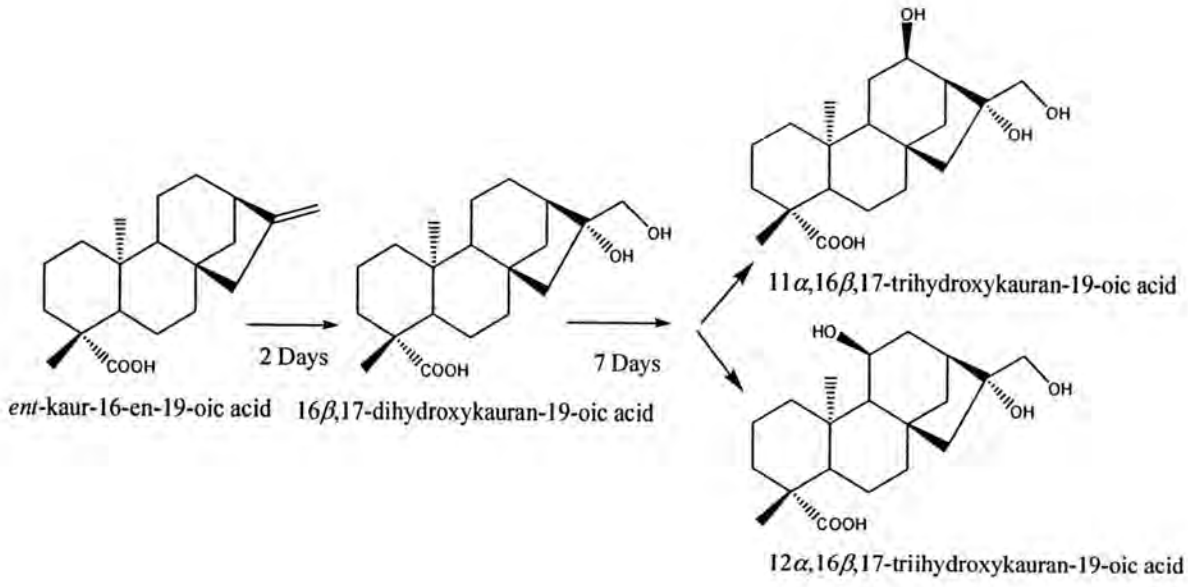
CHEPTER V

CONCLUSION

The biotransformation of *ent*-kaurenoic acid, *ent*-kaur-16-en-19-oic acid, from the stem bark of *Croton oblongifilius* Roxb. was carried using the fungus *Psilocybe cubensis*. After 2 days incubation the first product, compound **2**: *ent*-16β,17-dihydroxy-kauren-19-oic acid (21.1%), was afforded. After the longer period of incubation for 9 days three metabolites; compound **2** (11.5%) and the two novel metabolites, *ent*-12α,16β,17-trihydroxy-kauran-19-oic acid (**3**) and *ent*-11α,16β,17-trihydroxy-kauran -19-oic acid (**4**) at 8.3% and 2.8%, respectively, were isolated. The metabolites were identified by spectroscopic methods and X-ray crystallography. The biotransformation of *ent*-kaur-16-en-19-oic acid by *P. cubensis* was showed in scheme 8. The biological evaluation revealed that all products showed the lower level of biological activity on the cytotoxicity test toward 6 tumor cell lines; K562, SW620, BT474, KATO-3, HEP-G2, and CHAGO in comparison with *ent*-kaurenoic acid. The anti-bacteria were also observed against 4 bacteria; *Bacillus cereous*, *Staphyllococcus aureus* ATTC 25923, *Escherichia coli* ATTC 25922 and *Pseudomonas aeruginosa* ATTC 27853. The anti-bacterial activity showed that compound **2**, **3** and **4** were inactive against all tested bacteria. Over all results from the enzyme study concluded that this enzyme is an induced oxygenase enzyme located in microsomal fraction. NADPH and FAD were required as cofactors for catalysis where the reaction mixture consist of 0.15 mM *ent*-kaurenoic acid, 4 μM FAD, 0.15 mM NADPH and 200 μg protein sample at pH 7.6 in 800 ml total volume and the reaction was performed at 30°C. The results cannot identify the enzyme responsible for compound **2** production but have shown that the enzyme is NADPH dependent associated with the *ent*-kaurenoic acid oxygenation due to the method of NADPH consumption assay and the specific activity was found at 3.74 U/ug protein. The protein identification using MALDI/Tof MS and peptide mass mapping showed the matched peptide with the Cyt P450 of *Aspergillus fumigatus* Af293 at 12 % sequences coverage.



Scheme 8. Biotransformation of *ent*-kaur-16-en-19-oic acid by *P. cubensis*

Suggestions for future work

1. Since the biological activities of all products are very interesting, especially the anti-HIV reveres transcriptase. Further studies of the potential biological activities of the biotransformed products should be carried out.

2. The identification of the enzyme involved in this biotransformation process requires more studies to prove which type of enzyme as well as the determination of the kinetic mechanism.