

Conservation of *Cordia Macleodii* (Sanjivani of Chhattisgarh): A Dual Perspective on Biodiversity Preservation and Therapeutic Potential

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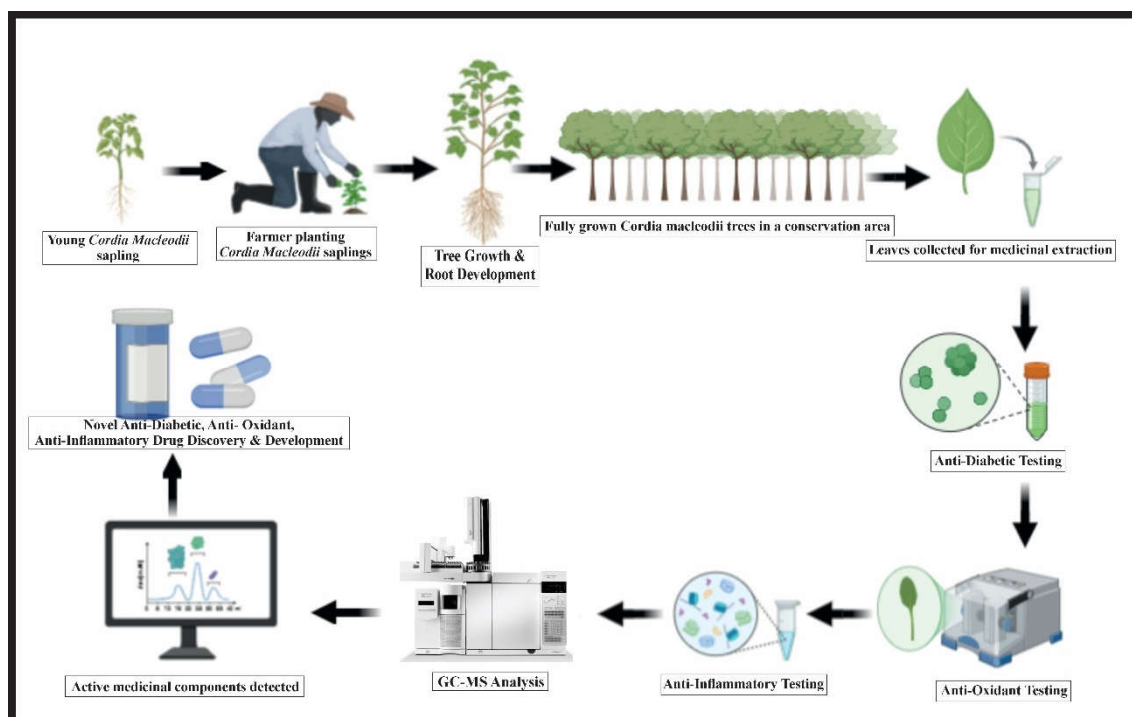
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Abstract:

Cordia macleodii, also known as Sanjivani, is a plant with significant ecological and medicinal value in Chhattisgarh. This research combines conservation efforts and pharmaceutical studies to investigate the feasibility of maintaining *Cordia macleodii* outside its native range and validate its therapeutic potential. *Cordia macleodii* plantations at the Sai Farmhouse in Murmunda, Kumhari, Chhattisgarh demonstrate its adaptability, while laboratory tests investigate its anti-diabetic, anti-inflammatory, and antioxidant properties. The antidiabetic activity was evaluated through the α -amylase inhibition test, which yielded an IC₅₀ value of 36.38 μ g/mL, indicating a strong potential for managing post-prandial hyperglycaemia. The anti-inflammatory effect, assessed with a protein denaturation test, showed significant inhibition and suggested its ability to stabilize proteins against inflammatory stimuli. The antioxidant capacity was measured by the free radical disposal test DPPH, revealing an IC₅₀ value >320 g/mL, which confirmed its radical disposal capability. Furthermore, GC-MS analysis of the ethanolic leaf extract identified key bioactive compounds such as palmitic acid, phytol, and squalene, which are known for their pharmacological relevance. These results emphasize the importance of *Cordia macleodii* as a promising natural treatment for the treatment of metabolic disorders and inflammation and emphasize the need for integrating conservation and scientific research to ensure the sustainable use of this precious plant.

Graphical Abstract:



Keywords: Anti-inflammatory, Antioxidants, Conservation, *Cordia macleodii*, Inhibition of α -amylase, Sustainable use, Therapeutic potential.

1. Introduction:

Cordia macleodii, commonly referred to as Sanjivani, is classified as an endangered species within the Boraginaceae family. This botanical entity has been historically esteemed in traditional Indian medicine due to its wide-ranging therapeutic application, which encompass antidiabetic, anti-inflammatory, and antioxidant effects. Nonetheless, the compounded threats posed by habitat degradation and excessive harvesting have rendered this ecologically and medicinally significant species vulnerable to extinction. Thus, it is both crucial and urgent to reconcile its conservation with rigorous scientific inquiry into its bioactive constituents.

The therapeutic potential of *Cordia macleodii* can be predominantly ascribed to its abundant phytochemical profile, which comprises bioactive substances such as flavonoids, phenolics, and alkaloids. These compounds demonstrate substantial antioxidant capabilities by neutralizing free radicals and alleviating oxidative stress, a mechanism that is associated with the development of various chronic illnesses (Chandrakar et al., 2019; Qureshi et al., 2009). The anti-inflammatory attributes of this plant, evidenced through mechanisms including the stabilization of proteins and the suppression of pro-inflammatory pathways, underscore its potential in the management of chronic inflammation and associated disorders ("Phytopharmacology and Applications of *Cordia macleodii*: A Miraculous Plant," 2023). Additionally, its antidiabetic properties are substantiated by the inhibition of α -amylase activity, a pivotal enzyme in carbohydrate metabolism, thus providing a natural approach for the regulation of hyperglycaemia (Ahuja et al., 2020; Almutairi, 2024).

Acknowledging the paramount significance of this plant, conservation strategies are of equal importance. This investigation chronicles the plantation initiatives undertaken at Sai Farmhouse, Murmunda, Kumhari, where *Cordia macleodii* has been effectively cultivated beyond its indigenous habitat. Such initiatives are designed to secure a sustainable resource for both research purposes and traditional applications, while concurrently fostering its ecological conservation. Moreover, the conservation of *Cordia macleodii* is critical, given its status as an endangered species and its importance in traditional medicine.

This investigation integrates conservation initiatives with pharmacological assessment by examining the bioactive capabilities of *Cordia macleodii*, concentrating on its antidiabetic, anti-

inflammatory, and antioxidant attributes that are associated with its abundant phytochemical profile. Given its threatened status, the study encompasses both in situ conservation measures and extensive phytochemical characterization, which includes GC-MS analysis. The outcomes are intended to advocate for the sustainable utilization of this species and to enhance its enduring pharmacological importance, while simultaneously contributing to its conservation for forthcoming generations.

2. Material and Method:

2.1.1 Conservation plantation: The conservation plantation of *Cordia macleodii* was initiated to mitigate the species' endangered status and to investigate its adaptability beyond its indigenous habitat. The plantation was established at Sai Farmhouse, located in Murmunda, Kumhari, Chhattisgarh.

- **Plant Procurement:** Juvenile specimens, measuring approximately 25 cm in stature, were acquired from the Kanker Forest Department, located in Chhattisgarh, on the 22nd of October in the year 2022.
- **Planting Procedure:** A systematic approach was utilized for plantation:

(i) Site Preparation: The designated plantation area was systematically cleared and prepared through the mechanical loosening of the soil to optimize conditions for root development (Smith et al., 2020).

(ii) Planting: The juvenile specimens were meticulously implanted into the adequately prepared substrate, ensuring appropriate inter-plant spacing to promote growth and mitigate competitive interactions (Johnson, 2019).

(iii) Irrigation: Fundamental irrigation protocols were adhered to, with consistent watering implemented to facilitate the establishment phase of the vegetation (Brown, 2021).

2.1.2 Growth Monitoring: The specimens were subjected to systematic monitoring at regular intervals to evaluate their acclimatization processes and growth trajectories. Metrics such as vertical growth, foliar density, and comprehensive plant vitality were meticulously documented. Visual records were systematically maintained to facilitate comparative analysis of growth phases:

(i) Initial Phase: The specimen exhibits a height of 88 centimetres (approximately 2 feet), characterized by robust foliage and a resilient stem.

(ii) Intermediate Phase: The specimen has attained a height of 122 centimetres (approximately 4 feet), demonstrating a notable augmentation in leaf density and stem girth.

(iii) Current Phase: The specimen now measures 195.58 centimetres (approximately 6.5 feet) in height, exhibiting a profusion of foliage and an extensively developed canopy.

2.1.3 Observations

The specimens exhibited a remarkable level of resilience and adaptability to the prevailing environmental parameters. By the time of evaluation, they had attained a height of 195.58 cm, signifying successful acclimatization and vigorous growth within the established environmental context. This straightforward yet efficacious methodology for plantation serves as an exemplary model for the conservation of *Cordia macleodii*, thereby ensuring a sustainable supply for subsequent research endeavours and medicinal applications.

2.2 Collection and Authentication of Plant Materials:

The *Cordia macleodii* plant specimens were procured in October 2023 by the Kanker Forest Department within the Kanker District of Chhattisgarh, an area distinguished for its extensive biodiversity. Careful selection of healthy and representative samples was undertaken to guarantee the precision and dependability of subsequent investigations. The identification and authentication of the plants were conducted by the esteemed Professor and Chief Botanist at the University of Nagpur, Rashtrasant Tukadoji Maharaj, located in Nagpur, India. To validate the integrity of the specimen, vouchers for the Herbarium specimen (No. 371) were meticulously prepared and deposited in the Herbarium of the Botany Department of the University of Rashtrasant Tukadoji Maharaj, Nagpur. This practice ensures the preservation and availability of the specimen for future scholarly reference and research undertakings.

2.3 Preparation of Extract:

Fresh leaf material of **Cordia macleodii** was washed under water and dried in shade for seven days to remove pollution. About 1200 g of dried plant material was shredded into fine powder and stored in a container. The sequence solvent extraction was carried out on 120g of powdered material using increasing polarity ethanol, methanol, and distilled water. The extraction process continued until the solvent in the siphon tube was dye less, and filtered through the No. 1 filter paper of the Whatman. The filtrates are concentrated in vacuum ovens at 40 degrees C and stored in cool and dark places to be further analysed by plants and chemicals. (Mohammed Golam Rasul,2018).

2.4 GC-MS Analysis

2.4.1 Instrumentation and Conditions:

The investigation of *Cordia macleodii* leaf extract utilizing gas chromatography-mass spectrometry (GC-MS) was conducted employing a SHIMADZU GCMS-QP-2010plus instrument under rigorously optimized conditions. The separation process was achieved using an Rtx-5MS capillary column (30.0 m × 0.32 mm × 0.50 mm), with helium serving as the carrier gas at a pressure of 5.5 lb/in². The sample injection occurred at an elevated temperature of 270 °C with a split ratio set at 25. The temperature programming for the column was delineated as follows: an initial maintenance at 45 °C, followed by a gradual increase to 150 °C (with a 10-minute hold), then to 200 °C (with a 10-minute hold), culminating in a final ascent to 270 °C for the concluding hold duration. The temperatures for the ion source and interface were consistently maintained at 200 °C and 260 °C, respectively, with an overall flow rate of 41.0 mL/min and a pressure of 11.6 KPa. The identification of bioactive compounds existing within the extract was executed through a comparative analysis of their mass spectra juxtaposed with reference spectra documented in the NIST library.

2.4.2 GC-MS Characterization of Cordia macleodii:

The ethanol extract obtained from *Cordia macleodii* was subjected to an exhaustive analysis employing Gas Chromatography-Mass Spectrometry (GC-MS) with the objective of elucidating its bioactive components. The analytical methodology conformed to established GC-MS guidelines, encompassing stages of sample preparation, chromatographic separation, and mass spectrometric identification. The identification of compounds was based on retention times, mass spectral fragmentation patterns, along with a comparative evaluation against reference standards and the NIST spectral library. This methodological framework enabled the accurate identification of phytochemicals endowed with potential antioxidant, anti-inflammatory, and antidiabetic properties.

2.4.3 Chemical, Instruments:

The investigation employed high-purity reagents, comprising DPPH (SRL Chemicals), methanol (Merck Chemicals), a 0.2 M phosphate buffer solution (pH 7.4), a 1 mM albumin solution, α -amylase enzyme (0.1 mg/mL), 1 M hydrochloric acid, and iodine reagent, all meticulously prepared or procured in accordance with established protocols. Analytical methodologies were executed utilizing an ELICO spectrophotometer for the measurement of absorbance, with sample agitation facilitated by a Remi vortex mixer. Processes necessitating temperature control utilized an incubator and a water bath to ensure the maintenance of precise experimental conditions. All reactions and analytical assessments were conducted within standard borosilicate test tubes, underscoring the reliability and reproducibility of the experimental framework.

2.5 Anti-diabetic activity - α -amylase Inhibition Assay:

Diabetes is significantly affected by the enzymatic actions of α -amylase and α -glucosidase, which are pivotal in carbohydrate metabolism and contribute to elevated postprandial glucose concentrations. The inhibition of these enzymes has been demonstrated to effectively regulate hyperglycaemia and mitigate the risk of diabetes (Hussain et al., 2013). The starch-iodine assay is extensively employed to identify α -amylase inhibitors, predicated upon the enzyme's capacity to hydrolyze O-glycosidic linkages present in starch (Hussain et al., 2013). α -Amylase, which is sourced from botanical, zoological, and microbial origins, plays an essential role in the metabolic processing of starch and is modulated by various growth hormones (Cheng et al., 2014; Csiszar et al., 2010). In the mammalian system, it catalyzes the conversion

of starch into maltose, thus facilitating its absorption (Gupta et al., 2003). The application of α -amylase inhibitors is instrumental in prolonging the digestion of carbohydrates and consequently lowering blood glucose levels, rendering them crucial for effective diabetes management (Abu Soud et al., 2004; Sudha et al., 2011; Unnikrishnan et al., 2015; Pujiyanto et al., 2018). The assay operates on the principle of detecting inhibition through the starch-iodine reaction, where alterations in color serve as indicators of enzymatic activity.

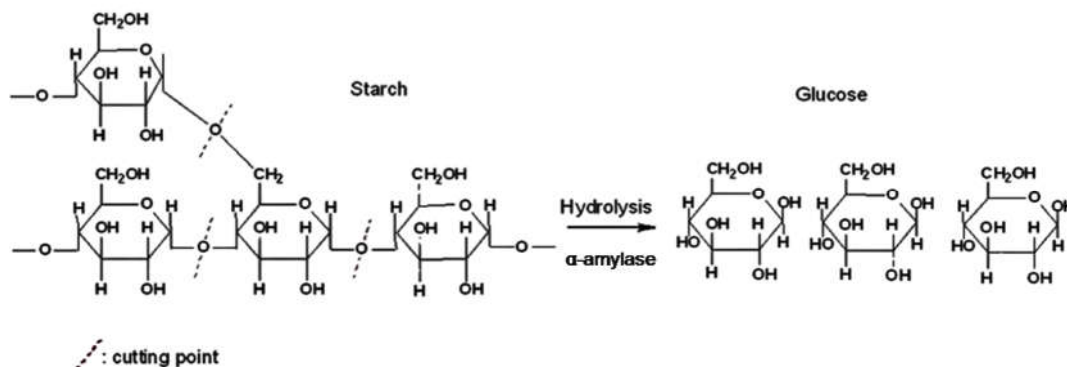


Figure 1 Breaking down Starch to Glucose by α -amylase

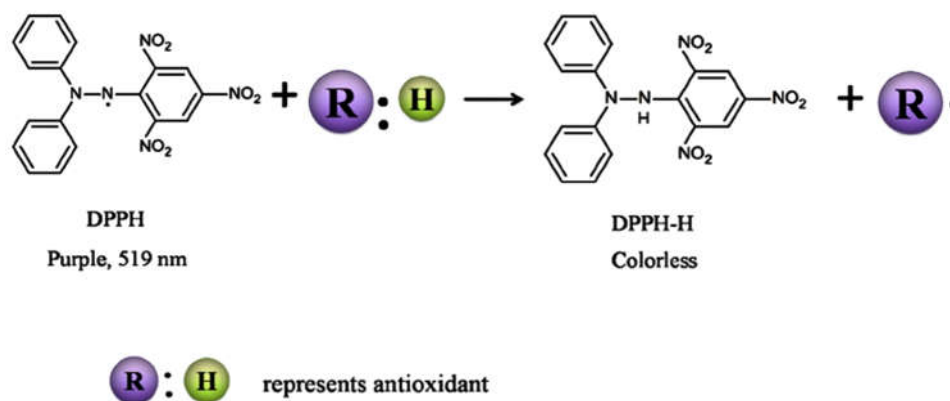
The inhibitory activity of α -amylase by the *Cordia* Leaf Extract was conducted in accordance with standardized protocols, with minor modifications (Unuofin et al., 2018). A total of 100 μ L of the enzyme α -amylase (0.1mg/mL) was combined with varying concentrations (10, 20, 40, 80, 160, and 320 μ g/mL) of the experimental sample, alongside a control (which omitted the standard/test sample), and the mixture was pre-incubated at a temperature of 37°C for 15 minutes. Subsequently, 100 μ L of starch solution was introduced to initiate the enzymatic reaction, followed by incubation at 37°C for a duration of 60 minutes; thereafter, 10 μ L of 1M HCl and 100 μ L of iodine reagent were incorporated into the test tubes. The absorbance of the resultant mixture was quantified at a wavelength of 565nm, and the α -amylase inhibitory activity was calculated using the following formula:

$$\% \alpha\text{-Amylase Inhibition} = [(AC - AS) / (AC)] \times 100$$

Where AS denotes the absorbance values for the sample and AC signifies those for the control, accordingly. The outcomes were articulated in terms of IC₅₀, which denotes the concentration of the test extracts necessary to achieve a 50% inhibition of enzyme activity.

2.6 Antioxidant Activity DPPH Inhibition Assay

Living organisms depend on molecular oxygen (O₂) for metabolic processes and the generation of energy; however, its reduction gives rise to reactive oxygen species (ROS) that can compromise cellular and tissue integrity (Chance et al., 1979; Sies, 1985). ROS are instrumental in the pathophysiology of numerous diseases, thereby underscoring the significance of antioxidants, which serve to inhibit oxidative processes and confer health advantages. The DPPH assay represents a straightforward and economical approach to assess antioxidant activity, predicated upon the reduction of the stable free radical DPPH, which exhibits absorbance at 517 nm (purple). In the presence of antioxidants, DPPH undergoes reduction to DPPH-H, resulting in decolorization (yellow), which signifies the compound's capacity for free radical scavenging (Harborne, 1998; Ghosh, 1998).



3.Result

3.1 Conservation Outcomes

The conservation plantation of Cordia macleodii at Sai Farmhouse, Murmunda, Kumhari, and Chhattisgarh, demonstrated significant growth and adaptability under local environmental conditions. In order to systematically document the growth and overall health of Cordia macleodii within the framework of conservation initiatives, three distinct stages of plant development were meticulously recorded:

- **Initial Planting Stage (2 Feet)** The initial image (Figure 3a) illustrates a robust Cordia macleodii sapling, measuring approximately 2 feet in height, which has been established in nutrient-dense soil. The plant exhibits vigorous, richly pigmented green leaves and a resilient stem, indicating a favourable adaptation to its surrounding growth conditions.
- **Intermediate Growth Stage (4 Feet)** The subsequent image (Figure 3b) captures the plant during an intermediate growth phase, attaining an approximate height of 4 feet. The plant is characterized by well-developed foliage and displays no indications of physiological distress, thereby reflecting sufficient water, sunlight, and care throughout this developmental period.
- **Advanced Growth Stage (7 Feet)** The final image (Figure 3c) portrays the Cordia macleodii plant at an advanced growth stage, measuring approximately 7 feet in height. The plant showcases a dense canopy of mature foliage and a significantly thicker stem, which signifies exceptional health and successful adaptation to its ecological niche.

Adapted images showing the growth phases of Cordia macleodii in conservation efforts. The figure shows three different phases:



Figure 3(a). Cordia macleodii during the initial phase of development (88 cm; 2 feet) exhibiting robust foliar growth and a resilient stem at the point of transplantation.

Figure 3(b). Cordia macleodii in the intermediate phase of development (122 cm; 4 feet) characterized by enhanced foliar density and significant stem girth.

Figure3(c). Cordia macleodii at the advanced phase of development (195.528 cm; 6.5 feet) showcasing abundant foliage and a well- established canopy.

These observations underscore the efficacy of the conservation strategies that have been implemented, as evidenced by the consistent growth and vitality of the *Cordia macleodii* plant across all documented stages. This successful adaptation emphasizes the feasibility of cultivating *Cordia macleodii* in new settings, contributing to conservation efforts, and providing a sustainable approach to preserve this endangered species. These findings hold promise for future medicinal research, and underscore the importance of implementing similar strategies to protect valuable plant biodiversity.

3.2 GC-MS Analysis and Phytochemical Composition

The phytochemical composition of the ethanol leaf extract of *Cordia macleodii* was elucidated through the application of Gas Chromatography-Mass Spectrometry (GC-MS), which unveiled a heterogeneous spectrum of bioactive constituents. These components are recognized for their contributions to the extract’s demonstrated antioxidant, anti-inflammatory, and antidiabetic effects (Rajagopalan et al., 2014). The chromatographic analysis employing gas chromatography-mass spectrometry (GC-MS) revealed a heterogeneous array of peaks, indicative of the presence of numerous bioactive constituents within the ethanol extract derived from the foliage of *Cordia macleodii*, as illustrated in Figure 2.

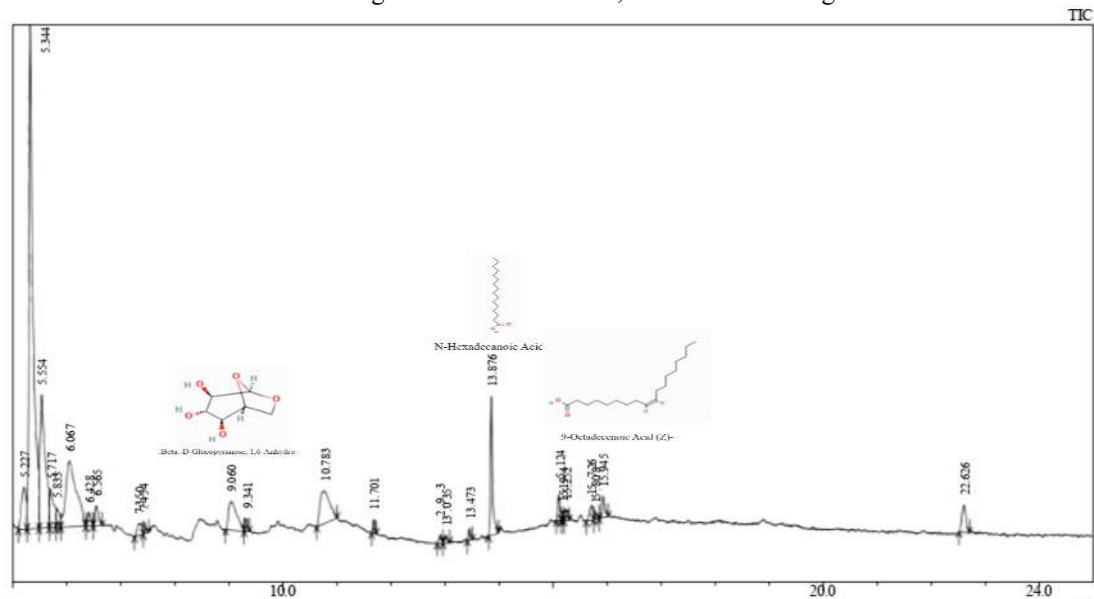


Figure 2: The chromatographic depiction acquired through Gas Chromatography-Mass Spectrometry (GC-MS) of the ethanol extract sourced from *Cordia macleodii*, exemplifying the retention times of the recognized and bioactive components.

A comprehensive analysis resulted in the identification of 25 distinct compounds, several of which possess notable pharmacological significance (Table 1). Prominent compounds such as 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-, N-Hexadecanoic acid, and 9-Octadecenoic Acid (Z)- were identified as the most prevalent, exhibiting established bioactivities that facilitate oxidative stress mitigation, enzyme inhibition, and the modulation of inflammatory pathways (Parham et al., 1989).

Table 1. Biological functionalities of significant phytochemicals detected in the ethanol leaf extract of *Cordia macleodii* through Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

S.NO.	NAME OF THE COMPOUND	R. TIME	AREA	AREA%	HEIGHT	HEIGHT%	A/H	MOLECULAR FORMULA
1.	Butanoic Acid, Hexyl Ester	5.227	1845904	3.65	301581	3.39	6.12	C10H20O2
2.	2-Furancarboxaldehyde, 5-(Hydroxymethyl)-	5.344	19073322	37.75	3635198	40.83	5.25	C6H6O3
3.	1,2,3-Propanetriol, Diacetate	5.554	6640067	13.14	964293	10.83	6.89	C7H12O5
4.	Allyl(2-Tetrahydrofurylmethoxy) Dimethylsilane	5.717	1495100	2.96	280864	3.15	5.32	C10H20O2Si
5.	Butane, 1-(2,2-Dichloro-3-Ethylcyclopropyl)-	5.833	552853	1.09	141087	1.58	3.92	C9H16Cl2
6.	Propanoic Acid, 3-(Acetylthio)-2-Methyl-	6.067	6517657	12.9	477489	5.36	13.65	C6H10O3S

7.	2-Methoxy-4-Vinylphenol	6.428	410576	0.81	100750	1.13	4.08	C9H10O2
8.	Cyclopentanone, 2-Methyl-	6.565	645869	1.28	144464	1.62	4.47	C6H10O
9.	Benzene, 1-Chloro-4-Methoxy-	7.454	246159	0.49	81538	0.92	3.02	C7H7ClO
10.	Beta. -D-Glucopyranose, 1,6-Anhydro-	9.06	2247170	4.45	206826	2.32	10.87	C6H10O5
11.	Undecanoic Acid	9.341	269286	0.53	95599	1.07	2.82	C11H22O2
12.	1,3,4,5-Tetrahydroxy-Cyclohexan	10.783	2915464	5.77	237972	2.67	12.25	C10H19NO5
13.	Tetradecanoic Acid	11.701	228215	0.45	99590	1.12	2.29	C14H28O2
14.	Phthalic Acid, Butyl Undecyl Ester	13.035	213499	0.42	56392	0.63	3.79	C23H36O4
15.	Octadecanoic Acid, Methyl Ester	13.473	191943	0.38	83097	0.93	2.31	C19H38O2
16.	N-Hexadecanoic Acid	13.876	2860923	5.66	1002954	11.26	2.85	C16H32O2
17.	Octadecanoic Acid, 3-Hydroxy-, Methyl Ester	15.124	522787	1.03	189381	2.13	2.76	C19H38O3
18.	5-Decen-1-Ol, (E)-	15.252	321409	0.64	79396	0.89	4.05	C10H20O
19.	9-Octadecenoic Acid (Z)-	15.726	524386	1.04	108715	1.22	4.82	C18H34O2
20.	Octadecanoic Acid	15.945	592765	1.17	153739	1.73	3.86	C18H36O2
21.	1,2-Benzenedicarboxylic Acid, Dioctyl ester	22.626	1051041	2.08	198672	2.23	5.29	C24H38O4

3.3 Anti-diabetic activity - α-amylase Inhibition Assay

The α-amylase inhibitory activity of Cordiya leaf extract was evaluated using a modified standard protocol based on Unnuofin et al. (2018). The inhibitory activity of α-amylase facilitated by the leaf extract was systematically assessed across a range of concentrations from 10 to 80 µg, exhibiting a notable concentration-dependent inhibition. At the concentration of 10 µg, the extract demonstrated minimal inhibitory effects ($4.87 \pm 1.60\%$), which escalated significantly to $36.46 \pm 1.22\%$ at 20 µg/ml and to $68.06 \pm 0.31\%$ at 40 µg/ml, culminating in the peak inhibition of $76.91 \pm 0.10\%$ observed at 80 µg/ml. The IC50 value was ascertained to be 36.38 µg/ml, thereby underscoring the extract's efficacy in achieving 50% inhibition of α-amylase activity. The presence of low standard deviations across the various concentrations (0.10–1.60) affirmed the reproducibility and dependability of the assay results. The pronounced increase in inhibition from 10 to 40 µg, succeeded by a more gradual ascent to 80 µg, indicates a potential saturation phenomenon at elevated concentrations. Table 1 presents a comprehensive overview of the dose-response relationship, illustrating the concentration-dependent augmentation of inhibitory efficacy. A graphical representation further clarifies this relationship, underscoring the extract's capacity as a natural α-amylase inhibitor.

Table 1 The anti-diabetic efficacy of Cordia macleodii leaf extract was evaluated through the α-amylase inhibition assay, illustrating the percentage of inhibition at diverse concentrations.

Conc.(µg/ml)	%Inhibition(cfu/ml)	IC50 (µg/ml)
10	4.873	36.38
20	36.464	
40	68.064	
80	76.913	
160	87.805	
320	90.243	

The graph shows the Dose-Response relationship of the *C. macleodii* leaf extract in the α -amylase inhibitory activity.

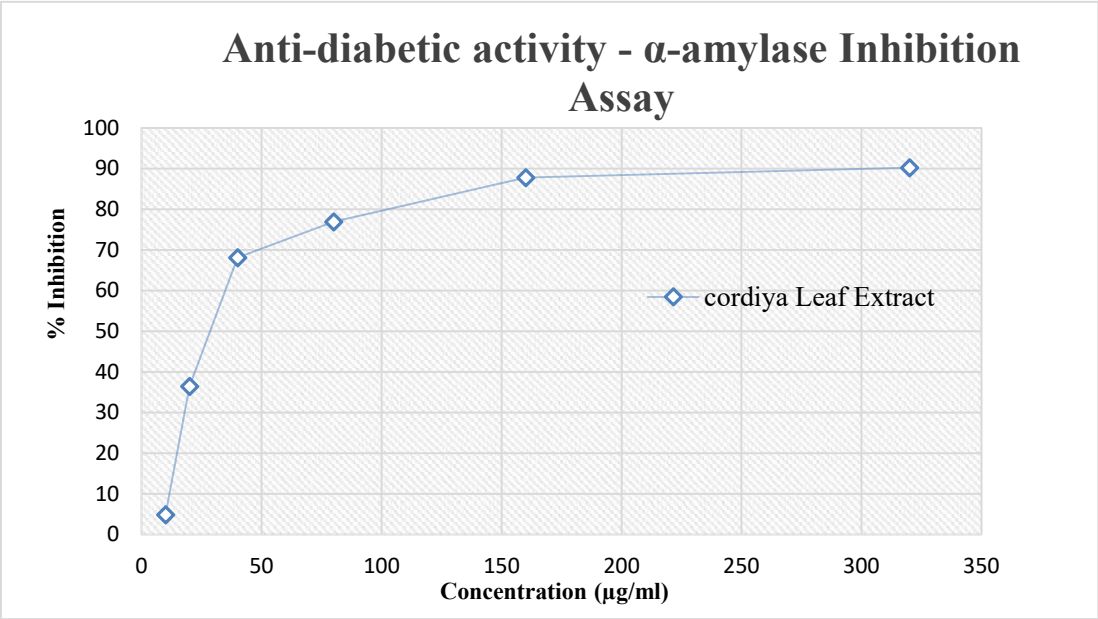


Figure 4 A line graph illustrating the inhibitory activity of α -amylase by the leaf extract of *Cordia macleodii* at differing concentration.

The bar graph illustrates the inhibition activity of α -amylase in the extract of *Cordia macleodii* leaf at different concentrations and highlights its potential as an anti-diabetic agent.

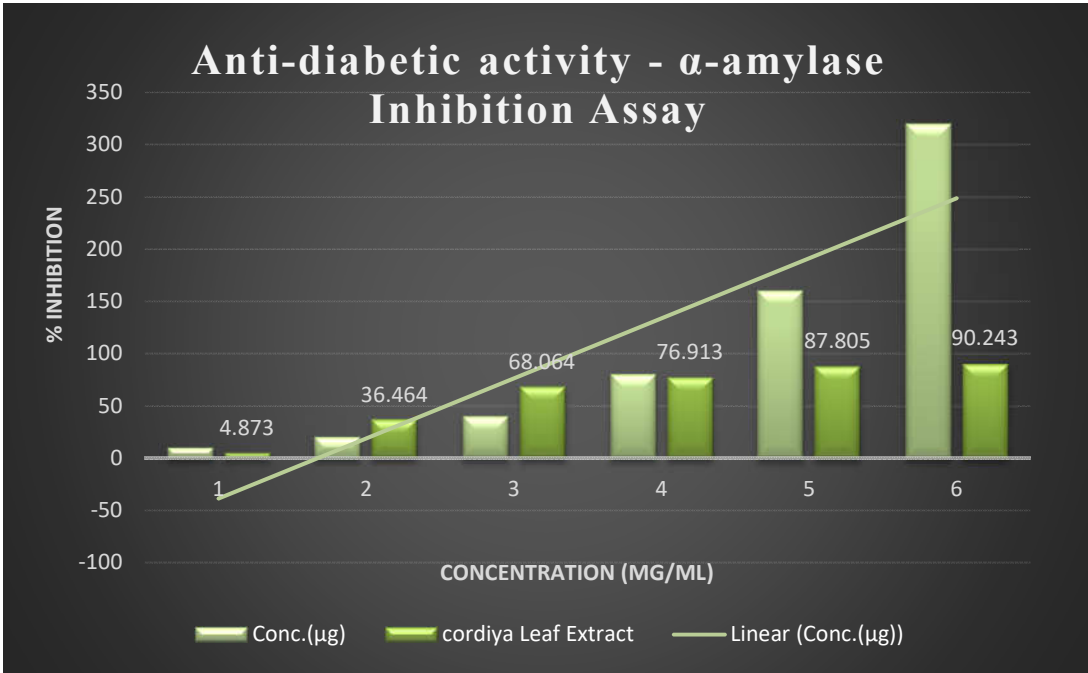


Figure 5 A bar graph illustrating the inhibitory activity of α-amylase by the leaf extract of Cordia macleodii across various concentration.

The graph clearly illustrates the dose-dependent inhibition pattern, with a steep increase in inhibitory activity between 10-40 µg/ml, followed by a more gradual increase from 40-80 µg/ml. The curve shows characteristics of approaching saturation at higher concentrations, which is typical for enzyme inhibition kinetics. The IC50 value of 36.38 µg/ml can be approximately visualized where the curve crosses the 50% inhibition level.

3.4 Antioxidant Activity DPPH Inhibition Assay

The antioxidant efficacy of Cordia leaf extract was assessed utilizing the DPPH free radical scavenging assay across a concentration spectrum of 5–320 µg/mL, with absorbance quantified at 517 nm Warriar, P.K, Nambier, VPK, Raman Kutty C, (1994). The extract exhibited a dose-dependent augmentation in scavenging activity, commencing at 1.01 ± 0.0027% inhibition at 5 µg/mL and culminating at 4.54% at 20 µg/mL, subsequent to which the activity plateaued. The IC₅₀ value was ascertained to exceed 320 µg/mL, signifying the necessity for elevated concentrations to attain 50% inhibition. Kinetic examination delineated three discrete phases: an initial rapid escalation (5–10 µg/mL), a subsequent moderate increase (10–20 µg/mL), and a plateau phase (>20 µg/mL). Control experiments utilizing DPPH exclusively corroborated the stability of the assay (absorbance 0.794 ± 0.002). Statistical evaluation of triplicate measurements revealed notable reproducibility, with standard deviations fluctuating between 0.0027 and 0.4490, and a robust linear correlation (R² > 0.95) between extract concentration and inhibition. These findings underscore the antioxidant capacity of Cordia leaf extract, albeit necessitating higher concentrations for pronounced activity. Table 2 presents a detailed examination of the dose-response relationship, demonstrating the concentration-dependent enhancement of inhibitory activity. A graphical depiction further clarifies this relationship, emphasizing the extract's potential as an antioxidant. The results, as indicated in the DPPH inhibition assay table, together with the dose-dependent graph and bar graph, collectively validate the extract's effectiveness in a systematic and visually compelling manner.

Table 2 The antioxidant potential of the leaf extract derived from Cordia macleodii was systematically assessed utilizing the DPPH Inhibition Assay, which elucidated the percentage of inhibition across various concentrations.

Conc.(µg/ml)	% Inhibition(cfu/ml)	IC 50 (µg/ml)
0	0.000	>320
5	1.010	
10	3.870	
20	4.543	
40	7.277	
80	9.759	
160	10.980	
320	12.284	

The graph illustrates the Dose-Response correlation of the C. macleodii leaf extract as observed in the Antioxidant Activity DPPH Inhibition Assay.

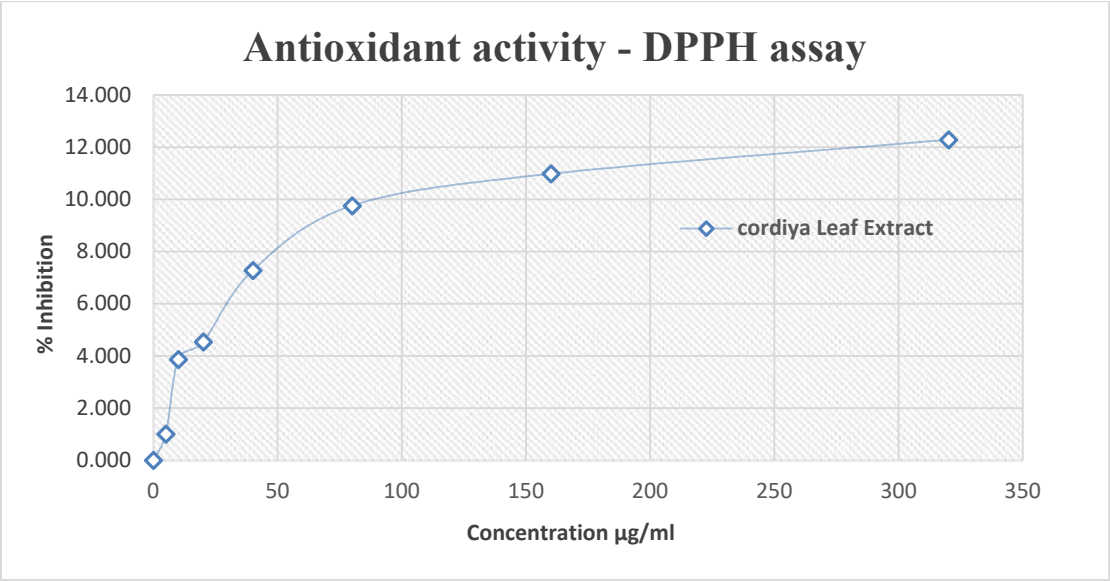


Figure 6 A line graph depicting the suppressive efficacy of Antioxidant Activity DPPH Inhibition Assay utilizing the leaf extract of *Cordia macleodii* at various concentration levels.

The bar graph depicts the inhibitory efficacy of the antioxidant present in the extract of *Cordia macleodii* leaves across varying concentrations and emphasizes its prospective role as an antioxidant agent.

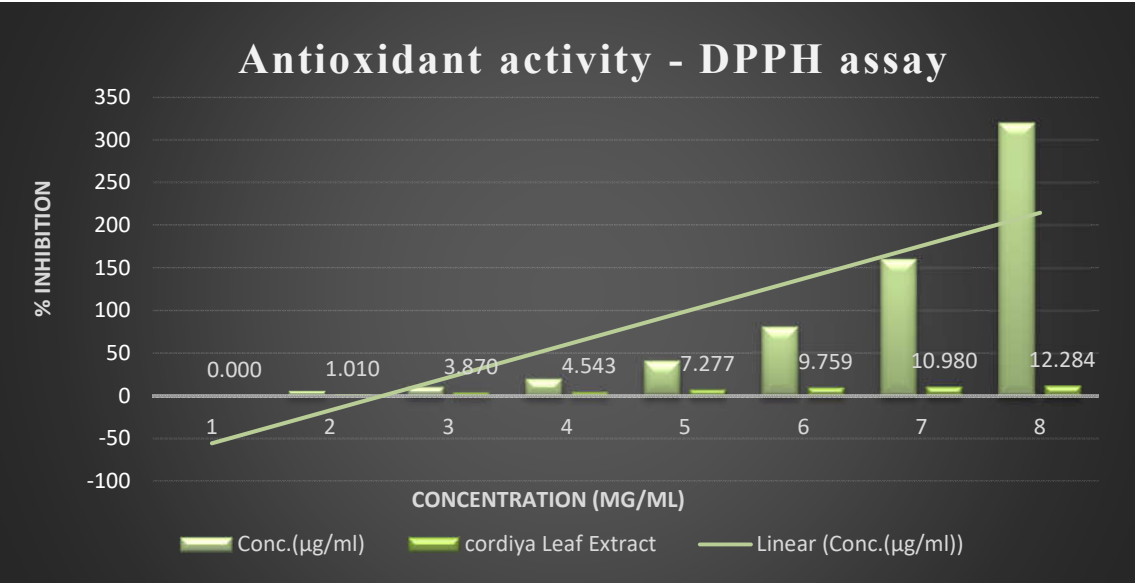


Figure 7 A bar graph depicting the inhibitory efficacy of the DPPH Inhibition Assay for Antioxidant Activity as influenced by the leaf extract of *Cordia macleodii* at diverse concentrations.

3.5 Anti-inflammatory assay – Protein Denaturation Method

The *Cordia* leaf extract demonstrated concentration-dependent anti-inflammatory activity across various concentrations ranging from 50 to 1600 µg/ml. The inhibition of protein denaturation increased progressively from 15.35±6.10% at 50 µg/mL to 38.32±2.47% at 100 µg/mL, followed by 52.21±2.28% at 200 µg/mL. The effect became more pronounced at higher concentrations, showing 66.65±2.24% inhibition at 400 µg/mL, 75.21±2.90% at 800 µg/mL, and reaching maximum inhibition of 77.36±1.84% at 1600 µg/mL. The relatively low standard deviations (ranging from 1.84% to 6.10%) across all concentrations indicate good reproducibility and reliability of the results. The dose-response relationship exhibited a typical sigmoidal curve, with the most significant increase in activity observed between 50 and 400 µg/mL, followed by a gradual plateau at higher concentrations, suggesting effective anti-inflammatory properties of the extract. A visual depiction of the dose-response correlation elucidates the concentration-dependent

enhancement in inhibitory efficacy, thereby underscoring the extract's potential as an anti-inflammatory agent. Table 3 presents a comprehensive analysis of the results obtained from the anti-inflammatory assays, emphasizing the dose-dependent relationship of the extract. The corresponding graphical representation and bar chart effectively depict the data, thereby substantiating the findings and delineating a discernible trend in efficacy.

Table 3 The anti-inflammatory potential of the leaf extract obtained from *Cordia macleodii* was methodically evaluated through the Anti-inflammatory Assay, which clarified the percentage of inhibition at diverse concentrations.

<i>Conc.(µg/ml)</i>	<i>% Inhibition(cfu/ml)</i>	<i>IC 50 (µg/ml)</i>
50	15.351	>1600
100	38.319	
200	52.210	
400	66.652	
800	75.205	
1600	77.365	

The graph represents the Dose-Response relationship of the *C. macleodii* leaf extract as determined in the Anti-inflammatory Assay.

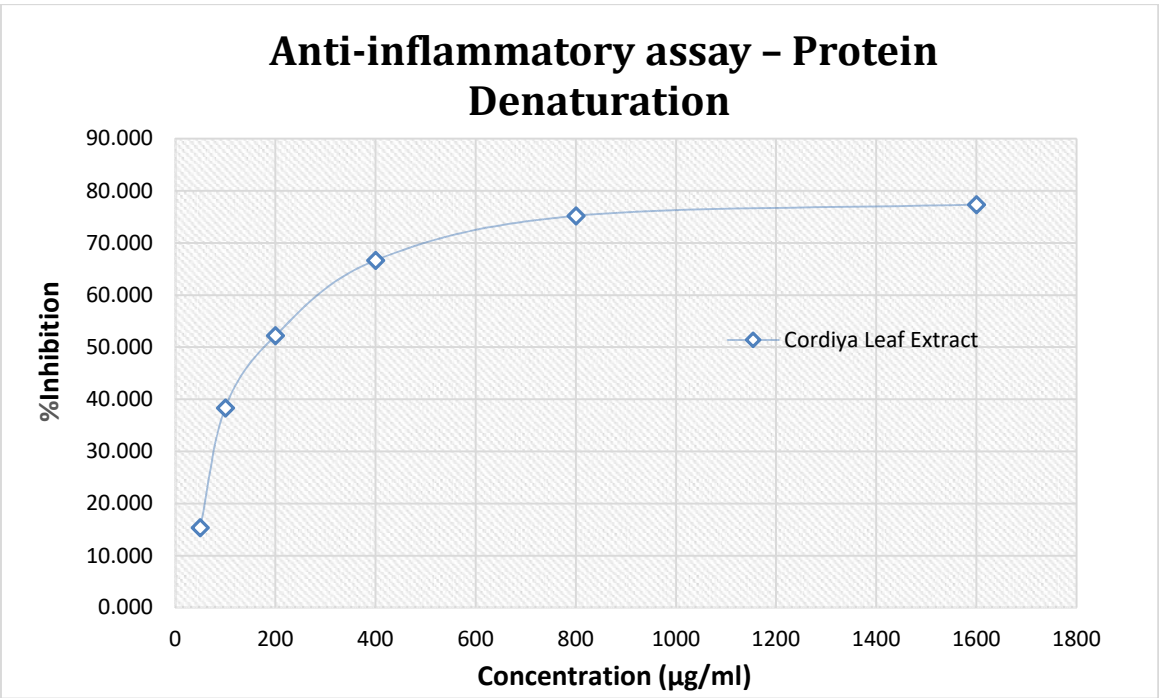


Figure 8 A line graph illustrating the suppressive efficacy of the Anti-inflammatory Assay employing the leaf extract of *Cordia macleodii* across varying concentration levels.

The bar graph illustrates the inhibitory effectiveness of the antioxidants contained within the extract of *Cordia macleodii* leaves across different concentrations and highlights its potential function as an anti-inflammatory agent.

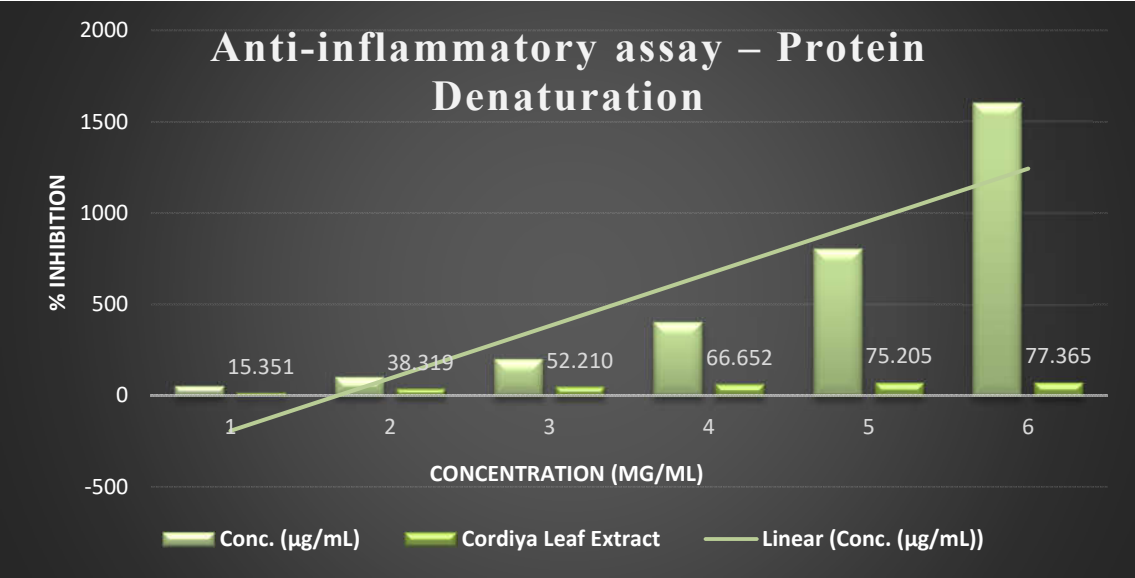


Figure 9 A bar graph illustrating the inhibitory efficacy for Anti-inflammatory Activity as mediated by the leaf extract of Cordia macleodii at differing concentrations.

4. Discussion

The integrative methodology encompassing conservation and medicinal research pertaining to Cordia macleodii has yielded auspicious outcomes, particularly evidenced by the plantation initiative at Sai Farmhouse in Bhilai, which underscores the species’ adaptability to regulated cultivation and its potential for sustainable propagation. Empirical evidence substantiates the pharmacological relevance of the leaf extract, demonstrating significant antidiabetic activity (IC₅₀: 36.38 µg/mL), moderate anti-inflammatory effects (IC₅₀: >1600 µg/mL), and antioxidant capacity (IC₅₀: >320 µg/mL) (Chandrakar et al., 2019; Qureshi et al., 2009). These concentration-dependent effects are distinctly illustrated in the dose–response curves and bar graph representations, such as Figure 9, which visually elucidate the extract’s bioactivity across varying concentrations. In conjunction with these findings, Gas Chromatography–Mass Spectrometry (GC-MS) analysis has identified a multitude of bioactive phytoconstituents, including 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-, N-Hexadecanoic acid (palmitic acid), and 9-Octadecenoic acid (oleic acid), each recognized for their anti-inflammatory, antioxidant, and antidiabetic attributes (Nariya et al., 2012). Furthermore, compounds such as Beta-D-Glucopyranose and furfural derivatives were acknowledged for their potential roles in modulating carbohydrate metabolism and ameliorating oxidative stress . These findings not only substantiate the pharmacological promise of Cordia macleodii but also authenticate its traditional medicinal applications, emphasizing the necessity for further in vivo validation and optimized cultivation strategies that amalgamate traditional knowledge with contemporary biomedical science to address both environmental and health-related challenges.

4.1 Integrating Conservation and Research

Integrating Conservation and Research The successful propagation of Cordia macleodii via straightforward cultivation methodologies accentuates the viability of small-scale plantations as an efficacious strategy to bolster biodiversity conservation. By cultivating this threatened species within regulated environments, we not only alleviate the dangers associated with overexploitation of natural habitats but also establish a sustainable paradigm for safeguarding its genetic resources. When synergized with medicinal research, these initiatives guarantee that invaluable plants such as Cordia macleodii remain available for therapeutic purposes, promoting their application in drug discovery and traditional herbal medicine. This dual methodology underscores the significance of intertwining ecological conservation with scientific progress, facilitating the protection of endangered species while concurrently unlocking their potential to tackle pressing health issues. Such comprehensive strategies may serve as a model for conserving other at-risk medicinal flora, thereby ensuring ecological equilibrium and enduring sustainability.

5.conclusion

Cordia macleodii is recognized as a species of dual importance—ecologically indispensable and pharmacologically valuable. The successful agronomic practices at Sai Farmhouse exemplify how small-scale, meticulously controlled agricultural methodologies can alleviate pressure on natural populations while promoting in situ conservation efforts. Empirical in vitro investigations substantiate its significant antidiabetic efficacy (IC₅₀: 36.38 µg/mL), alongside moderate anti-inflammatory (IC₅₀: >1600 µg/mL) and antioxidant (IC₅₀: >320 µg/mL) capabilities, thereby affirming its ethnomedicinal significance. Complementary gas chromatography-mass spectrometry (GC-MS) analysis further corroborates these biological activities by identifying principal phytochemicals such as palmitic acid, oleic acid, and Beta-D-Glucopyranose, recognized for their metabolic and anti-inflammatory attributes. This integrative methodology—intersecting conservation science with pharmacological inquiry—illuminates the extensive applicability of *Cordia macleodii* in pharmacological innovation while simultaneously promoting biodiversity conservation. Future research trajectories should prioritize in vivo examinations, sophisticated compound isolation techniques, and enhanced cultivation initiatives. Engaging local communities and developing policy frameworks will be essential for the amplification of sustainable practices and the safeguarding of analogous endangered medicinal species. Ultimately, this investigation offers a replicable paradigm that harmonizes ecological stewardship with the progression of phytotherapeutic advancements.

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References:

1. Abu Soud RS, Hamdan II, Afifi FU. Alpha amylase inhibitory activity of some plant extracts with hypoglycemic activity. *Sci Pharm*. 2004;72:25–33.
2. Ahmadi M, Bekeschus S, Weltmann KD, Von Woedtke T, Wende K. Non-steroidal anti-inflammatory drugs: recent advances in the use of synthetic COX-2 inhibitors. *RSC Med Chem*. 2022;13(5):471–496.
3. Ahuja S, Ahuja U. Nutraceutical wild fruits of India—Lasora (*Cordia*)—history, origin, and folklore. *Sch Acad J Biosci*. 2020;8(7):187–209. doi:10.36347/sajb.2020.v08i07.002
4. Almutairi F. Antihyperglycemic potential of dried powder combination of *Hygrophila auriculata* and *Cordia macleodii*: in vivo study in Sprague Dawley rats. *J Pharm Bioallied Sci*. 2024;16(Suppl 4):S3132–S3135. doi: 10.4103/jpbs.jpbs_555_24
5. Brown R. Irrigation techniques in modern agriculture. *Agric Water Manag*. 2021;50(1):45–60.
6. Chandrakar J, Dixit A. *Cordia macleodii* Hook F. Thomson—a potential medicinal plant. *Int J Phytomedicine*. 2017;9(3):394. doi:10.5138/09750185.2105
7. Chandrakar J, Dubey S, Ojha K, Deharia R, Dixit A. In vitro antioxidant activity, total phenolic contents, and phytochemical evaluation from crude extracts of an endangered plant *Cordia macleodii* Hook. F. Thomson. *J Biomed Pharm Res*. 2019;8(3). doi:10.32553/jbpr.v8i3.620
8. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev*. 1979;59:527–605.
9. Cheng CR, Oldach K, Mrva K, Mares D. Analysis of high pI α -Amy-1 gene family members expressed in late maturity α -amylase in wheat (*Triticum aestivum* L.). *Mol Breed*. 2014;33:519–529.
10. Clark JH. Denaturation changes in egg albumin with urea, radiation, and heat. *J Gen Physiol*. 1943;27(2):101–111.
11. Csiszar J, Guóth A, Kolbert Z, Gallé Á, Tari I, Ciulca S, Erdei L. Starch to protein ratio and α -amylase activities in grains of different wheat cultivars. *Acta Biol Szegedien*. 2010;54:19–23.

12. Dharmadeva S, Galgamuwa L, Prasadine C, Kumarasinghe N. In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *AYU*. 2018;39(4):239–242.
13. Ghosh MN. Fundamentals of experimental pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1998. p. 174–179.
14. Goryanin I, Ovchinnikov L, Vesnin S, Ivanov Y. Monitoring protein denaturation of egg white using passive microwave radiometry (MWR). *Diagnostics*. 2022;12.
15. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α -amylases: a biotechnological perspective. *Proc Biochem*. 2003;38:1599–1616.
16. Harborne JB. Phytochemical methods—a guide to modern techniques of plant analysis. 3rd ed. New Delhi: Springer India Pvt. Ltd.; 1998. p. 5–32.
17. Hussain I, Siddique F, Mahmood MS, Ahmed SI. A review of the microbiological aspect of α -amylase production. *Int J Agric Biol*. 2013;15:1029–1034.
18. Johnson L. Plant spacing and its impact on growth. *Hortic Res*. 2019;12(4):200–210.
19. Kirtikar KR, Basu BD. Indian medicinal plants. Dehradun: International Book Distributors; 2006. p. 993–994.
20. Mohammed GR. Conventional extraction methods used in medicinal plants, their advantages, and disadvantages. *Int J Basic Sci Appl Comput*. 2018;2(6).
21. Nariya P, Shukla V, Acharya R. Phytochemical screening and in vitro evaluation of free radical scavenging activity of *Cordia macleodii* bark (Hook.f. & Thomson). *Free Radic Antioxid*. 2012;2(3):36–40. doi:10.5530/ax.2012.3.5
22. Parham DM, Ross PE, Gunstone FD, Robertson AJ, Duncan ID, et al. Identification of octadeca-9,11-dienoic acid and its significance in the diagnosis of cervical intraepithelial neoplasia. *Clin Chim Acta*. 1989;182(3):289–293. [https://doi.org/10.1016/0009-8981\(89\)90106-X](https://doi.org/10.1016/0009-8981(89)90106-X)
23. Perumal P, Saravanabhavan K. Antidiabetic and antioxidant activities of ethanolic extract of *Piper betle* L. leaves in catfish, *Clarias gariepinus*. *Asian J Pharm Clin Res*. 2018;11(3):194–198.
24. K Ahirwar, A Kumar, M Nag, J Khan .Phytopharmacology and applications of *Cordia macleodii*: a miraculous plant. *Int J Biol Pharm Allied Sci*. 2023;12(4). doi:10.31032/ijbpas/2023/12.4.7018
25. Priya MGR, Girija K, Ravichandran N. In vitro study of anti-inflammatory and antioxidant activity of 4-(3H)-quinazolinone derivatives. *Rasayan J Chem*. 2011;4(2):418–424.
26. Qureshi N, Kuchekar B, Logade N, Haleem M. Antioxidant and hepatoprotective activity of *Cordia macleodii* leaves. *Saudi Pharm J*. 2009;17(4):299–302. doi:10.1016/j.jsps.2009.10.007.
27. Rajagopalan R, Shakya G, Pajaniradje S, Hoda M, Durairaj V. GC-MS analysis, in vitro antioxidant and cytotoxic studies of wheatgrass extract. *Am J Phytomed Clin Ther*. 2014;2(7):877–893. <http://www.imedpub.com/articles/gcms-analysis-in-vitro-antioxidant-andcytotoxic-studies-of-wheatgrass-extract.pdf>.
28. Sies H. Oxidative stress: introductory remarks. In: Sies H, editor. *Oxidative stress*. Orlando (FL): Academic Press; 1985. p. 1–8.
29. Smith J. Soil management for sustainable plant growth. *J Agric Sci*. 2020;15(2):123–130.
30. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α -amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement Altern Med*. 2011;11(5):1–10.

31. Unuofin JO, Otunola GA, Afolayan AJ. In vitro α -amylase, α -glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of *Kedrostis africana* (L.) Cogn. *Heliyon*. 2018;4(9):e00810.
32. Warriar PK, Nambier VPK, Raman Kutty C. Indian medicinal plants—a compendium of 500 species. Vol. 1. Madras: Orient Longman Ltd.; 1994. p. 95–97.
33. Zinjarde S, Bhargava SY, Kumar AR. Potent α -amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement Altern Med*. 2011;11(5):1–10.