Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 7 (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Investigation of Phytochemicals and Inflammatory Response Modulation by Himalayan Orchid Root Extract

Karishma P Duhijod[1], Priyanka Tiwari[2], J. Priyanga[3], Ramya A[4], Vijay Sharma[5], Farhad F Mehta[6], Ram Mohan Manda[7], Monisha J*[8]

[1]Assistant Professor, Quality Assurance Department, Nagpur College of Pharmacy, Wanadongri Higna Road Nagpur, Maharashtra 441110, India

[2]Assistant Professor, Department of Pharmaceutical Chemistry, KLE College of Pharmacy, 2nd Block, Rajajinagar, Bengaluru, KLE Academy of Higher Education and Research, Belagavi, Karnataka 560010, India

[3]Assistant Professor, Vels Institute of Science Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu 600117, India

[4]Assistant professor, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu 600117, India

[5]Professor, Faculty of Pharmacy, IFTM University, Moradabad, U.P 244002, India

[6]Assistant Professor, School of Pharmaceutical Sciences, UTD, RGPV University, Bhopal, Madhya Pradesh, 462033, India

[7]Associate Professor, School of Pharmacy, Anurag University, Hyderabad, Telangana 500082, India

[8]Assistant Professor, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu 600117, India

***Corresponding Author:**

Monisha J

Designation and Affiliation: Assistant Professor, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu 600117, India

Email ID: monisha.sps@vistas.ac.in

Cite this paper as: Karishma P Duhijod, Priyanka Tiwari, J. Priyanga, Ramya A, Vijay Sharma, Farhad F Mehta, Ram Mohan Manda, Monisha J, (2025) Investigation of Phytochemicals and Inflammatory Response Modulation by Himalayan Orchid Root Extract. *Journal of Neonatal Surgery*, 14 (7), 808-817.

ABSTRACT

Inflammations is body's natural physiological response that helps the body to protect the mechanism of the body from harmful agents and supports tissue repair. However, when prolonged or unregulated, it can lead to chronic conditions such as cardiovascular disorders, osteoarthritis, and neurodegenerative diseases. Although conventional anti-inflammatory drugs like NSAIDs and corticosteroids are effective, their long-term use is often associated with adverse side effects. This research investigates the soothing effect on inflammation of water-ethanol base extract obtained from the root system of *Dactylorhiza hatagirea* and the rhizome of *Curcuma angustifolia* rhizomes, both traditionally used in Indian and Nepalese medicine.

The plant materials were authenticated and extracted using a hydro-ethanolic solvent, followed by phytochemical screening that confirmed the presence of major bioactive constituents, including flavonoids, phenolics, saponins, and carbohydrates. Quantitative analysis demonstrated notable concentrations of total phenolics (21.25 mg GAE per 100 mg of plant extract), flavonoids (33.82 mg of QE per 100 mg of plant extract) and tannins (12.83 mg/mL TAE per 100 mg of plant extract). Anti-inflammatory response is studied using Carrageenan induced paw model in wistar rats. Both extracts showed a dose-dependent reduction in paw swelling, with *Curcuma angustifolia* producing up to 55.3% inhibition and *Dactylorhiza hatagirea* showing 52.9% inhibition at 200 mg/kg, compared to 66.8% for the standard drug, diclofenac sodium. Protein denaturation assay is carried out to assess invitro anti-inflammatory activity, showed concentration dependent inhibition, with *Dactylorhiza hatagirea* and *Curcuma angustifolia* extracts exhibiting maximum inhibition of 78.4% and 72.1% at 400 µg/mL, respectively, compared to 85.6% by diclofenac sodium. Additionally, herbal tablets were formulated using the active extracts and evaluated as per Indian Pharmacopoeia guidelines. The study demonstrates that both plant extracts possess notable anti-inflammatory activity, likely due to their rich phytochemical content. These results shows that the cultural medicinal use of these plants and indicate their potential as natural sources for developing anti-inflammatory therapies with potentially fewer side effects than conventional drugs.

Keywords: *Neurodegenerative, Hydro-Ethanolic Extracts, Flavonoids, Paw Edema Model*

1. INTRODUCTION

Inflammation is a vital biological response that helps maintain internal stability and shields the body from threats such as infections, tissue damage, and cellular disturbances. Under normal conditions, the inflammatory response is well-regulated and beneficial. However, when inflammation becomes chronic, it can be involved in the onset and worsening of several diseases such as osteoarthritis, cardiovascular conditions, Alzeimer's disease, age related muscular degeneration, COPD, multiple sclerosis, stroke, and different types of cancer[1-6].

The hallmark features of inflammatory response include protein denaturation, membrane disruption, increase in vascular permeation, mediator release, infiltration of leukocytes, edema and formation of granuloma[7-9]. Immune cells like B and T cells, macrophages, neutrophils play an important role in inflammation regulation, which produce a variety of signaling substances—including cytokines, chemokines, prostaglandins, and proteolytic enzymes[10-12].

Currently, NSAIDs are commonly prescribed for managing pain and inflammation. However, their overuse can lead do severe side effects like gastrointestinal problems, ulcers, internal bleeding, and, in some cases, fatal perforations[12]. As a result, there has been growing interest in plant-based therapies, which are often considered to have fewer side effects and have been used for centuries in various traditional healing practices.

Among the plants with traditional medicinal value, *Dactylorhiza hatagirea* (family: Orchidaceae), known for its aphrodisiac and nutritive properties, has been widely used in the Indian subcontinent and Nepal. Its tubers, known as Salep, are utilized both as restorative tonics and alternatives to European Salep[13]. Another important ethnomedicinal plant is *Curcuma angustifolia* Roxb (family: Zingiberaceae), used extensively in rural India. Historically, the rhizomes have been utilized to treat conditions like jaundice, fever, kidney issues, and inflammation, while also being appreciated for their role in boosting vitality and supporting bone health [14-18].

2. MATERIALS AND PROCEDURE

The *Dactylorhiza hatagirea* roots and the *Curcuma angustifolia* rhizomes were gathered from rural areas in Maharashtra, India. A certified botanist conducted the taxonomical identification and validation of plant species. Identification done on macroscopic and microscopic characteristics, as well as comparative analysis with standard floras such as **Flora of Maharashtra** and **The Flora of British India**. Additional confirmatory methods included **morphological examination, organoleptic evaluation**, and **phytochemical screening.**

Chemicals required

Carrageenan and other standard reagents required for the induction of inflammation and assay procedures wetre obtained fro SDFCL Mumbai, india. Additional chemical reagents and solvents used in extraction and analysis were procured from Cosmo Chemicals (Mumbai), Alpha Chemicals (Mumbai), and Bright Lab (Hyderabad). All chemical reagents used for analysis were of high analytical grade.

Preparation and isolation of plant extract

The *Dactylorhiza hatagirea* roots and the *Curcuma angustifolia* rhizomes were collected and carefully cleaned with distilled water to eliminate any adhering soil or impurities. Collected samples of plants were dried in shade at room temperature for 2 weeks. Dried samples of plants were ground into a coarse powder using a mechanical grinder and stored in airtight containers in a dry environment until needed for further procedures[16].

The powdered plant materials were subjected to extraction using the hydro-ethanolic method**.** Briefly, 100 gram of powdered plant sample was soaked in 70 % of ethanol in a ration of 1:10 w/v in a clean conical flask. Prepared mixture placed on a rotary shaker at room temperature for 72 hours to facilitate complete extraction. Following the maceration process, plant extracts were filtered through muslin cloth and then passed through whatman no. 1 filter paper. A rotary evaporator maintained at 40 to 45°C is used for resulting filterate concentration, yielding a semisolid residue. These dried crude extracts were then stored in sterile, airtight containers and refrigerated at 4°C until they were subjected to further analysis and pharmacological testing

Identification of Bioactive Compounds in the Plant Extract

Preliminary phytochemical screening of the hydro-ethanolic extracts derived from the root system of *Dactylorhiza hatagirea* and rhizomes of *Curcuma angustifolia* was carried out following the standard procedures outlined by Khandelwal and Kokate. The goal of this analysis was to identify different types of bioactive compound with known therapeutic properties [18].

The extracts were analyzed to determine the presence or absence of major phytochemical compounds like amino acids, proteins, carbohydrates, flavonoids, phenolic compound, glycosides, etc. Standard qualitative chemical tests, such as Mayer's and Dragendorff's for alkaloids, ferric chloride test for phenolics, foam test for saponins, and lead acetate test for flavonoids, were employed.

Quantification of Secondary Metabolites

The quantitative evaluation of secondary metabolites is vital for identifying the concentration of bioactive substances in plant extracts, which are responsible for their therapeutic properties. In this study, standard procedures were employed to assess the phenolic and flavonoid content water ethanol extract of *Dactylorhiza hatagirea* roots and *Curcuma angustifolia* rhizomes[19-20].

Determination of total phenolic Substances

To determine the TPC of hydro-ethanolic extracts from *Dactylorhiza hatagirea* roots and *Curcuma angustifolia* rhizomes, the method by Olufunmiso et al. was employed. In this method, A volume of 2 ml from each extract was mixed with 1 ml of Folin- Ciocaltu reagent(7.5g/L) and 1 ml sodium carbonate. This mixture was kept at ambient temperature for 15 minutes, allowing to form blue coloration. The absorbance of this solution was measured at 765 wavelengths in ultraviolet visible spectrophotometer. To find the phenolic content concentration a standard calibration curve was plotted[21-22].

Determination of total flavonoid substances

The TFC was determined by method given by Olufunmiso et al. 2 ml of each plant extract was blended with 2 % methanolic aluminum chloride. This mixture kept at room temperature for 1 hour. Following incubation, absorbance was recorded at 420 nm using Uv-Visible spectrophotometer. TFC was determined by comparing the values to a standard quercetin curve in milligrams of quercetin equivalents in mg/100 mg of plant extract[23].

Determination of Tannin substances

To determine the tannin content in the hydroethanolic extracts of roots of *Dactylorhiza hatagirea* and rhizomes of *Curcuma angustifolia*, the vanillin-HCl colorimetric method was employed. Accurately weighed amounts of the dried plant extracts were dissolved in a known volume of 70% ethanol to obtain a stock solution. 1 ml of plant extract was merged with vanillin reagent, vortexed and incubated for 20 minutes at room temperature for color development. Characteristic reddish color formed due to the reaction between condensed tannins and vanillin in an acidic medium was recorded using UV-visible spectrophotometer at 510nm. A blank solution was prepared in the same manner but without adding plant extract to eliminate background interference. All samples were analyzed in triplicate to ensure precision and accuracy[24].

Animals

Six Male Wistar rats weighed between 150 to 250 grams were selected for study in controlled environment with 12 hr. light/dark cycle at 25°C temperature and humidity range in between 50-60%. The short-term toxicity of the hydroethanolic extracts of roots of *Dactylorhiza hatagirea* and rhizomes of *Curcuma angustifolia* was assessed following the OECD Guidelines 423 for testing acute oral toxicity. Animals were kept on 4 hrs. of fasting period before experiment, though they had free access to water during the fasting period. In line with the OECD guidelines, the initial dose was selected to be the highest amount likely to cause mortality in some test animals. When no prior data is available on the substance, the starting dose is chosen from the fixed levels of 50, 100, 500, 1000mg per kg body weight. The acute toxicity was evaluated based on the standard procedure as outlined in earlier studies.

Investigation of Anti-inflammatory Properties using in-vivo model in Carrageenan-Induced Edema

0.1 ml of 1% carrageenan induced to the left paw of each rat to induce edema. Rats were categorized into groups with six rats in each group.

Table 1: Group distribution for Carrageenan induced paw edema study

Group	Treatment
I	Carrageenan control (no treatment)
II	Hydroalcoholic extract of *Dactylorhiza hatagirea* (100 mg/kg, orally)
III	Hydroalcoholic extract of *Dactylorhiza hatagirea* (200 mg/kg, orally)
IV	Hydroalcoholic extract of *Curcuma angustifolia* (100 mg/kg, orally)
V	Hydroalcoholic extract of *Curcuma angustifolia* (200 mg/kg, orally)
VI	Diclofenac sodium (10 mg/kg, orally) as standard reference

Paw thickness was recorded before and after carrageenan injection at 1,2, 3, 4 hours of injection using Vernier caliper. The anti-inflammatory response was assessed by calculating change in reduction in edema in treated groups with carrageenan control. The anti-inflammatory effect was eavaluated by measuring the percentage reduction in edema compared to carrageenan control group[25].

Following formula is used to calculate % inhibition in edema:

$$\% \text{ Inhibition} = T_o - T_t \quad X\ 100 / T_o$$

Where,

Where Tt is the rat paw thickness treated with the test extract at the corresponding time point, and To refers to control group rat's paw thickness at the same time

Investigation of Anti-inflammatory Properties using in-vitro model using Protein denaturation assay

The invitro inflammatory response of the plant extract was assessed using the protein denaturation assay, based on the principle that protein denaturation is well established cause to inflammation and compounds that inhibit protein denaturation may exhibit anti-inflammatory potential. The assay was performed using Bovine serum albumin (BSA) as the test protein. A control sample without the extract and a standard sample containing diclofenac sodium (or aspirin) at equivalent concentrations were included for comparison. All experiments are done in triplicate and protein denaturation's percentage inhibition was measured using following formula:

$$\text{Inhibition } (\%) = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

The invitro inflammatory activity of root system of *Dactylorhiza hatagirea* extract and rhizomes of *Curcuma angustifolia* extract was assessed using the protein denaturation assay, a well-established method based on the inhibition of thermally induced protein denaturation. The reaction mixture contained 0.45 ml of 5% BSA solution and 0.05 ml of plant extract in distilled water or DMSO at different concentrations (50,100,200,400 mg/ml). the pH was set at 6.3 using 1 N HCL. Samples were incubated at 37°C for 20 minutes, heated at 70°C FOR 5 minutes to induce denaturation, cooled and turbidity was measured at 660 nm using UV Visible spectrophotometer. Control samples and standard drug samples containing diclofenac sodium at similar concentrations were prepared and processed under the same conditions[26].

Statistical evaluation and Herbal Tablet Formulation

The findings were presented in mean and standard deviation. For statistical evaluation one way ANOVA test is done, followed by Dunnett's post hoc test for pairwise comparison. A p-value below 0.05 was considered statistically significant with all results compared against control group. Herbal tablets formulated with the active extracts were produced using the dry granulation technique. The powdered extracts were mixed with other excipients and thoroughly blended before undergoing granulation. The resulting granule mixture was then compressed into tablet using an 8-station tablet compression machine. The herbal tablets were assessed according to various parameters outlined in the IP.

Table 2: Contents for tablet formulation

Ingredient	Quantity Per Tablet (mg)
Dactylorhiza hatagirea	150
Curcuma angustifolia	150
Talc	100
MCC	50
Magnesium Stearate	10

3. RESULTS AND DISCUSSION

The preliminary phytochemical evaluation of Dactylorhiza hatagirea and Curcuma angustifolia identified the presence of multiple biologically active compounds. Both plant extracts were found to contain flavonoids, phenolics, carbohydrates, and saponins, which are associated with antioxidant, anti-inflammatory, and potential medicinal benefits. These observations

indicate that the extracts might be effective in managing health conditions related to oxidative stress and inflammation. In contrast, both plants tested negative for alkaloids, glycosides, amino acids, proteins, and oils, indicating a lack of these specific compounds.

Notably, *Curcuma angustifolia* showed the presence of diterpenes, which are linked to anti-inflammatory and anticancer activities, while *Dactylorhiza hatagirea* did not. These results highlight the differing phytochemical profiles of the two plants, with *Curcuma angustifolia* potentially offering more diverse therapeutic properties because presence of diterpenes. Overall, the plant-based metabolites present in both plants support their traditional use and potential as sources for developing natural therapeutic agents.

Estimation of total phenolic and flavonoid composition

The TPC of the plant extract was determined using gallic acid as standard. The hydroalcoholic extracts phenolic concentration was calculated using standard calibration curve, which followed the linear regression equation:

Y=0.0204X+0.0015, R2 =0.9998

Where Y represents the absorbance and X is the concentration of phenolic compounds, expressed as milligrams of gallic acid equivalent (GAE) per 100 mg of dry extract.

Similarly, the total flavonoid content was determined using quercetin as the standard. The TFC was derived by the quercetin calibration curve with the regression equation:

Y= 0.0133X + 0.0015, R2= 0.999

Here, Y represents the absorbance and X is the flavonoid content, expressed as mg quercetin equivalent (QE) per 100 mg of dry extract.

The high R^2 values (0.9998) indicate excellent linearity of the calibration curves, confirming the reliability of the analytical method. The detailed results of TPC and TFC for *Dactylorhiza hatagirea* and *Curcuma angustifolia* are provided in Table 2, and presented in graphical format in fig. 1 and fig. 2

Estimation of Tannin content

Based on the absorbance value obtained from the hydro-ethanolic plant extract, the tannin content was estimated using the standard calibration curve equation Y = 0.012X + 0.05, where Y represents the absorbance and X is the concentration of tannins, expressed in mg/mL. The measured absorbance of the extract was 0.204. Substituting this value into the equation and solving for X, we get:

0.204 = 0.012X + 0.05.

Rearranging the equation,

X = (0.204 − 0.05) / 0.012 = 12.83 mg/mL.

Thus, the tannin content in the tested extract was calculated to be 12.83 mg/mL, expressed as tannic acid equivalents. This value reflects the amount of tannins present in the extract solution and can be further used to compute the total tannin content per gram of dried extract, depending on the dilution factor and sample preparation.

This value, expressed as tannic acid equivalent (TAE), represents the tannin concentration in the tested plant extract solution. The final tannin content was then converted to mg/g of extract based on the dilution factor and sample weight.

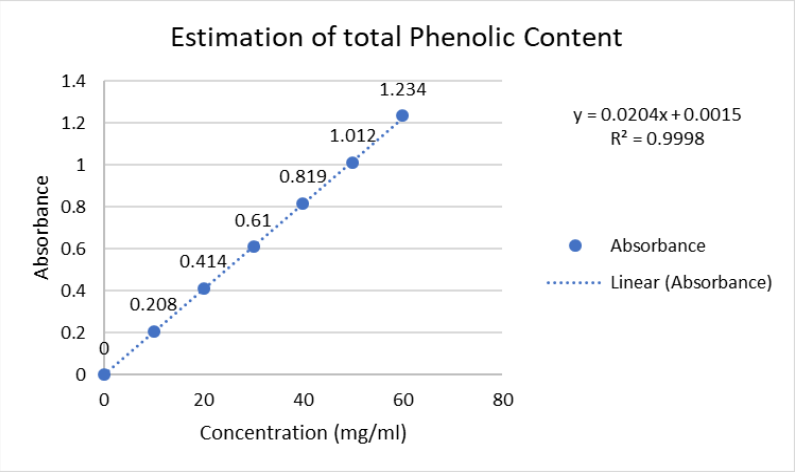

Figure 1: Total phenolic content estimation

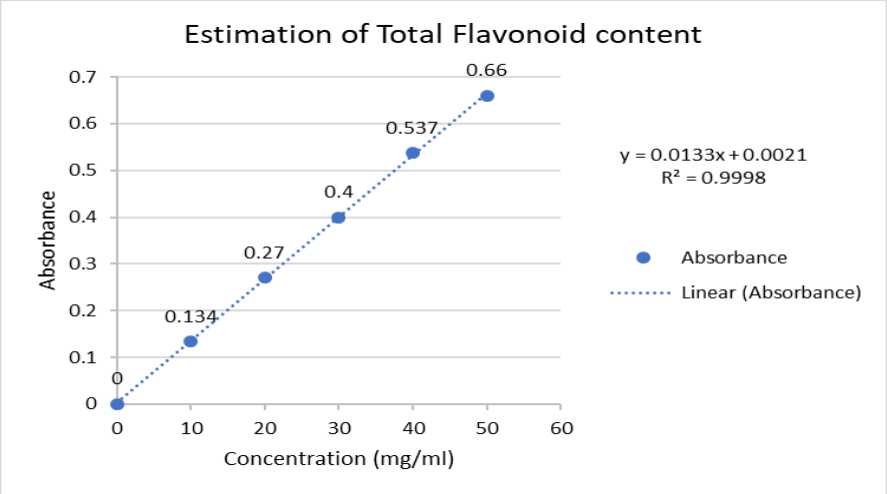

Figure 2: Total flavonoid content estimation

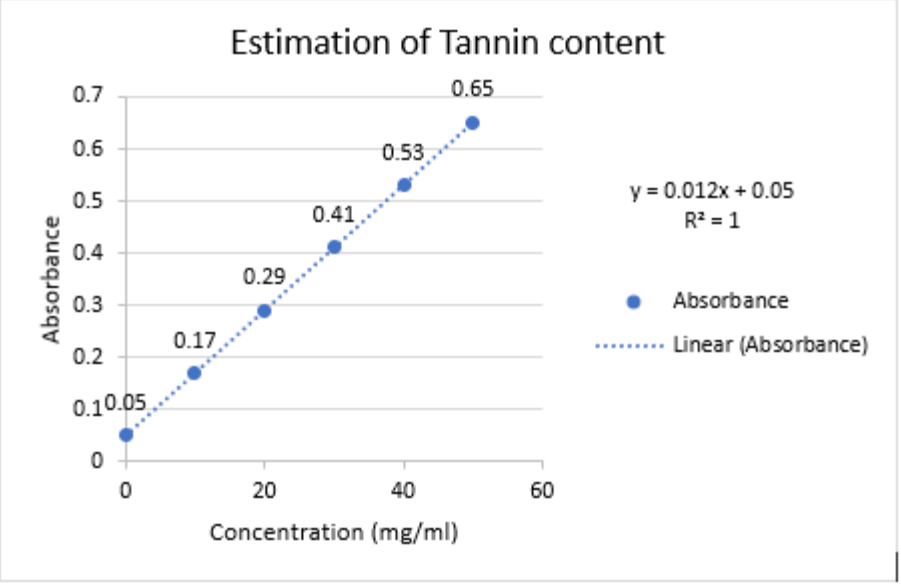

Figure 3: Estimation of Tannin content

Table 3: Result of total phenol and flavonoid composition

Phytochemical	**Absorbance Value**	**Content**
Total phenolic content (TPC)	0.435	21.25 mg of GAE/ 100 mg extract
Total Flavonoid content (TFC)	0.452	33.82 mg Quercetin equivalents /100 mg extract
Total Tannin content	0.204	12.83 mg/ml TAE / 100 mg extract

S. No.	Treatment Group	Dose (mg/kg, p.o.)	% Inhibition at 1 hr	2 hr	3 hr	4 hr
1	Carrageenan Control	—	0%	0%	0%	0%
2	Hydroalcoholic extract *Dactylorhiza hatagirea* (100 mg/kg)	100	18.30%	29.60%	35.80%	42.10%
3	Hydroalcoholic extract *Dactylorhiza hatagirea* (200 mg/kg)	200	24.80%	38.20%	45.50%	52.90%
4	Hydroalcoholic extract *Curcuma angustifolia* (100 mg/kg)	100	20.50%	31.40%	39.20%	46.70%
5	Hydroalcoholic extract *Curcuma angustifolia* (200 mg/kg)	200	27.20%	40.70%	48.90%	55.30%
6	Diclofenac Sodium (Standard Drug)	10	32.10%	49.30%	58.70%	66.80%

The hydroalcoholic extracts of both plant system indicate effective anti-inflammatory response in carrageenan induced paw model. At oral doses of 100 and 200 mg/kg, both extracts reduced paw swelling in a manner dependent on both the dose and time. After 4 hours, D. hatagirea showed 42.1% and 52.9% inhibition, while C. angustifolia demonstrated 46.7% and 55.3% inhibition at with respective doses of 100mg/kg and 200mg/kg. The Carrageenan control, which received only the inflammatory agent without any treatment, showed 0% inhibition, serving as the baseline for comparison. In contrast, the standard drug shows **66.8%** of inhibition at 4 hours. These results suggest both plant extracts possess promising anti-inflammatory potential. Results of developed tablet formulation were depicted in table 4.

Table 5: Table 5: *Dactylorhiza hatagirea* Root Extract and *Curcuma angustifolia* Rhizome Extract's invitro anti-inflammatory activity Based on Protein Denaturation Assay

Concentration (mg/mL)	*Dactylorhiza hatagirea* (%)	*Curcuma angustifolia (%)*	Diclofenac Sodium (%)
50	32.5 ± 1.8	28.4 ± 2.1	44.2 ± 1.5
100	49.7 ± 2.3	43.6 ± 2.0	62.5 ± 1.8
200	64.9 ± 2.6	58.3 ± 2.5	76.8 ± 2.1
400	78.4 ± 2.5	72.1 ± 2.8	85.6 ± 1.9

The results of the protein denaturation assay demonstrated that both *plant* extracts showed inhibition of protein denaturation that increased with concentration, indicative of anti-inflammatory activity. At the highest concentration tested (400 µg/mL), *Dactylorhiza hatagirea* extract showed a maximum inhibition of 78.4 ± 2.5%, while *Curcuma angustifolia* extract exhibited 72.1 ± 2.8% inhibition. In comparison, the standard drug diclofenac sodium produced 85.6 ± 1.9% inhibition at the same concentration. The IC_{50} values were observed to be 145.3 mg/ml for *Dactylorhiza hatagirea* and 167.8 mg/ml for *Curcuma angustifolia*, compared to 92.5 µm/mL for diclofenac sodium. Statistical analysis revealed that both extracts showed significant inhibition ($p < 0.05$) at concentrations ≥100 mg/mL when relative to control group.

The protein denaturation assay result showed that both *Dactylorhiza hatagirea* and *Curcuma angustifolia* possess notable invitro anti-inflammatory activity, likely because of the presence of phytoconstituents flavonoids, phenolic compounds, and tannins that are known to stabilize proteins and inhibit inflammatory processes. The ability of the extracts to prevent thermal denaturation of BSA suggests that they may suppress inflammation by protecting proteins from structural alterations commonly associated with inflammatory conditions. *Dactylorhiza hatagirea* exhibited slightly stronger activity than *Curcuma angustifolia*, which may be attributed to its higher content of secondary metabolites like ferulic acid, β-sitosterol, and flavonoids previously reported in the literature. Although the inhibition was lower than that of the standard drug diclofenac sodium, the extracts still demonstrated substantial activity, supporting their traditional use in inflammatory conditions. These findings warrant further mechanistic studies, including in vivo models and molecular docking approaches, to better understand their anti-inflammatory potential and active constituents.

Table 6: Assessment characteristics of developed herbal formulation

Sr. no.	Evaluation parameter	Result
1	Weight variation	±1.8%
2	Hardness	5.1 kg/cm²
3	Tablet friability	0.65%
4	Breakdown time (DT)	12 minutes
5	Thickness	4.6 mm
6	Diameter	8.9 mm
7	Moisture Content (LOD)	2.85%
8	Drug Content Uniformity	93.2% (DH), 95.8% (CA)
9	Wetting Time	1.8 minutes
10	pH of Tablet Dispersion (1% sol.)	6.5
11	In Vitro Dissolution (30 min)	85.7% (DH), 89.9% (CA)

4. CONCLUSION

The current study highlighted the notable anti-inflammatory effects of the hydroalcoholic extracts of *Dactylorhiza hatagirea* and *Curcuma angustifolia*, supported by both phytochemical profiling and pharmacological testing. Preliminary screening identified flavonoids, phenolics, carbohydrates, and saponins in both extracts, all of which are linked to antioxidant and anti-inflammatory properties. Additionally, C. angustifolia was found to contain diterpenes—bioactive compounds recognized for their anti-inflammatory and anticancer benefits—suggesting its therapeutic potential may be broader than that of D. hatagirea. The quantitative analysis showed significant amounts of TPC and TFC with D. hatagirea yielding 21.25 mg GAE/100 mg and 33.82 mg QE/100 mg, respectively and tannin content 12.38 TAE/100 mg plant extract. These bioactive compounds were closely associated with the observed anti-inflammatory response where both extracts effectively reduced inflammation in a dose-dependent fashion. At a 200 mg/kg dose, C. angustifolia achieved a higher inhibition rate (55.3%) compared to D. hatagirea (52.9%), with both extracts showing comparable effects to the standard drug. *Both* Dactylorhiza hatagirea *and* Curcuma angustifolia *demonstrated significant in vitro anti-inflammatory activity, with Dactylorhiza hatagirea showing stronger inhibition of protein denaturation, supporting their potential as natural anti-inflammatory agents*

In addition to pharmacological efficacy, the herbal tablets formulated from these extracts demonstrated excellent physical characteristics. Evaluation parameters such as weight variation (±1.8%), hardness (5.1 kg/cm²), friability (0.65%), and disintegration time (12 minutes) met pharmacopeial standards. Further assessments, including thickness, diameter, moisture content, pH of dispersion, and in vitro drug release, confirmed the formulation's quality, stability, and consistency.

Overall, the study validates the cultural use of these plants in inflammatory conditions and supports their potential development into safe, effective, and stable herbal formulations for therapeutic use.

REFERENCES

[1] Lee, Y. S., & Olefsky, J. M. (2021). Chronic tissue inflammation and metabolic disease. Genes & Development, 35(5–6), 307–328.

[2] Medzhitov, R. (2021). Inflammation: The good, the bad, and the ugly. Cell, 184(1), 1–13.

[3] Ridker, P. M., Everett, B. M., Thuren, T., MacFadyen, J. G., Chang, W. H., & Libby, P. (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. New England Journal of Medicine, 377(12), 1119–1131

[4] Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014;4(3):47-55.

[5] Vishvakarma P, Kumari R, Vanmathi SM, Korni RD, Bhattacharya V, Jesudasan RE, Mandal S. Oral delivery of peptide and protein therapeutics: challenges and strategies. Journal of Experimental Zoology India. 2023 Jul 1;26(2).

[6] Desplats, P., Gutierrez, A. M., Antonelli, M. C., & Frasch, M. G. (2019). Microglial memory of early life stress and inflammation: Susceptibility to neurodegeneration in adulthood. Frontiers in Neuroscience, 13, 1–13.

[7] Vane JR, Botting RM, Anti-inflammatory drugs and their mechanism of action, Inflamm Res, 1998;47:78–87.

[8] Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag NB, Iputo JE, An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation, J Med Plants Res, 2010; 4:789–795.

[9] Vishvakarma P, Kaur J, Chakraborthy G, Vishwakarma DK, Reddy BB, Thanthati P, Aleesha S, Khatoon Y. Nephroprotective Potential of Terminalia Arjuna Against Cadmium-Induced Renal Toxicity By In-Vitro Study. Journal of Experimental Zoology India. 2025 Jan 1;28(1).

[10] Vishvakarma P, Mohapatra L, Kumar NN, Mandal S, Mandal S. An innovative approach on microemulsion: A review. European Chemical Bulletin. 2023;12(4):11710-33.

[11] Li, R., Ye, J. J., Gan, L., Zhang, Z., Zhou, X., & Zheng, J. (2024). Traumatic inflammatory response: Pathophysiological role and clinical value of cytokines. European Journal of Trauma and Emergency Surgery, 50(6), 1313–1330.

[12] Griffin MR, Epidemiology of nonsteroidal anti-inflammatory drugassociated gastrointestinal injury, Am J Med, 1998; 104:23–29.

[13] Bhattarai NK, Some endangered medicinal plants of Nepal. In: Handa SS, Kaul MK (eds). Supplement to Cultivation and Aphrodisiac Activity of Dactylorhiza hatagirea Utilization of Medicinal Plants. Jammu: Regional Research Laboratory, 1996; 676–7.

[14] Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.

[15] Bhavna D, Khatri J, Gentella asiatica: The elixir of life. Inter J Pharmaceutical Res 2011; 2(2):431-438.

[16] Mukherjee PK, Quality Control of Herbal Drugs", 2nd Edition, Business Horizons, 2007; 2-14.

[17] Khandelwal KR, Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn, 2005; 15, 29, 149, 56.

[18] Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, 1994; 112,120.

[19] Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, Peltonen K, Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin, Food and chemical toxicology, 2013; 53:27-32.

[20] Winter CA, Risley EA, Nuss GW, Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs, Proc Soc Exp Biol Med, 1962; 111:544–547.

[21] Hagerman, A. E., & Butler, L. G. (1989). Choosing appropriate methods and standards for assaying tannin. Journal of Chemical Ecology, 15(6), 1795–1810.

[22] Castro-Montoya, J. M., Dickhoefer, U., & Müller, M. (2021). Rapid tannin profiling of tree fodders using untargeted mid-infrared spectroscopy and partial least squares regression. Plant Methods, 17, 1–13.

[23] Chand, M. B., Paudel, M. R., & Pant, B. (2016). The antioxidant activity of selected wild orchids of Nepal. Journal of Medicinal Plants Research, 10(9), 126–133

[24] Jurica, J., & Zovko Končić, M. (2017). Phytochemical profile and pharmacological activities of water and hydroethanolic dry extracts of Calluna vulgaris (L.) Hull. Herb, 62(3), 123–135.

[25] Sharma, S., Jain, P. K., & Parkhe, G. (2020). Extraction, phytochemical screening and anti-inflammatory activity of hydro-ethanolic extract of roots of Dactylorhiza hatagirea. Journal of Drug Delivery and Therapeutics, 10(3-s), 86–90.

[26] Dinis, T. C., *Oliveira*, A. M., *Almeida*, L. M., & *Rebelo*, P. A. (1994). Actions of flavonoids on the inhibition of protein denaturation in an in vitro assay of inflammation. *European Journal of Pharmacology*, *265*(3), 213-218.