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In vitro anthelmintic properties of root extracts of three *Musa* species

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Abstract

Helminth infections are among the commonest infections in the developing world. Development of resistance in helminthic against conventional anthelmintic drugs however, has become a foremost problem in the treatment of helminth diseases leading to the evaluation of medicinal plants as an alternative source of anthelmintic. Plant collection, authentication, air-drying and extraction with methanol were done. The sheep tapeworm (*Moniezia benedeni*), roundworm (*Ascaris lumbricoides*) and adult earthworm (*Esenia fetida*) were used for the assay. Methanol extracts from the roots of *Musa* species (*M. paradisiaca*, *M. sapientum* and *M. nana*) were investigated for their anthelmintic activity against the three different worms. The worms were placed in petri dishes in solutions of crude extracts at different concentrations, time of paralysis and death were recorded. Piperazine (20 mg/mL) was used as reference compound. The higher concentrations of plant extracts 200 mg/mL produced faster paralytic effects and showed shorter time of death. *Musa paradisiaca* was the most potent, requiring less time for paralysis 26.07±1.7, 57.08±1.32, 80.04±0.5 min and death 48.08±2.2, 121.04±0.9, 151.39±0.1 min of the worms of all the extracts. The results of the fractions obtained revealed that dichloromethane (DCM) fraction gave the best activity 20.36, 17.08 and 48.61 min, respectively compared to the other fractions. It can be concluded that *Musa* species could produce a potent anthelmintic agent in the near future based on this study. Further studies are required for the identification of bioactive component(s) responsible for anthelmintic activity and determining the mechanism of action of these plants.

Keywords: *Moniezia benedeni; Ascaris lumbricoides; Esenia fetida; Musa* species; Anthelmintic

INTRODUCTION

Parasitic diseases are often the major burden of tropical and subtropical communities especially in developing world. The environments offer climates that allow species to thrive and provide support to potential hosts of parasitic diseases [1]. In developing countries, helminths are the most infectious agents of humans. They produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia [2-4]. According to World Health Organization (WHO) statistics, over than two billion people harbour parasitic worm infections [5,6]. This is an indication of the prevalence of the infections. The major cause of helminth infections is poor hygiene, influenced by poor socioeconomic in several communities. This includes inadequate housing, low levels of education, low family income, poor health services, dirty environments, crowded household, poor/inadequate access to

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sanitation facilities and clean drinking water [7-9].

Anthelmintics are drugs that act locally to expel worms from the gastro-intestinal tract or systemically to eradicate adult helminths or developmental stages that invade organs and tissues [10]. They can also be defined as drugs that expel helminths and other internal parasites from the body by either stunning or killing them without causing significant damage to the host [11]. Currently, more than 50% of the drugs used in modern medicine are derived from plants, however except for ivermectin a macrocyclic lactone derived from *Streptomyces avermitilis*, there are very few success stories of plant drug related to anthelmintic activity [6]. Synthetic anthelmintic have been the only source for control. However, challenges such as high cost, harmful side effects and the development of the resistant populations, have led to the search for alternatives [12].

To eliminate the harmful side effects of the synthetic anthelmintic drugs and to provide newer and cheaper alternative, it is important for us to promote the studies of traditionally used anthelmintic plants, which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects [13-15].

EXPERIMENTAL

Description of *Musa* species. *Musa* species belongs to the family Musaceae (banana family). The genus *Musa* is given to these plants in the honour of a Roman physician, Antonia Musa of the first century. Plants are native to Southeast Asia and India including Pakistan and cultivated in tropical and sub-tropical regions. They are familiar tropical fruit [16]. Fruit develops parthenocarpically in the absence of seed development. It is a tree-like perennial herb that grows 9 – 30 m in height, with tuberous rhizome, and a hard, long pseudostem [17]. The banana plant is the largest herbaceous flowering plant [18]. The plant is used in inflammation, rheumatism, gripe, diabetes, diarrhea, cough, epileptic, hypertension, snakebite, tuberculosis and has anthelmintic properties [16,17,19].

Materials. Distilled n-hexane, Distilled ethyl acetate, Distilled dichloromethane, Distilled methanol, Distilled water, Normal saline and Piperazine. All the chemicals and drugs used were of analytical grade. Weighing balance, Rotary evaporator

Preparation of extracts. Plant extracts were obtained by macerating the powdered sample (1 kg each) of the plant with distilled methanol for 72 hours. The extracts were filtered and evaporated in vacuo using rotary evaporator at about 40^0C and finally dried over water bath. The yield was determined, and the extracts stored at 4^0C in the refrigerator.

Plant materials. *Musa paradisiaca* was collected from Forestry Research Institute of Nigeria, Jericho Ibadan (FRIN) and *Musa sapientum* was collected from Amina way, University of Ibadan. *Musa nana* was collected from NIHORT Idi-Ishin, Ibadan. The plants were identified and authenticated at Forest Herbarium Ibadan (FHI) with the FHI numbers 110120 (*Musa sapientum*), 1101121 (*Musa nana*) and 1101122 (*Musa paradisiaca*). A voucher specimen of each plant was deposited in the herbarium. The plants were cleaned after collection; the roots were cut into smaller pieces to hasten the drying and grinding process. They were air-dried under a shade, later dried at 40^oC, and ground into powder.

Collection of worms. The worms (*Ascaris lumbricoides* and *Moniezia benedeni*) were collected from Bodija market, Ibadan, and confirmed at the Faculty of Veterinary Medicine, University of Ibadan, Oyo State. The earthworm (*Esenia fetida*) was collected from Amina Way, University of Ibadan, Ibadan and confirmed at the Department of Zoology, University of Ibadan.

Anthelmintic activity assay. The anthelmintic assay was carried out according to [20], with minor modifications. The assay was performed on sheep tapeworm (*Moniezia benedeni*), roundworm (*Ascaris lumbricoides*) and adult earthworm (*Esenia fetida)* due to its anatomical and physiological resemblance with the intestinal roundworms parasite of human beings. Availability of earthworms has made its use wide spread for the initial evaluation of anthelmintic compounds *in vitro.* Methanol extracts from the roots of *Musa* species (*M. paradisiaca*, *M. sapientum* and *M. nana*) were investigated for their anthelmintic activity against the three different worms. The most active plant extract and fractions were subjected to bioassay-guided fractionation. Three worms were placed in petri dishes in solutions of crude extracts at different concentrations and in duplicate (25, 50, 100, and 200 mg/mL in distilled water), respectively. Mean times for paralysis (P, in minutes) were taken (the time when no movement of any type could be observed, except when the worms were shaken vigorously). Times of death of worms (D, minutes) were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C). Piperazine (20 mg/mL) was used as reference compound, while distilled water was included as negative control. All test solutions and standard drug solutions were prepared fresh before the experiments.

Partitioning of extracts. *Musa paradisiaca* extract, the most active was subjected to liquid-liquid partitioning by re-dissolving the extract in methanol/water mixture (3:1) which was partitioned successively with n-hexane, dichloromethane (DCM), ethyl acetate, and butanol. The fractions were concentrated, dried on water bath, the yields, determined and kept in the refrigerator.

RESULTS

Among the extracts of Musa species tested (*Musa paradisiaca*, *Musa sapientum* and *Musa nana*); the methanol extract of *Musa paradisiaca* was the most potent, requiring less time for paralysis and death of the worms. It showed a concentration dependent anthelmintic property (Table 2).

Musa paradisiaca methanol root extract was the most active extract against tapeworm with paralysis time 30.05 ± 1.32 and death time 118.04 ± 0.9 in minutes at 200 mg/mL while *Musa nana* was the least active with paralysis time 120.04 ± 0.81 and death time 174.15 ± 0.76 in minutes at 200 mg/mL (Table 2). All the extracts of Musa species displayed prominent activities against earthworm, *Musa paradisiaca* was still the most active with paralysis time 80.04 ± 0.5 and death time 151.39 ± 0.1 in minutes at 200 mg/mL while *Musa nana* still gave the least activity with time for paralysis 94.09 ± 3.23 and time of death 189.04 ± 2.5 in minutes at 200 mg/mL (Table 2).

In addition, the methanol root extracts of Musa species were active on roundworm. *Musa paradisiaca* maintained the best activity with paralysis and death times of 26.07 ± 1.7and 48.08 ± 2.2 min, respectively while *Musa nana* still had the least activity with the paralysis and death times of 68.05 ± 2.45 and 90.06 ± 0.87 minutes (Table 2).

Preliminary phytochemical screening of powdered plant samples revealed the presence of tannins, alkaloids, cardiac glycosides, saponins and flavonoid. The phytochemical screening also revealed the absence of terpenoids and anthraquinones. The preliminary phytochemical analysis of the extracts has shown the presence of phenolics like tannins. Others are saponins and alkaloids.

Dichloromethane showed the best activity on tapeworm and roundworm at a time of 48.61 and 35.09 min, respectively on the results of the fractions. It took a shorter

time for the paralysis and death of the worms 20.36 and 48.61 min, respectively at 100 mg/mL compare to the standard control, which gave the time of paralysis and time as 17.08 and 39.25 min. The ethyl acetate fraction gave the least activity on the fractions on tapeworms. The time for paralysis and death was 28.45 and 52.05 min, respectively at 100 mg/mL concentration as shown on Fig. 3 & 4.

The result of the fractions on roundworms also revealed that dichloromethane fraction gave the best activity compare to other fractions. The worms were also paralysed and died earlier in this fraction than in other fractions.

Table 1: Preliminary phytochemical screening of powdered plant material

Test	*Musa paradisiaca*	*Musa sapientum*	*Musa nana*
Alkaloids	+	++	+
Anthraquinones	-	-	-
Cardiac glycosides	+	+	+
Flavonoids	+	++	+
Saponins	+	++	-
Tannins	++	+	+
Terpenoids	-	-	-

+= positive, - = negative, ++ = very strong

Table 2: Anthelminthic activity of Methanol root extract of Musa species on *Moniezia benedeni* (tapeworm), *Esenia fetida* (earthworm) and *Ascaris lumbricoides* (roundworm)

Groups	Conc. (mg/mL)	*Moniezia benedeni* (tapeworm)		*Esenia fetida* (earthworm)		*Ascaris lumbricoides* (roundworm)	
		P	D)	P	D	P	D (min)
Normal control	-	-	-	-	-	-	-
Musa paradisiaca	25	87.38 ± 1.57	160.74 ± 0.8	135.06 ±1.2	238.04 ±0.2	57.71 ± 3.07	76.05 ± 2.92
	50	48.03 ± 0.98	125.05 ± 0.52	112.73 ±0.9	186 ±0.12	37.04 ± 1.89	60.05 ± 1.35
	100	32.06 ± 2.04	122.40 ± 1.95	96.39 ±2.17	167 ±1.93	35.72 ± 1.50	53.04 ± 1.22
	200	30.05 ± 1.32	118.04 ± 0.9	80.04 ±0.5	151.39 ±0.1	26.07 ± 1.7	48.08 ± 2.2
Musa nana	25	120.04 ± 0.8	174.15 ± 0.53	158.04 ±1.4	295 ±0.9	68.05 ± 2.45	90.06 ± 0.9
	50	104.13 ± 3.2	145.05 ± 2.66	137.05 ±1.9	238 ± 2.22	47.06 ± 0.75	78.08 ± 0.26
	100	97.07 ± 0.45	134.05 ± 3.45	126.71 ±0.7	215 ±0.24	38.05 ± 0.65	59.72 ± 0.35
	200	57.08 ± 1.56	129.06 ± 1.25	94.09 ±3.23	189.04 ±2.5	32.04 ± 0.34	53.04 ± 0.12
Musa sapientum	25	125.35 ± 1.9	161.18 ± 1.44	150.05 ±2.3	275 ±2.13	60.73 ± 1.54	82.04 ± 1.27
	50	49.04 ± 2.39	141.05 ± 2.10	127.04 ±4.2	227 ±2.17	49.8 ± 0.82	66.06 ±0.41
	100	35.06 ± 0.77	126.39 ± 0.97	106.07 ±1.6	176 ±1.02	35.05 ±1.12	53.38 ±0.73
	200	33.07 ± 2.57	121.04 ± 1.84	86.04 ±0.69	150 ±0.26	30.38 ±1.87	50.05 ±1.43
Piperazine citrate	20	17.08 ± 1.7	39.25 ± 3.24	63.76 ±0.5	135.33 ±0.2	25.03 ± 0.62	32.05 ± 0.32

P = Time taken for paralysis, D = Time taken for death

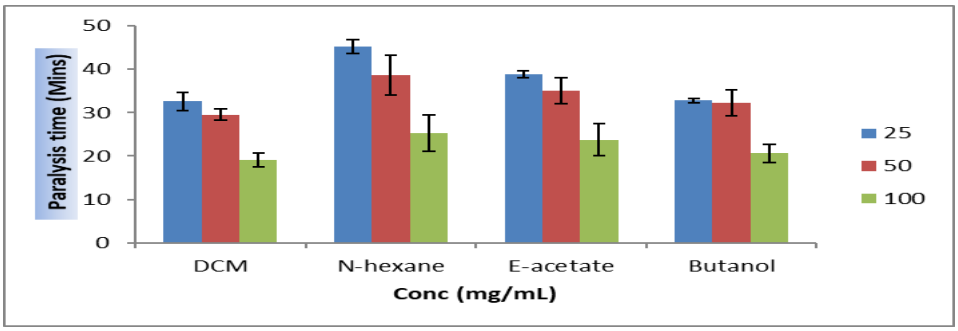

Fig.1: Anthelmintic activity of *Musa paradisiaca* fractions on *Ascaris lumbricoides (*roundworm)

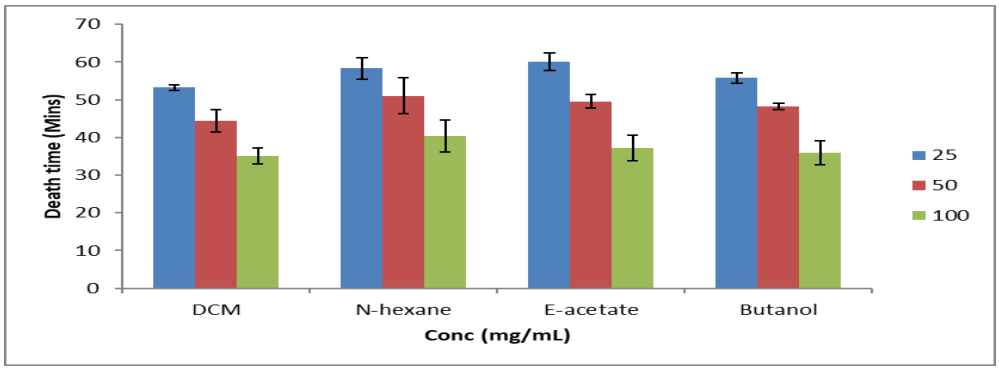

Fig. 2: Anthelmintic activity of *Musa paradisiaca* fractions on *Ascaris lumbricoides* (roundworm)

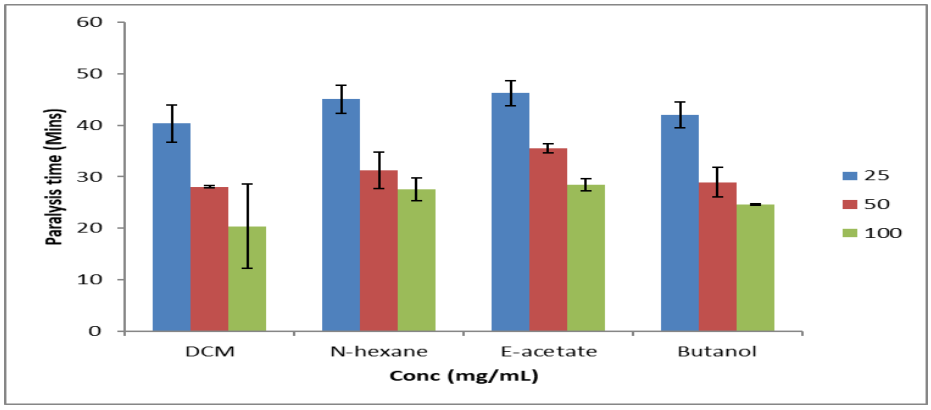

Fig. 3: Anthelmintic activity of *Musa paradisiaca* fractions on *Moniezia benedeni* (Tapeworm)

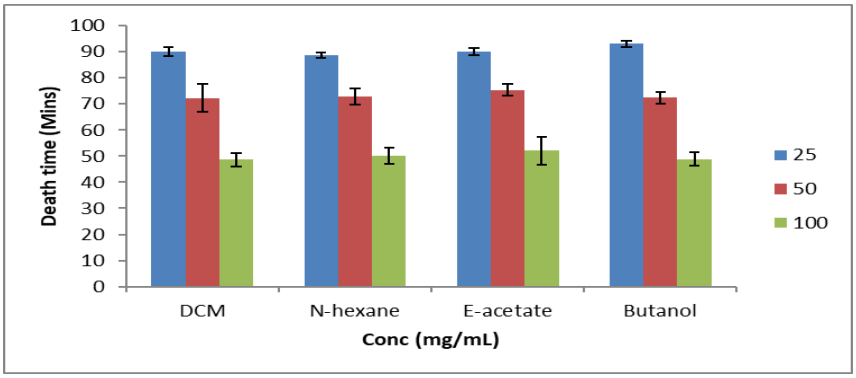

Fig. 4: Anthelmintic activity of *Musa paradisiaca* fractions on *Moniezia benedeni* (Tapeworm)

The time of paralysis and death was 19.05 and 35.09 min, respectively at 100 mg/mL. The fraction that gave the least activity was *n*-hexane fraction, which showed the time of paralysis and death as 25.22 and 40.38 min, respectively as shown in Fig. 1 and 2.

DISCUSSION

Results obtained from the anthelmintic assay indicated that higher concentrations of plant extracts produced faster paralytic effects and showed shorter time of death. The predominant effect of piperazine citrate on worm is to cause a flaccid paralysis that results in expulsion of the worm by peristalsis. Piperazine citrate acts by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads the muscle to relaxation and flaccid paralysis [21]. Methanol extracts of *Musa* species demonstrated paralysis as well as death of worms especially at higher concentration of 200 mg/mL while 25 mg concentration also showed activity but at longer time. *In vitro* assay was used in this present investigation in agreement with the findings of [22]. This provides cheaper, economical and rapid turnover in contrast of *in vivo* assays as far as anti-parasitic properties of plants and plant extracts are concerned [22]. The phytochemical constituent showing anthelmintic effects includes alkaloids, saponins, polyphenols, tannins, etc. Alkaloids suppress the transfer of sucrose from stomach to small intestine, diminish the support of glucose to the helminths, and act on the CNS causing paralysis. Saponins possess vacuolization and disintegration of teguments. Polyphenols and tannins increase the supply of digestible proteins by animals via forming protein complexes in rumen, interfere with energy generation in the helminth parasites by uncoupling the oxidative phosphorylation, cause a decrease in gastrointestinal metabolism which leads to paralysis and death of helminths [23].

Results obtained from the fractions obtained revealed that dichloromethane (DCM) fraction gave the best activity compared to every other fraction (ethyl acetate, butanol and N-hexane fractions). The activities were also concentration-dependent. The lowest concentration 25 mg/mL gave the highest time for the paralysis and death in all the fractions. However, in comparing the results of the crude methanol extracts with the fractions, DCM fraction showed the best activity. The time of death and paralysis was shorter in the DCM fraction compared to the other fractions and crude methanol extracts. This correlates with the findings of [24] on the leaves of *Musa* species, which revealed that active ingredient responsible for anthelmintic activity is relatively a polar compound.

Conclusion. One can conclude from the above results, that Musa species used traditionally to treat intestinal worm infections, showed anthelmintic activity suggesting the possible use of Musa species extracts for control of intestinal nematode and cestodes. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. Further studies are required for the identification of bioactive components responsible for anthelmintic activity and to determine the exact mechanism of action of these plants.

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