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BIOACTIVITY AND SAFETY EVALUATION OF LYOPHILIZED HYDROETHANOLIC *CISSUS QUADRANGULARIS* EXTRACT

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ABSTRACT: This study examined the phytochemical composition, bioactivity, and biosafety of the hydroethanolic extract of *Cissus quadrangularis*, a plant traditionally employed in the management of diabetes mellitus. Phytochemical analysis identified a rich presence of alkaloids, flavonoids, glycosides, and phenolic compounds, with the ethanolic fraction yielding a high total phenolic content (60.96 mg GAE/g). The *in-vitro* anti-inflammatory potential of the extract was assessed via the egg albumin denaturation assay across concentrations up to 1000 µg/mL and benchmarked against Diclofenac, a standard non-steroidal anti-inflammatory drug. In parallel, acute oral toxicity and biosafety were evaluated in female Sprague-Dawley rats over a 14-day period, with dosage escalation up to 5000 mg/kg body weight. The hydroethanolic extract exhibited significant, concentration-dependent anti-inflammatory activity, attaining a maximum inhibition of 69.30±1.57% at 1000 µg/mL, which was comparable to the 83.45±1.27% inhibition observed with Diclofenac. Acute toxicity evaluation indicated an excellent safety profile, with no mortality or adverse clinical signs, and normal behavior, food and water intake, and weight gain observed across all groups, including the highest dose cohort. In summary, the hydroethanolic extract of *Cissus quadrangularis* demonstrates marked anti-inflammatory efficacy and a high safety margin, supporting its traditional use and potential for development as a safe, effective therapeutic agent.

INTRODUCTION:

***Cissus quadrangularis*:** *Cissus quadrangularis* is widely adapted to arid conditions prevalent in tropical and subtropical regions, with its natural distribution notably encompassing coastal and lowland ecosystems¹. The species has been extensively documented throughout Africa and India, where it holds significant ethnomedicinal value¹.

Cissus quadrangularis is frequently encountered in thickets, open forests, scrub jungles, forest peripheries, riverbanks, and wastelands situated at low to moderate elevations². This resilient climbing plant is referred to by a range of vernacular and international names, such as veldt grape, adamant creeper, cactus vine, kangaroo vine, stemmed vine, and winged tree bine³.

Cissus quadrangularis is classified as a perennial herbaceous climber distinguished by its robust, succulent, quadrangular stems, which are deep green in color, glabrous, and exhibit constrictions at the nodes⁴. The aerial components of the plant comprise tendrils, leaves, inflorescences, flowers, and fruits¹. The leaves are simple, displaying ovate or reniform shapes with serrulate or dentate

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margins and variable lobation. Flowers, which emerge from the stem, are pink to white in coloration, cup-shaped, and possess four well-defined ovate-oblong petals. The fruit is represented by globose, red, acidic berries, each

containing a single, smooth, obovoid seed³. The principal flowering and fruiting phases occur during June and July, delineating the reproductive period of the species in its natural habitat¹.



FIG. 1: *CISSUS QUADRANGULARIS* PLANT AND FRUIT

Traditional uses of *Cissus quadrangularis*: A comprehensive review of traditional literature regarding *Cissus quadrangularis* reveals its widespread use in managing various health conditions affecting both humans and animals across different regions¹. The plant is commonly employed in the treatment of conditions such as fractures, pain, inflammation, osteoporosis, rheumatoid arthritis, and osteoarthritis⁵. The plant has been claimed to treat a range of conditions, such as diabetes, swelling, hemorrhage, anorexia, flatulence, dyspepsia, colic, chronic ulcers, hemoptysis, convulsions, skin diseases, leprosy, and helminthiasis⁴.

Anti-inflammatory and Antioxidant Secondary Metabolites: The plant extracts of *Cissus quadrangularis* show the presence of several notable secondary metabolites³. These include alkaloids, flavones and flavonoids, saponins, phytosterols, steroids, stilbenes, triterpenoids, tannins, carotene, cardiac glycosides, and vitamins, with an emphasis on vitamin C⁴. The secondary metabolites present in *Cissus quadrangularis* encompass a diverse range of bioactive compounds, many of which are recognized for their significant roles in diabetes management⁵. Alkaloids are a diverse group of naturally occurring compounds recognized for their substantial antidiabetic effects. Through multiple biochemical pathways, they enhance insulin secretion, facilitate glucose uptake, and suppress the process of

gluconeogenesis⁶. Glycosides exert hypoglycemic effects by enhancing insulin sensitivity and inhibiting carbohydrate conversion. Compounds such as myrciacitrins and myrciaphenones inhibit both aldose reductase and α -glucosidase, key enzymes in glucose metabolism⁴. Flavonoids, a major group of polyphenolic compounds, contribute to diabetes management by mimicking insulin, stimulating its secretion, and slowing down glucose digestion³.

MATERIALS AND METHODS:

Materials, Equipment and Facilities: All chemicals, equipment, associated reagents and facilities for the *in-vitro* and bioactivity assays were obtained from the laboratories at the University of Zimbabwe, Faculty of Medicine and Health sciences, Pharmacy and Pharmaceutical Sciences Department in 2026. This study was conducted with approval from the Joint Research and Ethics Committee of the University of Zimbabwe College of Health Sciences and Parirenyatwa Group of Hospitals (JREC/484/2025).

***Cissus quadrangularis* Plant Material Collection, Identification and Authentication:** The fresh plant of *Cissus quadrangularis* was collected from Makoni District, Rusape 18° 32' 7" S (Latitude) and 32° 8' 6" E (Longitude), during the month of November 2025 after carefully examining the location's surrounding. Samples of the plants were authenticated on 4 November 2025 and identified

at the National Herbarium and Botanical Garden, by the Research Officer and was identified as *Cissus quadrangularis*. The stem of the plant was used for this study. The plant was stored under the shed at room temperature.

Plant Preparation: The stem was sun dried for at least three weeks and were later pulverized with a mortar and pestle. The materials were then grounded into fine powder using a coffee grinder (Hamilton Beach Coffee Grinder Model- 80410). For phytochemical screening, 500g of the powdered plant material were macerated into 1000ml of 70% hydroethanolic solution in a sterile bottle. The mixture was macerated for 3 days with constant continuous shaking for 3 minutes, twice daily. Primary filtration was performed using a muslin cloth, followed by secondary filtration (vacuum pressure filtration), using a Whatman number 1 filter paper. The filtrate was then concentrated using the rotary vapor, (Rotavapor R300, Buchi, Switzerland) under reduced pressure,

56Pa, to remove excess ethanol. This was followed by lyophilization at low pressure of 177 mTorr and ultra-low temperature of -52 °C using a Heto Freeze dryer model three ³. The dried extract was stored in an airtight, sterile amber bottle at 4 °C. Method by Chifamba et al 2025 was used as a guide ⁷. The percentage yield obtained was calculated using the following equation.

Equation 1:

$$\text{Yield (\%w/w)} = \frac{\text{Obtained mass of plant extract (g)}}{\text{Amount of plant material soaked (g)}} \times 100$$

Phytochemical Screening of *Cissus ouadrangularis*: 3g of the hydroethanolic extract of *Cissus ouadrangularis* was dissolved in 50ml distilled water and subjected to various phytochemical screening techniques to confirm the presence or absence of the important phytoconstituents of pharmacological interest.

TABLE 1: PHYTOCHEMICAL SCREENINGTESTS

Test	Procedure	Reference
Test for flavonoids	To 2ml of the plant extract solution, 2mls of dilute ammonia solution was added, followed by a few drops of concentrated sulphuric acid	[7]
Test for alkaloids	To 2mls of the extract, a few drops of Wagner’s reagent were added along the sides of a test tube	[8]
Test for tannins	To 3mls of <i>Cissus quadrangularis</i> extract, added 4 drops of 10% ferric chloride solution	[8]
Test for glycosides	To 2ml of <i>Cissus quadrangularis</i> , 1.5ml of glacial acetic acid was added followed by 1 drop of 5% ferric chloride and 2 drops of concentrated sulphuric acid alongside of the test tube	[8]
Test for phenols	A few drops of ferric chloride were added to 2mls <i>Cissus quadrangularis</i> extract	[7]
Test for Saponins	To 2ml of the extract, 5mls of distilled water were added, the mixture was then shaken in a granulated cylinder for 10 minutes	[7]

Acute Oral Toxicity: The acute oral toxicity assessment of *Cissus ouadrangularis* was conducted following a modified version of OECD Guidelines 425 and study methods by Chifamba, et al., 2025 with a few modifications ^{7, 9}. This modification was done to estimate LD50 while using fewer animals. Six healthy, nulliparous and non-pregnant female Sprague Dawley rats aged 8-10 weeks and weighing 215g-220g were selected for the acute oral toxicity assessment. The rats were individually housed and acclimatized to the laboratory conditions for 10 days ⁷. They were maintained in well-ventilated cages with free access to commercial rodent feed and water to ensure nutritional adequacy. Their welfare was monitored by a professional throughout the study.

Study Design: The animals were fasted overnight with access to drinking water to ensure accurate absorption assessment ⁷. Each rat was dosed individually *via* oral gavage using a fixed volume of plant extract. The doses ranged from 50, 500, 1000, 2500 and 5000mg/kg body weight at 48-hour interval. The dose range follows a logarithmic escalation starting at a conservatively “sighting limit” level and a progressively increasing to a limit dose to efficiently and ethically identify the toxicological threshold and LD50 while minimizing unnecessary animal exposure.If the rate survived, the dose for the next rat was increased and if the animal died, the next dose was reduced. The doses were prepared by dissolving the lyophilised hydroethanolic *Cissus ouadrangularis*

extracts in distilled water to achieve the desired concentration. The control animal received an equivalent volume of distilled water.

Post Dosing Observations and Toxicity Monitoring: Post administration, each animal was closely monitored for the initial 30mins for any acute signs of toxicity, including tremor, convulsions, salivation, diarrhoea, drowsiness and urination frequency⁹. Subsequent observations were made twice daily for 14 days, during which clinical parameters including skin and fur, ocular and mucous membrane appearance, respiratory patterns, locomotor activity and food and water consumption were monitored and recorded. Body weights were measured on day 1, 7 and 14⁷. A veterinary specialist oversaw these observations to promptly identify and document any adverse clinical signs or mortality. This protocol allowed for the ethical assessment of the acute oral toxicity of *Cissus ouadrangularis*.

Euthanasia and Post-Study Handling: All surviving animals were humanely euthanised by cervical dislocation, adhering to AVMA guidelines for the ethical euthanasia of laboratory animals, at the end of the study period.

Anti-inflammatory Assay: The egg albumin test was used to determine the anti-inflammatory activities of lyophilizedhydroethanolic *Cissus ouadrangularis* extracts. Methods by Onyeka, et al 2022 and HDT 2023 were used as a guide^{10, 9}. Stock solution of the plant extracts were prepared at a concentration of 10mg/ml in 0.4% dimethyl sulfoxide (DMSO). Diclofenac stock solution was prepared at an equivalent concentration using the same solvent. The diclofenac stock solution was used as the positive control. Serial dilutions of both solutions were then performed to attain final concentration of 50, 100, 250, 500, 750 and 1000µg/ml in the reaction mixture. Test tubes were labelled and each test tube was allocated 0.4 ml of the fresh egg albumin, 0.5ml of either 0.5ml of either the lyophilizedhydroethanolic *Cissus ouadrangularis* extract or diclofenac, in addition to 3ml of phosphate-buffer saline (PBS). The negative control test tubes were prepared with 0.4ml of egg albumin, 0.5ml of 0.4% DMSO and 3ml of PBS. Following preparation, the mixtures were incubated at 37°C for 20 minutes and then subjected to a

water bath at 65°C for 30 minutes to induce protein denaturation. After cooling, absorbance readings were obtained at 660nm using a UV/Vis spectrophotometer with 0.4% DMSO employed as the blank. The percentage inhibition of protein denaturation was then calculated using the equation below:

Equation 2:

$$\text{Inhibition\%} = (\text{Abs}(\text{control}) - \text{Abs}(\text{sample}) / (\text{Abs}(\text{control}) \times 100$$

Abs (control) - The absorbance of the negative control (Reaction mixture containing phosphate-buffered saline and egg albumin). Abs (sample) - The absorbance of the test sample (Reaction mixture containing phosphate-buffered saline, egg albumin and the plant extract/Standard drug).

Anti-oxidation Assay:

Total Phenolic Content: The quantification of total phenolic content in the extracts was conducted using the Folin-Ciocalteu reagent assay, following established protocols with slight modifications¹¹. A gallic acid stock solution was prepared at a concentration of 5 mg/mL by dissolving 5 mg of gallic acid in 1 mL of distilled water. Serial dilutions of this stock solution were then performed to generate five different concentrations of gallic acid standard solutions: 0.04, 0.08, 0.12, 0.16, and 0.2 mg/mL. The lyophilizedhydroethanolic *Cissus ouadrangularis* extract was reconstituted in distilled water to a concentration of 10 mg/mL. Immediately prior to use, the Folin-Ciocalteu reagent was diluted 10-fold with deionized water. Aliquots of 0.25 mL of each gallic acid standard solution and 0.25 mL of each plant extract solution were transferred into separate test tubes.

To each test tube, 1.25 mL of the 10-fold diluted Folin-Ciocalteu reagent was added, and the mixture was allowed to react for 5 minutes at room temperature. Subsequently, 2 mL of 7.5% sodium carbonate combined with folin solution was added to each test tube, and the final volume in each test tube was adjusted to 5 mL with distilled water. The test tubes were then incubated in the dark at room temperature for 60 minutes to facilitate color development. After incubation, the absorbance at 760 nm was measured spectrophotometrically, using the Folin-Ciocalteu reagent combined with

sodium carbonate solution as a blank. All experiments were performed in triplicate. A calibration curve was generated by plotting the absorbance values of the gallic acid standard solutions against their corresponding concentrations. This calibration curve was used to determine the total phenolic content in lyophilized hydroethanolic *Cissus quadrangularis* extract by comparing the absorbance of the extract solution to the gallic acid calibration curve. The results were expressed as milligrams of gallic acid equivalents per gram of the sample. The total phenolic content was calculated using the formula derived from the gallic acid calibration curve:

Equation 3:

$$\text{Total Phenolic Content (mg GAE/g)} = C \times V/M$$

Where, C is the concentration of gallic acid determined from the calibration curve (mg/mL), V is the volume of the extract in mL, M is the weight of the dry plant extract in grams.

RESULTS AND DISCUSSIONS:

Percentage Yield of the Hydroethanolic Extract of *Cissus quadrangularis*: In this study, hydro-ethanolic extraction of *Cissus quadrangularis* produced a yield of 15 grams from 500 grams of dried plant material, corresponding to a 3% extraction efficiency. This result is lower than previously reported extraction yields for *Cissus quadrangularis*, which typically range from about 4% to 24%, depending on the solvent system and extraction methods employed¹². The elevated yield observed in the present work may be attributed to methodological optimizations such as refined maceration periods, controlled extraction temperatures, and carefully adjusted solvent-to-solid ratios. Additionally, the yield of plant extract is substantially influenced by the geographical origin of the collected material. Environmental parameters including soil composition, climate conditions, altitude, and seasonal changes play a crucial role in determining the biosynthetic capacity of the plant to produce secondary metabolites¹¹.

The qualitative phytochemical screening of the hydro-ethanolic extract of *Cissus quadrangularis* revealed a notably dense concentration of secondary metabolites, including alkaloids,

flavonoids, glycosides, and phenolic compounds, all demonstrating a strong presence (+++) **Table 2**. The effectiveness of the hydro-ethanolic solvent system is particularly significant in this context, as it efficiently partitioned both polar and semi-polar phytochemicals. This comprehensive phytochemical profile underscores the extract's substantial pharmacological potential¹. The high concentration of glycosides (+++) in the extract is aligned with mechanisms by which glycosides exert hypoglycemic effects, namely through the inhibition of carbohydrate conversion and the reduction of intestinal glucose absorption¹³. Similarly, the substantial presence of flavonoids (+++) and phenolic compounds (+++) indicates a strong potential for insulin-mimetic activity. Flavonoids such as quercetin and rutin are well-documented for their ability to enhance insulin signaling and decrease hepatic glucose output¹³.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *CISSUS QUADRANGULARIS*

Test	Presence in Hydro-ethanolic extract
Alkaloids	+++
Flavonoids	+++
Saponins	++
Phenols	+++
Tannins	++
Glycosides	+++

Key (+) present, (-) absent, (++) Present in moderate amounts, (+++) Present in abundance.

While this study focuses on qualitative phytochemical screening to identify the presence of bioactive classes such as flavonoids, tannins, and saponins, it is acknowledged that quantitative analysis such as the determination of total phenolic or flavonoid content and marker-based chromatographic profiling would provide a more granular understanding of the extract's composition. Within the scope of this research, the qualitative screening serves as a foundational step to confirm the existence of known therapeutic metabolites, which provides a basis for future bio-guided fractionation and quantitative characterization.

Acute Oral Toxicity Evaluation: The acute oral toxicity assessment was conducted in accordance with the OECD Technical Guideline 425. All observations and interpretations were performed under the supervision of a qualified veterinary expert from the Department of Animal Sciences. An initial dose of 250 mg/kg was selected based on

existing toxicological data, ensuring it remained below the estimated LD50 threshold. Following administration, rats were monitored twice daily for signs of mortality and morbidity. In addition, clinical manifestations of toxicity were recorded every hour for the first 12 hours post-dosing. Body weight was measured daily to identify any physiological changes. This systematic protocol provided a comprehensive evaluation of the extract's safety profile and maintained adherence to ethical standards for animal experimentation. The results demonstrated that administration of the extract at doses up to 5000 mg/kg body weight did not produce observable signs of toxicity or cause mortality in rats, indicating a favorable safety profile. Consistent increases in body weight throughout the study period suggest that the extract does not induce systemic toxicity or disrupt normal metabolic processes, even at high concentrations

Fig. 2 Table 3. Notably, no animals were withdrawn from the study for any reason during the observation period. Although no mortality occurred, pre-defined humane endpoints including

excessive weight loss (>20%), prolonged lethargy, or failure to access food and water were established to ensure ethical intervention if adverse clinical signs had appeared. These findings are consistent with those reported by Kavitha *et al.*, who also estimated the LD50 of *Cissus quadrangularis* extract to be more than 5000 mg/kg body weight⁵. Therefore, the results of this study indicate that even high concentrations of the extract can be used to achieve desired bioactive effects in *Cissus quadrangularis* L.-based treatments without posing a toxicity risk. Consequently, the safety profile of the lyophilized hydroethanolic *Cissus quadrangularis* extract was determined based on clinical observations, behavioral monitoring, and mortality rates over the 14-day observation period. While these clinical findings provide robust evidence of the extract's high margin of biosafety at the tested doses, future studies incorporating detailed histopathological examinations are recommended to confirm the absence of sub-acute or sub-chronic tissue-level changes.

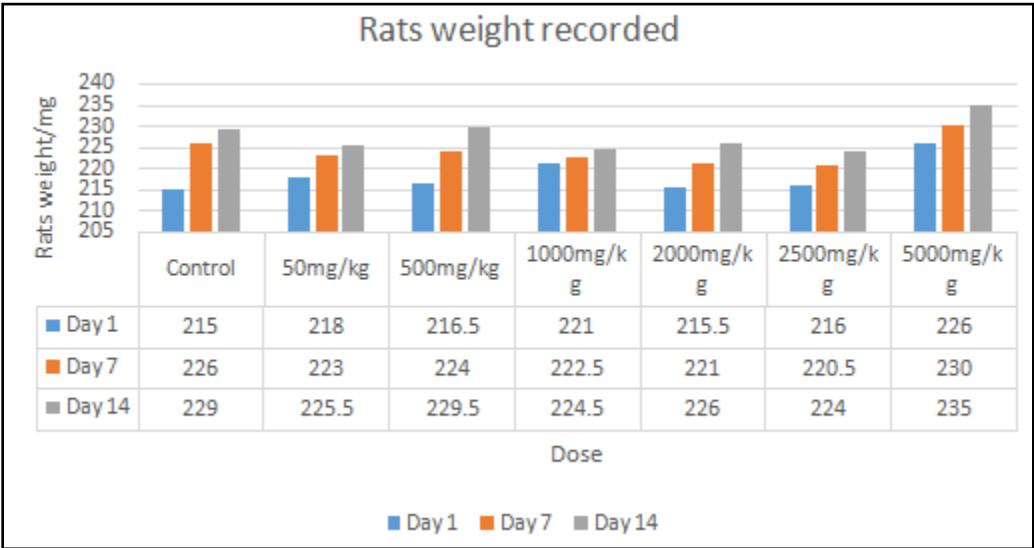


FIG. 2: WEIGHT GAIN RECORDED ON DAY 1, DAY 7 AND DAY 14

TABLE 3: BEHAVIOURAL OBSERVATIONS

Observed Parameter	Dose of <i>Cissus ouadrangularis</i> L. in mg/kg body weight						
	Control	50mg/kg	500mg/kg	1000mg/kg	2000mg/kg	2500mg/kg	5000mg/kg
Water intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Death	Alive	Alive	Alive	Alive	Alive	Alive	Alive
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Normal
defecation	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Erection of fur	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Anti-inflammatory Evaluations: The anti-inflammatory efficacy of the *Cissus quadrangularis* extract was evaluated and compared against the standard reference drug, Diclofenac, across a concentration gradient ranging from 50 to 1000 µg/mL **Table 4** and **Table 5**. Both substances exhibited a clear, dose-dependent increase in percentage inhibition. Diclofenac demonstrated superior inhibitory activity at all tested concentrations, yielding an IC₅₀ of 271 µg/mL, whereas *Cissus quadrangularis* showed an IC₅₀ of 492 µg/mL. At the maximum tested concentration of 1000 µg/mL, *Cissus quadrangularis* achieved 69.30 ± 1.57% inhibition, whereas Diclofenac reached 83.45 ± 1.27%. Furthermore, a two-way ANOVA revealed a statistically significant difference between the inhibition profiles of *Cissus quadrangularis* and Diclofenac (p < 0.05), with Sidak’s post-hoc analysis confirming that the standard drug significantly outperformed the plant extract across the dose range (p < 0.001).The findings of this study demonstrate that *Cissus quadrangularis*

extract possesses notable, dose-dependent anti-inflammatory activity, achieving up to nearly 70% inhibition at the highest concentration tested. Although Diclofenac exhibited a lower IC₅₀ and greater overall potency due to its status as a purified, single-entity pharmaceutical agent, the moderate IC₅₀ of the *Cissus* extract underscores its pharmacological relevance. In the broader context of *Cissus quadrangularis* research, its anti-inflammatory potential is well-documented; studies have shown that specific constituents can modulate complex biological processes, including the inhibition of cyclooxygenase enzymes and the downregulation of pro-inflammatory cytokines such as IL-6 and TNF-α⁴. The protein-stabilizing capacity observed in this study serves as a preliminary, non-cellular indication of the extract's bioactivity, which, when considered alongside the established phytochemical profile of *Cissus quadrangularis*, warrants further investigation using cell-based assays to confirm direct pathway modulation and therapeutic efficacy.

TABLE 4: RESULTS FOR CISSUS QUADRANGULARIS

Concentrationug/ml	% Inhibition Cissus ouadrangularis Reps				Mean ± SD
	1	2	3	4	
50	7.2	8.1	8.5	9.5	8.33±1.59
100	20.5	21.4	22.0	23.1	21.75±1.58
250	34.2	35.0	35.5	36.4	35.28±1.46
500	49.9	50.7	51.3	52.2	51.03±1.58
750	61.2	62.1	62.7	63.6	62.40±1.55
1000	68.1	69.0	69.6	70.5	69.30±1.57

TABLE 5: RESULTS FOR DICLOFENAC

Concentration ug/ml	% Inhibition Diclofenac Reps				Mean ± SD
	1	2	3	4	
50	14.4	15.0	15.5	16.2	15.27±1.24
100	28.2	28.9	29.5	30.2	29.20±1.42
250	46.6	47.4	48.0	48.4	47.61±1.32
500	64.7	65.5	66.1	66.5	65.69±1.32
750	76.4	77.2	77.8	78.0	77.35±1.26
1000	82.5	83.3	83.9	84.1	83.45±1.27

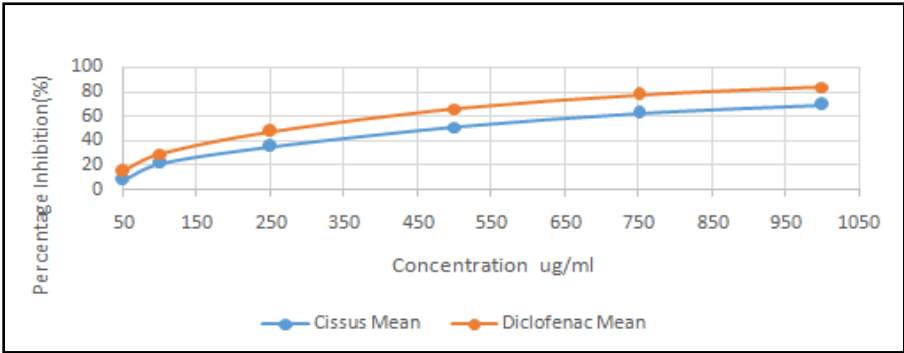


FIG. 3: DOSE-RESPONSE CURVE OF CISSUS QUADRANGULARIS AND DICLOFENAC

Antioxidation Evaluation: The substantial phenolic content quantified in *Cissus quadrangularis* extracts supports the plant's traditional use in managing Diabetes Mellitus³. The high concentration of aromatic hydroxyl groups in the phenolics of *Cissus quadrangularis* extracts enables these compounds to act as electron donors and neutralize free radicals, thus disrupting oxidative stress pathways¹⁴. Additionally, polyphenols in *Cissus quadrangularis* may protect vascular endothelial walls from advanced glycation end-products¹⁵. The hydroethanolic extract of *Cissus quadrangularis* yielded a total phenolic content of 60.96 mg GAE/g extract.

CONCLUSION: This study shows that hydroethanolic stem extract of *Cissus quadrangularis* contains a variety of important phytochemicals, including alkaloids, flavonoids, glycosides, and phenolics, with a total phenolic content of 60.96 mg GAE/g.

The extract demonstrated moderate concentration-dependent *in-vitro* anti-inflammatory activity, reaching $69.30 \pm 1.57\%$ inhibition at 1000 $\mu\text{g/mL}$, comparable to Diclofenac's $83.45 \pm 1.27\%$, suggesting its promise as a natural alternative for chronic inflammation. Acute oral toxicity tests in female rats revealed no negative effects or mortalities even at doses up to 5000 mg/kg, confirming high biosafety. Overall, the extract is potent and non-toxic, supporting its future pharmaceutical development for inflammatory and metabolic disorders.

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Authors' Contributions: This work was carried out in collaboration among all authors. Authors TK, SZ and RJM participated in data collection and drafting of the manuscript. Author JC reviewed the study design and approved the final manuscripts. All authors read and approved the final manuscript.

Ethical Approval: Prior to the investigations, animal use and research ethics approvals were obtained from the Joint Parirenyatwa Research Ethics Committee (JREC/484/2025) which is the

local research Institutional Review board for the University of Zimbabwe.

CONFLICTS OF INTERESTS: Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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