Chapter 9

General discussion, conclusions and recommendations

9.1 Less known genera of the Combretaceae

Previous work on the African Combretaceae has concentrated mainly on the genus *Combretum* and to a lesser extent on *Terminalia*. This is probably because they occur in larger numbers over a wide geographical area and consequently are easier to collect (Katerere, 2001). This study extended phytochemical and pharmacological investigations to other related genera of Combretaceae, *Pteleopsis* and *Quisqualis.* Initially, investigations involved extracts of *Pteleopsis myrtifolia* leaves and fruit as well as of *Quisqualis littorea* leaves, but later investigations involved only *P. myrtifolia* leaves. This was due to the fact that fruit were only available during a part of the season and not in large enough quantities. The material available from *Quisqualis,* was from a relatively young climber, and leaves were not available in large enough quantities.

9.2 Antibacterial activity of extracts from *P. myrtifolia* and *Q. littorea*

A primary objective of this investigation was to investigate the antibacterial activity of extracts of *P. myrtifolia* leaves and fruit and *Q. littorea* leaves. MIC values, thin layer chromatography and bioautography indicated that extracts of *P. myrtifolia* leaves and fruit and *Q. littorea* leaves contain several antibacterial compounds. *Q. littorea* leaf extracts exhibited good activity against the Gram-negative bacterium, *E coli*, in contrast to previous reports where the majority of plants did not have activity against Gram-negative bacteria (Vlietinck *et al*., 1995). Isolation of compounds from an extract of *Q. littorea* holds the potential to lead to an antibiotic for Gram-negative bacteria, but was not investigated due to lack of enough material.

9.3 Growth inhibition effect of *P. myrtifolia* leaf extracts on human cell lines

Another objective of this investigation was to investigate the cytotoxic activity of extracts of *P. myrtifolia* leaves. The growth of the cancerous cell line $WHCO_3$ (which was the most differentiated from the cell lines used) was inhibited by all plant extracts - 20 μg/ml had significant less growth inhibition than 60 μg/ml. The growth of cell lines MCF-7 and H157 were inhibited by all plant extracts except the methanol without tannin extract - the 20 μg/ml had significant less growth inhibition than the 100 μg/ml concentration. The HeLa cell line's growth was inhibited by all plant extracts except the hot water and chloroform extracts, with significant less growth inhibition at 20 μg/ml than 100 μg/ml.

Extracts with tannin inhibited the growth of the cancer cell lines MCF-7 and HeLa more than the same extracts without tannins. For the H157 cell line, extracts without tannin inhibited the growth more than extracts with tannins. A possible explanation could be that the H157 cells were more sensitive to tannins present than the other cell lines. After the tannins were removed, the active compound(s) had more pronounced activity. This was not the case for the other cell lines, as they were less sensitive to tannin or responded to other compounds in the extract.

GI_{50} values were reached for all extracts ≥ 100 μg/ml for the MCF-7 and $WHCO_3$ cell lines. All, except the chloroform extract attained GI_{50} values for the HeLa cell line. No extracts reached GI_{50} values for the MCF-12A cell line, and only the hot water, methanol and chloroform extracts exhibited their GI_{50} values for the H157 cell line. LC values were attained for the cold water, hot water, methanol and ethanol extracts for the MCF-7, $WHCO_3$ and HeLa cell lines. No extracts reached constant lethal concentration values for the cell lines H157 and MCF-12. Some extracts exhibited their GI_{50} values but not their LC values at a specific concentration for a specific cell line, especially with $WHCO_3$ cell line. This was the desired effect, since the ideal

would be to find plant extracts or compounds that inhibit cell growth, but that are not toxic (lethal). The cell lines differed in their sensitivity to the plant extracts and were most sensitive to the hot water and methanol extracts.

9.4 Antioxidant activity of *P. myrtifolia* leaf extracts

The antioxidant activity of extracts of *P. myrtifolia* leaves was also investigated. All *P. myrtifolia* leaf extracts gave positive scavenging capacity (antioxidant activity) with DPPH, but the quantity differed for all different extracts. Compared to black tea, where one gram of dry mass, had a vitamin C equivalent of 0.126 mg, the cold water extract of *P. myrtifolia* leaves, had a vitamin C equivalent of 0.34 mg, more than twice that of black tea. Further, the vitamin C equivalent of the methanol and hot water extracts were both more than that of black tea, 0.20 and 0.147 mg/g respectively.

9.5 Solvent-solvent separation to find best fraction for purification

Bioassay-guided fractionation by solvent-solvent separation identified that all fractions: *n*-hexane, carbon tetrachloride, chloroform, 35% water in methanol, *n*-butanol and water had antibacterial activity for at least two of the bacteria tested. The chloroform fraction was antibacterial to all bacteria tested, and seven areas of inhibition were observed. This fraction was chosen for further purification and isolation of compounds. The *n*-hexane fraction also had high antibacterial activity and contained mostly waxes and fat-like substances. It was not considered for further development, because waxes cannot be utilised in medicine due to the non-specificity for host or bacterial cells as well as their poor pharmacokinetic profile (Martini, 2002).

9.6 Pure compounds isolated from *P. myrtifolia* leaves

In total, 17 pure compounds were isolated from the chloroform fraction of *P. myrtifolia* leaves.

One compound, a triterpenoid taraxerol, was present in sufficient quantities for structure elucidation. It is not a novel structure, but is reported for the first time in this species. Taraxerol is usually bound in the leaf matrix of some plant's leaves, probably ester-bonded to cutin (Killops and Frewin, 1994). One might expect this rather non-polar compound to be associated with the *n*-hexane fraction, not the chloroform fraction which it was isolated from in this investigation. The presence of saponins offers an explanation to non-polar substances being soluble in the chloroform fraction.

Results found in this study contributed to knowledge of the phytochemistry of Combretaceae – that taraxerol occur in the leaves of *P. myrtifolia.* Taraxerol was isolated from another genus of Combretaceae, namely from *Terminalia glabrescens* leaves by Garcez *et al*., in 2003.

9.7 Bioactivity of taraxerol

When the bioactivity of taraxerol was investigated (the final objective for this study), no other reports about its antibacterial activity could be found. It had MIC values of 0.04, 0.016, 0.63 and 0.31 mg/ml for the bacteria *S. aureus*, *E. faecalis, P. aeruginosa* and *E. coli* respectively. In *Terminalia sericea* an extract which contained terminoic acid and had a MIC value of 0.033 mg/ml for *S. aureus*, was developed into a topical ointment for veterinary use (Kruger, 2004). In an experiment with mice, this ointment was found to be more effective than commercial Gentamycin cream. Since *T. sericea*'s activity is less than taraxerol's activity for *S. aureus*, taraxerol's commercialisation as an ointment may be even more effective than the ointment prepared from *Terminalia* extracts. This is however, not a viable option due to (as mentioned earlier), the low yield of taraxerol from leaves, limited distribution and numbers of *P. myrtifolia* trees and thus insufficient leaves. Before the spectrum of antimicrobial activity is further researched, a Pharmaceutical developer may not be convinced that it can be specific enough for identified bacteria. A leaf extract from *Pteleopsis* trees may find an application by rural

people who live in the vicinity of trees and who can gather leaves to apply a crude leaf extract (as an antibiotic) for topical infections.

Taraxerol did not in itself have free radical scavenging (antioxidant) activity, but the extract it was isolated from, had.

Taraxerol significantly inhibited growth of the human lung cancer cell line H157. With the examined concentrations of up to 100 µg/ml, the H157 cell line did not reach GI_{50} values or lethal concentrations. The activity of the extracts could have been due to synergy of more than one compound.

Results from this study indicated that extracts from *P. myrtifolia* and *Q. littorea* had antibacterial activity, that extracts from *P. myrtifolia* leaves had cytotoxic and antioxidant activity, and that a pure compound from *P. myrtifolia* leaves had antibacterial and cytotoxic activity.

9.8 Recommendations for future

Future research on antibacterial extracts will benefit by using apparatus that can handle large quantities of dried material throughout the extraction and chromatography process. According to a company representative of Shimoda in the Eastern Cape, they have developed an apparatus for investigating bulk material. When using samples of up to five kilograms of dried material to isolate pure compounds, it is highly probable that compounds will not be in sufficient quantities to determine their chemical structures. The complexity of plant material from Combretaceae as well as too small quantities of possible pure compounds have also been reported by Famakin (2002), Serage (2003) and Kruger (2004).

Where a large enough quantity of plant material from *Q. littorea* can be found, isolation of compounds from an extract with activity for Gram-negative bacteria should be persued, since

it holds the potential to lead to an antibiotic for Gram-negative bacteria.

The use of optical density of bacterial cultures should be explored to see of results of different laboratories or researchers might be more uniform.

An investigation of the antimicrobial activity of taraxerol against many bacteria and fungi is recommended. As more knowledge about its specific activity becomes available, it might be that, although it is a non-polar triterpenoid, its activity against specific bacterial cells can be very well defined and be commercialised. It is recommended that it be tested in *in vivo* experimental conditions in future.

9.9 Literature references

Famakin JO (2002) Investigation of antibacterial compounds present in *Combretum woodii*. MSc thesis Pharmacology, University of Pretoria, Pretoria

Garcez FR, Garcez WS, Miguel DLS, Serea AT, Prado FC (2003) Chemical constituents from *Terminalia glabrescens*. Journal of the Brazilian Chemical Society 14(3): 461-465

Katerere DRP (2001) Phytochemical and Pharmacological Studies of Species of African Combretaceae. PhD thesis, University of Strathclyde, Glasgow, Scotland, UK

Killops SD, Frewin NL (1994) Triterpenoid diagenesis and cuticular preservation. Organic Geochemistry 21:1193-1209

Kruger JP (2004) Isolation, chemical characterization and clinical application of an antibacterial compound from *Terminalia sericea.* PhD thesis, University of Pretoria, Pretoria

Martini ND (2002) The isolation and characterization of antibacterial compounds from

Combretum erythrophyllum (Burch.) Sond. PhD thesis Pharmacology, University of Pretoria, Pretoria

Serage A (2003) Isolation and characterization of antibacterial compounds present in *Combretum apiculatum* subsp *apiculatum*. MSc thesis, University of Pretoria, Pretoria

Vlietinck AJ, Van Hoof L, Totte J, Lasure A, Van den Berghe D, Rwangabo PC, Mvukiyumwami J (1995) Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. Journal of Ethnopharmacology 46: 31 - 47