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Isolation and Chemical Structural Characterization of the Mixture of Two Related Phytosterols from *Ricinus communis* L. (Euphorbiaceae) Leaves

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Authors' contributions

This work was developed in collaboration by the both authors. Author PM conceived the project and wrote the final draft of the manuscript and was responsible for the overall laboratory work, data analysis and data interpretations. Author VN did the isolation of active compounds and biological assays. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: *Ricinus communis*, a member of the family Euphorbiaceace, is widely used in South African traditional medicine for the treatment of wounds, sores and boils. The plant was selected based on a high antibacterial activity demonstrated in a previous *In vitro* study. There is a need to isolate compounds that are responsible for antibacterial activity of *R. communis* leaves.
Methodology: The leaves of *R. communis* were milled to a fine powder and sequentially extracted with n-hexane, dichloromethane, acetone, and methanol using a serial exhaustive extraction method. Thin layer chromatography was used to analyse the phytochemical components of the extracts and bioautography for the presence of antibacterial compounds. A pure compound was isolated using column chromatography coupled with preparative thin layer chromatography and the structure was elucidated by nuclear magnetic resonance.
Results: Hexane extracts had potent antibacterial activity against *Escherichia coli* and

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Enterococcus faecalis and low activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The average minimum inhibitory concentration value of hexane crude extracts was 0.47 mg/ml, followed by extracts of dichloromethane (0.94 mg/ml), acetone (0.39 mg/ml) and methanol (1.49 mg/ml). The bioassay guided fractionation of the hexane extract led to the successful isolation of compound 1 at the Rf value of 0.40. Compound 1 was identified as a mixture of stigmasterol and β-sitosterol.

Conclusion: Compound 1 lost its antibacterial activity during the purification process. This may lend support to the theory that some compounds present in plant extracts act synergistically to give a particular biological activity. This study represents the first report of the isolation of stigmasterol and β-sitosterol from the leaves of *R. communis*.

Keywords: Ricinus communis; antibacterial; minimum inhibitory concentration; bioautography; stigmasterol; β-sitosterol.

1. INTRODUCTION

Over thousands of years ago, people from rural areas especially in South Africa and other developing countries relied on herbs as a primary source of medicines. To date, large groups of people in South Africa still prefer the use of African herbal medicines over Western medicines provided by the public health sector. Moreover, medicinal plants still continue to serve as a source of new potent remedies and natural product drugs in modern medicines. Of all modern medicines, it is said there is more than 50% natural products and their derivatives presently used as modern drugs globally [1,2]. For example, Aspirin, digitalis, quinine, diosgenin and opium are some of the drugs isolated from different species of plants [3]. More specifically, diosgenin has been derived from *Dioscorea* species and used to synthesize contraceptive agents [3]. Quinine has been isolated from the barks of *Cinchona* species and used as a template for the synthesis of quinoline antimalarial drugs such as chloroquine, amodiaquine and mefloquine [4]. While, vinblastine, vincristine and taxol are antitumor drugs that were isolated from medicinal plants and these drugs are currently used for chemotherapy of some cancers [5].

Now a days, scientists are able to isolate bioactive compounds from plant parts such as leaves, roots, barks and stems used by traditional doctors as medicines. Plant extracts have successfully served as a source of drugs with various biological activities such as antibacterial, anti-inflammatory and antioxidant activity. Alkaloids, steroids, terpenoids and phenolic compounds are responsible for biological activities of plants. Isolation and purification of these compounds become a challenge because of a large number of phytochemical compounds present in plants [6-9]. The successful isolation of compounds with desired activity from plant extracts is determined through a number of bioassays. Even though, some compounds tend to lose activity with each step of purification because they may act synergistically to exhibit one biological activity. Once they are separated they lose activity.

Ricinus communis (Castor oil) belongs to the family Euphorbiaceace. The castor oil plant is originally from Africa and is now found in all tropical countries including South Africa, India, Brazil and Russia. It grows wild in waste places [10,11], and is known for its therapeutic properties and biological activities in India and Africa. Some of the most well-known uses of the castor oil plant include its use as laxative, hepatoprotective and antidiabetic. Moreover, in India, castor oil is also used for combating fungal infections, relieving menstrual pains when applied on the lower abdomen and reducing stretch marks [12]. Its leaves are used for headache, inflammation, liver disorder, warts, dropsy and as a contraceptive herbal drug [12,13]. In South Africa, *R. communis* leaves are used for the treatment of wounds and have been reported for antibacterial activity against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) [14]. A recent study on the chemical constituents of the *R. communis* leaves revealed the presence of alkaloids, saponins, flavonoids and steroids [12]. Quercitin, gallic acid, gentisic acid, Ricinine (1-methyl-3-cyano-4-methoxy-2-pyridine), rutin, and two flavonoid compounds; kaempferol-3-O-beta-d-rutinoside and kaempferol-3-O-beta-d-xylopyranoid are compounds that have been isolated from roots, stems and seeds of *R. communis* [15]. To date, there is no study which investigated the isolation of antibacterial

compounds in *R. communis* leaves. Hence, the present study was aimed at the isolation of antibacterial compounds from *R. communis* leaves through bioassay-guided fractions.

2. METHODS AND MATERIALS

2.1 Plant Collection

R. communis leaves were collected in February of 2011 from the University of Limpopo (Turfloop campus), South Africa. Plant identity was confirmed by a specialist from the University of Limpopo Herbarium, Dr Brownyn Egan .The herbarium voucher number for the plant was UNIN 11352. As already, indicated in the introduction, the plant was selected based on its high antibacterial activity as reported in a previous *In vitro* study [16].

2.2 Plant Storage

The leaves were separated from twigs and dried at room temperature. Most scientists tend to use dried plant material because there are fewer problems associated with large scale extraction of compounds? from dried plants rather than from fresh plant materials [17]. The dried leaves were milled to fine powder and stored at room temperature in closed bottles in the dark until used for the extraction.

2.3 Extraction Procedure

Dried plant materials were sequentially extracted by suspending 1.125 kg of plant material starting with 5 L of hexane then, dichloromethane thereafter acetone and last, methanol in big glass bottle. Each time during the process, the bottles were vigorously shaken for overnight at a high speed of 300 rpm. Plant residues were allowed to settle and the supernatant was filtered and evaporated using a rotary evaporator (BÜCHi Labotec rotavapormodel R-205, Germany) and transferred into pre-weighed labeled glass beakers. The process was repeated three times to exhaustively extract the plant material and the extracts for each solvent were combined. After that, the solvent was removed under a stream of cold air at room temperature. Plant extracts were reconstituted using acetone to a final concentration of 10 mg/ml.

2.4 Analysis of Extracts By TLC

Ten microliters of 10 mg/ml plant extracts were loaded on a thin layer chromatography plates. Three separation systems of varying polarities were used to analyse plant extracts by thin layer chromatography (TLC) (Fluka, silica gel F_{254} plates); benzene: ethanol: ammonium hydroxide (BEA) (36:4:0.4), chloroform: ethyl acetate: formic acid (CEF) (20: 16: 4) and ethyl acetate: methanol: water (EMW) (20:10.8: 8). Chromatograms were examined under ultraviolet light (254 and 365 nm) and sprayed with freshly prepared vanillin spray reagent (0.1 g vanillin, 28 ml, methanol, 1 ml sulphuric acid) to visualised separated compounds. The plates were carefully heated at 110°C for optimal colour development [18].

2.5 Antibacterial Activities

2.5.1 Test microorganisms

Four bacterial strains were obtained from the Department of Biochemistry, Microbiology and Biotechnology, Faculty of Science and Agriculture, University of Limpopo (Turfloop campus) and used as test organisms. Two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and two Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213) were maintained on nutrient agar slants. The organisms were sub-cultured on nutrient broth, incubated at 37°C for 24 hours and stored at 4°C as stock cultures. Thereafter, the freshly prepared organisms were used for the following antibacterial assays.

2.5.2 Qualitative antibacterial assay by bioautography

The antibacterial compounds present were determined by bioautography using a method described by Begue and Kline [19]. Twenty microliters of 10 mg/ml plant extract was loaded on the TLC plates. The plates were developed in mobile phases as described earlier. Chromatograms were dried at room temperature for about four days to remove solvents used to develop chromatograms. The chromatograms were sprayed with overnight cultures until completely wet and incubated at 37°C for 24 hours. Plates were sprayed with 2 mg/ml of *p*-iodonitrotetrazolium violet (INT) (Sigma®) and further incubated for 2 hours. White areas against pink background indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of tested bacteria.

2.5.3 Quantitative antibacterial assay by minimum inhibitory concentration

The MIC value of plant extracts was determined by a serial dilution method microplate assay method using *p*-iodonitrotetrazolium violet to indicate growth of bacteria as described by Eloff [16]. The MIC was recorded as the lowest concentration of the extract that inhibited bacteria growth after 24 hours.

2.6 Isolation by Column Chromatography

Hexane fraction (53.84 g), which had the highest total antibacterial activity, was subjected to fractionation though silica gel 60 in an open column chromatography (40 x 6 cm) and eluted with mixtures of increasing polarity of hexane/ethyl acetate and ethyl acetate/methanol (2 L/fraction). Eluted fractions were collected, concentrated and analysed by TLC and bioautography. Active fractions eluted by 70% hexane in ethyl acetate, 50% hexane in ethyl acetate and 80% ethyl acetate in methanol were combined and subjected to another column (31 X 6 cm) filled with silica gel 60 and eluted with 90% dichloromethane in ethyl acetate. Forty-eight fractions (250 ml) were collected and pooled together based on analytical TLC results. One group of fraction (13 - 25) was combined and subjected to repeated column chromatography filled with silica gel 60 (63- 200 mm) (Merck) and eluted with 90% dichloromethane in ethyl acetate. One hundred and one fractions were collected in test tubes, analysed and regrouped based on analytical TLC data. One group of fractions (1 - 5) was obtained and further purified by preparative TLC plates.

2.7 Preparative Thin Layer Chromatography Plates

About 2.3027 mg obtained from column chromatography was further purified by preparative TLC silica gel glass plates (Merck, Silica gel 60 F_{254}) separated with 90% dichloromethane in ethyl acetate. Separated bands were visualized under ultraviolet light (254 and 360 nm) before a small part of the plate was sprayed with vanillin sulphuric acid and heated at 110^{o}C. The rest of the compound on the plates was protected with glass and an aluminum foil against the damage by vanillin sulphuric acid spraying reagent and heat. The visualized band on the side of the plate was used as reference line for scraping the remaining compound on the plate with glass rod. The components were collected, crushed, subjected into a glass Pasteur pipette plugged with cotton cool, washed with 90% dichloromethane in ethyl acetate to remove mixture of compound 1. The process was repeated three to four times to until the compound was fully recovered from the powder silica gel. The solvents were evaporated, weighed and transferred into a vial labeled compound one.

3. RESULTS AND DISCUSSION

Crude extracts were quantitatively tested for antibacterial activity against *E. coli, E. faecalis, P. aeruginosa*, and *S. aureus* using microbroth dilution method to determine the minimum inhibitory concentration values (Table 1). The average minimum inhibitory concentration value of hexane crude extracts was 0.47 mg/ml, dichloromethane (0.94 mg/ml), acetone (0.39 mg/ml) and methanol (1.49 mg/ml). Bioassay-guided fractionation in open column chromatography resulted in the successful isolation of two related phytosterol compounds from *R. communis* leaves. The highest antibacterial activity against tested microorganisms was observed in hexane extracts. About 53.54 g of hexane extracts was fractionated as described and illustrated in Scheme 1. Out of 53.54 g of *R. communis* hexane extract used, 40.253 g was collected using different eluent systems. The fractions were separated by TLC plates developed in BEA and sprayed with vanillin spraying reagent to determine their composition (Fig. 1). Fractions were developed in the BEA separation system because more compounds were observed when using BEA in our previous study [16]. Fractions were also assayed for antibacterial activity against *E. coli* using bioautography (Fig. 2). We also tested fractions against *P. aeruginosa* and *S. aureus* and both organisms were resistant to all the fractions (data not shown). Antibacterial compounds were revealed in 90% hexane in ethyl acetate to 100% methanol. Fractions were pooled together based on the phytochemical results and bioautogram making three different fractions: 90% hexane in ethyl acetate to 80% hexane in ethyl acetate, 70% hexane in ethyl acetate to 80% ethyl acetate in methanol and 70% ethyl acetate in methanol to 100% methanol. About 10 g of second fraction 70% hexane in ethyl acetate to 80% ethyl acetate was further fractionated using 90% dichloromethane in ethyl acetate. These fractions were active against *E. coli* and *E. faecalis*.

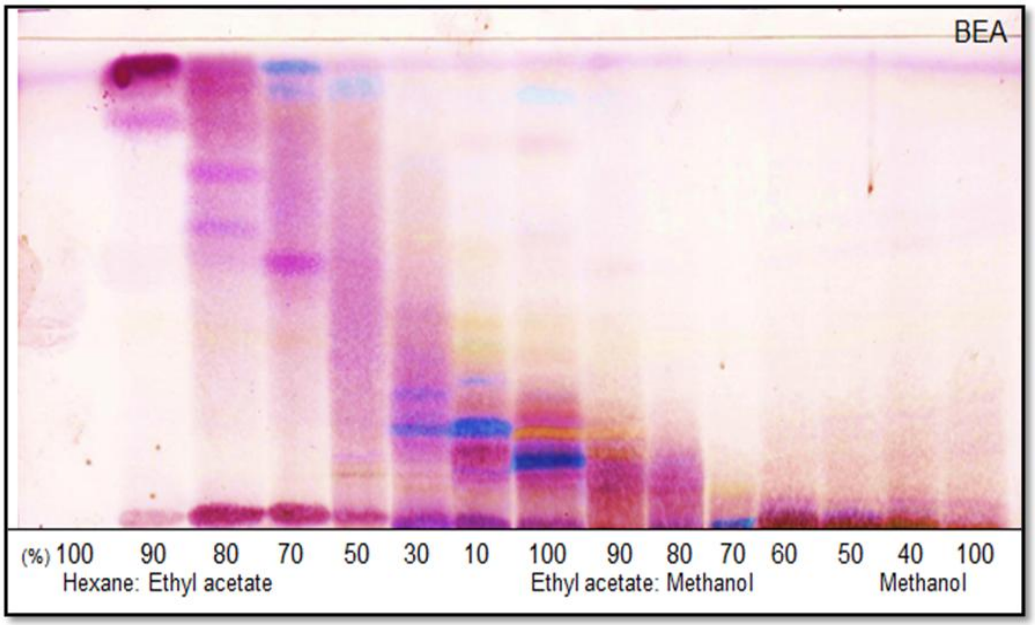


Fig. 1. Chromatogram of *Ricinus communis* hexane extracts fractionated by 100% hexane increasing polarity with ethyl acetate until 100% methanol. Chromatogram was developed in BEA (Benzene: ethanol: ammonium hydroxide) solvent system and sprayed with vanillin-sulphuric to show compounds isolated with different eluent systems

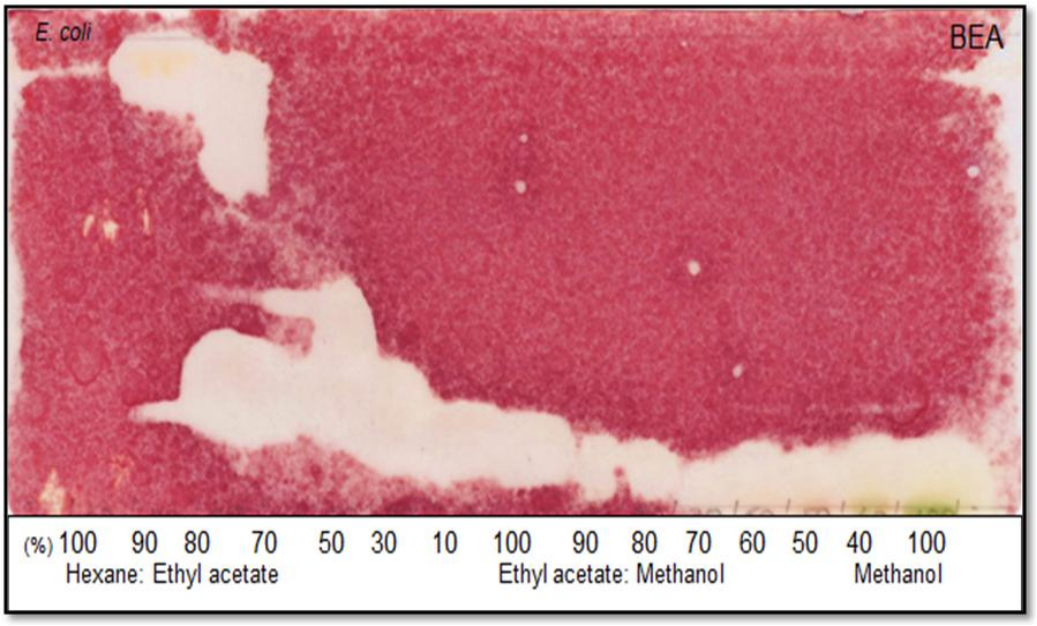
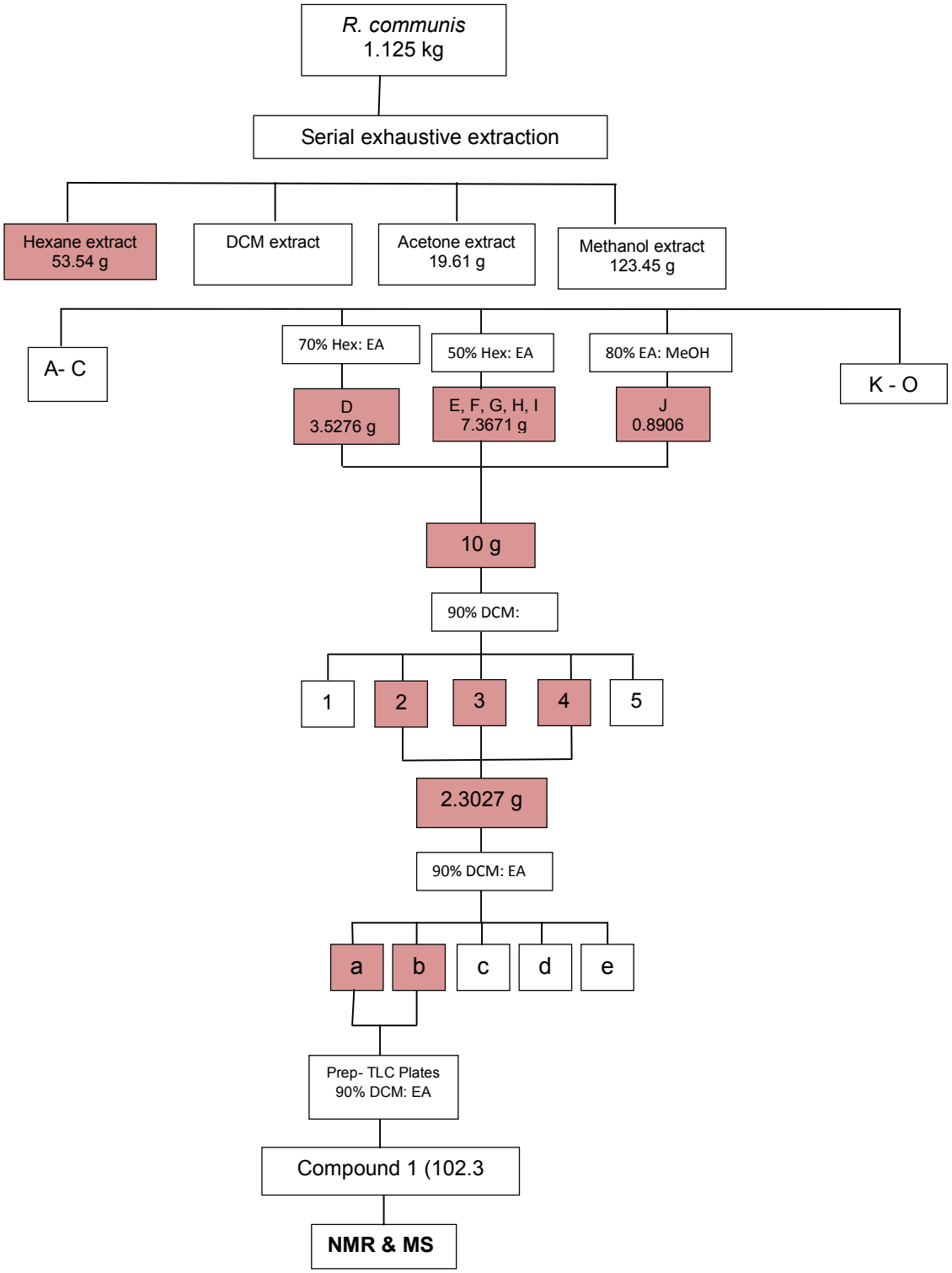


Fig. 2. Bioautogram of *Ricinus communis* hexane extracts fractionated by 100% hexane increasing polarity with ethyl acetate until 100% methanol. Chromatogram was eluted by BEA and sprayed with *Escherichia coli*. White areas indicate where reduction of p-iodonitrotetrazolium (INT) to the coloured formazan did not take place due to the presence of antibacterial compounds that inhibited the growth of *Escherichia coli*



Scheme 1. Overview of isolation process of compound 1

Table 1. Minimum inhibitory concentration (MIC) values of *R. communis* leaf extracts against four tested microorganisms after incubation at 37°C for 24 hours

Microorganisms	MIC values (mg/ml)												
	Hexane			Dichloromethane			Acetone			Methanol			Ampicillin
	I	II	III	I	II	III	I	II	III	I	II	III	
E. coli	0.31	0.47	0.31	1.25	0.94	0.94	0.31	0.31	0.47	0.94	1.56	3.75	0.08
P. aeruginosa	0.47	0.47	0.63	0.63	0.63	0.63	0.31	0.31	0.31	0.47	0.63	0.47	0.13
E. faecalis	0.63	0.63	0.63	1.25	0.63	1.25	0.31	0.47	0.63	1.25	1.88	1.88	0.16
S. aureus	0.31	0.31	0.31	0.94	0.94	1.25	0.47	0.47	0.31	1.25	1.25	2.50	0.16
Average	0.43	0.47	0.47	1.02	0.79	1.02	0.35	0.39	0.43	0.98	1.33	2.15	0.13
Total average	0.47			0.94			0.39			1.49			

The aim of this project was to isolate the antibacterial compound which was present in three fractions (Fig. 1). After the purification of the antibacterial compound, the active compound appeared pink on TLC plate after spraying with vanillin sulphuric acid reagent (Fig. 3). Consequently, the pink compound at R_f of 0.39 was targeted for isolation. Compound 1 was ultraviolet light active, fluorescing in wavelength 365 nm and quenching fluorescence (black colour) at a wavelength of 254 nm. Column chromatography followed by preparative TLC plates led to the successful isolation of compound 1 at the R_f value of 0.40, developed in 90% dichloromethane in ethyl acetate (Fig. 4). Compound 1 precipitated as a white powder after removing solvents (90% dichloromethane in ethyl acetate) under a stream of cold air. Compound 1 dissolved completely in both 100% chloroform and 90% dichloromethane in ethyl acetate. About 102.3 mg of compound 1 was obtained from 53.54 g *R. communis* hexane extract. Compound 1 was assayed for antibacterial activity and the activity was seen to be lost. After NMR analysis, compound 1 was revealed as the mixture of two related compounds. The structure of compound 1 was elucidated by using NMR data. Two types of peaks were observed from the DEPT–NMR data, labeled major (1a) and minor (1b) peaks and ^{1}H–NMR data of 1a and 1b were similar. DEPT–NMR data revealed that 1a and 1b possessed nine and eleven CH_2, eleven and nine CH, respectively. The ^{13}C–NMR data of compound 1 were similar to those recorded for stigmasterol (1a) and β-sitosterol (1b) [20] (Fig. 4). Spectroscopic data were analysed from Table 2.

Stigmasterol (Stigmasterin 1a) and β-sitosterol (Stigmast-5-en-3-ol 1b) are well known and have been previously isolated from *R. communis*. Stigmasterol was first isolated from ether extract of seeds *of R. communis* and reported for significant antifertility activity [21,22]. Stigmasterol and β-sitosterol have also been isolated from roots of *R. communis* [23,24], however in the present study it is the first time that the two compounds were isolated from the leaves of *R. communis*. Stigmasterol and β-sitosterols possess antitumor activity, cholesterol lowering activity, antibacterial activity and anti-inflammatory activity [25-27]. Stigmasterol and β-sitosterols were also isolated from the leaves of *Rubus suavissimus*, which has been used to make a beverage leaf tea in China [28]. β-sitosterol has been also isolated from the petroleum ether extract of *Abutilon indicum* and it revealed moderate larvicidal activity [27]. Gomes et al. [29] isolated the mixture of stigmasterol and β-sitosterols from the root extract of *Pluchea indica*, which has been used against snakebite. Saeidnia et al. [27] reported antibacterial activity of β-sitosterols against *E. coli, P. aeruginosa* and *S. aureus* using the disc diffusion method with the zone of inhibition ranging from 10 to 14 mm but there was no activity with MIC values above 200 µg/ml. From the literature, it is not clear that β-sitosterols have antimicrobial activity because of the contradiction of results between the studies conducted. Some studies did not report on the antimicrobial activity of this compound [27,30,31]. In the present study compound 1 (which is presented as mixture of 1a and 1b) lost its activity during the purification process. Our results agree with the studies reported that β-sitosterol has no antimicrobial activity against tested microorganisms. The lack of antibacterial activity of compound 1 may be the proof that some of compounds act synergistically to give a particular biological activity as when they are separated they lose activity.

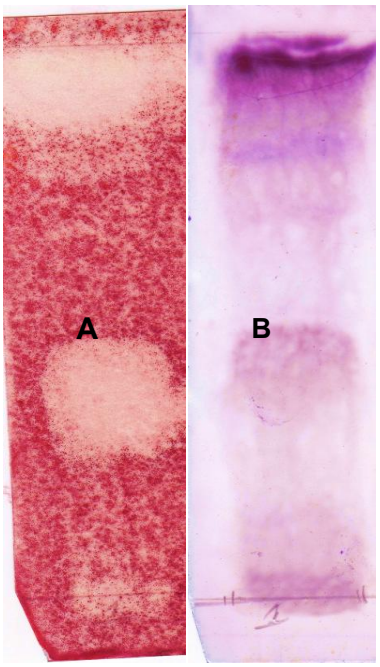
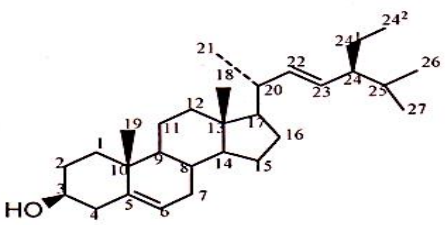


Fig. 3. Chromatograms were developed in 90% dichloromethane in ethyl acetate and sprayed with *Escherichia coli* (A) and vanillin sulphuric acid spraying reagent (B). The solvent (90% dichloromethane in ethyl acetate) was selected as the best elution system for isolation of targeted pink colour (antibacterial compound) at R_f value of 0.39 from *R. communis* hexane extracts

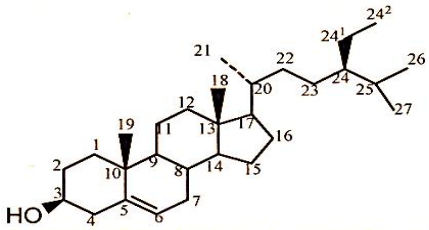
Table 2. ^{13}C-NMR data of isolated compound 1 (400 MHz, chloroform-d)

Position	Stigmasterol [20]	1a	β-Sitosterol [20]	1b
1	37.3	37.2	37.3	37.2
2	31.6	31.6	31.9	31.9
3	71.8	71.9	71.8	71.9
4	42.3	42.2	42.3	42.2
5	140.8	140.7	140.8	140.7
6	121.7	121.8	121.7	121.8
7	31.9	31.9	31.9	31.9
8	31.7	31.8	31.9	31.9
9	50.2	50.1	50.2	50.1
10	36.5	36.5	36.5	36.5
11	21.1	21.0	21.2	21.0
12	39.8	39.8	39.7	39.7
13	42.3	42.3	42.2	42.3
14	56.7	56.8	57.0	56.9
15	24.3	24.3	24.4	24.4
16	28.3	28.3	28.9	28.9
17	56.1	56.0	56.8	55.9
18	11.9	11.9	12.3	12.3
19	19.4	19.4	19.4	19.4
20	36.2	36.1	40.5	40.5
21	18.8	18.8	21.2	21.2
22	34.0	34.0	138.3	138.3
23	26.1	26.0	129.3	129.6
24	45.9	45.8	51.3	51.2
25	29.2	29.1	31.9	30.9
26	19.0	19.0	21.1	21.2
27	19.8	19.8	19.0	18.9
24^1	23.1	23.1	25.4	25.4
24^2	12.3	12.3	12.1	12.0

Fig. 4. Chromatogram of 5 µl of compound 1 developed in 90% dichloromethane in ethyl acetate and sprayed with vanillin sulphuric acid spraying reagent



(1a) Stigmasterol



(1b) β-Sitosterol

Fig. 5. Compound 1, a mixture of two related phytosterol compounds, which were stigmasterol (1a) and β-sitosterol (1b)

4. CONCLUSION

Antibacterial bioassay-guided fractionation led to successful isolation of compound 1 which was identified as a mixture of stigmasterol and β-sitosterols. This is the first time that these two compounds were isolated from leaves of *R. communis,* although the activity of compound **1** was lost during the purification process. It can be concluded that leaves of *R. communis* have similar compounds found in the roots. Therefore, to ensure that the plant species does not get extinct, traditional healers will be advised to use leaves instead of uprooting the plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Masoko P, Mdee LK, Mampuru LJ, Eloff JN. Biological activity of two related triterpenes isolated from *Combretum nelsonii* (Combretaceae) leaves. Nat Prod Res. 2008;22(12):1074-1084.
2. Mokgotho MP, Gololo SS, Masoko P, Mdee LK, Mbazima V, Shai LJ, Bagla VP, Eloff JN, Mampuru LJ. Isolation and chemical structural characterisation of a compound with antioxidant activity from the roots of *Senna italic*. Evidence-Based Compl Altern Med. 2013;1-6.
3. Shah HJ, Lele SS. Extraction of Diosgenin, a Bioactive Compound from Natural Source *Dioscorea alata* Var purpurae, Anal Bioanalyt Techn. 2012;3(4):1-3.
4. Kasali FM, Mahano AO, Nyakabwa DS, Kadima NJ, Misakabu FM, Tshibangu DST, Ngbolua KN, Mpiana PT. Ethnopharmacological Survey of Medicinal Plants Used against Malaria in Bukavu City (D. R. Congo), Eur J Med Plants. 2014; 4(1):29-44.
5. Talib WH, Mahasneh AM. Antiproliferative activity of plant extracts used against cancer in traditional medicine. Sci Pharm. 2010;78:33-45.
6. Karuppusamy S. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. J Med Plants Res. 2009; 3(13):1222-1239.
7. Patel VR, Patel PP, Kujal SS. Antioxidant activity of some selected medicinal plants in western Region of India. Adv Biol Res. 2010;4(1):23-26.
8. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha YL. Extraction, isolation and characterization of bioactive compounds from plant's extracts. Afri J Trad Compl Altern Med. 2011;8(1):10.
9. Patra JK, Gouda S, Sahoo SK, Thatoi HN. Chromatography separation, ^{1}H NMR analysis and bioautography screening of methanol extract of *Excoecaria agallocha L*. from Bhitarkanika, Orissa, India. Asian Pac J Trop Biomed. 2010;5-56.
10. Singh RK, Gupta MK, Singh AK, Kumar S. Pharmacognostical investigation of *Ricinus communis* stem. Int J Pharma Sci Res. 2010;1(6):89-94.
11. Jeyaseelan EC, Jashothan PTJ. *In vitro* control of *Staphylococcus aureus* (NCTC 6571) and *Esherichia coli* (ATCC 25922) by *Ricinus communis L*. Asian Pac J Trop Biomed. 2012;2(10):717–721.
12. Taur DJ, Waghmare MG, Bandal RS, Patil R. Antinociceptive activity of *Ricinus communis L*. leaves, Asian Pac J Trop Biomed. 2011;139-141.
13. Rekha D, Tamil-Selvi S, Bharathidasan R, Panneerselvam A, Ilakkiya R, Jayapal R. Study of medicinal plants used from koothanoallur and marakkadai, Thiruvarur district of Tamil nadu, India, Hygeia J Drugs Med. 2013;5(1):164-170.
14. Luseba D, Elgorashi EE, Ntloedibe DT, van Staden J. Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained

placenta in livestock. S Afri J Bot. 2007; 73:378–383.
15. Rana M, Dhamija H, Prashar B, Sharma S. *Ricinus communis L.* – A Review, Int J Pharm Tech Res. 2012;4(4):1706–1711.
16. Nemudzivhadi V. Isolation and characterization of antibacterial and antioxidant compounds from *Ricinus communis* leaves. Master's dissertation. University of Limpopo (Turfloop campus). 2014;75–78.
17. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica.1998;64:711-713.
18. Kotze M, Eloff JN. Extraction of antibacterial compounds from *Combretum* spp (Combretaceae). S Afri J Bot. 2002; 68:62–67.
19. Begue, WJ, Kline RM. The use of tetrazolium salts in bioautographic procedures. J Chromatogr. 1972;64: 182-184.
20. Kpoviessi DSS, Gdaguidi FA, Gbenou JD, Accrombessi GC, Haddad M, Moudachirou M, Quetin-Leclercq J. Allelopathic effects on cowpea (*Vigna unguiculata* (L) Walp) plant and cytotoxic activities of sterols and triterpene isolated from *Justicia anselliana* (NEES) T. Anders, Electr J Nat Subst. 2006;1:12–19.
21. Zhang X, Han F, Gao P, Yu D, Liu S, Bioassay-guided fractionation of antifertility components of castorbean (*Ricinus communis L.*) seed extracts. Nat Prod Res. 21(11):982–989.
22. Jena J, Gupta AK, *Ricinus communis* Linn: A phytopharmacological review. Int J Pharm Pharmaceut Sci. 2012;4(4):25–29.
23. Ali M, Mitta A. Fatty acid glycosides from the roots of *Ricinus Communis L.* American J Pharm Tech Res. 2012;2(6): 637–650.
24. Bayaso I, Nahunnaro H, Gwary DM. Effects of aqueous extract of *Ricinus communis* on radial growth of *Alternaria solani*. Afri J Agricul. 2013;8(37): 4541-4545.
25. Ghosh T, Maity TK, Singh J. Evaluation of antitumor activity of stigmasterol, a constituent isolated from *Bacopa monnieri* Linn aerial parts against Ehrlich Ascites Carcinoma in mice. Orient Pharm Exp Med. 2011;11:41-49.
26. Venkata-Raman B, Samuel LA, Pardha-Saradhi M, Narashimha-Rao B, Naga-Vamsi KA, Sudhakar M, Radhakrishnan TM. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum.* Asian J Pharm Clin Res. 2012;5(2):99-106.
27. Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The Story of Beta-sitosterol-A Review, Eur J Med Plants. 2014;4(5): 590–609.
28. Chaturvedula VS, Prakash I. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus*. Int Curr Pharm J. 2012;1(9): 239-242.
29. Gomes A, Saha A, Chatterjee A, Chakravart AK. Viper and cobra venom neutralization by beta-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less (Asteraceae), Phytomed. 2007;14:637-643.
30. Gohari AR, Saeidnia S, Shahverdi AR, Yassa N, Malmir M, Mollazade K, Naghinejad AR. Phytochemistry and antimicrobial compounds of *Hymenocrater calycinus*. Eur Asian J Bio Sci. 2009;3: 64- 68.
31. Mishra BB, Singh DD, Kishore N, Tiwari VK, Tripathi V. Antifungal constituents isolated from the seeds of *Aegle marmelos*. Phytochem, 2010;71:230-234.

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