown in Table 3.3.

Table 3.2: Zone of Inhibition in Agar Well Diffusion Method Against *F. oxysporum, T. harziainum, P. oryzae,* and *A. flavus*

Organisms	**Zone of Inhibition diameter (mm)**			
	Distilled water	**Methanol**	**Ethanol**	**Control**
Fusarium oxysporum	14	15	16	-
Trichoderma harzianum	14	14	17	-
Pyricularia oryzae	13	-	15	-
Aspergillus flavus	15	16	17	-

Table 3.3: Minimum Inhibitory Concentration

Organisms	Solvents	Concentration				
		100µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	Control
Fusarium oxysporum	Distilled water	-	-	+	+	+
	Methanol	-	-	+	+	+
	Ethanol	-	-	+	+	+
Trichoderma harzianum	Distilled water	-	-	-	+	+
	Methanol	-	-	+	+	+
	Ethanol	-	-	+	+	+
Pyricularia oryzae	Distilled water	-	-	+	+	+
	Methanol	-	-	+	+	+
	Ethanol	-	-	+	+	+
Aspergillus flavus	Distilled water	-	-	+	+	+
	Methanol	-	-	-	+	+
	Ethanol	-	-	-	+	+
Escherichia coli	Distilled water	-	-	-	+	+
	Methanol	-	-	-	+	+
	Ethanol	-	-	+	+	+
Staphylococcus aureus	Distilled water	-	-	+	+	+
	Methanol	-	-	+	+	+
	Ethanol	-	-	+	+	+
Pseudomonas aeroginosa	Distilled water	-	-	+	+	+
	Methanol	-	-	+	+	+
	Ethanol	-	-	+	+	+
Klebsiella pnemoniae	Distilled water	-	-	+	+	+
	Methanol	-	-	+	+	+
	Ethanol	-	-	-	+	+

Keys: - Negative; + Positive

4.0 Discussion

The Distilled water, methanolic and ethanolic extract of fruit content of *Adansonia digitata* was tested on eight different microorganisms (*Fusarium oxysporum, Trichoderma harzianum, Pyricularia oryzae, Aspergillus flavus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa,* and *Klebsiella pnemoniae*) in order to know their activity.

It was observed that the distilled water, methanol, and ethanol extract of *Adansonia digitata* were effective against all the test organisms used in this study which is in accordance with Abdallah *et al*. (2018) who extracted *A. digitata* using ethanol, distilled water, and chloroform and tested it against *Escherichia coli* and *Salmonella typhi*.

The *A. digitata* extract zone of inhibition of ethanol and distilled water were 11mm and 13mm respectively which was similar to the research conducted by Abdallah *et al.* (2018) that showed

zone of inhibition of 19mm for ethanol and 15mm for aqueous extract. Abdallah *et al.* (2018) also showed a larger zone of inhibition against the bacteria used in his work (*Escherichia coli* and *Salmonella typhi*) and revealed that the ethanol extract was more effective than aqueous extract which is a little bit different from the result shown by the bacteria isolate used in this study which may be due to the difference in the method used for the antimicrobial activity. The paper disk diffusion method was used against the bacteria isolate while Abdallah *et al*. (2018) used Agar well diffusion method. The effectiveness can probably be linked to the smaller volume of extract absorbed by the paper disk (0.015 mL) compared to the 0.lml that was injected into the well for Agar well diffusion.

The ethanol extract proved to be more effective against the fungi isolates (*Fusarium oxysporum, Trichoderma harzianum, Pyricularia oryzae*, and *Aspergillus flavus*) which is in agreement with Abdallah *et al*. (2018) although using bacteria isolates (*Escherichia coli* and *Salmonella typhi*).

The *Adansonia digitata* plant also demonstrated antimicrobial qualities as it showed zone of inhibition against some fungi which include *Aspergillus flavus, Fusarium oxysporum, Pyricularia oryzae* and *Trichoderma harzianum*. This result is in accordance with another investigation conducted by Samatha *et al.* (2017) using *Adansonia digitata* leaf extract, floral extract, fruit wall extract, seed extract and bark methanolic extract which showed that it was effective against fungi isolates (*Aspergillus sp* and *Penicillium sp*).

The ethanol extract of *Adansonia digitata* was more effective with 9mm – 18mm range of zone of inhibition which is in agreement with Magashi and Abdulmalik (2018) who revealed similar result showing that ethanol extract of *Adansonia digitata* was effective against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus mirabilis*, and *Streptococcus pneumoniae*. They further explained that the effectiveness of ethanolic extract was due to the difference in accumulation of phytochemical component, solubility of the bioactive metabolites and the polarity of the solvent which tends to affect its ability to recover compounds.

Magashi and Abdulmalik also revealed the presence of phytochemicals such as alkaloids, tanins, saponins, flavonoids, glucosides, steroids, triterpernoids and reducing sugars in the ethanol and aqueous extract of *Adansonia digitata*. These phytochemicals found in the plant have been confirmed to have antimicrobial activities by other research work conducted by Ajiboye *et al*. (2020) who revealed the presence of flavonoids, alkaloids, tannins and triterpenoids. Ajiboye also tested the *Adansonia digitata* aqueous stem bark against *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli* and *Candida albicans* revealing its effectiveness against them.

The MIC values obtained from this study showed that distilled water, methanol, and ethanol extract of *Adansonia digitata* were effective against all the test organisms used in this study at the concentrations of 50µg/ml and 25µg/ml which is a little bit different from the work of Bashir *et al*., (2021) who revealed that the MIC values at 25mg/ml for methanolic extract inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* which is not similar to the result of this work probably because of the differences in concentration values and the method used and the laboratory conditions during the study.

Many other plants in the family of Malvaceace are medicinal like *Adansonia digitata* such as *Hibiscus rosa sinensis*. The parts of *Hibiscus rosa sinensis* plant showed efficacy against *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus* using methanol solvent for the extraction process in the investigation conducted by Sangeetha *et al.*, (2009).

5.0 Conclusion

This research project hypothesizes that the various extracts of *Adansonia digitata* possess antimicrobial compounds that could be effective

in the development of antimicrobial medications to treat a variety of infectious diseases.

6.0 Recommendation

In view of these findings, I recommend that efforts should be made to investigate the antimicrobial activity of this plant in vivo and also to determine its MIC in a living system.

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