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In vitro Activity of *Ozoroa pulcherrima* Schweinf. Extracts and Fractions on *Schistosoma mansoni* Cercariae and Adult Worms

Nestor Gipwe Feussom[1,2], Hermine Boukeng Jatsa[1,2*], Mérimé Christian Kenfack[1,2], Emilienne Tienga Nkondo[1,2], Ulrich Membe Femoe[1,2], Joseph Bertin Kadji Fassi[1,2], Christelle Dongmo Tsague[3], Etienne Dongo[3], Paul Désiré Djomeni Dzeufiet[1] and Louis-Albert Tchuem Tchuente[2,4]

[1]Laboratory of Animal Physiology, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.
[2]Centre for Schistosomiasis and Parasitology, P.O.Box 7244, Yaoundé, Cameroon.
[3]Laboratory of Phytochemistry, Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.
[4]Laboratory of Biology, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors HBJ and LATT designed the study and wrote the protocol. Authors NGF, HBJ, MCK, ETN, UMF and JBKF performed in vitro experiments. Author ED designed and wrote the phytochemical protocol and author CDT performed it. Author PDDD managed the literature searches. Authors NGF and HBJ performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: mjatsa@yahoo.fr;*

ABSTRACT

Aims: Continuous attempts are being made to develop new and more effective drugs for the treatment of schistosomiasis. *Ozoroa pulcherrima* Schweinf. is a medicinal plant used in Africa for the treatment of dysmenorrhea, lower abdominal pain, dystocia and intestinal helminthiasis. This study provides findings on the cercaricidal and schistosomicidal activity of extracts and fractions of *Ozoroa pulcherrima* in *in vitro* assays.

Methodology: The aqueous and methanolic extracts from *Ozoroa pulcherrima* root parts (62.5 – 2000 µg/mL), as well as the methanol derived fractions (*n*-hexane and ethyl acetate: 31.25 – 1000 µg/mL) were tested on cercariae and adult worms of *Schistosoma mansoni*. Niclosamide-olamine 5% (1 µg/mL) and praziquantel (10 µg/mL) were respectively used as reference drugs. During the assays, the mortality of cercariae after 2 hours, and adult worms' mobility and mortality after 48 hours of incubation were evaluated.

Results: *Ozoroa pulcherrima* extracts and fractions significantly increased cercariae and worm mortality in a concentration-dependent manner. The methanolic extract was the most active on cercariae with a $LC_{50}$of 20.65 µg/mL after 30 minutes, while the *n*-hexane fraction was the most active on worm with a LC_{50} of 79.54 µg/mL (65.58 – 96.47 µg/mL) after 48 hours. Significant reduction of motor activity (18.47 to 100%) was recorded for surviving worms incubated in different concentrations of the extracts and fractions.

Conclusion: This study proves that *Ozoroa pulcherrima* extracts and fractions have cercaricidal and schistosomicidal activities. *Ozoroa pulcherrima* may have great potential as an anti-schistosomal agent for further research.

Keywords: Ozoroa pulcherrima; cercaricidal activity; schistosomicidal activity; Schistosoma mansoni.

1. INTRODUCTION

Schistosomiasis, caused by trematode flatworms of genus *Schistosoma* is one of the most significant neglected tropical diseases in the world. According to the World Health Organization [1], schistosomiasis affects more than 206.4 million people in tropical and sub-tropical areas. While chemotherapy with praziquantel (PZQ) is an important component in schistosomiasis control, health education, provision of safe water and sanitation, environmental management and snail control, are necessary for a comprehensive control program [2]. Despite the fact that praziquantel is effective against all forms of schistosomiasis, the dependence on this single drug is a concern because some strains can become resistant to it, as pointed out by some authors [3-6]. Praziquantel has little effect on immature worms [7], so pre-patent or newly acquired infections cannot be cured by praziquantel. In this context, the identification of new and effective schistosomicidal compounds is essential. The trend of using natural plant extracts as new, safe and effective drugs is promising and constitutes the basis for the development of lead chemicals for therapeutics [8,9].

Considered by the World Health Organization as a neglected tropical disease, little attention has been given to the research and development of new and effective antischistosomal drugs in the last decade [10]. Recently, several *in vitro* studies have been performed to search for new active compounds from medicinal plants against *S. mansoni* and promising results have been reported [11-14]. *Ozoroa pulcherrima* Schweinf. belongs to family Anacardiaceae is found in the far north regions of Cameroon, Guinea, Togo, Benin, Central Republic, Ethiopia and Sudan [15]. Plants of this genus are extensively studied regarding their chemical composition and biological activity, as antimicrobial and antitumor [16], schistosomicidal effects [17] and antiproliferative activities on breast cancer cells [18]. To treat intestinal helminthiasis, it is recommended to mix the roots of young tree with water boil and drink 250 mL of decoction once post-prandial [12]. Our previous studies demonstrated the schistosomicidal activity of *O. pulcherrima* methanolic extract *in vivo* models [19]. Research has been carried out to investigate the phytochemical and chemical composition of *O. pulcherrima*. Secondary metabolites identified in the methanolic extract include: anthraquinones, terpenoids, flavonoids, saponins, tannins, phenols, cardiac glycosides, alkaloids, triterpenes and lipids. Three alkylanacardic acids, namely, ozocardic A, ozorcardic A and ozorcardic B were isolated from the methanol-dichloromethane extract [19-21].

Thus, this study aimed to evaluate the *in vitro* schistosomicidal effect of *Ozoroa pulcherrima* aqueous and methanolic extracts and its

methanolic-derived fractions, which have not yet been described.

2. MATERIALS AND METHODS

2.1 Preparation of the Plant Extracts and Fractions

2.1.1 Plant material

Roots of *O. pulcherrima* were harvested in July 2012 in the locality of Wakwa near Ngaoundere in the Adamawa region of Cameroon. Botanical identification of a plant sample was performed at the "National Herbarium", Yaoundé, Cameroon and a voucher specimen n° 13667/SRF/Cam were deposited.

2.1.2 Extraction and fractionation

The roots of *O. pulcherrima* were dried at room temperature and powdered. The powder of *O. pulcherrima* was subjected to static maceration with distilled water (100 g/L) for 24 hours, at room temperature. The solution was filtered, frozen and then lyophilized to give the aqueous extract (*Op*AE), with a recovery rate of 13.6% w/w. Moreover, the powder (4130 g) was submitted to maceration in methanol during 48 h. The macerate was filtered under reduced pressure in a rotary evaporator (BÜCHI B-480) and dried in an oven at 50°C. Then, we obtained 138.12 g of *O. pulcherrima* roots methanolic extract (*Op*ME), with a recovery rate of 3.34% w/w. The methanolic extract was fractionated by partition between immiscible solvents, as follows. The dried methanolic extract was suspended and sequentially partitioned with equal volumes (2x 50 mL) of *n*-hexane and ethyl acetate. Solvents were removed in a rotary evaporator, at a maximum temperature of 50°C. The process allowed obtaining the *n*-hexane fraction (93.30 mg), the ethyl acetate fraction (38.23 g) and the methanolic residue.

2.2 *In vitro* Bioassays Against Cercariae Stage of *Schistosoma mansoni*

2.2.1 Preparation of the cercarial suspension

Infected *Biomphalaria pfeifferi (*snails)were obtained experimentally at the Centre for Schistosomiasis and Parasitology of Yaoundé (CSP). Snails know to shed cercariae were then pooled into a glass beaker containing 20 mL of distilled water and allowed to shed cercariae by exposing them to artificial light for 2 hours. An amount of 20 fresh cercariae were counted under inverted microscope and were used for cercaricidal evaluation.

2.2.2 Bioassays of *Ozoroa pulcherrima* extracts and fractions

A series of crude plant extracts and different fractions concentrations (31.25, 62.5, 125, 250, 500 and 1000 µg/mL) were freshly prepared in a 24 microtiter well plate and analyzed alongside with the positive control Niclosamide-olamine 5% (1 µg/mL) (Jiangsu Aijin Agrochemical Co., Ltd, China). Aqueous extract was dissolved in distilled water, while methanolic extract and its derived fractions (ethyl acetate and *n*-hexane) were dissolved in DMSO 0.5%. Approximately, 20 cercariae were subjected in each concentration and each well contained a final volume of 1 mL. The negative control contained 0.5% DMSO or distilled water. Afterward, cercariae were monitored from 2 hours at 30 minutes intervals and bioactivity was assessed based on mortality. All experiments were done in quadruplicate and at least two tests were performed. Surviving and dead cercariae were observed with an inverted microscope (Olympus CK 2). Cercariae were presumed dead when they were motionless and sank down and their tails went into part [22,23]. The LC_{50}value of the plant extracts and fractions on *Schistosoma mansoni*cercariae was determined by the log(agonist) vs. response - Variable slope (four parameters) regression model.

2.3 *In vitro* Bioassays of *Schistosoma mansoni* Adult Worms

2.3.1 *Schistosoma mansoni* worms recovery

Balb/c mice were infected with 130 cercariae of *S. mansoni* released from experimentally infected *Biomphalaria pfeifferi* at the Centre for Schistosomiasis and Parasitology of Yaoundé (CSP). After 7 weeks of infection, adult worms were recovered under aseptic conditions by perfusion of the mesenteric veins and liver accordingly to the method described by Pellegrino and Siqueira [24]. Adult *S. mansoni*worms (male and female) recovered from infected animals were washed three times in a Glasgow Minimum Essential Medium (GMEM) (Sigma, St Louis, USA) supplemented with an antibiotic-antimycotic solution (10,000 U/mL penicillin, 10,000 µg/mL streptomycin and 25 µg/mL amphotericin B (Atlanta Biologicals, Lawrenceville, USA) and gentamicine (40 µg/mL). To test the effect of *O. pulcherrima*crude extracts and derived fractions on *S. mansoni*adult worms, the bioassay followed the standard operating procedures that recommended at least 5 females and 5 males

per treatment [25]. In this bioassay, 5 males and 5 females adult worms were transferred to each well of a 24-well culture plate containing 1900 µL of complete GMEM culture medium (GMEM medium buffered to pH 7.5 containing 20 mM of HEPES, 40 µg/mL gentamicine, 50 µg/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL neomycin, 2 mM of L-glutamine and 5% heat-inactivated fœtal bovine serum). The plates were then incubated for 2 hours at 37˚C in a humid atmosphere containing 5% CO_2 prior addition of products.

2.3.2 Bioassays of *Ozoroa pulcherrima* extracts and fractions

Concentrations ranging from 10 µg/mL to 50 mg/mLwere generally used for *in vitro* screening of plants extracts or compounds for anti-schistosomal activity [11-14]. In this study, aqueous extract, methanolicextract and methanolic residue of *O. pulcherrima* were initially dissolved in distilled water and DMSO, respectively, filtered through a 0.2 µm sterile syringe filter and diluted in a complete GMEM culture medium to final concentrations of 2000, 1000, 500, 250, 125 and 62.5 µg/mL. The *n*-hexane andethyl acetate fractions were dissolved in DMSO 10% and diluted in the culture medium to final concentrations of 1000, 500, 250, 125, 62.5 and 31.25 µg/mL. It is important to mention that the final volume was 2 mL/well and themaximum concentration of DMSO in each well was 0.5% v/v. The positive control group was treated with a lethal concentration of praziquantel (10 µg/mL), whereas the negative control for the aqueous extract and methanolic residue was keptin GMEM medium, while GMEM medium containing 0.5% of DMSO was the negative control for organic fractions. Quadruplicate measurements were carried out for each concentration and two independent experiments were performed for each sample. Culture plates were kept at 37˚C for 48 h in a 5% CO_2 incubator. This test evaluated the motility and viability of the worms (males and females) at 24 h and48 h using an inverted microscope (Olympus CK2). Reduction of motor activity was defined as the absence of worm motility apart from gut movements and occasional movement of the head and tail of schistosome. Parasite death was defined as the absence of motoractivity during 2 minutes. The median lethal concentration (LC_{50}) was calculated using the Trimmed Spearman-Karber (TSK) method, version 1.5 software downloaded from the US Environmental Protection agency [26].

2.4 Statistical Analysis

Statistical analyzes were performed using GraphPad Prism 7.00 software (San Diego, CA, USA). Significant differences were determined by two-way analysis of variance (ANOVA) for cercaricidal activity and by one-way analysis of variance (ANOVA) for schistosomicidal activity. The multiple comparison Dunnett test was used as post-test and the level of significance was set at $P<.05$. The results were presented as mean ± SEM.

3. RESULTS

3.1 *In vitro* Cercaricidal Activity of *Ozoroapulcherrima*

The effect of *Ozoroa pulcherrima*roots extracts and fractions on the mortality of cercariae after 2 hours of incubation is depicted in Fig. 1. The mortality was both time and concentrations dependent for *O. pulcherrima* aqueous extract which exhibited the highest mortality rate at 500 µg/mL after 90 min of incubation. Following incubation with methanolic extract and its derived fractions at concentrations ranging from 31.25 µg/mL to 1000 µg/mL, cercariae mortality increased significantly within 30 min. Since there was no dead in the aqueous extract at the lowest concentration of 31.25 µg/mL after 30 min of incubation, mortality rates were 96, 91 and 64% for the methanolic extract, ethyl acetate and *n*-hexane fractions respectively. After 30 min of incubation, the cercaricidal activities of the methanolic extract (31.25 – 1000 µg/mL), the ethyl acetate fraction (31.25 – 1000 µg/mL) and *n*-hexane fraction (250 – 1000 µg/mL) were significantly higher than that of the reference molluscicideniclosamide-olamine5%. Otherwise, a 100% of cercariae mortality was registered after onehour of incubation in *O. pulcherrima*methanolic extract, ethyl acetate fraction as well as in *n*-hexane fraction at all concentrations (31.25 - 1000µg/mL).

3.2 *In vitro* Schistosomicidal Activity of *Ozoroa pulcherrima*

3.2.1 Mortality of *Schistosoma mansoni* adult worms

The schistosomicidal effect of the aqueous extract and the methanolic extract and its derived fractions was evaluated by *in vitro* bioassay on the viability of adult worms according to concentration and incubation time as

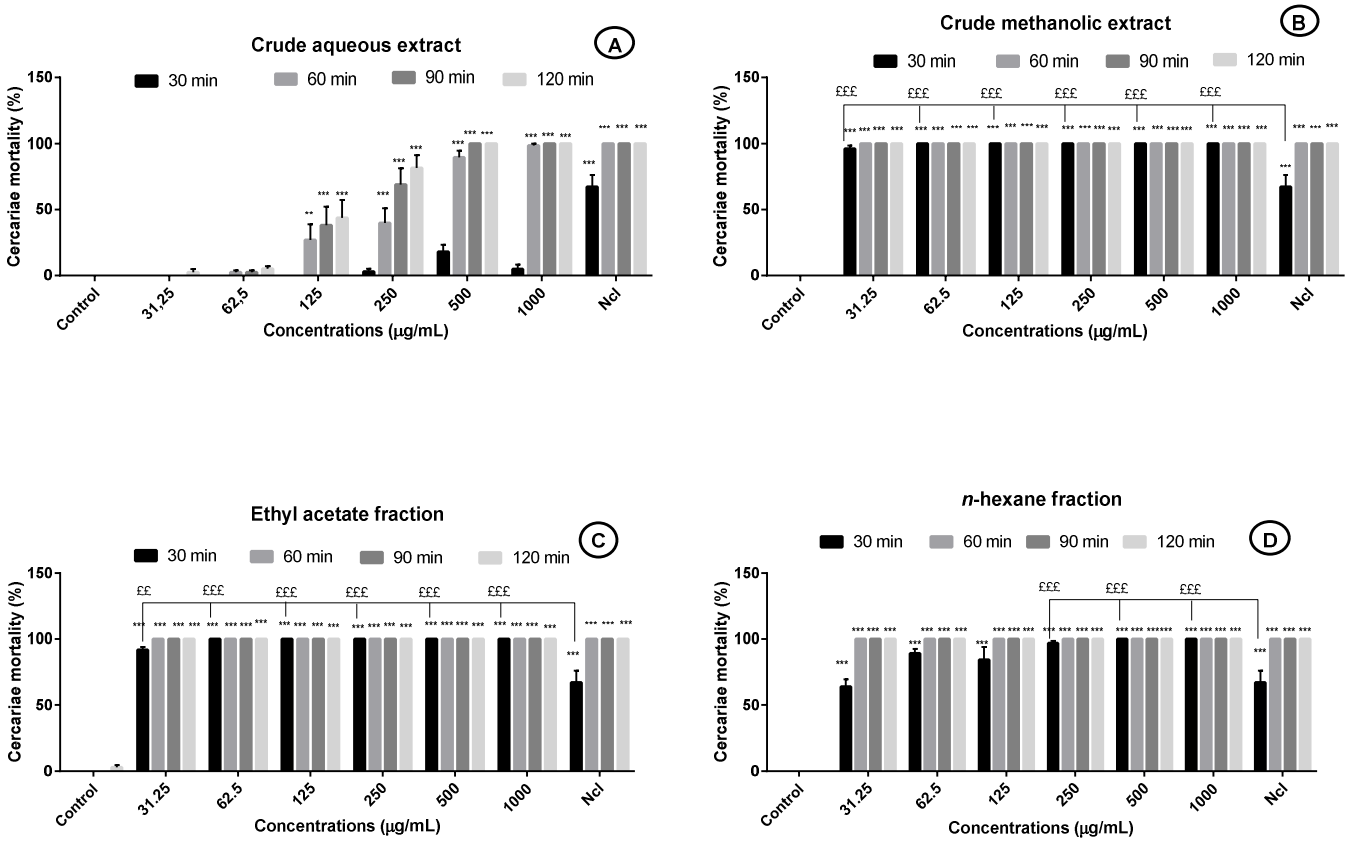

Fig. 1. *In vitro* effect of *Ozoroa pulcherrima* aqueous extract, methanolic extract and derived fractions on the mortality of *Schistosoma mansoni* cercariae after two hours of incubation

*All values are expressed as mean ± SEM. **P = .01; ***P = .001:significantly different from controls (distilled water or 0.5% DMSO).$^{££}$P = .01; $^{£££}$P = .001: significantly different from the referencemolluscicideniclosamide-olamine5% (Ncl)*

summarized in Fig. 2 and Fig. 3. There was a concentration-dependent increase in mortality of adult *S. mansoni* worms after incubation either with the aqueous extract or the methanolic extract and its derived fractions. After 24 hours of incubation of worms in *O. pulcherrima* aqueous or methanolic extracts, a low anti-schistosomal activity appears at the highest concentration of 2000 µg/mL with 13.30 and 57.81% mortality rate, respectively. Fractions from *O. pulcherrima* methanolic extract showed a better anti-schistosomal activity than their extract since mortality rates of 89.72 and 100% were recorded after incubation of worms in 1000 µg/mL of the ethyl acetate and *n*-hexane fractions, respectively (Fig. 2). Incubation of worms with the residue of the methanolic extract did not show any worms' death.

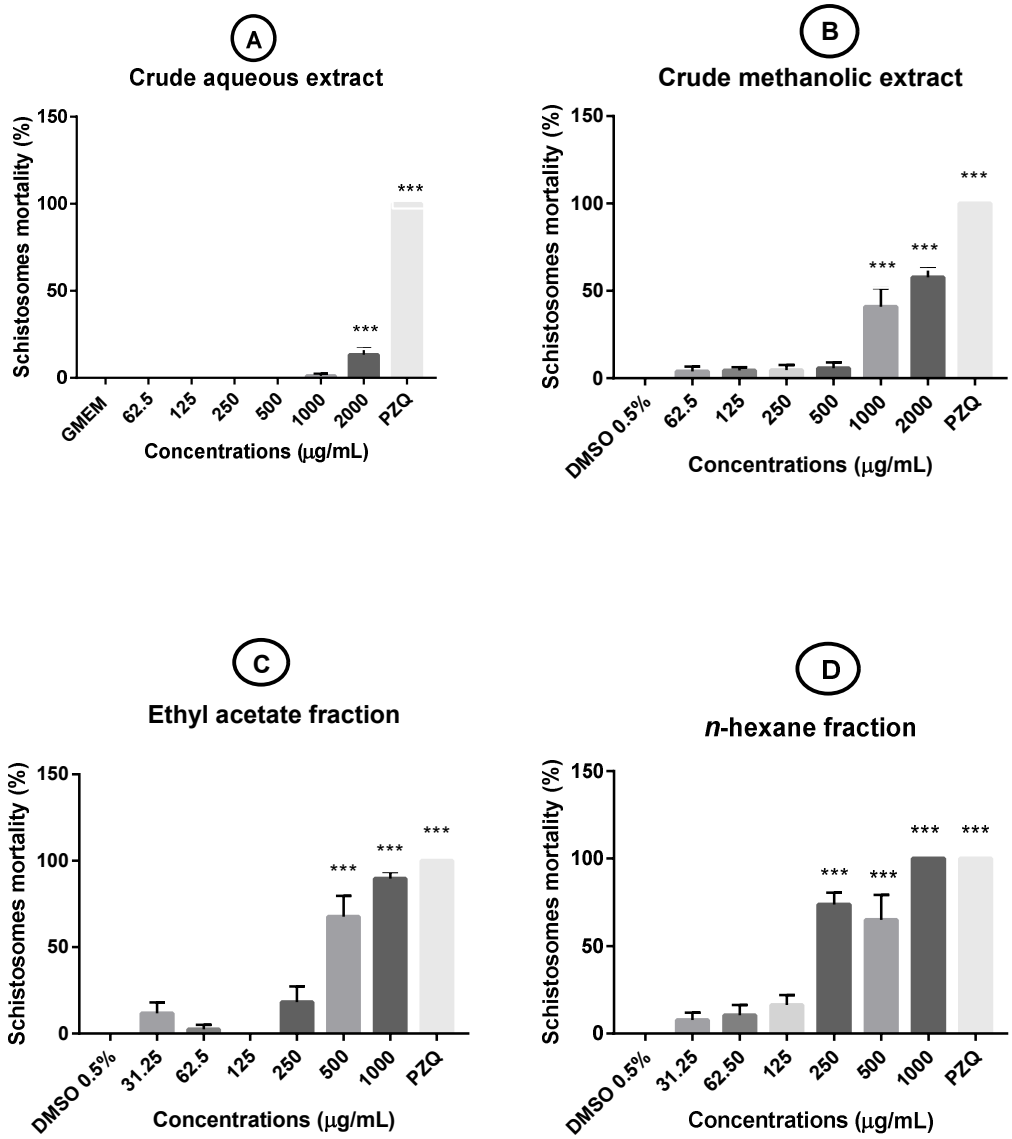

Fig. 2. *In vitro* effect of *Ozoroa pulcherrima* aqueous extract, methanolic extract and derived fractions on the mortality of *Schistosoma mansoni* adult worms after twenty-four(24) hours of incubation

*All values are expressed as mean ± SEM. ***P = .001: significantly different from controls (GMEM or DMSO0.5%).GMEM: Glasgow minimum essential medium; PZQ: praziquantel*

The schistosomicidal activity of *O. pulcherrima* was also time-dependent. In fact, after 48 hours of incubation of adult *S. mansoni* worms with 2000 µg/mL of the aqueous extract, the methanolic extract or its residue, 74.54, 100 and 7.50% mortality rates were recorded, respectively. Otherwise, incubation of worms in 250, 500 and 1000 µg/mL of the ethyl acetate fraction resulted in significant mortality of 56.28%, 88.75% and 98.61%, respectively. The *n*-hexane fraction was the most effective one with significant mortality rates of 41.14, 70, 79.43 and 100% recorded after incubation of worms in concentrations of 62.50, 125, 250 and 500 µg/mL, respectively (Fig. 3).

In the assays carried out with the control groups in which worms were kept in GMEM medium or 0.50%DMSO, they all remained alive for 48 hours. In the positive control group in which worms were treated with praziquantel, 100% of them died within a period of 24 hours at the lethal concentration of 10 µg/mL.

The evaluation of the medium lethal concentration (LC_{50}) of *O. pulcherrima* aqueous extract, methanolic extract and derived fractions was calculated after 48 h of incubation using Trimmed Spearman-Karber method. The schistosomicidal activity can then be establish as follow: *n*-hexane fraction > ethyl acetate fraction > methanolic extract > aqueous extract. It then appears that the *n*- hexane fraction from *O. pulcherrima*methanolic extract was the most active with a LC_{50} of 79.54 µg/mL (65.58 – 94.47 µg/mL) (Table 1).

3.2.2 Motor activity of *Schistosoma mansoni* adult worms

The motor activity of worms incubated in different concentrations of *O. pulcherrima* aqueous extract, methanolic extract and its various fractions was recorded after 24 and 48 hours and results are reported in Fig. 4. Control worms incubated only in the culture medium remained viable up to 48 hours even though some of them displayed weak reduced motor activity. The effect of the aqueous extract, the methanolic extract and its derived fractions on the motility of the worms was assessed as directly proportional to the concentration and the incubation period. In fact, after 24 hours of incubation, schistosome displayed slightly reduced motor activity and severely reduced motility after 48 hours of exposure, whatever the extract or the fraction. The reduction of motor activity reached 100% for worms exposed to *O. pulcherrima* methanolic extract at 2000 µg/mL or to its ethyl acetate fraction at 1000 µg/mL for 48 hours. With the most active schistosomicidal fraction *n*-hexane, a 62.92% reduction of motor activity was recorded at the concentration of 125 µg/mL. Schistosomes motor activity reduction was mainly marked by the weak movement of the suckers and occasional waves of the body.

4. DISCUSSION

The spread of schistosomiasis in endemic areas and increasing infection rate support the need for new drug discovery and development [27]. Research on natural products has provided remarkable new drugs or drug leads for the control of several diseases. To evaluate the schistosomicidal effect of a drug, it is important to understand the biology of the parasite. According to Moraes et al. [28], compounds with schistosomicidal activity can be effective in different ways: prophylactically (causing the death of cercariae and/or schistosomula), suppressively (inhibiting oviposition) and curatively (causing the death of the adult worm). Several biological activities have been described for species of genus *Ozoroa*, such as anti-proliferative, antimicrobial, anti-tumor and anthelmintic [16-18]. Jatsa et al. [19] have previously shown the *in vivo* schistosomicidal activity of *O. pulcherrima* methanolic extract. In recent years a number of studies have been developed through *in vitro* screening using crude extracts, essential oil and isolate compounds from medicinal plants to identify a leading substance that can be used in preclinical trials for the treatment of experimental schistosomiasis [11,17]. In this study, we soughtto investigate *in vitro* schistosomicidal potential ofthe *O. pulcherrim a*aqueous extract, methanolic

Table 1. Median lethal concentration (LC_{50}) values of *Ozoroa pulcherrima* aqueous extract, methanolic extract and derived fractions after forty-eight (48) hours of incubation

Ozoroa pulcherrima	LC_{50}(µg/mL)	95% low limit (µg/mL)	95 % upper limit (µg/mL)
Aqueous extract	1363.26	1120.39	1658.77
Methanolic extract	773.25	697.51	897.27
Ethyl acetate fraction	235.16	205.39	268.85
n-Hexane fraction	79.54	65.58	94.47

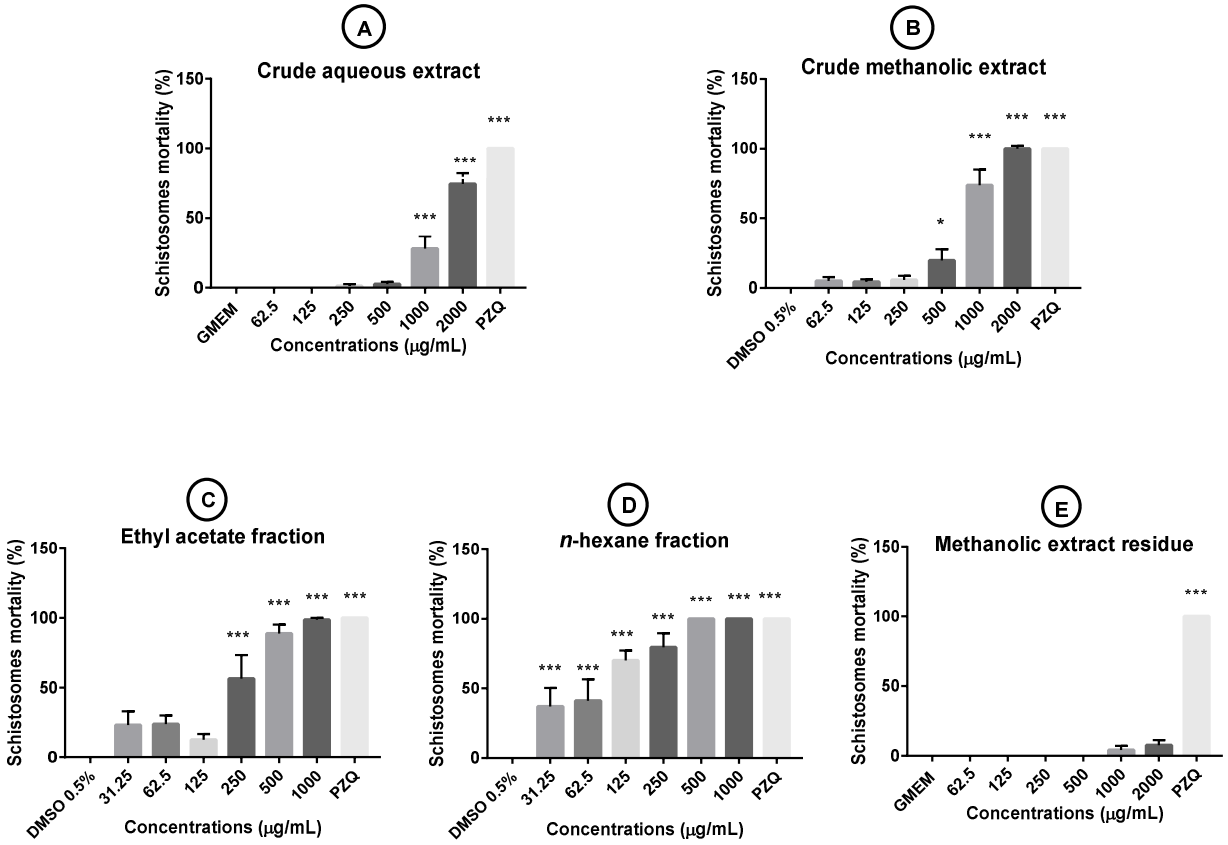

Fig. 3. *In vitro* effect of *Ozoroa pulcherrima* aqueous extract, methanolic extract and derived fractions on the mortality of *Schistosoma mansoni* adult worms after forty-eight (48) hours of incubation

*All values are expressed as mean ± SEM.*P = .05; ***P = .001: significantly different from controls (GMEM or DMSO0.5%).GMEM: Glasgow minimum essential medium; PZQ: praziquantel*

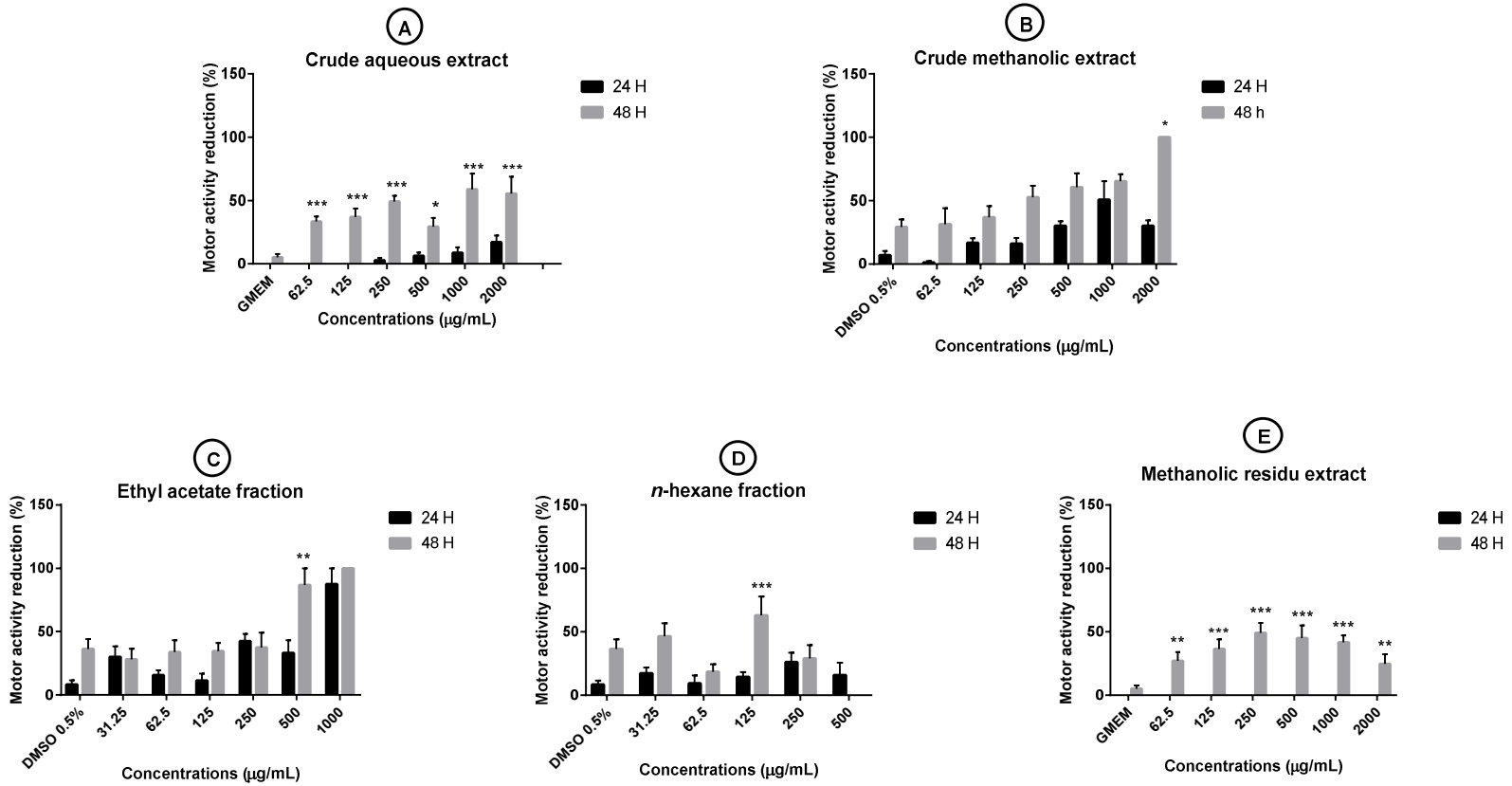

Fig. 4. *In vitro* effect of *Ozoroa pulcherrima*aqueous extract, methanolic extract and derived fractions on the motor activity of *Schistosoma mansoni* adult worms after forty-eight (48) hours of incubation

*All values are expressed as mean ± SEM.*P =.05;**P = .01; ***P = .001: significantly different from controls (GMEM or DMSO0.5%). GMEM: Glasgow minimum essential medium*

extract and derived fractions at different concentrations.

The present observation showed that aqueous and methanolic extracts and derived fractions possess cercaricidal activity against *S. mansoni* cercariae. Our results showed that the methanolic extract and its derived fractions were more potent than the aqueous extract. The activity was both time and dose dependent. Our findings are in line with the reported data for *Phytolacca dodecandra* by Obare et al. [22] and *Rauwolfia vomitoria* by Tekwu et al. [23] who reported the cercaricidal activity of the plants extracts. More precisely, the cercaricidal activity of some medicinal plants has been correlated to their secondary metabolites. Isoflavonoids from the seeds of *Millettia thonningii* and a penta-substituted pyridine alkaloid from the rhizome of *Jatropha elliptica* have in fact exhibited strong cercaricidal and schistosomicidal activities against *S. mansoni* [29,30]. Due to the presence of flavonoids and alkaloids in *O. pulcherrima* roots methanolic extract [19], we could say that its cercaricidal activity might be linked to these secondary metabolites.

Ozoroa pulcherrima aqueous extract, methanolic extract and its fractions caused the death of S.*mansoni*in a concentration and time dependent manner. Our results corroborate similar studies on the crude extracts of *Ozoroa insignis* [17], *Zanthoxylum naranjillo* [31], *Zingiber officinale* [32], *Sidapilosa* [33], *Rauwolfia vomitoria* [23]; essential oil of *Piper cubeba*(Piperaceae) [34], *Baccharis trimera* [35] and isolate compounds terpene nerolidol [36] and piplartine [37]. Lethal concentrations of *O. pulcherrima* aqueous extract (2000 µg/mL), methanolic extract (2000 µg/mL) and its derived fractions (1000 µg/mL) were in the range of lethal concentrations (0.6 to 25 mg/mL) of plant species popularly used against schistosomiasis [11,12,17,33]. In the present study, assessment of the medium lethal concentration (LC_{50}) of the aqueous extract, the methanolic extract and its derived fractions disclosed the *n*-hexane fraction as the most active, with the LC_{50} of 79.54 µg/mL. The potential effect of *O. pulcherrima* extracts and fractions may be due to the presence of bioactive chemical compounds. Our previous studies have identified the presence of reducing sugar, anthraquinones, terpenoids, tannins, phenols, flavonoids alkaloids, cardiac glycosides, triterpenes and lipid steroid in methanolic extract of *O. pulcherrima* [19]. Additionally, various terpenoids, flavonoids and new alkaloids have been isolated and characterized in its ethyl acetate fraction [38].

Terpenoids are the largest naturally occurring family of hydrocarbons with a very broad range of biological activities including antimalarial [39], anticancer [40] and antischistosomal [41] properties. Some terpenoids are known to kill adult *S. mansoni* worms [35,36]. Their schistosomicidal activity is probably due to their ability to alter the cholinergic nervous system of *S. mansoni*; acetylcholine being associated with the parasite motility [35]. Alkaloids are widely distributed and have been isolated from several plant species. Several of them have schistosomicidal activity by causing extensive disruption of tegument, sloughing the parasite motor activity, or the death of *S. mansoni* adult worms after *in vitro* or *in vivo* exposure [42-44]. Recently it has been shown that a fatty acid named arachidonic acid, killed juvenile and adult schistosomes *in vitro*. The arachidonic acid mediated killing is essentially due to excessive activation of schistosome magnesium-dependent neural sphingomyelinase, leading to the hydrolysis of sphingomyelin to ceramide and phosphorylcholine. Consequently, sphingomyelin hydrolysis elicits an increase in membrane permeability, bending, and aggregation as well as dramatic perturbations in the lipid content and rigidity of the schistosome [45].

Motor activity is often evaluated as indicator of biological activity of schistosome species. In this study, change in the motility of *S. mansoni* varied according to the concentration of the crude extracts and derived fractions of *O. pulcherrima* and exposure period. It was observed that the *n*-hexane fraction caused major reduction of the parasite motor activity after 48 h. Studies revealed that motility of *S. mansoni* is associate with important neuro transmittersor neuromodulators such as acetylcholine, serotonin, neuropeptides and glutamate [34,35, 46,47]. Absence of motility apart from guts movement and reduction of peristaltic waves along schistosome body after incubation in *O. pulcherrima* could be the consequence of the plant interference with the mechanism of concentration-relaxation of worm smooth muscles [48]. Motility reduction of adult *S. mansoni* exposed to *O. pulcherrima* might also be associated with alteration in the neurotransmitter system of the parasite. It has been in fact reported that flavonoids which moderately reduce *S. mansoni* motor activity *in*

vitro, were identified as selective inhibitors of the *S. mansoni* NAD^+ catabolizing enzyme (SmNACE). SmNACE is an important target localized in the outer tegument of the adult parasite and it is presumably involved in the parasite survival by manipulating the host's immune regulatory pathways. The discovery of flavonoids that inhibit SmNACE in the low micromolar range has led to the consideration of flavonoids as promising drug candidate for treating schistosomiasis [49].

5. CONCLUSION

From the present study, it can be concluded that *Ozoroa pulcherrima* methanolic extract and derived fractions possess antischistosomal properties against two life stages of *Schistosoma mansoni;* cercariae and adult worms. Both methanolic extract and derived fractions showed different activity with respect to both time and concentration. Our results reported the *n*-hexane fraction being more active. Further investigations are in progress to disclose the important biological effects of this plant, whereas *Ozoroa pulcherrima* has great potential as a source of active compounds against *Schistosoma mansoni*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures in this study followed the principles of laboratory animal use and care of the "European Community" guidelines (EEC Directive 2010/63/EEC) and were approved by the "Animal Ethical Committee" of the Laboratory of Animal Physiology of the Faculty of Sciences, University of Yaounde I Cameroon.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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