

ANTI-INFLAMMATORY ACTIVITY AND BRINE SHRIMP BIOASSAY OF *ALEURITOPTERIS ANCEPS*

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ABSTRACT

The main objective of current study was to find out the presence of anti-inflammatory activity and perform brine shrimp bioassay in the methanol extract of *Aleuritopteris anceps* (Ranisinka). The plant was collected from Kathmandu and Lalitpur district about 1300 meter altitude in months June to July. The plant was subjected to hexane, ethyl acetate and methanol extraction by maceration process. Previous studies on qualitative analysis revealed that different extract of the fern offered diverse phytochemical compounds such as alkaloids, saponins, glycosides, terpenoids, tannins, carbohydrates and phenols. This plant also possesses different biological activity such as antioxidant,

antimicrobial sensitivity test and anti-diabetic activity. The methanol extract of fern showed mild cytotoxic activity with LC_{50} 50.118 μ g/ml. There was dose dependent anti-inflammatory activity shows by 200mg/kg and 400 mg/kg concentration of methanol extract comparing with standard drug Indomethacin after 5 hour of egg albumin induce paw edema. The percentage of anti-inflammatory activity of 200mg/kg was found 24.31% 400mg/kg extract showed 26.18% where as standard Indomethacin showed 31.79%. Thus *Aleuritopteris anceps* showed significant anti-inflammatory activity.

KEYWORDS: *Aleuritopteris anceps*, Ranisinka, Brine shrimp bioassay, Cytotoxic activity, Anti-inflammatory activity.

INTRODUCTION

Traditional medicinal information is important not only for its prospective impact to the development as well as publics' health.^[1] The interest has been increased in the study of

medicinal plants and their traditional use in different parts of the world during the last few decades.^[2] According to World Health Organization 80% of the world's population typically folks of developing countries depend on plant derived medicines for their healthcare needs.^[3] In the previous study revealed lots of phytochemicals such as alkaloids, saponins, glycosides, terpenoids, tannins, carbohydrates and phenols which are rich source of antioxidant activity, antimicrobial activity, and diabetic activity. Reactive oxygen species and free radicals are produced as normal products of cellular metabolism. The rapid production of such free radicals in the body lead to oxidative damage to biomolecules and may cause various disorders such as cancer, cardiovascular disease, diabetes, inflammatory diseases, asthma, neurodegenerative diseases and premature aging.^[4] A study reported that pteridophytes are not infected by microbial pathogens, it may be one of the significant aspect for the evolutionary achievement of pteridophyte and information that they stay alive for more than 350 million of years.^[5] *Aleuritopteris anceps* is one of the well-known traditional medicinal plants, widely distributed in middle hilly region of Nepal. Thus, this study is a combinatorial approach to find potential bioactive compound. This study will help to explore the medicinal potential of the herb being focused on variations of chemical constituents, biological activity and give way for further exploration on this plant in Nepal.

MATERIALS AND METHOD

Study Design

Experimental and descriptive research design.

Plant Material

Whole plant was collected from Kathmandu and Lalitpur district about 1300 meter altitude in June-July and then it was identified as *Aleuritopteris anceps* in National Herbarium Center and Plant laboratory, Godawari, Nepal.

Chemicals and Apparatus

Methanol, Dimethyl sulphoxide, Indomethacin, Rotary shaker (Associated Scientific Technologies Delhi, India).

Brine Shrimp Bioassay

Introduction

Brine shrimp bioassay is based on the determination of the concentration of sample under consideration of extract which kills 50% of the laboratory –breed *Artemia salina* within 24

hours under the specified appropriate condition. This concentration is Known as LC₅₀ (Lethal dose or Lethal concentration) and is expressed in µg/ml.

Brine Shrimp Bioassay detection

Brine shrimp bioassay is a simple, inexpensive and attractive method because test in the micro well scale can be performed in low toxin amount.^[6] The brine shrimp bioassay is used to forecast the toxicity and probable presence of potential anticancer compounds.^{[7][8]}

The toxic levels from brine shrimp bioassay are as follows:

LC ₅₀ Value	Remarks
<1.0 µg/ml	Highly toxic
1-10.0 µg/ml	Toxic
10.0-30.0 µg/ml	Moderately toxic
30.0-100 µg/ml	Mildly toxic
>1000 µg/ml	Non-toxic

PROCEDURE

Hatching of brine shrimp^{[9][10]}

Brine shrimp eggs were hatched for 24 hours in a beaker containing artificial sea water. At first, Artemia cyst was mixed with sea water contained beaker which was well aerated with the aid of an air pump that reaches the bottom of beaker and mixes with the sea water well. A torch light was positioned over the culture in order to synchronize hatching. The whole set up was kept in incubator at 27⁰C up to 24 hours. Then the pump was turned off and light over the beaker was removed and brine shrimp was removed by needleless syringe.

Preparation of test and control samples

250 mg of methanol extract was dissolved in 1 ml of DMSO and volume made to 250 ml by sea water. This solution was stock solution of 1000 µg/ml. From the stock solution 100ml, 50ml, 25ml, 12.5ml, 7.5ml, 5ml and 2.5 ml were pipette out and diluted by sea water to volume make up 100 ml on each concentration. Different concentration of sample were prepared as 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 75 µg/ml, 50 µg/ml, 25 µg/ml. From the each 20 ml is transferred in triplicate test tube and Brine shrimp were transferred on each test tube. DMSO in sea water was used as negative control. The test tubes were incubated for 24 hours at 27⁰C.

Application of Brine shrimp Nauplii to the test tubes

From the each stock solution 20 ml is pipette out in triplicate test tube and the highly motile Nauplii were counted macroscopically in light background and 10 are transferred in each test tube. DMSO in sea water was used as negative control.

Counting of Nauplii

The test samples of Nauplii were kept for 24 hours at temperature of 27⁰C. After 24 hours the test tubes were observed and the number dead Nauplii in each triplicate test tub were counted against bright background. Nauplii were considered dead if they did not exhibit any movement during several seconds of observation.^[11] From the data the percentage mortality of brine shrimp Nauplii were calculated at each concentration of the experimental sample and control groups using following formula:

$$\% \text{ mortality} = \frac{\text{Number of death shrimp}}{\text{Number of dead + alive shrimps}} \times 100$$

Calculation of LC₅₀ value

LC₅₀ value of the extract was calculated by plotting mean % mortality against logarithm of Concentration.

Anti-inflammatory Activity

Introduction

An inflammation is a body's immune response against cells or tissues injury either due to chemical, thermal, mechanical or infections.^[12] Prostaglandins are the end product of arachidonic acid metabolism which is the key mediator of inflammation response.^[13] Redness, pain, heat, swelling and edema are the major sign of inflammation. Anti-inflammatory drugs are required to treat inflammatory diseases like arthritis, asthma and cardiovascular diseases. Long term sue of commonly used Non-steroidal Anti-inflammatory Drugs (NSAIDs) causes bleeding, GI ulceration and renal dysfunctions.^[14] There is need for search new anti-inflammatory drugs which do not present NSAIDs like side effects.

Procedure^{[15][16]}

- Anti-inflammatory activity of the methanol extract was studied in rats (150-200g). Rats were divided into 4 groups of 3 rats each.
- Standard group I: Given Indomethacin 5mg/kg given orally made in normal saline.

- Test group II: Given methanol extract orally at 200mg/kg dissolved in distilled water.
- Test group III: Given methanol extract orally at 400mg/kg dissolved in distilled water
- Control group IV: Given only distilled water.
- After half hour the rats were injected with 0.05 ml of fresh egg albumin into sub planter region of the right hind paw.
- The diameter of the paw of each rat was measured before and 6 hours after the egg albumin injection. The increase in thickness of paw was recorded. Paw thickness in the dorsal planter axis at the metatarsal level was measured by Varner caliper. The point of measurement was pre-marked on the top of the foot with a permanent marker pen for reference at successive measurements.

$$\text{Percentage anti-inflammatory activity} = (1 - W_e / W_c) \times 100$$

Where

W_e is paw circumference of test/standard

W_c is paw circumference of control

RESULTS

The result of brine shrimp bioassay as below

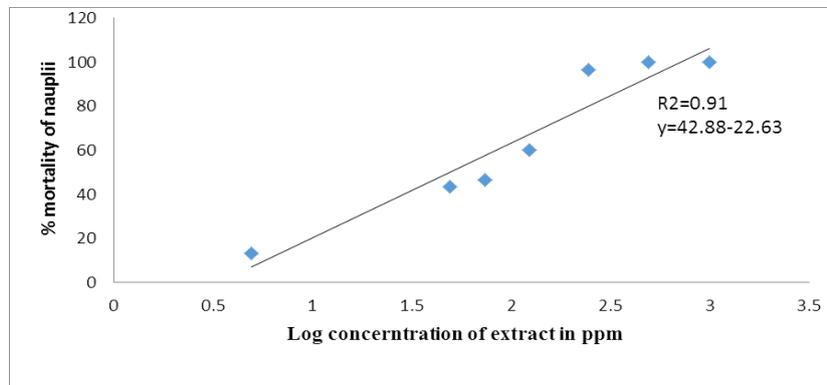


Figure 1: Mortality response of brine shrimp against methanol extract of plant.

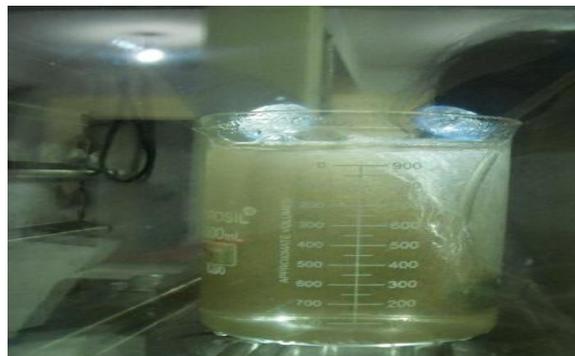


Photo 1: Hatching of Brine shrimp.

Anti-inflammatory activity in rats

The methanol extract of *Aleuritopteris anceps* inhibited fresh egg albumin induced acute paw edema in rat. After treatment of 200mg/kg, 400mg/kg methanol extract of *Aleuritopteris anceps* and 5mg/kg Indomethacin led to a dose dependent anti-inflammatory activity.

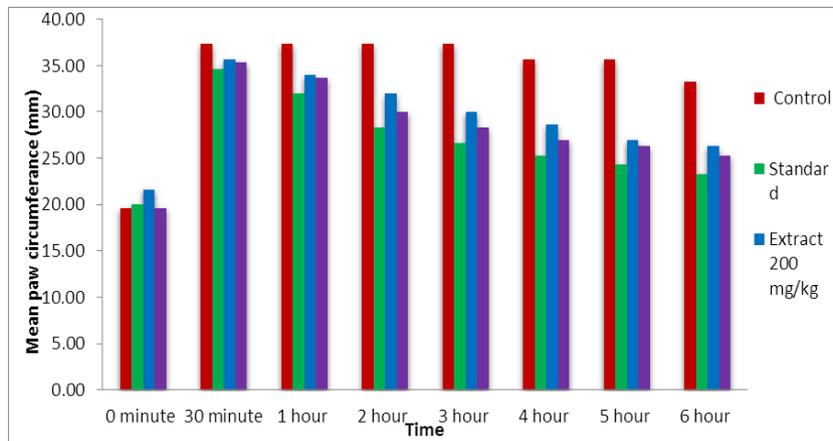


Figure 2: Mean paw circumference decrease by different concentration of extract and Indomethacin.

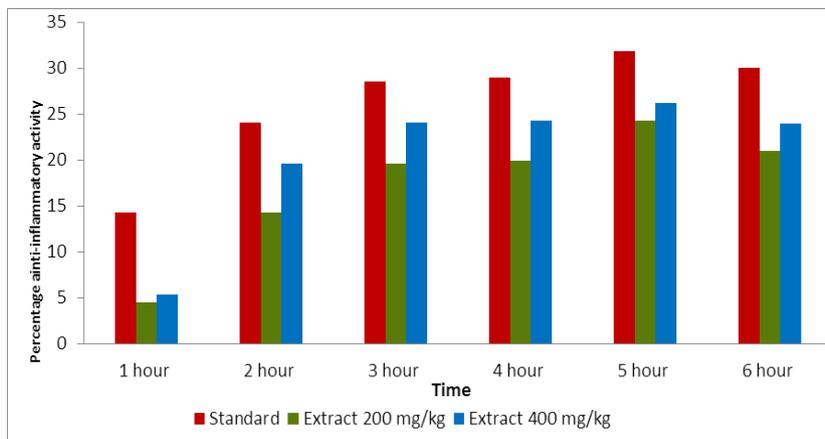


Figure 3: Percentage anti-inflammatory activity by different concentration of extract and Indomethacin.



Photo 2: Anti-inflammatory test



Photo 3: Anti-inflammatory test

CONCLUSION

From previous study, it was found that the plant possesses different phytochemical constituents and biological activities. It was found that the preliminary phytochemical screening exhibit positive test to alkaloid, saponins, glycosides, terpenoids, tannins, carbohydrate, flavonoids and phenols. The plant has considerable antioxidant activity with high amount of total phenolic and flavonoid content. This could be a good source of natural antioxidant. Brine shrimp lethality bioassay revealed that methanol extract of the plant *Aleuritopteris anceps* showed the mild cytotoxic activity. The LC₅₀ value of the brine shrimp was found to be 50.118µg/ml. The degree of lethality was found to be directly proportional to the concentration of the extract. In the evaluation of general toxicity using brine shrimp, maximum mortalities placed at a concentration of 1000 µg/ml whereas least mortality was 25 µg/ml concentrations. The LC₅₀ value of the extract was obtained by plot of percentage of the shrimp killed against the log concentration of the extracts. Both methanol extracts and Indomethacin showed anti-inflammatory activity on fresh egg albumin induced paw edema in rat maximum at 5 hour after treatment. Indomethacin showed 31.79% and 200mg/kg and 400mg/kg extract showed 24.31% and 26.18% anti-inflammatory activity respectively. There was dose dependent effect showed by different concentration of extracts comparing with standard drug Indomethacin. This study gives the information to further research in the technique of isolation and identification of the bioactive compounds from the plant. The whole plant along with rhizome an abundant source of phytochemicals as well as phenolic compounds and compacts to the value –added products from this fern, therefore enhance today's prospects in pharmaceuticals and food applications for human health.

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