OPEN ACCESS

World Journal of Applied Medical Sciences
ISSN: 3049-0200 (Online) | Vol. 3, No. 6, 2026
Website: https://wasrpublication.com/index.php/wjams/

"Comparative Evaluation of the Antimicrobial Efficacy of *Moringa oleifera* and *Ziziphus spina-christi* (Sidr) Extracts against Gram-Positive, Gram-Negative Bacteria, and Fungi: An In Vitro Study"

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Received: 15/04/2026 | Accepted: 06/06/2026 | Published: 26/06/2026

Abstract: The increasing prevalence of antimicrobial resistance has intensified the search for effective natural antimicrobial agents. This study aimed to compare the antimicrobial activity of *Moringa oleifera* and *Ziziphus spina-christi* (Sidr) leaf extracts against selected Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungi (*Candida albicans* and *Aspergillus niger*). Antimicrobial activity was evaluated using the agar well diffusion assay, while minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) were determined using standard microdilution methods. Statistical analysis was performed using two-way ANOVA followed by Tukey's HSD test. Both plant extracts exhibited broad-spectrum antimicrobial activity against all tested microorganisms; however, *Z. spina-christi* consistently demonstrated significantly greater efficacy than *M. oleifera*. Sidr produced larger inhibition zones, ranging from 10.2 ± 1.3 to 18.5 ± 1.2 mm, compared with 8.3 ± 0.9 to 14.2 ± 0.8 mm for Moringa. The strongest activity was observed against *S. aureus* (18.5 ± 1.2 mm) and *C. albicans* (17.4 ± 1.0 mm), whereas *P. aeruginosa* showed the lowest susceptibility. MIC values for Sidr ranged from 12.5 to 50.0 mg/mL, whereas Moringa required 25.0–100.0 mg/mL. Similarly, Sidr exhibited lower MBC/MFC values (25.0–100.0 mg/mL) than Moringa (50.0–200.0 mg/mL). The MBC/MIC ratio was 2.0 for both extracts against all microorganisms, indicating bactericidal and fungicidal activity. Two-way ANOVA revealed highly significant effects of plant type (F = 142.200, $p < 0.001$) and microbial category (F = 40.800, $p < 0.001$), whereas their interaction was not significant ($p = 0.382$). Tukey's HSD test confirmed the superior antimicrobial efficacy of Sidr over Moringa, with a mean increase in inhibition zone of 4.38 mm ($p < 0.001$), while Gram-positive bacteria were significantly more susceptible than Gram-negative bacteria and fungi. These findings demonstrate that *Z. spina-christi* possesses stronger antimicrobial activity than *M. oleifera* and represents a promising natural source of broad-spectrum antimicrobial compounds for potential pharmaceutical and food preservation applications.

Keywords: *Ziziphus spina-christi; Moringa oleifera;* antimicrobial activity; medicinal plants; Gram-positive bacteria; antifungal activity.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health threats, compromising the effectiveness of conventional antimicrobial therapies and increasing morbidity, mortality, and healthcare costs worldwide. According to the World Health Organization (WHO), bacterial antimicrobial resistance was directly associated with approximately 1.27 million deaths in 2019, while millions of additional deaths were linked to drug-resistant infections, highlighting the urgent need for novel antimicrobial agents [1]. Consequently, medicinal plants have attracted considerable scientific interest as valuable sources of natural antimicrobial compounds owing to their broad spectrum of bioactive secondary metabolites, including flavonoids, phenolic acids, tannins, saponins, alkaloids, and terpenoids, which exert antimicrobial effects through multiple mechanisms such as disruption of microbial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and suppression of biofilm formation [2. Among these plants, *Moringa oleifera*

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Lam. (family Moringaceae), commonly known as the miracle tree, is recognized for its exceptional nutritional and medicinal value, as its leaves are rich in proteins, vitamins, minerals, polyphenols, glucosinolates, isothiocyanates, and flavonoids that exhibit significant antibacterial and antifungal activities against clinically important pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [3]. Likewise, *Ziziphus spina-christi* (L.) Desf. (Sidr), a member of the family Rhamnaceae widely distributed throughout North Africa, the Middle East, and the Arabian Peninsula, has been extensively used in traditional medicine for the treatment of infectious and inflammatory disorders. Its leaves contain abundant flavonoids, triterpenoids, tannins, saponins, and other phenolic constituents that have demonstrated potent antibacterial, antifungal, antioxidant, and anti-inflammatory activities against a wide range of pathogenic microorganisms [4]. Although both plant species have been investigated independently for their antimicrobial potential, direct comparative studies employing standardized extraction procedures, identical microbial panels, and unified antimicrobial susceptibility assays remain scarce [5]. Therefore, the present study was designed to comparatively evaluate the antimicrobial efficacy of *M. oleifera* and *Z. spina-christi* leaf extracts against selected bacterial and fungal pathogens, determine their minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC), distinguish between bacteriostatic and bactericidal modes of action, and provide statistically robust comparisons to identify the plant with superior antimicrobial potential.

Materials and Methods

Fresh leaves of **Moringa oleifera** (Samno, Sabha, Libya) and **Ziziphus spina-christi** (Sidr; Green Mountain, Libya) were shade-dried, finely powdered, and extracted with 70% ethanol at a ratio of 1:10 (w/v). The extracts were filtered, concentrated under reduced pressure at ≤45°C, dried, and resuspended to obtain a stock solution of 200 mg/mL. Antimicrobial activity was evaluated against six reference microorganisms (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus niger*) using the agar well diffusion method with a 0.5 McFarland inoculum and 6 mm wells. Gentamicin and Nystatin served as positive controls for bacteria and fungi, respectively, while the extraction solvent served as the negative control. Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method according to CLSI guidelines, whereas minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined by subculturing from growth-free wells onto fresh agar. Bactericidal or fungicidal activity was defined as an MBC/MIC or MFC/MIC ratio ≤4. All experiments were performed in triplicate, and results are expressed as mean ± SD. Statistical analysis was performed using two-way ANOVA followed by Tukey's HSD test, with normality and homogeneity of variance assessed using the Shapiro–Wilk and Levene tests, respectively. Statistical significance was accepted at *p* < 0.05.

Results

As presented in Table 1, both *Moringa oleifera* and *Ziziphus spina-christi* (Sidr) extracts demonstrated measurable antimicrobial activity against all tested microorganisms, with variable inhibition zones depending on the organism. Sidr extract consistently showed larger zones of inhibition compared with Moringa across all microbial strains. For Gram-positive bacteria, S. aureus recorded 18.5 ± 1.2 mm for Sidr and 14.2 ± 0.8 mm for Moringa, while B. subtilis showed 16.3 ± 0.9 mm and 12.8 ± 1.0 mm, respectively. For Gram-negative bacteria, E. coli exhibited inhibition zones of 15.7 ± 1.1 mm (Sidr) and 11.5 ± 0.6 mm (Moringa), whereas P. aeruginosa showed lower sensitivity with 10.2 ± 1.3 mm and 8.3 ± 0.9 mm, respectively. In the antifungal assays, C. albicans showed inhibition zones of 17.4 ± 1.0 mm for Sidr and 13.6 ± 0.7 mm for Moringa, while A. niger recorded 12.6 ± 0.8 mm and 9.4 ± 1.1 mm, respectively. The positive controls (Gentamicin and Nystatin) exhibited substantially higher inhibition zones, while no activity was observed in the negative control (0.0 mm).

Table 1. Qualitative Antimicrobial Screening of Plant Extracts by Agar Well Diffusion Method

Test Organism	Moringa Extract (mm)	Sidr Extract (mm)	Positive Control (mm)	Negative Control (mm)
S. aureus (G+)	14.2 ± 0.8	18.5 ± 1.2	28.3 ± 0.5 (Gentamicin 10 µg)	0.0
B. subtilis (G+)	12.8 ± 1.0	16.3 ± 0.9	26.7 ± 0.8 (Gentamicin 10 µg)	0.0
E. coli (G−)	11.5 ± 0.6	15.7 ± 1.1	25.4 ± 0.6 (Gentamicin 10 µg)	0.0
P. aeruginosa (G−)	8.3 ± 0.9	10.2 ± 1.3	22.1 ± 0.7 (Gentamicin 10 µg)	0.0
C. albicans (Yeast)	13.6 ± 0.7	17.4 ± 1.0	24.8 ± 0.9 (Nystatin 100 IU)	0.0
A. niger (Mold)	9.4 ± 1.1	12.6 ± 0.8	21.5 ± 0.6 (Nystatin 100 IU)	0.0

Note: Values represent mean zone of inhibition (mm) ± SD (n = 3). Well diameter = 6 mm. G+ = Gram-positive; G− = Gram-negative.

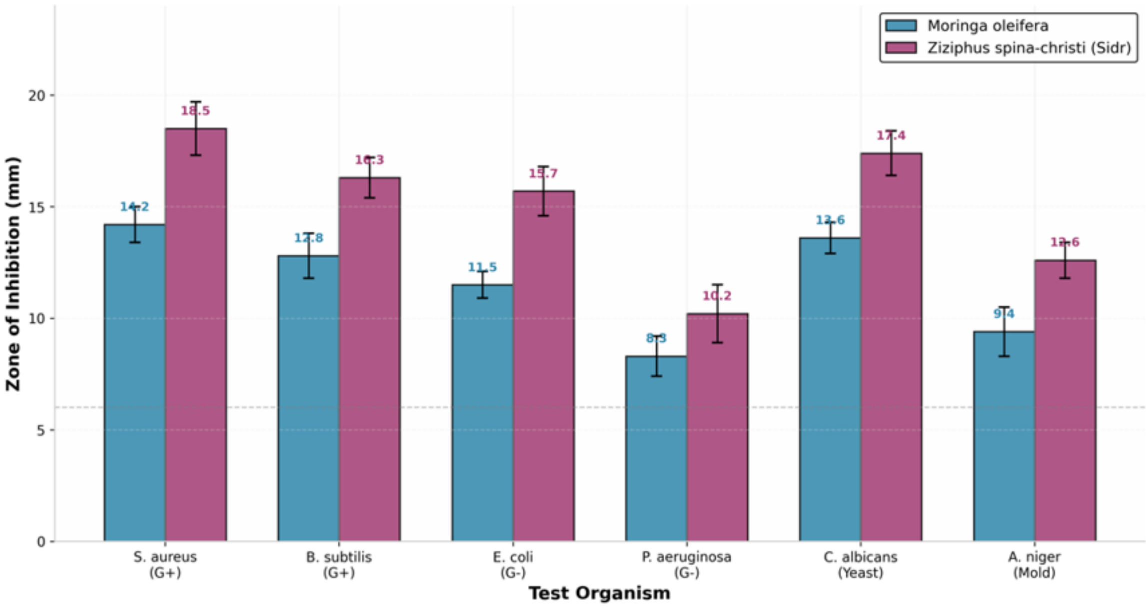

Figure 1. Comparative Zone of Inhibition of *Moringa* and Sidr Extracts Against Test Microorganisms. Bar chart showing mean inhibition zones (mm) ± SD for Gram-positive bacteria (*S. aureus*, *B. subtilis*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*), and fungi (*C. albicans*, *A. niger*). Sidr extract demonstrates consistently larger inhibition zones across all organisms.

As shown in Table 2 and Figure 2, the Minimum Inhibitory Concentration (MIC) results demonstrated that *Ziziphus spina-christi* (Sidr) extract exhibited stronger antimicrobial potency than *Moringa oleifera* across all tested microorganisms. Sidr showed lower MIC values, particularly against S. aureus and C. albicans (12.5 mg/mL), compared with *Moringa* (25.0 mg/mL) for the same organisms. For B. subtilis and E. coli, Sidr recorded an MIC of 25.0 mg/mL, while Moringa required 50.0 mg/mL. The highest MIC values were observed against P. aeruginosa and A. niger, where Sidr showed 50.0 mg/mL and *Moringa* reached 100.0 mg/mL, indicating lower sensitivity of these organisms to both extracts. In comparison, the standard antibiotics (Gentamicin and Nystatin) exhibited much lower MIC values, reflecting higher antimicrobial potency.

Table 2. Minimum Inhibitory Concentration (MIC) of Plant Extracts Against Test Microorganisms

Test Organism	Moringa MIC (mg/mL)	Sidr MIC (mg/mL)	Positive Control MIC (µg/mL)
S. aureus	25.0	12.5	1.56 (Gentamicin)
B. subtilis	50.0	25.0	3.12 (Gentamicin)
E. coli	50.0	25.0	3.12 (Gentamicin)
P. aeruginosa	100.0	50.0	12.5 (Gentamicin)
C. albicans	25.0	12.5	6.25 (Nystatin)
A. niger	100.0	50.0	25.0 (Nystatin)

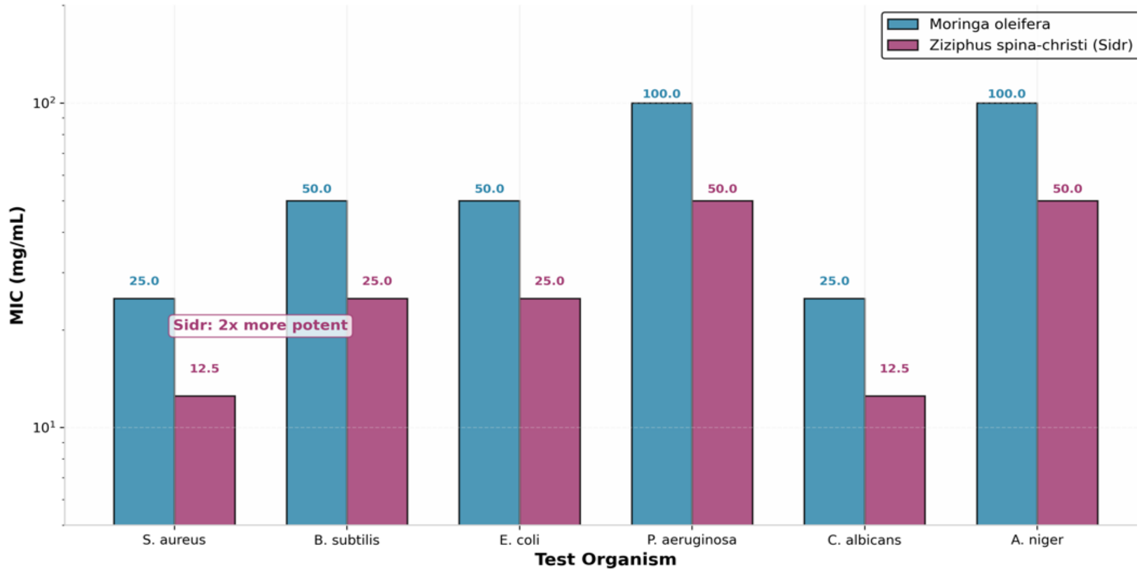

Figure 2. Minimum Inhibitory Concentration (MIC) Profile of Plant Extracts.

As shown in Table 3, the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values further confirmed the antimicrobial potency of both plant extracts. Ziziphus spina-christi (Sidr) consistently exhibited lower MBC/MFC values compared with Moringa oleifera, indicating stronger killing activity against all tested microorganisms. For S. aureus and C. albicans, Sidr showed MBC/MFC values of 25.0 mg/mL, whereas Moringa required 50.0 mg/mL. For B. subtilis and E. coli, Sidr recorded 50.0 mg/mL, compared with 100.0 mg/mL in Moringa. The highest values were observed against P. aeruginosa and A. niger, where Sidr reached 100.0 mg/mL and Moringa reached 200.0 mg/mL. Importantly, the MBC/MIC ratio for both extracts was consistently 2.0 across all organisms. Since all values were ≤ 4, both extracts demonstrated a bactericidal/fungicidal mode of action rather than bacteriostatic or fungistatic effects.

Table 3. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of Plant Extracts

Test Organism	Moringa MBC/MFC (mg/mL)	Sidr MBC/MFC (mg/mL)	MBC/MIC Ratio (Moringa)	MBC/MIC Ratio (Sidr)
S. aureus	50.0	25.0	2.0	2.0
B. subtilis	100.0	50.0	2.0	2.0
E. coli	100.0	50.0	2.0	2.0
P. aeruginosa	200.0	100.0	2.0	2.0
C. albicans	50.0	25.0	2.0	2.0
A. niger	200.0	100.0	2.0	2.0

MBC/MIC ratio ≤ 4 indicates bactericidal/fungicidal activity; ratio > 4 indicates bacteriostatic/fungistatic activity.

As shown in Table 4, the two-way ANOVA analysis of antimicrobial zone of inhibition data revealed highly significant effects of both plant type and microbial category. The effect of plant type was highly significant ($F = 142.200$, $p < 0.001$), indicating clear differences in antimicrobial activity between the tested extracts. Similarly, microbial category also showed a highly significant effect ($F = 40.800$, $p < 0.001$), demonstrating that the response varied significantly among different groups of microorganisms. In contrast, the interaction between plant type and microbial category was not statistically significant ($F = 1.064$, $p = 0.382$), suggesting that the pattern of antimicrobial activity across microbial groups was consistent for both plant extracts. The residual variation was relatively low, supporting the reliability of the observed effects.

Table 4. Two-Way ANOVA Results for Antimicrobial Zone of Inhibition Data

Source	Sum of Squares	Df	Mean Square	F	p-value	Significance
Plant Type	156.420	1	156.420	142.200	<0.001	***
Microbial Category	89.760	2	44.880	40.800	<0.001	***
Plant × Category	2.340	2	1.170	1.064	0.382	NS
Residual	19.840	18	1.102	—	—	—
Total	268.360	23	—	—	—	—

Note: *** $p < 0.001$ (highly significant); NS = Not Significant ($p > 0.05$).

As presented in Table 5, the post-hoc Tukey HSD analysis revealed statistically significant pairwise differences in antimicrobial activity. The comparison between *Ziziphus spina-christi* (Sidr) and *Moringa oleifera* showed a highly significant difference with a mean increase of +4.38 mm in favor of Sidr ($p < 0.001$), accompanied by a very large effect size ($\eta^2 = 0.887$). In addition, significant differences were observed among microbial categories. Gram-positive bacteria showed higher susceptibility compared with Gram-negative bacteria, with a mean difference of +5.42 mm ($p < 0.001$, $\eta^2 = 0.695$). Gram-positive bacteria also showed significantly higher activity compared with fungi (+3.18 mm, $p = 0.002$, $\eta^2 = 0.512$), while Gram-negative bacteria exhibited lower activity than fungi (−2.24 mm, $p = 0.041$, $\eta^2 = 0.298$), indicating a medium effect size.

Table 5. Post-hoc Tukey HSD Test for Pairwise Comparisons of Antimicrobial Activity

Comparison	Mean Difference (mm)	p-value (Tukey)	η²	Effect Size
Sidr vs. Moringa (Overall)	+4.38	<0.001***	0.887	Very Large
Gram+ vs. Gram−	+5.42	<0.001***	0.695	Large
Gram+ vs. Fungi	+3.18	0.002**	0.512	Large
Gram− vs. Fungi	−2.24	0.041*	0.298	Medium

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

The present study demonstrated that **Ziziphus spina-christi** (Sidr) leaf extract exhibited significantly stronger antimicrobial activity than **Moringa oleifera** against all tested bacterial and fungal strains, as evidenced by larger inhibition zones and lower MIC and MBC/MFC values. The superior efficacy of Sidr is likely attributed to its higher content of phenolic compounds, flavonoids, tannins, and saponins, which disrupt microbial cell membranes, interfere with essential cellular functions, and ultimately lead to cell death [6]. The MBC/MIC ratio of 2.0 for both extracts indicates a bactericidal and fungicidal mode of action rather than merely inhibiting microbial growth . These findings agree with previous reports describing the antimicrobial properties of both **M. oleifera** and *Z. spina-christi*, while confirming the greater potency of Sidr extract [7]. Gram-positive bacteria were the most susceptible microorganisms, followed by fungi and Gram-negative bacteria, which is consistent with differences in cell wall structure and the protective outer membrane of Gram-negative species [8-12]. Although both plant extracts were less potent than the standard antibiotics, their broad-spectrum antimicrobial activity highlights their potential as natural sources of bioactive compounds for pharmaceutical, food preservation, and agricultural applications. Nevertheless, further studies are required to isolate the active constituents, evaluate their cytotoxicity, investigate synergistic interactions with conventional antibiotics, and confirm their efficacy through in vivo experiments.

Conclusion

Both *Moringa oleifera* and *Ziziphus spina-christi* leaf extracts exhibited broad-spectrum antimicrobial activity against the tested bacteria and fungi. However, Z. spina-christi showed significantly greater efficacy, with larger inhibition zones and lower MIC and MBC/MFC values than M. oleifera. Both extracts demonstrated bactericidal and fungicidal activity (MBC/MIC ≤ 4). These findings suggest that Z. spina-christi is a promising natural source of antimicrobial compounds with potential pharmaceutical and food preservation applications, although further phytochemical, toxicological, and in vivo studies are needed.

References

1. **Allemailem, K. S. (2021). Antimicrobial potential of naturally occurring bioactive secondary** metabolites. *Journal of Pharmacy & Bioallied Sciences*, *13*(2), 155–162.
2. Berida, T. I., Adekunle, Y. A., Dada-Adegbola, H., Kdimy, A., Roy, S., & Sarker, S. D. (2024). Plant antibacterials: *The challenges and opportunities. Heliyon*, *10*(10), e30913.
3. Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology*, *15*, 51–62.
4. Isenberg, R. Y., Sackih, C. W., & Willett, J. L. (2025). Streptococcal natural products mediate interspecies competition with Gram-positive bacterial pathogens. bioRxiv. https://doi.org/10.1101/2025
5. Jones, R. N. (2006). Microbiological features of vancomycin in the 21st century: Minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clinical Infectious Diseases*, *42*(Suppl. 1), S13–S24.
6. Panova, N., Gerasimova, A., Gentscheva, G., Nikolova, S., Makedonski, L., Velikova, M., ... & Nikolova, K. (2025). Moringa oleifera Lam.: A nutritional powerhouse with multifaceted pharmacological and functional applications. Life, 15(6), 881.
7. Purnamayati, L., Riyadi, P. H., & Prayitno, S. B. (2021). Phytochemical analysis and antibacterial activities of Sidr leaf extract (Ziziphus spina-christi) against pathogenic bacteria in aquaculture. *Pertanika Journal of Tropical Agricultural Science*, *44*(4).
8. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023). Antimicrobial resistance: A growing serious threat for global public health. *Healthcare*, *11*(13), 1946. https://doi.org/10.3390/healthcare11131946
9. Khaled, F. A., Somya, A., Abdelmola, H. J., Badr, S,. Abdullah, M., & Salih, A. (2025). Hormonal protective potential of Moringa oleifera against potassium dichromate (Cr VI)-induced endocrine disruption and oxidative stress in male rabbits. *Carcinogenesis*, *24*(7 Suppl.), 474–480.
10. Aldeeb, O., & Abdulsalam, A. (2024). The impact of including Moringa oleifera leaves to lower potassium dichromate toxicity on rabbit reproductive outcomes underheat stress. *AlQalam Journal of Medical and Applied Sciences*, *7*(4), 556–565.
11. Khaled, F. A., & Ahmed, A. I. (2025). Experimental study on the effect of Ziziphus spina-christi leaves on blood plasma hormones in male rabbits. *World Journal of Applied Medical Sciences*, *2*(9), 1–5
12. Hammad, A. A., Khaled, F. A., & Hussien, S. M. (2026). GC- MS characterization of Ziziphus spina-christi essential oil and its antioxidant efficacy in rabbit models. *World Journal of Applied Medical Sciences*, *3*(1), 14–18.