4 The use of Thin Layer Chromatography (TLC) to identify medicinal plants

4.1 Introduction

In Chapter 3, Table 3.2 shows a summary of commonly used traditional medicines in the Pretoria area. However, the identities of those plant species have not been verified experimentally. The documented names of medicinal plants found in literature, have not been verified scientifically. Therefore, there is a need to establish the connection between documented African names and their scientific names.

The objective of this section is to identify traditional medicines traded in the Pretoria markets in the form of barks by developing a comparative Thin Layer Chromatography (TLC) profile of both the market species and reference species from Pretoria National Botanical Garden (PNBG) and Agricultural Research Council (ARC). To do this the TLC method was used, as described in Section 2.3. First a standard of botanically verified samples of plant believed to be those on the market was developed. This research continues from the findings in Chapter 3. Ethical names turn to be generic rather than specific.

Amongst the 20 highly ranked commonly used medicines in the Pretoria area (Table 3.2), 6 were chosen for further identification and biological analysis. These medicinal plants were selected because their reference samples were available in the PNBG and ARC. The medicinal plants selected are *Warburgia salutaris* (Molaka), *Peltophorum africanum* (Mosetlha), *Croton sylvaticus* (Umahlanganis), *Boophane haemanthooids* (Legwama), *Artemisia afra* (Lengana) and *Acacia caffra* (Mosetlhana).

The developed TLC plates were visualized under ultra violet (UV) light and fluorescent spots marked by HB pencil and then sprayed separately with *p*-anisaldehyde, vanillin-sulphuric acid and vanillin-phosphoric acid reagents. The results are shown in Figure 4.1 through to 4.6. In all cases the reference sample (denoted with A for ARC, B for PNBG or E for J. N Eloff, depending on the supplier of the samples used) were analyzed alongside the suspected market sample (denoted M for market). The suspected market sample was retrieved by literature search using the African names as shown in Table 3.2. The results of each species are discussed in detail in Section 4.2.1.

4.2 Results and discussion

4.2.1 Thin Layer Chromatography Identification

4.2.1.1 *Warburgia salutaris* (Molaka)

Figure 4.1 shows the TLC profiles of Molaka (market) and *Warburgia salutaris* (PNBG) extracts detected with *p*-anisaldehyde, vanillin, sulfuric acid and vanillin phosphoric acid spray reagents. The chromatographic profile of the market species extracted with ethanol (ME), acetone (MA) and hexane (MH) was generally similar to that of the reference material (RE, RA and RH) from a tree in the garden of J. N. Eloff.

However, there are variations in the fluorescence detected between the market and the reference extracts. For instance, a compound only found in reference extracts showed a red fluorescence that was not present in the market extract. This compound has an R_f value of 0.92 in the CEF [chloroform (4): ethyl acetate (3): formic acid (1)] profile. Another compound was found, only in the market BEA [benzene (80): ethanol (10): ammonium (1)] extract that had an R_f of 0.62 with a white fluorescence.

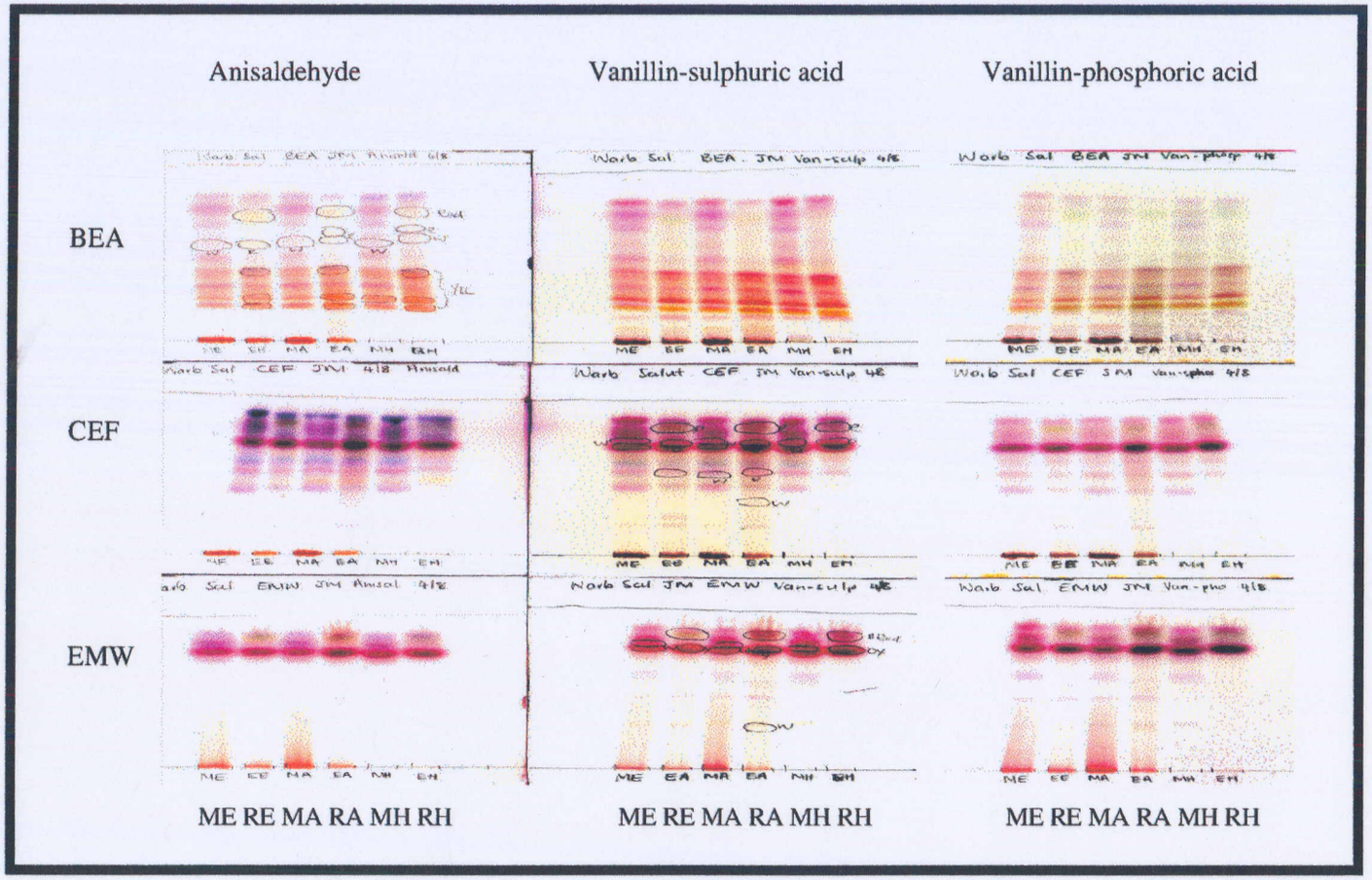

Figure 4.1 TLC profiles of Molaka (ME, MA and MH) and *Warburgia salutaris* (RE, RA and RH) showing chemical components sprayed with *p*-anisaldehyde, vanillin-sulphuric acid and vanillin-phosphoric acid respectively. Key: ME: market ethanol extract; RE: reference ethanol extract; MA: market acetone extracts; RA: reference acetone extract; MH: market hexane extract; RH: reference hexane extract; BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

Fluorescence can be a very sensitive way of detecting compounds separated and may indicate the presence of compounds in low concentrations. There was no great difference amongst the compounds detected with the three spray reagents. However, the intensity differences of the colour implied varying concentrations. The BEA profile of vanillin-sulphuric acid reagent shows a major variation in the detection of compounds falling between the R_f value range of 0.44 and 0.81. This may be the result of genetic variation or environmental factors (Solomon, *et al.* 1999).

Vanillin-sulphuric acid appears to be the best reagent for profiling this plant species because some compounds are more intensely detected by it compared to the other reagents. *P*-anisaldehyde, vanillin-sulphuric acid and vanillin-phosphoric acid spray reagents detected some compounds coloured in purple–brown in Figure 4.1. This plant species has steroid-like compounds because all three-spray reagents are expected to detect steroid (Table 2.2). The BEA separation system shows better separation of compounds for this plant species than CEF and EMW [ethyl acetate (10): methanol (1.35): water (1)]. This could mean than *Warburgia salutaris* and Molaka have more non-polar compounds than intermediate and polar compounds.

Based of the foregoing and not withstanding the variations noted, the market species Molaka is similar to the reference species *Warburgia salutaris* from the J. N Eloffs' garden. Therefore, the market sample is *Warburgia salutaris*.

4.2.1.2 *Peltophorum africanum* (Mosetlha)

Figure 4.2 shows the TLC profile of Mosetlha (market) and *Peltophorum africanum* (PNBG) extracts detected with *p*-anisaldehyde, vanillin-sulphuric acid and vanillin-phosphoric acid spray reagents. The TLC profile of Mosetlha from the market (ME, MA and MH) and

fingerprints of *Peltophorum africanum* from the PNBG (RE, RA and RH) show similar compounds for all extracts separated by all three systems (BEA, CEF and EMW), which indicates that they are the same species.

Like *Warburgia salutaris,* this species also has a red fluorescing compound that is restricted to the reference extracts circled in BEA (R_f = 0.72), CEF (R_f = 0.77) and EMW (R_f =0.81) TLC systems. In addition, a blue fluorescing compound with an R_f value of 0.62 separated by the EMW system of the ethanol and acetone extracts was detected in both samples (ME, MA, RE and RA). Furthermore, the hexane extracts showed a white compound of R_f value of 0.24, which was not detected with any of the chosen spray reagents. *P*- anisaldehyde seems to be the best reagent for profiling this plant species, since it detected the most compounds.

This species has polar compounds clearly shown as smears in the EMW profiles detected by all three reagents. These smears showed a blue fluorescing colour before the plates were sprayed with the detecting reagents. Therefore, the detected compound could be chromophores. It is also possible that some fluorescing compounds are present in low concentrations and may, therefore, not be significant for visualization. In conclusion, Mosetlha can be confirmed to be *Peltoforum africanum.*

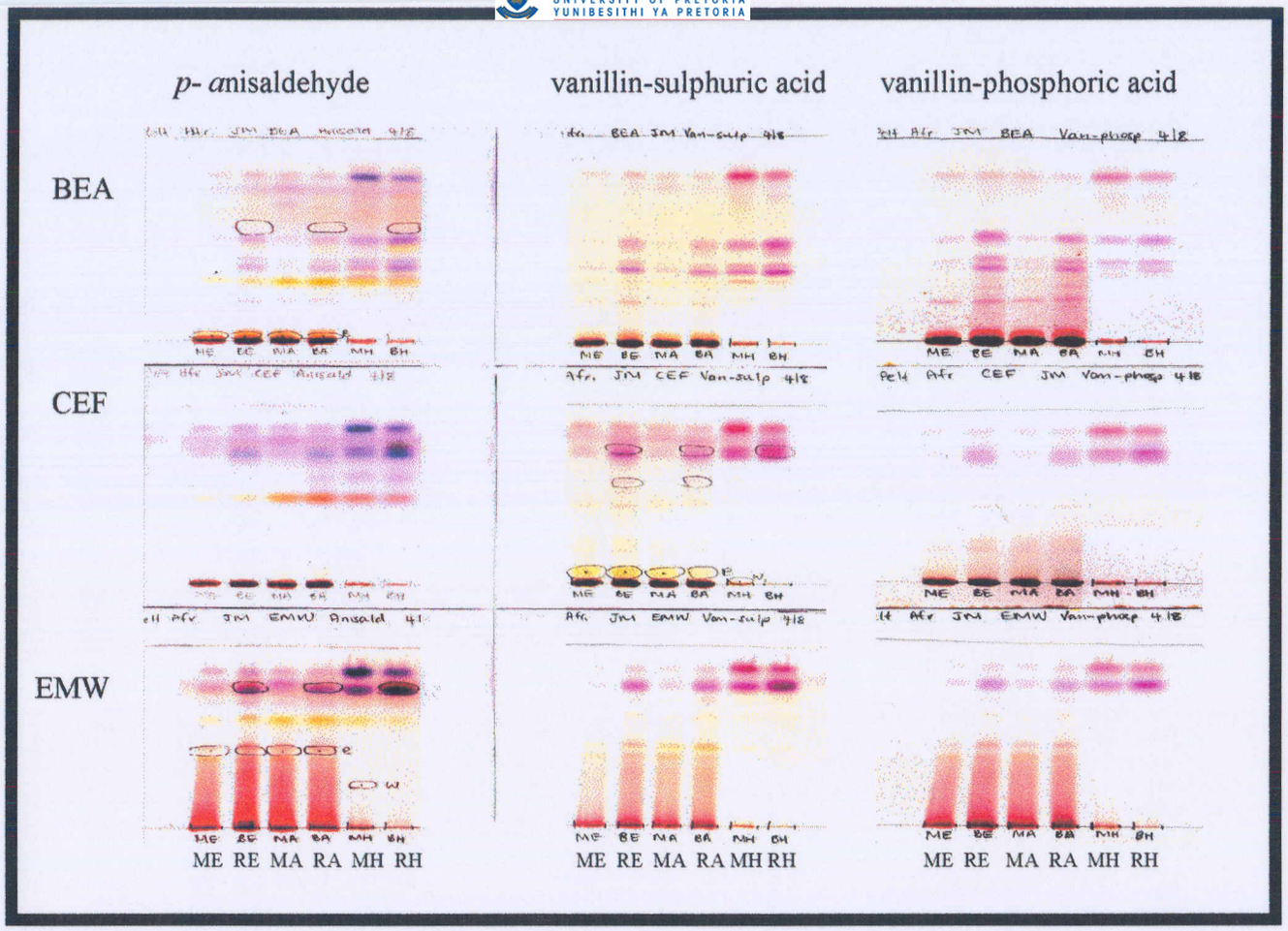

Figure 4.2 TLC profiles of Mosetlha (ME, MA and MH) and *Peltophorum africanum* (RE, RA and RH) extracts sprayed with *p*-anisaldehyde, vanillin sulphuric acid and vanillin phosphoric acid respectively. Key: ME: market ethanol extract; RE: reference ethanol extract; MA: market acetone extracts; RA: reference acetone extract; MH: market hexane extract; RH: reference hexane extract; .BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

4.2.1.3 *Croton sylvaticus* (Umahlanganisa)

Figure 4.3 show the TLC profiles of Umahlanganisa (market) and *Croton sylvaticus* (PNBG) extracts separated in the three systems described in Section 2.3. The TLC profiles for all the market extraction (ME, MA and MH) are completely different from the reference *Croton sylvaticus* (RE, RA and RH) extracts. The market extracts showed a blue colour under UV [R_f values 0.29 (BEA), 0.72 (CEF) and 0.8 (EMW)], which was not detected in the reference extracts. Furthermore, an orange-yellow compound was seen in the market (ME and MA) extracts [R_f values 0.16 (CEF) and 0.3 (EMW)] that was not detected in the reference sample (RE and RA) under UV and after spraying with spray reagents.

Overall, there are major differences in the chromatograms sprayed in the number of compounds separated, relative concentrations and R_f values. However, some compounds are present in both the market and the reference extracts and they were clearly detected with vanillin-sulphuric acid. These compounds have R_f values of 0.74, 0.29 and 0.26 in the BEA system: 0.87 and 0.83 in the CEF system and 0.9 in the EMW system. These results indicate that the market and reference samples belong to the same genus but are not of the same species or they may be totally different plant species with no relationship.

The traditional medicine traded as Umahlanganisa that was collected from the market is, therefore, not *Croton sylvaticus* as the African name would imply. Preliminary investigations conducted as part of this study indicated that the *Croton* commonly used is not *C. sylvaticus* but C. *gratissimus*. The experiments were conducted to determine the similarities in chemical profiles of Umahlanganisa and *Croton gratissimus.* The possible reason for the variations in the market and reference material profiling could, therefore, be because of the incorrectly labelled plant product that was received from the market.

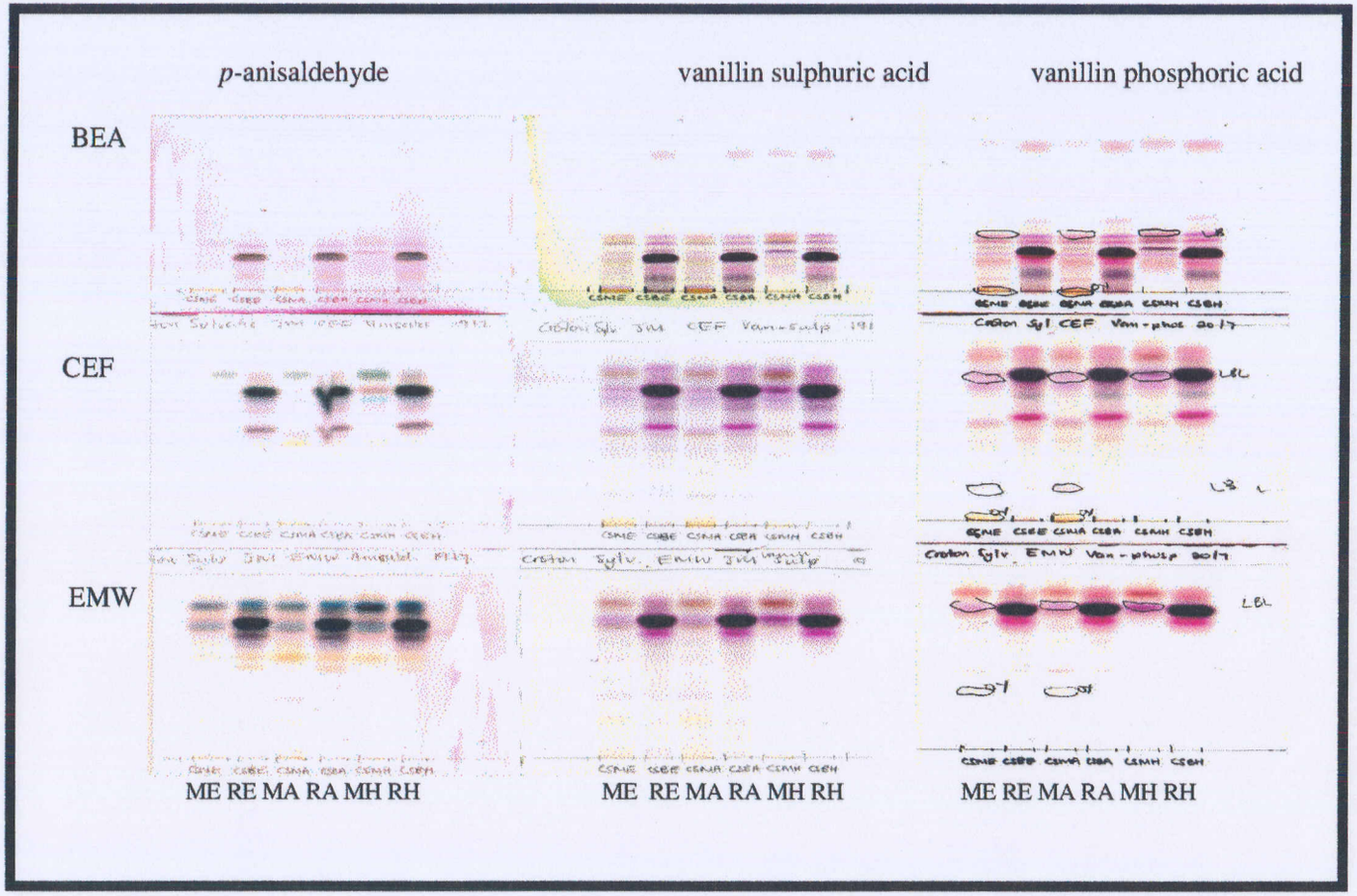

Figure 4.3 TLC profiles of market Umahlanganisa (ME, MA and MH) and reference *Croton sylvaticus* (RE, RA and RH) extracts sprayed with *p*-anisaldehyde, vanillin sulphuric acid and vanillin phosphoric acid spray reagents. Key: ME: market ethanol extract; RE: reference ethanol extract; MA: market acetone extracts; RA: reference acetone extract; MH: market hexane extract; RH: reference hexane extract; BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

The other reason may be because different species may be identified by the same name. A comparison of the chemical fingerprints of different *Croton* species can be used to resolve this issue.

4.2.1.4 *Boophane haemanthooides* (Legwama)

Figure 4.4 shows the TLC profiles of Legwama (market) and *Boophane haemanthoides* (ARC) detected with *p*-anisaldehyde and vanillin-sulphuric acid spray reagents. These two samples have similar compounds as shown in Figure 4.4. However, they showed some degree of variation in the compound intensity.

Best separation of compounds in both the analyte and reference was achieved by the BEA system and best detected with *p*-anisaldehyde spray reagent. Consequently, it is probable but not definite that Legwama is *Boophane haemanthooides.* According to documentations, *B. haemanthoides* only grows in the Cape while *B. disticha* is found all over the country. The market plant species is likely to be *B. disticha,* but since *Boophane* species are closely related, they cannot only be distinguished from each other by Botanists and not by TLC profiles during the flowering season (Du Plessis and Duncan, 1989).

4.2.1.5 *Artemisia afra (Lengana)*

Figure 4.5 shows the TLC profiles collected from the market and two reference species of *Artemisia afra* from ARC and PNBG. The solvent extracts of all three species from market, ARC and PNBG have similar chemical compositions detected by all three-spray reagents, although the intensity of detected compounds varies amongst the species. It was concluded that Lengana was *Artemisia afra*.

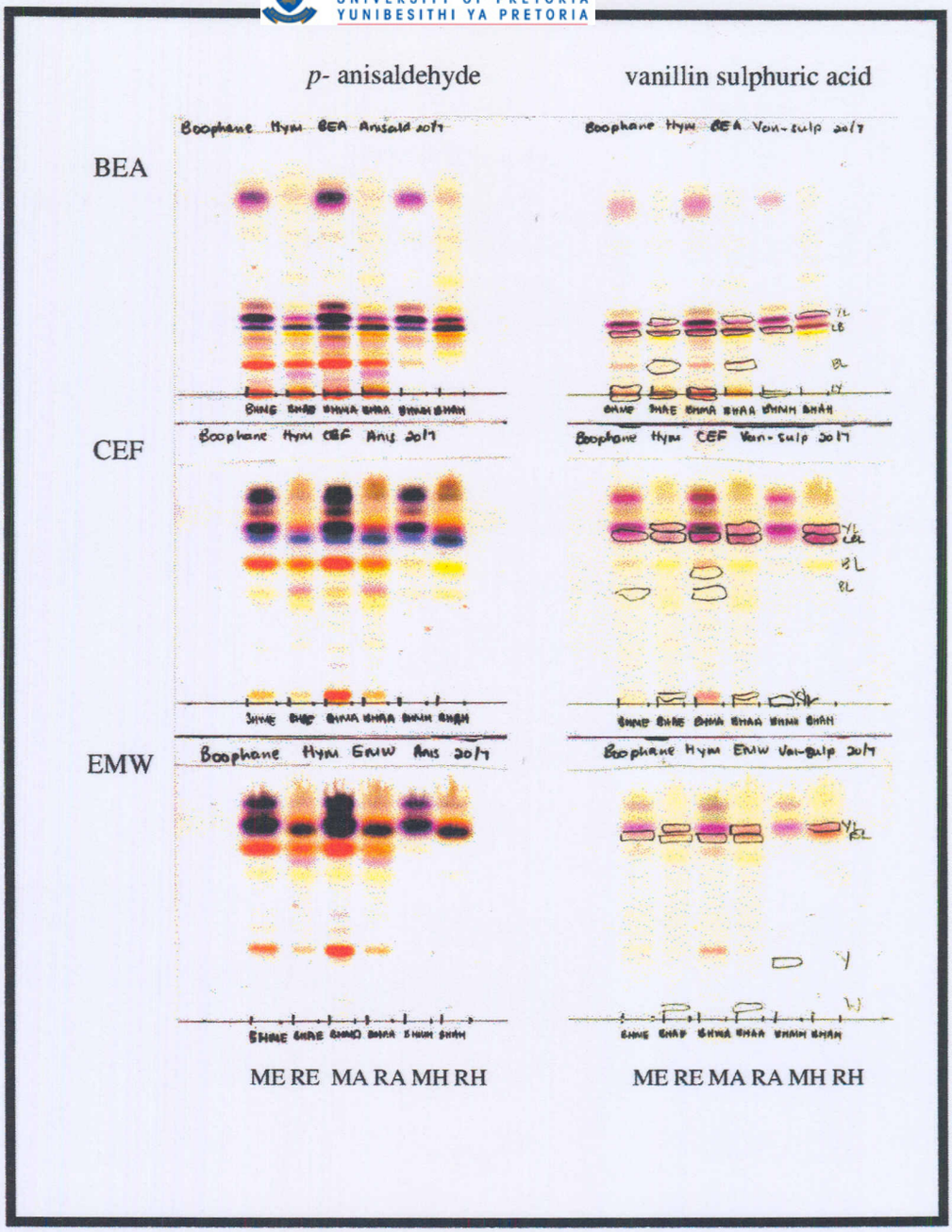

Figure 4.4 TLC profiles of Legwama and *Boophane haemanthooides* extracts sprayed with both *p*-anisaldehyde and vanillin-sulphuric acid. Key: ME: market ethanol extract; RE: reference ethanol extract; MA: market acetone extracts; RA: reference acetone extract; MH: market hexane extract; RH: reference hexane extract; BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

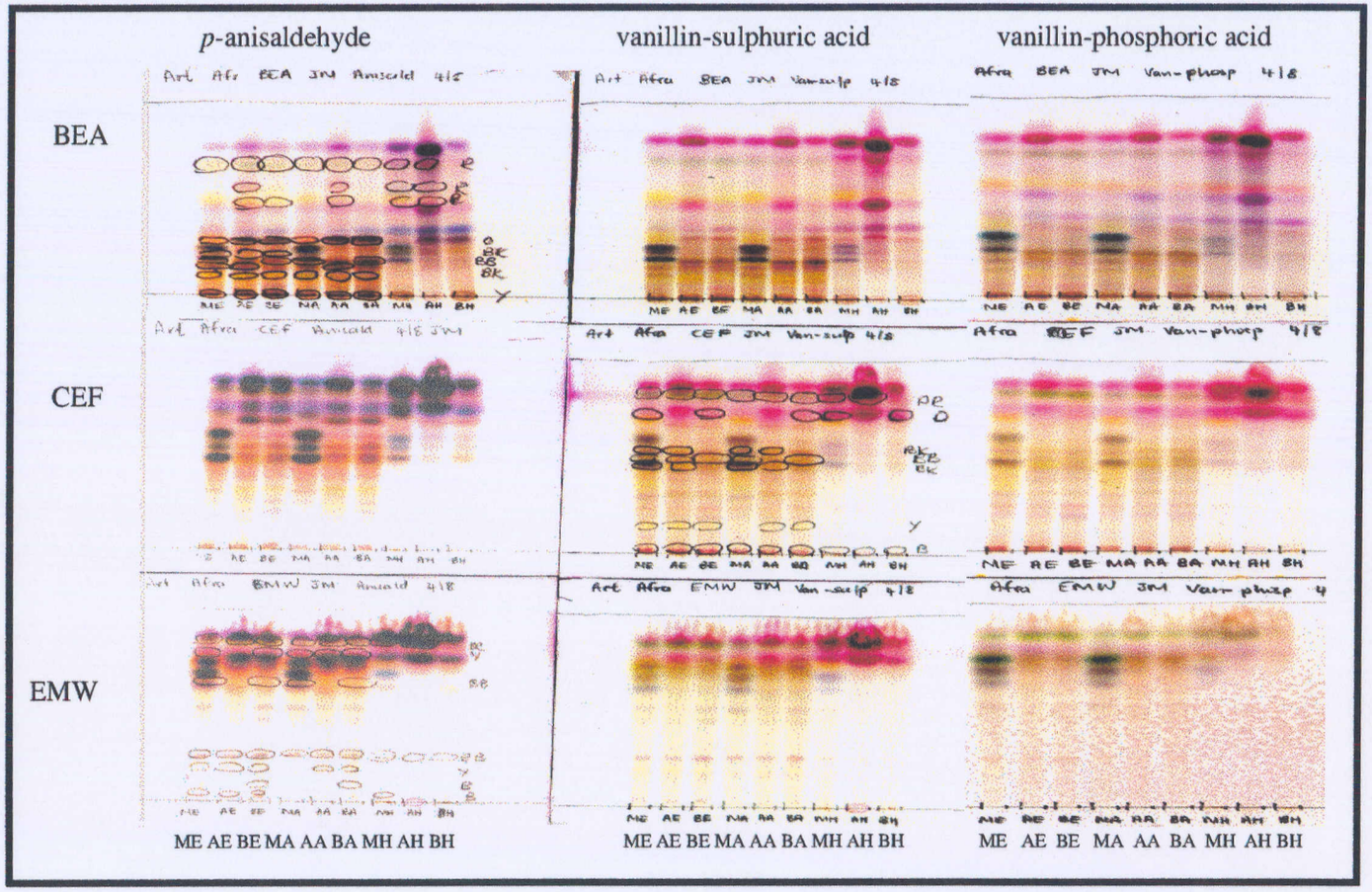

Figure 4.5 The TLC profiles of Lengana and *Artemisia afra* extracts sprayed with *p*-anisaldehyde, vanillin-sulphuric acid and vanillin-phosphoric acid respectively. Key: ME: market ethanol extract; AE: ARC ethanol extract; BE: PNBG ethanol extract; MA: market acetone extracts; AA: ARC acetone extract; BA: PNBG acetone extract; MH: market hexane extract; AH: ARC hexane extract; BH: PNBG hexane extract BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

4.2.1.6 *Acacia caffra (Mosetlhana)*

Figure 4.6 shows the TLC profiles of Mosetlhana (market) and *Acacia caffra* (PNBG) extracts that were detected with *p*-anisaldehyde, vanillin-sulphuric acid and vanillin-phosphoric acid spray reagents. The ethanol, acetone and hexane extracts reflect similar compound patterns for both species although there is a high variation in intensity amongst the extracts as indicated in Figure 4.6. However, it is clear that Mosetlhana and *Acacia caffra* have the same chemical profile and could be the same species.

In summary, this investigation showed that it is possible to use the chemical fingerprint of TLC as an identification tool for traditional medicines, although it may give equivocal results if closely related species are analyzed. Additionally, it was decided to investigate whether biological activity of species can be used to confirm the identity of market specimens. It was assumed that many, if not all, plant species traded in markets, as medicines are biologically active, although they do not undergo any assessment. Preliminary testing for biological activity was, consequently, undertaken. The bioassay chosen was for antibacterial activity. This was because the conditions for this assay had already been optimized in the laboratory where the experiment was conducted and because many plants have potential antibacterial activity and the assay is more convenient.

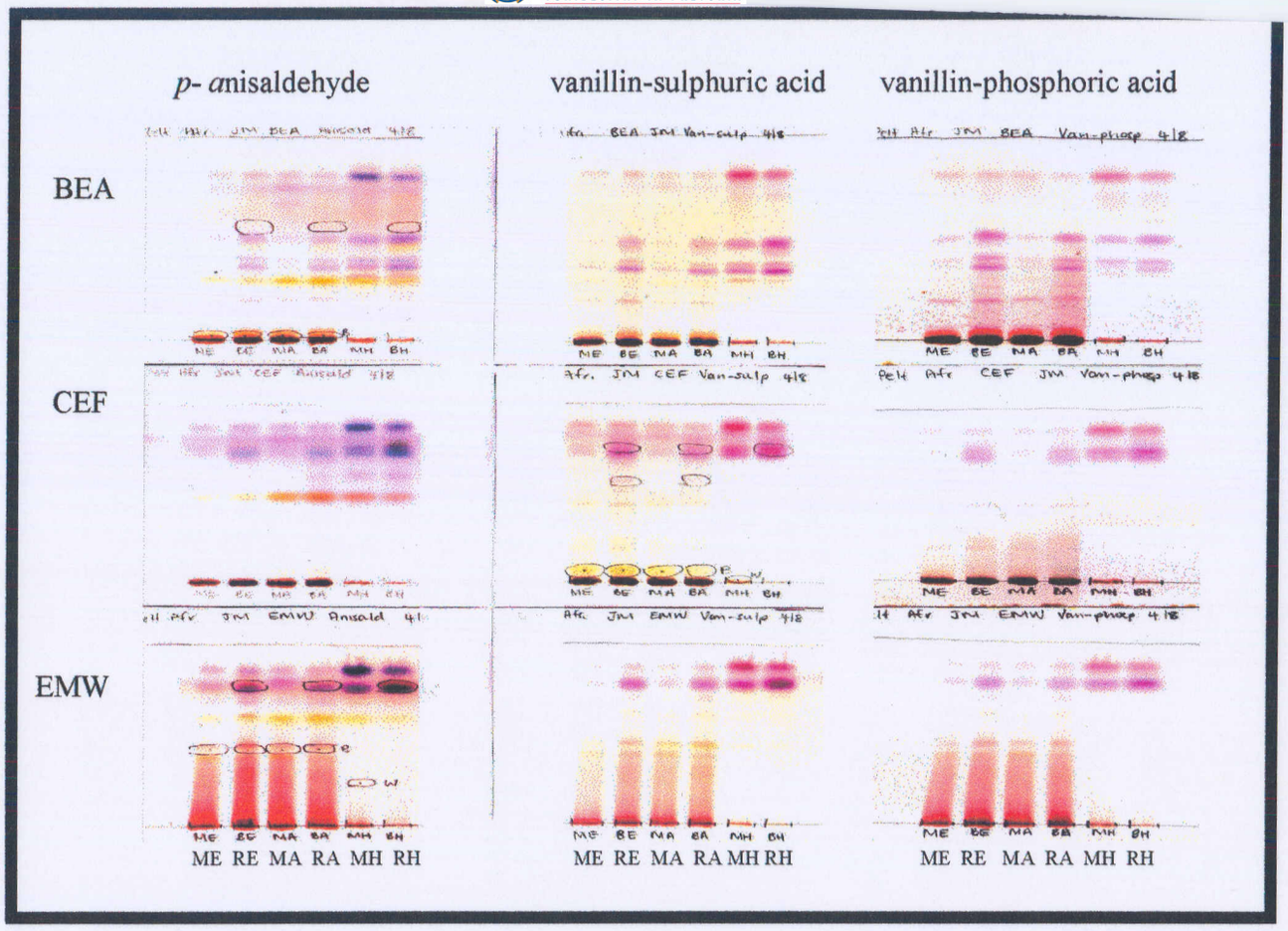

Figure 4.2 TLC profiles of Mosetlha (ME, MA and MH) and *Peltophorum africanum* (RE, RA and RH) extracts sprayed with *p*-anisaldehyde, vanillin sulphuric acid and vanillin phosphoric acid respectively. Key: ME: market ethanol extract; RE: reference ethanol extract; MA: market acetone extracts; RA: reference acetone extract; MH: market hexane extract; RH: reference hexane extract; .BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

4.2.2 Bio-autography

The method used is explained in detail in Section 2.5. The bacterial cultures used were *Enterococcus faecali (Entero)*, *Staphylococcus aureus (Staph)*, *Escherichia coli (E. coli)* and *Psuedonomas aeruginosa (Psuedo)*. Not all bio-autography results of the plant species identified in Section 4.2.1.1 to 4.2.1.6 are shown. This is because antibacterial inhibition with some of the organisms e.g. *Escherichia coli* and *Pseudonomas aeruginosa* could not be visualized on the TLC plates due to low or no inhibition rates. Photographs of biautograms treated with *W. salutaris* and *Croton sylvaticus* are shown in the Figures 4.7 and 4.8 respectively. *W. salutaris* showed remarkable bacterial inhibition. Umahlanganisa varied in its activity with *C. sylvaticus,* this being a confirmation of the major variation in chemical profiles of these species in Section 4.2.1.

W. salutaris and Molaka are the only species that showed high bacterial inhibition, especially against *Staphylococcus aureus* as indicated in Figure 4.7. These species like the rest tested have very low anti-*Escherichia coli* activity. The similarities in bioassays of Molaka and *W. salutaris* is a further confirmation of the chemical profile obtained in Section 4.2.1

In Figure 4.8, the reference species *C. sylvaticus* from Pretoria National Botanical Garden (PNBG) shows biological activity against all three organisms that was not detected for the market Mosetlhana species. A possible explanation for this could be the fact that these are two different species, which are collected from different regions. It is certain that the environment where a plant is grown has an effect on its chemical profile and consequently its biological activity (Solomon et al. 1999). The biological activity, therefore, confirmed the results obtained through the chemical fingerprints using the specific solvent systems and spray reagents.

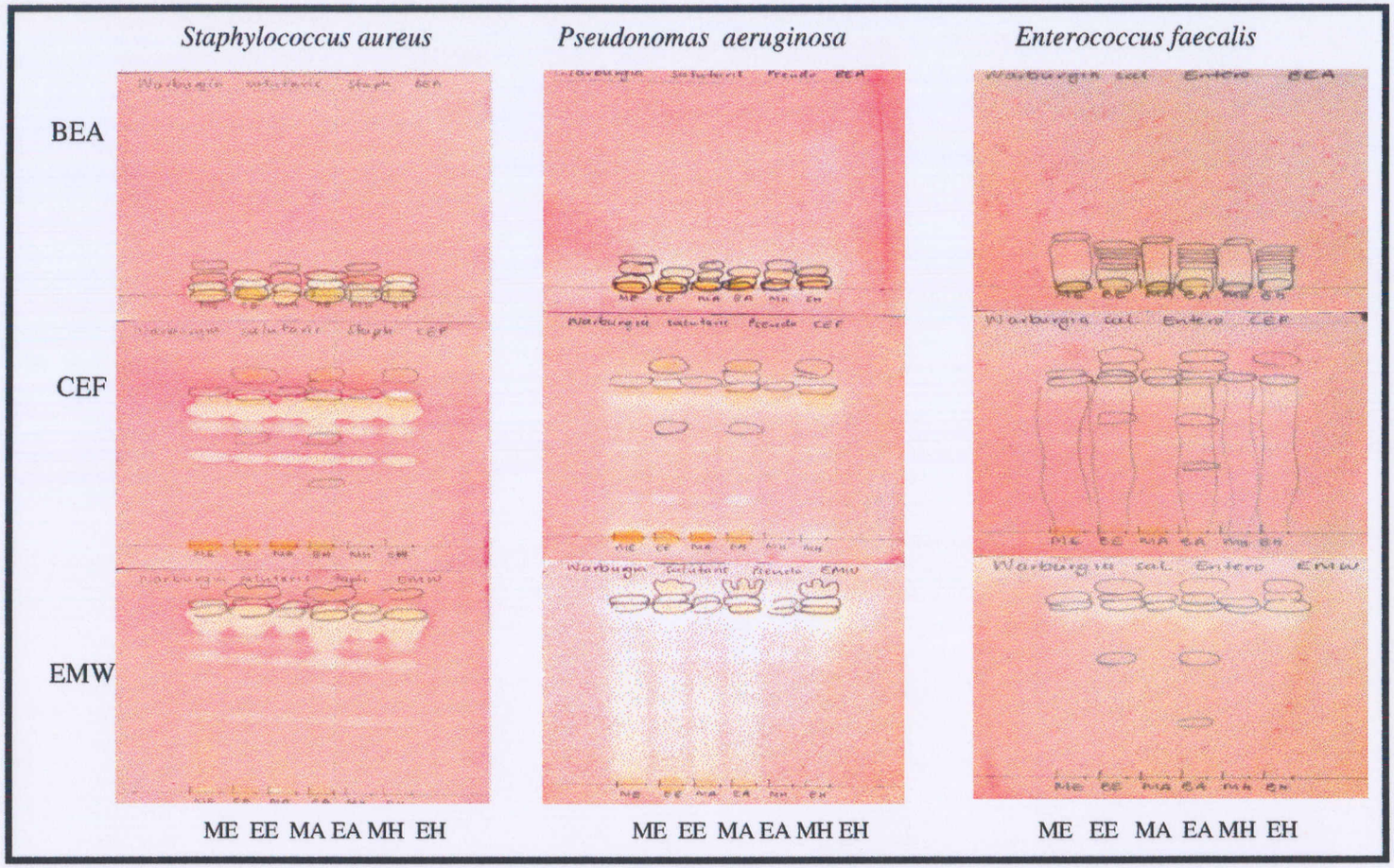

Figure 4.7 Bio-autograms of Molaka and *Warburgia salutaris* against *Staphylococcus aureus*, *Speudonomas aeruginosa* and *Enterococcus faecalis* respectively. Key: ME: market ethanol extract; EE: reference ethanol extract; MA: market acetone extracts; EA: reference acetone extract; MH: market hexane extract; EH: reference hexane extract; BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.

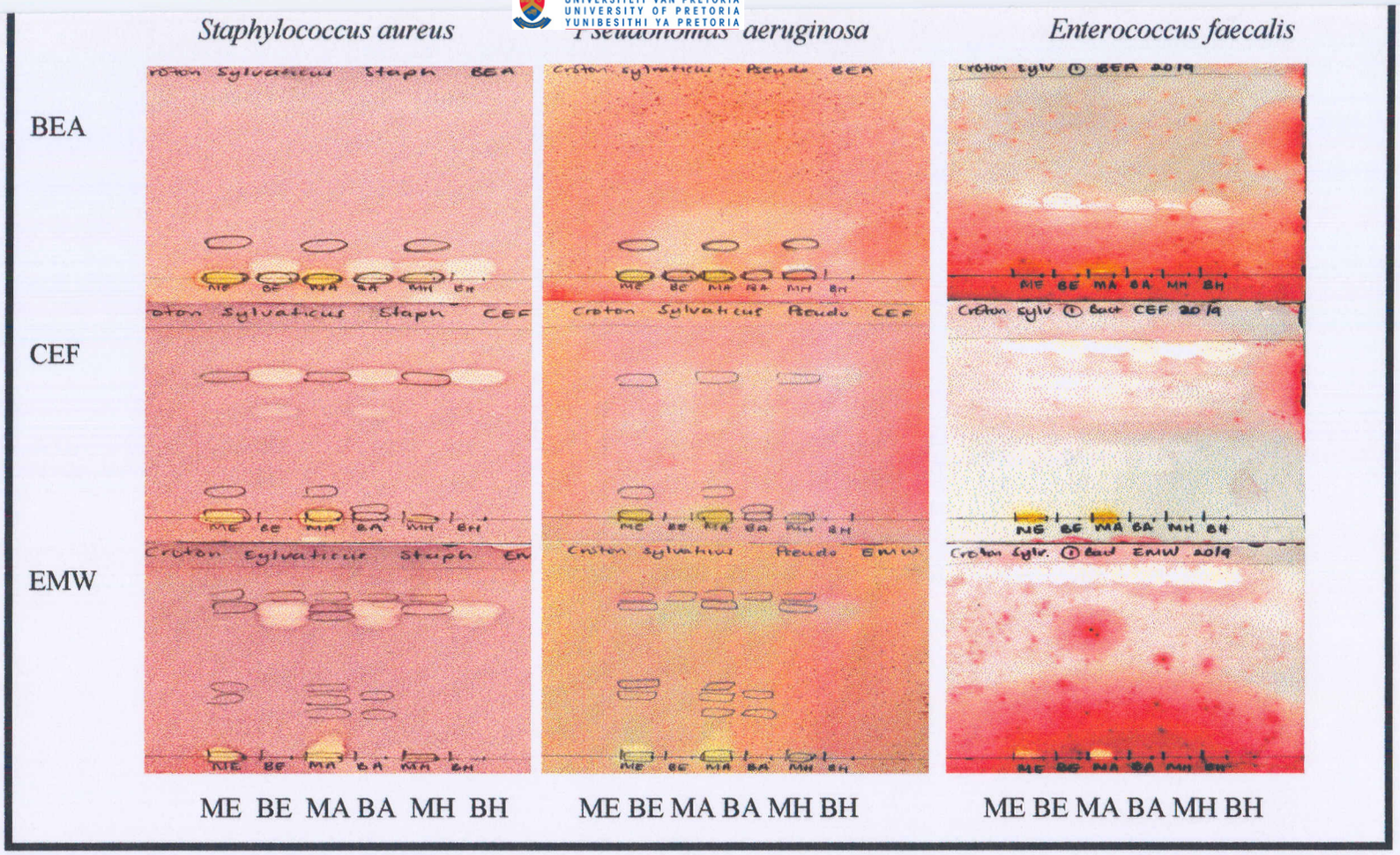

Figure 4.8 Bio-autograms of Mosetlhana and *Croton sylaticus* against *Staphylococcus aureus*, *Pseudonomas aeruginosa* and *Enterococcus faecalis* respectively**.** Key: ME: market ethanol extract; BE: reference (PNBG) ethanol extract; MA: market acetone extracts; BA: reference (PNBG) acetone extract; MH: market hexane extract; BH: reference (PNBG) hexane extract; BEA: benzene, ethanol and ammonium separation system; CEF: chloroform, ethyl acetate and formic acid separation system and EMW: ethyl acetate, methanol and water separation system.