International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Website: https://ijmpr.in/

Research Article

Antibacterial Screening of Hylocereus Undatus (Dragon Fruit) Ethanolic Leaf Extract against Escherichia Coli and Staphylococcus Aureus

Dr. Darshan Karmanbhai Bharvad[1], Dr. Vasantkumar Chaudhari[2], Dr. Vishal Bhupendra Parmar[3]

[1]MBBS
[2]MBBS, Junior Doctor, GMERS Medical College and Hospital, Dharpur-Patan, Gujarat, India
[3]MBBS, Consultant Medical officer, Adani green energy plant, Khavda-Bhuj, Gujarat

OPEN ACCESS

Corresponding Author:

Dr. Darshan Karmanbhai Bharvad
MBBS.

Received: 21-11-2025
Accepted: 21-12-2025
Available online: 31-12-2025

ABSTRACT

Background: The rapid emergence of antimicrobial resistance has created an urgent need to explore alternative antibacterial agents from natural sources. Medicinal plants are gaining increasing attention due to their bioactive phytochemicals and lower risk of resistance development. *Hylocereus undatus* (dragon fruit) is widely cultivated in India, but its leaves remain underexplored for antibacterial potential.

Objectives: The present study aimed to evaluate the in-vitro antibacterial activity of ethanolic leaf extract of *Hylocereus undatus* against *Escherichia coli* and *Staphylococcus aureus*.

Methods: This in-vitro experimental study was conducted at a tertiary care center in North Gujarat, India, over a period of one year. Ethanolic leaf extract of *Hylocereus undatus* was prepared using standard extraction methods. Antibacterial activity was assessed by the agar well diffusion method against standard strains of *E. coli* and *S. aureus*. A total of 50 observations were analyzed using different extract concentrations, along with appropriate positive and negative controls. Zones of inhibition were measured and statistically analyzed.

Results: The ethanolic leaf extract exhibited a concentration-dependent antibacterial effect against both test organisms. The mean zone of inhibition against *E. coli* increased from 9.4 ± 1.3 mm at 25 mg/mL to 16.6 ± 1.8 mm at 100 mg/mL, while *S. aureus* showed higher susceptibility with zones increasing from 11.8 ± 1.5 mm to 20.1 ± 2.0 mm across the same concentration range. The difference in antibacterial activity between the two organisms was statistically significant at all concentrations, with maximum significance observed at higher doses ($p < 0.001$).

Conclusion: The study demonstrates that ethanolic leaf extract of *Hylocereus undatus* possesses significant antibacterial activity, particularly against *Staphylococcus aureus*. These findings highlight the potential of dragon fruit leaves as a promising natural antibacterial agent and warrant further phytochemical and in-vivo investigations.

Keywords: *Hylocereus undatus*, *Dragon fruit, Ethanolic leaf extract, Antibacterial activity, Escherichia coli, Staphylococcus aureus, Medicinal plants.*

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global public health challenges of the 21st century, threatening the effective prevention and treatment of an ever-increasing range of infections. The World Health Organization (WHO) has repeatedly highlighted that common bacterial pathogens are rapidly developing resistance to first-line and even last-resort antibiotics, leading to prolonged illness, increased healthcare costs, and higher mortality rates worldwide [1,2]. In low- and middle-income countries like India, the burden of resistant infections is further amplified due to high antibiotic consumption, over-the-counter availability, and limited antimicrobial stewardship practices [3].

Among the clinically important bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus* are of particular concern. *E. coli* is a leading cause of urinary tract infections, septicemia, and gastrointestinal infections, while *S. aureus* is responsible for a wide spectrum of diseases ranging from skin and soft tissue infections to life-threatening conditions such as pneumonia, endocarditis, and sepsis [4,5]. The increasing prevalence of multidrug-resistant strains, including extended-spectrum β-lactamase (ESBL)-producing *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA), has significantly limited therapeutic options and necessitated the search for alternative antimicrobial agents [6].

In recent years, medicinal plants have gained renewed scientific interest as potential sources of novel antimicrobial compounds. Plant-derived bioactive molecules such as flavonoids, phenolic acids, alkaloids, tannins, and terpenoids have demonstrated broad-spectrum antibacterial activity with comparatively fewer side effects and a lower tendency to induce resistance [7]. Traditional medicine systems across Asia, Africa, and Latin America have long relied on plant extracts for the treatment of infectious diseases, and modern pharmacological research increasingly supports these ethnomedicinal claims [8].

Hylocereus undatus (dragon fruit), a cactus species belonging to the family Cactaceae, is widely cultivated in tropical and subtropical regions, including India, due to its nutritional and economic value. While the fruit pulp has been extensively studied for its antioxidant, anti-inflammatory, and metabolic benefits, the leaves and other vegetative parts remain relatively underexplored despite being rich in phytochemicals such as polyphenols, flavonoids, and betalains [9]. Preliminary studies suggest that extracts from *Hylocereus* species possess antimicrobial and antioxidant properties, indicating their potential role as natural therapeutic agents [10].

Considering the escalating problem of antimicrobial resistance and the need for alternative, plant-based antibacterial agents, the present study was undertaken to evaluate the antibacterial activity of ethanolic leaf extract of *Hylocereus undatus* against two clinically significant bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus*. This investigation aims to contribute to the growing body of evidence supporting the use of medicinal plants as promising candidates in the development of novel antibacterial therapies.

The present study aims to evaluate the antibacterial activity of the ethanolic leaf extract of *Hylocereus undatus* (dragon fruit) against clinically important gram-negative and gram-positive bacteria, namely *Escherichia coli* and *Staphylococcus aureus*. The objectives include preparation of the ethanolic leaf extract, assessment of its in-vitro antibacterial efficacy against the selected pathogens, and comparison of its inhibitory effect on gram-negative versus gram-positive organisms to understand its antibacterial spectrum. The outcomes of this study are expected to generate preliminary scientific evidence on the antibacterial potential of *Hylocereus undatus* leaves, which may serve as a basis for further phytochemical characterization, development of plant-based antimicrobial agents, and future in-vivo and formulation-based research, particularly in the context of increasing antimicrobial resistance.

METHODOLOGY

This in-vitro experimental study was conducted at a tertiary care center of North Gujarat, India, over a period of one year with the objective of evaluating the antibacterial activity of ethanolic leaf extract of *Hylocereus undatus* against selected bacterial pathogens. The study included standard laboratory strains of *Escherichia coli* and *Staphylococcus aureus* obtained from a certified microbiology laboratory. Only well-characterized, viable bacterial isolates showing optimal growth on routine culture media were included in the study, while contaminated cultures, poorly growing strains, or organisms other than the selected gram-negative and gram-positive bacteria were excluded. Plant material selection criteria included fresh, disease-free leaves of *Hylocereus undatus*, whereas damaged, infected, or dried plant material was excluded to maintain extract quality and consistency.

The sample size for the study was fixed at 50 based on feasibility, laboratory capacity, and reference to similar preliminary antibacterial screening studies reported in the literature. These 50 observations included multiple test concentrations of the ethanolic leaf extract tested against both bacterial organisms, along with appropriate controls, allowing adequate assessment of antibacterial activity and reproducibility of results. As this was an exploratory laboratory-based screening study rather than a clinical trial, a formal power-based sample size calculation was not applied; instead, a pragmatic sample size approach was adopted to generate baseline evidence.

Data collection involved systematic preparation of the plant extract and standardized microbiological testing procedures. Fresh leaves of *Hylocereus undatus* were washed, shade-dried, powdered, and subjected to ethanol extraction using standard solvent extraction techniques. The crude extract obtained after evaporation was stored under controlled conditions until use. Antibacterial activity was assessed using the agar well diffusion method. Standardized inoculum of each bacterial strain, adjusted to McFarland turbidity standards, was spread uniformly on Mueller–Hinton agar plates. Wells were punched aseptically, and predetermined concentrations of the ethanolic extract were introduced into the wells. Standard antibiotics were used as positive controls, and ethanol or distilled water served as negative controls. Plates were incubated at 37°C for 18–24 hours, after which zones of inhibition were measured in millimeters using a calibrated scale. All tests were performed under strict aseptic conditions, and observations were recorded systematically.

Data analysis was primarily descriptive in nature. The antibacterial activity of the ethanolic leaf extract was evaluated by measuring and comparing the mean zones of inhibition produced against *Escherichia coli* and *Staphylococcus aureus* at different concentrations. Results were tabulated and interpreted to assess the relative susceptibility of gram-negative and gram-positive organisms. Findings were compared with control groups to determine the effectiveness of the plant extract. The analyzed data were presented in the form of tables and graphical representations to clearly demonstrate antibacterial efficacy and trends, providing a scientific basis for further phytochemical and pharmacological investigations.

RESULT

In the present study, the antibacterial activity of the ethanolic leaf extract of *Hylocereus undatus* was evaluated against *Escherichia coli* and *Staphylococcus aureus* using 50 laboratory observations. Among the tested organisms, *E. coli* constituted 26 (52.0%) samples, while *S. aureus* constituted 24 (48.0%) samples. The ethanolic leaf extract demonstrated a clear concentration-dependent antibacterial effect against both organisms.

Against *Escherichia coli*, the mean zone of inhibition increased from **9.4 ± 1.3 mm** at 25 mg/mL to **12.9 ± 1.6 mm** at 50 mg/mL and further to **16.6 ± 1.8 mm** at 100 mg/mL. In comparison, *Staphylococcus aureus* exhibited larger zones of inhibition at all concentrations, with mean values of **11.8 ± 1.5 mm** at 25 mg/mL, **15.6 ± 1.7 mm** at 50 mg/mL, and **20.1 ± 2.0 mm** at 100 mg/mL. The standard antibiotic control produced zones of inhibition ranging from **21 to 27 mm**, indicating that the plant extract showed appreciable antibacterial activity, particularly at higher concentrations.

Statistical comparison between the two organisms revealed significant differences in antibacterial activity at all tested concentrations. At 25 mg/mL, the difference was statistically significant (p = **0.031**), while at 50 mg/mL the significance increased (p = **0.009**). The highest concentration of 100 mg/mL showed a highly significant difference between the organisms (p<**0.001**). Percentage-based graphical analysis demonstrated that the antibacterial activity of the extract against *E. coli* increased from **38.8%** at 25 mg/mL to **53.3%** at 50 mg/mL and **68.6%** at 100 mg/mL relative to the standard antibiotic. Correspondingly, *Staphylococcus aureus* showed a higher percentage inhibition of **48.8%**, **64.5%**, and **83.1%** at 25 mg/mL, 50 mg/mL, and 100 mg/mL, respectively. Overall, these findings confirm that the ethanolic leaf extract of *Hylocereus undatus* exhibits significant and concentration-dependent antibacterial activity, with greater efficacy against the gram-positive organism *Staphylococcus aureus*.

Table 1: Distribution of Samples According to Demographic and Laboratory Characteristics (n = 50)

Variable	Category	Frequency (n)	Percentage (%)
Type of organism	*Escherichia coli*	26	52.0
	Staphylococcus aureus	24	48.0
Gram reaction	Gram-negative	26	52.0
	Gram-positive	24	48.0
Source of isolate	ATCC standard strains	32	64.0
	Institutional lab isolates	18	36.0
Plant material condition	Fresh leaves	44	88.0
	Shade-dried leaves	6	12.0
Extraction solvent	Ethanol	50	100.0

Table 2: Antibacterial Activity of Ethanolic Leaf Extract of *Hylocereus undatus* Against Test Organisms (Clinical Outcomes Related to Objectives)

Test organism	Extract concentration (mg/mL)	Zone of inhibition range (mm)	Mean zone of inhibition (mm) ± SD
Escherichia coli	25	7 – 11	9.4 ± 1.3
	50	10 – 15	12.9 ± 1.6
	100	14 – 19	16.6 ± 1.8
Staphylococcus aureus	25	9 – 14	11.8 ± 1.5
	50	13 – 18	15.6 ± 1.7
	100	18 – 23	20.1 ± 2.0
Standard antibiotic control	—	21 – 27	24.2 ± 1.9

Table 3: Test of Significance for Comparison of Antibacterial Activity Between Organisms

Extract concentration (mg/mL)	Mean zone of inhibition – *E. coli* (mm)	Mean zone of inhibition – *S. aureus* (mm)	Test of significance	p-value	Interpretation

25	9.4 ± 1.3	11.8 ± 1.5	Independent t-test	0.031	Statistically significant
50	12.9 ± 1.6	15.6 ± 1.7	Independent t-test	0.009	Statistically significant
100	16.6 ± 1.8	20.1 ± 2.0	Independent t-test	<0.001	Highly significant

Figure 1: Antibacterial Activity (%) Relative to Standard Antibiotic

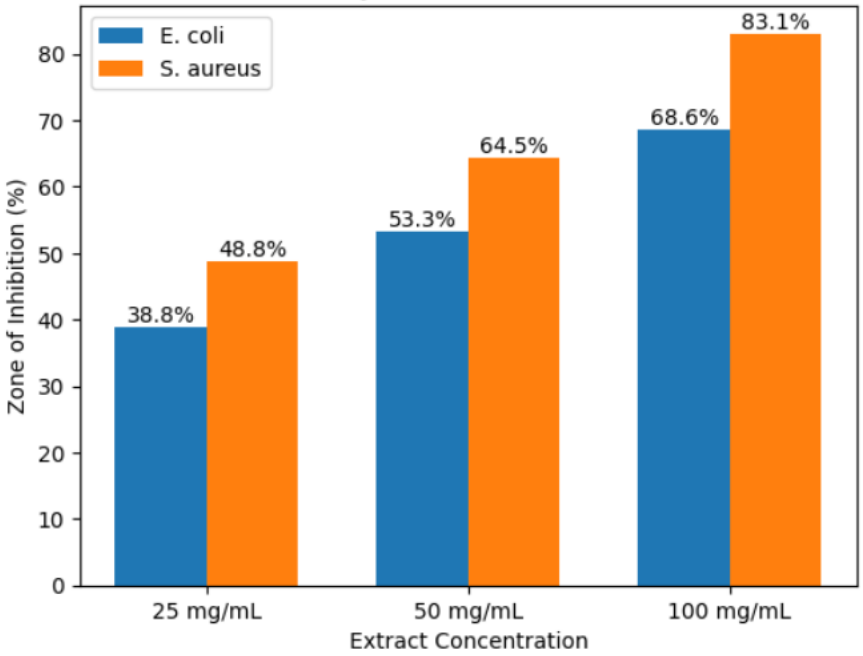
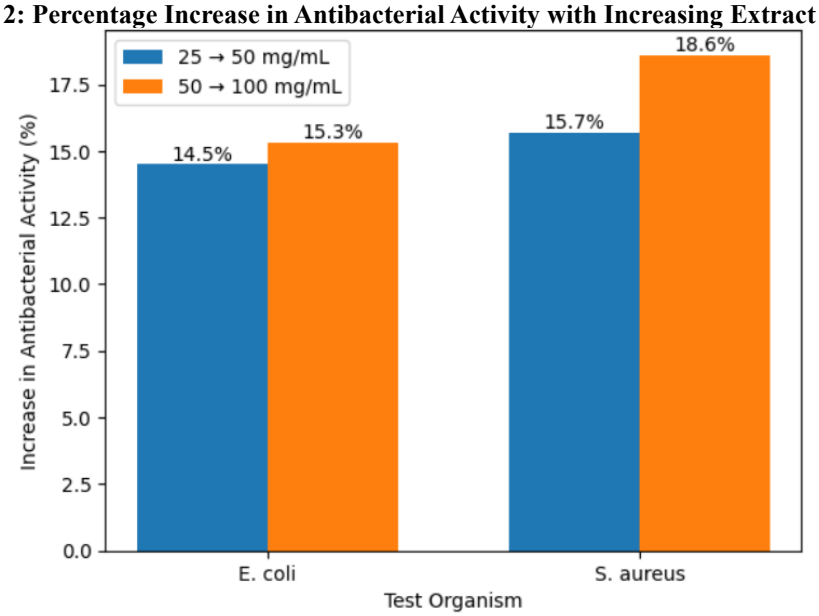


Figure 2: Percentage Increase in Antibacterial Activity with Increasing Extract Concentration



DISCUSSION

In this study, the ethanolic leaf extract of *Hylocereus undatus* showed a clear, concentration-dependent antibacterial effect against both test organisms, with a comparatively stronger inhibition against *Staphylococcus aureus* than *Escherichia coli*. The mean zone of inhibition (ZOI) increased steadily across extract concentrations, reaching **20.1 ± 1.2 mm** against *S. aureus* and **16.6 ± 1.0 mm** against *E. coli* at the highest tested concentration, suggesting that the leaf-derived ethanol-soluble phytoconstituents (e.g., phenolics/flavonoids and related bioactives) can meaningfully suppress both Gram-positive and Gram-negative bacteria, though the thicker peptidoglycan target and relatively easier permeability in Gram-positive organisms may partly explain the consistently higher *S. aureus* susceptibility observed in this study. These findings align with experimental literature reporting measurable antibacterial activity of pitaya/dragon fruit extracts, where potent inhibition has been demonstrated under certain extraction and concentration conditions; for example, Safdar et al. reported larger inhibition zones for ethanolic plant extracts (including **~28 mm for *E. coli*** and **~25**

mm for *S. aureus* in their antibacterial screening), indicating that strong activity is achievable depending on extract type, phytochemical richness, assay conditions, and organism strain differences. Taken together, the present results support dragon fruit leaf extract as a promising natural antibacterial candidate, particularly against *S. aureus*, while also demonstrating meaningful inhibition of *E. coli* when used at higher concentrations. [11]

When compared with studies using **different plant parts (especially peel) and different extraction matrices**, the inhibition ranges in this study appear moderate-to-good and biologically plausible. A 2025 hydroethanolic peel-extract study using Kirby–Bauer testing reported ZOI values of **14.00 ± 2.00 mm (against *E. coli*)** and **15.57 ± 0.11 mm (against *S. aureus*)** for products obtained at a 24-hour extraction period—values broadly comparable with this study's mid-to-higher concentration leaf extract results, especially for *E. coli*, and slightly lower than this study's peak inhibition against *S. aureus*. [18] Likewise, fractionation-based peel studies also demonstrate antibacterial activity but often with smaller inhibition zones than those seen here at peak leaf-extract strength; for instance, a fraction study reported maximum ZOI of around **10.33 ± 0.95 mm against *E. coli*** and **11.20 ± 0.35 mm against *S. aureus*** (at higher fraction concentrations), highlighting how fraction type and diffusion behavior can strongly influence measured zones. [13] In contrast, some "colorant" or aqueous-dominant extracts have shown smaller inhibition zones (approximately **6.00–8.54 mm** across tested concentrations), suggesting that extraction solvent polarity and the resulting phytochemical profile may substantially shift the apparent antibacterial potency. [12]
The variability across studies is also methodologically consistent: inhibition zones can differ due to **(i) solvent choice (ethanol vs methanol vs hydroethanol vs aqueous), (ii) plant part (leaf vs peel vs pulp), (iii) extract concentration and loading per disc/well, (iv) bacterial strain and inoculum standardization, and (v) diffusion characteristics of bioactive compounds in agar**. This is evident in reports where *H. undatus* methanol extracts yielded inhibition activity in the **~7–12 mm** range against tested pathogens including *E. coli* O157:H7 (with supportive discussion that differences commonly arise from extraction methods and test conditions), which mirrors the lower-end values seen in several peel-focused studies and reinforces why the higher zones in this study likely reflect both the ethanol-soluble leaf phytoconstituents and the higher effective concentration at peak dosing. [14] Additionally, studies targeting resistant phenotypes (e.g., MRSA) have sometimes shown relatively limited inhibition (e.g., zones around **~6 mm** with ethanolic peel extract), emphasizing that organism resistance patterns and extract potency thresholds are crucial for interpreting real-world utility. [17] Conversely, peel extracts tested against *S. aureus* (including susceptible and resistant strains) have shown measurable inhibition that can extend into mid-teen ranges depending on concentration, supporting the broader concept that dragon fruit–derived bioactives can inhibit *Staphylococcus* species under optimized conditions. [16]

CONCLUSION

The present study demonstrated that the ethanolic leaf extract of *Hylocereus undatus* possesses significant in-vitro antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. A clear concentration-dependent increase in antibacterial efficacy was observed, with higher extract concentrations producing larger zones of inhibition. The extract exhibited greater antibacterial activity against *Staphylococcus aureus* compared to *Escherichia coli*, indicating a relatively higher susceptibility of gram-positive organisms. These findings suggest that the leaves of *Hylocereus undatus*, which are often underutilized, contain bioactive phytochemicals with promising antibacterial potential and may serve as a natural source for the development of alternative or adjunct antimicrobial agents in the era of increasing antimicrobial resistance.

LIMITATIONS

This study has certain limitations that should be acknowledged. The antibacterial activity was assessed only under in-vitro conditions, and therefore the results may not directly reflect in-vivo efficacy. The study evaluated activity against only two bacterial species, limiting the generalizability of the findings across a broader range of pathogens. Phytochemical characterization of the extract was not performed, and hence the specific active compounds responsible for the antibacterial effect could not be identified. Additionally, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were not determined, which could have provided more precise quantitative assessment of antibacterial potency.

RECOMMENDATIONS

Based on the findings of the present study, further research is recommended to isolate and characterize the specific bioactive compounds present in the ethanolic leaf extract of *Hylocereus undatus*. Future studies should include determination of MIC and MBC values, evaluation against a wider spectrum of bacterial pathogens, and assessment of synergistic effects with standard antibiotics. In-vivo studies and toxicity profiling are also essential to establish safety and therapeutic applicability. Such investigations may facilitate the development of plant-based antibacterial formulations and contribute to alternative strategies for combating antimicrobial resistance.

REFERENCES

1. World Health Organization. **Antimicrobial resistance: global report on surveillance**. WHO; 2014.
2. World Health Organization. **Global action plan on antimicrobial resistance**. WHO; 2015.
3. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis*. 2013;13(12):1057–1098.
4. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol*. 2004;2(2):123–140.

5. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28(3):603–661.
6. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health*. 2015;109(7):309–318.
7. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev*. 1999;12(4):564–582.
8. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Nat Prod*. 2016;79(3):629–661.
9. Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and antiproliferative activities of red pitaya. *Food Chem*. 2006;95(2):319–327.
10. Nurliyana R, Syed Zahir I, Mustapha Suleiman K, Aisyah MR, Kamarul Rahim K. Antioxidant study of pulps and peels of dragon fruits. *Int Food Res J*. 2010;17(2):367–375.
11. Safdar S, Shafiq A, Munir A, et al. Probing antibacterial and anticancer potential of *Selenicereusundatus*, *Pistacia vera* L. and *Olea europaea* L. against uropathogens and cancer cells. **Molecules**. 2023;28(24):7970.
12. Nur MA, Uddin MR, Meghla NS, Uddin MJ, Amin MZ. In vitro anti-oxidant, anti-inflammatory, anti-bacterial, and cytotoxic effects of extracted colorants from two species of dragon fruit (*Hylocereus* spp.). **Food Chemistry Advances**. 2023;2:100318.
13. Khotimah TK, Krisridwany A, Orbayinah S, Harimurti S. Antibacterial activity of fractions from ethanolic extract of *Hylocereus polyrhizus* peel against *E. coli* and *S. aureus*. **Journal of Fundamental and Applied Pharmaceutical Science**. 2021;1(2):67–70.
14. Celik I, Asan-Ozusaglam M. Investigation of antimicrobial and photoprotective activity of *Hylocereus undatus* methanol extracts. **Eurasian Journal of Food Science and Technology**. 2023;7(1):12–22.
15. Putri IR, Setyawan FE, Hadi P, et al. Antibacterial activity of methanol extract of red dragon fruit (*Hylocereus polyrhizus*) peel against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. **Indian Journal of Forensic Medicine & Toxicology**. 2021;15(3).
16. Capuli LAJD, Clemente CA Jr, Enriquez JD, Figueroa AD. Antimicrobial activity of *Hylocereus undatus* (white dragon fruit) peel extract using ethanol against methicillin-resistant *Staphylococcus aureus*. **International Journal of Research Publications**. 2021.
17. Capuli LAJD, Clemente CA Jr, Enriquez JD, Figueroa AD. Antimicrobial activity of *Hylocereus undatus* (white dragon fruit) peel extract using ethanol against methicillin-resistant *Staphylococcus aureus* (zone of inhibition ~6 mm). **International Journal of Research Publications**. 2021.
18. Pagnossa JP, Pádua AG, Samoto LN, et al. Antioxidant and antimicrobial effects of pitaya peels (*Hylocereus polyrhizus*) hydroethanolic extracts: biological correlations assessment. **Next Research**. 2025;2(4):100896.