IN VITRO ACTIVITY OF FRACTIONS OF *Calotropis procera* METHANOLIC LEAF EXTRACT AGAINST *S. haematobium* ADULT WORM.

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ABSTRACT

Schistosomiasis is an important water borne disease caused by the digenetic trematode of the genus *Schistosoma.* Itis the commonest in Nigeria and it exert great burden on the health and economy of the people. The objective of this study was to evaluate fractions of methanolic extract of *Calotropis procera* (Ait) for antischistosomal activities *in-vitro* using viable, mature *Schistosoma haematobium* adult worms. The preliminary test at 100µg/ml revealed that methanolic leaf extract of *Calotropis procera*, was active with 100% mortality within 48 hours post exposure time. Secondary Bioassay of graded concentrations of 50, 40, 30, 20, 10µg/mls revealed time dependent activity of the extract with increasing mortality as the post exposure time increased from 24 hours to 72 hours. Mortality reduced with decreasing concentration of extracts with LC_{50} and LC_{90} of (10.1, 12.9 µg/ml. *In-vitro* study of the fractions of the crude extract at graded concentrations of 10, 8, 6, 4, and 2µg/mls showed that chloroform fraction (fraction B) and ethylacetate fraction (fraction C) of *C. procera* were effective against adult worms of *S. haematobiumin-vitro*, with LC_{50} and LC_{90} of (4.6, 11.9µg/mls) and (5.7, 15.2 µg/ml) respectively. The chloroform fraction (fraction B) of *C. procera* showed the stronger activity against *S. haematobiumin-vitro.* These fractions may represent additional source of bioactive materials that deserve further investigation for antischistosomal drug discovery.

Keywords: Antischistosomal, Calotropis, Chloroform, Ethylacetate, Methanolic and Praziquantel

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INTRODUCTION

Chemotherapeutic measures have been the mainstay in the control of schistosomiasis (Fenwick and Webster, 2006). Since 1970, Praziquantel (PZQ) remain the drug of choice against the three major human species of Schistosomes, namely *S. mansoni* (Sambom), *S. heamatobium* (Bilharz) and *S. japonicum* (Katsurada) (Doenhoff and Pica-Mattocia, 2006). It is a relatively safe, orally, administered drug that leads to reducing the prevalence of Schistosomiasis (Southgate *et al.,* 2005). Consequently a targeted as well as mass drug administration programme presently relies heavily on Praziquantel (PZQ) for the control of Schistosome induced morbidity (Eissa, *et al.,* 2011; El Bardicy *et al.,* 2012).With only one drug of choice and the emerging evidence of

the development of tolerance and/or resistance to Praziquantel, (Ndamba *et al.,* 1994; Brindley, 1994; Alonso *et al.,* 2006; Fallon and Doenhoff, 2008) there is need for the search and development of new drug for the prevention and cure of schistosomiasis. The search for alternative antischistosomal agent against the three species of schistosome (Allegereti *et al.,* 2012) is ongoing however, most of the plants parts and extracts that have been assayed were tested against adult *Schistosoma mansoni,* which is relatively easily procured as laboratory material from specialized laboratories (Yousif *et al.,* 2007; 2012; Ramirez *et al.,* 2007). With paucity of data on screening of antischistosomal agents against *S. haematobium* coupled with possible differences in sensitivity of schistosomes to the extract, there is the urgent need to specifically test more extracts against *S. haematobium,* first *in vitro* and then *in vivo* following the conventional bioactivity guided assay in drug discovery. *Calotropis procera* (Linn) also known as Giant Swallow wort, Ark, Milkweed or Sodom apple belong to the family *Asclepiadaceae.* It is native to North Africa, Tropical Africa particularly semi arid region of Bauchi, Borno, Kano, Kaduna and most parts of Northern Nigeria). The plant has been shown to have many ethnomedicinal uses (Sofowora, 1993). Uddin, *et al.* (2012) demonstrated the chemical constituents, antibacterial, antioxidant and analgesic properties of *Calotropis procera.*

MATERIALS AND METHODS

Calotropis procera (Linn) was identified and collected in Bosso, Minna. Vouchers were deposited at the Herbarium of Biological Sciences Department of Federal University of Technology, Minna Niger state Nigeria.

Parasites (*Schistosoma* spp)

Viable adult *S. haematobium,* were obtained from Schistosome Biological Supply Company, Theodore Bilharz Research Institute (SBSC/TBRI) Giza, Cairo. Egypt. The adult parasites were maintained in laboratory animal models namely Hamsters (*Mexoriatus aurantus*) and Balb BC. Mice (*Musmusculus).* Parasites were recovered from the animal model by Perfussion Techniques (Liang *et al.,* 1987).

RESULTS AND DISCUSSION

Result of the *in vitro* bioassay of the crude extract and fractions of *C. procera* tested against adult *S. haematobium* are presented in Tables 1 and 2.The crude methanolic extract and all the fractions possess reproducible and confirmed activity against adult of *S. haematobium in vitro.*

Results of *in vitro* schistosomal assay of graded concentration of the chloroform fraction (fraction B) of crude methanolic extract of leaf of *C. procera* reveal that the fraction possess strong activity against adult of *S. haematobium.* A 100% mortality was recorded at all concentrations from 24 hours post exposure. LC_{50} and LC_{90} were calculated. Similar result was obtained for ethylacetate fraction (fraction C) of the crude methanolic extract of *C. procera* (Table 2).

Table 1 Preliminary *In vitro* assay of crude methanolic extract of *C. procera* at 100µg/ml with LC_{50} and LC_{90} (µg/ml) of graded concentrations.

Extract	Worm mortality(%) at different exposure time (hours)			LC_{50}	LC_{90}
	24 hours	48 hours	72hours		
C procera	75	100	100	10.1	12.9
Praziquantel	72.7	90.9	100	0.8	0.12
DMSO	00	00	00	No effect	

Table 2 Activity of fractions of *C. procera* against *S. haematobium* adult worm

Extract	Worm mortality(%) at different exposure time (hours)			LC_{50}	LC_{90}
	24 hours	48 hours	72hours		
B fraction	50	70	100	4.6	11.9
C fraction	58.3	60	100	5.7	15.2
Praziquantel	00	00	00	0.8	0.8
DMSO	00	00	00	No effect	

Ramos *et al.* (2006) reported that fractions of the crude latex produced by the green parts of *C. procera* were effective to prevent egg hatching in *A. aegypti*. The fractions were also lethal to 3rd instars larva causing 100% mortality within 24 hours.

Allegretti (2012) observed that higher activity of the organic fraction from *Cardia verbenacea* (a medicinal plant native of Brazil commonly known as Maggy plant) compared with the crude aqeous as well as crude ethanolic extract. The organic fraction recorded 100% worm mortality at 400µg/ml within 24 hours of exposure of parasite. In similar vein, the same author reported that fractions 1 and 4 of the plant *Phyllanthus arnusus* showed strong antischistosomal activity against *S. mansoni in vitro* compared with other fractions 2 and 3. The fractions were obtained from crude ethanolic extract of leaf of the plants. The chloroform extract of *Curcuma longa (L)* (Zingeraceae) was lethal to *S. mansoni* worms after 24 hours incubation period (Abdul-Hameed, 2008). The LC_{50} calculated by authors were 28.92 and 31.58 for male and female worms respectively. Yemin *et al.* (2008) reported that *C. procera* possess both antioxidant and hepatoprotective activity in CCL_4 induced hepatic damage. Similar observation was also made by Qureshi *et al,* (2007) in CCL_4 – induced hepatic damage. It is therefore plausible that the activity of fraction B of *C. procera* in this study is based on similar interaction of the extract with the parasite *in vitro.* Phytochemical and pharmacological studies on the crude methanolic extract of

C. procera reveal high content of flavonoid in both leaf and latex of the plant (Uddin, 2012).

Singh *et al.* (2006), described the purification of a novel cysteine protease, procerain B, from *C. procera* with distinct characteristics compared to procerain. While Farid *et al.,* (2013) assessed cystane protease inhibitors (CPI) as chemotherapeutic agent for *S. mansoni* in mice. The authors reported that CPI can selectively arrest parasite replication without untoward toxicity to the host.

Effective exploitation of plant derived compound depends on sufficient supply of the plant materials. *Calotropis procera* is abundant in tropical and subtropical areas and also adapted to arid climates. Therefore exploitation is feasible.

The importance of plants to medicine as sources of natural product bioactive molecules lies not only on their pharmacological or chemotherapeutic effect, but also on their role as template molecules for the production of new drug substances (Yousif *et al.,* 2001). This study is a step towards this direction and introduces some new antischistosomal sources.
These fractions could represent additional promising bioactive sources that deserve further investigation for drug discovery against *S. haematobium* infection.

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