7.0. CHAPTER 5

ANTIMICROBIAL AND WOUND HEALING ACTIVITIES OF

S. POLYCYSTUM SODIUM ALGINATE

7.1. INTRODUCTION

Invasion of the body by disease causing organism that become established, multiply and produce symptoms. Bacteria and viruses cause most diseases, also some of the diseases are caused by other microorganism, protozoans and other parasites (Anathanarayan and Paniker, 2006). A less common route of entry of microbes is through the skin, either by contamination of an open wound or by penetration of the intact skin surface (Brook, 2004). In pus infection breaking of the host protective layer- the skin and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (Collier, 2003). Infection of the wound is the successful invasion and proliferation by one or more species of microorganisms anywhere within the body’s sterile tissues. The nature of the infection is the attachment of microorganisms to the host cells and they proliferate, colonize and become better placed to cause damage to the host tissues, and sometimes resulting in pus formation (Collier, 2003). Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites (Bowler et al., 2001). The common pus forming pathogens are Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella sp., Proteus sp., etc. (Wren et al., 1977; Mordi and Momoh, 2009).
Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious process and an understanding of the pharmacology and pharmaco kinetics of the intended therapeutic agents (Kelwin, 1999). To protect from the microorganisms today a best option is antibiotic therapy, now different generation of antibiotics are present. The routine use of antibiotics in both medical and veterinary medicine has resulted in wide spread antibiotic resistance and development of antibiotic resistance genes (Sengupta et al., 2001). Hence the treatments against infections are necessary to select the right kind of antibiotics, appropriate concentrations to be used and also the duration of the antibiotic treatment (Rajalakshmi and Amsaveni, 2012).

There are different sources for getting antibacterial agents from terrestrial or aquatic environments including plants, animals and also from symbiotic microbes. Marine environment is a major part constitutes different varieties of plants, animals and microbes, which yield innumerable numbers of phytotherapeutic agents including antibiotic substances. Seaweeds are marine plants, from which, lot of therapeutic potentials have been derived. Kolanjinathan and Stella (2011) have studied the antimicrobial activity of Ulva reticulate and U. lactuca against human pathogens such as Staphylococcus aureus, Streptococcus epidermis, S. pyogenes, Bacillus subtilis, B. cereus, E. coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumonia and Enterobacter aerogenes. The antimicrobial effect of crude marine mollusc extracts has been reported against human pathogenic bacteria such as E. coli, P.aeruginosa, K. oxytoca, Proteus mirabilis and Serratia liquefaciens (Degiam and Abas, 2010). Ramasamy et al.
(2011) have investigated the antimicrobial potential of polysaccharide from cuttlebone and methanolic extract from body tissue of Sepia prashadi against ten human pathogenic bacteria such as V. cholerae, P. aeruginosa, K. pneumoniae, V. alginolyticus, S. aureus, V. parahaemolyticus, Streptococcus sp., S. pneumoniae, Salmonella sp. and E. coli. Mohanarji et al. (2012) have studied the antimicrobial activity of Lignosus rhinoceros extracts against human pathogens. Govindasamy et al. (2011) studied the in vitro antimicrobial activities of seaweeds such as Gracillaria corticata, Padina tetrastromatica and Halomeda macroloba extracts against human pathogens.

Since there is a need for improved pharmacokinetic properties, which necessitate continued research for novel antimicrobial compounds for the development of drugs (Al Haj et al., 2009). Consequently, pharmaceutical industries are turned their concentration to the traditional sources of compounds derived from plants and animals (Solomon and Santhi, 2008). Many chemically distinctive compounds of marine origin with different biologically activity have been isolated and a number of them are under investigation and/or are being developed as new pharmaceuticals (Blunt et al., 2009; Ravikumar et al., 2009; Lin et al., 2010; Spavieri et al., 2010; Gori and Nzel, 2011; Ravikumar et al., 2011). Marine algae are the potential and diverse source of bioactive compounds, therefore they have been studied as valuable biocidal and pharmaceutical agents (Rangaiah et al., 2010). The benzene extract of G. corticata showed antibacterial activity against S. typhi and E. coli, whereas, the methanol and chloroform extracts showed antibacterial activity against P. aeruginosa (Sastry and Rao, 1994). Moreover, certain marine resources
such as mangroves, seaweeds, sponges and sea grasses have already been proved on their antibacterial and antifungal potentials (Ravikumar et al., 2009 and 2010).

Microbial pathogens, which may invade in to the human body by the way of inflammation and wound on the skin, breathing, drinking water, etc. Among these, would on the skin of human is the best way to invade pathogen in to the human body. Hence, the wound healing is considered as major problem in human being.

Wound healing is a complex biological process including blood coagulation, inflammation, fibroplasia, collagen deposition, and wound contraction (Gurtner et al., 2008). Since the early 1980s, numerous wound dressings have been developed to promote wound healing (Paddle-Ledinek et al., 2006). The ideal dressing needs to ensure that the wound remains moist with exudates, but not macerated, and free of infection, while fulfilling prerequisites concerning structure and biocompatibility (Purna and Babu, 2000). Furthermore, they should be non-cytotoxic and non-antigenetic, guarantee uniform cell distribution, maintain cell viability and phenotype, and should induce migration and proliferation of epithelial cells, fibroblasts and endothelial cells, as well as the synthesis of extracellular matrix components required for wound repair (Balakrishnan et al., 2005). In addition, wound dressings should exhibit ease of application and removal, and proper adherence, in order to ensure that there will no areas of non-adherence left to create fluid-filled pockets for bacterial proliferation (Quinn et al., 1985).

The employment of natural polymers with different mechanical, physical and biological properties as wound dressings has been widely studied based on the many advantages of using these macromolecular agents, such as biocompatibility and their
non-irritant and nontoxic properties (Kordestani et al., 2008; Thomas, 2008). In addition, wound dressings are thought to prevent loss of body fluid, to prevent exudate buildup, to protect the wounds from external contamination, to have sufficient bactericidal effects to inhibit infection and to prepare an optimum wound bed for autografting (Lee et al., 2001).

Alginate has been used in a number of biomedical applications, such as wound dressing, tissue engineering and drug delivery. Several reports have suggested that certain alginate dressings (e.g., Kaltostat) can enhance wound healing by stimulating monocytes to produce elevated levels of cytokines such as interleukin-6 and tumor necrosis factor-a (Thomas et al., 2000). Production of these cytokines at wound sites results in pro-inflammatory factors that are advantageous to wound healing. Sodium alginate (AL) is a natural linear polysaccharide consisting of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) units. Alginate exhibits excellent biocompatibility, non-toxicity, non-immunogenicity, biodegradability and can be easily gelled with divalent cations such as calcium ion (George and Abraham, 2006). Alginate wound dressing is fabricated by introducing calcium ions into sodium alginate solution, and has been used clinically to absorb excess exudates to maintain an appropriate moist environment at the wound surface (Qin, 2008).

Commercial alginate-based dressings include Algisitew M (non-woven calcium alginate fibre, Smith and Nephew), Algosterilw (calcium alginate, Beiersdorf), Kaltocarbw (calcium alginate fibre, Conva Tec.), Kaltogelw (calcium/sodium alginate gelling fibre, Conva Tec.), Kaltostatw (calcium alginate fibres in
non-woven pads, Conva Tec.), Melgisorbw (calcium/sodium alginate gelling fibre, Molnlycke), Seasorbw (calcium/sodium alginate gelling fibre, Coloplast), Sorbalgonw (calcium alginate, Hartman) and Sorbsanw (calcium alginate fibres in non-woven pads, Maersk) (British National Formulary, 2001; Kennedy et al, 2001). Consequently there are numerous patents detailing the production of alginate fibres and dressings (Tong, 1985; Thompson, 1996; Fenton et al., 1997; Mahoney and Howells, 1998; Barikosky, 1999; Kershaw and Mahoney, 1999; Mahoney et al., 1999; Mahoney and Walker, 1999; Horsler, 2000; Qin and Gilding, 2000).

Considering the importance of the above, the present study was carried out to determine the antibacterial activity and wound healing effect of *S. polycystum* polysaccharide- sodium alginate on male Wistar albino rats with the following objectives.

**Objectives**

1. To screen the antibacterial activity of *S. polycystum* polysaccharide- sodium alginate against wound pus forming pathogens.
2. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. polycystum* sodium alginate against wound pathogens.
3. To evaluate the burned wound healing properties of *S. polycystum* sodium alginate on male Wistar albino rats.
7.2. MATERIALS AND METHODS

7.2.1. Screening of antibacterial activities against pus forming human pathogens

7.2.1.1. Preparation of bacterial inocula

The wound pus forming human pathogens such as *E. coli*, *Staphylococcus aureus*, *Klebsilla pneumoniae*, *Streptococcus pyogenes* and *Pseudomonas aerogenosa* were collected from Microbiology laboratory of CMST, M. S. University. The pathogens were individually cultured on nutrient agar plates for 24 h at 37ºC. The cultures were transferred individually in to 10ml of nutrient broth and the broths were incubated at 37ºC for 24h as stock cultures for further experiment.

7.2.1.2. Antibacterial effect of sodium alginate and commercial antibiotics by agar well diffusion method

The antibacterial activity of sodium alginate was performed by agar well diffusion assay (Cappuccino, 1986). The nutrient agar medium was prepared, sterilized and poured in to the sterilized petriplates. After solidification, 0.1ml each of individual test organisms such as *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pyogenes* and *P. aerogenosa* (diluted to an OD 600 of 1) were taken from the respective stock broth and swabbed on agar plates by using sterilized buds. The wells (5mm) were made on the agar plates by using a sterilized well cutter. The different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100mg/ml) of sodium alginate was prepared individually. Then 0.1ml each of individual concentration of sodium alginate was poured in to the wells by using sterilized pipettes and the plates were incubated at 37º C for 24h. After incubation, the antibacterial activity of the test
materials was observed through zone of inhibition (mm diameter) on the plates (Plates 7.1-7.5). Simultaneously, the antibacterial activity of commercial antibiotics such as Amikacin (30mg/ml), Ciprofloxacin (10mg/ml), Cephalexin (30mg/ml), Erythromycin (15mg/ml) and Chloramphenicol (30mg/ml) was also screened against the same pathogens by the above said agar well diffusion methodology.

7.2.1.3. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of sodium alginate was determined according to Lennette et al. (1974). The sodium alginate was solubilized in Muller Hinton Broth (MHB) at the concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.625 mg/ml and was taken in individual test tubes. Then 100µl each of bacterial broth cultures of *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pyogenes* and *P. aerogenosa* (diluted to an OD 600 of 1) were added individually in to the serially diluted test substances. The MHB without sodium alginate was used as control. The inoculated test tubes were incubated at 35º C for 24h. After incubation period, the turbidity was evaluated. The MIC is defined as the lowest antibacterial concentration of the test compound, which inhibits bacterial growth.

7.2.1.4. Minimum Bactericidal Concentration (MBC)

To determine the minimum bactericidal concentration, aliquots of one loopful of the above serially diluted and incubated concentrations were streaked individually on petriplates containing Muller Hinton Agar and incubated at 35º C for 24h. Then the plates were evaluated by comparing them with control plates containing test bacteria without test compounds. The lowest concentration that has no visible growth was considered as MBC.
7.2.2. Wound healing activity of sodium alginate

7.2.2.1. Preparation of hydrogel sheet with sodium alginate

The hydrogel sheet with sodium alginate was prepared as per the methodology described by Murakami et al. (2010). The *S. polycystum* sodium alginate powder at three different concentrations of 100, 500 and 1000 mg were individually spreaded on Whatman No. 1 filter papers, and distilled water was sprayed onto the powder to generate a paste on the powder surface. The paste and supporter were soaked in 50% ethanol for 5 sec, followed by distilled water for 1 min, in order to cause the paste to swell. Swelled paste and the supporter were treated with 2 M CaCl$_2$ for 20 sec in order to produce calcium alginate hydrogel on the paste surface, and were then treated with 5% acetate. Hydrogel sheets were spiked using a needle-pointed flower holder (8.6 needles (0.9 mm in diameter) at 1 cm$^2$:330 pin holes/sheet), and were then soaked in distilled water for 1 min. Following treatment with 1% NaOH for 1 min, sheets were rinsed with distilled water and soaked with distilled water for 3 min. Hydrogel sheets were then treated with 2 M CaCl$_2$ for 20 sec in order to produce calcium alginate hydrogel on the surfaces and holes of the pastes. After soaking in phosphate-buffered saline (PBS) for 1 min, sheets were soaked in distilled water for 10 min.

7.2.2.2. Creation of burn wound

The rats were anesthetised with an intraperitoneal injection of ketaminexylazine (100 mg/kg) and 1 cm$^2$ burn wounds were created on the backs of the animals. This was accomplished using a brass probe that had been immersed in boiling (100°C) water until thermal equilibrium was achieved, which was then
placed without pressure for 20 sec on the previously shaved dorsal region of the rats (Hosnuter et al., 2004). The animals were handled in accordance with aseptic principles in order to avoid exogenous bacterial contamination. Subsequently, the rats were randomly assigned in to four groups of six animals each, according to the treatment of the burn wounds: untreated (Control), and experimental groups dressed with sodium alginate hydrogel sheet of three different concentrations of 100, 500 and 1000mg. After treatment, the wound closure was measured at every 3 days intervals up to 21 days. The percentage of wound closure was calculated as follows by using the initial and final area of wound during the experiment. The wound healing percentage was calculated as follows (Yates et al., 2007)

\[
\text{Wound healing percentage (\%) = } \frac{A_0 - A_t}{A_0} \times 100
\]

where \(A_0\) is the original wound area at the beginning of treatment, and \(A_t\) is the area of wound at the time of closing on every 3 days intervals up to 21 days.

7.2.2.3. Histopathological analysis

At the end of the experiment (21st day), the closed wound tissues (skin) were collected from individual group of treatment and the samples were fixed separately in 10% formalin solution, embedded in paraffin and sectioned in 4 mm (Yamato Kohki Inc., Asaka, Saitama, Japan). Sections were made perpendicular to the anterior-posterior axis and perpendicular to the surface of the wounds. Sections were then positioned on a glass slide and stained with hematoxylin-eosin (HE). In each section, a randomized area (microscopic field, magnification x 200) showing granulation and capillary formation were photographed, and the thickness of
granulation formation and number of capillary lumens per photomicrograph were evaluated.

7.2.3. Statistical analysis

The data obtained in the present study were expressed as Mean ± SD and were analyzed using one way ANOVA at 5% level of significance. Further a multiple comparison test (Tukey’s test) was conducted to compare the significant differences among the parameters using computer software Statistica 6.0 (Statsoft, UK).
7.3. RESULTS

The present study was carried out to determine the antibacterial activity against pus forming pathogens and wound healing effect in Wistar albino rats by *S. polycystum*- sodium alginate. The detailed results are given below.

7.3.1. Antibacterial activity of commercial antibiotics against pus forming human pathogens

The result on antibacterial effect of commercial antibiotics against pus forming human pathogens is given in Table 7.1. The zone of inhibition observed was 15.0 to 18.33 mm by Amikacin (30mg/ml), 14.66 to 18 mm by Ciprofloxacin (10mg/ml), 7.33 to 10.33mm by Cephalexin (30mg/ml), 8.66 to 12.66 by Erythromycin (15mg/ml) and 17.33 to 21.33mm by Chlorampheniciol (30mg/ml), respectively against *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pyogenes* and *P. aerogenosa*.

7.3.2. Antibacterial activity of sodium alginate against pus forming human pathogens

The result on antibacterial effect of sodium alginate of *S. polycystum* against pus forming human pathogens is given in Table 7.2. At lowest concentration (10mg/ml) of sodium alginate, all the organisms were resistant. Whereas at 20 mg/ml concentration of sodium alginate, the growth of the only pathogen *E. coli* was inhibited at a lesser level with the zone size of 2.66mm. At the same time at 30mg/ml concentration of sodium alginate, the growth inhibition rate noted against all the tested pathogens was ranged between 2.0 to 3.0 mm except *P. aerogenosa*. 
Subsequently, when the concentration level of sodium alginate increased, correspondingly the growth inhibition rate of pathogens was also increased. Finally, at 100 mg/ml concentration, the zone of inhibition observed was 11.66, 9.33, 9.0, 8.0 and 6.33 mm against the tested pathogens such as *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pyogenes* and *P. aerogenosa*, respectively (Plates 7.1-7.5).

7.3.3. Minimum Inhibitory Concentration (MIC) of sodium alginate against pus forming human pathogens

The result on Minimum Inhibitory Concentration (MIC) of sodium alginate against the tested pathogens is given in Table 7.3. The MIC of sodium alginate observed was 12.5 mg/ml against *E. coli* and 25 mg/ml each against *S. aureus*, *K. pneumoniae* and *S. pyogenes*, whereas it was 50 mg/ml against *P. aerogenosa*.

7.3.4. Minimum Bactericidal Concentration (MBC) of sodium alginate against pus forming human pathogens

The MBC result displayed with minus (−) sign indicates no growth in the petriplates with Muller Hinton Agar. Single plus (+) sign indicates very less growth of pathogens. Double plus (+++) indicates moderate growth of pathogens and triple plus (++++) indicates normal growth. Accordingly in the present study, the MBC of sodium alginate observed was 12.5 mg/ml against *E. coli*, 25 mg/ml each against *S. aureus*, *K. pneumoniae* and *S. pyogenes*, whereas 50 mg/ml against *P. aerogenosa* (Table 7.4).
7.3.5. Wound healing effect of *S. polycystum* sodium alginate

At the beginning of the experiment (0 day), there was no wound healing activity observed. On 3rd day of experiment, the wound healing rate observed was 10 and 20% at 500 and 1000mg concentrations of sodium alginate treated rats, respectively. However, the wound healing percentage increased with increasing concentrations of sodium alginate and duration of the experiment. Accordingly, at the end of the experiment (21st day), the wound healing rate recorded was 70, 90 and 100%, respectively in 100, 500 and 1000mg of sodium alginate treated groups. At the same time in control group, the wound healing rate observed was only 30% (Table 7.5 and Plate 7.6). The Two way ANOVA test conducted for the data on wound healing percentage as a function of variation between control and different concentrations of sodium alginate treated groups as well as variation between different days intervals of experiment were statistically more significant (F= 24.09474 and 26.33684; P< 0.0001) (Table 7.5a).

7.3.5.1. Histopathological observation of closed wound

The histopathology of closed wound of control and experimental groups of rats are shown in Plate 7.7 (a to d). After 21 days of wound healing experiment, the epithelialisation rate and blood vessel contents were significantly higher in different concentrations (100, 500 and 1000mg) of sodium alginate hydrogel treated groups than control group. Among the experimental groups, the rats treated with highest concentration (1000mg) of sodium alginate hydrogel, the result on the epithelialisation rate and blood vessel content were significantly higher than the lower concentrations (100 and 500mg) of sodium alginate hydrogel treated groups.
7.4. DISCUSSION

Seaweed polysaccharides have prominent antimicrobial activity against bacterial pathogens. For example, *S. muticum* extracts inhibit the growth of Gram positive and Gram negative bacteria of marine forms (Hellio *et al.*, 2001). Chotigeat *et al.* (2004) evidenced that the crude extract of fucoidan extracted from *S. polycystum* inhibited both gram negative (*V. harveyi* and *E. coli*) and Gram positive (*S. aureus*) bacteria. Similarly, Pholdaeng and Pongsamart (2010) stated that the polysaccharide gel extracted from the fruit of durian (*Duriozibethinus*) has better antibacterial activity against *V. harveyi*. Li *et al.* (2006) reported the antimicrobial effect of purified polysaccharide from the seaweed *Lygodium japonicum* against human pathogens. Toppazzini *et al.* (2011) and Benavides *et al.* (2012) have studied the antibacterial activity of antimicrobial peptide LL-37 and alginate against human pathogens.

In the present study also, the antibacterial effect of *S. polycystum* sodium alginate was screened against human pathogens (wound pus forming pathogens) by agar well diffusion method. The maximum zone of inhibition observed was 11.66, 9.33, 9.0, 8.0 and 6.33mm diameter against *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pyogenes* and *P. aerogenosa*, respectively at the highest concentration of 100 mg/ml. Similarly, Vijayabaskar and Shiyamala (2011) have studied the antibacterial effect of methanolic extract of brown seaweeds such as *S. wightii* and *Turbinaria ornata* against human bacterial pathogens. They observed that the *T. ornata* showed 20, 16, 14 and 15mm zone of inhibition against *B. subtilis*, *E. coli*, *S. flexnerii* and *S. aureus*, respectively. *S. wightii* strongly inhibited the growth of *E. coli* (18mm)
and *Aeromonas hydrophila* (15mm) and moderately inhibited the growth against *B. subtilis* (12mm) and *P. aeruginosa* (12mm). Similarly, Chotigeat *et al.* (2004) have reported the antibacterial effect of fucoidan of *S. polycystum* against bacterial pathogens *V. harveyi, S. aureus* and *E. coli* by agar well diffusion method. They reported that the zone of inhibition was 13, 10 and 9 mm, respectively against *V. harveyi, S. aureus* and *E. coli* at 12 mg/ml concentration of fucoidan. Pholdaeng and Pongsamart (2010) have studied the antibacterial activity of polysaccharide gel extracted from *D. zibethinus* against bacterial pathogen *V. harveyi* through agar well diffusion test. The result inferred that the inhibition zone of sharp and clear margin with a diameter of 20.43, 16.47, 12.15, 10.70 and 8.88 mm on the agar plates with various concentrations such as 50.0, 25.0, 12.5, 6.3 and 3.1 mg/ml of polysaccharide gel, respectively.

In the present study, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sodium alginate observed was 12.5 mg/ml each against *E. coli*, 25 mg/ml each against *S. aureus, K. pneumoniae*, and *S. pyogenes* and 50mg/ml each against *P. aerogenosa*. Similarly, Ramasamy *et al.* (2011) have studied the MIC and MBC of polysaccharide of cuttlebone and methanolic extract of body tissue of cuttle fish *Sepia prashadi* against human pathogens. They reported the MIC values of cuttle bone polysaccharide against bacterial strains such as *V. cholera, K. pneumonia, V. alginolyticus, S. aureus, V. parahaemolyticus* and *Streptococcus* sp. were reported as 80, 100, 80, 80, 60 and 80 mg/ml, respectively. Whereas the methanolic extract of cuttle fish tissue showed, the MIC value inbetween 80 and 100mg/ml against *V. cholera, S. aureus, V. parahaemolyticus* and *E. coli*, respectively. Similarly Chotigeat *et al.* (2004) have
reported the MIC value of 6 to 12mg/ml of *S. polycystum* fucoidan against bacterial pathogens like *V. harveyi*, *S. aureus* and *E. coli*, respectively. Likewise, Pholdaeng and Pongsamart (2010) stated that the MIC and MBC values of polysaccharide gel (PG) extracted from *D. zibethinus* were 6.3mg/ml and 12.5mg/ml against bacterial pathogen *V. harveyi*.

Kolanjinathan and Stella (2011) have studied the MIC of extracts of *Ulva reticulata* and *U. lactuca* against human pathogens. They found that the MIC value of *U. reticulata* against bacteria was ranged between 2.50 to 80 mg/ml. The lowest MIC (2.50 mg/ml) value was recorded against *S. aureus, B. subtilis, B. cereus, K. pneumoniae* and *E. aerogens*. Likewise, the MIC value of *U. lactuca* against the tested bacteria was ranged between 1.25 to 80mg/ml and the lowest MIC value of 1.25 mg/ml was recorded against *K. pneumoniae*. Results obtained in the present study indicated that the extracted sodium alginate of *S. polycystum* may have potential antibacterial applications against human pathogens.

Considering the fact that, the use of dressing films is very important in order to prevent the entry of pathogenic microbes in to the human body and avoid external bacterial contamination (Kordestani *et al.*, 2008; Thomas, 2008; Albuquerque-Junior *et al.*, 2009). Various studies have suggested that chitin/chitosan (Ishihara *et al.*, 2001; 2002; Obara *et al.*, 2003; 2005) and alginate (Quinn *et al.*, 1985; Thomas *et al.*, 2000; Balakrishnan *et al.*, 2005) accelerate wound healing, and remedies on using chitin/chitosan or alginate in wound treatments are already in the market. However, chitin/chitosan and alginate are currently used as filaments, fibers, powders, granules, sponges or composites with cotton or polyester
In the present study, the wound healing activity of sodium alginate was determined on burning wound created rats for 21 days. In control group, only 30% wound healing rate was observed on 21st day of experiment. But in the experimental groups treated with different concentrations (100-1000mg) of sodium alginate hydrogel sheet, the wound healing rate recorded was 70 – 100%, respectively on 21st day of experiment. Here the wound healing rate of sodium alginate was recorded as increased with increasing concentrations of sodium alginate. Similarly, Dantas et al. (2011) have investigated the effect of combination of sodium alginate and chitosan based films on burn wound healing. They observed that on 8th day, the inflammatory infiltrate was acute in the control group and sub acute in the experimental groups. Within 14 days, the infiltration was chronic in all the groups. The severity of the inflammatory infiltration was ranged from intense to moderate within 8 days and from moderate to absent on 14th day. On 8th day, the inflammatory profile was significantly (P< 0.05) more intense in the control group than in the laser-irradiated (LT), dressed with cellulose & laser-irradiated (CLLT) and dressed with sodium alginate/chitosan-based film (SCLT), but no other significant (P> 0.05) difference was evident among the groups, either on 8th or 14th day mark. Murakami et al. (2010) have studied the effect of hydrogel blends of chitin/chitosan, fucoidan and alginate (ACF-HC) on wound healing effect in rats. They pointed out that the positive effects on wound closure, progress of wound contraction and re-epithelialization were observed only in ACF-HC-treated wounds during the 18th day of observation. Lee et al. (2012) have investigated the effect of alginate hydrogel dressing on wound healing in diabetic rats. They observed that the
wound area treated by alginate-chitosan-calcium PGA powder (AL–CS–PGA) seemed to be smaller than other samples on 7th and 14th days after wounding. After 3rd, 7th, 9th, 14th, and 21st days, a significant increase (P< 0.05) in the rate of wound closure was noticed in the AL–CS–PGA when comparing to the commercial product. Comparing with other groups, the wound treated with AL–CS–PGA exhibited the fastest wound closure.

In the present study, the histological observation of closed wound skin showed significantly increased epithelialisation rate and blood vessel content in experimental groups than control. Similarly, Dantas et al. (2011) have studied the histopathology of burn wound healed skin treated by combining sodium alginate and chitosan based films and low level laser therapy. They reported that the epithelialisation rate and blood vessel content of burn wound healed skin treated by combining sodium alginate and chitosan based films and low level laser therapy was significantly increased than control group after 8 and 14 days of experiment. The advantages of the use of cellulose dressing films on burn wounds are well established in experimental models and clinical trials (Thomas, 2008; Alves et al., 2009).

The results from animal testing on wound healing in rat models indicated that S. polycystum sodium alginate hydrogel can be used as wound dressing that can accelerate wound healing. The application of sodium alginate hydrogel sheet significantly stimulated repair of burned healing-impaired wounds in rats.
7.5. SUMMARY

The present study was undertaken to screen the antibacterial activity against pus forming bacterial pathogens and wound healing activities of *S. polycystum* – sodium alginate on burned wound rats. The highlights of the study are summarized below.

- The antibacterial effect of sodium alginate extracted from *S. polycystum* against human pus forming bacterial pathogens was screened by agar well diffusion method. In this study, the maximum zone of inhibition observed was 11.66, 9.33, 9.0, 8.0 and 6.33mm diameter against *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pyogenes* and *P. aerogenosa*, respectively at the highest sodium alginate concentration of 100 mg/ml.

- The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of sodium alginate against bacterial pathogens were determined. The MIC and MBC of sodium alginate were observed as 12.5 mg/ml each against *E. coli*, 25 mg/ml each against *S. aureus*, *K. pneumoniae*, *S. pyogenes* and 50mg/ml against *P. aerogenosa*.

- The wound healing activity of sodium alginate was determined on buring wound created rats for 21 days. In control group, only 30% wound healing rate was observed on 21\textsuperscript{st} day of experiment. But in the experimental groups of rats treated with different concentrations (100 - 1000mg) of sodium alginate hydrogel sheet, the wound healing rate recorded was 70 – 100%, respectively on 21\textsuperscript{st} day of experiment. The wound healing rate in
experimental rats was increased with increasing concentrations of sodium alginate.

- The histopathology of closed wound skin was analyzed at the end of the experiment (21 days). The epithelialization rate and blood vessel content of closed wound skin were significantly increased in experimental groups than control