CHAPTER V
CONCLUSIONS

Plant cell culture of *Thunbergia laurifolia* leaves could create secondary metabolite, rosmarinic acid, which usually found in the leave part of normal plant. The media for callus subculture was MS media with hormones NAA 1 mg/L and BA 2 mg/L. A small amount of rosmarinic acid was found in the ethanol extract of callus and analyzed by HPLC with the validated method.

The processes of microencapsulation in this study were spray drying and freeze drying process and using sodium alginate and polysaccharide gel extracted from durian fruit hulls (DG) as polymer. Spray drying process created spherical shape microparticles where as freeze drying process created irregular flatted-shape powders. The DG polymer caused shrinking on the particles obtained from SD process more than AG. The surface of microparticles from AG was smoother than DG while the mixture of polymer produced smoother surface than those using pure DG. Crosslinked spray dried microparticles created larger size than those of noncrosslinked spray dried microparticles. The microparticles from SD process were very small as compared to the powders obtained from FD process. DG polymer made the particles larger than those of AG polymer. The mixed polymer of AG and DG affected the reduction of particle size compared to those formulations using pure DG. In case of solid state characterization, rosmarinic acid was incorporated into the polymer. An entrapment efficiency of crosslinked spray dried formulations provided much more lower of rosmarinic acid amount than noncrosslinked since rosmarinic acid lost in the crosslinking process and caused the reducing of %EE of spray dried crosslinked microparticles significantly to the lower than 5%. The crosslinked spray dried formulations were not chosen to study their release and need further study. Furthermore, the entrapment efficiency of the formulations processed from freeze dry were higher than spray dry. Thus in entrapment efficiency case, the FD process provided the better than SD process. However, in case of morphological aspect, SD provided the spherical-shaped products.

As for the release and stability of rosmarinic acid from the microparticles, the increasing of DG polymer resulted in the lower release of RA and the increasing of polymer concentration caused the lower release as well. In the case of polymer types, DG yielded slower release than AG. For stability, AG seemed to be able to increase the stability through spray drying process while DG through freeze drying process.

So in encapsulated RA microparticles, the process affected the morphology and particle size, entrapment efficiency and stability as mentioned above that SD made the particles smaller and more spherical than FD. However, FD provided higher entrapment efficiency and better stability than SD process. While the two processes did not show much different in release studies. The polymer types also effected to the morphology, size, entrapment and release study as AG made smoother surface, smaller size, less entrapment efficiency in SD and faster release profile while AG provided better stability in SD and DG in FD. When comparing the stability of the two processes, FD process showed better stability result than SD process.

In the encapsulated TC microparticles studies, *T. laurifolia* callus extract was prepared, harvested and extracted. The extract was analyzed and no rosmarinic acid found in the water extract part. While in ethanol extract of *T. laurifolia* callus was found but in a small amount thus further experiment needs to be developed for higher producing of rosmarinic acid as secondary metabolite from callus culture.

As for the formulations for encapsulated TC microparticles, five formulations chosen before were performed in this study. DG microparticles showed rougher surface than AG. The higher polymer concentration provided the larger size of the particles. In the case of using pure polymer, DG formulations showed larger particle size than AG formulation. The mixed polymer of AG and DG in formulation TC-FC-2DGAG1:1 showed smaller particle size than that using the pure polymer at the same concentrations. For the solid state case, the callus extract was incorporated into the polymer. However, TC-SN-1AG showed the peak at 124 $^{\circ}$C which was the exothermic peak so recrystallization occurred at this temperature. In freeze drying process, DG polymer exhibited better incorporation of the extract into polymer structure than AG. Moreover, entrapment efficiency of the freeze dried microparticles showed significantly higher than that of spray drying process. On the other hand, the result of release study in encapsulated TC microparticles was different from the encapsulated RA microparticles as AG caused slower release more than DG and TC-SN-1AG showed the slowest release rate. The TL callus extract exhibited the decreasing of rosmarinic acid stability much more than those of all formulations in all conditions and TC-FC-2DGAG1:1 showed the best result of rosmarinic acid stability.

So in encapsulated TC microparticles, the process and polymer types affected the same results as in encapsulated RA microparticles studies in the part of morphology and particle size, entrapment efficiency as mentioned above but the release studies results were different and the mixed polymer was able to improve the most in stability.

TC MPs-loaded freeze dried patches showed more slower release profiles than the TC- loaded microparticles as rosmarinic acid needed to release through the patch layer. The TC microparticles exhibited slower release than TC. The particles processed through AG spray drying process was observed to be the slowest release. The TC MPs-loaded patch improved the stability of rosmarinic acid in all storage conditions and PTC-FC-2DGAG1:1 formulation provided the best stability result at the end of storage. So the mixed polymer of 2% DG with AG at the ratio 1:1 prepared by FD process created the most protective property for protecting rosmarinic acid which was included in TC.

In conclusions, *T. laurifolia* callus extract using rosmarinic acid as chemical marker, was used as a model of natural extract obtained from plant callus culture. The extract could be encapsulated in the microparticles made of sodium alginate and DG using spray drying and freeze drying process. The obtained microparticles and the wound dressing patch containing the microparticles of callus extract provided suitable release profile and improved stability of active substance. So this wound dressing system will provide a great potential to pharmaceutical and biomedical applications in the area of wound healing.