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THE PRELIMINARY ASSESSMENT OF ANTI-MICROBIAL ACTIVITY OF HPLC SEPARATED COMPONENTS OF *KIRKIA WILMSII*

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Abstract

Background: Most communities in developing countries rely on traditional medicines for the treatment of diseases. In South Africa, the Limpopo province, within the Lebowakgomo district, uses tuberous roots of *Kirkia wilmsii*, after infusion in water for the treatment of a wide range of diseases by Sotho communities.
Materials and Methods: The main objective of the study was to assess the anti-microbial activity of separated aqueous components of the *Kirkia wilmsii* tuberous roots. The clear aqueous extracts that were obtained after a 0.45 µm membrane filtration (Millipore Millex-HV Hydrophillic PVDF filter), were then injected into a preparative high performance liquid chromatography instrument in which pure components, as shown by peaks, were collected and evaluated for anti-microbial activity against a range of microorganisms.
Results: The eight separated components were obtained, out of which four components showed anti-microbial activity (AMA). The freeze dried components were re-dissolved in deionised water and then evaluated for AMA against *Vibrio cholerae, Shigella dysenteriae, Aeromonas hydrophilia, Salmonella typhi Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Candida albicans and Enterobacter aerogenes.* Component one exhibited antimicrobial activity against *Shigella dysenteriae, Aeromonas hydrophilia, Salmonella typhi, Proteus mirabilis, Escherichia coli* and *Staphylococcus aureus* with a minimum inhibitory concentration (MIC), of 3.445 mg/ml. Component five was only active against *Proteus mirabilis* with a MIC of 0.08 mg/ml. Component 7, was active against *Shigella dysenteriae, Staphylococcus aureus* and *Escherichia coli* with a MIC of 0.365 mg/ml against both *Shigella dysenteriae* and *Staphylococcus aureus* and 0.091 mg/ml against *Escherichia coli*. Component 8, was active against *Shigella, Aeromonas hydrophilia, Salmonella, Proteus mirabilis, Escherichia coli* with a MIC of 155 mg/ml.
Conclusion: Only four out of eight aqueous extracts showed AMA against both gram negative and positive bacteria and showed no AMA against *Candida albicans, Enterobacter aerogenes* and *Vibrio cholerae*. Therefore the *Kirkia wilmsii* plant root may be used as a broad spectrum antibiotic.

Keywords: *Kirkia wilmsii*, method development, gradient elution, biological activity.

Introduction

Plant preparations have been used for thousands of years as remedies for many ailments all over the world, especially in developing countries where primary health care facilities are limited (Balunas and Kinghorn, 2005). Some of these are used with little or no processing at all, which could be in the form of tinctures, teas, powders, poultices and other forms (Balick and Cox, 1997). There are several hundreds of thousands of plant species that are found in many places around the world, however only a small fraction of these plants has been phytochemically and pharmacologically investigated (Hostettmann, 1999). A direct consequence of this fact is that there are many plants that can be investigated, and hence efficient systems for the rapid chemical and biological screening of the plants must be put in place.

According to the World Health Organisation (WHO), about 80% of the population in developing countries of the world depend on traditional medicines for the treatment of diseases, using plant extracts (WHO, 2008; Harvey, 2000). They are about 4 billion people in the world who rely on the use of herbal plant formulations (Farnsworth, 1988). Farnsworth (1988) also states that Hong Kong is the biggest herbal market in the world, with Japan and China also claiming large chunks of the herbal market. The WHO now encourages the use of and/or research into the target molecules (TM) that originate from traditional medicines, so that the TM can be used safely and effectively. By so doing the use of the TM can be regulated when all the information about the TM becomes available, backed by research and scientific facts. An analysis of the new drugs that were introduced between 1982 and 2002 showed that of the new chemical entities (NCE), 52% came from traditional medicines also known as natural products (Chin et al., 2006). This clearly shows that natural products are important factor/s in the production of new drugs.

The use of TM for the development of new drugs has been necessitated by the advent of new diseases, for example Acquired Immune Deficiency Syndrome (AIDS), and related illnesses, untreatable diseases such as cancer and the need for cheaper medicines. Closely related to this was the emergence of drug resistant tuberculosis (TB), strains (Pauli et al., 2005). Thus in the quest to finding new drugs, natural products present as alternative source of drugs. A good example in question is the occurrence of opportunistic infections which are more pronounced in immune compromised individuals, for example candidiasis, cryptococcosis and others for which there are limited number of effective anti-fungal (Hosttetmann, 1999). Natural products can then be used as a solution for such problems.

The separation of the components of natural products has been done by column chromatography, which was very tedious, and time consuming. Further complete separation has to be confirmed through other methods such as thin layer chromatography (TLC). Most often, the separations are not completed in a single run, thus necessitating multiple runs. The column chromatography/TLC cycle has to be repeated several times until satisfactory results are obtained. Recent advances in HPLC have seen the development of fraction collectors which can be attached to an HPLC system that can collect the separated components. The advantages associated with the use of HPLC in the analysis of natural products include; accuracy, precision and the method is not limited by the nature of the sample, that is, volatility or stability of the sample (Jandera et al., 2007; Lee et al., 2008). HPLC has been widely used in the quality control regulation of medicines and herbal preparations (Pothitirat and Gritsanapan, 2009), and therefore the HPLC method development is an important step in the separation and analysis of drugs. However TLC can

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be used as a preliminary technique before HPLC is employed, to ascertain the polarity of each component and to design the mobile phase composition (Mahler and Thomason, 2007).

There is limited research that has been carried out on the *Kirkia wilmsii* tree. The leaves of the *Kirkia wilmsii* were investigated and the dichloromethane/methanol and water extracts were found to have anti-plasmodial properties (Clarkson et al., 2004; Pillay et al., 2008). The leaves of the same tree showed biological activity against *Staphylococcus aureus* (acetone, methanol, hexane and dichloromethane extracts), *Enterococcus faecalis* (acetone, methanol and hexane extracts), *Escherichia coli* (acetone, hexane and dichloromethane extracts), *Pseudomonas aeruginosa* (acetone, hexane and dicholoromethane extracts), *Sporothrix schenckii* (methanol and hexane extracts), *Microsporum canis* (acetone extract), *Cryptococcus neoformans* (acetone, hexane and dichloromethane extracts) and *Candida albicans* (acetone and hexane extracts) (Suleiman et al., 2010(a and b)). The leaves of the *Kirkia wilmsii* further showed biological activity against the animal fungal pathogen *Aspergillus fumigatus* and the MIC ranged from 0.17 to 2.11 mg/ml (Suleiman et al., 2010(b)). The hexane, dichloromethane and methanol extracts of the dried and milled bark of the *Kirkia wilmsii* was investigated and were found to contain some secondary metabolites (Mulholland et al., 2003). The metabolites were then identified as lignans, isocoumarins, flavonoids and nor-carotenoids; however there were no antimicrobial studies that were performed.

The search for new plant drugs has followed a wide range of routes. One of the most effective methods has been found to be the testing of plants that are currently in use in certain communities. In this way, the chances of success are very high subject to the availability of enough funding for further research (Fansworth, 1988). The plant of choice, *Kirkia wilmsii* (Sotho name is Legaba or Modumela), is being used by Sotho communities within the Lebowakgomo district located in Limpopo province, South Africa. The tuberous roots of *Kirkia wilmsii* have not been fully investigated and yet it has been widely used amongst the Sotho communities for the treatment of a wide range of diseases. In our opinion, this translates to suggesting that the plant cures many ailments and might become a broad spectrum drug. Thus the isolation and biological activity of the active compounds were investigated.

Materials and Methods

Plant materials

The roots of the *Kirkia wilmsii* plant were collected from the Lebowakgomo region in Polokwane district, which was situated in the Limpopo province of South Africa. The plant name was identified by the Department of Botany at the University of Venda and the name was further confirmed by the National Herbarium (Voucher number MPT00112), in Pretoria, South Africa.

Sample preparation

Only the tuberous bulb of the *Kirkia wilmsii* tree was used in the study. The bark was removed from the bulbs as the indigenous people removed the bark from the tubers before eating them. The peeled roots were first cut into small pieces using ordinary kitchen knife and a small amount of deionised water, (Milli-Q Millipore, 0.054 µS/cm), was added to the cut pieces. This mixture was transferred into a heavy duty Russell Hobbs blender which produced a fine paste. More deionised water was added to completely immerse the fine paste. This was left to stand at room temperature for 48 hrs in order to complete sample extraction and the aqueous extracts were stored in the refrigerator at 4°C for further use. The aqueous extracts were filtered through a 0.45 µm membrane (Millipore Millex-HV Hydrophillic PVDF filter), to remove the residues before separation analysis on the HPLC.

High performance liquid chromatography (HPLC) analysis

The HPLC analysis was performed on the Waters HPLC 2535 with a quaternary Gradient Elution Module, Water 2707 Auto-sampler, Waters Fraction Collector III, Photo Diode Array (PDA), Detector and a Waters 2707 Auto-sampler was used for the separation of the components of the extracts. A gradient elution method for the separation of the components from the plant extract was developed. The method consists of two mobile phases: Mobile phase A; 0.5%v/v trifluoroacetic acid (TFA), in deionised water whereas mobile phase B was 0.25%v/v TFA in acetonitrile (Table 1). The PDA was set to run from 200 to 800 nm and the total run time was 60 minutes. The gradient elution programme was then adapted to the preparative mode using the prep calculator. The conditions obtained from the prep calculator where then used for the analysis. The following prep method was used. The chromatographic system consisted of Waters BEH300 19 x 150mm 5µm Prep C18 column, PDA detector set to scan from 200 to 800nm and the run time was 130 minutes. The chemicals used in the chemistry laboratory were purchased from Sigma-Aldrich Chemicals. The separated components were then freeze dried on a Vacutec Flexi-Dryer µp Freeze Dryer.

Table 1: Final optimised gradient elution method

Time(min)	Flow(ml/min)	Pump A (%)	Pump B (%)
Initial	2.0	45	55
48.00	2.0	80	20
50.00	2.0	25	75
54.00	2.0	25	75
55.00	2.0	75	25
70.00	2.0	75	25
71.00	2.0	25	75
75.00	4.0	25	75
88.00	4.0	25	75
89.00	2.0	25	75
90.00	4.0	50	50
110.00	4.0	50	50
110.01	2.0	75	25
129.00	2.0	75	25
130.00	0.2	75	25

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Preparation of stock solution and media

Iodonitrotetrazolium chloride (INT), nutrient broth (NB), and the brain heart infusion broth (BHIB), were purchased from Sigma-aldrich Chemicals. The INT solution was prepared at a concentration of 20 mg/100ml in deionised water. Sterile NB and BHIB were prepared by dissolving a requisite amount of powder in deionised water as per manufacturer's instruction. The MacFarland standard was prepared in Muller Hinton Broth according to National Committee for Clinical Laboratory Standards (NCLS) standards (http://www.bd.com/europe/regulatory/Assets/IFU/US/8808421%280205%29_en.pdf).

Culture of microorganisms

The microorganisms that were used to assess the antimicrobial activity of the plant extracts were both Gram positive and negative bacteria and yeast (Table 2). The microorganisms were cultured in NB and BHIB. Prior to the antimicrobial test, the bacterial cell count was estimated by comparing with the MacFarland 0.5 standard (http://www.bd.com/europe/regulatory/Assets/IFU/US/8808421%280205%29_en.pdf). Then 1 ml of the broth was added in to 9 ml fresh sterile media (BHIB), to dilute the organisms during susceptibility testing and adjust to 1 McFarland standard.

Table 2: List of the microorganisms used in the study

Microorganisms	Origin	Type of organism
Escherichia coli	ATCC 8739	G-negative
Proteus mirabilis	Clinical isolate	G-negative
Salmonella typhi	Clinical isolate	G-negative
Shigella dysenteriae	Clinical isolate	G-negative
Staphylococcus aureus	Clinical isolate	G-positive
Vibrio cholerae	Clinical isolate	G-negative
Aeromonas hydrophilia	Clinical isolate	G-negative
Candida albicans	Clinical isolate	Yeast
Enterobacter aerogenes	Clinical isolate	G-negative

Evaluation of Anti-microbial activity of separated plant components

The minimum inhibitory concentration (MIC), determinations were carried out as per procedure of O'Donnell et al. (2010), with the use of a 96 well micro-plates. The freeze dried components (a known weight), was re-dissolved in 2 ml deionised water. A different component was added to the wells in the first rows and the serial dilutions for each extract were done four times to determine the MIC. For the first row, 20µl of each component was added to a different well, and then 180µl of BHIB broth was added to all the wells and mixed thoroughly. Serial dilutions were performed from the first row by taking 20µl each time until the last row. When all the dilutions were done, 100µl of the different microorganisms were added to all the wells such that each component of the extract was tested against all the nine available microorganisms. This covered the first 8 columns of the micro-plate.

The positive control was Kanamycin and was dissolved in deionised water (0.25mg/ml), and then 20µl added to column 10. Then 180µl of BHIB and NB were added to all the wells in column 9, in order to stimulate the growth of microorganisms. Serial dilutions were made and the microorganisms added. The negative controls were 180µl of NB and microorganisms were added to column 10 and 180µl of BHIB and microorganisms were added columns 11 and 12. Lastly 100 µl of media (BHIB or NB), was added to all wells to give the total volume in each well of 400 µl. The micro-plates were covered with parafilm and then incubated 37 °C for 24 h in a Labcon incubator. Thereafter 20µl iodonitrotetrazolium chloride (INT), was added to all the wells in all the micro-plates. The results were observed visually. The wells which exhibited a purple pink colour showed that there was microbiological activity going on. The wells where the microorganisms were dead indicate there was lack of colour (colourless). The wells were kanamycin was added were completely clear to show that organisms were killed as can be seen in Figure 2 below.

Data analysis

Yield was calculated by subtracting the tare mass from the mass of the samples in the containers. Molar absorptivity values were calculated from the Beer-Lambert Law.

Results

HPLC Separation of components

The study found eight components that were HPLC separated and collected (Figure 1), but only four components showed antimicrobial activity (Table 2). The 8 separated components were identified by the retention times and were then labelled as components 1 to 8 (corresponding to peak 1 to 8).

Assessment of anti-microbial activity

The 8 purified extracts exhibited different biological activity and half exhibited anti-microbial activity against a wide range of micro-organisms (Table 2). For components 2, 3, 4 and 6, did not show any anti-microbial activity. None of the 8 components were exhibited anti-microbial activity against *Enterobacter aerogenes* and *Candida albicans*

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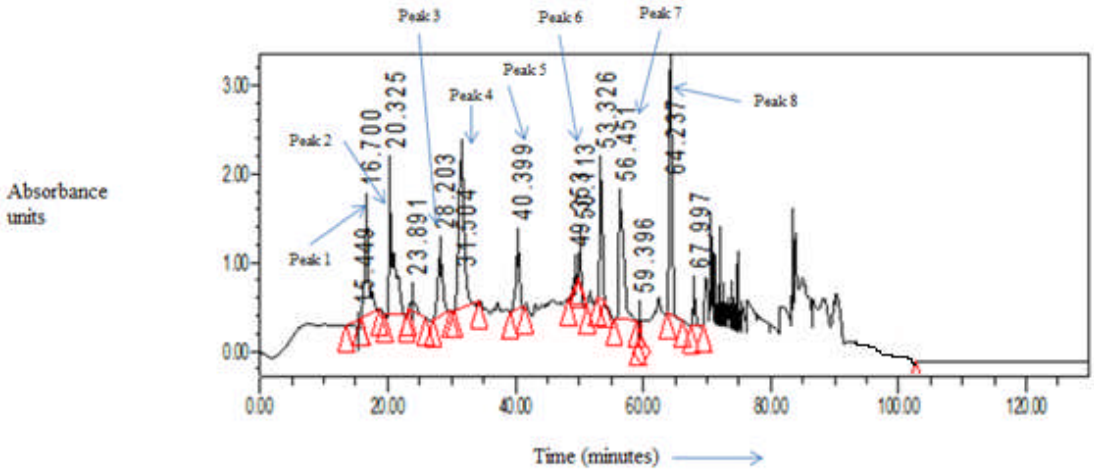


Figure 1: Final optimized chromatogram from *Kirkia wilmsii* extract

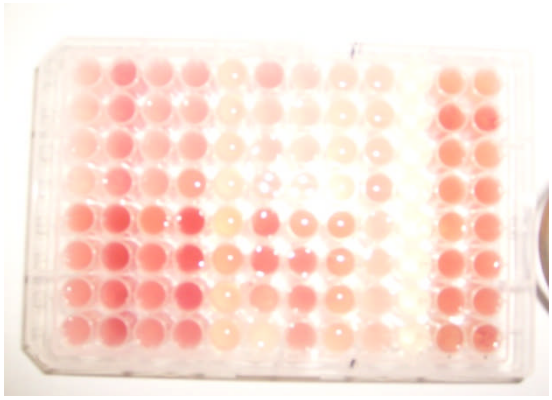


Figure 2: Micro-titre plate soon after incubation and visualisation

Table 2: Antimicrobial activity results for separated components

	*Vibrio cholera	*Shigella dysenteriae	*Aeromonas hydrophilia	*Enterobacter aerogenes	*Salmonella typhi	*Proteus mirabilis	*Escherichia coli	Candida albicans	**Staphylococcus aureus
Component 1	--	++	++	--	++	++	++	--	++
Component 2	--	--	--	--	--	--	--	--	--
Component 3	--	--	--	--	--	--	--	--	--
Component 4	--	--	--	--	--	--	--	--	--
Component 5	--	--	--	--	--	++	--	--	--
Component 6	--	--	--	--	--	--	--	--	--
Component 7	--	++	--	--	--	--	++	--	++
Component 8	--	++	++	--	++	++	++	--	--

Microbiological activity (++) means component exhibited anti-microbial activity; Or (--) means the component did not exhibit any anti-microbial activity. * *Gram negative; **Gram positive

The four components that exhibited anti-microbial activity, the yield of the freeze dried individual components were determined (Table 3); with variable levels of the minimum inhibitory concentration (MIC), (Table 4), and the ultra violet visible (UV-Vis) spectra were also obtained from the chromatograms since the PDA detector was used (Figure 2).

Table 3: Results of dried components which were biologically active

	Component 1	**Component 5**	**Component 7**	**Component 8**
Retention time (min)	16.87	31.98	56.13	65.92
Yield (mg)	70.9	1.6	7.3	3.1
Initial concentration mg/ml)*	35.45	0.80	3.65	1.55
Maximum absorption	0.20	0.79	1.95	2.60
Molar absorptivity ($M^{-1}cm^{-1}$)	0.0056	0.99	0.53	1.68

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*The dried components were weighed and each component was re-dissolved in 2 ml of deionised water to give different concentrations.

The resultant solutions were subjected to the anti-microbial activity tests and the determination of minimum inhibitory concentration was done and the MIC results are shown in Table 4.

Table 4: Calculated MIC (mg/ml) values for the active compounds

	*Shigella dysenteriae	*Aeromonas hydrophilia	*Salmonella typhi	*Proteus mirabilis	*Escherichia coli	**Staphylococcus aureus
Component 1	3.445	3.445	3.445	3.445	3.445	3.445
Component 5				0.080		
Component 7	0. 365				0.091	0.365
Component 8	0.155	0.155	0.155	0.155	0.155	

*Gram negative; **Gram positive

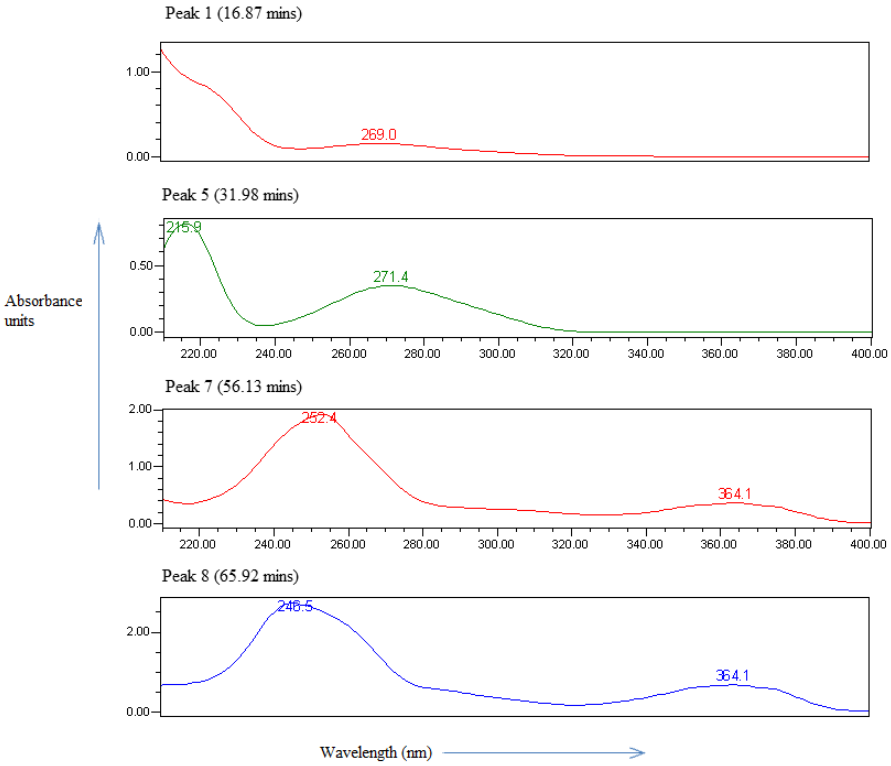


Figure 3: The UV-Vis spectrum of the separated biologically active components

Table 5: HPLC reproducibility of retention times (min)

	Inj 1	Inj 2	Inj 3	Inj 4	Inj 5	Inj 6	Mean	%RSD
Peak #	Rt (min)	Rt (min)	Rt (min)	Rt (min)	Rt(min)	Rt (min)	Rt (min)	
1	16.700	16.569	16.722	16.651	16.593	16.658	16.649	0.356
2	20.3250	20.372	20.327	20.429	20.407	20.360	20.370	0.206
3	28.203	29.380	28.852	28.358	28.743	28.732	28.711	1.437
4	31.504	31.415	31.199	32.048	31.159	31.583	31.485	1.024
5	40.399	40.772	39.863	41.300	40.442	41.287	40.677	1.375
6	49.353	49.571	49.936	49.842	49.480	49.652	49.639	0.443
7	56.451	57.162	56.653	55.489	57.725	57.711	56.865	1.503
8	64.237	62.930	63.916	63.911	64.681	64.061	63.956	0.904

Discussion

The results show that the components of *Kirkia wilmsii* can be separated by using an HPLC gradient elution method (Figure 1). The programme was developed by using two eluents; one organic (acetonitrile buffered with TFA), and the other one aqueous (water also buffered with TFA), and the peaks were detected using the PDA detector (200-400nm), (Figure 2). The mixture gave a good resolution of the peaks of

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interest in the *Kirkia wilmsii* extract. The peak retention times of six consecutive injections were reproducible, RSD < 2.0%, showing that the precision of the developed method (Table 5).

The aqueous plant extracts were tested for antimicrobial activity against a range of microorganisms. Our results show that only four aqueous extracts exhibited potent microbial activity (Table 2). This is in contrast to the studies of Lall and Meyer (2000), and Shale et al. (1999) who reported that the aqueous extracts exhibited low microbial activity towards Gram negative bacteria.

Component 1 exhibited activity against *Shigella dysenteriae, Aeromonas hydrophilia, Salmonella typhi, Proteus mirabilis, Escherichia coli* and *Staphylococcus aureus* and MIC was calculated to be 3.445 mg/mL for this extract against all these microorganisms**.** Suleiman et al., 2010b also reported activity against *Escherichia coli and Staphylococcus aureus* on the organic extracts of the leaves. However, they reported MIC values ranging from 0.16 to 1.25 mg/ml as compared to 3.445mg/ml for the tuber. This shows that the tuber's component 1 is more potent than the leaves.

Component 5 was only active against Proteus mirabilis and the MIC was calculated to be 0.08 mg/ml which is not reported in literature. The MIC is also relatively low. Component 7 was active against *Shigella dysenteriae, Escherichia coli* and *Staphylococcus aureus*. The MIC for component 7 against *Shigella dysenteriae* was 0.365 mg/ml, and 0.091 mg/ml against *Escherichia coli.* Similar activity was reported (Suleiman et al., 2010b). The MIC values are much lower as compared to the reported values, which shows that component 7 is not as potent as component 1.

Component 8 was active against *Shigella dysenteriae, Aeromonas hydrophilia, Salmonella, Proteus mirabilis* and *Escherichia coli.* The MIC against all these microorganisms was found to be 0.155 ng/µl. Organic extracts from leaves of *Kirkia wilmsii* have been shown to be active against *E. coli* (Suleiman et al., 2010b) and all the other organisms that were active in our tuber are not reported. In their work, Suleiman et al. (2010b), reported MIC values ranging from 0.15 to 1.25 mg/ml of which their minimum value of 0.16 mg/ml is very close to our value of 0.155 mg/ml.

The MIC values obtained from our results ranged from 0.08 to 3.445 mg/ml (Table 4). The minimum MIC values are lower than the minimum obtained values with organic extracts of the *Kirkia wilmsii* leaves which ranged from 0.17 to 2.11 mg/ml (Suleiman et al., 2010b). However 3.445 mg/ml was much higher than the reported maximum of 2.11 mg/ml. This comparison shows that the tuberous bulb might be more potent than the leaves.

Our findings showed that the tuberous bulb extracts exhibited high antimicrobial activity of 3445 µg/ml which was higher than the concentration of 100 µg/ml reported by Rios et al. (1988), a concentration at which the plant extracts are considered to have good potency of antimicrobial level when using the micro-plate dilution method. Therefore these extracts have to be pursued as they can be a good source of bioactive compounds.

The UV-Vis spectrum of the bioactive compounds was obtained. The UV –Vis scans were from 20 nm to 400 nm, and it was shown that component 1 had a maximum absorption at 269nm; component 5 at 271.4nm; component 7 at 252.4nm and component 8 at 246.5nm. The corresponding molar absorptivity's were found to be 0.0056; 0.99; 0.53 and 1.68 $M^{-1}cm^{-1}$ respectfully. The molar absorptivity is generally used to measure the extent to which molecules can absorb energy. Therefore the results show that compound one absorbs the least amount. Furthermore, the molar absorptivity information gives an indication of the types of bonds in the molecules, especially π and σ bonds. As the number of π bonds increases, the molar absorptivity also increases. This information can be used in structural determination.

Conclusion

The eight separated components were obtained but only four components exhibited anti-microbial activity against a wide range of microorganisms. A weighed aliquot of the dried components was re-dissolved in deionised water and then evaluated for anti-microbial activity against *Vibrio cholerae, Shigella dysenteriae, Aeromonas hydrophilia, Salmonella typhi Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Candida albicans and Enterobacter aerogenes*. Component 1 exhibited antimicrobial activity against *Shigella dysenteriae, Aeromonas hydrophilia, Salmonella typhi, Proteus mirabilis, Escherichia coli* and *Staphylococcus aureus.* The minimum inhibitory concentration (MIC), was calculated to be 3.445 mg/mL for this extract against all these microorganisms**.** Component 5 was only active against *Proteus mirabilis* and the MIC was calculated to be 0.08 mg/ml. Component 7 was active against *Shigella, Escherichia coli* and *Staphylococcus aureus*. The MIC for component 7 against *Shigella* was 0.365 mg/ml, and 0.091 mg/ml against *Escherichia coli*. Component 8 was active against *Shigella dysenteriae, Aeromonas hydrophilia, Salmonella, Proteus mirabilis, Escherichia coli*. The MIC for component 8 against all these microorganisms was found to be 0.155 mg/ml. Some components of *Kirkia wilmsii* possess antimicrobial properties. The aqueous extracts showed anti-microbial activity against both Gram negative and positive bacteria and showed no anti-microbial activity against *Candida albicans, Enterobacter aerogenes* and *Vibrio cholerae.* Therefore the *Kirkia wilmsii* plant root may be used as a broad spectrum antibiotic by the Sotho communities in the Lebowakgomo district, Limpopo province of South Africa.

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References

1. Balunas, M.J., and Kinghorn, A.D. (2005). Drug discovery from medicinal plants. Life Sciences 78(5): 431-441.
2. Chin, Y.W., Balunas, M.J., Chai, H.B., and Kinghorn, A.D. (2006). Drug Discovery from Natural Sources. The American Association of Pharmaceutical Scientists journal. 8 (2): E239-E240.
3. Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J., and Folb, P.I. (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of Ethnopharmacology. 92: 177-191.
4. Fabricant, D.S., and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives. 109: 69-75.
5. Farnsworth, N.R. (1988). Screening plants for new medicines. Biodiversity. National Academy Press pp 83-97.
6. Farnsworth, N.R., Akerele, O., Bingel, A. S., Soejarto, D. D., and Guo, Z. (1985). Medicinal Plants in Therapy. Bulletin of the World Health Organisation no. 63 (6): 965-981.

http://dx.doi.org/10.4314/ajtcam.v11i3.38
7. Harvey, L.A. (2000). Strategies for Discovering Drugs from Previously Unexplored Natural Products. Drug Discovery Today no. 5: 294-300.
8. Hellstrom, J.K., and Mattila, P.H. (2008). HPLC Determination of Extractable and Unextractable Proanthocyanidins in Plant Materials. Journal of Agriculture and Food Chemistry no. 58:7617 – 7624.
9. Hostettmann, K. (1999). Strategy for the Biological and Chemical Evaluation of Plant Extracts. Pure and Applied Chemistry.70:1-8.
10. Jachak, S.M., and Saklani, A. (2007). Challenges and Opportunities in Drug Discovery from Plants. Current Science no. 92 (9): 1251-1257.
11. Jandera, P., Skeifikova, V., Rehova, L., Hajek, T., Baldrianova, L., Skopova, G., Kellner, V., and Horna, A. (2005). RP-HPLC Analysis of Phenolic Componds and Flavonoinds in Beverages and Plant Extracts Using Coul Array Detector. Journal of Separation Science no. 28:1005-1022.
12. Lee, S.T., Gardner, D.R., Chang, C.W., Panter, K.E., and Molyneux, R.J. (2008). Separation and measurement of plant alkanoids enantiomersby RP-HPLC Analysis of their Fmoc-Alanine Analogs. Phytochemistry Analysis no. 19:395 – 402.
13. Mahler, M., and Thomason, V. (2007). Purification of Natural Products, Case Study 1: *Scutellaria baicalensis*. Chromatography Application Note AN25: 1-4.
14. Mulholland, D.A., Cheplogoi, P., and Crouch, N.R. (2003). Secondary Metabolites from *Kirkia acuminata* and *Kirkia wilmsii* (kirkiaceae). Biochemical Systematics and Ecology no. 3: 793-797.
15. O'donnell, F., Smyth, T. J. P., Ramachandran, V. N., and Smyth, W. F. (2010). A study of the antimicrobial activity of selected synthetic and naturally occurring quinolines. International Journal of Antimicrobial Agents, 35, 30-38.
16. Pauli, G.F., Case, R.J., Inui, T., Wang, Y., Cho, S., Fischer, N.H., and Franzblau, S.G. (2005). New Perspectives on Natural Products in TB Research. Life Sciences no. 78: 485-494.
17. Phillipson, J.D. (2007). Phytochemistry and Pharmacognosy. Phytochemistry no. 68: 2960-2972.
18. Pillay, P., Maharaj, V.J., and Simth, P.J. (2008). Investigating South African Plants as a Source of New Antimalarial Drugs. Journal of Ethnopharmacology no. 119:434-454.
19. Pothitirat, W., and Gritsanapan, W. (2009). HPLC Quantitative Analysis Method for the Determination of α-Mangostin in Mangosteen Fruit Rind Extract. Thai Journal of Agricultural Science 42:7-12.
20. Rios, J.L., Asha, M.K., Amit, A. (2001). Screening methods for natural products with antimicrobial activity: A review of literature. Journal of Ethnopharmacology, 23, 127-149.
21. Suleima, M.M., McGaw, L.J., Naidoo, V., and Eloff, J.N. (2010a). Detection of Antimicrobial Compounds by Bioautography of Different Extracts of Leaves of Selected South African Tree Species. African Journal of Traditional, Complementary and Alternative Medicines no. , 7(1):64-78.
22. Suleiman, M.M., McGaw, L.J., Naidoo, V., and Eloff, J.N. (2010b). Evaluation of Several Tree Species for Activity Against Animal Fungal Pathogen *Aspergillus fumigatus*. South African Journal of Botany no. 76: 64-71.