ANTHELMINTIC EFFECT OF AQUEOUS EXTRACT OF *ANOGEISSUS LEIOCARPUS* STEM-BARK ON THE EGGS OF *HAEMONCHUS CONTORTUS* OF SHEEP

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Abstract

Haemonchus contortus (H. contortus) is a very important gastro intestinal nematode that has been recognised as one of the most significant causes of anaemia in small ruminants in many countries of the world. The main method of control for *H. contortus* has been the use of anthelmintics. However, due to continuous and inappropriate use of these drugs, it has resulted in the loss of their effectiveness and the development of anthelmintic resistance. *Anogeissus leiocarpus* aqueous stem-bark extract (AAE) was investigated for its egg hatch inhibitory activity against *H. contortus*. The extract was prepared by cold maceration and five different concentrations of the extract were studied (4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml). Albendazole and distilled water were used as positive and negative control respectively. The IC_{50} of AAE for the egg hatch assay was 1.48 mg/ml. Increasing concentrations of the extract exhibited increasing activity up to 2 mg/ml, after which the activity started to decrease. *Anogeissus leiocarpus* (*A. leiocarpus*) could find application in the treatment of *H. contortus* infection in ruminants.

Keyword

Anthelmintic, stem-bark, *Anogeissus leiocarpus, Haemonchus contortus,* eggs.

Introduction

Haemonchus contortus is a nematode parasite that belongs to the family Trichostrongylidae later classified as Haemonchidae (Palevich *et al*., 2019). It is a blood-sucking nematode that feeds on blood from capillaries in the abomasum of ruminants. (Hoberg & Zarlenga, 2016). It is a major obstacle encountered in sheep farming in developing countries. Infection with *H. contortus* occurs all over the world particularly in the tropical and subtropical regions (Naeem *et al.*, 2021). Damages caused by *H. contortus* can lead to billions of economic losses to the breeding industry (Goel *et al*., 2020).

Globally, anthelmintics are conventionally used for the treatment of *H. contortus* (Calvete *et al*., 2020). Over the years, due to continuous and inappropriate use, these drugs have lost their effectiveness. This has also led to a drastic level of anthelmintic resistance (Fissiha & Kinde, 2021)

Anogeissus leiocarpus (*A. leiocarpus*) is commonly known as African birch or Axle wood tree. It belongs to the family Combretaceae and is widely distributed in Africa. Traditionally, the plant has been reported to be used for the treatment of different ailments. (Ahmad, 2014). Studies by various scientists has shown that *A. leiocarpus* has a lot of biologically active compounds with several pharmacologic effects, one of which is its anthelmintic effects reported by Ademola & Eloff, 2011.

The aim of this study was to investigate the egg inhibitory effect of aqueous stem-bark extract of *A. leiocarpus* extract against Haemonchus contortus of sheep.

Materials and methods

Plant material

The plant material was collected from Panhawya, Giwa local government area of Kaduna state. The flower, leaves and seeds of the plant were taken to the Herbarium, Department of Botany, Faculty of life sciences Ahmadu Bello University, Zaria, Nigeria, for identification and a voucher specimen No. 0900389 was deposited. The stem-bark was dried to constant weight in open air at room temperature for 3weeks. The dried sample was then ground into powder form.

Preparation of extract

500 g of the powdered stem-bark was extracted with water over a period of 72 hours with agitation of the mixture at intervals. A solvent to dry weight ratio of 5:1 was used. The extract was filtered into a beaker using Whatman filter paper No.1 and then concentrated in an evaporating dish placed in an oven at 40^{o}C. The extract which yielded 29.3g i. e 5.86% (W/W) was then stored in a refrigerator until further required.

Phytochemical screening

Phytochemical screening of the crude methanol stem-bark extract of *A. leiocarpus* was conducted to determine the presence of secondary metabolites using standard procedure as described by Evans (2009).

***Haemonchus contortus* egg recovery**

H. contortus eggs were recovered from faeces according to Hubert & Kerboeuf (1992). Fresh faeces (10 g) were collected from sheep experimentally infected with mono-specific larval suspensions of *H. contortus*. Faecal sample was suspended in water and cleared of debris by filtration through a 100 µm sieve and the eggs were collected on a 25 µm sieve. The eggs were concentrated in a flotation medium (Sodium chloride) and then centrifuged at 1000 × g for 5 mins. The supernatant was filtered through 100 µm sieve then washed in water and collected on a 25 µm sieve. This procedure of centrifuging and washing was repeated twice. An average of 10 counts were taken and the eggs were estimated at 120 eggs/ 0.1 ml.

Egg hatch assay

The in vitro egg hatch assay was based on the method described by Coles *et al*. (1992) with slight modification. The egg suspension (0.1 ml) containing 120 fresh eggs were dispensed into a 48-well microtitre plate. To each well an equal volume of plant extract dissolved in distilled water at concentrations of 4 mg/ml in 4 serial dilutions. Albendazole (99.8% pure standard reference) (Divine laboratories, Gujarat, India) was used as a positive control. The albendazole was dissolved in distilled water and diluted at concentrations of 10 µg/ml in 4 serial dilutions. Distilled water was used as the negative control. The experiment was done in triplicates. The microtitre plate was incubated for 48 h at 27 °C and 70% relative humidity. After this time a drop of Lugol's iodine solution was added to stop the eggs from hatching. All the eggs and first-stage larvae (L1) in each plate were counted.

Statistical analysis

The best-fit IC_{50} value was calculated with 95% confidence interval using GraphPad Prism version 5.0 for Windows.

The relation below gives the percentage egg hatch inhibition parameter:

$$\frac{\text{Mean No. of eggs hatched in the control well} - \text{Mean No of eggs hatched in the treated wells}}{\text{Mean No of eggs hatched in the control well}} \times 100$$

Results

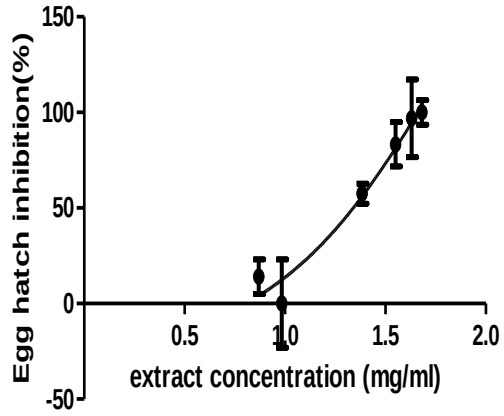
Phytochemical screening

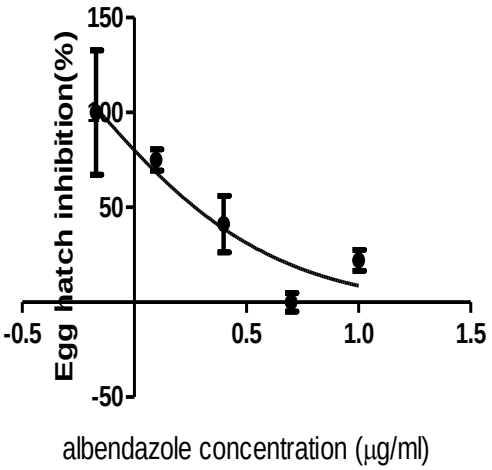
The presence of alkaloids, saponins, tannins and anthraquinones were detected in *Anogeissus leiocarpus* aqueous stem-bark extract.

Egg hatch inhibition

Anogeissus leiocarpus aqueous stem-bark extract showed concentration-dependent egg hatch inhibitory activity up to 2 mg/ml, after which the activity started to decrease (figure 1). The IC_{50} is 1.48mg/ml for the plant extract, while IC_{50} of albendazole is 0.316µg/ml or 0.003mg/ml

Figure 1: A non-linear regression graph of EHI assay of *Anogeissus leiocarpus* aqueous stem-bark and albendazole at various concentrations.





IC_{50}=1.48mg/ml

IC_{50}= 0.316µg/ml

Discussion

Phytochemical screening of the aqueous stem bark extract of *A. leiocarpus* showed the presence of Saponins, Anthraquinones, Alkaloids and Tannins. This is similar to the result obtained by Orlando *et al*., 2019, though they also detected flavonoids. *Mann et al.*, (2010) reported the presence of tannins, alkaloid, steroid, saponin and phenol.

The IC_{50} obtained for the EHI assay of AAE was 1.48 mg/ml. This result is slightly similar to that obtained by Akkari *et al*., 2014 using *Artemisia campestris* aqueous extracts which had an IC_{50} of 1 mg/ml. Adama *et al*., 2009 reported IC_{50} value of 0.409mg/ml using *A. leiocarpus* leaves aqueous extracts, while Ademola and Eloff, 2011 reported 0.196 mg/ml for the water in methanol fraction. This variation in the efficacy of the plant could be attributed to several factors such as geographical location, climate, extraction technique e. t. c. (stefanucci *et al*., 2018).

This anthelmintic effect of the plant extract may be attributed to its phytochemical constituents, since they are considered the sources of chemical components responsible for wide therapeutic activities of several medicinal plants (Debella, 2002). Several studies have demonstrated the anthelmintic activity of tannins, flavonoids, and alkaloids (Wang *et al.*, 2010)

Conclusions and Recommendations

These results indicate that the aqueous extract of *A. leiocarpus* possesses an anthelmintic property and may justify its use in traditional medicine for the treatment of gastrointestinal parasites. However further *in vitro* and *in vivo* studies is recommended to determine the extent of its activity.

References

Ademola, I.O. & Eloff, J.N. (2011). *In vitro* anthelmintic Effect of *Anogeissus Leiocarpus* (DC.) Guill. And Perr. Leaf Extracts and Fractions on Development Stages of *Haemonchus contortus. African Journal Traditional, Complementary and Alternative Medicine*, 8(2), 34-43.

Adama, K., Gaston, B. A. M., Hamidou, T. H., Amadou, T. & Laya, S. (2009). In vitro anthelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniellia oliveri*) on *Haemonchus contortus*, an abosomal nematode of sheep in Burkina Faso. *African Journal of Biotechnology*, 8 (18), 4690-4695.

Ahmad, H.A. (2014). Review on *Anogeissus leiocarpus* a potent African traditional drug. *International Journal of Research In Pharmacy And Chemistry*, 4(3), 496-500

Akkari, H., Rtibi, K., B'chir, F., Rekik, M. Darghouth, M.A. & Gharbi, M. (2014). In vitro evidence that the pastoral *Artemisia campestris* species exerts an anthelmintic effect on *Haemonchus contortus* from sheep. *Veterinary Research communications*, 38 (3).

Calvete, C., González, J.M., Ferrer, L.M., Ramos, J.J., Lacasta, D., Delgado, I., & Uriarte, J. (2020). Assessment of targeted selective treatment criteria to control subclinical gastrointestinal nematode infections on sheep farms. *Veterinary Parasitology*, 277, 109018.

Coles, G.C & R.T. Roush, 1992. Slowing the spread of anthelmintic resistant nematodes of sheep and goats in the United Kingdom. *Veterinary Research*, 130, 505- 510.

Debella, A. (2002). Manual for Phytochemical Screening of Medicinal Plants, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, 2002.

Evans, W.C. (2009). Trease & Evans *Pharmacognosy: Phytochemistry,* pp. 135. Edinburgh: Saunders Elsevier.

Fissiha, W. & Kinde, M.Z. (2021). Anthelmintic Resistance and its Mechanism: A review. *Infection and Drug Resistance*, 14, 5403-5410.

Goel, V., Singla, L.D., & Choudhury, D. (2020). Cuminaldehyde induces oxidative stress-mediated physical damage and death of *Haemonchus contortus*. *Biomedicine & Pharmacotherapy*, 130.

Hoberg, E.P. & Zarlenga, D. S. (2016). Evolution and biogeography of *Haemonchus contortus*: linking faunal dynamics in space and time. *Advances in Parasitology,* 93, 1-30.

Hubert, J. & Kerboeuf, D. (1992) A Microlarval Development Assay for the Detection of Anthelmintic Resistance in Sheep Nematode. *Veterinary Record*, 130, 442-446.

Mann, A., Barnabas, B.B. & Daniel, I.I. (2010). The Effect of Methanolic Extracts of *Anogeissus Leiocarpus* and *Terminalia avicennioides* on the growth of some Food-borne Microorganisms. *Australian Journal of Basic and Applied Sciences*, 4(12), 6041-6045.

Naeem, M., Iqbal, Z. & Roohi, M. (2021). Ovine haemonchosis: a review. *Tropical Animal Health and Production*, 53 (19), 1-11.

Orlando, G., Ferrante, C. Zengin, G., Sinan, K. I. … & Menghini, L. (2019). Qualitative Chemical Characterization and Multidirectional Biological Investigation of Leaves and Bark Extracts of *Anogeissus leiocarpus* (DC.) Guill. & Perr. (Combretaceae). *Antioxidants*, 8, 343.

Palevich, N., Maclean, P., Baten, A., Scott, R., & Leathwick, D.M. (2019). The complete mitochondrial genome of the New Zealand parasitic roundworm *Haemonchus contortus* (Trichostrongyloidea: Haemonchidae) field strain NZ_Hco_NP, *Mitochondrial DNA Part B Resources*, 4, 2208-2210.

Stefanucci A, Zengin G, Locatelli M, Macedonio G, Wang CK, Novellino E, Mahomoodally MF & Mollica A (2018). Impact of different geographical locations on varying profile of bioactives and associated functionalities of caper (*Capparis spinosa* L.). *Food and Chemical Toxicology*

Wang, G.X., Zhou, Z., Jiang, D.X., Han, J… & Li, J. (2010). *In vivo* anthelmintic activity of five alkaloids from *Macleaya microcarpa* (maxim) fedde against *Dactylogyrus intermedius* in *carassius auratus, Veterinary parasitology*, 171(3), 305-313.