



## Article

# In Vitro Assessment of Antiplasmodial and Antitrypanosomal Activities of Chloroform, Ethyl Acetate and Ethanol Leaf Extracts of *Oedera genistifolia*

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**Featured Application:** *Oedera genistifolia* could serve as a source of isolating lead compounds for the development of new antiplasmodial and antitrypanosomal agents.

**Abstract:** The high resistance evolution of protozoans to the existing antiparasitic drugs has necessitated the quest for novel and effective drugs against plasmodium and trypanosome parasites. As a result, this study aimed to assess the antiplasmodial and antitrypanosomal potentials of chloroform, ethyl acetate and ethanol leaf extracts of *Oedera genistifolia*. Standard biochemical procedures were explored for the plant extraction and gas chromatography-mass spectroscopy (GCMS) was used to identify the bioactive compounds in the crude extracts. The cytotoxic effects of the crude extracts were assessed against human cervix adenocarcinoma (HeLa cells) and their antiparasitic activities were investigated against *Plasmodium falciparum* strain 3D7 and *Trypanosoma brucei brucei*. GCMS analyses of the crude extracts revealed the bioactive compounds that could be responsible for the biological activities. The extracts had no cytotoxic effect on HeLa cells and demonstrated good antiplasmodial activity (chloroform extract:  $IC_{50} = 11.6 \mu\text{g}\cdot\text{mL}^{-1}$ , ethyl acetate extract:  $IC_{50} = 3.3 \mu\text{g}\cdot\text{mL}^{-1}$  and ethanol extract:  $IC_{50} = 3.7 \mu\text{g}\cdot\text{mL}^{-1}$ ). Likewise, they showed excellent antitrypanosomal activity with  $IC_{50} = 0.5 \mu\text{g}\cdot\text{mL}^{-1}$  for chloroform and ethyl acetate extracts and  $IC_{50} = 0.4 \mu\text{g}\cdot\text{mL}^{-1}$  for the ethanol extract. Findings from the present study indicated that *O. genistifolia* could be a good source of strong antiplasmodial and antitrypanosomal agents.

**Keywords:** *Oedera genistifolia*; bioactive compounds; cytotoxicity; antiplasmodial; antitrypanosomal

## 1. Introduction

Malaria has been known as one of the tropical diseases with a devastating effect on the well-being of people, especially from developing countries in the world. Although considerable progress has been made in the fight to combat malaria over the last two decades, the disease burden has been prevalent in Africa, where global cases and deaths have increased to about 82% and 90%, respectively [1]. The report further signposts that children below age 5 years are more vulnerable to this disease, which account for about 78% of 90% malaria death cases reported, thus making malaria one of the most dreadful diseases affecting the quality of life lived by an individual in tropical countries. Current malarial drugs include artemether-lumefantrine (coartem), artesunate-amodiaquine, quinine sulfate, mefloquine and primaquine phosphate. Although these drugs have been recognized to be effective, the side effects on

long usage cannot be neglected and are coupled with other demerits such as high cost, drug failure and a high resistance rate. Consequently, these aforementioned reasons have underscored the unending and urgent need for the discovery and development of potent, cost-effective and innocuous antimalarial drugs [2–4].

African trypanosomiasis is a parasitic disease caused by *Trypanosoma brucei brucei* which has affected the lives of about 48 million cattle and 60 million people worldwide [5,6]. Regrettably, antitrypanosomal drugs are not economical and their side effects, as well as the rate at which a new strain of these parasites develops resistance to the existing drugs, have rather caused them to be ineffective [7]. Consequently, the search for safe and cheap therapeutic drugs from plant sources could be considered as an alternative. Nevertheless, some studies on the antitrypanosomal activity of plant extracts and plant derivatives have been recently documented [8–10].

Historically, plants and their derivatives have been reported to be the foundation for the discovery of leads used in the designing of drugs to treat various types of human diseases [2]. It is appraised that 66–85% of the world's population relies on folkloric herbs and the search for drugs derived from plants has been increasing in the last two decades [11–16]. African medicinal plants are known to contain a large variety of bioactive compounds and extracts of these plants have been screened for various biological activities, in the pursuit for prospective novel therapeutic drugs against the different human and animal illnesses [17,18]. Several plant bioactive compounds such as tannins, flavonoids, glycosides, alkaloids, anthraquinones and alkaloids have been labeled with antiplasmodial and antitrypanosomal activities [2].

*Oedera genistifolia* (“Klein Perdekaroo”) is a prickly shrublet that is grouped to the daisy family (Compositae or Asteraceae). They are usually found in the Southwestern Cape, South Africa (Namaqualand to Grahamstown) and mostly present on the lower north-facing slopes, especially on rocky clay and shale-based soils. The aqueous leaf extract of *O. genistifolia* has been explored for green silver nanoparticles and their antibacterial activity has been reported in our previous studies [19]. From our literature search, there is no document reporting on the antiparasitic activity of organic solvent crude extracts of *O. genistifolia*. Therefore, the present study aimed to investigate the in vitro antiplasmodial and antitrypanosomal potentials of chloroform, ethylacetate and ethanol leaf extracts of *O. genistifolia* collected from the Eastern Cape Province of South Africa.

## 2. Materials and Methods

### 2.1. Extraction of Plant Material

Plant collection and identification have been reported in our previous study [19]. The leaves were rinsed with distilled water to remove any form of impurity and shade-dried at room temperature for 14 days. The extraction was carried out following the procedure of Mzena et al. [4]. Briefly, the dried leaves were made to powder with an electric blender and 100 g of the powdered leaves were soaked in 500 mL of organic solvents (chloroform, ethyl acetate and ethanol) contained separately in 1 L of a volumetric flask and agitated in a shaker for 72 h. Thereafter, the mixtures were filtered with a Whatman filter paper no 1 to obtain the filtrates from each extraction, and thereafter the rotary evaporator was used to remove the solvents and the crude extracts were left under a fume hood to dry and kept at 4 °C until further use.

### 2.2. GCMS Analysis

The crude extracts of *O. genistifolia* were subjected to GCMS (Shimadzu Europa GmbH, Duisburg, Germany) as described by Tonisi et al. [20]. The crude extracts (1 µL) were injected into a GCMS system consisting of Agilent 7890B GC System coupled with 5977A mass spectra data. An HP-5 fused silica capillary column (30 m × 0.320 mm i.d. and 0.250 µm film thickness) was used. The injector was set at 250 °C (temperature) and 48.745 kPa (pressure) and it operated at pulse splitless mode. The carrier gas helium (99.999% purity) was run at an average velocity: 36.262 cm/s. Oven temperature

programming was started from 40 °C (held for 1 min), ramped to 240 °C at 3 °C/min. The total runtime was 67.667 min. Mass Hunter software was used for the data analysis and the identity of the bioactive compounds was known by comparing their retention times with those of the standards.

### 2.3. Biocompatibility Study

The cytotoxic effects of chloroform, ethyl acetate and ethanol leaves extracts of *O. genistifolia* against HeLa cells (Cellonex, Johannesburg, South Africa) were evaluated as described by Okaiyeto et al. [19]. Briefly, 50 µg·mL<sup>-1</sup> of the crude extracts was added into 96-well plates containing HeLa cells for 48 h. The number of cells surviving the extracts was determined using resazurin-based reagent and reading resorufin fluorescence in a Spectramax M3 microplate reader. Subsequently, the results were expressed as % viability—the resorufin fluorescence in extract-treated wells relative to untreated controls. These extracts were tested in duplicate and standard deviation (SD) was derived. Emetine (which induces cell apoptosis) was used as a control drug standard.

### 2.4. Antiplasmodial Activity

Antiplasmodial potential of the studied extracts of *O. genistifolia* against chloroquine-sensitive *P. falciparum* (strain 3D7) was evaluated as described by Okaiyeto et al. [21] using parasite lactate dehydrogenase (pLDH) activity as a biomarker. Malaria parasites (*P. falciparum* strain 3D7) were maintained in RPMI 1640 medium containing 2 mM L-glutamine and 25 mM hepes (Lonza, Basel, Switzerland). The medium was further supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg·mL<sup>-1</sup> gentamycin and 2% hematocrit human red blood cells. The parasites were cultured at 37 °C under an atmosphere of 5% CO<sub>2</sub>, 5% O<sub>2</sub> and 90% N<sub>2</sub> in a sealed T75 culture flask.

To determine the antiplasmodial activity of the extracts, serial dilutions of the extracts were added to in vitro cultures of *P. falciparum* (strain 3D7) in 96-well plates and incubated for 48 h. Thereafter, an aliquot of 20 µL of culture was removed from each well combined with 125 µL of a mixture of Malsat and NBT/PES solutions in a fresh 96-well plate. These solutions measure the activity of the parasite lactate dehydrogenase (pLDH) enzyme in the cultures. A purple product was formed when pLDH was present, and this product was quantified in a Spectramax M3 microplate reader (Abs<sub>620</sub>). The Abs<sub>620</sub> reading in each well is thus an indication of the pLDH activity and hence the number of parasites present. For the IC<sub>50</sub> determination, the extracts were tested in a range testing from 100 µM·mL<sup>-1</sup> and extended using a 3-fold serial dilution. For each extract, percentage parasite viability—the pLDH activity in extract-treated wells relative to untreated controls—was calculated. The extracts were tested in duplicate and standard deviation (SD) was derived. For each compound and each extract, percentage viability was plotted against Log (extract concentration) and the IC<sub>50</sub> (50% inhibitory concentration) obtained from the resulting dose–response curve by non-linear regression. For comparative purposes, chloroquine (a standard antimalarial drug) was used as a control.

### 2.5. Antitrypanosomal Activity

Antitrypanosomal activity of chloroform, ethyl acetate and ethanol extracts of *O. genistifolia* was assessed against *T. b. brucei* as previously described by Okaiyeto et al. [21]. Briefly, 50 µg·mL<sup>-1</sup> of the crude extracts was treated with the parasites in a 96-well plate incubated for 48 h alongside pentamidine (control drug). Subsequently, the viable parasites were mixed with resazurin-based reagents to quantify those that survived the treatment. Resazurin is reduced to resorufin (a fluorophore (EXC560/Em59)) in viable cells and was quantified in a Spectramax M3 microplate reader. Results were expressed as % parasite viability—the resorufin in extract-treated wells relative to untreated controls. The extracts were tested in duplicate and standard deviation (SD) was derived. Extracts that reduced the parasite viability to <20% were considered for further testing (dose–response assay). To determine the trypanocidal potency of the tested extracts, serial dilutions of the extracts were added to in vitro cultures of *T. b. brucei* in 96-well plates. After a 48-h incubation, parasites surviving the extracts were

enumerated by adding a resazurin-based reagent and the IC<sub>50</sub> value of each extract was evaluated as previously described elsewhere.

### 2.6. Statistical Analysis

All the toxicity assays were carried out in triplicates, and IC<sub>50</sub> values were derived by non-linear regression analysis using GraphPad Prism.

## 3. Results and Discussion

### 3.1. GCMS Analysis

Plant bioactive compounds have played a substantial role in the drug discovery struggles for the treatment of human ailments [22]. In reality, most antimalarial, anticancer, antibacterial, antioxidant and anti-inflammatory agents are based on natural products, thus suggesting the need to identify novel bioactive compounds from plant origin. Folkloric medicine continues to be an important task in the search for therapeutic drugs for human use [23,24]. According to the report of Ajaiyeoba et al. [25], the antimalarial activity of *Annona senegalensis* might be due to bioactive compounds such as alkaloids, quassinoids and canthin. In recent years, the importance of phytochemicals has been emphasized due to their noteworthy health benefits [26]. In the present study, GCMS analysis of the chloroform leaf extract of *O. genistifolia* (Table 1) revealed the existence of the following most significant bioactive compounds, among others: (R)- $\alpha$ -pinene, camphene,  $\gamma$ -terpinene, eucalyptol, (+)-2-bornanone, bornyl acetate, caryophyllene, aromandendrene, alloaromadendrene, (+)-ledene, (+)- $\delta$ -cadinene, spatulenol,  $\beta$ -eudesmol,  $\alpha$ -eudesmol, acorenone 1, n-hexadecanoic acid,  $\alpha$ -linolenic acid, ferruginol, D-homopregn-17a(20)-ene, (5.alpha., 17aE)- and (.+/-)-demethylsalvicanol.

Similarly, the GCMS profile of the ethyl acetate leaf extract of *O. genistifolia* showed the presence of the following bioactive compounds, among others (Table 1): sabinene ( $\beta$ -thujene), eucalyptol, (+)-2-bornanone, bornyl acetate, caryophyllene, aromandendrene, alloaromadendrene, viridiflorene,  $\delta$ -cadinene, (+)-, spatulenol,  $\beta$ -eudesmol, 2-(4a,8-dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol, acorenone 1, n-hexadecanoic acid,  $\alpha$ -linolenic acid, ferruginol, benzene, ethenylpentaethyl-6-Iodo-2-methylquinazolin-4(3H)-on and (.+/-)-demethylsalvicanol.

The GCMS profile of the ethanol leaf extract of *O. genistifolia* showed these bioactive compounds (Table 1): eucalyptol, (+)-2-bornanone, bornyl acetate, caryophyllene, aromandendrene, spatulenol,  $\beta$ -selinenol,  $\gamma$ -eudesmol, acorenone 1, n-hexadecanoic acid, ferruginol, D-homopregn-17a(20)-ene and (5.alpha., 17aE)-. A comprehensive information about the three crude extracts are presented in the Supplementary data (Tables S1–S3). These bioactive compounds have been acknowledged to be responsible for the different biological activities demonstrated by these crude extracts [27,28]. From the GCMS profiles of the crude extracts, we observed that the organic solvents used were able to extract similar bioactive compounds, which further buttress analogous biological and pharmacological activities exhibited by the three extracts in our present study. For example, phytol was present in all three extracts and previous studies have highlighted that it has antioxidant, anticancer and antinociceptive effects [29–32]. In the same vein, hexadecanoic acid is known to exhibit strong antimicrobial and anti-inflammatory activity [33]. Similarly, ferruginol and caryophyllene were also present in all the extracts and their antimalarial activity has been well documented previously [1]. Therefore, fractionation and isolation of pure compounds from the crude extracts are necessary because of numerous bioactive compounds that support the medicinal application of *O. genistifolia*.

**Table 1.** GCMS profiles of chloroform, ethyl acetate and ethanolic extracts of *O. genistifolia*.

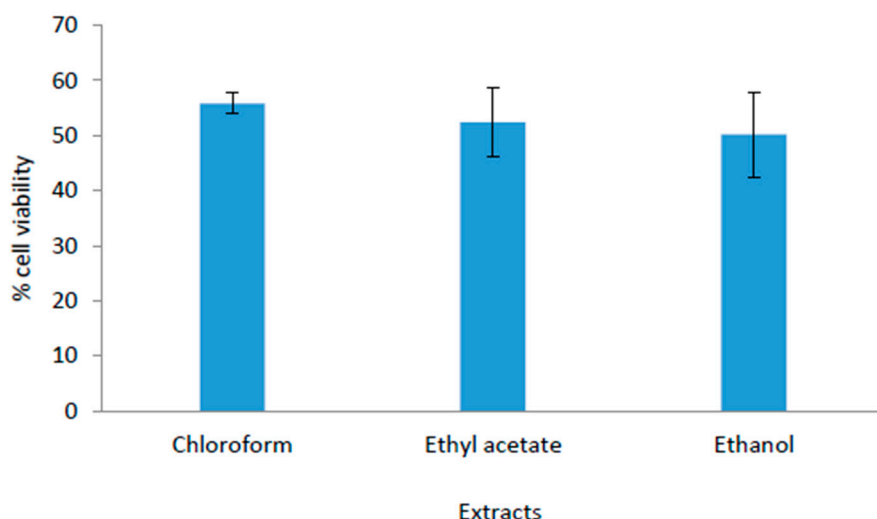
s/n	RT	Area	Qual (%)	Chloroform Extract	Ethyl Acetate Extract	Ethanol Extract
1	8.909	1.54	96	(R)- $\alpha$ -Pinene	-	-
2	9.455	1.53	97	Camphene	-	-
3	12.844	1.77	91	$\gamma$ -Terpinene	-	-
4	12.875	1.37	91	-	Sabinene ( $\beta$ -Thujene)	-
5	13.025	3.19	94	Eucalyptol	-	-
6	13.003	2.06	94	-	Eucalyptol	-
7	13.284	1.43	98	-	-	Eucalyptol
8	18.033	1.65	98	(+)-2-Bornanone	-	-
9	18.037	1.51	98	-	(+)-2-Bornanone	-
10	18.137	1.30	98	-	-	(+)-2-Bornanone
11	24.562	4.10	99	-	-	Bornyl acetate
12	24.583	4.42	94	Bornyl acetate	-	-
13	24.612	4.50	94	-	Bornyl acetate	-
14	30.178	6.54	99	-	-	Caryophyllene
15	30.265	7.69	99	Caryophyllene	-	-
16	30.311	8.58	99	-	Caryophyllene	-
17	30.910	1.64	99	-	-	Aromandendrene
18	30.964	2.19	99	Aromandendrene	-	-
19	30.997	2.42	-	-	Aromandendrene	-
20	31.784	1.09	99	Alloaromadendrene	-	-
21	31.804	1.20	99	-	Alloaromadendrene	-
22	33.225	1.17	99	(+)-Ledene	-	-
23	33.258	1.35	98	-	Virdiflorene	-
24	34.380	1.05	99	(+)- $\delta$ -Cadinene	-	-
25	34.401	1.13	99	-	$\delta$ -Cadinene, (+)-	-
26	36.429	1.12	99	-	-	Spatulenol
27	36.464	1.25	99	Spatulenol	-	-
28	36.502	1.37	99	-	Spatulenol	-
29	39.154	1.72	99	-	-	$\beta$ -Selinenol
30	39.215	2.01	99	$\beta$ -Eudesmol	-	-
31	39.291	2.38	96	-	$\beta$ -Eudesmol	-
32	39.298	2.67	95	-	-	$\gamma$ -Eudesmol
33	39.371	2.82	95	$\alpha$ -Eudesmol	-	-
34	39.427	2.92	93	-	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol	-
35	44.148	3.02	83	-	-	Acorenone 1
36	44.214	3.62	83	Acorenone 1	-	-
37	44.266	4.06	62	-	Acorenone 1	-
38	50.332	1.05	99	n-Hexadecanoic acid	-	-
39	50.495	1.37	99	-	n-Hexadecanoic acid	-
40	55.541	1.03	99	-	-	n-Hexadecanoic acid
41	55.739	2.19	99	$\alpha$ -Linolenic acid	-	-
42	55.843	1.90	99	-	$\alpha$ -Linolenic acid	-
43	58.879	1.72	60	-	-	Estra-1,3,5(10)-trien-17-one, 3-hydroxy-2-methoxy-2(1H)-Phenanthrene, 6-(acetyloxy)-3,4,4a,9,10,10a-hexahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-
44	59.930	1.38	83	-	-	Ferruginol
45	60.401	1.55	99	-	-	-
46	60.434	1.37	99	Ferruginol	-	-
47	60.486	1.59	99	-	Ferruginol	-
48	61.746	4.29	95	-	-	D-Homopregn-17a(20)-ene, (5.alpha., 17aE)-
49	61.864	6.32	94	D-Homopregn-17a(20)-ene, (5.alpha., 17aE)-	-	-
50	61.906	6.17	56	-	Benzene, ethenylpentaethyl-	-
51	62.537	5.40	60	-	6-Iodo-2-methylquinazolin-4(3H)-on	-
52	62.487	5.65	90	2,5-Methano-2H-thieno [3,2-b]thiopyran-8-ol, hexahydro-, acetate, (2.alpha.,3a.beta.,5.alpha.,7a.beta., 8R*)-	-	-
53	67.280	1.34	70	-	-	4-[3-Pyridyl]-3-thiosemicarbazonepiperonal
54	67.332	1.07	99	(+/-)-Demethylsalvicanol	-	-
55	67.397	1.05	95	-	(+/-)-Demethylsalvicanol	-

s/n—serial number, RT—retention time, Quali—quality (%).

### 3.2. Biocompatibility Study

Any chemotherapeutic research aims to obtain bioactive compounds that can selectively reduce or destroy cancerous cells or microbes with negligible side effects on the host cells [34]. This principle is known as selective toxicity and is the main goal for chemotherapeutic treatment [35]. There are different methods to assay for the cytotoxicity effect of compounds, among which 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and resazurin-based reducing assays are the most commonly used. Therefore, in the present study, a resazurin-based reducing assay was employed. The lactate dehydrogenase (LDH) assay is commonly employed to evaluate tissue or cellular damage, which can be monitored in the extracellular medium and is a good biomarker for cellular toxicity [36,37].

In the present study, the cytotoxic effect of the crude extracts (chloroform, ethyl acetate and ethanol) was evaluated. The percentage cell viability was  $55 \pm 1.88$ ,  $52.31 \pm 6.31$  and  $50.14 \pm 7.72$  for chloroform, ethyl acetate and ethanol extracts at  $50 \mu\text{g}\cdot\text{mL}^{-1}$ , respectively (Figure 1). From the results, it was observed that the cell viability in all the extract-treated cells was more than 50% and this indicates that the extracts were not cytotoxic (moderate) because values below 50% are considered cytotoxic to the cells. Although the cell viability was almost half of the initial numbers, the extracts may be considered as safe for human use. Similar to our present findings, Satish and Sunita [38] recorded that the organic extracts (chloroform, ethyl acetate and ethanol) of *Pongamia pinnata* (L.) Pierre had no cytotoxic effect against *P. falciparum* (3D7 strain) and *P. berghei* (ANKA). Paudel et al. [27] reported that the chloroform extract of *Dendrobium crepidatum* inhibited the growth of 81.49% of HeLa cells at  $800 \mu\text{g}\cdot\text{mL}^{-1}$ . Widiyastuti et al. [39] reported that the ethanolic extracts (flowers, stems and leaves) of *Stachytarpheta jamaicensis* (L.) Vahl had no toxic effect on HeLa cells. On the contrary, Al-Sheddi et al. [40] reported the cytotoxic effect of ethyl acetate, chloroform and ethanol extracts of *Lavandula coronopifolia* against MDA-MB-321 cells and in the same vein, the crude leaves ethanol extract of *Lasiosiphon eriocephalus* exhibited a high toxicity effect against HeLa cells [41]. In vitro cytotoxicity studies revealed that ethanol extracts of leaves of *Tamilnadia uliginosa* exhibited toxic effects on HeLa cells [42]. Okoro and Tor-Anyiin [43] also documented that the ethyl acetate of stem bark extracts from *Pycnanthus angolensis* showed a significant cytotoxic effect against HeLa cells.



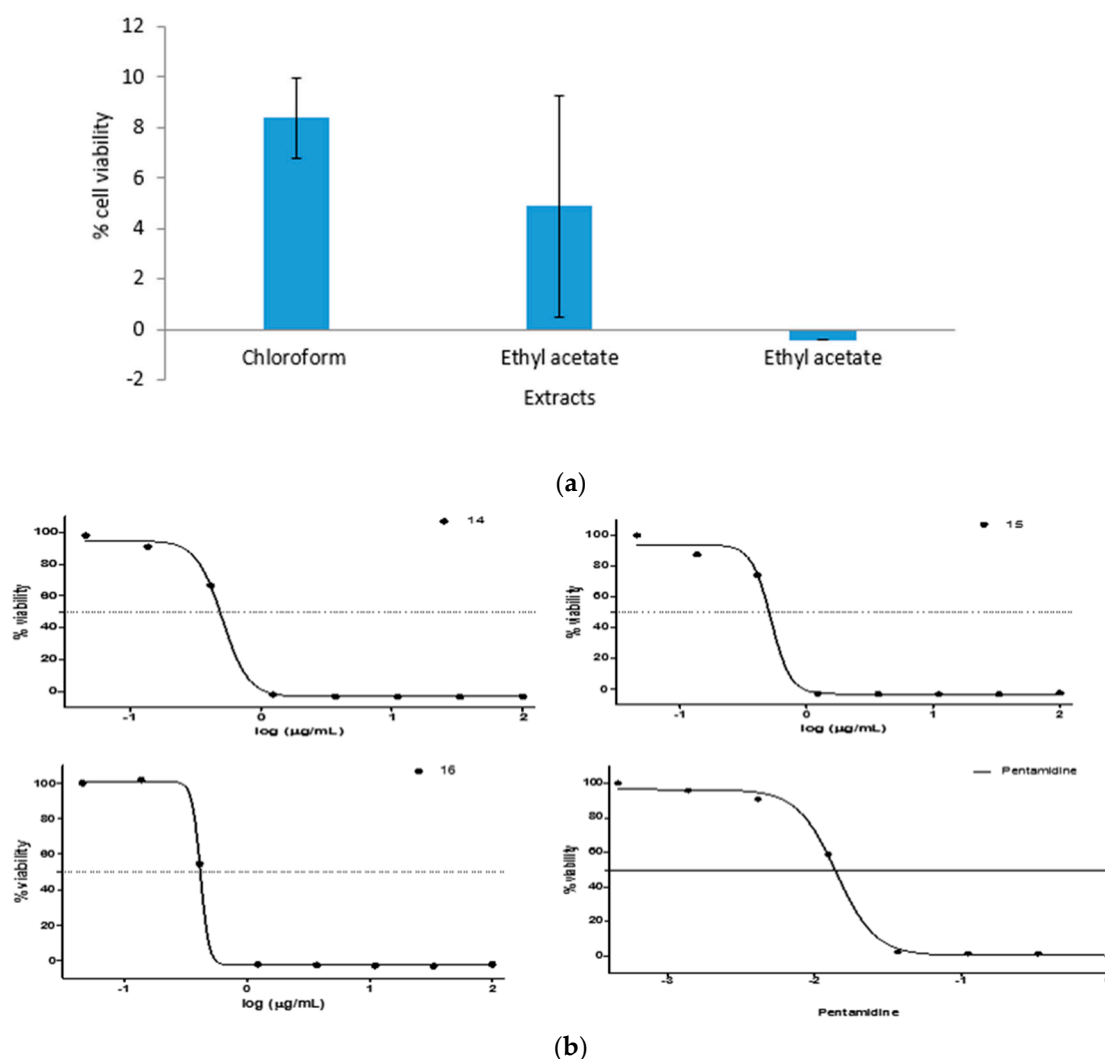
**Figure 1.** Cytotoxic effect of chloroform, ethyl acetate and ethanol extracts of *O. genistifolia*.

### 3.3. Antiplasmodial Study

In the present study, the antiplasmodial activity of solvent extracts (chloroform, ethylacetate and ethanol) of *O. genistifolia* was investigated against *P. falciparum* 3D7 and the results are depicted in Figure 2 and Table 2. The chloroform extract of *O. genistifolia* demonstrated the lowest antiplasmodial activity with  $\text{IC}_{50} = 11.6 \mu\text{g}\cdot\text{mL}^{-1}$ , followed by ethanolic extracts ( $\text{IC}_{50} = 3.7 \mu\text{g}\cdot\text{mL}^{-1}$ ), and the highest



was observed with ethyl acetate ( $IC_{50} = 3.3 \mu\text{g}\cdot\text{mL}^{-1}$ ). The weight of scientific evidence has highlighted that the variation in the antiplasmodial activity exhibited by these crude extracts could be due to their differences in chemical composition as a result of the variation in the polarity of solvents used for the plant extraction [13,44]. In comparison with other antiplasmodial activities of plant extracts from previous studies around the Africa region (Table 3), our findings revealed that *O. genistifolia* extracts showed good antiplasmodial activity. Antiplasmodial potentials of some plant extracts have been documented in the literature.



**Figure 2.** Antiplasmodial activity of chloroform, ethyl acetate and ethanol extracts of *O. genistifolia* (a), dose-dependent curves of the antiplasmodial activity of chloroform (14), ethyl acetate (15) and ethanol (16) extracts of *O. genistifolia* (b).

**Table 2.** Antiplasmodial and antitrypanosomal activities of chloroform, ethyl acetate and ethanol extracts of *O. genistifolia* against *P. falciparum* strain 3D7 and *Trypanosoma brucei brucei*.

Solvent	Antiplasmodial Activity ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Antitrypanosomal Activity ( $\mu\text{g}\cdot\text{mL}^{-1}$ )
Chloroform extract	11.6	0.5
Ethyl acetate extract	3.3	0.5
Ethanol extract	3.7	0.4
Chloroquine (control)	0.05 $\mu\text{M}$	-
Pentamidine (control)	-	0.02 $\mu\text{M}$

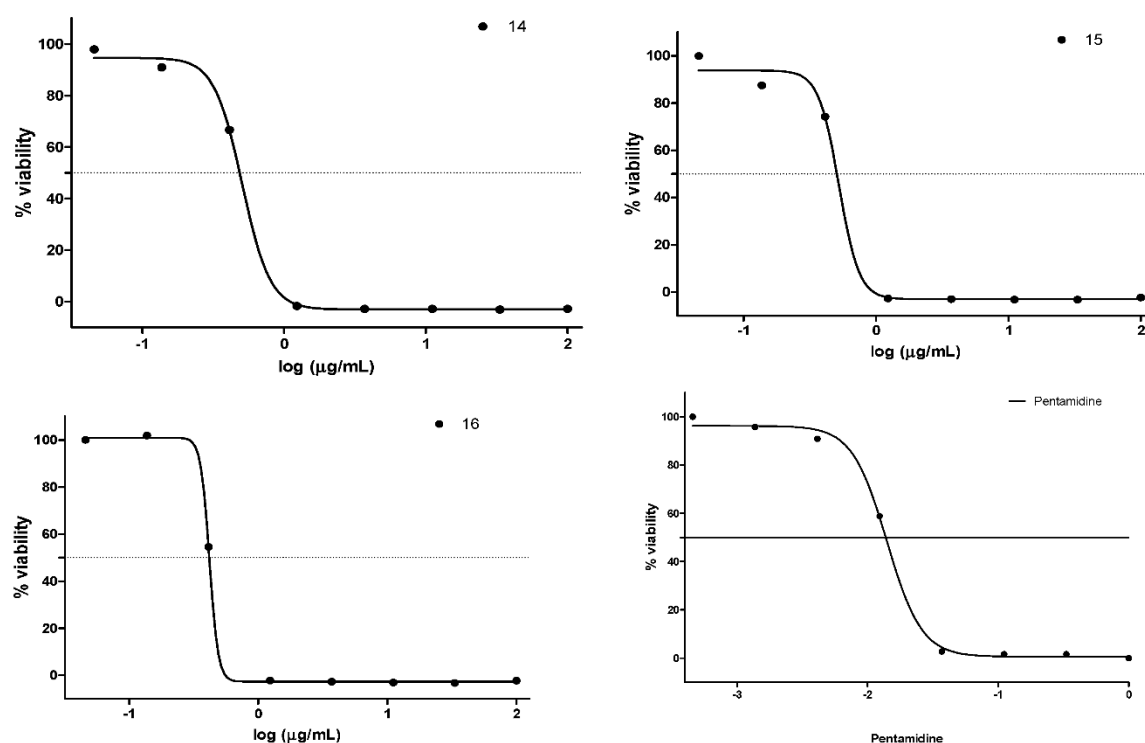
**Table 3.** Some examples of reported studies on antimalarial activities of plant extracts from different regions in Africa.

Family	Plants	Plant Parts	Solvent	Parasite	IC <sub>50</sub> (mg/mL)	Country	Reference
Asteraceae	<i>O. genistifolia</i>	Leave	Chloroform Ethyl acetate Ethanol	<i>P. falciparum</i> strain 3D7	11.6 (chloroform extract); 3.3 (Ethyl acetate extract) and 3.7 (ethanol extract)	South Africa	Present study
Euphorbiaceae	<i>Mellotus oppositifolius</i>	Leaves	Chloroform: methanol (1:1)	pf F32	40 at 10	Cameroon	Harikrishna et al. [45]
Asteraceae	<i>Artemisia Absinthium</i>	Leaves	Ethanol	pf D6	52	Egypt	Shimaa et al. [46]
Balanitaceae	<i>Balanites aegyptiaca</i>	Stem	Methanol	pfDd2	5.2	Sudan	El-Tahir et al. [47]
Fuerstia africana	<i>Fuerstia africana</i>	Leaves	Dichloromethane/MeOH	pf 3D7	6.9/40.2	Rwanda	Muganga et al. [48]
Alangiaceae	<i>Alangium chinense</i>	Aerial parts	Hexane/Dichloromethane/Methanol	FcB1	>50/6.15/2.8	Kenya	Sylvain et al. [49]
Fabaceae	<i>Afzelia africana</i>	Leaves	Methanol	3D7/K1	31.55/39.72	Nigeria	Shuaibu et al. [50]
Passifloraceae	<i>Adenia cissampeloides</i>	Whole plant	Ethanol	pf3D7	8.52	Ghana	Annan et al. [51]
Rutaceae	<i>Agathosma puberula</i>	Roots	Dichloromethane	P. f D10	33	South Africa	Clakson et al. [52]
Meliaceae	<i>Vismia guinensis</i>	Stem bark	Methanol	pf DD2	43.42	Cameroon	Zofou et al. [53]
Fabaceae	<i>Stylosanthes erecta Swartzia</i>	Aerial parts	Dichloromethane/Methanol	Pfk1	21.9/23.3	Mali	Bah et al. [54]



### 3.4. Antitrypanosomal Study

African trypanosomiasis is a disease with devastating adverse effects, which is difficult to control, thereby imposing a high risk to public health [55]. Studies have shown that trypanosomes are developing high resistance to the existing antitrypanosomal agents and, remarkably, plants have been used in time immemorial for the treatment of diseases because of the enormous bioactive compounds they possess [56]. In the present study, the antitrypanosomal activity of chloroform, ethyl acetate and ethanol extracts of *O. genistifolia* was investigated and results obtained from this experiment showed their exceptional antitrypanosomal potential (Table 2). The extracts of the studied plant (chloroform extract  $IC_{50} = 0.5 \mu\text{g}\cdot\text{mL}^{-1}$ , ethyl acetate extract  $IC_{50} = 0.5 \mu\text{g}\cdot\text{mL}^{-1}$  and ethanol extract  $IC_{50} = 0.4 \mu\text{g}\cdot\text{mL}^{-1}$ ) were more effective against the parasites as compared to those reported from previous studies (Figure 3). Similarly, crude hexane and ethyl acetate extracts of *Crateva adansonii* displayed good antitrypanosomal potential against *T. b. brucei* ( $IC_{50} 12.5 \mu\text{g}\cdot\text{mL}^{-1}$ ) [57]. On the other hand, Abu et al. [58] reported a very good antitrypanosomal activity ( $IC_{50} = 2.08 \mu\text{g}\cdot\text{mL}^{-1}$ ) from the ethyl acetate extract of *Ocimum gratissimum*. Other researchers have also reported the antitrypanosomal potentials of some medicinal plants such as *Moringa oleifera*, *Terminalia ivorensis*, *Waltheria indica*, *Artemisia maciverae*, *Eucalyptus camaldulensis* and *Psidium guajava* [56,59–63].



**Figure 3.** Dose-dependent curves of the antitrypanosomal activity of chloroform (14), ethyl acetate (15) and ethanol (16) extracts of *O. genistifolia*.

However, the mechanism by which chloroform, ethyl acetate and ethanol leaf extracts of *O. genistifolia* demonstrated exceptional antitrypanosomal effects against the parasite is vague and we are yet to find ways to describe the biological activity shown. However, facts highlighted in the study of Bala et al. [56] proposed that the bioactive compounds in some plants have the potential by generating free radicals that can cause peroxidative damage to trypanothione reductase which consequently alters the redox balance of the parasites. The report of Shaba et al. [64] highlighted that some bioactive compounds from *Q. borealis* can bind with the DNA of the plasmodium parasite and subsequently activate events that could lead to the parasite's death.

#### 4. Conclusions

In vitro antiplasmodial and antitrypanosomal activities of chloroform, ethyl acetate and ethanol leaf extracts of *O. genistifolia* were reported for the first time in this study. The high antiparasitic activities demonstrated by these extracts could be due to the presence of bioactive compounds. As a result, this study could serve as a potential reference point for the discovery of novel bioactive compounds that could be used as lead substrates for the development of new antiplasmodial and antitrypanosomal drugs against these dreadful parasites. Findings from the present study revealed that these extracts had no cytotoxicity effect against HeLa cells in vitro, however, additional in vivo toxicity studies using animal models are highly recommended in future studies to validate the safety of these extracts on mammalian cells. Lastly, further study on the isolation of pure compounds from these extracts is ongoing in our lab.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2076-3417/10/19/6987/s1>, Table S1. GCMS analysis of chloroform crude extract of *O. genistifolia*. Table S2. GCMS analysis of Ethylacetate crude extract of *O. genistifolia*. Table S3. GCMS analysis of ethanolic crude extract of *O. genistifolia*.

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