

6 The use of planar chromatography to identify over-exploited medicinal plants

6.1 Introduction

Uncontrolled utilization of bark for traditional medicine is of great concern to the conservation officials, researchers and traditional healers in South African. Conservation groups are currently conducting case studies to explore the extent of the threat posed to popular medicinal species in South Africa (Zschocke *et al.*, 2000b).

The most popular medicinal plants have been identified as *Cryptocarya woodii*, *Cryptocarya myrtifolia*, *Ocotea bullata*, *Prunus africana*, *Rapanea melanophloeas* and *Zanthoxylum dayvi*. *Prunus africana* (African Stinkwood) is of worldwide interest since it is heavily exploited for its bark for use in Benign Prostatic Hypertrophy (Traffic network, 2001 and Achieng, 1999). However, more plant species in Africa are endangered due to overuse such as *Warburgia salutaris* and Hypoxis. From the work reported in Section 5.2.1, it was concluded that TLC is limited when it comes to distinguishing closely related species. An attempt was made to use the method to profile medicinal plants that appear to be endangered.

Fresh bark samples of five over-exploited species were collected and are described in the next section. The bark material was ground up, extracted with ethanol, acetone and hexane and then separated on TLC plates in three systems (Chapter 2). Chemical components of the extracts were visualized with *p*-anisaldehyde, vanillin sulphuric acid and vanillin phosphoric acid spray reagents. Antibacterial activities of these species were

detected despite the fact that some species like *Ocotea bullata* and *Cryptocarya* are mainly used for headaches.

6.2 Results and discussion

6.2.1 TLC analysis of over-exploited traditional medicines

6.2.1.1 *Cryptocarya myrtifolia*

Common names: kanferboom (Afrikaans), camphor tree (English), igqeba or umkhondweni (Zulu)

The bark is used as a substitute for *Ocotea bullata* (Hutchings et al. 1996), however, the main constituents of *O. bullata* are not found in any of the *Cryptocarya* species (Zschocke et al., 2000a). Since *O. bullata* bark remedies are used for treating headaches, van Staden and Zschocke (2000) investigated the analgesic activity of *O. bullata* and *Cryptocarya* species. Their findings were similar for both plant species (van Staden and Zschocke, 2000). Figure 6.1 shows the TLC profile of *Cryptocarya myrtifolia* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid.

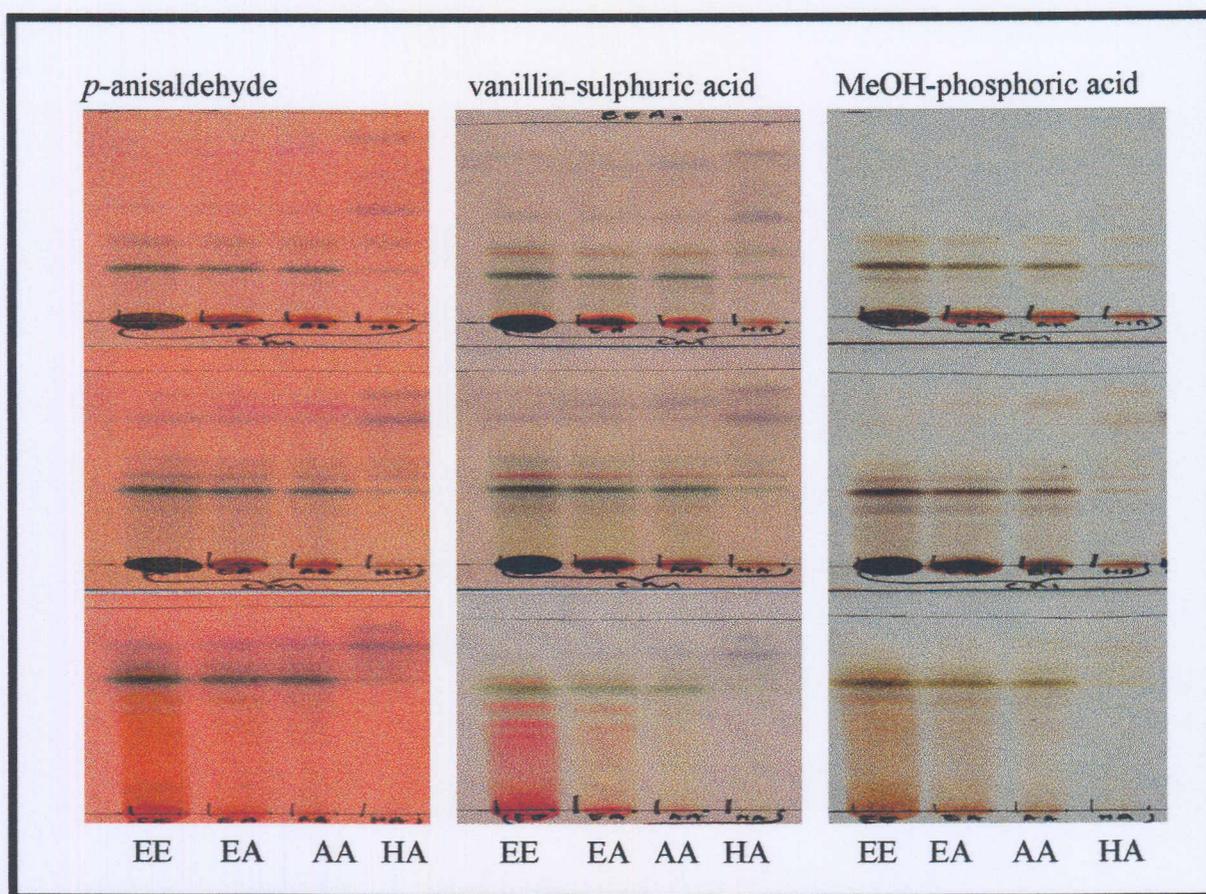


Figure 6.1. TLC profiles of *Cryptocarya myrtifolia* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid. Key: EE: ethanol-ethanol extract; EA: ethanol-acetone extract; AA: acetone-acetone extract and HA: hexane-acetone extract.

The ethanol-ethanol (EE) extract contain more compounds than the ethanol-acetone (EA) extract and acetone-acetone extract (AA). This is because, not all compounds extracted with ethanol can dissolve in acetone as these solvents have different polarities. Vanillin sulphuric acid spray reagent is a better detection reagent for this species and it detected compounds that were also detectable with *p*-anisaldehyde and methanol phosphoric acid spray reagents.

6.2.1.2 *Ocotea bullata*

Common names: stinkhout (Afrikaans) black stinkwood (English) and unukani (Zulu),

The stem bark of *Ocotea bullata* is one of the most frequently used traditional medicines in South Africa (Mander, 1997). The use of this plant species by traditional healers for a wide-range of ailments including headaches, back-ache, urinary tract problems and magical purposes, is well documented (Hutchings et al. 1996 and van Wyk et al. 1997). However, it has not been possible to associate any of the known compounds with specific biological activity such as cyclo-oxygenase-1 (COX-1) inhibitory activity (Jäger et al., 1996). It is also used for producing high quality furniture and its popularity has made it rare species.

This plant species is widely distributed in the forests along the southern and eastern parts of South Africa, from the Cape Peninsula eastwards to the Southern Cape, Eastern Cape, KwaZulu-Natal, Mpumalanga and the Limpopo Province (van Wyk et al. 1997). *O. bullata* is now protected in KwaZulu-Natal as it has become an endangered species. Its importance to the herbal medicine trade has attracted the attention of conservationist and natural products chemists. Currently, several new neolignans have been isolated from the bark of this species, e.g. ocobullenone (Zschocke et al., 2002). Its leaves are assumed to have unidentified volatile compounds such as monoterpenoids. The biological activity of this plant is suspected to result from the neolignans in the bark (van Wyk et al. 1997). Figure 6.2 illustrates the fingerprints of *Ocotea bullata* sprayed with *p*-anisaldehyde, vanillin sulphuric acid and methanol-phosphoric acid spray reagents.

6.2.1.3 *Rapanea melanophloeas*

Common names: isiqalaba-sehlathi (Xhosa and Zulu), isiqwane-sehlathi (Xhosa), umaphipha (Zulu), Cape beech (English) and Kaapse boekenhout (Afrikaans)

The tree grows naturally along the east coast of South Africa. Bark infusions are more frequently used than roots as expectorants, emetics and treatment of muscular pain, stomach and heart disorders. The bark and leaves are known to contain tannins and triterpenoid saponins, respectively. There is no readily available information about the pharmacological activity of this plant (Hutchings et al. 1996 and Wyk et al., 1997). Figure 6.3 shows the TLC profile of *Rapanea melanophloeas* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid spray reagents.

Lesser compounds were detected with the chosen sprays. Therefore, conclusions about its chemical composition cannot be drawn except that it contains the compounds detected by three spray reagents.

6.2.1.4 *Zanthoxylum davyi*

Common names: isimungumabele (Zulu) knobthorn (English), knoppiesdoring (Afrikaans)

Its bark is used for snakebites, chronic coughs, boils, toothache, pleurisy and as emetic; the leaves are used for chest pains and roots for sore throats, mouth ulcers, venereal diseases and aphrodisiacs. Figure 6.4 shows the TLC profile of *Zanthoxylum davyi* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid spray reagents.

6.3 Conclusion

In this chapter, TLC fingerprints of plant materials over-harvested were shown and may be used in future for adulteration investigation. In conclusion, planar chromatography can be used to compile fingerprints of traditional medicines as references. The next chapter gives a summary of all investigations conducted in this study and concludes by recommending future work.

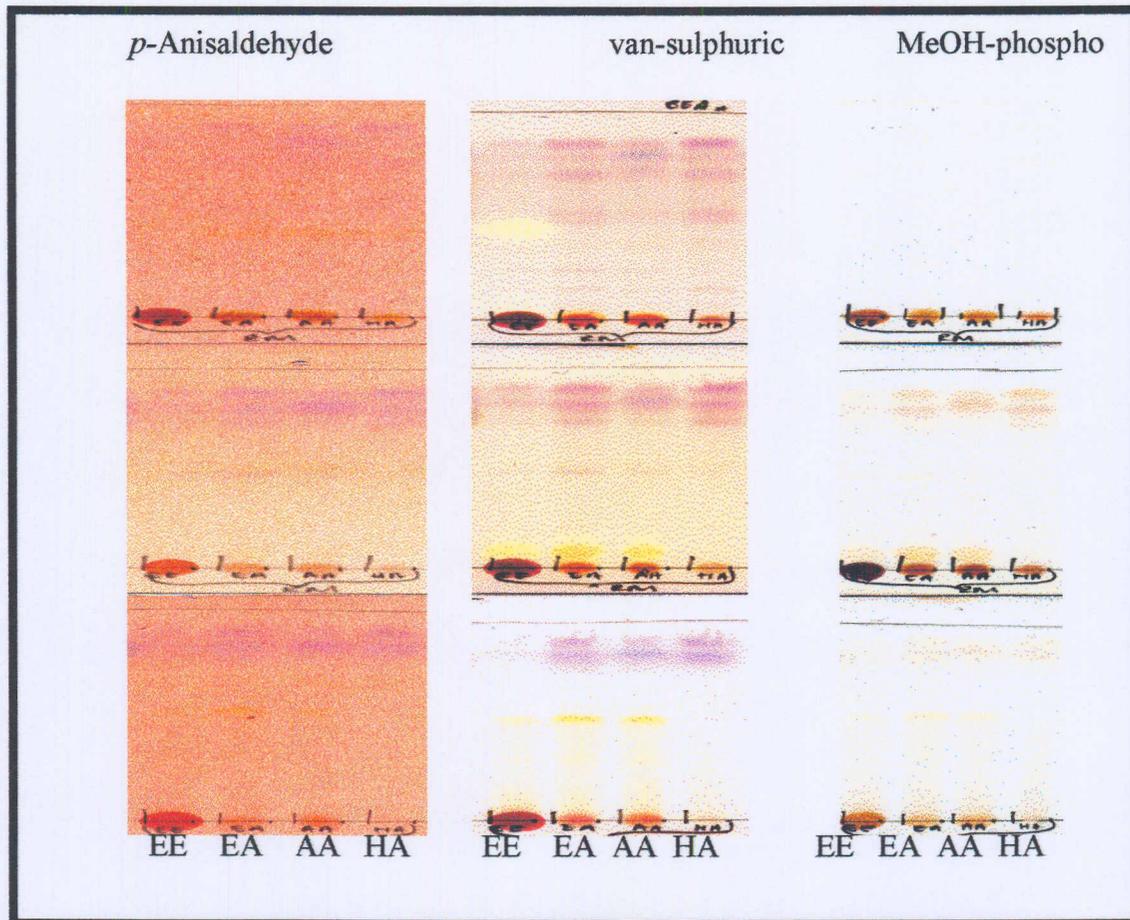


Figure 6.3 TLC profiles of *Rapanea melanophloeas* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid spray reagents. Key: EE: ethanol-ethanol extraction; EA: ethanol-acetone extract; AA: acetone-acetone extract and HA: hexane-acetone extract.

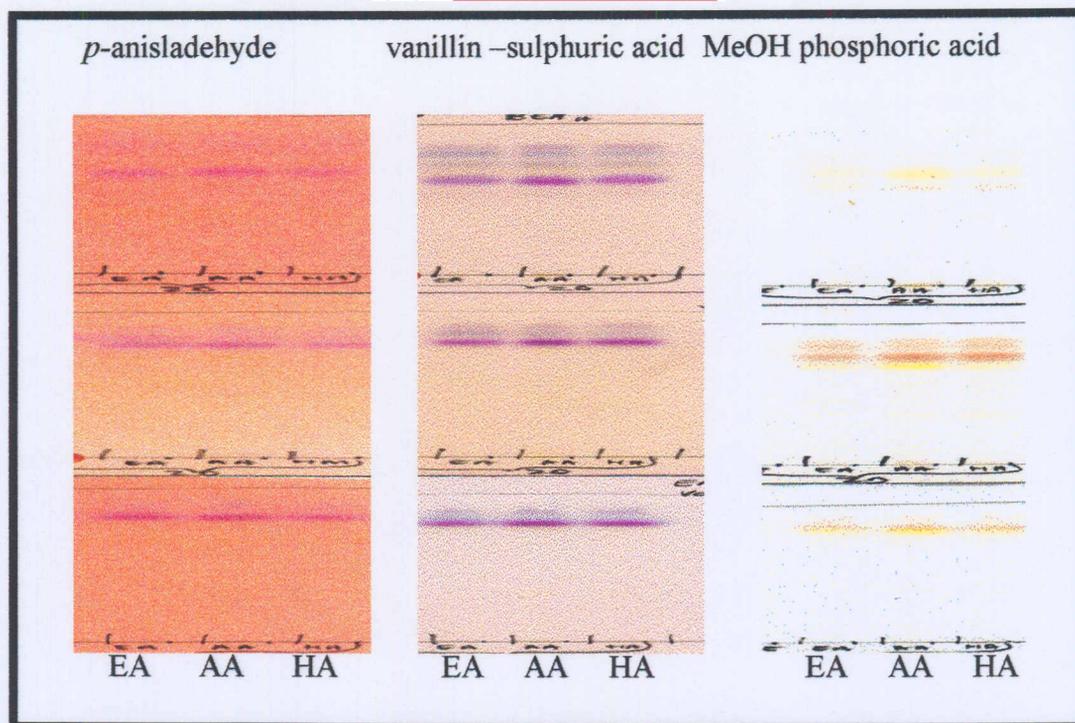


Figure 6.4 TLC profile of *Zanthoxylum davyi* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid spray reagents. Key: EA: ethanol-acetone extract; AA: acetone-acetone extract and HA: hexane-acetone extract.

The ethanol (EA), acetone (AA) and hexane (HA) extracts show similar chemical fingerprints for all extracts in Figure 6.4. This characteristic appears to be unique to this species when comparing it to all species analyzed. Like the rest of the over-exploited species analyzed in this chapter, it contains few compounds that are detectable using the spray reagents. The detected purple colour could be terpenoid.

6.2.1.5 *Prunus africana*

Common names: inyazangoma-elimnyama (Zulu), umkakase (Xhosa), rooistinkhout (Afrikaans) and red stinkwood (English)

The stem bark is the only plant part used for medicinal purposes such as chest pains (decoction) and benign prostate hypertrophy (lipid and phytosterol extracts). Previous investigations have revealed the presence of phytosterols (β -sitosterol and campesterol) and cyanogenic glycosides (amygdalin) from bark extracts. Pentacyclic triterpenoid esters and various linear aliphatic alcohols together with their ferulic acid esters were also observed in the extracts. Biological activity against prostatic adenoma has been reported as resulting from β -sitosterols. Other components may contribute to the beneficial biological activity. Its present exploitation has led to exceptional shortage in South Africa and Cameroon (Ndibi and Kay, 1997). Figure 6.5 shows the TLC profile of *Prunus africana* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid spray reagents.

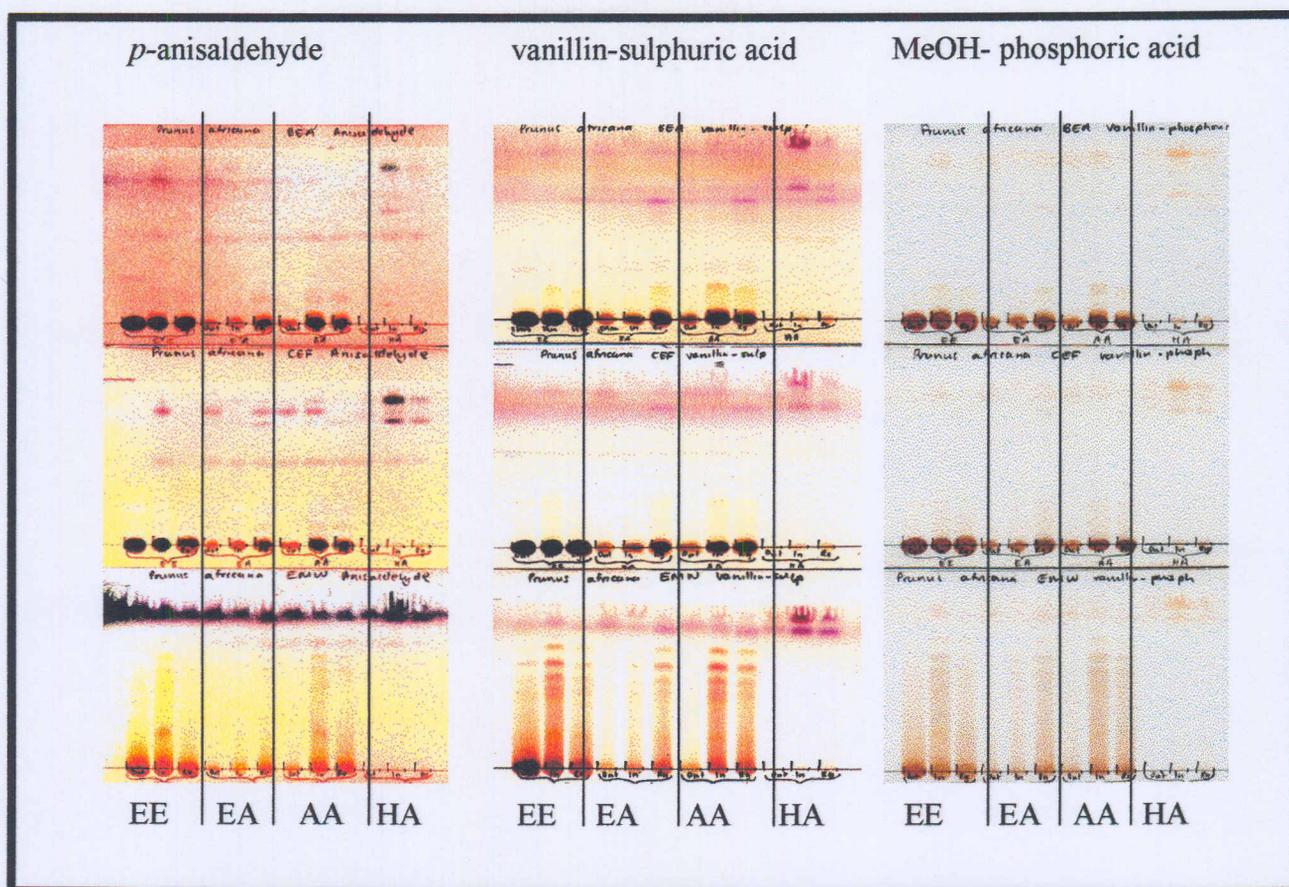


Figure 6.5 TLC profile of *Prunus africana* sprayed with *p*-anisaldehyde, vanillin-sulphuric and methanol-phosphoric acid spray reagents. Key: EE: ethanol-ethanol extraction; EA: ethanol-acetone extract; AA: acetone-acetone extract and HA: hexane-acetone extract.

The ethanol-ethanol (EE) extract of *Prunus africana* was similar to the acetone-acetone (AA) extract, whereas the ethanol-acetone (EA) extract showed little compound extraction. This observation could imply that compounds extracted with ethanol should be dissolved in ethanol not in acetone because they may appear insoluble in acetone.

6.2.2 Antibacterial activity analysis

Determination of the biological activity of the exploited species was conducted, because the techniques and material were available and also because many traditional medicines may have potential antibacterial activity. Figure 6.6 shows graphical illustrations of total activity of the plant species investigated in this section.

The MIC value for the ethanol-ethanol extract of *Rapanea melanophloeas* was 0.02 mg/ml. In this investigation we have compiled TLC fingerprints of over-exploited medicinal plants. *Cryptocarya myrtifolia* sample extracted with ethanol showed high sensitivity to *Staphylococcus aureus*. However, *Cryptocarya myrtifolia* was less sensitive to the other organisms. *Zanthoxylum dayvi* was less sensitive to all microorganisms. *Ocotea bullata* ethanol and acetone extracts were slightly sensitive to *Enterococcus faecalis*. In general, these species are vulnerable for over-exploitation and have shown poor antibacterial inhibition against the microorganisms used in the test.

During the extraction process it was noticed that the ethanol-extracted components were decreased drastically when the ethanol crude extract was dissolved in acetone. It was then decided to keep the extract compounds in ethanol although ethanol takes time to be absorbed into the silica plates.

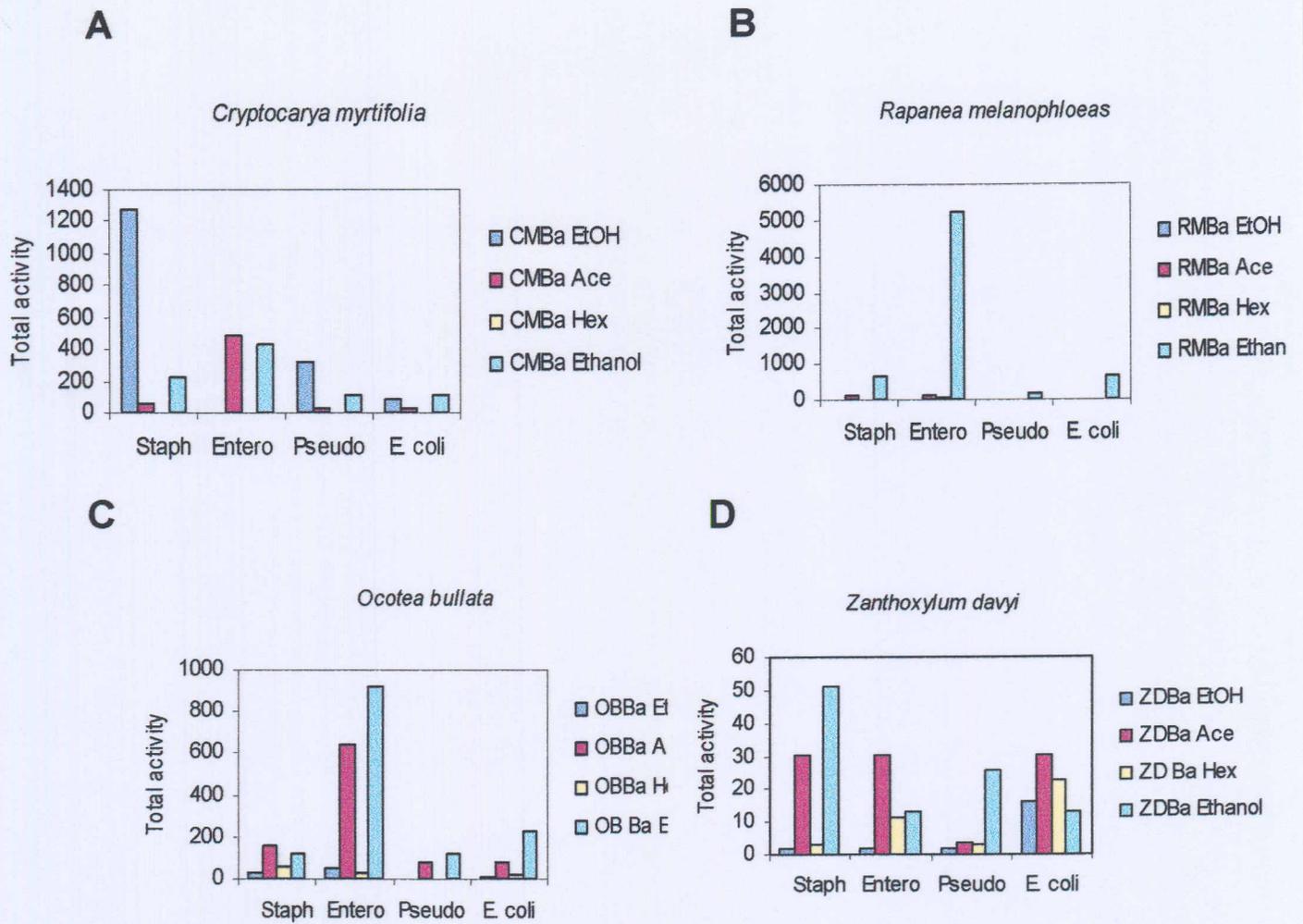


Figure 6.6 Total activity of the over-exploited species A: *Cryptocarya myrtifolia*, B: *Rapanea melanophloeas*, C: *Ocotea bullata*, and D: *Zanthoxylum davyi* tested against *E. faecalis* (Entero) *S. aureus* (Staph), *P. aeruginosa* (Pseudo) and *E. coli* microorganisms.

6.3 Conclusion

In this chapter, TLC fingerprints of plant materials over-harvested were shown and may be used in future for adulteration investigation. In conclusion, planar chromatography can be used to compile fingerprints of traditional medicines as references. The next chapter gives a summary of all investigations conducted in this study and concludes by recommending future work.

7 General Conclusions

The separation systems used BEA (benzene (8): ethanol (1): ammonium system ratio (0.1)), CEF (chloroform (4): ethyl acetate (3): formic acid system (1)) and EMW (ethyl acetate (10): methanol (1.35): water system (1)) were suitable for separating compound in all plant extracts. The study showed that planar chromatography could be used to identify traditional medicines by comparing chemical fingerprints of unknown plant species to reference species. However, this application did not appear suitable for identification of closely related plant species. The chemical fingerprint of the market species traded as isibhaha (IsiZulu), Mosetlha (SeSotho), Legwama (SeSotho), mosetlhana (SeSotho) and Lengana (SeSotho) were in agreement with those of *Warburgia salutaris*, *Peltophorum africanum*, *Boophane hymanthoides*, *Acacia caffra*, and *Artemisia afra*, respectively. However, the market species traded as umahlanganisa (Zulu) was identified as neither *Croton sylvaticus* nor *C. gratissimus*. It is possible that the *Croton* sample was substituted with a different species or that the Zulu name used for the medicinal plant refer to a different species. For future study, further identification of this species need to be confirmed.

Although the technique applied in this investigation was adequate for this study. It was shown that the Thin Layer Chromatography (TLC) method was unable to differentiate amongst closely related plant species of the Fabaceae family.

The application of TLC showed chemical and antibacterial activity variation amongst the samples analyzed. It was found that the environment where a plant is grown may influence its chemical composition, especially when treated with pesticides. This investigation confirmed that *Artemisia afra* has high diversity. Therefore, this study

indicated that the environment where the plant is grown play a minor role in changing the chemical composition and biological activity of the plant, unless treated with pesticides. This study has also shown that adulteration in the Pretoria market is not a problem. Planar chromatography can also be used to compile fingerprints of traditional medicines that can be used to identify plant species in poisoning incidences or during uncertainties in identifying plant part.