RESEARCH ARTICLE

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Phytochemical analysis and antimicrobial activity of baobab (*Adansonia digitata*) leaves and steam bark extracts on *Staphylococcus aureus* and *Escherichia coli*

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Article info:
Received: 10 August 2017
In revised form: 9 September 2017
Accepted: 9 September 2017

ABSTRACT

The phytochemical analysis and antibacterial activity of methanolic and ethanolic leaf and stem bark extracts of baobab tree on *Escherichia coli* and *Staphylococcus aureus* were carried out using agar well diffusion method. The clinical bacterial isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from Microbiology laboratory, Kaduna State University, Kaduna. The bacteria isolates were re-confirmed and identified based on their morphology, cultural characteristics and biochemical tests. The bacteria isolates were confirmed to be *Escherichia coli* and *Staphylococcus aureus.* The phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, tannins and terpenoids. The methanolic leaf extract showed a wide range of activity on test isolates, with varying zones of inhibitions as 12 mm, 10 mm, 7 mm, and 4 mm against *Staphylococcus aureus* and 13 mm, 9 mm, 7 mm, and 3 mm against *Escherichia coli* at concentration of 1000 mg/ml, 500 mg/ml, 200 mg/ml and 100 mg/ml respectively. The ethanolic leaf extract also showed a wide range of activity on test isolates with varying zones of inhibitions, such as 11 mm, 6 mm, 5 mm and 3 mm against *S. aureus* and 8 mm, 7 mm, 5 mm, and 4 mm against *E. coli* at the concentration of 1000 mg/ml, 500 mg/ml, 200 mg/ml and 100 mg/ml for each respectively. The methanolic stem bark extract showed less antibacterial activity against the test isolates with the inhibition of 5mm and 4 mm against *S. aureus* and 4 mm and 3 mm against *E. coli* at concentrations of 1000 mg/ml and 500 mg/ml respectively with no zones of inhibition at concentrations of 200 mg/ml and 100 mg/ml. The ethanolic stem bark extract also showed no antibacterial activity with no zones of inhibition against the test isolates at concentration of 1000 mg/ml, 500 mg/ml, 200 mg/ml and 100 mg/ml. The methanolic leaf extract inhibited the growth of *S. aureus* and *E. coli* at concentration of 100 mg/ml with minimum bactericidal concentration at 100 mg/ml. The ethanolic leaf extract inhibited the growth of *S. aureus* and *E. coli* at the concentration of 100 mg/ml with a minimum bactericidal concentration of 100 mg/ml. The relationship between the phytochemical profile/constituents and biological activity of the extracts at different concentrations on the test isolates could be as a result of the effects exhibited by the extract constituents/bioactive substances. This result showed that the ethanolic and methanolic leaf extract of *Adansonia digitata* contained bioactive substances that may likely be used against some pathogenic bacteria when medically used.

Key words: phytochemical, baobab, extract, bacteria, inhibition, bioactive substances

Introduction

In an attempt to combat the various forms of the disease that have continued to plague humans from time immemorial to this day, different types of antimicrobials have been developed to fight the pathogens responsible for these diseases. Antimicrobials, which are substances that kill or inhibit the growth of microorganisms, could be in the form of antibiotics, which are products of microorganisms or synthesized derivatives (Cowan, 2002), antimicrobial peptides produced by complex organisms as well as some

microbes (Jenssen et al., 2006) and medicinal plants, which appear to be the focus of mainstream medicine today (Cowan, 2002). They are widely employed to reduce the microbial load on animate and inanimate surfaces or in the cure of diseases associated with microorganisms mostly bacteria and fungi. The action of these agents could either irreversibly inhibit the growth of bacteria and hence are said to be "bactericidal" or reversibly inhibit the growth of a microorganism due to continuous contact with the agent it is referred to as being "bacteriostatic" (Rajesh & Rattan, 2008). Drug reaction and side effects, increased risk of malignancy, fake and adulterated drugs have added to the problem of antibiotic resistance (Green, 2007). The emergence of pathogens resistant to antibiotics as a result of their excessive use in clinical and veterinary applications represents a serious public health concern (Keymanesh et al., 2009).In the last three decades, pathogenic resistant bacteria caused major health problems throughout the world although the pharmacological industries produced quantities of antibiotics. Unfortunately, the resistance of bacteria to these drugs is increasingly important. The search for plants with antibacterial activity has gained increasing importance in recent years due to the development of resistance. A typical example is *Adansonia digitata* (generally known as the African Baobab) is one of the eight species of baobab in the genus *Adansonia* L, and the only one that occurs naturally on mainland Africa. It is found throughout most of Africa, south of the Sahara (Baum, 1995).

The Baobab tree belongs to the Malvaceae family (Bremer et al., 2003). It has been the subject of investigation as a potential source of numerous antimicrobial compounds. Agbafor (2011) and Kubmarawa et al. (2007) reported that Phytochemicals such as Alkaloid, saponins, Flavonoids, tannins and terpenoids are chemical bioactive components that could be responsible for antibacterial activities in the plant. Results of antibiotic studies show that ethanolic, methanolic and aqueous extract of *A. digitata* stem bark or leaves have antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis, Escherichia coli* and *Mycobacterium phlei* (Anani et al., 2000). Methanolic extract from the stem of *A. digitata* has also been reported to have anti-trypanosomal activity against *Trypanosomacongolense* and *T. brucei* (Atawodi et al., 2003). The baobab is mainly used for food. The fruits, flowers, leaves, shoots, roots of seedlings and even the tree roots are edible. The leaves can be used either fresh, as a cooked vegetable, or dried and powdered as a functional ingredient (thickener) of soups and sauces. The flowers, shoots and roots of seedlings are eaten (Bosch et al., 2004). The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern. Treatment of diseases caused by bacteria with antibiotics often results in drug resistance in both enteric and non-enteric bacteria (Fhogartaigh & Edgeworth, 2009). This is most likely related to the frequent unrestricted use of over-the-counter drugs without medical supervision. At present, the continued development of new antimicrobials, other than antibiotic ones, particularly those used for the treatment of pathogenic Bacteria, is critically important. This research will be carried out to reveal the active components of leaves and stem bark extracts of baobab tree on selected strains of pathogenic bacteria and to validate traditional antimicrobial uses of baobab tree. This research is aimed at assessing the phytochemical compounds and antibacterial activity of leaves and stem bark extracts of the baobab tree on *Staphylococcus aureus* and *Escherichia coli*.

Materials and Methods

Plant collection and identification

The leaves of baobab *(Adansonia digitata)* were plucked and the Stem bark was scraped using a sterile knife at Kaduna North Local Government Area of Kaduna State. The plant parts were authenticated in the Botanical laboratory of the department of biological sciences, Kaduna State University, Kaduna. The scrapings and leaves were washed and air-dried and ground into fine powder using mortar and pestle in the laboratory as described by Mukhtar and Tukur (2000).

Collection of test organisms and re-confirmation

The clinical bacterial isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from Microbiology laboratory, Kaduna State University, Kaduna, Kaduna State, Nigeria. The bacteria isolates were re-confirmed and identified based on their morphology, cultural characteristics and biochemical tests as described by Cheesbrough (2000), Parija (2006) and Oyeleke and Manga, (2008).

Preparation of ethanolic and methanolic extract of leaves and stem bark of Adansonia digitata

Adansonia digitata leaves and stem bark plant extracts were prepared by cold maceration method as described by Basri and Fan (2005) and Elkady (2012). Both ethanolic extract (A) and Methanolic extract (B). Ethanolic extracts of the leaves and stem bark of *Adansonia digitata* were prepared by soaking 50 g of finely grounded powder of leaves and stem bark of *Adansonia digitata* in 250 ml of ethanol for 24 hours. After 24 hours, the extracts were filtered through Whatman No. 1 filter Paper and the residual matter was again soaked with 150 ml of ethanol for another 24 hours. The extract was filtered through Whatman No. 1 filter paper and the two extracts were pooled together and the combined extracts were then concentrated in a water bath set at 70 °C. The prepared extract was transferred into clean and dried

universal bottle and stored in the refrigerator until needed for analysis.

Methanolic extracts of the leaves and stem bark of *Adansonia digitata* were prepared by soaking 50 g of finely grounded powder of leaves and stem bark of *Adansonia digitata* in 250 ml of methanol for 24 hours. After 24 hours, the extracts were filtered through Whatman No. 1 filter Paper and the residual matter was again soaked with 150 ml of methanol for another 24 hours. The extract was filtered through Whatman No. 1 filter paper and the two extracts were pooled together and the combined extracts were then concentrated in a water bath set at 70 °C. The prepared extract was transferred in clean and dried universal bottle and stored in the refrigerator until needed for analysis.

Phytochemical screening of leaves and stem bark extracts of baobab tree

Phytochemical screening of the extracts was done as described by Sofowora (2008).

Detection of alkaloids

A fraction of the extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml distilled water) and observed for the formation of reddish brown coloured precipitate.

Detection of flavonoids

Sulphuric acid test. A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange color.

Aqueous sodium hydroxide test. A fraction of the extract was treated with aqueous NaOH solution and observed for the formation of yellow-orange coloration.

Detection of terpenoids

To 1 ml of chloroform, was added to 2.5 ml of each extract and were mixed, and concentrated sulphuric acid was added drop wise to form a layer. The presence of reddish brown coloration at the interface indicates the presence of terpenoids.

Detection of saponins

Twenty milliliters of distilled water was added to 0.5 g of the extract in a graduated cylinder and shaken gently for 15 minutes. The formation of 1 cm layer of foam indicates the presence of saponins.

Detection of tannins

One percent gelatin solution containing sodium chloride was added to each of the extracts separately. The formation of white precipitate indicates the presence of tannins.

Characterization and identification of bacterial isolates

Cultural identification of bacterial isolates

The two bacterial isolates collected (*Escherichia coli* and *Staphylococcus aureus*) were subcultured on eosin methylene blue agar (EMB) and mannitol salt agar (MSA) respectively in order to purify and obtain a pure culture of the isolates.

Characterization of bacterial isolates

The procedures of Parija (2006) and Oyeleke and Manga (2008) were employed for the biochemical tests. The biochemical tests performed include: Gram's staining, Catalase test, Coagulase test (Slide test), indole test, methyl-red Voges-Proskauer test (MR-VP) for bacteria characterization and identification.

Gram's staining

A smear of the isolates were prepared on glass slide separately which were then stained with crystal violet solution for 60 seconds after which each slide was washed distilled water and was well-drained to avoid diluting the mordant. The slides were flooded with iodine solution (mordant) for 60 second and washed with water. The slides were then decolorized with acetone by adding drop wise until all free colour were removed and washed with water. The slides were flooded with water. The slides were flooded with neutral red (counter stain) for 30 seconds, washed and allowed to dry. All slides were examined under the oil immersion objectives lens (Oyeleke & Manga, 2008).

Catalase test

A drop of hydrogen peroxide was placed on a clean grease-free slide separately and emulsified with the test organism picked using a sterile wire loop then observed for bubble formation. (Oyeleke & Manga, 2008).

Coagulase yest (Slide test)

Clean glass slides were marked into two halves by a marker. Two drops of sterile saline were added on two halves of the glass slides. Colonies of *S. aureus* to be tested were picked up from agar culture and gently emulsify with drops of saline. A drop of undiluted plasma was added to the bacterial suspension and mixed with a wire loop. Another drop of saline was added in another half of the slide as a control. The slides were rock back and forth, and observed for the prompt clumping of the bacterial suspension within 10-15 seconds (Parija, 2006).

Indole test

The organisms were grown in 5 ml of peptone water for 24 hours. After 24 hours of incubation Kovac's indole reagent of about three to eight drops were added. It was tilted gently and a positive test was indicated by the development of a red colour in the reagent surface layer above, within 10 minutes while negative reaction retained its yellow colour (Parija, 2006).

Methyl-red Vogues-Proskauer test (MR-VP)

Five milliliters of MR-VP broth were inoculated with the isolates and incubated at 37 °C for 48 hours. After

incubation, 1ml of the broth each were transferred to a small test tube separately and about 2-3 drops of methyl – red were added. A red colour on addition of the indicator signified a positive test while a yellow colour signified negative test. To the rest of the broth in the original tube 5 drops of 40% potassium hydroxide followed by 15 drops of 5% naphthol in ethanol were added. It was shaken and the cap of the tube was loosened and the tubes were placed in a sloping position. The development of a pink color starting from the liquid – air interface within 1 hour indicated a VP positive test. No color change occurs in a VP negative test (Parija, 2006).

Preparation of concentrations of leaves and stem bark extract of baobab tree

Using sterile dilution technique, 2 g of the ethanolic and methanolic extracts were dissolved separately in 2 ml of water to give a concentration of 1000 mg/ml (highest stock solution) followed by serial dilution with distilled water to give various concentrations of 500 mg/ml, 200 mg/ml and 100 mg/ml. The tubes containing the various concentrations were labeled and used immediately. Ciprofloxacin was used as the standard drug (500 mg/ml) (Ekwe & Elenglam, 2005).

Preparation of media

Muller-Hinton agar was prepared according to the manufacturer's instruction in a sterile conical flask and it was then sterilized at 121 °C for l5 minutes and allowed to cool to about 45 °C before dispensing into sterile Petri plates.

Preparation of 0.5 McFarland turbidity standard

One percent solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 into 99 ml of water. One percent solution of barium chloride ($BaCl_2$) was also prepared by dissolving 0.5 g of dehydrated barium chloride in 50 ml distilled water. 0.5 ml of Barium chloride solution was added to 9.5 ml of sulphuric acid solution to yield 1.0% barium sulphate suspension. The turbid solution formed was transferred into a test tube as the standard for comparison.

Preparation and standardization of inoculum suspension

Bacterial inoculum suspension was prepared as described by Coyle (2005). Using sterile cotton swab, enough material from an over-night broth culture of the test organisms were transferred into a tube containing 2.0 ml normal saline and turbidity were adjusted to match 0.5 McFarland turbidity standard (Corresponding to approximately 1.5 x 10^8 CFU/ml) for each isolate respectively. The suspension was spread over the agar having a sterile cotton swab.

Determination of antibacterial activity using agar well diffusion assay

The agar well diffusion technique as described by Biradar et al. (2007) was employed to test the antimicrobial effects of methanolic and ethanolic leaves and stem bark extract of the baobab tree. The Muller-Hinton agar was poured into sterilized Petri-dishes, allowed to solidify for 30 minutes. The test organisms were inoculated onto the sterile agar plates using a sterile cotton swab. After 15 minutes of the inoculation, five wells of 5.0 mm in diameter each were aseptically bored using a sterile cork borer on each agar plate. On each agar plate, about 0.3 ml of the extract of varying concentration (1000/ml, 500/ml, 200/ml, and 100/ml) were dispensed into 4 of the wells and to the last well, ciprofloxacin was dispensed as a positive control. The plates were then incubated at 37 °C for 24 hours. Effect of the extract was assessed by measuring the diameters of zones of inhibition to the nearest millimeter as described by National Committee for Clinical Laboratory Standards (2003) and were then compared with the standard ciprofloxacin.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of the aqueous and ethanolic extracts of the leave and stem bark were determined by the broth dilution method using serially diluted plant extracts according to the National Committee for Clinical Laboratory Standards protocol (National Committee for Clinical Laboratory Standards, 2003) as described by Lar et al. (2011). Six tubes labeled 1-6 were used for the determination of MIC of the ethanolic extract. The six tubes contained 5 ml of Muller-Hinton broth. One ml of the crude extract in the concentration of (100mg, 50 mg, 25 mg and 12.5 mg/ml) were introduced into tube 1-4 respectively and were mixed thoroughly. To each of the test tubes (1-5), 0.l ml of broth cultures (equivalent of approximately 1.5 x 10^8 cfu/ml) of the test organism (*E. coli* and *S. aureus)* were added to 4 tubes with the last tube serving as broth control for each respectively. All the tubes were incubated at 37 °C for 24 hours, after which they were examined for bacterial growth. The minimum inhibitory concentration (MIC) of the crude extract is the lowest concentration of the extract that is capable of inhibiting the growth of specified inoculum of the test organisms.

Determination of minimum bactericidal concentration

Five milliters of prepared Mueller-Hinton broth were dispensed into sterile test tubes equivalent to the number of tubes that showed no visible growth from the determination of Minimum Inhibitory Concentration. From the tubes that showed no visible growth 0.1 ml of the culture were transferred separately to each tube containing the 5 ml Mueller-Hinton broth. The tubes were labeled and kept in a test-tube rack. Prepared Mueller-Hinton agar was poured into sterile Petri dishes equivalent to the number of culture tubes that showed no visible growth, it was allowed to solidify. Using sterile pipette, 0.1 ml were transferred from each tube to the surface of the agar. The inoculums were spread out using a smooth sterile bent glass rod for each respectively.

Both tubes and plates were incubated at 37 °C for 24 hours. Turbidity or cloudiness was observed in the broth culture and also bacteria growth colonies were observed on the solid medium plate (Vollekova et al., 2001).

alkaloids, tannins, flavonoids, saponins and terpenoids are presented as shown in Table 2.

Antibacterial activity of ethanolic and methanolic leaf and stem bark extracts of Adansonia digitata

Table 1. *Cultural and biochemical characteristics of bacterial isolates.*

Test isolates	Gram reaction	Morphology	Catalase	Coagulase	Indole	MR	VP
Escherichi coli	-	Bacilli	+	-	+	+	-
Staphylococcus aureus	+	Cocci	+	+	-	+	+

Legend: "+" – confirmed to be positive; "-" – confirmed to be negative; MR – methyl red test; VP – Voges-Proskauer test.

Table 2. *Phytochemical constituents of ethanolic and methanolic leaves and stem bark extracts of baobab tree.*

Phytochemical compounds	Ethanolic leaf extract	Ethanolic stem bark extract	Methanolic leaf extract	Methanolic stem bark extract
Alkaloids	+	+	+	-
Saponins	+	+	+	+
Flavonoids	+	-	+	+
Tannins	-	-	+	-
Terpenoids	+	-	+	-

Legend: "+" – confirmed to be present; "-" – confirmed to be absent.

Results

Cultural and biochemical characteristics of Staphylococcus aureus and Escherichia coli

The growth of the two bacterial isolates (*Staphylococcus aureus* and *Escherichia coli*) on the different media used revealed the following cultural characteristics: Metallic green sheen was observed on eosin methylene blue agar (EMB) plate which was presumed to be *E. coli* as it ferment lactose in the medium, *E. coli* produce strong-acid by-product causing a color change in the colony; on mannitol salt agar (MSA) plate, yellow colonies with yellow zones were observed which was expected to be mannitol fermenters such as *S. aureus*. *E. coli* was confirmed to be catalase positive, MR positive, and VP negative. The presumptive *Staphylococcus aureus* was confirmed to be negative for indole test and positive for catalase, MR, VP and coagulase test. Also**,** their Gram reaction revealed the following morphology: *Escherichia coli* were observed to be Gram negative rod while *Staphylococcus aureus* appeared to be gram positive cocci in clusters (Table 1).

Phytochemical constituents of the extract

The result obtained for the bioactive components such as

The results of the antimicrobial activity of ethanolic and methanolic leaf and stem bark extracts at different concentrations (1000 mg/ml, 500 mg/ml, 200 mg/ml, and 100 mg/ml) against *Escherichia coli* and *Staphylococcus aureus* are presented in Table 3A-D.

Minimum inhibitory concentration (MIC)

The result of the minimum inhibitory concentration (MIC) of the ethanolic and methanolic leaf extract is presented in Table 4A and 4B. The MIC of the methanolic leaf extract was observed at 100 mg/ml and 50 mg/ml for both *Escherichia coli* and *Staphylococcus aureus* respectively, and 100 mg/ml for ethanolic leaf extract for both *Escherichia coli* and *Staphylococcus aureus* which indicates that the extract was able to inhibit the growth of the organism at these concentrations, therefore no turbidity was observed.

Minimum bactericidal concentration

The result of minimum bactericidal concentration (MBC) of the extract is shown in the Table 5A and 5B. The minimum bactericidal concentrations were found to be 100 mg/ml for both ethanolic and methanolic leaf extracts of *Adansonia digitata* on *Escherichia coli* and *Staphylococcus aureus*.

Table 3A. *Antibacterial sensitivity test of ethanolic leaf extract on test organisms.*

Test organisms	Zones of inhibition (diameter in mm) at different concentrations				
	Concentration (mg/ml)				
	1000	500	200	100	500 (ciprofloxacin)
Escherichia coli	8	7	5	3	19
Staphylococcus aureus	11	6	5	4	17

Table 3B. *Antibacterial sensitivity test of methanolic leaf extract on test organisms.*

Test organisms	Zones of inhibition (diameter in mm) at different concentrations				
	Concentration (mg/ml)				
	1000	500	200	100	500 (ciprofloxacin)
Escherichia coli	13	9	7	3	17
Staphylococcus aureus	12	10	7	4	15

Table 3C. *Antibacterial sensitivity test of ethanolic stem bark extract on test organisms.*

Test organisms	Zones of inhibition (diameter in mm) at different concentrations				
	Concentration (mg/ml)				
	1000	500	200	100	500 (ciprofloxacin)
Escherichia coli	-	-	-	-	17
Staphylococcus aureus	-	-	-	-	16

Legend: "-" – no zones of inhibition.

Table 3D. *Antibacterial sensitivity test of methanolic stem bark extract on test organisms.*

Test organisms	Zones of inhibition (diameter in mm) at different concentrations				
	Concentration (mg/ml)				
	1000	500	200	100	500 (ciprofloxacin)
Escherichia coli	4	3	-	-	18
Staphylococcus aureus	5	4	-	-	17

Legend: "-" – no zones of inhibition.

Table 4A. *The minimum inhibition concentration of ethanolic leaf extract of Adansonia digitata on test organisms.*

Test organism	Concentration (mg/ml)				
	100	50	25	12.5	MIC (mg/ml)
Escherichia coli	-	+	+	+	100
Staphylococcus aureus	-	+	+	+	100

Legend: "-" – no growth observed; "+" – growth observed.

Table 4B. *The minimum inhibition concentration of methanolic leaf extract of Adansonia digitata on test organisms.*

Test organism	Concentration (mg/ml)				
	100	50	25	12.5	MIC (mg/ml)
Escherichia coli	-	-	+	+	50
Staphylococcus aureus	-	-	+	+	50

Legend: "-" – no growth observed; "+" – growth observed.

Table 5A. *The minimum bacterial concentration of methanolic leaf extract of Adansonia digitata on test organisms.*

Test organism	Concentration (mg/ml)		
	100	50	MBC (mg/ml)
Escherichia coli	-	+	50
Staphylococcus aureus	-	+	50

Legend: "-" – no growth observed; "+" – growth observed.

Table 5B. *The minimum bacterial concentration of ethanolic leaf extract of Adansonia digitata on test organisms.*

Test organism	Concentration (mg/ml)	
	100	MBC (mg/ml)
Escherichia coli	-	100
Staphylococcus aureus	-	100

Legend: "-" – no growth observed.

Discussion

Many plants in different location have been recognized as a source of cure for ailment in their region of existence. These facts arrived by trial and error and thereafter, their importance became obvious. The plant part mostly used include the seeds, leaves, bark, oil and root. Agbafor (2011) and Kubmarawa et al. (2007) reported thatphytochemicals such as alkaloid, saponnins, flavonoids, tannins and terpenoids are chemical bioactive components could be responsible for antibacterial activities in the plant, hence the need to determine such for the purpose of development of drugs by pharmaceutical companies. The presence of tannins, saponnins, flavonoids, alkaloids and terpenoids in methanolic leaf extract of baobab were observed while tannins was absent in ethanolic leaf extract. Both alkaloids and saponins were present in ethanolic stem bark extract while saponnins and flavonoids were present in methanolic stem bark extract.and these indicated that the plant is of pharmacological importance. The presence of flavonoids in this research indicates that the naturally occurring phenolic compound, with beneficial effect in the human diet as antioxidants and neutralizing free radicals. This is similar to the findings of Djeridane et al. (2006), Wong *et al*. (2006). extract of *Adansonia digitata* against *Escherichia coli* and *Staphylococcusaureus* at different concentrations were

observed. These extracts inhibited considerable level of inhibition against the Gram negative and Gram positive organisms which indicates the presence of broad spectrum antibiotic compound in the plant which corresponds to the findings of Khanna and Kannabiran (2008). The extracts exhibited an appreciable level of inhibition against *Escherichia coli* and *Staphylococcus aureus*, with the highest activity on *E. coli* followed by *S. aureus* at all concentrations, this result supports the findings of Anani et al. (2000). Result reported by Adeleke et al. (2006) suggested that diameter of zone of inhibition of 10 mm were considered active. The zones inhibition were higher at 1000 mg/ml, 500 mg/ml, 200 mg/ml and 100 mg/ml respectively. This implies that concentration and zones of inhibition thus have a direct relationship. This means that the higher the concentration, the higher the zones of inhibition and vice versa which supports the findings of Anani et al. (2000) and Doughari (2006). The activity of these extracts against *Escherichia coli* and *Staphylococcus aureus* which are the potential causative agent of abdominal ailment agreed with previous work reported by Cowan (1999), Anani et al. (2000) and Doughari (2006). The decoction of the leaf has been established to exhibit significant reduction in intestinal motility. Methanolic extract of baobab in this study had higher solubility for more bioactive compounds, consequently having the highest antibacterial activity which contradict the findings of Anani et al. (2000) and Doughari (2006) in which they demonstrated that ethanolic extract have higher solubility for more bioactive compunds thus, having the highest antibacterial activity.

The antibacterial activity of ethanolic and methanolic stem bark extract of *Adansonia digitata* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations were observed. The methanolic stem bark extract inhibited the growth of the test organisms at concentration of 1000 mg/ml and 500 mg/ml with no zones of inhibtion at 200 mg/ml and 100 mg/ml. The ethanolic stem bark extract had no effect on the test organisms with no zones of inhibition at all concentration. This is in conformity with the findings of Khanna and Kannabiran (2008). This low antibacterial activity shown by the stem bark extracts indicated that the active compound(s) does not extract by the cold maceration method as reported by Anani et al. (2000) and Doughari (2006).

The minimum inhibitory concentration (MIC) showed that the methanolic leaf extract inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* at concentrations of 100 mg/ml and 50 mg/ml and the ethanolic leaf extract inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* at concentration of 100mg/ml as reported by Palombo (2006). The minimum bactericidal concentration (MBC) result showed that the bactericidal effects of the methanolic and ethanolic extract were at concentration of 100 mg/ml for both *Escherichia coli* and *Staphylococcus aureus*. This is in conformity with the statement reported by Cowan (1999), Anani et al. (2000) and Doughari (2006). The minimum bactericidal concentration obtained showed that the leaf extracts from the plant were very active even at lower concentrations against the important clinical human pathogens tested for, in this research works, which support the findings of Cowan (1999), Anani et al. (2000) and Doughari (2006).

In conclusion, based on the results obtained from this study, *Adansonia digitata* commonly known as "Baobab tree" has effective and important ingredient in the treatment of wide variety of diseases. The facts that the extract produced inhibitory activities against the test organisms provide some scientific basis for some of the folkloric claims. The findings in this work have justified the potent use of this plant in ethno-medicinal treatment of stomach ache, diarrhea, which are caused by *Escherichia coli* and *Staphylococcus aureus* used in this study. Also, the antibacterial activity displayed by the isolates justified its ethno-botanical uses for the treatment of ophthalmic, coughs, colic and hemorrhoids. *Adansonia digitata* could be taken alongside some synthetic drugs pending on the severity of the illness during the treatment of diseases caused by *Escherichia coli* and *Staphylococcus aureus.*

Conclusion

The following recommendations were made:

1. It can be established that methanolic and ethanolic leaf extract of *Adansonia digitata* can be recommended for treatment of different ailments.

2. Further research work should be carried out on root bark, seed, fruit pulp and aerial part to be able to ascertain which of these parts is more effective on a particular ailment.

3. Further characterization and isolation of bioactive constituent(s) of the extracts of this plant should be carried out.

4. Also, synergistic studies with no antibiotic should be carried out to determine the efficacy of treatment of the ailment with this plant.

References

Adeleke OE, Afolabi RO, Adekunle JM, Ojo OP. 2006. Antimicrobial activities of extracts of *Garcinia kola* on agents of respiratory tract infections. Nig. J. Microbiol., 20: 1184-1190.

Agbafor KN, Nwachukwu N. 2011. Phytochemical analysis and antioxidant property of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. Intl. J. Biochem. Res., 2: 7-9.

Anani K, Hudson JB, de Souza C, Akpagana K, Tower GHN, Amason JT, Gbeassor M. 2000. Investigation of medicinal

plants of Togo for antiviral and antimicrobial activities. J. Pharm. Biol., 38(1): 40-45.
Atawodi SE, Bulus TS, Ibrahim DA, Ameh AJ, Nok M, Mamman M, Galadima M. 2003. *In vitro* trypanocidal effect of methanolic extracts of some Nigerian savannah plants. Afri. J. Biotechnol., 2(9): 317-321.
Basri DF, Fan SH. 2005. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Ind. J. Pharmacol., 37(1): 26-29.
Baum DA. 1995. The comparative pollen and floral biology of baobab (*Adansonia bombacaceae*). Ann. Misso. Bot. Gard., 82(2): 322.
Biradar YS, Jagatap S, Khandelwal KR, Singhania SS. 2007. Exploring of antimicrobial activity of *Triphala mashi* - an *Ayurvedic* formulation. Oxf. J., 5(1): 107-113.
Bosch CH, Sié K, Asafa BA. 2004. *Adansonia digitata* L. Record from Protabase. – In: Grubben, GJH and Denton OA (eds). PROTA (Plant Resources of Tropical Africa / Ressourcesvégétales de l'Afriquetropicale), Wageningen, Netherlands, p. 520-522.
Bremer B, Bremer K, Chase MW, Reveal JL, Soltis DE, Soltis PS, Stevens PF, Anderberg, AA, Fay MF, Goldblatt P, Judd WS, Kallersjo M, Karehed J, Kron KA, Lundberg J, Nickrent DL, Olmstead RG, Oxelman B, Pires JC, Rodman JE, Rudall PJ, Savolainen V, Sytsma KJ, Van der Bank M, Wurdack K, Xiang JQY, Zmarzty S. 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc., 141(4): 399-436.
Cheesebrough M. 2000. Antimicrobial sensitivity testing. District Laboratory Practice in Tropical Countries, 2: 132-143.
Clinical Laboratory Standard Institute (National Committee for Clinical Laboratory Standards). 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A6. 6th ed. Wayne, Pa, USA: National Committee for Clinical Laboratory Standards. p. 18-22.
Cowan MM. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12(4): 564-582.
Cowan MM. 2002. Plant product as antimicrobial agent revolution. Clin. Microbiol. Rev., 12(4): 561-582.
Coyle MB. 2005. Manual of antimicrobial susceptibility testing. American Society for Microbiology.
Djeridane A, Yousfi M, Nadjemi B, Boutassouna, D, Stocker P and Vidal N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. J. Food Chem., 97(4): 654-660.
Doughari JH. 2006. Antimicrobial activity of *Adansonia digitata*. Linn Tropics. J. Pharm. Res., 5: 597-603.
Elkady IA. 2012. Crude extract of *Nigella sativa* inhibits proliferation and induces apoptosis in human cervical carcinoma HeLa cells. Afri. J. Biotechnol., 11(64): 12710-12720.
Ekwe UN, Eleglam N. 2005. Antibacterial activity of ginger (*Zingiber officinale*) and garlic (*Alluimsativum* L.) extracts on *Escherichia coli* and *Salmonella typhi.* J. Mol. Med. Adv. Sci., 1(4): 411-416.
Fhogartaigh CN, Edgeworth JD. 2009. Bacterial gastroenteritis. J. Bact. Med., 37(11): 586-593. Food and Agriculture Organization, FAO. 2011. Grassland Index. A searchable catalogue of grass and forage legumes. p. 101-111.
Green BO. 2007. Significance and efficacy of medicinal plants in the Niger Delta. Cont. J. Pharm. Sci., 1(3):23-29.
Jenssen H, Hamill P, Hancock REW. 2006. Peptide antimicrobial agents. Clin. Microbiol. Rev., 19(3): 491-511.
Keymanesh K, Hamedi J, Moradi S, Mohammad F, Sardari S. 2009. Antibacterial, antifungal and toxicity of rare Iranian plants. Intl J. Pharm. Sci., 5(1): 81-85.
Khanna VG, Kannabiran K. 2008. Antimicrobial activity of saponnin fractions of leaves of *Gymnemasyl vestre* and *Eclipta prostrate*. World. J. Microbiol. Biotechnol., 24: 2737-2740.
Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afric. J. Biotechnol., 6: 1690-1696.
Lar PM, Ojile EE, Dashe E, Oluoma JN. 2011. Antibacterial activity of *Moringa oleifera* seed extracts on some gram negative bacterial isolates. Afric. J. Nat. Sci., 14: 57-62.
Mukhtar MD, Tukur A. 2000. *In vitro* screening for activity of *Pistia stratiotes* extracts. Nig. Soc. Exptl. Biol. J., 1(1): 51.
Oyeleke SB, Manga BS. 2008. Essentials of laboratory practical in microbiology (1st ed.). – Tobest Publishers, Minna, Nigeria p. 28-62.
Palombo EA. 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phyt. Res., 20(9): 717-724.
Parija SC. 2006. Textbook of practical microbiology (1st ed.). – Ahuja Publishers, Bangalore, New Delhi, India. p. 13-18.
Rajash B, Rattan LI. 2008. Essentials of medical microbiology (4th ed.). Jaypee Brothers Medical Publishers Limited, New Delhi, India, p. 500.
Sofowora A. 2008. Phytochemical Screening. Medicinal Plants and Traditional Medicine in Africa, 3rd ed. Spectrum books limited, Ibadan, Nigeria, p. 199-204.
Vollekova A, Kostalove D, Sochorbva R. 2001. Idoquinone, alkaloids from *Mahonia aquitolium* stem bark active against *Malassezia* sp. Folia Microbiol., 46(2): 107-111.
Wong C-C, Li H-B, Cheng K-W, Chen F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. J. Food Chem., 97(4): 705-711.