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Full Length Research

Anthelmintic effects of four medicinal plants using *in vitro* L3 larva stage

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ABSTRACT: *In vitro* anthelminthic activity of *Vernonia amygdalina, Ocimum grattissimum, Nicotiana tabacum* and *Talinum triangulare* using L3 stage larva was investigated. Standard procedure was followed for larva culturing, preparation of extract and for *in vitro* assay. The concentration of *Vernonia amygdalina* was effective at 1.0 mg/ml at 1 and 2 hours, *Ocimum grattissimum* at 0.5 mg/ml at 1 hour, *Nicotiana tabacum* at 0.125 mg/ml at 1 hour and *Talinum triangulare* at 0.125 mg/ml at 2 hours. The result showed that there was anthelminthic activity in the four plants after 3 hours of the experiment. *Nicotiana tabacum* showed a faster anthelmintic effect at a lower concentration of 0.125 mg/l and at a lower hour (1 hour) while *Talinum triangulare* showed a slower anthelmintic effect compared to other medicinal plants at 2 hours. The health status of ruminants can be improved through the consumption of these four medicinal plants.

Keywords: Anthelminthic, health, larva, medicinal plants.

INTRODUCTION

Helminths such as nematodes (*Haemonchus contortus, Trichostrongylus sp, strongloides sp* and so on) have caused diseases such as constipation, diarrhoea, anaemia and loss of appetite. The use of herbal extract in the treatment of parasites has increased over the years (Rates, 2001). *Vernonia amgydalina and Ocimum grattisimum* had been used in the treatment of diarrhoea, which is a common disease of ruminants in Ekiti State. Diarrhoea is caused by nematodes and the infective stage of the parasite is larvae L3, which is mostly ingested through vegetation in the field.

Vernonia amygdalina young twigs are used as toothpicks or chewing sticks (Edegbai and Anoliefo, 2019) and are found in West and Central Africa. *Ocimum grattissimum* serves as a condiment in human food and is also widely known and used for its therapeutic properties. It is a native of tropical Africa. *Nicotiana tabacum*, in Brazil, the leaf juice is taken orally to induce vomiting and narcosis. *Talinum triangulare,* has been found in several countries in west and central Africa, the leaves are used in the preparation of slightly slimy soups and stews to complement the starchy main dish.

Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare had been used to treat different helminthes/parasites causing diseases (Nalule *et al*., 2013; Pessoa *et al*., 2002, Iqbal *et al*., 2001). The use of herbal extract to treat parasites is being used because of the resistance of the parasite to synthetic drugs. *In vivo* study by Adeola *et al*. (2015b) have shown anthelminthic activity of *Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare* on L3 stage larva in goat. The anthelminthic activity of *Talinum triangulare* have been studied by (Iwalewa *et al*., 2005). An *in vitro* study of *Vernonia amygdalina* in earthworms (Adeola *et al*., 2016) and adult *Haemonchus contortus* egg had been studied by (Alawa *et al*., 2003).

The phytochemical analysis of *Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare* had been shown by (Adeola *et al*., 2015a) which include saponins, tannin, alkaloid and flavonoid. Some can be responsible for the anthelminthic activity of these medicinal plants.

There are few studies on the effect of plants on the larva of nematodes. And due to diarrhoea diseases caused by nematode affecting most ruminants in rural areas, this study now investigates the anthelmintic activity of four medicinal plants on L3 stage larva of nematode.

MATERIALS AND METHODS

Study area

The medicinal plants and faecal samples were collected from specific houses in Ado-Ekiti. The culturing and *in vitro* assay were carried out in the Agricultural Science Laboratory, Federal University of Technology Akure.

Collection of plants

Plant materials (*Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare*) were collected from around the house in Ado-Ekiti and dried for 2 months. They were authenticated in the Department of Plant Science at Ekiti State University Ado-Ekiti. The medicinal plants were chosen because they were the most easily available to rural people to treat the diarrhoea diseases of their ruminants.

In vitro effects of medicinal plants on nematodes

Four nematodes were used for the study which includes *Trichostrongyle sp, Haemonchus contortus, Strongloides papillous, and Bunostomum sp*. Nematodes were chosen because are the most cause of diarrhoea, which affect most ruminants in Ekiti State.

Preparation of extract

The leaves (2 kg) of the medicinal plants were air dried for 2 months. After which they were grinded into powder using a counter top blender. The extraction method follows that described by (Edeoga *et al.* 2005). 74.8 g of *Vernonia amygdalina* Del was soaked in 262 ml ethanol, 74.64 g of *Ocimum grattisimum L* was soaked in 262 ml ethanol while 21.57 g of *Talinum triangulare* Wild was soaked in 75ml of ethanol. They were soaked for 72 hours. After this the mixture was filtered using Watman filter paper No 45 (125 mm). The filtrate was left to stand for 4 days for the ethanol to evaporate leaving the extract of the plants.

The stock solution was prepared from the extracts. For *Vernonia amgydalina*, 2 g was weighed and put in 200 ml of distilled water to give the stock solution of 80 mg/ml. For *Ocimum grattisimum*, 2.629 g was weighed and 10 ml of distilled water was added to make stock solution of 263 mg/ml. For *Nicotiana tabacum*, 1.847 g was weighed and 10 ml of distilled water was added to make stock solution of 184.7 mg/ml. For *Talinum triangulare*, 0.864 g was weighed and 10 ml of distilled water was added to make stock solution of 86.4 mg/ml.

Culturing of faecal sample

Culturing and harvesting of parasites followed the method described by Van Wyk and Mayhew (2013). The faecal samples were collected from rectum of free-range goats around Ado-Ekiti because the animals would have been infected with worms. They were then mixed with sawdust, to allow for aeration of the faecal samples and put inside 250 ml beakers, covered with paper foil and kept in the dark cupboard for 7 days.

Harvesting of parasites

After 7 days the faecal samples were put inside muscline cloth tied with stick on top to allow it suspended inside the beaker. Warm water was poured on it, to enable the parasite flow from the faecal sample inside the cloth to the water, and left for 45 minutes, to allow migration of the L3 stage larval of *Trichostrongyle spp, Haemonchus contortus, Strongloides papillous, and Bunostomum sp* from the faecal sample into the water. After 45 minutes, the tied cloth was removed and the water containing the parasite was decanted and the parasites were recovered from the bottom of the beaker. Parasites recovered were viewed under microscope for the identification of the parasite third stage larva (L3 stage larval) of nematodes present. 1500 larvae was contained in 10 ml of the solution, this gives 150 larvae contained in 1 ml of the solution and 15 larvae contained in 1 drop of the solution.

In vitro assay

A drop of the larvae solution containing 15 larvae was placed in triplicate on a slide and 1, 0.5, 0.25, 0.125 mg/ml of each extract was added to the larvae solution in triplicate. The positive control containing 0.55 mg/ml albendazole and negative control containing saline solutions were set up. These were observed under the microscope for 1, 2, and 3 hours for the anthelmintic effects of the extracts on the larvae and observations were recorded in the results section.

Statistical analysis

For the in vitro assay, T-test was used at $p<0.05$ significant level to test the significance between the control and experimental values.

RESULTS

In vitro study, showed that the ethanolic extracts of *Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum* and *Talinum triangulare* at concentrations of 0.125, 0.25, 0.5, 1.0 mg/ml showed antihelminthic effects

Table 1. Anthelmintic effects of *Vernonia amgdaylina in vitro.*

Extract	0h	1h	2h	3h
0.125 mg/ml	15±0.0	12±2.1	10±0.0	0.0±0.0
0.25 mg/ml	15±0.0	12±1.3	7±5.3	0.0±0.0
0.5 mg/ml	15±0.0	13±0.82	10±0.6	0.0±0.0
1.0 mg/ml	15±0.0	3±0.0*	0.0±0.0	0.0±0.0
Negative control (Saline)	15±0.0	12±2.8	12±2.8	12±2.8
Positive control (Albendazole)	15±0.0	12±2.8	11±0.0	0.0±0.0

N.B. *significance difference from negative control at p<0.05 using t-test.

Table 2. Anthelmintic effect of *Ocimum grattisimum in vitro.*

Extract	0h	1h	2h	3h
0.125 mg/ml	15.0±0.0	8.0±5.0	3.0±0.0*	0.0±0.0
0.25 mg/ml	15.0±0.0	9.0±0.6	3.0±1.2*	0.0±0.0
0.5 mg/ml	15.0±0.0	2.0±0.0*	0.0±0.0	0.0±0.0
1.0 mg/ml	15.0±0.0	11.0±1.0	7.0±4.0	0.0 ±0.0
Negative control (Saline)	15.0±0.0	12.0±2.8	12.0±2.8	12.0 ±2.8
Positive control (Albendazole)	15. 0±0.0	12.0±2.8	11.0±0.0	0.0±0.0

N.B. *significance difference from negative and positive control at p<0.05 using t-test.

Table 3. Anthelmintic effect *of Nicotiana tabacum in vitro.*

Extract	0h	1h	2h	3h
0.125 mg/ml	15.0±0.0	6.0±1.4*	3.0±0.0*	0.0±0.0
0.25 mg/ml	15.0±0.0	8.3 ±0.9	2.0±0.1	0.0±0.0
0.5 mg/ml	15.0±0.0	4.0 ±0.0	0.0±0.0	0.0±0.0
1.0 mg/ml	15.0 ±0.0	6.0±1.4*	3.0±0.6*	0.0±0.0
Negative control (Saline)	15.0±0.0	12.0±2.8	12.0 ±2.8	12.0±2.8
Positive control Albendazole	15.0±0.0	12.0 ±2.8	11.0±0.0	0.0±0.0

N.B. *significance difference from negative and positive control at p<0.05 using t-test.

Table 4. Anthelmintic effect of *Talinum triangulare in vitro.*

Extract	0h	1h	2h	3h
0.12 5mg/ml	15.0±0.0	13.0±0.6	10.3±0.9	0.0±0.0
0.25 mg/ml	15.0±0.0	8.0±0.0	5.0±0.6*	0.0±0.0
0.5 mg/ml	15.0±0.0	12.0±0.6	10.0±0.6	0.0±0.0
1.0 mg/ml	15.0±0.0	9.0±0.0	3.3±0.9*	0.0±0.0
Negative control (Saline)	15.0±0.0	12.0±0.0	12.0±2.8	12.0±2.8
Positive control (Albendazole)	15.0±0.0	12.0 ±0.0	11.0±0.0	0.0±0.0

N.B. * significance difference from negative and positive control at p<0.05 using t-test.

in vitro over L3 stage larval of nematode when compared to control with distilled water at 3 hours, as evident from the mortality of the worms by 3 hours (Tables 1 to 4). *Vernonia amgydalina* at 1.0 mg/ml caused statistically significance difference in the number of larvae after 1h from both negative and positive control at p<0.05 but there was no significant difference with other concentrations (0.125, 0.25 and 0.5 mg/ml). *Ocimum grattisimum* at 0.5 mg/ml caused statistically significance difference in the number of larva after 1h from both negative and positive control at p<0.05 significance level. While *Ocimum grattisimum* at 0.125 and 0.25 mg/ml caused statistically

significance difference in the number of larva after 2 hours from both negative and positive control at p<0.05 significance level. But no significance difference at 1mg/ml at p<0.05. *Nicotiana tabacum* at the lowest and highest concentration (0.125 and 1 mg/ml) caused statistically significant difference in the number of larva after 1 and 2 hours at p<0.05. But no significance difference at 0.25 and 0.5 mg/ml at p<0.05. *Talinum triangulare* at 0.25 mg/ml and 1 mg/ml caused statistically significant difference in the number of larva after 2 hours from both negative and positive control, but no significant difference at 0.125 and 0.5 mg/ml concentration at p<0.05 significance level.

DISCUSSION

The result showed that all the worms exposed to albendazole, a standard antihelminthic agent, were found dead at 3 hours, whereas none of the worms were found dead or paralysed in saline solution which acted as the negative control (Tables 1 to 4). This agrees with previous study on in vivo and *in vitro* study in goat and earthworm (Adeola *et al*., 2015b; Adeola *et al*., 2016).

In vitro anthelmintic effects of *Vernonia amygdalina* on L3 stage larvae was supported by (Nalule *et al*., 2013), *Nicotiana tabacum* was supported by (Iqbal *et al*., 2006), while *Ocimum gratisimum and Talinum triangulare* also showed anthelmintic effect. The anthelmintic effect could be due to the action of tannins, alkaloids and flavonoid present in the plants on the L3 larval of nematodes (Adeola *et al*., 2015a). The alkaloid present in the plants could have contributed to the death of the worms because nematocidial activity of the alkaloid have been demonstrated by (Satou *et al*., 2002) when they used two rats models for human nematodes. The nematocidal activity of tannin extracts has also been reported with (Dawson *et al.,* 19991; Athanasiadou *et al*., 2001). The study under reviews showed that *Vernonia amgydalina* was significantly from both negative and positive control by 1hr which indicated *Vernonia amygdalina* was effective *in vitro* at 1mg/ml. *Ocimum grattisimum* at 0.5 mg/ml by 1hr and at 0.125 and 0.25 mg/ml after 2 hours was different from both controls which indicated *Ocimum grattisimum* was effective *in vitro* at these concentrations. This shows dose-dependent effect of *Ocimum grattissimum* on L3 larva stage at 2 hours

Nicotiana tabacum at 0.125 and 1.0 mg/ml by 1 and 2 hours was different from controls. Nicotiana tabacum was effective at 0.125, 0.25, 0.5 and 1.0 mg/ml against L3 larva stage at 2 hours, which dose dependent ability of *Nicotiana tabacum* on L3 larva stage. This means that *Nicotiana tabacum* was faster reactivity at 1 hour and at the lowest concentration *in vitro,* which indicates high amount of alkaloid that can be responsible for the anthelmintic effect shown. The result showed that *Talinum triangulare* at 0.25 and 1.0 mg/ml by 2 hours was significant from controls. This indicated that it took Tt a bit longer (2 hours) to have effect compared to other extracts at 1 hour. This shows that *Nicotiana tabacum* is a faster anthelmintic while *Talinum triangulare* is a slower anthelmintic compared to other three medicinal plants (Adeola *et al*., 2016).

Conclusion and Recommendation

These four medicinal plants were effective botanical anthelmintic *in vitro*. This can provide a balance of what is happening in the whole organism. The study can help to improve the economy value of ruminants in the country.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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