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Anti-Schistosomal Activity of *Chenopodium ambrosoides* Extracts in Adult Worms *In vivo* and *In vitro*

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Abstract
Plants may contain ingredients that have anti-parasitic activity against parasites of medical significance. *Chenopodium ambrosoides* (Wormseed) a wide spread herb in the Family Chenopodiacea was investigated for anti-schistosomal activity using, the human trematode parasite, *Schistosoma mansoni*, as the target. The plant is well known for its vermifuge and anti-helminthetic properties. The root, stem, leaves and fruit of the plant were extracted sequentially using *n*-hexane, dichloromethane, methanol and distilled water as solvents and tested for anti-schistosomal activity. The crude extracts of leaves and fruits wereremarkableand showedsignificant activity that resulted in significant egg counts reduction, compared to untreated controls ($P < 0.05$). Among the plant extracts (*n* – hexane, dichloromethane, methanol and aqueous), aqueous (leaf) and methanol (fruit) extracts showed responses closest to PZQ. Aqueous (leaf) had 46% worms reduction, methanol (fruit) had 23% worms reduction and Praziquantel had 34% worms reduction ($P > 0.05$). The *in vitro* results showed methanol (fruit) extract killed more adult worms of *S. mansoni* than the aqueous (leaf) extract. The effect of both methanol (fruit) and aqueous (leaf) extracts on *S. mansoni* adult worms showed that methanol (fruit) extract had better potency than aqueous (leaf) extract. The killing effect of methanol (fruit) and aqueous (leaf) extracts were statistically similar to Praziquantel.
Keywords:*Chenopodium ambrosoides* (Wormseed), *In vivo* and *In vitro*

1. INTRODUCTION
Plants have been used for medicinal purpose since ancient Egyptian civilization(Hamed, 2009). The plant C. *ambrosoides* has been used for medicinal purposes (Sagrero *et. al., 1995*). *Chenopodium ambrosoides* is an erect herb that grows to a height of 40 cm, often branched and is distributed through out the world (Guether, 1952). In parts of South East Asia, e.g. in Java it occurs at 1600 – 2000 m altitude (Guenther, 1952). At least 250 species of *Chenopodium* are known to exist *(*Agnew *et al*., 1994*)*.The plant *Chenopodium ambrosoides* occurs abundantly along roadsides and in waste places, sometimes also in upland rice fields. In Kenya, the distribution and availability of *C. ambrosoides* is variable. It occurs mainly in the highlands of Aberdares, Kitale, Machakos, Narok and Nairobi (Agnew *et al*., 1994).*C. ambrosoides* is an effective anthelmintic with a long history of use (Bliss, 1925). It is effective against hookworms in humans (*Ancylostoma duodenale and Necator americanus*), roundworms (*Ascaris ambricoides*) and whipworms (*Trichuris trichiura*) (Kliks*, 1985;* Zulane *et al., 2012*). *Chenopodium ambrosoides* aqueous extract is effective against gastrointestinal parasitic nematodes (Salifoce *et al.,* 2013). Cathy Wong, (2012) has also indicated that *C. ambrosoides* is used as a herbal remedy in the tropics to treat roundworms, hookworms and tapeworms. *In vitro*, studies with oil of *C. ambrosoides* extracts have been shown to inhibit egg development of parasites and maturation of larva but these tests have not been confirmed in *in vivo* studies (Sagrero et *al.,* 1995; Ketzi and Brown, 1998).
The saponin of *Chenopodium ambrosoides*is reported to have anti-fungal activity (Kishore *et al.,* 1993). The essential oil and its main component ascaridole of *C. ambrosoides* is reported to be a potent inhibitor of plasmodial growth in lower *c*oncentrations and kill malarial parasites in higher concentrations (Okuyama *et al*., 1993). Using essential oils of *Chenopodium ambrosoides* for intraperitoneal treatment at dose 30mg/kg in BALB/c infected mice with *leishmania amazonesis*, the data demonstrated that the essential oils had better anti-leishmanial effect than the reference drug (amphotericin B at 1mg/kg) suggesting that the essential oils could be used as a drug (Monzote *et al*., 2007). Anti-schistosomal properties of *C. ambrosoides* methanol extract against *S. mansoni* infected mice were assessed (pi) and the treatment raised reduction rates of worm load/mouse to 66.3% and the oval/g tissue to 76.9% Kamel *et al*., (2011).

2. Materials and Methods
2.2*In vivo* Test
The plant *C. ambrosoides* test materials (root, stem, leaves and fruits) were collected, dried in room temperature and then ground into powder form using a Mekon Micromealers Single Phase grinder. Extraction of the powdered materials was done with a range of solvents (*n*-hexane, dichloromethane, methanol and aqueous)

starting with compounds which eluted non-polar to more polar extract components using method of Harborne (1984). Groups of BALB/c mice were infected with *S. mansoni* and each group consisting of (3 males and 3 females) were used to test extracts from different parts of the plant for worms reduction. Aqueous extract and praziquantel were prepared as suspension in distilled water and *n*- hexane, dichloromethane, methanol extracts were prepared as a suspension in 10% Tween 80 solution. Each suspension was administered at 150mg/body wt, and praziquantel at (450mg/body wt. was used as a reference drug. A control group (infected but not treated) consisting of 3 males and 3 females was also set up. Each mouse from the test group was administered 200 µl of extract suspension /30g body wt. using a 200 µl micropipette. Each experiment for each extract was repeated. At week 6 mice were perfused for worm recovery

2.3 *In vitro* Test

In vitro test was done using *S.mansoni* worms perfused from BALB/c mice infected with *S. mansoni* cercariae. Perfusion of mice was done at wk 5 to recover the adult worms using a modified method of Smithers and Terry (1965; Yole *et al.*(1996). Groups of five male and five female adult worms were collected and separated into Petri dishes. Methanol (fruit) and aqueous (leaf) extracts which were efficacious against *S. mansoniin vivo*were each weighed, diluted with distilled water into test concentration of 0.05 mg/ml, 0.15 mg/l. and 0.3 mg/l.2ml of each test concentrate was dispensed into Petri dishes containing the worms. These were kept at room temperature and monitored at 5, 10, 15, 20 and 30 min. in each of the test concentration. The number of dead worms were noted and recorded. A repeat of dosing procedure on the same number of adult worms was carried out.

3. RESULTS

3.1 In vivo

The results of *in vivo* are shown in Tables 1, 2 and Fig 1. Aqueous(leaf) extract had the lowest mean worm counts of 16.75 ± 2.87 and methanol (leaf) extract had a mean worm counts of 23.60 ± 2.04. Methanol (fruit) extract had a mean worm counts of 24.00 ± 1.31 and aqueous (fruit) extract had a mean worm count of 24.50 ± 3.07. Praziquantel had a mean worm counts of 20.50 ± 1.71 and the infected untreated control had a mean worm counts of 31.20 ± 3.23.

Worms reduction showed that aqueous (leaf) extract had the highest worms reduction of 46.3%, methanol (leaf) extract was second with 24.3% worms reduction and methanol (fruit) extract was third with 23.% worms reduction and aqueous (fruit) extract was fourth with 21.4% worms reduction and Praziquantel had 34.2% worms reduction. Aqueous (leaf) extract had the best worms reduction. When aqueous (leaf), methanol (leaf) and methanol (fruit) were compared with Praziquantel, the results showed there was not a statistically significant difference ($P < 0.05$). When aqueous (leaf), methanol (leaf) and methanol (fruit) were compared with the infected controls, the results showed there was a statistically significant difference ($P > 0.05$).

Aqueous (leaf) extractwas the best while methanol (fruit) extract was the second best in worms reduction.The experiments showed both the aqueous (leaf) and methanol (fruit) extracts were relatively effective for treatment of *S. mansoni* parasites in BALB/c mice

Table 1: Anti-schistosomal activity of *C.ambrosoides* parts (root, stem, leaf and fruit) extracts in different solvents using *S. mansoni* as target parasite in BALB/c mice

Solvent	**Root**	**Stem**	**Leaf**	**Fruit**
n-Hexane	32.86 ± 2.27	35.14 ± 3.56	33.40 ± 4.35	32.83 ± 2.27
Dichloromethane	36.25 ± 5.02	34.40 ± 2.79	37.00 ± 8.51	37.00 ± 3.89
Methanol	32.50 ± 1.61	40.33 ± 4.09	23.60 ± 2.04	24.00 ± 1.31
Aqueous	37.17 ± 2.18	39.60 ± 5.14	16.75 ± 2.87	24.50 ± 3.07
Praziquantel	20.50 ± 1.71			
Infected untreated control	31.20 ± 3.23			

Table 2: Mean worm counts and percentage worms reduction of biologically active extracts

Extract	**Mean ± S.E**	**%Worms Reduction**
Aqueous(leaf)	16.75 ± 2.87	46.3
Methanol(leaf)	23.60 ± 2.04	24.35
Methanol(fruit)	24.00 ± 1.31	23.07
Aqueous(fruit)	24.50 ± 3.07	21.47
Praziquantel	20.50 ± 1.71	34.29
Infected untreated control	31.20 ± 3.23	

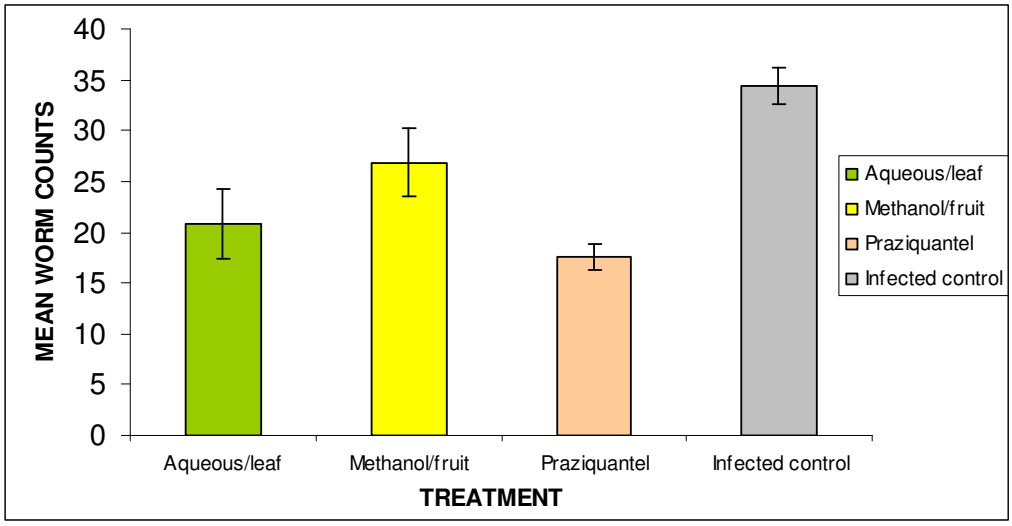

Fig.1. Mean worm counts after treatment with both aqueous (leaf) and methanol (fruit) extracts of *C. ambrosoides*

3.2 *In vitro*

The *in vitro* results are shown in Table 3. When methanol (fruit) extract was used to treat adult worms, the result showed that 100% adult worms died at 5th min. in doses 0.05 mg/ml,0.15mg/ml and 0.3 gm/ml. Aqueous (leaf) extract was used to treat adult worms, the result showed that in doses 0.05 mg/ml and 0.15 mg/ml, 100% adult worms died at 5th min. while in the subsequent dose 0.3 gm/ml, only 20% adult worms had died at the 30th min. For the controls, the results showed 90% adult worms died at 10th min. and all the adult worms died at 15th min. in praziquantel. No worms died in the phosphate buffered saline. When repeat experiments were done for both methanol (fruit) and aqueous (leaf) extracts, similar results were obtained.

Table 3: Adult worms mortality using different time and concentrations of methanol (fruit) and Aqueous (leaf) extracts (n= 10).

Concentration	Time	Methanol(fruit) % Dead worms	Aqueous(leaf) % Dead worms	PZQ % Dead worms	PBS % Dead worms
0.05 mg/ml	5 min.	100	100	0*	0**
	10 min	-	-	90	-
	15 min	-	-	100	-
	20 min	-	-	-	-
	30 min	-	-	-	-
0.15 mg/ml	5 min	100	100	100	-
	10 min	-	-		-
	15 min	-	-		-
	20 min	-	-		-
	30 min	-	-		-
0.3 mg/ml	5 min	100	20	100	-
	10 min	-	20		-
	15 min	-	20		-
	20 min	-	20		-
	30 min	-	20		-

Key: * = sluggish movement of *S. mansoni* worms
** = Live and active *S. mansoni* worms
PBS = Phosphate Buffered Saline
PZQ = Praziquantel

4.DISCUSSION

4.1 In vivo

Percentage worms reduction showed that aqueous (leaf) and methanol (fruit) extracts had the highest worms reduction. The results of mean worm counts showed that aqueous (leaf) extract had the lowest mean worm counts followed by methanol (fruit) extract. Among all the 16 extracts, tested for anti-schistosomal activity, only

the 2 extracts had results closest to Praziquantel in terms of worms reduction showing that the extracts were able to invoke protection of the mice against *S.mansoni* infection. Praziquantel was used as a positive control since it is effective against *S. mansoni* (Cioli, 2000; Hagan *et al*., 2004). Praziquantel is the drug of choice for the treatment of Schistosomiasis (WHO, 2010). It is known to cause titanic contractions and tegumental vacuoles which cause the worms to detach from the walls of the veins and die (Ross *et al*., 2002).

The infected untreated controls had the highest mean worm counts compared to the rest of the extracts. When the results of aqueous (leaf) extract, methanol (fruit) extract and Praziquantel were compared with the infected untreated controls, there was significant difference (Anova;'t'- test, $p < 0.05$). There was no significant difference between the infected untreated controls with the other extracts (Anova; $p > 0.05$) demonstrating that these were not protective against *S. mansoni* infection in the mice. In this study, it can therefore be concluded that both aqueous (leaf) and methanol (fruit) extracts of *Chenopodium ambrosoide* had anti-schistosomal active ingredients against *S. manoni* infection in BALB/c mice.

4.2 In vitro

In this in vitro experiment, anti-schistosomal activity of*Chenopodium ambrosoidies* on *S. mansoni* adult worms was tested.Praziquantel and physiological buffered saline acted as controls in both experiments and the repeat experiment. Aqueous (leaf) extract killed more worms at lower concentration than the higher concentrations. Killing effect could depend on oinization of the extract; the higher the dilution (i.e lower concentration) the higher the oinization and the higher the wormicidal effect. The results showed methanol (fruit) extract killed more adult worms of *S. mamsoni* than the aqueous (leaf) extract. Methanol (fruit) extract potentency was similar in the 3 concentrations. The effect of both methanol (fruit) and aqueous (leaf) extracts on *S. mansoni* adult worms showed that methanol (fruit) extract had better potency than aqueous (leaf) extract. The killing effect of methanol (fruit), and aqueous (leaf) extracts were similar to Praziquantel.

Chenopodum ambrosoides has been used to treat a variety of intestinal worms (Kliks 1985), Different parts of *C, ambrosoides* plant eg. the roots have biochemical products which have been used treat hookworms and ascarids in humans (Kishore *et aL,* 1989). *In vitro* studies carried out on the infectivity of *S. mansoni* cereariae to albino mice exposed to methanol extract *of Chenopodum ambrosoides* showed that the number of worms recovered from infected mice was less than those of infected control group (Kamel *et aL,* 2010).

5. CONCLUSION AND RECOMMENDATION

Both methanol (fruit) and aqueous (leaf) extracts had similar efficacy to praziquantel, in terms of worm reduction showing that the extracts were able to invoke protection of the mice against *S. mansoni* infection.*In vitro* treatment using both methanol (fruit) and aqueous (leaf) extracts on *S. mansoni* adult worms, showed methanol (fruit) extract was a better wormicidal than aqueous (leaf) extract. Time exposure in either lower or higher concentrations of both methanol (fruit) and aqueous (leaf) extractsdid not influence the mortality rate of adult worms.

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