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Isolation of antileishmanial, antimalarial and antimicrobial metabolites from *Jatropha multifida*

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PEER REVIEW

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Comments

As a whole this is a good study to unveil the medicinal value of the plant, *J. multifida* that has been reported to exhibit high medicinal value especially against leishmaniasis. Moreover the known metabolites, microcyclic lathyrane diterpenoids, multifidone and multifidinol that are isolated from the plant have exhibited inhibition of antileishmanial, antimalarial and antimicrobial actions against the tested organisms. Such class of compounds has been proved to be promising antileishmanial and antimalarial agents.

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ABSTRACT

Objective: To investigate the antileishmanial, antimicrobial and antimalarial activities of the pure metabolites from *Jatropha multifida* used in African ethnomedicine.

Methods: The methanolic stem bark extract of *Jatropha multifida* used in Nigerian folk medicine as remedy against bacterial infections was subjected to column chromatography and HPLC analyses to obtain three known metabolites, microcyclic lathyrane diterpenoids (1-3). Structures were confirmed by comparison of 1D and 2D spectral data with literature.

Results: The three compounds exhibited inhibition of antileishmanial, antimalarial and antimicrobial actions against the tested organisms with compouds 2 and 3 active against *Cryptococcus neoformans* at IC_{50} of 8.2 and 8.7 μg/mL, respectively.

Conclusions: The research lends support to the ethnomedicinal use of the plant in combating microbial infections, leishmaniasis and malarial infections.

KEYWORDS

Jatropha multifida, Stem bark, Antimalarial, Leishmaniasis, Antimicrobial

1. Introduction

A large portion of the world population, especially in developing countries depends on the traditional system of medicine against a variety of diseases. Several hundred genera are used medicinally, mainly as herbal preparations in the indigenous systems of medicine in different countries and are sources of very potent and powerful drug which

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have stood the test of time and modern chemistry has not been able to replace most of them. The World Health Organization reported that 80% of the world's population rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents[1]. Due to the indiscriminate use of antimicrobial drugs the microorganisms have developed resistance to many antibiotics. This has created immense clinical problems in the treatment of infectious diseases[2]. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hyper sensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions[3]. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties.

Malaria is the major tropical disease due to parasites, responsible for significant morbidity and mortality in the world. A dramatic recrudescence of malaria is ongoing due to the increasing resistance of vectors to insecticides and the progressive resistance of the parasite, mainly *Plasmodium falciparum* (*P. falciparum*), to drugs. These developments and the difficulty of creating efficient vaccines underline the urgent need for new antimalarial drugs. In endemic countries, accessible treatments against malaria are mainly based on the use of traditional herbal remedies. Indeed, indigenous plants play an important role in the treatment of many diseases and 80% of people worldwide are estimated to use herbal remedies[4]. *P. falciparum* is responsible for more than 200 million episodes of clinical malaria, mostly from tropical and subtropical zones, and results in over one million deaths per year in Africa[5]. Malaria causes high mortality and morbidity in tropical regions of Africa, Asia and South America[6]. Natural products from plants or other organisms, represent a virtually inexhaustible reservoir of molecules, most of which are hardly explored and can constitute lead molecules for new antimalarial drugs, such as artemisinin, initially isolated from *Artemisia annua*[7].

Leishmaniasis is a major public health problem and it can cause many different clinical manifestations in humans. It is caused by Trypanosomatidae of the *Leishmania* genus spread in Africa, Asia, Europe, North and South America. It has been estimated that 12 million people are infected in the tropical and subtropical areas of five continents, and that 2 and 0.5 million cases of cutaneous and visceral leishmaniasis are reported each year[8]. The lack of an effective anti-leishmanial drug has led to a renewed interest in the study of traditional remedies as sources for the development of new chemotherapeutic compounds with better activity and less toxic effects. The drugs currently used for the treatment of leishmaniasis are still based on pentavalent antimonials as sodium stibugluconate (Pentostam®) and meglumine antimoniate (Glucantime®). However, these drugs are relatively toxic and expensive and the treatment duration is usually long and unbearable. Furthermore, resistance to these compounds was observed. Quite a lot of funds and resources have gone into the search of novel leads from natural products with emphasis on medicinal plants for combating them.

In our search for hepatitis C virus inhibitors rRNA inhibitors and antileshmanial agents from traditional medicines, we investigated extracts and fractions of *Jatropha multifida* (*J. multifida*) from five different traditional Nigerian medicines. *J. multifida* is used in Nigerian folk medicine for the treatment of parasitic infections, cancer and hepatitis[9]. It is worthy to note that traditional medical practitioners have achieved success with the use of this plant as remedies against hepatitis and leishmaniasis.

J. multifida otherwise known as coral bush is a fast growing evergreen shrub or small tree belonging to the Euphorbiaceae family. The roots, stems, leaves, seeds and oil of the plant have been widely used in African folk medicine for the treatment of oral candidiasis, gonorrhoea, fever, astriction, wounds and skin infections[10-12].

2. Materials and methods

2.1. Plant materials

Fresh stem bark of *J. multifida* was collected from Edo State, Nigeria between January and June, 2013. It was identified and authenticated by Mr. Ugbogu OA and Shasanya OS of the Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimen FHI 93265 is deposited in the herbarium.

2.2. Extraction and isolation

The powdered stem bark (100 g) material was extracted with 500 mL methanol for 48 h (3×) by cold maceration, filtered and the filtrate was evaporated to dryness under reduced pressure to obtain the crude extract of *J. multifida*. The total extract was subjected to VLC with hexane: ethylacetate 50%, ethylacetate 100%, ethylacetate: methnol 50%, and methanol 100%. The hexane: ethylacetate fraction (3 g) was chromatographed on LH-20 sephadex eluting with dichloromethane: methanol (1:1) isocratically to obtain fraction A1 to A23. Fractions A1 to A5 was further purified by reversed-phase high performance liquid chromatography (diameter, 10 mm × length, 250 mm, 10 μm) eluted with CH_3OH: H_2O (60:40) to afford compounds 2 (tR 4.3 min, 6 mg) and 3 (tR 6 min, 4 mg). Compound 1 (9 mg) was obtained as colourless oil in fraction A10. The chemical structures of compounds 1, 2 and 3 were established by 2D nuclear magnetic resonance data and comparison of their spectral data with previously reported literature.

2.3. Antimicrobial testing

In vitro antimicrobial activity against a panel of microorganisms, including fungi: *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *Cryptococcus neoformans* (ATCC 90113) and *Aspergillus fumigatus* (ATCC 204305) (*A. fumigatus*); and bacteria: *Staphylococcus aureus* (ATCC 29213) (*S. aureus*), methicillin-resistant *S. aureus* (ATCC 33591), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068) (*M. intracellulare*), was determined using modified versions of the CLSI/NCCLS methods[13,14]. *M. intracellulare* and *A. fumigatus* was tested using an Alamar Blue method[15]. All organisms were obtained from the American Type Culture Collection (Manassas, VA). Samples, dissolved in dimethylsulfoxide, were serially diluted in saline and transferred in duplicate to 96 well micro plates. Susceptibility testing was performed for all organ cate to 96-well flat bottom micro plates. Microbial inocula were prepared by correcting the OD630 of microbe suspensions in incubation broth to afford final target inocula. Controls [fungi: amphotericin B; bacteria: ciprofloxacin (ICN Biomedicals, OH)] were included in each assay. All plates were read at 530 or 544 (ex)/590 (em) nm (*M. intracellulare* and *A. fumigatus*) prior to and after incubation. Percent growth was plotted versus test concentration to afford the IC_{50} using XLFit (Alameda, CA).

2.4. Antimalarial/Parasite lactate dehydrogenase assay

The *in vitro* antimalarial assay procedure utilized was an adaptation of the parasite lactate dehydrogenase assay[16]. The assay was performed in a 96-well microplate and included two *P. falciparum* clones [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. In primary screening the crude plant extracts were tested, in duplicate, at a single concentration of 15.9 ífg/mL only on the chloroquine-sensitive (D6) strain of *P. falciparum*. The extract showing >50% growth inhibition of the parasite was subjected to screening. For bioassay-guided fractionation, the column fractions were also tested only at single concentration. The pure compounds were subjected to additional testing for determination of IC_{50} values. The standard antimalarial agents chloroquine and artemisinin were used as positive controls, with dimethylsulfoxide (0.25%) as the negative (vehicle) control. The selectivity indices (SI) were determined by measuring the cytotoxicity of samples on mammalian cells (VERO; monkey kidney fibroblast). All experiments were carried out in duplicate.

3. Results

The ^{1}HNMR-guided fractionation of a methanol extract of *J. multifida* afforded three known compounds 1, 2 and 3 all of which are macrocyclic lathyrane diterpenoids, multifidone and multifidinol respectively. The molecular formula of 1 was determined to be $C_{20}H_{30}O_4$ from the HRESIMS data, with *m/z* 357 [M+Na]. The ^{13}CNMR and ^{1}HNMR (Table 1) revealed the presence of a lathyrane diterpene in agreement with literature report[17]. The presence of the cyclopropane moieties in the compounds is diagnostic of the compounds. The compound was established as 14-deoxy-1β-hydroxy-4(4E)-jatrogrossidentadione. Compound 2 was obtained as colourless solid, with a molecular formula of $C_{20}H_{32}O_4$ from its HRESIMS data. The presence of hydroxyl and carbonyl groups in the molecule was diagnosed in infrared radiation. The ^{13}CNMR and ^{1}HNMR (Table 1) also showed the structure to be related to the lathyrane diterpenoid. Compound 2 was named as 15-deoxy-1β-hydroxy-4(4E)-jatrogrossidentadione (Figure 1). Compound 3 was structurally similar to 2 except that the ring A of 3 was unsaturated while that of 2 was saturated (Figure 1). Compounds 1, 2 and 3 were diterpenoids having a lathyrane-diterpenoid skeleton in seco-form. Diterpenoids in *Jatropha* species have been known to possess anticancer activity[18,19]. Three metabolites are known compounds.

Table 1

^{1}HNMR and ^{13}CNMR spectral ($CDCl_3$, 400 MHz) data of compounds 1-3.

	Compound 1		Compound 2		Compound 3	
Position	^{1}HNMR	^{13}CNMR	^{1}HNMR	^{13}CNMR	^{1}HNMR	^{13}CNMR
1	6.70	155.5	1.90, 1.63	42.0	5.40	128.6
2	-	143.8	1.94	40.4		148.6
3	-	195.7	4.04	82.9	4.90	80.2
4	-	137.6		145.3		145.4
5	6.55	141.8	5.68	134.6	6.40	136.8
6	-	74.6		74.5		74.6
7	2.15	42.7	1.82	41.6	1.82	42.1
					1.79	
	1.80		1.60		1.79	19.4
8	1.84	20.6	1.54	19.5	0.80	
	1.68		0.83		0.44	
9	0.96	26.2	0.46	27.3		27.5
	0.44					17.1
10	-	17.0		17.8	0.64	19.4
11	1.16	21.5	0.64	19.3	1.79	28.5
	1.52	24.8	1.73	28.4		
	1.13		1.43			
13	1.70	30.5	3.06	38.9	2.84	38.6
14	3.84	81.4		210.6		211.6
15	-	79.8		84.6		88.8
16	1.86	10.4	1.22	17.2	1.96	13.9
17	1.40	29.3	1.26	29.8	1.28	29.4
18	0.99	28.5	0.98	28.5	0.99	29.9
19	o.69	14.3	0.72	14.6	0.72	14.6
20	1.21	21.4	1.23	17.2	1.14	16.8
-OH	5.56	-	5.26		5.52	
					4.14	

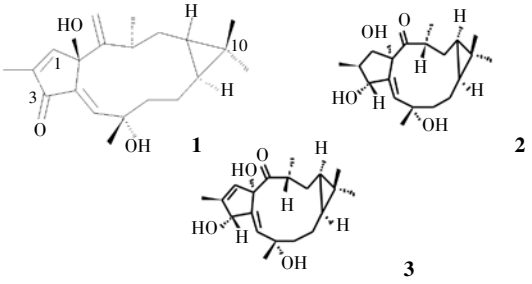

Figure 1. Chemical structures of compounds 1-3.

Compounds 2 and 3 exhibited strong *in vitro* inhibition against *P. falciparum* at IC_{50} values ranging from 1 485 and 47 600 μg/mL. Of the 3 metabolites tested (Table 2), compound 1 was considered to be the most active against the two clones of *P. falciparum*in culture with IC_{50} values 7 231.5 μg/mL and 7 805.6 μg/mL respectively.

The results of the antimicrobial activity of compounds 1-3 shown in Table 3 revealed potent activity of compounds 1, 2 and 3 against the panel of microorganisms used in the study. The result of the antileishmanial activity of the compounds are presented in Table 4. All the compounds 1-3 were active against *Leishmania donavoni* at the tested concentrations. The most potent compound was 2 with IC_{50} and IC_{90} of 4.69 and 6.28 μg/mL, respectively.

Table 2

Activity of metabolites 1-3 against *P. falciparum*.

Metabolites	*P. falciparum* D6		*P. falciparum* W2		VERO
	IC_{50} (μg/mL)	SI	IC_{50} (μg/mL)	SI	
1	7 231.5	>6.6	7 805.6	> 6.1	>47 600
2	>14 875.0	>3.2	25 052.5	>1.9	>47 600
3	>47 600.0	1.0	>47 600.0	1.0	>47 600

Table 3

Antimicrobial activities of metabolites 1-3.

Test organism	IC_{50} (μg/mL)				
	1	2	3	Amphotericin B	Ciprofloxacin
CA	>200.0	32.2	167.9	0.27	NT
CG	130.5	30.6	23.5	0.39	NT
CK	>200.0	15.5	>200.0	0.65	NT
AF	>200.0	14.7	155.6	1.18	NT
CN	>200.0	8.7	8.2	0.24	NT
SA	34.8	23.6	>200.0	NT	0.120
MRSA	28.4	55.2	>200.0	NT	0.100
EC	>200.0	>200.0	>200.0	NT	0.006
PA	>200.0	183.5	>200.0	NT	0.090
MI	>200.0	>200.0	>200.0	NT	0.400

CA: *Candida albicans*, CG: *Candida glabrata*, CK: *Candida krusei*, AF: *A. fumigatus*, CN: *Cryptococcus neoformas*, SA: *S. aureus*, MRSA: methicillin-resistant *S. aureus*, EC: *Escherichia coli*, PA: *Pseudomonas aeruginosa*, MI: *M. intracellulare*. NT: Not tested.

Table 4

In vitro antileishmenial (*Leishmania donovani*) activity of metabolites 1-3.

Metabolites	*Leishmania donavoni*		Test concentration (μg/mL)
	IC_{50} (μg/mL)	IC_{90} (μg/mL)	
1	11.90	22.67	40-1.6
2	4.69	6.22	40-1.6
3	4.56	6.31	40-1.6
Amphotericin B (control)	0.28	0.46	40-1.6

IC_{50} and IC_{90} are the sample concentration that kills 50% and 90% cells compared to vehicle control.

4. Discussion

The phytochemical investigation of the stems of *J. muiltifida* led to the isolation of three known constituents which were established by the unequivocal 1D and 2D NMR experiments. The structures were also identified by comparison of data with reported data in literature. Hence, the three known diterpenoid compounds possessed a lathyrane nucleus. The lathyrane diterpenoids have been known to possess a number of interesting biological activities such as cytotoxic and anticancer properties. Hence, it was considered necessary to subject the compounds to antimalarial, antileishmanial and antimicrobial activities.

The strong inhibition of compound 1 against chloroquine-sensitive and the chloroquine-resistant *P. falciparum* isolates could be due to the presence of the exo methylene group in 1. The highest antifungal activity was consistently observed with compounds 2 and 3 (IC_{50} values of 8.7 and 8.2 μg/mL respectively) against *C. neoformans*. A comparison of activities of compounds 2 and 3 suggests that the unsaturation of the ring A in 3 considerably increases the antifungal activity. Compounds 1 and 2 showed marked activity against methicillin-resistant *S. aureus* at IC_{50} values of 28.4 and 55.2 μg/mL respectively, suggesting that the lathyrane diterpenoid nucleus could be responsible for the antibacterial activity.

The preliminary results of this investigation appear to indicate that a number of Nigerian medicinal plants have high potential of antileishmanial, antimicrobial and antimalarial activities.

This study, to the best of our knownledge is the first report of the antileishmanial and antimalarial activities of this class of compounds. Further work will be necessary to determine *in vivo* antileishmanial, antimicrobial and antimalarial activities, using experimental animals.

From this study only those compounds that are effective for both chloroquine-resistant and chloroquine-sensitive strains and which have low IC_{50} values should be developed further. The biological activities lend supports to the ethnomedicinal usage of the plant for which they are known and used for.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was in part supported by the Fulbright Senior Scholar Program granted to Dr A. Falodun to study at the School of Pharmacy, University of Mississippi. Funding was also by NIH, NIAID, Division of AIDS, Grant No. AI 27094 (antifungal) and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-1-603 (antibacterial). TETFUND/DESS/RP/UNIV/BENIN/VOL.111 2013 and URPC VC.23.

Comments

Background

The ^{1}HNMR-guided fractionation of the methanolic stem bark extract of *J. multifida* was investigated for phytochemicals resulting into the isolation of three known metabolites, microcyclic lathyrane diterpenoids, multifidone

and multifidinol. The three pure compounds exhibited inhibition of antileishmanial, antimalarial and antimicrobial actions against the tested organisms. According to the authors, this is the first report of the antileishmanial and antimalarial activities of this class of compounds.

Research frontiers

The present research work represents the antileishmanial, antimalarial and antimicrobial activities of the known microcyclic lathyrane diterpenoids isolated and purified from the stem bark of *Jatropha multifida*.

Related reports

The phytochemical investigation of the stems of *J. muiltifida* led to the isolation of three known constituents which were established by the unequivocal 1D and 2D NMR experiments. The preliminary results of this investigation appear to indicate that a number of Nigerian medicinal plants have high potential of antileishmanial, antimicrobial and antimalarial activities.

Innovations and breakthroughs

The traditional Nigerian medicines made of *J. multifida*, are used in Nigerian folk medicine for the treatment of parasitic infections, cancer, hepatitis and leishmaniasis. According to the authors this is the first report of the antileishmanial and antimalarial activities of microcyclic lathyrane diterpenoids that are isolated from the plant.

Applications

According to the literature the roots, stems, leaves, seeds and oil of the plant, *J. multifida*, have been widely used in African folk medicine for the treatment of oral candidiasis, gonorrhoea, fever, wounds and skin infections, and it is also used as purgative. Furthermore the local practitioners have achieved success with the use of this plant as remedies against hepatitis and leishmaniasis. The present experimental results prove the importance of the plant against leishmaniasis.

Peer review

As a whole this is a good study to unveil the medicinal value of the plant, *J. multifida* that has been reported to exhibit high medicinal value especially against leishmaniasis. Moreover the known metabolites, microcyclic lathyrane diterpenoids, multifidone and multifidinol that are isolated from the plant have exhibited inhibition of antileishmanial, antimalarial and antimicrobial actions against the tested organisms. Such class of compounds has been proved to be promising antileishmanial and antimalarial agents.

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