



Original article

Phytochemical screening and antibacterial activity of leaf and stem bark extracts of *Adansonia digitata* on *E.coli*, *S. aureus* and *S. typhi*

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ABSTRACT

Background: *Adansonia digitata* L (Baobab) contains different classes of bioactive compounds which were identified from various parts of the plant such as seed, leaves, and roots and also stem bark. The study was designed to determine the antibacterial activity of *Adansonia digitata* leaf and stem bark extracts. **Methods:** The plant material was extracted using aqueous, ethanol and methanol; and their activity against the three clinical isolates *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Salmonella typhi* (*S. typhi*) was ascertained using agar well diffusion method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the different extracts were also determined. One way analysis of variance was conducted using Stata/SE11.1 and t-test to determine the significant difference between the effects at $p \leq 0.05$. **Results:** The extracts were found to be effective against the tested organisms. The methanolic extracts showed significantly higher activity against the test organisms compared to aqueous and ethanolic extracts ($p = 0.000$). The result also demonstrated that the leaf extract is more active than the stem bark extract with significant difference ($p=0.000$). The methanolic and ethanolic leaf extracts exhibited highest inhibitions zone of 19mm and 16mm against *E. coli* at concentration of 1000mg/mL respectively. The MIC result of the study showed that the methanolic and ethanolic extracts inhibited the growth of the organisms at 25 mg/ml. The methanolic and ethanolic extracts have MBC at 25 mg/mL. **Conclusion:** The methanolic and ethanolic leaf extracts have significant effect against the test organisms at all concentration tested.

Introduction

The plant "*Adansonia digitata*" (*A. digitata*) also known as Baobab tree is native to Africa [1] and typically present in dry, hot grasslands of sub-Saharan Africa. Baobab tree is

called "Kuka" in Hausa (Nigeria), the leaves are used to make "kuka soup". The leaves are dried and stored for future use; they can also be grounded and sieved to fine powdered particles which is the most common form found in the markets. The tree is big

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and can be 25 m in height, deciduous in nature, and it can survive for many years and used for medicinal purpose [2]. The plants part such as leaves, bark, and seeds are used for traditional medicine in most parts of Africa to treat diseases [3].

The use of plants to heal diseases, including many infectious illnesses, has been extensively applied in indigenous medicine [4] and the medicinal plants are of great potential for treatment [5]. The plant leaf is a good source of proteins, and its infusions are used in the treatment of various diseases like diarrhea, fever, inflammation, kidney disease, and asthma. The leaf is also a good source of proteins [6]. The antibacterial efficacy of *A. digitata* could be associated with availability of different phytochemical constituents like alkaloid, flavonoid, saponins, tannins, terpenoids, reducing sugar and steroid [6,7]. Several studies conducted on its phytochemistry revealed the presence of important bioactive constituents.

Microorganisms cause life-threatening infections and severe diseases all over the globe. Many human infections are caused by bacteria like *E. coli*, *S. aureus*, *S. typhi* etc.; these infections range from foodborne disease associated with gastroenteritis, typhoid fever, stomach upset, diarrhea, and dysentery and these diseases occurring mostly in African due to low environmental hygiene, limited access to clean water, etc. Hence, the present study ascertains the phytochemicals properties presents in baobab tree and also determine the antibacterial activity of the baobab leaf and stem bark extracts.

Materials and Methods

Collection and identification of leaf and stem bark of *A. digitata*

Adansonia digitata L (Baobab) (family Bombacaceae) leaves were collected by flocking and stem bark was collected by scraping the back of the baobab tree using a sterile knife early in the morning [8]. The leaves and stem bark of the plant were identified by a botanist (F. K. Channya) of the department of plant science, Modibbo Adama University of technology Yola, Adamawa state, Nigeria. The specimen voucher number is MAUTECH/PLS/0899.

Preparation of leaf and stem bark of *A. digitata*

The leaves and stem bark of *A. digitata* were dried at room temperature under shed for a period of two weeks [9]. Thereafter, they were grounded into

powder using sterile mortar pestle and then stored for future use [10].

Preparation of leaf and stem bark stock extracts of *A. digitata*

Adansonia digitata leaves and stem bark extracts were prepared by cold maceration method. Aqueous, ethanolic and methanolic extracts of the leaves and stem bark of the plant were prepared by soaking 50 g of finely grounded powder of leaves and stem bark in 250 mL of the solvents for 24 hours. After 24 hours the extracts were filtered using Whatman No. 1 filter paper; the residue on the filter paper was soaked in 150 mL of the solvents for another 24 hours and then filter. The two extracts was pool together and the combine extracts was concentrated at 50°C using water bath. The prepared extract was stored in clean universal bottles in a refrigerator until needed for analysis [11, 12].

Phytochemical screening of leaves and stem bark extracts of *A. digitata*

The presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, tannins, and saponins present in the leaf and stem bark extracts of *A. digitata* was investigated by using procedure described by Sofowora [13].

Collection of test organisms and confirmation

The test organisms *E. coli*, *S. aureus* and *S. typhi* were collected from specialist hospital Yola, Adamawa state, and they were confirmed based on their cultural and biochemical characteristics as described by Cheesbrough [14], the isolates were maintained on nutrient agar slants inside a refrigerator prior to use.

Preparation of 0.5 McFarland turbidity standard

Turbidity standard equivalent to McFarland 0.5 mL was prepared. This was achieved by preparing 1% v/v solution of sulfuric acid by adding 1mL of concentrated sulfuric acid to 99 mL of distilled water and mix well. Then 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 50 mL of distilled water. Thereafter, 0.6 mL of the barium chloride solution was added to 99.4 mL of the sulfuric acid solution and the solution was mixed. A small volume of the turbid solution was transferred to a screw-cap bottle of the same type as used for preparing the test and control inoculum [14].

Determination of antibacterial activity using agar well diffusion assay

The organisms were grown on Muller-Hinton agar and the activity of aqueous, ethanolic and

methanolic extracts of leaves and stem bark of *A. digitata* was tested against the organisms by agar-well diffusion technique using the procedure described by **Biradar et al.** [15]. After inoculation of the inoculum of the test organisms on Muller-Hinton agar, five wells of 5.0 mm were bored using a sterile corked borer on the agar plates. 0.3 mL of different concentration (1000, 500, 200, and 100 mg/mL) of the plant extracts was dispensed into 4 of the wells containing Muller-Hinton agar and the inoculum of the test organisms, and to the last well, ciprofloxacin was dispensed as a positive control. Agar plates were incubated at 37°C for 24 hours. The effects of the extracts against the test organisms was assessed by measuring the diameter the zones of the inhibition to the nearest millimeter was measured using a transparent ruler and recorded, the zones of inhibition was compared with the inhibition zones of standard ciprofloxacin.

Determination of minimum inhibitory concentration (MIC)

Broth dilution was used to determine the MIC of the plant extracts as described by **Lar et al.** [16]. Six tubes containing 5 mL of Muller-Hinton broth were prepared. 1 mL of the crude extract from (100, 50, 25 and 12.5 mg/mL) was introduced into tube 1-4 respectively and was mix thoroughly. Thereafter, 0.1 mL of broth cultures of the test organisms was added to 4 tubes with the last tube serving as broth control for each respectively. The inoculated tubes are kept at 37°C for 24 hours in an incubator. Ciprofloxacin 500 mg/mL was used as the positive control. The lowest concentration that showed no growth is considered as the MIC.

Determination of minimum bactericidal concentration (MBC)

Five milliliter of prepared Mueller-Hinton broth was dispensed into sterile test tubes equivalent to the number of tubes that show no visible growth from the MIC. Then 0.1 mL of the broth culture was transferred to tubes containing the 5 mL Mueller-Hinton broth. The tubes were labeled and kept in a test-tube rack. Prepared Mueller-Hinton agar was pour into sterile Petri plate and allowed to solidify. Using a sterile pipette, 0.1 mL was transferred from each tube to the surface of the agar. The inoculum was spread out using a smooth sterile bent glass rod. Both tubes and plates were kept at 37°C for 24 hours in an incubator. Presence or absence of turbidity or cloudiness in the broth culture and also bacteria growth colonies in solid agar medium was observed [17].

Results

Phytochemical properties of leaf and stem bark extracts of *A. digitata*

Bioactive components such as alkaloids, tannins, flavonoids, saponins and terpenoids were detected are presented in **table (1)**. Aqueous, ethanolic and methanolic stem bark extracts contain alkaloid, saponins, tannins and terpenoids.

Antibacterial activity of methanolic, ethanolic and aqueous leaf extracts on the test organisms in milliliter (mm)

The activity of methanolic, ethanolic and aqueous leaf extract of *A. digitata* against the test isolates is presented in **table (2)**; with methanolic leaf extract having a higher zone of inhibition of 19 mm at concentration of 1000 mg/mL against *E. coli*. It is the lowest activity is observed against *S. typhi* with 8 mm at concentration of 100 mg/mL. The ethanolic leaf extracts showed less activity than the methanolic leaf extract. It also has a higher activity against *E. coli* with 16 mm at 1000 mg/mL, and 5 mm as the lowest zone of inhibition at 100 mg/mL against *S. typhi*. None of the aqueous leaf extract showed activity against the organisms tested.

Antibacterial activity of methanolic, ethanolic and aqueous stem bark extracts on the test organisms in milliliter (mm)

The result of the antibacterial activity of stem bark extracts revealed that higher zone of inhibition was obtained against *E. coli* with 8 mm and 4 mm at 1000 mg/mL for methanolic and ethanolic extracts respectively, as described in **table (3)**. The aqueous stem bark demonstrated that the extract is effective at higher concentration (1000 mg/ml) with a zone of inhibition ranging from 4 mm to 5 mm against all the test organisms. However, at 500 mg/mL of the extract it has no inhibition against *S. typhi*.

Minimum inhibitory concentration (MIC) of the extracts against the test organisms in mg/ml

At 25 mg/mL the methanolic leaf extract inhibit the growth of all the test organisms, while the ethanolic leaf extracts showed absence of growth of the test organisms at both 25 mg/mL and 50 mg/mL. However, **Table 4** revealed that the leaf extracts of *A. digitata* are more effective with low MIC when compared to stem bark extract.

Minimum bactericidal concentration (MBC) of the extracts against the test organisms in mg/ml

Table 5 demonstrated the MBC of the leaf and stem bark extracts of *A. digitata* against the test

isolates. The result illustrated that both methanolic and ethanolic leaf extracts showed no growth of the

test organisms at concentrations ranges from 25 mg/mL to 100 mg/mL.

Table 1. Phytochemical constituents of leaf and stem bark extracts of *A. digitata*.

Phytochemicals	ELE	ESE	MLE	MSE	ALE	ASE
Alkaloids	+	+	+	-	+	+
Saponins	+	+	+	+	+	-
Flavonoids	+	-	+	+	+	+
Tannins	+	-	+	-	-	+
Terpenoids	+	-	+	-	+	-

Key: + = Present of phytochemical, - = Absence of phytochemicals, ELE = Ethanol Leaf Extract, ESE = Ethanol Stem Bark Extract, MLE = Methanolic Leaf Extract, MSE = Methanolic Stem Bark Extract, ALE = Aqueous Leaf Extract, ASE = Aqueous Stem Bark Extract.

Table 2. Antibacterial activity of methanolic, ethanolic and aqueous leaf extract on the test organisms in milliliter (mm).

Test organisms / Solvent used	1000mg/mL	500mg/mL	200mg/mL	100mg/mL	Control (50mg/mL)	
<i>E. coli</i>	Methanol	19	15	12	9	21
	Ethanol	16	11	9	7	19
	Aqueous	-	-	-	-	18
<i>S. aureus</i>	Methanol	16	15	12	7	19
	Ethanol	14	10	7	6	17
	Aqueous	-	-	-	-	17
<i>S. typhi</i>	Methanol	17	14	10	8	20
	Ethanol	15	12	10	5	18
	Aqueous	-	-	-	-	19

Key: - = no diameter zones of inhibition

Table 3. Antibacterial activity of methanolic, ethanolic and aqueous stem bark extract on the test organisms in millimeter (mm).

Test organisms/ Solvent used	1000mg/mL	500mg/mL	200mg/mL	100mg/mL	Control (50mg/mL)	
<i>E. coli</i>	Methanol	8	5	-	-	19
	Ethanol	4	3	-	-	19
	Aqueous	5	3	-	-	19
<i>S. aureus</i>	Methanol	6	3	-	-	19
	Ethanol	3	-	-	-	17
	Aqueous	4	2	-	-	17
<i>S. typhi</i>	Methanol	7	3	-	-	20
	Ethanol	3	-	-	-	19
	Aqueous	5	-	-	-	18

Key: - = no diameter zones of inhibition

Table 4. Minimum inhibitory concentration (mic) of the extracts against the test organisms in mg/ml.

Extracts	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>
ML	25	25	25
MSB	+	+	+
EL	25	50	50
ESB	+	+	+
ASB	+	+	+

Key: + = presence of turbidity, ML = Methanolic Leaf, MSB = Methanolic Stem Bark, EL = Ethanol Leaf, ESB = Ethanol Stem Bark, ASB = Aqueous Stem Bark.

Table 5. Minimum bactericidal concentration (mbc) of the extracts against the test organisms mg/ml.

Extracts	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>
ML	25	50	50
MSB	+	+	+
EL	50	50	100
ESB	+	+	+
ASB	+	+	+

Key: + = presence of turbidity, ML = Methanolic Leaf, MSB = Methanolic Stem Bark, EL = Ethanol Leaf, ESB = Ethanol Stem Bark, ASB = Aqueous Stem Bark.

Discussion

Plants have been identified as a good source of cure for different diseases [18]. The plant parts mostly used include the seeds, leaves, bark, oil and root [19]. Phytochemicals such as alkaloid, saponins, flavonoids, tannins and terpenoids are chemical bioactive components could be responsible for antibacterial activities in the plant. The presence of bioactive compounds such as flavonoids in plants indicates the presence of naturally occurring phenolic compound with beneficial effect in the human diet as an antioxidant that neutralized free radicals [18].

Methanolic extract of *A. digitata* in this study had higher solubility for more bioactive compounds than ethanol and aqueous extracts; this consequently account for its higher antibacterial activity [18]. However, this contradicts the findings of **Anani et al.** [2] and **Doughari** [20] where they demonstrated that ethanolic extract having higher solubility for more bioactive compounds thus, having the highest antibacterial activity. All the stem bark extracts (aqueous, methanolic and ethanolic) showed zone of activity at concentration of 1000 mg/mL and 500 mg/mL against all the test isolates; but there are no zones of inhibitions at concentrations 200 mg/mL and 100 mg/mL for all the isolate tested.

The methanolic leaf and stem bark had much activity against all the test organisms with 1000 mg/mL concentration having the highest activity followed by 500 mg/mL concentration of the extract. This low antibacterial activity shown by the ethanolic stem bark extracts may be an indication that the active compound(s) were poorly extracted by the cold maceration method [2,20]. Aqueous extracts was also found to be effective against the test organism's only at higher concentration (1000 and 500 mg/mL) and *S. typhi* showing zone of inhibition only at 1000 mg/mL. This outcome is similar to those observed by **Uzama et al.** [21]. The findings of the study demonstrated

that leaf extracts of *A. digitata* have much more activity than the stem bark because it has activity against the test organisms at all concentration; while the stem bark extract showed activity only at 1000 and 500 mg/mL.

The MIC obtained showed that both methanolic and ethanolic leaf extracts were very active even at lower concentration against the test organisms and this supports the findings of other researchers like **Anani et al.** [2], **Doughari** [20], and **Cowan** [22]. However, methanolic extract is even more effective than the ethanolic extract because it inhibited the growth of the test organisms at a concentration of 25 mg/mL which is lower than 50 mg/mL concentration inhibited by ethanolic extracts. The aqueous extracts did not inhibit the test organisms at all concentration used. Hence, the methanolic and ethanolic extracts inhibited all the test organisms (*E. coli*, *S. aureus* and *S. typhi*) at concentrations ranging from 25 to 50 mg/mL.

Adansonia digitata leaf extracts have higher bactericidal activity compared with the stem bark extracts. The MBC showed that the methanolic extracts eliminated *E. coli* at 25 mg/mL than ethanolic extracts which eliminated *E. coli* at 50 mg/mL. *Salmonella typhi* was only destroyed when the ethanolic extracts concentration reached 100 mg/mL. Hence, the methanolic extracts are more effective than the ethanolic extracts. The minimum bactericidal concentration obtained in the study indicated that leaf extracts of *A. digitata* plants are very active even at lower concentrations especially when solvents such as methanol and ethanol were used for extraction.

Conclusion

Adansonia digitata possesses an important ingredient that helps in the treatment of diseases. The leaf of the plant was found to have more activity against the tested organisms than the stem bark of the plant. However, methanolic extract showed

higher antibacterial activity than the ethanolic and aqueous extracts of *A. digitata*.

Recommendations

Adansonia digitata could be taken alongside some synthetic drugs pending on the severity of the illness during the treatment of diseases caused by *E. coli*, *S. typhi* and *S. aureus*. Also, methanol should be the best solvent when it comes to extraction of *A. digitata* leaf or stem bark.

Conflict of interest

The authors wishes to report that there is no conflict of interest

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Author contribution statements

The study was carried out in collaboration among all the authors. Authors MB, and AI conceptualize and designed the study and carried out the laboratory experiment. Author HI manage the data analysis. The literature search was managed by RA, KHS, and II. The first draft of the manuscript was written by MB, and MBy. However, all the authors read and approved the final draft of the manuscript to be submitted to the microbes and infectious disease.

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