Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221−1691.261822 Impact factor: 1.59

Antimicrobial, cytotoxicity, anticancer and antioxidant activities of *Jatropha zeyheri* Sond. roots (Euphorbiaceae)

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ARTICLE INFO

Article history:
Received 26 March 2019
Revision 15 April 2019
Accepted 20 June 2019
Available online 5 July 2019

Keywords:
Antimicrobial activity
Selectivity index
Jatropha zeyheri
GC-MS
Antioxidant activity
Anti-proliferative effect

ABSTRACT

Objective: To evaluate the antimicrobial, antioxidant, cytotoxicity and anticancer activity of fractions from *Jatropha zeyheri* roots and to explore the phytochemical profile of the most biologically active fraction.

Methods: Fractions from *Jatropha zeyheri* ethyl acetate extract were investigated for antimicrobial activity against a plethora of pathogenic microorganisms of different origins. The cytotoxicity studies of fractions were evaluated *in vitro* using tetrazolium-based calorimetric assay against human dermal fibroblast, colon adenocarcinoma (Caco-2), breast cancer (MCF-7) and lung cancer (A547) cell lines. The anti-oxidant activity of fractions was determined *in vitro* against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and chelation of iron (Fe^{2+}). Gas chromatography mass spectrometry analysis was performed to detect phytochemical constituents in fraction with potent biological activity.

Results: Fraction 2 of *Jatropha zeyheri* roots exhibited the lowest minimum inhibitory concentration of 40 μg/mL against *Klebsiella pneumoniae* and 80 μg/mL against *Candida albicans*, *Staphylococcus aureus* and *Mycoplasma hominis*. The fractions revealed some varying degrees of cytotoxicity against human dermal fibroblasts yielding LC_{50} values ranging from 28.96 to 166.52 μg/mL. Fraction 3 exhibited the highest selectivity index value of 2.08 against *Klebsiella pneumoniae*. Moreover, fraction 2 selectively inhibited the growth of Caco-2 with LC_{50} of 8.83 μg/mL, compared to other cancerous cell lines. Fraction 2 of *Jatropha zeyheri* further exhibited IC_{50} of 19.66, 22.63 and 1.82 μg/mL against DPPH, ABTS and Fe^{2+}, respectively. Gas chromatography mass spectrometry analysis revealed the presence of cyclotetracosane (10.08%), 9-hexacosene (9.40%), hexadecanoic acid (3.90%), (Z)-9-octadecenamide (3.63%), octacosane (2.27%), 11-n-decylheneicosane (2.23%), ethyl vallesiachotamate (2.17%), heneicosanoic acid (2.10%), and octadecanoic acid (2.08%) in fraction 2 of *Jatropha zeyheri*.

Conclusions: These identified compounds, particularly cyclotetracosane (hydrocarbon), may well explain the biological activity of fraction 2 of *Jatropha zeyheri*, which possesses higher biological activity than other fractions. These compounds can be further investigated for use in treating various bacterial and fungal opportunistic infections associated with HIV-AIDS and related cancers.

How to cite this article: Mongalo NI, Soyingbe OS, Makhafola TJ. Antimicrobial, cytotoxicity, anticancer and antioxidant activities of *Jatropha zeyheri* Sond. roots (Euphorbiaceae). Asian Pac J Trop Biomed 2019; 9(7): 307-314.

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1. Introduction

Most HIV-AIDS and cancer patients undergo a much less competent immune system throughout the infection, resulting in numerous opportunistic infections which may become difficult to treat due to alarming resistant microbes to numerous antibiotics used in most developing countries[1,2]. In the process, the free radicals may also accumulate and deplete the immune system further. Cancer patients undergo chemotherapy, which may not well distinguish between cancerous and normal cell line responsible for various body functioning and maintenance of immunity. Pain and inflammation may also become extremely prevalent in such patients. Medicinal plants and related products with potent anticancer, selective cytotoxicity, antimicrobial activity, coupled with noteworthy antioxidant activity may be of critical importance in curbing such health problems and may be of great therapeutic importance.

Jatropha is a genus that is comprised of approximately 170 known species distributed all over the world, mostly in tropical areas[3]. Besides being edible and consumed by humans, some members of the genus are known to possess a wide variety of important pharmacological activities[4–10]. *Jatropha zeyheri* (*J. zeyheri*) is one of the most important South African medicinal herb which grows up to 300 mm in height and possesses leaves which are either palmate or runcinated in shape. The leaves are alternate, sessile and have short petioles. The flowers are yellow in color, and mostly appear in clusters. The roots may be used in the treatment and management of various infections including sore eyes, gynaecological complaints, diarrhoea, sexually transmitted infections and other ethno-veterinary infections[11–17]. Pharmacologically, the extracts from both roots and leaves are reported to possess noteworthy antimicrobial, anti-inflammatory and mutagenic effects[18–20]. In the current work, the antimicrobial, antioxidant and anti-proliferative activities of fractions from *J. zeyheri* roots were investigated. The phytochemical profile of the fraction with noteworthy biological activity was explored using gas chromatography mass spectrometry (GC-MS) analysis while cytotoxicity potential was evaluated against human dermal fibroblast (HDF). In additon, the anti-proliferative effect of fractions was evaluated against colon adenocarcinoma (Caco-2), breast cancer (MCF-7) and lung cancer (A547) cell lines. In our hypothesis, cancer associated with HIV results in many complications within a patient. However, plant-derived products with potent antimicrobial, antioxidant and anticancer activities may well curb such infections including oxidation and improve the immune system of such patients. Such plant-derived products should further possess selective cytotoxicity.

2. Materials and methods

2.1. Collection and identification of plant materials

Roots from *J. zeyheri* were collected from Pickum farm in June 2018, with the help of a traditional healer, Mr Benjamin Mokgehle. The specimens were chopped using axe into small pieces, which were washed with tap water to remove all the adhering soil debris. Specimens were then packaged with newspapers and then dried on the laboratory bench in the absence of light. Dried plant materials were ground into thin powder using a Scientec hammer mill (Germany). The voucher specimen (MNI-29) was also collected and lodged at University of South Africa (Science Campus, Florida) herbarium.

2.2. Extraction and fractionation of ethyl acetate extract

Ethyl acetate was a solvent of choice because of its reported noteworthy antimicrobial activity[19]. Powdered *J. zeyheri* roots (1.5 kg) were macerated in 6 L ethyl acetate (AR grade, Merck, South Africa) and left in a shaker incubator (Already Enterprise Inc., Taiwan, Model: LM-600 RD) at 100 rpm for 2 d. The resulting extract was filtered through Whatmans No1 filter paper and then evaporated using Buchi rotary evaporator. The crude extract of 18.88 g was subjected to column chromatography using silica gel 60 (0.2-0.5 mm) obtained from Merck, South Africa. A column of 4.0 mm thickness was packed to a height of 25 mm and allowed to settle overnight. Crude extract was loaded and different solvents made up of hexane and ethyl acetate (300 mL), which differ in polarity were used to obtain fractions for biological assays (Figure 1). The resulting crude fractions JZ1, JZ2, JZ3 and JZ4 revealed 166.88, 119.68, 198.68 and 132.46 mg, respectively.

2.3. Antimicrobial activity

2.3.1. Selected microorganisms

Microorganisms such as *Moraxella catarrhalis* (*M. catarrhalis*), *Proteus vulgaris* (*P. vulgaris*), *Cryptococcus neoformans* (*C. neoformans*), *Candida albicans* (*C. albicans*) and *Staphylococcus aureus* (*S. aureus*) isolated from HIV-AIDS patients were selected for the study. Reference strains of *Bacillus cereus* (*B. cereus*) (10702), *Escherichia coli* (*E. coli*) (ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 10031), *Klebsiella pneumoniae* (*K. pneumoniae*) (ATCC 13883) and *Mycoplasma hominis* (*M. hominis*) (ATCC 15488) were also selected for the study for comparison purposes. All the bacterials strains were maintained on Muller Hinton agar slants, except *M. hominis*, which was maintained on *Mycoplasma* agar supplemented with *Mycoplasma* G, while fungal strains were maintained on Potato Dextrose agar. All the growth medium were obtained from Sigma Aldrich (Germany).

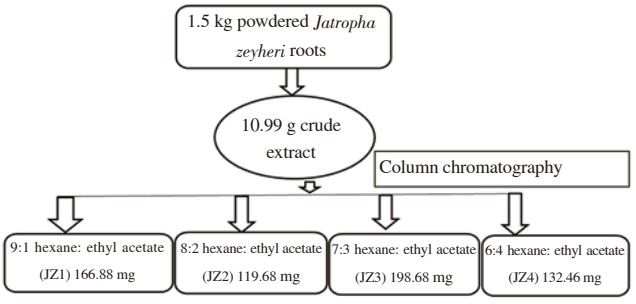

Figure 1. Schematic presentation of fractions from ethyl acetate extract of *Jatropha zeyheri* roots.

2.3.2. Antibacterial activity

The antimicrobial activity of fractions from *J. zeyheri* was investigated using the broth micro-dilution assay[21] with slight modification. The overnight culture was diluted with fresh Mueller Hinton broth to a concentration of about 1.1×10^7 cfu/mL. A volume of 100 µL of fractions [25 mg/mL in 5% dimethyl sulfoxide (DMSO)] was added to a multi well plate containing 100 µL of sterile distilled water and two-fold serially diluted. Bacterial culture was added (100 µL) to each well. Gentamycin was used as a positive control. Plates were then incubated overnight at 37 ℃. Plates for *M. hominis* were incubated for 24 h while other bacterial strains were incubated overnight. About 40 µL of 0.2 mg/mL freshly prepared *p*-iodo-nitro-tetrazolium chloride (Fluka) was added to each well and incubated for 30 min at the same temperature. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract to inhibit bacterial growth.

2.3.3. Antifungal activity

The antifungal activity was determined using the method adopted from Masoko *et al*.[22]. Shortly, the MIC values of the fractions were determined from the stock solution of 25 mg/mL. About 0.2 mg/mL of the freshly prepared *p*-iodo-nitro-tetrazolium chloride was added into the diluted plant extracts and overnight cultured as in the antibacterial assay. The results were read after 48 hours of incubation period. Amphotericin was used as a positive control.

2.4. Cytotoxicity studies

The cytotoxic effects of fractions from *J. zeyheri* were evaluated against human dermal fibroblast (HDF), colon adenocarcinoma (Caco-2), lung cancer (A547) and breast cancer (MCF-7) cell lines using the MTT assay as described by Mosmann[23]. Cells of a sub-confluent culture of each cell line were grown on Minimal Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). A cell suspension of about 200 µL containing 5×10^4 cells per mL was pipetted into each well of columns 2 to 11 of a sterile 96-well micro-titre plate in a Laminar Flow. MEM (200 µL) was added to wells of columns 1 and 12 to minimize the "edge effect" and maintain humidity.

The plates were incubated for 24 h at 37 ℃ in a 5% CO_2 incubator, until the cells were in the exponential phase of growth. The MEM was aspirated from the cells which were then washed with 150 µL phosphate buffered saline (Whitehead Scientific) and replaced with 200 µL of the fractions at different concentrations ranging from 7.5 to 1 000 µg/mL. The serial dilutions of the test fractions were prepared in MEM. The micro titre plates were incubated at 37 ℃ in a 5% CO_2 incubator for 48 h with test fractions. Untreated cells and positive control (doxorubicin chloride, Pfizer Laboratories) were included in the assay. Each experiment was repeated three times independently. The LC_{50} (concentration of the plant extract that inhibits 50% of cell growth) values were determined from the graphs of the concentration *vs* % inhibition using the formula below: Percentage cell inhibition =[1-Abs (Sample) / Abs (Control)] × 100[24].

Selectivity index (SI) values were calculated using formula below: SI= LC_{50}/MIC (both units in µg/mL)[25].

2.5. Antioxidant activity

2.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Free radical scavenging activity of the fractions from *J. zeyheri* roots was determined against DPPH using method adopted from Opoku *et al*[26] with slight modification. DPPH solution was prepared by dissolving DPPH into methanol (Sigma Aldrich, Germany) to make a working solution of 2 mg/100 mL. Shortly, plant extracts at 6 mg/100 mL (150 µL) were serially diluted with DMSO in 96 well plates. Later, DPPH solution (150 µL) was added to all the wells and the plate was placed on a shaker at 80 motions per minute (IKA® KS, Laboratory equipment, Germany) for 10 min covered with a foil and ten incubated in the dark for 50 min. Wells containing DPPH 1:1 DMSO: acetone were used as sample blank while ascorbic acid was as a positive control. Absorbance was read at 517 nm using microplate reader (Varioshskan Flash, Thermo Scientific Amsterdam).

2.5.2. 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

Free radical scavenging activity of the fractions from *J. zeyheri* roots was determined against ABTS, using method from Re *et al*[27] with slight modification. Shortly, 7 mM of ABTS tablet was diluted with 3 mL sterile distilled water and 2.45 mM potassium persulfate and the mixture which was then incubated at room temperature in the dark for 16 h. Equal amounts of serially diluted fractions as in DPPH assay were reacted with 150 µL of ABTS solution and absorbance was measured at 734 nm using micro plate reader (Varioshskan Flash, Thermo Scientific Amsterdam) after 6 minutes of vigorous shaking at 80 motions per minute (IKA® KS, Laboratory equipment, Germany). Ascorbic acid was used as positive control.

2.5.3. Iron chelating activity

The Fe^{2+} chelating effect of the fractions from *J. zeyheri* roots was evaluated according Decker and Wielch[28], with slight modifications. Plant extracts as in DPPH assay were serially diluted with DMSO. The extracts in all wells were further diluted with 100 µL of sterile distilled water, 10 µL of $FeCl_2$ (2 mM) and 20 µL of disodium; 4-[3-pyridin-2-yl-6-(4-sulfonatophenyl)-1,2,4-triazin-5-yl]benzenesulfonate (5 mM). The mixture was covered with foil and placed on a shaker at 80 motions per minute (IKA® KS, Laboratory equipment, Germany) for 10 min and the absorbance was measured at 562 nm using microplate reader (Varioshskan Flash, Thermo Scientific, Amsterdam).

Percentage of inhibition in all the three antioxidant assays was calculated as

% Scavenging Inhibition = $[1-A_t/A_0] \times 100$

Where A_t represents the absorbance of the test sample, while A_0 absorbance of blank solution. The IC_{50} values, concentrations of plant sample that inhibit 50% of test radical, were extrapolated from graphs drawn from concentrations *vs*. % inhibition using Graph Pad Prism software 7.

2.6. Phytochemical analysis of fractions from J. zeyheri roots by GC-MS

Fraction 2 exhibited the most noteworthy biological activity compared to other fractions, hence selected for phyto-constituents identification. The fraction was dissolved in dichloromethane (GC-MS grade, Sigma Aldrich, Germany) to a lowest concentration and then the separation of compounds was performed on a gas chromatograph (6890N, Agilent technologies network) coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL auto-sampler. Separation was performed on a non-polar DB-5MS (30 m, 0.25 mm ID, 0.25 μm film thickness capillary column).

Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 250 ℃. One μL of the sample was injected in split-less mode. The oven temperature was programmed as follows: 50 ℃ for 2 min, ramped up to 70 ℃ at a rate of 2 ℃/min for 6 min and finally ramped up to 320 ℃ at 20 ℃/min and held for 5 min. The mass selective detector was operated in a full scan mode and the source and quad temperatures were maintained at 230 ℃ and 150 ℃, respectively. The transfer line temperature was maintained at 250 ℃. The mass spectrometer was operated under electron impact mode at ionization energy of 70 eV, scanning from 35 to 500 *m/z*. The compounds were identified using two different libraries (NIST 95 and WILLEY275) for compound matches.

2.7. Statistical analysis

The antimicrobial experiments were done in triplicates and the mean value of results was expressed as a mean only. In other experiments, mean±SEM was reported. The data were subjected to statistical one-way analysis of variance and samples differences were extrapolated by Duncan's multiple range test, where it is applicable. Mean values of results were considered statistically significant when $P < 0.05$.

3. Results

3.1. Antimicrobial activity

The antimicrobial activities of fractions from *J. zeyheri* roots are shown in Table 1. JZ2 exhibited the lowest MIC value of 40 μg/mL against *K. pneumoniae* and of 80 μg/mL against *M. hominis*, *S. aureus* and *C. albicans*. Besides, JZ4 revealed a MIC value of 80 μg/mL against both *K. pneumoniae* and *P. vulgaris*, while JZ3 revealed a similar MIC value against *K. pneumoniae*.

3.2. Cytotoxicity studies

3.2.1. Cytotoxicity studies against HDF cell line

JZ4 significantly ($P<0.05$) inhibited the growth of HDF cell line with LC_{50} value of 28.96 μg/mL, compared to the other fractions. In brief, the fractions inhibited the growth of HDF cell line yielding LC_{50} values ranging from 28.96 to 166.52 μg/mL, while doxorubicin yielded LC_{50} value of 1.11 μg/mL.

3.2.2. SI value

JZ3 exhibited the highest SI values of 2.08 against *K. pneumoniae* and 1.04 against both *M. catarrhalis* and *S. aureus*. Moreover, JZ4 exhibited the lowest SI value of 0.02 against *M. hominis* (Table 2).

3.2.3. Anti-proliferative effect

The anti-proliferative effects of fractions against Caco-2, MCF-7 and A547 are shown in Table 3. JZ2 had the lowest lethal concentration (LC_{50}) value of 8.83 μg/mL ($P<0.05$), while JZ1 and JZ4 had LC_{50} value of >1 000 μg/mL against MCF-7. Furthermore, JZ3 and JZ4 had LC_{50} values of 72.59 and 32.71 μg/mL against Caco-2, respectively. Although JZ1 revealed LC_{50} value of >1 000 μg/mL against Caco-2, other fractions generally inhibited Caco-2 much better than MCF-7 and A547.

Table 1. Antimicrobial activity (MIC in μg/mL) of fractions from *Jatropha zeyheri* root extract.

Microorganisms	JZ1	JZ2	JZ3	JZ4	Amphotericin B	Gentamycin
Moraxella catarrhalis	630	310	160	630	ND	13
Escherichia coli	2 500	630	630	630	ND	01
Proteus vulgaris	1 250	160	310	80	ND	01
Pseudomonas aeruginosa	2 500	310	1 250	630	ND	13
Klebsiella pneumoniae	630	40	80	80	ND	08
Mycoplasma hominis	1 250	80	630	1 250	ND	04
Staphylococcus aureus	630	80	160	160	ND	03
Bacillus cereus	1 250	310	630	630	ND	08
Cryptococcus neoformans	630	160	310	310	06	ND
Candida albicans	1 250	80	1 250	630	02	ND

ND-not done. JZ1, JZ2, JZ3 and JZ4: crude fractions of *Jatropha zeyheri*.

Table 2. Selectivity index of selected fractions.

Microorganisms	JZ1	JZ2	JZ3	JZ4
Moraxella catarrhalis	0.21	0.11	1.04	0.05
Escherichia coli	0.05	0.05	0.26	0.05
Proteus vulgaris	0.11	0.21	0.54	0.36
Pseudomonas aeruginosa	0.05	0.11	0.13	0.05
Klebsiella pneumoniae	0.21	0.84	2.08	0.36
Mycoplasma hominis	0.11	0.42	0.26	0.02
Staphylococcus aureus	0.21	0.42	1.04	0.18
Bacillus cereus	0.11	0.11	0.26	0.05
Cryptococcus neoformans	0.21	0.21	0.54	0.09
Candida albicans	0.11	0.42	0.13	0.05

Table 3. Anti-proliferative effect (LC_{50} in μg/mL) of fractions from *Jatropha zeyheri* roots extract.

Fractions	Caco-2	MCF-7	A547
JZ1	>1 000[a]	>1 000[a]	466.53±4.82[a]
JZ2	8.83±0.00[b]	224.48±0.01[b]	102.88±2.17[b]
JZ3	72.59±1.16[c]	393.00±4.23[c]	246.62±1.99[c]
JZ4	32.71±0.01[d]	>1 000[a]	>1 000[d]
Doxorubicin	2.76±0.06[e]	1.26±0.03[d]	2.18±0.02[e]

Means with different letters are significantly different ($P < 0.05$) from each other along the column.

3.3. Antioxidant activity

The anti-oxidant potential of fractions from *J. zeyheri* roots is presented in Table 4. The fractions exhibited antioxidant activity in the order Fe^{2+}>ABTS>DPPH. JZ2 showed the most potent antioxidant activities ($P<0.05$) with IC_{50} values of 19.66, 22.63 and 1.82 μg/mL against DPPH, ABTS and Fe^{2+}, respectively.

3.4. GC–MS analysis of JZ2

JZ2 fraction revealed a total of 73 compounds with varying percentage areas and retention times (Table 5). Cyclotetracosane (10.08%), 9-hexacosene (9.40%), hexadecanoic acid (3.90%), (Z)-9-octadecenamide (3.63%), octacosane (2.27%), 11-n-decylheneicosane (2.23%), ethyl vallesiachotamate (2.17%), heneicosanoic acid (2.10%), and octadecanoic acid (2.08%) were the major compounds with % area greater than 2. The GC-MS chromatogram shows that the phyto-contituents appear in higher quantities from 26 to 34 min and that cyclotetacosane is the major compound.

Table 4. Antioxidant activity of fractions from *Jatropha zeyheri* root extract (μg/mL).

Fractions	DPPH	ABTS	Fe^{2+}
JZ1	>50[a]	44.940±0.001[a]	2.640±0.001[a]
JZ2	19.660±0.011[b]	22.630±0.001[b]	1.820±0.002[b]
JZ3	>50[a]	>50[c]	1.730±0.005[c]
JZ4	>50[a]	33.430±0.001[d]	3.550±0.000[e]
Ascorbic acid	1.360±0.041[c]	3.120±0.006[e]	0.096±0.001[d]

Means with different letters are significantly different ($P < 0.05$) from each other along the column. DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.

Table 5. Phyto-contituents of fraction 2 identified using GC-MS analysis.

Retention time	Library identification	Area percent
19.47	Nonanal	0.78
24.43	9-oxononanoic acid	0.74
24.80	Octanedioic acid	0.15
24.93	Dodecanoic acid	0.19
25.45	Nonanedioic acid	0.61
27.23	**Hexadecanoic acid**	**3.90**
27.63	1-Dodecene	0.11
27.69	Heptadecanoic acid	0.50
27.82	1-Octadecanol	0.40
28.02	1,3-Dimethoxy-5-methylanthraquinone	0.23
28.07	(Z)-9-Octadecenoic acid (Oleic acid)	0.64
28.18	**Octadecanoic acid**	**2.08**
28.29	(Z)-9-Octadecenamide	1.62
28.47	Linoleic acid	1.17
28.62	Nonadecanoic acid	0.64
28.73	Calarene	0.73
29.09	Eicosanoic acid	0.75
29.12	**(Z)–9–Octadecenamide**	**3.63**
29.49	**Heneicosanoic acid**	**2.10**
29.55	N,N-Diisopropyl-N'-(trifluoracetyl)carbamide	0.53
29.61	Eicosane	1.05
29.89	Docosanoic acid	1.57
30.00	Heneicosane	0.79
30.28	Octadecanoic acid	1.04
30.38	**11–n–Decylheneicosane**	**2.23**
30.66	Docosanoic acid	0.91
30.75	**Octacosane**	**2.27**
31.09	1,5-Dimethoxy-2,4-bis(3-methylphthalidyl)benzol	0.75
31.16	Nonacosane	0.93
31.35	(+)-Otochilone	0.72
31.38	1-Eicosene	0.49
31.58	1-Nonadecene	1.35
31.64	Cyclotetracosane	1.19
32.07	Octacosane	1.08
32.17	**9–Hexacosene**	**9.40**
32.20	**Ethyl vallesiachotamate**	**2.17**
32.26	Octacosanoic acid, methyl ester	1.04
32.37	**Cyclooctacosane**	**2.28**
32.73	**Cyclotetracosane**	**10.08**
33.26	Eicosane	0.82
33.35	1-Eicosanol	1.41
34.09	**Tricosane**	**2.38**

Bold faceted compounds appear abundantly with % area greater than 2 %.

4. Discussion

Cancerous illnesses associated with HIV-AIDS and the prevalence of persistent opportunistic infections are of paramount importance in various health care systems. These infections are lethal. The antimicrobial activity of fractions from *J. zeyheri* roots was investigated in this study. Fraction JZ2 revealed the lowest MIC value of 40 μg/mL against *K. pneumoniae* and 80 μg/mL against *M. hominis*, *C. albicans* and *S. aureus*. These microorganisms are well implicated as the possible agents of various sexually transmitted related infections in humans, along with other microbes such as *Treponema pallidium*, *Neisseria gonorhoeae*, *Chlamydia trachomatis*, *Gardnerella vaginalis* and various *Ureaplasma* and *Streptococcus*

species[29-31]. It is important to note that the general consensus on the plant derived antimicrobial agents is that the fractions and plant extracts should reveal a MIC value of 100 μg/mL or less to be referred to as potent and worth of isolating possible antimicrobial agents[32,33]. These may well suggest that the antimicrobial compounds from *J. zeyheri* roots are prevalent in fraction 2 (JZ2). In the current study, the fractions yielding MIC values ranging from 101 to 350 μg/mL are moderately active while those >350 μg/mL are less active. These may well suggest that the antimicrobial activity of the fractions is in the order JZ2>JZ4>JZ3>JZ1. It is also important to note that JZ4 revealed a potent antimicrobial activity yielding a MIC value of 80 μg/mL against both *P. vulgaris* and *K. pneumoniae*. Along with *E. coli*, various *Citrobacter* species and *P. aeruginosa*, these microorganisms have been recently isolated in most cancer patients in hospitals with higher resistance to commonly used antibiotics[34-36]. However, some authors propose the use of such drugs in combination with plant-derived antimicrobials to curb multi-resistance, treat and manage various acute and chronic infections and relieve side effects associated with synthetic drugs[37,38].

Although other authors recently reported the antimicrobial activity of some extracts from *J. zeyheri* roots against related pathogens[20,39], these may not well compare with the current study due to difference in extraction methods and the nature and origin of selected microorganisms.

In the cytotoxicity studies against HDF, the fractions revealed some cytotoxicity against the selected normal human skin cell line. JZ4 and JZ2 significantly revealed 50% lethal dose (LC_{50}) value of 28.96 and 33.45 μg/mL, respectively against HDF. Although some authors refer to a LC_{50} value of less than 100 μg/mL to be toxic[40-42], the American National Cancer Institute refer to an LC_{50} of less than 30 μg/mL to be toxic[43]. These may well suggest that JZ2 and JZ4 revealed some higher degree of toxicity towards HDF cell line. These results are worrying, considering that the plant species is also used as a cosmetic agent, applied directly to the skin among Xhosa ethnic group. Furthermore, JZ3 revealed the highest SI value of 2.08 against *K. pneumoniae*, and a notable SI value of 1.04 against *M. catarrhalis* and *S. aureus*. Higher SI values are indicative of higher safety margin. However, the *in vitro* results may not always translate into *in vivo* studies. There is a need therefore to carry the *in vivo* studies, validating the use of the plant species in the treatment of skin related infections including ache.

The anti-oxidative potential of *J. zeyheri* fractions was also studied. The fractions chelate iron better than inhibiting the free radicals such as ABTS and DPPH. JZ2 revealed the lowest IC_{50} of 19.66 and 22.63 μg/mL against DPPH and ABTS, respectively, while JZ3 had IC_{50} value of 1.73 μg/mL in the iron chelating assay. In agreement with our findings, Khamtache-Abrerrahim *et al*[44] reported fractions from other plant species possessed better inhibition against ABTS than DPPH. The plant-derived products with higher antioxidant activity against at least two radicals or more assays that are different are likely to prevent cellular damage and excess oxidative injury arising from such free radicals[45]. In the current work, only JZ2 fits into this category.

Moreover, in the anti-proliferative assay, JZ2 fraction revealed the lowest LC_{50} value of 8.83 μg/mL against Caco-2 cell line. Contrarily, other authors reported plant-based fractions inhibited other cancerous cell lines better than Caco-2[46-48]. Fractions, compounds and extracts from other *Jatropha* species also revealed some noteworthy anti-proliferative effect against a variety of cancerous cell lines[49-51]. When using the standards documented by American National Cancer Institute that refer to an LC_{50} of less than 30 μg/mL to be a potent anti-proliferative agent[43], only JZ2 revealed a potent activity against Caco-2, while all the fractions revealed less activity against both MCF-7 and A547. Besides, the LC_{50} value of 40 μg/mL was referred to as having potent anti-proliferative activity[52]. Cyclotetracosane (saturated hydrocarbon) and 9-hexacosene (Alpha-olefin) were the two major compounds obtained from JZ2 fraction which exerted a noteworthy antimicrobial, antioxidant and selective anti-proliferative effect against Caco-2. These compounds have been identified using GC-MS from other plant-based food sources worldwide and reported to exert a potential antimicrobial and antioxidant activity[53,54]. These compounds, both individually or in combination, may well explain the biological activity of JZ2 and in a way validate the use of the plant species in African traditional medicine. But other authors reported the phenolic acids and flavonoids to be responsible for most biological activities[55-59].

J. zeyheri is a rich source of anti-bacterial, antioxidant and anti-cancer phytocompounds, with the major saturated hydrocarbon (cyclotetracosane) and an alpha-olefin (9-hexacosene). JZ2 revealed a potential antimicrobial and antioxidant activity. The fraction further revealed a selective inhibition of Caco-2. However, the fraction revealed some degree of toxicity to a normal skin cell line (HDF), suggesting that it may be harmful to human skin. Further *in vivo* studies need to be done to validate such toxicity against other normal human cell line. Furthermore, the mode of action of these fractions against both cancerous cell lines and microorganisms still needs to be further explored. The antioxidant activity of the fraction may well explain that it could curb oxidation in immune-compromised individuals and improve the general well-being of patients thereof. There is also a need to explore the antimicrobial activity of the fractions and/or identified compounds against agents of sexually transmitted infections and multi-drug resistant microbes that may severely threaten human life. The anti-inflammatory activity of fractions and extracts from the plant species also needs to be explored.

Generally, cancers associated with HIV may result in various inflammation from different body parts of a patient. For this reason, there is a need to explore the anti-inflammatory activity of the fractions, extracts and compounds from *J. zeyheri* roots.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

Authors are thankful for Stellenbosch University (Mr Lucky Mokoena) for assisting with the running of samples for GC-MS (chemical profile).

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