Available online on 15.06.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Exploring the Therapeutic Potential of *Mammillaria beneckei* C. Ehrenb. Stem Extracts: In-Vitro Anti-diabetic and Anti-inflammatory Activities

Rupak Das, Bapi Ray Sarkar *

Department of Pharmaceutical Technology, University of North Bengal, Raja Rammohunpur, Darjeeling, West Bengal-734013, India

Article Info:

Article History:

Received 23 March 2025
Reviewed 01 May 2025
Accepted 04 June 2025
Published 15 June 2025

Cite this article as:

Das R, Sarkar BR, Exploring the Therapeutic Potential of *Mammillaria beneckei* C. Ehrenb. Stem Extracts: *In-Vitro* Anti-diabetic and Anti-inflammatory Activities, Journal of Drug Delivery and Therapeutics. 2025; 15(6):126-131 *DOI: http://dx.doi.org/10.22270/jddt.v15i6.7220*

*Address for Correspondence:

Bapi Ray Sarkar, Associate Professor, Department of Pharmaceutical Technology, University of North Bengal, Darjeeling, India

Abstract

Background: The present study investigates the *In-Vitro* Anti-inflammatory and Anti-diabetic properties of different solvent extracts of *Mammillaria beneckei* C. Ehrenb. Stem, this cactus species is traditionally used in folk medicine. It belongs to the family Cactaceae and the genus *Mammillaria*, which is known for its globular or cylindrical shape, and most importantly, its flowers.

Methods: Firstly, Phytochemical screening is required to establish the work. After performing phytochemical screening, many of the compound I have already found like- Alkaloids, Flavonoids, Glycosides, and Tannins etc., Pet. Ether, Methanol, Chloroform, and aqueous extracts were prepared using standard extraction techniques. Anti-inflammatory activity was evaluated through inhibition of albumin denaturation assays, while Anti-diabetic potential was assessed via α-amylase enzyme inhibition methods.

Result & Discussion: All extracts exhibited dose-dependent biological activities, with the methanol extract showing the highest inhibition in both anti-inflammatory and anti-diabetic assays. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which may contribute to the observed pharmacological effects.

Conclusion: These findings suggest that *Mammillaria beneckei* stem extracts possess significant Anti-inflammatory and Anti-diabetic potential, warranting further investigation for their use in the development of natural therapeutic agents. Further studies are warranted to explore its bioactive compounds and their mechanisms of action.

Keywords: Cactaceae, Albumin denaturation, Bioactive compounds, Phytochemical screening.

INTRODUCTION:

Medicinal plants have long been served as a valuable source of therapeutic agents, and traditional practices which offers as a natural remedy for a broad spectrum of diseases. Among the diverse botanical families, cacti have gained increasing attention due to their rich phytochemical composition and potential pharmacological properties. *Mammillaria beneckei* C. Ehrenb., a lesser-explored member of the Cactaceae family, the genus Mammillaria, holds traditional significance in folk medicine; however, its pharmacognostic and pharmacological attributes remain largely uncharacterized. [1] This cactus is a generally small, slow-growing plant, usually characterized by a rounded body with dense clusters of spines, often pale or white in colour, which makes it famous, this species of cactus is mainly found in central region of Mexico, and Rajasthan, in India. It is known for its shape, mainly globular or cylindrical and it's very famous for the attractive flowers. The current investigation aims to comprehensively evaluate the stem of *Mammillaria beneckei* through a multi-faceted approach, beginning with the optimization of extraction techniques to ensure efficient recovery of bioactive constituents. Extraction methodology is a critical step in natural product research, as the choice of solvent and technique significantly influences the phytochemical yield and biological efficacy of plant extracts. [2]

In the *In-Vitro* evaluation of anti-inflammatory and anti-diabetic activities forms the core of the biological screening in this study. Inflammatory disorders are global health concerns, and the search for alternative treatments with fewer side effects has intensified interest in plant-based therapeutics. By employing established assays such as protein denaturation inhibition and α-amylase inhibition. Phytochemical profiling helps to identify and quantify of bioactive compounds present in the crude drugs (that means plant), such as Alkaloids, Glycosides, Terpenoids, Amino acids, Carbohydrates, Protein, Flavonoids, Tannin, & Phenolic compounds, Steroids which is present or not. The extraction process is mainly obtaining that what type of bioactive compounds is present in the crude drugs, in this research purposes here we follow the Successive

extraction process. In here we first authenticate the plant, then we collect the mature bulb of *Mammillaria beneckei* C. Ehnerb. Stem in rainy season, dried under room temperature (shed drying), we use the solvents like non polar to polar like; Pet. Ether, Chloroform, Methanol, Hydro alcohol & Water, after that dried it on Rotary vacuum evaporator with their respective temperature. [3]

The present study demonstrated that various extracts of *Mammillaria beneckei* stem possess promising *In-vitro* anti-inflammatory and anti-diabetic activities. Among the above tested extracts, Methanol extracts & Chloroform extracts showed the most significant inhibition of key inflammatory mediators, such as protein denaturation and membrane stabilization, as well as notable α-amylase inhibitory activity, indicating its potential for blood glucose regulation. [4]

Figure 1: Stem of cactus (*Mammillaria beneckei* C. Ehrenb)

Review of Literatures:

- Govindappa M, Naga Sravya S., Poojashri M. N., Sadananda T. S. and Chandrappa C. P, et al. antimicrobial, antioxidant and in vitro antiinflammatory activity of ethanol extract and active phytochemical screening of Wedelia trilobata (L.) Hitchc. Journal of Pharmacognosy and Phytotherapy, 2011, 3(3): 43-51.
- R. Manikandan, A.Vijaya Anand1 and G. Durai Muthumani, et al. Phytochemical and in vitro antidiabetic activity of methanolic extract of Psidium guajava leaves International Journal of.Current Microbiolgy and Applied Science, 2013, 2(2):15-19.
- Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey, Sanjib Bhattacharya , et al. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine. 2012,178-180.
- Saltos MB, Puente BF, Faraone I, Milella L, De Tommasi N, Braca A (2015) Inhibitors of α-amylase and α-glucosidase from Andromachia igniaria Humb. & Bonpl. Phytochem Lett 14:45–50.
- Yesmin S, Paul A, Naz T, Rahman AA, Akhter SF, Wahed MI, Emran TB, Siddiqui SA (2020) Membrane stabilization as a mechanism of the anti-infammatory activity of ethanolic root extract of Choi (Piper chaba). Clin Phytosci 6(1):1–10.

Objectives:

To prepare and characterize various extracts such as-methanolic, aqueous from the stem of *Mammillaria beneckei* C. Ehrenb. using standard extraction techniques. Then, to perform preliminary phytochemical screening of the extracts to identify the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, and tannins. Then evaluate the *In-vitro* anti-inflammatory activity of the different extracts using suitable assays, like Protein denaturation assay and assess the *In-vitro* anti-diabetic activity of the extracts using standard enzyme inhibition assays, mainly α-amylase inhibition assay.

MATERIALS AND METHODS:

Plant materials:

Stem of Cactus is selected for this research experiments (*Mammillaria beneckei* C. Ehrenb)

Authentication & Collection:

The plant was collected in rainy season then it transformed for herbarium as per guidelines and submitted to the Botanical Survey of India. After authentication of plants, it was collected from my native area in bulk quantities for further processing.

Plant preparation:

Once the plants were collected, then it cleaned using tap water to get rid of any undesired contaminants. After that, it was cut into little pieces for easier drying and allowed to dry for a few days in the shade. Following thorough drying, the plant components were ground into a coarse powder using a mechanical grinder, sieved, and then placed in a tightly sealed container for storage.[5]

Preparation of extracts:

The powder form of this plants being shade-dried below 40 °C. After five to six days, 215 grams of powdered crude drug were extracted using a series of non-polar to polar solvents, including Pet. Ether, Chloroform, Methanol, Hydro alcohol (alcohol: water = 70:30), and water respectively. After filtering, the extracts were dried out in a rotary evaporator before being placed in a water bath at the appropriate solvent temperature. [6]

Chemicals and reagents:

The major analytical grade chemicals were used as per the requirements like Pet. Ether, chloroform, methanol, Benedict's reagent, Ninhydrin soln., Dragendroffs' reagent, Mayers' reagent, Hagers' reagent, Acarbose, Diclofenac etc. [7]

Preparation of standard:

The *Mammillaria beneckei* C. Ehrenb. extracts and standard (Acarbose acid & Diclofenac) were prepared at

a concentration of 100, 300, 500, 800 µg/mL & 100, 200, 300, 400, 500 µg/mL using distilled water.

Phytochemical screening:

Mammillaria beneckei C. Ehrenb. extracts were tested for their phytochemical composition using conventional techniques using Petroleum ether, Chloroform, Methanol, Hydro alcoholic and Water extracts. [8]

Anti-inflammatory activity:

Inhibition of Protein Denaturation: The *In-vitro* Anti-inflammatory activity of *Mammillaria beneckei* C. Ehrenb was determined by egg albumin denaturation test. Diclofenac sodium was used as the standard drug in the following extracts amounts. 1mL of various concentrations (1.2 µg/ml, 2.5 µg/ml, 5 µg/ml 10 µg/ml in distilled water) was mixed with 2ml of egg albumin and 3 ml of phosphate-buffered sol. which pH is 6.5. [9] Then the mixtures were incubated for 15 min at 37°C. After that it was heated for 12 min at 66°C, and after that cooling, the absorbance was recorded at 665 nm.[10] The percentage inhibition of egg albumin denaturation was estimated using the following formula,

$$\% \text{ Inhibition} = [(A_0-A_1)/A_0] \times 100$$

Where, A_0 = the absorbance of the control

A_1= the absorbance of the extract/standard.

Here, the standard was diclofenac. The extract/drug concentration for 50% inhibition (IC_{50}) was discovered. [11]

Anti-diabetic activity:

Inhibition of α-amylase: The α-amylase inhibition of various extracts of cactus *Mammillaria beneckei* C. Ehrenb was measured using DNSA method. The samples were mixed with 0.5 mL α-amylase solution (0.5 mg/mL) and incubation is needed for 10 min at 25 °C temperature. Then, 0.5 mL sodium were mixed with α-amylase in 0.1 M phosphate buffer (100 µL) containing pH 6.9 and incubated at 25 °C for 5 min. The reaction mixtures were then incubated at 25 °C for 10 minutes. The reaction was stopped with 50 µl of 96 mM 3,5-dinitrosalicylic acid (DNSA) colour reagent (formula: 1 g DNSA, 200 mg crystalline phenol, and freshly prepared 50 mg sodium sulphite dissolved in 1 % w/v NaOH) and then incubated in a boiling water bath for 5 minutes. [12] After incubation it would be cooled at room temperature. Absorbance was measured at 540 nm.

The following formula was used to calculate extract inhibitory activity.

$$\% \text{ inhibitory activity} = [(A_0 - A_1) / A_0] \times 100$$

Where,

A_0 = absorbance of the control (100% enzyme activity)

A_1 = absorbance of the sample.

The concentration of the test sample required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated. The study helps to determine the IC_{50} value. [13]

RESULTS AND DISCUSSION:

Authentication and Collection:

Mammillaria beneckei C. Ehrenb, Cactus were selected on its traditional uses. Initially, it was discovered in the corner of our property, and these plants had been collected in substantial quantities from our region, the Sundarbans in West Bengal on the month of August, 2024. It was collected and ready for the herbarium as per guidelines, we submitted the sample to the Botanical Survey of India, (CENTRAL NATIONAL HERBARIUM) Howrah, Shivpur with specimen no. – UNB/RD-01 on 24.10.2024. The herbarium specimen copy is attached below on the Figure 2.

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
फैक्स/ Fax: (033)26686226
दूरभाष/ Phone: (033)26683235/3364
ई-मेल/ E-mail: calherbarium@yahoo.co.in

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या/No.: CNH/Tech.II/2024/132 दिनांक/Date: 24.10.2024

To,
Mr. Rupak Das
M. Pharm.,
Department of Pharmaceutical Technology
University of North Bengal
Darjeeling-734013

Sub.: Identification of one plant specimen – reg.

Dear Mr. Das,

Please refer to your letter no. PT/BRS/100 dated 26th September 2024 along with a plant specimen for identification. It is to inform you that the specimen is incomplete bearing only vegetative plant parts, without any flower or fruit. The specimen has been tentatively identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	UNB/RD–01	**Mammillaria beneckei** C.Ehrenb.	Cactaceae

The receipt of ₹ 250/- (Rupees two hundred fifty only) Transaction Ref. No. 2210240043399 dated 22.10.2024 payment made via bharatkosh.gov.in is enclosed herewith.

Your specimen is returned herewith.

Yours sincerely

24.10.2024

(R.K GUPTA)
Scientist –'F' & Head of Office

Figure 2: Herbarium specimen copy of *Mammillaria beneckei* C. Ehrenb

Phytochemical screening:

Table 1: Phytochemical analysis of various extracts prepared from the powder of *Mammillaria beneckei* C. Ehrenb (Stem)

Sl. No	Test	Pet. Ether extracts	Chloroform extracts	Methanol extracts	Hydro alcohol extracts (70:30)	Water extracts
1.	Alkaloids	-	+	+	+	-
2.	Flavonoids	-	++	+	-	-
3.	Glycosides	+	+	+	-	-
4.	Carbohydrates	-	+	-	-	-
5.	Tannins	-	+	++	+	-

(++) sign = strongly positive results, (+) sign = positive results, (-) sign = negative results[14]

Anti-inflammatory activity:

Inhibition of Protein Denaturation:

The protein denaturation bioassay was selected for *In-vitro* assessment of Anti-inflammatory property of various extracts of *Mammillaria beneckei* C. Ehrenb. Diclofenac, used as a standard anti-inflammatory drug, showed comparatively maximum anti-inflammatory activity (46.92% at 500 µg/mL) at 1 mg/mL concentration. The Methanolic and Chloroform extract had a maximal inhibition of 48.64 % and 49.54 % at 500 µg/ml. As compare to diclofenac, methanol and chloroform extracts shown the good result [**Fig: 3** shown this graph]. [15]

This was further supported by comparing the IC_{50} values of the methanolic and chloroform extract to the diclofenac sodium. It demonstrates that anti-inflammatory activities is may present in this plants part, mainly two various extracts. [16]

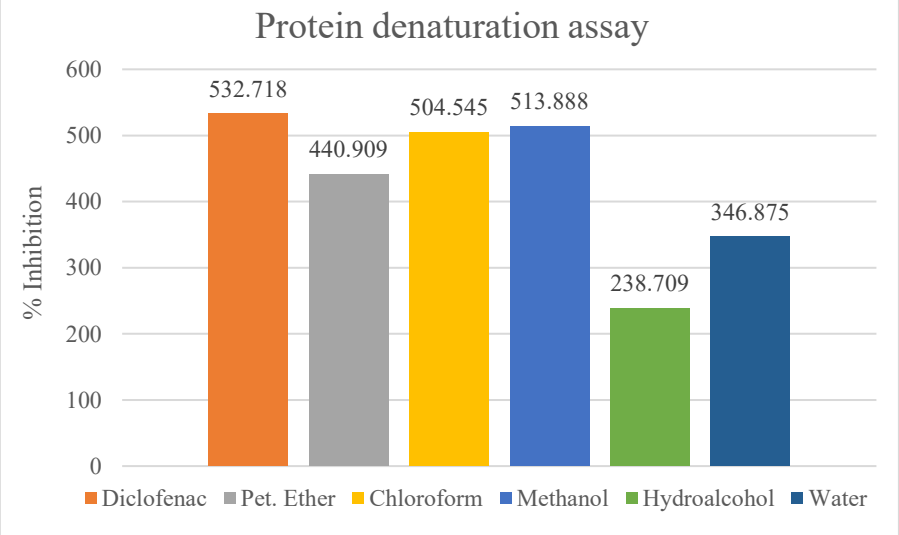

Figure 3: Protein denaturation test by using standards and extracts.

Anti-diabetic activity:

Inhibition of α-amylase:

α -amylase, an intestine digestive enzyme, is essential for the breakdown of carbohydrates. α -amylase enzyme inhibition is one of the Anti-diabetic treatment strategy that lowers blood postprandial glucose levels. These may be a crucial tactic in blood glucose control. According to the *In-vitro* α-amylase inhibitory tests, exhibits strong anti-diabetic properties. Here, we use the concentration like- 100, 200, 300, 400 & 500. As per standard, we got the best result in Chloroform extracts and Methanol extracts that we can see in the below graph [**Fig: 4**] [17].

At a concentration of 100 µg/ml of *Mammillaria beneckei* C. Ehrenb extracts Chloroform and Methanol showed a percentage inhibition 88.09 % and 90.59 %, Where the standard Acarbose 88.13 %. The IC_{50} value of Chloroform & Methanol extracts is 56.75 µg/mL and 55.19 µg/mL where the standards IC_{50} value is 56.72 µg/mL. The IC_{50} value revealed that the plant extracts of *Mammillaria beneckei* C. Ehrenb showed potent α-amylase inhibitory activity. [18]

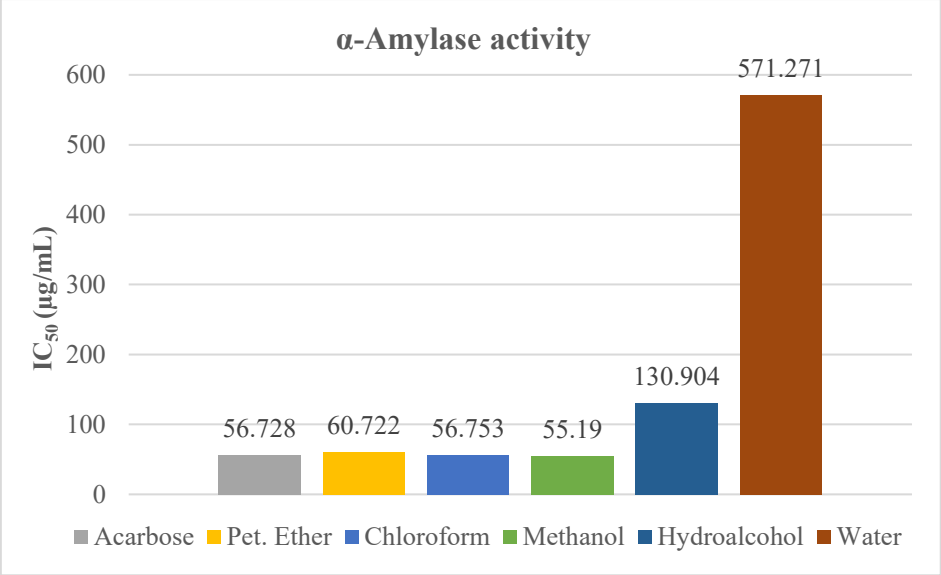

Figure 4: α-Amylase inhibition assay of standards and extracts.

DISCUSSION:

After performing the phytochemical screening Alkaloids, Flavonoids & Glycosides may be present in the Chloroform and Methanol extract. In pharmacological studies mainly in *In-vitro* Anti-inflammatory activity chloroform & methanol extracts showing good results as compare to standards Diclofenac sodium[19]. Another one in Anti-diabetic activity, methanol extract and chloroform extracts showing good results as per the Acarbose standard, so we can conclude that Anti-diabetics and Anti-inflammatory activity may be present in this plants part. Mainly in two Chloroform and Methanol extracts. Further thorough of investigations are required. [20]

CONCLUSION:

The present study demonstrated that various extracts of *Mammillaria beneckei* C. Ehrenb. Stem exhibit promising in-vitro anti-inflammatory and anti-diabetic activities. The preliminary phytochemical shows that the maximum number of secondary metabolites was present in the methanol and chloroform extract followed by another extract. Among the tested extracts, prominently methanol extract showed the most significant inhibitory activity in both protein denaturation assays (for anti-inflammatory activity) and α-amylase inhibition assays (for anti-diabetic activity), indicating strong potential as a natural therapeutic agent. These findings support the traditional use of this cactus species in folk medicine and highlight its potential for development into plant-based treatments for inflammatory and metabolic disorders. However, further *In-vivo* studies are required to isolate active constituents and fully understand the mechanisms of action.

Conflict of Interest: There are no conflicts of interest for the authors and co-authors in relation to this research purposes. This thesis paper's writing and content are solely the authors' responsibility.

Acknowledgement: First and foremost, I would like to sincerely thank my research supervisor, Dr. Bapi Ray Sarkar, for his invaluable guidance, and Mr. Ganesh Dey, a research scholar in the University of North Bengal, Department of Pharmaceutical Technology, for his insightful suggestions, ongoing support, and valuable guidance during this research study. Their knowledge and support were crucial to this study project's successful conclusion. I also want to express my gratitude to our department's faculty.

Lastly, I would like to thank my parents for their unwavering love, patience, and support, which gave me strength and motivation throughout the project.

Author Contributions: All authors have equal contribution in the preparation of manuscript and compilation.

Source of Support: Nil

Funding: The authors declared that this study has received no financial support.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethical approval: This study does not involve experiments on animals or human subjects.

REFERENCES:

1. Oome, S., Variable cotyledon numbers in Mammillaria beneckei (Cactaceae). 2005.

2. Harpke, D., et al., Phylogenetic evaluation of chloroplast trnL-trnF DNA sequence variation in the genus Mammillaria (Cactaceae). Schlechtendalia, 2006;14:7-16.

3. Breslin, P.B., M.F. Wojciechowski, and L.C. Majure, Molecular phylogeny of the Mammilloid clade (Cactaceae) resolves the monophyly of Mammillaria. Taxon, 2021;70(2):308-323. https://doi.org/10.1002/tax.12451

4. Cervantes, C.R., et al., Evaluating the monophyly of Mammillaria series Supertextae (Cactaceae). PhytoKeys, 2021;177:25. https://doi.org/10.3897/phytokeys.177.62915 PMid:33967580 PMCid:PMC8099837

5. Dewanjee, S., et al., Study of anti-inflammatory and antinociceptive activity of hydroalcoholic extract of Schima wallichii bark. Pharmaceutical Biology, 2009;47(5):402-407. https://doi.org/10.1080/13880200902758824

6. Das, M.J. and B.R. Sarkar, Evaluation of in vitro anti-inflammatory activity of methanolic extract of Impatiens bracteata Colebr. ex wall.

7. Akbar, S., et al., Pharmacognostic studies of stem, roots and leaves of Malva parviflora L. Asian Pacific journal of tropical biomedicine, 2014;4(5):410-415. https://doi.org/10.12980/APJTB.4.2014C1107 PMid:25182728 PMCid:PMC3985058

8. Alam, T., et al., Pharmacognostical parameters, sphytochemical screening, TPC, TFC and evaluation of in-vitro anti-oxidant activity of leaves of Cymbidium aloifolium L (SW). Research Journal of Pharmacy and Technology, 2024;17(12):5743-5749. https://doi.org/10.52711/0974-360X.2024.00874

9. Osei Akoto, C., et al., Anthelminthic, Anti-Inflammatory, Antioxidant, and Antimicrobial Activities and FTIR Analyses of Vernonia camporum Stem-Bark. Journal of Chemistry, 2021;2021(1):3328073. https://doi.org/10.1155/2021/3328073

10. Akoto, C.O., et al., In vitro anthelmintic, anti-inflammatory, antioxidant activities and FTIR analysis of Sclerocarya birrea root. 2020.

11. Osei Akoto, C., et al., Anti-Inflammatory, Antioxidant, and Anthelmintic Activities of Ocimum basilicum (Sweet Basil) Fruits. Journal of Chemistry, 2020;2020(1):2153534. https://doi.org/10.1155/2020/2153534

12. Bagchi, A., B.R. Sarkar, and P. Pal, An antidiabetic and antioxidant activity of an unexplored plant of Diapensia himalaica in Alloxan-induced diabetic rats, African Journal of Biological Sciences. 2024;6(5):1646-1658

13. Chakraborty, R., S. Roy, and V. Mandal, Assessment of traditional knowledge of the antidiabetic plants of Darjeeling and Sikkim Himalayas in the context of recent phytochemical and pharmacological advances. Journal of Integrative Medicine, 2016;14(5):336-358. https://doi.org/10.1016/S2095-4964(16)60267-4 PMid:27641606

14. Sarkar, B.R., Phytochemical Screening and Proximate exploration of Diapensia himalacia leaf extract.

15. Azab, A., A. Nassar, and A.N. Azab, Anti-inflammatory activity of natural products. Molecules, 2016;21(10):1321. https://doi.org/10.3390/molecules21101321 PMid:27706084 PMCid:PMC6274146

16. Vallianou, N.G., et al., Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. Gen Med (Los Angel), 2014;2(132):1-5. https://doi.org/10.4172/2327-5146.1000132

17. Chirumamilla, P. and S. Taduri, Assessment of in vitro anti-inflammatory, antioxidant and antidiabetic activities of Solanum khasianum Clarke. Vegetos, 2023;36(2):575-582. https://doi.org/10.1007/s42535-022-00410-6

18. Singh, A.K., et al., Evaluation of antidiabetic activity of dietary phenolic compound chlorogenic acid in streptozotocin induced diabetic rats: Molecular docking, molecular dynamics, in silico toxicity, in vitro and in vivo studies. Computers in biology and medicine, 2021;134:104462. https://doi.org/10.1016/j.compbiomed.2021.104462 PMid:34148008

19. Singh, K.K., S.S. Singh, and C.L. Sharma, Antidiabetic and antioxidant properties of important traditional medicinal plants from Northeastern India. Int J Pharm Sci & Res, 2024;15(6):1728-36.

20. Ginwala, R., et al., Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants, 2019;8(2):35. https://doi.org/10.3390/antiox8020035 PMid:30764536 PMCid:PMC6407021