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Safety and Antioxidant Potential of *Ziziphus mauritiana* Leaf Extract: An Acute Toxicity and DPPH Scavenging Study

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ABSTRACT

The current study aimed to assess the toxicological profile and antioxidant potential of the ethanolic extract of *Ziziphus mauritiana* (*Z. mauritiana*) leaves, evaluating its safety and therapeutic properties. The toxicological assessment followed the Organization for Economic Cooperation and Development 423 guidelines, using female Wistar rats to evaluate acute oral toxicity. The extract was administered in doses of 5, 50, 300, and 2000 mg/kg body weight, with physiological responses and mortality observed over 14 days. Antioxidant potential was assessed through total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) assays. TPC and TFC were measured using gallic acid and quercetin as standards, respectively. No signs of toxicity or mortality were observed across all dose levels, indicating the extract's safety even at high doses. Antioxidant analysis revealed significant TPC and TFC values, reaching 5.24 mg gallic acid equivalent per gram (GAE/g) and 4.17 mg quercetin equivalent per gram (QE/g), respectively. The extract exhibited strong DPPH RSA, with a half-maximal inhibitory concentration (IC50) value of 128.17 µg/mL, indicating potent antioxidant efficacy. The ethanolic extract of *Z. mauritiana* leaves demonstrated substantial antioxidant activity and an absence of acute toxicity, supporting its potential use as a natural antioxidant agent in medicinal applications. Future studies should explore its bioactive components and effects in disease models.

Keywords: *Ziziphus mauritiana*, antioxidant, toxicology, phenolic content, flavonoid content, DPPH radical scavenging

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INTRODUCTION

Plants have long served as significant sources of new drugs, with various species from the genus *Ziziphus* traditionally utilized to treat numerous ailments and bodily disorders [1-3]. *Ziziphus mauritiana (Z. mauritiana)*, commonly known as ber or Indian jujube, is a tropical fruit-bearing tree classified under the Rhamnaceae family. This species is characterized as a spiny, evergreen shrub or small tree, featuring drooping branches, glossy green leaves, and small yellow flowers. Its fruit, a drupe with sweet, tangy flesh, is widely consumed across the Indian subcontinent and beyond [4-7]. Originally native to the Indo-Malaysian region, *Z. mauritiana* has spread across tropical regions, including Southern Africa, the Middle East, China, and the Pacific Islands [5,6,8].

Bioactive molecules in plant extracts can exert both pharmacological and toxicological effects [9]. Toxicity studies on bioactive plant extracts are essential for understanding animal responses and correlating them with human outcomes, providing insights into pharmacological safety and determining effective doses [1,10]. Animal models are recommended for toxicological evaluations in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines to ensure standardization and reliability [1].

Antioxidants protect cells from damage caused by reactive oxygen species (ROS), commonly referred to as free radicals. These radicals, produced during normal metabolism, can damage cells by initiating oxidation. Antioxidants neutralize these radicals by interrupting chain reactions that lead to oxidative stress. Medicinal plants are rich in bioactive compounds, such as carotenoids, flavonoids, phenolics, and tannins, which exhibit strong antioxidant activities through free radical scavenging and stabilization [11]. Phenolic compounds play a critical role by donating hydrogen or electrons to free radicals, acting as stable intermediates during oxidation and helping prevent cellular damage and oxidative stress [12].

Evaluating the antioxidant activity of plant extracts helps establish their therapeutic potential and highlights their value as natural antioxidant agents. Among the methods commonly employed for antioxidant assessment, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay quantifies an extract's ability to neutralize free radicals [13]. Extracts from various parts of *Z. mauritiana*—including fruits, leaves, and seeds—have been reported to possess antioxidant properties [14-19].

Previous studies have focused on various aspects of *Z. mauritiana* extracts. For instance, Dahiru D *et al*. investigated the effects of aqueous *Z. mauritiana* leaf extract on alcohol-induced liver damage, measuring biochemical markers, antioxidant enzyme levels, and histopathological changes in the liver [20]. Conversely, Owolarafe T *et al*. examined the phytochemical composition and subchronic toxicity of aqueous leaf extract, offering insights into its safety profile [21]. However, both studies primarily used aqueous extracts, with limited exploration of the toxicological and biochemical properties of ethanolic extracts of *Z. mauritiana* leaves.

In contrast, the current research focuses on the acute toxicity and antioxidant potential (total phenolic content [TPC], total flavonoid content [TFC], DPPH assay) of the ethanolic extract of *Z. mauritiana* leaves. Ethanolic extraction is significant as it can yield different bioactive compounds compared to aqueous extraction, potentially offering distinct pharmacological benefits. While aqueous and methanolic extracts have been extensively studied [22,23], the antioxidant activity and toxicological profile of ethanolic extracts remain underexplored. This study fills this gap by using standardized protocols to evaluate total phenolic and flavonoid content and assessing the extract's free radical scavenging activity (RSA) using the DPPH assay. This comprehensive approach aims to establish a detailed understanding of the therapeutic potential of ethanolic extract of *Z. mauritiana* leaves.

MATERIALS AND METHODS

Plant Material and Extraction

Collection and Identification: The leaves of *Z. mauritiana* were collected from Hatlai Ghat (Hills) in Dharashiv (Osmanabad), Maharashtra, India. The plant material was identified and authenticated by the Professor and Head of the Department of Botany at Venkatesh Mahajan Senior College in Dharashiv, Maharashtra (voucher specimens were deposited with herbarium no. 128(B) 2022-23), affiliated with Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhaji Nagar (Aurangabad), Maharashtra, India.

Preparation of Extract: The collected leaves of *Z. mauritiana* were thoroughly washed with water from a reverse osmosis purifier and dried at room temperature for 21 days until they became completely dry and brittle. The dried leaves were ground into powder using an electric grinder. A total of 500 g of powdered leaves were extracted by maceration in ethanol (absolute ethanol [99.5% ethanol]). This mixture was stored at room temperature and shaken 4 to 6 times daily

for 3 days. The mixture was then filtered, and the extract was concentrated using a rotary evaporator to yield the ethanolic extract

Toxicological Evaluation

Animal Model: For toxicological evaluation, 15 female Wistar rats aged 6 to 8 weeks were selected, given their well-documented use in toxicological studies and physiological relevance to human metabolic processes. The rats were housed under standard laboratory conditions in accordance with OECD 423 guidelines, including proper bedding, cage size, and environmental enrichment to ensure animal welfare [24]. Ethical approval for this study was obtained from Scitesla Private Limited, Navi Mumbai, Maharashtra (approval no. SCI/IAEC/2024-25/107, dated 17-Jul-2024). The rats were provided with a standard diet and water *ad libitum*, in line with OECD 423 guidelines.

Organization for Economic Cooperation and Development 423 Guidelines: The acute oral toxicity study followed OECD 423 guidelines, specifically using the Acute Toxicity – Acute Toxic Class Method. The procedure involved administering the test substance incrementally, using three animals of a single sex per step. The ethanolic extract of *Z. mauritiana* leaves was administered orally at doses of 5, 50, 300, and 2000 mg/kg body weight to separate groups of animals. A control group consisting of three animals of the same sex received an equivalent volume of distilled water, serving as the vehicle for the test extract.

All animals, including the control group, were observed individually for signs of toxicity, including changes in eyes, fur, mucous membranes, and skin, as well as effects on the autonomic, central nervous, circulatory, and respiratory systems. Behavioral patterns, somatomotor activity, and parameters such as convulsions, coma, diarrhea, lethargy, salivation, sleep, and tremors were closely monitored.

Animals were observed at least once during the first 30 minutes after administration, periodically during the first 24 hours, and daily thereafter over a total period of 14 days. The control group served as a baseline for comparison with the test groups, enabling differentiation of any effects specifically attributable to the plant extract [24].

Antioxidant Evaluation

Total Phenolic Content: The TPC in the ethanolic extract of *Z. mauritiana* leaves was determined using the Folin-Ciocalteu (FC) assay, with gallic acid as the standard. The gallic acid standard curve was prepared using proportional dilutions of gallic acid ranging from 20 µg/mL to 240 µg/mL. The ethanolic extract was prepared at fixed concentrations of 100 µg/mL and 200 µg/mL to ensure its absorbance fell within the range of the standard curve.

The reaction mixture consisted of 0.5 mL of samples/standards, 3 mL of distilled water, and 0.25 mL of FC reagent (diluted 1:10 with distilled water). After shaking, the mixture was incubated in the dark for 5 minutes, followed by the addition of 1 mL of 7.5% sodium carbonate. The mixture was further incubated at room temperature in the dark for 60 minutes. A reagent blank was prepared using ethanol.

Absorbance was measured against the reagent blank at 760 nm using a double-beam Ultraviolet–visible (UV-Vis) spectrophotometer. TPC was calculated using the standard curve equation ($y = 0.0107x + 0.0277$, $R^2 = 0.9679$) and expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (mg GAE/g). All measurements were conducted in triplicate to ensure accuracy [25-28].

The TPC was calculated using the following equation:[29]

$TPC = (C \times V) / M$

Where:

C = concentration of phenolics in the extract solution, typically obtained from a standard calibration curve using gallic acid, expressed in mg/mL

V = volume of the extract solution used in the assay, in mL

M = mass of the extract used in the assay, in grams

Total Flavonoid Content: The TFC in the ethanolic extract of *Z. mauritiana* leaves was determined using the aluminum chloride (AC) assay, with quercetin as the standard. The quercetin standard curve was prepared using proportional dilutions of quercetin ranging from

20 µg/mL to 240 µg/mL. The ethanolic extract was prepared at fixed concentrations of 100 µg/mL and 200 µg/mL.

The reaction mixture consisted of 0.5 mL of samples/standards, 2 mL of distilled water, and 0.15 mL of 5% sodium nitrate. After a 5-minute incubation, 0.15 mL of 10% AC was added, followed by 1 mL of 1 M sodium hydroxide after 1 minute. The final volume was adjusted to 5 mL with distilled water, and the reaction mixture was left to stand for 10 minutes at room temperature. A reagent blank was prepared using ethanol.

Absorbance was measured against the reagent blank at 510 nm using a double-beam UV-Vis spectrophotometer. TFC was calculated using the standard curve equation ($y = 0.0005x + 0.0062$, $R^2 = 0.9587$) and expressed as milligrams of quercetin equivalents (QE) per gram of sample (mg QE/g). All measurements were conducted in triplicate to ensure accuracy [25-28].

The TFC was calculated using the following equation:[29]

$TFC = (C \times V) / M$

Where:

C = concentration of flavonoids in the extract soluti
usually determined from a standard calibration curv
quercetin, expressed in mg/mL

V = volume of the extract solution used in the assay,

M = mass of the extract used in the assay, in grams

DPPH Assay: The antioxidant activity of the ethanolic extract of *Z. mauritiana* leaves was evaluated using the DPPH method, following a modified protocol by Khorasani Esmaeili et al. (2015) [30]. A mass of 19.70 mg of DPPH was dissolved in a small amount of ethanol and then diluted to 500 mL with ethanol, achieving a final concentration of 0.1 mM DPPH solution. The control was prepared by mixing 3 mL of ethanol and 2 mL of DPPH solution.

A stock solution of ascorbic acid was prepared by dissolving 50 mg of ascorbic acid in 50 mL of ethanol. Proportional dilutions were performed to prepare standard solutions at concentrations of 1, 10, 25, 50, 100, 250, 500, and 1000 µg/mL. Similarly, the ethanolic extract was prepared at various concentrations to compare their antioxidant activities.

For each sample/standard, 2.4 mL of 0.1 mM DPPH in ethanol was mixed with 1.6 mL of respective sample or standard solution at different concentrations (1-1000 µg/mL). The reaction mixtures were incubated in the dark at room temperature for 30 minutes. After incubation, absorbance was measured at 517 nm using a double-beam UV-Vis spectrophotometer.

The percentage of DPPH RSA was calculated using the formula [25-27,29,30]:

$RSA\ (\%) = ((A_{control} - A_{sample}) / A_{control}) \times 100$

Where:

$A_{control}$ = absorbance of the control sample (DPPH) (without the antioxidant)

A_{sample} = absorbance of the sample (*Z. mauritiana* extract) with the antioxidant

The half-maximal inhibitory concentration (IC50) value, representing the concentration of the sample required to inhibit 50% of the DPPH radicals, was determined from the dose-response curve by plotting the percentage of RSA against the concentration of the extract or standard. This value was estimated within the linear range of the curve, with each concentration analyzed in triplicate to ensure accuracy and reproducibility. Results were expressed as milligrams of ascorbic acid equivalent (AAE) per gram of sample (mg AAE/g) [25-27,29].

RESULTS

Toxicological Evaluation

Acute oral toxicity: The acute oral toxicity of the ethanolic extract of *Ziziphus mauritiana* leaves was assessed following OECD 423 guidelines using 15 female Wistar rats, including a control group of three animals. The extract was administered orally at doses of 5, 50, 300, and 2000 mg/kg body weight, and the rats were monitored for 14 days for any signs of toxicity.

Across all dose levels, no significant indicators of toxicity were observed. The animals maintained normal physiological functions, with no noticeable changes in eyes, fur, mucous membranes, skin, circulatory, or respiratory systems. Behavioral observations showed no abnormalities in the autonomic or central nervous systems, and somatomotor activity and

behavioral patterns remained consistent across all groups. The control group, which received distilled water, exhibited baseline physiological and behavioral parameters closely aligned with those observed in the experimental groups, confirming that the vehicle did not contribute to any effects. There were no instances of mortality in any of the groups. Body weight measurements recorded at regular intervals showed no significant deviations from baseline values, and all animals displayed healthy weight gain appropriate to their age and sex.

At the end of the study, necropsy examinations revealed no gross pathological changes in either the experimental or control groups. The ethanolic extract was well tolerated at all administered doses, with no signs of acute toxicity observed, even at the highest dose of 2000 mg/kg body weight.

Antioxidant Evaluation

Total Phenolic Content: The TPC of the ethanolic extract from *Z. mauritiana* leaves was determined using the FC assay, with gallic acid serving as the standard reference. Results are expressed in mg GAE/g and are presented in Table 1. The TPC was quantified using a gallic acid standard curve and demonstrated a concentration-dependent increase, reaching a peak of 5.24 mg GAE/g at 200 µg/mL of extract.

Table 1. Total Phenolic Content of Ethanolic Extract of *Z. mauritiana* Leaves

Concentration (µg/mL)	GAE (µg/mL)	TPC in mg GAE/g Extract Mean ± SD
100	41.35	2.07 ± 0.0007
200	104.78	5.24 ± 0.0012

Total Flavonoid Content: The TFC of the ethanolic extract of *Z. mauritiana* leaves was determined using the AC assay, with quercetin as the standard reference. Results are expressed as mg QE/g and are displayed in Table 2. The TFC was quantified using a quercetin standard curve and showed a progressive increase with higher concentrations of the extract, reaching a maximum of 4.17 mg QE/g at 200 µg/mL.

Table 2. Total Flavonoid Content of Ethanolic Extract of *Z. mauritiana* Leaves

Concentration (µg/mL)	QE (µg/mL)	TFC in mg QE/g Extract Mean ± SD
100	25.20	1.26 ± 0.0100
200	83.40	4.17 ± 0.0100

The TPC and TFC of the ethanolic extract at various concentrations are illustrated in Figure 1.

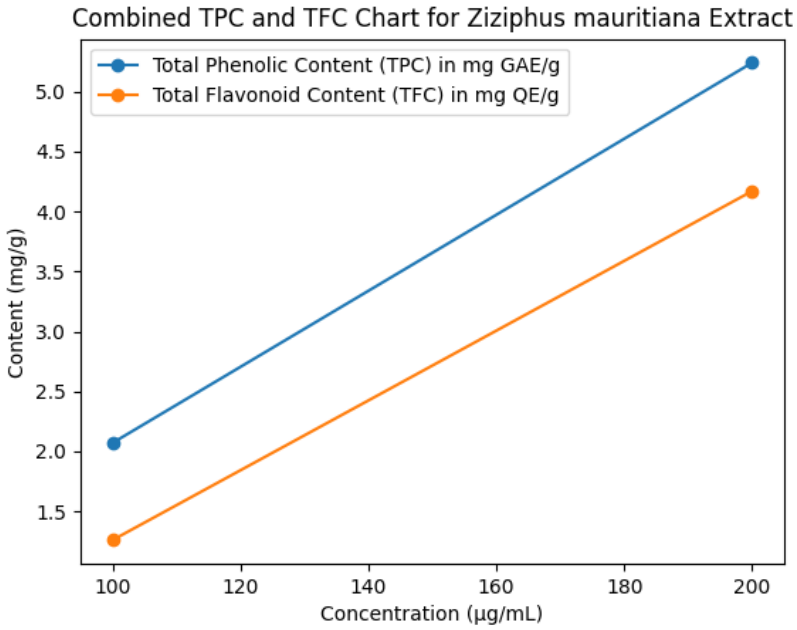

Figure 1. Total Phenolic Content and Total Flavonoid Content of the Ethanolic Extract of *Z. mauritiana* Leaves

DPPH Radical Scavenging Activity

The antioxidant potential of the ethanolic extract of *Z. mauritiana* leaves was evaluated using the DPPH radical scavenging assay, with ascorbic acid used as the reference standard for comparison. This method measures the percentage of radical scavenging activity (% RSA) and determines the IC50 value, representing the concentration required to inhibit 50% of DPPH radicals.

The ethanolic extract demonstrated a concentration-dependent increase in DPPH scavenging activity, achieving maximum inhibition of 94.16% at a concentration of 1000 µg/mL. The IC50 value of the extract was calculated to be 128.17 µg/mL, indicating substantial antioxidant potential. By comparison, ascorbic acid exhibited a significantly lower IC50 value of 24.96 µg/mL, reflecting greater efficacy at lower concentrations.

The % RSA values for both the ethanolic extract and ascorbic acid at different concentrations, along with their respective IC50 values, are presented in Table 3, offering a comparative overview of their antioxidant activities.

Table 3. DPPH Radical Scavenging Activity of Ethanolic Extract of Z. mauritiana Leaves

Concentration (µg/mL)	Ascorbic acid		Z. mauritiana	
	RSA (%)	IC50 (µg/mL)	RSA (%)	IC50 (µg/mL)
1	6.07	24.96	2.24	128.17
10	27.87		19.40	
25	37.94		31.54	
50	60.73		51.13	
100	94.95		84.35	
250	96.08		90.96	

500	96.64		92.39	
1000	96.92		94.16	

The DPPH RSA (% RSA) of the ethanolic extract of *Z. mauritiana* leaves compared to ascorbic acid across varying concentrations is illustrated in Figure 2. The data demonstrates a dose-dependent increase in antioxidant activity, as evidenced by the increasing percentage of DPPH radical inhibition.

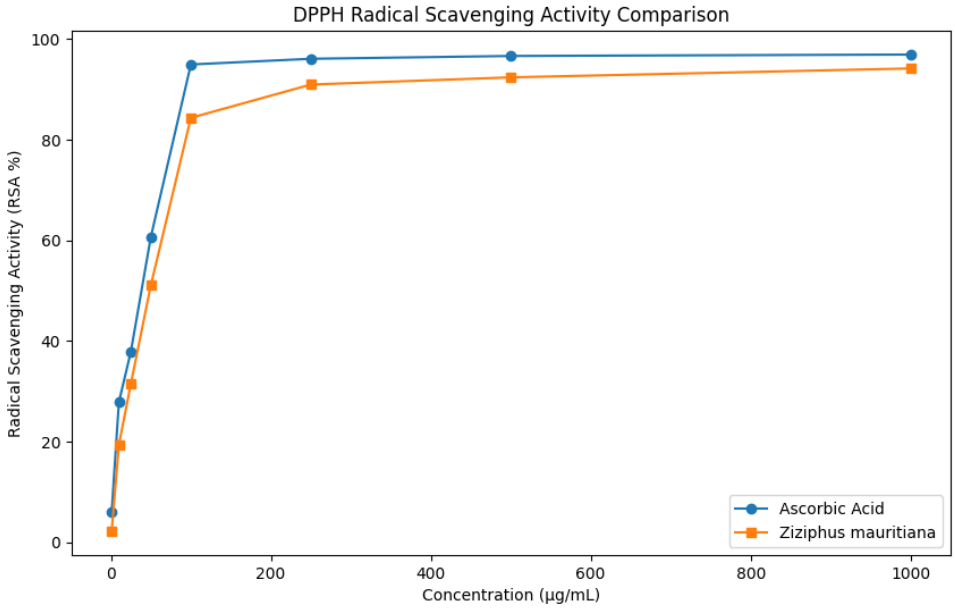

Figure 2. DPPH Radical Scavenging Activity of Ethanolic Extract of *Z. mauritiana* Leaves Compared to Ascorbic Acid

DISCUSSION

The current study's findings are consistent with previous reports on the antioxidant and toxicological profiles of *Z. mauritiana*. Javed et al. documented that methanolic extracts of *Z. mauritiana* leaves demonstrated significant antioxidant activity, as evidenced by the DPPH radical scavenging assay [31]. Correspondingly, the current study observed that the ethanolic extract of *Z. mauritiana* leaves achieved a maximum inhibition of 94.16% at a concentration of 1000 µg/mL, with an IC50 value of 128.17 µg/mL. This strong antioxidant potential was further validated by high TPC and TFC observed, both of which are established contributors to the plant's antioxidant capacity.

The strong antioxidant activity in the extract is attributed to its rich content of phenolic compounds and flavonoids, bioactive molecules capable of neutralizing ROS by donating hydrogen atoms or electrons. This property aids in preventing oxidative stress and protecting cells from damage—a fundamental mechanism implicated in various chronic diseases [31,32]. The absence of acute toxicity in this study corroborates findings related to other *Ziziphus* species. Sakna et al. highlighted that many species within the genus, including *Z. jujuba* and *Z. spina-christi*, exhibit non-toxic profiles and have been traditionally employed for medicinal purposes [32]. This supports the safe profile of *Z. mauritiana* while emphasizing its potential therapeutic applications.

The non-toxic nature of the extract may partly stem from bioactive compounds such as saponins and triterpenoids, which are known for stabilizing cellular membranes and augmenting endogenous antioxidant defenses. This dual benefit of antioxidant activity coupled with a lack of toxicity underlines the plant's therapeutic value.

Future research directions include evaluating the therapeutic effects of the ethanolic extract of *Z. mauritiana* in disease models driven by oxidative stress. For instance, studies examining its impact on metabolic disorders, including dyslipidemia and hyperlipidemia induced by high-fat diets, could provide valuable insights. Additionally, isolating and characterizing specific bioactive constituents responsible for the observed antioxidant effects may lead to novel phytopharmaceutical applications. Research exploring long-term toxicity and repeated exposure safety would further solidify the extract's profile for chronic use.

The current study suggests that the ethanolic extract of *Z. mauritiana* leaves holds substantial therapeutic promise due to its robust antioxidant properties and absence of acute toxicity. Its potential as a natural antioxidant supplement could support health and prevent diseases linked to oxidative stress, such as cardiovascular disorders, diabetes, and neurodegenerative conditions. Moreover, traditional applications of *Z. mauritiana* for treating various ailments gain scientific validation through this work, opening the possibility for broader therapeutic utilization. As a plant-based remedy, it could cater to the rising demand for natural products in the pharmaceutical and nutraceutical sectors.

This study has limitations, such as focusing solely on acute toxicity and antioxidant properties, without addressing chronic toxicity or long-term safety. Further studies are recommended to investigate potential long-term toxic effects to offer a complete safety profile. Despite its limitations, the study has strengths, including a comprehensive evaluation of toxicological and antioxidant properties and the use of established assays, such as the DPPH, FC, and AC assays, which lend reliability to the findings. Adherence to OECD guidelines ensures international compliance for the toxicological evaluation.

In summary, this research supports traditional medicinal uses of *Z. mauritiana* and emphasize its potential for novel therapeutic applications, providing a foundation for future investigations.

CONCLUSION

The current study offers a comprehensive analysis of the toxicological and antioxidant properties of the ethanolic extract of *Z. mauritiana* leaves. Results indicate significant antioxidant activity, demonstrated by high total phenolic and flavonoid contents and potent DPPH radical scavenging activity. The absence of acute toxicity in Wistar rats further supports the safety of the extract, even at elevated doses.

These findings imply that *Z. mauritiana* extract holds considerable promise as a natural antioxidant agent with potential therapeutic applications. Its phenolic and flavonoid content contributes substantially to its antioxidant properties, potentially mitigating oxidative stress and protecting against associated diseases. The extract's non-toxic nature further enhances its viability for use in medicinal and nutraceutical applications.

Future research should concentrate on long-term toxicity studies and isolating specific bioactive compounds to further elucidate the therapeutic potential of *Z. mauritiana*. Investigating its effects in disease models related to oxidative stress may pave the way for new treatments and health supplements.

In conclusion, the ethanolic extract of *Z. mauritiana* leaves presents as a promising candidate for natural antioxidant therapies, offering a safe and effective avenue for health enhancement and disease prevention.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AAE, ascorbic acid equivalent; AC, aluminum chloride; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FC, Folin-Ciocalteu; GAE, gallic acid equivalents; OECD, Organization for Economic Cooperation and Development; IC50, half-maximal inhibitory concentration; QE, quercetin equivalents; ROS, reactive oxygen species; RSA, radical scavenging activity; TFC, total flavonoid content; TPC, total phenolic content; UV-Vis, Ultraviolet–visible; *Z. mauritiana*, *Ziziphus mauritiana*.

REFERENCES

[1] Rehman MH, Saleem U, Ahmad B, Rashid M. Phytochemical and toxicological evaluation of Zephyranthes citrina. Frontiers in Pharmacology. 2022 Sep 23; 13:1007310.

[2] Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two Ziziphus Mill. species. PloS one. 2020 May 19;15(5): e0232599.

[3] Chen J, Liu X, Li Z, Qi A, Yao P, Zhou Z, Dong TT, Tsim KW. A review of dietary Ziziphus jujuba fruit (Jujube): Developing health food supplements for brain protection. Evidence-Based Complementary and Alternative Medicine. 2017(1):3019568.

[4] O'Brien CJ, Campbell S, Young A, Vogler W, Galea VJ. Chinee apple (Ziziphus mauritiana): a comprehensive review of its weediness, ecological impacts and management approaches. Plants. 2023 Sep 8;12(18):3213.

[5] Khadivi A. Morphological characterization and interspecific variation among five species of Ziziphus genus to select superiors in Iran. BMC Plant Biology. 2023 Nov 8;23(1):550.

[6] Naaz F, Agari N, Singh A. Medicinal Properties of Ziziphus mauritiana: A Review Article. Int. J. Pharm. Pharm. Res. 2020; 19:219-30.

[7] Ziziphus mauritiana. Atlas of Florida Plants, Institute of Systematic Botany. Website. https://florida.plantatlas.usf.edu/plant.aspx?id=255 Accessed October 02, 2024.

[8] Ziziphus mauritiana var. mauritiana. Royal Botanic Gardens Kew. Plants of the World Online. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77225453-1 Accessed October 02, 2024.

[9] Yang L, Wu X, Luo M, Shi T, Gong F, Yan L, Li J, Ma T, Li R, Liu H. Na+/Ca2+ induced the migration of soy hull polysaccharides in the mucus layer in vitro. International Journal of Biological Macromolecules. 2022 Feb 28;199: 331-40.

[10] Anwar F, Saleem U, Rehman AU, Ahmad B, Ismail T, Mirza MU, Ahmad S. Acute oral, subacute, and developmental toxicity profiling of naphthalene 2-yl, 2-chloro, 5-nitrobenzoate: Assessment based on stress response, toxicity, and adverse outcome pathways. Frontiers in Pharmacology. 2022 Jan 20;12: 810704.

[11] Muflihah YM, Gollavelli G, Ling YC. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. Antioxidants. 2021 Sep 27;10(10):1530.

[12] Pang Y, Ahmed S, Xu Y, Beta T, Zhu Z, Shao Y, Bao J. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chemistry. 2018 Feb 1; 240:212-21.

[13] Chaves N, Santiago A, Alías JC. Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity

and hierarchization based on the method used. Antioxidants. 2020 Jan 15;9(1):76.

]14] Gupta N. Morphological and physico-chemical characterization of ber (Ziziphus mauritiana Lamk.) genotypes in semi-arid zone of Punjab. Int J Chem Stud. 2018;6(5):2353-6.

[15] Dahiru D, Obidoa O. Pretreatment of albino rats with aqueous leaf extract of Ziziphus mauritiana protects against alcohol-induced liver damage. Tropical Journal of Pharmaceutical Research. 2007 Jul 31;6(2):705-10.

[16] Bhatia A, Mishra T. Free radical scavenging and antioxidant potential of Ziziphus mauritiana (Lamk.) seed extract. J Compl Integr Med. 2009;8(1):42–46.

[17] Ndhlala AR, Mupure CH, Chitindingu K, Benhura MA, Muchuweti M. Antioxidant potentials and degrees of polymerization of six wild fruits. Scientific Research and Essay. 2006 Dec 1;1(3):87-92.

[18] Dahiru D, William ET, Nadro MS. Protective effect of Ziziphus mauritiana leaf extract on carbon tetrachloride-induced liver injury. African Journal of Biotechnology. 2005;4(10).

[19] Mishra T, Khullar M, Bhatia A. Anticancer potential of aqueous ethanol seed extract of Ziziphus mauritiana against cancer cell lines and Ehrlich ascites carcinoma. Evidence-Based Complementary and Alternative Medicine. 2011;2011(1):765029.

[20] Dahiru, D., & Obidoa, O. (2007). Evaluation of the antioxidant effects of *Ziziphus mauritiana* Lam. Leaf extracts against chronic ethanol-induced hepatotoxicity in rat liver. African journal of traditional, complementary, and alternative medicines: AJTCAM, *5*(1), 39–45. https://doi.org/10.4314/ajtcam.v5i1.31254

[21] Owolarafe, T., Ihegboro, G., Salawu, K., Ononamadu, C., Fadılu, M., & Musa, B. (2022). Toxicological investigation of aqueous extract of *Ziziphus mauritiana* leaves on wistar rats. International Journal of Traditional and Complementary Medicine Research, *3*(2), 91-100.

[22] Yahaya T, Shehu K, Isah H, Oladele E, Shemishere U. Toxicological evaluation of the leaves of Guiera senegalensis (JF Gme), Cassia occidentalis (Linn), and Ziziphus mauritiana (Lam). Beni-Suef University Journal of Basic and Applied Sciences. 2019 Dec;8: 1-9.

[23] Owolarafe T, Ihegboro G, Salawu K, Ononamadu C, Fadılu M, Musa B. Toxicological investigation of aqueous extract of Ziziphus mauritiana leaves on wistar rats. International Journal of Traditional and Complementary Medicine Research. 2022;3(2):91-100.

[24] OECD. Test No. 423: acute oral toxicity-acute toxic class method. OECD Guidelines for the Testing of Chemicals, Section 4. 2002:14.

[25] Ayele DT, Akele ML, Melese AT. Analysis of total phenolic contents, flavonoids, antioxidant and antibacterial activities of Croton macrostachyus root extracts. BMC chemistry. 2022 May 12;16(1):30.

[26] Haile K, Mehari B, Atlabachew M, Chandravanshi BS. Phenolic composition and antioxidant activities of cladodes of the two varieties of cactus pear (Opuntia ficus-indica) grown in Ethiopia. Bulletin of the Chemical Society of Ethiopia. 2016;30(3):347-56.

[27] Seifu T, Mehari B, Atlabachew M, Chandravanshi B. Polyphenolic content and antioxidant activity of leaves of Urtica simensis grown in Ethiopia. Latin American Applied Research. 2017 Jan 1;47(1):35-40.

[28] Rahman MM, Islam MB, Biswas M, Khurshid Alam AH. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC research notes. 2015 Dec;8: 1-9.

[29] Akanda MK, Hasan AN. Characterization of pharmacological properties of methanolic seed and stem bark extracts of Ziziphus mauritiana (BAU Kul) using in-vitro and in-vivo animal (Swiss albino male mice) model. Clinical Phytoscience. 2021 Dec;7: 1-3.

[30] Khorasani Esmaeili A, Mat Taha R, Mohajer S, Banisalam B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from in vivo and in vitro grown Trifolium pratense L. (Red Clover). BioMed Research International. 2015;2015(1):643285.

[31] Javed M, Bibi R, Nazir K, Hussain S. Phytochemistry of Ziziphus mauritiana; its antioxidant and antimicrobial potential. Advancements in Life Sciences. 2022 Aug 27;9(2):157-62.

[32] Sakna ST, Maghraby YR, Abdelfattah MS, Farag MA. Phytochemical diversity and pharmacological effects of triterpenes from genus Ziziphus: a comprehensive review. Phytochemistry Reviews. 2023 Dec;22(6):1611-36.