African Journal of Biotechnology Vol. 5 (18), pp. 1625-1647, 18 September 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Bioautography indicates the multiplicity of antifungal compounds from twenty-four southern African *Combretum* species (Combretaceae)

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Accepted 22 June, 2006

Dried ground leaves of 24 Combretum spp were extracted with hexane, dichloromethane, acetone and methanol and analysed by bioautography to determine the number of antifungal compounds against five animal fungal pathogens (*Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Microsporum canis* and *Sporothrix schenckii*). There was some similarity in the chemical composition of the non-polar components of extracts using extractants of varying polarity. Acetone extracted the most antifungal compounds from *Combretum* spp. *Combretum* spp. in the section *Hypocrateropsis, C. celastroides* ssp. *celastroides* and *C. clelastroides* ssp. *orientale* had 62 different antifungal zones of inhibition compared to the 7 to 8 of *C. microphyllum* and *C. paniculatum* in the *Connivetaia* section. *C. collinum* subspecies were not active against all the tested pathogens. *C. neoformans* was the most sensitive organism against all *Combretum* species, with 367 zones of inhibition using different TLC solvent systems and extracts. *A. fumigatus* was the most resistant (192 zones of inhibition). The antifungal activity and number of active antifungal compounds were high enough to consider the use of extracts for clinical application and to isolate antifungal compounds from the extracts. Based on the R_f values of the antifungal compounds determined using solvents of varying polarity, activity is not only be attributable to tannins found in *Combretum* extracts as was previously postulated.

Key words: *Combretum* species, Combretaceae, bioautography, R_f value.

INTRODUCTION

The increasing incidence of mycoses associated with AIDS and also those arising after treatment by immunosuppressive drugs have given fresh impetus to the search for novel antifungal agents (Hostettmann et al., 2000). Due to an increase in antimicrobial resistance in the public health sector, there is a need to investigate and search for more therapeutic agents. There are few really effective antifungal preparations currently indicated for the treatment of systemic mycoses. These mycoses are very difficult to eradicate constituting a challenge for healthcare providers (Meyers, 1990). Many of the drugs have undesirable effects, e.g. they are very toxic (amphotericin B), produce recrudence, show drug–drug interactions (azoles) or lead to the development of resistance (fluconazole, 5-flucytosine) (White et al., 1998).

The search of new antifungal agents with potent and broad spectrum fungicidal activities needed for the effective management of these infections led us to the screening of antifungal compounds in *Combretum* species (Combretaceae). The selection of this genus is based on uses in traditional medicine, since *Combretum* species are widely used medicinal plants both in Africa and in Asia. Traditional healers throughout Africa use species of the Combretaceae for many medicinal purposes. This includes treating fever, headaches, abdominal disorders, abdominal pains, gallstones, diarrhoea, dysen-

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tery, gastric ulcers, bilharziasis, hookworm, nosebleeds, sore throats, colds, chest coughs, pneumonia, conjunctivitis, dysmenorrhoea, infertility in women, venereal diseases including syphilis, earache, fattening babies, leprosy, scorpion and snake bites, swelling caused by mumps, toothache, heart diseases, cleansing the urinary system, backache, jaundice, stomach and gastric problems, blennorhagia, constipation and general weakness (Hutchings et al., 1996; Neuwinger, 1996; Iwu, 1993; Oliver-Bever, 1986). Some of these uses may be related to antifungal activity of extracts.

The Combretaceae consists of 18 genera, the largest of which are *Combretum*, with about 370 species, and *Terminalia*, with about 200 species (Lawrence, 1951). Species from the genus *Combretum* and to a lesser extent *Terminalia* are most widely used for medicinal purposes. As they are common and widely distributed throughout western and southern Africa, (Rogers and Verotta, 1996), they are readily available for use. The leaves and bark of the *Combretum* species are predominantly used.

Members of our group have worked and isolated a number of antibacterial compound from the *Combretum* species. In 1998, Martini and Eloff demonstrated the presence of at least 14 different unidentified bacterial inhibitors, of widely differing polarity, in leaves of *Combretum erythrophyllum* (Martini and Eloff, 1998). Seven of these compounds have been isolated, their structure determined (Martini et al., 2004a) and several biological activities of five of these flavonoids were determined (Martini et al., 2004b). Eloff (1999) examined other members of the Combretaceae, namely 27 species of *Combretum*, *Terminalia*, *Pteleopsis* and *Quisqualis*. Acetone leaf extracts of all the species inhibited the growth of the test bacteria to varying degrees. Eloff et al. (2005a) have demonstrated that *C. woodii* leaf extracts have high activity against Gram-negative and Gram-positive bacteria. The main antibacterial compound from the leaves of *C. woodii* is combrestatin B5, a stilbene (Eloff et al., 2005b). MacGaw et al. (2001) have investigated some of the biological activity (anti-inflammatory, anthelmintic, anti-bilharzia (antischistosomal) and DNA-damaging) of *Combretum* species. Significant activity in more than one bioassay was exhibited by *C. apiculatum*, *C. hereroense*, *C. molle* and *C. mossambicense.*

Alexander et al. (1992) found several antibacterial components in some of the 12 *Combretum* species they investigated and they found up to five different inhibitors in extracts of other *Combretum* spp. using different test organisms. In preliminary bioassays their extracts were active against *Staphylococcus aureus* but there was no activity in subsequent assays. Breytenbach and Malan (1989) isolated three antibacterial compounds from *Combretum zeyheri* and proposed structures for two of them. All of these compounds had antibacterial activity against *S. aureus.*

Baba-Moussa et al. (1999) reported that seven species of the West African Combretaceae had a degree of antifungal activity. These researchers suggested that the tannins and saponins, present in the plant extracts, may be responsible for the antifungal activity. We have reported that extracts of South African *Terminalia* species (another member of the Combretaceae) have substantial antifungal activities, with MICs as low as 20 µg/ml (Masoko et al., 2005) and we have also reported their diversity of compounds responsible for the antifungal activity (Masoko and Eloff, 2005).

Hostettmann et al. (2000) have reported that it is impossible to do direct bioautography with yeast like *Candida albicans*. We improved and developed a technique for bioautography of fungal extracts using extracts of six *Terminalia* spp. (Masoko and Eloff, 2005). Bioautography is a valuable detection method for a new or unidentified antifungal compounds, because it is based on the biological effects of the substances under study. Bioautography also worked with *C. albicans*. Bioautography in different solvent systems makes it possible to dereplicate the isolation of compounds previously isolated from related taxa.

The widespread use of *Combretum* species in indigenous medicine for many different ailments, the significant antimicrobial and other activity exhibited by several species and the need for effective antifungal agents, justifies the further investigation of the antifungal activity of *Combretum* species. Some difficulties in the bioautographic method could be resolved and the technique made it possible to select the most promising species for isolation and *in vivo* activity studies.

MATERIALS AND METHOD

Plant collection

Leaves were collected, in Summer, from plants in the Lowveld National Botanical Garden in Nelspruit in 2003. Voucher specimens and provenance of the trees are kept in garden herbarium. Plants used are listed in Table 1. More information on the origin and references of these plants are presented elsewhere (Eloff, 1999).

Plant drying and storage

Leaves were separated from stems, and dried at room temperature. Most scientists have tended to use dried material because there 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.

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 centrifuge tubes. These

Table 1. *Combretum* species used for antifungal screening. Infra generic classification from Carr (1988).

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
	C. moggii Excell
	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

tubes were vigorously shaken for 3 - 5 min in a Labotec model 20.2 shaking machine at high speed. After centrifugation at 3500 rpm for 10 min, the supernatant was decanted into labelled containers. This process was repeated 3 times to exhaustively extract the plant material and the extracts were combined. The solvent was removed under a stream of air in a fume cupboard at room temperature before dissolving extracts in acetone to a concentration of 10 mg/ml, to quantify the assay. Preliminary experiments have shown that acetone diluted according to the MIC bioassay procedure does not inhibit the growth of any of the fungi tested.

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 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/ammonium hydroxide (90:10:1): [BEA] (non-polar/basic) (Kotze and Eloff, 2002). Development of the chromatograms was in a closed tank in which the atmosphere had been saturated with the eluent vapour by lining the tank with filter paper wetted with the eluent.

To detect the chemical components of each extract, 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.

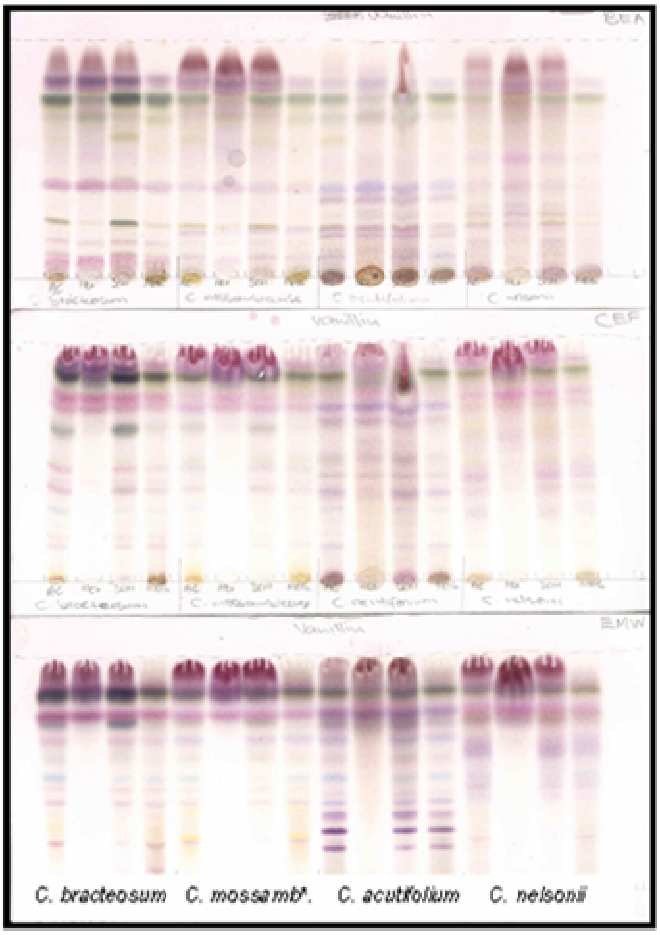
Fungal test organisms

Five fungi were obtained from the central microbiology laboratory Faculty of Veterinary Science, University of Pretoria and used as test organisms. These fungi represent the different morphological forms of fungi, namely yeasts (*Candida albicans* and *Cryptococcus neoformans*), thermally dimorphic fungi (*Sporothrix schenckii*) and moulds (*Aspergillus fumigatus* and *Microsporum canis*) and are the most common and important disease-causing fungi of animals. *C. albicans* was isolated from a Goldian finch, *C. neoformans* from a cheetah, and *A. fumigatus* from a chicken, all of which suffered from a systemic mycosis. *M. canis* was isolated from a cat with dermatophytosis and *S. schenckii* from a horse with cutaneous lymphangitis. Not one of the animals had been treated prior to sampling. All fungal strains were maintained on Sabouraud dextrose agar (Oxoid, Basingstoke, UK).

Bioautographic methods

TLC plates (10 x 20 cm) were loaded with 100 µg (5 µl of 20 mg/ml) of each of the extracts. The prepared plates were developed in the three different mobile systems used: CEF, BEA and EMW. The chromatograms were dried for up to a week at room temperature under a stream of air to remove the remaining solvent.

Cultures were grown on Sabouraud agar for 3 - 5 days. Sabouraud broth was prepared in 250 ml bottles. Cultures were transferred into broth from agar with a sterile swab. The TLC plates developed were inoculated with a fine spray of the concentrated suspension containing approximately 10^{9} organisms per ml of actively growing fungi e.g. conidia for filamentous fungi and yeast cells for the other fungi in a Biosafety Class II cabinet (Labotec, SA) cupboard. The plates were sprayed until they were just wet, incubated overnight and then sprayed with a 2 mg/ml solution of *p*-iodonitrotetrazolium violet (Sigma®) (INT) (Begue and Klein, 1972) and further incubated overnight or longer in the case of *S. schenckii* and *M. canis* at 35°C in a clean chamber at 100% relative humidity in the dark. White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of tested fungi. We tried to use digital photography to record results but it did not work well. Good results were obtained after using a scanner (HP scanjet



C. mossamb*.= C. mossambicense

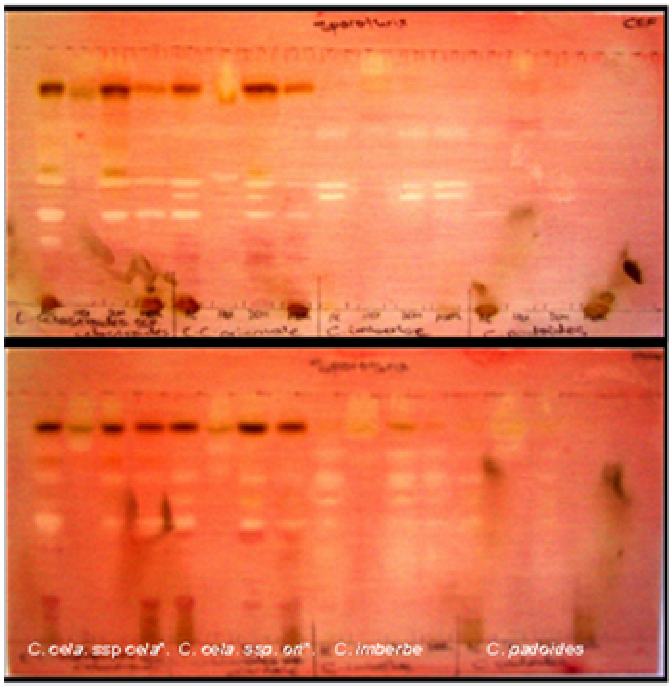
Figure 1. Chromatograms of *Combretum* species developed in BEA (top), CEF (centre), and EMW (bottom) solvent systems and sprayed with vanillin–sulphuric acid to show compounds extracted with acetone (Ac), hexane (Hex), dichloromethane (D) and methanol (Met), in lanes from left to right for each group.

5470c). To minimize fungal spreading and infections in our laboratory, the bioautograms were sealed in the clear plastic envelopes before scanning in for a permanent record.

RESULTS AND DISCUSSION

Number of compounds separated by TLC

There were differences in compounds extracted with different extractants (Figure 1). From the extractants used, acetone and methanol extracted more chemical compounds from leaves of the *Combretum* species than the other extractants, but the methanol extract may contain highly polar tannin-like compounds that are not that interesting for clinical application. Baba-Moussa (1999) has found that methanol extracts of Combretaceae members 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.



C. cela. ssp. cela. = C. celastroide ssp celastroides
C. cela. ssp. ori. = C. celastroides ssp. orientale

Figure 2. Bioautography of *Combretum* species extracted with acetone (Ac), hexane (Hex), dichloromethane (D) and methanol (Met) in lanes from left to right for each group, separated by CEF (top) and EMW (bottom) and sprayed with *S. schenckii*. White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *S. schenckii.*

Saponins are extracted by acetone and methanol and they react well with the vanillin sulphuric acid spray reagent. The fact that few compounds appear to be present at the origin of the most polar solvent system used (EMW), indicates that there were hardly any saponins extracted (Figure 1).

The separated compounds on TLC plates were made visible by spraying with vanillin-sulphuric acid. There was some similarity in the chemical composition of the non-polar components of extracts using extractants of varying polarity (Figure 1).

Number of antifungal bands observed

Bioautography was used to separate the antifungal compounds to obtain more information on the diversity of antifungal compounds present in different *Combretum* species extracts. Inhibition zones of antifungals are observed as white lines on a purple-red background (Figure 2). White areas indicate where reduction of INT to

the coloured formazan did not take place due to the presence of compounds that inhibited the growth of tested fungi.

To compare the different parameters, the number of inhibition zones encountered with the different Combretum species, the different extractants, the different solvent systems and the different pathogens were counted. Many of these were most likely due to the same compounds. The number of inhibition zones does, therefore, not mean that there are those numbers of different antifungal compounds present.

A representative bioautograms of some *Combretum* extracts against the different fungi is shown (Figure 2). To make it easier to evaluate the results of the biochromatograms in the data has been tabulated (Tables 2 to 23).

Terminalia species (Masoko et al., 2005) and *Combretum* species (Masoko et al., 2006) occurring in southern Africa have substantial antifungal activity. Most of the crude extracts had MIC values of c. 0.08 mg/ml, and some had MIC's as low as 0.02 mg/ml, especially against *C. neoformans*, *S. schenckii* and *M. canis*. The methanolic extracts of *C. moggii* and *C. petrophilum* were very active against all the tested pathogens. All extracts of *C. nelsonii* were very effective against all the pathogens. Acetone and methanol extracts of *C. acutifolium* were active against all pathogens after 24 h of incubation, with MIC values ranging from 0.02 and 0.04 mg/ml (Masoko et al., 2006).

Chemotaxonomic comparison

To determine if there any relationship between antifungal activity and taxonomy based on morphological parameters (Carr, 1988) (Table 1), information for the different sections of the genus was compared. Extracts of *Combretum* species in the Section *Hypocrateropsis* had high number of potentially different active antifungal compounds, ranging from 56 to 62 (Table 24). These values were high because the zones of inhibition were counted for the different solvent systems used. A compound with an R_f of 0.47 in BEA may e.g. be the same compound with an R_f of 0.36 in CEF. For comparative purposes we counted all antifungal bands. That explains the high numbers reported. *C. celastroides* ssp. *celastroides* and *C. clelastroides* ssp. *orientale* had 62 active bands each. Section *Angustimarginata* follows section *Hypocrateropsis* had active compounds ranging from 37 to 43, with more active compound in *C. nelsonii* (43).

The *Metallicum* section, which is made up of *C. collinum* ssp. *suluense* and *C. collinum* ssp. *taborense* did not contain active compounds against all tested microorganisms. *C. zeyheri* in *Spathulipetala* section contained 26 active compounds. In *Ciliatipetala* section, *C. albopunctactum* contained more active compounds than other species in the same section, with 43 compounds. It is followed by *C. apiculatum* sp. *apiculatum* and *C. petrophilum* with 20 and 14 compounds, respectively. In *Breviramea* section we had only one species, *C. hereroense,* with 16 active compounds. *Connivetaia* section species, *C. microphyllum* and *C. paniculatum* had a similar number of active compounds, 7 and 8, respecttively. *C. mossambicense* and C. *acutifolium* in *Poivrea* section, had the most active compounds, which were 25 for each species, and *C. bracteosum* had 14. That was the biggest difference in the section as compared to others where species in the section had almost the same number of compounds.

Hypocrateropsis section had high average number of active compounds, followed by *Angustimarginata* and *Ciliatipetala* sections, which were 59, 40 and 26, respectively. *Metallicum* section did not have active compounds. *Breviramea* and *Connivetia* sections had low average number of active of active compounds, which were 17 and 8, respectively (Table 25).

Combretum species are divided into two subgenera *Combretum* and *Cacoucia*. Subgenus *Combretum* had 581 zones of inhibition for all species, extracts, pathogens and solvent systems compared to the 79 of subgenus *Cacoucia* (Table 24). It therefore appears as if there is a correlation between the taxonomy and number of antifungal compounds.

Sensitivity of fungal pathogens

C. neoformans was sensitive against the most compounds in all *Combretum* species, with 367 zones of inhibition for all the extracts and solvent systems used active against it. This was followed by *C. albicans* (339), S*. schenckii* (314) *M. canis* (298) and *A. fumigatus* with 192 compounds active against it (Table 26).

Comparison of extractants

Success in isolating compounds from plant material is largely dependent on the type of the solvent used in the extraction procedure. The total number of compounds extracted using different solvents (acetone, hexane, DCM and methanol) is shown in Table 24. Acetone and dichloromethane were the best extractants, extracting 199 active compounds, followed by methanol (189) and hexane (73). This difference is apparently not related to the sectional division of the species (Carr, 1988).

Comparison of eluent systems

Lastly we determined which TLC solvent system separated the highest number of active compounds (Table 27). EMW was the best solvent (548) followed by CEF (506) and BEA (456), active compounds were separated. This implies that polar systems separated less active compounds compared to non-polar system. The wide diversity in polarity of the antimicrobial components may provide clinically useful leads.

In some cases organisms did not grow too well and it was difficult to detect inhibition zones especially with *A. fumigatus*. *A. fumigatus* was inhibited by some extracts;

Table 2. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. celastroides* ssp. *celastroides* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent system	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.81								√												
	0.63								√					√					√		
	0.57	√	√	√								√	√				√	√	√		
	0.52	√	√	√								√	√	√			√	√	√		
	0.47	√	√	√								√	√	√			√	√			
	0.17				√										√					√	
	0.09				√										√					√	
CEF	0.82	√	√	√								√	√				√	√			
	0.80	√	√	√								√	√				√	√			
	0.50				√										√					√	
	0.45	√	√	√	√							√	√	√	√		√	√	√	√	
	0.36	√	√	√	√							√	√	√	√		√	√	√	√	
	0.20			√	√				√					√					√		
EMW	0.92	√	√	√					√			√	√	√			√	√	√		
	0.85	√	√	√					√	√		√	√	√			√	√	√		
	0.78	√	√	√					√			√	√	√			√	√	√		
	0.74	√	√	√	√							√	√		√					√	
	0.62		√	√	√								√	√	√		√	√	√	√	
	0.56				√										√					√	
	0.51			√	√									√	√				√	√	

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 3. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. celestroides* ssp. *orientale* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.81								√												
	0.63								√					√					√		
	0.57	√	√	√								√	√				√	√	√		
	0.52	√	√	√								√	√	√			√	√	√		
	0.47	√	√	√								√	√	√			√	√			
	0.17				√										√					√	
	0.09				√										√					√	
CEF	0.82	√	√	√								√	√				√	√			
	0.80	√	√	√								√	√				√	√			
	0.50				√										√					√	
	0.45	√	√	√	√							√	√	√	√		√	√	√	√	
	0.36	√	√	√	√							√	√	√	√		√	√	√	√	
	0.20			√	√				√					√					√		
EMW	0.92	√	√	√					√			√	√	√			√	√	√		
	0.85	√	√	√					√	√		√	√	√			√	√	√		
	0.78	√	√	√					√			√	√	√			√	√	√		
	0.74	√	√	√	√							√	√		√		√	√		√	
	0.62		√	√	√								√	√	√			√	√	√	
	0.56				√										√					√	
	0.51			√	√									√	√				√	√	

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 4. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. imberbe* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.81								√												
	0.63								√					√					√		
	0.57	√	√	√								√	√				√	√	√		
	0.52	√	√	√								√	√	√			√	√	√		
	0.47	√	√	√								√	√	√			√	√			
	0.17				√										√					√	
	0.09				√										√					√	
CEF	0.71	√	√		√							√	√		√		√	√		√	
	0.50	√	√	√	√							√	√	√	√		√	√	√	√	
	0.45	√	√	√	√							√	√	√	√		√	√	√	√	
	0.36				√										√					√	
	0.20				√																
EMW	0.92	√	√	√					√			√	√	√			√	√	√		
	0.85	√	√	√					√	√		√	√	√			√	√	√		
	0.78	√	√	√					√			√	√	√			√	√	√		
	0.74	√	√	√	√							√	√		√		√	√		√	
	0.62		√	√	√								√	√	√			√	√	√	
	0.56				√										√					√	
	0.51			√	√									√	√				√	√	

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 5. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. padoides* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.81								√					√							
	0.63								√					√							
	0.57	√	√									√	√				√	√			
	0.52	√	√									√	√				√	√			
	0.47	√	√									√	√				√	√			
	0.17				√										√					√	
	0.09				√										√					√	
CEF	0.71	√	√		√							√	√		√		√	√		√	
	0.50	√	√	√	√							√	√	√	√		√	√	√	√	
	0.45	√	√	√	√							√	√	√	√		√	√	√	√	
	0.36				√										√					√	
	0.20				√																
EMW	0.92	√	√	√					√			√	√	√			√	√			
	0.85	√	√	√					√	√		√	√	√			√	√			
	0.78	√	√	√					√			√	√	√			√	√			
	0.74	√	√	√	√							√	√		√		√	√		√	
	0.62		√		√										√					√	
	0.56				√										√					√	
	0.51			√	√										√					√	

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 6. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. caffrum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.24			√	√									√	√				√	√	
	0.20	√	√	√	√	√						√	√	√	√	√	√	√	√	√	√
	0.13	√	√	√	√							√	√	√	√		√	√	√	√	
	0.10	√	√	√		√								√		√					√
	0.04	√	√	√	√	√								√	√	√					√
CEF	0.78			√										√					√		
	0.53	√	√	√	√	√						√	√	√		√	√	√		√	√
	0.44	√	√									√	√				√	√			
	0.38	√	√	√	√	√						√	√	√		√				√	
EMW	0.84	√	√	√	√	√						√	√	√	√	√	√	√	√	√	√
	0.70	√	√		√							√	√		√		√	√		√	
	0.64	√	√		√										√		√	√		√	
	0.04			√	√	√															√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 7. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. erythrophyllum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent system	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.24			√	√									√	√					√	
	0.20	√	√	√	√							√	√	√	√		√	√	√	√	
	0.13	√	√	√	√	√						√	√	√	√	√	√	√		√	√
	0.10	√	√			√										√					√
	0.04					√									√	√					√
CEF	0.78			√										√							
	0.53	√	√			√						√	√			√	√	√			√
	0.44	√	√	√	√							√	√	√			√	√		√	
	0.38	√	√	√	√	√						√	√	√		√				√	
EMW	0.84	√	√	√	√	√						√	√	√	√	√	√	√	√	√	√
	0.70	√	√		√							√	√		√		√	√		√	
	0.64	√	√		√										√		√	√		√	
	0.04					√															√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 8. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. kraussii* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent system	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.24			√	√									√	√					√	
	0.20	√	√	√	√							√	√	√	√		√	√	√	√	
	0.13	√	√	√	√	√						√	√	√	√	√	√	√		√	√
	0.10	√	√			√										√					√
	0.04	√	√			√									√	√					√
CEF	0.78			√										√							
	0.53	√	√			√						√	√			√	√	√			√
	0.44	√	√	√	√							√	√	√			√	√		√	√
	0.38	√	√	√	√	√						√	√	√		√				√	√
EMW	0.84	√	√	√	√	√						√	√	√	√	√	√	√	√	√	√
	0.70	√	√		√							√	√		√		√	√		√	
	0.64				√										√					√	
	0.59	√	√														√	√			
	0.24																√	√		√	
	0.04					√											√	√		√	√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 9. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. woodii* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.24				√	√									√	√				√	√
	0.20	√	√		√							√	√		√		√	√		√	
	0.13	√	√		√	√						√	√		√	√	√	√		√	√
	0.10	√	√			√										√					√
	0.04	√	√			√										√					√
CEF	0.78			√										√							
	0.53	√	√	√		√						√	√			√	√	√	√		√
	0.44	√	√	√	√							√	√	√			√	√	√	√	√
	0.38	√	√	√	√	√						√	√	√		√				√	√
EMW	0.84	√	√	√	√	√						√	√	√	√	√	√	√	√	√	√
	0.70	√	√		√							√	√		√		√	√		√	
	0.64	√	√		√										√					√	
	0.04					√														√	√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 10. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. zeyheri* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent system	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.34		√		√		√				√		√			√				√	
	0.18			√	√											√				√	√
	0.07	√	√					√				√	√					√			
CEF	0.81	√					√	√				√	√	√		√					
	0.75			√	√									√	√						
	0.57				√						√	√		√	√	√					
	0.01		√															√			
EMW	0.90	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 11. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. albopuntactum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent system	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.80	√								√		√									
	0.67					√															
	0.18			√	√	√								√	√	√			√	√	√
	0.08	√	√	√		√						√	√	√		√		√	√		
	0.05	√	√		√							√	√		√					√	
CEF	0.91					√					√					√					
	0.81	√				√						√		√			√				
	0.76					√			√			√		√	√						
	0.65								√						√						
	0.57	√	√	√	√	√								√	√	√	√			√	√
	0.53	√											√								
	0.41	√	√	√	√		√											√			√
EMW	0.93	√	√		√	√	√			√	√	√	√		√	√	√	√		√	
	0.84	√		√				√				√		√			√				
	0.77		√		√								√		√						
	0.61	√	√	√	√																

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 12. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. capitulatum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Solvent system	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.35															√					√
	0.08				√								√							√	
CEF	0.81	√					√						√								
	0.76				√		√					√				√				√	
	0.56												√				√				
	0.1										√					√					
EMW	0.90	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 13. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. edwardsii* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum edwardsii																					
Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.08							√													
	0.01		√																		
CEF	0.91															√					
	0.81						√					√					√				
	0.76									√					√						
EMW	0.89	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 14. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. moggii* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum moggii																					
Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
CEF	0.81	√																			
	0.76									√					√						
EMW	0.93		√		√			√		√	√	√			√	√	√			√	

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 15. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. molle* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum molle																					
Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.05	√	√	√	√	√											√	√	√	√	√
CEF	0.05	√	√	√	√												√	√	√	√	
EMW	0.89						√		√		√	√		√		√	√				√
	0.05	√																			

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 16. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. petrophilum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum petrophilum																				
Solvent systems	**R_f values**	**Acetone**					**Hexane**					**DCM**					**Methanol**				
		C.a	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**
BEA	0.72							√					√								
	0.05	√	√	√	√	√											√	√	√	√	√
CEF	0.92							√					√								
	0.63												√								
	0.05	√	√	√	√												√	√	√	√	
EMW	0.89		√				√	√	√		√	√	√	√							
	0.1	√	√			√											√	√			√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 17. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. hereronse* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum hereronse																				
Solvent systems	**R_f values**	**Acetone**					**Hexane**					**DCM**					**Methanol**				
		C.a	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**
BEA	0.48																	√			
	0.05	√	√		√	√											√	√		√	√
CEF	0.92						√														
	0.85				√		√			√											
	0.81						√														
	0.72												√								
	0.41												√								
	0.27																	√			
	0.05	√	√		√												√	√		√	
EMW	0.95								√		√			√		√					
	0.68	√				√											√				√

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 18. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. microphyllum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum microphyllum																					
Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
CEF	0.90					√		√		√											
	0.72												√								
EMW	0.89						√		√			√		√							
	0.80							√										√			

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 19. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. paniculatum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum paniculatum																					
Solvent systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.72							√					√								
CEF	0.90					√				√					√						
EMW	0.90								√					√			√				

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 20. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. bracteosum* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum bracteosum																					
Solvent Systems	R_f values	Acetone					Hexane					DCM					Methanol				
		C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f	C.a	C.n	M.c	S.s	A.f
BEA	0.87								√					√							
	0.20								√					√					√		
CEF	0.71		√										√								
	0.67						√					√					√				
	0.49		√															√			
	0.35	√															√				

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 21. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. mossambesiaca* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum mossambesiaca																				
Solvent systems	**R_f values**	**Acetone**					**Hexane**					**DCM**					**Methanol**				
		C.a	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**
BEA	0.36					√			√		√				√	√					√
	0.22			√		√										√					√
	0.16		√										√					√			
CEF	0.81		√										√					√			
	0.71						√					√	√				√	√			
	0.52					√								√		√					√
	0.49	√	√		√							√	√		√		√	√		√	
EMW	0.89			√		√								√		√			√		√
	0.75													√					√		

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 22. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. acutifolium* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum acutifolium																				
Solvent systems	**R_f values**	**Acetone**					**Hexane**					**DCM**					**Methanol**				
		C.a	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**
BEA	0.36					√			√							√					√
	0.22			√		√								√		√					√
	0.11					√															
CEF	0.81		√					√					√					√			
	0.71		√					√					√					√			
	0.67						√					√					√				
	0.53			√															√		
	0.49	√	√	√								√	√					√			
EMW	0.89			√															√		

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 23. The bioautographic qualitative inhibition of tested fungi growth by extracts of *C. nelsonii* separated by TLC with BEA, CEF and EMW as eluents. R_f values of active components.

Combretum nelsonii																				
Solvent systems	**R_f values**	**Acetone**					**Hexane**					**DCM**					**Methanol**				
		C.a	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**	**C.a**	**C.n**	**M.c**	**S.s**	**A.f**
BEA	0.36			√	√	√			√						√	√			√	√	√
	0.29		√			√		√	√				√			√		√			√
	0.06	√	√		√	√						√	√		√	√	√	√		√	√
CEF	0.81		√					√					√					√			
	0.71		√					√					√					√			
	0.63	√	√		√	√	√	√		√		√	√		√	√	√	√		√	√
	0.52		√	√	√	√							√	√	√	√		√	√	√	√
	0.44	√			√	√						√			√	√	√			√	√
	0.35	√										√					√				
EMW	0.93		√										√					√			
	0.88	√	√	√	√	√			√	√		√	√	√	√	√	√	√	√	√	√
	0.75	√	√	√	√				√	√		√	√	√	√		√	√	√	√	

C.a, *C. albicans*; C.n, *C. neoformans*; M.c, *M. canis*; S.s, *S. schenckii* and A.f, *A. fumigatus*.

Table 24. Number of antifungal bands present for all tested organisms against *Combretum* species, TLC solvent systems and extractants.

Combretum species	Solvent systems	Extractants				Total	Grand-total	Section
		Acetone	Hexane	DCM	Methanol			
C. celastroides ssp. *celastroides*	BEA	5	2	6	6	**19**	**62**	**H**
	CEF	6	1	6	6	**19**		
	EMW	7	3	7	7	**24**		
C. celastroides ssp. *orientale*	BEA	5	2	6	6	**19**	**62**	**H**
	CEF	6	1	6	6	**19**		
	EMW	7	3	7	7	**24**		
C. imberbe	BEA	5	2	6	6	**19**	**57**	**H**
	CEF	5	1	4	4	**14**		
	EMW	7	3	7	7	**24**		
C. padoides	BEA	5	2	7	5	**19**	**56**	**H**
	CEF	5	0	4	4	**13**		
	EMW	7	3	7	7	**24**		
C. caffrum	BEA	5	0	5	5	**15**	**39**	**A**
	CEF	4	0	4	4	**12**		
	EMW	4	0	4	4	**12**		
C. erythrophyllum	BEA	5	0	5	5	**15**	**39**	**A**
	CEF	4	0	4	4	**12**		
	EMW	4	0	4	4	**12**		
C. kraussii	BEA	5	0	5	5	**15**	**40**	**A**
	CEF	4	0	4	3	**11**		
	EMW	5	0	3	6	**14**		
C. woodii	BEA	5	0	5	5	**15**	**37**	**A**
	CEF	4	0	4	3	**11**		
	EMW	4	0	3	4	**11**		
C. nelsonii	BEA	3	2	3	3	**11**	**43**	**A**
	CEF	6	3	6	6	**21**		
	EMW	3	2	3	3	**11**		
C. collinum ssp. *suluense*	BEA	0	0	0	0	**0**	**0**	**M**
	CEF	0	0	0	0	**0**		
	EMW	0	0	0	0	**0**		
C. collinum ssp. *taborense*	BEA	0	0	0	0	**0**	**0**	**M**
	CEF	0	0	0	0	**0**		
	EMW	0	0	0	0	**0**		
C. zeyheri	BEA	3	2	3	3	**11**	**26**	**S**
	CEF	4	2	4	1	**11**		
	EMW	1	1	1	1	**4**		
C. albopunctactum	BEA	5	1	4	3	**13**	**43**	**C**
	CEF	7	3	6	3	**19**		
	EMW	4	2	3	2	**11**		
C. apiculatum ssp. *apiculatum*	BEA	1	0	2	2	**5**	**20**	**C**
	CEF	2	2	4	3	**11**		
	EMW	1	1	1	1	**4**		

Table 24. Contd.

	BEA	1	1	0	0	**2**		
	CEF	0	2	3	1	**6**	**12**	**C**
C. edwardsii	EMW	1	1	1	1	**4**		
	BEA	1	1	1	0	**3**		
	CEF	1	1	1	1	**4**	**7**	**C**
C. moggi	EMW	0	0	0	0	**0**		
	BEA	1	0	0	1	**2**		
	CEF	1	0	0	1	**2**	**8**	**C**
C. molle	EMW	1	1	1	1	**4**		
	BEA	1	1	1	1	**4**		
	CEF	1	1	2	1	**5**	**14**	**C**
C. petrophilum	EMW	2	1	1	1	**5**		
	BEA	1	0	0	2	**3**		
	CEF	2	3	2	2	**9**	**16**	**B**
C. hereroense	EMW	1	1	1	1	**4**		
	BEA	1	1	1	0	**3**		
	CEF	0	2	1	1	**4**	**7**	**Co**
C. microphyllum	EMW	0	0	0	0	**0**		
	BEA	0	1	1	0	**2**		
	CEF	1	1	1	0	**3**	**8**	**Co**
C. paniculatum	EMW	0	1	1	1	**3**		
	BEA	0	2	2	1	**5**		
	CEF	3	1	2	3	**9**	**14**	**P**
C. bracteosum	EMW	0	0	0	0	**0**		
	BEA	3	1	3	3	**10**		
	CEF	4	1	4	4	**13**	**25**	**P**
C. mossambicense	EMW	1	0	0	1	**2**		
	BEA	3	1	2	2	**8**		
	CEF	4	3	4	4	**15**	**25**	**P**
C. acutifolium	EMW	1	0	0	1	**2**		
TOTAL		**199**	**73**	**199**	**189**			

H, *Hypocrateropsis*; A, *Angustimarginata*; M, *Metallicum*; C, *Ciliatipetala*; B, *Breviramea*, Co, *Connivetia*; P, *Poivrea*

Table 25. Average number of active compounds in different sections.

***Combretum* sections**	**Average Number of active compounds**
Hypocrateropsis	59
Angustimarginata	40
Metallicum	0
Ciliatipetala	26
Breviramea	17
Connivetia	8
Poivrea	21

the inhibition was only detected early during incubation, subsequent growth of mycelia on top of an active band, made it difficult to see inhibition. The non-activity of the other *Combretum* extracts by using bioautography assays could be explained by a weak activity of the extracts against the microorganisms selected, with the disruption of synergism between active constituents caused by TLC separation, or the low concentration of the active compounds in the crude extract under the tested conditions. There were a few cases where fungi did not grow well on certain parts of the bioautograms, making it difficult to evaluate the number of antifungal

Table 26. Number of antifungal bands present for all *Combretum* species against tested organisms, TLC solvent systems and extractants.

Fungi	Solvent Systems	Extractants				Total	Grand-total
		Acetone	Hexane	DCM	Methanol		
C. albicans	BEA	37	1	25	24	**87**	**339**
	CEF	39	10	41	37	**127**	
	EMW	39	7	38	41	**125**	
C. neoformans	BEA	39	5	30	31	**105**	**367**
	CEF	43	8	44	39	**134**	
	EMW	44	7	37	40	**128**	
M. canis	BEA	28	14	27	21	**90**	**298**
	CEF	35	4	31	17	**87**	
	EMW	37	22	35	27	**121**	
S. schenckii	BEA	32	1	29	30	**92**	**314**
	CEF	40	6	26	31	**103**	
	EMW	36	9	36	38	**119**	
A. fumigatus	BEA	27	2	26	27	**82**	**192**
	CEF	19	3	18	15	**55**	
	EMW	16	8	16	15	**55**	
TOTAL		**511**	**107**	**459**	**433**		

Table 27. Number of antifungal bands present for all plant species and extractants against tested organisms.

Extractants	Fungi					
	C. albicans	***C. neoformans***	***M. canis***	***S. schenckii***	***A. fumigatus***	**Total**
BEA	87	105	90	92	82	**456**
CEF	127	134	87	103	55	**506**
EMW	125	128	121	119	55	**548**
TOTAL	**339**	**367**	**298**	**314**	**192**	

compounds. In the cases where CEF was used as eluent, the most likely explanation is that there were still traces of formic acid left on the chromatogram that inhibited the fungal growth.

In other cases there were growth, but no inhibition was observed even though MIC values indicated antifungal activity (Masoko et al, 2005). The non-activity of these extracts in bioautography may possibly be explained by evaporation of active compounds during removal of the TLC eluents or by the disruption of synergism between active constituents caused by TLC separation.

Selection of most promising extracts to work with

This study showed that there are 660 zones of inhibition detected with the different extracts, fungi and solvent systems used in *Combretum* extracts. Activity may not only be attributable to tannins found in *Combretum,* as was previously suggested (Baba-Moussa et al., 1999). The results obtained here are in line with the low MIC values obtained in different extracts in previous work (Masoko et al., 2005, 2006). *C. imberbe*, *C. albopunctactum* and *C. nelsonii* were selected for the next studies because of their broad spectrum activity against the tested fungi.

Conclusion

Acetone was the best extractant as was found previously with antibacterial compounds (Eloff 1998b); it extracted more antifungal compounds from *Combretum* spp. Most compounds were extracted in intermediate polarity solvent (acetone), fewer in high (methanol) and least in low polarity (hexane). Therefore antifungal activity was not mainly due to tannins. A number of plants used in this study showed promising biological activity. This may indicate that the use of these plants in traditional medi-

cine is of value. Antifungal compounds from selected species have been isolated and *in vivo* animal studies have been carried out with these compounds and some extracts (Masoko, 2006).

ACKNOWLEDGEMENTS

The National Research Foundation (NRF) provided funding and Dr. Picard provided fungal pathogens. Rudi Kotze and Johan Hurter allowed us to collect plant material from the Lowveld National Botanical Garden.

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