MedCrave
Step into the World of Research

Horticulture International Journal

Research Article

Open Access

CrossMark

In Vitro gardicidal and amoebicidal activity of *Anogeissus leicarpus* leaves extracts

Abstract

This study was carried out to evaluate gardicidal and amoebicidal activity of *Anogeisus leicarpus* variety supreme court leaves petroleum ether and methanol extracts *in vitro* tests were perform using four concentrations: (1000ppm, 500ppm 250ppm and 125ppm). The highest activity against *Giardia lamblia,* from petroleum ether leaves extract of *Anogeisus leicarpus* exhibit 77.02% mortality at concentration (1000ppm) after 72hours. On the other hand, the lowest antigiardial activity was recorded by methanolic extract 67.28% mortality with 1000ppm concentration in 27hours. The highest the activity against *Entamoba histolytica*, with respect to time, was obtained from methanolic extract which exhibited 70.06% mortality within 72h with a concentration of 1000 ppm. On the other hand, the lowest antiamobic activity was recorded by petroleum ether extract 61.35% mortality with 1000 ppm concentration within 72hours. This was compared with Metronidazole which gave 83.42% inhibition at concentration 312.5µg/ml at the same time. Our results revealed a pharmacological activity against *G. lamblia* and *E. histolytica* we suggested that the extracts have the potential of being used in parasitic infection.

Keywords: *Anogeissus leicarpus*, gardicidal, amoebicidal, gentisic, protocatechuic, gallicacids, chebulagic acid, chebulinic acid, cathecin, quercetin, is quercetin, rutin, vitexin, kaempferol

Volume 2 Issue 5 - 2018

Mohamed Ismail Garbi,[1] Ahmed Saeed Kabbashi,[1] El-Badri Elamin Osman,[1] Waleed Sayed Koko,[2] Mahmoud Mohammed Dahab,[3] Ahmed Abdelhafiz Elshikh[1]

[1]Department of Microbiology, International University of Africa, Sudan

[2]College of Science and Arts in Rass, Qassim University, Saudi Arabia

[3]Nutritional Immunology and Functional Foods, Key Laboratory of Tropical Plant Resources, China

Correspondence: Mohamed Ismail Garbi, Department of Microbiology, Faculty of Medical Laboratory Science, International University of Africa, Khartoum, Sudan, Email mogh511@gmail.com

Received: October 26, 2017 | **Published:** September 07, 2018

Introduction

The infection of intestinal parasite is one of the most familiar in the outgrowth countries its negatively effects on the feed and healthy case of human.[1] Some of the intestinal parasites cause sudden and acute diarrhea continues for many days as in cases of giardiasis and amoebiasis.[2] The intestinal parasites may be caused anemia and a different grade of malnutrition.[3] *Giardia lamblia* and *Entamoeba histolytica* are microaerophilic protists, which cause dysentery and diarrhea, respectively.[4,5] Each is a single cell protist with a motile trophozoite stage and an immotile cyst stage. In many other ways amoebae and giardia are quite dissimilar. Amoebae have a single diploid nucleus, while giardia has two similar nuclei. While amoebae move along surfaces by an actinimyosin-mediated crawl, giardia swim by the synchronous beating of flagella and adhere to surfaces by means of a unique ventral disc.[6] Giardiasis is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries. *Gisardia lamblia* is considered to be one of the leading causative agents of diarrhea in both children and adults.[7–11] Giardiasis is the most common cause of parasitic gastro-intestinal disease and it is estimated that up to two hundred million people are chronically infected with *Giardia lamblia* globally, and 500,000 new cases reported annually.[12] The prevalence of the disease varies from 2% 5% in developed to 20% 30% in developing countries. The variation in prevalence depends on factors such as the geographical area, the urban or rural setting of the society, the age group composition and the socio-economic conditions of the study subject,[13] mortality world-wide.[14]

Amebiasis may have been first recognized as a deadly disease by Hippocrates (460 to 377 B.C.), who described a patient with fever and dysentery.[15] From that time onwards, invasive amebiasis is one of the world most prevalent and fatal infectious diseases. Around 500 million people are infected worldwide while 75,000 die of the disease annually. Behind malaria and schistosomiasis, amebiasis ranks third on the list of parasitic causes of death worldwide. The infection is common in developing countries and predominantly affects individuals with poor socioeconomic conditions, non-hygienic practices, and malnutrition.[16] *Anogeissus leiocarpus* (DC) Guill and Perr (family: Combretaceae). Many traditional uses have been reported for the plant. In Sudanese traditional medicine the decoction of the barks is used against cough.[17] Rural populations of Nigeria use sticks for or dental hygiene, the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum.[18] Ivory Coast traditional practitioners use the plant for parasitic disease such as Malaria, Trypansomiasis, Helminthasis and dysenteric syndrome.[19] In Togolese traditional medicine it used against fungal infections such as dermatitis and Mycosis, also the decoction of leaves is used against stomach infections.[20] The plant is also used for the treatment of diabetic ulcers general body pain, blood clots, asthma, coughing and tuberculosis.[21]

The plant for the major secondary constituents showed that, the plant was rich in tannins and having appreciable quantities of flavonoids, terpenes andsaponins, however it was devoid of alkaloids and anthraquinones.[22,23] Polyphenolic compounds such as 3,3,4-tri-Omethylflavellagicacid, 3,3,4-tri-O-methylflavellagic acid-4-Dglucoside, gentisic, protocatechuic, gallicacids, chebulagic acid, chebulinic acid and ellagic acid were isolated. Flavogallonicacidbislactone, castalagin and ellagic acid were isolated from the bark.[24–26] Eight flavonoids, namely, *4H-1-Benzopyran-4-one, 7- [(6-deoxy-α-Lmannopyranosyl) oxy]-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)*, *cathecin*, *quercetin, is quercetin, rutin, vitexin, kaempferol* and *procyanidin B2* were isolated from the leavesof the plant Five triterpens and triterpeneglycosides were isolated, namely sericoside, itsrelated aglyconesericic acid, rachelosperoside; itsrelated aglyconerachelosperogenin, andarjungenin.[27] The present study was conducted to investigate the gardicidal and amoebicidal activities of *A. leicarpus* (leaves) in Sudan.

Materials and methods

Plant materials

The plant used in this study was collected from Al Sunut Jungle in the middle of Khartoum State central of Sudan collected between January and February 2018. The specimens were taxonomically identified by the member of Herbarium in Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Centre for Research, Khartoum, Sudan. A voucher specimen was deposited at the herbarium of the institute. The leaf was air-dried and coarsely ground to powder.

Preparation of crude extracts

30 grams if the coarsely ground material of the leaves were successively extracted by soxhlet apparatus using petroleum ether, and methanol. The extracts ware then filtered and evaporated under reduced pressure using rotator evaporator apparatus.

Preparation of extracts solutions

Using a sensitive balance 5mg of each extracts were weighed and put in eppendorf tubes. 50µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution. The concentrates were stored at -20°C for further analysis.

Parasite isolate

G. lamblia and *E.histolytica* used in all experiments were taken from patients of Ibrahim Malik Hospital (Khartoum). All positive samples were examined by wet amount preparation. Then the positive sample was transported to the laboratory in RPMI 1640 medium. Trophozoites of *G.lamblia* were maintained in RPMI 1640 medium containing 5% bovine serum at 37±1°C. The trophozoites were maintained for the assays and were employed in the log phase of growth.

In vitro susceptibility assay

In vitro susceptibility assays used the sub- culture method of.[28] Which is being described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoba histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis*[29]).Sterile 96-well microtiter plate was used for different plant extracts, positive control and negative control. Three out of 8 columns of microtiter plate wells (8columns×12rows) were chosen for each extract, 40µl (micro-liters) of an extract solution (5mg/ml) were added to the first column wells C-1: On the other hand, twenty µl of complete RPMI medium were added to the other wells the second column and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking twenty µl of extract to the second column wells and taking 20µl out of the complete solution in C-2 wells to C-3 wells and discarding 20µl from the total solution of C-3 to the remaining 20µl serial solutions in the successive columns. 80µl of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100µl. In each test metronidazole (trichomonocide) pure compound [(1-(2-hydroxyethl)-2-methyl-5 nitroimidazole], a was used as positive control in concentration 312.5ppm, whereas untreated cells were used as a negative control (culture medium plus trophozoites). For counting the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 0, 24, 48, and 72h. The mortality % of parasite for each extracts activity was carried out according to the following formula:

$$Mortality\ of\ parasite\ (\%) = \frac{(Control\ negative\ -\ tested\ sample\ with\ extract)}{Control\ negative} \times 100\%$$

Statistical analysis

All data were presented as means±S.D. Statistical analysis for all the assays results were done using Microsoft Excel program 2010.

Results and discussion

In this study the yield % of *A. Leicarpus* (leaves) methanol and petroleum etherextracts was 20.1 and 9.27g respectively. Moreover, the activity of *Anogeissus leicarpus* exhibit 67.28% mortality against *G. lamblia* at concentration (1000ppm) after 72hours. On the otherhand the activity of *Anogeissus leicarpus* exhibit 77.02 % mortality against *G. lamblia* in concentration (1000ppm) after 72hours; this was compared with Mertronidazole powder (the reference control) which gave 83.42% mortality at concentration 312.5µg/ml in the same time. Figure 1, Figure 2. The *A. leicarpus* (leaves) was extracted by methanol and petroleum ether with different concentrations (1000, 500, 250 and 125ppm) and Mertronidazole powder (the reference control) with concentration (312.5µg/ml) to be investigated against *Entamoeba histolytica*, trophozoites *in vitro*. The antiamoebic potential of *Anogeissus leicarpus* petroleum ether extract exhibit 61.35% mortality against *Entamoeba histolytica* at concentration (1000ppm) after 72 hours the activity of *Anogeissus leicarpus* methanol extract exhibit 67.01% mortality against *Entamoeba histolytica* at concentration (1000ppm) after 72 hours. Figure 3, Figure 4. Comprehensive screening of Sudanese medicinal plants were previously studied for their antiprotozoal activity[30–33] In the present study, this plant was shown for the first time as Antigiardial and antiamobical activity *in vitro* and was screened for their antiparasitical activity against *Entamoeba histolytica* and *Giardia lamblia*. One reference drug metranidazole (Flagyl®) was used as suitable drug for treatment Giardiasis and Amobiasis. In the present study, the mortality of the parasite was shown to be dependenton time.

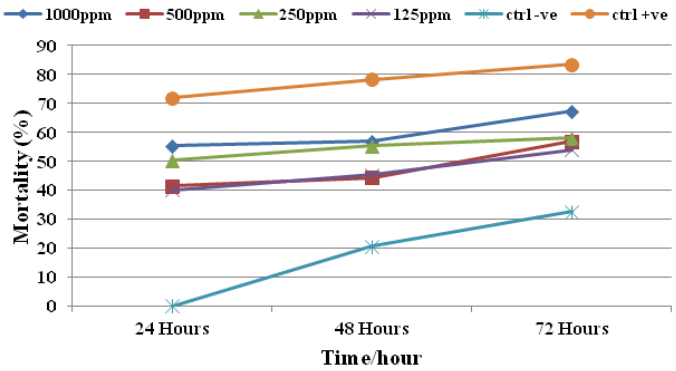

Figure 1 *In vitro* activity of *A. Leicarpus* methanol extract against *G.lamblia*.

Citation: Garbi MI, Kabbashi AS, El-Badri Elamin O, et al. *In Vitro* gardicidal and amoebicidal activity of *Anogeissus leicarpus* leaves extracts. *Horticult Int J.* 2018;2(5):232–235. DOI: 10.15406/hij.2018.02.00057

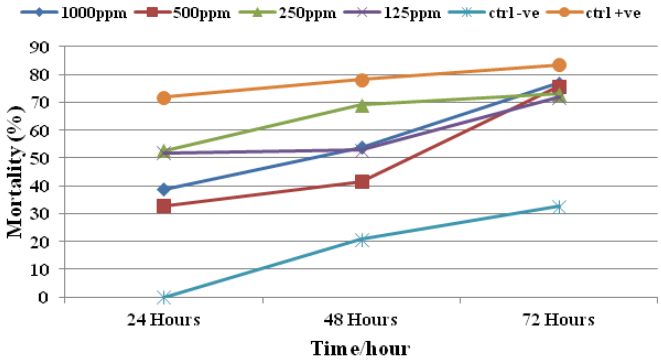

Figure 2 *In vitro* activity of *A. leicarpus* petroleum ether extract against *G.lamblia.*

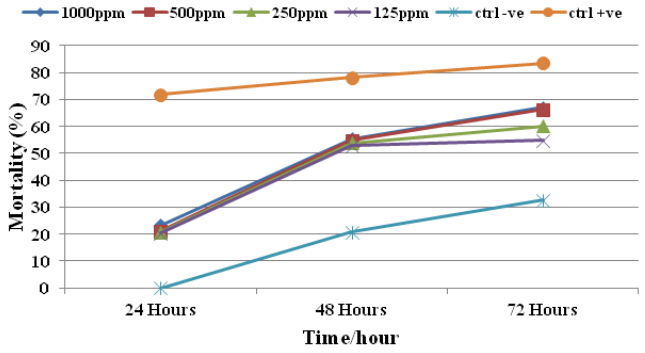

Figure 3 *In vitro* activity of *A. leicarpus* petroleum ether extract against *E.histolytica*.

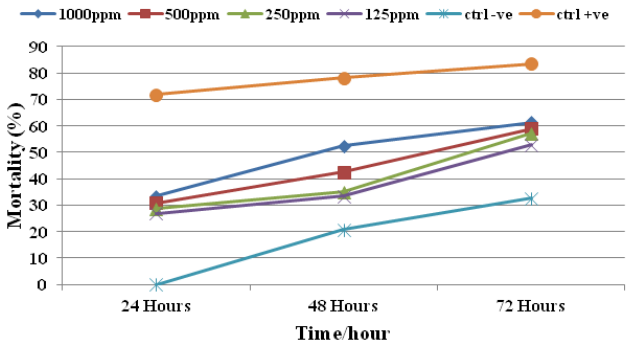

Figure 4 *In vitro* activity of *A.leicarpus* methanol extract against *E.histolytica.*

A. leicarpus In, none of the extracts tested were as active as the positive control against *Entameba hostaletica.* Meanwhile, petroleum ether extract of *Anogeissus leicarpus* leafs have an equally activity with metronidazole against *Giardia lamblia* at the same period. Screening of West African plants for anthelmintic activity revealed significant effect of *Anogeissus leiocarpus* against *Nippostrongylus brasiliensis.*[34] Also Mann *et al* found *in vitro* antifungal activities against *Aspergillus niger*, A*spergillus fumigatus, Penicillium* species, *Microsporum audouinii* and *Trichophyton rubrum* using radial growth technique appears.[35] Investigated for Antimalarial, leishmanicidal, trypanocidal, antihelminthiasis and ant scabies activities were determined.[36] Other study found the methylene chloride extract of *A. leiocarpus* exhibited high inhibition of the growth of *Plasmodium falciparum.*[19] This study confirms the effectiveness of the plant at least against *Giardia lamblia.*

Conclusion

Our results revealed a moderate pharmacological activity against *G. lamblia* and *E. histolytica* we suggested that the extracts have the potential of being used in parasitic infection. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

References

1. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol.* 2009;9(5):313–323.
2. Garcia LS. Diagnostic medical parasitology. *Am Soc Microbiol Press.* 2006.
3. Pullan R, Brooker S. The health impact of polyparasitism in humans: are we under-estimating the burden of parasitic diseases? *Parasitology.* 2008;135(7):783–794.
4. Leitsch D. Drug resistance in the microaerophilic parasite *Giardia lamblia*. *Curr Trop Med Rep.* 2015;2(3):128–135.
5. Huston CD, Petri WA. Emerging and reemerging intestinal protozoa. *Curr Opin Gastroenterol.* 2001;17(1):17–23.
6. Ghosh SK, Rogers R, Samuelson J. How giardiae swim and divide. *Infect Immun.* 2001;69(12):7866–7872.
7. Noor Azian MY, San YM, Gan CC, et al. Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia. *Trop Biomed.* 2007;24(1):55–62.
8. Dib HH, Lu SQ, Wen SF. Prevalence of *Giardia lamblia* with or without diarrhea in South East, South East Asia and the Far East. *Parasitol Res.* 2008;103(2):239–251.
9. Addy PAK, Antepim G, Frimpong EH. Prevalence of pathogenic *Escherichia coli* and parasites in infants with diarrhoea in Kumasi, Ghana. *E Afr Med J.* 2004;81(7):353–357.
10. Ayeh Kumi PF, Quarcoo S, Kwakye Nuako G, et al. Prevalence of Intestinal Parasitic Infections among Food Vendors in Accra, Ghana. *J Trop Med Parasitol.* 2009;32(1):1.
11. Nyarango RM. The risk of pathogenic intestinal parasite infections in Kisii Municipality, Kenya. *BMC Public Health*. 2008;8:237.
12. Garbi MI, Osman EE, Dahab MM, et al. Antigiardial, Amoebicidal and Cytotoxic activity of the plant *Prosopis juliflora* leave extracts. *Merit Res J Biochem Bioinform.* 2014;2(2):1–7.
13. Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin.* 2011;61(2):69–90.
14. Samuel L, Stanley J, Sharon L, et al. Microbes and microbial toxin: paradigms for microbial- mucosal. Interactions VI. *Entamoeba histolytica:* parasite- host interactions. *Am J Phy Gastrointest Liver Physiol.* 2001;280(6):1049–1054.

15. Alam F, Salam MA, Hassan P, et al. Amebic liver abscess in northern region of Bangladesh: sociodemographic determinants and clinical outcomes. *BMC Res Notes.* 2014;7(1):625.

16. Rahim K, Saleha S, Zhu X, et al. Bacterial contribution in chronicity of wounds. *Microb Ecol.* 2017;73(3):710–721.

17. El Ghazali GEB, Abdalla WE, Khalid HE. Medicinal Plants of the Sudan, part V, Medicinal Plants of Ingassana Area. *Sudan Nat Cen Res;* 2003.

18. Arbab AH. Review on *Anogeissus leiocarpus* a potent African traditional drug. *Int J Res Pharm Chem.* 2014;4(3):496–500.

19. Okpekon T. Antiparasitic activities of medicinal plants used in Ivory Coast. *J Ethnopharmacol.* 2004;90(1):91–97.

20. Batawila K. Antifungal activities of five Combretaceae used in Togolese traditional medicine. *Fitoterapia.* 2005;76(2):264–268.

21. Victor YA. *In Vitro* Assessment of Antioxidant and Antimicrobial Activities of Methanol Extracts of Six Wound Healing Medicinal Plants. *J Nat Sci Res.* 2013;3(1):74–82.

22. Elegami AA. Antimicrobial activity of some species of the family Combretaceae. *Phytother Res.* 2002;16(6):555–561.

23. Salau AK, Yakubu MT, Oladiji AT. Cytotoxic activity of aqueous extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* root barks against Ehrlich Ascites Carcinoma cells. *Indian J Pharmacol.* 2013;45(4):381–385.

24. Shuaibu MN. Castalagin from *Anogeissus leiocarpus* mediates the killing of Leishmania *in vitro*. *Parasitol Res.* 2008;103(6):1333–1338.

25. Shuaibu MN. The use of microfluorometric method for activity guided isolation of antiplasmodial compound from plant extracts. *Parasitol Res.* 2008;102(6):1119–27.

26. Shuaibu MN. Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennioides*. *Parasitol Res.* 2008;102(4):697–703.

27. Chaabi M. Triterpenes and polyphenols from *Anogeissus leiocarpus* (Combretaceae). *Biochem Systematic Ecol.* 2008;36(1):59–62.

28. Flores Carrillo P, Velázquez López JM, Aguayo Ortiz R, et al. Synthesis, antiprotozoal activity, and chemoinformatic analysis of 2-(methylthio)-1H-benzimidazole-5-carboxamide derivatives: Identification of new selective *Giardicidal* and *Trichomonicidal* compounds. *Eur J Med Chem.* 2017;137:211–220.

29. Arguello Garcia R, Cruz Suto M, Romero Montoya L, et al. Variability and variation in drug susceptibility among *Giardia duodenalis* isolates and clones exposed to 5-nitromidazoles and benzimidazoles *in vitro*. *J Antimicrob Chemother.* 2004;54(4):711–721.

30. Samia H. Abdurrahman, Elmalik H, Khalid HS. Biochemical changes in rates experimentally infected with *Trypanosoma evansi*. *J Animal Veterinary Adv*. 2004;3(7):483–486.

31. Muddathir AM, Yamauchi K, Batubara I, et al. Anti-tyrosinase, total phenolic content and antioxidant activity of selected Sudanese medicinal plants. *South African J Bot.* 2017;109:9–15.

32. Koko SW. Antimalarial Activity of *Xanthium brasilicum* Vell. *In vitro, In vivo* and Toxicological Approaches. *Recent Progress Med Plants*. 2005;15:1–10.

33. Koko SW, Ahmed Mesaik M, Yousaf S, et al. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. *J Ethnopharmacol.* 2008;118(1):26–34.

34. Ademola IO. The potential of Nigerian bioactive plants for controlling gastrointestinal nematode infection in livestock. *Anim Health Res Rev.* 2016;17(2):85–91.

35. Mann A, Banso A, Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. *Tanzania J Health Res.* 2008;10(1):34–38.

36. Vonthron Sénécheau C, Weniger B, Ouattara M, et al. *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. *J Ethnopharmacol.* 2003;87(2-3):221–225.

Citation: Garbi MI, Kabbashi AS, El-Badri Elamin O, et al. *In Vitro* gardicidal and amoebicidal activity of *Anogeissus leicarpus* leaves extracts. *Horticult Int J.* 2018;2(5):232–235. DOI: 10.15406/hij.2018.02.00057