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# Exploring the anti-inflammatory abilities of the medicinal plants used by Tharu tribes in India's Dudhwa National Park

**Abhay Kumar Mishra[1,*], Kamran Javed Naquvi[1], Sambit Kumar Parida[2]**
[1]Department of Pharmacy, Institute of Biomedical Education and Research, Mangalayatan University, Aligarh-202146, India
[2] Amity Institute of Pharmacy, Amity University Rajasthan, Jaipur, India
**Corresponding Author:** Abhay Kumar Mishra [1,*]
**Affiliation:** Department of Pharmacy, Institute of Biomedical Education and Research, Mangalayatan University, Aligarh-202146, India
**Email id:** abhaycps05@gmail.com

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**Abstract:**
Inflammation & pain management is done mostly by using narcotic & non-narcotic drugs like opioids, salicylates, corticosteroids etc. These medications have known negative effects and can be harmful. The aim of our present study was to find out inflammation reducing quality of some specific herbs used by tharu tribes living around Dudhwa National Park, India by following *in-vitro* methods. Two specific herbs, i.e. leaves of *Cleome viscosa* (Hurhur) & roots of *Tephrosia purpurea* (Sarphonk) were extracted successively with solvents like pertroleum ether, ethyl acetate and methanol. These plant extracts were evaluated further regarding inflammation reducing capability by following established *in vitro* test models like impediment of denaturation of protein, heat instigated hemolysis supported test for membrane-stabilization and antiprotease activity at different concentrations. The selected plant extracts showed significant inhibition of protein denaturation EETP-63.72 & EECV-61.92 %, at their maximum dose, in comparison to the standard ibuprofen was 67.26 %. Similarly, all extracts between 0.1 to 0.5 mg/mL decreased amount of heat instigated RBC hemolysis to some extent. Maximum inhibitory effects were reported at 0.5 mg/ml concentration (EETP-69.59, EECV-64.78 % comparable to Ibuprofen (72.26%). These plant extracts' substantial levels of flavonoid may be responsible for their excellent anti-inflammatory attributes. The outcome of our current investigation shows that our selected plants are suitable candidates for proceeding for further detailed anti-inflammatory sources as suggested by tribals of Dudhwa National Park.

Keywords: NSAIDs, Anti-inflammatory, protein denaturation, Membrane stabilization, RBC hemolysis

## Introduction:

Because of their ability to effectively reduce pain and inflammation, non-steroidal anti-inflammatory medicines are widely used throughout the world. NSAIDs use is unavoidable due to the increase in musculoskeletal issues, according to data from the 2016 Global Burden of Disease.[1]. No philosophy serves as the foundation for allopathic treatment. just the use of chemicals to interfere with the body's physiological and metabolic processes. In this kind of contemporary therapy system, the patient is not treated as an individual and the treatment is essentially mechanical. As per the WHO estimation, natural medicine continues to be the primary source of healthcare for most people in most countries. Herbal medications do have certain drawbacks like sub-standard labeling as well as quality standards, insufficient patient information and scanty awareness of possible negative effects[2].

Uttar Pradesh's Dudhwa Tiger Reserve is well-known for its wildlife and nature. However, the Tharu community is essential to Dudhwa mythology. This tribe is largely people from east-Asian ancestry having certain non-Mongolian characteristics also[3]. Within the buffer zone of the park, which is in the Terai region on the Indian-Nepal border, they live in 46 villages. Their holistic knowledge, customs, and way of life are well-known. This tribe treats a range of illnesses with ethnomedicines, such as circulatory system problems, respiratory problems, digestive problems, and inflammation[4]. Some of the important plants like *Cleome viscosa* (commonly known as Hurhur) *& Tephrosia purpurea* (commonly known as Sarphonk) are native to India but has become widespread in many tropical and sub- tropical parts of the world. It is used as a medicinal plant and grows as a weed in in hilly roadsides and in open terai grass lands of Dudhwa National Park[5]. These two plants were used widely by tribals in this area to get rid of inflammation. But this claimed activity has not received scientific validation, according to the literature search. We therefore felt it worthwhile to provide a scientific basis for the said activity with a systematic approach.

## Materials & Methods

We have procured all essentials chemicals and all associated compounds/kits (Evan's blue, egg albumin, trypsin, casein etc) from SRL (Mumbai) and Ibuprofen from IOL chemicals & Pharmaceuticals Ltd (Ludhiana).

The selected plants for our proposed research work were identified and authenticated by Taxonomist. The proposed plant parts (Leaves of *Cleome viscosa* & Roots of *Tephrosia purpurea*) were collected from Surma & Kheri village, Kishanpur Range, Dudhwa National Park respectively in the month of July 2023. We have gone for proper proximate analysis after which we go for preparation of exact plant materials for solvent extraction methods. We carried out our extraction process by selecting various solvents according to increasing order of polarity. All the powdered materials of different plant parts were extracted successively by taking solvents according to polarity (i.e. petroleum ether, ethyl acetate & methanol) using Soxhlet apparatus. All the extracts were subjected to preliminary phytochemical analysis[6] after proper encoding as:
**Leaves of *Cleome viscosa***: **-PECV**–Pet. Ether Extract; **EECV**-ethyl acetate extract; **MECV**--

Methanol extract

**Roots of *Tephrosia purpurea*- PETP**–Pet. Ether Extract; **EETP**-ethyl acetate extract; **METP**--Methanol extract

These plant extracts were evaluated further regarding inflammation reducing capability by following established in vitro test models like impediment of denaturation of protein, heat instigated hemolysis supported test for membrane-stabilization and antiprotease activity at different concentrations.

**Protein denaturation retardation**

We have conducted a study for exploration of inflammation reducing capability of extracts of our selected plants by following the *in-vitro* method of denaturation of protein inhibition. In this method, 1ml test solution with various conc$^{n}$ (0.1-0.5 mg/ml) of selected extract mixed to 1 mililitre solution of egg-albumin (1mM with pH-6.8). After fifteen more minutes of incubation at 37°C, this mixture was put in a water bath set at 70°C for 10 minutes to cause denaturation.

Turbidity was measured three times with a spectrophotometer at 660 nm after 10 minutes of cooling in running tap water, and the average was determined[7,8].

The protein denaturation's inhibition percentage was determined by comparing it to the control through the following calculation:

$$\%\ Denaturation\ Inhibition\ = \frac{(Control\ Absorbance - Sample\ Absorbance)}{Control\ Absorbance} \times\ 100$$

At varying quantities, it was discovered that almost every plant was efficient in suppressing the results demonstrating the denaturation of albumin due to the heat. The maximum reduction was detected in all EA parts (EETP-63.72 & EECV-61.92 %, with an $IC_{50}$ of 300.2, 347.5) at their maximum dose, the inhibition level of the standard NSAID medication ibuprofen was 67.26 % at 100 µL, which was higher than that of the one being compared to it.

**Tab. 1: Albumin Denaturation Data (n=3)**

| Conc$^{n}$ (mg/ml) | STD. | EECV | MECV | PECV | EETP | METP | PETP |
|---|---|---|---|---|---|---|---|
| 0 | **0** | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | **67.26** | 34.43 | 15.55 | 13.56 | 36.34 | 17.65 | 14.24 |
| 0.2 | | 40.12 | 20.38 | 17.04 | 41.33 | 21.24 | 19.68 |
| 0.3 | | 47.18 | 29.6 | 20.78 | 49.47 | 31.86 | 22.66 |
| 0.4 | | 54.88 | 37.72 | 24.84 | 56.22 | 39.94 | 27.24 |
| 0.5 | | **61.92** | 41.2 | 28.53 | **59.92** | 36.14 | 26.56 |

**Heat Induced Hemolysis based Membrane Stabilization**

The anti-inflammatory effect of different extracts from chosen plants was further studied by performing an updated version of the RBC membrane stabilization test[9,10]. For this experiment blood from healthy human volunteers (who had not used NSAIDS in the last 14 days) was collected and put in a heparinized centrifuge tube. This sample was centrifuged thrice with NaCl buffer sol$^{n}$ (154mM)

with Na3PO4 buffer sol$^{n}$ (10mM) for 10 min in 3000 RPM.
Plant extract (0.1–0.5 mg/ml) is present in 0.5 ml of stock erythrocytes (RBC) suspended in hypotonic solution (5 ml, 50 mM NaCl) in 10 mM sodium phosphate-buffered saline (pH 7.4). 0.5 cc of RBC suspended alone in a hypotonic buffered saline solution served as the control sample. After 30 minutes of incubation at 370°C, the test mixtures were centrifuged for 20 minutes at 3000 rpm. The Using spectrophotometry, the hemoglobin content of the supernatant solution was measured at 560 nm. Three repetitions of each experiment were made, and the average was determined. The following formula was used to calculate the percentage suppression of hemolysis or membrane stabilization, using ibuprofen (100 mg/ml) as a reference standard.

$$\%\ Inhibition\ of\ haemolysis = \frac{(A1-A2)}{A1} \times\ 100$$

Where, Hypotonic buffered saline solution absorption is represented by A1, while test sample absorption in a hypotonic solution is represented by A2.

The table showed that all extracts between 0.1-0.5 mg/mL decreased the amount of heat- induced RBC hemolysis to a certain degree. Maximum inhibitory effects were reported at 0.5 mg/ml concentration (EETP-69.59, EECV-64.78 % with $IC_{50}$ values of 251.5, 307 respectively) comparable to standard medication Ibuprofen (72.26%) at 0.1 mg/ml conc$^{n}$.

**Table 2 : Various Extracts' Impact on Membrane Stabilizing Activity (n=3)**

| on$^{n}$(mg/ml) | STD. | EECV | MECV | PECV | EETP | METP | PETP |
|---|---|---|---|---|---|---|---|
| 0 | **0** | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | **72.26** | 36.12 | 26.33 | 19.06 | 39.5 | 29.24 | 22.66 |
| 0.2 | | 42.62 | 30.35 | 26.38 | 45.67 | 34.57 | 29.28 |
| 0.3 | | 49.08 | 36.34 | 29.86 | 53.44 | 39 | 34.53 |
| 0.4 | | 57.7 | 41.02 | 37.48 | 61.36 | 46.62 | 40.58 |
| 0.5 | | **64.78** | 48.22 | 44.33 | **69.59** | 51.56 | 46.45 |

**Inhibitory Action of Proteinase**

The experiment was carried out in accordance with a well-accepted procedure[11,12]. 2 milliliters of a reaction mixture was prepared which is composed of 0.06 milligram trypsin, 1 milliliter of Tris-HCl buffer, and 1 milliliter plant extracts with concentrations from 0.1-0.5 mg/ml. This mixture was taken for incubation for 5 minutes under 37-degree Centigrade temperature. After adding 1 mL casein sol$^{n}$ ( 0.8% w/v), this mixture was incubated further for 20 minute. Finally, 2 milliliter perchloric acid was added to this mixture for stopping this process of reaction. The hazy sample centrifuged; supernatant part was taken for absorbance study at 210 nm against blank. We have carried out this study three times.
Proteinase inhibitory activity was determined as percentage of calculation.

$$\%\ Denaturation\ Inhibition\ = \frac{(Control\ Absorbance - Sam\quad Absorbance)}{Control\ Absorbance} \times\ 100$$

All ethyl acetate parts shown remarkable anti-proteinase result. These extracts showed maximum inhibition. The best results of inhibition were observed at 0.5 mg/mL conc$^{n}$ using all EA extracts (EETP-59.59 % & EECV-54.55% with $IC_{50}$ value of 381.5 & 437.3) which was near- about like Ibuprofen (63.33 %).

**Tab. 8. 3- Proteinase Inhibitory result using all plant samples (n=3)**

| Conⁿ(mg/ml) | STD. | EECV | MECV | PECV | EETP | METP | PETP |
|---|---|---|---|---|---|---|---|
| | - | 0 | 0 | 0 | 0 | 0 | 0 |
| .1 | **63.33** | 26.45 | 16.12 | 9.06 | 29.51 | 19.21 | 12.15 |
| .2 | - | 32.62 | 20.35 | 16.27 | 35.43 | 24.5 | 19.28 |
| .3 | - | 39.18 | 26.22 | 19.33 | 43.18 | 29.87 | 24.34 |
| .4 | - | 47.36 | 31.72 | 27.48 | 51.36 | 36.54 | 30.34 |
| .5 | - | **54.55** | 38.43 | 34.12 | 59.59 | 41.13 | 36.82 |

## CONCLUSION

There was not a single one of the test samples that did not create responses that were really intriguing. It was noticed that these selected plants are good alternatives for the search of best anti-inflammatory options from natural source in India. According to the observed $IC_{50}$ result, ethyl acetate extracts of the two chosen plants exhibit potent anti-inflammatory properties that are on par with the reference drug i.e. ibuprofen. It can be strongly advocated that due to the presence of specific secondary metabolites like tannins, alkaloid, flavonoids; we could be able to get this positive result as targeted. Thus, based on the current findings of our study, it may be concluded that *Tephrosia purpurea* and *Cleome viscosa* have potent inflammation reducing properties. To determine the mechanism and specific component causing the anti-inflammatory action, more research will be conducted by us in future.

## References

1. Bindu S, Mazumder S, Bandopadhyay U. 2020. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochemical Pharmacology. 180, 114147.
2. Anonymous, 2023. Traditional medicine. World Health Organization.
3. Singh LR, The Tarai region of UP A study in human geography, Ram Narain Lal Beni Prasad, Allahabad, India, 1965.
4. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
5. Khanna KK. 2018. Angiospermic Plants of Terai region, Uttar Pradesh, India. Biobulletin, 4(2), 26-102.
6. Odebiyi, A, Sofowora AE. 1999. Phytochemical screenings of Nigerian medicinal plants part. Lyodia, 44, 234-246.
7. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
8. 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.

9. Samina Y, Arkajyoti P, Tarannum N, ABM. Atiqur R, Sarkar Farhana A, MII Wahed, Talha Bin E, Shafayet Ahmed S. 2020. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). International Journal of Phytomedicine and Phytotherapy. 6, 59.
10. Douglas BA, Daniels K, Isaac TH, Martins E. 2021. Protective Effect of Bergapten against Human Erythrocyte Hemolysis and Protein Denaturation In Vitro. International Journal of Inflammation, Article ID 1279359, 1-7.
11. Assiry AA, Bhavikatti SK, Althobaiti FA, Mohamed RN, Karobari MI. 2022. Evaluation of In Vitro Antiprotease Activity of Selected Traditional Medicinal Herbs in Dentistry and Its In Silico PASS Prediction. Biochemistry Research International, Article ID 5870443, 1-8.
12. Godswill NA, Azeezat AO,Babafemi O. 2019. Evaluation of anti-inflammatory activity of Justicia secunda Vahl leaf extract using in vitro and in vivo inflammation models. International Journal of Phytomedicine and Phytotherapy, 5, 49.