

**A PRELIMINARY STUDY OF  
CHEMISTRY AND TOXICOLOGY OF  
A NATURAL ANTIPROTOZOAL AGENT**

**A thesis submitted for the degree of  
Master of Rural Science of the University of New England**

**by**

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**March, 1994**

## PREFACE

The studies presented in this thesis were completed by the author while a post graduate student in the Department of Biochemistry, Microbiology and Nutrition, the Faculty of Science, the University of New England, Armidale, N.S.W., Australia. Assistance given by other persons is indicated in the text or in the list of acknowledgements. All references cited are included in a bibliography. The work is otherwise original.

\* \* \*

*I certify that the substances of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.*

*I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.*

*March, 1994*

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## ACKNOWLEDGMENTS

I wish to express my most sincere gratitude to my supervisor, Dr. R.G. Gerdes, for his advice, guidance and encouragement over the past three years. I am indebted to him for stimulating my initial interest in the studies presented here, for introducing me to the joys of research and for his ability in criticism of the manuscript. I am also indebted to Dr. R.P. Learmonth as my acting supervisor while Dr. R.G. Gerdes was overseas on study leave (between September 1991 and September 1992).

Thanks are also due, through the Rector of Andalas University, Padang, Indonesia, to the Indonesian Government for leave during my candidature in the University of New England Armidale, Australia. I am also indebted to the Australian International Development Assistance Bureau (AIDAB) for financial assistance in the form of an AIDAB Scholarship.

Dr. D. Tucker from the Department of Chemistry, is gratefully acknowledged for purification of *Enterolobium* leaf extract. I am thankful to Dr. S.H. Bird for his advice regarding the technique for enumeration of rumen protozoa.

Thanks are also due to Mrs. J Dawson, Mrs. F. MacDonald, Mrs. J. Baker, Mrs. R. Busby, Mr. R. Wicks, and Mr. E. Thomson for their invaluable assistance with many routine laboratory procedures. Special thanks are also due to Mr. R. Woodgate for his aid in feeding the experimental animal, to Mr. J. Hanlan for ordering and supply of chemicals and equipment, and to Mrs. R. Fox and Mrs. C. Davies for library and administration assistance.

I also wish to thank to my fellow students Mrs. V. Rao, Mr. M. Dornbusch and Mr. G. Moran for many useful and stimulating discussions. My particular thanks to Dr. D.R. Evans and Ms. R. Heyman for their willingness to read the first draft of the manuscript. Thanks are also due to the Indonesian Student Association of Armidale (HKMIA) for a constructive atmosphere and assisting the progress of study by warm and friendly relationships.

Finally, I dearly wish to thank my parents, brothers and sisters for their moral support and for their enduring patience.

## SUMMARY

A survey of some tree forage supplements with potential to manipulate rumen protozoa found that *Enterolobium cyclocarpum* Griseb. showed antiprotozoal activity (Leng *et al.*, 1992). However, there is no further information available on the active agent(s) present in this plant.

Therefore, a preliminary study on the chemistry and toxicology of the active agent present in the extract of *Enterolobium* leaf was carried out. Working on the hypothesis that the active agent was a saponin, the active agent was isolated by maceration of the water-soluble compounds from the dried leaves, followed by partitioning between water and n-butanol layer with the butanol layer containing the active fraction. Further fractionation of the leaf extracts was guided by two bioassays, haemolytic assay and antiprotozoal assay.

A haemolytic assay using sheep erythrocytes has been developed to test the presence of saponins. The purified fractions showed strong haemolytic activity, although comparative studies with white saponin and Alkanate 3SL3 (sodium lauryl diethoxysulphate) indicated that haemolytic activity may not reflect a parallel ability to lyse rumen protozoa. White saponin, the most potent agent observed to lyse red blood cells, has been found to have no activity on protozoa, whereas Alkanate 3SL3, the second in these comparative studies, has been found to effectively eliminate protozoa from the rumen of lambs (Burggraaf, 1980). Therefore, the antiprotozoal assay using rumen protozoa was developed. The antiprotozoal assays were conducted under two different conditions, aerobic and anaerobic systems.

Recent *in vitro* results suggest that those purified fractions exhibiting a pronounced ability to immobilise rumen protozoa tend to produce high lytic activity with these cells. Study on the possible synergistic interaction effects among components in the leaf extract indicated that activity was not affected after fractionation. It appears that the results are consistent within experiments but not between experiments and this may be due to the number of protozoa fluctuating widely between samples from the donor animal.

Gas-Liquid Chromatography analysis of trimethylsilyl derivatives of monosaccharides liberated by acid hydrolysis of leaf components was employed to help elucidate the structure of the active agents. The proposed structures of the active agents were reported. Since the nature of the aglycone was not studied in this project, it was assumed that the analysed fractions are composed of saponins containing the single aglycone machaerinic acid lactone, as identified in *Enterolobium contortisiliquum* (Delgado *et al.*, 1984). It appears that the structure of the active agent is unlikely to be substituted with more than one sugar. Glucose seems predominant followed by rhamnose, arabinose and xylose, while galactose is not present in all fractions.

In summary, the purified fractions obtained after extensive reverse-phase HPLC were, in general, still unresolved mixtures of saponins.

## ABBREVIATIONS

Ara	Arabinose
ATP	Adenosine triphosphate
DHPS	Dihydropterolate synthetase
Gal	Galactose
GLC	Gas Liquid Chromatography
Glc	Glucose
HPLC	High Performance Liquid Chromatography
MAL	Machaeinic acid lactone
NMR	Nuclear Magnetic Resonance
PABA	Para amino benzoic acid
PExt	Partitioned extract (crude butanol extract)
Rha	Rhamnose
TMS	Trimethylsilylation
tRNA	transport Ribonucleic Acid
VFA	Volatile Fatty Acids
Xyl	Xylose

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