



ANTIBACTERIAL EFFECT OF *Adansonia digitata* LEAF EXTRACT AGAINST *Pseudomonas aeruginosa* AND *Streptococcus* SPECIES ISOLATED FROM INFECTED WOUNDS

MUSA, F.M.¹, UMAR, F.J.^{1*}, AND ATTAHIRU, M.²

¹Department of Microbiology, Faculty of Life Sciences, Kaduna State University, Kaduna, Nigeria

²Department of Applied Biology, Kaduna Polytechnic, Kaduna, Nigeria

ABSTRACT

Adansonia digitata is among many plant species which have been reported to have ethnomedicinal uses and has been widely used in the traditional systems of medicine. This study investigated the Antibacterial activities of *Adansonia digitata* leaf extract against *Pseudomonas aeruginosa* and *Streptococcus* species isolated from infected wounds using standard plate technique. Extraction of the plant leaf was carried out using maceration technique. Phytochemical screening of *Adansonia digitata* leaf was determined by qualitative technique and antibacterial activity of the plant was carried out using agar well diffusion with the concentrations of 62.5, 125, 250 & 500 mg/ml while commercially obtained ciprofloxacin was used as a control. The leaf extract indicated the presence of tannin, saponin, glucoside, quinones, phenol, terpenoid and steroid. The extract showed significant inhibitory effects at $p < 0.05$ against the reference isolates. Both ethanol leaf extract and aqueous leaf extract demonstrated a high sensitivity against the test isolates at 500 mg/ml. The minimum inhibitory concentration (MIC) and the minimum bacterial count (MBC) of aqueous leaf extract against the isolates were 62.5 mm/ml respectively. This result is an indication that the *Adansonia digitata* leaf extract has potential benefit in wound care.

Keywords: *Adansonia digitata*, antibacterial, *Pseudomonas aeruginosa*, *Streptococcus* species

***Correspondence:** umar.fatima@kasu.edu.ng, 07038286347

INTRODUCTION

Africa has an abundant novel plant species which are known to be rich in health-promoting compounds, many of which remain undiscovered or unused by the western society [1]. The name *Adansonia digitata* was given by Linnaeus, the generic name honouring Michel Adanson who had been to Senegal in the eighteenth century and described Baobab. It is locally referred to as “kuka” in Hausa, the Igbo called it Enga and is called IweItumọ-Oro by Yorubas. It belongs to the family of Bombacaceae a Sub-family of the Malvaceae. The history of the African baobab is well documented in Baum [1]. Darwin documented baobab trees on the St Jago in the Cape Verde Islands in 1832 and he commented on their size and longevity [1]. *Adansonia digitata* leaf is the most widely spread of the *Adansonia* species on the African continent which belongs to the family of Bombacaceae a sub family of the Malvaceae. *Adansonia* species comprises of 8 different species with large, spectacular, nocturnal flowers. One of these species is the *A. digitata* L., it occurs throughout the drier parts of Africa. A second species is restricted to North-Western Australia (*A. gibbosa*), and the remaining six species are endemic to Madagascar [1].

African baobab is a very long-lived tree with multipurpose uses. It is thought that some trees are over 1000 years old since it was not grown agronomically nor properly domesticated. It has been introduced to areas outside Africa and grown

successfully [1]. The tree provides food, shelter, clothing and medicine as well as material for hunting and fishing. The baobab has an extensive root system and high-water holding capacity. Its mean annual temperature range is 20–30°C, but it can tolerate well high temperatures up to 40–42°C (in West Africa), it's resistant to fire, and survive low temperature as long as there is no frost. It is drought tolerant and frost sensitive. This adaptation allows it to grow in zones with 100–1000 mm annual rainfall, but trees are often stunted in the lower rainfall areas. The tender root, tubers, twigs, fruit, seeds, leaf and flowers are all edible and they are common ingredients in traditional dishes in rural areas in Africa [1].

Adansonia digitata is among many plant species which have been reported to have ethnomedicinal uses and has been widely used in the traditional systems of medicine. According to Sodimu *et al.* [1] the fruit pulp of *A. digitata* is traditionally used for the treatment of fever, diarrhea, dysentery, haemoptysis and small pox in humans. Leaf infusions are used as treatment for diarrhea, fever, inflammation, kidney and bladder diseases, blood clearing and asthma in humans. Some work has been done to validate the efficacy of *A. digitata* and identify its bioactive constituents. For example, experiments performed on rats showed that, administration of fruit pulp extract at a dose between 400 and 800 mg/ml had a marked anti-inflammatory effect and reduce formalin-induced oedema in the animals. These effects are comparable with those produced by 15 mg/ml of phenylbutazone, a

common anti-inflammatory drug used as an internal standard [2]. Equally, extracts from *A. digitata* fruit pulp have analgesic (pain killing) and antipyretic (temperature reducing) activities [2]. This activity could be due to the presence of sterols, saponins and triterpenes in the fruit pulp [2]. Moreover, a study conducted on 160 children demonstrated that an aqueous solution of *A. digitata* fruit is almost as significant as the traditional “WHO solution” for dehydration of children affected with diarrhea. Tannins, mucilage, cellulose, citric acid and other typical constituents of the fruit pulp may be responsible for its effect against diarrhea [2].

MATERIALS AND METHODS

Collection, identification and authentication

Adansonia digitata leaf was collected from Unguwan Waziri, Chikun LGA, Kaduna State on the 2nd November 2021. The leaf of the plant was identified by a botanist of the Department of Biological Science Kaduna State University, Voucher Number 2347 was assigned.

Preparation of leaf extract of *A. digitata*

The leaf of *A. digitata* was dried at room temperature under shade for a period of two weeks after which it was grounded into coarse powder using sterile mortar and pestle before being used for the experiment [3]. The leaf extract of *A. digitata* leaf was prepared by cold maceration method. The aqueous and ethanolic extract of *A. digitata* were prepared by suspending 50 g each of finely ground powder of leaf in two separate 500ml conical flasks, containing 250ml of distilled water and 250ml of 70% ethanol solvent. Each flask was kept for 24 hours with constant shaking. Thereafter, each extract was filtered using Whatman's No. 1 filter paper. Each residue on the filter paper was soaked in 150 ml each of the solvents for another 24 hours and then filtered and concentrated at 50°C using water bath. Each crude extract was stored in clean universal bottles and kept in the refrigerator and maintained at 4°C until required.

Phytochemical screening of leaf extract of *A. digitata*

The presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, tannins, and saponins present in the leaf extracts of *A. digitata* was investigated according to the method described by Sofowora [4].

Collection of clinical isolates

The isolates were obtained from General Hospital, Sabon Tasha using a slant bottle containing media (specify media used). It was then transported to

Microbiology laboratory, Kaduna State University and it was kept in the refrigerator at 4°C until required [5].

Confirmation of the bacterial isolate

The isolates were sub cultured onto Blood agar and Cetrimide agar and subsequently incubated in an incubator at 37°C for 24 hours. The resulting colonies were gram stained and further re-confirmed using biochemical tests such as Catalase, oxidase, citrate utilization, coagulase, indole and Voges Proskeur test [5].

Gram staining

A drop of saline was placed on a clean grease free slide after which a wire loop was passed over a flame and was used to pick a viable colony to make a smear on the slide. The preparation was swiftly passed over flame and thereafter air dried. The slide was flooded with crystal violet and left for a period of 60 seconds. After which mordant was added and left for 60 seconds and it was rinsed off with a decolorized acetone after which it was washed. Safranin was added for 60 seconds and rinsed off. The back of the slide was dried. The slide was examined under a compound light microscope using the oil immersion objectives and the results interpreted appropriately [5].

Biochemical tests

The biochemical tests conducted were; Catalase Test, Methyl Red Test, Citrate Utilization test, Oxidase Test, Vogas-Proskaeur Test, Coagulase Test, Indole Test and Motility Test [5].

Preparation of 0.5 McFarland turbidity standard

A 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 dehydrate 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 [6].

Standardization of inoculum

The method of El Khadi [6] was used to standardize the organism. The isolates were cultured on Muller Hinton Agar (MHA) that was prepared according to manufacturer's instruction. Colonies from the overnight growth on the MHA were inoculated into a tube containing Muller Hinton Broth until the turbidity

was equivalent to the density of 0.5 McFarland standards (1.5×10^8 CFU/ml).

Preparation of extract concentration

One gram of ethanol and aqueous extract of *Adansonia digitata* was dissolved in 10 ml dimethyl sulphoxide (DMSO) to obtain 100 mg/ml as stock concentration. Subsequently, the concentration was diluted to obtain 500, 250, 125 and 62.5 mg/ml for the sensitivity test.

Determination of antibacterial activity using agar well diffusion assay

Pseudomonas aeruginosa and *Streptococcus* specie were grown on Muller-Hinton agar and the activity of aqueous and ethanol extracts of leaf of *A. digitata* was tested against the organisms using the agar well diffusion technique in a procedure described by Khaira et al. [7]. After inoculating the test organisms on Muller Hinton agar, five wells of 5.0 mm were bored using a sterile corkborer on the agar plates. 0.3 ml of different concentrations (500, 250, 125 and 62.5 mg/mL) of the plant extracts was dispensed into 4 of the wells but to the last well, ciprofloxacin was dispensed as a positive control. The agar plates were incubated at 37°C for 24 hours. The effects of the extracts against the test organisms were assessed by measuring the diameter of the zones of the inhibition to the nearest millimeter measured using a transparent ruler and recorded. The zones of inhibition were 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 extract as described by Bashir et al. [2]. Six tubes containing 5 mL of Muller-Hinton broth were prepared. 1 mL of the crude extract from (500, 250, 125 and 62.5 mg/ml) was introduced into tube 1-4 respectively and was mix thoroughly. Thereafter, 0.1 mL broth cultures of the test organisms were added to 4 tubes with the last tube containing broth to serve as control. 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 milliliters 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 poured 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 in solid agar medium was observed [2].

RESULT

Table 1 indicates the phytochemical constituents of leaf extract of *Adansonia digitata*. The phytochemical constituent of the aqueous leaf extract of *Adansonia digitata* indicated the presence of Tannins, Saponins, Quinones, Phenols and Steroids. The phytochemical constituents of ethanol leaf extract of *Adansonia digitata* were Tannins, Saponins, Phenols and Steroids.

Table 1: Phytochemical constituents of leaf extracts of *Adansonia digitata*

Bioactive compounds	Aqueous Extract	Ethanol Extract
Tannins	+	+
Saponins	+	+
Alkaloids	-	-
Glycosides	+	-
Quinones	+	-
Phenols	+	+
Terpenoids	+	-
Cardiac glycosides	-	-
Steroids	-	+
Phlobotannins	-	-

Key: +: positive, -: negative

Table 2 revealed the Gram reaction and biochemical characteristics of the isolates. *Pseudomonas aeruginosa* was gram negative rod, and was positive for Catalase, Methyl red, Citrate, Oxidase and Motility while *Streptococcus species* is a Gram-positive coccus, methyl red positive, Citrate positive and Coagulase positive organism.

Table 2: Identification and characterization of *Pseudomonas aeruginosa* and *Streptococcus* species from clinical isolates

Test	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus</i> species
Gram Reaction	- rod	+ cocci
Catalase	+	-
Methyl Red	+	+
Citrate	+	+
Oxidase	+	-
Voges-Proskauer	-	-
Coagulase	-	+
Motility	+	-

Key: +: positive, -: negative

Table 3 shows antibacterial susceptibility test of *Adansonia digitata* leaf extracts against *Streptococcus* species and *Pseudomonas aeruginosa*. *Streptococcus* species and *Pseudomonas aeruginosa* were sensitive to the extracts at all concentrations (500, 250, 125 and

62.5 mg/mL). The test organisms were sensitive to the ethanol leaf extract at 500 and 250 mg/ml. Both test organisms were sensitive to the aqueous leaf extract at 500, 250 and 125 mg/ml concentrations.

Table 3: Antibacterial susceptibility of *Adansonia digitata* leaf extracts against *Pseudomonas aeruginosa* and *Streptococcus* species

Isolate	Extracts	Concentration mm/ml/Zone of Inhibition				Control
		500	250	125	62.5	Ciprofloxacin
<i>Streptococcus</i> sp.	EL	23.75 ±1.06 ^b	15.25±0.35 ^b	12.75±0.35 ^{ab}	9.5±0.71 ^d	19.5±1.41 ^d
	AL	23.25 ±0.35 ^b	17.57±0.71 ^{ca}	15.5±0.71 ^d	9.57±0.35 ^{ab}	16.75±0.35 ^c
<i>Pseudomonas aeruginosa</i>	EL	20.25±0.35 ^c	17.0±0.00 ^b	13.75±1.06 ^a	13.75±0.35 ^b	22.0 ±0.00 ^{ab}
	AL	19.5±0.71 ^a	18.25±0.35 ^{cb}	14.0 ±1.41 ^{ab}	8.5 ±0.71 ^{bd}	14.5±0.71 ^b

Key: EL: Ethanol leaf extract, AL: Aqueous leaf extract and Different superscripts across the column indicate a significant ($p < 0.05$) difference.

Table 4 presents the Minimum Inhibitory Concentration of *Adansonia digitata* leaf extract against *Streptococcus faecalis*. No turbidity was recorded at 500 mm/ml concentration of N-butanol and aqueous extracts of leaf. The MIC of aqueous leaf extract was 125 mm/ml while the MIC of N-butanol leaf extract was 250mm/ml.

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of *Adansonia digitata* leaf extract against *Pseudomonas aeruginosa* and *Streptococcus* species

Isolates	MIC (mg/ml)
<i>Pseudomonas aeruginosa</i>	62.5
<i>Streptococcus species</i>	250

Table 5 indicates the Minimum Inhibitory Concentration of *Adansonia digitata* leaf and extract against *Pseudomonas aeruginosa*. The MIC of aqueous leaf extract was 125 mg/ml while the MIC of N-butanol leaf extract was 250 mg/ml.

Table 5: Minimum Inhibitory Concentration of *Adansonia digitata* leaf extracts against *Pseudomonas aeruginosa*

Concentration	Aqueous Extract	N-Butanol Extract
500	–	–
250	–	–
125	–	+
62.5	+	+

Keys: - = no growth + = growth

Table 6 shows the Minimum Bactericidal Concentration of *Adansonia digitata* leaf extracts against *Streptococcus faecalis*. The MBC of aqueous leaf extract was 250 mm/ml while the MBC of Ethanol leaf extract was 500 mm/ml.

Table 6: The Minimum Bactericidal Concentration of *Adansonia digitata* leaf extracts against *Streptococcus* species

Concentration	Aqueous Extract	Ethanol Extract
500	–	–
250	–	+
125	+	+
62.5	+	+

Key: -: no visible growth, +: visible growth

Table 7 shows the Minimum Bactericidal Concentration of *Adansonia digitata* leaf extract against *Pseudomonas aeruginosa*. The MBC of aqueous leaf extract was 250 mm/ml while the MBC of N-butanol leaf extract was 500 mm/ml.

Table 7: Minimum Bactericidal Concentration of *Adansonia digitata* leaf extracts against *Pseudomonas aeruginosa*

Concentration	Aqueous Extract	Ethanol Extract
500	–	–
250	–	+
125	+	+
62.5	+	+

Key: -: no visible growth, +: visible growth

DISCUSSION

The phytochemical constituents identified could be responsible for antibacterial activities of *Adansonia digitata* leaf against *Streptococcus* species and *Pseudomonas aeruginosa* isolates of wound. 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, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties [8]. Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and antibiotics properties [9]. 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 [10].

The variation in susceptibility of isolates to varied concentration of extracts may be due to the differences in the cell wall of the bacteria. *Streptococcus* species been gram positive bacteria with no lipopolysaccharide tend to allow diffusion of the active components, whereas *Pseudomonas aeruginosa* been gram negative bacteria possess lipopolysaccharide that might have regulated penetration of active components of extracts. *Pseudomonas aeruginosa* was more susceptible than *Streptococcus* species which may be attributed to the fact that *Streptococcus* species are usually resistant to most antibiotics due to permeability barriers afforded by its outer membrane that is composed of lipopolysaccharide. Also, its tendency to colonize surfaces in a biofilm form makes the cell impervious to therapeutic concentration of antibiotics [11].

Similar findings were reported by World Health Organisation [11] where *Adansonia digitata* leaf was used against *Pseudomonas aeruginosa* and *Streptococcus* species showing high susceptibility. This is also in line with the work of Fagbemi *et al.* [12], where higher zones of inhibition were observed against gram negative bacteria than gram positive bacteria, but contradicted the work of Warra [13] where gram positive bacteria show more susceptibility than gram negative bacteria. However, all activities of the extract were dose dependent which is similar to the research conducted by Ozioma and Chinwe [14] where it was observed that at high concentration, exhibited more antibacterial activity.

The low Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) exhibited by the plant against the isolates were attributed to effectiveness of the plant extracts. This is similar to the study reported by Kar and Bhattacharjee [15] where similar MIC and MBC were obtained. Also, the reports of Adefolaju [16] were similar to that of this

study where MIC and MBC of 62.5 mg/ml and 125 mg/ml were obtained against *P. aeruginosa* and *Streptococcus* species. The slight differences observed could be due to geographical location where the plant was collected and method of extraction.

The plant extracts in this study inhibited the clinical isolates of *Pseudomonas aeruginosa* and *Streptococcus* species obtained from wounds of patients. This is an indication that the *Adansonia digitata* leaf extract is beneficial in wound care. Wound infection has been identified as one of the most important factors that delays wound repair process and outcome [17].

CONCLUSION

In this study, important phytochemical compounds such as tannin, saponin, glucoside, quinones, phenol, terpenoid and steroid were detected in the leaf extract of *Adansonia digitata*. The extracts exhibited significant antibacterial activities against wound isolates of *Streptococcus* species and *Pseudomonas aeruginosa* at varied concentrations. *P. aeruginosa* was more susceptible to the extract at MIC and MBC of 62.5 mg/ml and 125 mg/ml. The presence of phytochemicals detected in the plant extract could be responsible for the observed antibacterial activities.

The antibacterial activity of ointment containing *Adansonia digitata* leaf extract may contribute remarkably to the faster wound healing rate. The inhibition of the microbial contaminants of wounds will allow the normal tissue repair process to occur. Further research on fractionation, should be done on the leaf of *Adansonia digitata* to its high antibacterial activity and if possible, other parts of the plant should be explored.

The toxicity profile of this plant should be carried out so as to establish its safety/therapeutic index. The antibacterial activity of ointment should be developed using *Adansonia digitata* leaf extract since it contributes remarkably to the faster wound healing rate.

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