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CYTOTOXIC ACTIVITY OF ISOLATES FROM *TETRAPLEURA TETRAPTERA* TAUBERT (MIMOSACEAE) POD AGAINST BREAST CANCER CELLS AND *LEISHMANIA MAJOR* PROMASTIGOTES

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ABSTRACT

The cytotoxic effects of *Tetrapleura tetraptera* pod *in vitro* and *in vivo* has been reported. This work examined the cytotoxic effect of its compounds obtained through Vacuum Liquid chromatography, Column chromatography and High-Pressure Liquid chromatography. Compounds TT2 and TRA obtained were tested on AU565 and MDA-MB231 breast cancer cell lines respectively, at 50 µM, and analysed with 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. Antileishmanial activity of compound TRA on *Leishmania major* promastigotes was also examined. TT2 and TRA gave +14.36 and +19 % inhibitions on AU565 and MDA-MB231 breast cancer cells respectively. Significant activity was observed on *L. major* with compound TRA, having an IC_{50} value of 35.04 ± 0.17 µM. On the basis of the mass spectrometric (MS) data and comparison of ^{1}H and ^{13}C-NMR data, the compounds TT2 and TRA were identified as 22-hydroxyisohopane and 3-O-[β-D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid (aridanin) respectively. These results show the potential of this plant for usage against these diseases.

Keywords: Breast cancer, Cytotoxic, Leishmanicidal, *Tetrapleura tetraptera*,

INTRODUCTION

Cancer is a chronic, deteriorating, and advancing disease that accounts for the second most common cause of mortality after cardiovascular disease worldwide (Uchendu, 2019). There are currently more than a hundred distinct forms of cancer that can be divided based on etiology and natural history. The most common malignant diseases in women are breast and cervical cancers, among other types of cancer (Jedi-Agba *et al*., 2012).

Numerous variables, such as sex, age, race, genetic predisposition, and exposure to environmental carcinogens, influence the incidence, geographic distribution, and behavior of particular forms of cancer. In addition to surgery, chemotherapy has been shown to be effective in treating a variety of cancer forms, but it is not without drawbacks. Some of these adverse effects make it difficult to continue receiving therapy, which ultimately results in advanced cancer stages and fatality. As a result, there is an ongoing search for safer and more effective anticancer substances, and both synthetic and natural products show promise in this regard (Schirrmacher, 2019).

Due to their significant chemical components, which serve as a significant reservoir of possible leads in the creation of novel medications, medicinal plants are

widely employed in the global healthcare system as alternative remedies for a variety of disorders (Happi *et al*., 2022). Through numerous studies, there has been significant advancement in the pharmacological evaluation of plants employed in traditional medical systems and their isolated constituents (Chikezie *et al*., 2019).

Insects transmit the infectious disease leishmaniasis, which is common in Europe, Africa, Asia, and the Americas. It is a parasitic protozoan infection caused by more than 20 species of the intracellular parasite Leishmania, with the most notable being *Leishmania major*, *L. mexicana*, *L. tropica*, *L. braziliensis*, *L. donovani*, and *L. infantum* (Alvar *et al.*, 2012; Barrett and Croft, 2012; Ngouateu and Dondji, 2022). It is an important public health issue since it significantly increases morbidity and mortality (Amato *et al*, 2008). Leishmaniasis is endemic in 98 countries (Alvar *et al.*, 2012), 82% of which are low-income countries. Its management still faces significant obstacles due to toxicity and the establishment of resistance to conventional antileishmanial drugs. As a result, finding and creating new, less harmful chemotherapeutic drugs has received a lot of interest (Pitzer *et al*, 1998). In an ongoing search for better and more affordable leishmanicidal agents, plant-derived products represent an attractive option. Around 80% of people around the world, according to the World Health Organization (WHO), rely on traditional medicines for their medical care (Dutta *et al*, 2007). The plants *Azadirachta indica*, *Maytenus senegalensis* and *Eucalyptus globulus* have been shown to have leishmanicidal properties with IC_{50} values of 11.5, 55 and 78 μg/ml, respectively (Tahir *et al*., 1998) on *L. major* promastigotes at a concentration of 0.5 mg/ml. Other medicinal plants as well as their isolates have been investigated and some have shown activity against various forms of the *leishmania* parasites (Kayser and Kiderlen, 2001; Rocha *et al*, 2005; Atta-ur-Rahman *et al*, 2008).

Tetrapleura tetraptera Taubert (Mimosaceae) is a medicinal plant found in Uganda, Mali, Burkina Faso, Mauritania and countries from Gambia to Nigeria. It's a tree which thrives most luxuriantly in the rain forest, reaching 20-25 m in height, with a girth of about 1.2-3 m (Adesina *et al*., 2016). It is locally known as *Uyayak* in Ibibio, *Edeminang* in Efik, *Osakirisa* or *Oshosho* in Igbo, *Dawo* in Hausa, *Ighimiakia* in Bini, and *Aridan* in Yoruba (Ojewole and Adewunmi, 2004). It is commonly used in soups of nursing mothers to prevent postpartum contractions (Nwaiwu and Akali, 1986), to cure feverish conditions, constipation, and as an emetic. The whole fruit is often taken as an infusion. It has also been found to be effective in the treatment of microbiological infections, leprosy, inflammation, rheumatic pains, arthritis, diabetes, epilepsy, jaundice, hypertension, and schistosomiasis (Adesina *et al*. (2016). Kemigisha *et al*. in 2018 also reported its usage in the treatment of breast cancer.

Pharmacological studies have reported its antitumor, anti-inflammatory properties and also inhibition of HIV replication due to the presence of caffeic acid in the fruits (Adesina, *et al*, 1980). Activity-guided fractionation of the fruit methanol extract revealed saponins, which exhibited strong molluscicidal property against the schistosomiasis-transmitting snails *Biomphelaria Glabrata* (Maillard *et al*. 1989). Pharmacological examination of the extract through *in vitro* and *in vivo* experiments showed that it possesses hypotensive effects in anaesthetized rats (Ojewole and Adesina, 1983).

Okochi *et al*. (1999) reported the trypanocidal effect of the water extract of *T. tetraptera* against *Trypanosoma brucei*. Anti-inflammation and hypoglycaemia were also observed effects of the fruit aqueous extract in rats (Ojewole and Adewunmi, 2004). Furthermore, Aderibigbe *et al*. (2007a) documented its anticonvulsant, analgesic and hypothermic activities in mice.

The soft parts of the fruit are known to contain sugars, tannins, traces of saponin and amino acids (Adesina *et.al*., 1980). Activity-directed fractionation of the fruit has yielded several compounds including scopoletin and aridanin (Essien *et al*., 1983; Adesina and Reisch, 1985; Fleischer *et al*., 2006). This work aims to isolate and characterize active ingredients from *T. tetraptera* pod extract and determine their cytotoxic activity against breast cancer cells (MDA-MB231 and AU565) and *Leishmania major* (CNRL) promastigotes due to its numerous ethnomedicinal and pharmacological capabilities.

METHODS

Collection and identification of plant material

Tetrapleura tetraptera pod was collected between September and November 2016 in Ibadan, Nigeria. Its identity was confirmed by Dr Shasanya Olufemi, the Plant Taxonomist at the Forest Research Institute of Nigeria (FRIN) where herbarium specimen number FHI 110614 was assigned to it.

Extraction of plant material

T. tetraptera pod (1.5 kg) was air-dried for one week before being oven-dried at 50 to 60ºC. With the use of an electric mill, it was later reduced to powder. Using a Soxhlet apparatus, the powdered material was extracted with absolute methanol (5 L) and concentrated using rotary evaporator maintained at 50°C.

Isolation of compounds from the methanol extract of *T. Tetraptera* pod

The pod extract (60g) was re-dissolved in methanol-water (1:1) and further partitioned exhaustively with hexane and chloroform. The chloroform fraction (31 g) was later subjected to vacuum liquid chromatography (VLC) with silica gel G (60-120 µm). Gradient elution was done with C_6H_{14} (100%)- A, C_6H_{14}-$CHCl_3$ (1:1)- B, $CHCl_3$ (100%)- C, $CHCl_3$-$CH_3COOC_2H_5$ (1:1)- D, $CH_3COOC_2H_5$ (100%)- E, $CH_3COOC_2H_5$-CH_3OH (1:1)- F, CH_3OH (100%)- G, CH_3OH-H_2O (1:1)- H. The various fractions (A-H) were reduced to dryness under pressure.

VLC fractions C and D (1 g) were bulked together based on similarities in their TLC profile. The combination was subjected to column chromatography using silica gel (200-400 mesh). Gradient elution was done with 200 mL of C_6H_{14} (100%), C_6H_{14}-$CH_3COOC_2H_5$ (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) and CH_3OH (100%). Ninety-six (96) fractions collected were differently bulked based on their TLC profile and coded C1 (3-20), C2 (21-34), C3 (35-37), C4 (38-50), C5 (51-68), C6 (69-96). Fraction C2 was subjected to normal phase Prep-HPLC. The column specifications are internal diameter (10 mm), length (250 mm), containing silica with particle size of 4 µm, UV detector (UV – 50), RI detector (RI – 5), flow rate (4 mm/min). Isocratic elution was carried out with C_6H_{14}- $CH_3COOC_2H_5$ (7:3). Five (5) fractions were obtained with fraction 4 producing white precipitates (6 mg). It had one spot on commercial TLC plate and was coded as TT2.

VLC fractions F and G (4 g) were also bulked together based on similarities in their TLC profile. The combination was subjected to column chromatography using silica gel (200-400 mesh). Gradient elution was done with 200 mL of C_6H_{14} (100%), C_6H_{14}-$CHCl_3$ (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90), $CHCl_3$ (100%), $CHCl_3$-$CH_3COOC_2H_5$ (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90), $CH_3COOC_2H_5$ (100%) and $CH_3COOC_2H_5$-CH_3OH (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90). Seventy-eight (78) fractions were collected and bulked based on their TLC profile and coded F1 (1-13), F2 (14-15), F3 (16-20), F4 (21-24), F5 (25-28), F6 (29-34), F7 (35-39), F8 (40-45), F9 (46-53), F10 (54-57), F11 (58-62), F12 (63-66), F13 (67-69), F14 (70-74) and F15 (75-78).

Fraction F11 produced white precipitates which were rinsed repeatedly with ethyl acetate. The washed precipitate (170 mg) was subjected to reversed phase Prep-HPLC with column specifications of internal diameter (10 mm), length (250 mm), particle size (4 µm), octadecyl silane H80, UV detector (UV – 50), RI detector (RI – 5), flow rate (4 mm/min). Isocratic elution was carried out with CH_3OH-H_2O (9:1). Seven (7) fractions were obtained with fraction 6 (35 mg) showing one spot on commercial TLC plate. It was reduced to dryness and coded as TRA.

Compounds TT2 and TRA were subjected to spectroscopic analyses using ^{1}NMR and mass spectroscopy. Their cytotoxic activity on breast cancer cell lines **(**AU 565 and MDA-MB231 respectively) at 50 µM concentration was determined. Leishmanicidal activity on *L. major* promastigotes was also determined for compound TRA.

Cytotoxic effect of compounds TT2 and TRA on breast cancer cell lines

The cytotoxic activity was analysed with MTT assay on breast cancer cells. Compounds TT2 and TRA were tested on AU565 and MDA-MB231 cell lines respectively. The cancer cells were obtained from the molecular bank of the International Center for Chemical and Biological Sciences (ICCBS) at the University of Karachi, Pakistan. They were placed in 96-well plates at a density of 10,000 cells/well/100 µL and allowed to incubate for 24 h in complete media at 37 °C and 5% CO_2 for the healthy growth of the cells. The stock solution of compounds TT2 and TRA were prepared in sterile DMSO and later diluted to 50 µM concentration which was used for the experiment. Doxorubicin at 50 µM was used as the standard and the experiment was performed in triplicates. After treatment, the cell culture was allowed to incubate for 48 hours at 37 °C and in humidified atmosphere of 5 % CO_2 after which 200µL of MTT (0.5mM) dye was added in each well and then incubated for another 3-4 h. The resulting formazan crystals were dissolved in 100µL of DMSO. The absorbance of the resulting solution was measured at 570 nm [33]. IC_{50} was calculated by EZ-Fit software.

Leishmanicidal activity of compound TRA on *Leishmania major*

Compound TRA (1 mg) was dissolved in 50µL of DMSO and diluted with 950µL of RPMI-1640 media. *Leishmania major* parasite promastigotes were cultured in liquid medium RPMI-1640 supplemented with 10% fetal bovine serum. Parasites at the log phase were centrifuged at 2000 rpm for 10 min and the supernatant discarded. Parasites were diluted with fresh culture medium to a final density of 1×10^6 cells/mL. In a 96-well microtiter plate, 100 µL of media was added in all wells except in

the first column which received 180µL media. Stock of test compound (20µL) was placed in the first well and mixed well after which serial dilutions were made. 100 µL of parasite culture were added in each well. To serve as negative control, medium without a test compound was used. Amphotericin B and pentamidine were used as standards. Plates were incubated in the dark at 25 ºC. After 72 hours, activity of standards/test compounds were assessed microscopically on a Neubauer counting chamber. IC_{50} values were calculated using EZ-Fit software. All assays were performed in triplicates (Verma and Dey, 2004; Ebrahimisadra *et al*, 2013).

Statistical analysis

Data obtained were expressed as mean ± SEM and analyzed with one way analysis of variance (ANOVA) using SPSS 21. $P< 0.05$ was regarded as significant.

RESULTS

Result of chromatographic procedure

Vacuum liquid chromatography of the chloroform fraction of *T. tetraptera* pod extract, followed by column chromatography and preparative HPLC led to the isolation of compounds TT2 (R_f 0.48 in C_6H_{14} – $CH_3COOC_2H_5$ 7:3) and TRA (R_f 0.29 in CH_3Cl-CH_3OH 8:2). Their NMR results are shown below (Table 1 and 2).

The ^{13}C-NMR spectrum of compound TT2 showed that there are 30 carbon atoms in the molecule which makes it most likely a triterpenoid. The signals at δ 51.1, 32.7, 40.9, 23.7, 33.9, 18.0, 21.3, 33.9, 26.1, 40.2, 20.6, indicate the presence of the methylenes (CH_2) in the molecule. Five methines (CH) resonances (55.5, 53.8, 48.6, 49.9, 62.7), eight methyl resonances (33.4, 16.5, 16.7, 16.8, 15.9, 22.3, 30.8, 28.9) and six quaternary carbons (49.5, 43.6, 34.4, 41.5, 38.4, 71.6) were also observed in the spectrum. Due to the presence of a hydroxyl group at position 22, ^{13}C NMR showed two signals downfield at 62.7 (C-21) and 71.6 (C-22) which are methine and a quaternary carbon respectively.

The broadband has a signal at 71.6. This signal did not show in both dept 90 and 135 suggesting that it is a quaternary carbon, which supports the proposed structure.

The presence of a signal downfield at 4.089 shows that a hydroxyl group is present as revealed by the ^{1}H NMR spectra. The proton NMR shows the presence of tall signals which are indicative of methyl signals.

The above data shows that compound TT2 is a triterpene with a hydroxyl group attached at position 22. The molecular ion peak (M^+) at 426.3 (M±2) is consistent with the molecular formula $C_{30}H_{52}O$.

On the basis of the MS data and comparison of ^{1}H and ^{13}C-NMR data the structure of the compound was identified as 2-(5a,5b,8,8,11a,13b-Hexamethyl-icosahydro-cyclopenta[a]chrysen-3-yl)-propan-2-ol, also known as 22-hydroxyisohopane (Figure 1).

Compound TRA was isolated as a white powder which gave a positive reaction to the test for amines. A carboxylic acid signal was obtained at δ 178.6 (C-23) and a double bond signal at δ 121.5 (C-12) and δ143.9 (C-13). The absence of a carbon signal in DEPT at C-23 and C-13 further confirms this. An O- glycosidic bond is present at position 3 of the steroid nucleus with a resonance of δ 76.7. An anomeric carbon signal at δ 103.5 indicated the presence of a single monosaccharide moiety. This indicates a β-glucose anomeric carbon. An amide group is present in the sugar portion (C-7',δ 168.7) which is further confirmed by the absence of carbon in the DEPT. EIMS did not show the sugar portion due to its polarity and bulky nature.

Table 1: NMR Data of Compound TT2 (600 MHz for ^{1}H and 150 MHz for ^{13}C) in DMSO

Carbon No.	δ_C (ppm)	DEPT	δ_H (ppm)	Multiplicity, *J* (Hz)
1	51.1	CH_2	1.15	M
2	32.7	CH_2	1.57	M
3	40.9	CH_2	1.33	M
4	49.5	C	-	-
5	55.1	CH	1.89	M
6	23.7	CH_2	1.52	M
7	33.9	CH_2	1.41	M
8	43.6	C	-	-
9	53.8	CH	2.07	M
10	34.4	C	-	-
11	18.0	CH_2	1.42	M
12	21.3	CH_2	1.44	M
13	48.6	CH	1.91	M
14	41.5	C	-	-
15	33.9	CH_2	1.31	Q
16	26.1	CH_2	1.54	M
17	49.9	CH	1.88	M
18	38.4	C	-	-
19	40.2	CH_2	1.22	M
20	20.6	CH_2	1.59	M
21	62.7	CH	1.62	M
22	71.6	C	-	-
23	33.4	CH_3	1.07	S
24	16.5	CH_3	0.79	D
25	16.7	CH_3	0.91	D
26	16.8	CH_3	0.85	S
27	15.9	CH_3	1.07	S
28	22.3	CH_3	1.03	M
29	30.8	CH_3	1.07	S
30	28.9	CH_3	0.85	S
-OH	-	-	4.08	Q

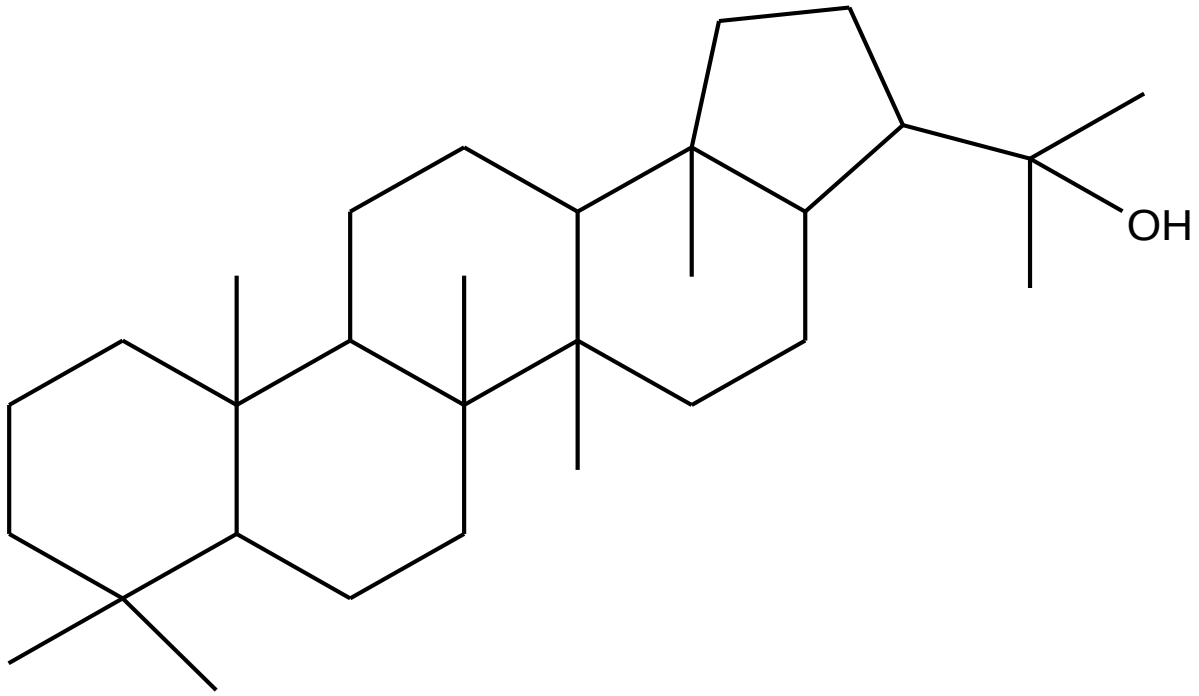

Figure 1: Structure of compound TT2 (2-(5a,5b,8,8,11a,13b-Hexamethyl-icosahydro-cyclopenta[a]chrysen-3-yl)-propan-2-ol) (22-hydroxyisohopane)

The molecular ion peak (M +) at 456.2 is consistent with the molecular formula $C_{30}H_{48}O_3$ which is the aglycone portion of the structure thus confirming the presence of an extra mass of 204 g/mol which is obviously from a hexose sugar (glucose) in addition to a nitrogen-containing moiety. The chemical shift for fructose was ruled out because of the absence of a carbonyl signal in the ^{13}C-NMR spectra. The ^{13}C-NMR spectrum of compound TRA showed that there are 38 carbon atoms in the molecule. With these data, compound TRA is likely a glycoside of triterpene. The signals at δ 17.8, 33.3, 22.9, 23.1, 38.0, 25.3, 25.5, 27.2, 32.4, 32.8, and 61.2 indicate the presence of methylenes in the molecule. The ten methine resonances at δ 76.7, 45.7, 47.1, 121.5, 54.9, 103.5, 88.1, 74.0, 70.8, 55.8 as well as the methyl resonance at δ 25.5, 27.5, 23.4, 23.1, 16.9, 15.1, 16.3, and 32.8 were due to C-3, C-5, C-9, C-12, C-18, C-1', C-2', C-3', C-4', C-5' and C-24, C-25, C-26, C-27, C-28, C-29, C-30, and C-8' respectively on the molecule. Signals at δ 39.7 (C-4), 41.3 (C-8), 39.8 (C-10), 143.9 (C-13), 48.6 (C-14), 45.5 (C-17), 40.0 (C-20), 178.6 (C-23), 168.7 (C-7') correspond to the 28 quaternary carbons of the moiety. The fragmentation of the molecule is shown by the EIMS; the difference between peak 456.2 and 439.3 shows that an hydroxyl group has been lost while the difference between 456.2 and 410.3 shows that a carboxylic acid group has

been lost. After comparing with the information in literature, and on the basis of the MS data and comparison of ^{1}H and ^{13}C-NMR data the compound was identified to be 3-O-[β-D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid, also known as aridanin.

Table 2: NMR Data of Compound TRA (600 MHz for ^{1}H and 150 MHz for ^{13}C) in DMSO

Carbon No.	δ_C (ppm)	DEPT	δ_H (ppm)	Multiplicity, *J* (Hz)
1	17.8	CH_2	1.23	m
2	33.3	CH_2	1.29	m
3	76.7	CH	3.40	m
4	39.7	C	-	-
5	45.7	CH	1.89	m
6	22.9	CH_2	1.40	m
7	23.1	CH_2	1.45	m
8	41.3	C	-	-
9	47.1	CH	2.72	dd (10.2)
10	39.8	C	-	-
11	38.0	CH_2	2.96	dd (3.6, 10.8)
12	121.5	CH	5.13	s
13	143.9	C	-	-
14	48.6	C	-	-
15	25.3	CH_2	1.47	m
16	25.5	CH_2	1.47	m
17	45.5	C	-	-
18	54.9	CH	3.03	m
19	27.2	CH_2	1.49	m
20	40.0	C	-	-
21	32.4	CH_2	1.61	m
22	32.8	CH_2	1.65	m
23	178.6	C	-	-
24	25.5	CH_3	0.87	s
25	27.5	CH_3	0.87	s
26	23.4	CH_3	0.88	s
27	23.1	CH_3	1.08	s
28	16.9	CH_3	0.85	s
29	15.1	CH_3	0.71	s
30	16.3	CH_3	0.65	s
1’	103.5	CH	7.65	d (9.0)
2’	88.1	CH	4.80	d (5.4)
3’	74.0	CH	3.64	dd (5.4, 11.4)
4’	70.8	CH	3.15	d (4.8)
5’	55.8	CH	4.07	q (4.8)
6’	61.2	CH_2	3.39	m
1’’	168.7	C	-	-
2’’	32.8	CH_3	1.80	s

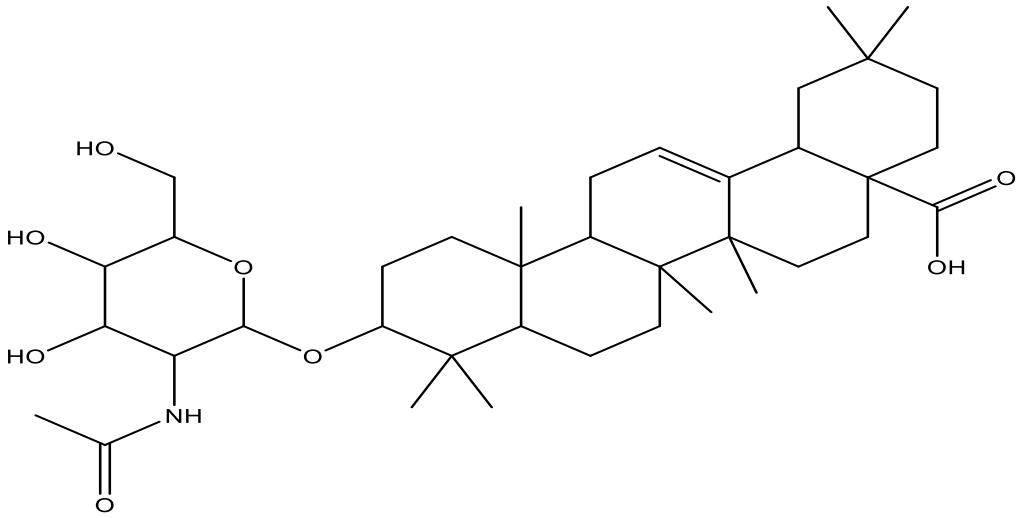

Figure 2: Structure of Compound TRA (3-O-[β-D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid) (Aridanin)

Inhibition effects of compounds on breast cancer cell lines and *L. major* promastigotes

TT2 and TRA gave +14.36 and +19 % inhibitions with AU565 and MDA-MB231 breast cancer cells respectively (Table 3) which were lower than that of doxorubicin ($P<0.05$). Against *L. major*, significant activity was observed with TRA having an IC_{50} value of 35.04 ± 0.17 µM (Table 4).

Table 3: Inhibitory effect of compounds TT2 and TRA on breast cancer cell lines

Compound	Concentration (µM)	% Inhibition	IC_{50} (µM) ± SEM
TT2 (AU 565)	50	+14.36	-
TRA (MDA-MB231)	50	+19	-
Doxorubicin	50	+75.5	0.5 ± 0.07

.

Table 4: Leishmanicidal activity of compound TRA on *L. major*

Samples	IC_{50} (µM) ± SEM
TT2	ND
TRA	35.04 ± 0.17
Amphotericin B	0.39 ± 0.005
Pentamidine	3.16 ± 0.015

DISCUSSION

Triterpenoids are one of the most distributed class of compounds throughout natural sources with more than 20,000 known structures (Happi *et al*., 2022). The triterpenoid 22-hydroxyhopane has previously been isolated from leaves of the fern *Adiantum latifolium* and revealed to possess larvicidal activity against the larvae of the coconut pest *Oryctes rhinoceros* (Kumar *et al*, 2019). It exhibited histolysis of midgut tissues, demonstrated antibacterial action against symbiotic gut bacteria, and blocked the release of digestive enzymes like protease, amylase, and trehalase, which caused the larvae to lose weight (Kumar *et al*, 2019). It has also been cited to have multitargeted activity against SARS-CoV-2 (Kumar and Saddique, 2022). Though this compound's cytotoxic action on AU565 cancer cell line from this study was low to moderate, other hopane-type triterpenoids have been found to show significant cytostatic activity on HeLa and A549 human tumor cell lines with IC_{50} values of 3.93 ± 0.10 and 7.90 ± 0.31 µM, respectively (Yu *et al*., 2019).

Aridanin has previously been identified in *T. tetraptera* fruit and has been reported to have anticonvulsant, analgesic, and hypothermic activities (Aderibigbe *et al*., 2007a). Aridanin isolated from *Diospyros conocarpa*, was tested against *Trypanosoma brucei* strain and it showed strong trypanocidal activity with an IC_{50} value of 0.1 µM, and only mildly inhibited HIV-1 integrase with an IC_{50} of 18.32 µM (Fouokeng *et al*., 2019). Its antibacterial activity was comparable to ciprofloxacin in some cases with MIC value range of 0.78−6.25 µg/ml with the highest potencies recorded against *E. coli, E. smartii,* and *E. aeroginese* (Lunga *et al*., 2014).

A recent study found that aridanin inhibited the proliferation of CCRF-CEM and HepG2 cells with IC_{50} values of 3.18 and 9.56 µM, respectively. However, in the present investigation, aridanin demonstrated low to moderate growth inhibitory action on MDA-MB231 cell line. It was shown to have much lower IC_{50} values than doxorubicin when used against the drug-resistant CEM/ADR5000 cells and melanoma cell lines (MaMel-80a, Mel-2a, MV3, and SKMel-505) (Mbaveng *et al*., 2020). According to Tameye *et al*. (2020), the L5178Y mouse lymphoma cell line was highly sensitive to the cytotoxic effects of aridanin, which was isolated from the roots of *Ficus exasperata*.

Generally, the mechanisms of action that medicinal plants use to combat cancer include cytotoxicity, the ability to suppress cancer cell proliferation, effective tumor volume reduction, and the capacity to shield DNA from dangerous radiation, all of which lead to higher survival rates. (Saeed *et al*., 2019; Islam *et al*., 2020). Previous studies have shown that the chloroform fraction of *T. tetraptera* pod extract has strong cytotoxic effects on AU565 breast cancer cell line, with an IC_{50} value of 9.63 ± 3.61 µg/mL (Imade *et al*., 2022). The fraction showed a concentration-dependent growth inhibitory effect on *Sorghum bicolor* radicles in the same investigation, with an inhibition of 81.98 % observed at 5 mg/mL, demonstrating its antiproliferative property. Its potent cytotoxic activity on the cell line is the basis for the isolation exercise carried out in this study.

The compound aridanin demonstrated notable effectiveness on *L. major*, with an IC_{50} of 35.04 ± 0.17 µM being achieved. The disruption of electron transport chain, which alters the energy level of the strains tested, as well as the induction of apoptosis

are two of the mechanisms by which natural compounds with antileishmanial activity work (Inuoe *et al*, 1994). Leishmanicidal activities have also been linked to the inhibition of DNA topoisomerase, the key enzyme in DNA compaction. Any of these methods, individually or in combination, may be how aridanin works.

CONCLUSION

The compounds, 22-hydroxyisohopane and 3-O-[β-D-glucopyranosyl-2'-acetamido-2'-deoxy]-oleanolic acid isolated from *T. tetraptera* pod were observed to have low to moderate cytotoxic activities against breast cancer cells while significant activity was seen with 3-O-[β-D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid on *L. major* promastigotes. These results indicate the potential of this plant for usage against these diseases.

Conflict of Interest

The authors declare that they have no conflict of interest.

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