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Activity of Sterols Isolated from the Leaves of *Commiphora swynnertonii* (Burtt) Against Some Nosocomial Infectious Bacteria

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ABSTRACT

Commiphora swynnertonii (Burtt) is traditionally used for medicinal purposes both in humans and animals in Tanzania. Previously, its leaves extract has been demonstrated antibacterial activity. However, there is limited information on identification of pure compounds responsible for its observed antibacterial activity. Therefore, this study was conducted to isolate and evaluate antibacterial activity of pure compounds isolated from its leaves extract. Colum chromatographic separation of its dichloromethane leaves extract afforded the isolation of two sterols namely, Cholest-7-en-3β-ol (1) and β-sitosterol (2). Their structures were deduced by using NMR data experimentally obtained and comparison with spectral data available in literatures. The sterols were evaluated for activity against four nosocomial infectious bacteria namely; *Staphylococcus* aureus, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* to determine Minimum Inhibitory Concentrations (MIC) by broth microdilution method. Cholest-7-en-3β-ol (1) demonstrated strong activity against *S. aureus (*MIC= 0.5 mg/ml) and weak activities (MIC >2mg/ml) against other bacteria. β-sitosterol (2) exhibited weak activities (MIC >2mg/ml) against all tested bacteria. Therefore, presence of antibacterial active compound(s) such as Cholest-7-en-3β-ol (1) among others in the leaves of *C. swynnertonii* can justify its earlier reported antibacterial activity and further confirms the claimed traditional uses of this plant in the treatment of bacterial infections.

Keywords: β-sitosterol, Burseraceae, Cholest-7-en-3β-ol, *Commiphora swynnertonii*, Nosocomial Infectious Bacteria, Sterols.

INTRODUCTION

Despite the presence of synthetic antibiotics for treatment of bacterial infections, the latter are still threatening human health globally [1-3]. Emergence of antibiotic resistance and related side effect issues are reported to be limiting the use of synthetic antibiotics currently present [3]. World Health Organization (WHO) reported the death of 9.2 million people worldwide in 2013 due to antibiotic resistance infections and this number is projected to be 10 million yearly by 2050 [1,4,5].

Occurrence of antibiotic resistance is reported to be the response towards indiscriminate and irrational use of synthetic antibiotic drugs frequently used for infectious diseases treatment [6,7]. As their defense mechanism, bacteria acquire antibiotic resistance by means of genetic mutation and Horizontal Gene Transfer (HGT) through mobile genetic elements such as chromosomes, plasmids and transposons [8].

Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* and Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* are some of the bacteria strains which are the major cause of nosocomial infections where antibiotic resistance is a high risk to human health [8]. Currently, there is no evidences for existence of effective antibiotic drugs against antibiotic resistance bacterial infections. Hence, prompting research for novel antibiotic drugs against resistant strains from medicinal plants due to their comparable safety, efficacy and possession of secondary metabolites such as alkaloids, coumarins, flavonoids, sterols, terpenoids, tannins and essential oils, among others [3,6,8].

In Tanzania, *Commiphora swynnertonii* Burtt. (Family Burseraceae) is an important medicinal plant which is used traditionally for treatment of various bacterial infections in humans such as skin infections, respiratory infections, sexual transmitted diseases and diarrhea [9,10]. The leaves and resin extracts from *C. swynnertonii* have previously demonstrated antibacterial activities [11,12].

Plant species in the genus *Commiphora* have been reported to possess various classes of secondary metabolites such as alkaloids, phenolics, flavonoids, terpenoids, sterols, lignans, cardiac glycosides,

saponins and long chain aliphatic derivatives, among others [9,11,12]. The classes of secondary metabolites implicated with antimicrobial activities in this genus are sterols/steroids, alkaloids, saponins, flavonoids, terpenoids, lignans and cardiac glycosides among others [11,12].

Regardless of the crude extract from the leaves of *C. swynnertonii* to demonstrate antibacterial activity, so far there is limited information on isolation of pure compound (s) responsible for this observed activity. The few existing information regarding compounds from *C. swynnertonii* is the isolation of five furan sesquiterpenoids, namely; 6-oxodendrolasin, (E)-6-oxoisodendrolasin, (Z)-6-oxoisodendrolasin, crassifolone and 7,8-dihydroisodendrolasin from its bark exudate [13]. all of which have not been reported to have antibacterial activities to date.

Hence, the aim of this study was to investigate antibacterial activity of the two isolated sterol compounds, namely; Cholest-7-en-3β-ol (1) and β-sitosterol (2) from the leaves of *C. swynnertonii*.

MATERIALS AND METHODS

General Chemicals and Materials used

Solvents used in this study include dichloromethane (DCM) and dimethylsulphoxide (DMSO) obtained from Loba Chemie, Mumbai-India, petroleum ether (PE) and methanol (MeOH) from or Finar Chemical, Gujarat-India, silica gel 60 (70-230 mesh) and precoated thin layer chromatography Aluminum sheets (TLC, silica gel 60 F_{254}) from Merck KGaA group, Darmstadt, Germany, standard antibiotic used as positive control was gentamicin from Sigma-Aldrich, Germany and Mueller Hinton agar and Mueller Hinton broth from HIMEDIA® (Himedia Laboratories Pvt Ltd, Mumbai-India).

Infectious Bacteria strains

Four representative nosocomial infectious bacteria used for this study are *Staphylococcus aureus* American Type Culture Collection (ATCC 29213), *Enterococcus faecalis* (ATCC 51559), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 278553) which were obtained from Microbiology laboratory in the Department of Biosciences- Sokoine University of Agriculture (SUA).

Identification, Collection and Preparation of Plant Materials

Plant samples were identified by a botanist from the Department of Botany, University of Dar es Salaam (UDSM). The leaves of *C. swynnertonii* were collected from Mirer ani village (03°36′ to 03°14.73′ S and 36°50′ to 36°18.05′ E) in Manjiro District- Manyara region, Tanzania in February 2020 and the voucher specimen no. 3673 was stored in the herbarium of the Department of Botany- UDSM. Plant leaves were air dried under shade at room temperature, then ground into fine powder (approximately 2 mm particle size) by using a milling machine type Y (Hangyu®, China).

Extraction of Plant Materials

About 1 kg powder of plant leaves were exhaustively extracted with 100% dichloromethane by using Soxhlet method described previously [14]. The filtrate collected was evaporated by rotary evaporator (Büchi Labor Technik, Flawil, Switzerland) at 40°C.

Isolation and Purification of Bioactive Compounds

About 35 g of 100% DCM leaves extract was chromatographed on silica gel by gradient elution using PE, DCM and MeOH. Solvent system for use in isolation process was initially established by TLC analysis of the crude extract. The column was eluted by solvent having 25% DCM in PE to obtain 9 fractions (*Fr. 1-9*), 50 ml each. Solvent polarity was increased to 50% of DCM in PE to give 11 fractions (*Fr. 10-20*). Thereafter, the solvent polarity was further increased to 75% of DCM in PE to yield 4 fractions (*Fr. 21-24*) followed by 100% DCM to give 11 fractions (*Fr. 25-35*). Solvent polarity was increased to 10% of MeOH in DCM to give 10 fractions (*Fr. 36-45*), finally increased to 20% of MeOH in DCM to afford 8 fractions (*Fr. 46-53).*

On TLC analysis, seven fraction combinations with similar chemical profiles were developed, i.e., *Fr. 1-9, Fr. 10-20, Fr. 21-24, Fr. 25-30, Fr. 31-35, Fr. 36-45* and *Fr. 46-53*. After settling at room temperature for 24 h, the fourth (*Fr. 25-30*) and fifth (*Fr. 31-35*) fraction combinations were observed to form precipitates. The precipitates were filtered by washing with 100% MeOH and dried to obtain pure compound 1 and 2 respectively which showed single spot on TLC. All fraction combinations which did not form precipitates were stored for later further purification.

Structure Determination

The structures of compound 1 and 2 were deduced by using experimental ^{1}H NMR and ^{13}C NMR data and by comparison with NMR data available in literatures. The NMR data were recorded on a 600 Megahertz Bruker Avance NMR spectrometer. Chemical shifts (δ) were expressed relative to the standard, tetramethyl silane (TMS) in parts per million (ppm). Obtained data were processed by Topspin software version 3.6.3.

Antibacterial Activity Evaluation

Activities of compound 1 and 2 were tested against four representative nosocomial infectious bacteria (*S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*). With some slight modifications, Minimum Inhibitory Concentrations (MIC) were determined by using two-fold microdilution method to evaluate antibacterial activity of compound 1 and 2 according to the previous described procedure [15,16]. In briefly, the bacteria were tested against each compound in duplicate by using a sterile 96-well polystyrene microtiter plate. Two rows of the 96-well microtiter plate were labeled for DMSO solvent (negative control), two for gentamicin (positive control), two rows for only broth and bacteria, two rows for compound 1 and other two rows for compound 2. Then, each well of the plate was loaded with 50 µL of Mueller Hinton broth followed by addition of 50 µL DMSO solvent in the negative control rows, 50 µL of 0.1 mg/ml of gentamicin in the positive control rows, 50 µL of 2 mg/ml of compound 1 in the rows for compound 1 and 50 µL of 2 mg/ml of compound 2 in the rows for compound 2 making a total volume of 100 µL.

Then, 50 µl was drawn from the first two rows of each category and transferred down to the subsequent rows until the last rows where 50 µL of the mixtures from the last rows were discarded. Later, 50 µL of the bacterial suspension equivalent to 0.5 MacFarland standard turbidity (1.5×10^{6} CFU mL^{-1}) was added to each well. The two rows with only broth and bacteria mentioned above were used as growth control. The inoculated microtiter plate was incubated at 37ºC for 24 h. Thereafter, MIC values were determined by using Iodonitrotetrazolium chloride (INT) indicator which change its color from pink to yellow where there is bacteria growth. 40 µl of a 0.2% INT was added in each well followed by incubation for 1 h at 37°C. The lowest concentration without bacteria growth was considered as MIC value. Grading of antibacterial activity was done basing on the previous described criteria [15]. as follows: MIC ≤ 0.5 mg/ml (strong activity), MIC = 0.6-1.5 mg/ml (moderate activity) and MIC >1.5 mg/ml (weak activity).

RESULTS AND DISCUSSION

Isolation of Bioactive Compounds

Compound 1 and 2 were isolated as white powder from 100% DCM leaves extract of *C. swynnertonii* with weights of 40 and 85 mg respectively. The two compounds indicated single spot on TLC analysis. Furthermore, both were tested negative under Ultraviolet

lamp (UV-lamp) indicating that they have no conjugation chemical structures.

Structure Determination

Chemical structures of compound 1 and 2 (Fig. 1) were determined by using NMR spectral data and with comparison with spectral data available in literatures as follows:

Compound 1

The experimental ^{13}C NMR (150 MHz, $CDCl_3$) spectra of compound 1 indicated 27 carbon signals at chemical shifts, δ_C (ppm) as follows: 31.7 (C-1), 29.9 (C-2), 71.3 (C-3), 38.2 (C-4), 43.6 (C-5), 21.9 (C-6), 117.6 (C-7), 139.9 (C-8), 49.7 (C-9), 40.5 (C-10), 24.1 (C-11), 36.3 (C-12), 39.8 (C-13), 55.3 (C-14), 28.2 (C-15), 28.1 (C-16), 56.4 (C-17), 23.0 (C-18), 23.2 (C-19), 19.1 (C-20), 34.4 (C-21), 36.4 (C-22), 22.8 (C-23), 39.7 (C-24), 37.4 (C-25), 13.3 (C-26) and 12.1 (C-27).

The experimental ^{1}H NMR (600 MHz, $CDCl_3$) spectra showed the presence of five methyl signals that appeared as two methyl singlets at δ 0.51 (3H, *s*, CH_3-18) and 0.77 (3H, *s*, CH_3-19); three methyl doublets that appeared at δ 0.84 (3H, *d*, *J* = 3.4 Hz, CH_3-26), 0.85 (3H, *d*, *J* = 3.4 Hz, CH_3-27) and 0.90 (3H, *d*, *J* = 6.5 Hz, CH_3-21); proton attached to carbon bearing hydroxyl group attachment at δ 3.57 (1H, *m*, CH-3) and one olefinic proton at δ 5.14 (1H, *t*, CH-7). The comparison of experimental ^{13}C NMR and ^{1}H NMR spectra with the literature ^{13}C NMR and ^{1}H NMR data cited [17-19]. for compound 1 are shown in Table 1 and 2 respectively. Hence, the structure of compound 1 was deduced to be Cholest-7-en-3β-ol (1).

Compound 2

The experimental ^{13}C NMR (150 MHz, $CDCl_3$) spectra of compound **2** indicated 29 carbon signals at chemical shifts, δ_C (ppm) as follows: 37.5 (C-1), 31.9 (C-2), 72.0 (C-3), 42.5 (C-4), 141.0 (C-5), 121.9 (C-6), 32.0 (C-7), 32.1 (C-8), 50.4 (C-9), 36.7 (C-10), 21.3 (C-11), 40.0 (C-12), 42.5 (C-13), 57.0 (C-14), 24.5 (C-15), 28.5 (C-16), 56.3 (C-17), 12.1 (C-18), 19.6 (C-19), 36.4 (C-20), 19.0 (C-21), 34.1 (C-22), 26.3 (C-23), 46.1 (C-24), 29.4 (C-25), 20.0 (C-26), 19.3 (C-27), 23.3 (C-28) and 12.2 (C-29).

The experimental ^{1}H NMR (600 MHz, $CDCl_3$) spectra showed the presence of six methyl signals that appeared as two methyl singlets at δ 0.99 (3H, *s*, CH_3-18) and δ 0.66 (3H, *s*, CH_3-19); three methyl doublets that appeared at δ 0.90 (3H, *d*, *J* = 6.2 Hz, CH_3-21), δ 0.80 (3H, *d*, *J* = 6.6 Hz, CH_3-27) and 0.81 (3H, *d*, *J* = 6.6 Hz, CH_3-29); and a methyl triplet at δ 0.82 (3H, *t*, *J* = 7.1 Hz, CH_3-26); a proton connected to the carbon bearing hydroxyl group attachment at δ 3.50 (1H, *m*, CH-3) and one olefinic proton at δ 5.33 (1H, *t*, CH-6). Comparison of experimental ^{13}C NMR and ^{1}H NMR spectra with the literature ^{13}C NMR and ^{1}H NMR data cited [20,21]. for compound 2 are shown in Table 3 and 4 respectively. Therefore, the structure for compound 2 was deduced to be β-sitosterol (2).

This study succeeded to isolate Cholest-7-en-3β-ol (1) and β-sitosterol (2) from 100% DCM leaves of *C. swynnertonii*. Previously only 6-oxodendrolasin, (*E*)-6-oxoisodendrolasin, (*Z*)-6-oxoisodendrolasin, crassifolone and 7,8-dihydroisodendrolasin have been isolated from its bark exudate [13].

Therefore, this study reports the isolation of Cholest-7-en-3β –ol (1) and β-sitosterol (2) from this plant for the first time. However, Literature search showed that Cholest-7-en-3β-ol (1) and β-sitosterol (2) have also been isolated from other plant species of the genus *Commiphora*. For instance, Cholest-7-en-3β –ol (1) has been previously isolated from the resin of *Commiphora* species such as *Commiphora eminii*, *Commiphora habessinica* and *Commiphora kua* [17,18,22]. Likewise, β-sitosterol (2) has been isolated from other *Commiphora* species such as *Commiphora molmol*, *Commiphora Mukul* and *Commiphora myrrha* [23-28].

Antibacterial Activity

Activity of Cholest-7-en-3β-ol (1) and β-sitosterol (2) against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* was conducted, the MIC values were determined. Only Cholest-7-en-3β-ol (1) exhibited strong activity against gram-positive bacteria *S. aureus* with MIC value of 0.5 mg/ml and weak activities (MIC > 2 mg/ml) against other bacteria strains. On the other hand, β-sitosterol (2) exhibited weak activities (MIC >2mg/ml) against all tested bacteria strains (Table 5).

Literature search revealed that, sterols are widely found in plants that have various of biological functions [26]. Likewise, Cholest-7-en-3β-ol (1) and β-sitosterol (2) are reported to possess various biological activities. For instance, Cholest-7-en-3β-ol (1) has antimycobacterial activity [18]. and antiproliferative activity [17]. while β-sitosterol (2) possesses anti-benign prostatic hyperplasia, anti-inflammatory, anti-tuberculosis, anti-asthma, anti-cholesterol, diuretic and anti-arthritic activities [27,28]. Therefore, this study reports the activity of Cholest-7-en-3β-ol (1) against *S. aureus* for the first time.

In looking to establish for antibacterial structure activity relationship (SAR) of Cholest-7-en-3β-ol (1); several studies previously conducted on its activity were reviewed [17,18,22,29].These studies reported that, both cholestane skeleton and position of the double bond in the tetracyclic ring structure of sterols which differ from one another affect their activities [17,29]. The double bond of Cholest-7-en-3β-ol (1) is at C-7/C-8 in its tetracyclic ring structure (Fig. 2). From these viewpoints, it can be deduced that cholestane skeleton and position of the double bond on Cholest-7-en-3β-ol (1) at C-7/C-8 might have favored its observed activity against *Staphylococcus aureus*.

Table 1: Comparison of experimental and literature ^{13}C NMR chemical shifts (150 MHz, $CDCl_3$, δ_C in ppm) for compound 1

Position (C)	Experimental δ	Literature δ [17-19].
1	31.7	31.4
2	29.9	29.6
3	71.3	71.0
4	38.2	37.9
5	43.6	43.3
6	21.9	21.5
7	117.6	117.4
8	139.9	139.6
9	49.7	49.4
10	40.5	40.2
11	24.1	23.9
12	36.3	36.1

13	39.8	39.5
14	55.3	55.0
15	28.2	28.0
16	28.1	27.9
17	56.4	56.1
18	23.0	22.5
19	23.2	22.9
20	19.1	18.8
21	34.4	34.2
22	36.4	36.2
23	22.8	22.5
24	39.7	39.4
25	37.4	37.0
26	13.3	13.0
27	12.1	11.8

Table 2: Comparison of experimental and literature ^{1}H NMR chemical shifts (600 MHz, $CDCl_3$, δ_H in ppm) for compound 1

Position (H)	Splitting pattern, number of Hydrogen	Experimental δ	Literature δ [17-19].
C-3	*m*, 1H	3.57	3.58
C-7	*t*, 1H	5.14	5.17
C-18	*s*, 3H	0.51	0.54
C-19	*s*, 3H	0.77	0.80
C-21	*d*, *J* = 6.5 Hz, 3H	0.90	0.94
C-26	*d*, *J* = 3.4 Hz, 3H	0.84	0.82
C-27	*d*, *J* = 3.4 Hz, 3H	0.85	0.89

Table 3: Comparison of experimental and literature ^{13}C NMR chemical shifts (150 MHz, $CDCl_3$, δ_C in ppm) for compound 2

Position (C)	Experimental δ	Literature δ [20,21].
1	37.5	37.3
2	31.9	31.7
3	72.0	71.9
4	42.5	42.3
5	141.0	140.8
6	121.9	121.7
7	32.0	31.9
8	32.1	31.9
9	50.4	50.2
10	36.7	36.5
11	21.3	21.1
12	40.0	39.8
13	42.5	42.3
14	57.0	56.8
15	24.5	24.3
16	28.5	28.2
17	56.3	56.1
18	12.1	11.9
19	19.6	19.4
20	36.4	36.1
21	19.0	18.8
22	34.1	34.0
23	26.3	26.2

24	46.1	45.9
25	29.4	29.2
26	20.0	19.8
27	19.3	19.0
28	23.3	23.1
29	12.2	12.2

Table 4: Comparison of experimental and literature ^{1}H NMR chemical shifts (600 MHz, $CDCl_3$, δ_H in ppm) for compound 2

Position (H)	Splitting pattern, number of hydrogens	Experimental δ	Literature δ [20,21]
C-3	*m*, 1H	3.50	3.53
C-6	*t*, 1H	5.33	5.36
C-18	*s*, 3H	0.99	1.01
C-19	*s*, 3H	0.66	0.68
C-21	*d*, *J* = 6.2 H z, 3H	0.90	0.93
C-26	*t*, *J* = 7.1 H z, 3H	0.82	0.84
C-27	*d*, *J* = 6.6 H z, 3H	0.80	0.81
C-29	*d*, *J* = 6.6 H z, 3H	0.81	0.83

Table 5: Antibacterial activity expressed as Minimum inhibitory concentrations (MICs)

Sample name	MIC (mg/ml)			
	S. aureus	***E. faecalis***	***E. coli***	***P. aeruginosa***
Cholest-7-en-3β-ol **(1)**	0.5	> 2 mg/ml	> 2 mg/ml	> 2 mg/ml
β-sitosterol **(2)**	> 2 mg/ml	> 2 mg/ml	> 2 mg/ml	> 2 mg/ml
Gentamicin	0.002	0.002	0.004	0.004

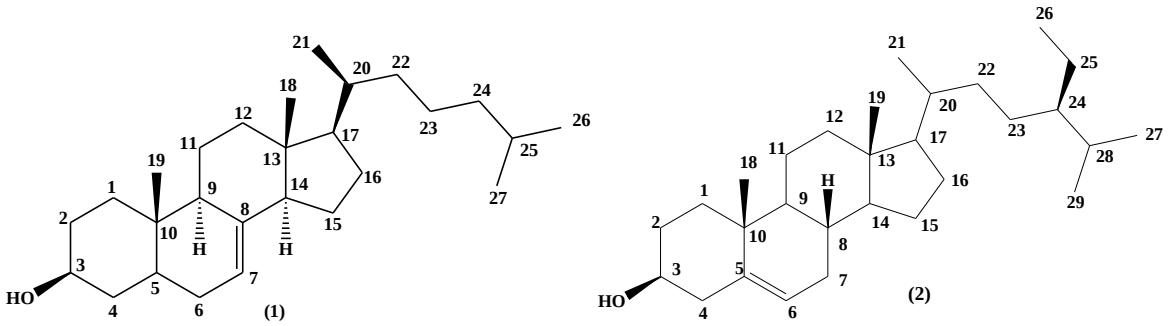

Figure 1: Chemical structures of compound 1 and 2

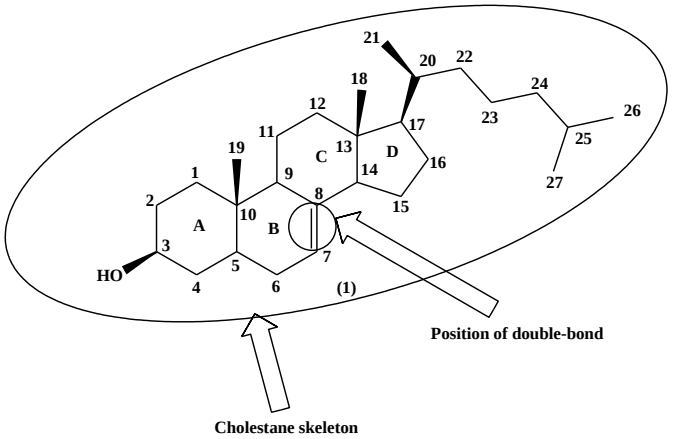

Figure 2: Cholestane skeleton and position of double bond of compound 1

CONCLUSION

Presence of antibacterial active compound(s) like Cholest-7-en-3β-ol (1) among others in the leaves of *C. swynnertonii* may justify its earlier reported antibacterial activity and further confirms the claimed traditional uses of this plant in the treatment of bacterial infections. Furthermore, Cholest-7-en-3β-ol may be considered as A lead compound for synthesis of drugs against *Staphylococcus aureus*.

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Conflict of Interest

None declared.

Supplementary information

Supplementary figures (Figure 1-4) can be downloaded by following the given link.

Link:
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