

Original Research Article

In vitro Cytotoxicity and Antiplasmodial activity of fractions from *Anthocleista djalensis* A. Chev. Acetone root extract

Abstract

AIM: Malaria caused by *Plasmodium falciparum* is one of the killer diseases in Africa today and the uncontrollable spread of drug resistance and limited drugs with therapeutic efficacy makes it necessary to discover agents against this deadly parasite. Traditionally *Anthocleista djalensis* root extract is used in the treatment of Malaria in many parts of Africa and has demonstrated to be a source of antiplasmodial agents. This study aims at identifying possible antiplasmodial agents from chromatographic root fractions of *Anthocleista djalensis* of the Genetianaceae family as well as to evaluate their cytotoxicity against HeLa cells.

Place and Duration of study: The study was undertaken in the Department of Organic Chemistry, Rhodes University, Grahamstown, South Africa. The duration period was between March and July 2016.

Methodology: The *Anthocleista djalensis* roots were collected from Arochukwu, Abia State, Nigeria. The acetone extract was obtained from successive maceration of the methanolic crude extract with hexane, ethyl acetate and acetone. The concentration (0.01-100 µg/mL range) of the chromatographic fractions from acetone root extract of *Anthocleista djalensis* were tested for antimalarial activity against *Plasmodium falciparum* (*P. falciparum*). Cytotoxicity against HeLa cells was also evaluated using resazurin based assay.

Results: The Five fractions obtained from the chromatographic fractionation of acetone extract labelled A1, A2, A3, A4, and A5 with percentage yield (13.02, 26.66, 24.70, 0.05 and 26.66 % respectively) showed excellent antiplasmodial activity. The antimalarial bioassay test showed fractions A1, A2, A3, A4 and A5 with IC₅₀ value of 0.0360 ± 0.0100, 8.1299 ± 2.0358, 46.2482 ± 1.2720, 0.0151 ± 0.0010, and 9.8013 ± 0.8171 µg/mL respectively. CC₅₀ values of 44.2010 ± 8.6790, 50.0000 ± 5.6412, 71.6221 ± 2.9600, 36.7212 ± 5.8900 and 0.5132 ± 3.770 µg mL⁻¹ were recorded for fractions A1, A2, A3, A4 and A5 respectively. Fractions were classified as marginally active (A3) showing SI of 1.540 ± 0.0091, partially active (A2 and A5) with SI 6.150 ± 0.0200 and 4.133 ± 0.015 and as active (A1, A4,) exhibiting SI of 1227.805 ± 8.210 and 2431.867 ± 1.589 respectively. A1 and A4 showed SI > 10 and IC₅₀ < 10 µg/mL. Chloroquine, used as a reference antimalarial drug, tested in parallel had an IC₅₀ of 0.0125 ± 0.0001 µM and was comparable with A1 and A4.

Conclusion: The chromatographic fractions from acetone root extract of *Anthocleista djalensis* are potential sources for antimalarial agents of lead compounds for the development of antiplasmodial drugs.

12 **Keywords:** Antiplasmodial activity; Cytotoxicity; IC₅₀; Fractionation; *Anthocleista*
13 *djalonensis*

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17 **1. INTRODUCTION**

18
19 Medicinal plants contain chemical substances or constituents that have pharmacological activities [1-

20 7]. These activities include anti-cancer [8,9] anti-tumor[10]anti-oxidant[11] and anti-microbial
21 activities[12, 21]. Healing with medicinal plants is as old as mankind itself. The connection between
22 man and his search for drugs in nature dates from the far past. Awareness of medicinal plants usage is
23 a result of the many years of struggles against illnesses by which man learned to pursue drugs in
24 barks, seeds, fruit bodies, and other parts of the plants [13]. Contemporary science has acknowledged
25 their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin,
26 known by ancient civilizations and used throughout the millennia. The knowledge of the development
27 of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased
28 the ability of pharmacists and physicians to respond to the challenges that have emerged with the
29 spreading of professional services in facilitation of man's life [13].

30 The medicinal plant *Anthocleista djalonensis*, A. Chev –Gentianaceae is a large tree which grows up
31 to 20 feet; bole up to 4cm in diameter, stilt-rooted, twig sometimes erect, spines above the leaf axils
32 and with white flowers that are scented [14]. Traditionally, the plant is used to treat wound, malaria,
33 constipation, dysentery, diarrhea, hepatitis, skin infection, and inflammation [15]. Ethnobotanical
34 investigation revealed the use of *Anthocleista djalonensis* for the treatment of cancer [16]. Three
35 compounds (monoterpene diol, djalonenol and iridoid glucoside djalonenoside) were isolated
36 from *Anthocleista djalonensis* [17]. These compounds when tested in addition with six
37 semisynthetic derivatives for cytotoxicity of constituents, djalonenol and iridoid glucoside
38 demonstrated cytotoxic effect on the brain tumor fibroblasts (18). The root of *A. djalonensis*
39 are used traditionally to treat malaria jaundice, diabetes and abscesses [15] Reports of

antibacterial and wound healing activity [19,15], *in vitro* anthelmintic [20] and antimalarial activity[21, 22] of the plant have been published.

Malaria, the most prevalent and most pernicious parasitic disease of humans, is estimated to kill between one and two million people (mainly children) each year [23]. One of the greatest obstacles to the control of malaria has been the spread of resistance to drugs used on a large scale [24]. Chloroquine, though effective as a blood schizontocidal drug, is ineffective or partially effective in resistant cases [25]. The emergence of strains of *Plasmodium falciparum* resistant to chloroquine and many other drugs in succession have stimulated efforts to identify new anti- malarial agents [25].

Previous pharmacological and bioactivity study of *Anthocleista djalensis* root extracts has necessitated this investigation. In this study, the activity of chromatographic fractions from the acetone root extract against *P. falciparum* was assayed and the cytotoxicity on HeLa cells was also evaluated in order to determine the selectivity of anti-malarial action. *Plasmodium falciparum* responsible for malaria in human was quite suitable for this study.

2. MATERIAL AND METHODS

2.1. Plant materials

The roots of *Anthocleista djalensis* were obtained from Arochukwu, Abia state, Nigeria. The taxonomic classification of the selected plant was established by Mr Ibe Ndukwe of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Voucher samples of the plants are deposited in the Herbarium of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The roots were dried under shade for three weeks.

2.2. Acetone extract preparation

The dried plant samples were pulverized to coarse powder using a laboratory mill (Model 4 Arthur Thomas, USA). The 93 g (AF00) was extracted successively with Hexane (4 x 100 mL), Ethyl acetate (4 x 100 mL), acetone (4 x 100 mL) by maceration. The extracts were

concentrated individually with Rota vapor at 35°C to give AF01, AF02, AF03, respectively with AF00 as the crude extract.

2.3. Fractionation of Acetone extract

Acetone extract being the most active of all the extracts was made candidate for column chromatography (The activity of this extract will be Published elsewhere). About 30 g of concentrated acetone extract was fractionated using chromatography column. Packaging was done using an 80 g column of silica gel. Best eluent (hexane, dichloromethane and methanol) were used as mobile phase with a gradient system. Eluates were collected in several bottles of 50 mL, each of which was given a number then analyzed by TLC. The spots separated in TLC were observed with 254 nm UV light. The R_f and eluates which had the same pattern spots appearance on TLC were combined as one fraction then concentrated. Percentages of fractions were calculated using the formula:

$$\% \text{ Fraction} = \text{Weight of Fraction (g)} / \text{Weight of plant extract} \times 100$$

2.4. HeLa cell culture and treatment [26]

Human cervix adenocarcinoma cells (HeLa) obtained (from ATCC CCL-2 LGC standard Wesel, Germany) were cultured in a 5%CO₂ incubator at 37°C in DMEM medium supplemented with 10% fetal bovine serum and antibiotics (penicillin/streptomycin/fungizone). The cells were split every 3-5 days (when the cells have reached close to full confluency), and were detached from the culture flask surface using trypsin/EDTA, and the majority aspirated off. Medium was added to the flask and the remainder of the cells, and the flask returned to incubation. The confluency and state of the cells were regularly assessed using an inverted light microscope. Cells were cryo-preserved by detaching the cells from the culture flask in trypsin/EDTA, pelleting the cells, transferring them to cryo-tubes in 10% DMSO in fetal bovine serum, and placing the tubes in a -80

freezer. For the cytotoxicity assay a range of concentrations of extract (1-1000 $\mu\text{g mL}^{-1}$) was used for 24 h treatment for the determination of 50% cytotoxic concentration (CC_{50}).

2.5. *In vitro* Cytotoxicity assay

Cytotoxic activity was determined by resazurin reduction based assay [27]. *HeLa* cells were used for the determination of the CC_{50} value of the cytotoxicity of chromatographic fractions from *Anthocleista djalensis*. To assess the overt cytotoxicity of the compounds, extracts were incubated at various concentrations in 96-well plates containing *HeLa* (human cervix adenocarcinoma) cells for 24 hours. The numbers of cells surviving on drug exposure were also determined by using the resazurin based reagent and reading resorufin fluorescence in a multiwell plate reader. Reagent was prepared by dissolving high purity resazurin in DPBS (pH 7.4) to 0.15 mg/mL. The resazurin solution was filtered and sterilized through a 0.2 μm filter into a sterile, light protected container. The resazurin solution was stored and protected from light at 4 °C for frequent use or at -20 °C for long term storage. Cells and test compounds were prepared in opaque-walled 96-well plates containing a final volume of 100 μL /well. An optional set of wells were prepared with medium only for background subtraction and instrument gain adjustment. This was incubated for desired period of exposure. 20 μL resazurin solution was added to each well. This was incubated for 1 to 4 hours at 37 °C. The fluorescence was recorded using a 560 nm excitation / 590 nm emission filter set.

2.6. *Plasmodium falciparum* cultivation

Chloroquine sensitive and resistant strains of *P. falciparum* 3D7, respectively, obtained from Rhodes University Grahamstown, South Africa was kept in continuous *in vitro* culture

117 according to the modified candle-jar method of Trager and Jensen [28]. Human red blood
118 cells (blood type A+) in RPMI 1640 medium (Sigma Chemical Co., USA) supplemented with
119 25 mM HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (Sigma), 0.2 per cent
120 sodium bicarbonate (Sigma), and 15 per cent complement inactivated human AB+ serum
121 were used for parasite culture.

122 **2.7. *In vitro* antiplasmodial assay**

123 Anti-malarial test was carried out using the Immunocapture parasite lactate dehydrogenase
124 (IcpLDH) assay method as described by Makler et al., [29]. Extracts with concentration
125 (0.01-100 ug/mL) were added to parasite cultures in 96-well plates and incubated for 48 h in
126 a 37 °C CO₂ incubator. After 48 h the plates were removed from the incubator. Twenty µL of
127 culture was removed from each well and mixed with 125 µL of a mixture of Malstat solution
128 and NBT/PES solution in a fresh 96-well plate. These solutions measure the activity of the
129 parasite lactate dehydrogenase (pLDH) enzyme in the cultures. A purple product was
130 formed when pLDH was present, and this product was quantified in a 96-well plate reader by
131 absorbance at 620 nm (Abs₆₂₀). The Abs₆₂₀ reading in each well is thus an indication of the
132 pLDH activity in that well and also the number of parasites in that well. Each sample was
133 tested in duplicates. For each concentration, % parasite viability – the PLDH activity in
134 treated wells relative to untreated controls was calculated.

135 **2.8. Data analysis**

136 All experiments were performed in duplicates and presented as the Mean ± SD. Statistical
137 analysis of the data was carried out by one way ANOVA (Graph Pad Prism 5.02 Software).
138 A value of p< 0.05, p<0.01, p<0.001 and p<0.0001 were considered to be significant, very
139 significant and highly significant, respectively. Linear regression analysis was used to
140 calculate CC₅₀ and IC₅₀. The antiplasmodial activities of fractions were expressed by the
141 inhibitory concentrations (IC₅₀) of the drug that induced 50% reduction in parasitaemia

142 compared to the control (100 %) parasitaemia. The activity was analysed according to the
143 classification for antiparasmodial activity by Valdes [30]. Therefore extract exhibiting IC_{50}
144 *P.falciparum* > 100 µg/mL was considered inactive. Extract showing IC_{50} *P.falciparum* < 100 µg/mL
145 was classified as follows: - Marginally active at SI < 4, partially active at SI 4-10 and active at
146 SI > 10. Active extract showing IC_{50} *P.falciparum* < 10 µg/mL was to be selected for further
147 bioassay-guided fractionation. Selectivity index was calculated as the ratio of cytotoxicity of
148 extract on *HeLa* cell line (cytotoxicity) to the IC_{50} of the extract against *P. falciparum*
149 (antiparasmodial activity) strains.

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153 3. RESULTS AND DISCUSSION

154 3.1. Fractionation of acetone extract

155 Fractionation of the acetone extract using column chromatography produced five fractions
156 (Table 1). Fraction grouping was based on the TLC analysis using hexane, dichloromethane
157 and methanol as eluents. Based on Table 1, the yield of fractions obtained ranged from
158 0.05-26.66%. Fraction number A2 and A5 had the highest yield of 26.66% followed by
159 fraction A3, A1 and A4. The smallest yield was fraction A4 (0.05%). From the TLC analysis
160 all fractions had many number of spots and none with a single spot. The fractions were
161 observed to have different colours visually. This may be due to different types of constituents
162 found in each fraction.

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167 Table 1: Yield of Fractions of *A.djalonensis* Acetone root extract

Fractions	Weight(g)	% Yield	Colour of Fraction	Rf
A1	3.90	13.02	White	0.66
A2	8.00	26.66	light yellow	0.56
A3	7.43	24.70	Yellow	0.62
A4	1.50	0.05	Brown	0.70
A5	8.00	26.66	Dark brown	0.61

168 Rf= Retardation factor

169 3.2. **In vitro** assays

170 Cytotoxicity and antimalarial activity was determined from CC₅₀ and IC₅₀ value of the
 171 fractions. The CC₅₀ and IC₅₀ value is always inversely proportional to the cytotoxicity and anti
 172 plasmodial activity respectively. This meant the higher the CC₅₀ and IC₅₀ values, the lower
 173 the activities and **vice versa**. The results showed that there was a positive correlation
 174 between the concentrations of fractions with the percentage of *HeLa* cells and *P. falciparum*
 175 parasite cell viability (Fig. 6 and 12).

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182 Table 2 shows the CC₅₀ and IC₅₀ values of all five chromatographic fractions of
183 *A.djalensis* acetone extract.

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Fractions	<i>P.falciparum</i> (IC ₅₀)	HeLa Cells(CC ₅₀)	SI	Classification
	µg/mL	µg/mL		
A1	0.0360 ± 0.0100	44.2010 ± 8.6790	1227.8055 ± 8.2100**	Active
A2	8.1299 ± 2.0358*	50.0000 ± 5.6412**	6.150 ± 0.0200****	Partially active
A3	46.2482 ± 1.2720**	71.6221 ± 2.9600**	1.540 ± 0.0091****	Marginally active
A4	0.0151 ± 0.001	36.7212 ± 5.8900	2431.8675 ± 1.5890****	Active
A5	9.8013 ± 0.8171**	40.5132 ± 3.7700*	4.133 ± 0.0150****	Partially active
CON	0.0125 ± 0.0001	18.6430 ± 1.6969	1491.2000 ± 3.009	Active

185 A1-A5 = fractions from acetone root extract of *Anthocleista djalensis*. CON = control, IC₅₀ = The half
186 maximal inhibitory concentration, CC₅₀ = The 50% Cytotoxic concentration, SI = selective index.
187 Results were expressed as mean ± STD. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared to
188 positive control. Marginally active at SI < 4, partially active at SI 4-10 and active at SI > 10

189 3.2.1 *In vitro* Cytotoxicity assay

190 The test results on *HeLa* cells indicated growth inhibition by the fractions of acetone root
191 extract from *A.djalensis*. All Fractions A1, A2, A3, A4 and A5 had CC₅₀ ≤ 100. The highest
192 cytotoxicity activity was demonstrated by A4 with CC₅₀ value of 36.72 µg/mL followed by A5,
193 A1 and A2 with CC₅₀ value of 40.51 µg/mL, 44.20 µg/mL and 50.00 µg/mL respectively.
194 Fraction A3 showed the lowest cytotoxicity with CC₅₀ value of 71.62 µg/mL (Fig1-6).

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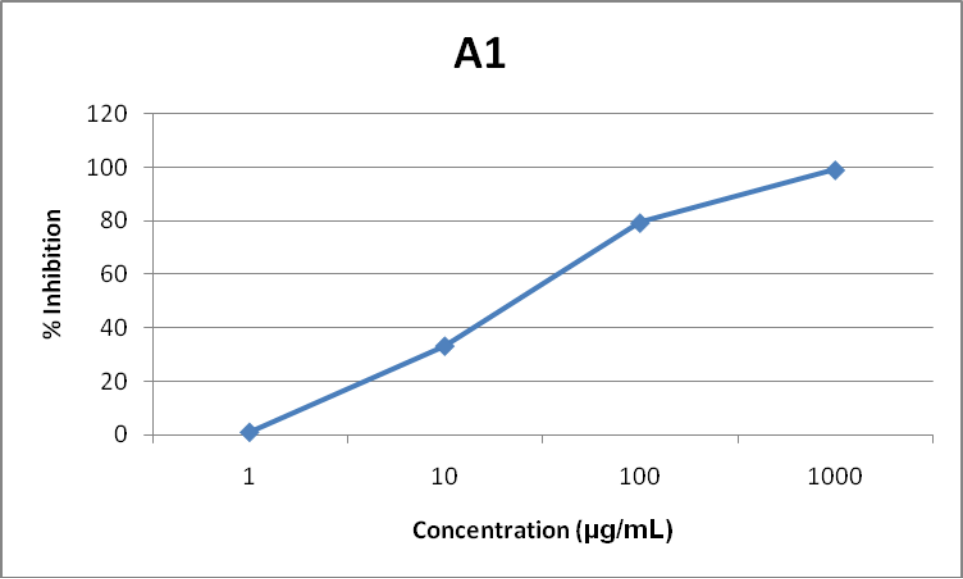


Fig 1: Cytotoxicity of fraction A1 against *HeLa* cells at different concentration (µg/mL)

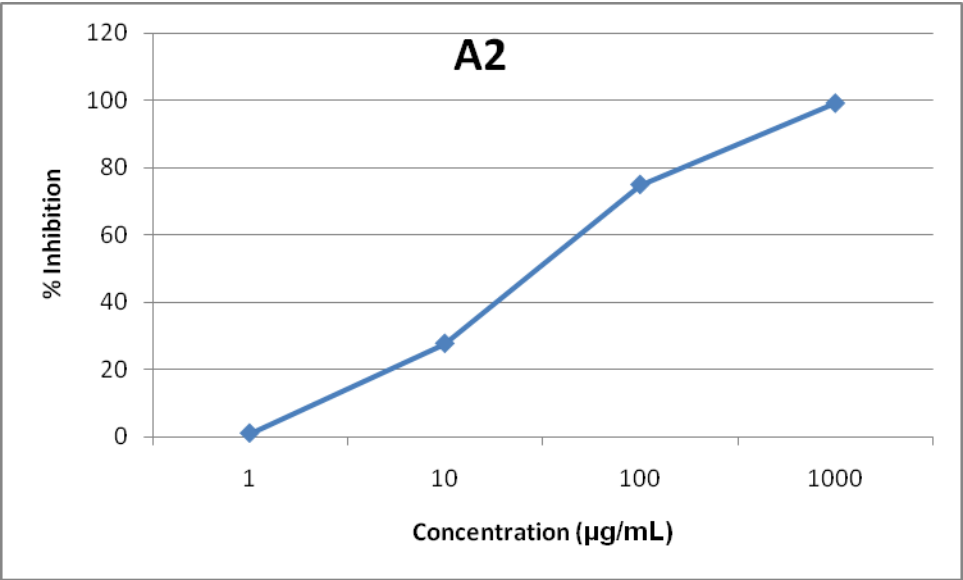


Fig 2: Cytotoxicity of fraction A2 against *HeLa* cells at different concentration (µg/mL)

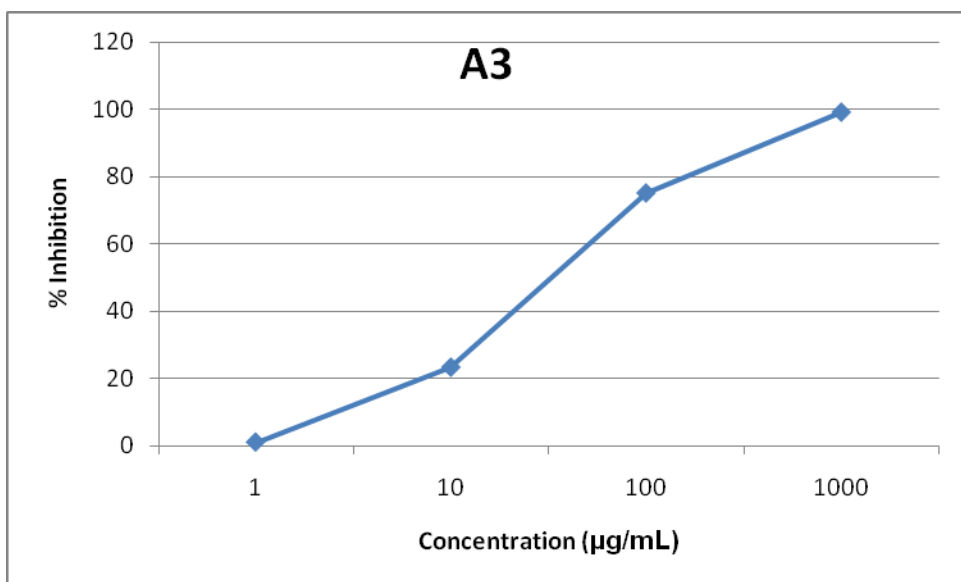


Fig 3: Cytotoxicity of fraction A3 against *HeLa* cells at different concentration (µg/mL)

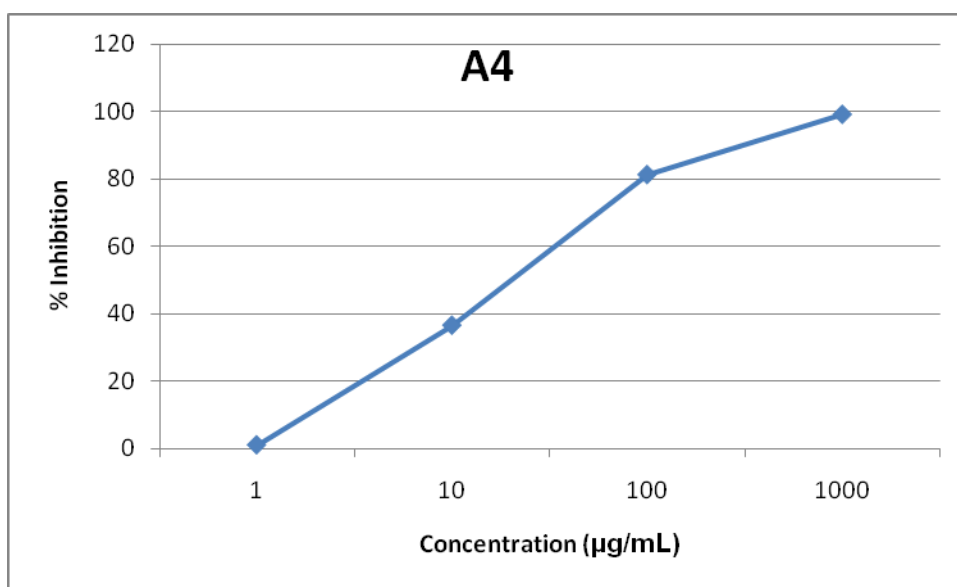


Fig 4: Cytotoxicity of fraction A4 against *HeLa* cells at different concentration (µg/mL)

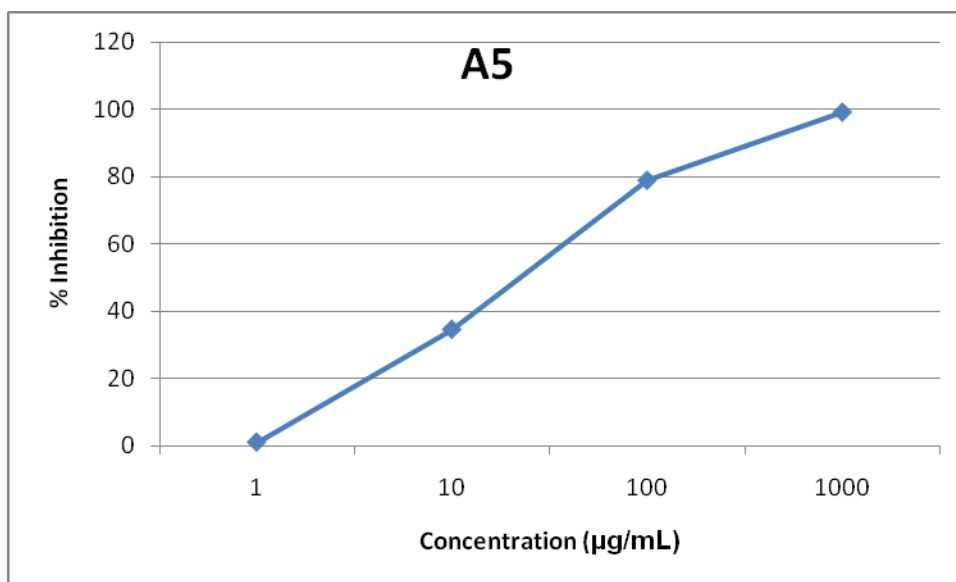


Fig 5: Cytotoxicity of fraction A5 against *HeLa* cells at different concentration (µg/mL)

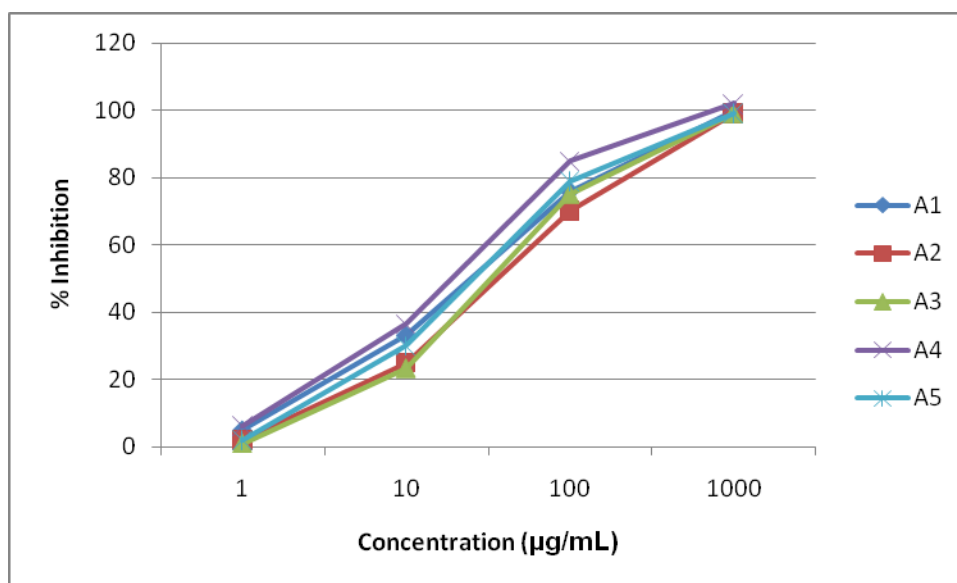
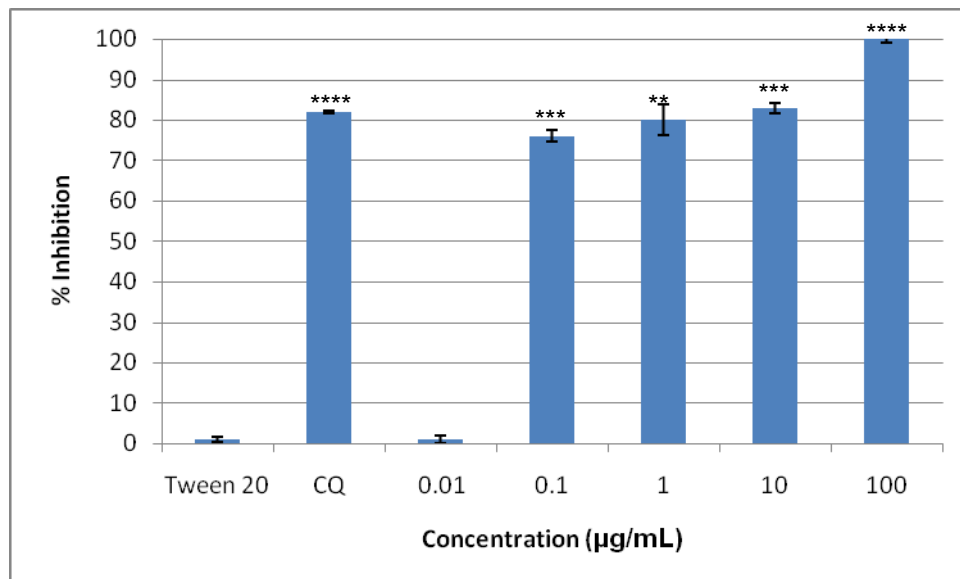


Fig 6: Cytotoxicity of all five fractions against *HeLa* cells at different concentration (µg/mL)

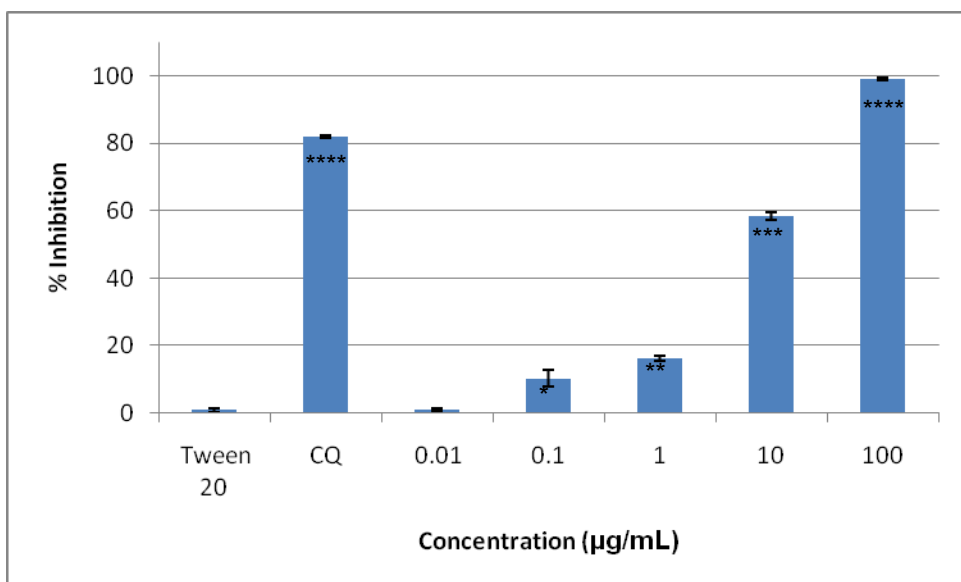
232 3.2.2. *In vitro* anti-malarial activity

233 The test results of *in vitro* anti-malarial activity showed that all fractions of *A.djalensis*
 234 acetone extract had the ability to inhibit the growth of *P. falciparum* (Fig 7-11). The IC₅₀
 235 values and selectivity indices (SI = ratio of cytotoxicity to anti-malarial activity) of extracts are
 236 shown in Table 2. The IC₅₀ values for fractions A1, A2, A3, A4, and A5 were 0.036 µg/mL,
 237 8.129 µg/mL, 46.248 µg/mL, 0.0151 µg/mL and 9.801 µg/mL respectively. Fractions were
 238 classified as marginally active (A3) showing SI of 1.540, partially active (A2 and A5) with SI
 239 of 6.150 and 4.133 respectively and as active (A1 and A4) exhibiting SI of 1227.805 and
 240 2431.867 respectively. Furthermore, A1 and A4 showed SI > 10 and IC₅₀ < 10 µg/mL.
 241 Chloroquine, used as a reference anti-malarial drug, tested in parallel had an IC₅₀ of 0.0125
 242 µM and was comparable with A1 and A4 (IC₅₀: 0.0360 µg/mL and 0.0151 µg/mL). This
 243 showed A1 and A4 as being very prospective fractions to be developed as anti-malarial
 244 agents.



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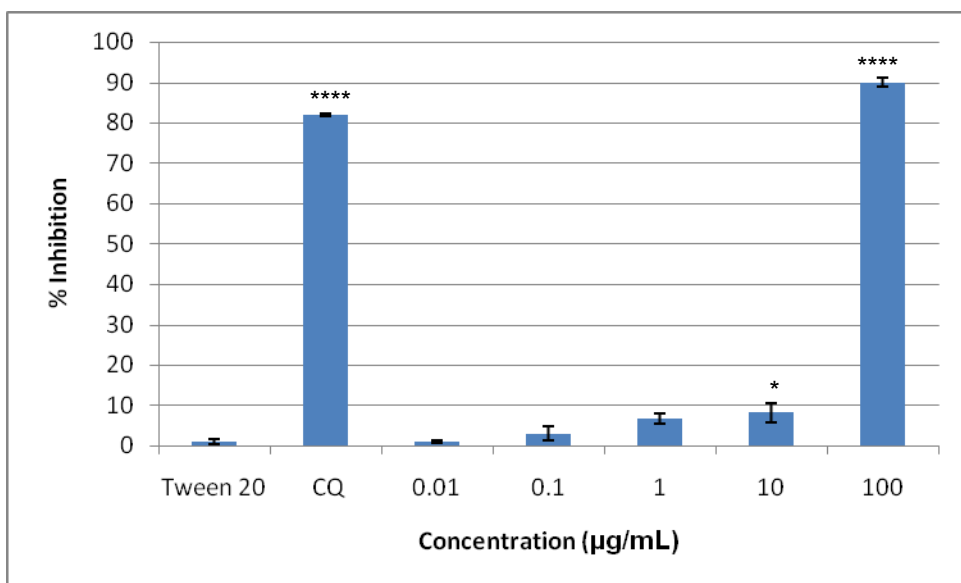
246 **Fig 7: Activity of fraction A1 against *P.falciparum* at different concentration of**
 247 **0.01,0.1,1,10,100 µg/mL.** CQ (0.1µg/mL) was used as positive control and negative control
 248 was treated with tween 20. Results were expressed as mean ± STD. **p<0.01,
 249 ***p<0.001and ****p<0.0001 compared to negative control



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251 **Fig 8: Activity of fraction A2 against *P.falciparum* at different concentration of**
 252 **0.01,0.1,1,10,100 µg/mL.** CQ (0.1ug/mL) was used as positive control and negative control
 253 was treated with Tween 20. Results were expressed as mean ± STD. *p<0.05, **p<0.01,
 254 ***p<0.001and ****P<0.0001 compared to negative control.

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259 **Fig 9: Activity of fraction A3 against *P.falciparum* at different concentration of**
 260 **0.01,0.1,1,10,100 µg/mL.** CQ (0.1ug/mL) was used as positive control and negative control
 261 was treated with tween 20. Results were expressed as mean ± STD. **p<0.05, ****p<0.0001
 262 compared to negative control.

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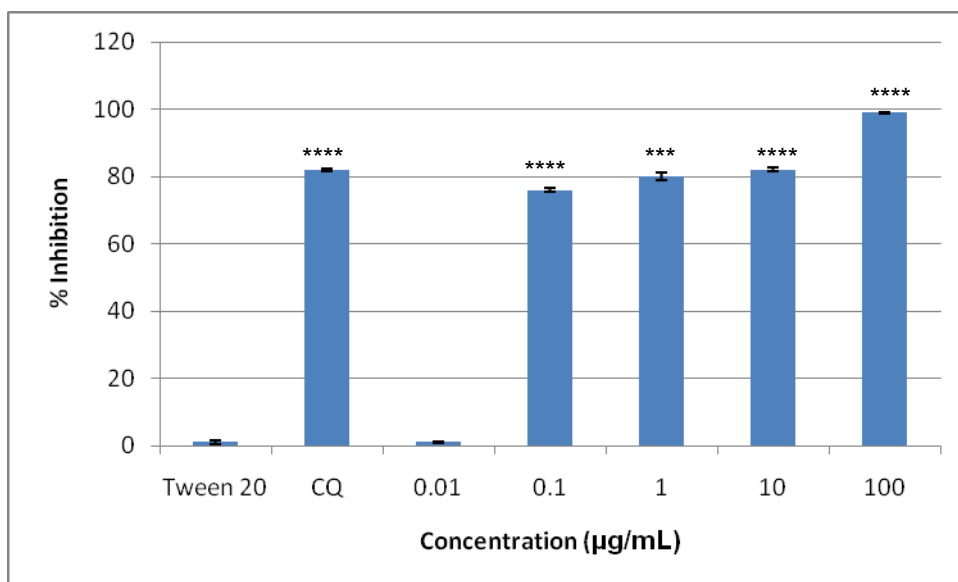


Fig 10: Activity of fraction A4 against *P.falciparum* at different concentration of 0.01,0.1,1,10,100 µg/mL. CQ (0.1ug/mL) was used as positive control and negative control was treated with tween 20. Results were expressed as mean $\pm$ STD. *** $p < 0.01$ and **** $p < 0.0001$ compared to negative control.

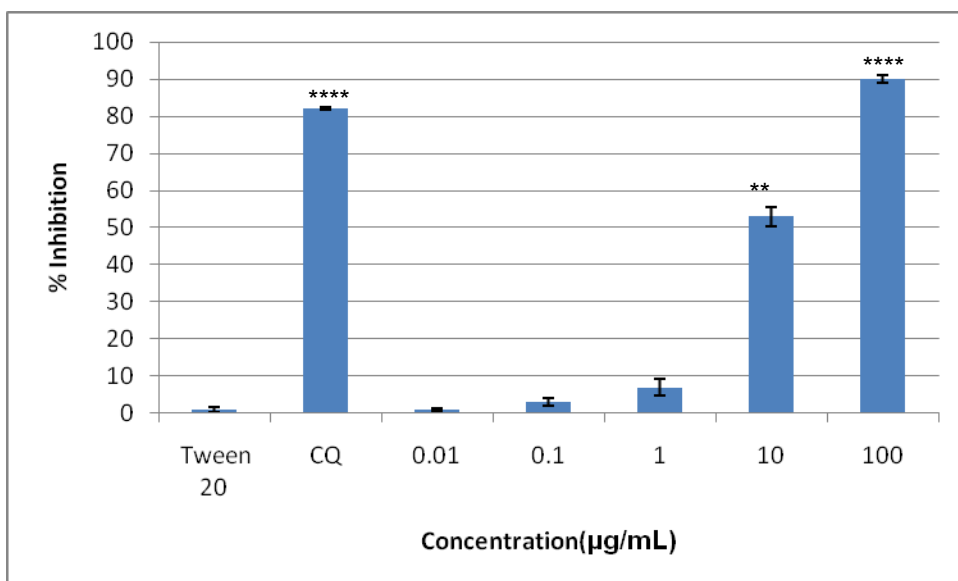


Fig 11: Activity of fraction A5 against *P.falciparum* at different concentration of 0.01,0.1,1,10,100 µg/mL. CQ (0.1ug/mL) was used as positive control and negative control was treated with tween 20. Results were expressed as mean $\pm$ STD. ** $p < 0.01$ and **** $p < 0.0001$ compared to negative control

280 3.3 Discussion

281 Resistance to anti-malarial drugs has often threatened malaria elimination efforts and
282 historically has led to the short-term resurgence of malaria incidences and deaths [31]. Anti-
283 malarial drug resistance develops when spontaneously occurring parasite mutants with
284 reduced susceptibility are selected, and are then transmitted [32]. Chloroquine resistance is
285 associated with mutations in the polymorphic gene encoding a putative chloroquine
286 transporter and located on chromosome “7” [31]. Chloroquine-resistant *Plasmodium*
287 *falciparum* malaria is a major health problem. *P. falciparum* infections acquired in most of
288 Africa, and some parts of Asia and South America cannot be treated with chloroquine [33].
289 Increasing drug resistance in *plasmodium falciparum* and a resurgence of malaria in tropical
290 areas have effected a change in treatment of malaria [34]. A combination of antimalarial
291 drugs is responsive to *P.falciparum* with high grade resistance to chloroquine. Artemisinin
292 and its semi-synthetic derivatives are anti-malarial drugs effective against CQ-resistant *P.*
293 *falciparum* as single therapeutic agents. However, to minimize the risks of recrudescence
294 and the development of resistance, a combination treatment with a second antimalarial drug
295 is recommended [35]. Newer drug combination of compounds from biodiversity to combat
296 malarial disease and drug resistant strain are urgently needed. The long-established use of
297 quinine and the more recent introduction of artemisinin and its derivatives as highly effective
298 antimalarials demonstrates that plant species are an important resource for the discovery of
299 new antimalarial agents[36]. *Anthocleista djalensis* of Gentianeaceae family is one plant
300 with diverse medicinal uses. Some activities of the leaf stem [22] and roots [15, 21] parts of
301 the plant against malaria have been reported. The phytochemical screening of the root
302 extract of *Anthocleista djalensis* carried out indicated the presence of saponins,
303 flavonoids, tannins, reducing sugar, steroids, phlobatanins, volatile oils and alkaloids which
304 are active components present in the plant that makes it medicinal [36].

305 *In vivo* activity of the root extracts and fractions against *P. berghei* may have been reported
306 [21], yet this is the first scientific study of the fractions from the root of *Anthocleista*
307 *djalonensis* on *Plasmodium falciparum* (the parasite responsible for human malaria). In this
308 study we investigated the anti-malarial and cytotoxicity activity of acetone chromatographic
309 fractions of *A.djalonensis*. Fractions A2, A3, and A5 with low SI (1.33, 1.68 and 1.30)
310 revealed that the anti-malarial activity was dependent on the cytotoxicity and independent on
311 the activity against the parasites. While A1 and A4 with high SI (441.25 and 1560.03) meant
312 that activity against the parasites was attributed to the parasites themselves and not
313 cytotoxicity. According to Soh [37], high selective index means safer therapy. Hence a cutoff
314 point of 4 certified safe anti-malarial use. Whereas, SI greater than 10 and IC₅₀ values below
315 10 µg/mL should be promising sources of anti-malarial molecules. Activity against *HeLa* cells
316 and *P. falciparum* in a high percentage of tested fractions were observed. Activity was
317 directly proportional to the concentration. Obviously, *in vitro* study presumes a direct action
318 on the parasite [38].

319 Although, anti-malarial activity has been detected in some parts of *A.djalonensis* plants like
320 the stem and leaves [22], this is the first report for the chromatographic fractions of acetone
321 root of *A.djalonensis* plant. Phytochemical analysis of these fractions from acetone root
322 extract of *A.djalonensis* suggests the presence of triterpenoids, flavonoids and
323 anthraquinones [17] as chemical classes with widely demonstrated effective antimalarial
324 activity [39]. Flavonoids act by inhibiting the fatty acid biosynthesis (FAS II) of the parasite
325 [40,41] as well as inhibiting the influx of L-glutamine and myoinositol into infected
326 erythrocytes [43]. Antimalarial activity of anthoquinone could be due to nitric oxide
327 generation from macrophages using polysaccharide. In combination of high concentration of
328 this oxide with sub-optimal doses of chloroquine, the parasitaemia in chloroquine resistant
329 malarial infection was suppressed [43]. While triterpenoids mechanism of action is in the
330 arresting of parasite development, inhibition of the hemozoin polymerization on the parasite

331 [44, 45] and the lactase dehydrogenase of the *Plasmodium falciparum* (an essential enzyme
332 used to generate energy within the parasite) [46, 47].

333 The high activity of fraction A1 and A4 is an indication that these fractions are promising
334 sources as anti-malarial agents.

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338 4. CONCLUSION

339 The chromatographic root fractions of *Anthocleista djalensis* showed potent anti-malarial
340 and cytotoxic activities. The results highlighted the safety in the use of the plant of which
341 can become a source of lead compounds of high therapeutic efficacy for malarial diseases.

342 The remarkable anti malarial activity of *A.djalensis* encourages the investigation of native
343 and naturalized African plants to explore as a potential source of anti malarial drugs.
344 However, fractions were selected for further purification, isolation and identification of active
345 chemical classes with proved efficacy against *P. falciparum*.

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350 COMPETING INTERESTS

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352 Authors have declared that no competing interests exist.

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