Journal of Scientific and Innovative Research 2022; 11(3): 58-62
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2022; 11(3): 58-62
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Received: 24-08-2022
Accepted: 16-09-2022

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Isolation and Cytotoxicity Evaluation of Long Chain Bioactive Compounds from *Commiphora swynnertonii* (Burtt)

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DOI: 10.31254/jsir.2022.11304

Abstract

Commiphora swynnertonii (Burtt) is a medicinal plant with diverse traditional uses in Tanzania. However, there is limited information on isolation and cytotoxicity properties of bioactive compounds responsible for its pharmacological activities. Hence, this study attempted to isolate and assess toxicity properties of bioactive compounds from its leaves, stem barks and whole root. Isolation of bioactive compounds was done by column chromatography method. There structures were deduced with the aid of NMR and GC-MS data and comparison with spectral data available in the literatures. Cytotoxicity evaluation was done by brine shrimp lethality test (BSLT) method. Chromatographic separation led to isolation of Hexacosane (1), Ethyl octadecanoate (2) and Octacosanol (3) from the leaves; Ethyl pentadecanoate (4) and Hexacosanoic acid (5) from the stem barks and Ethyl tetradecanoate (6), Methyl pentadecanoate (7) and Ethyl hexadecanoate (8) from the whole root. BSLT evaluation revealed that all compounds were non-toxic to brine shrimp larvae (LC_{50} >100 µg/ml).

This study reports the isolation of these compounds for the first time from this plant, moreover, their presence demonstrates the usefulness of *C. swynnertonii* as a good source of pharmacological active reported compounds which authenticate its diverse uses in traditional medicine. Furthermore, the revealed non-toxic effect of these compounds shows safety and possibly its traditional use has no cytotoxicity effect. Yet, further studies on toxicity of these compounds are recommended.

Keywords: Bioactive Compounds, Brine Shrimp Test, Burseraceae, *Commiphora swynnertonii,* Long-Chain Compounds, Toxicity.

INTRODUCTION

Obviously medicinal plants have been used by humans as alternative for treatment and management of various diseases since time immemorial [1]. According to World Health Organization (WHO), over three quarter of people in third world countries depend on traditional medicines derived from plants as their primary healthcare [2]. Currently, about 25% of all medicines available in the world market originate from medicinal plants either direct or indirect [3].

In Tanzania, *Commiphora swynnertonii* Burtt. (Family Burseraceae) is one of the valuable medicinal plants used traditionally for treatment of various diseases such as skin infections, respiratory infections, urinary tract infections and sexual transmitted diseases in humans and repel ticks and other insects in animals [2,4,5]. The leaves, barks, roots and bark exudates are said to be the useful parts of this plant for medical purposes [5]. Bioactivities which have been demonstrated by this plant include anti-Newcastle disease virus [6], anti-coccidial [7], acaricidal [8], and antimicrobial [9]. Long chain compounds, alkaloids, phenolics, flavonoids, terpenoids, sterols, lignans, cardiac glycosides and saponins are among of the classes of secondary metabolites responsible for bioactivities in the genus *Commiphora* [5,9,10].

Regardless of its usefulness in traditional medicines, there is limited information on isolation of bioactive compounds responsible for pharmacological activities of *C. swynnertonii*. The available information regarding isolation of bioactive compounds from this plant is the isolation of 6-oxodendrolasin (E)-6-oxoisodendrolasin, (Z)-6-oxoisodendrolasin, crassifolone and 7,8-dihydroisodendrolasin from its bark exudate [11]. Furthermore, despite its huge traditional uses this plant has been previously implicated to exhibit moderate toxicity brine shrimp larvae [2].

Therefore, this study was done to further isolate and evaluate toxicity properties of bioactive compounds from the leaves, stem barks and whole root of this plant.

MATERIALS & METHODS

Chemicals

The solvents used were dichloromethane (DCM), and petroleum ether (PE) from Loba Chemie, Mumbai-India and methanol (MeOH) from Finar Chemical, Gujarat-India. Silica gel 60 (70-230 mesh, 60 angstrom pore size) and precoated thin layer chromatography Aluminum sheets (TLC, silica gel 60 F_{254}) were obtained from Merck KGaA group, Darmstadt, Germany whereas, brine shrimp's eggs and sea salt were obtained in the Department of Chemistry and Physics, Sokoine University of Agriculture-Tanzania.

Identification, Collection and Preparation of Plant Materials

Plant identification was done by a botanist from the Department of Botany, University of Dar es Salaam (UDSM). The leaves, stem barks and whole root of *C. swynnertonii* were collected from Mirerani village (03° 36′ to 03° 14.73′ S and 36° 50′ to 36° 18.05′ E) in Simanjiro 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 parts 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 each part of the plant was exhaustively extracted with 100% dichloromethane (DCM) by Soxhlet method described previously [12]. The filtrates collected were evaporated by rotary evaporator (Büchi Labor Technik, Flawil, Switzerland) at 40°C.

Isolation and Purification of Bioactive Compounds

Solvent system for isolation of compounds from crude extract of each part was initially established through TLC analysis. About 35 g of 100% DCM leaves extract was subjected to column chromatography over silica gel by gradient elution using solvent systems of varying polarities starting with PE, DCM and MeOH and the fractions (*Fr*), each of 50 ml were collected. By using TLC to monitor separations, the column was eluted by using 75% PE in DCM to give 9 fractions (*Fr 1-9*) followed by 50% of DCM in PE to give 11 fractions (*Fr 10-20*). It was further increased to 75% of DCM in PE to obtain 4 fractions (*Fr 21-24*), then to 100% DCM to yield 11 fractions (*Fr 25-35*). It was then increased to 10% of MeOH in DCM to obtain 10 fractions (*Fr 36-45*), finally increased to 20% of MeOH in DCM to give 8 fractions (*Fr 46-53*). TLC analysis resulted into development of seven fraction combinations with similar chemical profiles (*Fr 1-9*, *Fr 10-20*, *Fr 21-24*, *Fr 25-30*, *Fr 31-35*, *Fr 36-45* and *Fr 46-53*). The first (*Fr 1-9*), second (*Fr 10-20*) and third (*Fr 21-24*) fraction combinations indicated single spot on TLC, hence, purified by precipitation using 100% MeOH. Precipitates obtained were filtered by washing with 100% MeOH and dried to give compound 1, 2 and 3 respectively.

Similarly, about 35 g of 100% DCM stem bark extract was chromatographed over silica gel using PE, DCM and MeOH. Column was eluted as follows: 75% PE in DCM gave 11 fractions (*Fr 1-11*), 50% of DCM in PE yielded 10 fractions (*Fr 12-21*), 75% of DCM in PE produced 15 fractions (*Fr 22-36*), 100% DCM yielded 7 fractions (*Fr 37-43*), 10% of MeOH in DCM gave 7 fractions (*Fr 44-50*) and 20% of MeOH in DCM generated 6 fractions (*Fr 51-56*). TLC analysis led to form eight fraction combinations (*Fr 1-11*, *Fr 12-13*, *Fr 14-21*, *Fr 22-25*, *Fr 26-36*, *Fr 37-43*, *Fr 44-50* and *Fr 51-56*). On TLC analysis, the third (*Fr 14-21*) and sixth (*Fr 37-43*) fraction combinations showed single spot, thus, precipitated by 100% MeOH followed by precipitates filtration by washing with 100% MeOH and dried to obtain compound 4 and 5 respectively.

Finally, about 35 g of 100% DCM whole root extract was also chromatographed over silica gel using PE, DCM and MeOH as follows: 75% PE in DCM produced 4 fractions (*Fr 1-4*), 50% of DCM in PE generated 7 fractions (*Fr 5-11*), 75% of DCM in PE gave 9 fractions (*Fr 12-20*), 100% DCM yielded 19 fractions (*Fr 21-39*), 10% of MeOH in DCM generated 6 fractions (*Fr 40-45*) and 20% of MeOH in DCM produced 7 fractions (*Fr 46-52*). TLC analysis led to develop eight fraction combinations (*Fr 1-4*, *Fr 5-11*, *Fr 12-16*, *Fr 17-20*, *Fr 21-27*, *Fr 28-39*, *Fr 40-45* and *Fr 46-52*). On TLC analysis, the first (*Fr 1-5*), second (*Fr 5-11*) and sixth (*Fr 28*-39) fraction combinations indicated single spot, so, precipitated by 100% MeOH, then precipitates were filtered by washing with 100% MeOH and dried to obtain compound 6, 7 and 8 respectively.

All fraction combinations which did not indicate single spot on TLC analysis were left for future further purification.

Structure Determination

There structures of compounds were determined with the aid of NMR and GC-MS data and comparison with spectral data available in the literatures. The experimental ^{1}H and ^{13}C NMR data were recorded on a 600 Megahertz Bruker Avance NMR spectrometer with tetramethyl silane (TMS) as internal standard. The MS data were recorded in electron impact (EI) mode by an Agilent 5973 Triple Quadrupole GC-MS system with the National Institute of Standards and Technology (NIST) spectral library (version 2002). Hence, the MS data were used to confirm the structure of each compound by using the molecular ion peak, $[M]^+$. Based on the value of mass to charge (*m/z*) ratio of $[M]^+$, the structure of each compound was suggested by the NIST spectral library.

Cytotoxicity Test

Cytotoxicity evaluation of compounds 1-8 was done by Brine Shrimp Lethality Test (BSLT) method described previously [13]. with some minor modifications. In brief, 40 mg/ml stock solution of each compound was prepared in 20% dimethylsulphoxide (DMSO). Then, five concentration levels (1000, 800, 600, 400 and 200 μg/mL) were made by drawing different volumes from the stock solutions and added into vials, each having ten brine shrimp larvae. Then, volume of vial was adjusted to 5 mL with artificial seawater prepared by dissolving 3.8 g of sea salt in 1 L of distilled water. Each concentration was tested in duplicate. The negative control containing artificial seawater, brine shrimp and 20% DMSO was made. The vials were then incubated under light for 24 h. The number of died brine shrimps for each vial after incubation were counted and their mean at each concentration was determined. Then, mean percentage of mortality at each concentration was determined using the equation:

Percentage mortality = (number of dead nauplii/number of nauplii added in a vial) x 100 [14].

The mean percentage mortality was subjected to regression analysis using Microsoft Excel (version 2016). The mean percentage mortality was plotted against logarithm of concentrations. The regression equations obtained from the graphs were used to determine the lethal concentrations for 50% mortality of the larvae (LC_{50}) and the 95% confidence interval (95% CI) values according to the previous described procedure [13-15]. The results of brine shrimp toxicity were interpreted as follows: $LC_{50} < 1.0$ μg/ml as highly toxic; LC_{50} 1.0-10.0 μg/ml as toxic; LC_{50} 10.0-30.0 μg/ml as moderately toxic; LC_{50} >30 <100 μg/ml as mildly toxic and LC_{50} > 100 μg/ml as non-toxic as described previously [15].

RESULTS AND DISCUSSION

Isolation and Purification of Bioactive Compounds

Compound 1, 2 and 3 were isolated from 100% DCM leaves extract with 318, 178 and 686 mg respectively. Compound 4 and 5 were isolated from 100% DCM stem bark extract with 30 and 40 mg respectively while compound 6, 7 and 8 were isolated from 100% DCM whole root extract with 28, 188 and 60 mg respectively. All compounds indicated single spot on TLC analysis to confirm their purity.

Structure Determination

Based on experimental MS and NMR data and comparison with other published spectroscopic data, chemical structures of compounds 1-8 (Fig. 1) were determined as follows:

Compound 1: EI-MS *m/z* 366.7 $[M]^+$ ($C_{26}H_{54}$) corresponding to Hexacosane (1) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD_2Cl_2): δ (ppm) 0.88 (-CH_3, *t, J* =7.0 Hz) and 1.26-1.52 (-CH_2, *s*). ^{13}C NMR (150 MHz, d-CD_2Cl_2): δ (ppm) 13.86 (CH_3), 22.69, 29.35, 29.67 and 31.93 (each, –CH_2). The above experimental spectral data are in consistent with the literature data [16].

Compound 2: EI-MS *m/z* 312.5 $[M]^+$ ($C_{20}H_{40}O_2$) corresponding to Ethyl octadecanoate (2) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD_2Cl_2): δ (ppm) 0.88 (-CH_3, *t*, *J* = 6.8 Hz), 1.27-153 (-CH_2, *s*), 1.61 (-CH_2, *m*), 2.26 (-CH_2, *t*, *J* = 7.5 Hz) and 4.02 (-CH_2, *t*, *J* = 6.7 Hz). NMR (150 MHz, d-CD_2Cl_2): δ (ppm) 13.87 (-CH_3), 22.68, 25.02, 25.94, 28.67, 29.13, 29.25, 29.28, 29.35, 29.48, 29.53, 29.57, 29.60, 29.65, 29.68, 31.92 (each, –CH_2), 34.29 (-CH_2-C=O), 64.20 (-CH_2-O) and 173.63 (-C=O). The experimental spectral data above are in consistent with the literature data [17].

Compound 3: EI-MS *m/z* 410.8 $[M]^+$ ($C_{28}H_{58}O$) corresponding to Octacosanol (3) as suggested by NIST library. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 0.86 (-CH_3, *t*, *J* = 6.9 Hz), 1.54 (-CH_2, *m*) and 3.62 (2H, *t*, *J* = 6.6 Hz, -CH_2OH). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 14.33 (-CH_3), 22.92, 25.97, 29.59, 29.66, 29.93, 32.15, 33.05 (each, –CH_2) and 63.34 (-CH_2OH). The experimental spectral data above are in consistent with the literature data [18].

Compound 4: EI-MS *m/z* 270.5 $[M]^+$ ($C_{17}H_{34}O_2$) corresponding to Ethyl pentadecanoate (4) as suggested by NIST library. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 0.86 (-CH_3, *t*, *J* = 7.1 Hz), 1.23-153 (-CH_2, *s*), 1.58 (-CH_2, *m*), 2.27 (-CH_2, *t*, *J* = 7.5 Hz) and 4.03 (-CH_2, *t*, *J* = 6.7 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 14.34 (-CH_3), 22.92, 25.27, 26.17, 28.89, 29.39, 29.49, 29.59, 29.93, 32.15 (each, –CH_2), 34.66 (-CH_2-C=O), 64.63 (-CH_2-O) and 174.25 (-C=O). The experimental spectral data above are in consistent with the literature data [19].

Compound 5: EI-MS *m/z* 396.7 $[M]^+$ ($C_{26}H_{52}O_2$) corresponding to Hexacosanoic acid (5) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD_2Cl_2): δ 0.88 (-CH_3, *t*, *J* = 5.4 Hz), 1.26 (-CH_2, *s*), 1.61 (-CH_2, *m*), 2.34 (-CH_2, *t*, *J* = 5.8 Hz) and 4.67 (OH, *s*). ^{13}C NMR (150 MHz, d-CD_2Cl_2): δ (ppm) 14.45 (-CH_3), 23.27, 25.30, 29.63, 29.82, 29.94, 30.04, 30.18, 30.27, 32.51 (each, -CH_2), 34.09 (-CH_2-C=O), and 177.87 (-C=O). The experimental spectral data above are in consistent with the literature data [20].

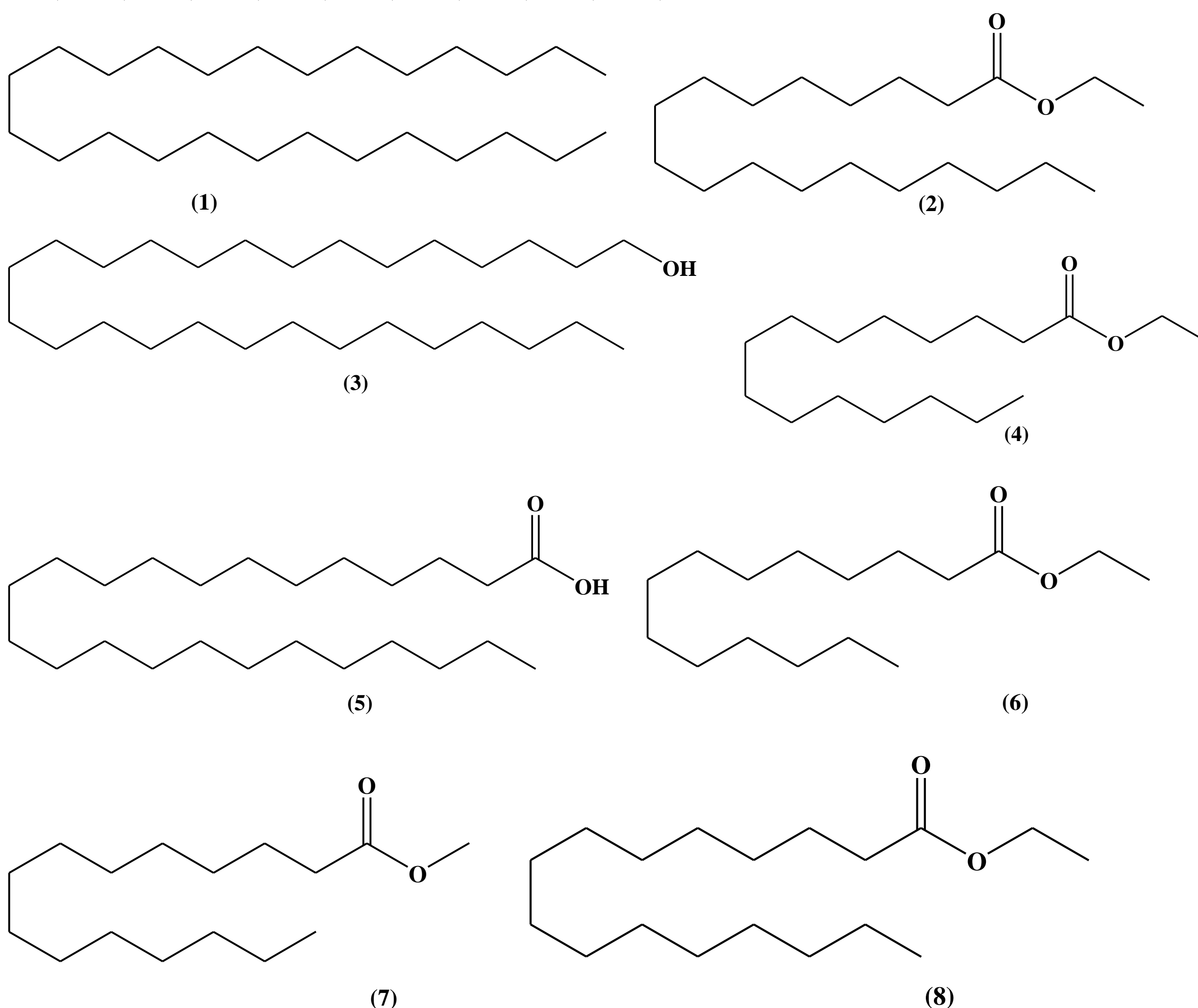

Figure 1: Chemical structures of compounds 1-8

Compound 6: EI-MS *m/z* 256.4 $[M]^+$ ($C_{16}H_{32}O_2$) corresponding to Ethyl tetradecanoate (6) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD_2Cl_2): δ (ppm) 0.80 ($-CH_3$, *t*, *J* = 6.6 Hz), 1.18-1.45 ($-CH_2$, *s*), 1.51 ($-CH_2$, *m*), 2.18 ($-CH_2$, *t*, *J* = 7.5 Hz) and 3.94 ($-CH_2$, *t*, *J* = 6.6 Hz). ^{13}C NMR (150 MHz, d-CD_2Cl_2): δ (ppm) 13.11 ($-CH_3$), 21.93, 24.28, 25.19, 27.92, 28.38, 28.50, 28.53, 28.60, 28.93, 31.17 (each, $–CH_2$), 33.55 ($-CH_2$-C=O), 63.46 ($-CH_2$-O) and 172.89 (-C=O). The experimental spectral data above are in consistent with the literature data [21].

Compound 7: EI-MS *m/z* 256.4 $[M]^+$ ($C_{16}H_{32}O_2$) corresponding to Methyl pentadecanoate (7) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD_2Cl_2): δ (ppm) 0.88 ($-CH_3$, *t*, *J* = 6.9 Hz), 1.26 ($-CH_2$, *s*), 1.59 ($-CH_2$, *m*), 2.28 ($-CH_2$, *t*, *J* = 7.6 Hz) and 3.63 ($-CH_3$, *s*). ^{13}C NMR (150 MHz, d-CD_2Cl_2): δ (ppm) 14.46 ($-CH_3$), 23.28, 25.54, 29.72, 29.86, 29.95, 30.06, 30.28, 32.52 (each, $–CH_2$), 34.57 ($-CH_2$-C=O), 64.81 ($-CH_2$-O) and 174.61 (-C=O). The experimental spectral data above are in consistent with the literature data [22].

Compound 8: EI-MS *m/z* 284.5 $[M]^+$ ($C_{18}H_{36}O_2$) corresponding to Ethyl hexadecanoate (8) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD_2Cl_2): δ (ppm) 0.88 ($-CH_3$, *t*, *J* = 7.0 Hz), 1.27 ($-CH_2$, *s*), 1.59 ($-CH_2$, *m*), 2.26 ($-CH_2$, *t*, *J* = 7.5 Hz) and 4.02 ($-CH_2$, *t*, *J* = 6.7 Hz). ^{13}C NMR (150 MHz, d-CD_2Cl_2): δ (ppm) 14.46 ($-CH_3$), 23.28, 25.52, 26.54, 29.27, 29.73, 29.85, 29.88, 29.95, 30.28, 32.52 (each, $–CH_2$), 34.89 ($-CH_2$-C=O), 64.80 ($-CH_2$-O) and 174.23 (-C=O). The experimental spectral data above are in consistent with the literature data [21].

This study reports the isolation of the long chain compounds 1-8 for the first time from *C. swynnertonii*. Long chain compounds and their derivatives are among of the classes of secondary metabolites responsible for bioactivities in the genus *Commiphora* [5,10]. Compounds 1-8 have been also isolated from other plant species previously and some of them revealed to exhibit various pharmacological activities. For example, hexacosane (1) has been demonstrated antimicrobial activity [16]. Ethyl octadecanoate (2) has shown antidiabetic activity [17]. Octacosanol (3) has exhibited antibacterial and antidermatophytic activities [23]. Ethyl pentadecanoate (4) has displayed antibacterial activity [24]. Hexacosanoic acid (5) has demonstrated antifungal activity [25]. Ethyl tetradecanoate (6) has shown anti-hypercholesterolemic activity [24]. Methyl pentadecanoate (7) has demonstrated antibacterial and antifungal activities [26]. and Ethyl hexadecanoate (8) has exhibited larvicidal and insecticidal [27], antioxidant and anti-inflammatory activities [28]. Therefore, existence of these long chain compounds in *C. swynnertonii* demonstrates its potential as a good source of active compounds which authenticate its traditional uses in treatment of numerous illnesses.

Cytotoxicity Test

The results of BSLT revealed that all compounds were non-toxic to brine shrimp larvae ($LC_{50} > 100$ μg/ml) as shown in Table 1. BSLT is a simple bioassay for testing plant extracts bioactivity which significantly correlates with cytotoxic and antitumor properties [29]. Therefore, the observed non-toxic effect of these compounds shows no cytotoxicity.

Table 1: Brine Shrimp Toxicity of compounds 1-8

Compound name	LC_{50} (μg/ml)	95% Confidence interval (μg/ml)	
		Lower limit	Upper limit
Hexacosane (1)	1.13×10^4	-124.48	40.98
Ethyl octadecanoate (2)	1.14×10^4	-124.38	41.27
Octacosanol (3)	2.79×10^5	-211.87	51.87
Ethyl pentadecanoate (4)	2.98×10^5	-211.79	51.68
Hexacosanoic acid (5)	1.27×10^4	-124.48	40.32
Ethyl tetradecanoate (6)	2.76×10^5	-211.74	51.64
Methyl pentadecanoate (7)	2.54×10^5	-211.61	51.52
Ethyl hexadecanoate (8)	2.78×10^5	-211.72	51.61

CONCLUSION

In this study, eight long chain compounds were isolated from *C. swynnertonii*, namely; Hexacosane (1), Ethyl octadecanoate (2) and Octacosanol (3) from its leaves; Ethyl pentadecanoate (4) and Hexacosanoic acid (5) from the stem bark and Ethyl tetradecanoate (6), Methyl pentadecanoate (7) and Ethyl hexadecanoate (8) were the whole root. All compounds were non-toxic to brine shrimp larvae. The results of this study are useful to reveal the potential of this plant as the good source of pharmacological active compounds which justifies its traditional uses in treatment of several diseases. The observed non-toxic effect of these compounds shows safety and perhaps its traditional use has no cytotoxicity effect though further studies on toxicity properties of these compounds are recommended.

Acknowledgement

The authors wish to acknowledge Danish International Development Agency (DANIDA) through Green Resources Innovations for Livelihood Improvement (GRILI) project (grant number DPRTC//R//126//266) for funding this study.

Conflict of Interest

None declared.

Supplementary information

Supplementary figures (Figures 1-16) can be downloaded by following the given link.

Link: http://www.jsirjournal.com/Vol11_Issue3_04_SupFig.pdf

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