Chapter 6

Discussion and Conclusion

6.1 Selection of plant

In a preliminary experiments it could be shown that leaf extracts of *Terminalia* species have antibacterial activity against several humans pathogenic bacteria (Eloff, 1999b). Because *Terminalia* species grow widely in South Africa and the research group of the Phytomedicine Programme at the University of Pretoria has developed substantial expertise in isolation and characterization of antibacterial compounds from members of the Combretaceae the aim of this study was to isolate antibacterial compounds from a *Terminalia* species and to determine whether such a compound can be used to treat animals or humans against infections. Hopefully this may lead to an inexpensive treatment of infections in humans in poor rural populations.

6.2 Extraction

The first step in the process was to determine the best extractant. Several extractants of varying polarity and selectivity were used to determine the quantity extracted, the chemical fingerprint of the extract and the antibacterial activity of the different extracts were determined. The results were in the main similar to the results obtained with

Combretum microphyllum (Kotze and Eloff, 2001). Tetrahydrofuran (91), acetone (64) and ethanol (126) had the highest total antibacterial activity (Table 2.6). Tetrahydrofuran (60.6 mg), methanol (39 mg), acetone (40.6 mg) and ethanol (52.6 mg) had the highest yield in terms of mass extracted from the 500g of leaf material that was started of with. Despite not scoring the highest, acetone was selected as extractant because of its miscibility with water, its non-toxicity to the microorganisms, its ease of removal and its safety in laboratory use as noted in earlier work on *Combretum erytrophyllum.* (Eloff, 1999b).

Seven *Terminalia* species occurring in southern Africa were analyzed to determine which was the most promising to use for isolating an antibacterial compound.
A second aspect was to confirm antibacterial activity with other members of the *Combretaceae.* The minimum inhibitory concentration (MIC) values for antibacterial activity of *T. sericea (*0.16 mg/ml*), T. prunoides (*0.13 mg/ml*), T. phanerophlebia (*0.11 mg/m*l)* and *T. gazenzis* (0.06 mg/ml) was as low as that of the positive control, gentamicin (0.16 mg/ml), against *S. aureus.* Against *E. coli, T. sericea (*0.31 mg/ml*), T. phanerophlebia (*0.21 mg/ml*), T. sambesiaca (*1.02 mg/ml*)* and *T. brachystema* (0.56 mg/ml) had the lowest MIC values. The results against the Gram-negative organisms showed *T. gazenzis* (0.25 and 0.25 mg/ml*)* and *T. sericea* (0.63 and 0.16 mg/ml) had the best antibacterial effect.

The *T. sericea* extract had on average the second best antibacterial activity and was chosen as the target plant for further investigation. This plant also had the advantage

that it is the most widely distributed in South Africa of the plants tested in this study. Development of an active product could benefit rural people in need of a cheap, effective antibacterial agent.

6.3 Isolation of terminoic acid

In the approach to isolate the active compound, a series of fractionating and isolating procedures was employed. The plant material was first extracted with acetone, and fractionated, before being subjected to VLC crude separation, and finally column chromatography. During these processes, the chemical composition and qualitative antibacterial activity was determined by TLC and bioautography on a continuous basis in order to search for single compounds and to confirm antibacterial activity. As a result of this, one sample, JK 3-2, was identified as a single, pure compound with antibacterial activity and was submitted to NMR and MS analyzes.

The NMR and MS spectra confirmed the identity as the triterpenoic acid, 1α, 2β-, 18 trihydroxy-olean 12-en-29-oic acid with a molecular mass of 488 and molecular formula of $C_{30}H_{48}O_5$ and the common name terminoic acid. This is the first time that this compound (terminoic acid) has been isolated from *T. sericea,* but it has previously been identified from *T. arjuna*.

It is likely that the most active compound was not isolated from *T.sericea* as can be seen from Fig. 4.6 where the chromatogram revealed higher levels of activity that was not pursued because of the complexity of the relevant fractions.

6.4 Animal study

An important final step was to perform an *in vivo* evaluation of the active compound and a crude extract. A system was developed in which two treatments as well as a positive and a negative control were applied to the same animal on infected skin lesions of rats. The results proved that both the 20% crude extract preparation as well as 1% terminoic acid had better clinical activity against *S. aureus* than the positive control, commercial 0.1% gentamycin cream on the three parameters investigated *i.e.*, formation of exudate, erythema and the size of the lesion.

6.5 Conclusion

If one looks at the original aim of the study, the third objective was to determine if the isolated compound can be used to treat superficial skin infections caused by *Staphylococcus aureus* pathogens, the objective certainly was met in the animal study and could be extrapolated for use in humans.

The plant leaves could be used with a simplified method of extraction like ethanol or just the macerated leave extract applied as a paste to the wound. This confirmed the hearsay evidence of the traditional healer that plant leaves were used in circumcision

ceremonies. The mechanism of action would still have to be cleared up as well as toxicity studies and systemic side effects. In the short term however the practical use would be limited to the crude extract to be incorporated in a cream and communicated to traditional healers to be used for mild, superficial septicaemia.