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Original article

Bacteriostatic and Haemolytic Activities of Extracts and Compounds of *Commiphora swynnertonii*

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Abstract

Background and objective: *Commiphora swynnertonii* (Burtt) is used in traditional medicine to treat infectious diseases. Previous studies have reported antimicrobial activity of this plant; however, the activity of compounds that are present in extracts of this plant has not been thoroughly documented. Likewise, the primary mode of action (bactericidal or bacteriostatic) and the possible toxicity on red blood cells have not been reported. **Methods:** Extracts of leaves, whole root, root bark, root wood, whole stem, stem bark and stem wood, were produced using hexane, dichloromethane, methanol and water. Cold and hot extraction methods were employed. Antibacterial activity of extracts was tested against selected medically important Gram-positive and Gram-negative bacteria by growth inhibition, minimum inhibitory concentrations and time kill assays. Moreover, haemolytic activity against sheep red blood cells was determined in vitro. **Results:** The hexane extracts of whole root and root bark, methanol extracts of root wood, and dichloromethane extracts from the leaves of *C. swynnertonii* inhibited the growth of *S. aureus*. MIC values for the extracts and compounds, indicated moderate activity against Gram-positive bacteria (*Staphylococcus aureus, Streptococci* species and *Enterococci* species) while the activity against Gram-negative bacteria (*Escherichia coli, Klebsiella* species, *Pseudomonas aeruginosa, Salmonella* species, *Shigella sonnei* and *Yersinia enterocolitica*) was weak. Time kill profiles showed the extracts have bacteriostatic activity against *S. aureus*, and low haemolytic effect, except for extracts of whole root and leaves at the concentration of 1000 µg/mL. **Conclusion:** Extracts of *C. swynnertonii* showed bacteriostatic activity against Gram-positive bacteria with low toxicity on red blood cells.

Keywords: antibacterial; *Commiphora swynnertonii* ; medicinal plant; red blood cells

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Introduction

Ethnopharmacological use of medicinal plants have been practiced since pre historical time for treatment of diseases [1]. As such, plant medicines contain substances that could be used in modern medicine directly for therapeutic purposes, or as precursors for the synthesis of useful drugs [2]. *Commiphora swynnertonii* (Burtt) is a medicinal plant used in traditional medicine to prevent and control health disorders, including infectious diseases [3].

Commiphora swynnertonii is identified as "oltemwai" in Dorobo (sub-ethnic group of Masaai tribe) of Tanzania. It belongs to the family Burseraceae. The ethnobotanical practice of the plant by Dorobo people shows that it is used as a first-aid remedy for treating infections, inflammations and cancer [4]. The crushed leaves are used as an insect repellent and the root decoction is used to treat swollen legs and the bark decoctions are used specifically for the treatment of general health issues. The dilution of exudates in water or milk is used to treat diarrhoea, cough, sexual transmitted infections and wounds. Previous research has shown that *C. swynnertonii* has antibacterial activity against both Gram-positive and Gram-negative bacteria [5,6]. Moreover, earlier findings have reported that *C. swynnertonii* possesses secondary metabolites, such as terpenoids, steroids, flavonoids, phenols, saponins and tannins, and these metabolites have been suggested to be the reason for antimicrobial activity [7–9].

Secondary metabolites are present in medicinal plants and have the potential to produce harmful effects such as cytotoxicity, allergic reactions, irritation of the gastrointestinal tract, and injury to vital body organs such as the heart, liver, kidney, and also carcinogenicity [10]. As the interest in plant-based medicine increases, it is essential to ensure that active ingredients from plants be non-toxic, and haemolytic activity represents as one of the first most commonly employed toxicity assessments [11]. Also, because red blood cells come into direct contact with any substance that is absorbed or injected intravenously, they are the main targets of toxicological actions [12].

Apart from the previous studies that reported the phytochemical and pharmacological actions of *C. swynnertonii*, the activity of compounds isolated from the plant have never been thoroughly characterized, and the primary mode of action (bacteriocidal or bacteriostatic) and the possible toxicity of extracts as well as compounds have not been determined. Thus, the aim of the current study was to characterize the antibacterial activity, primary mode of action, and putative toxicity of extracts and compounds of *C. swynnertonii*.

Material and Methods

Ethical considerations

This study was approved by ethical committee of National Institute of Medical Research, Dar es salaam, Tanzania (Approval number: NIMR/HQ/R.8a/Vol.IX/3066).

Chemicals

Hexane, dichloromethane (DCM) and methanol (MeOH), Mueller Hinton agar (MHA) and broth (MHB) were purchased from Sigma Aldrich, USA). Dimethyl-sulphoxide (DMSO), tetracycline and gentamicin were purchased from Sigma Aldrich, Germany.

Bacteria strains and growth media

Bacteria strains (n=95) were used in the current study including Gram-positive (*Staphyllococcus aureus*, *Streptococci* species and *Enterococci* species) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella* species, *Shigella* species, *Yersinia enterocolitica*). Reference strains and isolates bacteria were included in this study; details on these strains have been given in supplementary Table S1 and S2. Briefly, the strains were obtained from the Department of Veterinary and Animal Science, University of Copenhagen, Denmark; Department of Microbiology, Sokoine University of Agriculture, Tanzania; and Muhimbili University of Health and Allied Sciences, Tanzania.

Plant material

Commiphora swynnertonii was identified and confirmed by a botanist Mr. Frank Mbagho from the University of Dar es salaam. The plant was harvested in 2018 from Simanjiro district, Manyara Region in the Northern Highlands of Tanzania (S03^0 34' 49.9", E 037' 00' 05"). The voucher specimen number FMM 3897 was deposited at the Herbarium of Botany

Department, College of Natural and Applied Sciences, University of Dar es Salaam, Tanzania. During harvesting of leaves, whole root, root bark, root wood, stem, stem bark and stem wood were cut into small size and dried at 20 °C at Tanzania Tree Seed Agency Laboratory, Morogoro, Tanzania. The dried samples were milled in particle size of 2mm using milling machine (Christy Hunt Engineering Ltd, England). The cold (total and sequential) and hot methods of extraction with slight modifications from Vidyapeethet et al., 2017 and Azwanida, 2015 were done using hexane, dichloromethane and methanol [13,14]. With hot method of extraction, powdered materials were boiled in a Soxhlet extractor to obtain liquid extracts. For the decoction method, powdered samples were boiled in a charcoal burner for 30 minutes and then allowed to cool down, then filtered. For cold-total extraction, powdered materials were soaked in the solvent for 48 hours then filtered and for cold-sequential method, residue from dichhloromethane were resoaked with methanol for 48 hours and filtered. The filtration process was done using Whatman^{TM}qualitative filter paper1, 24.0cm (GE Healthcare Life Sciences, China). The filtrates from organic solvents were concentrated in a rotary evaporator (BüchiLabortechnik, Switzerland) at 40-55°C to obtain the dry extracts. Moreover, aqueous extracts were frozen at -20 and dried in a freeze dryer. The dried extracts were stored at -20°C. The extracts from different parts of the plant and with different extraction solvents are summarized in supplementary Table S3. The extract of DCM leaves from Soxlet extraction was used to isolate compounds **(1-3)**. Hexacosane **(1)** $C_{26}H_{54}$(m/z366.7), ethyl octadecenoate **(2)** $C_{20}H_{40}O_2$(m/z312.5) and octacosanol **(3)** $C_{28}H_{58}O$ (m/z410.8) were directly obtained from fractions eluted with 25%, 50% and 75% DCM/PE respectively. The extract of DCM stem bark from Soxlet extraction was used to isolate compounds **(4** and **5).** Hexacosanoic acid **(4**) $C_{17}H_{34}O_2$ (*m/z* 270.5) and ethyl tetradecanoate **(5)** $C_{26}H_{52}O_2$ (*m/z* 396.7) were directly obtained from fractions eluted with 50% DCM/PE and 10% Me/DCM, respectively. The extract of DCM whole root from Soxlet extraction was used to isolate compounds **(6-8).** Ethyl tetradecanoate **(6)** $C_{16}H_{32}O_2$(m/z256.4), methyl pentadecanoate **(7)**$C_{16}H_{32}O_2$ (m/z256.4) and ethyl hexadecanoate **(8)**$C_{18}H_{36}O_2$ (m/z284.5) were directly obtained from fractions eluted with 25% and 50% DCM/PE and 100% DCM, respectively. More details of the isolation and characterization process of compounds (**1-8**) were reported previously [7].

Growth inhibition assay

The growth inhibition assay of all extracts was performed in honey comb multi well plate using the Bioscreen C machine (Thermo Fischer Scientific, Finland). The plates were incubated for 24 hours with continuous shaking at 37 °C and OD_{600} readings at intervals of 20 minutes. Extracts were dissolved in 5% dimethyl sulphoxide to make concentrations of 1500 µg/mL and 150 µg/mL in MHB. The inoculums were prepared by growing the bacteria in MHB overnight. The inoculums were diluted by adding MHB to the desired optical density (OD_{600nm}) equal to 0.10. The experiment was performed in three independent determinants and negative controls (wells with bacteria and solvent used for dissolving the extracts and wells with broth and bacteria alone) were included in the plates. Positive controls, consisting of wells with bacteria with tetracycline and gentamicin at 4 µg/mL were also included. The activity of extracts was determined by generating growth curves of $\log_{10}$ optical density against time, and data were visualized using GraphpadPrism8 software (San Diego, USA).

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of extracts and compounds against strains of bacteria were determined by two folds micro-dilution technique. The active extracts identified in growth inhibition assay were used to determine the MIC against Gram-positive (*S. aureus*, *Streptococci s*pecies and *Enterococci* species) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *K. pneumonia*, *Salmonella* species, *Shigella* species, *Y. enterocolitica*) (supplementary Table S1). Additionally, MIC of compounds was determined against *S. aureus* ATCC25923, methicillin-resistant *S. aureus* CC398, methicillin-resistant *S. aureus* JE2 and *E. coli* ATCC25922. This study was conducted according to Wiegand et al., 2008 with slight modifications [15]. Primarily, about 50 µL of MHB was placed into 96 well plates. To each well of the first row, 50 µL of the extract solution or gentamicin or positive control was added.

Serial dilution was done for each test sample and positive controls to make a range of concentration starting with 3000 µg/mL to 24 µg/mL and 16 µg/mL to 0.125 µg/mL for extracts and antibiotics, respectively. Solvent control was used in a ratio of 1:5. Fifty µL of the test organism in a concentration of approximately 5 x 10^6 CFU/mL was added to each well. Sterility control contained blank culture medium and the growth control with bacteria and broth only were included in the plate. The microtitre plates were incubated at 37 °C for 16-18 hours. Wells with the lowest concentration with no observed bacterial turbidity were considered as MIC. The analyses were repeated three times. Activities were categorized as strong, moderate and weak [16].

Time kill assay

The time-kill kinetics of the active extracts identified from growth inhibition assay was determined by culturing bacteria in MHB with extracts concentrations in a doubling dilutions ranging (12000-375 µg/mL) as previously described by NCCL1999 with some modifications [17].One mL of MHB was added to test tube 1-9. Then 1 mL of plant extracts was added to test tube 1 followed by serial two-fold dilutions up to the 5^{th} tube leaving a volume of 1 mL in each test tube. To the 6^{th} test tube 1 mL of gentamicin at 8 µg/mL was added. The remaining tubes were set as controls of MHB with no bacteria, with bacteria and with DMSO solvent, respectively. One mL of MHB was added to tubes 1–5 to make a total volume of 2 mL. One hundred µL of a bacteria suspension of approximately 7.5x10^6 CFU/mL was added to all tubes except tube 7 to make an initial inoculum of approximately 0.5x10^5 CFU/ mL. Aliquots of 0.1 mL of the medium were taken at time intervals of 0, 2, 4, 6, 8, 10, 12 and 24 hours and inoculated into MHA petri dishes. The agar petri dishes containing inoculum were incubated at 37 °C for 24 h. The colony forming unit at each time interval were determined and graphs of the $\log_{10}$ CFU/ mL were plotted against time using GraphpadPrism8 software. The experiment was done in three independent experiments.

Haemolytic activity

The haemolysis activity of the active extracts identified in growth inhibition was performed according to Souza-Melo et al., 2021 in three independent experiments [18].The separation of RBCs from sheep blood was done by centrifugation at 3500 rpm for 5 minutes followed by three times wash with phosphate buffer pH=7.4. Two mL of 2% of RBCs suspension prepared in phosphate buffer was mixed with extracts at the concentrations of 100, 200, 500, 1000 µg/ mL to reach a final volume of 4 mL. The solvent (DMSO) used to dissolve the extracts was included as negative control and 3% of hydrogen peroxide (H_2O_2) used to induce total haemolysis on RBCs as positive control. The mixtures were incubated for 30 min at a temperature of 25±2. Afterwards, centrifugation of the mixture was done at 3500 rpm for 10 minutes; the supernatant was measured spectrophotometrically at the absorbance wavelength of 540 nm. Determination of percentage haemolysis was calculated by the subtraction of the negative control absorbance from the extracts absorbance relative to the positive control absorbance, and then multiplied by 100. The interpretation of low haemolytic activity corresponds to percentage haemolysis between 0-40% as compared to positive control [18].

Statistical analysis

The MIC data were expressed as the median and interquartile range (IQR). In cases where there were growths in all dilutions, the MIC value was set to 3000 µg/mL for statistical purposes. Shapiro-Wilk test was used to test the normality of the data. Comparison of the groups was performed using a Mann-Whitney U test. Comparison of more than two groups was performed by the Kruskal-Wallis test. The IBM Statistical Package for Social Sciences (SPSS) programme, version 20 was used. A two-tailed p-value of < 0.05 level was considered statistically significant.

Results and Discussion

The *C. swynnertonii* extracts were screened for their ability to inhibit the growth of *S. aureus* and *E. coli*. Cold-total hexane extracts of whole root and root bark, cold-total methanol extracts from root wood and hot-total dichloromethane extracts from the leaves at the concentration of 150 µg/mL interfered the growth of *S. aureus* (Figure 1A) while, *E. coli* was less affected even at the concentration of 1500 µg/mL (Figure 1B). Both of the antibiotic controls tetracycline and

gentamicin inhibited the growth of *S. aureus* and *E. coli*. Additional graphs for all the tested extracts can be found in supplementary Figure S1. The extracts which displayed growth inhibition were chosen to determine their MIC against collections of Gram-positive (*S. aureus, Streptococci* species and *Enterococci* species) and Gram-negative bacteria (*E. coli, P. aeruginosa, K. pneumonia, Salmonella* species, *Shigella* species, *Y. enterocolitica*). The median MIC of extracts for Gram-positive bacteria [1130 µg/mL (IQR 375-3000 µg/mL, n = 152)] was significantly lower than the MIC for Gram-negative bacteria [3000 µg/mL (IQR 3000->3000 µg/mL), n = 228)], p<0.0001. Extracts of whole root and root bark [MIC = 1500 µg/mL (IQR 750->3000 µg/mL for both extracts)] exhibited lower antibacterial activity than root wood and leaves [MIC = 750 µg/mL (IQR 375->3000 mg/mL for both groups)]P<0.001.

According to the definitions by Sartoratto et al., 2004 [16], the extracts from the whole root, root bark, root wood and leaves exhibited moderate activity against *S. aureus* and *Enterococci* spp., while they showed weak activity against *Streptococci* spp. All extracts from *C. swynnertonii* demonstrated weak activity against Gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa, Salmonella* spp., *Shigella* spp. and *Y. enterocolitica* with median MIC of >3000 µg/mL, and all extracts showed lower activity than gentamicin (p<0.0001) (Table 1).

A number of compounds were isolated from leaves and root bark, and the MIC of these compounds against selected strains of *S. aureus* and *E. coli* were determined (Table 2). None of the compounds exhibited activity corresponding to the crude extract, suggesting that these compounds were not the reason for the antibacterial activity of the crude extracts.

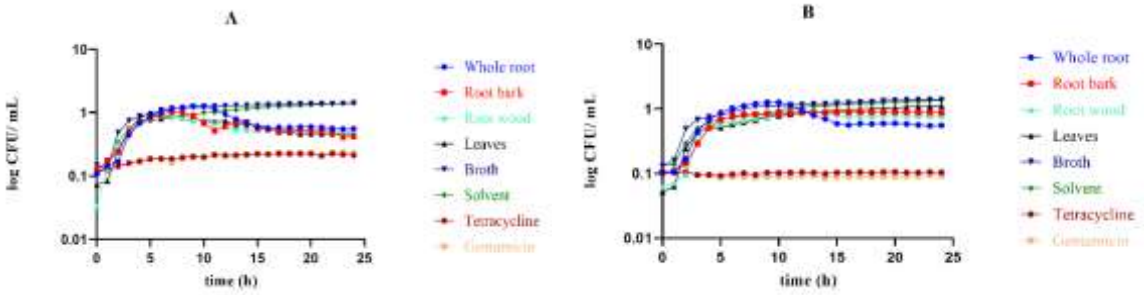

Figure 1. The growth curves of the selected extracts of *Commiphora swynnertonii* at the concentration of 150 µg/mL against *S. aureus* ATCC29213 (A) and 1500 µg/mL in MHB against *E. coli* ATCC25922 (B); 5% DMSO was used as solvent control, and tetracycline and gentamicin were used as antibiotic controls at the concentrations of 4 µg/mL Data are presented from three independent experiments as mean ± SD

Table 1. MIC (µg/mL) of *Commiphora swynnertonii* extracts against selected bacterial species

Bacteria strains	Median MIC (IQR) extracts and gentamicin (µg/mL)				
	Whole root	Root bark	Root wood	Leaves	Gentamicin
S. aureus,n=21	560 (375-3000)	750 (375-3000)	750 (375-3000)	750 (375-3000)	2(2-4)**
Enterococci spp, n=10	1120 (375-3000)	750 (375-3000)	750 (375-1310)	750 (375-3000)	8(4-8)**
Streptococci spp, n=7	3000 (375-3000)	3000 (750-3000)	3000 (375-3000)	3000 (375-3000)	8(4-16)**
E. coli, n=15	>3000	>3000	>3000	>3000	4(4-4)**
K. pneumoniae, n=13	>3000	>3000	>3000	>3000	8(4-12)**
P. aeruginosa,n=11	>3000	>3000	>3000	>3000	4(2-4)**
Salmonella spp.,n=12	>3000	>3000	>3000	>3000	4(4-14)**
Shigella spp., n=4	>3000	>3000	>3000	>3000	6(3-8)**
Y. enterocolitica,n=2	>3000	>3000	>3000	>3000	4(4-14)**

MIC: minimum inhibitory concentrations; IQR: interquartile range; **: significant levels at p<0.0001 when compared with whole root, root bark, root wood or leaves; *S. aureus: Staphyllococcus aureus; Streptococci* spp: *Streptococci* species; *Enterococci* spp*: Enterococci* species; *E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa*; *K. pneumoniae: Klebsiella pneumoniae*; *Salmonella* spp*: Salmonella* species; *Shigella* spp: *Shigella* species; *Y. enterocolitica: Yersinia enterocolitica*.

Time kill curves of the crude extracts of *C. swynnertonii* was determined against *S. aureus*. The extracts of root wood showed a significant growth inhibition (P <0.05) of number of viable cells of *S. aureus* when treated at the concentration of 6000 µg/mL and above [4.82±0.33 CFU/ml (95% CI, 4.54-5.10)] compared to controls group [broth 7.13±1.53CFU/mL (95% CI, 5.85-8.42)] after 24 hours. The number of bacteria left after treatment with gentamicin [1.05±2.00 CFU/mL (95% CI, 0.62-2.72)] was significantly lower than in tubes treated with plant extracts (P <0.05) (Figures 2 A-D). The extracts from whole root, root bark and root wood of *C. swynnertonii* showed no significant reduction of the number of viable cells at the tested concentrations.

The extracts from whole root, leaves, root bark and root wood showed haemolytic activity which was significantly higher than the negative control (5% DMSO) with p<0.05 which indicated that there was no observed toxic effect from negative control. More details of the comparison between extracts and negative control are found in supplementary Figure S2. Extracts from the root bark and leaves at the concentration of 1000 µg/mL showed haemeolysis of >40% compared to the positive control (Figure 3). Generally, the trend of haemolytic activity was concentration dependent.

Table 2. MIC (µg/mL) of compounds from *Commiphora swynnertonii* against *Staphyllococcus aureus* strains and *Escherichia coli*

	***S. aureus* ATCC25923**	**Methicillin-resistant *S. aureus* CC398**	**Methicillin-resistant *S. aureus* JE2**	***E. coli* ATCC 25922**
Leaves	188	750	375	>3000
Hexacosane**(1)**	>3000	>3000	>3000	>3000
Ethyl octadecanoate**(2)**	>3000	>3000	>3000	>3000
Octacosanol**(3)**	>3000	>3000	>3000	>3000
Stem bark	>3000	>3000	>3000	>3000
Ethyl pentadecanoate**(4)**	>3000	>3000	>3000	>3000
Hexacosanoic acid **(5)**	>3000	>3000	>3000	>3000
Whole root	188	375	375	>3000
Ethyl tetradecanoate**(6)**	>3000	>3000	>3000	>3000
Methyl pentadecanoate**(7)**	>3000	>3000	>3000	>3000
Ethyl hexadecanoate**(8)**	>3000	>3000	>3000	>3000
Gentamicin	4	4	4	2

MIC: Minimum Inhibitory concentration; Compounds hexacosane **(1)**, ethyl octadecenoate **(2)**) and octacosanol **(3)** were isolated from leaves; hexacosanoic acid **(4**) and ethyl tetradecanoate **(5)** were isolated from stem bark; **e**thyl tetradecanoate **(6)**, methyl pentadecanoate **(7)** and ethyl hexadecanoate **(8)** were isolated from whole root.

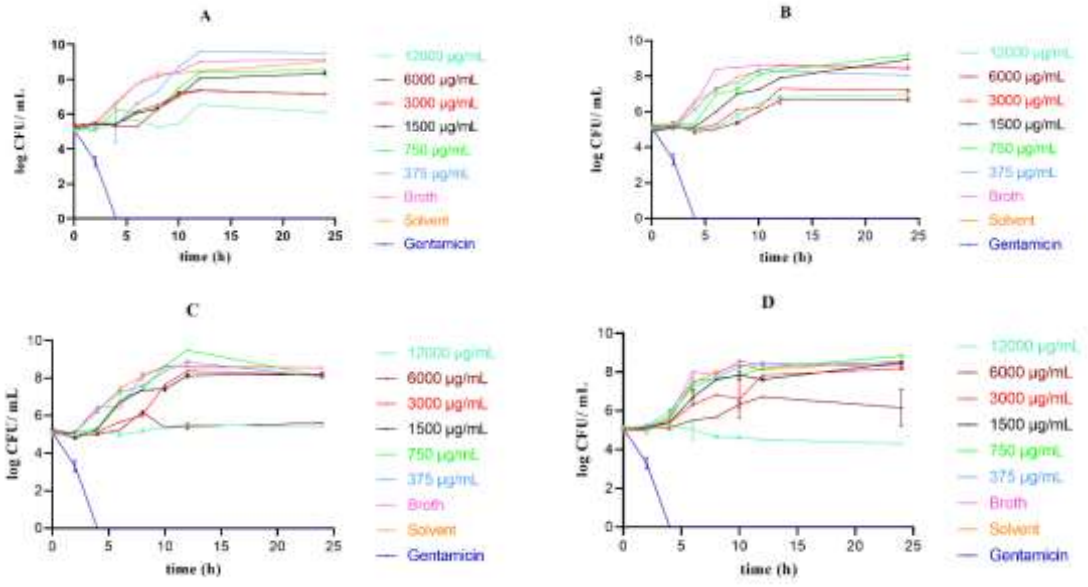

Figure 2. Time kill curves of *Staphyllococcus aureus* ATCC25923 treated with increasing concentrations of extracts from (A) whole root, (B) root bark, (C) root wood, (D) leaves; bacteria treated with gentamicin (4 µg/mL) and vehicle (5% DMSO) were included as controls. Data are presented as mean and standard deviations of the three independent experiments

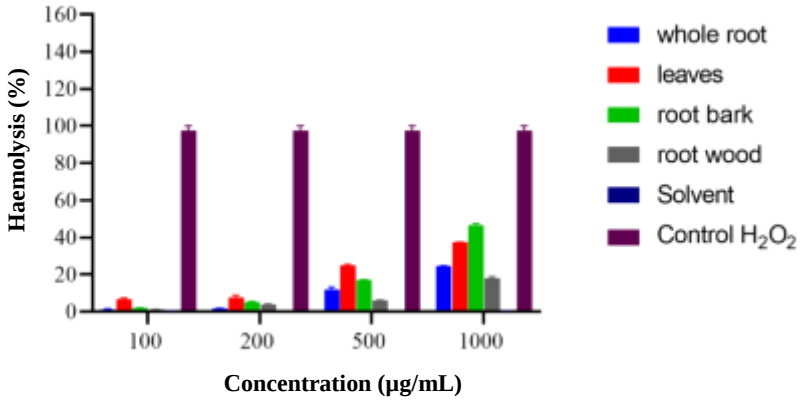

Figure 3. Haemolysis percentage of sheep RBCs treated with extracts of whole root, leaves, root wood and root bark of *Commiphora swynnertonii* as compared to the positive control; Error bars represent standard deviation from the mean. Data are presented as mean and standard error from three independent experiments.

The initial screening for the growth inhibition of *C. swynnertoni* extracts showed that the whole root, leaves, root wood and root bark extracts that inhibited the growth of *S. aureus* than *E. coli*. The weak activity of the extracts, could either be due to the absence of biologically active antibacterial compounds or due to the presence of growth factors in the extract that stimulated the growth of bacteria, negating the effect of possible growth inhibitory substances that might have been present [19].

The study investigated antimicrobial activity of crude extracts and compounds isolated from extracts of *C. swynnertonii* against medically important bacteria. Moderate antibacterial activity was observed for hexane extracts of whole root and root bark, methanol extracts of root wood, and dichloromethane extracts from the leaves against *S. aureus* and other Gram-positive bacteria. In contrast, no activity was observed for the tested compounds, and no activity was observed for extracts and compounds against the *E. coli* and other Gram-negative strains. The order of susceptibility among Gram-positive bacteria was *S. aureus*>*Enterococci* spp.>*Streptococci* spp. Antimicrobial activity in extracts of *C. swynnertonii* from exudates, leaves and root bark have previously been reported Bakari et al., 2012; however, based on different bioassays, different strains, other parts of plant and different methods of extraction [3]. It is therefore, possible that the current study has tested another set of active substances. The low activity against Gram-negative bacteria could probably be due to the specialized cell wall structure that make it impermeable to many antibiotics [20,21]. Similar to our study, Paraskeva et al. (2008), reported poor activity of South African *Commiphora* species against Gram-negative bacteria including *P. aeruginosa* [22].

The tested compounds (long chain of fatty acids, alcohols and esters) from *C. swynnertonii* had high MIC values against *S. aureus* strains and *E. coli* that showed weak antibacterial activity. These findings are comparable with the previous report on weak antimicrobial activity of long chain fatty acids and their derivatives such as hexacosane, hexacosanoic acid, methyl tetradecanoate, methyl hecadecanoate, octadecanol against *S. aureus* and *E. coli* [23–25]. Another study that worked on growth inhibition activity of fatty acid and their esters against oral microorganisms, including Gram-positive *Streptococcus mutans*, also described weak antimicrobial activity [26]. These findings indicated that, the obtained pure compounds from this plant need an enrichment or some modifications on their structures in order to potentiate them. But also, it indicated that, the activity shown by extracts from leaves and whole root might be due to synergistic effect contributed by the compounds present.

In time kill assays, *S. aureus* experienced the stagnant growth in the first 8 hours of incubation with extracts, which indicated a considerable bacteriostatic activity, which appear to be concentration dependent. Nevertheless, the

bacteriostatic or bactericidal action of this plant is not sufficiently researched. On the other hand the *Commiphora marlothii* stem extracts have displayed bactericidal efficacy against *S. aureus* [22]. This difference could be attributed by differences in plant morphology, geographical location and species, as well as differences in the extraction protocols, leading to different active substances.

Test for haemolytic activity showed that extracts of whole root and root wood had low haemolytic activity at the relevant concentrations for use as antibacterial. Extracts with haemolytic activity of <40 % of positive controls have weak haemolytic effect and can be considered as good lead molecules from this perspective [18]. The study noted that the use of root bark and leave extracts should be used sensibly especially at the concentration above 1000 µg/mL. This study might be presenting the haemolytic activity of *C. swynnertonii* for the first time. Apart from cytotoxicity in red blood cells, the cytotoxicity test in brine shrimp found that extracts from the leaves of *C. swynnertonii* were highly [3], and had the similar toxic effect on red blood cells as reported in this study.

Conclusion

This study has demonstrated that *C. Swynnertonii* only extracts had moderate to weak activity against Gram-positive bacteria, and no activity against Gram-negative ones. A number of compounds of active extracts were tested for activity, but none of them showed activity comparable with crude extracts, so the active molecules still remain to be unidentified. The time kill profiles for the crude extracts against *S. aureus* indicated that the effect was bacteriostatic and most of the active extracts showed low cytotoxicity at relevant concentrations. Therefore, further studies are recommended to test the combination of the compounds to explore their synergistic effects.

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Author contributions

Zaituni Msengwa contributed in plant collection, extraction, biological experiments, data analysis, wrote and review the manuscript; David Credo provided the pure compounds; Magesa Mafuru assisted in data analysis; James Mwesongo assisted in biological experiments; Faith Philemon Mabiki, Beda John Mwang'onde, Madundo Mkumbukwa Mtambo, Lughano Jeremy Kusiluka, Robinson Hammerthon Mdegela and John Elmerdahl Olsen provided professional advice, reviewed and edited the manuscript, provided supervision, and acquired funding; All authors made significant contributions to this article and participated in the conception and design of the experiments, reading, and approving the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

ATCC: American type culture collection; CFU: colony forming unit; DCM: dichloromethane; DMSO: dimethyl sulphoxide; MHA: Mueller-Hinton agar; MIC: minimum inhibitory concentration; NCCLS: the National Committee for Clinical Laboratory Standards; OD: optimum density; IQR: interquartile range; m/z: mass to charge ratio