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# Analgesic activity of methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera

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**Abstract**
In the present study, it was explored the analgesic activity of methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera, collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India. The dried and powdered *Lobophora variegata* was extracted in methanol to estimate the analgesic activity. The analgesic activity was assessed on intact mice by tail immersion method. Diclofenac Sodium in the dose of 100mg/kg was used as standard drug. Methanolic extracts of *Lobophora variegata* were given in the doses of 200 and 400mg/kg. Control group received normal saline solution. All the doses were given orally. Results observed that both the doses of methanolic extracts of *Lobophora variegata* had potent analgesic activity. From the observations, it was noted that methanolic extract of *Dictyopteris australis* at 200mg/kg was found to have more effect as compared to 400mg/kg methanolic extract.

**Keywords:** Analgesic, algae, *Lobophora variegata*, methanolic extracts, hare Island

## Introduction
Marine macro algae have been used as function food for long years, direct food for man and animals, remedies, organic fertilizer, industrial materials (agar, alginate, carrageenan), production of biofuel, etc [1]. In the last few decades, marine macro algal metabolites presenting various biological activities have been increasingly discovered. Such compounds have shown antibacterial, cytotoxic and anticoagulant activities, capability of agglutinating red blood cells and stimulating cell migration, anticancer properties, effects on the immune response and anti-inflammatory activity [2]. Many studies on marine algae had been published on nutritional values such as *Ulva* sp., *Porphyra* sp., *Sargassum* sp., *Gracilaria* sp., *Kappaphycus alvarezii*, etc [3, 4].
The extracts of marine macro brown algae namely *Sargassum fulvellum* and *Sargassum thunbergii* were examined for antipyretic, analgesic and anti-inflammatory activities on mice [5]. Nowadays, searches of natural medicinal herbs against analgesic activity, especially from marine organisms including marine algae with certain advantages are attracting the attention of many scientists and other countries in the world. Hence the present was focused to examine the analgesic activity using a tail immersion method conducted on Wistar albino rats. The animal model offers a distinct advantage for testing new substances under controlled conditions.

## Materials and methods
### Sample Collection and Preparation of Extract
The marine brown algae (*Lobophora variegata* (Lamour.) Womersley ex Oliviera) was collected from Island, Thoothukudi in the south east coast of Tamil Nadu. The brown algal species was identified based on their scientific names and authenticated. The collected algal specimens were washed with fresh water to remove epiphytes and salts [6]. A voucher specimen of each was deposited in St. Xavier's College Herbarium (XCH), Centre for Biodiversity and Biotechnology (CBB), St. Xavier's College, Palayamkottai, Tamil Nadu, India. For the convenience, the algal tissue was dried at room temperature until moisture content was reduced to 20 to 30% and then, ground to powder for 5 min using a coffee grinder. The powder was stored at -20°C until use. For 30 g algal powder, 500 ml of methanol was used to exhaustively extract solvent-soluble fraction by shaking at room temperature for 2 h and filtered. The crude extracts were concentrated *in vacuo* at 45 to 50°C to dryness. To remove salt from the extracts, the extraction was repeated several times until the amount of salt was visibly negligible [7].

### Experimental Animals
Wistar albino rats (160-200g) of either sex were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The selected animals were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 35±1°C, relative humidity 45-55% and light/dark cycle 12/12h. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% *Arachis* oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conduct between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain [8]. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Acute toxicity test
Acute oral toxicity study was performed as per OECD-423 guidelines [9]. Albino mice (n=6) of either sex selected through random sampling technique was used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract (50% methanolic extract) was administered orally at the dose level of 5 mg/Kg body weight by gastric intubation and observed

for 14 days. If mortality is observed in 2 out of 3 animals, then the dose administered would be assigned as toxic dose. If mortality is observed in 1 animal, then the same dose would be repeated again to confirm the toxic dose. If mortality is not observed, the procedure would be repeated for further higher doses such as 50, 300 and 2000 mg/Kg body weight. According to the results of acute toxicity test, the doses were chosen for experiments.

**Analgesic activity by Tail immersion method**

In the present study, analgesia was assessed according to the method of Luiz *et al.* [10]. Mice divided in the groups of six each were held in position in a suitable restrainer with the tail extending out. 2-3cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cutoff time for immersion was 180 seconds to avoid the injury of the tissues of tail. 0.2 ml of 0.9% NaCl solution was administered to control animals; plant extracts in doses of 200 and 400mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs and then 1h, 2h, 3h and 4h after the administration. The criterion for analgesia was post drug latency which was greater than two times the predrug average latency as reported by Janssen *et al.* [11]. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

**Results and Discussion**

In the tail immersion test, the standard analgesic drug (100mg/kg Diclofenac sodium) as well as the test drugs of methanolic extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera obtained the doses of (200 and 400mg/kg) showed a significant reduction in the number of tail flick of mice as compared to the control mice. Acute toxicity studies showed that the methanolic extracts did not cause any mortality up to 2000 mg/Kg and were considered as safe. The control group at pre-analgesic, 1h, 2h, 3h and 4h showed hot water reaction time in sec is 2.00±0.20, 2.25±0.13, 2.25±0.13, 2.25±0.13 and 2.50±0.15 respectively. The corresponding mean volumes in Diclofenac sodium (100 mg/kg) treated group were 1.75±0.12, 3.00±0.20, 5.75±0.47, 8.50±0.11 and 6.50±0.11 respectively indicating the significant analgesic activity of Diclofenac sodium from 1h onwards when compared to control. Methanolic extract of *Lobophora variegata* in both the doses of 200mg/kg and 400mg/kg had produced significant increase in hot water reaction time in dose depended manner from 1h to 4h. 200mg/kg methanolic extract of *Lobophora variegata* has taken 5.25±1.08 sec in 3h whereas 400mg/kg methanolic extract showed 3.25±1.08 sec at 2h. The methanolic extract of *Lobophora variegata* in both doses 200mg/kg and 400mg/kg had also produced significant analgesic effect with the mean hot water reaction time in dose dependent manner (Table-1). Among the two different concentration of methanol extract studied, 200mg/kg concentration of methanolic extract showed the highest activity compared to 400mg/kg methanolic extract of *Lobophora variegata*.

**Table 1:** Analgesic activity of methanol extract of *Lobophora variegata* (Lamour.) Womersley ex Oliviera

| Animal groups | Pre-analgesic (seconds) | 1 hour (seconds) | 2 hour (seconds) | 3 hour (seconds) | 4 hour (seconds) |
|---|---|---|---|---|---|
| Control | 2.00±0.20 | 2.25±0.13 | 2.25±0.13 | 2.25±0.13 | 2.50±0.15 |
| 100mg/kg Diclofenac sodium | 1.75±0.12 | 3.00±0.20 | 5.75±0.47 | 8.50±0.11 | 6.50±0.11 |
| 200mg/kg Methanol extract | 1.25±0.43 | 3.0±0.70 | 4.75±1.08 | 5.25±1.08 | 3.5±0.5 |
| 400mg/kg Methanol extract | 2.25±0.5 | 3.25±0.43 | 3.25±1.08 | 4.0±1.87 | 3.0±1.22 |

**Conclusion**

In conclusion, the present study demonstrated that methanolic extracts of *Lobophora variegata* (Lamour.) Womersley ex Oliviera has a potent analgesic effect, without any serious toxic effect at highest possible doses. The results of the study recommended that the methanolic extract of *Lobophora variegata* possesses the analgesic activity in both the doses of 200mg/kg and 400mg/kg. Among the two doses, 200mg/kg methanolic extract was found to be the best result. These findings reinforce the claims of the health care industry and indigenous medicine that those seaweeds can be used as remedies for inflammation related symptoms.

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