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Phytochemical and Anthelmintic Activity of *Terminalia Catappa* (Linn) Leaves

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ABSTRACT: Helminthiases is one of the tropical neglected diseases (NTDs) caused by soil transmitted helminths (STHs). The development of resistance to anthelmintics was observed and so there is an urgent need to discover novel drugs. This study investigates the anthelmintic activity of the *Terminalia catappa.* Dried fallen leaves of the plant was extracted with petroleum ether, dichloromethane, ethyl acetate, methanol and water using microwave assisted extraction (MAE). Anthelmintic activity of the crude extracts were investigated against *Haemonchus concortus*. Various concentrations (6.25, 12.5, 25, 50 and 100 mg/ml) of each extract and Albendazole were tested in the egg hatch assay and larval mortality assay. The dichloromethane extract displayed the highest egg hatch inhibition percentage of 98.94% at 6.25mg/ml while the methanol extract showed the lowest inhibition of 95.77% at the same concentration. The dichloromethane extract also showed 100% larval reduction at a concentration of 12.5mg/ml after 3 days and 98.9% at the least concentration of 6.25mg/ml. The preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extract. This result confirm the use of *Terminalia catappa* by traditional healers for the treatment of worm infections.

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Helminth infections are common in man. They affect a large proportion of the world's population especially in developing countries posing a threat to public health and contributing to the prevalence of malnutrition, anemia, eosinophilia and pneumonia (Lukhob *et al.,* 2006). The conventional method for the treatment of helminth infections is by using anthelmintic drugs such as Albendazole, Omeprazole, Piperazine (Wahab, 2003). However, the disadvantages of these drugs include non-availability in some developing countries, high cost, drug resistance, environmental pollution and food residues (Hammond *et al.,* 1997). Anthelmintic drugs from natural sources provide an alternative in overcoming some of these problems especially drug resistance. Studies on plant based anthelmintics are becoming urgent because of increased anthelmintic resistance around the world (Waller, 1997; Jackson and Coop, 2000) and also because they are sustainable and environmentally friendly (Hammond *et al.,* 1997; Githiori, 2004). Based on ethnobotanical information, *Terminalia catappa* was used as the experimental plant in this study. It is a large tree and an extensively used medicinal plant of the Combretaceae family growing majorly in the tropical regions of Asia, Africa and Australia. The leaves of this plant is used in traditional medicine in India and Philippine to treat dermatitis, helminthiases and hepatitis (Fan *et al.,* 2004). To the best of our knowledge, the study of *Terminalia catappa* against *Haemonchus concortus* is very limited. The current study utilized a different plant extraction approach and evaluated the dichloromethane, ethyl acetate, methanol and aqueous extract against the eggs and infective larva (L_3) of *Haemonchus concortus*.

MATERIALS AND METHODS

Plant material collection: The dried fallen leaves of *Terminalia catappa* were collected in Zaria, Kaduna State, Nigeria, in February, 2015 and its voucher number 01556 deposited there. The leaves were taxonomically identified at the Herbarium unit of the Biological Science Department, A.B.U, Zaria, Nigeria.

Extract preparation: Terminalia catappa dried fallen leaves were pulverized mechanically and the powdered leaves (500 g) were extracted successively with petroleum ether, dichloromethane, ethyl acetate, methanol and water using Microwave Assisted Extraction with some modifications (Dubey and Goel, 2013). The extracts were filtered using whatmann filter paper and concentrated using rotary evaporator at a temperature of 40 °C and later subjected to air

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drying to give the dried crude extracts which were weighed and recorded.

Phytochemical screening of extracts: The petroleum ether, dichloromethane, ethyl acetate, methanol and aqueous extracts were subjected to phytochemical screening using standard techniques (Harborne, 1998). The following metabolites were tested for; carbohydrates, flavonoids, anthraquinones, tannins, saponnins, cardiac glycosides, terpenes, alkaloids and steroids.

*Determination of Anthelmintic activity: Egg recovery***:** Eggs used to perform the egg hatch test (EHT) and also for the culture of infective larvae (L_3) for larval mortality test (LMT) were obtained from adult female parasites of *Haemonchus concortus* collected from the abomasum and intestinal contents of sheep at Zango slaughter house in Zaria, Kaduna state. The intestinal contents were washed with distilled water and the adult worms selected. The adult worms were crushed gently to liberate the eggs and debris of the mixture was passed through a sieve (mesh size 80µm). The eggs were recovered from the filtrate.

Egg Hatch assay: Egg hatch assay of the plant extract was conducted according the method describe by Al-Shaibani *et al.,* (2009) with minor modifications. The concentration of eggs was estimated using the McMaster technique as described by Urquhart *et al.,* (2000) by counting the number of eggs in 3 aliquots of 0.5ml of the suspension in a microscope slide and the mean number of eggs per 0.5ml was determined. The stock solutions were prepared by dissolving the crude extract in dimethyl sulfoxide (DMSO) to improve its solubility in water. Different concentrations (6.25, 12.5, 25.0, 50.0 and 100 mg/ml) of the crude aqueous, methanol, ethyl acetate and dichloromethane extract were prepared from stock solution. Also, Albendazole (Zentel™, GlaxoSmithKline) of same concentration with the crude extracts was utilized as positive control while distilled water was used as negative control. Test was performed using test-tubes. Egg suspension containing approximately 200 eggs per 0.5ml was pipetted into the test-tubes and mixed with 2ml of different concentrations of the extracts of *Terminalia catappa*. The entire experiment was carried out for 48hrs at 27°C and the number of unhatched egg and first larvae were counted under dissecting microscope. The percentage of inhibition of egg hatching was calculated for each concentration using the formula by Coles *et al.,* (1992).

$$\% \text{ inhibition} = 100(1 - \frac{X1}{X2})$$

Where X1 is the number of eggs in test extracts, X2 is the respective number in distilled water (negative control).

Larval Mortality Assay: For evaluation of the mortality of third-stage larvae (L_3), eggs were initially cultured according to the method of Ueno and Goncalves (1998) with modifications. The eggs were homogenized with sterile feces in a glass jar and incubated for 7 days at room temperature, L_3 were recovered by spontaneous migration using warm water (37°C). The Larval mortality test of the extract was carried out using the method described by McGaw *et al.,* (2001) and Zafar *et al.,* (2006) with modifications. Suspensions containing an average of 200L_3 was introduced into the different concentrations of the extracts in test tubes. Albendazole was used as positive control and distilled water as negative control. The experiment was carried out for 24hr at 27°C and the number of motile (alive) and non-motile (dead) larvae was counted consecutively for three days. Death was ascertained by absence of motility after being observed for 5-6 sec. The percentage of larval mortality was calculated using the formula described by Coles *et al.,* (1992) and Bizimenyera *et al.,* (2006):

$$\% \text{ inhibition} = 100(1 - \frac{X1}{X2})$$

Where X_1 is the initial number of larvae in test extracts pre-treatment and X_2 is the number of larvae that obtained post-treatment.

RESULTS AND DISCUSSION

Phytochemical screening of the crude extracts of *Terminalia catappa* (Table 1) revealed the presence of carbohydrates, cardiac glycosides and combined reducing sugars in the petroleum ether, dichloromethane, ethyl acetate, methanol and aqueous extracts while alkaloids, triterpenes and saponins were present only in the dichloromethane, ethyl acetate, methanol and aqueous extracts. Tannins and phenolics were present in the ethyl acetate, methanol and aqueous extracts while flavonoids were present only in the methanol and aqueous extracts.

The result of the egg hatch assay (Figure 1) shows that the anthelmintic activity of the plant extract is concentration dependent. There is a corresponding decrease in anthelminthic activity as the concentration decrease. The dichloromethane, ethyl acetate, methanol and aqueous extract showed 100% inhibition of the eggs at a concentration of 50-100mg/ml. At decrease concentration of 6-25mg/ml, the percent inhibition of all the extracts also decreased. The dichloromethane extract displayed the highest egg hatch inhibition percentage of 98.94% at 6.25mg/ml

which is similar to that of the standard drug while the methanol extract showed the lowest inhibition of 95.77% at the same concentration. The dichloromethane extract showed similar activity with Albendazole (positive control).

Table 1: Phytochemical screening of the leaf extracts of *Terminalia catappa*

Phytochemicals	PE	DCM	ET	ME	AQ
Flavonoids	-	-	-	+	+
Alkaloids	-	+	+	+	+
Tannins	-	-	+	+	+
Cardiac glycosides	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Triterpenes	-	+	+	+	+
Saponins	-	+	+	+	+
Phenolic	-	-	+	+	+
Carbohydrate	-	+	+	+	+
Reducing sugar	-	+	+	+	+

Key: + = present, - = absent, PE = Petroleum ether extract, DCM = Dichloromethane extracts, ET = Ethyl acetate extracts, ME = Methanol extracts, AQ = Aqueous extract

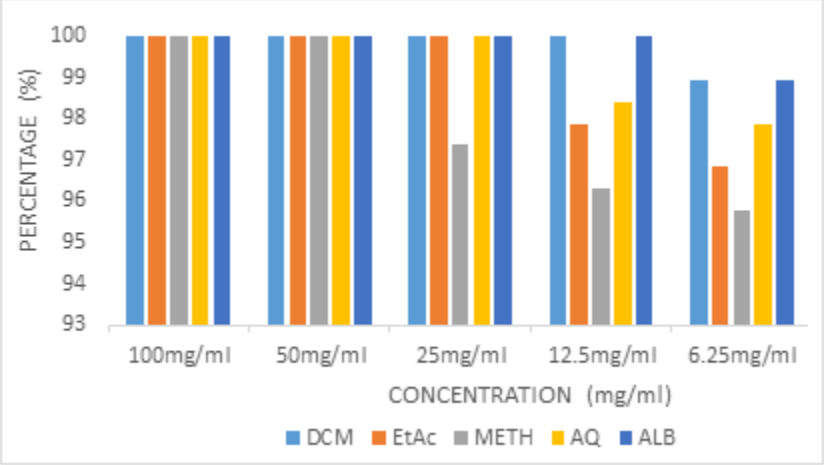

Fig 1: Egg hatch inhibition of the extract of *T. catappa* leaves

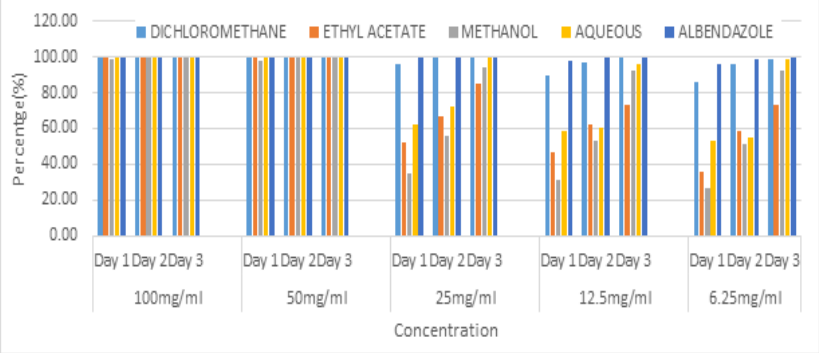

Fig 2: Larval mortality of the extract of *T. catappa* leaves

The result of the larval mortality assay (Figure 2) revealed that the activity of the extracts was dependent on concentration and also time of exposure. The higher the concentration of the extract, the higher the inhibition of the extracts. Also, the longer the time of exposure of the extract to the larvae, the more effective the inhibition will be. All the plants extracts had significant activity which was comparable to the standard drug. However, the dichloromethane extract showed significant anthelmintic activity at highest concentration of 100mg/ml. It also showed 100% larval reduction at a concentration of 12.5mg/ml after 3 days and 98.9% at the least concentration of 6.25mg/ml. This was comparable to the result obtained with the standard drug. Anthelmintic activity of all the plant extract shows maximum efficacy at a concentration of 100mg/ml after 2 days. This activity may be due to the presence of steroids, triterpenes and

other phytochemicals in the plant. Steroidal saponins and triterpene saponins have been reported to have a certain degree of antimicrobial and antiparasitic properties (Wink and van Wyke, 2008; Wink, 2008). Sorajini *et al.* (2011) and Patel *et al.* (2011) reported that the anthelmintic effects of the plants *Saraca indica* and *Lantana camara,* respectively were due to the presence of alkaloids. Khan and Diaz-Hermandex, (2000) have also reported the anthelmintic activity of condensed tannins (proanthocyanidins). According to Powers *et al.,* (1982), anthelmintic agents that are effective should inhibit egg hatching and larval motility by more than 90%. Inhibition of 80-90% should be considered moderately effective. Thus, the *in vitro* results of the *Terminalia catappa* leave extracts against *H. concortus* eggs and larvae obtained in this study particularly at higher concentrations allows the classification of the tested extracts as effective.

Conclusion: In conclusion, the results suggests that the extracts of *Terminalia catappa* leaves possess concentration dependent anthelminthic activity due to the presence of the phytochemicals observed.

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