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EVALUAION OF ANTIMICROBIAL ACTIVITY AND ANALGESIC ACIVITY OF DENDROCNIDE SINUATA (BLUME)

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Abstract-

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. *Dendrocnide sinuata* (Blume) is an evergreen large shrub to small trees, 3–7meter tall; stems and branch lets are densely covered with stinging hairs. Results of proximate analysis of dried leaves of *Dendrocnide sinuata* (Blume) revealed that the plant extract has low moisture content (8.5%) and a high ash value (5.46%). It also contains moderate concentration of protein (4.07%) and low concentration of fat (1.23%). As part of the investigation on the mechanism of the Analgesic activity was studied. It was effective in acetic acid induced writhing method. The different concentration of the extract were being made for anti microbial activity. It showed inhibition of gram positive and gram negative bacteria at the highest concentration 150 µg/ml. Phytochemical screening of the extract of *Dendrocnide sinuata* (Blume) showed the presence of various phytochemical constituents alkaloids, flavanoids, triterpenes, saponin, cardiac gylycosides, resins and tannins.

INTRODUCTION

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. The Indian Traditional Medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness[1]. An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic[2].

Dendrocnide sinuata is an evergreen large shrubs to small trees, 3–7meter tall; stems and branch lets are densely covered with stinging hairs. The bark is white and smooth with lenticellate blaze. Leaves are simple, alternate, spiral, with stipule caduceus and leaving scar. Petiole is 2-6cm long, terete, with glandular stinging hair. Lamina parts of the leaf are 9.5 - 34cm x 2 - 11.5cm , narrow oblanceolate to elliptic, apex acuminate, base attenuate-cuneate to obtuse, margin subentire or crenulate, coriaceous,withgrandular stinging hair; midrib raised above; secondary nerves 8-11 pairs; tertiary nerves distantly obliquely percurrent[3].

MATERIALS AND METHODS

Collection, Authentication and Drying the plant material

The *Dendrocnide sinuata* (Blume) Chew was collected from Pathalipahar area, Lakhimpur district of Assam (A north-eastern part of india) and duly authenticated from Botany Department of Gauhati University, Assam. The plant leaves were shed-dried, pulverized and stored in an airtight container for further extraction.

Fig.1- *Dendrocnide sinuata* (Blume)

Fig.2-Soxhlet extraction of *D.sinuata (Blume)*

Extraction process

The powdered sample (140 g) was extracted with an ethanol solvent (260 ml) by Soxhlet method for 3 days. After extraction, the sample was filtered by using a Bruckner funnel. The ethanol solvent was evaporated by using a rotary evaporator under reduced pressure at 40 °C for 1 h.

Animal Husbandry & Maintenance

Healthy adult wister strain albino rats (weighing 210-250 g, 5-8 weeks of age, male) were obtained from animal house facility, NETES institute of pharmaceutical science, Guwahati, Assam, India. The animals were placed in polypropylene cages with free access to standard laboratory diet (Pranav Agro Industries limited, Sangli, Maharastra, India) and provided water ad libitum.Each individual animal was clinically examined upon arrival and identified by fur marked with picric acid. Animals were grouped and housed in an environmentally-controlled room with temperature of 22±3℃ and 40-70% relative humidity with a 1 h light/dark cycle, and ventilation of 15-21 air changes /h for an acclimation period of 7 days to laboratory conditions prior to the beginning of the experiment in order to adjust the new environment and to overcome stress incurred during their transit .Only healthy animals were assigned for these studies. Approval to carry out these studies was obtained from the Institutional Animal Ethics Committee (IAEC) under a subproject and an experiment was performed in compliance with the principles of Laboratory Animal Care (NIH publication 85-23, revised 1985).All of the animal experimental protocols were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA)[4].

Proximate analysis and phytochemical screening

The parameters determined for proximate analyses include moisture, ash content, crude protein and fat. The analysis was carried out using the modified method described by oluduro et al.based on method of Association of Official Analytical Chemists (AOAC, 1990)[5]. The preliminary phytochemical evaluation of the plant extract for alkaloids, carbohydrates, reducing sugar, cardiac glycosides, flavonoids, saponins, phytosterols, terpenes, phenols, proteins and amino acids, tannins and steroids were determined by using the standard procedures[6].

Antimicrobial Activity

The dried plant extract of the plants were dissolved in ethanol i.e required amount of extract was dissolve in ethanol so that final concentration became 70,100,150 µg/ml. Antimicrobial tests were then carried out by well diffusion method using MHA as nutrient media. Bacteria cultured at 37°C over night for 24 hours were used as inoculums.

Muller-Hinton agar was prepared in sterile conical flask,autoclaved for 30 minute and poured into sterile petriplates.In each petriplates uniform wells were made with the well borer. He plates were the inoculated with the gram positive and gram negative bacteria and the well were filled with 50 microliters of the plant extracts and allow to diffuse. The plates were inoculated with the bacteria and the waells were filled with 50 µl of the plant extracts and allowed to diffuse. The plates were incubated at 37°C for 24 hours.

Acetic acid induced writhing method

The writhing model represents a chemical nociceptive test based on the induction of peritonitis like condition in animals by injecting irritant substances i.p. After 30 minutes of drug administration, 0.1 ml of 1% acetic acid solution was injected i.p. Mice were placed individually into glass beakers and five minutes were allowed to elapse. They were then observed for a period of ten minutes and the numbers of writhes were recorded in each animal. For scoring purpose, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Percentage inhibition was calculated using the following formula:

$\%\ \text{inhibition} = \{W_c - W_t) \times 100\} / Wc$

Where, W_c = No. of writhes in control group, W_t = No. of writhes in test group

Compounds with less than 70% inhibition were considered to have minimal analgesic activity.

Histology

On 15 day of necroscopy, rat skins were preserved in 10% neutral phosphate- buffered formalin solution. Skin from each group of an animal was removed and investigated for histological changes through microscopy analysis.

STATISTICAL ANALYSIS

All experimental data's were expressed as mean and standard deviation (±S.D) values. All the obtained results were statistically analyzed by statistical unpaired t-test, to compare between control and treated group. Graph Pad Instat Software (Version 3.05, USA) was used to perform the statistical analysis of data. P-value less than 5% was considered as statistically significant compared to control and treated group.

RESULTS & DISCUSSION

Proximate analysis

Results of proximate analysis of dried leaves of Dendrocnide sinuata (Blume) are demonstrated in Table 1. The results revealed that the plant extract has low moisture content (8.5%) and a high ash value (5.46%). It also contains moderate concentration of protein (4.07%) and low concentration of fat (1.23%).

Table-1-Physicochemical Parameters

Sl.No	Parameter	Value (%)
1	Moisture Content	8.5
2	Total ash	5.46
3	Protein	4.07
4	Fat	1.23

Phytochemical screening

Table 2 reveals the quantitative phytochemical analysis of Dendrocnide sinuata (Blume). The preliminary phytochemical evaluation of the plant extract confirmed the presence of alkaloids, flavanoids, triterpenes, saponin,cardiac gylycosides,Resins and tannins

Sl.No	Phytochemical Groups	Ethanolic Extract
1	Alkaloids	++
2	Flavanoids	++
3	Triterpenes	+++
4	Saponin	+
5.	Cardiac glycosides	+
6	Resins	+
7.	Tannins	+++

Table-2- Phytochemical composition of ethanolic extract of Dendrocnide sinuata (Blume)

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

Anti-microbial Activity:

1. Anti microbial activity of Dendrocnide sinuata (Blume) against E. Coli:

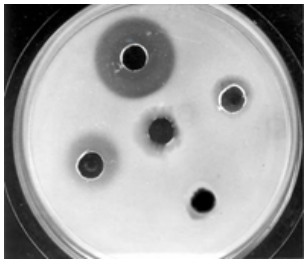

Fig.3: Zone of inhibition of various concentration against E.Coli

Table-3: Zone of inhibition against E.Coli

Sl.no	Mico-organism	Cocentration of sample (µg/ml)	Zone of inhibition (mm) +SEM
1	E.coli	70	121.73
		100	132.88
		150	160.58

2.Anti-microbial activity of Dendrocnide sinuata (Blume) against S. aureus.:

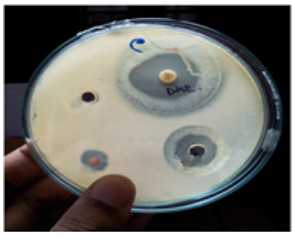

Fig.4: Zone of inhibition of various concentration against S. aureus

Table-4: Zone of inhibition against S. aureus

Sl. no	Mico-organism	Cocentration of sample (µg/ml)	Zone of inhibition(mm)+SEM
1	S. aureus	70	2±1.73
		100	7±2.88
		150	14±0.58

Acetic acid – induced writhing method

Table-5: Acetic acid induced writhing method

Groups	**Onset of writhing (in minutes)**	**Number of writhes (in 10 minutes)**	**Percentage analgesia**
Control	2.833±0.3073	26.17±1.400	-
Ethanolic extract (200 mg/kg bw)	5.667±0.4954	10.50±0.6938	59.87

Ethanolic extract (400 mg/kg bw)	4.167±0.3073	16.65±0.6912	36.30
Aspirin	6.667±0.3333	3.83±0.3073	85.36

Histopathology

The gross histological analysis of necropsy skin samples for both the control and treated group were shown almost no alteration of skin architectural pattern with almost no significant toxic effects, haemorrhage, skin appendages etc. The gross microscopic appearances of both the skins were found mostly normal concerning to normal skin morphology.

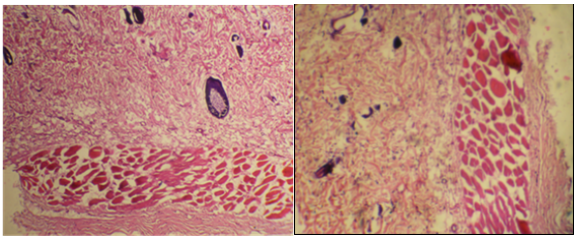

Fig.5: Gross histological investigation between control and ethanolic extract of *Dendrocnide sinuata* (Blume) group (Hematoxylin-eosin Stain with a magnification of 100X).

CONCLUSION

As part of the investigation on the mechanism of the Analgesic activity was studied. It was effective in acetic acid induced writhing method. The different concentrations of the extract were being made for anti microbial activity. It showed inhibition of gram positive and gram negative bacteria at the highest concentration 150 µg/ml. The ciprofloxacin was used as a standard drug for the test. Phytochemical screening of the extract of Dendrocnide sinuata (Blume) showed the presence of various phytochemical constituents alkaloids, flavanoids, triterpenes, saponin, cardiac gylycosides, resins and tannins.

Conflict of Interest

The authors declare no conflict of interest

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