academicJournals

Vol. 7(7), pp. 113-121, August 2013
DOI: 10.5897/AJBR12.098
ISSN 1996-0778 © 2013 Academic Journals
http://www.academicjournals.org/AJBR

African Journal of Biochemistry Research

Full Length Research Paper

A pentacyclic triterpene from the leaves of *Combretum collinum* Fresen showing antibacterial properties against *Staphylococcus aureus*

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Accepted 10 July, 2013

Leaf extracts of six different plants, many of which are used by traditional healers, were screened for antibacterial activity. These include *Adina microcephaha*, *Combretum aurea*, *Combretum bracteosam, Combretum collinum, Filicium decipiens* and *Ficus moraceae*. The highest mass of 118 mg was extracted with acetone from *F. decipiens* and the lowest mass of 18 mg was extracted from *F. moraceae*. The bioautography showed antibacterial activity of the dichloromethane and acetone extracts of *C. collinum* subspecies *suluense* and *F. decipiens* against *Escherichia coli*. It also showed antibacterial activity of the acetone extract of *C. collinum* subspecies *suluense* against *Staphylococcus aureus*. Two compounds were isolated from these extracts and characterized. Their minimum inhibitory concentrations were determined. One compound from *C. collinum* subspecies *suluense* had MIC value of 0.5689 mg/ml and compound E had 1.479 mg/ml. The chemical structure proposed for the compound was determined by correlation of HNMR and GC-MS data. The chemical structure of the other compound was not proposed.

Key words: *Combretum collinum* subspecie *suluense*, acetone extracts.

INTRODUCTION

Traditional medicine based on plant-derived remedies is the primary source of relief from a variety of diseases in Southern African cultures; it should be promoted, investigated and its potential developed for wider use and benefit to mankind (WHO, 1978). Primary health care has been adopted by all WHO member states, including those in the African continent, as the appropriate strategy, for developing national health systems (Akerele, 1988). In South Africa, about 60 to 80% of the population relies on medicinal plants to treat various illnesses (Hutching and van Staden, 1994) although traditional medicine is not fully accepted in the mainstream primary health care system. For full acceptance, the chemical components of the medicines, the safety and quality of their preparations must be investigated and validated to comply with the Medicines and Related Substances (MRS) Act 101 of 1965.

From a study conducted in northwestern Ethiopia to compile knowledge on the use of medicinal plants for treatment or prevention of human ailments large, proportions of medicinal plants were found to have been used for the treatments of gastro-intestinal complaints (26%), skin diseases (24%) and malaria (22%). The species *Croton macrostachyus*, *Calpurnia aurea*, *Clematis hirsuta* and *Plumbago zeylanica* were found to have the highest diversity of medicinal applications (Giday et al., 2007).

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Table 1. Diseases treated by traditional healers using *Combretum* species.

Specie	Traditionally used to cure	References
Combretum appiculam	Snake and scorpion bite, bloody diarrhea, leprosy, abdominal disorders, conjunctivitis and weakness.	Hutching, 1996
Combretum erythrophyllum	Fattening tonic for dogs. To reduce the size of the vaginal orifice.	Gelfland et al., 1985
Combretum hereroense	Bilharzia, headache and infertility in women.	Van Wyk and Van Wyk, 1997
Combretum molle	Hookworm, stomachache, snakebite, leprosy, fever, dysentery, chest complaints and as an anti-helminthic.	Watt and Breyer–Brandwijk, 1962
Combretum zeyheri	Toothache, cough, scorpion bite, bloody diarrhea, arrest menstrual flow, eye lotion, embrocating and abdominal disorders.	Hutching, 1996

Several plants have been tested for anti-bacterial properties; hydro alcoholic extracts of *Acokanthera schimperi*, *Calpurnia aurea*, *Kalancho epetitiana*, *Lippia adoensis*, *Malva parviflora*, *Olinia rochetiana*, *Phytolacca dodecandra* and *Verbascum sinaiticum*, traditionally used in the treatment of various skin disorders were screened for antimicrobial activity against different strains of bacteria and fungi which are known to cause different types of skin infections (Tadeg et al., 2005).

Several olean type saponins with antibacterial activity have been isolated from extracts of the dried bark of *Hippocratea excels* including 11β,21β-dihydroxy-olean-12-ene-3-one, 3α,11α,21β-trihydroxy-olean-12-ene, 3α,21β-dihydroxy-11α-methoxy-olean-12-ene, 3α,21β-dihydroxy-olean-9(11)-1,2-diene, 3α,21β-dihydroxy-olean-12-ene, 3α,21β-dihydroxy-11-α-methoxy-urs-12-ene and 11α,21-β-dihydroxy-olean-12-ene-3-one (Cáceres-Castillo et al., 2008).

Traditional healers throughout southern Africa employ species of Combretaceae and use them for many medicinal purposes, which include the treatment of abdominal pains, backache, chest coughs, colds, conjunctivitis, diarrhea, dysmenorrheal, earache, fever, headache, hookworm, infertility in women, leprosy, pneumonia, scorpion and snake bite, swelling caused by mumps, syphilis and general weakness (Watt and Breyer-Brandwijk, 1962). According to Njoroge and Bussmann (2006), Traditional healers use the dried leaves of *Combretum collinum* to manage throat and nose diseases in central Kenya. Some of the other uses of the dried leaves of *Combretum* species are listed in Table 1, to show that the species are widely used in African traditional healthcare systems.

According to Hedberg et al., (1982), *C. collinum* has various medicinal uses in African traditional medicine; the roots are used together with bark and roots of *Kigelia Africana* for the treatment of excessive menstrual bleeding. The hot water extract from the same plant is used for the treatment of diarrhea and anal bleeding. Haerdi (1964) found that traditional healers prescribed the hot water extract from leaves and roots to the patient to drink, as the treatment of malaria. Investigation by Kokwaro (1976) showed that snakebite patients chew the roots of *C. collinum* as treatment. Adjanounhoun et al. (1986) reported that three spoons of decoction of leaves are used for the treatment of malaria. Some compounds from *Combretum* species have shown anti-inflammatory, anti-helminthic, anti-bilharzia and anti-DNA damaging activity. Significant activity in more than one bioassay was exhibited by *Combretum apiculatum*, *Combretum hereroense*, *Combretum molle* and *Combretum mossambicense* (MacGaw et al., 2001).

C. collinum is one of the trees that produce a mean charcoal kiln efficiency of 23%. Most of the industrial wood energy is consumed by small-scale industries which include food processing industries and service sectors such as brewing, fish smoking, salt production, baking, restaurants, schools, hospitals, vending, agro-processing industries such as tobacco curing, tea drying and bees wax processing and production of building materials such as burnt bricks, lime, pottery and ceramics. These industry and domestic activities which rely uon wood energy provide employment and income for rural people particularly during off-season in agricultural production (Monela and Kihiyo, 1999).

PROBLEM STATEMENT

According to Warrier et al. (2001), medicinal plants provide accessible and largely safe sources of primary health care to the majority of the population in India. Poor people who are unable to financially afford formal health care systems are dependent on herbal medicine. Governments are constantly haggling with pharmaceutical companies because they are constantly increasing prices of their medicines. There is a growing interest in the value and efficacy of medicinal plants based on local and indigenous health systems as a way of meeting the current and future health care needs of the people (Warrier et al., 2001). Many investigators have reported that the dried leaves of *C. collinum* sub specie *suluense* have antibacterial activity (Pegel and Rogers, 1986). However, its chemical components have not been identified. Many traditional healers use water extracts of the dried leaves of *C. collinum* which means there is a need to characterize

Figure 1. A fresh twig of *C. collinum* ssp *suluense* (Neilspruit National botanical garden).

its chemical components. Many traditional healers prescribe water extracts of the dried leaves of *C. collinum* without specifying the concentration; this means there is a need to test the dried leaves of this plant against different organisms and measure the minimum effective concentration.

The aim of this study was to isolate and characterize antibacterial compounds from the dried leaves of *C. collinum* sub species *suluense*, shown in Figure 1, and to propose their chemical structures based on spectroscopic evidence. The research therefore involved extraction of compounds from the dried leaves of *C. collinum* sub specie *suluense* using sequential solvent extraction, followed by determination of the antibacterial activities of the extracts. The antibacterial activities of the extracts were used in the selection of extracts from which to isolate active compounds. The isolation of compounds from the extracts was achieved using chromatographic techniques, mostly column and preparative scale thin layer chromatography. In this study, the minimum inhibitory concentration of chemical components that have antibacterial activities was determined. The chemical structure of one antibacterial compound is proposed on the basis of its mass spectrum and comparison of its proton nuclear magnetic resonance data with that of several olean type saponins that have been isolated from extracts of the dried bark of *Hippocratea excels* (Cáceres-Castillo et al., 2008).

MATERIALS AND METHODS

Acetone, dichloromethane, hexane, chloroform, ethyl acetate, methanol, formic acid, vanillin, methanolic sulphuric acid mixture and *p*-iodonitrotetrazolium violet (INT) were obtained from Sigma-Aldrich as analytical reagents. The orbital shaker used was an MRC with twelve positions for 250 to 500 ml Erlenmeyer flasks. The Büchner funnel and the Whatman No. 1 filter paper, thin layer chromatography (TLC) plates (20 x 20 cm), Schott Duran bottle (500 ml) were obtained from Arcos. The rotary evaporator was a Buchi R-205 fitted with a vertical water cooled condenser. Luria Bertani (LB) and Muller Hinton (MH) broth cultures, were purchased from Arcos. The autoclave was an Optima B class from Prestige Medical. The water was triple distilled and passed through a deionizing column before use. The *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa* were kindly donated by Dr. LJ McGaw of the Phytomedicine Programme at the University of Pretoria, South Africa.

Plant collection drying and storage

Leaves of six plants, namely *Calpurnia aurea* (438 g), *C. collinum* (174 g), *Combretum bracteosam* (116 g), *Adina mcrocephaha* (239 g), *Ficus moraceae* (54 g), and *Filicium decipiens* (247 g) were collected from the National Botanical garden in Nelspruit, Mpumalanga during summer of 2009 - 2010. The leaves of the six plant species were air-dried in the dark for three weeks. The dried leaves were ground to fine powder using a mortar and pestle and placed in glass containers until further use (Rogers and Verotta, 1996; Eloff, 1998).

Extraction

The ground leaves (1 g) of each of the six different plant species were suspended in 10 ml of each of acetone, dichloromethane and hexane for preliminary screening against *S. aureus, E. coli, E. faecalis* and *P. aeruginosa* (Masoko et al., 2006) using the bioautography method. The mixtures were shaken for 30 min and filtered using Whatman no.1 filter paper. The extracts were air-dried and the mass of the extracts was measured and recorded (Abegaz et al., 1993; Masoko et al., 2006). From this preliminary screening, two compounds with antibacterial activity against *S. aureus* and *E. coli* were found in the leaves of *C. collinum* (Figure 3). For this reason, the investigation focused on *C. collinum.* The dried ground leaves of this plant (172 g) were extracted with acetone, hexane, chloroform, ethyl acetate and methanol, respectively (Shai et al., 2008).

Thin layer chromatography

A thin layer chromatography plate (TLC) (20 x 20 cm) spotted with100 µg of hexane, acetone and dichloromethane extracts was developed in a tank saturated with a mobile phase consisting of chloroform, ethyl acetate and formic acid (5:4:1) (CEF). The chemical components were visualized by spraying the plates with a vanillin-c sulphuric acid reagent [vanillin (0.1 g): methanol (28 ml): sulphuric acid (1 ml)], followed by gentle warming on a hot plate to 110°C until the color of the spots was fully developed (Kotze and Eloff, 2002).

Bioautography

Bacterial cells were cultured in a sterile Schott Duran bottle (500 ml) in Luria Bertani (LB) broth at 37°C (Reynolds, 2005). The bacterial cultures were prepared for 14 h, prior to antibacterial activity assays. The TLC plates (20 x 20 cm) were spotted with each extract and developed as already described. The chromatograms were dried up at room temperature under a stream of air to remove the remaining solvent. Cultures were grown in LB broth solution overnight. The plates were sprayed with cultures of the selected bacteria until wet and incubated overnight at 37°C. The plates were

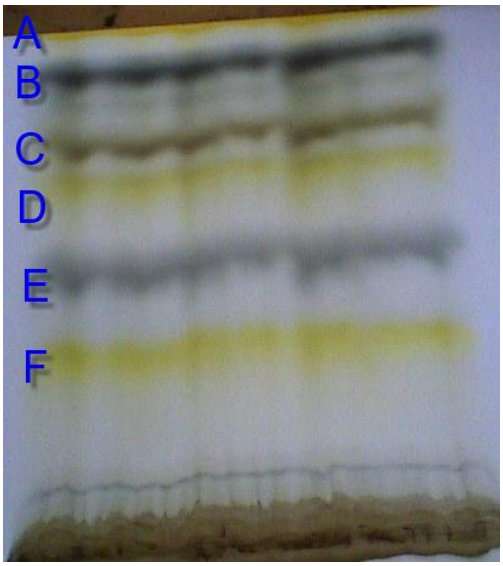


Figure 2. The preparative TLC plate of 2 mm thickness was developed using hexane and ethyl acetate in 2:1 ratio.

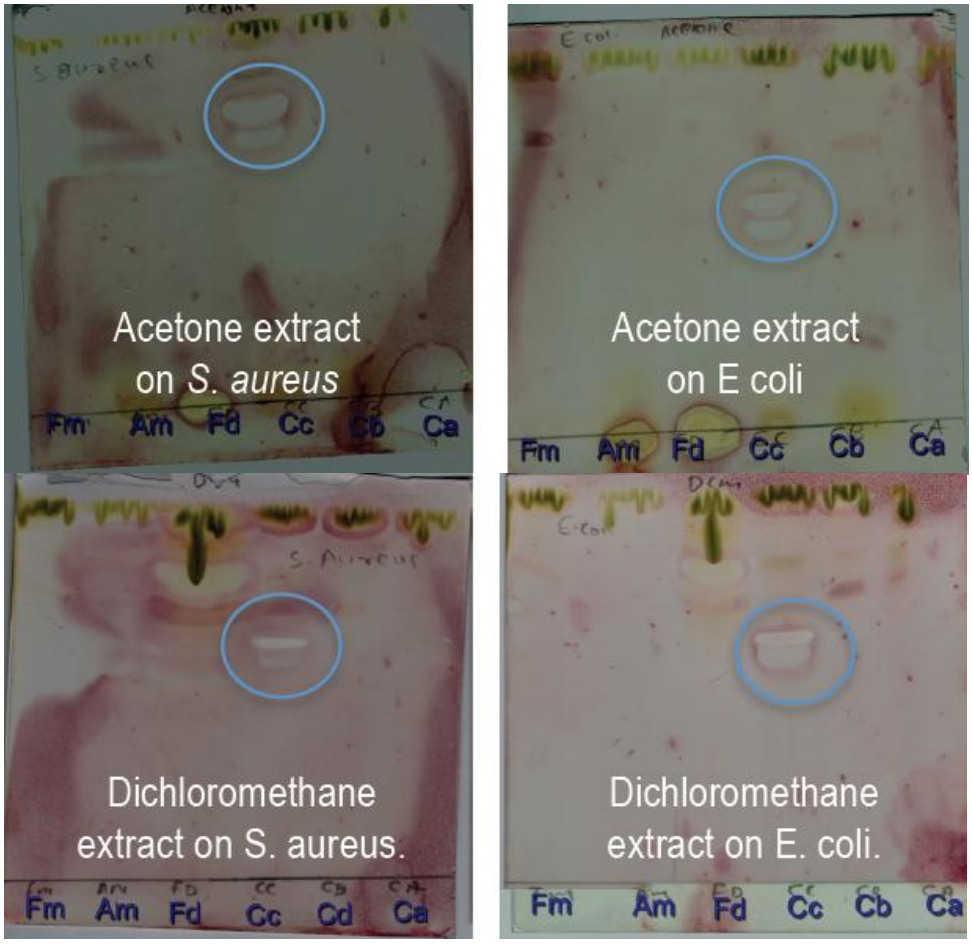


Figure 3. The bioautography screening of the plant extracts showed zones of inhibition on two spots on the plate. Fm = *Ficus moraceae*, Am = *Adina microcephaha*, Fd = *Filicium decipiens*, Cc = *Combretum collinum*, Cd = *Combretum bracteosam*, Ca = *Combretum aurea.*

Table 2. Mass of extracts from a gram of each of the six different plants and the percentage yield of each extract.

Name of plants	Mass of extracts (mg)		
	Acetone	DCM	Hexane
Adina microcephaha	74	86	37
Combretum aurea	35	20	63
Combretum bracteosam	27	47	33
Combretum collinum	40	30	30
Filicium decipiens	118	75	52
Ficus moraceae	44	30	18

sprayed with 2 mg/ml of *p*-iodonitrotetrazolium violet (INT) and further incubated at 37°C for color development (Begue and Kline, 1972).

Minimum inhibitory concentration

The microplate dilution method was used to determine the minimum inhibitory concentration (MIC) of the extracts against each test bacterial species (Eloff, 1998). The plant extracts or compounds were constituted to 10 mg/ml with acetone and 100 µl portions were serially diluted to 50% with water in a 96-well micro-plate. Muller Hinton (MH) broth culture was inoculated (1%) with the test bacteria, incubated at 37°C overnight and 100 µl of the resulting culture were added to each well (Eloff, 2004). The negative growth control contained MH broth and test organism with no plant material. The micro-plates were sealed and incubated at 37°C at 100% relative humidity for 18 h. A 0.2 mg/ml solution of INT (40 µl) in water was used as an indicator for bacterial growth and the solution was added to the micro-plate wells and incubated at 37°C for a further 30 min. The MIC was recorded as the lowest concentration of extract at which bacterial growth was inhibited (Eloff, 2004) as shown by total lack of the purple red formazan colour in the well. Lack of color in the well shows that the bacteria were inhibited from growth and a purple red or pink color indicated that there was bacterial growth (Begue and Kline, 1972).

Preparative thin layer chromatography (PTLC)

The acetone extract (1.0 g) was dissolved in acetone (15 ml), and loaded on a line drawn 2 mm from the bottom of the preparative plate as shown in Figure 2. The mobile phase used to separate the compounds was 2:1 ethyl acetate and hexane. The PTLC plate used was 20 x 20 cm in size. The bands shown in Figure 2 were scrapped from the PTLC plates and placed in separate beakers. The support material with adsorbed components from the scrapped bands was suspended in ethyl acetate to dissolve the adsorbed components. The adsorbed components were isolated by filtration by washing several times with the ethyl acetate. The ethyl acetate was removed by evaporation on the rotary evaporator.

The components separated using this procedure was examined by spotting on an analytical TLC plate, developing with a mixture of chloroform ethyl acetate and formic acid (5:4:1). The material obtained from the bands A, B and E of the PTLC chromatograms showed only one spot per band on the analytical TLC plates, whereas material obtained from bands C and D showed more than one spot. Thus, the material obtained from the bands A, B and E of the PTLC chromatograms was considered to be separated to analytical TLC purity, whereas the material obtained from bands C and D was considered not to have been sufficiently separated and was not used any further in this study.

Mass spectrometry

The instrument used for GC-MS spectra consisted of a Hewlett Packard 5973 mass selective detector, connected to a HP 6890 series GC system, Agilent Technologies Automatic Liquid Injection system 7683 series, at the Council for Scientific and Industrial Research in Pretoria (CSIR). The column used was HP-5MS (30 m x 0.25 mm x 0.25 µm). The column temperature programmed was 120°C with a 1 min ramp to 320 at 15°C/min thereafter held for 5 min at 320°C. The carrier gas was helium, electron energy was 70 eV, emission current 34.6 Ua, injector temperature was 250°C, the transfer line temperature was 25°C and the quadrupole temperature was 150°C The total flow rate was 14 ml/min, with a purge flow at a split vent of 0.1 ml/min, the acquisition mode was full scan and the scan range was 35 to 750 amu.

Nuclear magnetic resonance spectroscopy

The instrument used for NMR spectroscopy was mercury 300 MHz from University of Limpopo, Medunsa campus, at ambient temperature. The solvent used to dissolve the compound (18 mg) in the NMR tube was acetone-D6. The compound was relaxed and delayed by 1.000 second in the NMR instrument. The acquisition time was 1.997 s. The observation frequency was 300 MHz. The total time spent by the compound in the NMR instrument was 59 min and 1 s.

RESULTS AND DISCUSSION

Extraction

The yields obtained from the extraction of one gram of the dried and pulverized leaves of each of *Adina microcephaha, C. aurea, C. bracteosam, C. collinum, F. decipiens, F. moraceae* are shown in Table 2. Acetone extracted the highest mass of 118 mg from *F. decipiens* when compared with the extracts from the other five plants and hexane extracted the smallest amount of 18 mg from *Ficus moraceae*. This indicates that *C. collinum* has more moderate polar compounds than non-polar compounds since acetone is a polar solvent that extracts mainly polar and polar compounds.

The bioautography screening

The bioautograms in Figure 3 show two distinct zones of inhibition from the acetone and dichloromethane extracts

of *C. collinum* against *S. aureus* and *E. coli*. Clearly, the dichloromethane and acetone extracts of *C. collinum* were active against *S. aureus* and *E. coli*, and there are two compounds with antibacterial activity from these extracts. The bioautograms in Figure 3 also show that the retardation factors of the active compounds are very close. This meant that the separation of these two compounds would be a challenge. Although, higher yields could have been obtained, separation of these compounds using column chromatography would have been expensive and time consuming. Separation using preparative scale thin layer chromatography was chosen and it was quick and inexpensive, albeit with lower yields.

Chromatographic isolation

Two unknown compounds were isolated from the acetone extracts of *C. collinum* subspecies *suluense*. These compounds were isolated with preparative thin layer chromatography on 20 x 20 cm silica gel plates using 2:1 ethyl acetate and hexane as the development solvent mixture and re-crystallized from ethanol. After isolation using preparative thin layer chromatography, each compound showed one spot on the analytical TLC; with retardation factor values of 0.7 and 0.6, respectively. The two unknown compounds were also isolated from the dichloromethhane extracts of *C. collinum* subspecies *suluense* using preparative scale thin layer chromategraphy. Preparative thin layer chromatography of the acetone extract (40 mg) gave a higher yield of 12 and 9 mg (55%) for the two compounds while the dichloromethane extract (30 mg) gave a much lower yield of 4 and 3 mg (23 %), respectively.

Minimum inhibitory concentration

The minimum inhibitory concentration refers to the lowest concentration at which an antimicrobial agent inhibits the visible growth of a microorganism after overnight incubation (Andrews, 2001). The MIC of the one compound against *S. aureus* was 0.569 mg/ml and the MIC of the other compound against *S. aureus* was 1.479 mg/ml. This is in contrast with Netshiluvhi (2012) who found that the MIC of the leaf extracts of *C. collinum*, against *S. aureus*, ranged from 0.10 to 0.39 mg/ml, and also that these increased significantly with increase in the rate of annual rainfall. The lower activity of the isolated compounds when compared to the extracts suggests possible synergistic activities since the extract contains other secondary metabolites.

Structure elucidation

The chemical structure proposed for one of the compounds isolated from *C. collinum* subspecies *suluense* was determined by correlation of ^{1}H-NMR and GC-MS data. The compound was re-crystallized from 98% ethanol, dried and analyzed using ^{1}H-NMR (300 MHz), using tetramethylsilane (TMS) as a reference and acetone-D_6 as solvent. Correlation of the observed ^{1}H-NMR data with literature data is shown in Table 3. The comparison shows that the observed data correlates well with literature data for a known compound, 21-α-hydroxy-olean-12-ene-3-one. However, the observed ^{1}H-NMR data could not be conclusive mainly because of the poor quality of the spectrum due to the low concentration of the analyte (<0.1 mg/ml).

Compelling evidence for the structure proposed for compound came from the GC-MS data. The calculated molecular ion of this compound was observed at 424 m/z. The fragmentation pattern of the proposed structure is shown in Table 4. The molecular ion fragmented by loss of one of the methyl groups to give a fragment ion at M/z = 409. The rest of the fragmentation pattern shows the breaking of multiple carbon-carbon bonds at various positions of the molecule to give several ion fragments that match the observed mass spectrum. A comparison of the observed MS data with calculated data is shown in Table 4. The mass spectrum of the compound is shown in Figure 4. The molecular ion peak is significant at m/z = 424, in perfect agreement with the proposed structure. The peak at m/z = 409 corresponds to the loss of a methyl group; the proposed structure has two geminal dimethyl groups and four tertiary methyl groups. The fragmentation reaction 2 in Table 4 shows a possible loss of a methyl group; one of eight possibilities. The reactions 3 to 19 shown in Table 4 are high energy fragmentations involving the breaking of two carbon–carbon bonds, and they are all in good agreement with calculated fragmentations based on the proposed structure. Thus, the mass spectral data provides strong support for the proposed molecular structure. The chemical structure of the other compound was not proposed.

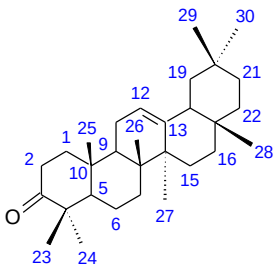
Conclusion

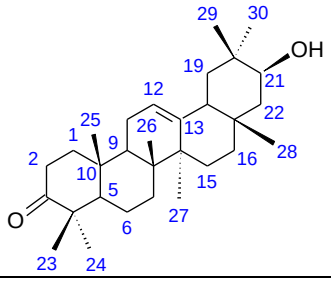
The proposed compound, olean-12-ene-3-one extracted with acetone and dichloromethane from *C. collinum* subspecies *suluense* is reported here for the first time. It shows antibacterial activity against *S. aureus* and *E. coli* with MIC = 0.5689 mg/ml. The structure of the other compound could not be characterized. Observed ^{1}H-NMR and MS data are in support of the proposed structure. However, the MS data is significantly more compelling. The lower antibacterial activity of the proposed compound when compared to the activity of the extract reported by Netshiluvhi (2012) suggests that there could be synergistic effects since the extract contains other secondary metabolites. These synergistic effects will be investigated.

ACKNOWLEDGEMENTS

This work was supported by a generous research

Table 3. H-NMR results of compound A.





H	Isolated compound	21α-hydroxy-olean-12-ene-3-one (Cáceres-Castillo et al., 2008)	
	Chemical shift	Chemical shifts	Coupling constants
1	0.887 m, 1.984 m	1.41 m, 190 m	-
2	2, 881 dd, 2.914 dd	2.36 ddd, 2.55 ddd	(16, 6.8,3.7), (16, 11, 7.3)
3	-	-	-
4	-	-	-
5	1.284 m	1.33 m	-
6	1.588 m, 1.601 m	1.48, 1.55 m	-
7	1.312 m, 1.333 m	1.35, 1.52 m	-
8	-	-	-
9	1.655 dd	1.65 dd	(11.5, 6.3)
10	-	-	-
11	2.044 dd, 2.058 dd	1.88, 1.97 m	-
12	5.133 t	5.24 t	(3.5)
13	-	-	-
14	-	-	-
15	1.051 m, 1.692 m	1.00, 1.76 m	-
16	1.113 m, 1.136 m	1.00, 1.96m	-
17	-	-	-
18	2.082 dd	2.00 dd	(14.8, 3.5)
19	1.655, 1.672 m	1.15, 1.75 m	-
20	-	-	-
21	2.914, 2.323 dd	3.53 dd	(12, 4.7)
22	1.42 m	1.37, 1.50 m	-
23	0.997 s	1.10 s	-
24	1.091 s	1.06 s	-
25	1.113 s	1.07 s	-
26	0.931 s	1.01 s	-
27	0.887 s	1.14 s	-
28	0.861 s	0.88 s	-
29	0.875 s	0.97 s	-
30	0.858 s	0.86 s	-

Table 4. GC-MS fragmentation of the proposed structure of isolated compound.

Fragment (M/z)	Found (M/z)	Calculated (M/z)
1 (M^+)	424	424.4 (100.0%), 425.4 (33.4%), 426.4 (5.6%)
2	409	409.4 (100.0%), 410.4 (33.0%), 411.4 (5.2%)
3	299	300.2 (100.0%), 274.2 (21.6%), 275.2 (2.4%)
4	273	273.2 (100.0%), 274.4 (21.6%), 275.2 (2.4%)
5	232	233.0 (100.0%), 234.2 (18.2%), 235.2 (1.8%)

Table 4. Contd.

6	218	218.2 (100.0%), 219.2 (17.1%), 220.2 (1.6%)
7	203	203.1 (100.0%), 204.1 (15.9%), 205.2 (1.2%)
8	190	190.1 (100.0%), 191.1 (14.8%), 192,1 (1.2%)
9	175	175.1 (100.0%), 176.1 (13.6%), 177.1 (1.1%)
10	161	161.1 (100.0%), 162.1 (12.5%)
11	147	147.1 (100.0%), 148.1 (11.3%)
12	135	135.1 (100.0%), 136.1 (10.2%)
13	122	122.1 (100.0%), 123.1 (9.1%)
14	109	109.1 (100.0%), 110.1 (8.0%)
15	95	95.0 (100.0%), 96.1 (6.8%)
16	81	81.0 (100.0%), 82.0 (5.7%)
17	69	68.0 (100.0%), 69.0 (4.5%)
18	55	55.0 (100.0%), 56.0 (3.4%)
19	41	41.0 (100.0%), 42.0 (2.3%)

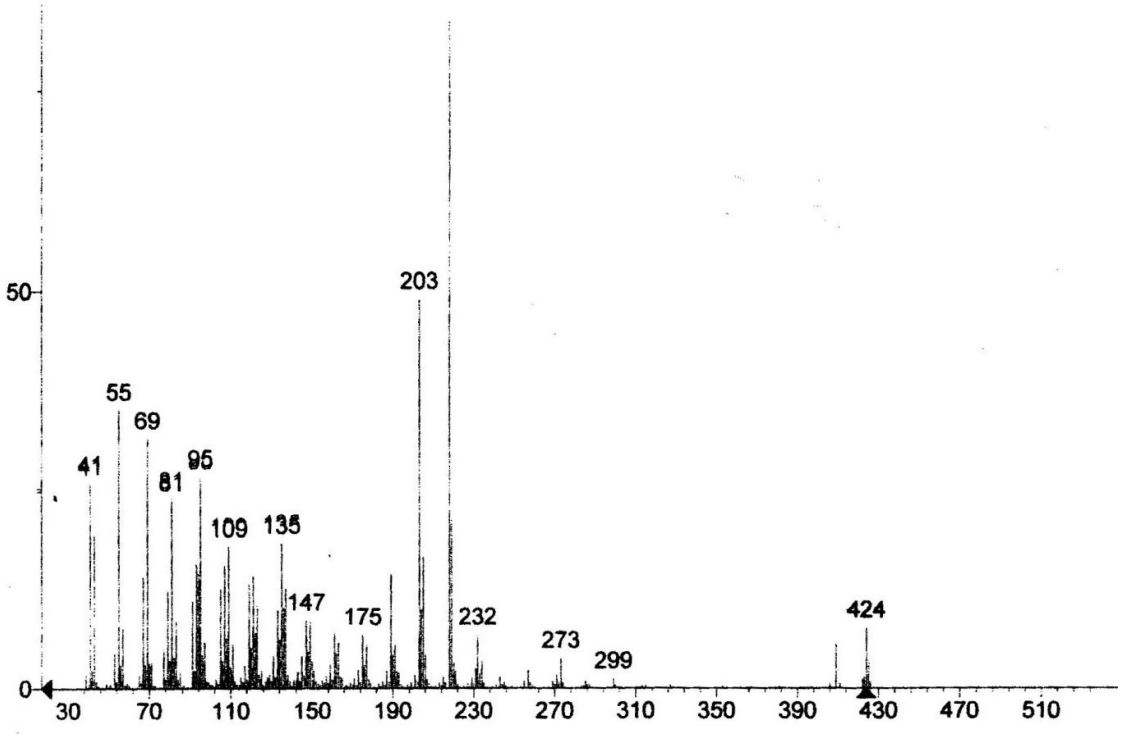


Figure 4. Mass spectrum of the proposed structure of isolated compound.

development grant from Walter Sisulu University for researchers in management as well as the NRF.

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