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Original article

Phytochemical Screening and antibacterial activity of the leaf extract of *Adansonia digitata* against *Eschericia coli* and *Staphylococcus aureus*

*[1]Bello, A. A., [1]Adamu, M., [2]GaGman, H. A. and [2]Zigau, Z. A.

[1]Department of Biology, Faculty of Natural and Applied Science, Nigerian Army University Biu, Borno State, Nigeria
[2]Department of Biological Science, Faculty of Science, Bauchi State University Gadau, Bauchi State Nigeria

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ABSTRACT

The phytochemical analysis and antibacterial activity of methanol and chloroform leaf extracts of *Adansonia digitata on Escherichia coli* and *Staphylococcus aureus* were carried out using disc diffusion method. The clinical bacterial isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from General Hospital Biu, Borno State Nigeria. The phytochemical analysis of the methanol extract revealed the presence of alkaloids, saponins and tannins while the chloroform revealed the presence of alkaloids and saponins. The antibiotic showed a wide range of inhibition which ranged between (24.0mm to 18.0 mm) and (26.0mm to 20.0 mm) *Escherichia coli and Staphylococcus aureus* respectively, followed by the chloroform leaf extract of *Adansonia digitata* with (18.0mm to 10.0 mm) and (14.0mm to 9.0 mm) against *Escherichia coli* and *Staphylococcus aureus* respectively. Methanol extract had a zone of inhibition of (16.0 to 11.0 mm) and (13.0 to 8.0 mm) against *Staphylococcus aureus* at 100 mg/ml to 25 mg/ml concentrations respectively. The result of the MIC was revealed at 75 mg/ml and 100 mg/ml on *Escherichia coli* and *Staphylococcus aureus* while the minimum bactericidal concentration shows the presence of turbidity at both the concentrations. Therefore, the *Adensonia digitata* leaves would be recommended for the treatment of any disease caused by *Escherichia coli* and *Staphylococcus aureus.*

Keys: Phytochemical Analysis, *Adensonia digitata*, Disc diffusion and Zone of inhibition

Corresponding author's email: abdullahihabubello@gmail.com

INTRODUCTION

Baobab tree (*Adansonia digitata)* is a large deciduous tree, which is native to the African continent. It is also known as the African baobab, and it is one of the most iconic trees in Africa, playing a vital role in the social, cultural, and economic aspects of life in many African communities [3]. *Adansonia digitata* is one of the nine species that belong to the Adansonia genus, and it is the most widespread tree species in the genus. It also has several other names such as “upside-down tree, "monkey bread tree," and “tree of life”. Baobab tree *Adansonia digitata* is characterized by a massive trunk that can grow up to 25 meters in diameter and up to 30 meters in height [10]. The trunk, which can store up to 120,000 liters of water, allows the tree to survive in the harsh arid environments of the African continent [3]. The bark is thick, fibrous, and gray, and it is used for making rope, baskets, and clothing by African communities [11]. The leaves are compound and deciduous, falling during the dry season, and have a feathery-like appearance. Baobab tree *Adansonia digitata* has a rich cultural and medicinal history, and it has been used by African communities for various purposes. In traditional medicine, the bark is used for treating fever, diarrhea, and dysentery, among other ailments. The fruit pulp is used for treating respiratory infections, fever, and malaria, among others [1]. In recent years, scientific studies have confirmed the potential health benefits of the baobab tree *Adansonia digitata*. It has been found that the leaf extract of baobab tree *Adansonia digitata* has strong antioxidant and antimicrobial properties. The results indicated that the extract could be used as natural preservatives and antimicrobial agents in the food and pharmaceutical industries. The leaves, bark, fruit pulp, and seeds of the African baobab have been reported to possess various bioactive compounds with medicinal properties [1]. These bacteria are common causes of infections in humans and can lead to severe illness or even death if left untreated. The seeds are a source of protein, essential fatty acids, and tocopherols [10]. The leaves contain phenolic compounds, including saponins, tannins, and alkaloids, which exhibit various biological activities [3]. Baobab tree *Adansonia digitata* has several properties that make it useful in traditional medicine and modern pharmacology. The fruit pulp has antibacterial, antifungal, antiviral, anti-inflammatory, and analgesic properties. The seed oil has been reported to have anti-inflammatory, antioxidant, and moisturizing properties, making it useful in skin care products [10]. The leaves have been reported to have antidiabetic, antioxidant, and wound healing properties [3]. The chemical constituents and properties of baobab tree *Adansonia digitata* have been studied extensively in recent years. For example, a study by [1] evaluated the chemical profile of the baobab pulp extract using high-performance liquid chromatography (HPLC). The results indicated the presence of several bioactive compounds, including as corbic acid, gallic acid, ellagic acid, and quercetin, which contribute to the antioxidant activity of the extract. The study found that the juice has significant antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi* [4]. The aim of this study was to investigate the antibacterial activity of *Adansonia digitata* leaf extract on *Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Study Area, Collection and Identification of *Adansonia digitata* Sample

This study was conducted in the Biology Laboratory, Faculty of Natural and Applied Science, Nigeria Army University Biu, Borno State Nigeria. The leave of baobab tree was collected in Biu Local Government Area behind UBA bank Borno State Nigeria; and transported for identification by laboratory technologist in Biology Department, Nigeria Army University Biu where the voucher specimen was also deposited.

Preparations of The Leaf Extract *Adansonia digitata*

The collected plant leaves were shade dried at room temperature for five days. The leaves were grinded into powder using mortar and pestle in the laboratory. 50 g of finely grinded powder of leaves were added into a conical flask of 500 ml of methanol and chloroform respectively, and then kept at room temperature for about seven days with regular shaking. The extracts were filtered through what man filter paper and the filtered was evaporated to dryness using rotary evaporator to obtain the extracts. The extracts were stored in sterilized bottle for further use.

Phytochemical Screening of the Leaves Extract

The presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, tannins, and saponins present in the leaf extract of *Adansonia digitata* were investigated by using procedure described by [11].

Detection of Alkaloids

Zero-point five gram of the leaf extract was treated with Wagner's reagent (few drop of iodine and potassium iodide in 100 ml distilled water) and observed for the formation of reddish-brown colored precipitate.

Detection of Flavonoids

Zero-point five gram of the leaf extract was treated with concentrated sulphuric acid and observed for the formation of orange color.

Detection of Terpenoids

One mil of chloroform was added to 0.5 g of the extract, and a drop of concentrated sulphuric acid was added. The presence of reddish-brown coloration at the interface indicates the presence of terpenoids.

Detection of Saponins

Twenty milliliters of distilled water were 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 the 1 g of extracts in a test tube. The formation of white precipitate indicates the presence of tannins.

Collection of Bacterial Isolates

The *Escherichia coli* and *Staphylococcus aureus* were collected from General Hospital Biu-Borno State, Nigeria and transported to the Biology Department under 4^{o}C as described by [5].

Concentration of Plant Extract and Antibiotics

The following concentrations of the plant extract were made (25 mg/ml, 50 m/ml, 75 mg/ml, and 100 mg/ml) and the same procedures for erythromycin (25 mg/ml, 50 m/ml, 75 mg/ml, and 100 mg/ml) were used as a positive control.

Sensitivity Test

The disk diffusion method was use in accordance with the method of (Johnson and Brown, 2015). The sterilized paper discs were impregnated with (25 mg/ml, 50 m/ml, 75 mg/ml, and 100 mg/ml) of the methanol and chloroform extract. The impregnated discs were placed onto prepared nutrient agar plate, each plate contained a culture of either *Escherichia coli* or *Staphylococcus aureus*. The same procedure was carried out using antibiotic as positive control and a control disc without the extract was served as negative control, the plates were incubated at 37°C for 48 hours. After incubation, the plates were examined for the presence or absence of a clear zone of inhibition. The clear zones of inhibition were measured by using a transparent ruler. All the experiment was conducted in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

Six tubes containing 5 ml of nutrient broth were prepared. 1ml of the leaves extract was introduced into tube containing 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml and mix thoroughly. Thereafter, 0.1 mL of broth cultures of the test organisms were added to 4 tubes with the last tube serving as broth control for each respectively. The inoculated tubes were kept at 37°C for 24 hours in an incubator. Erythromycin was used as the positive control. The lowest concentration that showed no growth is considered as the MIC. [9].

Determination of Minimum Bactericidal Concentration (MBC)

Five milliliter of prepared nutrient broth was dispensed into sterile test tubes equivalent to the number of tubes that show no visible growth from the minimum inhibitory concentration (MIC). Then 0.1 ml of the broth culture was transferred to tubes containing the 5 ml nutrient broth. The tubes were labeled and kept in a test-tube rack. Prepared nutrient 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 inoculums were 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 absences of bacteria growth culture were observed [12].

Data Analysis

The values obtained were subjected to one-way analysis of variance (ANOVA) using statistical package for Social Science version 2021. Results were considered as significant when p values were less than 0.05 ($P<0.05$).

RESULTS

Phytochemical Components

The following secondary metabolites alkaloids, tannins, and saponins were detected from the methanol leaves extract of *Adansonia digitata* while the chloroform extracts of *Adansonia digitata* leave revealed the presence of alkaloids and saponins but flavonoid, terpenoid were absent.

Table 1: Qualitative Phytochemical Composition of the Leaf Extracts of *Adansonia digitata*

Phytochemicals	*Adansonia digitata*	
	Methanol Leaf Extracts	Chloroform Leaf Extracts
Saponins	+	+
Flavonoids	-	-
Tanins	+	+
Alkaloids	+	+
Terpenoids	-	-

Key: + = Present, - = Absence,

Antibacterial Activity of Methanol and Chloroform Leaves Extracts on *E. coli*

The activity of the methanol and chloroform leaves extract of *Adansonia digitata* against *E. coli* presented in table 2. Among the plant extracts, methanol leaf extract had the highest zone of inhibition of 16 mm at concentration of 100 mg/mL against *E. coli*. The lowest activity was observed on the chloroform leaves extract of *Adansonia digitata* (10 mm) at 25 mg/ml against *S. aureus*. The Erythromycin as positive control showed the highest activity of 24 mm at concentration of 100 mg/mL against *E. coli* compared to the plant extracts.

Table 2: Antibacterial Effect of Four Different Concentration Levels of the Leaf Extracts of *Adansonia digitata* on *Escherichia coli* (mm)

Extracts / Solvents used	100 mg/ml	75 mg/ml	50 mg /ml	25 mg/ml
Methanol	16.0	14.0	13.0	11.0
Chloroform	18.0	16.0	14.0	10.0
Control (+)	24.0	22.0	20.0	18.0

Antibacterial Activity of Methanol and Chloroform Leaves Extracts on *S. aureus*

The activity of the methanol and chloroform leaves extract of *Adansonia digitata* against *Staphylococcus aureus* is presented in table 3. Among the plant extracts, chloroform leaf extract had the highest zone of inhibition of 14 mm at concentration of 100 mg/mL against *Escherichia coli*. The lowest activity was observed on the chloroform leaves extract of *Adansonia digitata* (6 mm) at 25 mg/ml against *Staphylococcus aureus*. The Erythromycin as positive control showed the highest activity of 24 mm at concentration of 100mg/mL against *Escherichia Coli* compared to the plant extracts.

Table 3: Antibacterial Effect of Four Different Concentration of the Leaf Extracts of *Adansonia digitata* on *Staphylococcus aureus* (mm)

Extracts / Conc.	100 mg/ml	75 mg/ml	50 mg /ml	25 mg/ml
Methanol	13.0	11.0	10.0	8.0
Chloroform	14.0	12.0	11.0	9.0
Control (+)	26.0	24.0	22.0	20.0

Minimum Inhibitory Concentration (MIC)

The result of the minimum inhibitory concentration (MIC) of the methanol and chloroform leaves extract are presented in Table 4. The MIC of the methanol leaf extract was observed at 100 mg/ml for *Escherichia coli* but the clear turbidity was observed in both the methanol leaves extract concentrations against *Staphylococcus aureus* while the MIC chloroform leaves extract are 75 mg/ml and 100 mg/ml for *Escherichia coli* and *Staphylococcus aureus* respectively. The minimum inhibitory concentration (MIC) of the positive control (erythromycin) was 50 mg/ml for both *Escherichia coli* and *Staphylococcus aureus* respectively.

Table 4: Minimum Inhibitory Concentration (MIC) of the Leaf Extracts Against *Escherichia coli* and *Staphylococcus aureus*

Extracts / Solvents used	*Escherichia coli*	*Staphylococcus aureus*
Methanol	100 mg/ml	+
Chloroform	75 mg/ml	100 mg/ml
Control (+)	50 mg/ml	50 mg/ml

Key: + = presence of turbidity.

Minimum Bactericidal Concentration

The result of minimum bactericidal concentration (MBC) of the extracts was shown in Table 5. The minimum bactericidal concentration for the methanol and chloroform leaves extracts shows the presence of turbidity for both *Escherichia coli* and *Staphylococcus aureus.*

Table 5: Minimum Bactericidal Concentration (MBC) of the Leaf Extracts Against *Escherichia coli* and *Staphylococcus aureus.*

Extracts / Solvents used	*Escherichia coli*	*Staphylococcus aureus*
Methanol	+	+
Chloroform	+	+
Control (+)	+	+

Key: + = presence of turbidity.

DISCUSSION

Quantitative Phytochemical Screening

This study revealed that the leaf of *Adansonia digitata* possess antibacterial properties; this is due to the presence of some secondary metabolites in the plants leaf [6]. The *Adansonia digitata* leaf extract possess higher antimicrobial activity, erythromycin had the highest zone on inhibition, which show that *Adansonia digitata* was sensitive to *Escherichia coli* and *Staphylococcus aureus. Adansonia digitata* leaf extract revealed the presence of the following secondary metabolite; alkaloid, saponins, tannins while flavonoids, and terpenoids were absence in both the extract [8].

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Antibacterial Activity

The result showed that the erythromycin had highest zones of inhibition of 24.0 mm at 100 mg/ml against *Escherichia coli*. This activity revealed that erythromycin demonstrated the bactericidal activity, followed by the Chloroform extract of the *Adansonia digitata* at different concentration which give a highest zone of inhibition of 18. 0 mm at 100 mg/ ml and the lowest zone of inhibition of 12.0 mm at 25 mg/ml against the isolate; this supports the findings of [2,7]. And also Methanol extract show the highest zone of inhibition of 16.0 mm at 100 mg/ml against *E. coli.* This result supports the previous findings of [2]. Chloroform extract have a highest zone of inhibition than the methanol extract, this antibacterial activity of the plant extract, might be due to the present of the phytochemical compounds (alkaloid, saponins, flavonoids and tannins) of the plant extracts [1]. The bacterial isolate was resistant to the negative control (distilled water) which revealed (0.00mm) zone of inhibition.

Minimum Inhibitory Concentration and Minimum Bactericide Concentration

The minimum inhibitory concentration (MIC) showed that the methanol leaf extract inhibited the growth of *Escherichia coli* at 100 mg/ml while *Staphylococcus aureus* did not inhibit the growth, and the chloroform leaf extract inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* at concentration of 75 mg/ml and 100mg/ml as reported by [4]. The MBC obtained showed that the minimum bactericidal concentration for the methanol and chloroform leaves extracts shows the presence of turbidity for both *Escherichia coli* and *Staphylococcus aureus.* This study was similar to the finding of [2, 7].

CONCLUSION

The Chloroform and methanol extracts of *Adansonia digitata* leaves inhibited the growth of the bacteria and could provide a therapeutic agent for the treatment of a disease caused by *Escherichia coli* and *Staphylococcus aureus*. Chloroform extract showed higher antibacterial effect compare to methanol extracts of *Adansonia digitata* but less than the positive control.

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