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BIOASSAY-GUIDED ISOLATION OF OCHROLIFUANINE: A BISINDOLE ALKALOID OF *DYERA COSTULATA* ACTIVE ON *TRYPANOSOMA BRUCEI BRUCEI*

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Dyera costulata, locally known as jelutong, have shown promising *in vitro* inhibitory activity against *Trypanosoma brucei brucei* strain BS221. The methanolic leaf extract, evaluated using Alamar Blue Assay, showed strong antitrypanosomal activity (IC_{50} 0.58 ± 0.01 µg mL^{-1}) and high selectivity index (SI value 169.5) towards the protozoa. While, the hexane leaf extract was moderately active (IC_{50} 4.4 ± 0.05 µg mL^{-1}; SI value of 22.7). Total alkaloids extracted from the active methanolic fraction showed strong activity and high selectivity (IC_{50} 0.08 ± 0.07 µg mL^{-1}, SI value 890) towards *T. b. brucei*. Bioassay-guided fractionation of the total alkaloid extract yielded an active compound **1** (IC_{50} 0.06 ± 0.03 µg mL^{-1}, SI value 76.3). Compound **1** was identified as ochrolifuanine from the bisindole alkaloid group, based on spectral analysis (MS, 1D and 2D NMR). The presence of alkaloids in *D. costulata* leaves may be indicative of the various bioactivities observed in this plant. Previously, ochrolifuanine has been reported for antimalarial and antimicrobial activity. This study reports, for the first time, on the antitrypanosomal activity of ochrolifuanine from *D. costulata*.

Keywords: Antitrypanosomal activity, Apocynaceae, total alkaloid extract, selectivity index (SI)

INTRODUCTION

Trypanosomiasis is a group of parasitic diseases affecting both man and animals, causing major health and economic problems in rural Sub-Saharan Africa. The causative agents of the disease are flagellated protozoa from the genus *Trypanosoma: Trypanosoma brucei rhodesiense* and *T. b. gambiense* cause human sleeping sickness, and *T. b. brucei* is morphologically and biochemically related to these two subspecies. Therefore *T. b. brucei* is used to study the *in vitro* antitrypanosomal activities in samples. The parasites are transmitted between vertebrate hosts by the tsetse fly (Brun et al. 2010, WHO 2013). Like other neglected diseases, it is a major health problem resulting from inadequate therapy and lack of an effective vaccine (Barret & Croft 2012). Moreover, many of the registered trypanocide drugs are toxic, require lengthy parenteral administration, lack efficacy and are unaffordable for most of the patients (Torres et al. 2014).

Chemists have synthesised a number of drugs which can be used against many target diseases, but not for all endoparasites (Wink 2012). Many of these drugs are old, developed many years ago, and some parasitic strains have become resistant towards them. Unfortunately, the development of new antiparasitic drug was not a priority investment for pharmaceutical companies because many of the parasitic diseases occur in poor countries where patients could not afford to pay a high price for the treatment. In view of these challenges, the search has been on-going to explore additional or alternative remedies from natural products which have great potential to eradicate trypanosomiasis diseases.

Nature is a potential source of such new drugs since it contains countless quantities of molecules with great variety of structures and pharmacological activities. Natural products are important leads for drug development and the database of natural products provide a convenient source for virtual molecular docking screening against drug targets (Harvey et al. 2015). This include lead compounds for

developing drugs for parasitic protozoal disease (Schmidt et al. 2012a, Schmidt et al. 2012b, Annang et al. 2016, Ogungbe & Setzer 2016). Medicinal plants have been a profit producer of important therapeutic agents, and many of the drugs developed today are plant-derived natural products or their derivatives (Kinghorn et al. 2011, Newman & Cragg 2012). Therefore, as an alternative to synthetic drug, natural products from plant species are potential sources of novel trypanocidal compounds (Butler 2008, Hannaert 2011, Wink 2012, Zimmermann et al. 2012).

In a previous study, methanolic extracts of a total of 119 Malaysian plant species were screened for antitrypanosomal activity. Leaf extract of *Dyera costulata* (Apocynaceae) exhibited the highest antitrypanosomal activity against *T. b. brucei* strain BS221 (Norhayati et al. 2013, Abd. Latif et al. 2015). Various studies have shown that plants from the Apocynaceae family have potential bioactivities such as anticancer, antimalarial, anti inflammatory and anti diabetic activity (Nurhanan et al. 2008), (De Aquino et al. 2013), (Wong et al. 2013), (Pereira et al. 2015). In traditional medicine, many species from this family have been used to treat fever, malaria, pain, diabetes and ectoparasitic diseases in the Asia Pacific regions (Omino & Kokwaro 1993, Wiart 2006, Wink 2012). Some of these species such as *Vallaris glabra*, *Aspidoperma desmanthum* and *Allamanda schottii* have been reported to possess anti-parasitic properties. (Andrade-Neto et al. 2007), (Wong et al. 2011), (Filho et al. 2013).

Dyera costulata, known as jelutong in Malaysia, is a tall forest tree spread in Southern Thailand, Malaysia and Sumatra. Six bisindole alkaloids (ochrolifuanine A, ochrolifuanines E and F, and 18-dehydroochrolifuanines A, E and F) have been isolated from the leaf extracts of *D. costulata* (Mirand et al. 1983). However, the bioactivities of these compounds have not yet been fully studied. Therefore, the present study reports for the first time on the antitrypanosomal activity of the bisindole alkaloid isolated from *D. costulata*.

MATERIAL AND METHODS

Chemicals and general methods

Chemicals such as vanillin and dragendorff's reagent and analytical grade solvents such as n-hexane, dichloromethane (DCM), chloroform, ethyl acetate (EtOAc), and methanol (MeOH) were purchaced from Merck Chemical (Germany). While acetic acid glacial, sodium chloride and ammonium hydroxide were purchased from Sigma-Aldrich (USA). The standard compound, pentamidine was purchased from Sigma-Aldrich (USA). Thin Layer Chromatography (TLC) was performed on 0.20 mm recoated silica gel aluminum sheet (Merck Kieselgel 60 F_{254}). Visualisation of TLC spots was carried out under UV light at 254 or 365 nm, spraying with Dragendorff reagent. Vacuum liquid chromatography (VLC) was carried out using Merck silica gel 60 (230–400 mesh) and solid phase extraction (SPE)-reverse phase (RP) was carried out using Merck, Lichroprep RP-18 (25-40 µm). The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data were recorded on Nuclear Magnetic Resonance (NMR) spectrometer with deuterated methanol (MeOD) as a solvent. Whereas ESIMS and ESIMS/MS were obtained on Thermo Finnigan LCQ Deca XP Plus mass spectrometer using electrospray ionization (ESI) voltage at 4500V. Chemical structure of the isolated compound was elucidated using NMR and mass spectrometry. All Chemicals for bioassay were purchased from Sigma-Adrich (USA), except for minimal essential medium (MEM) and fetal bovine serum (FBS) were purchased from Gibco (Australia).

Plant material

Leaf samples of *D. costulata* (3 kg) were collected from Forest Research Institute Malaysia (FRIM), Malaysia in the month of September 2012. The taxonomic identification of plant leaves was authenticated by a botanist at Forest Biodiversity Division, FRIM and was comparable with reference specimens at FRIM Herbarium (Voucher no: FMS 29764).

Preparation of the crude plant extract

Plant samples were dried in the drying oven for 5 days at 50 °C and ground to powder. The dried powder (555 g) was successively extracted by Soxhlet extraction apparatus using 5 L of hexane for 18 h. The mixture was then filtered with Whatman filter paper (grade no. 1) and evaporated to yield hexane extract (13.4 g, 2.41%). The residue was extracted again using 5 L of methanol with same extraction protocol to obtain methanol extract (164.6 g, 29.7%) as shown in Figure 1 (Siti Syarifah et al. 2011). All extracts were kept at 4 °C.

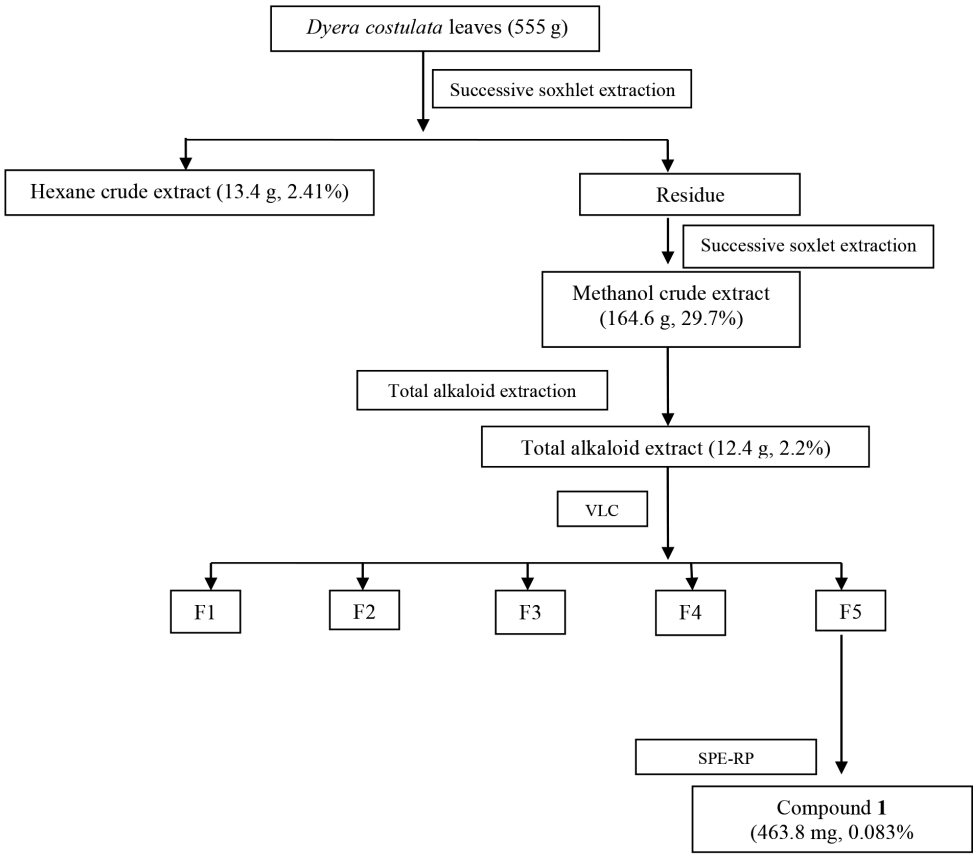

Figure 1 Bioassay-guided isolation of compound **1** from *Dyera costulata* leaf extract; VLC = vacuum liquid chromatography, F = fraction, SPE-RP = solid phase extraction-reverse phase

Preparation of total alkaloid extract

Crude alkaloids were extracted according to Harbornes (1973) with some modifications. A total of 164.6 g of methanol extract was added with 10% acetic acid for 24 h. Then the extract solution was adjusted to pH 10 with NH_4OH (25%, v/v) and further partitioned with chloroform. The chloroform layer was washed with sterile deionised water to neutralise the pH, dried with NaCl and concentrated to dryness under reduced pressure to obtain total alkaloid extract, 12.4 g, 2.2% (Figure 1).

Bioassay-guided isolation

The resulting extracts obtained were tested for antitrypanosomal activity and the selectivity index was determined by cytotoxicity against Vero cells. The total alkaloid extract (12 g) was then fractionated by using vacuum liquid chromatography (VLC) on silica gel 60 (230–400 mesh), eluted with mixture of increasing polarity *n*-hexane-ethyl acetate (100:0, 50:50, 0:100) followed by dichloromethane-methanol (100:0, 50:50, 0:100) to obtain 46 fractions. These fractions were combined based on similarity of TLC profile to yield 5 fractions, (F1–F5); F1 (0.52 g, 0.093%), F2 (0.8227 g, 0.15%), F3 (0.494 g, 0.089%), F4 (0.5514 g, 0.099%) and F5 (3.3821 g, 0.61%), which were then tested for antitrypanosomal activity. Fraction 5 which exhibited the most potent antitrypanosomal activity was then further separated using SPE-RP technique with mobile phase of H_2O/MeOH/DCM gradient (60:40:0, 10:90:0, 0:100:0, 0:90:10, 0:50:50) to obtain compound **1** (463.8 mg, 0.083%) as shown in Figure 1.

Parasite, cell line and culture conditions

Trypanosoma brucei brucei strain BS221, a derivative of S427, also known as MiTat 1.2/221, was obtained from the Swiss Tropical and Public Health Institute, Basel, Switzerland. The bloodstream form parasites were cultured in Balz minimal essential medium (BMEM) containing MEM supplemented with glucose

1 gL^{-1}, 25 nM HEPES, $NaHO_2$ 2.2 gL^{-1} and 10 mL MEM non-essential amino acids L^{-1}. A 0.14% (v/v) mercaptoethanol solution mixed with 10% (v/v) heat-inactivated FBS, 1 mM sodium pyruvate and 0.1 mM hypoxanthine were further added into the medium (Baltz et al. 1985). The parasites were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

Normal kidney (Vero) cell was purchased from the American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 5% (v/v) heat-activated FBS, penicillin (50 IU mL^{-1}) and streptomycin (50 µg mL^{-1}). The cell lines were incubated in a humidified atmosphere of 5% CO_2 at 37 °C (Siti Syarifah et al. 2011).

Antitrypanosomal activity

The extracts, fractions and isolated compound were evaluated for antitrypanosomal activity using methods described by Norhayati et al. (2013) with some modifications. The test samples were dissolved in absolute ethanol to produce final concentrations ranging from 0.03 to 6.25 µg mL^{-1} (extracts and fractions) and 0.001 to 0.100 µg mL^{-1} (compound), while the positive control pentamidine was dissolved in 5% dimethyl sulfoxide (DMSO). Solvents, 5% DMSO and 25% ethanol, and blank (sterile deionised MiliQ water) were used as negative control in the experiment. All assays were performed in a flat bottom 96-well microtiter plate. A 5 µL aliquot of pre-diluted extracts, fractions, isolated compound, pentamidine and negative control were added to each well of a 96-well microtiter plate. Subsequently, 95 µL of the trypanosome suspension at density 2.0×10^4 parasites mL^{-1} were added to each wells in triplicate plates. After 72 h of incubation at 37 °C, 10 µL of Resazurin dye was added into each well and plates were re-incubated for 4–5 h according to Alamar Blue assay method described by Räz et al. (1997). Plates were analysed using the Tecan Infinite M200 fluorescent plate reader at excitation wavelength 528 and emission wavelength 590 nm and Magellan™ data analysis software and the optical density (OD) was determined. The OD values were transferred into graphic program (Excel) and were evaluated to determine IC_{50} values by dose-response curve (Räz et al. 1997, Otoguro et al. 2008).

Antitrypanosomal activities of samples were classified into three categories; strongly active ($IC_{50} \leq 0.78$ µg mL^{-1}); moderate active (0.78 µg $mL^{-1} < IC_{50} \leq 6.25$ µg mL^{-1}) and not active ($IC_{50} >$ 6.25 µg mL^{-1}).

Cytotoxicity assay and selectivity index (SI)

The selectivity of test samples towards the parasite was evaluated based on cytotoxicity against Vero (Monkey normal kidney) cells. An aliquot of 90 µL cells in DMEM supplemented with 10% heat-inactivated FBS were seeded at the density cell of 4.0×10^4 cells mL^{-1} into wells on a 96 well microtiter plates. The plates were incubated at 37 °C in humidified atmosphere of 5% CO_2 for cell growth and to allow cell attachment. After 24 h, the medium was removed and added with fresh medium. Ten µL of pre-diluted test samples at concentration ranging from 0.1–100 µg mL^{-1} was added to the wells. Test plates were incubated for 72 h at 37 °C. The plates were assayed using Alamar Blue assay as described before, except the incubation time was slightly shorter within one to two hours. After incubation, plates were read at excitation wavelength 528 and emission wavelength 590 nm using fluorescent plate reader and Magellan™ data analysis software. The IC_{50} was determined as the concentration of test sample required to reduce 50% of cell growth, compared to control. Based on the cytotoxicity results, calculation of SI was determined using the formula:

$$SI = \frac{IC_{50} \text{ value of cytoto (Vero cells)}}{IC_{50} \text{ value of antitrypanosomal (}\textit{T. b. brucei}\text{)}}$$

The SI was classified into three categories, selective ($SI \geq 100$), moderate ($10 < SI < 100$) and not selective ($1 < SI < 10$) (Otoguro et al. 2008).

Statistical analysis

Samples were assayed in triplicate plates and the experiment was repeated at least three times. Excel was used to calculate the IC_{50}, which represents a 50% reduction in viability compared to the results seen in non treated cells, by linear interpolation method of Hills as explained by Huber and Koella (1993). The data were represented as means ± standard deviations (SD).

RESULTS

The antitrypanosomal activity of methanol, hexane and total alkaloid extracts of *D. costulata* against *T. b. brucei* and cytotoxicity on Vero cell (monkey kidney) are given in Table 1. Crude methanolic extract, which was the most active, was selected for total alkaloid extraction based on the literature review of *D. costulata* phytochemistry studies, that showed the present of alkaloid in this plant species. From the successive soxhlet extraction (Figure 1), methanol extract showed strong antitrypanosomal activity with IC_{50} 0.58 ± 0.01 µg mL^{-1}, compared to hexane extract which showed moderate activity (IC_{50} 4.4 ± 0.05 µg mL^{-1}). Cytotoxicity of both extracts exhibited an IC_{50} value of >100 µg mL^{-1}. However, the methanolic extract had a higher selectivity towards the parasite (SI value 169.5) compared to hexane extract (SI value 22.7). Therefore, the methanolic extract was further studied using TLC to determine the presence of alkaloid compounds. The extract was subjected to total alkaloid extraction to yield active alkaloid extract (IC_{50} 0.08 ± 0.07 µg mL-1). The alkaloid extract was then subjected to chromatographic fractionation using VLC to obtain five sub-fractions (F1–F5). All sub-fractions were tested for antitrypanosomal activity and the results are tabulated in Table 1. Active sub-fraction F5 exhibited strong activity with IC_{50} 0.07 ± 0.03 µg mL^{-1} and high SI value 731. All other sub-fractions were not active (IC_{50} > 6.25 µg mL^{-1}). Sub-fraction F5 was subjected to SPE-RP chromatographic technique to isolate compound **1** which showed potent antitrypanosomal activity with an IC_{50} value of 0.06 ± 0.03 µg $mL^{-1,}$ and SI value 76.3.

By correlating spectral data (^{1}H, ^{13}C NMR and MS) from literature (Mirand et al. 1983), compound **1** was identified as ochrolifuanine, as illustrated in Figure 2. Compound **1** was

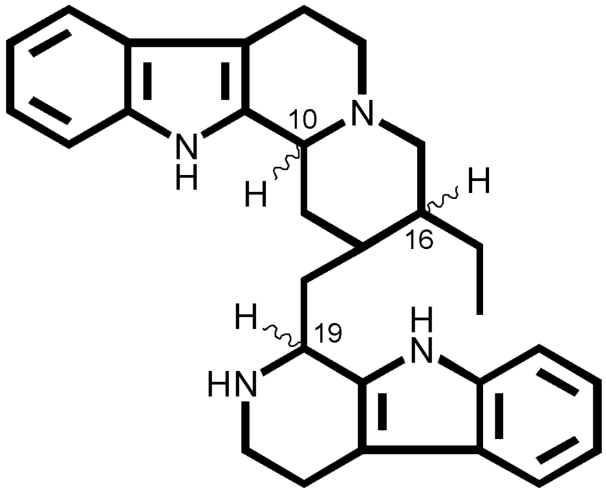

Figure 2 Structure of compound (**1**)

Table 1 Antitrypanosomal activity of *Dyera costulata* extract, fractions and compound **1** against *Trypanosoma brucei brucei* BS221 and cytotoxicity on Vero cell

Fraction	IC_{50} µg mL^{-1} ± SD*		SI value
	Antitrypanosomal	Cytotoxicity	
	T.b.brucei (BS221)	Vero	
Hexane extract	4.4 ± 0.05	> 100	> 22.7
Methanol extract	0.58 ± 0.01	> 100	> 172
Total alkaloid extract	0.08 ± 0.07	71.2 ± 0.07	890
Sub-fractions			
F1	> 6.25	NT	NT
F2	> 6.25	NT	NT
F3	> 6.25	NT	NT
F4	> 6.25	NT	NT
F5	0.07 ± 0.03	51.2 ± 0.21	731
Compound **1**	0.06 ± 0.03	4.58 ± 0.2	76.3
Pentamidine (antitrypanosomal drug)	0.00435 ± 0.03	27.60 ± 0.09	> 1000

*SD = standard deviation, NT= not tested, SI= selectivity index; antitrypanosomal activity determined as strong active (IC_{50} ≤ 0.78 µg mL^{-1}), moderate active (0.78 µg mL^{-1} < IC_{50} ≤ 6.25 µg mL^{-1}) and not active (IC_{50} > 6.25 µg mL^{-1})

obtained as a white solid with a molecular formula $C_{29}H_{34}N_4$ on the basis of ESI-MS data ($[M+H]^+$ mz^{-1} 439.5 calcd 438.619). The IR spectrum showed the presence of an amine (3470 cm^{-1}) and aromatic (1480 cm^{-1}). This compound was analysed spectroscopically using ^1H and ^{13}C NMR and its chemical shifts were summarised in Table 2.

DISCUSSION

Dyera costulata belongs to the family Apocynaceae which is one of the largest plant families with 250 genera and 2000 species. The bark and leaves of *D. costulata* have been traditionally used for the treatment of fever and inflammation (Subhadhirasakul et al. 2003). It was also

Table 2 ^1H and ^{13}C NMR chemical shifts (δ) of compound **1** (400, 100 MHz in MeOD)

Position	^1H	^{13}C
1	7.51, dd, *J* = 2.4, 11.2 Hz	110.97
2	7.16, t, *J* = 7.6 Hz	122.353
3	7.36, dd, *J* = 4.4, 8 Hz	119.38
4	7.51, dd, *J* = 1.6, 7.6 Hz	117.80
5	-	126.16
6	-	136.98
7	-	-
8	-	134.88
9	-	106.35
10	4.74, m	60.81
11	-	-
12	3.55, m	52.83
13	2.99, m	18.14
14	3.16, m	32.15
15	1.76, m	33.86
16	2.56, m	45.33
17	3.65, m	56.92
18	3.08, m	34.83
19	4.82, m	50.53
20	-	-
21	3.89, m	42.66
22	3.38, m	18.88
23	-	106.37
24	-	134.91
25	-	-
26	-	137.30
27	-	126.15
28	7.54, dd, *J* = 2.4, 11.2 Hz	117.91
29	7.11, dd, *J* = 0.8, 7.6 Hz	119.52
30	7.22, t, *J* = 1.6, 7.6 Hz 7.6	122.52
31	7.42, dd, *J* = 4.8, 8 Hz	111.16
32	3.29, m	31.43
33	1.04, t, *J* = 6.8 Hz	9.12

dd = doublet of doublets, t = triplet, m = multiplet, *J* = coupling constant in Hz

reported that leaf extracts of this plant showed antiplasmodial activity and *in vivo* analgesic effect in mice (Reanmongkok et al. 2002, Wong et al. 2011). Literature survey revealed that there is limited published information on the bioactivity of *D. costulata,* particularly on antiprotozoal activity. Wong et al. (2011) reported on the antiplasmodial activity of methanolic, dichloromethane/methanol and dichloromethane extracts of this plant against *Plasmodium falciparum* strain 3D7 with an IC_{50} value ranging 2.13 to 8.31 µg mL^{-1}, respectively.

Results in Table 1 showed that the methanolic extract of *D. costulata* leaves demonstrated strong antitrypanosomal activity and high selectivity. This activity is still lower compared to the antitrypanosomal standard drug, pentamidine (IC_{50} 0.00439 ± 0.07 ng mL^{-1}). However, since the crude extracts have mixtures of compounds, fractionation to isolate the active fraction increased this activity (Table 1). Freiburghaus (1996) classified those extracts with $IC_{50} \leq 10$ µg mL^{-1} as active**,** while Hoet et al. (2004a) considered extracts with $IC_{50} \leq 19$ µg mL^{-1} as active. However, it depends on the aim of the research when selecting the cut-off point of activity in these bioassays. The SI value was determined to identity extracts that were very selective to trypanosome parasites and at the same time had low toxicity on normal cells. The index was calculated based on the ratio of cytotoxicity (IC_{50}) on Vero cells to antitrypanosomal activity (IC_{50}). Extracts that show SI ≥100, is considered to have high selectivity and will be chosen for bioassay-guided isolation of active compound (Otoguro et al. 2008).

The total alkaloid extract tested exhibited strong antitrypanosomal activity of about 10-fold higher than the crude methanol extract (Table 1). It also presented a higher selectivity towards protozoa, compared to the methanolic extract. Results suggested that the active component in *D. costulata* leaves may correspond to the alkaloids present and are responsible for the antitrypanosomal activity observed. The observation supported the findings from previous studies which reported on the wide spectrum of antiprotozoal activity observed in alkaloids (Hoet et al. 2004b, Feng et al. 2010, Schmidt et al. 2012b, Kato et al. 2012, Scotti et al. 2015).

As mentioned earlier, natural product plays an important role in therapy. From 1981–2010, more than 50% of the drugs prescribed worldwide originated from nature (Newman & Cragg, 2012). The alkaloid is one of the most important and largest groups of plant secondary metabolites from a natural product that provided for human use (Wink 2015). Plant alkaloids are used as chemotherapeutic agents due to their capability to depolymerise the microtubules and thus inhibit cell division. Other reports have provided evidence that alkaloids possessing an indole moiety could display important antiprotozoal activities.

In the present study, bioassay-guided isolation was carried out to isolate the active antitrypanosomal compound from *D. costulata*. Compound **1** was identified as ochrolifuanine from the bisindole alkaloid group (IC_{50} 0.137µM; SI 76.3). This compound was first discovered from *Ochrosia lifuana* Guillaumin (Apocynaceae) by Peube-Locou et al. (1972) and in the leaves of *D. costulata* by Mirand et al. (1983). Ochrolifuanine has also been reported to be present in the root bark of *Strychnos potatorum* by Massiot et al. (1992). The ^{1}H-NMR spectral data showed aromatic protons (δ 7.11–7.54, 8 H), a methyl CH_3 at (δ 1.04, t, J = 6.8Hz) and eight methylene protons were displayed at (δ 3.1–3.89, 16 H). The ^{13}C-NMR spectrum displayed (δ 106.35–126.16, 8 C) attributing eight quaternary carbons on ochrolifuanine. All the chiral carbons signals were shown at δ 33.86 (C-15), δ 45.33 (C-16), δ 50.53 (C-19) and δ 60.81 (C-10). Meanwhile, the methyl carbon was observed at δ 9.12. The structure of compound **1** was further confirmed by two-dimensional spectroscopy experiment, heteronuclear multiple quantum coherence (HMQC). From this experiment, all the aromatic protons connected to its aromatic carbons (C-1 to C-4; C-28 to C-31). The presence of anomeric protons, (H-10, H-15, H-16 and H-19) were confirmed by their correlation to its carbons respectively.

Frederich (2002) reported of antiplasmodial activity in ochrolifuanine against *Plasmodium falciparum* (IC_{50} 0.5 µM). Even though compound **1** is a known compound, there is no report prior to this study on the antitrypanosomal activity of ochrolifuanine against *T. b. brucei*. The SI value of compound **1** was found to be 76.3 which falls under the moderately selective category (Otoguro et al. 2008). However, Hoet et al. (2004a) showed that compounds with SI ≥ 20 are considered as selective towards parasites. The significant antitrypanosomal activity and high selectivity exhibited by compound **1** warrants

further investigation into its mode of action to inhibit the parasite growth.

To our knowledge, this is the first report which described the antitrypanosomal activity of ochrolifuanine. Indole alkaloids are widely distributed in plants belonging to the families of Apocynaceae, Loganiaceae, Rubiaceae and Nyssacea (Hamid et al. 2017). Bisindole alkaloids contain a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. The pyrrole ring makes them particularly pharmacologically active (El-Sayed & Verpoorte 2007). Rosekranz & Wink (2008) demonstrated a sensitivity of *T. brucei* to isoquinoline alkaloid berberine and emetine at IC_{50} values of 0.5 and 0.04 µM. Other groups of alkaloids such as harmine and harmaline (indole alkaloid) have been reported for antiproliferative effects towards parasites of the genus *Trypanosoma* (Rosekranz & Wink, 2008), while other monomeric indole derivatives like ellipticine and olivacine showed antitrypanosomal activity on *T. cruzi* (Fournet et al. 1996, Gantier et al. 1996). Wink (2012) reported on the antitrypanosomal effects of vinblastine with an IC_{50} value of 0.21 µM against *T. b. brucei*. While bisindole alkaloid staurosporine showed potent antitrypanosomal activity with an IC_{50} value of 0.022 µM (Pimentel-Elardo et al. 2010). Based on the findings from the present study, the antitrypanosomal effects of ochrolifuanine (**1**) was found to be comparable with other reported bisindole alkaloid compounds. The high levels of antitrypanosomal activity observed in ochrolifunine (**1**) and staurosporine suggest that these compounds may share the same target site in the parasites.

CONCLUSIONS

In conclusion, the results presented here showed that ochrolifuanine (**1**) isolated from *D. costulata* leaves exhibited promising antitrypanosomal activity. These findings provided evidence that plants, a source of alternative therapy, are potential candidates for the discovery of novel drugs to combat trypanosomiasis diseases. Further studies are currently carried out to determine isomers of ochrolifuanine using optical rotation, α_D to identify them as isomers A, E or F. Evaluation of its *in vivo* effects in animal model will also be carried out to develop this compound as a new drug lead.

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