



Received on 27 December 2022; received in revised form, 11 April 2023; accepted 30 May 2023; published 01 August 2023

PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY OF *DENDROCNIDE SINUATA* (BLUME) LEAF EXTRACT

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Keywords:

Dendrocnide sinuata (Blume), Anti-inflammatory, Diclofenac, HRBC, Phytochemicals, Assam

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ABSTRACT: *Dendrocnide sinuata* (Blume) has been widely used in the treatment of various diseases. The plant *Dendrocnide sinuata* (Blume) belonging to the family Urticaceae. Many indigenous peoples used this herb to treat swelling, fever, and urinary disorders. The karbis tribes also used it as a hypersensitivity therapy. The Bodo people of Assam gather the flowers of *Dendrocnide sinuata* (blume) and use them as a portion of food with fish. The plant extract was subjected to a preliminary phytochemical analysis, which revealed the presence of carbohydrate, tannin, flavanoid, phenol, terpenoids, cardiac glycoside, alkaloid. As part of the investigation on the mechanism of the anti-inflammation activity, plant extract's ability to stabilize human red blood cells membrane was studied. It was effective in the stabilization of the human red blood cells membrane. Maximum stabilization of 65.03% was observed at 150µg/ml. Diclofenac, a standard anti-inflammation drug, showed a maximum stabilization of 54.08% at 150 µg/ml concentration compared with control.

INTRODUCTION:

Inflammation: It can be defined as the reaction of the body's immune system to an irritant. A foreign object, such as a splinter in the finger, or a germ, could be the irritant. The key features of inflammation are redness, warmth, swelling & pain. Now a day's it is understood that inflammation is a non-specific immunological response ¹.

This results in the release of various substances known as inflammatory mediators. These include the hormones histamine and bradykinin. They dilate the tissue's small blood vessels, permitting more blood to reach the damaged tissue. Because of this, the inflamed portions become red and hot to the touch.

The two main mechanisms for the metabolism of arachidonic acid are the cyclooxygenase (COX) pathway which produces thromboxane and prostaglandins (PGs), and the 5-lipoxygenase pathway, which produces 5S-hydroxy-6E, 8Z, 11Z, and 14Z-eicosatetraenoic acid (5-HETE) and leukotrienes ². Until the end of the nineteenth century, inflammation was considered an

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.14(8).4124-27</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(8).4124-27</p>
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unfavorable reaction that was bad for the host, even though it was formerly understood to be a necessary component of the healing process³.

Signs of Inflammation: The different signs of inflammations are: redness (rubor), swelling (tumour), heat (calor; only applicable to the body extremities), pain (dolor), and loss of function (functiolaesa).

Types of Inflammation: Acute and chronic inflammations are the two phases of inflammation. The complex processes of acute and chronic inflammation are carried on by various chemical messengers, including prostaglandins, platelet-activating factor, leukotrienes. Anti-inflammatory medicines work by preventing the release of the aforesaid inflammatory mediators.

Acute Inflammation: When vascular permeability increases, cells infiltrate the tissue, causing oedema to form from the extravasation of fluid and proteins, and leukocytes accumulate temporarily at the site of the inflammation, this is termed an acute inflammation⁴.

Chronic Inflammation: Chronic inflammation occurs when there is an insufficient acute response, preventing the release of pro-inflammatory agents. Fibroblasts multiply, neutrophils infiltrate and fluid exudes as a consequence of chronic inflammation. Proliferative cells that can either spread or form granulomas are what cause it to happen. Recurrent tissue injury and chronic inflammation can also happen when there is a persistent antigen or infection⁵.

Macrophages play a key role in managing a variety of immunopathological phenomena, including the excess production of inflammatory mediators and proinflammatory cytokines produced by activated COX-2 and iNOS, in chronic (or acute) inflammatory processes. Chronic (or acute) inflammation is a variety of processes mediated by activating immune or inflammatory cells.

Through adhesion molecule activation signal, immune cells are prompted to migrate to inflamed tissue during inflammatory conditions, which ultimately leads to heterotypic cell clustering involving endothelial cells, inflamed cells and immune cells. Chronic inflammation may result

from the complication of acute inflammation or due to the primary condition of slow onset⁶.

MATERIALS AND METHOD:

Literature Survey: Based on the literature survey, traditionally *Dendrocnide sinuata* (Blume) plants are used as an anti-inflammatory. Many tribal peoples used this plant for fever, urinary disorders, and swelling. It is also used for the treatment of hypersensitivity by the karbis communities. Flowers of *Dendrocnide sinuata* (Blume) are collected and used as vegetable with fishes, consider as medicinal by Bodo tribes of Assam.

Collection & Authentication of Plant Material:

The *Dendrocnide sinuata* (Blume) plant was collected from Changsari, Kamrup, Assam, India. Herbarium of *Dendrocnide sinuata* (Blume) was identified by its vernacular name and authenticated at Assam Bio-Resources Centre, Madan Kamdev, Kamrup, Assam; under Assam Science Technology and Environment Council, Bigyan Bhawan, Govt. Of Assam; Vide Accession no. ABRC/1033/21.

Extraction of Crude Drug: The whole *Dendrocnide sinuata* (Blume) plant was shaded and dried, and then these are made into coarsely powdered form using a dry grinder. The dry powder of the plant (50gm.) was packed in the Soxhlet apparatus and continuously extracted with ethanol till complete extraction. The extraction process continued for 72 hours. The solvent was eliminated by distillation; once the extraction process was complete, the concentrated extract was dried under reduced pressure using a rotary evaporator at a temperature not exceeding 40°C and then given moderate heating on a water bath. The yield of the extract was found to be 15 % w/w. The extract was reddish brown in colour and sticky in nature⁷.

Phytochemical Screening of the Ethanolic

Extract: All the phytochemical evaluation of the plant extract for alkaloids, carbohydrates, reducing sugar, flavonoids, saponins, cardiac glycosides, phytosterols, terpenes, phenols, proteins and amino acids, tannins, and steroids were determined by using the standard procedure^{8,9}.

Determination Anti-Inflammatory Activity:

Human Red Blood Cell Membrane Stabilization Assay (HRBC): First, venous blood was drawn

from a healthy adult female who had not used any anti-inflammatory or contraceptive medication in the two weeks before the sample's collection. Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water) was combined with an equal volume of blood. After centrifuging the mixture at 3000 rpm for 10 minutes, the supernatant was removed, and the packed cells were then washed three times with an isosaline solution (0.9% pH 7.2). The assay mixture was made by combining 1 milliliter of phosphate buffer (pH 7.4), 2 milliliters of hyposaline solution (0.36%) and 0.5 milliliters of HRBC suspension (10% v/v) with 1 milliliter of each plant extract of different concentrations (50, 100, or 150 µg/ml) or the reference drug diclofenac sodium (50, 100, or 150 µg/ml), as appropriate. Instead of using plant samples for the reaction mixture, distilled water was utilized as the control, and phosphate buffer served as the blank. After 30 minutes of incubation at 37° C, the mixture was centrifuged at 3000 rpm. At 560 nm, the amount of hemoglobin in the supernatant solution was calculated spectrophotometrically. The amount of hemolysis that would be produced in the presence of distilled water would be 100%. The formula was used to determine the percentage of stability of the HRBC membrane¹⁰.

Percentage of stabilization: $\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$

RESULTS & DISCUSSION:

Preliminary Phytochemical Analysis: *Dendrocnide sinuata* (Blume) quantitative phytochemical analysis is shown in **Table 1**. The plant extract was subjected to a preliminary phytochemical analysis, which revealed the presence of carbohydrate, tannin, flavanoid, phenol, terpenoids, cardiac glycoside, alkaloid.

TABLE 1: LIST OF PHYTOCHEMICALS PRESENT IN THE ETHANOLIC EXTRACT OF DENDROCNIDE SINUTA (BLUME)

Sl. no.	Phytochemical groups	Ethanollic Extract
1	Alkaloids	++
2	Carbohydrates	+
3	Cardiac glycosides	++
4	Flavanoids	++
5	Phenols	++
6	Terpenoids	+
7	Tannins	++

*The sign “-” means absent and “+” means present; higher the number of “+” higher is the concentration of phytochemicals.

In-vitro Anti-Inflammatory Activity: Human Red Blood Cells Membrane Stabilization Assay (HRBC):

TABLE 2: STABILIZATION OF HUMAN RED BLOOD CELLS MEMBRANE

Name of the sample	Concentration (µg/ml)	% stabilization (Mean ± SEM)
Test	50	39.78 ± 0.0112
	100	46.07 ± 0.0143
	150	65.03 ± 0.0231
Standard	50	34.31 ± 0.0051
	100	47.45 ± 0.0375
	150	54.08 ± 0.0478

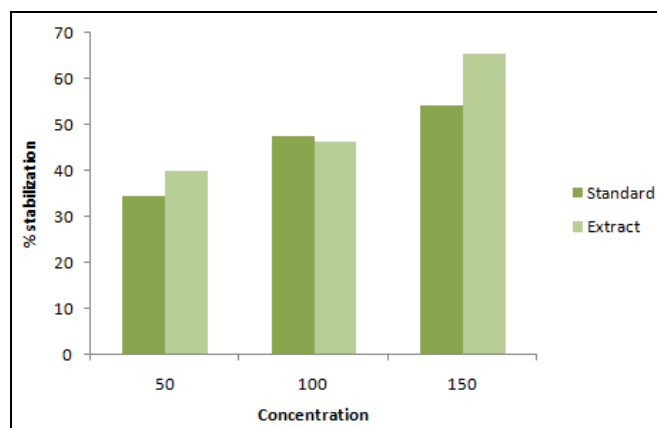


FIG. 1: STABILIZATION OF HUMAN RED BLOOD CELLS MEMBRANE

CONCLUSION: The leaf of the *Dendrocnide sinuata* (Blume) was collected and analyzed as per various standardization parameters. Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the drug. The tests were performed using ethanolic extracts. Phytochemical test revealed the presence of carbohydrate, tannin, flavonoid, phenol, terpenoids, cardiac glycoside, and alkaloid.

As part of the investigation on the mechanism of the anti-inflammation activity, plant extract's ability to stabilize human red blood cells membrane was studied. It was effective in the stabilization of the human red blood cell membrane. Maximum stabilization 65.03% was observed at 150 µg/ml. Diclofenac, a standard anti-inflammation drug, showed a maximum stabilization 54.08% at the concentration 150 µg/ml compared with the control.

ACKNOWLEDGEMENT: This work was supported by NETES Institute of Pharmaceutical Sciences, Mirza, Kamrup, Assam.

CONFLICTS OF INTEREST: The author declares no conflict of interest.

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How to cite this article:

Borah R, Dutta KN, Nath L, Lahkar M and Phukan S: Phytochemical analysis and *in-vitro* assessment of anti inflammatory activity of *Dendrocnide sinuata* (blume) leaf extract. *Int J Pharm Sci & Res* 2023; 14(8): 4124-27. doi: 10.13040/IJPSR.0975-8232.14(8).4124-27.

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