Original Research Article

Phytochemical Properties *and In Vitro* Antimicrobial Activity of Methanolic Leaf Extract of*Durio zibethinus*Murr. on Selected Clinical Isolates

ABSTRACT

Bacterial and viral infections represent the most challenging diseases to treat in humans due to resistance to most of the therapeutic agents. The advent of drug resistance factors that have challenged the effectiveness of all antimicrobial agents prompted the investigation of antimicrobial activity studies of the methanolic leaf extract of *Durio zibethinus*Murr. This study is focused on evaluating the phytochemical and antimicrobial activity of the leaf extract of *D. zibethinus*Murr. on selected clinical isolates: *Staphylococcus aureus* and *Staphylococcus epidermidis* (gram-positive bacterial strains), *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella typhi* (gram-negative bacterial strains) while *Candida albicans* and *Microsporum audouinii* (fungi strains) using the standard agar disc diffusion technique. Alkaloids, saponins, flavonoids, terpenoids, steroids, glycosides, and phenolic chemicals have all been identified via phytochemical investigation. The result indicated that the leaf extract exhibited antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia, Candida albicans* and *Microsporum audouinii* at a range of 10.34–16.02 mm at a concentration of 150 mg/mL relative to standard gentamicin and fluconazole antibiotics. The minimum inhibitory concentration (MIC) for *S. aureus* was 0.250 mg/mL; for *E. coli, it* was 0.125 mg/mL; whereas it was 0.250 mg/mL for the two fungal strains, *C. albicans* and *M. audouinii*. Our investigation revealed that the *D. zibethinus*Murr. leaf extract exhibited the highest antibacterial activity against *S. aureus* and *E. coli* whereas the extract exhibited the highest antifungal activity against *M. audouinii*.Our research concluded that the Durio zibethinus Murr. leaf extract had potent antibacterial and antifungal properties, suggesting that it could be utilized to treat various pathogenic diseases.

Keywords: Durio zibethinus.; antibacterial; antifungal; antibiotics.

1. INTRODUCTION

Antimicrobial agents play acrucial in reducing the global epidemic of transmissible diseases [1, 2]. But the excessive usage of antimicrobial agents has a negative impact on the environment, the ecosystem, and the overall well-being of people. It could also raise the frequency 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 for public health. [4, 5].

As a result of the rapid global growth 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 quantity of these plants is medicinal and have been reported as

important sources of naturally occurring antimicrobial compounds that may be used as alternative therapies that can be successful in treating these troubling bacterial infections. [10, 11].

*Durio zibethinus*Murr.is one the medicinal plants that can be used to overcome the problem of antibiotic resistance to bacteria. *Durio zibethinus*Murr. (Family Bombacaceae) is a type of tropical fruit plant called durian that has been widely cultivated in Malaysia and other Southeast Asian countries. Researchers have reported that antioxidant, anticancer, antidiabetic, anti-lipoxygenase, anti-heart disease, and anti-obesity properties can be attributed to durian [12, 13]. One of the purported medicinal and therapeutic benefits of durian fruit is its capacity to fortify the immune system. Its fruit pulp may be a reliable source of fiber, dietary fat, proteins, and carbohydrates. [14]. It has also been revealed that durian seed, pulp, and peel flour are endowed with nutritional, structural, anti-inflammatory, and antioxidant properties [15, 16]. Taking into consideration the tremendouspotential of plants as sources for antimicrobial drugs, this study aimed at investigating the *in vitro* antibacterial and antifungal activity of extracts from *D. zibethinus*Murr. against some selected clinical isolates.

2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

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

2.2 Preparation and Extraction of Plant Materials

Plant extract was prepared in accordance with the methods described by Ibrahim and Kebede [17], with minor modifications. After the fresh plant material was carefully cleaned and washed with distilled water, it was allowed to air dry at room temperature (26°C) for several weeks. An electrical grinder was then used to grind the material into a uniform powder. 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 filtrate was then concentrated in a vacuum using Rotary Evaporator at 30-40 °C. For three to four days, the methanol extract was kept at room temperature in a labeled vial to allow the solvents to evaporate. After that, the dried extract was place in a sterile bottle and refrigerated until further use.

2.3 List of Bacteria and Fungi Strains

The strains of bacteria and fungi used include Staphylococcus aureus, Staphylococcus epidermidis, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, and *Microsporum audouinii*. All the bacteria and fungi are clinical strains, obtained from Department of Microbiology &Parasitology,Igbinedion University Teaching Hospital, Okada, Nigeria.

2.4 Antibacterial Bioassay

*D. zibethinus*Murr. leaf extract was screened for antibacterial activities using two Gram-positive bacteria strains (*Staphylococcus aureus*and*Staphylococcus epidermis*) and three Gram-negative bacteria strains (*Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhi*) that are clinical strains obtained from the Department of Microbiology and Parasitology,Igbinedion University Teaching Hospital, Okada, Nigeria.

The agar diffusion procedure described by Asmerom *et al*. [18] was undertaken to test antibacterial activity of the extract, with minor modifications. The bacterial strains that were preserved in nutrient agar slant at 4 °C were allowed to grow in 50 mL of nutrient broth at 37 °C. The suspension culture of the five bacterial strains that was left overnight was then spread on the Mueller-Hinton agar (MHA) plate in a

100 mm diameter sterile petri dish. The thick lawn growth of seeded media was then dry at ambient 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 150 mg/mL, 125 mg/mL, 100 mg/mL, 75 mg/mL, 50 mg/mL, and 25 mg/mL of the solutions of the plant extract dissolved in 1% dimethyl sulfoxide (DMSO) and filled into the corresponding wells. The commercial antibiotic gentamicin (30 µL) was utilized as a positive control to determine the activity of the bacterial strains. The plates were placed in an incubator after being left undisturbed for almost 2 hours at ambient temperature to allow them diffuse onto the inoculated agar. The inoculated plates were then cultured for 24 hours at 37 ^{o}C. Using a metal caliper, the diameter of the zone of inhibition was measured and recorded in millimeters (mm).The experiment was performed in triplicate for each bacterium. The average zone of inhibition for both the standard antibiotics and each test sample were calculated

2.5 Antifungal Bioassay

*D. zibethinus*Murr. leaf extract was screened for antifungal activities using two fungi strains, that is, *Candida albicans*and*Microsporum audouinii* (both clinical strains) obtained from the Department of Microbiology and Parasitology,Igbinedion University Teaching Hospital, Okada, Nigeria.

The agar diffusion procedure described by Asmerom *et al*. [18] was undertaken to test antifungal activity of the extract, with minor modifications.The fungal strains that were preserved in nutrient agar slant at 4 °C were allowed to grow in 50 mL of nutrient broth at 37 °C.The suspension culture of the two fungal strains that was left overnight was then spread on the Mueller-Hinton agar (MHA) plate in a 100 mm diameter sterile petri dish. The thick lawn growth of seeded media was then dry at ambient 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 150 mg/mL, 125 mg/mL, 100 mg/mL, 75 mg/mL, 50 mg/mL, and 25 mg/mL of the solutions of the plant extract dissolved in 1% dimethyl sulfoxide (DMSO) and filled into the corresponding wells. The commercial antibiotic fluconazole (50 µL) was utilized as a positive control to determine the activity of the fungal strains. The plates were placed in an incubator after being left undisturbed for almost 2 hours at ambient temperature to allow them diffuse onto the inoculated agar. The inoculated plates were then cultured for 24 hours at 37 ^{o}C.Using a metal caliper, the diameter of the zone of inhibition was measured and recorded in millimeters (mm).The experiment was performed in triplicate for each bacterium.The average zone of inhibition for both the standard antibiotics and each test sample were calculated.

2.6 Minimum Inhibitory Concentration Assay (MIC)

The method described by Jensen et al. [19] was adopted with minor modifications. Into sterile test tubes was placed 1 mL of nutrient broth. Into one tube, 1 mL of the 1 mg/mL test solution was transferred and serially diluted, ranging from 0.05 mg/mL to 0.75 mg/mL.After that, 0.1 mL of inoculum was inoculated, and the tubes were then culturedfor 24 hours at 37 ^{o}C. The formation of turbidity in the tubes was observed visually. The MIC was determined by considering the lowest concentration of *Durio zibethinus*Murr. methanolic extract that inhibited bacterial and fungal growth, which was established by the absence of turbidity in the broth [21, 22].

2.7 Statistical Analysis

An analysis of variance (ANOVA) was performed to test for significant differences ($p < 0.05$) after determining the mean and standard deviation of the experiments.

3. RESULTS AND DISCUSSION

The results of the antimicrobial and phytochemical properties of the leaf extract of *Durio zibethinus*Murr are shown in Tables 1–4. The secondary metabolites detected in *Durio zibethinus*Murr. leaf extract can be seen in Table 1, which shows that the leaves contain alkaloids, saponins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds. From the results, it was shown that the leaf extract did not contain any tannins. This variation may be due to environmental conditions, which can alter the phytoconstituents of the plant leaves [23]. According to reports, secondary metabolites are known to be antimicrobial compounds against a wide range of microbes [24, 25, 26]. This study showed that *Durio zibethinus*Murr. leaf 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. [27], who reported the existence of alkaloids in the durian leaf, but contradicts the findings of Siburian et al. [24], who gave an account that the durian leaf did not contain any alkaloids.

Table 1. Content of secondary metabolites of the methanolic leaf extract of *Durio zibethinus*Murr.

No	Test type	Reactor	Result	Information
1.	Alkaloid	Mayer Dragendorff's	Orange precipitate formed Reddish-brown precipitate formed	+ +
2.	Saponin	Liquefied in purified water	Honey comb foam formed	+
3.	Tannin	Ferric chloride solution	No colouration formed	-
4.	Flavonoid	Dil. NaOH + HCl	Yellow solution that turned colourless	+
5.	Terpenoid	Salkowski's test	Reddish-brown colouration at the interface	+
6.	Steroid	Lieberman's test	No colour changes from purple to blue to green	+
7.	Glycoside	Keller-Killiani's test	Formation of greenish ring on top of brown ring and below is violet ring	+
8.	Phenolic compound	Liquefied in purified water + drops of 1% lead acetate	Bulky white precipitate formed	+

Presence = +; absence = -

The antibacterial and antifungal assays of *Durio zibethinus*Murr. leaf was tested against five clinical bacterial strains and two clinical fungal strains. Among the bacterial strains tested, two are gram-positive and three are gram-negative. The results illustrated in Table 2 revealed the antibacterial activity of the methanolic leaf extract of *D. zibethinus*Murr. From the results, the methanolic leaf extract exhibited antibacterial activity against *S. aureus* and *E. coli* with maximum inhibition zones of 16.02 ± 0.02 mm, 14.07 ± 0.02 mm at the highest concentration of 150 mg/mL, respectively, while the growth of other bacteria was not inhibited by this extract. The results obtained for the *D. zibethinus*Murr. methanolic leaf extract against *E. coli* suggest that the antimicrobial activity may be favorably matched with the commercially available antibiotic Gentamicin (14.00 mm). The results obtained were in agreement with the report of Jamal et al. [28], who tested the antibacterial activities of *D. zibethinus*Murr. against *Salmonella* and *Bacillus cereus* bacteria.Although some researchers have reported the antibacterial activityof durian fruit skin fleshextract [29] and durian rindsextract [30, 31] but little or no work has been reported for antibacterial of *D. zibethinus*leaf extract.

Table 2. Antibacterial activity of *Durio zibethinus*Murr. leaves

Concentrations (mg/mL)	Diameter of zone of inhibition (mm)/ Bacterial Strains				
	S. aureus	***S. epidermidis***	***K. pneumonia***	***E. coli***	***S. typhi***
150	16.02 ± 0.02	0	0	14.07 ± 0.02	0

125	15.67 ± 0.01	0	0	13.02 ± 0.02	0
100	14.23 ± 0.01	0	0	11.85 ± 0.01	0
75	13.94 ± 0.02	0	0	10.63 ± 0.01	0
50	13.46 ± 0.02	0	0	10.39 ± 0.01	0
25	12.24 ± 0.02	0	0	9.83 ± 0.03	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

The results illustrated in Table 3 revealed the antifungal activity of the methanolic leaf extract of *D. zibethinus*Murr. From the results, the methanolic leaf extract exhibited antifungal activity against *C. albicans* and *M. audouinii,* with maximum inhibition zones of 10.34 ± 0.01 mm, 10.67 ± 0.01 mm at the highest concentration of 150 mg/mL, respectively. The results obtained for the *D. zibethinus*Murr. methanolic leaf extract against *C. albicans* suggest that the antifungal activity may not be considered moderate when compared with the commercially available antibiotic Fluconazole drug of 20.00 mm, but the antifungal activity of the leaf extract against *M. audouinii* may be expressed as moderate when compared with the commercially available Fluconazole drug of 14.00 mm. However, the results indicated that the inhibition of bacteria and fungi depend on the concentration of the extract, which suggests that as the concentration of the extract increases the quantity of secondary metabolites contained in the test sample increases. As far as we are aware, there has been little to no research done on the antifungal activity of *D. zibethinus*Murr. leaf extract against fungal strains.

Table 3. Antifungal activity of *Durio zibethinus*Murr. leaves

Concentrations (mg/mL)	**Diameter of zone of inhibition (mm)/ Fungal Strains**	
	C. albicans	***M. audouinii***
150	10.34 ± 0.01	10.67 ± 0.01
125	10.12 ± 0.01	9.97 ± 0.01
100	9.76 ± 0.01	9.12 ± 0.01
75	8.95 ± 0.01	8.77 ± 0.01
50	8.04 ± 0.01	8.06 ± 0.01
25	7.56 ± 0.01	7.48 ± 0.01
Fluconazole (+)	20.00	14.00
DMSO (-)	0	0

Values are expressed in mean ± SD (n=3). SD: Standard Deviation. 0 = no growth

The minimum inhibitory concentration (MIC) results are presented in Table 4. The results disclosed that the MIC for *S. aureus* was 0.250 mg/mL and for *E. coli* was 0.125 mg/mL, whereas it was 0.250 mg/mL for the two fungal strains, *C. albicans* and *M. audouinii*. The results obtained were in contravention of the report of Chigurupati et al. [29], who determined the minimum inhibitory concentration (MIC) of some Gram-positive and Gram-negative bacteria using D. zibethinus Murr. ethanolic leaf extract. This disparity could be due to the solvent used for extraction because methanol is known to be more polar than ethanol, so it might be possible to extract more polar secondary metabolites that have antifungal and antibacterial properties.

Table 4. Minimum inhibitory concentration (MIC) of *Durio zibethinus*Murr. leaves

Microorganisms	**MIC (**mg/mL**)**
S. aureus	0.250
E. coli	0.125
C. albicans	0.250
M. audouinii	0.250

4. CONCLUSION

According to this study, the *D. zibethinus*Murr. leaf extract possesses some essential phytochemicals, such as, alkaloids, saponins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds. These phytochemicals serve as the basis for the antibacterial and antifungal activities of the extract against some clinical isolates. The study also revealed that the leaf extract exhibited the highest antimicrobial activity against *S. aureus*, *E. coli*, *K. pneumonia, C. albicans* and *M. audouinii*. Hence,it is therefore suggested that *D. zibethinus*Murr. leaf extract could be a viable therapeutic agent as an alternative against bacterial and fungal diseases as the antimicrobial medications are so costly these days.However, with the aim of fully utilizing the antimicrobial potential *of D. zibethinus*Murr. leaf, it is imperative that the active components be isolated and thoroughly characterized. Such active compounds can be utilized to develop novel, potent antimicrobial drugs.

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