CHAPTER 4

CONCLUSION

A new assay was developed for steviol glycosides. A novel method of processing extracts of *Stevia rebaudiana* leaves, based on fungal fermentation, was standardized in the laboratory.

4.1 COLORIMETRIC ASSAY FOR STEVIOL GLYCOSIDES

1. A colorimetric assay for steviol glycosides was developed on the basis of selective hydrolysis of the glycosides at the carboxyl attached glucose in C19.

2. The assay is specific since the carboxyl attached glucose is the only glucose cleaved on alkaline hydrolysis.

3. The assay is simple and does not require expensive equipment.

4. It is capable of scaling up to be a high throughput method since samples can be processed simultaneously.

5. The use of glucose as a standard ensures that expensive standards do not have to be procured.

6. Total steviol glycoside content of the samples are assayed rather than individual glycosides, to give a complete picture of the steviol glycoside content of the samples.
7. Preparation of leaf extracts was necessary since interfering substances were causing false high values.

8. The proposed methodology is specific, sensitive, robust and easy to standardize.

9. Future developments include standardizing methods of extraction of the glycosides so as to prevent the purification step.

4.2 FERMENTATIVE PURIFICATION OF STEVIOL GLYCOSIDES

1. A biological method for decolorisation of stevia leaf extracts, which contain color and odor causing impurities, was developed.

2. The method uses white-rot fungi, *Pleurotus ostreatus*, to remove the colored polyphenols and pigments of the extract.

3. Tannin acyl hydrolase and Gallic acid decarboxylase activity was followed during the course of the fermentation.

4. The activities of the enzymes were correlated with the reduction in total polyphenol content of the ferments.

5. A maximum of 22% and 40% color reduction at 470nm and 670nm was achieved within 72 hours.

6. The free radical scavenging activity of the extract was maintained despite the color reduction.

7. The addition of dextrose influenced the production of enzymes and decolorisation positively.
8. This method represents a green approach to phytochemical purification.

9. Future work may include attempts to increase the efficiency of the process in terms of total decolorisation and fermentation time required.

4.3 FUTURE DIRECTION OF WORK

1. Evaluate solid phase extraction as an alternative to TLC for purification in the assay.

2. Characterization of phenolics by molecular techniques

3. Improvement of fungal strain for better decolourisation