CHAPTER 2

Extraction and TLC profiles

2.1. Introduction

Many solvents can be used to extract antifungal compounds. Since the extracts are intended for use in microbial bioassay and *in vivo* study on rats, it was necessary to select solvents that are non-toxic or otherwise would be easy to remove by evaporation before subsequent assays. The choice of solvent also depends on the ability of solvents to extract the largest quantities of material while also extracting high antifungal and antioxidant activity. An important factor governing the choice of solvents used in an extraction is the type of phytochemical groups that are to be extracted (Houghton and Raman, 1998). In this case it is not known if antibacterial triterpenoids (Angeh, 2005), flavonoids (Martini *et al*., 2004b) or bibenzyls (Eloff *et al*., 2005b) isolated from other Combretaceae may be responsible for antifungal activity.

Several researchers have used different solvents while extracting compounds from plants, for example 80% ethanol in water solution (Vlietinck *et al.*, 1995), ethanol-water (50:50,v/v), methanol (Taylor *et al.*, 1996), petroleum ether, chloroform, ethanol, methanol and water (Salie *et al,* 1996). Cowan (1999) indicated that water, ethanol, methanol, chloroform, methylene dichloride and acetone have been used to isolate antimicrobial compounds from plants. Eloff (1998a) evaluated several solvents and concluded that acetone was the best extractant for antibacterial compounds from *C. erythrophyllum* and *Anthocleista grandiflora*.

The Combretaceae is particularly rich in triterpenoids, flavonoid and stilbenes (Rogers and Verotta, 1996). These compounds are intermediate polar compounds and as such would be extracted by intermediate polar solvents like diethyl ether, ethylacetate, acetone, ethanol and methanol. However, most antibacterial compounds isolated from the Combretaceae are non-polar (Kotze and Eloff, 2002), while most antioxidant compounds are polar (Re *et al*., 1999) hence the need to extract with a wide range of solvent polarities if antifungal and antioxidant compounds are to be extracted.

2.1.1. Extraction

The extraction step is the least developed part of most analytical procedure, and today Soxhlet extraction (developed by F. Soxhlet in 1879) is still used in many routine laboratories. In the last decade there has been an increasing demand for new extraction techniques, amenable to automation, with shortened extraction times and reduced organic solvent consumption — preventing pollution in analytical laboratories and reducing sample

preparation costs (Wan and Wong, 1996). Driven by these purposes advances in sample preparation have resulted in a number of techniques such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurised liquid extraction ((PLE, Dionex trade name ASE, for accelerated solvent extraction) (Björklund *et al*., 2000).

The most commonly studied parameters that have an effect on the extraction process are solvent composition, solvent volume, extraction temperature, extraction time and matrix characteristics such as water content.

2.1.2. Choice of solvents

A correct choice of solvent is fundamental for obtaining an optimal extraction process. When selecting a solvent consideration should be given to the interaction of the solvent with the matrix and the analyte solubility in the solvent. Preferably the solvent should have a high selectivity towards the analyte of interest and exclude unwanted matrix components (Eskilsson and Björklund, 2000). Another important aspect is the compatibility of the extraction solvent with the analytical method used for the final analysis step. Optimal extraction solvents cannot be deduced directly from those used in conventional procedures. We need to know the type of compounds we are targeting in terms of polarity, if not, we must use solvents of different polarities to have a wide range of compounds.

Traditional doctors in southern Africa and all over the world use water extract to cure different diseases. Some are using boiling water, but that can lead to denaturing of some of the compounds, which are heat sensitive. This naturally sets some limitations to the type and amount of compound to be extracted relative to their polarity (Eloff, 1998a).

2.1.3. Solvent volume

The important factor is the ratio of extractant to the sample to be extracted. The solvent volume must be sufficient to ensure that the entire sample is immersed, especially when having a matrix that will swell during the extraction process. Generally in conventional extraction techniques a higher volume of solvent will increase the recovery, because the extraction depends on the partition between the phases. A larger extractant phase leads to a lower consequently better partition from the sample. It is also better to extract repeatedly with a smaller volume than once with a larger volume (Eloff, 1998b). For efficient extractant a rates of 5 – 10 ml of extractant per gram of sample repeated three times extracts practically all soluble compounds from Combretum species (Kotze and Eloff, 2002). Hydrocarbons

have been extracted from sediment samples in the range of 1–15 g with solvent volumes between 10 and 30 ml (Vázquez Blanco *et al*., 2000).

2.1.4. Temperature

Extraction temperature is one of the parameters, which is not surprising since an increase in temperature shortens the establishment of the time needed for partition equilibrium, for all extraction techniques. When extraction is conducted at high temperature, the temperature may reach well above the boiling point of the solvent. This can only occur at high pressures in closed containers. These elevated temperatures result in improved extraction efficiencies, since desorption of analytes from active sites in the matrix will increase (Chee, 1997). Additionally, solvents have higher capacity to solubilize analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively. Room temperature was found to be optimum temperature to extract different compounds, usually there is little effect when heat is used, only when compound of interest are volatile, there is a need to use less heat (Lopez-Avila *et al*., 1994).

2.1.5. Extraction time and shaking

There is an inverse relationship between the time required for efficient extraction and size of the sample particles (Kotze and Eloff, 2002). Extraction times differs depending on the amount of the sample used. Often 10 min are sufficient for extracting 1 to 3 g samples, which is exemplified by the extraction of organic pollutants (Lopez-Avila *et al*., 1994), but even 3 min have been demonstrated to give full recovery for pesticides from soils and sediments (Onuska and Terry, 1993). In the extraction of sulfonylurea herbicides from soils it was demonstrated that increasing the extraction time from 5 to 30 min did not adversely affect the recovery (Font *et al*., 1998). This was also found by Stout *et al*. (1998) when extracting the fungicide dimethomorph from soil. No difference in recovery was found using 3 or 45 min extraction time. When extracting amino acids from food, no improvement in the extraction efficiency was observed applying longer irradiation times (Kovács et *al*., 1998). Additionally there was no evidence of breakdown or alteration of the amino acids caused by longer extraction times. With thermolabile compounds, long extraction times may result in degradation. The optimum time for most plants extracts is 10 minutes for 1 –2 grams sample (Kotze and Eloff, 2002).

2.1.6. Analysis of compounds in extracts

Chromatography and other related techniques are used to analyze plants extracts. The diversity of compounds and their content vary with not only the species but also with the growing conditions, the season when plants are harvested, the process methods and storage duration.

Although there is a wide choice of other chromatographic methods for plant extracts analysis (GC, HPLC), thin layer chromatography remains a valid and simple analytical procedure for semi-qualitative detection and quantitative determination of plant extracts and their metabolites in the environmental samples (Sherma, 2000).

Thin layer chromatography (TLC) is widely used to analyze compounds recovered from natural materials. TLC does not require expensive instrumentation, nor do samples generally need extensive purification prior to analysis and several extracts can be run on at a time. Compounds can be separated with good resolution, and methods are readily adaptable for applications ranging from high throughput to preparative-scale work. Both normal and reversed-phase adsorbents have been used with a variety of mobile-phase solvent systems.

Separated substances are visualized by UV absorption, chromogenic reaction with spray reagents, or bioautography, in which suspensions of indicator organisms in agar or broth are overlaid on chromatograms to detect bioactive spots (Homans and Fuchs, 1970). Compound identity is confirmed by appearance, distance traveled relative to the solvent front (R_f value), and co-chromatography with standards in at least two different solvent systems. Quantities can be estimated from spot size and intensity, or size of the inhibition zone for bioautography, at various dilutions relative to known amounts of standards run on the same plate (Fried and Sherma, 1982).

2.2. Materials and Methods

2.2.1. Plant collection

Leaves of *Combretum* and *Terminalia* species were collected in the Lowveld National Botanical Gardens (LNBG) in Nelspruit, South Africa in Summer of 2003. Summer is a good period to collect leaves, because new leaves are growing in number during this season. Voucher specimens and origins of the trees are kept in the Garden Herbarium. *Combretum* species collected are listed in **Table 2.1** and *Terminalia* species in **Table 2.2** below. More

information on the origin and references of these plants are presented elsewhere (Eloff, 1999).

Table 2.1. *Combretum* species collected for antifungal and antioxidant screening.

***Combretum* L**	
Section	**Species**
***Hypocrateropsis* Engl. & Diels**	*C. celastroides* Welw. Ex Laws (i) *C. celastroides* ssp. *celastroides* (ii) *C. celastroides* ssp. *orientale*
	C. imberbe Wawra
	C. padoides Eng. & Diels
***Angustimarginata* Engl. & Diels**	*C. caffrum* (Eckl. & Zeyh) Kuntze
	C. erythrophyllum (Burch.) Sond.
	C. kraussii Hochst
	C. woodii Duemmer
	C. nelsonii Duemmer
***Metallicum* Excell & Stace**	*C. collinum* Fresen (i) *C. collinum* ssp. *suluense* (ii) *C. collinum* ssp. *taborense*
***Spathulipetala* Engl. & Diels**	*C. zeyheri* Sond.
***Ciliatipetala* Engl. & Diels**	*C. albopunctactum* Suesseng.
	C. apiculatum Sond. (i) *C. apiculatum* ssp. *apiculatum*
	C. edwardsii Exell (provisional)
	C. moggii Excell (provisional)
	C. molle R. Br.
	C. *petrophilum* Retief
***Breviramea* Engl. & Diels**	*C. hereroense* Schinz
***Conniventia* Engl. Diels**	*C. microphyllum* Klotzsch
	C. paniculatum Vent.
***Poivrea* (Comm. Ex DC)**	*C. bracteosum* (Hochst)
	C. mossambicense (Klotzsch)
	C. acutifolium

Table 2.2. *Terminalia* species collected for antifungal and antioxidant screening.

***Terminalia* L.**	
Section	Species
Abbreviate Exell	*T. prunioides* M.A.Lawson
Psidiodes Exell	*T. brachystemma* Welw. ex Hiern
	T. sericea Burch ex DC
Platycarpae Eng. Diels emend Exell	*T. gazensis* Bak.f.

	T. mollis Laws
	T. sambesiaca Engl.&Diels

2.2.2. Plant storage

Leaves were separated from stems, and dried at room temperature. Most scientists have tended to use dried material because they are fewer problems associated with large-scale extraction of dried plants rather than fresh plant material (Eloff, 1998a). The dried plants were milled to a fine powder in a Macsalab mill (Model 200 LAB, Eriez®, Bramley), and stored at room temperature in closed containers in the dark until used.

Some of the reasons dried leaves were chosen to work with were, the time delay between collecting plant material and processing it makes it difficult to work with fresh material because differences in water content may affect solubility or subsequent separation by liquid-liquid extraction. The secondary metabolic plant components should be relatively stable especially if it is to be used as an antimicrobial agent, and many if not most plants are used in the dried form by traditional healers (Eloff, 1998a).

2.2.3. Extractants

When choosing the extractants the following parameters were considered, polarity (polar, intermediate or non-polar); the ease of subsequent handling of the extracts; the toxicity of the solvent in the bioassay process, the potential health hazard of the extractants (Eloff, 1998b). The following extractants, methanol (polar); acetone (intermediate polarity); dichloromethane (intermediate polarity) and hexane (Non-polar) were chosen on the basis of the above parameters.

2.2.4. Extraction procedure

Plant samples from each species were individually extracted by weighing four aliquots of 1 g of finely ground plant material and extracting with 10 ml of acetone, hexane, dichloromethane (DCM) or methanol (technical grade- Merck) in polyester centrifuge tubes. Tubes were vigorously shaken for 3-5 minutes in a Labotec model 20.2 shaking machine at high speed. After centrifuging at 1643 x g for 10 minutes the supernatant was decanted into pre-weighed labeled containers. The process was repeated 3 times to exhaustively extract the plant material and the extracts were combined. Extraction was done at room temperature,

because temperature was found not to have effect on antibacterial activity of Combretaceae species (Eloff, 1999). The solvent was removed under a stream of air in a fume cupboard at room temperature, to quantify the extraction.

2.2.5. Phytochemical analysis

Chemical constituents of the extracts were analyzed by thin layer chromatography (TLC) using aluminium-backed TLC plates (Merck, silica gel 60 F_{254}). The TLC plates were developed under saturated conditions with one of the three eluent systems developed in our laboratory that separate components of Combretaceae extracts well i.e.:
Ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral);
Chloroform/ethyl acetate/formic acid (5:4:1): [CEF] (intermediate polarity/acidic);
Benzene/ethanol/ammonia hydroxide (90:10:1): [BEA] (non-polar/basic) (Kotze and Eloff, 2000).

The dried extracts of the solvents were reconstituted to a concentration of 10 mg/ml in acetone. Acetone was the solvent of choice owing to its wide extraction capacity and low toxicity towards the test organisms in the bioassay procedures (Eloff, 1998b).

Approximately 100 µg aliquots (10 µl of a 10 mg/ml solution) of each of the extracts were loaded in 1 cm bands on three 10 x 10 cm TLC plates (Merck, silica gel 60 F_{254}) and each of these was developed with EMW, CEF or BEA. The extracts were applied approximately 1 cm from the bottom of the plates with a micropipette and allowed to develop for 8 to 9 cm in a tank containing eluent. The atmosphere in the tank was saturated by placing filter paper wetted with the eluent against the walls of the tanks, which were then sealed with lids.

Once developed, the separated compounds were observed under Camac Universal TL-600 UV light at 360 nm and 254 nm and the fluorescing (360 nm) or quenching (254 nm) compounds marked. To detect the separated compounds, vanillin-sulphuric acid (0.1 g vanillin (Sigma): 28 ml methanol: 1 ml sulphuric acid) was sprayed on the chromatograms and heated at 110 ^{o}C to optimal colour development.

2.3. Results

2.3.1. Extraction of raw material

The mass that each solvent extracted from 1 g leaf material of *Terminalia* species was

determined, calculated as % extracted and recorded in **Table 2.3** and of *Combretum* species in **Table 2.4**.

Table 2.3. The percentage mass (%) of *Terminalia* species extracted with four extractants from dried powdered leaves.

***Terminalia* species**	**Percentage mass residue extracted (%)**				
	Acetone	**Hexane**	**DCM**	**Methanol**	**Average**
T. prunioides	7.3	1.1	1.8	27.3	**9.4**
T. brachystemma	7.5	2.5	2.8	26.8	**9.9**
T. sericea	5.5	1.6	2.2	31.4	**10.2**
T. gazensis	2.4	0.3	0.6	3.3	**1.7**
T. mollis	4.7	0.6	0.9	47.3	**13.4**
T. sambesiaca	11.2	1	0.9	22.5	**8.9**
Average	**6.43**	**1.18**	**1.53**	**26.43**	**8.9**

Methanol (26.43 %) extracted the most material in terms of mass from all six *Terminalia* species, followed by acetone with average mass of 6.43 %; hexane and DCM extracted the lowest mass, which were 1.18 and 1.53 % respectively. The highest average mass extracted was from *T. mollis* (13.4 %) and *T. sericea* (10.2 %), and the lowest yield was from *T. gazensis* with 1.7 %. The total percentage mass extracted was 8.9 %. The 47.3 % extracted by methanol from *T. mollis* leaves is an extraordinary high value.

Table 2.4. The percentage mass (%) of *Combretum* species extracted with four extractants from dried powdered leaves.

***Combretum* species**	**Percentage mass residue extracted (%)**				
	Acetone	**Hexane**	**DCM**	**Methanol**	**Average**
C. celastroides ssp. *celastroides*	3.8	1.2	1.7	12.9	**4.9**
C. celastroides ssp. *orientale*	4.2	1.5	2.4	18.2	**6.6**
C. imberbe	5.1	0.9	2.9	9.6	**4.6**
C. padoides	3.5	1.5	2.5	24.5	**8.0**
C. caffrum	18.8	2.1	3.7	17.1	**10.4**
C. erythrophyllum	8	2.7	4.6	8.9	**6.1**
C. kraussii	6.5	0.9	1.7	24.9	**8.5**
C. woodii	5.9	1.2	4.1	20.4	**7.9**
C. collinum ssp. *suluense*	2.2	1.8	1.4	7.1	**3.1**
C. collinum ssp. *taborense*	4.7	1.8	2.1	18	**6.7**
C. zeyheri	3.4	0.9	1.9	18.8	**6.3**
C. albopunctactum	3	1.1	2.1	9.6	**4.0**
C. apiculatum ssp. *apiculatum*	8.9	1.7	2.3	35.2	**12.0**
C. edwardsii	2.9	0.8	1.1	23	**7.0**
C. moggi	7.2	1.5	2	24.2	**8.7**
C. molle	20.9	1.4	2.4	24	**12.2**
C. petrophilum	12.7	2.3	3	40.6	**14.7**

C. hereroense	8	0.7	1.2	32.9	**10.7**
C. microphyllum	1.7	1	1	29.9	**8.4**
C. paniculatum	1.5	0.6	1.5	17.7	**5.3**
C. bracteosum	2.2	1	1.9	8.9	**3.5**
C. mossambicense	5.8	2.8	3.8	11.8	**6.1**
C. acutifolium	4.1	1.4	2.3	18.9	**6.7**
C. nelsonii	5.8	2.3	4.3	18.9	**7.8**
Average	**6.28**	**1.46**	**2.41**	**19.83**	**7.5**

Methanol (19.83 %) extracted the most material in terms of mass from twenty-four *Combretum* species as in *Terminalia* species. This was followed by acetone with an average extracted mass of 6.28 %, almost similar to that of *Terminalia* species. Hexane and DCM extracted the lowest mass, which were 1.46 % and 2.41 % respectively. The highest average mass extracted was from *C. petrophilum* (14.7 mg), *C. molle* (12.2 %) and *C. apiculatum* ssp. *apiculatum* (12.0 %), and the lowest yields were from *C. collinum* ssp. *suluense* and *C. albopunctactum* with 3.1 and 4.0 % extracted respectively. The total percentage mass extracted was 7.5 %, lower than that of the *Terminalia* species with a difference of 1.4 %. In this case methanol extracted an extraordinary high concentration of 40.6 % from *C. petrophilum* leaves.

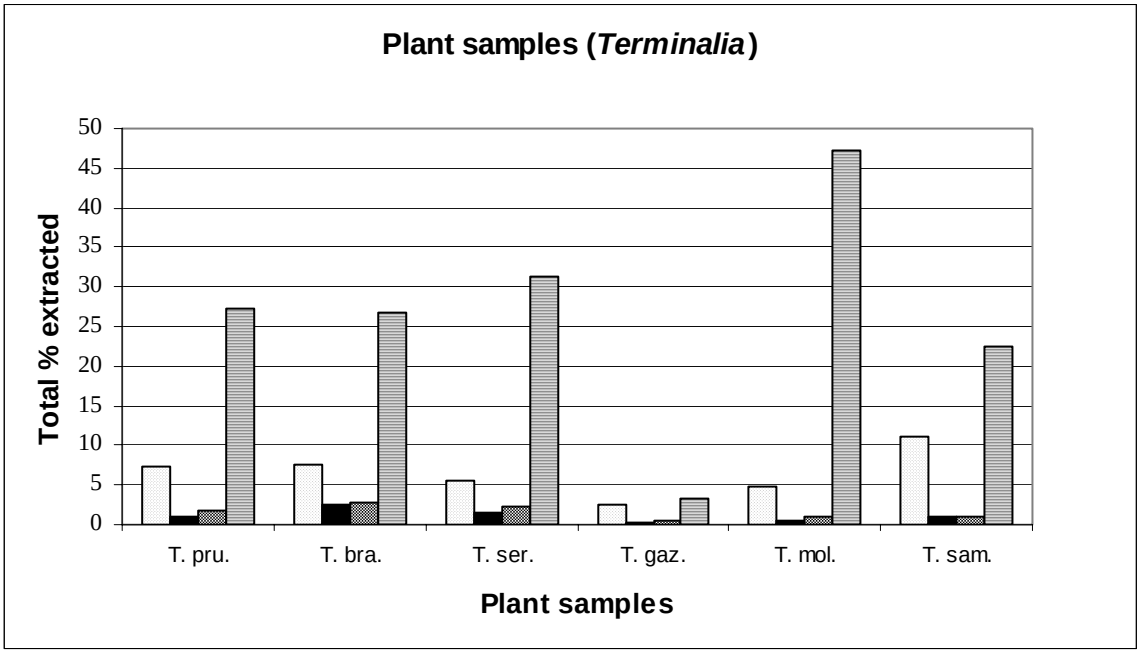


Fig 2.1. Percentage of powdered Terminalia leaf samples extracted by acetone ⬚ , hexane ■ , dichloromethane ▩ ,and methanol ▤ from the six *Terminalia* species: T. pru. = *T. prunioides*, T. bra. = *T. brachystemma*, T. ser. = *T. sericea*, T. gaz. = *T. gazensis*, T. mol = *T. mollis* and T. sam = *T. sambesiaca.*

The total percentages extracted of the *Combretum* species using different solvents (acetone, hexane, DCM and methanol) are shown in **Figure 2.2.** Methanol was the best extractant,

extracting a greater quantity of plant material than any of the other solvents. Total percentages extracted with methanol of *C. apiculatum* ssp. *apiculatum*, *C. petrophilum*, *C. hereroense* and *C. microphyllum* were between 25 and 41%. From **Figure 2.2** it appears that hexane and dichloromethane are more selective extractants for *Combretum* species, because for all the species, the total percentage extracted was below 5%. The total percentage extracted with acetone was better in 10 of the *Combretum* species tested, ranging from 5 to 21%.

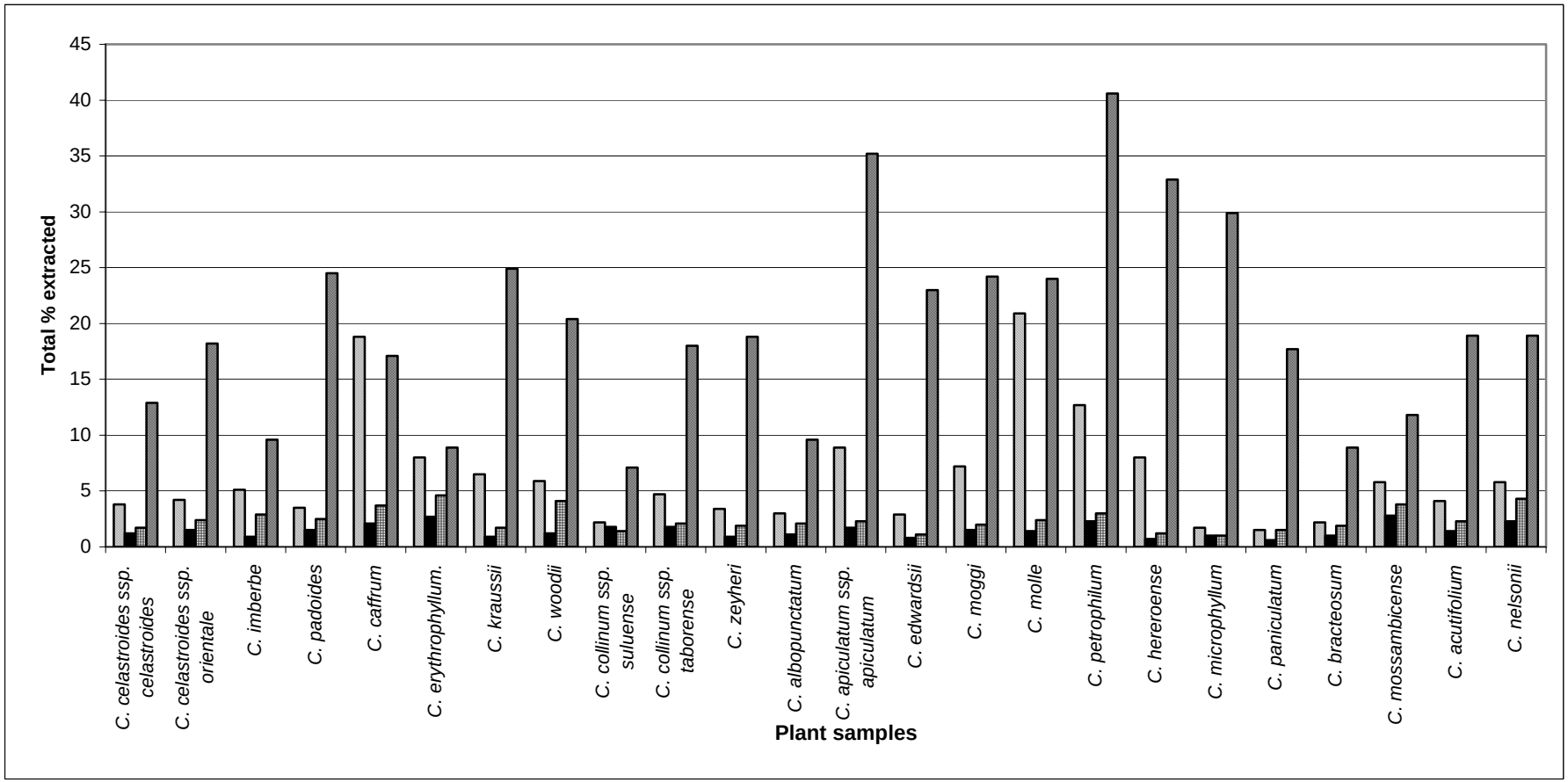


Figure 2.2. Percentage of powdered *Combretum* species leaf extracted by acetone , hexane , dichloromethane , and methanol .

2.3.2. Phytochemical analysis

In all the *Terminalia* and *Combretum* extracts vanillin spray reagent was chosen for visualization of compounds (**Figure 2.3a** to **Figure 2.3g)**.

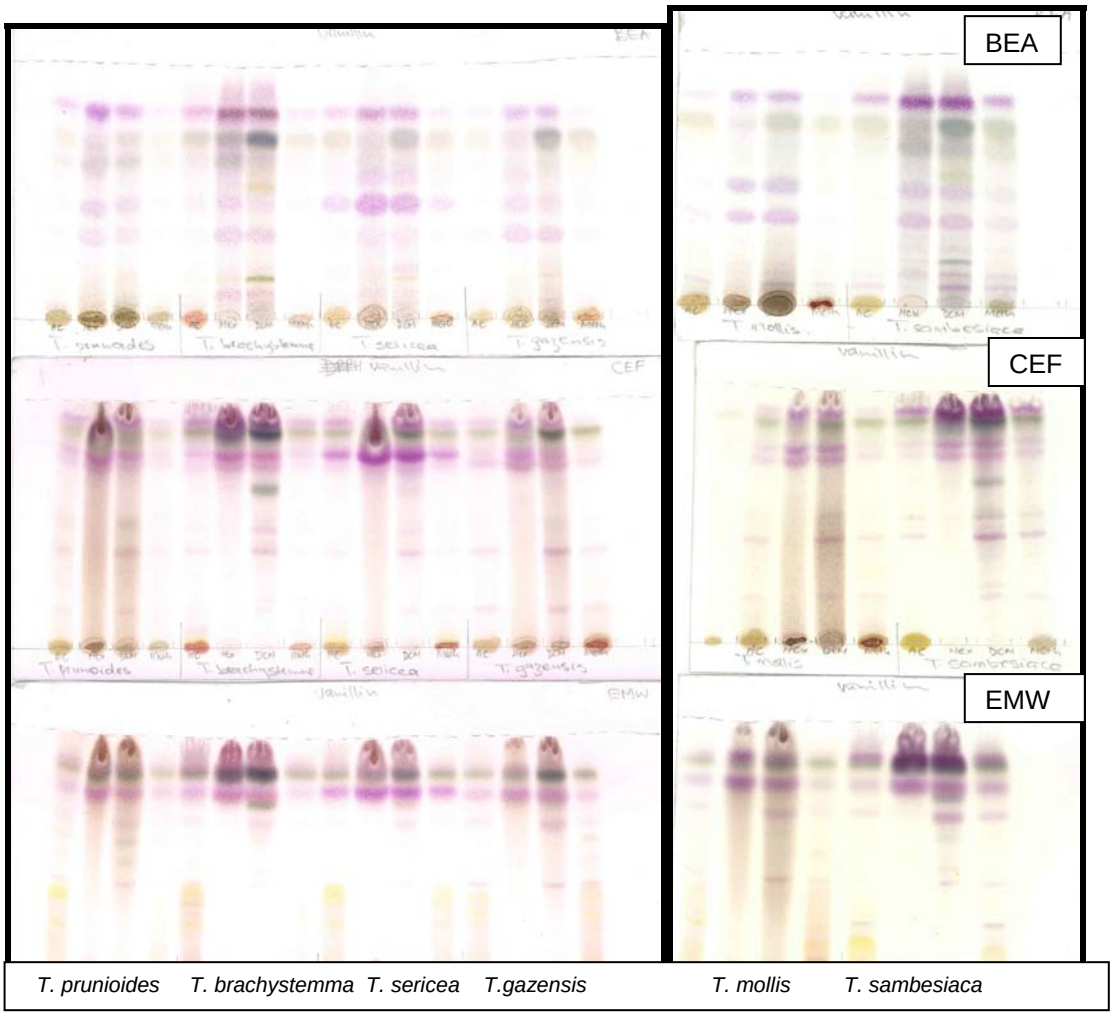
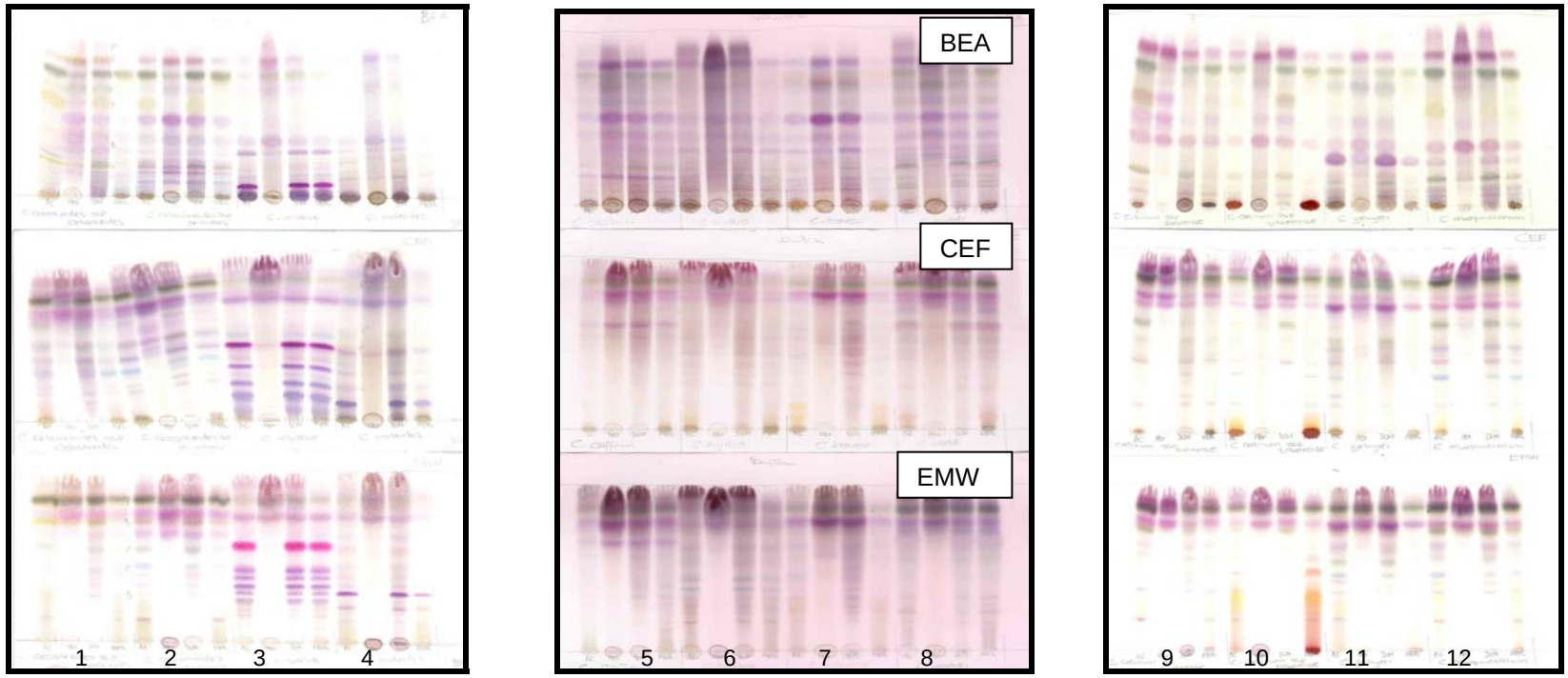


Figure 2.3a. Chromatograms of *Terminalia* species developed in BEA (top), CEF (centre), and EMW (bottom) solvent systems and sprayed with vanillin–sulphuric acid to show compounds extracted with acetone (Ac), hexane (Hex), dichloromethane (D) and methanol (Met), in lanes from left to right for each group.

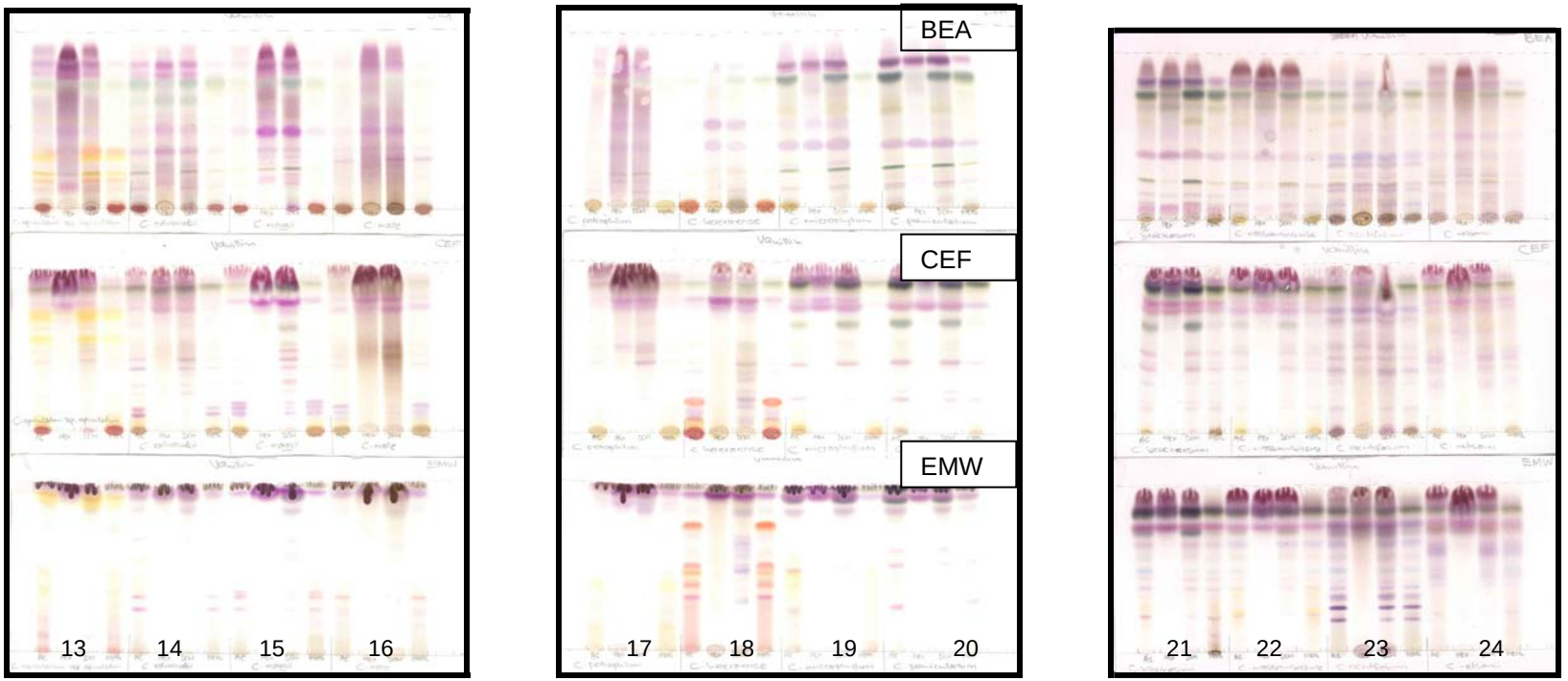


1 *C. celastroide* ssp *celastroides*
2 C. celastroides ssp*. orientale*
3 *C. imberbe*
4 C. padoides

5 *C. caffrum*
6 *C. erythrophyllum*
7 *C. kraussii*
8 *C. woodii*

9 *C. collinum* ssp. *suluense*
10 *C. collinum* ssp. *taborense*
11 *C. zeyheri*
12 *C. albopunctatum*

Figure 2.3b. Chromatograms of *Combretum* species developed in BEA (top), CEF (centre), and EMW (bottom) solvent systems and sprayed with vanillin–sulphuric acid to show compounds extracted with acetone (Ac), hexane (Hex), dichloromethane (D) and methanol (Met), in lanes from left to right for each group.



13 *C. apiculatum* ssp. *apiculatum*
14 *C. edwardsii*
15 *C. moggii*
16 *C. molle*

17 *C. petrophilum*
18 *C. hereroense*
19 *C. microphyllum*
20 *C. paniculatum*

21 *C. bracteosum*
22 *C. mossambicense*
23 *C. acutifolium*
24 *C. nelsonii*

Figure 2.3c. Chromatograms of *Combretum* species developed in BEA (top), CEF (centre), and EMW (bottom) solvent systems and sprayed with vanillin–sulphuric acid to show compounds extracted with acetone (Ac), hexane (Hex), dichloromethane (D) and methanol (Met), in lanes from left to right for each group

2.4. Discussion

Six *Terminalia* species and twenty-four *Combretum* species were selected for antifungal activity and antioxidant screening based on their use in traditional medicinal treatments for both domestic animals and humans in southern Africa, as well as their availability.

The majority of traditional healers use water to isolate active compounds from these plants, because water is not harmful to domestic animals and humans and is generally the only extractant available. Water extracts were not used as test substances in our study because in all our previous work water extracts had no antimicrobial activity and it is tedious to remove water from extracts. This naturally sets some limitations to the type and amount of compound to be extracted relative to their polarity (Eloff, 1998). Using only water leads to difficulties in extracting non-polar active compounds. Success in isolating compounds from plant material is largely dependent on the type of the solvent used in the extraction procedure (Lin *et al*., 1999).

The total percentages of the *Terminalia* species extracted using different solvents (acetone, hexane, DCM and methanol) are shown in **Figure 2.1.** Methanol was the best extractant, extracting a greater quantity of plant material than any of the other solvents. There was a major difference in the methanol extractability of *T. gazensis* leaves compared with all the other species. This difference is not related to the sectional division of the species (Carr, 1988).

After evaporation of extracting solvents, the hexane, dichloromethane and methanol extracts were redissolved in acetone because this solvent was found not to be harmful towards bacteria (Eloff, 1998b). I found that acetone was also not harmful towards fungi at concentrations used in the plant extracts (**Chapter 4**).

Of the four solvents used, methanol extracted more chemical compounds from leaves of the *Terminalia* and *Combretum* species, but the extract probably contained highly polar compounds that may not that interesting for clinical application. Baba-Moussa *et al* (1999)has found that methanol extracts of Combretaceae family contains tannins. Because tannins have low bioavailability, the potential value of tannins as a systemic antifungal compound is low. Some scientists have concluded

that there is therefore not much scope for investigating the Combretaceae for antimicrobial compounds.

There was similarity in the chemical composition of the non-polar compounds of extracts using extractants of varying polarity. This may indicate the presence of saponin-like compounds in the leaves. Saponins are a vast group of glycosides, widely distributed in higher plants. Their surface-active properties are what distinguish these compounds from other glycosides. They dissolve in water to form colloidal solutions that foam upon shaking. Saponins have also been sought after in the pharmaceutical industry because some form the starting point for the semi-synthesis of steroidal drugs. They are believed to form the main constituents of many plant drugs and folk medicines, and are considered responsible for numerous pharmacological properties (Estrada *et al*., 2000).

In all the extracts a number of compounds were observed. Because of the number of samples (120 extracts) I decided not to count all the compounds visualized, but present only the chromatograms. Thin layer chromatography (TLC) was used to fingerprint the plant extracts. This allowed for a comparison of the R_f values and thus aided in the identification of biologically active bands on the chromatograms, used for bioautography. The R_f value can however provide corroborative evidence to identity of a compound. If the two or more compounds have the same R_f values in several solvent systems they are most likely, although not necessarily, the same compounds.

The three eluent systems (**Section 2.2.5**) differed in separating the different polarity compounds. The EMW mobile system separates polar and neutral compounds well, the BEA mobile system separates non-polar compounds best and the CEF mobile system separates intermediate polarity and acidic compounds best. Before spraying the TLC plates with vanillin spray reagent, the plates were observed under UV and visible light, which identified fluorescent-quenching compounds in herbal extracts. UV light usually identifies fluorescing compounds with many double bonds and the visible light only detects coloured compounds, usually with conjugated bonds. Compounds containing aromatic rings adsorb UV light at 254 nm and therefore quench the florescence of the pigment included in the silica gel.

TLC can be used for qualitative as well as semi-quantitative analysis of crude extracts for identification of constituents (Houghton and Raman, 1998). Qualitative

analysis is done by comparing the retardation factor (R_f value) on the TLC against a reference value of a standard.

$$R_f \text{ value} = \frac{\text{distance moved by analyte}}{\text{distance moved by solvent front}}$$

After spraying the TLC plates with vanillin-sulphuric acid, many different compounds could be observed. It is difficult to identify certain compounds on these plates, but one can compare the R_f values of the compounds seen on the plates with the R_f values of compound isolated from *Combretum* or *Terminalia* species, to check resemblance between specific compounds in an extract. Different factors influence the chemical composition of the material and subsequently also influence the results of this study. Such factors may include the season when the plant has been harvested, together with effects of variation in growth conditions. As our study is based on the leaves only, and other parts of the plants were not considered, the results may underestimate the activity of the plant species involved.