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ASSESSMENT OF ANTI-INFLAMMATORY ACTION OF VITEXIN USING IN VITRO MODELS

Abhishek Kashyap, Faizan Ali

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Abhishek Kashyap, Faizan Ali
RB Science, Bhopal, Madhya Pradesh, India

Correspondence

Abhishek Kashyap
RB Science, Bhopal, Madhya Pradesh, India

Email: rbsofficework@gmail.com

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ABSTRACT

In the present work the in vitro anti-inflammatory potential of vitexin was evaluated using two different models of inflammation viz. inhibition of protein denaturation and inhibition of protease action. Both these models are based on the chemical mediators of inflammation and evaluating the drug on these models is self-evident of its anti-inflammatory property. Vitexin is a natural flavanol glycoside with iron chelating and antioxidant properties. It has diverse actions at the cellular and physiological levels, demonstrating anti-inflammatory, anti-cancer, and anti-fibrosis effects in animal models. The presence of peaks at 1727, 3407, 1462 and 2842 are indicative of the presence of carbonyl, hydroxyl, aromatic ring and aliphatic carbon respectively. The results of solubility analysis indicate that vitexin is soluble in organic solvents easily and is partially or sparingly soluble in aqueous solutions. The capacity of different concentrations of vitexin to inhibit protein denaturation of albumin was ranging from 20.33 ± 0.129 % to 69.63 ± 0.213 % in the assay and hence provides yet another evidence for its excellent anti-inflammatory property. The capacity of different concentrations of vitexin to inhibit serine protease was found to be ranging from 18.12 ± 0.214% to 75.21 ± 0.315% in the assay and therefore reinforces its tremendous anti-inflammatory property.

Key words: Vitexin, anti-inflammatory, albumin denaturation, serine protease, flavanol.

1 INTRODUCTION

Inflammation is a complex biological response to harmful stimuli such as pathogens, damaged cells, or irritants, and while it is essential for host defense, its dysregulation contributes to chronic diseases including arthritis, cardiovascular disorders, and cancer [1]. Natural flavonoids have gained attention for their ability to modulate inflammatory pathways through antioxidant and immunoregulatory mechanisms [2]. Vitexin, a flavone glycoside commonly found in medicinal plants, has demonstrated promising pharmacological effects including antioxidant, anti-cancer, and anti-inflammatory activities [3].

Recent studies have revealed that vitexin mitigates oxidative stress, mitochondrial damage, and pyroptosis in sepsis-associated acute lung injury, highlighting its role in regulating epigenetic pathways such as the SNHG1/DNMT1/miR-495 axis [4]. Similarly, vitexin has been shown to alleviate hyperthermia-induced oxidative stress and inflammation in buffalo mammary epithelial cells, suggesting its potential in veterinary and agricultural applications [5].Furthermore, vitexin, in combination with verapamil, has been reported to downregulate efflux pump P-glycoprotein and promote macrophage polarization from M1 to M2 phenotype via the TLR4-NF-κB-TNFR2 pathway, thereby reducing inflammatory responses in lipopolysaccharide-induced sepsis models [6].

These findings underscore vitexin's multifaceted role in modulating inflammatory mediators and cellular signaling pathways. Given its broad spectrum of activity, evaluating vitexin in in vitro models such as albumin denaturation and protease inhibition provides further evidence of its therapeutic potential against inflammation.

2. MATERIAL AND METHODS

Vitexin was purchased from TCI Chemicals (India) Pvt. Ltd and its identity was confirmed by IR spectroscopy prior to use. The purity of the drug was confirmed by the supplier as 96.3%. It was used as obtained without any processing for purification. All other chemicals used in the study were of AR grade and were procured from the local chemical supplier.

2.1 Physicochemical characterization of Vitexin

In order to confirm the identity of the Vitexin, Infra-Red spectroscopy of the sample was performed and the presence of functional groups therein was confirmed. A few other physicochemical properties (melting point, organoleptic features and solubility) of the sample were also evaluated.

2.2 Evaluation of *in vitro* anti-inflammatory activity

2.2.1 Inhibition of albumin denaturation

The technique of inhibition of albumin denaturation reported by Kumari *et al* [8] was used with slight modifications. The volume of each component of the reaction mixture was reduced to half its volume. Vitexin was dissolved in DMSO and appropriately diluted to prepare solutions of 62.5, 125, 250, 500 and 1000 mM concentration. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control. The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solution (vitexin). Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of vitexin solution. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

2.2.2 Antiprotease action

The technique of antiprotease action reported by Oyedepo *et al* [9] and Sakat *et al* [10] was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 µg/mL). The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition of protease inhibitory activity was calculated by the following formula:

Percentage inhibition = (Abs control –Abs sample) x 100/ Abs control

2.3 Statistical Analysis

All the experiments were performed in triplicate and the results are expressed as mean ± standard deviation. The difference between the experimental groups was compared by one way ANOVA followed by Dunnets multiple comparison test using Graph Pad Instat software.

3. RESULTS

3.1 Physicochemical characterization of vitexin

The identity of the purchased vitexin was confirmed by its IR spectra. The peaks of the functional groups were compared with that of the standard data available at pubchem [11].

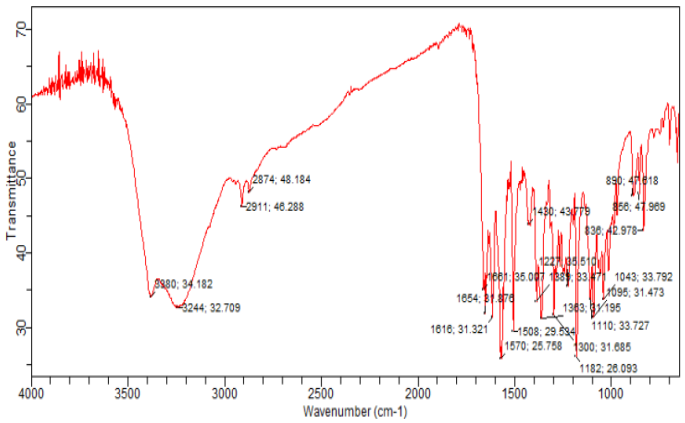

Figure 1 FT-IR Spectra of Vitexin

The purchased sample of vitexin was of yellow color and the texture appeared to be fine and crystalline. The data from pubchem and clearsynth also confirms the crystalline structure of vitexin. Solubility of vitexin was observed in different solvents and the results of solubility analysis are depicted below.

Table 1 Solubility of Vitexin

S.No	Solvent	Solubility
1	Water	Partially soluble
2	5% NaOH	Partially soluble
3	5% HCl	Partially soluble
4	Methanol	Soluble
5	Ethanol	Soluble
6	Dimethyl formamide	Soluble
7	DMSO	Freely soluble

The melting point was performed using an open capillary method and was found to be in the range of 425-428°C which was in consortium with the standard data available at clearsynth [12].

3.2 Anti-inflammatory activity

The anti-inflammatory activity was assessed using the in vitro models and the results of each model were statistically evaluated for significance.

3.2.1 Albumin Denaturation Inhibition

All the concentration levels of vitexin exhibited inhibition of albumin denaturation (Table 2). The concentration of 500 µg/mL vitexin had shown the greatest inhibition capacity with 69.63 ± 0.213% whereas the lowest inhibition capacity of 20.33 ± 0.129 % was exhibited by 200 µg/mL concentration. The inhibition protein denaturation by 100 µg/mL solution was not significant while the inhibition capacity of the positive control (Ibuprofen) was found to be 68.43 ± 0.001%.

Table 2 Albumin denaturation inhibition activity

S.No	Test Group	Conc (µg/mL)	Abs	% Inhibition
1	Negative Control	-	0.4153	0
2	Vitexin	100	0.3216	12.56 ± 0.051
3	Vitexin	200	0.3018	20.33 ± 0.129**
4	Vitexin	300	0.2647	27.87 ± 0.033**
5	Vitexin	400	0.1582	48.33 ± 0.102**
6	Vitexin	500	0.1334	69.63 ± 0.213**
7	Positive Control	100	0.1311	68.43 ± 0.001**

** p<0.01 considered as extremely significant

3.2.2 Antiprotease action

Vitexin was found to inhibit protease activity in all the tested concentrations (Table 3). The highest inhibition capacity was exhibited by vitexin solution of 500 µg/mL concentration, inhibiting 75.21 ± 0.315% while the 100 µg/mL vitexin solution was able to inhibit only 18.12 ± 0.214% protease activity. Aspirin solution of 100 µg/mL concentration was able to inhibit 48.78 ± 0.211% of protease activity.

Table 3 Antiprotease activity of vitexin solution

S.No	Test Group	Conc (µg/mL)	% Inhibition
1	Negative Control	-	0
2	Vitexin	100	18.12 ± 0.214*
3	Vitexin	200	23.21±0.318*
4	Vitexin	300	37.61±0.201**
5	Vitexin	400	59.10±0.113**
6	Vitexin	500	75.21 ± 0.315*
7	Positive Control	100	48.78 ± 0.211**

*p<0.05, **p<0.01

4. DISCUSSION

4.1 Physicochemical characterization

The presence of peaks at 1727, 3407, 1462 and 2842 are indicative of the presence of carbonyl, hydroxyl, aromatic ring and aliphatic carbon respectively. These peaks are consistent with the data of pubchem, confirming the identity of the molecule. The intensive bands between 1600 and 1580 are due to vibrations of the aromatic ring. The strong band at 2911 can be assigned to the C-H asymmetric stretching vibration and the peak at 2874 is due to the C-H symmetric stretching.

The results of solubility analysis (Table 2) indicate that vitexin is soluble in organic solvents easily and is partially or sparingly soluble in aqueous solutions. A similar solubility profile of vitexin was reported by Raghu and Agrawal [13]. According to their study vitexin was found to be soluble in organic solvents including methanol, ethanol and freely soluble in DMSO. The solubility profiling is done to prepare solution of the drug samples for analytical estimation as well as for pharmacological screening.

4.2 Albumin denaturation inhibitory activity

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis [14]. According to Opie [15], tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity.

The capacity of different concentrations of vitexin to inhibit protein denaturation of albumin was ranging from 27.33 ± 0.001% to 67.82 ± 0.002 in the assay and hence provides yet another evidence for its excellent anti-inflammatory property. The statistical analysis has shown that there is no significant difference ($p<0.01$) between the mean of the different concentrations of vitexin except 62.5 mM concentration which was not found to be significantly correlating.

4.3 Antiprotease activity

Neutrophils are known to be a rich source of serine protease and are localized at lysosomes. It has been previously reported that leukocytes protease play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by protease inhibitors [16]. Although mast cell protease-dependent proteolysis is critical to host defense against invading pathogens, regulation of these hydrolytic enzymes is essential to limiting self-induced damage as well. Indeed, dysregulated release of mast cell proteases is now recognized to contribute to the pathogenesis of a number of inflammatory conditions including asthma, abdominal aortic aneurysm formation, vessel damage in atherosclerosis and hypertension, arthritis, and ischemia/reperfusion injury [17].

The capacity of different concentrations of vitexin to inhibit serine protease was found to be ranging from 20.36 ± 0.214% to 77.53 ± 0.138 in the assay and therefore reinforces for its tremendous anti-inflammatory property. The statistical analysis has shown that there is no significant difference between the mean of the different concentrations of vitexin. The inhibition of protease was very much comparable to the standard drug Aspirin used in the study.

5. CONCLUSIONS

Vitexin compound has demonstrated promising anti-inflammatory potential by in vitro method suggesting the possible mechanism of interfering with the chemical mediators of inflammation. Increasing awareness, promotion and utilization of this flavonol compound will serve as a natural anti-inflammatory compound. A few more in vitro models could be studied for establishing the complete mechanism of action of vitexin at the cellular and molecular level in treating inflammation.

REFERENCES

1. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul;454(7203):428-35. doi:10.1038/nature07201.
2. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016 Dec;5:e47. doi:10.1017/jns.2016.41.
3. Xiao J, Chen X. Vitexin and isovitexin: chemical and pharmacological properties. Phytochem Rev. 2021 Mar;20(1):1-15. doi:10.1007/s11101-020-09695-3.
4. Zaki A, Mohsin M, Khan S, Khan A, Ahmad S, Verma A, Ali S, Fatma T, Syed MA. Vitexin mitigates oxidative stress, mitochondrial damage, pyroptosis and regulates SNHG1/DNMT1/miR-495 axis in sepsis-associated acute lung injury. Inflammopharmacology. 2024 Dec;32(6):2157-72. doi:10.1007/s10787-024-01609-6.
5. Senthamilan S, Aggarwal A, Grewal S, Rani S, Vats P, Pal P, Jaswal S, Arya A, Alhussien MN. Pre-treatment but not co-treatment with vitexin alleviates hyperthermia induced oxidative stress and inflammation in buffalo mammary epithelial cells. Journal of Reproductive Immunology. 2023 Aug 1;158:103979.
6. Kundu A, Ghosh P, Bishayi B. Vitexin along with verapamil downregulates efflux pump P-glycoprotein in macrophages and potentiates M1 to M2 switching via TLR4-NF-κB-TNFR2 pathway in lipopolysaccharide treated mice. Immunobiology. 2024 Jan;229(1):152767. doi:10.1016/j.imbio.2023.152767.
7. Osman NI, Sidik NJ, Awal A, Adam NAM, Rezali NI. In vitro xanthine oxidase and albumin denaturation inhibition assay of Barringtonia racemosa L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. J Intercult Ethnopharmacol. 2016;5(4):343-9. doi:10.5455/jice.20160922064205.
8. Kumari S, Yasmin N, Hussain MR, Babuselvam M. In vitro anti-inflammatory and anti-arthritic property of Rhizophora mucronata leaves. Int J Pharm Sci Res. 2015;6(2):482-5.
9. Oyedepo OO, Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of Fagra zanthoxiloides, Olax subscorpioides and Tetrapleura tetraptera. Int J Pharmacogn. 1995;33(1):65-9. doi:10.3109/13880209509088191.
10. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Int J Pharm Pharm Sci. 2010;2(1):146-55.
11. PubChem. Vitexin ATR-IR spectra. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Vitexin#section=ATR-IR-Spectra [Accessed 31 Aug 2026].
12. Clearsynth. Vitexin CST62759. Available from: https://www.clearsynth.com/en/CST62759.html [Accessed 31 Aug 2026].
13. Raghu MG, Agrawal P. The isolation and structural determination of flavonoids from Justicia gendarussa. IOSR J Pharm Biol Sci. 2016;11(2):73-9. doi:10.9790/3008-11227379.
14. Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. J Exp Med. 1962;115(3):597-608. doi:10.1084/jem.115.3.597.
15. Saso L, Valentini G, Casini ML, Grippa E, Gatto MT, Leone MG. Inhibition of heat-induced denaturation of albumin by nonsteroidal anti-inflammatory drugs (NSAIDs): pharmacological implications. Arch Pharm Res. 2001;24(2):150-8. doi:10.1007/BF02975129.
16. Das S, Chatterjee S. Long-term toxicity study of ART-400. Indian Indg Med. 1995;16(2):117-23.
17. Dai H, Korthuis RJ. Mast cell proteases and inflammation. Drug Discov Today Dis Models. 2011;8(2-3):47-55. doi:10.1016/j.ddmod.2011.06.004.