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ANTI-INFLAMMATORY, ANTIPYRETIC, WOUND HEALING PROPERTIES OF MARINE BROWN ALGAE *SARGASSUM WIGHTII*

Pharmacology

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ABSTRACT

Seaweeds are one of the most important marine resources of the world and being used as human food, animal feed and raw material for many industries. Brown algae are a large group of marine multicellular algae mainly present in mandapam coast, Gulf of mannar. the present study the methanolic extract of marine *Sargassum wightii* has significant antipyretic, anti-inflammatory and wound healing activities without any serious toxic side effects. These findings suggested that bioactive potential metabolites present in *Sargassum wightii* possessed potential biomedical importance and could be used as potential drugs.

KEYWORDS

Sargassum wightii, macroalgae, phytoconstituents, FTIR,

INTRODUCTION:

In recent years, with an increase in awareness of functional ingredients from foods much attention has been focused on polysaccharides isolated from natural sources. During the last decade, numerous bioactive polysaccharides with interesting functional properties have been discovered from seaweeds. India presently harvests only about 20,000 tonnes of macroalgae annually.(Verma *et al.*, 2012). The uses of marine algae include traditional cosmetics, as antipyretic ,anti inflammatory and antiseptic compounds, vermifuges and as treatments for sunstroke, coughs, haemorrhoids, stomach ailments, nose-bleeds, goiter and urinary disease (Ahmad., 2003). Hence in the present Investigation much focus of attention was given to evaluate the seaweed and its phytoconstituents for a renewed spirit of finding novel applications such as antipyretic activity, wound healing activity, anti-inflammation activity.

MATERIALS AND METHOD

Animal study was carried out in Venture institute of Biotechnology and Bioinformatics Research(Registered Research Organization), Madurai.

COLLECTION OF SAMPLE

Brown seaweed belonging to *chlorophyceae* member shows much attention in the recent years, *sargassum wightii* species was collected from Gulf of mannar.

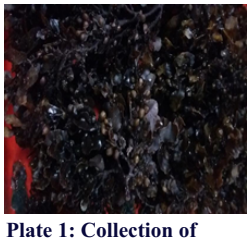

Plate 1: Collection of brown seaweed from Gulf



Plate 2: Extraction of Brown algae

EXPERIMENTAL ANIMALS

White albino rats weighing (150-240 gm) were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30 – 35 %) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water.

WOUND HEALING ACTIVITY

Two groups of white albino rats, with four animals in each group, were anesthetized with chloroform and the animals were depilated on the back. One excision wound was inflicted by cutting away 500 mm^2 full thickness of skin from the depilated area



Plate. 3. Burn wound model



Plate. 4. Ointment Preparation



Plate.5. Application of seaweed extract ointment in wound heal mice

PROTEIN PROFILE BY SDS – PAGE

PREPARATION OF PROTEIN SAMPLE

Wound tissues were chopped into small pieces and collected in Tris buffer (50Mm/P^H 6.8)1, with P^H6.8 containing protease inhibitor phenyl methyl sulfonyl fluoride (PMSF) are 10il ml^{-1}. Tissues were homogenized and homogenization, the samples were spun at 3000 × g at 4°C.



Plate.7. Removal of tissues in wound healed mice to study protein profile by SDS

ANTIPYRETIC ACTIVITY

Treatment was carried out as:

Group I: Control group animals (Normal saline 5 ml / kg); Group II: Paracetamol (10 mg /kg)

Group III: 200 mg /kg methanol extract ; Group IV: 400 mg /kg methanol extract

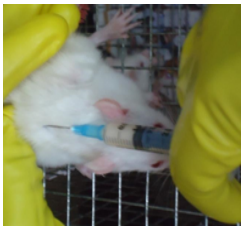

Plate.8 Induction of yeast suspension at the nape of neck for antipyretic study



Plate.9 Determination of rectal temperature in mice

INFLAMMATORY STUDY
EXPERIMENTAL ANIMAL
Group I- Control animals; Group II- Paracetamol (Standard); Group III- Methanol extract;

FORMALIN- INDUCED RAT PAW OEDEMA
The percentage of anti-inflammatory activity calculated by = (vc –vt / vc) × 100. Vc = control, vt – test sample.



Plate.10. Injection of formalin on the right Hind paw of mice for anti-inflammatory studies



Plate.11.Determination of paw oedema using formalin and oral administration of brown of brown seaweed extract in mice model

FTIR ANALYSIS OF SEAWEED
The Oven-dried seaweeds were ground into a fine powder by an agate mortar. Two milligrams of the sample were mixed with 200mg KBr (FT-IR grade) and pressed into a pellet. The pellet was immediately put into the sample holder and FT-IR spectra were recorded in the range 4000-400 cm^{-1}.

RESULT
WOUND HEALING ACTIVITY
The rate of wound contraction was expressed as the percentage of wound area that had healed was shown in Table 1. A significant reduction in the wound area was observed in control and experimental treated mice. The wound contraction was measured in all treatment groups with difference in the days such as 1st, 2nd, 4th, 6th, and 8th of post wounding days. The control mice revealed 3.7cm of wound area whereas treatment with plant extract showed 3.3cm. During 2nd day, the control mice was found to exhibit 3.5 cm contraction of wound whereas extract treated mice revealed 3 cm. On the 4th day, the control and experimental mice revealed wound contraction with 2.9 cm and 2.5 cm respectively. Similarly, the result obtained on 6th day revealed 2.5 cm for control mice and 2.1 cm for experimental mice. On the 8th day mice of experimental groups completely healed with 1.3 cm rather compared with control mice which exhibited 2 cm of wound contraction respectively.

TABLE 1.
WOUND HEALING ACTIVITY OF SEAWEED EXTRACT ON WOUND CLOSURE

S.no	Posting Wounding days	Area in sq cm control	Treatment group (30% gelly+seaweed extract)
1	1	3.7	3.3
2	2	3.5	3.0
3	4	2.9	2.5
4	6	2.5	2.1

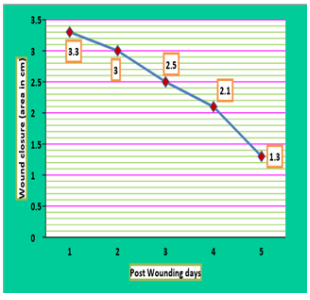

Fig. 1. Wound healing activity of seaweed extract on wound closure

MOLECULAR WEIGHT DETERMINATION IN SDS – PAGE BURN WOUND HEALED MICE
In burn wound model, granulation of tissues were excised after 8th day of wounding. In burn wound model, the molecular weight of protein was variable in control mice whereas similar protein was observed in both the brown algae extract treated mice. In brown algae seaweed treated groups as well as in standard drugs treated wound protein profile was identical and particular protein of molecular weight of 69 KDa were expressed in burn wound mice. A prominent appearance of protein with molecular weight of 19 KDa was absent in other groups.

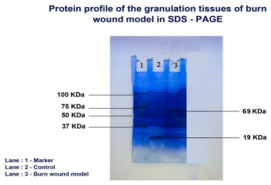

Plate.13.

ANTIPYRETIC EFFECT OF METHANOLIC EXTRACT OF *SARGASSUM WIGHTII*
Antipyretic potential of methanol extract of *sargassum wightii* was evaluated by determining its effect on yeast induced pyrexia in albino mice (2). Fever was induced in mice by subcutaneous injection of brewer's yeast suspension at a dose of 100 mg/kg body weight of mice, below the nape of the neck. The site of injection was then massaged to spread the suspension into the tissues. The rectal temperature was recorded for all groups of mice before yeast administration. The rectal temperature was markedly elevated to 38.6°C, 40.9°C, 40.1°C and 39.1°C for control, paracetamol and seaweed extract (100mg and 200mg treated groups). At that specific time (18 hours after yeast administration), drug were administered. As shown in Table 2, the temperature was decreased to 39.8°C and 38.9 within 1 hour of methanol extract (100mg and 200mg/kg) of *sargassum wightii* treatment respectively. After 4 hours,100 mg/kg and 200mg/kg of seaweed extract revealed a significant reduction in rectal temperature with 38.2°C and 37.4°C where compared to untreated control groups which exhibited a temperature of 41.3°C after 4 hours. Similarly, the rectal temperature was also monitored for paracetamol treated group which revealed a temperature of 38.4°C after 4 hours of administration. This results revealed that methanolic extract of *sargassum wightii* have marked antipyretic activity rather compared with standard paracetamol.

Table.2.
ANTIPYRETIC EFFECT OF METHANOL EXTRACT OF *SARGASSUM*

S. no	Groups	Recal temperature	Initial		Time after administration		
		Initial body temperature	18 hrs after yeast injection		1hr	2hr	3hr
1	Control	37	38.6	39.5	40.2	40.5	41.3
2	Paracetamol (standard) Exp:I	41.3	40.9	40.3	39.9	38.7	38.4
3	100mg/kg methanol extract Exp:II	37.5	40.1	39.8	38.7	38.3	38.2
4	200mg/kg methanol extract Exp:III	37.6	39.1	38.9	38.2	37.5	37.4

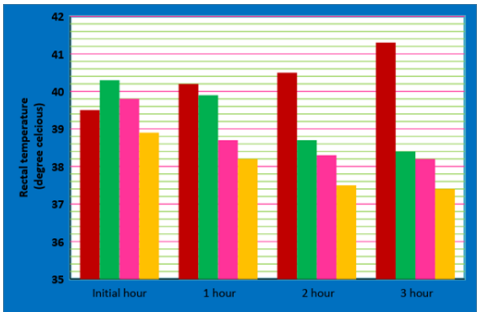

Fig. 2. Antipyretic effect of methanol extract of *Sargassum wightii*

Expt.1 – Paracetamol (standard) group, Expt 2 – seaweed extract (100 mg/kg) ,

Expt 3 – seaweed extract (200 mg /kg)

ANTI-INFLAMMATORY ACTIVITY OF SEAWEED EXTRACT IN MICE MODEL

Seaweed extract was subjected for their anti-inflammatory activity using formalin induced paw oedema method. Seaweed extract exhibited promising anti-inflammatory activity by oral administration at a dose level of 100 mg/kg compared to the reference standard drug paracetamol (100mg/kg). The results for percentage of inhibition of paw oedema was depicted in table 4.

The revealed percentage of oedema induced inhibition by the test compound seaweed extract was recorded after 1, 2, 3 and 4th hours with 17%, 30%, 50% and 60% respectively. While the reference standard drug paracetamol (100mg/kg) revealed a less percentage of inhibition of paw oedema with 23%, after 4 hours. The above results indicated that the seaweed extract (100mg/kg) have a significant anti-inflammatory effect on the formalin induced paw oedema in mice when compared with standard drug paracetamol (100mg/kg).

Table.3. ANTI-INFLAMMATORY ACTIVITY OF SEAWEED COMPOUNDS

S. no	Treatment of animal	Before formalin induced paw size	After formalin induced paw size	Percentage of inhibition (%)			
				1h	2h	3h	4h
1	Control	1 cm	3 cm	-	-	-	-
2	Test100mg/kg (Seaweed)	1.4 cm	2.5 cm	17%	30%	50%	60%
3	Standard 100mg/kg (paracetamol	1.6 cm	2.9 cm	3%	10%	16%	23%

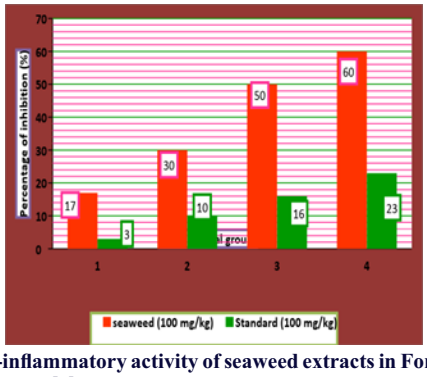

Fig. 3. Anti-inflammatory activity of seaweed extracts in Formalin induced mice model

FTIR ANALYSIS OF BROWN ALGAE

An intensive spectroscopic analysis was carried out in brown algae *sargassum wightii*. To identify the frequency of functional groups changes in the samples, FTIR technique was performed. The main functional groups involved are seaweeds carboxyl, sulfhydryl and hydroxyl which are prime constituents of the marine brown algae. The bands appeared with the Peak value of 2335 cm^{-1} indicated C-H and C-O vibrations of amines, hydroxyl and carboxylic groups. Another peak of weak band of 2368.59 cm^{-1} corresponds to C-O stretching bands. Another absorption band of 1649.14 cm^{-1} revealed C-O stretching and N-O asymmetric stretching. Another absorption band 1234 cm^{-1} are due to S=O. The marine *sargassum wightii* contains absorption band at 1035cm^{-1} which is due to S=O stretching vibration.

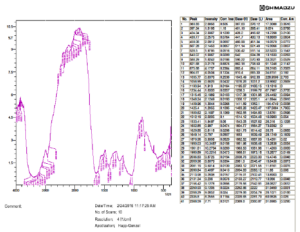

Fig.4.

DISCUSSION

Wounds occur due to agents that induce stress or injury. Wound healing is the primary response to tissue injury with different phases like contraction, granulation, epithelisation and collagenation which is mainly achieved by connective tissue matrix synthesis (Mukherjee, 2003). The present study was focused with much attention to evaluate the potential wound healing properties of methaolic extracts of *Sargassum wightii.*

The normal healing process begins with an orderly process of haemostasis and fibrin deposition, which leads to an inflammatory cell cascade, characterized by neutrophils, macrophages and lymphocytes within the tissue (Mast *et al*, 1992). In the present study, the methanolic extract of brown seaweed exhibit a remarkable wound healing activity in mice.

Wound healing in mice leads to granulation tissue formation. In the present study in burn wound model, Similarity was observed in protein profile of granulation tissue of *Sargassum wightii* as control group it was assumed that the extract obtained from marine *Sargassum wightii* elicited the wound healing process. The protein with the molecular weight 69 KDa was expressed in *Sargassum wightii* extract treated group indicated that this particular protein might be involved the wound healing activity.

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclo oxygenase and consequently reducing the levels of PGE2 with in the hypothalamic region (Rajani, 2011).

In the present study, the methanolic extract of *Sargassum wightii* showed more pronounced effect in lowering the hyperthermia, thus the standard drug paracetamol at 4th hour of administration. The extracts are likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine (Jude, 2010).

The results of present study indicated that the methanol crude extract of *Sargassum wightii* possesses significant antipyretic effect compared to the effect of standard drug paracetamol on yeast induced pyrexia in rats. This may be attributed to the presence of various secondary metabolites present in the methanol extract which may be involved in inhibition of prostaglandin synthesis.

Brown seaweed extract as well as structurally diverse compounds obtained have been shown to inhibit inflammation (Kang *et al*., 2008); Hoang *et al*., 2007). The compounds in the seaweed extracts may play as competitive inhibitors of cyclooxygenase and lipoxygenase in an inflammation reaction, resulting in decreased production of prostaglandins and leukotrienes (James *et al*., 2000).

The anti-inflammatory performance of the seaweed extract was confirmed for the structural relative activity. Seaweed extract was tested for anti-inflammatory study with paw oedema test. The result showed that the extract possessed promising anti-inflammatory activity. The formalin induced paw oedema is one of most important commonly employed techniques for evaluation of anti-inflammatory drug. It is based upon the ability of drug to inhibit oedema induced hind paw of mice in the plantar tissue of paw. In general, brown seaweed extract exhibited promising anti-inflammatory activity by oral administration at a dose of 100mg/kg compared to the reference drug paracetamol and relative percentage inhibition of oedema recorded values after 1 hour was 3% and after 4 hours revealed 23%. It was noteworthy to emphasize a maximum inhibition with 60% after 4 hours was recorded for seaweed extract which revealed an excellent anti-inflammatory activity. This may be attributed due to substitution of phenyl ring at fourth position which give rise to an increased anti-inflammatory activity (Razi ahmad *et al*., 2009).

In the present investigation, FTIR is a powerful technique to identify

the frequency of functional groups present in the brown seaweed *sargassum wightii* which exhibited the characteristic finger print band features. These strong absorption bands at 2335 cm^{-1} indicated the C-O stretching band. The absorption peak of weak band 2368.59 cm^{-1} corresponds to C-O stretching bands, which is a characteristic peak for carboxylic group (Rasasekar *et al*, 2013). The strong absorption peak of 1649.14 cm^{-1} of the spectrum is due to the C-O stretching and N-O asymmetric stretching indicated the ester group (Stewark, 1995). Another absorption peaks at 1234 cm^{-1} and 1035 cm^{-1} is due to S=O stretching vibration. (Sulfate ester) (Silva *et al*, 2005).

In conclusion, the present study demonstrated that the methanolic extracts of marine *Sargassum wightii* has potential antipyretic, anti-inflammatory and wound healing activities without any serious toxic side effects. These findings reinforce the claims of health care industry and indigenous medicine that these brown seaweeds can be used as remedies of biomedical importance.

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