

ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF *ZIZIPHUS MUCRONATA* AND *RICINUS COMMUNIS* LEAVES EXTRACTS

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Abstract

Background: Plants have always been a successful source of remedy from nature. With the widespread use of medicinal plants by indigenous people, the search for biologically active agents is relevant as these plants have the potential to provide pharmacologically active compounds. This study aimed for investigating the effect of antioxidant and antibacterial properties of *Ziziphus mucronata* Willd (Rhamnaceae) and *Ricinus communis* L. (Euphorbiaceae).

Materials: aluminum-backed TLC plates (Fluka, silica gel F254), vanillin-sulphuric acid reagent, Fehling's solution, concentrated sulphuric acid, ferric chloride, Drangendorff's reagent, acetic anhydride, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), *p*-iodonitrotetrazolium violet,

Methods: Antibacterial activity was evaluated using microdilution assay and bioautography. Antioxidant activities were determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). In vitro cytotoxicity was determined using the tetrazolium-based colorimetric assay.

Results: *R. communis* leaves had eight secondary metabolites. Quantitative assay for *R. communis*, chloroform and methanol extracts had very high antioxidant activity compared to vitamin C. Plants extracts from all solvents exhibited high antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* with minimum inhibitory concentration (MIC) values between 0.21 and 1.05 mg/ml. Most of the antibacterial compounds observed on bioautography had R_f values ranging from 0.21 to 0.88. *Z. mucronata* had LC50 of 105.5 µg/ml and *R. communis* 131.8 µg/ml on Vero cells.

Conclusion: This study revealed that both plants had free radical scavenging and antibacterial activities.

Key words: Minimum inhibitory concentration, bioautography, medicinal plants, R_f value, antibacterial.

Introduction

African populations depend on the use of medicinal plants as a source of medicines to improve their health and also to cure infectious diseases. Use of medicinal plants in South Africa and other African countries has been passed on from our ancestors to the current generation (Masoko et al., 2005). Traditional medicinal plants are extensively used in Africa since they are affordable and easily accessible (Shai et al., 2008). Different parts of medicinal plants used as medicines or raw drugs include roots, stem, leaves, flowers and fruits. The extracts collected from different parts of medicinal plants contain a variety of medicinal properties such as antimicrobial and antioxidant activities (Mahesh and Satish, 2008). Antimicrobials are known as drugs that inhibit growth at a concentration which is safe for the host, and some may also be applied as chemotherapeutic agents to prevent bacterial infection (Kota and Manthri, 2011). Antioxidants play an important role in living organisms (animal or human) to control levels of free radicals and other reactive molecules or species in the body to reduce oxidative damage. Phenolic compounds are known as effective free radical scavengers (Ebrahimzadeh et al., 2010). Some African indigenous plant species have been subjects of scientific screening for potential sources of pharmaceutical drugs (Shai et al., 2008). Throughout the worldwide, higher plants are considered as a source of new antimicrobial properties and used to treat several common infectious diseases such as diarrhea and fever (Eloff, 1998).

R. communis L. (Euphorbiaceae) is a soft-wooded small tree found or native throughout tropics and warm temperature regions of the world (Lavarasan et al., 2006). The leaves of the plant are primary food for Eri silkworms (Mukhopadhyay et al., 2011). In India, leaves, roots and seed oils of *R. communis* are used to treat inflammation and liver disorders (Lavarasan et al., 2006). *Z. mucronata* Willd (Rhamnaceae) is a shrub or medium-sized tree with crooked trunks, spreading branches and branching well above ground or near the base. The plant is a hardy species, most commonly found in dry areas and resistant to both frost and drought. The leaves, roots and barks of *Z. mucronata* are traditionally applied in South African as a medicine for chest complaints, boils, wounds, pain relief, skin infections and syphilis (Orwa et al., 2009). The aim of the study was to investigate the effect of biological properties of *Z. mucronata* and *R. communis* against selected bacterial species.

Materials and Methods

Plant materials collection and storage

The leaves of *Z. mucronata* and *R. communis* were collected at the University of Limpopo (Turfloop campus), South Africa during Summer in 2011. Voucher specimens in the garden herbarium and tree labels verified the identity of the plants. Plants were confirmed by Dr Bronwyn Egan (Herbarium). She also provided plants accession details of *Z. mucronata* (UNIN 11353) and *R. communis* (UNIN 11352). Leaves of both plants were separated from twigs and dried at room temperature. Most scientists have tended to use dried plant material because there are fewer problems associated with large-scale extraction of dried plants rather than fresh plant material (Eloff, 1998). The dried plant materials were ground to fine powder and stored at room temperature in closed containers in the dark until used for the extraction.

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Preparation of extracts

The leaves of *Z. mucronata* and *R. communis* were extracted by weighing 1.0 g of finely ground plant material and macerate in 10 ml of n-hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M) in glass tubes. Tubes were vigorously shaken for 10 min at a high speed and extracts were filtered into pre-weighed empty bottles to give extracts after removal of the solvents under a stream of cold air at room temperature. The process was repeated three times to exhaustively extract the compounds, and filtrates were combined.

Phytochemical analysis

Extracted chemical components were analyzed by separation with thin layer chromatography (TLC) using aluminum-backed TLC plates (Fluka, silica gel F254). TLC plates were developed in saturated chambers using mobile phases of different polarities, namely benzene/ethanol/ammonia hydroxide (BEA) (non-polar/basic) (18:2:0.2), chloroform/ethyl acetate/formic acid (CEF) (intermediate polarity/acidic) (10:8:2), and ethyl acetate/methanol/water (EMW) (polar/neutral) (10:5.4:4) (Kotze and Eloff, 2002). Separated compounds on the TLC plates were examined under ultraviolet light (254 and 365 nm) then sprayed with vanillin-sulphuric acid reagent [0.1 g vanillin (Sigma ®): 28 ml methanol: 1 ml concentrated sulphuric acid] and heated at 110°C for optimal color development.

Phytochemical analysis test of extracts

Test for reducing sugars

The aqueous extract was developed by dissolving 0.5 g of plant powder into 5 ml of water. The aqueous solution was added to a boiling Fehling's solution [Fehling A was prepared by dissolving 7 g of hydrated copper (II) sulphate into 100 ml of distilled water]. Fehling B was prepared by dissolving 35 g of potassium sodium tartrate and 10 g of sodium hydroxide in 100 ml of distilled water. Equal volumes of Fehling A and B were mixed to form a deep blue solution of Fehling's solution in a test tube. The solution was observed for a colour reaction (Ayoola et al., 2008).

Test for anthraquinones

Anthraquinone content in plant extracts was tested by weighing 0.5 g of ethanol extracts and boiled with 10 ml of 97% sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube to which 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes (Ayoola et al., 2008).

Test for terpenoids (Salkowski test)

The ethanol extract was tested for terpenoids by weighing 0.5 g of the extract and dissolve into 2 ml of chloroform, and 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids (Borokini and Omotayo, 2012).

Test for flavonoids

The presence of flavonoids were tested in the aqueous extracts by adding 5 ml of diluted ammonia solution to a portion of the aqueous filtrate of plant extracts, followed by addition of 1 ml of concentrated sulphuric acid. A yellow colouration that disappears on standing indicates the presence of flavonoids (Borokini and Omotayo, 2012).

Test for saponins

The persistent frothing test was used to test for saponins by weighing 1 g of plant powdered leaf sample and mixed with 30 ml of tap water. The mixture was vigorously shaken and heated at 100°C. The sample was observed for formation of persistent of froth to draw inference (Odebiyi and Sofowora, 1978).

Test for tannins

The tannins were tested by boiling 0.5 g of powdered leaf in 5 ml of distilled water in a test tube, then cooled and filtered. A few or three drops of 0.1% ferric chloride was added to 1 ml of solution in a test tube and observed for brownish green or a blue - black colouration (Trease and Evans, 1989).

Test for alkaloids

Drangendorff's reagent was used to test for the alkaloids by weighing 0.2 g of ground powdered leaves and extract with 95% ethanol using soxhlet extractor. The extracting solvent was evaporated to dryness using a vacuum evaporator at 45°C. The plant residues were dissolved in 5 ml of 1% hydrochloric acid and 5 drops of drangendorff's reagent was added. Reddish-brown colour change was observed (Harborne, 1973).

Test for cardiac glycosides (Keller- Killiani test)

The Keller- Killiani test was employed to test for cardiac glycosides by weighing 0.5 g of plant extracts and dilute to 5 ml of water. The mixture of 2 ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution was added to diluted plant extracts. This mixture was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of a deoxysugar characteristic of cardenolides (Borokini and Omotayo, 2012).

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Test for steroids

Steroids were tested by adding 2 ml of acetic anhydride to 0.5 g of plants extracts and followed by addition of 2 ml of sulphuric acid to the mixture. Blue or green change was observed to draw inference (Borokini and Omotayo, 2012).

Test for phlobatannin

Phlobatannin was tested by weighing 0.2 g of powdered leaf sample into 10 ml of distilled water and filtered. The filtrate was boiled with 2% hydrochloric acid solution. Sample was observed for the formation and red colour of precipitate to draw inference (Borokini and Omotayo, 2012).

Antioxidant activity

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

TLC plates were used to separate extracts as described earlier (phytochemical analysis). To detect antioxidant activity, chromatograms were sprayed with 0.2% (w/v) 2,2-diphenyl-1-picrylhydrazyl (Sigma®) (DPPH) in methanol as an indicator (Deby and Margotteaux, 1970).

Quantitative 2, 2-diphenyl-1-picrylhydrazyl assay

Quantitative DPPH assay was done on the *R. communis* extracts only by adding 100 µl of distilled water into each well of 96-welled plate. The plant extracts were prepared at a concentration of 10 mg/ml and 100 µl of plant extracts were transferred into the first well of the 96 plate and extracts in these wells were serially diluted 50%. Vitamin C at a concentration of 2 mg/ml was used as the antioxidant standard or positive control. An additional 15 µl of 0.2% of 2, 2-diphenyl-1-picrylhydrazyl (sigma®) in methanol was added into each well of microtiter plate and plate was kept in the dark for 15 min. The absorbance was measured at 540 nm using a microtiter plate reader (DTX 880 multimode detector; Beckman coulter, Inc.) (Ayoola et al., 2008). Percentage scavenging activity was calculated as follows: % scavenging activity (DPPH reduced) = 100% - DPPH oxidized

$$\% \text{ scavenging activity} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{experiment}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Microorganisms

The test organisms were supplied by the Department of Biochemistry, Microbiology and Biotechnology section at the University of Limpopo (Turfloop campus). Two Gram-positive (*S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) and two Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) bacterial strains were used in this study. The organisms were sub-cultured on nutrients broth, incubated at 37°C for 24 h and stored at 4°C in the refrigerator as stock cultures.

Antibacterial activity

Quantitative antibacterial activity assay by minimum inhibitory concentration

Minimum inhibitory concentration (MIC) values were determined using the serial microbroth dilution method developed by Eloff (1998). MIC is described as the lowest concentration of the compounds inhibiting the growth of tested microorganisms. Dried crude extracts were reconstituted in acetone to a concentration of 10 mg/ml crude extracts. The plant extracts were serially diluted 50% with water in 96 well microtiter plates. Bacterial cultures were sub-cultured and transferred into fresh nutrient broth and 100 µl of the culture were transferred into each well and acetone was included blanks. The microtiter plate was incubated at 37°C for 24 h. After incubation, 20 µl of 2 mg/ml p-iodonitrotetrazolium violet (Sigma®) (INT) dissolved in water was added to each microplate wells as an indicator of growth. The covered microtiter plates were incubated for 30 min at 35°C and 100% relative humidity. All determinations were carried out in triplicate. Microorganism growth led to the emergence of a purple-red colour resulting from the reduction of INT into formazan. Clear wells indicated the presence of compound in the extracts that inhibited the growth of the microorganisms tested. MIC was recorded as the lowest concentration of the extract that inhibited bacterial growth after 24 h.

Qualitative antibacterial activity assay by bioautography

For bioautographic analysis, 20 µl of each extract (10 mg/ml) was loaded on the TLC plates. The plates were developed in mobile phases as described earlier. The chromatograms were dried at room temperature for about four days to remove the solvents used to develop chromatograms. The chromatograms were sprayed with overnight cultures of the bacterial species until completely wet and were incubated at 37°C in a humidified chamber for 24 hours. The plates were sprayed with 2 mg/ml of INT (Sigma®) and incubated for a further 2 hours (Begue and Kline, 1972).

In vitro cytotoxicity assay

Viable cell growth after incubation with test extracts was determined using the tetrazolium-based colorimetric (MTT) assay described by Mosmann (1983). Normal Vero kidney cells (monkey) of a subconfluent culture were used. The LC50 value was calculated as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells.

Results and Discussion

Seven different solvents were used to extract bioactive compounds from the leaves of *Z. mucronata* and *R. communis* because success of isolating compounds from plant materials is highly dependent on the type of solvent used for extraction (Masoko et al., 2008). Non-polar compounds

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can only be extracted by non-polar solvents, while polar compounds are extracted by polar solvents. Methanol extracts had high yields of extraction in both *Z. mucronata* and *R. communis*, while lowest were obtained from hexane extracts. Therefore, high amounts of polar compounds were extracted in

Table 1: Phytochemical constituents of leaf extracts of *R. communis*.

Compounds	<i>R. communis</i>
Anthraquines	+
Alkaloids	+
Tannins	+
Saponins	-
Steroids	+
Phlabatannins	-
Terpenoids	+
Flavonoids	+
Cardiac glycosides	+
Reducing sugar	+

(+): Presence, (-): Absence

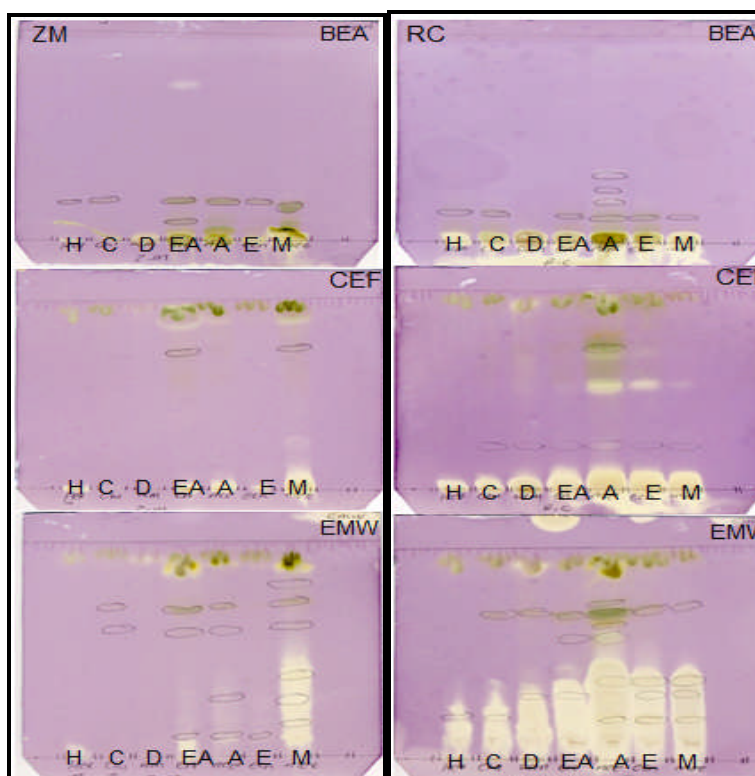


Figure 1: Chromatograms developed in BEA, CEF, EMW, and sprayed with 0.2% DPPH to show the antioxidant activity (clear zones) of *Z. mucronata* (ZM) and *R. communis* (RC) compounds extracted with hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

both plants since methanol is a polar solvent. Plants extracts from different solvents were reconstituted in acetone since acetone has been tested and reported to dissolve many of hydrophilic and lipophilic components, is miscible with water and is less toxic to bacteria (Eloff, 1998). In phytochemical analysis for both *Z. mucronata* and *R. communis*, more bands were observed on BEA followed by CEF then EMW, separation of extracts on thin layer chromatography plates indicate that more of non-polar compounds were extracted. Results in Table 1 showed the presence of anthraquines, alkaloids, tannins, steroids, terpenoids, flavonoids, cardiac glycosides, and reducing sugar in *R. communis* extracts and Saponins and phlabatannins were absent. For qualitative antioxidant activity, yellowish spot observed on TLC plates indicate presence of antioxidant activity (Figure 1). The ethyl acetate extracts of BEA and methanol extracts of EMW chromatograms of *Z. mucronata* had antioxidant activity, while the CEF had slight antioxidant activity on the methanol extract.

R. communis extracts separated by EMW had antioxidant activities in all the extracts and on the CEF chromatogram, acetone and ethanol extracts also have antioxidant activity. However, the chromatogram of *R. communis* separated by BEA only show antioxidant activity at the base where sample were loaded in the entire extracts. Quantitative antioxidant activity was done only on *R. communis* because it showed more bands on

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qualitative analysis and all tested crude extracts had free radical scavenging activity (Figure 2). Vitamin C was used as a positive control for qualitative antioxidant activity. Chloroform and methanol extracts were observed to have high antioxidant activity compare to the vitamin C. Methanol extracts was also found to have effectual antioxidant activity by Rao et al. (2013). Hexane and ethanolic extracts showed to have no antioxidant activity at concentration of 0.039 mg/ml. Dichloromethane, acetone and ethyl acetate extracts also showed antioxidant activity; however, the activity was less compared to vitamin C. Minimum inhibitory concentration values performed on both Gram negative bacteria and Gram positive bacteria were checked after 24 h (Tables 2 and 3). High inhibitory activity of extracts is indicated by the lowest concentration of extracts that inhibit bacterial growth. Kota and Manthri (2011) used agar diffusion method and revealed that ethanol extract of *R. communis* leaf had shown effective antibacterial activity.

In this study, ethanol extract of *R. communis* had better average activity against all tested organisms with the MIC of 0.31 mg/ml (Table 3), while hexane extract had better average MIC value of 0.50 mg/ml for *Z. mucronata* (Table 2). In Table 2, hexane extract showed an MIC value of 0.21 mg/ml against *E. coli*, 0.43 mg/ml against *P. aureginosa*, 0.53 mg/ml against *S. aureus* and 0.84 mg/ml against *E. faecalis*. Dichloromethane extracts

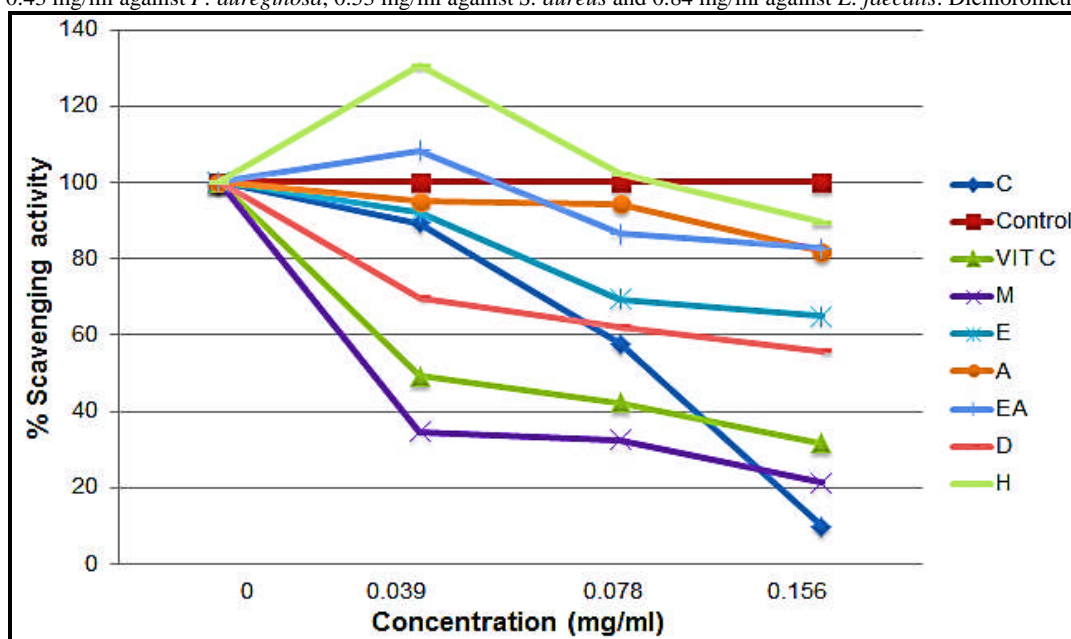


Figure 2: DPPH scavenging activity (%) of leaves extracts of *R. communis* at different concentrations at 540 nm. Negative control (control), Vitamin C (VIT C) positive control. Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

Table 2: Minimal inhibitory concentration (MIC) values (mg/ml) and total activity values (ml/g) of extracts of leaves of *Z. mucronata* against four bacterial test organisms.

Bacteria species	Plant extracts and MIC values (mg/ml)						
	H	C	D	EA	A	E	M
<i>E. coli</i>	0.21	0.27	0.64	0.84	0.64	0.53	0.74
<i>P. aureginosa</i>	0.43	0.84	0.64	0.43	0.32	0.74	0.64
<i>S. aureus</i>	0.53	1.05	0.27	0.43	0.32	0.53	0.53
<i>E. faecalis</i>	0.84	0.74	0.74	0.53	1.05	0.84	1.05
Averages	0.50	0.73	0.57	0.56	0.58	0.66	0.74
Bacteria species	Total activity (ml/g)						
	H	C	D	EA	A	E	M
<i>E. coli</i>	82	180	119	32	60	71	146
<i>P. aureginosa</i>	40	58	119	63	121	51	169
<i>S. aureus</i>	33	46	282	63	121	71	204
<i>E. faecalis</i>	21	66	103	52	37	45	103
Averages	44	88	156	53	85	60	156

Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

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showed a lowest concentration of 0.27 mg/ml against *S. aureus*, 0.53 mg/ml of ethyl acetate against *E. faecalis* and 0.32 mg/ml of acetone against *P. aureginosa*. In Table 3, the lowest MIC value for *R. communis* was observed in hexane extract (0.13 mg/ml) against *P. aureginosa* while highest concentration was observed in hexane extract (1.05 mg/ml) against Gram positive *S. aureus*. Hexane extract showed an MIC value of 0.43 mg/ml against *E. coli*, 0.13 mg/ml against *P. aureginosa*, 1.05 mg/ml against *S. aureus* and 0.84 mg/ml against *E. faecalis*. Dichloromethane and ethyl acetate showed the lowest concentration of 0.27 mg/ml against *E. coli*, 0.13 mg/ml of hexane and ethanol extract against *P. aureginosa*, 0.16 mg/ml of ethanol extract against *S. aureus* and 0.53 mg/ml of dichloromethane, ethyl acetate and acetone against *E. faecalis*.

Total activity of plants is defined as the amounts of material extracted from a single gram of plant dried material divided by the MIC value (ml/g). Total activity indicates largest volume to which biologically active compounds extracted from 1 g of plant material can be diluted and still inhibits growth of tested organisms. Total activity is important when evaluating potential use of plant extracts for treating fungal and bacterial infections (Eloff, 2004). Bioautography had bands of compounds that inhibited growth of tested organisms (results not shown). White spots against pink colour on the bioautograms plates indicate zone of inhibition of the tested organisms by certain compounds on the plant extracts. Dichloromethane extracts of *Z. mucronata* had high antibacterial activity in CEF and EMW against *E. coli*, *P. aureginosa* and *S. aureus*. High antibacterial active compounds of *R. communis* were observed on the CEF and EMW against *E. coli* and *S. aureus*. Slightly antibacterial inhibition was observed on the plates sprayed with *P. aureginosa* especially on dichloromethane extracts. Bioautography for *E. faecalis* for both plants were omitted due to the poor quality of results. The R_f values (Tables 4 to 9) of qualitative antimicrobial compounds were calculated and some of the antimicrobial compounds had high antimicrobial activity while other compounds were slightly actives.

Table 3: Minimal inhibitory concentration (MIC) values (mg/ml) and total activity values (ml/g) of extracts of leaves of *R. communis* against four bacterial test organisms

	Plant extracts and MIC values (mg/ml)						
Bacteria species	H	C	D	EA	A	E	M
<i>E. coli</i>	0.43	0.43	0.27	0.27	0.16	0.32	0.32
<i>P. aureginosa</i>	0.13	0.37	0.19	0.21	0.32	0.13	0.32
<i>S. aureus</i>	1.05	0.32	0.32	0.37	0.32	0.16	0.37
<i>E. faecalis</i>	0.84	0.74	0.53	0.53	0.53	0.64	0.84
Averages	0.61	0.47	0.33	0.35	0.33	0.31	0.46
	Total activity in (ml/g)						
Bacteria species	H	C	D	EA	A	E	M
<i>E. coli</i>	39	167	373	110	200	92	487
<i>P. aureginosa</i>	128	194	530	141	100	227	487
<i>S. aureus</i>	16	224	314	80	100	184	421
<i>E. faecalis</i>	20	97	190	56	60	46	186
Averages	51	171	352	97	155	137	395

Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

Table 4: Inhibition of tested microorganisms by the bioactive compounds of the *Z. mucronata* and *R. communis* extracts using bioautography separated by BEA. R_f values of bands of the extracts and degree of inhibition are indicated.

<i>E.coli</i>															
BEA[Benzene/Ethanol/Ammonia hydroxide]															
<i>Ziziphus mucronata</i>								<i>Ricinus communis</i>							
R _f values	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.86										x	x				

Relative degree of inhibition: X = Slight inhibition, XX = moderate inhibition, XXX = high inhibition; Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), Ethanol (E) and methanol (M). Empty box indicated no activity.

In Table 4, *E. coli* was slightly inhibited by chloroform and dichloromethane extracts at the R_f value of 0.86 while for *P. aureginosa* and *S. aureus*, no inhibition was found. In Table 5, most of the compounds separated by CEF had high inhibitory activity against *E. coli*, and *S. aureus*, and slightly inhibition for *P. aureginosa* at the R_f value of 0.87 and 0.64. Total numbers of the antimicrobial compounds found to inhibit *E. coli* from both plants were two and zero for *P. aureginosa* and *S. aureus* in BEA (Table 6). Thirteen antimicrobial compounds exhibit antimicrobial activity against *E. coli* on the CEF plates, four against *P. aureginosa* and sixteen against *S. aureus* (Table 8). In Table 9, thirteen antimicrobial compounds were found to inhibit *E. coli* growth, seven against *P. aureginosa* and eight against *S. aureus* in EMW mobile phase.

Conclusion

The study revealed that both plants have free radical scavenging activity and antibacterial activity against tested bacteria. Methanol and

<i>E. coli</i>															
CEF[Chloroform/ethyl acetate/formic acid]															
<i>Ziziphus mucronata</i>								<i>Ricinus communis</i>							
R _f values	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.86		xx	xxx	xxx	xxx	xx	xx								
0.63		xxx	xx	xxx	xx	xxx	xxx								
0.53											xxx				
<i>P. aureginosa</i>															
CEF[Chloroform/ethyl acetate/formic acid]															
<i>Ziziphus mucronata</i>								<i>Ricinus communis</i>							
Rf values	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.87			x	x	x										
0.64											x				
<i>S. aureus</i>															
CEF[Chloroform/ethyl acetate/formic acid]															
<i>Ziziphus mucronata</i>								<i>Ricinus communis</i>							
Rf values	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.88											xxx				
0.87		xx	xxx	xx	xx	Xx									
0.70			xx												
0.66		xx		xx	xx	Xx									
0.29				x	x	X									

Table 6: Inhibition of tested microorganisms by the bioactive compounds of the *Z. mucromata* and *R. communis* extracts using bioautography separated by EMW. R_f values of bands of the extracts and degree of inhibition are indicated.

E. coli															
EMW[Ethyl acetate/methanol/water]															
Ziziphus mucronata								Ricinus communis							
R _f Values	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.71											xxx				
0.62			xxx												
0.54				xx	xx	Xx									
0.51										x	xxx	x	x	x	
0.43			xxx												
0.39											xx				
P. aureginosa															
EMW[Ethyl acetate/methanol/water]															
Ziziphus mucronata								Ricinus communis							
R _f	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.85		x	x												
0.65											x				
0.55		x	x	x	x	X									
0.54											x				
0.44			x												
S. aureus															
EMW[Ethyl acetate/methanol/water]															

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<i>Ziziphus mucronata</i>								<i>Ricinus communis</i>							
R _f values	H	C	D	EA	A	E	M		H	C	D	EA	A	E	M
0.88		x	x	x	x										
0.64										x	x	x	x		

Relative degree of inhibition: X = Slight inhibition, XX = moderate inhibition, XXX = high inhibition; Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M). Empty box indicated no activity.

Table 7: Number of antimicrobial bands at different R_f values of *Z. mucronata* and *R. communis* separated by BEA, inhibiting the growth of tested microorganisms using bioautography.

BEA [Benzene/Ethanol/Ammonia hydroxide]																				Total		
	<i>E. coli</i>							<i>P. aureginosa,</i>							<i>S. aureus</i>							
R _f values	H	C	D	EA	A	E	M	H	C	D	EA	A	E	M	H	C	D	EA	A	E	M	
0.86		1	1																			2
Total	1 1																					
Grand values	2							0							0							

Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

Table 8: Number of antimicrobial bands at different R_f values of *Z. mucronata* and *R. communis* separated by CEF, inhibiting the growth of tested microorganisms using bioautography.

CEF [Chloroform/Ethyl acetate/Formic acid]																				Total		
R _f values	<i>E. coli</i>							<i>P. aureginosa</i>							<i>S. aureus</i>							
	H	C	D	EA	A	E	M	H	C	D	EA	A	E	M	H	C	D	EA	A	E	M	
0.88																	1					1
0.87										1	1	1				1	1	1	1	1		8
0.70																	1					1
0.86		1	1	1	1	1	1															6
0.66																1		1	1	1		4
0.64										1												1
0.63		1	1	1	1	1	1															6
0.53			1																			1
0.29																		1	1	1		3
Total	0	2	3	2	2	2	2	0	0	2	1	1	0	0	0	2	3	3	3	3	0	
Grand values	13							4							16							

Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

Table 9: Number of antimicrobial bands at different R_f values of *Z. mucronata* and *R. communis* separated by EMW, inhibiting the growth of tested microorganisms using bioautography.

EMW[Ethyl acetate/Methanol/water]																							Total
	<i>E. coli</i>							<i>P. aureginosa</i>							<i>S. aureus</i>								
R _f values	H	C	D	EA	A	E	M	H	C	D	EA	A	E	M	H	C	D	EA	A	E	M		
0.88																1	1	1	1			4	
0.85		1	1																			2	
0.71			1																			1	
0.65										1												1	
0.64																1	1	1	1			4	
0.62			1																			1	
0.55									1	1	1	1	1									5	
0.54				1	1	1				1												4	
0.51		1	1	1	1	1																5	
0.43			1																			1	
Total	0	2	5	2	2	2	0	0	1	3	1	1	1	0	0	2	2	2	2	0	0		
Grand values	13							7							8								

Hexane (H), chloroform (C), dichloromethane (D), ethyl acetate (EA), acetone (A), ethanol (E) and methanol (M).

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