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Full Length Research Paper

Biological activities of *Typha capensis* (Typhaceae) from Limpopo Province (South Africa)

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Dried ground leaves and rhizomes of *Typha capensis* were extracted with different solvents of varying polarity (hexane, dichloromethane, acetone, methanol) to determine the best extractant for subsequent isolation and characterization of antibacterial compounds. Some extracts were active against *Escherichia coli* and *Enterococcus faecalis*, with at least one of them exhibiting minimum inhibitory concentration values of 0.04 mg/ml. Methanol was the best extractant with an average minimum inhibitory concentration (MIC) value of 0.75 mg/ml (rhizome) and 0.21 mg/ml (leaves) for the four pathogens. *E. coli* and *E. faecalis* were the most sensitive with the average MIC values of 0.53 and 0.42 mg/ml, respectively. *Staphylococcus aureus* was the most resistant pathogen. The MIC values for the positive control (ampicillin) were ranging from 0.08 to 0.16 mg/ml. Average total activity, a measure of potency, was highest for methanol (4498 ml/g) leaves and (1838 ml/g) rhizomes extract followed by acetone (1795 ml/g) leaves and (1075 ml/g) rhizome extracts. The most active compounds were at the R_f value of 0.47 against all pathogens. In some extracts the antibacterial activity was high enough to consider extracts for isolation and characterisation of antibacterial compounds.

Key words: *Typha capensis*, typhaceae, antibacterial, bioautography.

INTRODUCTION

The treatment of many infections is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes. In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions (Ahmad et al., 1998). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources such as medicinal plants.

The family typhaceae have one genera and 10 to 15 species. The characteristic inflorescence gives the family the common name 'cat tails'. Individuals are tall and can reproduce clonally, by submerged rhizomes, forming dense stands. The leaves are diuretic (Duke and Ayensu, 1985). The pollen is astringent, desiccant, diuretic, haemostatic and vulnerary (Duke and Ayensu, 1985). Pollen is used in the treatment of nose bleeds, haematemesis, haematuria, uterine bleeding, dysmenor-rhoea, postpartum abdominal pain andgastralgia, scrofula and abscesses (Yeung, 1985). It is also contradicated for pregnant women (Yeung, 1985). The seed down is haemostatic (Duke and Ayensu, 1985). The rootstock is astringent and diuretic (Chopra et al., 1986). Gautam et al. (2007) have reported that *Typha angustufolia* (Watt) syn. *Typha elephantine* (Roxb) and *Typha latifolia* (Edgew) are used as the sources of antimycobacterial agent in India. The current study, however is focused on *Typha capensis*.

T. capensis (Rohrb.) N.E.Br, known as bulrush (English), *Lesehu* (Sepedi/North-Sotho), *papkuil* or *matjiesriet* or *palmiet* (Afrikaans.), *Ibhuma* (Zulu, Swazi), *Ingcongolo* (Xhosa) and *Motsitla* (Sesotho) is a very common plant in wet places and shallow or stagnant water. It is mostly distributed in Northwestern Cape region but very common in the rest of South Africa (van Wyk et al., 1997). It is most common in aquatic situations whether in standing or slow-flowing waters. Marshes, stream banks, dams and lakes are most commonly inhabited by *T. capensis.* The muddy substrate of these water bodies helps the plant to anchor its rhizomes firmly.

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The thick fleshy rhizomes are harvested to make decoctions for many uses such as in treatment of venereal diseases, or during pregnancy to ensure an easy delivery, as well as for stomach ailments and to promote fertility in women and libido in men, to improve circulation and for diarrhoea and dysentery (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). The presence of steroid-like constituents is very interesting, and there are indications that this group of phytosteroids can be metabolised to either androgen or oestrogen-like substances.

Apart from its important medicinal uses, *T. capensis* also has value as a source of starch. The spongy rhizomes may be pounded to a meal; furthermore, the pollen may be used as a high-protein food. Pancakes can be made from flour and bulrush pollen. Leaves are also used to make hand brooms and are sometimes used in weaving and thatching in Sekhukhuneland (South Africa).

Nevertheless, there is no information about the antibacterial property of *T. capensis* leaves and rhizomes. Thus, and as part of our ongoing project on the study of South African medicinal plants used in Limpopo Province, we report here the antibacterial activity of extracts of *T. capensis* leaves and rhizomes.

MATERIALS AND METHODS

Plant collection

T. capensis (Rohrb.) was collected from the river banks in Ga-Mashabela and Jane Furse regions (Sekhukhuneland) in Limpopo Province, South Africa. The plant was identified by Prof Mampuru. Leaves were separated from the rhizomes and dried at room temperature and milled into powder with grounding machine (ML90L4) (Monitoring and control laboratories (Pty) Ltd) at the School of Agricultural and Environmental Sciences (University of Limpopo). The powder was stored at room temperature in the dark in tightly closed plastic bags until used.

Microorganisms used

Staphylococcus aureus ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 species are the major cause of nosocomial infections in hospitals (Sacho and Schoub, 1993) and are mainly the strains recommended for use by the National Committee for Clinical Laboratory Standards (NCCLS, 1992).

Extraction procedure

To determine the efficiency of different extractants, 1 g samples of finely ground leaves and rhizomes were extracted in 10 ml in each of acetone, hexane, dichloromethane (DCM) or methanol (technical grade-Merck), respectively in centrifuge tubes. These tubes were vigorously shaken for 3 - 5 min in a Labotec model 20.2 shaking machine at high speed. After centrifugation at 959 x g for 10 min, the supernatant were 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.

Phytochemical analysis

Chemical constituents of the extracts were analyzed by thin layer chromatography (TLC) using aluminium-backed TLC plates (Merck, silica gel 60 F_{234}). The TLC plates were developed with one of the three eluent systems, 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 done 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.

TLC analysis of the extracts

Visible bands were marked under daylight and ultraviolet light (254 and 360 nm, Camac Universal UV lamp TL-600) before spraying with freshly prepared *p*-anisaldehyde (1 ml *p*-anisaldehyde, 18 ml ethanol, 1 ml sulphuric acid) or vanillin (0.1 g vanillin, 28 ml methanol, 1 ml sulphuric acid) spray reagents (Stahl, 1969). The plates were carefully heated at 105ºC for optimal colour development.

Qualitative 2,2 -diphenyl-1-picrylhydrazyl (DPPH) assay on TLC

TLCs were used to separate extracts as described earlier. The plates were dried in the fumehood. To detect antioxidant activity, chromatograms were sprayed with 0.2% 2,2-diphenyl-2-picrylhydrazyl (Sigma®) (DPPH) in methanol, as an indicator (Deby and Margotteaux, 1970). The presence of antioxidant compounds were detected by yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

Quantitative DPPH radical scavenging activity assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *T. capensis* fractions were determined according to the method described by Katsube et al. (2004). The assay involves the measurement of the disappearance of the coloured free radical, DPPH, by spectrophotometric determination. Serial dilution from 2 to 250 µg/ml was done using dH_2O; 50 µl of the prepared concentrations was pipetted into a 96-well plate. An equivalent volume of ascorbic acid (Vit C) at a concentration of 250 µg/ml was used as a positive control. Hundred and eighty five microliters (185 µl) of DPPH solution dissolved in a 50% methanol solution was added to each well and the plate gently shaken for 20 min at room temperature. The change in absorbance at 550 nm was measured using a microtiter plate reader.

Quantitative antibacterial activity assay by minimum inhibitory concentration (MIC)

The microplate serial dilution method (Eloff, 1998a) was used to determine the minimum inhibitory concentration (MIC) of extracts against *S. aureus, P. aeruginosa*, *E. faecalis* and *E. coli*. Extracts (10 mg/ml) were dissolved in acetone and serially diluted with sterile water in microplates in a laminar flow cabinet. The same volume of an actively growing culture of the test bacteria was added to the different wells and cultures were grown overnight in 100% relative humidity at 37°C. The next morning tetrazolium violet was added to all the wells. Growth was indicated by a violet colour of the culture. The lowest concentration of the test solution that led to an inhibition of growth was taken as the MIC. The negative control acetone had no influence on the growth at the highest concentration used (25%).

Total activity of the extracts

The total activity in ml/g was calculated by dividing the MIC value with the quantity extracted from 1 g of plant material. The resultant value indicates the volume to which the extract can be diluted and still inhibit the growth of the bacterial isolate (Eloff, 2004).

Qualitative antibacterial activity assay by bioautography

The bioautography procedure described by Begue and Kline (1972) was used. TLC plates were prepared and developed in the different solvent systems, dried for 2 to 3 days under a stream of air to remove residual solvent, which might inhibit bacterial growth. The plates were sprayed with one of four bacterial cultures listed above. Ten milliliters (10 ml) of highly dense fresh bacteria culture was centrifuged at 5300 x g for 20 min to concentrate the bacteria. The supernatant was discarded and the combined pellet resuspended in 2 – 4 ml of fresh Müller-Hilton broth. The plates were sprayed with the concentrated suspension until they were just wet, air-dried to remove excess liquid, and incubated overnight at 37ºC in 100% relative humidity. After incubation, plates were sprayed with a 2 mg/ml solution of *p*-iodonitrotetrazolium violet (Sigma Chemicals). Clear zones on chromatograms indicated inhibition of growth after incubating for about 1 h at 37ºC (Begue and Kline, 1972).

RESULTS AND DISCUSSION

T. capensis was selected for antibacterial activity screening based on its use in traditional medicinal treatments for both domestic animals and humans in southern Africa and for its availability. Drugs used by traditional healers are mostly prepared by some form of extraction with water, as the healers do not usually have access to more lipophilic solvents. This is of concern, as it is possible that healers do not extract all the active compound(s) that might be present in the plant and consequently the prepared drug might not contain all the pharmacologically active compounds. Success in isolating compounds from plant material is largely dependent on the type of the solvent used in the extraction procedure. Mass extracted from the leaves and rhizomes of *T. capensis* using different solvents of varying polarity (hexane, dichloromethane, acetone and methanol) are shown in Figure 1. Methanol was quantitatively the best extractant, extracting a greater quantity of plant material from leaves than any of the other solvents, with the hexane extracting the least amount of the material. Acetone was best extractant for the roots.

After evaporation of the 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). The separated compounds on TLC plates were made visible by spraying with vanillin-sulphuric acid reagent. There was some similarity in the chemical composition of the non-polar components of extracts using extractants of varying polarity. There were more bands separated with the non-polar BEA compared to very few bands with the intermediate polarity CEF and few bands with the more polar EMW solvent system (Figure 2). Acetone leaf extract had many prominent bands in the EMW system.

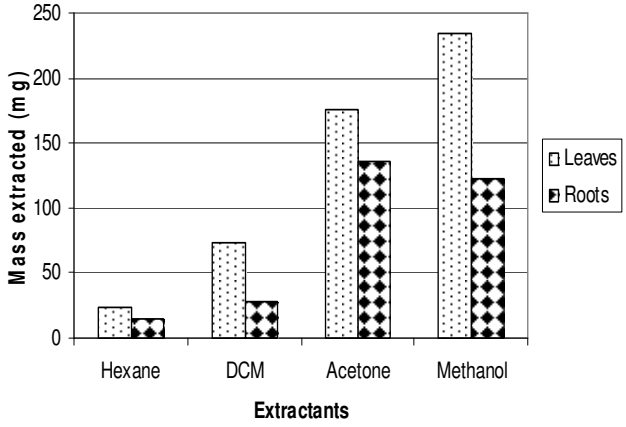

Figure 1. Powder mass of *T. capensis* leaves and roots samples extracted with hexane, dichloromethane, acetone and methanol.

All the leaves and rhizome extracts did not have any antioxidant activity after spraying the chromatogram with 0.2% DPPH (results not shown). The DPPH spectrophotometric assay is considered a valid and easy assay to evaluate scavenging potentials of antioxidants. The percentage scavenging activities of the *T. Capensis* are shown in Table 1. All the plant parts extracted with different solvents did not show significant scavenging powers. Leaf fractions prepared with different solvents gave values with less activity than expected. The roots did not have any activity. Positive control (Vit. C) had 100% scavenging activity. The results correlated with the TLC chromatogram results (results are not shown).

To determine the MIC values, bacterial growth was checked after 24 h (Table 2). Amphicillin was used as a positive control. Most of the extracts were outside the expected cut-off range of 0.02 - 0.16 mg/ml. Methanolic extracts of the rhizome and leaves had better average activity against all tested bacteria, with the average MIC of 0.75 and 0.21 mg/ml, respectively. Hexane extracts of the rhizome had no activity but the leaf extracts had an average MIC of 1.57 mg/ml. In hexane leaf extract, activity was observed against *E. coli* and *E. faecalis*; both have the MIC value of 0.64 mg/ml. *E. coli* and E. *faecalis* were the most sensitive bacteria, with the average MIC values of 0.53 and 0.42 mg/ml, respectively. According to Vlietinck et al. (1995), gram-positive bacteria are significantly more susceptible to the extracts tested than the gram-negative ones. In this experiment we observed the opposite, with gram-negatives being more suscep-tible. Generally the leaf extracts were more active than the rhizome extracts. Dichloromethane, acetone and methanol extracts displayed a better activity against *E. coli* and *E. faecalis*, with MIC values of 0.16, 0.08, 0.08 mg/ml, respectively for *E. coli,* and 0.04, 0.04 and 0.02 mg/ml, respectively for *E. faecalis* compared to amphicillin which had the MIC values of 0.16 mg/ml for both *E. coli* and *E. faecalis*. *S. aureus* was the most resistant bacteria.

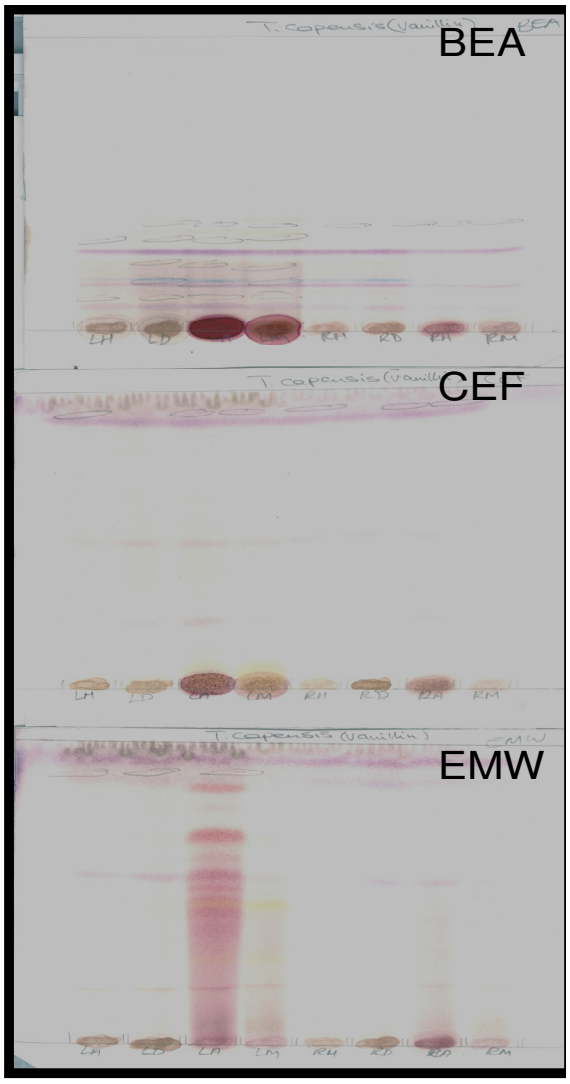

Figure 2. Chromatograms of *T. capensis* developed in benzene/-ethanol/ammonium hydroxide (90:10:1): [BEA] (non-polar/basic) chloroform/ethyl acetate/formic acid (5:4:1): [CEF] (intermediate polarity/acidic); ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral) solvent systems and sprayed with vanillin-sulphuric acid reagent. RH, Rhizome extracted with hexane; RD, rhizome extracted with dichloromethane; RA, rhizome extracted with acetone; RM, rhizome extracted with methanol; LH, leaf extracted with hexane; LD, leaf extracted with dichloromethane; LA, leaf extracted with acetone; LM, leaf extracted with methanol.

The quantity of antibacterial compounds present was also determined as shown in Table 3. To determine which extract can be used for further testing and isolation, not only the MIC value is important, but also the total activity. Because the MIC value is inversely related to the quantity of antibacterial compounds present, an arbitrary measure of the quantity of antibacterial compounds present was calculated by dividing the quantity extracted in milligrams from 1 g leaf by the MIC value in mg/ml. This value indicates the volume to which the biologically active compound present in 1 g of the dried plant material can be diluted and still kill the bacteria (Eloff, 2004). Extracts with higher values were considered the best to work with. From Table 3, substantial total activity was observed against *E. faecalis* followed by *E. coli* after 24 h; *S. aureus* was relatively resistant. Average total activity, a measure of potency, was highest for methanol (4498 ml/g) leaves and (1838 ml/g) rhizomes extract followed by acetone (1795 ml/g) leaves and (1075 ml/g) rhizome extracts.

Although little is known about the antimicrobial activity of the *T. capensis* extracts or compounds isolated from it, the ethnomedicinal usage of some *Typha* species in terms of antimicrobial activity were broadly considered. Dichloromethane roots extracts of *T. latifolia* have shown activity against *Bacillus subtilis* (ATCC 6633) and no activity against *P. aeruginosa* (ATCC 27853), methicillin resistant strain of *S. aureus* (MRSA), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25422). Leaf extract of *T. latifolia* did not have any activity against all the mentioned bacteria (Eduardo et al., 2006). It was also reported that *T. angustifolia* did not have activity against *S. aureus*, *E. coli*, *Erwimia carotovora* and *Phytomonas tumefaciens* (Hayes, 1947). *Typha domingensis* also did not have activity against *E. coli* and *S. aureus* (Mele'ndez and Capriles, 2006). Comparing activity of *T. capensis* with reported activities of some of *Typha* species, our findings suggest that *T. capensis* had better activity and this might validate its wide use in traditional medicine.

The bioautography method worked well with *E. coli*, *S. aureus* and *E. faecalis* but not against *P. aeruginosa*. EMW and BEA yielded better results in bioautography probably due to the difficulty of removing residual formic acid in CEF chromatograms. There were two major antibacterial compounds (Rf value of 0.47 in EMW) against *E. coli* (Figure 3). The same two compounds from non polar extractants were also active against *E. faecalis* and *S. aureus* (results not shown). Shode et al. (2002) have reported the examination of rhizomes of *T. capensis* collected in Pinetown near Durban, S.A. which yielded the novel compounds typhaphthalide and typharin as well as sitosterol, afzelechin, epiafzelechin, catechin and epicatechin. Several flavones and other phenolic compounds, long chain hydrocarbons as well as various triterpenoids with a steroidal skeleton have also been isolated from *T. capensis* (Chapman and Hall, 1996). Some of these compounds are known to have antibacterial activity e.g. typhaphthalide have shown antibacterial activity (Brady et al., 2000), Afzelechin and epiafzelechin were reported to have antibacterial activity (Xie and Dixon, 2005). We can therefore speculate that the antibacterial activity observed in the present study may be due to some of the compounds mentioned, but studies are still underway to isolate and confirm this speculation.

In most cases plant extracts are active against gram-positive pathogens (Vlietinck et al., 1995), but *T. capensis* extracts had substantial activity against gram-negative

Table 1. *In vitro* free radical scavenging activity of *T. capensis* extracts using DPPH spectrophotometric assay.

Extractants	Plant parts	µg/ml	% Scavenged capacity
Hexane	Leaves	31	5.3
		250	12.8
	Roots	31	ND
		250	ND
DCM	Leaves	31	10.6
		250	12.5
	Roots	31	ND
		250	ND
Ace	Leaves	31	9
		250	5.7
	Roots	31	ND
		250	ND
MeOH	Leaves	31	0
		250	5
	Roots	31	ND
		250	ND
	Vit.C	31	100
		250	100

ND, Not Detected.

Table 2. Minimum inhibitory concentration (MIC) of *T. capensis* extracts after 24 h incubation at 37°C.

Microorganism	Minimum Inhibitory Concentration (MIC) (mg/ml)									
	RH	RD	RA	RM	LH	LD	LA	LM	Average	AMP
P. aeruginosa	2.5	0.32	0.32	0.16	2.5	0.16	0.32	0.08	**0.80**	0.13
E. coli	2.5	0.16	0.32	0.32	0.64	0.16	0.08	0.08	**0.53**	0.16
S. aureus	2.5	2.5	2.5	2.5	2.5	2.5	2.5	0.64	**2.27**	0.08
E. faecalis	2.5	0.08	0.04	0.02	0.64	0.04	0.04	0.02	**0.42**	0.16
Average	**2.50**	**0.77**	**0.80**	**0.75**	**1.57**	**0.72**	**0.74**	**0.21**		

RH, Rhizome extracted with hexane; RD, rhizome extracted with dichloromethane; RA, rhizome extracted with acetone; RM, rhizome extracted with methanol; LH, leaf extracted with hexane; LD, leaf extracted with dichloromethane; LA, leaf extracted with acetone; LM, leaf extracted with methanol. AMP, Ampicillin.

Table 3. Total activity in ml/g of *T. capensis* extracts after 24 h incubation at 37°C.

Microorganism	Total activity (ml/g)								
	RH	RD	RA	RM	LH	LD	LA	LM	Average
P. aeruginosa	6	88	425	769	10	456	547	2938	655
E. coli	6	175	425	384	38	456	2188	2938	826
S. aureus	6	11	54	49	10	29	70	367	75
E. faecalis	6	350	3400	6150	38	1825	4375	11750	3487
Average	**6**	**156**	**1076**	**1838**	**24**	**692**	**1795**	**4498**	

RH, Rhizome extracted with hexane; RD, rhizome extracted with dichloromethane; RA, rhizome extracted with acetone; RM, rhizome extracted with methanol; LH, leaf extracted with hexane; LD, leaf extracted with dichloromethane; LA, leaf extracted with acetone; LM, leaf extracted with methanol.

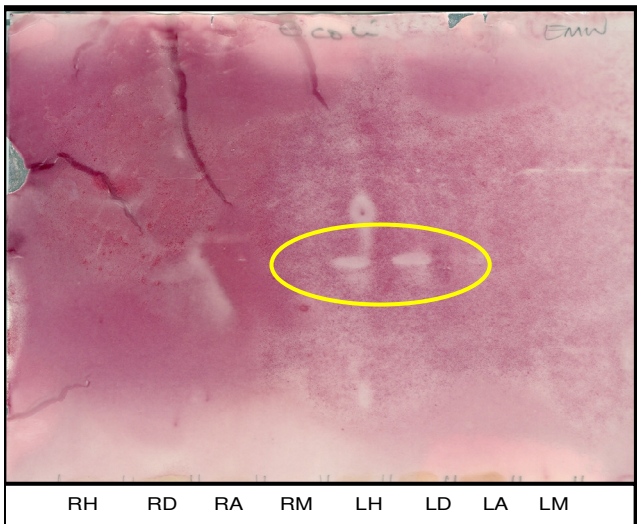

Figure 3. Bioautogram of *T. capensis* extracted with hexane, dichloromethane, acetone and methanol, separated by ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral) solvent system and sprayed with *E. coli*. Circled white areas indicates activity. RH, Rhizome extracted with hexane; RD, rhizome extracted with dichloromethane; RA, rhizome extracted with acetone; RM, rhizome extracted with methanol; LH, leaf extracted with hexane; LD, leaf extracted with dichloromethane; LA, leaf extracted with acetone; LM, leaf extracted with methanol.

bacteria as well. The activities of most of the plant extracts were higher than that of the positive controls. It will be reasonable therefore to consider the extracts for isolation of active compounds.

In conclusion this study validates and documents, in a systematic way, the antibacterial properties of *T. capensis* used for many years by the people of South Africa. The study also provides valuable information for further phytochemical isolation and characterization studies of active compounds, with potential for the development of new drugs.

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