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Cercaricidal Efficacy of Plant Extract: Evidence from the Methanolic Leaves and Bark Extracts of *Anacardium ocidentale* (Linnaeus)

Adeniyi, K. A[1,2*]., Ibeh, E. O[3]. Oyibo-Usman, K. A[4]., Shariff, M. A[5]., Ismail, A[6]., Omalu, I. C J[6]. Sadiq, A. A[7]. and Salami, K. D[8], Yusuf L.[9]

[1]Department of Biological Sciences,
Federal University Dutse,
Dutse, Jigawa State, Nigeria

[2]Parasitology and Public Health Unit,
Center for Arid Zone Ecology,
Federal University Dutse,
Dutse, Jigawa State, Nigeria

[3]Department of Biotechnology and Environmental Biology,
Veritas University Abuja

[4]Zoology Unit. Department of Biological Sciences,
Federal University Gusau,
Zamfara, Nigeria

[5]Department of Hospital Service,
Jigawa State Ministry of Health,
Jigawa State, Nigeria

[6]Applied Entomology and Parasitology Unit,
Department of Animal Biology,
Federal University of Technology, Minna,
Niger State, Nigeria

[7]Department of Biological Sciences,
Sule Lamido University Kafin Hausa,
Jigawa State, Nigeria.

[8]Department of Forestry and Wildlife Management,
Federal University Dutse,
Jigawa State

Department of Microbiology
Prince Abubakr Audu University
Ayingba, Kogi State, Nigeria

**Author for Correspondence*

Abstract

Schistosomiasis continues to be a public health problem causing in unsatisfactorily high level of morbidity. Praziquantel is a recent medicine for treatment but unable to kill emerging schistosomes. It does not prevent re-infection and its constant application may lead to the advent of drug-resistant parasites. Qualitative phytochemical screening of the methanol crude extract of A. occidentale revealed the presence of Tannins, Flavonoids and Saponins. Cercaricidal activity on Gymnocephalous cercaria, after 120 minutes of contact in a concentration of 0.10 mg/mL, showed that there was 92 and 34% death when exposed to the methanol leave and bark extracts of A. occidentale leaves, and A. occidentale bark respectively. There was 99% and 98.5% mortality between the methanol bark extract of A. occidentale and the leaf of A. occidentale plant respectively in a concentration of 0.60mg/mL. Lethal concentration on Gymnocephalous cercariae showed that A. occidentale methanolic bark extract had the highest LC_{50} and LC_{84} of 0.151 and 0.204 mg/mL respectively while A. occidentale methanol leaf extract had the lowest LC50 and LC84 of 0.123 and 0.162 respectively. A. occidentale methanol bark extract had the lowest LC50 of 0.237 and A. occidentale methanol leaf extract had the lowest LC 84 of 0.316 mg/ml. The correlation coefficient of the plant parts showed a strong positive correlation between the log concentrations and probit mortality. The extracts had an effect on the cercariae tested. A. occidentale leaves extract showed highest cercaricidal activity. Gymnocephalous cercariae was more susceptible to the plant parts than the Brevifurcate cercaria

Keywords: Cercaricide, *Anacardium occidentale, Gymnocephalus cercaria, Brevifurcate cercaria*

INTRODUCTION

Schistosomiasis also referred to as snail fever is a tropical parasitic disease caused by blood fluke of the genus *Schistosoma* (Nafiu *et al*., 2016). Hamed (2010) reported that about 200 million individuals are affected in 74 countries. Approximately 90% of those requiring treatment for schistosomiasis lives in Africa (WHO, 2014). In Africa where the greatest prevalence of infection occurs, it has been estimated that 150,000 people die each year due to schistosomiasis-related causes (WHO, 2011). In Nigeria, one of the most severely affected countries in Africa, it is estimated that 101.28 million people are at risk of infection while 25.83 million are infected (Chitsulo *et al*., 2000, Ibeh *et al*., 2019). Aiming towards the treatment of the disease, praziquantel the only drug currently available, is unable to kill developing schistosomes, it does not prevent re-infection and its continued extensive use may result in the future emergence of drug-resistant parasites (Angaye, 2016). In a bid to complement chemotherapy, niclosamide was among many molluscides produced (Yang *et al*., 2010). However, the high cost of niclosamide, difficulty in formulation and its biocidal nature which affects non-target organism in the snail's habitat have caused researchers to seek for an alternative means to control the disease (Otarigho and Morenikeji, 2012; Adewumi *et al*., 2013). Despite past efforts, effective vaccine has not been produced to prevent schistosomal infection (Doenhof *et al*., 2008). Consequently, the only efficacious strategy for the control of the disease relies solely on mass drug administration (MDA) of praziquantel (PZQ). Although the drug is active against adult forms of all schistosome species, it is inefficient against larval and juvenile worms and such, unable to prevent reinfection and block further transmission (Vale *et al*., 2017). Besides, the repeated use of the drug (i.e., mass drug administration with praziquantel) has caused the parasite to develop resistance against the drug; hence the worry of therapeutic failure has emerged especially in the disease endemic regions. These, among others, have called for aggressive search for better and more efficient alternative, especially against the larvae, cercariae, for effective interruption of the parasite life cycle. Plant and plant materials have been well documented to be effective against parasites and vector (Tienga *et*

al., 2022). Development of Cercaricidal agent with effective plant could help in disrupting the parasites life cycle. This will serve a vital role in controlling the diseases. Several plants have both mortality activities of both molluscidal and cercarcidal. This study aimed at examining the effect of cercaricidal efficacy for both bark and leaves of *A. occidentale*
Changing the parasites life cycle with different plants will serve a vital role in controlling the diseases. Several plants have both mortality activities of both molluscidal and cercarcidal. This study aimed at examining the effect of cercaricidal efficacy for both the bark and leaves of *A. occidentale*.

MATERIALS AND METHODS

Study Area

This research was carried out in Niger state, Bosso, Local Government Area. The mean annual rainfall is 133 mm, and the highest rainfall was recorded in August and September Mohammed (2018). Temperature is highest in March (30 ^{0}C) while the lowest in August (22 ^{0}C). Bosso LGA. exhibits tropical wet and dry seasons. A study on snails' species was conducted in some parts of Bosso LGA; Tsuami, Gidan Kwano and Garatu. Several human contact activities were taking place in the water bodies in Bosso LGA.

Plant Extraction

The active components of the leaves and bark of the cashew were extracted using soxhlet apparatus with methanol as solvent. Fifty gramme (50g) powder of leaves and bark was put into soxhlet apparatus. Fifty gramme (50 g) each of the milled leaves and bark of cashew was measured on the digital weighing balance. 250 mL of methanol was put into boiling flask, the filter paper was mounted into the flask and soxhlet apparatus was arranged for easy reflux for 3 hours until the solvent was clear and transparent. The solvent was evaporated using the steam bath to get the crude extract of the various plant parts (Adetunji and Salawu, 2010). The extracts were then crushed and laid in a little tube-like structure of 50 mL and then placed at 4 ^{0}C.

Plate 1: Cashew [*Anacardium occidentale* (L.)
(**Source**: Field photograph, 2016.)

Phytochemical Screening of Plant Samples
This was done to assess the qualitative and quantitative chemical composition of the methanol crude extract using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as saponin, alkaloids, flavonoids, tanins (Sofowara, 2008).

Preparation of Snails
Biomphalaria pfeifferi snails were collected from the irrigation canals around Federal University of Technology, Minna, from different localities in Gidan-kwanu, Minna, Niger State. Groups of 24-30 infected *Biomphalaria pfeifferi* snails were placed in 50 mL beakers and washed with distilled water to remove fecal and food particles from the field (Al Hamshary *et al*., 2018). A sample of 0.5 mL of water was viewed with used of microscope. The number of cercariae was recorded and their identities were determined according to the methods of Hira and Muller (1966) and Akufongwe *et al*., (1995).

Determination of Cercariae Number
This was done using Neubaeur counting chamber and calculated using the formula:

$$\frac{\text{Number of cercariae in a square} \times \text{Volume of square}}{\text{Number of big square}} = \frac{1200 \times 2}{24} \quad = 100\%$$

Dilution Preparation and In-vitro Experiment
Firstly, 10% stock solution concentration was made by dissolving 1 g of the crude extract in 10 mL in distilled water and preserved at 4 ^{0}C. During the cercaricidal activity, 24 well plates were used to test the efficacy of the plants extracts. The following concentrations were used 0.00(control), 0.60, 0.40, 0.30, 0.20, 0.15, 0.10, 0.05 and 0.03 mg/mL. All test concentrations were replicated. The mortality of the cercariae was observed by its morphological and physiological change for a period of 120 minutes. Dead cercariae were counted and percentage mortality was determined for each concentration. Lethal concentration (LC_{50}) of the leaves and bark of the plants were also determined.

Data Analysis
The percentage mortalities of cercariae was calculated and transformed into probit mortality. Data collected on Lethal concentrations (LC_{50}), was subjected to the probit regression analysis (Finney, 1971). All the analysis was considered significant when the P value is less than 0.05.

RESULTS

Phytochemical of leaves and bark of *A. occiddentale*
The results obtained from the phytochemical analyses of leaves and bark of *A. occiddentale* are presented in Table 1. The phytochemical screening revealed tannin, flavonoid, anthraquinanes and saponin as the active components present in both leaves and bark of the plant. Additionally, alkaloid, phlobatanin, cardiac, glycoside and reducing sugar were present in *A. occidentale* bark while steroid was present in *A. occidentale* leaves.

Table 1: Phytochemical analysis of the leaves and bark of *A. occidentale*

Phytochemical constituents	*A. occidentale* leaves	*A. occidentale* bark
Tanins	+	+
Flavanoids	+	+
Alkaloids	-	+
Saponins	+	+
Steroids	+	-
Phlobatanins	-	+
Cardiac glycosides	-	+
Anthraquinones	+	+
Reducing sugar	-	+

Key: + = present; - = Absent

Phytochemical Constituents of *A. occidentale* leaf and bark

The result of Phytochemical constituent of *A. occidentale* leaves and bark (mg/100g) is presented in table 2. Quantitative phytochemical analyses of *A. occidentale* leaves and bark revealed the presence of flavonoids, phenols and alkaloids. However, the concentrations of these constituents were higher in the extract of the leaves when compared with the bark. It also showed that tannins and saponins were more abundant in *A. occidentale* bark extract than in *A. occidentale* leaves extract. Statistical analysis revealed significant differences in the phytochemical constituents among the plant parts except for saponins. No significant difference (P>0.05) in the quantity of saponins present among the plant parts.

Table 2: Phytochemical constituents of *A. occidentale* leaves and bark

Phytochemicals	Concentration (mg/100g)	
	A. occidentale leaves	*A. occidentale* bark
Flavonoids	373.33±3.27^{b}	109.09±1.20^{a}
Phenols	603.65±1.85^{b}	522.40±6.20^{a}
Tannins	26.30±0.57^{a}	70.93±1.27^{b}
Alkaloids	36.64±1.32^{b}	22.08±0.01^{a}
Saponins	7.25±0.02^{a}	8.47±0.61^{a}

Values followed by different superscript alphabets on the same row are significantly different at $p < 0.05$ level of significance. Values are in mean ± standard error of mean of two determinations.

Effect of Extract of *A. occidentale* on Cercariea mortality

The result of **effect of *A. occidentale* on the mortality of Cercariea** is presented in Table 3. Number of mortality was recorded when the cercariea was exposed to 0.03 mg/mL and 0.05 mg/mL concentration at different time interval for both *Gymnocephalus cercaria* and *Brevifurcate cercaria.* at 0.10 mg/mL for *Gymnocephalus cercaria* there were 4, 14 and 34 % mortality at 45 minutes, 60 minutes, and 120 minutes while at same concentrations, for Brevifurcate, no mortality was recorded at both different time intervals. The highest mortality rate for both of cercaciae was recorded when exposed to 0.4 and 0.60 mg/mL. Gymnocephalous recorded 60 %, 80 % and 100 % mortality, at both 0.4 mg/mL and 0.60 mg/mL while for Brevifurcate, the recorded mortality was 51.5, 71.5 and 91.5% at 0.4 mg/mL and 59, 79 and 99 % when exposure to 0.60 mg/mL, respectively.

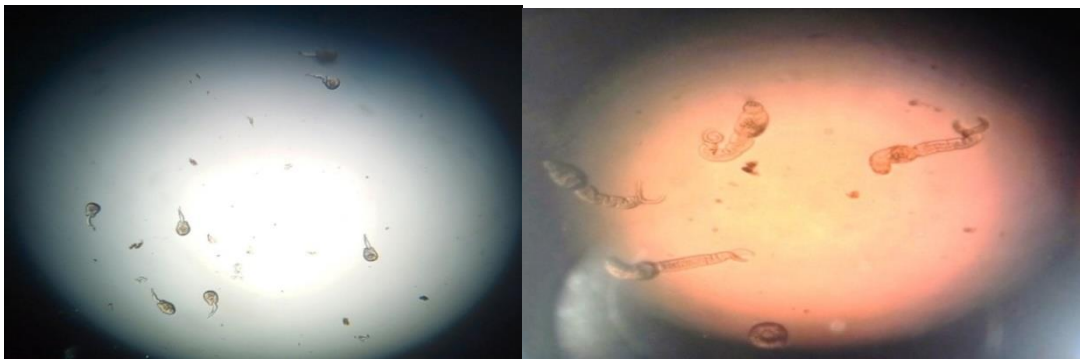

Plate 2: *Gymnocephalous cercariae* (x10) **Plate 3:** *Brevifurcate cercariae* (x10)
Source: Field photograph, (2016)

Table 3: Effect of bark extract of *A. occidentale* (Methanol) of plant mortality on cercariea

Conc.(mg/ml)	Biological test	**45 mins**	**60mins**	**120mins**
0.03	*Gymnocephalous*	0	0	0
	Brevifurcate	0	0	0
0.05	*Gymnocephalous*	0	0	0
	Brevifurcate	0	0	0
0.10	*Gymnocephalous*	4	14	34
	Brevifurcate	0	0	0
0.15	*Gymnocephalous*	38	58	78
	Brevifurcate	21	41	61
0.20	*Gymnocephalous*	56.5	76.5	96.5
	Brevifurcate	38	58	78
0.30	*Gymnocephalous*	59.5	79.5	99.5
	Brevifurcate	44.5	64.5	84.5
0.40	*Gymnocephalous*	60	80	100
	brevifurcate	51.5	71.5	91.5
0.60	*Gymnocephalous*	60	80	100
	Brevifurcate	59	79	99
Control	*Gymnocephalous*	0	0	0
	Brevifurcate	0	0	0

Mortality effects of cashew leaves extract (Methanol)

The result of cashew leaves on mortality effect on cercariea is presented in table 4. No mortality rate was recorded when for both cercariea when they are exposure to 0.*03mg*/ml and 0.05mg/mL concentration. 52%, 72% and 92% mortality rate were recorded when *Gymnocephalous cercariea* was exposure to 0.1mg/ml while no mortality rate was recorded at same concentration for Brevifurcate. The highest mortality rates were recorded at 0.4mg/ml and 0.6mg/ml for *Gymnocephalous* and *Brevifurcate cercariea.*

Table 4: Mortality effects of cashew leaves extract (Methanol)

Conc (mg/mL)	Biological test	45 minutes	60 minutes	120 minutes
0.03	*Gymnocephalous*	0	0	0
	Brevifurcate	0	0	0
0.05	*Gymnocephalous*	0	0	0
	Brevifurcate	0	0	0
0.10	*Gymnocephalous*	52	72	92
	Brevifurcate	0	0	0
0.15	*Gymnocephalous*	56	76	96
	Brevifurcate	22.5	42.5	62.5
0.20	*Gymnocephalous*	58	78	98
	Brevifurcate	32.5	52.5	72.5
0.30	*Gymnocephalous*	60	80	100
	Brevifurcate	43	63	83
0.40	*Gymnocephalous*	60	80	100
	Brevifurcate	53	73	93
0.60	*Gymnocephalous*	60	80	100
	Brevifurcate	58.5	78.5	98.5
Control	*Gymnocephalous*	0	0	0
	Brevifurcate	0	0	0

Effects of Lethal concentration (LC50 and LC84) of *A. occidentales* on *Gymnocephalous cercariae.*

The study revealed that the LC50 and LC84 values of bark extraction of *A. occidentale* plant against *Gymnocephalous cercariea* was 0.151 and 0.204mg/Ml respectively with an upper and lower 95% confidence limit of 0.041 and 0.151mg/mL. Analysis also showed that *A. occidentale* methanol; leaves extract had a LC50 and LC84 value of 0.123 and 0.162mg/mL with the upper and lower limit calculated to be 0.042 and 0.357 mg/mL respectively (Table 5). (r^2=0.943 and 0.863) were correlation coefficient for the bark and leaves extracts of *A. occidentale* respectively. There was higher positive correlation among mortality and log concentration. Highest LC50 and LC84 were recorded in a bark extract while lower LC50 and LC84 were recorded in leaf extract.

Table 5: Lethal concentration of *A. occidentale* leaves and bark extracts on *Gymnocephalus cercaria*

Plants	LC_{16}	LC_{50}	LC_{84}	UL	LL	R_2	Regression Equation
AOL	0.056	0.123	0.162	0.042	0.357	0.865	Y=7.69x+12.04
AOB	0.104	0.151	0.204	0.041	0.551	0.943	Y=7.69x+11.51

Keys:
AOL= *Anacardium occidentale* leaf; AOB= *Anacardium occidentale* bark
UL= Upper Limit; LL = Lower Limit

Lethal concentration (LC50 and LC84) from extract of part of *A. occidentales* on *Brevifurcate cercariae*. The study revealed that the lethal concentration (LC50 and LC84) of bark extract on Brevifurcate had a LC50 and LC84 values of 0.237 and 0.323mg/ml with higher and lower of confidence limit as found to be 0.0065 and 0.858 mg/ml respectively. *A. occidentale* methanol leaves extract had its LC50 and LC84 values to be 0.239 and 0.316 mg/mL with higher and

lower 95% confidence limit found to be 0.065 and 0.882mg/mL respectively. The relationship coefficient of bark and leaves was found to be (r2= 0.838 and 0.841). It revealed higher positive correlation among the log concentration and probit mortality. Obtained results showed that bark extract had the least LC50 and leaves extract had the least LC84. Probit analysis revealed that lesser test concentration of *A. occidentale* methanol bark extract was required to achieve 50% mortality of *Brevifurcate cercaria parasites.*

Table 6: Lethal concentrations of *A. occidentale* leaves and bark extracts on *Brevifurcate cercaria*

Plants	LC_{16}	LC_{50}	LC_{84}	UL	LL	R_2	Regression Equation
A.O.L	0.165	0.239	0.316	0.065	0.882	0.841	Y=6.631x+9.21
A.O.B	0.162	0.237	0.323	0.065	0.858	0.838	Y=6.638x+9.23

Keys: AOL= *Anacardium occidentale* leaf; AOB= *Anacardium occidentale* bark; UL= Upper Limit; LL = Lower Limit

DISCUSSION

The qualitative and quantitative results of *A. occidentale* revealed that all the plant parts tested contained phytochemicals such as tannin, flavonoid, and saponin. The higher quantity of flavonoid, phenol and alkaloid contained in the leaves extract of *A. occidentale* could be responsible for the high rate of mortality recorded for *Gymnocephalus cercaria.* The leaves and bark of *A. occidentale* is used traditionally for the cure of venereal diseases, intestinal colic, leishmaniasis and syphilis-related skin disorders (Franca *et al*., 1993).

The study showed that plant extract used in this study had cercaricidal potential effect on cercariae. Observed mortality may be an indication of possible usefulness of the plant extract as cercaricidal agent. Cercaricidal activities indicated that higher concentration result to the increases in the mortality rate in a short time. When *Gymnocephalus cercaria*was subjected to the leaf extract of *A. occidentale* in 0.30mg/mL concentration result to 100% mortality while 99.5% rate of mortality was observed for the bark of *A. occidentale* after 120 mins exposure time. The result revealed that the leaf of *A. occidentale* plant possess more effect on *Gymnocephalous cercariae* than the bark. This result is in line with the works of Mohamed *et al*. (2005), who reported the cercaricidal activity of the crushed seed of *Nigella sativa* to be both time and concentration dependent. On exposure of *Brevifurcate cercaria* to the plant extracts, 99 and 98.5% mortality was recorded between the bark and leaves extract of *A. occidentale* respectively in a concentration of 0.60mg/mL after 120 mins exposure time. The major finding of this study showed the bark had more effect on *Brevifurcates cercaria.* With regard to susceptibility cercariae of Gymnocephalous were higher sensitive than Brevifurcate because shorter time required to achieve mortality. This may also be related to genetic constituents of the parasites. This finding is also in accordance with the reported data for *Milletia thoningii* by Perret *et al*. (1994) and *Irishgermanica* by Singaba *et al*. (2006). The observed mortalities could be as a result of the active phytochemical components present in the plant parts such as Flavonoids, Saponins and Tannins (Adebanjo, 1983).

From the results obtained the LC50 and LC84 on Gymnocephalous revealed that the bark extract of *A.occidentale* recorded higher LC50 and LC84 while leaf extract recorded lower LC50 and LC84. implying that littlequantity of the leaf extract was required to achieve 50% mortality while the bark of *A. occidentale* required much more quantity of extract to achieve 50% mortality. It was also revealed that the lethal concentration of the cashew bark extract had the

least LC50 and *cashew* leaf extract have least LC84 on *Brevifurcate cercaria*, requiring little extract to achieved 50% mortality rate.

More so, the efficacy of cercaricidal activities followed a model for *Gymnocephalous cercaria* in which the LC reduced with time and between the two plant parts used but this model was not similar with *Brevifurcate cercaria*. The cercaricidal potential for *Brevifurcate cercaria* was not stable and fluctuated between the leaf and bark. This pattern does not agree with most literature (Perret *et al*., 1994; Abdel-Gawad *et al*., 2004; Singaba *et al*., 2006; Fayez, 2011; Kiros *et al*., 2014). Possibilities of this fluctuations could be that at some point, the *Brevifurcate cercaria* became resistant to the plant extract tested and also as a result of the difference in their genetic makeup. It is also possible the leaves extract may have more concentration of phytochemical properties which resulted to their toxicity thereby requiring little concentration of the extract to achieve mortality. The compounds present in the plant parts have significant application against human pathogens including those that cause infections (Franca *et al*., 1993; Kim *et al*., 2006).

CONCLUSION

Efficacy of toxicity on cercarie the leaves and bark methanol extracts had toxicity effect on *Gymnocephalus cercaria* and *Brevifurcate cercaria* tested. More so, methanol leaves and bark extracts had cercaricidal effect on *Brevifurcate cercaria* On *Gymnocephalous cercaria A. occidentale* leaves extract was more active than *A. occidentale* bark extract. *Gymnocephalous cercaria* was more susceptible while *Brevifurcate cercaria* were more resistant to the plant parts extracts. Tannin, flavoid and saponin observed during phyto chemical analysis might be the responsible cercaricidal agents witnessed in this study. Further study is needed to determine the active compounds responsible for the cercaricidal activities of the plant parts used and to also determine the synergistic activities of the plant.

RECOMMENDATIONS

Research should be carried out to determine the active compounds responsible for the cercaricidal activities of the plant parts in this study. Again, the synergistic activities of the plant are recommended.

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