Annals of Microbiology and Infectious Diseases
Volume 2, Issue 1, 2019, PP 31-37
ISSN 2637-5346

Antibacterial Activity of Leaf and Stem Bark Extracts of Adansonia Digitata Against Escherichia Coli and Salmonella Typhi Grown in Potiskum, Yobe State Nigeria

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ABSTRACT

Adansonia digitata is a tree of nutraceutical importance as source of protein and as well can be used for treatment of different types of diseases. The research was conducted to determine the secondary metabolites and antibacterial efficacy of Adansonia digitata stem bark and leaf extracts. The agar well diffusion method was used to determine the antibacterial activity of leaf and stem bark of Adansonia digitata extracted using water, ethanol and chloroform against Escherichia coli and Salmonella typhi. The preliminary phytochemical screening of the leaf and stem bark of the plant showed the presence of alkaloid, saponin, tannin, Flavonoid, terpenoid and steroid. The antibacterial activity of the leaf and stem bark extract of the plants showed that the plant extracts used were effective against the isolates tested. The ethanol extracts of the plant parts showed higher antibacterial efficacy against the test isolates when compared to aqueous and chloroform extracts. The results also demonstrated that the leaf extract is more active than stem bark extracts. Statistical analysis from the results obtained showed that an average zone of inhibition of 9.04 mm and 7.83 mm for E. coli and S. typhi respectively were found. Minimum inhibitory concentration (MIC) of the extracts ranged between 10 – 40 mg/mL Based on the findings of this study, the decoction of plant parts can be used for medicinal purposes.

Keywords: *Antibacterial activity, Adansonia digitata, Escherichia coli, Salmonella typhi*

INTRODUCTION

Adansonia digitata, the Baobab, belongs to family Malvaceae and it is the most widespread tree species in the genus *Adansonia*, the baobabs. The plant is native to Africa and typically present in dry, hot grasslands of sub-Saharan Africa. The tree is massive and grow up to 25m high, deciduous in nature which may survive for hundreds of years and used for medicinal purpose [1]. The plant's leaf infusions are important in the treatment of several diseases such as diarrhea, fever, inflammation, kidney disease, and asthma. The leaf is also a good source of proteins [2]. The antibacterial efficacy of *A. digitata* may be attributed to the availability of secondary metabolites it contained. Several studies conducted on its phytochemistry revealed the presence of important bioactive constituents. Yusha'u et al. [3] found that the back extracts of *A. digitata* contain alkaloid, flavonoid, tannin, reducing sugar and steroid which were active against some pathogenic bacteria; *E. coli, S. aureus, Klebsiella* and *Proteus mirabilis.*

Kubmarawa et al. [2] 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. Findings on antibacterial studies indicated that ethanolic, methanolic and aqueous leaf and stem bark extracts of *A. digitata* demonstrated antibacterial activity against some pathogenic bacteria such as *E. coli, Bacillus subtilis, Staphylococcus aureus, Mycobacterium phlei* and *Streptococcus faecalis* [1]. According to Yusha'u et al. [3], the stem bark extracts of the plant contain secondary metabolites which are responsible for antimicrobial activity of the crude aqueous andethanol extracts. *Vitex doniana* is used by traditional healers alone or in a combination with stem bark of *A. digitata* to treat diarrhea, leprosy and dysentery [2]. It was also found that extracts of leaf and root bark possessed antiviral properties due to the presence of sterol, saponins and triterpenes [3].

The leaf of the plant is used for treatment of bladder and kidney diseases, general fatigue, diarrhea, asthma, insects bite, inflammations and guinea worm [4]. A decoction of the bark deteriorates rapidly due to the mucilaginous substances present [5]. Baobab bark is used in Europe as a febrifuge (antipyretic). In some African countries such as Ghana, the stem bark extract of *A. digitata* is used for curing malaria fever in place of quinine [6]. In Indian medicine, baobab bark is used internally as a refrigerant, antipyretic and antiperiodic [5]. In Nigeria, the bark of the plant is certainly used for the treatment of fever [4]. The antibacterial activity of *A. digitata* may be attributed to the presence of secondary metabolites it contained. The present study was aimed to screen for phytochemical constituents and antibacterial efficacy of leaf and stem bark extract of *A. digitata* against clinical isolates of *E. coli* and *S. typhi*.

Materials and Methods

Test Organisms

Clinical isolates of *E. coli* and *Salmonella typhi* were collected from Microbiology laboratory of Potiskum General Hospital Yobe State. The isolates were transported in Nutrient agar slant to the laboratory of Biological Science Yobe State University Damaturu for identification. The bacterial isolates were identified using different methods including Gram staining, bacteriological analysis and biochemical characterization (Indole, Methyl red, Vogues Proskauer and citrate utilization test) as described by Holt et al. [7] and Cheesbrough [8]. The bacterial isolates were refrigerated at 4^0C for further use.

Collection and Identification of Leaf and Stem Bark

The leaves and stem-bark were collected at Potiskum local Government of Yobe State Nigeria, with coordinates Longitude $11^0$04', Latitude 11^0 43'and identified by a taxonomist at the Department of Biological Sciences Yobe State University, Damaturu. The samples were deposited at Herbarium with Voucher specimen number BH 0125 and BH 0126 respectively. The leaf and stem bark were washed thoroughly with distilled water and air-dried in a shade for two weeks, then cut into smaller pieces and grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use.

Extraction of Leaf and Stem Bark

Water, ethanol and chloroform were used for extraction of secondary metabolites of the plant part. For aqueous extraction, water extraction method as described by Fatofe [9] was used, 50 g of each of the grounded leaf and stem back were extracted by successive soaking for 3 days using 250 mL of distilled water in a sterile conical flask.

The extracts were filtered using Whatman filter paper and the filtrate was evaporated to dryness in water bath at 60°C until a solid residue is obtained.

The solid concentrated filtrate, now the extracts were then stored in universal bottles in the refrigerator at 4°C before use. For ethanol and chloroform, 50 g of the powdered plant part was extracted by soaking the powder in 250 mL of methanol and chloroform for 2 days with intermittent shaking.

The mixture was filtered using Whatman No.1 filter paper and the filtrate was evaporated to dryness using rotary evaporator until solid residue is obtained. The solid residues obtained were reconstituted in 30% DMSO at stock concentration, stored in the refrigerator at 4 ^{0}C until used.

Phytochemical Screening

This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem back of *A. digitata.* Presence of Alkaloid, saponin, Glycoside, Tannin, flavonoids, resin, steroid, terpenoid, Anthraquinones, Protein and amino acid were determined using procedure described by Sofowora [10].

Test for Alkaloids

Wagner's test: To 0. l mL of the extract in a test tube, 3 drops of Wagner's reagent (Iodine in Potassium iodide) was added. Formation of brown/ reddish precipitate indicates the presence of Alkaloids.

Test for Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. The formation of yellow colored precipitate indicates the presence of flavonoids.

Test for Saponins

Foam test: Half gram (0.5 g) of the powdered sample was dispensed in a test tube and 5 mL of distilled water was added and shaken vigorously. Persistent froth (foam) that lasted for about 10 minutes indicated the presence of saponin.

Test for Steroids

To 2 mL of the sample, 2 mL of acetic acid was added and the solution was kept under ice for cooling for few minutes. Then 2 mL of concentrated Tetraoxosulphate (VI) acid was added carefully. Color changes, from violet to blue/bluish green indicated the presence of steroids.

Test for Tannin

Gelatin test: To 2 mL of the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

Test for Terpenoid

Salkowski test: About 5 mL of extract was added with 2 mL of chloroform and 3 mL of concentrated Tetraoxosulphate (VI) acid. Reddish brown colour at the interface indicates the presence of terpenoids.

Antibacterial Activity of the Plant Extracts

The antibacterial activity of the extracts was determined by using agar well diffusion method as described by Abdallah et al. [11] with modifications. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (1.5 x 10^6 CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A 6 mm diameter sterile cork borer was used to make five (5) wells into the agar medium. The wells were filled with about 0.1mL different concentration of the extracts namely; 20, 30, 40 and 50 mg/mL. The inoculated plates were allowed to stand on laboratory bench for a period of 1 hour in order to allow proper diffusion of the extracts into the agar medium. The plates were incubated at 37^0C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured. Amoxicillin 25 mg/mL (Micro Lab limited) was served as positive control for the study.

Minimum Inhibitory Concentration of the Extracts

The Minimum inhibitory concentration (MIC) of the extracts was evaluated by means of broth dilution technique. A double fold serial dilution of each extract was prepared by adding 2 mL of 100mg/mL of the extract into a test tube containing 2mL of Nutrient broth, thereby producing a solution containing 50mg/mL of the extract. The process was continues serially to the next five test tubes using the following concentrations; 40, 20, 10, 5, 2.5 mg/mL. The last test tube (No. 6) does not contain the extract and serve as negative control. Exactly 0.5 mL of standards of test organisms (0.5 MacFarland) were introduced into the test tubes and incubated at 37^0 C for 24 hours. The test tubes were observed for growth by checking for turbidity [12].

Results and Discussion

Phytochemical Screening of the Extracts

The phytochemical constituents of the aqueous, ethanolic and chloroform leaves extracts of *Adansonia digitata* is presented in the table below (Table 1). From the table, the result showed that the aqueous, ethanolic and chloroform leaves extracts contain alkaloid, saponin, tannin, Flavonoid, terpenoid and steroid.

Table 1. *Phytochemical Constituent of leaves Extracts of A. digitata*

S/N	Phytochemicals	Aqueous extract	Ethanolic extract	Chloroform extract
1	Alkaloids	+	+	+
2	Flavonoids	+	+	-
3	Tannin	+	+	+
4	terpenoids	+	+	+
5	Saponin	+	-	-
6	Steroid	+	-	-

Key: + = *Present of phytochemical*, - = *absent of phytochemical*

The phytochemical constituents of aqueous, ethanolic and chloroform stem bark extracts of *Adansonia digitata* is presented in Table 2. The result showed that the aqueous, ethanolic and chloroform stem bark extracts contain alkaloid, saponin, tannin and steroid.

Table 2. *Phytochemical Constituent of Stem bark Extracts of A. digital*

S/N	Phytochemicals	Aqueous extract	Ethanolic extract	Chloroform extract
1	Alkaloids	+	+	+
2	Flavonoids	-	-	-
3	Tannin	+	+	+
4	terpenoids	-	-	-
5	Saponin	+	-	-
6	Steroid	+	+	-

Key: + = *Present of phytochemical*, - = *absent of phytochemical*

Antibacterial Activity of the Extracts

The antibacterial activity of the aqueous, ethanol and chloroform leaves and stem bark of *A. digitata* against *E. coli* is presented in Table 3. The result showed that the extracts were active against the isolate and the ethanol leaf extract was more effective with zone of inhibition of 21 mm at concentration of 50mg/mL, followed by aqueous leaves extract (19 mm). Aqueous and chlorofextracts of the stem bark were in effective at a concentration of 20 and 30 gm/mL. The activity shown by the control was found to be 21 mm.

Table 3. *Antibacterial activity of the extracts against Escherichia coli*

Concentration (mg/mL)/ Zone of Inhibition (mm)					
Extracts	**20**	**30**	**40**	**50**	**Control**
Aqueous leaf extract	10	14	15	19	21
Aqueous stem bark extract	00	00	07	11	
Ethanol leaf extract	13	15	19	21	
Ethanol stem bark extract	00	12	13	14	
Chloroform leaf extract	00	09	10	11	
Chloroform stem bark extract	00	00	04	10	

The antibacterial activity of the aqueous, ethanol and chloroform leaves and stem bark of *A. digitata* against *S. typhi* is presented in Table 4. The result showed that the extracts were active against the isolate and the ethanol leaf extract was more effective with zone of inhibition of 19 mm at concentration of 50mg/mL, followed by aqueous leaves extract (15 mm). Most of the extracts from the stem bark were in effective at a concentration of 20 and 30 gm/mL. The activity shown by the control was found to be 20 mm.

Table 4. *Antibacterial activity of the extracts against Salmonella typhi*

Concentration (mg/mL)/ Zone of Inhibition (mm)					
Extracts	**20**	**30**	**40**	**50**	**Control**
Aqueous leaf extract	08	13	13	15	20
Aqueous stem bark extract	00	00	07	09	
Ethanol leaf extract	10	12	15	19	
Ethanol stem bark extract	00	08	13	13	
Chloroform leaf extract	00	03	07	10	
Chloroform stem bark extract	00	00	06	07	

Table 5. *Minimum Inhibitory Concentration (MIC) of the Extracts against the Isolate*

Isolates/MIC (mg/mL)		
Extracts	***E. coli***	***S. typhi***
Aqueous leaf extract	10	20
Aqueous stem bark extract	40	40
Ethanol leaf extract	10	10
Ethanol stem bark extract	20	40
Chloroform leaf extract	40	40
Chloroform stem bark extract	40	40

Minimum Inhibitory Concentration of the Extracts

The Minimum inhibitory concentration (MIC) of the extracts against the test isolates is presented in Table 5. From the table, the result showed that the extracts were able to inhibit the growth of the isolates at a concentration ranged between 10 – 40 mg/mL. Leaves extracts were more effective with low MIC when compared to stem bark extract.

The preliminary phytochemical screening of *A. digitata* leaves and stem bark indicated the presence of alkaloids, flavonoids, tannin, terpenoids, saponins, and steroids were present in the extracts. The findings of the present study showed that more phytochemical were found in the leaves extracts than the stem bark extracts. Similarly, ethanol and water were found to be more effective solvents for extracting phytochemicals than chloroform. The difference is due to differences in polarity of the extracting solvents and that of the phytochemicals [13]. Moreover, this finding supported the work of Imaran Khan and Rodriguez [14] who screened for the phytochemical constituents of *Adansonia* leaves using different solvent such as aqueous, chloroform and methanol, and the result showed the presence of terpenoid, alkaloid and fatty acids. Early studies proved ethanol as the most efficient solvent for extracting broad spectrum of antibacterial compounds from plants. It also reported that the ethanolic extract of *A. digitata* whole plant shows presence of flavonoids and tannin [14]. The finding of this study agrees with that of Yusha'u et al. [3] who found that the back extracts of *A. digitata* contain alkaloid, flavonoid, tannin, reducing sugar and steroid Alkaloids are known to play some metabolic roles and control development in living system [15]. It also interferes with cell division, hence the presence of alkaloids in *A. digitata* could account for their use as antimicrobial agents. Aboaba *et al*. [16] had reported that the antimicrobial properties of substances are desirable tools in food spoilage and food safety. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, anti-parasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immune modulatory properties [17]. Flavonoids are also present in the extract as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [18, 19]. It also helps in managing diabetes induced oxidative stress. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production [20]. Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and antibiotics properties [21]. In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells [22, 23]

The result of antibacterial activity of A. digitata extracts against E. coil and S. typhi (Table 2 and 3) shows that the ethanol extracts is more effective against the tested isolates than aqueous and chloroform extracts. E. coli was found to be more susceptible to the extracts in comparison with the S. typhi each with average zone of inhibition of 14.97 and 13.84 mm respectively. The result of antimicrobial activity of A. digitata in this study was inconformity with the study conducted by many researchers. The antibacterial activities of the extracts are expected due to the presence of compounds such as alkaloid, flavonoids and tannin.

The results obtained in this study corroborate with the work conducted by Al-Bakri [24] reveals that the leaves of boabab have antimicrobial activity against E-coli and salmonella. The result of antibacterial activity of the stem bark extracts of this study supported that of Yusha'u et al. [3] who found that both ethanol and chloroform extracts were active against E. coli, Klebsiella, Proteus spp and Staphylococcus with ethanol extract having higher activity than chloroform extract. Similarly, the finding of this study correlates with that of Samatha et al. [25] who study the antibacterial activity of methanol extracts of various parts of Adansonia digitata such as leaves, fruits and flower. They found that the extracts were active against some pathogenic bacteria isolates namely; E. coli, Klebsiella, Proteus spp, Enterobacter and Staphylococcus The Minimum inhibitory concentration of the extracts against the isolates revealed that the extracts were able to inhibit the growth of the isolates at a concentration ranged between 10 – 40mg/mL. Leaves extracts were more effective with low MIC when compared to stem bark extracts.

CONCLUSION

The phytochemical screening of leaf and stem bark extract of *A. digitata* showed the presence

of alkaloid, saponin, flavonoid, steroid, tannin and terpenoid. The antibacterial activity of the extracts against *E. coli* and *Salmonella typhi* indicated that the extracts were active against the isolates. The Minimum inhibitory concentration (MIC) of the extracts against the test isolates showed at a higher concentration of 40mg/mL, the aqueous, ethanol and chloroform extracts of *A. digitata* leaves and stem bark can inhibit growth of the isolates.

ACKNOWLEDGEMENT

The authors hereby wish to acknowledge to the staff of Microbiology Department of Potiskum General Hospital Yobe State for sample provision. Thanks to the technical staff of Biological Science Department Yobe State University, Damaturu for the use of laboratory facilities.

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Citation: *Abdallah, M Salihu and Muhammad Ali" Antibacterial Activity of Leaf and Stem Bark Extracts of Adansonia Digitata against Escherichia Coli and Salmonella typhi Grown in Potiskum, Yobe State Nigeria". Annals of Microbiology and Infectious Diseases 2(1), 2019, pp.31-37.*