



CHAPTER V

DISCUSSION AND CONCLUSION

Scaphium scaphigerum fruits have been used not only as traditional medicine for relieving cough and conjunctivitis but also as refreshing beverage which is the well known local product from Chantaburi province. It has been reported that polysaccharide PP III and histamine were the main constituents of the water extract of these fruits (Hayman *et al.*, 1988: 338, Chen *et al.*, 1996: 39-41). Polysaccharide was also identified as a component of the water extract of *S. scaphigerum* fruit in this study. Mounting evidences have been reported that many polysaccharides from plants possess immunomodulating and antitumor activities (Hildebert *et al.*, 1988: 119-126; Wagner, 1990: 1217-1222; Guo, 2000: 307-316; Paulsen, 2001: 939-950; Kim *et al.*, 2003: 1281-1292; Shao *et al.*, 2004: 1103-1111; Guo *et al.*, 2004: 1124-1132). In addition, a preliminary study showed that the water extract of *S. scaphigerum* fruits demonstrated potential immunostimulatory effect on human PBMCs. Therefore this study aimed to investigate the immunostimulatory activities of the water extract of *S. scaphigerum* fruits on immune cells of both specific and nonspecific immunities. *In vitro* T lymphocyte activation and macrophage functions were used to evaluate the effect of the extract.

The water extract metabolically stimulated human PBMCs in a concentration dependent manner by MTT assay. The positive result from the MTT assay represents cell activation as well as cell proliferation (Gerlier and Thomasset, 1986: 57-63; Weichert *et al.*, 1991: 139-144). The stimulatory effect of the extract on PBMCs was confirmed by tritiated thymidine incorporation assay, which determines cell proliferation. When compared to 10 $\mu\text{g/ml}$ PHA, it was observed that the water extract could metabolically activate PBMCs in a higher magnitude than stimulate the cell proliferation. It is suggested that the water extract of *S. scarphigerum* fruits possesses immunostimulatory activities. So, the water extract was investigated in the next step for its effects on both types, the innate and the adaptive, of immune responses. *In vitro* T cell activation and macrophage function were used for evaluating immunostimulatory activities of the

extract by determining activated marker expression and functions of activated immune cells.

T cells are central to the specific immune response by providing helper activity, proinflammatory cytokine secretion (e.g., TNF- α), suppressor (regulatory) function through direct cellular interactions and/or inhibitory cytokine secretion (e.g., IL-10 and TGF- β), and cytotoxic effector function. Standard laboratory methods for assessing *in vitro* T-lymphocyte function include measurement of lymphoproliferation, assessment of cytokine secretion after T-cell activation, and evaluation of surface antigens, such as CD25 or CD69, expressed on activated T-cells (Julian, 1998: 1-20). The expression of CD69 on T-cell activation in response to the *S. scaphigerum* fruit extract was performed in this study by flow cytometry using antibodies against CD3 and CD69. It was demonstrated that the extract slightly stimulated CD69 expression on T lymphocytes. It could increased the CD69 expression on CD3⁺ cells only 3 folds at 600 $\mu\text{g/ml}$ while 10 $\mu\text{g/ml}$ PHA could increased the expression up to 58 folds. It is suggested that the extract have weak stimulatory activity on T cells activation. However, this activation should be confirmed by determining markers of other T cell activation such as cytokines. Furthermore, the immunostimulating activity of the extract on humoral immune response should also be evaluated because many polysaccharides were reported to activate B lymphocytes *in vivo* (Matsumoto *et al*, 2003: 111-118; Kim *et al*, 2003: 1281-1292; Guo *et al*, 2004: 1124-1132) The pharmacological effect of the extract on B cell activation should be done in the future.

Biological activities of polysaccharides from plants have been one of the attractive studies in biochemical and medical fields because of their immunomodulating and anti-tumor effects. Many polysaccharides from medicinal plants have been demonstrated immunostimulation both *in vivo* and *in vitro*. Most of them were reported to stimulate innate immunity, including complement activation, phagocytosis of neutrophils and macrophages (Shin *et al.*, 2002: 469-482; Kun *et al.*, 2004: 1029-1038; Kun and Young, 2005: 1225-1233). This drew the interest for this study to investigate the effect of the water extract of *S. scaphigerum* fruits, which also contains polysaccharide, on innate

immunity. It is showed that the extract stimulated phagocytic activity of murine macrophages J774A.1 in a concentration dependent manner. The extract could also stimulate nitric oxide production from these cells. It is postulated that the polysaccharide in the extract can induce macrophage activation *in vitro*. This macrophage activation should not come from histamine; another constituent of the water extract from *S. scaphigerum* fruits, because it was reported that histamine could inhibit macrophage function *in vitro* (Azuma *et al.*, 2001: 1867-1875).

Phagocytosis via macrophages or neutrophils is triggered by interaction of pathogens with specific receptors on the phagocyte cell surface, including Fc γ receptor (Fc γ R), complement receptor (CR), mannose receptor and Toll-like receptor (TLR). It is less likely that Fc γ R or CR would play role in the stimulatory effect of the water extract on macrophage because the signal transduction pathways activated by FcRs or CR involving antibody-dependent mechanism (Sanchez and Rosales, 1998: 521-533; David and Adrian, 2002: 825-852). It is possible that mannose receptor, which primarily presents on macrophages and recognizes a range of carbohydrates on antigens, may involve in the activation effect of the extract (Alan *et al.*, 1990: 1785-1794). Interacting with a TLR could be the other possibility of the extract on macrophage J774A.1 cells because there were reported that plant polysaccharides could activate macrophages via TLRs (Han *et al.*, 2003: 1301–1312; Yoon *et al.*, 2004: 1477-1487.; Shao *et al.*, 2004: 1103-1111; Nakaya *et al.*, 2004: 93-100). This possibility should be investigated in the future.

In conclusion, it is demonstrated for the first time that the water extract of *Scaphium scaphigerum* fruits exerted *in vitro* immunostimulatory activities. The extract could definitely stimulate macrophage phagocytosis and nitric oxide production. However, it had slight effect on T cell activation. It is postulated that the polysaccharide PPIII plays a role in this immunostimulation. There is still much to be investigated for the effect of this extract in the immune response, both *in vitro* and *in vivo*.