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INSIGHTS INTO THE IN-VITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC STEM BARK EXTRACT FROM DURIO ZIBETHINUS MURR. AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIAL STRAINS

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ABSTRACT

Since most medications for treatment are ineffective against bacterial and viral infections, they are the most difficult diseases to cure in humans. The appearance of medication resistance factors that have challenged the efficiency of all antibacterial agents prompted the research on the antibacterial properties of methanol stem bark extract of D. zibethinus. This research aims to take a look at the phytochemical and antibacterial properties of the stem bark extract of D. zibethinus Murr. against various bacterial strains, including gram-positive strains such as Staphylococcus aureus and Staphylococcus epidermidis, also gram-negative strains like Escherichia coli, Salmonella typhi and Klebsiella pneumonia. Making use of qualitative analysis, the availability of phytoconstituents in methanol stem bark extract of D. zibethinus Murr. were examined, while the standard agar disc diffusion technique was utilized to evaluate the antibacterial activity. The results obtained showed the availability of phenolic compounds, alkaloids, flavonoids, tannins, saponins, terpenoids, steroids and glycosides in the D. zibethinus Murr. stem bark. Furthermore, it demonstrated that the stem bark extract showed antibacterial activity against S. aureus, S. epidermidis, E. coli, and S. Typhi, with inhibition zones ranging from 15.88 to 18.72 mm at the highest concentration of 200 mg/mL compared to the standard gentamicin antibiotic. The minimum inhibitory concentrations (MIC) for S. aureus and S. epidermidis was 0.250 mg/mL, while E. coli had MIC of 0.125 mg/mL. The findings of our investigation revealed that the stem bark extract of D. zibethinus Murr. exhibited the most potent antibacterial activity against E. coli. Our conclusion is that the D. zibethinus Murr. stem bark extract could be a viable therapeutic agent as an alternative.

Keywords: Phytochemicals, Antibacterial, *Durio zibethinus*, Minimum inhibitory concentration.

Introduction

Antimicrobial agents are crucial in reducing the global epidemic of transmissible diseases (Baker et al., 2022, Salam et al., 2023). However, excessive usage of antibiotics has adverse consequences on the environment, ecosystem, and the overall well-being of people has adverse consequences on the environment, ecosystem, and the overall well-being of people. It might increase the extent of infections resistant to drugs (Pulingam et al., 2022).

However, the emergence and spread of multidrug resistant (MDR) strains of pathogenic bacteria have become a major public health problem due to the lack of efficient antimicrobial medications for infections caused by these bacteria. (Cameron et al., 2022, Walsh et al., 2023).

As a result of the speedy global growth of resistant clinical isolates, new antimicrobial medications must be discovered in intention to completely eradicate the antibiotic resistance that the clinical isolates exhibit (Aslam et al., 2021, Emeraud et al., 2023). Plants are well-endowed with many secondary metabolites, which have been shown to have abundant antimicrobial traits (Chassagne et al., 2021, Hossaini et al., 2021). Many of these plants are medicinal and have been reported as important sources of naturally occurring antimicrobial compounds that might be utilized as alternative remedies that could be successful in treating these troubling bacterial infections (Alaoui Mdarhri et al., 2022, Iseppi et al., 2022).

D. zibethinus Murr is among the medicinal plants that could be employed to overcome the problem of antibiotic resistance to bacteria. D. zibethinus Murr is a fruit bearing tropical plant type called durian that has been extensively grown in Southeast Asian countries, majorly in Malaysia. Researchers have stated that antioxidant, anticancer, antidiabetic, anti-lipoxygenase, anti-heart disease, and anti-obesity properties can be attributed to durian (Adeniyi et al., 2024a, Siburian et al., 2019). The ability of durian fruit to give strength to the immune system is one of its alleged medical and therapeutic advantages (Husin et al., 2018). Its fruit pulp might be a reliable source of fiber, dietary fat, proteins, and carbohydrates (Adeniyi et al., 2019). It has also been revealed that durian seed, pulp, and peel flour are endowed with nutritional, structural, anti-inflammatory, and antioxidant properties (Permatasari et al., 2022, Charoenphun and Klangbud, 2022). Taking into consideration the tremendous potential of plants as sources for antibacterial agents, this study sought to determine the in vitro antibacterial properties of the stem bark extract of D. zibethinus Murr. against gram-positive and gram-negative bacterial strains including S. aureus, S. epidermidis, E. coli, K. pneumonia and S. Typhi in an attempt to detect new source of antibacterial agent.

Materials and Methods

Sample collection and authentication

In August 2023, D. zibethinus Murr stem bark from Fig. 1 was collected at Igbinedion University's Crown Estate in Okada, Edo State, Nigeria. Identification and authentication of the plant sample was done by the Taxonomist at the Plant Biology Department, University of Ilorin, Nigeria. The plant sample was assigned Voucher No. UILH/001/1371. At ambient temperature, the sample was air-dried and then ground into a powder before being extracted.

Fig. 1 Image of fruits bearing branch of a durian plant

Preparation and Extraction of Plant Material

The plant extract was made using the techniques outlined by Ibrahim and Kebede (2020) with little modifications. The fresh plant sample was washed with distilled water and then left to air dry for eight weeks at room temperature (26°C), after which an electric herb grinder (MODEL-750, LEJIEYIN China) was used to ground it into a uniform powder.

2.0 kg of each powdered plant material was soaked in 10.0 L of methanol for 72 hours at room temperature in order to produce the extract. This extract was filtered using Whatmann No. 1 filter paper, and a Rotary Evaporator (RE-52A, LabScience England) was used to vacuum-concentrate the filtrates at 30 to 40 °C. After transferring the methanol extract to labelled vials, the remaining solvents were left to evaporate for three to four days at room temperature. Then the dried extract was stored in sterile bottles and kept in a refrigerator until further use.

Tested Microorganisms

The Department of Microbiology & Parasitology at Igbinedion University Teaching Hospital in Okada, Nigeria, provided the test cultures. The test cultures used in this study include *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. coli* ATCC 25922, *K. pneumonia* ATCC 27729 and *S. Typhi* ATCC 13311 (Microbiologics Inc., St. Cloud, MN, USA).

Phytoconstituent Analysis

Alkaloids

To obtain the alkaloidal base, 0.5 gram of extract was diluted with ten milliliters of acid alcohol, heated, and then filtered. To the five milliliters of filtrate, two milliliters of diluted ammonia and five milliliters of chloroform were added and shaken gently. To the mixture, 10 mL of acetic acid was added in order to extract the chloroform layer, this was divided into portions 1 and 2 in which Mayer's reagent was added to portion 1 and to portion 2, Draggendoff's reagent was added. Alkaloids were considered to be present when a cream formed with Mayer's reagent or a reddish-brown precipitate formed with Draggendoff's reagent (Nortjie et al., 2022).

Saponins

In a test tube, 5 mL of extract was mixed with a drop of Na_2CO_3 solution. After vigorous shaking, it was allowed to rest for five minutes. Foam formation was deemed to be positive for saponins (Dubale et al., 2023).

Tannins

In a test tube, ten milliliters of water were used to dissolve 0.5 gram of the extract, which was then boiled and filtered. Drops of 0.1% $FeCl_3$ were added to the filtrate. The presence of tannins was deemed to be indicated by blue-black or brownish green colour (Kancherla et al., 2019).

Flavonoids

After dissolving 0.5 gram of each plant extract in diluted NaOH, HCl was added. A yellow solution that revolves to colorless was regarded as positive for flavonoids (Adil et al., 2024).

Terpenoids (Salkowski Method)

Two millimeters of chloroform was added to 0.5 grams of each extract, and the mixture was combined with 2 milliliters Conc. H_2SO_4, thereby forming a layer. Reddish-brown coloration appearing at the interface was considered as positive for terpenoids (Octiara et al., 2021).

Steroids

Two millimeters of acetic anhydride were added to each of 0.5 grams of methanol extract, and the mixture was then combined with 2 milliliters of H_2SO_4. When the colour of sample changed from violet to blue or green in some samples, the test was considered to be positive for steroids (Alemu et al., 2024).

Cardiac Glycosides

A single drop of ferric chloride solution, 2 milliliters of glacial acetic acid and 0.5 grams of extract that had been diluted to 5 milliliters in water were mixed together before 1 milliliter of concentrated sulfuric acid was added to the resultant solution. The appearance of a brown ring at the interface follow by development of greenish ring on top of brown ring and violet ring below was considered positive for glycosides (Bakir Çilesizoglu et al., 2022).

Phenolic Compounds

0.5 milliliters of extract and 1 mL of lead tetra acetate solution were mixed together. The formation of a large, white precipitate was regarded positive for phenolic compounds (Warsi et al., 2023).

Antibacterial Activity Assay

Two gram-positive and three gram-negative bacterial strains including *S. aureus*, *S. epidermis*, *E. coli*, *K. pneumonia*, and *S. Typhi* were used to evaluate the antibacterial activity of the methanol stem bark extract of *D. zibethinus* Murr.

With little modifications, the agar diffusion method was used to evaluate the antibacterial activity of the extract as reported by Asmerom et al. (2020). The bacterial strains were maintained in a nutrient agar slant at 4 °C after being cultivated in 50 milliliters of nutrient broth at 37 °C. In a sterile petri dish with a diameter of 100 mm, the Mueller-Hinton agar (MHA) plate had been coated with an overnight suspension culture of the five bacterial strains. The uniformly thick lawn-growing seeded media was allowed to dry at room temperature for about 30 minutes.

The wells on individual plate were punched and assigned numbers using a sterile borer with a 6 mm diameter. Twenty microliters of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL solutions of the plant extracts diluted in 1% dimethyl sulfoxide (DMSO) were added to the respective wells and the disc was impregnated. The activities of the bacterial strains were assessed using the commercial antibiotic gentamicin as a positive control. Afterward, the plates were transferred into an incubator after being left undisturbed for about 2 hours to give enough time to diffuse on the inoculated agar at room temperature. After incubation at 37 °C for 24-hour period, the zone of inhibition was measured using a metal caliper and reported in millimeters (mm). The experiment was conducted in triplicate for each microorganism. The average zone of inhibition was calculated for each test sample and the standard antibiotics.

Minimum Inhibitory Concentration Assay (MIC)

The method described by Jensen et al. (2020) was adopted with little modifications. A fresh stock solution of D. zibethinus methanol stem bark extract was prepared in 0.02 M HCl with a final concentration of 102.4 µg ml^{-1}. The minimum inhibitory concentration (MIC) was determined in a 96-well plate. Bacterial strain cultures were diluted overnight 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 set at 5 × 105 CFU/mL in Mueller-Hinton broth (MH; Oxoid CM0405) in wells containing standard twofold plant extract dilutions in a final volume of 100 µL. The plates were shaken at 300 rpm as well as incubated for 24 hours at 37°C. Every experiment was carried out three times. The non-appearance of turbidity in the broth indicates that at the lowest concentration, the methanol stem bark extract of *D. zibethinus* suppressed the bacterial inhibition, which was used to evaluate the MIC (Nava-Solis et al., 2022, Tankeshwar, 2022, Mokhtar et al., 2023).

Data Analysis

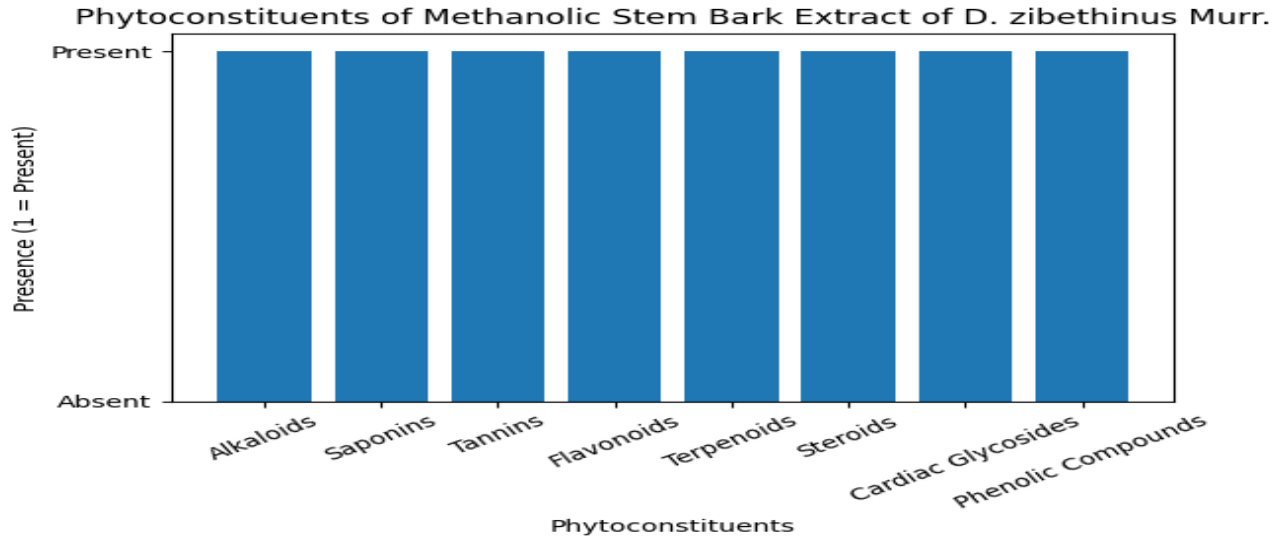
The experimental mean and standard deviation were determined. An analysis of variance (ANOVA) was used to check for significant differences ($p < 0.05$) in the antibacterial study.

Results

Phytochemical Analysis

Phytochemical Analysis Table 1 shows results of the phytoconstituents of the stem bark extract of D. zibethinus Murr. The secondary metabolites detected in the stem bark extract from D. zibethinus Murr, are given as follows: alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds.

Bar chart for Table 1 showing the **presence of all tested phytoconstituents**



Brief interpretation (for Results section)

The bar chart indicates the presence of all tested phytoconstituents in the methanolic stem bark extract from *D. zibethinus* Murr. Alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, and phenolic compounds were all detected, suggesting that the extract contains a wide range of bioactive compounds that may contribute to its observed antibacterial activity.

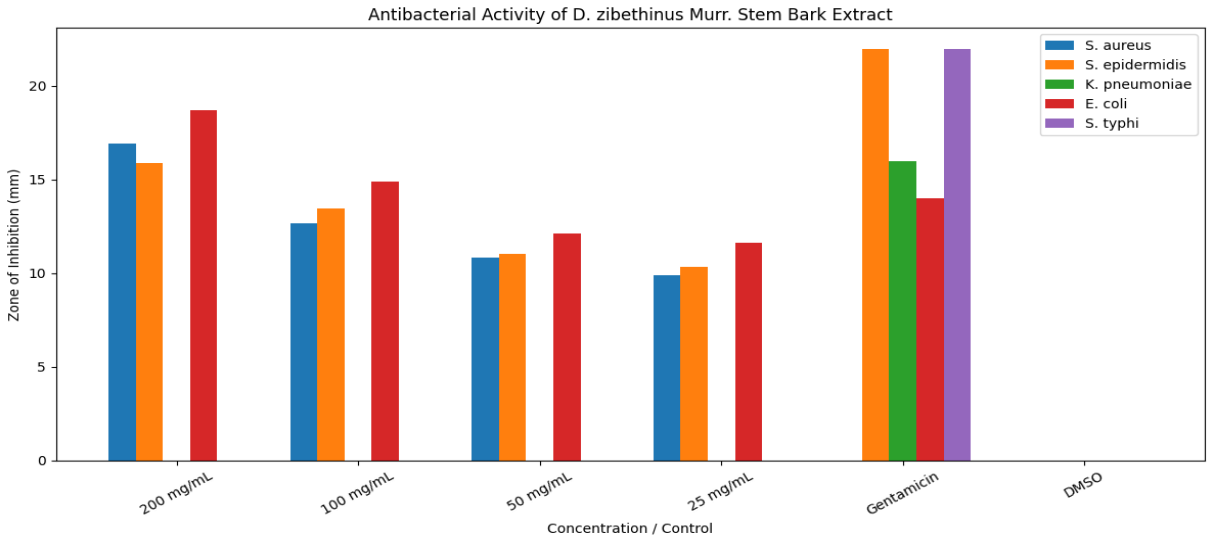
Table 2 illustrates results of in vitro antibacterial activity of the methanol stem bark extract from D. zibethinus Murr, against S. aureus (ATCC 25923), S. epidermidis (ATCC 12228), K. pneumonia (ATCC 27729), E. coli (ATCC 25922), and S. typhi (ATCC 13311). The stem bark extract demonstrated antibacterial activity against S. aureus (ATCC 25923), S. epidermidis (ATCC 12228), and E. coli (ATCC 25922) at the highest concentration of 200 mg/mL, with maximum inhibition zones of 16.92 ± 0.01 mm, 15.88 ± 0.01 mm, and 18.72 ± 0.01 mm, respectively. However, the D. zibethinus stem bark extract did not inhibit the growth of K. pneumonia (ATCC 27729) and S. Typhi (ATCC 13311).

Table 1. Phytoconstituents of methanolic stem bark extract of D. zibethinus Murr.

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 = -

The chart in Table 2 clearly shows the Antibacterial activity of *D. zibethinus* Murr. stem bark



Antibacterial activity of *D. zibethinus* Murr, stem bark

- **Dose-dependent activity** against *S. aureus*, *S. epidermidis*, and *E. coli*
- **No inhibitory effect (0 mm)** against *K. pneumoniae* and *S. typhi* at all extract concentrations
- **Gentamicin (positive control)** shows strong activity across susceptible organisms
- **DMSO (negative control)** shows no activity, confirming extract validity

Table 2. Antibacterial activity of *D. zibethinus* Murr. stem bark

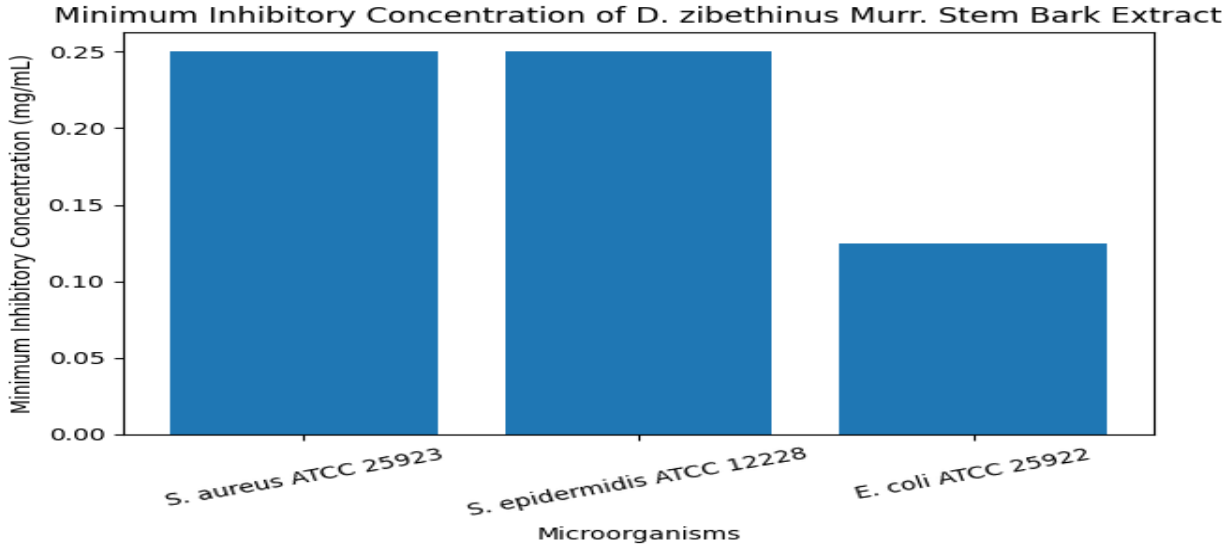
Diameter of zone of inhibition (mm) / Bacterial Strains

Concentration (mg/mL)	*S. aureus* ATCC 25923	*S. epidermis* ATCC 12228	*K. pneumonia* ATCC 27729	*E. coli* ATCC 25922	*S.* typhi ATCC 13311
200	16.92 ± 0.01	15.88 ± 0.01	0	18.72 ± 0.01	0
100	12.69 ± 0.01	13.48 ± 0.02	0	14.87 ± 0.01	0
50	10.85 ± 0.01	11.02 ± 0.01	0	12.11 ± 0.01	0
25	9.87 ± 0.01	10.34 ± 0.01	0	11.63 ± 0.01	0
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 growth

Minimum Inhibitory Concentration (MIC)

Bar chart showing the Minimum Inhibitory Concentration (MIC) of *D. zibethinus* Murr. stem bark extract against the three bacterial strains.



Results/Discussion

The bar chart shows that *S. aureus* and *S. epidermidis* had the same MIC value (0.250 mg/mL), indicating similar susceptibility to the extract, while *E. coli* exhibited a lower MIC (0.125 mg/mL), suggesting higher sensitivity to the methanolic stem bark extract.

Table 3 summarizes the results of minimum inhibitory concentration (MIC) of stem bark extract of *D. zibethinus* Murr. The result disclosed that the MIC for *S. epidermidis* and *S. aureus* was 0.250 mg/mL, whereas it was 0.125 mg/mL for *E. coli* respectively.

Table 3. Minimum inhibitory concentration of *D. zibethinus* Murr. stem bark

Microorganism	Minimum inhibitory concentration (mg/mL)
S. aureus ATCC 25923	0.250
S. epidermis ATCC 12228	0.250
E. coli ATCC 25922	0.125

Discussion

The results shown in Table 1 were in agreement with the reports of Mohiuddin (2019) as well as Adeniyi et al. (2024b). According to reports, secondary metabolites have been identified as antimicrobial compounds against a variety of microbes (Siburian et al., 2019, Hidayah et al., 2022, Sabir, 2005). This study demonstrated that the stem bark of *D. zibethinus* Murr. is a potential wellspring of antibacterial agents due to the presence of secondary metabolites like flavonoids, phenols, and alkaloids. This result aligns the studies of Adeniyi et al. (2024b) and Manurung et al. (2022).

According to the results in Table 2, methanol stem bark extract from *D. zibethinus* Murr, has shown antibacterial efficacy against every bacterium except *K. pneumonia* ATCC 27729 and *S. Typhi* ATCC 13311. According to the findings, the extract has stronger antibacterial properties against *S. aureus* ATCC 25923 (16.92 mm) and *E. coli* ATCC 25922 (18.72 mm), whereas the activity may be considered moderate against *S. epidermidis* ATCC 12228 (15.88 mm) in contrast to the commercially available antibiotic Gentamicin (0 mm, 14.00 mm and 22.00 mm respectively). The results obtained were in agreement with the report of Alkandahri et al., (2021) who examined the antibacterial efficacies of *D.* zibethinus Murr.

leaves and fruit against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus* bacteria. Although some researchers have reported the antibacterial properties of durian fruit skin flesh extract (Jamal et al., 2019) and the rinds extract durian (Fitrianingsih et al., 2019, Octiara et al., 2023). However, little or no report has been given on the antibacterial properties of D. zibethinus stem bark extract.

The MIC of the extract was determined for only bacteria that were inhibited as reported in Table 3. The results disclosed that the MIC for *S. epidermidis* and *S. aureus* were 0.250 mg/mL respectively, whereas it was 0.125 mg/mL for *E. coli*. To the best of our knowledge, little or no report has been recorded for MIC of D. zibethinus Murr. stem bark extract.

Conclusion

In our findings, the D. zibethinus Murr. stem bark extract possesses some essential phytochemicals, such as, alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds. These phytochemicals serve as the basis for the antibacterial properties of the extract against some bacterial strains. Additionally, the study also showed that the strongest antibacterial properties of the stem bark extract were noticed against *S. aureus* ATCC 25923, *S. epidermis* ATCC 12228, and *E. coli* ATCC 25922. The two most susceptible bacterial species were found to be *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

In recent time, researchers have reported the antibacterial properties of *D. zibethinus* Murr. against some bacterial strains; nevertheless, our study found that the stem bark extract had the strongest antibacterial activity. Hence, it is therefore suggested that stem bark extract of *D. zibethinus* Murr. could be a viable therapeutic agent as a substitute against bacterial diseases as antimicrobial medications are so costly these days. However, with the purpose of fully utilizing the antibacterial potential of stem bark of *D. zibethinus* Murr., it is imperative that the active components be isolated and thoroughly characterized. Such active compounds may be employed to develop novel, potent antimicrobial drugs.

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Conflict of Interest

No conflict of interest is declared.

Authors Contribution

Sunday Adegoke Adeniyi and Gabriel Ademola Olatunji planned and designed the research experiments, Olubunmi Stephen Oguntoye approved the final version of the article, Omolade Abiodun Akpa wrote the research article and Ezekiel Olatoye Solanke performed the research experiments.

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