

## Original Research Article

### **Phytochemical Properties and In Vitro Antimicrobial Activity of Methanolic Leaf Extract of *Duriozibethinus* 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 pneumoniae*, *Escherichia coli*, *Salmonella typhi* (gram-negative bacterial strains) while *Candida albicans* and *Microsporium 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 pneumoniae*, *Candida albicans* and *Microsporium 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 a crucial role 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 of 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 tremendous potential 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.

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## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Authentication

The plant material, *Durio zibethinus* Murr. leaves, were collected in August, 2023 at 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 placed 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 pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, and

*Microsporium 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

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*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 °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

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*D. zibethinus* Murr. leaf extract was screened for antifungal activities using two fungi strains, that is, *Candida albicans* and *Microsporium 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 °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 cultured for 24 hours at 37 °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
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1.	Alkaloid	Mayer Dragendorff's	Orange precipitate formed Reddish-brown precipitate formed	+
2.	Saponin	Liquefied in purified water	Honey combfoam 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  $16.02 \pm 0.02$  mm,  $14.07 \pm 0.02$  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.

**Comment [R4]:** Add some of references extra to discussion part

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

Concentrations (mg/mL)	Diameter of zone of inhibition (mm)/ Bacterial Strains				
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>K. pneumonia</i>	<i>E. coli</i>	<i>S. typhi</i>
150	$16.02 \pm 0.02$	0	0	$14.07 \pm 0.02$	0
125	$15.67 \pm 0.01$	0	0	$13.02 \pm 0.02$	0
100	$14.23 \pm 0.01$	0	0	$11.85 \pm 0.01$	0
75	$13.94 \pm 0.02$	0	0	$10.63 \pm 0.01$	0
50	$13.46 \pm 0.02$	0	0	$10.39 \pm 0.01$	0
25	$12.24 \pm 0.02$	0	0	$9.83 \pm 0.03$	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  $10.34 \pm 0.01$  mm,  $10.67 \pm 0.01$  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	
	<i>C. albicans</i>	<i>M. audouinii</i>
150	$10.34 \pm 0.01$	$10.67 \pm 0.01$
125	$10.12 \pm 0.01$	$9.97 \pm 0.01$
100	$9.76 \pm 0.01$	$9.12 \pm 0.01$
75	$8.95 \pm 0.01$	$8.77 \pm 0.01$
50	$8.04 \pm 0.01$	$8.06 \pm 0.01$
25	$7.56 \pm 0.01$	$7.48 \pm 0.01$
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)
<i>S. aureus</i>	0.250
<i>E. coli</i>	0.125
<i>C. albicans</i>	0.250
<i>M. audouinii</i>	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. Because antimicrobial medications are so costly these days, it is therefore suggested that *D. zibethinus* Murr. leaf extract could be a viable therapeutic agent as an alternative against bacterial and fungal diseases. 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.

**Comment [R5]:** Mention the benefits of plant extracts affect in different microorganisms studies in this paper

#### REFERENCES

1. Baker RE, Mahmud AS, Miller IF, Rajeev M, Rasambainarivo F, Rice BL, Takahashi S, Tatem AJ, Wagner CE, Wang Lin-Fa, Amy Wesolowski A, Metcalf CJE. Infectious disease in an era of global change. *Natural Reviews Microbiology*. 2022; 20:193–205. Available: <https://doi.org/10.1038/s41579-021-00639-z>
2. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MAA. Antimicrobial resistance: A growing serious threat for global public health. *Healthcare (Basel)*. 2023; 11(13):1946. Available: <https://doi: 10.3390/healthcare11131946>.
3. Pulingam T, Parumasivam T, Gazzali AM, Sulaiman AM, Chee JY, Lakshmanan M, Chin CF, Sudesh K. Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. *European Journal of Pharmaceutical Sciences*. 2022; 170:106103. Available: <https://doi: 10.1016/j.ejps.2021.106103>.
4. Cameron A, Esiovwa R, Connolly J, Hursthouse A, Henriquez F. Antimicrobial resistance as a global health threat: The need to learn lessons from the COVID-19 pandemic. *Global Policy*. 2022; 13:179–192. Available: <https://doi.org/10.1111/1758-5899.13049>
5. Walsh TR, Gales AC, Laxminarayan R, Dodd PC. Antimicrobial resistance: Addressing a global threat to humanity. *PLoS Medicine*. 2023; 20(7):e1004264.

Available:<https://doi.org/10.1371/journal.pmed.1004264>

6. Emerald C, Mahamat A, Jousset AB, Bernabeu S, Goncalves T, Pommier C, Girlich D, Birer A,

Rodriguez C, Pawlotsky JM, Naas T, Bonnin RA, Dortet L. Emergence and rapid dissemination of

highly resistant NDM-14-producing *Klebsiella pneumoniae* ST147, *Eurosurveillance*. 2023; 28(42):2300095. Available:<https://doi.org/10.2807/1560-7917.ES.2023.28.42.2300095>.

7. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, Shah T, Chaudhry TH,

Rasool MH, Shahid A, Xueshan X, Baloch Z. Antibiotic resistance: One health oneWorld outlook.

*Frontiers in Cellular and Infection Microbiology*. 2021; 11:771510.

Available:<https://doi.org/10.3389/fcimb.2021.771510>

8. Hossaini F, Das NC, Hossaini F, Acharjee M, Munshi SK. Antimicrobial traits of different medicinal

plants locally available in Bangladesh. *Biomedical and Biotechnology Research Journal*. 2021; 5:1-6.

Available:[https://doi.org/10.4103/bbrj.bbrj\\_198\\_20](https://doi.org/10.4103/bbrj.bbrj_198_20)

9. Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, Marquez L, Salam AM, Shabih S,

Farrokhi DR, Quave CL. A systematic review of plants with antibacterial activities: A taxonomic and

phylogenetic perspective. *Frontiers in Pharmacology*. 2021; 11:586548.

Available:<https://doi.org/10.3389/fphar.2020.586548>

10. Alaoui Mdarhri H, Benmessaoud R, Yacoubi H, Seffar L, Guennouni Assimi H, Hamam M, Boussettine R, Filali-Ansari N, Lahlou FA, Diawara I, Ennaji MM, Kettani-Halabi M. Alternatives

therapeutic approaches to conventional antibiotics: Advantages, Limitations and Potential application in Medicine. *Antibiotics (Basel)*. 2022; 11(12):1826.

Available:<https://doi.org/10.3390/antibiotics11121826>.

11. Iseppi R, Sabia C. Editorial: Natural substances: A new weapon against antibiotic-resistant bacteria

in the clinical and veterinary field. *Frontiers in Microbiology*. 2022; 13:987615.

Available:<https://doi.org/10.3389/fmicb.2022.987615>.

12. Siburian R, Aruan DGR, Barus T, Haro G, Simanjuntak P. Phytochemical screening and antidiabetic activity of n-hexane, ethyl acetate and water extract from Durian leaves (*Durio zibethinus* L.), *Oriental Journal of Chemistry*. 2019; 35(1):487-490.

Available:<http://dx.doi.org/10.13005/ojc/350166>

13. Adeniyi SA, Oyeku JS, Owan T. GC-MS analysis and biological roles of phytochemical compounds in n-Hexane extract of *Durio zibethinus* Murr. seeds. *South Asian Research Journal of Natural Products*. 2024; 7(1):9-19.

14. Adeniyi SA, Oke VJ, Olatunji GA. GC-MS analysis of phytochemical constituents in methanol extract

of wood bark from *Durio zibethinus* Murr. *International Journal of Medicinal Plants and Natural Products*. 2019; 5(3):1-11. Available:<http://dx.doi.org/10.20431/2454-7999.0503001>

15. Charoenphun N, Klangbud WK. Antioxidant and anti-inflammatory activities of Durian (*Durio zibethinus* Murr.) seed, pulp and peel flour. *Peer J*. 2022; 10:e12933. Available:<https://doi.org/10.7717/peerj.12933>
16. Permatasari ND, Witoyo JE, Masruri M, Yuwono SS, Widjanarko SB. Nutritional and structural properties of Durian seed (*Durio zibethinus* Murr.) flour originated from West Kalimantan, Indonesia. *IOP Conference Series: Earth and Environmental Science*. 2022; 1012:012038. Available:<https://doi.org/10.1088/1755-1315/1012/1/012038>
17. Ibrahim N, Kebede A. *In vitro* antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria. *Saudi Journal of Biological Sciences*. 2020; 27(9):2261-2268. <https://doi.org/10.1016/j.sjbs.2020.06.047>
18. Asmerom D, Kalay TH, Tafere GG. Antibacterial and antifungal activities of the leaf exudate of *Aloe megalacantha* Baker. *International Journal of Microbiology*. 2020; 2020(8840857):6 pages. <https://doi.org/10.1155/2020/8840857>
19. Jensen CH, Vestergaard M, Dalsgaard A, Frees D, Leisner JJ. Nisin damages the septal membrane and triggers DNA condensation in methicillin—Resistant *Staphylococcus aureus*. *Frontiers in Microbiology*. 2020; 11:1007.
20. Nava-Solis U, Rodriguez-Canales M, Hernandez-Hernandez AB, Velasco-Melgoza DA, Moreno-Guzman BP, Rodriguez-Monroy MA, Canales-Martinez MM. Antimicrobial activity of the methanolic leaf extract of *Prosopis laevigata*. *Scientific Reports*. 2022; 12(20807):10 pgs. <https://doi.org/10.1038/s41598-022-25271-6>
21. Tankeshwar A. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). *Microbeonline Journal*. 2022; 2–6.
22. Mokhtar LM, Salim IA, Alotaibi SN, Awaji EA, Alotaibi MM, Doman AO. Phytochemical screening and antimicrobial activity of methanolic extract of *Cymbopogon schoenanthus* (L.) (azkhar) collected from Afif City, Saudi Arabia. *Life (Basel)*. 2023; 13(7):1451. doi: 10.3390/life13071451.
23. Mohiuddin AK. Impact of various environmental factors on secondary metabolism of medicinal plants. *Journal of Pharmacology and Clinical Research*. 2019; 7(1):555704. DOI: [10.19080/JPCR.2019.07.555704](https://doi.org/10.19080/JPCR.2019.07.555704)
24. Siburian R, Aruan DGR, Barus T, Haro G, Simanjuntak P. Phytochemical Screening and antidiabetic activity of n-hexane, ethyl acetate and water extract from Durian leaves (*Durio zibethinus* L.). *Oriental Journal of Chemistry*. 2019; 35(1):487-490.
25. Hidayah N, Nurdin GM, Ameliani NA. Antibacterial activity of Acacia leaf extract (*Acacia auriculiformis*) on the growth of *Bacillus* sp., *Staphylococcus aureus* and *Escherichia coli* bacteria.

Bionature. 2022; 23(2):126. Available:<https://doi.org/10.35580/bionature.v23i2.37593>

26. Sabir A. Antibacterial activity of flavonoids *Trigona sp. propolis* against *Streptococcus mutans*

(*In-vitro*).Dental Journal (Majalah Dentistry). 2005;38(3):135.

Available:<https://doi.org/10.20473/j.djmk.v38.i3.p135-141>

27. Manurung H, Susanto DWI, Kusumawati EKO, Aryani R, Nugruho RA, Ratna Kusuma R, Rahmawati Z, Sari RD. Phytochemical, GC-MS analysis and antioxidant activities of leaf methanolic

extract of Lai (*Durio kutejensis*), the endemic plant of Kalimantan, Indonesia. Biodiversitas. 2022;

23(11):5566-5573. DOI: 10.13057/biodiv/d231104.

28. Jamal KP, Muhaimin, Fitrianiingsih. Antibacterial activities of ethanol extracts of durian fruitskin

(*Durio zibethinus* Murr.) on *Salmonella bacteria* in ATCC 14028 and *Bacillus cereus* ATCC11778

cause of diarrhea. Indonesian Journal of Pharma Science. 2019; 1(1):1-6.

29. Chigurupati S, Mohammad JI, Vijayabalan S, Vaipuri ND, Selvarajan KK, Nemala AR. Quantitative

estimation and antimicrobial potential of ethanol extract of *Durio zibethinus*Murr. leaves. Asian

Journal of Pharmaceutical and Clinical Research. 2017; 10(9):251-254.

DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i9.19767>

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