



## Phytochemical and antioxidant potential of *Cestrum diurnum* L. leaves

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### Abstract

*Cestrum diurnum* L., a member of the Solanaceae family, is a widely distributed shrub that is known for its rich phytochemical composition and pharmacological significance. This study aimed to evaluate the phytochemical profile and antioxidant potential of *C. diurnum* leaf extract. Preliminary phytochemical screening confirmed the presence of alkaloids, saponins, flavonoids, tannins, and phytosterols, which contributed to their therapeutic potential. Quantitative analysis revealed a high concentration of total phenolics ( $127.23 \pm 0.99$  mg GAE/g), flavonoids ( $80.12 \pm 1.44$  mg RE/g), and tannins ( $165.47 \pm 1.11$  mg TAE/g), indicating strong antioxidant properties.

The antioxidant potential of *C. diurnum* was assessed using DPPH and nitric oxide (NO) free-radical scavenging assays, with ascorbic acid as the standard. The extract exhibited dose-dependent radical scavenging activity, with an IC<sub>50</sub> value of 36.29 µg/mL for DPPH and 37.85 µg/mL for NO scavenging, suggesting its potent antioxidant efficacy. These findings indicate that *C. diurnum* has strong free radical neutralization capacity, which may be beneficial for oxidative stress-related disorders, including osteoporosis.

The presence of calcitriol, a biologically active form of vitamin D, highlights its potential role in bone metabolism and calcium homeostasis. These findings support the pharmacological importance of *C. diurnum* for the development of natural antioxidant and anti-osteoporotic therapies.

**Keywords:** *Cestrum diurnum*, phytochemical, antioxidant activity, oxidative stress, osteoporosis

### Introduction

*Cestrum diurnum*, also known as day-opening jasmine, is an evergreen perennial shrub belonging to the Solanaceae family (Fakhrah *et al.*, 2025) [11]. Originally from the West Indies, it was introduced and widely established in tropical and subtropical regions, including India (Bahgat *et al.*, 2023) [4]. It thrives in diverse ecosystems, from coastal uplands to flatwoods and disturbed areas, showing its adaptability and widespread presence (Satapathy *et al.*, 2024) [18].

Phytochemical studies have identified various bioactive compounds in the flowers and leaves of this plant, including alkaloids, saponins, flavonoids, terpenoids, tannins, and phytosterols, all of which contribute to its broad pharmacological potential (Khatun *et al.* 2022) [13]. A unique feature of *C. diurnum* is its high calcitriol content (1,25-dihydroxyvitamin D<sub>3</sub>), the biologically active form of vitamin D<sub>3</sub>, which plays a crucial role in calcium homeostasis and bone health (Wasserman *et al.*, 1976) [21]. This suggests its potential application in the treatment of osteoporosis, a condition associated with reduced bone mass and increased fracture risk (Chennaiah *et al.*, 2004) [9].

The pharmacological profile of *C. diurnum* demonstrates a diverse range of bioactivities, including antioxidant, anti-inflammatory, antiviral, and antimicrobial (Alrabayah *et al.*, 2022) [2]. Plant extracts have been utilized in the synthesis of silver and zinc oxide nanoparticles, which have shown significant antiviral and antibacterial effects, supporting their modern therapeutic applications (Chakraborty *et al.*, 2022) [8]. Additionally, its anti-inflammatory activity is linked to the inhibition of NF-κB activation and other inflammatory mediators that play a role in the progression

of osteoporosis (Ghosh *et al.*, 2006; Prasad *et al.*, 2013) [6, 17]. Since oxidative stress and chronic inflammation are major contributors to bone loss, the antioxidant and immunomodulatory effects of *C. diurnum* may help protect bone health and manage osteoporosis (Blagov *et al.*, 2024; Sibony *et al.*, 2024) [7, 19].

This study aimed to investigate the ability of *C. diurnum* leaf extract to modulate oxidative stress in an experimental model. By exploring its mechanism of action, this research could provide valuable insights into its potential as a herbal remedy for osteoporosis, while also assessing its efficacy and safety for future therapeutic use.

### Materials and Methods

#### 1. Plant material

*C. diurnum* leaves were collected from Jadcherla, Telangana, in November 2022, and authenticated by the Botanical Survey of India (BSI/DRC/2022-23/Identification/38), Deccan Region, Hyderabad, India.

#### 2. Extraction

Leaves were collected, washed, and dried in the shade. Phytochemicals from the dried leaf powder were extracted using Soxhlet extraction with ethanol at 60°C for 24 h. The crude extract was collected and filtered, and the solvent was evaporated using a rotary evaporator at 40°C (Dokuparthi *et al.*, 2021) [10].

#### 3. Phytochemical study

Preliminary phytochemical analysis of *C. diurnum* leaf extract was performed according to standard protocols (Sujatha *et al.*, 2023) [20].

## Quantitative Phytochemical Screening

### 1. Determination of Total Phenolic Content (TPC)

The total phenolic content of the sample was determined using the Folin-Ciocalteu method with gallic acid as the reference standard. A 0.5 mL aliquot of the extract was mixed with Folin–Ciocalteu reagent (0.5 mL) and allowed to react for 5 min. Then this, 2.5 mL of 7% sodium carbonate solution was added, and the final volume was adjusted to 25 mL with distilled water. The mixture was incubated at room temperature for 90 min and the absorbance was measured at 550 nm using a UV-visible spectrophotometer. A standard calibration curve of gallic acid (200–1000 µg/mL) was used for quantification. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (Patil *et al.*, 2023; Sujatha *et al.*, 2022)<sup>[16]</sup>.

### 2. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was analyzed using an aluminum chloride colorimetric assay, with rutin serving as the standard. 1 mL aliquot of the extract was placed in a 10 mL volumetric flask and mixed with 4 mL of distilled water. After 5 minutes, 0.3 mL of a 5% sodium nitrite solution was added, followed by 0.3 mL of a 10% aluminum chloride solution after another 5 min. After an additional 6 min, 2 mL of 1 M sodium hydroxide solution was introduced, and the final volume was adjusted to 10 mL using distilled water. The solution was incubated at room temperature for 30 min and the absorbance was measured at 510 nm using a UV-visible spectrophotometer. The flavonoid content was calculated based on a rutin standard curve (100–1000 µg/mL) and expressed as milligrams of rutin equivalent (RE) per gram of extract (Abbagoni *et al.*, 2021)<sup>[1]</sup>.

### 3. Determination of Total Tannin Content (TTC)

The total tannin content was assessed using the vanillin-hydrochloric acid (HCl) method, with tannic acid as the reference standard. A 400 µL aliquot of the diluted extract was mixed with 3 mL of a 4% methanolic vanillin solution and concentrated HCl (1.5 mL of concentrated HCl). The mixture was incubated at room temperature for 15 minutes. The absorbance was recorded at 500 nm using a UV-visible spectrophotometer. Tannin content was quantified using a standard calibration curve and expressed as milligrams of tannic acid equivalent per gram of extract (Kuchana *et al.*, 2021)<sup>[14]</sup>.

## Antioxidant activity

### 1. DPPH Free Radical Scavenging Assay

The antioxidant activity of the extracts was assessed using a DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A 1 mL aliquot of the extract was mixed with 3 mL of 0.5 mM methanolic DPPH solution. The reaction mixture was then incubated in the dark at room temperature for 30 min. The absorbance was recorded at 517 nm using a UV-visible spectrophotometer (Mahalakshmi *et al.*, 2021; Aryal *et al.*, 2019)<sup>[3, 15]</sup>. The percent inhibition of DPPH radicals was calculated using the following formula:

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

Ascorbic acid was used as a positive control, and the IC<sub>50</sub> values were determined.

### 2. Nitric Oxide (NO) Free Radical Scavenging Assay

Nitric oxide (NO) scavenging activity of the extract was evaluated using sodium nitroprusside as the NO donor. The reaction mixture consisted of 1 mL of 10 mM sodium nitroprusside solution in phosphate-buffered saline (pH 7.4) and 1 mL of the extract at varying concentrations. The mixture was incubated at 25°C for 150 minutes. After incubation, 1 mL Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 2% phosphoric acid) was added to 1 mL of the reaction mixture. The absorbance was measured at 546 nm using a UV-visible spectrophotometer. The percentage inhibition of NO radicals was calculated using the same formula as that used for the DPPH assay. Ascorbic acid was used as a reference standard (Barajas *et al.*, 2023; Benjamaa *et al.*, 2024)<sup>[5, 6]</sup>.

## Results

### 1. Phytochemical screening

Preliminary phytochemical analysis of the ethanolic extract of *C. diurnum* leaves revealed several secondary metabolites including alkaloids, saponins, flavonoids, and tannins. (Table 1).

**Table 1:** Phytochemical profile of *C. diurnum*

Phytochemicals	Ethanol extract
Alkaloids	-
Glycosides	+
Saponins	+
Flavonoids	+
Steroids	+
Tannins	+

Present (+)/absent (-)

### 2. Quantitative Phytochemical Screening

#### Total Phenolic Content (TPC)

The total phenolic content (TPC) of *C. diurnum* leaf extract was measured at 127.23 ± 0.99 mg GAE/g, indicating a high concentration of phenolic compounds. These phenolics are well known for their strong antioxidant properties, which help to neutralize free radicals and protect cells from oxidative damage. TPC was quantified using a calibration curve based on gallic acid (Figure 1), which established a linear relationship between absorbance and concentration, following the equation  $y = 0.0072x + 0.056$  ( $R^2 = 0.9917$ ). This strong correlation confirmed the accuracy of phenolic content estimation. The high TPC value suggests that the *C. diurnum* leaf extract is rich in bioactive compounds with significant pharmacological potential, particularly in managing oxidative stress-related conditions.

#### Total Flavonoid Content (TFC)

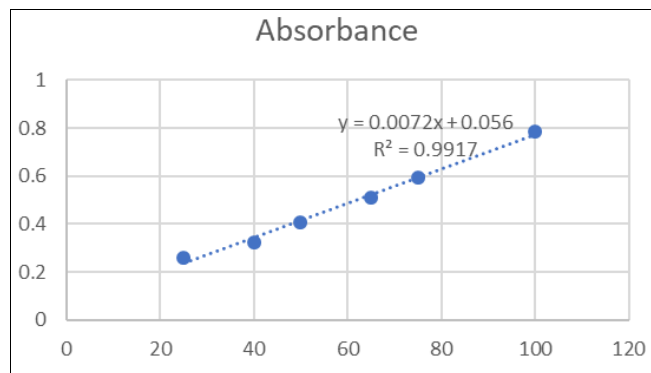
The total flavonoid content (TFC) of *C. diurnum* leaf extract was determined to be 80.12 ± 1.44 mg RE/g, reflecting a substantial presence of flavonoids. These secondary metabolites play crucial roles in immune modulation, anti-inflammatory processes, and antimicrobial defenses. Flavonoid content was quantified using a calibration curve based on rutin (Figure 2), which followed the equation  $y = 0.0086x - 0.0328$  ( $R^2 = 0.9963$ ), showing a highly linear correlation. This confirms the accuracy and reliability of flavonoid estimation. The considerable TFC value suggests that the *C. diurnum* leaf extract may help enhance immune responses, regulate cytokine production, and mitigate oxidative stress, further supporting its potential therapeutic applications.

**Total Tannin Content (TTC)**

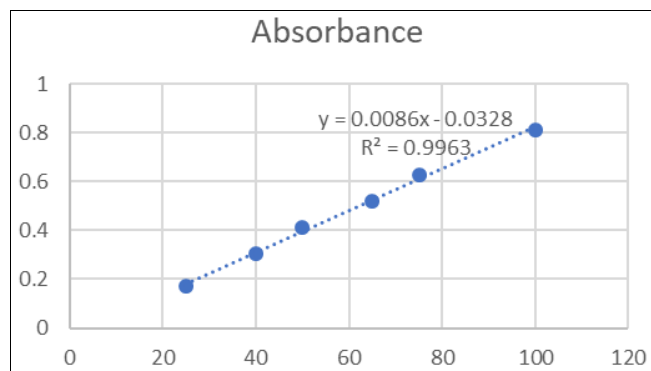
The total tannin content (TTC) of *C. diurnum* leaf extract was found to be  $165.47 \pm 1.11$  mg TAE/g, highlighting a high concentration of tannins, which are well known for their antimicrobial, astringent, and gastroprotective properties. Tannins play an essential role in gut health by inhibiting microbial growth, regulating digestive enzyme activity, and protecting against gastrointestinal disorders. The tannin content was quantified using a calibration curve based on tannic acid (Figure 3) using the equation  $y = 0.0079x - 0.0429$  ( $R^2 = 0.982$ ), ensuring the precision of the measurement. The significant tannin content suggests that the *C. diurnum* leaf extract may have potential applications in preventing intestinal infections and promoting digestive health.

**Table 2:** Quantitative Phytochemical analysis of *C. diurnum*

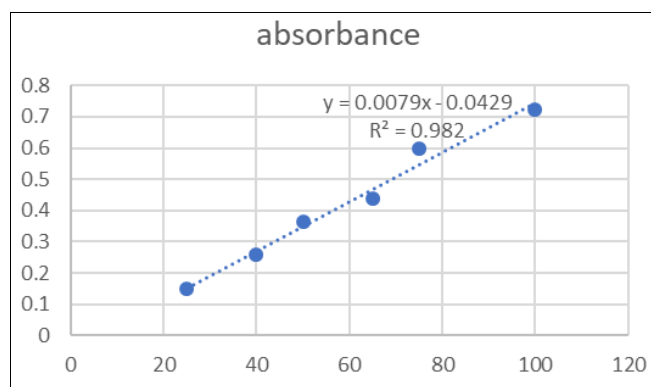
Total phenolic content	Total Flavonoid content	Total Tannin content
103.114±0.87mgGE/g	72.74±1.26mgRE/g	92.22±1.34mgTAE/g



**Fig 1:** Calibration curve of Gallic acid



**Fig 2:** Calibration curve of Rutin



**Fig 3:** Calibration curve of Tannic acid

**3. Antioxidant Activity**

**3.1. DPPH Free Radical Scavenging Activity**

**DPPH Free Radical Scavenging Assay**

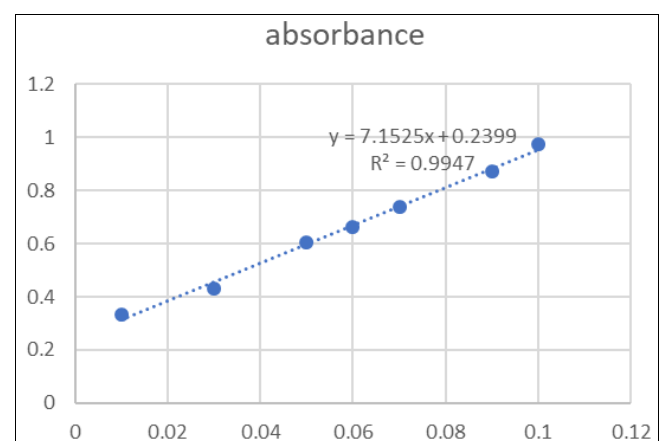
The antioxidant potential of the *C. diurnum* leaf extract was evaluated using the DPPH free radical scavenging assay, with ascorbic acid as the standard for comparison. The extract exhibited a dose-dependent increase in free radical inhibition, with inhibition values ranging from 18.01% at 10 µg/mL to 72.49% at 75 µg/mL. In comparison, ascorbic acid displayed a higher inhibition rate, reaching 85.41% at 75 µg/mL and 66.09% at 50 µg/mL, indicating its superior radical scavenging efficiency.

Interestingly, the IC<sub>50</sub> value of *C. diurnum* extract (36.29 µg/mL) was lower than that of ascorbic acid (39.92 µg/mL), suggesting that the extract has greater potency in scavenging DPPH radicals. The calibration curve for ascorbic acid (Figure 4) was used as a reference for precise absorbance-based quantification, confirming the reliability of the measurements.

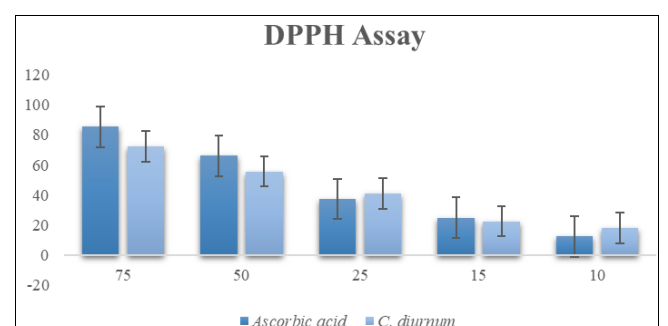
The significant antioxidant activity of the *C. diurnum* leaf extract suggests the presence of phenolics and flavonoids, which play a key role in neutralizing free radicals and mitigating oxidative stress. This finding supports its potential as a natural antioxidant source with pharmacological benefits.

**Table 3.** DPPH Free Radical Scavenging Assay

Concentration µg/ml	Ascorbic acid	<i>C. diurnum</i>
75	85.41	72.49
50	66.09	55.69
25	37.33	41.01
15	24.95	22.54
10	12.38	18.01
0	0	0
IC <sub>50</sub>	39.92	36.29



**Fig 4:** Calibration curve of Ascorbic acid



**Fig 5:** DPPH Free Radical Scavenging Assay

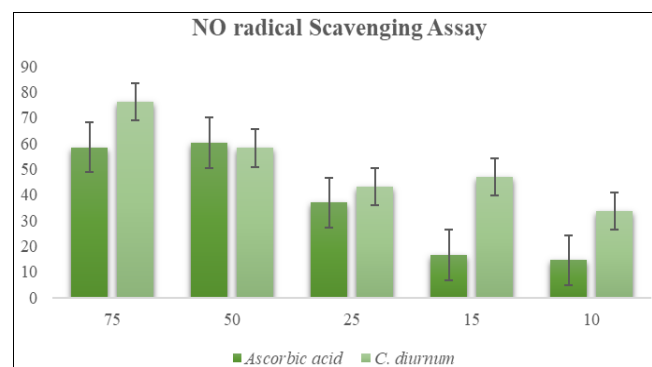
### 3.1. Nitric Oxide (NO) Free Radical Scavenging Activity

The nitric oxide (NO) scavenging assay revealed a clear trend, where *C. diurnum* leaf extract exhibited higher NO scavenging activity than ascorbic acid across most tested concentrations. At 75 µg/mL, the extract showed an impressive 76.12% inhibition, surpassing ascorbic acid, which showed 58.44% inhibition. Similarly, at 50 µg/mL, the extract displayed 58.12% inhibition, whereas ascorbic acid showed a comparable 60.12% inhibition.

Notably, the IC<sub>50</sub> value of *C. diurnum* extract (37.85 µg/mL) was significantly lower than that of ascorbic acid (52.18 µg/mL), indicating that the extract has greater efficiency in scavenging NO radicals. This suggests that the bioactive compounds present in the *C. diurnum* extract interact effectively with NO radicals, potentially contributing to its anti-inflammatory and vasoprotective properties. Furthermore, the calibration curve of ascorbic acid reinforces the accuracy of absorbance-based quantification, ensuring that the data interpretation is reliable and precise. These findings highlight the therapeutic potential of *C. diurnum* for combating oxidative stress and inflammation-related conditions.

**Table 4:** NO Free Radical Scavenging Assay

Concentration µg/ml	Ascorbic acid	<i>C. diurnum</i>
75	58.444	76.1235
50	60.116	58.121
25	36.879	43.149
15	16.5205	46.892
10	14.4685	33.5065
IC <sub>50</sub>	<b>52.18</b>	<b>37.85</b>



**Fig 6:** NO Free Radical Scavenging Assay

### Summary

This study explored the phytochemical composition and antioxidant potential of *C. diurnum* leaf extract. Preliminary phytochemical screening confirmed the presence of alkaloids, saponins, flavonoids, tannins, and phytosterols, thereby reinforcing their bioactive potential. Quantitative analysis revealed high levels of total phenolic content ( $127.23 \pm 0.99$  mg GAE/g), total flavonoid content ( $80.12 \pm 1.44$  mg RE/g), and total tannin content ( $165.47 \pm 1.11$  mg TAE/g), highlighting its strong antioxidant activity. These secondary metabolites are essential for neutralizing free radicals and reducing oxidative stress, and play a key role in preventing oxidative stress-related disorders, including osteoporosis. Additionally, the presence of calcitriol, the biologically active form of vitamin D<sub>3</sub>, emphasizes the importance of plants in calcium homeostasis and bone metabolism.

The antioxidant activity of the *C. diurnum* extract was evaluated using DPPH and nitric oxide (NO) scavenging assays, with ascorbic acid serving as the reference standard. The DPPH assay exhibited a dose-dependent increase in free radical inhibition, where *C. diurnum* extract demonstrated an IC<sub>50</sub> value of 36.29 µg/mL, confirming strong antioxidant activity compared to ascorbic acid (IC<sub>50</sub> = 39.92 µg/mL). The NO scavenging assay further showed that *C. diurnum* extract had a greater inhibitory effect than ascorbic acid at most concentrations, with an IC<sub>50</sub> value of 37.85 µg/mL, indicating superior nitric oxide scavenging capability. These findings suggest that *C. diurnum* is a potent natural antioxidant, with potential applications in preventing oxidative stress-related disorders.

### Conclusion

The results of this study confirm that the *C. diurnum* leaf extract has strong antioxidant activity and a rich phytochemical profile. High concentrations of phenolics, flavonoids, and tannins significantly contribute to free radical scavenging, making it a promising candidate for therapeutic applications. Furthermore, the presence of calcitriol supports its potential role in bone health and osteoporosis management, positioning it as a natural alternative to maintain calcium homeostasis. Additionally, its strong nitric oxide scavenging activity reinforces its anti-inflammatory and vascular-protective functions, further supporting its potential therapeutic benefits. These findings highlight *C. diurnum* leaf extract as a valuable natural antioxidant with potential applications in managing oxidative stress, inflammation, and bone health-related disorders.

### Acknowledgments

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### Conflict of interest

The authors declare no conflict of interest relevant to this article.

### References

1. Abbagoni S, Edupuganti S, Rani GJ. Phytochemical and antioxidant screening of *Cocculus hirsutus* and *Calycopteris floribunda*. *Int J Health Sci*,2021;5(1):576–84. doi:10.53730/ijhs.v5nS1.13642
2. Alrabayah IN, Elhawary S, Kandil ZA, Abd El-Kadder EM, Moemen YS, Saleh AM, El Raey MA. Green synthesized zinc oxide nanoparticles based on *Cestrum diurnum* L. of potential antiviral activity against human Corona 229-E virus. *Molecules*,2022;28(1):266. doi:10.3390/molecules28010266
3. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*,2019;8(4):96. doi:10.3390/plants8040096
4. Bahgat DMR, Gad HA, Al-Sayed EM, Mahmoud SH, Mostafa A, Mahfouz NM, Eldahshan OA, Singab ANB. Essential oil of *Cestrum diurnum* L.: GC/MS analysis, *in vitro* and *in silico* anti-HCoV-229E effects and inhibitory activity against LPS-induced inflammation. *Chem Biodivers*, 2023, 20(4). doi:10.1002/cbdv.202201045

5. Barajas-Ramírez JA, Cabrera-Ramírez AH, Aguilar-Raymundo VG. Antioxidant activity, total phenolic, tannin, and flavonoid content of five plants used in traditional medicine in Penjamo, Guanajuato. *Chem Biodivers*,2023;20(1):e202200834. doi:10.1002/cbdv.202200834
6. Benjamaa R, Elbouny H, Errati H, Moujanni A, Kaushik N, Gupta R, Ennibi O, Nasser B, Choi EH, Kaushik NK, Essamadi A. Comparative evaluation of antioxidant activity, total phenolic content, anti-inflammatory, and antibacterial potential of *Euphorbia*-derived functional products. *Front Pharmacol*,2024;15:1345340. doi:10.3389/fphar.2024.1345340
7. Blagov AV, Summerhill VI, Sukhorukov VN, Zhigmitova EB, Postnov A, Orekhov A. Potential use of antioxidants for the treatment of chronic inflammatory diseases. *Front Pharmacol*, 2024. doi:10.3389/fphar.2024.1378335
8. Chakraborty S. Genotoxic efficacy of fruit extract of *Cestrum diurnum* using *Allium cepa* assay. *Appl Biol Res*,2022;24(3):393–8. doi:10.5958/0974-4517.2022.00044.1
9. Chennaiah S, Qadri SS, Rao SV, Shyamsunder G, Raghuramulu N. *Cestrum diurnum* leaf as a source of 1,25(OH)<sub>2</sub> Vitamin D<sub>3</sub> improves eggshell thickness. *J Steroid Biochem Mol Biol*,2004;89-90(1–5):589–94. doi:10.1016/j.jsbmb.2004.03.101
10. Dokuparthi SK, Reddy TRM. Antioxidant and nephroprotective activity of flavonoid-rich fraction of *Alphonsea sclerocarpa* Thw. *Int J Pharm Sci Drug Res*,2021;13(4):384–94. doi:10.25004/IJPSDR.2021.130404
11. Fakhrah S, Mohanty CS, Kumari A. The current status and challenges of two overlooked medicinal plants *Cestrum diurnum* and *Cestrum nocturnum*: A review. *Pharmacogn Rev*,2025;18(36):101–10. doi:10.5530/phrev.20241937
12. Ghosh A, Chandra G. Biocontrol efficacy of *Cestrum diurnum* L. (Solanaceae: Solanales) against the larval forms of *Anopheles stephensi*. *Nat Prod Res*,2006;20(4):371–9. doi:10.1080/14786410600661575
13. Khatun A, Rahman M, Nesa ML. Analgesic, anti-inflammatory, and NF-κB inhibitory activity of aerial parts of *Cestrum diurnum*. *Clin Phytoscience*, 2022, 8(1). doi:10.1186/s40816-022-00340-5
14. Kuchana P, Sujatha E. Phytochemical investigation, antioxidant and cytotoxic potential of *Dracaena reflexa* Lam. *Int J Biol Pharm Allied Sci*,2021;10(9):698–708. doi:10.31032/IJBPAS/2021/10.9.1054
15. Mahalakshmi TV, Sujatha E, Ramadevi B. Total phenolic content, flavonoid content, and antioxidant activity of *Alternanthera ficoidea* (L.) P. Beauv. *Int J Biol Pharm Allied Sci*,2021;10(9 Suppl.):453–60. doi:10.31032/IJBPAS/2021/10.9.1034
16. Patil AS, Salve SP, Phatak SR, Chivate ND. Quantitative estimation of total phenolic, total flavonoid content and assessment of *in-vitro* antioxidant capacity of *Psidium guajava* L. leaves extracts. *Res J Pharm Technol*,2023;16(5):1028–32. doi:10.52711/0974-360X.2023.00172
17. Prasad MP, Prabhu A, Thakur MS, Ruparel YM. Phytochemical screening, antioxidant potential, and antimicrobial activities in three species of *Cestrum* plants. *Int J Pharma Bio Sci*, 2013, 4(2). Available from: <https://espace.library.uq.edu.au/view/UQ:ded9114>
18. Satapathy T, Banjare B, Sahu H. A comprehensive analysis of *Cestrum nocturnum*: Its phytochemical composition, pharmacological applications, and toxicity profile in the context of traditional medicinal practices. *J Drug Deliv Ther*,2024;14(9):215–22. doi:10.22270/jddt.v14i9.6771
19. Sibony RW, Segev O, Dor S, Raz I. Overview of oxidative stress and inflammation in diabetes. *J Diabetes*, 2024, 16(10). doi:10.1111/1753-0407.70014
20. Sujatha E, Fatima M. Phytochemical profile, antioxidant and cytotoxic activities of aquatic weed *Landoltia punctata* (G. Mey.) Les & D. J. Crawford. *Ann Phytomed*,2023;12(1):1–6. Available from: <http://dx.doi.org/10.54085/ap.2023.12.1.33>
21. Wasserman RH, Corradino RA, Krook L, Hughes MR, Haussler MR. Studies on the 1 $\alpha$ , 25-dihydroxycholecalciferol-like activity in a calcinogenic plant, *Cestrum diurnum*, in the chick. *J Nutr*,1976;106(4):457–65. doi:10.1093/jn/106.4.457