CHAPTER V
CONCLUSION

In these studies, the specifically polyclonal antibody against MuA was produced, characterized and used as high sensitivity, simple and rapid tool to determine MuA accumulations of various samples by indirect competitive ELISA with the optimal concentration range of assay extends from 0.17-15.62 µg/ml, the maximum RSD of intra- and inter-assay were 4.92 % and 4.85%. The percentages of recovery in the range of 97.6–101.4% and correlation between the results from the ELISA and HPLC exhibited a high coefficient of determination (R^2=0.994).

The callus cultures were induced from leaves of *M. alba* on MS medium supplemented with 0.1 mg/l TDZ and 1 mg/l NAA. Root cultures were regenerated from calli of *M. alba* on MS medium contains 1 mg/l NAA. After inductions, cell and root cultures were transferred into each liquid medium. The results show that 3-week-old cell and root cultures exhibited high growth rate and production period. Various elicitors including yeast extract (YE), methyl jasmonate (MJ), chitosan (CT), salicylic acid, extracts of *Phoma* sp., *B. subtillis* and *Trichoderma* sp. were used in these studies. After addition of elicitors on the high growth rate and production period, the maximum enhancement of total MuA level in cell suspension culture of *M. alba* was occurred in the 100 µM SA treated with 48 h exposure time group (37.95±1.54 mg/g DW, increased 3.71 folds). Whereas, maximum increasing percentage of extracellular MuA in the cell cultures was obtained by addition of 2% TE (8.58±1.56 mg/g DW, increased 9.61 folds). All selected elicitors can significantly increase total MuA in cell suspension culture except CT. In root cultures, the highest elicitation effect on enhancement of total MuA (23.81±0.28 mg/g DW, increased 6.56 folds) and extracellular MuA (6.69±0.08 mg/g DW, increased 10.29 folds) were obtained by addition of 2 mg/ml YE. Almost elicitors can significantly enhance total MuA productions in the root cultures except CT and TE. These results show that 3-week-old *M. alba in vitro* cultures treated with YE and SA as elicitors can

produce high yield of MuA compared with several years old intact *M. alba* root (22.39±1.06 mg/g DW) and root bark (26.86±2.69 mg/g DW).

The optimum inoculum size of cell cultures (approximately 9 g of cell initial FW) was accumulated higher total MuA level (82.69±3.76 mg/g DW, 3.08 folds higher) than the intact root bark. Therefore, optimization of the inoculum size is an important high potential strategy to enhance the MuA accumulation in *in vitro* cultures of *M. alba*.

Beside that both of *in vitro* cultures showed strong tyrosinase and α-glucosidase inhibition. Especially the root culture extracts exerted a higher anti-α-glucosidase activity compared with 1-deoxynojirimycin (58 folds, approximately). According to high accumulations of MuA and bioactivities, both *in vitro* cultures of *M. alba* are potential sources for production of MuA and secondary metabolites in large scale cultures.

Endophytic microbial were isolated from *M. alba*. The positive strains for enhancement of MuA production from *in vitro* cultures of *M. alba* were identified as *B. subtillis*, *Phoma* sp. and *Trichoderma* sp. These endophytes are effective elicitors in both cell suspension and root cultures. In addition, not only increase extracellular MuA levels in cell suspension culture of *M. alba* but the *Trichoderma* sp., endophytic fungus isolated from this plant can also produce compounds with the same or similar structures as MuA together with anti-tyrosinase and anti-α-glucosidase compounds. The results suggest that *Trichoderma* sp. is an alternative valuable source of bioactive secondary metabolite compounds. Therefore, isolation and identification of secondary metabolites from *Trichoderma* sp. are interesting for future study.