J. Afr. Ass. Physiol. Sci 5 (2): 111-120, December 2017

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Biological Activities of a Macrocyclic Diterpenoid Isolated from the Roots of *Jatropha gossypiifolia*

J.C. Ogbonna[1], I. Igbe[2]*, O. Erharuyi[1], V.O. Imieje[1] and A. Falodun[1]

[1]Departments of Pharmaceutical Chemistry and [2]Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Keywords:
Jatropha gossypiifolia, jatrophone, antiprotozoal, analgesic, cytotoxic activity

ABSTRACT

Background: Extracts of *Jatropha gossypiifolia* L. (Euphorbiaceae) have been used ethnomedicinally for the treatment of various ailments. The present study was to investigate the biological activity of Jatrophone, a macrocyclic diterpenoid isolated from the roots of *J. gossypiifolia*. **Methods:** Phytochemical studies followed by chromatographic separation of the methanol root bark extract led to the isolation of a macrocyclic diterpenoid, identified as jatrophone on the basis of NMR and mass spectra data and by comparison with literature. The isolated compound, jatrophone was evaluated for its antiprotozoal activity against *Plasmodium falciparum* strains [D6 (chloroquine sensitive) and W2 (chloroquine-resistant)], *Leishmania donovani* and *Trypanosoma brucei*. The antimicrobial activity was evaluated against type culture of selected fungi and bacteria. Analgesic activity was investigated using the acetic acid-induced writhing and hot plate model in mice. The cytotoxicity was assessed against VERO cell line (monkey kidney fibroblast) using neural red uptake method. **Results:** Jatrophone showed significant antiplasmodial and antileishmanial activities with IC_{50} of 0.55, 0.52 and < 0.4 µg/mL for *P. falciparum* (D6 strain), *P. falciparum* (W2 strain) and *L. donovani* respectively. Compound 1 was highly cytotoxic to VERO cell line with IC_{50} of 0.43 µg/mL. Jatrophone demonstrated significant analgesic effect with a percentage reduction in acetic acid-induced writhes of 54.03% and 66.35% at 5 and 10 mg/kg respectively. No significant antimicrobial activity was observed against the test organisms. **Conclusion:** The present study has shown that jatrophone possess antimalarial, antileishmanial, and analgesic activities.

INTRODUCTION

Jatropha gossypiifolia L. (Euphorbiaceae) commonly called *"bellyache bush"* or *"black physic nut"* belongs to the family, Euphorbiaceae. It is a pantropical species originating from South America but widely distributed in countries of tropical, subtropical, and dry tropical weather and tropical semiarid regions of Africa and the Americas (Parvathi *et al*., 2012). The common names in Nigeria include Ake mgogho (Igbo), Lapalapa pupa (Yoruba), Binidizugu (Hausa) (Odebiyi and Sofowora, 1978).

*Address for correspondence:
E-mail: Igbe.ighodaro@uniben.edu
Tel. +234 8035023349

Different parts of the plant including the leaves, stems, roots, seeds, and latex, are used ethnomedicinally in different forms of preparation in the treatment of variety of ailments. The leaves are used in the treatment of boils, carbuncles, eczema, stomach ache, itches, venereal diseases and as a febrifuge (Dhale and Birari, 2010; Parvathi *et al*., 2012). The roots are employed against leprosy, as an antidote for snakebite and in urinary complaints, while a decoction of the bark is used as an emmenagogue (Kirtikar and Basu, 1996).

J. gossypifolia has been reported for its analgesic, anti-inflammatory (Panda *et al*., 2009; Nagaharika *et al*, 2013), antiprotozoal (Sabandar *et al.*, 2013), neuropharmacological, anti-diarrheal (Apu *et al*., 2012), antimicrobial (Ogundare, 2007), molluscicidal, insect repellent (Khumrungsee *et al*., 2009), larvicidal (Bullangpoti *et al*., 2012), and anti-coagulating activities (Oduola *et al.*, 2005). Previous phytochemical investigation of the plant has reported the presence of tannins, lignins, phenolic compounds, flavonoids,

including the isolation of diterpenes and triterpenes, such as jatrophone, jatropholones A and B, jatrophatrione, jatrodien, apigenin, cyclogossine A and B, vitexin, isovitexin, gossypiline (Matsuse *et al*., 1999; Oduola *et al*., 2005; Khumrungsee *et al*., 2009).

Jatrophone is a macrocyclic diterpene isolated from *J. gossypiifolia* and *J. elliptica* (Kupchan *et al.,* 1975; Calixto and Sant'Ana, 1987). The natural derivatives of jatrophone, termed as hydroxyl jatrophones (2α-OH jatrophone, 2β-OH jatrophone and 2β-OH-5, 6-isojatrophone) were isolated from the roots of *J. gossypiifolia*. Jatrophone has been shown to possess multiple biological activities including inhibition of insulin release, inhibition of lymphocytes activation, and inhibition of tumor cells, molluscicidal activity and gastroprotective effects (Taylor *et al*., 1983; Pertino *et al*., 2007; Theoduloz *et al*., 2009). In the present study, jatrophone isolated from the root of *J. gossypiifolia* was evaluated for its possible antiprotozoal, antimicrobial, analgesic and cytotoxic activities.

MATERIALS AND METHODS

General experimental procedure

Optical rotations were measured on a Perkin-Elmer P-2000 polarimeter. Melting points were determined on a capillary melting point apparatus (M-560, Buchi labtechnik, Switzerland). UV spectra were recorded in MeOH on Evolution 300BB UV-Visible spectrophotometer (Thermo-Scientific, England). IR spectra were recorded on KBr discs on FTIR-8900 spectrophotometer (Shimadzu, Japan). The NMR spectra were determined on a Bruker Avance AV-500 spectrometer. Chemical shifts were expressed in parts per million (ppm) using TMS as internal standard. HR-EI-MS was recorded on a double focusing Magnetic sector mass analyzer (JEOL JMS-600H, Japan) at ionizing voltage of 70eV (direct probe). TLC analyses were performed on precoated silica gel 60 F_{254} plates. The plates were visualized under UV (254 and 366 nm) and by spraying with vanillin-sulphuric acid reagent, and drying.

Collection and Identification of Plant Material

Fresh roots of *Jatropha gossypiifolia* were collected from Iguosa estate, Benin City in September, 2014. The plant (fruits, flowers, and leaves) was identified and authenticated in the Forestry Research Institute of Nigeria (FRIN), Ibadan and a voucher specimen number FHI109968 was issued.

Extraction and Isolation

Powdered root bark (2.2 kg) was macerated in methanol (3 L) for 48 h. The methanol extract was concentrated at reduced pressure. Crude extract (107.3 g) was fractionated with 100% petroleum ether (2 L), petroleum:ether:ethyl acetate (1:1, 2 L), 100% ethyl acetate (2 L), ethyl acetate:methanol (1:1, 2 L) using vacuum liquid chromatography.

The petroleum ether-ethyl acetate fraction (11.6 g) was subjected to column chromatography [silica gel (150 g), 200-400 mesh] eluting with petroleum ether and increasing polarity with ethyl acetate up to 60% ethyl acetate at 2% increment. A total of 150 fractions were collected. Fractions 55-65 were combined after TLC analysis and re-chromatographed over silica gel eluting with petroleum ether:ethyl acetate:methanol (5:0.5:0.5) to obtain a colourless crystal.

Evaluation of Antiprotozoal Activity

Antimalarial assay

The *in vitro* antimalarial activity of the jatrophone was measured by a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity (Makler *et al*., 1993; Samoylenko *et al*., 2009). The assay was performed in 96-well microplate and included two *P. falciparum* strains [Sierra Leone D6 (chloroquine sensitive) and Indochina W2 (chloroquine-resistant)]. Briefly, a suspension of red blood cells infected with *P. falciparum* (D6 or W2) strains (200 µL, with 2% parasitemia and 2% hematocrit in RPMI - 1640 medium supplemented with 10% human serum and 60 µg/mL amikacin) was added to the wells of a 96-well plate containing 10 µL of test samples at various concentrations. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2, in a modular incubation chamber (Billups-Rothenberg, 4464 M) and incubated at 37°C, for 72 h. Plasmodial LDH activity was determined by using Malstat™ reagent (Flow Inc., Portland, OR). The IC_{50} values were computed from the dose response curves generated by plotting percent growth against test concentrations. DMSO, artemisinin and chloroquine were included in each assay as vehicle and drug controls, respectively. The selectivity index (SI) of antimalarial activity was determined by measuring the cytotoxicity of samples towards mammalian cells (VERO; monkey kidney fibroblasts).

Antileishmanial assay

The Antileishmanial activity of jatrophone was tested *ex vivo* using the parasite-rescue and transformation assay with differentiated THP1 cells infected with *Leishmania donovani* as described by Jain *et al*., 2012. In a 96-well microplate assay, the compound with appropriate dilution was added to the *Leishmania promastigotes* culture (2×10^6 cells/mL). The plates were incubated at 26°C for 72 hours and growth of Leishmania promastigotes was determined by Alamar blue assay. Pentamidine and amphotericin B were used as standard antileishmanial agents.

Trypanosomicidal assay

The isolated compound, jatrophone was screened again *T. brucei* using a method previously described by Jain *et al*., 2016. Briefly, a 2-day old culture of *T. brucei* in the exponential phase was diluted with IMDM medium to 5 X 10^3 cells/mL and dispensed in 384 well culture plates with 98 µL in each well plus 2 µLof test samples and incubated at 37ºC in a 5% CO_2 incubator for 48 hr . After 48 hr, 5 µL of alamarBlue was added to each well and the plates were incubated further for 24 hr. Standard fluorescence was measured on a Fluostar Galaxy fluorometer (BMG LabTechnologies) at 544 nm ex, 590nm em.

IC_{50} and IC_{90} values were computed from dose response growth inhibition curve.

Antimicrobial Assay

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* (ATCC), *Cryptococcus neoformans* (ATCC 90113) and *Aspergillus fumigatus* (ATCC 90906) and the bacteria *Staphylococcus aureus* (ATCC), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC), *Klebsiella pneumoniae* (ATCC) and *M. intracellulare* (ATCC 23068). Susceptibility testing was performed using a modified version of the CLSI methods (NCCLS, 1998; 2000a; 2000b). Susceptibility testing of *M. intracellulare* using the modified Alamar Blue™ procedure as described by Franzblau *et al*. Samples (dissolved in DMSO) were serially-diluted in 20% DMSO/saline and transferred (103L) in duplicate to 96 well flat bottom microplates. Microbial inocula were prepared in assay medium to afford target CFU/mL after addition to the samples. Growth, solvent and media controls were included on each test plate. Assay plates were read at 630 nm or 544 ex/590 em (*A. fumigatus* and *M. intracellulare*) before and after incubation using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Vermont) or the Polarstar Galaxy Plate Reader (BMG Lab Technologies), respectively. Percentage growth was plotted vs. test concentration to afford the IC_{50} (Franzblau *et al*., 1998). The minimum inhibitory concentration (MIC) is defined as the lowest test concentration that allows no detectable growth. The minimum fungicidal or bactericidal concentrations (MFC/MBCs) were determined by removing 5 µL from each clear well, transferred to agar and incubated until growth was seen. The MFC/MBC is defined as the lowest test concentration that allows no growth on agar. Drug controls [ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi] were included in each assay.

Cytotoxicity Assay

Cytotoxicity assay was performed in 96-well microtiter plates using neural red uptake method as previously described (Borenfreund and Puerner, 1985; Repetto *et al*., 2008). The cytotoxicity of the isolated compound was assessed against VERO cell line (monkey kidney fibroblast) cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 0.2% $NaHCO_3$ at 37ºC in an atmosphere of 95% humidity, 5% CO_2. Concentration ranges tested were between 0.19 – 48µg/mL. IC_{50} was calculated from dose-response curve as earlier described. The selectivity indices (SI) [the ratio between the cytotoxicity (VERO cells) and antiparasitic activities] were determined by measuring the cytotoxicity of samples on mammalian cells (VERO; monkey kidney fibroblast).

Antinociceptive Activity

Animals

Albino mice (20-30 g) of either sex were obtained from the Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were allowed to acclimatize for two weeks. They were fed with standard pellet diet (Bendel Feeds and Flower Mill, Ewu, Edo state, Nigeria) and allowed free access to water. The study was carried out following the approval from the Ethics Committee on the Use and Care of Animals, University of Benin, Benin City, Nigeria.

Acetic acid-induced writhing

The acetic acid-induced writhing test was done based on a modified method. Mice were randomly divided into four groups. Different groups received jatrophone (5 and 10 mg/kg) or acetyl salicylic acid (100 mg/kg) or distilled water (5 mL/kg) orally. The animals were treated 1 h prior to injection of 0.6% v/v acetic acid (10 mL/kg) intraperitoneally. The number of writhes by each mouse was counted immediately after acetic acid administration at intervals of 5 min for a period of 30 min (Igbe *et al*., 2009).

Hot Plate test

The hot plate test was used to measure the latencies of pain response according to a previously described method (Eddy and Leimback, 1953). Mice were divided into four groups. The animals were individually placed on a hot plate maintained at a constant temperature of 55 ± 1°C, the time interval from placement and shaking/licking of the paw or jumping was determined and the cut off time was set at 30 sec. Different group of mice received jatrophone (5 and 10 mg/kg) or distilled water (5 mL/kg) orally. Morphine (4 mg/kg s.c.) served as standard. The mice were placed at

30, 60, 90 and 120 min after treatment and response time recorded.

Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M) of three replicates. Data were subjected to one way analysis of variance (ANOVA), and difference between means was determined by Duncan's multiple range tests. Analysis was done using graph pad prism version 5.0. Results were considered significant when $P < 0.05$.

Table 1: NMR Data of Jatrophone (500 MHz for ^{1}H and 150 MHz for ^{13}C) in $CDCl_3$

Carbon No.	δ_C (ppm)	δ_H (Multiplicity, J in Hz
1	42.5	2.12 (dd, 5.5, 14.0) 1.83 (m)
2	38.3	2.96 (m)
3	147.1	5.77 (br s)
4	137.1	----
5	123.7	5.79 (br s)
6	141.8	----
7	202.1	----
8	128.7	5.98 (d, 16.5)
9	159.1	6.43 (d, 16.5)
10	36.6	----
11	41.2	2.84 (d, 14.5) 2.39 (d, 15.0)
12	183.3	----
13	114.1	----
14	203.9	----
15	99.8	----
16	18.9	1.06 (d, 7.0)
17	20.8	1.85 (s)
18	30.4	1.21 (s)
19	26.9	1.33 (s)
20	6.1	1.72 (s)

RESULTS

Chromatographic fractionation of the methanol extract of *Jatropha gossypifolia* root bark followed by silica gel column chromatographic separation of the petroleum ether-ethyl acetate fraction led to the isolation of a macrocyclic diterpenoid. On the basis of NMR, Mass spectra data and comparison with literature, compound was identified as jatrophone (Figure 1). Table 1 shows the NMR spectra data of jatrophone. Other physical and spectra characteristics of jatrophone are presented below.

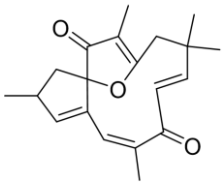

Fig. 1: Chemical structure of diterpenoid (Jatrophone) from the roots of *Jatropha gossypiifolia*

Jatrophone

Colourless needles, m.p. 152-154°C, $[\alpha]_D^{25}$ + 342° (c = 0.13, $CHCl_3$). Molecular formula $C_{20}H_{24}O_3$ from HREIMS: m/z = 312.1731 [M^+] (cald. 312.1725), UV (MeOH) λ_{max} 222 nm, IR (KBr) cm^{-1} 2960.5 (C-H), 1656.7-1618.2 (C=C), and 1693.4 (C=O).

The antiprotozoal activity of jatrophone was evaluated *in vitro* against *Plasmodium falciparum* [D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains], *L. donovani* (promastigotes, axenic amastigotes and intracellular amastigotes in THP1 cells) and *T. brucei* trypomastigote form.

Jatrophone exhibited strong inhibition against *P. falciparum*. As presented in table 2, jatrophone was active against the two clones of the plasmodial parasite with IC_{50} values of 0.55 µg/mL and 0.52 µg/mL against D6 (chloroquine sensitive) and W2 (chloroquine resistant) clone, respectively.

The results of the antileishmanial and trypanomicidal activities of jatrophone are presented in Table 3. Jatrophone was active against the promastigote and amastigote forms of *Leishmania donavoni* as well as the trypomastigote form of *Trypanosoma brucei* at the tested concentrations. The IC_{50} ranged from < 0.4 – 1.04 µg/mL against *L. donovani* and 4.42 µg/mL against *T. brucei*.

Table 4 presents the antimicrobial activity of jatrophone. Jatrophone produced no significant antimicrobial activity against the panel of microorganisms used in this study.

Jatrophone exhibited potent analgesic effect both in the acetic acid-induced writhing test and the hot plate test. Table 5 represents the cumulative number of writhes in the acetic acid induced writhing test. Jatrophone (5 and 10 mg/kg) and aspirin (100 mg/kg) significantly (p < 0.05) reduced the writhing reflex (contraction of abdominal muscle and stretching of hind limbs) in a dose-dependent manner compared to control (distilled water). The percentage inhibition of mouse writhes after 30 minutes was 54.03% and 66.35% at 5 mg/kg and 10 mg/kg respectively. The effects of Jatrophone and morphine on the latency times of mice in the hot plate test are presented in table 6. Jatrophone (5 and 10 mg/kg) and morphine (4 mg/kg) significantly (P < 0.05) increased the reaction time of mice after 30 minutes of administration compared to control (distilled

Table 2: Antimalarial activity (IC_{50} values are in ng/mL) of jatrophone

Compound	Conc. tested	*P. falciparum*				VERO cells IC_{50}
		D6 IC_{50}	SI	W2 IC_{50}	SI	
Jatrophone	528.9 - 4760 ng/mL	<528.9	>9	<528.9	>9	>4760
Jatrophone repeat	19.59 - 4760 ng/mL	549.5	0.8	521.2	0.8	431.9
Chloroquine	26.4 – 238 ng/mL	15	>317	15	>28.7	NC
Artemisinin	26.4 - 238 ng/mL	10	476	10	43.2	NC

D6=Chloroquine sensitive clone; W2=Chloroqine resistant clone; SI= Selectivity Index=VERO IC_{50}/IC_{50} *P. falciparum;* NC= Not cytotoxic

Table 3: Primary and Secondary antileishmanial screening (IC_{50} and IC_{90}) of jatrophone

Compounds/drugs	***L. donvani* Promastigote**		***L. donvani* Amastigote**		***L. donovani Amastigote* + THP**		***T. brucei***		**THP**		**Test Conc. (µg/mL)**
	IC_{50}	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	
Jatrophone	<0.4	-	<0.4	<0.4	1.04	1.33	4.42	7.76	0.67	1.27	0.4 - 10 µg/mL
Amphotericine B	0.202	0.376	0.848	1.215	0.128	0.215	NT	NT	-	-	0.08 – 2.0µg/mL
Pentamidine	0.976	2.367	-	-	0.784	1.278	NT	NT	-	-	0.4 – 10 µg/mL
DMFO	-	-	-	-	-	-	-	-	4.919	9.100	0.8 - 20 µg/mL
Primary screening results of compound 1 showing percentage inhibition											
Jatrophone	97		98		96		96		98		

Table 4: Antimicrobial activity of jatrophone (IC_{50}/MIC (µg/mL)

Compound/drug	C. albicans IC_{50}	A. fumigatus IC_{50}	C. neoformans IC_{50}	S. aureus IC_{50}	MRSA IC_{50}	E. coli IC_{50}	P. aeruginosa IC_{50}	K. pneumoniae IC_{50}	VRE IC_{50}	Test Conc.
Secondary Screening (jatro)	5	>20	>20	-	>20	>20	>20	>20	>20	0.8-20 µg/mL
Tertiary Screening (jatro)	>20	>20	>20	-	>20	>20	>20	>20	>20	0.8-20 µg/mL
MIC	>20	>20	>20	-	>20	>20	>20	>20	>20	0.8-20 µg/mL
Amphotericine B	0.133	0.35	0.153	-	>100	>100	>100	>100	>100	4-100 µg/mL
Ciprofloxacin	>10	>10	>10	-	9.235	<0.01	0.419	>10	>10	0.4-10 µg/mL

Jatro = jatrophone

water). There was no significant difference in the mean reaction time between the two doses tested.

DISCUSSION

Jatropha gossypiifolia is a well-known medicinal plant, parts of the plant particularly the leaves, stem barks and roots are employed in various herbal preparations for the treatment of fever, pain, as laxatives, antitoxin, antimalarial and various kinds of microbial infections (Balee, 1994; Asprey and Thornton, 2005; Burkhill, 1994; Lans *et al.*, 2001; Dash and Padhy, 2006; Dabur *et al.*, 2007; Kayode and Omotoyinho, 2008; Ogundare, 2009).

In the present study, the root bark of *Jatropha gossypifolia* was extracted and the extract was subjected to pre-fractionation by vacuum liquid

Table 5: Time course of the effect of jatrophone on acetic acid-induced mouse writhes

Group		Number of Writhes						
		0-5 min	5-10 min	10-15 min	15-20 min	20-25 min	25-30 min	% Inhibition after 30 min
Control	-	6.8±0.50	20.8±0.52	48.2±0.59	71.8±1.04	88.6±0.96	104.0±0.92	-
Jatrophone	5mg/kg	1.4±0.22*	7.6±0.67*	18.4±0.88*	28.6±1.04**	46.6±1.32*	47.8±1.95**	54.03
	10mg/kg	1.2±0.16*	6.6±0.22*	16.4±0.54**	22.0±0.40**	30.4±0.46**	35.0±0.49**	66.35
Aspirin 100mg/kg		1.2±0.23*	8.0±0.57*	17.6±0.46**	20.8±0.66**	22.8±0.88**	26.4±0.83**	74.60

Data represent mean ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control (n = 5 for each group)

Table 6: Effect of Jatrophone on reaction time of mice in the hot plate test

Group	Dose (mg/kg)	Reaction time (secs)				
		0 min	30 min	60 min	90 min	120 min
Control	-	3.8±0.66	4.0±0.64	6.1±0.22	6.3±1.00	5.1±0.69
Jatrophone	5	3.7±0.07	10.3±0.17**	11.4±0.34*	10.5±0.36*	10.3±0.10**
	10	4.1±0.32	11.8±0.33**	12.9±0.22**	10.1±0.21*	9.4±0.15*
Morphine	4	4.4±0.22	14.4±0.48**	15.8±0.18**	14.9±0.18*	13.0±0.34**

Data represent mean ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control (n = 5 for each group)

chromatography to obtain four fractions (petroleum ether, petroleum:ether-ethyl acetate (1:1), ethyl acetate and ethyl acetate-methanol). Further chromatographic separation of the petroleum ether-ethyl acetate fraction resulted in the isolation of a macrocyclic diterpenoid (jatrophone). Jatrophone was obtained as a colourless needles with molecular formulae $C_{20}H_{24}O_3$ deduced from the high-resolution mass spectrometry (HREIMS) with molecular ion [M^+] peak at m/z = 312.1731. The UV spectrum exhibited absorption at λ_{max} 222 nm, Infrared spectrum showed absorptions at 1656.7-1618.2 and 1693.4 which were suggestive of a highly conjugated carbonyl functionality. The ^{1}H-NMR spectrum of **1** displayed signals for four vinylic proton at δ_H 6.43 (d, *J* = 16.5, H-9), 5.98 (d, *J* = 16.5, H-8), 5.79 (br s, H-5) and 5.77 (s, H-3), and five methyl protons at δ_H 1.85 (s), 1.72 (s), 1.33 (s), 1.21 (s) and 1.06 (d, *J* = 7.0) (Table 1). The ^{13}C-NMR displayed signals for twenty (20) carbon resonances including two carbonyl carbons at δc 202.1 (C-7) and δc 203.9 (C-14), eight olefinic carbons in the region δc 114.1 – 183.3, five methyl carbons (δc 6.1 – 30.4), two methylene carbons at δc 42.5 (C-1) and 41.2 (C-11), one methine carbon at δc 38.3 (C-2) and two quaternary carbons resonating at δc 36.6 (C-10) and δc 99.8 (C-15, oxycarbon). The isolated compound was characterized as the macrocyclic diterpenoid jatrophone (Figure 1) based on the spectral data and by comparison to data reported in the literature. A number of macrocyclic diterpenoids with the lathyrane, daphnane, tigliane, dinorditerpene, rhamnofolane and the pimarane moiety have been isolated from the genus Jatropha. These compounds have been shown to possess interesting pharmacological activities such as gastroprotective, cytotoxic, antitumor and antimicrobial activities (Pertino *et al*., 2007a; Devappa *et al*., 2011; Falodun *et al*., 2012; Falodun *et al*., 2014). Jatrophone has been shown to possess gastroprotective (Pertino *et al*., 2007b) and cytotoxic activity against in number of cancer cell lines (Sahidin, 2013; Sabandar *et al*., 2013; Asep *et al*., 2017).

In the present study, jatrophone was evaluated for its antiprotozoal activity against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum*, the promastigotes and amastigotes of *Leishmania donovani* and the trypomastigote form of *Trypanosoma brucei.*

The antimalarial evaluation of jatrophone revealed significant inhibition of *plasmodium* parasites growth in chloroquine sensitive *P. falciparum* (D6) and chloroquine resistant (W2) clones. As shown in table 2, jatrophone demonstrated potent antiplasmodial activity with IC_{50} values of 0.55 µg/mL and 0.52 µg/mL against D6 and W2 strains of *P. falciparum*, respectively. Although, this activity was quite remarkable, but was much less compared to that of the positive controls

chloroquine and artemisinin which gave IC_{50} values of 0.015 µg/mL and 0.010 µg/mL, respectively for both the D6 and W2 strains. For the antileishmanial and antitrypanosidal assays, jatrophone also exhibited significant leishmanicidal and trypanosomicidal activities against *L. donovani* promastigotes and amastigotes, and *T. brucei* blood stage trypamastigotes. The primary screen results showed percentage parasite inhibition of 96 – 98% (Table 3). The IC_{50} value of jatrophone was < 0.40 µg/mL for both the promastigote and amastigote forms of *L. donovani* while the IC_{50} was 1.04 µg/mL for the amastigotes of *L. donovani* in THP cells. THP cells are spontaneously immortalized monocyte-like cell line, derived from the peripheral blood of a childhood case of acute monocytic leukemia (M5 subtype) (Tsuchiya *et al*., 1980). *L. donovani* in THP cells represents an *in vitro* model developed for studying host-parasite cellular interactions in visceral leishmaniasis. The culture system has been shown to supports the growth of *Leishmania donovani* amastigotes with different parasite/macrophage ratios for up to 2 weeks (Chang and Dwyer, 1978). The present results indicated that the "forms" of the amastigotes (isolated or in host cells) had no significant effect on the leishmanicidal activity of jatrophone. The higher IC_{50} value in THP cells (1.03 µg/mL) compared to that for the isolated amastigotes (< 0.4 µg/mL) may be due to lesser penetrability of the compound through the host cell membrane and other interfering components of the host cells which may reduce the concentration of the compound that interacted with the parasites. Jatrophone also showed moderate activity against *T. brucei* with IC_{50} value of 4.42 µg/mL. The drugs Amphotericin B and Pentamidine were used as standards and the IC_{50} values ranged from 0.13 to 0.85 µg/mL for Amphotericin B and from 0.78 – 0.98 µg/mL for Pentamidine against the promastigote and amastigote forms of *L. donovani* (Table 3).

Jatrophone was subjected to cytotoxicity evaluation. As shown in table 2, jatrophone exhibited high cytotoxic effect against monkey kidney fibroblast (VERO) cell line with IC_{50} of 0.43 µg/mL. The compound was also toxic to THP cells with IC_{50} of 0.67 µg/mL (Table 3). The selectivity index (SI) of jatrophone was 0.8 which indicated almost equal level of toxicity against target cells (in this case the protozoal parasites) and normal/healthy cells. This observation may suggest a low margin of safety of Jatrophone. On the other hand, the positive control drugs (artemisinin and chloroquine) were nontoxic to VERO cells, with a high margin of safety as indicated by their high SI values. The SI for chloroquine was > 317 and > 28.7 for D6 and W2 clones of the plasmodial parasite, while the SI for artemisinin was 476 and 43.2 for the D6 and W2 clones respectively.

In the present study, jatrophone did not show any significant antimicrobial activity, the MIC value was > 20 µg/mL in the tertiary screen against the test organisms (Table 4).

In continuation of the investigation of jatrophone for biological activity, the compound was investigated for its analgesic activity using the acetic acid induced writhing and hot plate test in mice. The acetic acid induced writhing test has been widely used to assess the peripheral analgesic effect of analgesic agents ((Gene *et al*., 1998; Neves *et al*., 2007, Igbe *et al*., 2012). This model allows for easy observation, and presents great sensitivity to various analgesic, nonsteroidal, and steroidal anti-inflammatory drugs, as well as morphine-like compounds and other analgesic substances that act centrally or peripherally (Borges *et al*., 2013). It has been shown that the intraperitoneal injection of acetic acid causes the release of inflammatory mediator such as prostaglandins which excites nociceptors (Bose *et al*., 2007). Hence, the assay is used in screening for both peripheral and centrally acting analgesic agents. The result shows that jatrophone caused a significant ($P < 0.05$) and dose-dependent reduction in the number of cumulative writhing comparable to aspirin, and this was sustained over the 30 minutes period. After 30 minutes, the percentage reduction in the number of writhes was found to be 54.03% and 66.35% at 5 and 10 mg/kg of jatrophone, respectively while that of aspirin was 77.60% at a dose of 100 mg/kg (Table 5). The comparable analgesic action of jatrophone and that of aspirin may suggest similarity in the mechanism of action. Aspirin, a non-steroidal anti-inflammatory drug (NSAID), acts by inhibiting the synthesis of the pain-mediating autacoids prostaglandins (Simmons *et al*., 2004).

The Hot plate method is one of the most common heat nociception model use for evaluating the effectiveness of central acting drugs (Mandegary *et al*., 2004). Pain induced by thermal stimulus is usually selective for the centrally acting analgesics (Heidari *et al*., 2009; Khan *et al*., 2010). In this model, sensory nerves sensitize the nociceptors with minimal involvement of endogenous substances like the prostanoids. The results of the hot plate test as presented in table 6 showed that jatrophone demonstrated a significant ($P < 0.05$) and dose dependent increase in the mean reaction time up to the 60^{th} minutes compared to the control (distilled water). At the 90^{th} and 120^{th} minute, the increase in reaction latency was sustained over the 2-hour period and was slightly higher at the lower dose (5 mg/kg) compared to the higher dose (10 mg/kg). Although, the reaction latency was slightly reduced beyond the 60^{th} minute, but no statistical significant difference in the latency period at the 60^{th}, 90^{th} and 120^{th} minute. Furthermore, the increase latency time exhibited by jatrophone was comparable to that of the positive control (morphine at

4 mg/kg). The opioid analgesic such as morphine exert their analgesic effects via supra spinal and spinal receptors (Nemirovsky *et al*., 2001). The present result showed that the isolated compound (Jatrophone) exhibit antinociceptive effect at comparable level to that of morphine and this may suggest that the compound may act via a similar mechanism (Le Bars *et al*., 2001). Findings in both the acetic acid induced writhing and the hot plate tests suggest that Jatrophone maybe exerting its antinociceptive effect via both peripheral and central mechanisms.

Conclusion

The result presented in this study shows that the macrocyclic diterpenoid, Jatrophone from the root of *J. gossypiifolia* possess antiplasmodial, antileismanial and analgesic activities. The compound maybe responsible for the antimalarial, analgesic and anti-inflammatory activities of the extracts of *J. gossypiifolia* reported previously, hence, it may serve as a new source of bioactive natural products.

Acknowledgements

The authors would like to acknowledge the Natural Product Research Laboratory, Department of Pharmaceutical Chemistry and the department of Pharmacology and Toxicology, University of Benin for their support in this work. The authors would also like to thank the National Center for Natural Product Research (NCNPR), Institute of Pharmaceutical Sciences, University of Mississippi for the use of their laboratory for part of this work.

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