



Evaluation of *In Vitro* Antibacterial Efficacy of Methanolic Root Extract from *D. zibethinus* Murr. Against Gram-positive and Gram-negative Bacterial Strains

Sunday Adegoke Adeniyi^{*1}, Peter Agbomire Agbugui¹, Obhioze Augustine Akpoka¹, Ezekiel Olatoye Solanke¹

¹ College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria.

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ABSTRACT

Bacterial and viral infections represent some of the most difficult diseases to treat in humans due to resistance to most of the therapeutic agents. The emergence of drug resistance factors that have threatened the efficacy of all antibacterial agents prompted the investigation of antimicrobial activity studies of methanol extracts from *Durio zibethinus* Murr. This study is focused on evaluating the phytoconstituents, and antibacterial efficacy of methanolic root extract of *D. zibethinus* Murr. against gram-positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram-negative bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella Typhi*). Phytochemical properties of methanolic root extract of *D. zibethinus* Murr were investigated using qualitative analysis, while the antibacterial was evaluated using standard agar disc diffusion technique. The results revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds in the *D. zibethinus* Murr. root. It also indicated that the root extract exhibited antibacterial activity against *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *S. Typhi* at a range of 12.47 – 24.56 mm at the highest concentration of 200 mg/mL relative to standard gentamicin antibiotic. The minimum inhibitory concentration (MIC) for *S. aureus* was 0.250 mg/mL, whereas it was 0.125 mg/mL for *S. epidermidis*, *K. pneumoniae* and *S. Typhi*. In the present study, root extract of *D. zibethinus* Murr. showed the highest antibacterial activity against *S. aureus*. Hence, *D. zibethinus* Murr. root can be used as new source for antibacterial substance.

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1. INTRODUCTION

Antimicrobial agents play an important role in reducing the global epidemic of transmissible diseases [1, 2]. But the overuse of antimicrobial agents has a negative impact on the environment, ecosystem, and the overall well-being of people. It could also raise the prevalence of infections resistant to drugs [3]. However, since there are fewer or often no effective antimicrobial drugs available for an infection caused by pathogenic bacteria, the development and spread of multidrug resistant (MDR) strains in pathogenic bacteria have become a serious concern to public health [4, 5].

As a result of the rapid global dissemination of resistant clinical isolates, new antimicrobial agents must be discovered in

order to completely eradicate the antibiotic resistance that the clinical isolates exhibit [6, 7]. Plants are well-endowed with many secondary metabolites which have been shown to have abundant antimicrobial traits [8, 9]. A large number of these plants are medicinal and have been reported as important sources of naturally occurring antimicrobial compounds that may be used as alternative therapies which can be successful in treating bacterial infections [10, 11].

Among the medicinal plants is *Durio zibethinus* Murr. *Durio zibethinus* (Family Bombacaceae), a tropical fruit plant, also known as durian, has been widely cultivated in Malaysia and other Southeast Asian countries.

*Corresponding author:

E-mail address: Sunday Adegoke Adeniyi <adeniyi.sunday@iuokada.edu.ng>.

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Researchers have reported antioxidant, anticancer, antidiabetic, anti-lipoxygenase, anti-heart disease and anti-obesity properties in durian [12, 13]. The ability to strengthen the immune system is one of the claimed medicinal and therapeutic benefits of durian fruit [14]. Its fruit pulp may be an excellent source of fibre, dietary fat, proteins, and carbohydrates [15]. It has also been revealed that durian seed, pulp, and peel flour were endowed with nutritional, structural, anti-inflammatory, and antioxidant properties [16, 17]. Taking into consideration the enormous potential of plants as sources for antibacterial agents, this study aimed to investigate *in vitro* antibacterial activity of extract from *D. zibethinus* M against Gram-positive and Gram-negative bacterial strains like *S. aureus*, *S. epidermidis*, *K. pneumonia*, *E. coli* and *S. Typhi* in order to detect new source of antibacterial agent.

2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

The plant material, *Durio zibethinus* Murr. root was collected at Crown Estate of Igbinedion University, Okada, Edo State, Nigeria in the month of August, 2023. The plant material was identified and authenticated at the Taxonomy section of Department of Plant Biology, University of Ilorin, Nigeria. The Voucher No. UILH/001/1371 was assigned. The sample was pulverized and extracted after being air-dried at ambient temperature.



Fig. 1. Images of the (A) fruit, (B) seeds and (C) crushed seeds used for this study

2.2 Preparation and Extraction of Plant Materials

The plant extract was prepared in accordance with the methods described by Ibrahim and Kebede [18] with minor modifications. The fresh plant material was washed and cleaned thoroughly with distilled water and air-dried at room temperature (26 °C) for 8 weeks, after which it was milled into uniform powder with the aid of an electric herb grinder (MODEL-750, LEJIEYIN China).

The extract was processed by soaking 2.0 kg of each powdered plant material in 10.0 L of methanol at ambient temperature for 72 hours. The extract was filtered with Whatmann No.1 filter paper and the filtrates were then separately concentrated in a vacuum using Rotary Evaporator (RE-52A, Labscience England) at 30-40 °C. The methanol extract was transferred carefully to labelled vials and allowed to permit evaporation of residual solvents at room temperature for 3-4 days. Then the dried extract was stored in sterile bottles and kept in a refrigerator until further use.

2.3 Tested Microorganisms

The test cultures were obtained from the Department of Microbiology & Parasitology, Igbinedion University Teaching Hospital, Okada, Nigeria. The test cultures that were used

throughout the study are *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumonia* ATCC 27729, *Escherichia coli* ATCC 25922 and *Salmonella Typhi* ATCC 13311 (Microbiologics Inc., St. Cloud, MN, USA).

2.4 Phytoconstituent Analysis

2.4.1 Alkaloids

0.5 g of extract was diluted with 10 mL of acid alcohol, boiled and filtered. To 5 mL of the filtrate was added 2 mL of dilute ammonia. 5 mL of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Dragendoff's reagent) was regarded as positive for the alkaloids [19].

2.4.2 Saponins

A drop of Na_2CO_3 solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation was deemed to be a positive test for saponins [20].

2.4.3 Tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration was considered positive for the tannins [21].

2.4.4 Flavonoids

About 0.5 g of each plant extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless was considered a positive test for flavonoids [22].

2.4.5 Terpenoids (Salkowski method)

To 0.5 g each of the extract was added 2 mL of chloroform. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown colouration of the interface was regarded as positive for the terpenoids [23].

2.4.6 Steroids

Two millimeters of acetic anhydride was added to 0.5 g of ethanol extract of each sample with 2 ml H_2SO_4 . The colour changed from violet to blue or green in some samples was deemed to be a positive test for steroids [24].

2.4.7 Cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 mL in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A formation of greenish ring on top of brown ring and hours below is violet ring was considered a positive test for glycosides [25].

2.4.8 Phenolic compounds

Lead tetra acetic acid test. One milliliter of lead tetra acetate solution was treated with 0.5 mL of extract, bulky white precipitate formation was regarded as positive for the phenolic compounds [26].

2.5 Antibacterial Assay

An extract of *D. zibethinus* M was screened for antibacterial activity using two Gram-positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus epidermis*) and three Gram-negative bacterial strains (*Klebsiella pneumonia*, *Escherichia coli* and *Salmonella Typhi*).

The antibacterial activity of the extract was undertaken by following the agar diffusion procedure described by Asmerom et al. [27] with minor modifications. The bacterial strains were grown in 50 mL of nutrient broth at 37 °C and maintained in a nutrient agar slant at 4 °C. An overnight suspension culture of the five bacterial strains was spread on the Mueller–Hinton agar (MHA) plate in a 100 mm diameter sterile petri dish. The uniform thick lawn growth of the seeded media was then allowed to dry at room temperature for about 30 minutes.

On each plate, the wells were punched using a 6 mm diameter sterilized borer and assigned numbers. The disc was impregnated with 20 µL of 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL of the solutions of the plant extracts dissolved in 1% dimethyl sulfoxide (DMSO) were filled into the corresponding wells. The commercial antibiotic gentamicin was used as a positive control to determine the activities of the bacterial strains. Afterward, the plates were left undisturbed for about 2 hours at room temperature to give sufficient time to diffuse on the inoculated agar, and the plates were transferred into an incubator. The inoculated plates were incubated at 37 °C for 24 hours, and the diameter of the zone of inhibition was measured using a metal calliper and recorded in millimetres (mm). The experiment was carried out in triplicate for each bacterium. The average zone of inhibition was calculated for each test sample and the standard antibiotics.

2.6 Minimum Inhibitory Concentration Assay (MIC)

The method described by Jensen et al. [28] was adopted with minor modifications. A fresh stock solution of *Durio zibethinus* methanolic root extract was prepared in 0.02 M HCl with a final concentration of 102.4 µg ml⁻¹. Minimal inhibitory concentration (MIC) was determined in a 96-well. Overnight cultures of bacterial strains were diluted in physiological saline (0.9% NaCl) to reach turbidity of 0.5 McFarland (corresponding to ~10⁸ CFU/mL; Sensititre[®] nephelometer and the Sensititre[®] McFarland Standard). The bacterial suspensions were adjusted to 5 × 10⁵ CFU/mL in Mueller–Hinton broth (MH; Oxoid

CM0405) in wells containing standard twofold dilutions of plant extract in a final volume of 100 µL. The plates were incubated for 24 hours with shaking (300 rpm) at 37°C. All experiments were performed in triplicate. The MIC was determined by the lowest concentration of *Durio zibethinus* methanolic root extract that suppressed bacterial inhibition which was determined by the absence of turbidity in the broth [29, 30, 31].

2.7 Statistical Analysis

The mean and standard deviation of the experiments were determined. Analysis of variance (ANOVA) on antibacterial analysis was performed to test for significant differences (p < 0.05).

3. RESULTS

The results of the phytoconstituents of the root extract of *Durio zibethinus* Murr are shown in Table 1. The secondary metabolites detected in *Durio zibethinus* Murr. root extracts are alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds.

Table 2 presents the results of the antibacterial activity of the methanolic root extract of *D. zibethinus* Murr. From the results, the methanolic root extract exhibited antibacterial activity against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *K. pneumonia* ATCC 27729 and *S. Typhi* ATCC 13311 with maximum inhibition zones of 24.01 ± 0.01 mm, 12.07 ± 0.01 mm, 16.03 ± 0.01 mm and 14.03 ± 0.02 mm at the highest concentration of 150 mg/mL, respectively, while the growth of *E. coli* ATCC 25922 was not inhibited by this extract.

The minimum inhibitory concentration (MIC) of *D. zibethinus* Murr. root extract results were presented in Table 3. The result disclosed that the MIC for *S. aureus* was 0.250 mg/mL, whereas it was 0.125 mg/mL for *S. epidermidis*, *K. pneumonia* and *S. Typhi* respectively.

Table 1. Phytoconstituents of methanolic root extract of *Durio zibethinus*

S/N	Phytoconstituents	Inference
1	Alkaloid	
	(a) Mayer's test	+
	(b) Dragendorff's	+
2	Saponin	+
3	Tannin	+
4	Flavonoid	+
5	Terpenoid	+
6	Steroid	+
7	Cardiac Glycoside	+
8	Phenolic compounds	+

*Presence = +, Absence = -

Table 2. Antibacterial activity of *Durio zibethinus* root

Concentrations (mg/mL)	Diameter of zone of inhibition (mm)/ Bacterial Strains				
	<i>S. aureus</i> ATCC 25923	<i>S. epidermidis</i> ATCC 25923	<i>K. pneumonia</i> ATCC 12228	<i>E. coli</i> ATCC 25922	<i>S. Typhi</i> ATCC 13311
200	24.56 ± 0.01	12.47 ± 0.01	16.83 ± 0.01	0	14.52 ± 0.02
100	21.63 ± 0.01	11.08 ± 0.01	14.96 ± 0.01	0	12.83 ± 0.01
50	20.23 ± 0.01	9.75 ± 0.16	12.76 ± 0.01	0	10.74 ± 0.01
25	20.23 ± 0.01	9.25 ± 0.01	11.24 ± 0.01	0	9.88 ± 0.01
Gentamicin (+)	0	22.00	16.00	14.00	22.00
DMSO (-)	0	0	0	0	0

*Values are expressed in mean ± SD (n=3). SD: Standard Deviation. 0 = no zone of inhibition

Table 3. Minimum inhibitory concentration (MIC) of *Durio zibethinus* root

Microorganisms	Minimum Inhibitory Concentration (mg/mL)
<i>S. aureus</i> ATCC 25923	0.250
<i>S. epidermidis</i> ATCC 12228	0.125
<i>K. pneumonia</i> ATCC 27729	0.125
<i>S. Typhi</i> ATCC 13311	0.125

4. DISCUSSIONS

The results obtained in Table 1 were in agreement with the report of Mohiuddin [32]. According to reports, secondary metabolites are known to be antimicrobial compounds against a wide range of microbes [33, 34, 35]. This study showed that *Durio zibethinus* Murr. root is a potential wellspring of antimicrobial agents as a result of the availability of secondary metabolites like flavonoids, phenols, and alkaloids. This finding is in line with the study of Manurung *et al.*[36].

From the results in Table 2, the methanolic root extract of *D. zibethinus* Murr. exhibited antibacterial activity against all the bacteria with the exception of *E. coli* ATCC 25922 which is known to be resistant to many antibiotics [37]. The results suggested that the extract exhibited more antimicrobial activity against *S. aureus* ATCC 25923 and *K. pneumonia* ATCC 27729, whereas the activity may be considered moderate against *S. epidermidis* ATCC 12228 and *S. Typhi* ATCC 13311 when compared to the commercially available antibiotic Gentamicin (16.00 mm). The results obtained were in agreement with the report of Alkandahri *et al.*, [38] who tested the antibacterial activities of *D. zibethinus* Murr. fruit and leaves against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* bacteria. Although some researchers have reported the antibacterial activity of durian fruit skin flesh extract [39] and durian rinds extract [40, 41] but little or no work has been reported for antibacterial activity of *D. zibethinus* root extract.

5. CONCLUSION

In our study, the *D. zibethinus* Murr. root extract possesses some essential phytochemicals, such as, alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds. These phytochemicals serve as the basis for the antibacterial activity of the extract against some bacterial strains. The study also revealed that the root extract exhibited the highest antibacterial activity against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *K. pneumonia* ATCC 27729 and *S. Typhi* ATCC 13311. Among all the bacterial species, *S.*

aureus ATCC 25923 was found to be the most sensitive bacterium. In recent time, researchers have reported the antibacterial activities of *D. zibethinus* Murr. against some bacterial strains but based on our study it was found that the root extract exhibited the highest antibacterial activity. Hence, it is therefore suggested that *D. zibethinus* Murr. root extract could be a viable therapeutic agent as an alternative against bacterial diseases as antibiotic medications are so costly these days. However, with the aim of fully utilizing the antibacterial potential of *D. zibethinus* Murr. root, it is imperative that the active components be isolated and thoroughly characterized. Such active compounds can be utilized to develop novel, potent antibiotic drugs.

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