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ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS OF *Adansonia digitata* LEAVES ON CLINICAL ISOLATES OF *Staphylococcus aureus, Escherichia coli* AND *Bacillus* specie

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ABSTRACT
Treatment failure as a result of resistance strains of pathogens in circulation and the high cost of orthodox drugs relative to herbal preparations has made people, especially in developing countries, gravitate towards herbal medicine. These herbal preparations are commonly hawked on the streets of major cities in Nigeria and consumed by citizens either as a prophylactic or therapeutic agent due to claims that such preparations provide cures to most, if not all, ailments. Advances in the field of medicine and science have made it possible to confirm the ethnopharmacological claims made by herbal medicine practitioners by testing the antibacterial activity of common herbal plants to provide scientific evidence to either support or refute the claims made by the users of such herbal medicine. The antibacterial activity of the aqueous extract of Adansonia digitata against clinical isolates of Staphylococcus aureus, E. coli, and Bacillus spp. was carried out following standard microbiological techniques. Three (3) clinical isolates each of Staphylococcus aureus, E. coli, and Bacillus spp. collected from Shehu Muhammad Kangiwa Medical Center, Kaduna Polytechnic, were subjected to antibacterial susceptibility testing. The phytochemical Screening of the extract revealed the presence of Alkaloids, terpeniods, flavonoids, and steroids, while tannins and saponins were absent. There was an absence of antibacterial activity at all concentrations tested (100, 50, 25, and 12.5mg/ml), so no Minimum inhibitory concentration (MIC) or Minimum bacteriocidal concentration (MBC) values were recorded. From the results of this study, it is evident that the aqueous extract of Adansonia digitata has an abundance of phytochemicals that can be explored for medicinal uses; however, the extract of Adansonia digitata showed no antibacterial activity against the test isolates at the concentrations tested.
Keywords: Adansonia digitata, Staphylococcus aureus, Bacillus spp, Escherichia coli

INTRODUCTION

Adansonia digitata (A.digitata) the African baobab, is the most widespread tree species of the genus Adansonia, The African Baobab is widespread in tropical savannas (Kempe *et al.,* 2018) .It is found in drier climates, is sensitive to water logging and frost and is not found in areas where sand is deep (Descriptions and articles, 2015).It is native to the African continent between 16° and 26° north latitude (Patrut *et al*., 2015) .The tree has also been introduced to many other regions including Australia and Asia (Curious Kimberly, 2018)People have traditionally valued trees as sources of food, water, medicine or shelter. Baobab is a traditional edible plant in Africa, but little known elsewhere.(Hankey and Andrew, 2004)Baobab leaves can be eaten as a relish. Young fresh leaves are cooked in a sauce and sometimes are dried and powdered. The leaves are used in the preparation of a soup termed " Miyan kuka "in Northern Nigeria and are rich in phytochemicals and minerals (Ogbaga *et al*., 2017).Oil can be extracted from the seeds for cooking (Williams, 2002).

According to a report by The Lancet, antimicrobial resistance is a global health threat, with at least 1.27 million deaths and nearly 5 million deaths in 2019 (Antimicrobial resistance collaborators, 2022).

Based on predictive statistical modelling, there were approximately 4.95 million (3.62–6.57) deaths from bacterial AMR in 2019, including 1.27 million (95% CI 0.911–1.71) deaths from bacterial AMR. Regionally, the estimated all-age mortality rate for resistance was highest in sub-Saharan West Africa, 27.3 deaths per 100,000 (20.9–35.3), and lowest in Australia, 6.5 deaths (4.3- . 9.4) up to 100,000. The six leading pathogens for deaths associated with resistance (*Escherichia coli,* followed by *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa*) were responsible for 929 000 (660 000-1 270 000) deaths attributable to AMR and 3·57 million (2·62-4·78) deaths associated to AMR in 2019 (Antimicrobial resistance collaborators, 2022).

The search for new antimicrobials should be a continuous process, so as to have a stock pile of replacement drugs to help solve the problems of drug resistance if and when it occurs This is important bearing in mind that as living things, microorganisms will continuously look for ways of surviving in the presence of anything targeted against it, including drugs.

Since ancient times, the plant has been considered one of the main sources of drugs, especially in Asia and Africa. Almost all parts of the plant are believed to have medicinal properties. According to WHO, 80% of African population use herbal medicine for primary health care (WHO, 2002). The cost of orthodox drugs and treatment failure as a result of resistance strains of pathogens in circulation has made people especially in developing countries gravitate towards herbal medicine. These herbal preparations are commonly hawked on the streets of major cities in Nigeria and consumed by citizens either as a prophylactic or therapeutic agent due to claims that such preparations provides cure to most if not all ailments. Some of these plants are called miracle plant, tree of live etc because of the believe that such plants can provide cure to almost all ailments. Advances in the field of medicine and science has made it possible to confirm the ethnopharmacological claims made by herbal medicine practitioners by testing the antibacterial activity of common herbal plants to provide scientific evidence to either support or refute the claims made by the users of such herbal medicine. The aim of this study is to determine the antibacterial activity of aqueous extract of *Andosonia digitata* leaves against clinical isolates of *E coli, bacillus spp a*nd *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection and preparation of samples

Matured fresh-disease free leaves of *Adosonia digitata* were collected from Bagodo Yakowa express way, Kaduna state. The leaves were authenticated in Kaduna State University (KASU) and authentication number Kasu/BSH/187 was issued.

Plant materials

The leaves were shade dried, ground into powder and extracted by maceration with a water solvent. 200g of each finely ground leaf was soaked in 5000 mL of water for 72 h with vigorous shaking. The extract was evaporated to dryness in a water bath. The extracts were then stored in sterile wide-mouth containers

Test Organisms

The test organisms used were clinical pathogens. The test isolates were *Escherichia coli, Bacillus* spp *and Staphylococcus aureus* from Shehu Muhammad Kangiwa Medical Centre. Isolates were collected on sterile agar plates and then subcultured on fresh medium plates incubated at 37°C for 24h. Isolates were confirmed by Gram stain and biochemical tests. The isolates were then stored as stock cultures in a refrigerator at 40 °C.

Phytochemical Screening of Crude Extract

Standard screening test were carried out on the powered leaves for various phytochemical constituents (Trease and Evans, 2002)

Test for Alkaloids

Meyers test

About 2 drop of Meyer's reagent was added to 2ml of the plant extract. Yellow precipitate indicates the presence of alkaloids. (Trease and Evans, 2002)

Test for steroids

0.5g of plants extract was mixed in 2ml of chloroform and three drop of concentrated H_2SO_4 was added to form a lower layer. A reddish brown colour at the interface indicates a positive result

Test for Tannins

0.5g of plant extract was boiled with water separately and filtered. Two drop of ferric chloride was added to the filtrate. A blue black, green precipitate indicates presence of tannins (Trease and Evans, 2002).

Test for Saponins

Fronthing test

About 0.5g of plant extract was shaken separately in a test tube, frothing which persisted for 15 minutes or when warm on water bath indicates the presence of saponins. (Trease and Evans, 2002)

Test for Flavonoids

0.5g of plant extract was boiled with water separately and filtered. To 2ml of the filtrate, 2 drop of ferric chloride (freshly prepared) solution was added. A green blue or violet colouration indicates the presence of phenolic hydroxyle group. (Trease and Evans, 2002).

Test for terpenoids

5g of the plant sample was mixed with 2ml of $CHCL_3$ in a test tube. 3ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer; an interface with a reddish-brown coloration is formed if Terpeniods constituent is present. (Trease and Evans, 2002)

Standardization of the Inoculum

The clinical isolates were sub-cultured in 5ml nutrient broth for 18-24 hours. A loopful of the overnight culture was used to prepare a tenfold serial dilution in three tubes each containing 9ml normal saline with a last tube containing 4ml normal saline which resulted in a concentration of 1:10, 1:1000 and 1:5000. The turbidity of the last tube containing the concentration of 1:5000 matched the 0.5 McaFarland standard (Cappuccino and Sherman, 2011).

Preparation of varied concentrations of crude extracts

The aqueous leaf extract of was prepared in accordance with the dilution method . The stock solution was prepared by dissolving 1g of the aqueous leaf extract of *Adansonia digitata* in 10ml of distilled water separately, to make a concentration of 100mg/ml. This concentration (100mg/ml) was then further diluted to make working concentrations of 50mg/ml, 25mg/ml and 12.5mg/ml.

Antibacterial activity test

Susceptibility of test organisms to aqueous extracts of *Adansonia digitata* was determined using the agar well diffusion method described by (Ofokansi and Esinmone, 2005). Agar plates containing 20ml of Nutrient agar were seeded with 0.1ml of the standardized bacterial preparation in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of the organisms. Wells of 7.0mm diameter were bored on the agar plates using a sterile cork bore. 0.1ml of the extracts of different concentrations was used to fill each well. The Petri-dishes were allowed to stand for about 30minutes at room temperature to allow for proper diffusion of the extracts to take place. The plates were then incubated at 37^0C for 24 hours. The zones of inhibition if any were measured in millimeters and recorded.

Determination of the Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC)

The MIC of the extracts was measured using the agar dilution method (Lorian and Victor, 2005). 0.1ml of the varied concentrations of the extract was incorporated into nutrient agar medium, poured into Petri dishes and allowed to solidify. The standardized inoculum was inoculated with the solidified medium using point inoculation techniques on paper disc. The plates were then incubated at 37°C for 24 hours. After the period of inoculation, the lowest concentration of the extract which showed no visible growth was recorded as the MIC. The plates which showed no visible growth was then sub cultured onto fresh medium with no extract, incubated at 37^0C for 24 hours. The concentration which showed no visible growth was then recorded as the MBC.

RESULTS AND DISCUSSION

The Phytochemical screening of the aqueous extract of *Adansonia digitata* showed the presence of terpenoids, Alkaloids, Steroids and Flavonoids.(Table i).Ogbaga *et al.,* (2017); Abiona *et al*., (2015); Zagga *et al.* (2018); Faleyemu Oluwalana (2008); however reported the presence of tannins and saponins in aqueous leave extract of *Adansonia digitata*. The slight variations in the phytoconstituents of *Adansonia digitata* reported in this study from that of previous studies could be attributed to differences in drying method employed and differences in climatic and environmental factors of the area where the leaves was gotten from. According to Chadare *et al*.,(2008) variations might be due to quality, age and origin of sample. It might also be due to treatment before analysis, storage conditions, processing methods, soil structure, chemical composition of the soil and genetic variation (De Caluwe, 2010). The aqueous extract of *Adansonia digitata s*howed no antibacterial activity against clinical isolates of *Staphylococcus aureus, Bacillus spp and E coli* at concentrations tested (Table ii). Kubamarwa *et al.,*2007); Bamidele *et al.,* (2013); Sanaa, (2008) reported absence of antibacterial activity of *Adansonia digitata* aqueous leave extract. However, Abiona *et al*. (2015) reported antibacterial activity of the aqueous extract of *Adansonia digitata* against some gram positive and gram negative bacteria. These differences in findings could be attributed to the method of extraction, time of collection of plant and maturity level of plant as at the time of collection. The absence of antibacterial activity in the aqueous extract of *A.digitata* reported in this study could also be attributed to the absence of phytoconstituents (tannins and

saponins) in the leaves of *Adansonia digitata* studied. Aliyu (2008) reported that plants with pharmacologically active metabolites such as saponin and flavonoids have been found in vitro to have anti-microbial activity. The solvent used for extraction also contribute to the presence or absence of antimicrobial activity. According to Dirar *et al.*, (2019) ;Ouafae *et al.*, (2022) extraction solvents had variable effect on the content of secondary metabolites and bioactivities.

Table i: Phytochemical Screening of the aqueous extract of *Adonsonia digitata* Leaves

Phytochemicals	*Adonsonia digitata*
Alkaloids	+
Tannins	-
Saponins	-
Terpeniods	+
Steroids	+
Flavonoids	+

Key : - = Absence ; += Presence

Table ii: Antibacterial activity of the aqueous extract of *Andosonia digitata* against clinical isolates of *Staphylococcus aureus, Bacillus spp and Escherichia coli*.

Isolates.	Concentration(mg/ml) 100 50 25 12.5 Zones of inhibition (mm)	CONTROL(Gentamicin 40mg/ml) (mm)
E coli 1	_ _ _ _	40
E coli 2	_ _ _ _	35
E coli 3	_ _ _ _	40
Staphylococcus aureus 1	_ _ _ _	45
Staphylococcus aureus 2	_ _ _ _	50
Staphylococcus aureus 3	_ _ _ _	40
Bacillus spp 1	_ _ _ _	25
Bacillus spp 2	_ _ _ _	30
Bacillus spp 3	_ _ _ _	30

Key:-= absence of zone of inhibition

CONCLUSION

The aqueous extract of *Adansonia digitata* leaves has an abundance of phytoconstituents. therefore it can be explored for medicinal uses, however the extract of *Adansonia digitata* showed no antibacterial activity against clinical isolates of *Staphylococcus aureus, E. Coli and Bacillus spp* at the concentrations tested.

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