

CHAPTER 6

DISCUSSION AND CONCLUSION

The aim of this study was to isolate and characterize antibacterial compounds from *C. apiculatum*. After determining the best extractant, an acetone extract was fractionated by solvent-solvent fractionation. The chloroform fraction had the highest antibacterial activity. By bio-assay guided fractionation three antibacterial compounds were isolated using silica gel column chromatography. The chemical structure and antibacterial activity of the isolated compounds were determined.

The need to develop new antimicrobial agents has become indispensable. Many scientists in different disciplines have embarked on the screening and analysis of plants for pharmacological activity (Alexander, 1992).

Member of the genus *Combretum* is used widely in traditional medicines. Members of this genus have shown the following biological activity including, anti-inflammatory, hypotensive, antifungal, molluscicidal and antimicrobial (Hutching et al 1996). This prompted the need to investigate the antibacterial activity of *C. apiculatum*. The leaves were extracted with different solvents of varying polarities. The solvents used were hexane (HE), isopropyl ether (IE), ethyl ether (EE), methylene dichloride (MD), ethyl acetate (EA), tetrahydrofuran (TH), acetone (AC), methanol (ME), ethanol (ET) and water (WA). Acetone, tetrahydrofuran, ethyl acetate and ethanol had a better extraction efficiency compared to the other extractants. TH and AC gave a good yield because they are moderately polar. Water was not a good extractant.

The solvent systems that resulted in the best chromatographic resolution were the EMW and CEF. With regard to the bioassay, IE, EE, EA, ME and MD had good inhibition on all microorganisms, (their MIC's ranging between 0.04 mg /ml and 0.6 mg /ml). TH and AC extracts were reasonably active against all microorganisms except *P. aeruginosa*. Total activity was good against *E. coli*, *E. faecalis* and *S. aureus*, while *P. aeruginosa* showed resistance towards almost all extracts. The average total activity for the extracts, EE, IE, EA, ME and MD was 354 ml. TH and AC extracts both inhibited *E. coli* and *S. aureus*.

These extracts were less complex and had a higher extraction yield than the other extracts. Compounds partitioning into this fraction also showed good activity against *S. aureus* and *E. coli*. Isolation work was carried out on the chloroform fraction. Seven antibacterial compounds which inhibited the *S. aureus* and *E. coli* were seen in bioautography. These compounds had R_f values of 0.4 and 0.8 and were later isolated in column chromatography. Even though the crude extracts inhibited *S. aureus*, *E. faecalis* and *E. coli*, most of the activity was seen using *S. aureus*. *S. aureus* was therefore used for the bioassay guided fractionation of three antibacterial compounds. It is possible that there could be other active compounds against the rest of the bacteria. This phenomenon could be due to the possibility of synergistic effects among the active compounds in the crude extracts.

Three compounds were isolated by column chromatography and elucidated in this study. They were identified as alpinetin (5-hydroxy-7-methoxyflavanone), pinocembrin (5,7-dihydroxyflavanone), and flavokawain-A (4'-hydroxy-2',4'-dimethoxychalcone), which is a chalcone. These compounds were active against, *E. coli*, *E. faecalis*, *S. aureus*, and *P. aeruginosa*.

The biological activity found in propolis has been associated with pinocembrin that is one of the 38 flavonoids found in propolis and responsible for its wide therapeutic values. A large number of studies have confirmed propolis to possess antimicrobial activity on at least 21 species of bacteria, 9 species of fungi, 3 species of protozoa and a large range of viruses including the herpes and influenza (Anonymous, 2001). Apart from the antimicrobial value it has also shown to carry a range of other therapeutic properties like, anti-inflammatory, antioxidant, anti-allergic action (Van Wyk, 2000). Pinocembrin has also been found to act as a nutritional supplement, as a sweetener and a flavoring agent.

Flavokawain-A has been found as a constituent of *Piper methysticum*. It is known of anti-inflammatory and antiseptic activity has been used for the treatment of upper respiratory tract infections and cystitis (anonymous, 2001). It is also known for its antioxidant action, and the ability to inhibit cyclooxygenase enzyme. Anti-bacterial activity against *S. aureus* by this compound has been reported without providing MIC values (Bremner and Meyer, 1998).

The three active flavonoids isolated in this study have activities not much higher than crude extracts. The R_f values of the isolated compounds were similar to the R_f value of the active compound in the crude extract. The unexpected low antibacterial activity is therefore not because minor active compounds were isolated but rather indicates a synergistic effect in the crude extract.

Although all of these compounds are known, not one of them has been isolated from the Combretaceae and the MIC values were not known.

There is little prospect of developing pharmaceutical products based on these compounds. The bioassay was limited to only four microorganisms and there were no control experiments in this study. This study however, justifies the use of *C. apiculatum* for diarrhoea and conjunctivitis in traditional medicines. Further studies on the use of crude extracts in rural communities where the tree occurs, may be useful