

EFFECTS OF CRUDE ROOT EXTRACTS OF *SENNA DIDYMOBOTRYA* ON CAECAL AMOEBIASIS IN MICE

Lydia Bonareri Nyamwamu* ¹, Moses Ngeiywa ¹, Margaret Mulaa ¹, Agola Eric Lelo ², Johnstone Ingonga ², Albert Kimutai ³, Michael Gicheru⁴.

¹University of Eldoret, Department of Biological Sciences, P.O. BOX 1125-30100, Eldoret, Kenya.

²Kenya Medical Research Institute, Centre for Biotechnology Research and Development P.O. BOX 54840-00200, Nairobi, Kenya.

³University of Kabianga, Department of Biological Sciences, P.O. BOX 2030-20200, Kericho, Kenya.

⁴Kenyatta University, Department of Zoological Sciences, P.O. Box 43844-00100, Nairobi, Kenya

*Corresponding Author

Lydia Bonareri Nyamwamu

E mail: lydianyamwamu@yahoo.com

P.O. BOX 8165-30100, Eldoret, Kenya.

Tel no. +254 710 454 181

ABSTRACT

Anti-amoebic effects of crude root extracts of *Senna didymobotrya* against *Entamoeba histolytica* infecting caecum of mice were studied. Caecal amoebiasis was induced by injection of *Entamoeba histolytica* trophozoites directly into the caecum. Mice were treated orally with the root dichloromethane extracts, ethyl acetate extracts, methanol total extracts, methanol successive extracts, hexane extracts, water extracts, metronidazole and normal saline for five consecutive days and examined on the sixth day. At a dose of 500mg/g/day, root extracts of dichloromethane, ethyl acetate, methanol total, methanol successive, hexane and water had a curative rate of 50, 66.7, 100, 66.7, and 83.3%, each, respectively. At a concentration of 1mg/g/day, dichloromethane, ethyl acetate, methanol total, water, hexane extracts were effective in 33.3 each, 83.3 each and 66.7% of the cases respectively, while methanol successive extract at a dose of less than 500mg/kg/day did not cure any mice. Metronidazole at a concentration of 125mg/kg/day had a curative rate of 100%. Severity of caecal wall ulceration reduced in mice which received extracts and metronidazole compared to negative control animals.

Keywords: Amoebiasis; Antiamoebic, *Entamoeba histolytica*; *Senna didymobotrya*.

1.0 INTRODUCTION

Amoebiasis is a disease caused by *Entamoeba histolytica*, a parasite protozoan that infects humans and is responsible for 40,000 to 110,000 deaths per year ^[1]. Ten percent of infected persons exhibit clinical symptoms; 80% to 98% of which are intestinal, and 2% to 20% are extra intestinal^[2]. *Entamoeba histolytica* is the pathogenic and the etiologic agent of amoebic colitis and liver abscess ^[2] ^[3]. Differential diagnosis between *E. histolytica* and *Entamoeba dispar* species is essential both for treatment decision and public health knowledge ^[4]. The World Health Organization (WHO) suggested that *E. histolytica* should be specifically identified and, if present, treatment is crucial

[5]. The incidence of amoebiasis has decreased significantly in recent years because of increased sanitation in many countries and the use of effective therapeutic agents. The World Health Organization and the Pan-American Health Organization recommend the treatment of all patients with confirmed *E. histolytica* infection, regardless of the presence of symptoms [6]. In spite of the effective therapeutic agents that are available for the treatment of amoebiasis, it still constitutes a global health problem [7]. The prevalence of amoebiasis varies from 1% in industrialized countries to 50%–80% in tropical countries [1].

Among parasitic infections, amoebiasis ranks third worldwide in lethal infection, after malaria and schistosomiasis [8]. Although it is asymptomatic in 90% of cases, about 50 million people are estimated to suffer from the symptoms of amoebiasis [9]. These infections result in 40 000–110 000 deaths annually [1]. Prevalence rates of amoebiasis are highest in developing countries in Asia, particularly the Indian subcontinent and Indonesia, the sub-Saharan and tropical regions of Africa, and areas of Central and South America [10]. The estimated number of infected cases may be much higher due to the lack of a sensitive and specific diagnostic test [11].

The current treatments of choice are either one of a family of nitroimidazoles (usually metronidazole), nitrofurans, quinacrine or paromomycin [12]. However, this drug has been reported to cause mutagenicity in bacteria [13] and is carcinogenic in rodents [14]. It has been reported not only metronidazole and also its hydroxy metabolite is potentially genotoxic and carcinogenic [15]. Moreover, it seems to act as an immunosuppressive agent in experimental rats, both in cell-mediated and humoral immune responses [16]. Therefore, there is need to develop a safe and effective alternative antiamoebic agent. For people in developing countries, medicinal plants are popular because their products are safe and widely available at low cost [17]. Some compounds extracted from medicinal plants already play an important role against infectious diseases for instance quinine from *Cinchona* species, and artemisinin from *Artemisia annua*; both are effective against malaria [18].

Senna didymobotrya (Fres.) Irwin & Barneby (syn *Cassia didymobotrya*) belonging to the family Fabaceae (Leguminosae) is a widely used medicinal plant in East Africa [19]. It is a potential medicinal plant and the medicinal values are explored well in many parts of the world by traditional practitioners. In Kenya, traditionally Kipsigis community has been using these plants to control malaria as well as diarrhea [19]. In addition, it has been used to treat skin conditions of humans and livestock infections as well [20]. In Congo, Rwanda, Burundi, Kenya, Uganda, Tanzania, root decoction of these plants was used for the treatment of malaria, other fevers, jaundice and intestinal worm [19]. In addition, root or leaf mixed with water or decoction of fresh parts was used to treat abscess of the skeletal muscle and venereal diseases [21]. The plant is also useful for the treatment of fungal, bacterial infections, hypertension, haemorrhoides, sickle cell anemia, a range of women's diseases such as inflammation of fallopian tubes, fibroids and backache, to stimulate lactation and to induce uterine contraction and abortion [22]. In the present study, *S. didymobotrya* root extract was selected because this species was routinely used to cure diarrhea in the Kipsigis traditional medical practice. It is, therefore, of interest to scientifically evaluate its effect on amoebiasis for potential antiamoebic activity *in vitro* and *in vivo*.

2.0 MATERIALS AND METHODS

2.1 *Entamoeba histolytica* trophozoite culture

Entamoeba histolytica HM-1: IMSS strains were used in all experiments. The parasite and culture media was obtained from University Boulevard, Manassas, USA. The trophozoites were cultured axenically in screw-capped tubes at 35.5⁰C on LYI-S-2 medium, supplemented with 10% (v/v) heat-inactivated bovine serum ^[23]. Subcultures were performed routinely at 48hr intervals by replacing the medium without detaching the monolayer. Cells were harvested by replacing the medium with a fresh one, chilling on ice for 20 min, and inverting gently to detach the monolayer.

2.2 Collection of *Senna didymobotrya* roots

The roots of *S. didymobotrya* were collected randomly during the month of October-November, 2012 and were authenticated. The plant materials were taxonomically identified by a taxonomist and the voucher specimens were preserved at the Centre for Biotechnology Research and Development for future reference.

2.3 Preparation of extracts

The roots were washed, cut into small pieces and air-dried for three weeks under a shed. The dried specimens were shred using an electrical mill in readiness for extraction. The sample preparation and extraction procedure were carried out as described by Harbone, (1994) ^[24]. Cold sequential extraction was carried out on plant material with distilled water, Ethyl acetate, hexane and Methanol as the solvent system.

2.3.1 Solvent Extraction

300g of dried powder were taken in 600ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hrs. After 24hrs the supernatant was collected and the solvent evaporated. The residue obtained was collected and stored at 4°C in airtight bottles. The process was repeated sequentially for ethyl acetate, dichloromethane, methanol and hexane.

2.3.5 Aqueous Extraction

300g of dried powder were added to 600ml of distilled water in a conical flask and boiled on slow heat for 2hrs. It was then filtered using No. 1 Whitman filter paper and centrifuged at 5000rpm for 10min. After 6hrs, the supernatant was collected at an interval of every 2 hrs pooled together and concentrated using a rotary evaporator. The residue obtained was collected and stored at 4°C in airtight bottles.

2.4 Inoculation procedure

The BALB/c mice were prepared for *E. histolytica* infection according to the method described by Ray and Chatterjee (1981) ^[25] with a slight modification. Briefly, 24h before the commencement of the surgery, the mice (25–35g) were starved, and, in the morning and evening, the mice were pretreated orally with 0.5ml of 25% MgSO₄ in distilled water. On the next day, the mice were anesthetized by an intraperitoneal injection of pentobarbital sodium 40mg/g. Laparotomy was performed to expose the caecum. The suspension of actively motile *E. histolytica* containing 2×10^6 trophozoites was injected directly into the caecum. The caecum was then returned into the peritoneal cavity, the abdominal muscle closed and the skin sutured. Rat pellets and drinking water were provided ad libitum. The mice were randomly selected for the treatment and control groups.

2.5 Effects of crude extracts and metronidazole on amoebiasis in mice

The extracts of *S. didymobotrya* and the standard drug, metronidazole in tablet form, were suspended in distilled water. All treatments were administered daily by peroral using a feeding tube, for five consecutive days, beginning 24h after infection with *E. histolytica*. The doses that were used for the plant extracts are 1 and 500mg/g body weight per day. The control animals were treated with normal saline 5ml/g body weight and metronidazole 125mg/g per day. Six animals were used for each treatment.

On the sixth day, the animals were sacrificed by use of chloroform and the caecum carefully examined macroscopically for lesions and the content structure. The severity of infection was scored according to the method described by Neal, ranging from 0 for normal to 4 for severe structure destruction^[26]. The presence of *E. histolytica* trophozoites in the caecum was observed under an inverted light microscope. In the absence of *E. histolytica* trophozoites, a small amount of caecum content was transferred into a fresh medium and cultured for 24–48hr and this was then examined for trophozoites under an inverted light microscope.

3.0 RESULTS AND DISCUSSION

3.1 Extraction of compounds of *Senna didymobotrya*

Three hundred grams of *S. didymobotrya* yielded 1.77g of dichloromethane, 3.85g of ethyl acetate, and 7.35g of methanol total, 6.23g methanol successive, 1.52g of hexane extract and 7.5g of water extract (Figure 1). There was a significant difference ($p < 0.05$) in the yields obtained. Water extraction produced the highest yields compared to the other solvents (Figure 5). This is because water is more polar compared to the organic solvents hence its able to extract more compounds from a plant material^[27]. Kigundu, (2007),^[28] also found out that water extract produced the highest yields as compared to organic solvents. Methanol total produced the highest yields compared to the other organic solvents (Figure 1). This was also observed in a study carried out by Korir *et. al.*, (2012)^[27].

3.2 Effects of crude extracts of *Senna didymobotrya* and metronidazole on amoebiasis in mice

The effects of extracts from *S. didymobotrya* against experimental caecal amoebiasis in mice are shown in Table 1. The results from the present study demonstrate that methanol total extract is more effective against *E. histolytica* in mice as evaluated by the number of mice cured and the reduction of severity of the mice caecal content and caecal wall lesions in comparison to the untreated mice (Table 1). The anti-amoebic effects of all extracts are clearly dose-dependent.

This study shows that the selected medicinal plant extracts also reduce the severity of caecum due to *E. histolytica* infection in mice. The pooled controls of 6 mice were all positive for amoebae at the time of sacrifice (Table 1). This amoebic infection generally produced score of caecal content and caecal wall ranging between 4 and 4 with the average of 4 and 4, respectively (Table 1). This indicates the virulence of the strain of *E. histolytica* used in this study. It is generally known that axenic strain of *E. histolytica* becomes non-invasive after prolonged cultivation in vitro^[29]. This study demonstrates that the amoebae isolated from the control mice infected with this strain of *E. histolytica* was still virulence and could be used subsequently.

In the present study, mice treated with metronidazole at a concentration of 125mg/g per day for 5 days were successfully cured from amoebiasis, confirming that this strain of *E. histolytica* was still sensitive to this drug (Table 1). The results on efficacy of metronidazole were similar to the studies of several investigators whose studies on caecal amoebiasis were performed, both in rats and mice models [30] [31] [32]. The extract from methanol total appeared to be the most effective at a concentration of 500mg/g per day after 48 hours, as this dose cleared all *E. histolytica* from the intestine of mice on the day of examination. This is comparable to metronidazole at the dose 125 mg/kg per day. Although treatment with the extract from methanol total at a concentration of less than 500 mg/g per day did not cure all animals, the caecal content and caecal wall of these mice appeared normal indicating the effectiveness of the extract against the parasites. The use of this extract to treat amoebiasis may at least help in reducing severity that occurred in the intestine.

The presence of *E. histolytica* trophozoites in the caecum was observed under an inverted light microscope. A small amount of caecum content was transferred into a fresh medium and cultured for 24–48hr and this was then examined for *E. histolytica* trophozoites under light microscope. There was a significant difference between the different extracts as well as the different concentrations ($p < 0.05$; Table 2). Methanol total extract had a negative result for *E. histolytica* trophozoites and compared favorably well with the current drug of choice metronidazole at a concentration of 500mg/ml (Table 2), whereas dichloromethane, methanol successive, hexane and water extracts had trophozoite levels ranging from moderate to low respectively. The activity of the *S. didymobotrya* crude extracts is dose dependent however; ethyl acetate extract was inactive even at a higher concentration by having high to very high trophozoite levels (Table 2). This study shows that the effect of methanol total on *E. histolytica* are consistent with those previously reported that the methanol extracts from *Piper sarmentosum* root and *Quercus infectoria* nut gall appeared to be effective against caecal amoebiasis in mice at a concentration of 1000mg/ml per day [33], ethanol extract of *Piper longum* fruit at a concentration of 1000mg/ml per day can cure 90% of rats infected with *E. histolytica* [32].

CONCLUSION

From the findings of this study concerning the antiamoebic property of *Senna didymobotrya*, it can be concluded that the methanolic total crude root extract seems to be a good antiamoebic candidate for amoebiasis treatment. Further investigations are therefore needed to identify an active compound of this extract and to determine whether the alteration of enzyme activity is the target mode of action of this extract.

ACKNOWLEDGEMENTS

The authors are very grateful to Ms Emily Wanjiku and Milkah Mwangi for their assistance during data collection.

REFERENCES

1. Araujo J, Garc'ia ME, Diaz-Su'arez O, Urdaneta H. "Amoebiasis: importance of the diagnosis and treatment. Mini review," *Invest Clin*, 2008; 49: 265–271.
2. Wong-Baeza I, Alc'antara-Hern'andez M, Mancilla-Herrera I, Ram'irez-Sald'ivar I, Arriaga-Pizano L, Ferat-Osorio E, L'opez-Mac'ias C, Isibasi A. Review Article. The Role of Lipopeptidophosphoglycan in the Immune Response to *Entamoeba histolytica*. *J. Biomed. Biotechnol*, 2010; 10:1155.

3. Al-Harthi SA, Jamjoom MB. Diagnosis and Differentiation of Entamoeba Infection in MakkahAl Mukarramah Using Microscopy and Stool Antigen Detection Kits. *WJMS*, 2007; 2: 15-20.
4. Fotedar R, Stark D, Marriott D, Ellis J, Harkness J. *Entamoeba moshkovskii* infections in Sydney, Australia. *Eur J Clin Microbiol Infect Dis*, 2008; 27:133–137.
5. Pritt BS, Clark GC. “Amoebiasis,” *Mayo Clin Proc*, 2008; 83: 10: 1154–1160.
6. WHO/PAHO/UNESCO (1997). A report of a consultation of experts on amoebiasis (1997). *WER*. 72:97–9.
7. Stanley Jr SL. “Amoebiasis,” *The Lancet*; 2003; 361: 1025–1034.
8. Jackson TFHG. Amebiasis. London: Imperial College Press; 2000. p. 47
9. Bansal D, Ave P, Kerneis S, Frileux P, Boche´ O, Baglin CA, Dubost G, Leguern AS, Prevost MC, Bracha R, Mirelman D, Guille´n N, Labruye`re E. An ex-vivo Human Intestinal Model to Study *Entamoeba histolytica* Pathogenesis. *NTD*, 2009; 3: e551.
10. Petri WA Jr, Singh U. Diagnosis and management of amoebiasis. *Clin Infect Dis*, 1999; 29: 1117–25.
11. Petri Jr, WA, Haque R, Lyerly D, Vines RR. Estimating the impact of amoebiasis on health. *Parasitol Today*, 2000; 16: 320–321.
12. Tracy JW, Webster Jr LT. Drugs used in the chemotherapy of protozoal infection. In: Hardman, J.G., Limbird, L.E. (Eds.), *The Pharmacological Basis of Therapeutics*, 9th ed. McGraw-Hill, New York, 1996; 987–1008.
13. Cantelli-Forti G, Aicardi G, Guerra MC, Barbaro AM, Biagi GL. Mutagenicity of a series of 25 nitroimidazoles and two nitrothiazoles in *Salmonella typhimurium*. *Teratog Carcinog Mutagen*.
14. Dobias L, Cerna M, Rossner P, Sram R. Genotoxicity and carcinogenicity of metronidazole. *Mutat Res*. 1994 Jun; 317(3):177-94.
15. Saxena A, Chugh S, Vinayak VK. Modulation of host immune responses by metronidazole. *IJMR*, 1985; 81: 387–390.
16. Menendez D, Bendesky A, Rojas E, Salamanca F, Ostrosky-Wegman P. Role of P53 functionality in the genotoxicity of metronidazole and its hydroxy metabolite. *Mutat Res*. 2002 Apr; 501(1-2):57-67.

17. Rani D. Plant Extracts with Antiamobic Properties: A Theoretical Study with reference to *Entamoeba histolytica*. *J Pharm Tech & Res*, 2011; 3: 1113-1117.
18. Hidayathula S, Chandra KK, Chandrashekar KR. Phytochemical evaluation and antibacterial activity of *Pterospermum diversifolium blume*. *Int J Pharm Sci*, 2011; 3: 165-167.
19. Nagappan R. Evaluation of aqueous and ethanol extract of bioactive medicinal plant, *Cassia didymobotrya* (Fresenius) Irwin & Barneby against immature stages of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pac J Trop Biomed*, 2012; 707-711.
20. Njoroge GN, Bussmann RW. Ethnotherapeutic management of skin diseases among the Kikuyu of central Kenya. *J Ethnopharmacol*, 2007; 111: 303-307.
21. Kamatenesi-Mugisha M. Medicinal plants used in reproductive health care in Western Uganda: documentation, phytochemical and bioactivity evaluation. Ph.D. thesis. Makerere University, Kampala, Uganda, 2004; 228.
22. Tabuti JRS. *Senna didymobotrya* (Fresen.) H.S. Irwin & Barneby. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). *Prota 11(1): Medicinal plants/Plantes médicinales 1*. PROTA, Wageningen, Netherlands, 2007.
23. Diamond LS, Harlow DR, Cunnick CC (1978). A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Transactions of the Royal Society of Trop Med Hyg*, 1978; 72:431-432.
24. Harborne JB. *Biochemistry of Phenolic compounds*. Academic Press. London, 1994.
25. Chatterjee A., Ghosh S.K., Jang K., Bullitt E., Moore L., Robbins P.W., and Samuelson J. Evidence for a "wattle and daub" model of the cyst wall of *Entamoeba*. *PLoS Pathogens*, 2009; 5: e1000498.
26. Neal RA. Some observations on the variation of virulence and response to chemotherapy of strains of *Entamoeba histolytica* in rats. *Trans. R. Soc. Trop. Med. Hyg*, 1951; 44: 439-452
27. Korir RK, Mataj C, Kiiyukia C, Bii C. Antibacterial activity and safety of two medicinal plants traditionally used in Bomet district of Kenya. *J. Med. Plants Res*, 2012; 6: 370-387.
28. Kigonda EVM. Phytochemical and anti-parasitic activity of selected medicinal plants from Kenya. Msc. Thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya, 2007.
29. Phillips BP. *Entamoeba histolytica*: Concurrent irreversible loss of infectivity/pathogenicity and encystment potential after prolonged maintenance in axenic culture in vitro. *Exp Parasitol*, 1973; 34:163-167.

30. Bhopale KK, Pradhan KS, Masani KB, Kaul CL. A comparative study of experimental caecal amoebiasis and the evaluation of amoebicides. *Ann Trop Med Parasitol*, 1995; 89: 253–259.
31. Sohni YR, Kaimal P, Bhatt R.M. The antiamebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica* in vitro and in vivo. *J Ethnopharmacol*, 1995; 45: 43–52.
32. Ghoshal S, Krishna Prasad BN, Lakshmi V. Antiamebic activity of *Piper longum* fruits against *Entamoeba histolytica* in vitro and in vivo. *J Ethnopharmacol*, 1996; 50: 166–170.
33. Sawangjaroen N, Sawangjaroen K, Poonpanang P (2004). Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. *J Ethnopharmacol*, 2004; 91: 37–360.

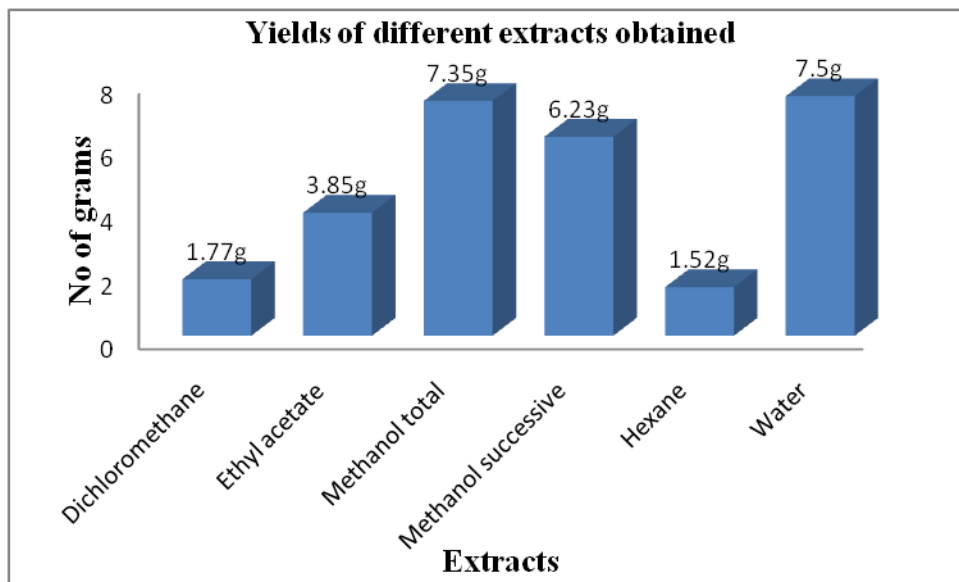


Figure 1. Yields of different extracts of *Senna didymobotrya* obtained.

Table 1. Effects of crude root extracts of *Senna didymobotrya* and Metronidazole on caecal amoebiasis in mice

Test materials	Dose	No of mice treated	Average caecal score (range)

	(mg/g/day)	(% cured)	Contents	Walls
Dichloromethane	1	(2/6) 33.3	3 (3-4)	3 (3-4)
	500	(3/6) 50	3 (3-4)	1 (1-2)
Ethyl acetate	1	(2/6) 33.3	4 (4-4)	3 (3-4)
	500	(4/6) 66.7	2 (2-3)	1 (0-1)
Methanol total	1	(5/6) 83.3	0.5 (0-1)	1 (0-1)
	500	(6/6) 100	0 (0-0)	0 (0-0)
Methanol successive	1	(0/6) 0	4 (4-4)	4 (4-4)
	500	(4/6) 66.7	3 (2-3)	1 (1-2)
Hexane	1	(4/6) 66.7	3 (2-3)	1 (0-1)
	500	(5/6) 83.3	0.5 (0-1)	0.5 (0-1)
Water	1	(5/6) 83.3	0.5 (0-1)	0.5 (0-1)
	500	(5/6) 83.3	0.5 (0-1)	0.5 (0-1)
Metronidazole	125	(6/6) 100	0 (0-0)	0 (0-0)
Normal saline	5	(0/6) 0	4 (4-4)	4 (4-4)

Caecal scores were graded upon the following criteria (Neal, 1951). Wall: normal, 0; slight thickening, 1; marked local thickening and contraction, 2; extensive thickening and contraction, 3; caecum shapeless (extensive ulceration with abscess formation), 4. Contents: normal, 0; slightly less solid than normal, 1; slightly mucoid, 2; mucoid (some solid matter present), 3; no solid matter (white or yellow mucus only), 4.

Table 2. Trophozoite levels after treatment of mice with Metronidazole and *Senna didymobotrya* crude root extracts

Extract	Dose (mg/ml)	Trophozoite levels
---------	--------------	--------------------

Dichloromethane	1	+++
	500	++
Ethyl acetate	1	++++
	500	+++
Methanol total	1	+
	500	-
Methanol successive	1	++++
	500	++
Hexane	1	+++
	500	+
Water	1	+++
	500	++
Metronidazole	125	-
Normal saline	5	++++

Trophozoite levels were graded upon the following criteria: -, Absent; +, Low; ++, Moderate; +++, High; +++++, Very high.