
Srilekha, V., ¹ Krishna, G., ²Seshasrinivas, V. ³ & Singaracharya, M.A. ²

¹Department of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad 500085, India
²Department of Microbiology, Kakatiya University, Warangal-506009, India
³Department of Biotechnology, SNIST, Ghatkesar, Hyderabad-501301, India

[E.mail: srilekamicrobiology@gmail.com ]

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Pigmented marine bacteria were isolated from seawater collected from different coastal areas of Nellore by spread plate technique using Zobell marine agar. Among 29 pigmented bacterial isolates, the one with intense yellow pigmentation was selected for further study. Selected isolate was identified as *Micrococcus sp* by using morphological and biochemical and molecular analysis. Pigment was extracted from bacterial pellet using methanol as a solvent and the crude yellow pigment extract was subjected to anti-inflammatory and wound healing activity. The *in vivo* wound healing activity was studied using excision wound model. Ointment of extract was prepared and used for assessment of wound healing activity in albino rats. Topical application of the ointment improved wound contraction when compared with control group of rats. Methanolic extract of yellow pigment reduced the carrageenan induced rat paw edema by 31.18 % on oral administration of 100 mg / kg body wt.

**Keywords**: Anti-inflammatory; Carrageenan; Edema; Wound healing; Wound contraction; Excision wound.

Introduction

Wound is a physical injury, especially one in which the skin or another external surface is torn, pierced, cut or broken with disruption of anatomical and functional continuity of structures. The most common symptoms of wounds are bleeding, loss of feeling below the wound site, painful or throbbing sensation, heat and redness around the wound, swelling of tissue in the area and pus like drainage. Wounds result in the loss of continuity of epithelium with or without the loss of underlying connective tissue. Proper healing of wounds is required for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Unhealed wounds constantly produce inflammatory mediators that cause pain and swelling at the site of wound. Chronic wounds may even sometimes lead to multiple organ failure or death of the patient. Wound healing is an intricate process initiated in tissue injury that involves a complex set of cellular, physiological, and molecular events targeted towards the restoration of the structural and functional integrity of the damaged tissue. Wound healing process involves three stages namely inflammation, proliferation and lastly the remodelling phase which determines the potency and appearance of the healed tissue. Inflammation is induced subsequent to wound occurrence due to the release of leukotrienes, prostaglandins, and free radicals. Cellular proliferation is the second stage and encompasses collagen deposition, epithelization, and angiogenesis which are regulated by growth factors. Finally, tissue remodelling occurs with proliferation of fibroblasts and synthesis/accumulation of collagen fibres. Drugs of natural origin are an important source for the treatment of many diseases worldwide.

Inflammation is a natural complicated protective biological response by the living organism to harmful stimuli such as pathogens, damaged cells or irritants and thus initiates the healing process for tissues. Inflammation is the reaction of a normal healthy living tissue to local injury and it plays a key role in the defence mechanism which helps to protect us from injury or infection. Currently, there is significant evidence that various types of inflammatory tissue injury are mediated by reactive oxygen species (ROS) from activated neutrophil and macrophage. Oxidants such as superoxide anion ($O_2^{-}$), and hydroxyl radicals (OH$^-$), hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HOCl), are formed at sites of inflammation, and appear to
Contribute to the damage in some acute and chronic inflammatory diseases. This over production leads to tissue injury damaging the macromolecules and lipid peroxidation of membranes. Inflammatory diseases including different types of rheumatic diseases are major cause of morbidity throughout world. This has been called the ‘King of Human Miseries’. Oedema formation, leukocyte infiltration and granuloma formation are main manifestations of inflammation. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability or the mediators that increase blood flow. However, if the inflammation is left untreated or uncontrolled, it will lead to onset of many acute and chronic human diseases. Drugs which are in use presently for the management of pain and inflammatory conditions are conventional anti-inflammatory drugs, such as steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs). It has been suggested that most of the anti-inflammatory drugs might exert some of their effects by scavenging oxidants and decreasing formation of ROS by activated phagocyte. However, prolonged use of these drugs may produce several adverse effects, including gastrointestinal disorders, immunodeficiency and humoral disturbances.

There is a clinical need to identify new compounds that are safe, for the treatment and prevention of inflammatory diseases. Drugs available currently can’t affect all phases of wound healing, making it necessary for developing new drugs from different sources.

Marine microorganisms have been investigated over the last 20 years with the goal of discovering new drugs and are considered as unique biological sources of potently active secondary metabolites. The Ocean represents a rich source of both biological and chemical diversity. Although this diversity is the source of unique chemical compounds, its potential for pharmaceutical applications remains still underexplored. Marine microorganisms exhibit unique metabolic and physiological capabilities conferring them the ability to survive in extreme conditions and consequently produce novel metabolites that cannot be found elsewhere. Present study was to assess the wound healing activity of crude pigment extract of pigmented marine bacteria. Now it is a growing concern all over for the development of new safe, potent, less toxic anti-inflammatory drugs. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.

**Materials and Methods**

Surface Sea water samples were collected from Nellore Seacoast, Andhra Pradesh. The samples were collected in sterile containers at 20 meters off the shore line at a depth of about 40 cm from the top and brought to the lab in an ice-box.

The collected sea water samples were spread onto the surface of Zobell agar medium (peptone 5.0 g, yeast extract 1.0 g, ferric citrate 0.1 g, sodium chloride 19.45 g, magnesium chloride 8.8 g, sodium sulfate 3.24 g, calcium chloride 1.8 g, potassium chloride 0.55 g, sodium bicarbonate 0.16 g, potassium bromide. 0.08 g, strontium chloride 34.0 mg, boric acid 22.0mg, sodium silicate 4.0mg, sodium fluoride 2.4 mg, ammonium nitrate 1.6 mg, disodiumphosphate 8.0 mg and agar 15.0 g) and the petri dishes were then incubated at room temperature and the colonies were observed up to 3 days. All the pigment producing single cell colonies that appeared on the agar plates were picked, purified by streak plate method on the same medium. The colonies with coloured pigments were then selected and purified. These were purified by repeated streaking and were sub cultured onto Zobell Agar slants. The marine bacterial isolates so obtained were preserved at 4°C, for further studies.

The criteria employed for selection of potential strain included production of intense color i.e., considerable amount of pigmentation in solid medium and in the liquid medium was selected. The selected strain was identified according to the morphological, cultural and a biochemical characteristic, as outlined in Bergey’s Manual of Systematic Bacteriology and confirmed by molecular analysis. The isolate, which showed the intense pigmentation, was chosen for molecular identification. Sequencing was outsourced at Xcelries laboratories; Ahmedabad. The 16s rRNA gene sequence was compared for similarity with the reference species of bacteria contained in genomic database banks using NCBI BLAST. Phylogenetic tree was constructed by using the sequence and was deposited in GenBank with accession number HF 565033.

The isolated and selected bacterial strain was inoculated into 50 ml of Zobell marine broth and incubated on a shaker at 120 rpm for 72 hrs. At the
end of incubation period about 2 ml of culture broth was taken in a test tube and centrifuged at 10000 rpm for 10 minutes to separate cell mass from fermentation medium in a cooling centrifuge. Approximately a quantity of 2 ml of methanol was added to the pellet and centrifuged at 10000 rpm for 10 minutes at 4°C to extract the pigment from obtained pellet. The colored supernatant thus obtained was filtered through Whatman no.1 filter paper and concentrated by evaporation. Different solvents like methanol, acetone, and ethanol were used to check for the maximum solubility of pigments.

Wistar albino rats weighing about 8-10 weeks (150–250 g), were used for the study (Mahaveer Agencies, Hyderabad). The rats were fed with standard rodent pellet diet and were housed in polypropylene cages maintained under standard conditions of temperature (25 ± 3 °C), light dark cycles of (12-hour light - dark cycle) and relative humidity of (35–60%). The animals were left for ten days to the laboratory environment for acclimatization. A minimum of six animals were taken in each group. The experimental rats used and the protocols followed in this study were reviewed and approved by the Institutional Animal Ethical Committee (44/SPIPS/IAEC/13) before the commencement of the experiment.

For assessment of wound healing activity by excision wound model the extract was formulated in the form of ointment. The ointment is prepared by fusion method. For preparation of simple ointment, the ingredients used includes wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin which were mixed and heated gently according to increasing order of their melting point and mixed gently with stirring followed by cooling and then packed in a wide mouth container. In same manner 10% ointment yellow pigment was prepared and packed in a wide mouth container. The cutaneous excision model was used to assess the wound healing activity of the yellow pigment extract. The wound was inflicted at dorsal side on the rats as described in the literature. Before inflicting the excision wounds, the experimental rats were anesthetized by intraperitoneal administration of ketamine (70 mg/kg body weight), and the fur on the dorsal side of the animals was shaved using an aseptic surgical blade and disinfected with 40% of ethanol. A circular excision wound, of 300 mm² and 0.2 cm depth, was inflicted with a surgical blade on the dorsal surface at the thoracolumbar region of each of the experimental rats, under sterile conditions. To each experimental animal, 10% formulation yellow pigment extract ointment was applied topically twice a day on the wound until they are completely healed. The progressive changes in wound were monitored planimetrically by tracing the wound margin on graph paper every alternate day. Epithelialisation time was noted as a number of days after wound required for the scar to fall off leaving no raw wound behind. From the healed wound, a specimen sample of tissue is isolated from each group of rats for histopathological examination.

The effect of yellow pigment extract on wound healing activity was studied in male Wistar rats. A total of 24 rats weighing 150–200 g was randomly selected and divided into four groups consisting of six rats in each group. Rats in each of the different experimental groups topically received an application of cream the following compounds:

- Group I: Control group Received no treatment and served as control
- Group II: Ointment base Received the treatment of simple ointment base
- Group III: Standard Received application of standard drug ointment (2% soframycin ointment)
- Group IV: Test group Received application of 10% yellow pigment extract ointment served as test group

The wound indices were measured after every 3 days of wound formation following a random scoring system. The healing property was evaluated as percentage of wound contraction, measuring the length and size of the wound with digital callipers following the Walker and Mason formula. Significance in wound healing of the test groups was derived by comparing the healed wound area, on the respective days, with the healed wound area of the control group. The rate of wound contraction was calculated using the given formula:

\[
\% \text{Wound contraction} = \frac{\text{Initial area wound} - \text{n-th day area of wound}}{\text{Initial area of wound}} \times 100
\]

This study was performed to observe if there is any skin irritation for the animal model. The rabbits were shaved on three sites about 300 mm² on the dorsal side of the rat and were kept under rat holder. One side served as control and the other two sides were applied with standard ointment and extracted 10% yellow pigment ointment and observed for skin irritation.
The histological changes, i.e. epithelialization, granulation tissue formation, and cell migration, were observed during the process of wound healing in individual experimental rats that were treated with yellow pigment extract. On the 18th day of post wounding, all the animals were sacrificed, and the granulation tissue formed on and around the excision wounds of the untreated and treated rats was carefully dissected with a sterile surgical knife and carefully collected without any folding, and weighed. Later, the sample tissues were fixed in 10% buffered formalin solution (pH 7.4) and stored. After the usual processing of the tissue in dehydrated alcohol, these tissues were cleared in xylene and were embedded in paraffin wax (melting point 55 °C). Skin samples from wound healing sites were taken for histopathological studies. These tissues were stained with haematoxylin-eosin stain and viewed under microscope for histopathological examination. The sections were then observed under a light microscope (Olympus BX51) for qualitative assessment of the degree of necrosis, epithelialization, collagen formation and fibroblast proliferation in the wound tissues. Congestion, edema, PNL, mononuclear cells, fibroblasts and vascularization were also qualitatively evaluated for treated and untreated rats. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.

The values were calculated as mean ± SEM. Significance of the difference of the mean value with respect to control group was analyzed by one-way ANOVA followed by Dunnet’s t- test using statistica 8.0. Statistically significant at a level of P<0.05 or above was considered to be significant.

Wistar Albino rats of male sex weighing 150 – 200 gm procured from Mahaveer Enterprises, Reg No. 177/99/CPCSEA, Hyderabad were used in the present study. They had free access to food and water and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. Animals were acclimatized to laboratory conditions for 2 days before behavioral studies. All the observations were recorded between 10 a.m. and 5 p.m. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India with a registration No.2011/10/1/12.

The anti-inflammatory activity of the yellow pigment extract was determined by carrageenan induced paw oedema method according to the method suggested by Wintret et al. (1962) and as reported by Ibrahim et al., (2012) with slight modifications. In this experiment, carrageenan-induced rat hind paw edema was used as the animal model of acute inflammation. In this method, rats were divided into 3 groups of 6 animals in each group. The animals were divided into 3 different groups of 6 animals each. Rats were divided into 3 groups (6 animals in each group). Animals were fasted for 24 hours before starting the experiment with free access to water. Animals were treated with drugs and yellow pigment extract.

Group I served as control and received vehicle (0.9% Normal saline) by oral route.
Group II served as standard and received indomethacin (10 mg/kg) + 0.1 ml of 1% carrageenan orally.
Group III, received treatment with yellow pigment extract (100 mg/kg, respectively) + 0.1 ml of 1% carrageenan orally.

After 1 h, the edema was induced in animals of all the groups by injecting with 0.1 ml of 1% carrageenan in 0.9% normal sterile saline (w/v), subcutaneously into the sub-plantar region of the rat right hind paw of the animals. Group I animals (carrageenan control) received vehicle p.o., one hour prior to carrageenan injection. Group II, the standard reference group was given p.o., an aqueous solution of indomethacin (10 mg/kg), one hour prior to carrageenan injection. Group III received p.o., 100 mg/kg of prepared methanol extract suspension, 1 hour prior to carrageenan injection, respectively. The measurement of the paw volume was repeated using the plethysmometer an hour after carrageenan injection. The paw volume of the rats was measured plethysmographically just before and upto 5 hours after administration of carrageenan. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group. The percentage (%) inhibition of edema volume between treated and control group was calculated using the formula as follows:

\[
\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where, \(V_c\) and \(V_t\) represented mean increase in paw volume in control and treated groups, respectively.
Statistical Analysis

Data obtained from animal experiments was expressed as the mean standard error (±SEM). The significance of the difference of the mean value with respect to control group was analyzed by one-way ANOVA followed by Dunnet’s t-test using statistica 8.0. Statistically significant at a level of P<0.05 or above was considered to be significant.

Results

Marine water samples collected from Nellore coastal area were used to isolate pigment producing microbial strains using Zobell marine agar plates. A total of 29 different pigmented bacterial colonies were isolated and incubated at room temperature, purified and preserved. Among the isolated strains, the strain which produced intense yellow pigment was selected for further studies and identified as Micrococcus sp according to morphological and biochemical characteristics and further confirmed by 16s RNA sequence method. Among various solvents used for pigment extraction, Methanol was found to be most efficient solvent for the extraction of the yellow pigment from the bacterial pellet. In this study wound healing and anti-inflammatory activity of methanol extract of yellow pigment from marine pigmented Micrococcus sp. was evaluated by in vivo screening methods.

Excision wound model was used to determine the wound healing activity of yellow pigment extract. In this method, the wound of 300 mm² was induced and applied with yellow pigment extract ointment two times a day. The ointment of yellow pigment extract, reference standard was applied to wound twice, until recovery to respective group of animals. The observation of percentage wound closer was made on 3rd, 6th, 9th, 12th, 15th, 18th and 21st post wounding days. From the results, it was observed that pigment extract ointment showed better and fast wound healing activity when compared to untreated control. Percentage of wound contraction with 10% (w/w) yellow pigment ointment treated groups showed a better wound healing from sixth day onwards. The wound closure time was lesser as well as the percentage of wound contraction was more with the yellow pigment ointment treated group when compared to ointment base group and control group. A better healing pattern with complete wound closure was observed in rats treated with yellow pigment extract within 14 days for the yellow pigment extract where as it was 21 days in the control animals for complete healing. Almost 100% wound contraction was observed with standard 2% soframycin ointment and test group treated with yellow pigment ointment when compared with control group and ointment base. The percentage of wound contraction in standard and test is 100 percentage and in control group the percentage of wound contraction was comparatively less because the wound was left open so it has taken more time to get healed completely (Figure 2). Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. There was a reduction in wound area from day three onwards in treated mice and also on later days the closure rate was much faster than when compared with control mice. Table 1 shows the effect of pigment extract on excision wound model in mice.

Further observation revealed that the wound contracting ability of the 10% (w/w) yellow pigment extract ointment treated groups showed significant wound healing from the sixth day onwards. The wound closure time was lesser, as well as the percentage of wound contraction was more with the 10% yellow pigment extract ointment treated group.

Histopathological studies of the wound tissues of the rats that received yellow pigment extract ointment showed morphological changes, such as higher collagen content, granulation tissue formation, and increased migration of macrophages and fibroblasts cells, which aid in granulation tissue formation and wound tissue repair. Antimicrobial property of yellow pigment extract massively reduced the bacterial population, thereby indirectly reducing the inflammatory cells on the wound site. Complete wound epithelialization with increased collagen formation was observed on the 16th day of wound healing in the experimental rats that received yellow pigment extract. The visual observation of wound
confirmed that growth of the granulation tissue initiated from the base of the wound and proceeded to fill the entire wounds following treatment with both the yellow pigment extract and softramycin. The histopathological slides obtained from the animals during wound healing process also showed clear cut differences in between control and treatment (Figure 3). The granulation tissue formed was pink-red in color, moist, and shiny. The percentage of wound contraction and reepithelialization after topically administered yellow pigment extract was promising when compared with the control group studied. Thus, the study clearly demonstrated that the yellow pigment ointment accelerated the process of
wound healing compared with the untreated animals. Furthermore, all the three (ointment base, 10% yellow pigment extract ointment and 2% soframycin) was shown negative results for skin irritation studies.

Table 1 — Wound healing studies of methanolic extract of yellow pigment showing percentage reduction of wound size in rats (% contraction)

<table>
<thead>
<tr>
<th>Post-wound healing days</th>
<th>Wound in (mm²) (Mean±SEM) and percentage of wound contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>503.66±2.9857 (0%)</td>
</tr>
<tr>
<td>3</td>
<td>478.33±3.7749 (30.02%)</td>
</tr>
<tr>
<td>6</td>
<td>275.33±1.3336 (61.55%)</td>
</tr>
<tr>
<td>9</td>
<td>184±1.1549 (87.89%)</td>
</tr>
<tr>
<td>12</td>
<td>60.66±1.202 (90.97%)</td>
</tr>
<tr>
<td>15</td>
<td>25.33±1.7641 (95.64%)</td>
</tr>
<tr>
<td>18</td>
<td>26.00±0.012 (97.56%)</td>
</tr>
<tr>
<td>21</td>
<td>0±0.0 (100%)</td>
</tr>
</tbody>
</table>

The anti-inflammatory property of the methanolic extract of pigment was investigated using the carrageenan-induced rat paw edema. The yellow pigment extract, standard drug and control were given to the rat orally an hour before injecting with 0.1ml of 1% w/v carrageenan solution in sterile normal saline in the sub-planter region of the right hind paw of each rat to induce acute paw edema. The paw volume was measured plethysmometrically before administering carrageenan and 1, 2, 3, 4 and 5 hours after carrageenan injection respectively. Measurement of paw thickness was taken before carrageenan injection that is, at “0 hour” and then 1, 2, 3,4h and 5h after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at “0 hour” and paw thickness at respective hours. The paw volumes and percentages of inhibition by the methanolic extract of yellow pigment and standard drugs are shown in Table 2. There was a gradual increase in paw edema volume in the control group. Standard drug, indomethacin at 10 mg/kg and yellow pigment extract inhibited the inflammation significantly at all time intervals.

Fig. 3 — Histo pathological evaluation of wound tissues in excision wound model of yellow pigment extracts showing wound healing process
The anti-inflammatory activity of the methanolic extract of yellow pigment extract of marine pigmented bacterium. Wound is defined as the physical disruption of the cellular and anatomic or functional continuity of a living tissue. Wound contraction was measured in each 2 days interval, until complete wound healing and expressed in percentage of healed wound area. All treated groups were compared with the control groups. Wound healing is an intricate and continual cascade of events, with various cellular and biochemical processes, ultimately resulting in the reconstruction and regeneration of damaged tissue. In general, the wound healing process is characterized by dynamic, interactive events involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells that result in the permanent restoration of anatomic and functional integrity. The yellow pigment extract possessed potent wound healing capacity as evident from the wound contraction, increased tensile strength.

The anti-inflammatory activity of the methanolic extract of yellow pigment of marine bacteria was evaluated by carrageenan induced rat paw oedema method. Inflammation is the complex biological response of vascular tissues to a variety of noxious stimuli including pathogens, irritants or damaged cells resulting local accumulation of plasma fluid and blood cells. Inflammatory tissue damages could be due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites. It is one of the most important processes involved in the defence of an organism against local injury and infections; however, it often progresses to a chronic disease requiring pharmacological treatment. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs that have been used frequently to assess the anti-edematous effect of natural products. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation study. Carrageenan induced oedema of rat paw is used widely as a standard working model of inflammation for investigating or evaluating new anti-inflammatory drug agents, and appeared to be the basis of the discovery of Indomethacin, the anti-inflammatory drug. Carrageenan is a phlogistic agent of choice for investigating or evaluating new drug for anti-inflammatory drugs, as it is not known to be antigenic and is devoid of any apparent systemic effects. This phlogistic agent, when injected locally into the rat paw, induced a severe inflammatory reaction, discernible with in 30 min. Carrageenan is widely used to induce hind paw edema for the discovery and evaluation of anti-inflammatory drugs. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability or the mediators that increase blood flow. Moreover, the experimental model exhibits a high degree of reproducibility and is least affected by non-specific factors and variations in strain, sex or body weight. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and is commonly used to induce acute inflammation. Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents; therefore, it has a significant predictive value for anti-inflammatory agents acting through mediators of acute inflammation.

The oedema which develops in rat paw after carrageenan injection is generally reported to be a biphasic event or response. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin and increased synthesis of prostaglandins. The first phase of inflammation in carrageenan model occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. In the first phase, during the first hour, histamine, serotonin and bradykinin are the mediators involved, while prostaglandins are implicated in the second phase...

Table 2 — Anti-inflammatory activity of the yellow pigment extract

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time (h)</th>
<th>Volume variation (μL)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>1</td>
<td>1.53 ± 0.12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.84 ± 0.17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.22 ± 0.24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.44 ± 0.26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.11 ± 0.21</td>
<td>0</td>
</tr>
<tr>
<td>Standard</td>
<td>1</td>
<td>1.17 ± 0.01</td>
<td>23.53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.35 ± 0.01</td>
<td>26.63</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.14 ± 0.02</td>
<td>48.6**</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.03 ± 0.02</td>
<td>57.79***</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.89 ± 0.01</td>
<td>57.82***</td>
</tr>
<tr>
<td>Yellow pigment</td>
<td>1</td>
<td>1.27 ± 0.04</td>
<td>14.99</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.44 ± 0.06</td>
<td>23.74</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.49 ± 0.04</td>
<td>23.8*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.45 ± 0.07</td>
<td>30.57*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.22 ± 0.08</td>
<td>31.18*</td>
</tr>
</tbody>
</table>

Discussion

The present study was carried out to investigate in vivo wound healing properties and anti-inflammatory activity of yellow pigment extract of marine pigmented bacterium. Wound is defined as the physical disruption of the cellular and anatomic or functional continuity of a living tissue. Wound contraction was measured in each 2 days interval, until complete wound healing and expressed in percentage of healed wound area. All treated groups were compared with the control groups. Wound healing is an intricate and continual cascade of events, with various cellular and biochemical processes, ultimately resulting in the reconstruction and regeneration of damaged tissue. In general, the wound healing process is characterized by dynamic, interactive events involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells that result in the permanent restoration of anatomic and functional integrity. The yellow pigment extract possessed potent wound healing capacity as evident from the wound contraction, increased tensile strength.

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The oedema which develops in rat paw after carrageenan injection is generally reported to be a biphasic event or response. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin and increased synthesis of prostaglandins. The first phase of inflammation in carrageenan model occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. In the first phase, during the first hour, histamine, serotonin and bradykinin are the mediators involved, while prostaglandins are implicated in the second phase...
(3-5 h) and continuity between the two phases is provided by kinins. Edema induced by carrageenan is believed to be biphasic: the first phase (1 h) involves the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products. Carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. Enzymes and reactive oxygen species may be released into the extracellular environment where it acts as mediators of inflammation. Such mediators are mainly arachidonic acid metabolites, generated through Cyclooxygenase and Lipoxygenase pathways. Most of the anti-inflammatory drugs are targeted on these pathways.

Conclusions
The present study revealed that the isolated marine strain has a great efficiency to produce the pigment which has potential as anti-inflammatory and wound healing agent. The wound healing activity of the pigment may be assisted by antibacterial property of the pigment. Application of 10% pigment revealed reduced wound closer time and complete wound contraction compared to that of control. Pigment containing ointment application resulted in effective reduction of congestion, oedema, mononuclear leukocyte infiltration and necrosis along with mild vascular proliferation and reduction of accessory skin structures along with increase in the dermal collagen content over control. The methanol extract showed better wound healing activity on in vivo animal models. In this study, it was found that topical application of yellow pigment extract ointment accelerates wound healing process in an excision wound model. Present study clearly showed that the methanol crude pigment extract from marine pigmented bacteria had a better wound healing property and could be a good source of wound healing compound.

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