Research Article

An Evaluation of *In vitro* anti-inflammatory Activity of Ethnomedicinal Plants *Dendrocnide sinuata* (Blume) Chew and *Chenopodium ambrosioides* (L.)

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ABSTRACT

Traditionally *Dendrocnide sinuata* and *Chenopodium ambrosioides* are used by Assamese people to treat inflammation related ailments. The aim of the present study was to investigate and validate the *in-vitro* anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides.* The anti-inflammatory property was studied by Human red blood cell (HRBC) membrane stabilization method. The phytochemicals were analyzed according to the techniques given elsewhere. Both the plant extracts were found to have membrane stabilizing activity with *Chenopodium ambrosioides* showing higher efficacy than *Dendrocnide sinuata* as compared to standard drug diclofenac. The activity of the extracts was dose-dependent and highest at 5,000 µg/ml for both the plant extracts. The phytochemical analysis showed the presence of alkaloids, flavonoids, triterpenes, saponins, cardiac glycosides, resins. The study shows the anti-inflammatory efficacy of both the plants.

Keywords: Inflammation, diclofenac, HRBC, *Dendrocnide sinuata, Chenopodium ambrosioides.*

INTRODUCTION

Inflammation is a non-specific defence of the body in respond to tissue malfunction and is a property of both innate and adaptive immune system to keep in check any pathogenic intruders.[1] However, chronic inflammation leads to autoimmune diseases and cancer.[2,3] The anti-inflammatory drugs to treat ailments are known to come with a lot of side effects like mucosal damage, bleeding, renal failure etc.[4] The side effects, cost of medication, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of plants as medicines.[5] Phytoconstituents of plants like flavonoids and triterpenoids are potent anti-inflammatory agents.[6] Therefore, one of the latest trends in biological research is to scientifically extract these phytoconstituents that may be a prospect for drug development in near future.[7]

Assam, one of the states of North East India has 200 plants documented and scientifically validated to have medicinal property.[8,9,10] Recently, the research and documentation of traditional knowledge of North Eastern states have increased, but looking at the vast source of indigenous knowledge, still there is dearth of study.[11] Many of the ethno medicinal plants have been investigated for validation of their anti-inflammatory property by *in vivo* and *in vitro* methods. Plants such as *Balanites aegyptiaca*,[12] *Ipomoea staphylina*,[13] *Mikania micrantha*,[14] *Ficus hispida*,[15] *Mikania glomerata* and *Mikania laevigata* [16] was found to have anti-inflammatory property by *in vivo* carrageenan induced rat-paw oedema method. On the other hand, by *in-vitro* human RBC membrane stabilisation method plants such as *Cassia occidentalis*,[17] *Centella asiatica*,[6] *Gendarussa vulgaris*,[18] *Erioglossum rubiginosum*,[19] *Centratherum punctatum*,[20] were found to have anti-inflammatory property. In our previous study two ethnomedicinal plants of Sikkim *Viscum articulatum* and *Acorus calamus* were observed to have anti-inflammatory property by human RBC membrane stabilization method.[7]

Dendrocnide sinuata is used by various ethnic communities of North East and has been found to have anti-microbial activity.[21] Both *Dendrocnide sinuata* and *Chenopodium ambrosioides* have been explored for anti-inflammatory property by carrageenan induced rat-paw oedema method.[22,23] However, both these plants have not been explored in *in-vivo* models. Even though *Chenopodium ambrosioides* have been investigated previously by TrivellatoGrassi et al. (2013)[22] from Nigeria but it has not been investigated from Assam and it is expected that phytoconstituents of a plant species may vary considerably according to its geographical location which are involved in anti-inflammatory property.[24] Therefore, the present study was undertaken to investigate the anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides* from Assam by human RBC membrane stabilization method. The assay is useful for studying anti-inflammatory property as the RBC membrane resembles the lysosomal membrane[25] and rupture of RBC membrane resemble the burst of the lysosomes in inflammatory reaction.[26]

MATERIALS AND METHODS

Plant collection and processing

Leaves of *Dendrocnide sinuata* and *Chenopodium ambrosioides* were collected from the Sivasagar district of upper Assam during the month of December 2017 and January 2018. The cleaned leaves were dried in the shed

for a period of two weeks. The leaves were then ground to powder and brought to the laboratory of the Department of Zoology, Sikkim University.

Extract Preparation

The plant extract was prepared in Soxhlet apparatus by mixing 10gm powdered plant materials in 400 ml of methanol. The extract was then evaporated using a rotary evaporator. Finally, a thick sticky plant extract was obtained which was then stored at - 20 ˚C until use.

Preparation of stock solutions

Stock solutions of plants and the standard drug diclofenac were prepared by adding 1ml. of water to 80 mg of plant extracts, constituting the solution to be of 80 mg/ml. This solution was further diluted to make 10 mg/ml concentration which was further used to make appropriate dose with specific dilution.

Preparation of Human Red Blood Cell (HRBC) suspension

Fresh 5 ml. blood sample was collected from the investigator who was free from a nonsteroidal anti-inflammatory drug (NSAIDS) for two weeks. The collected blood was mixed with an equal volume of sterilized Alsever's solution. The blood was centrifuged at 3000 revolutions per minute (rpm) for 10 minutes and the packed cells were measured and reconstituted as 10% v/v suspension with isosaline.

Hypotonicity induced haemolysis

The concentrations of 1000, 2000, 3000, 4000, and 5000 µg/ml of plant extract and standard drug were prepared in a test tube using distilled water. To each concentrations 2ml hyposaline, 1ml of PBS, and 0.5 ml HRBC suspension was added. The controls were prepared by omitting the extracts and standard drug. These were incubated at 37˚C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The haemoglobin content in the supernatant solution was read at 560 nm using a spectrophotometer.

The percentage of haemolysis of HRBC membrane was calculated as follows: -

$$\% \text{ Haemolysis} = \frac{\text{Optical density of the Test samples}}{\text{Optical density of Control}} \times 100$$

The percentage of protection of HRBC membrane stabilization was calculated as follows:-

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of Test samples}}{\text{Optical density of Control}} \times 100$$

Phytochemical analysis

The phytoconstituents was studied using the crude powder and methanolic extract of plants as described by Parekh & Chanda (2007).[27]

Statistical Analysis

Statistical analysis was done by SPSS version 23, IBM Crop. ANOVA was performed, followed by Spearman's correlation test. P-value ≤ 0.05 was considered significant.

RESULTS

The results of the assay have been presented in table 1. It was observed that both the plant extracts have an inhibitory effect on haemolysis comparable to that of the drug diclofenac. One-way ANOVA analysis between plant extracts and drug showed no significant difference in protection to haemolysis. The highest percentage of haemolysis recorded from the extract of *D. sinuata* was 29.6%, *C. ambrosioides* was 36.8% and standard drug Diclofenac was 32%. However, *D. sinuata* was found to provide slightly more protection to haemolysis than *C. ambrosioides* as compared to the standard drug.

Table 1: Effect of the methanolic extract of *Dendrocnide sinuata*, *Chenopodium ambrosioides* and drug on HRBC membrane haemolysis and protection.

Concentration (µg/ml.)	Haemolysis (in %)			Protection (in %)		
	Chenopodium ambrosioides	*Dendrocnide sinuata*	Diclofenac	*Chenopodium ambrosioides*	*Dendrocnide sinuata*	Diclofenac
1000	36.8 ±0.04	29.6±0.02	32±0.07	63.2±0.05	70.4±0.04	68±0.01
2000	32.8±0.05	28.±0.01	29±0.01	67.2±0.02	72±0.06	71±0.02
3000	32.8±0.03	24±0.02	27±0.02	67.2±0.06	76±0.07	73±0.01
4000	30.4±0.09	19.2±0.05	23±0.01	69.6±0.08	80.8±0.07	77.6±0.07
5000	24.8±0.02	16.8±0.02	20±0.04	75.2±0.08	83.2±0.01	80±0.01

*Values are mean ±SD; n=3 in each concentration

The Spearman's correlation analysis test indicates that the protection provided by the drug and *D. sinuata* and *C. ambrosioides* is dependent on concentration i.e. with an increase in concentration the protection also increases (Table 2).

The results of the phytochemical analysis are provided in table 3. The preliminary phytochemical screening revealed the presence and absence of certain phytochemicals in both *D. sinuata* and *C. ambrosioides.* Out of the eleven phytochemicals screened, test for flavonoids was positive in both extracts.

Table 2: Spearman's correlation analysis of dose dependency of diclofenac, *Dendrocnide sinuata* and *Chenopodium ambrosioides* with concentration.

Treatment	r-value	P-value
Diclofenac vs. Concentration	0.948	0.05
Dendrocnide sinuata vs. Concentration	0.989	0.05
Chenopodium ambrosioides vs. Concentration	0.993	0.05

Table 3: Phytochemical composition of plant extracts.

Phytochemicals	*Dendrocnide sinuata*	*Chenopodium ambrosioides*
Alkaloids	-	++
Flavonoids	++	+
Tannins	+++	-
Phlobatanins	-	-
Triterpenes	+++	+
Steroids	-	-
Saponins	++	+
Glycosides	-	-
Cardiac glycosides	+++	+
Anthraquinones	-	-
Resins	+	++

*The sign '–' means absent and '+' means present; higher the number of '+' higher is the concentration of phytochemicals

DISCUSSION

In traditional medicinal system *Dendrocnide sinuata* and *Chenopodium ambrosioides* have been used by Assamese people to treat inflammation associated ailments.[21,28] As far as literature review is concerned the present investigation is the first-time approach to assess the anti-inflammatory activity of *Dendrocnide sinuata* and *Chenopodium ambrosioides* of Assam by Human red blood cell membrane stabilization method.

In the present study methanolic extract of *Dendrocnide sinuata* was found to have anti-inflammatory activity higher than the standard drug Diclofenac. The result of the present investigation is in agreement with the findings by Angom *et al.* (2015).[22] However, it was based on rat paw oedema model while the present study analysed anti-inflammatory activity based on *in vitro* method of HRBC membrane stabilization. Another difference between the present and the study conducted by Angom et al. (2015)[22] is the solvent medium used for preparing the plant extract. Nevertheless, both the studies confirm the anti-inflammatory effect of *D. sinuata*. On the other hand, *Chenopodium ambrosioides* was found to inhibit haemolysis of human red blood cell membrane lesser than that of the standard drug. The results of the present study corroborated with the findings of *in vivo* studies.[29,23] In the *in vivo* condition, *Chenopodium ambrosioides* have been found to be effective to reduce inflammation induced by different phlogistic agents in both acute and chronic phases.[29] As the acute inflammation is associated with the release of histamine, serotonin, prostaglandins and leukotrienes, and the chronic inflammation is characterised by neutrophil exudation,[22,29,30] *Chenopodium ambrosiodes* and *Dendrocnide sinuata* was speculated to inhibit inflammation by regulating the biosynthesis of these inflammatory molecules.[22,29] Since the membrane of HRBC is analogous to lysosomal membrane and rupture of lysosomes is associated with release of enzymes,[31] the HRBC membrane protection observed in the present study by methanolic extract of *Dendrocnide sinuata* and *Chenopodium ambrosioides* supports the effectiveness of these plants in resolving the condition of acute and chronic inflammation. Further, for both *Dendrocnide sinuata* and *Chenopodium ambrosioides* with a successive increase in concentration for the extract from 1000µg/ml to 5000µg/ml an increase in the protection to HRBC membrane was observed. Similar, dose-dependent anti-inflammatory activity was observed in *in vitro* study by TrivellatoGrassi et al. (2013).[22] Hence it can be supposed that the potency of the plant corresponds to dose levels and is strongest in the highest dose level.

The phytochemical analysis reveals the presence of important bioactive components such as flavonoids, triterpenes, saponins etc. in *Dendrocnide sinuata* and *Chenopodium ambrosioides* which are proven anti-inflammatory agent.[6] On the other hand, phlobatanin, glycosides and anthraquinones were absent. Flavonoids, especially quercetin has been reported to inhibit both COX-2 and 5-LOX enzymes involved in the production of eicosanoids from arachidonic acid. Nonsteroidal anti-inflammatory drug (NSAID) are also known to execute anti-inflammatory activity by preventing the metabolism of arachidonic acid to prostaglandins which in turn is responsible for the release of lysosomal enzymes indirectly by maintaining the cAMP levels high.[32] Thus, it can be speculated that both the plants may have comparable activity to NSAIDs in providing an anti-inflammatory effect. In addition, flavonoids have also been reported to inhibit inflammation by their ability to scavenge NO (Nitric Oxide) rather suppressing the translation and transcription of iNOS which is responsible for NO production.[33]

In the present study, only a single assay has been performed to investigate the anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides*. In addition, only six phytochemicals were screened qualitatively and the quantitative phytochemical analysis was not performed. Considering these limitations, the present study provides the evidence for an anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides.*

CONCLUSION

The results of the present study approve the traditional claims of anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides.* Besides, it is evident from the study that *Dendrocnide sinuata* and *Chenopodium ambrosioides* have the potential to be a very important raw material for drug development against inflammation. Future investigations with more sensitive assays will provide an insight into the possible mechanism of action of *D. sinuata* and *C. ambroisoides* as an anti-inflammatory agent.

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