



Preliminary Phytochemical Screening and *In Vitro* Anthelmintic Activity of *Detarium Senegalense* J.f Gmel (Fabaceae) Leaf Methanol Extract

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ABSTRACT

Detarium senegalense is an importance medicinal plant used in North-east Nigeria and some African countries in traditional medicine. This present study evaluated the preliminary phytochemical screening and anthelmintic activity of methanol leaf extract. Phytochemical screening was carried out by solvent-solvent partitioning of leaf extract in chloroform, n-hexane, dioxane, ethyl acetate, acetone and methanol. The results showed that methanol fraction contained alkaloids, tannins, saponins, and cardiac glycosides while the ethyl acetate fraction contained only flavonoids with no anthracenes. Ethanol fraction demonstrated the highest anthelmintic activity with a decreased time of paralysis and death in the various groups of earthworm (*Lumbricus terrestris*) in dose-dependent manner at concentrations of 10 mg/mL, 20 mg/mL, 30 mg/mL, and 40 mg/mL. The time for paralysis and death for Group VII 400 mg/mL of extract were 5.0 ± 0.3 min and 10.0 ± 1.02 min respectively. This result was compared with that of a standard anthelmintic drug piperazine citrate (USP) whose values were 12 ± 1.01 min and 16 ± 1.02 min respectively for time of paralysis and death at $p \leq 0.05$ (one-way ANOVA). The results showed that *D. senegalense* leaf methanol extract possessed anthelmintic activities by osmotic shock at the clitellum portion of the earthworms, and this justifies the use of the plant extract in herbal medicine for the eradication of worms.

Keywords: *Detarium senegalense*, Phytochemicals, Anthelmintic, Earthworms, Piperazine citrate.

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How to cite this article:

Ukwubile et al., Preliminary Phytochemical Screening and In Vitro Anthelmintic Activity of *Detarium Senegalense* J.f Gmel (Fabaceae) Leaf Methanol Extract. American Journal of Biotechnology and Bioinformatics, 2017; 1:2 .

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Website: <http://escipub.com/>

Introduction

Infections with helminthes are among the widest spread infection in humans and their domestic animals affecting a large number of world population. Helminthes infections are commonly found among rural dwellers in Nigeria , and recognized as cause of much acute as well as chronic illness among the various human being as well as livestock especially sheep, cattle, and goats. More than half of the population of the world suffers from various types of infections related to helminthes, and majority of livestock suffer from worm infection ¹.

However, the high cost of the modern anti-helminthes has limited the effective control of these parasites. In some cases, widespread intensive use of sometimes low quality anti-helminthes has led to development of resistance to helminthes infection, hence a reduction in the usefulness of available anthelmintic ². Although, the use of alternative drugs has also been devoted as a measure to avoid the development of resistant strains of helminthes parasite. This is a means of reducing the cost of controlling helminthes ^{3,4,5,6}.

Medicinal plants are of great importance to the health of individuals and livestock. Many of these indigenous medicinal plants are used as food supplements, nutraceuticals, and food plants. Today most pharmaceutical anthelmintic drugs derived from excellent ingredients in medicinal plants are being used to treat worm infection. Many naturally derived anthelmintic drugs have the potential to treat many diseases and disorder, one of which one is *Detarium senegalense*. *Detarium senegalense* is locally called “*Taura*” in Hausa language and “*Ofor*” in Igbo language in Nigeria and it is a medium size tree that may grow up to 40 m high. The plant is known to provide rich sources of botanical anthelmintic drug in traditional medicine.

Detarium senegalense commonly called Tallow tree belonging to the family Fabaceae is a leguminous tree in the sub-family *Detarioideae*. Unlike most members of the family, it produces

globular fruits. Its other common name include “*Didax*, *Ditakh*, and *Detar*” tree. The tree is of value for several reasons; it produces nutritious fruits. As its linear name indicates, it is native to Senegal and the surrounding countries of West Africa. *D. senegalense* is distributed also in The Gambia, East to Western Sudan, and South to Northern Congo DR ⁷. It is propagated as fruit tree and ornamental shade tree within its area of distribution and has been planted in the Caribbean region for its fruit.

Like many tree in the *Detarioideae*, they have thick, irregularly placed branches. The trunks of mature tree typically range from 60-100 cm in diameter. The fruit is globular to dark-green drupe with fibrous pulp and a single seed. They are similar to the fruits of tamarind which belong to *Tamarindus*, another genus in Sub-family *Detarioideae* ^{7, 8, 9}. The sweet and sour flavor of the fruits is popular and shelf life is good because of the hard pericarp and dry pulp to establish the tree more widely as an agricultural line.

Bark decoction is use in preparation for the expulsion of placenta after birth and for the treatment of anemia, wounds, skin problems, pneumonia, stomach ache and digestive disorders ¹⁰, and the leaf and shoot mixture have been use in the treatment of helminthes, dysentery, conjunctivitis, arthritis, etc. The seed infusion have been applied to control blood-glucose levels in diabetic individual, as well as for the treatment of mosquito bite and as an antidote against arrows poison and snake bite. In South-east Nigeria, nutritionally “*ofor*” flour made from the seeds is often use as a soup thickener while the pulp from the fruits form is eaten directly and also made into sherbets juices. The timber from the tree is referred to as “African mahogany” and is characterized by its dark reddish-brown shade ¹¹.

This present study evaluated the phytochemical constituents and anthelmintic activity of *Detarium senegalense* leaf ethanol extract.

Materials and Methods

Plant collection and identification

Fresh leaves of *Detarium senegalense* plant material was collected from a forest in Bali, Taraba State, Nigeria. The plant was authenticated by a taxonomist in the Department of Science Laboratory Technology, Biology Unit, Federal Polytechnic Bali, Nigeria, where a voucher number MEL002 was deposited for the plant.

Preparation of plant

The fresh leaves were air-dried under shade for two weeks. It is then shade dried for two weeks. Furthermore, the leaves were grind into fine powder using electronic blender. 500 g and then extracted in 1000 mL absolute ethanol (Sigma Aldrich) using Soxhlet apparatus. The filtrate was concentrated *in vacuo* using rotary evaporator for 8 hours to obtain a dark paste-like extract. This was then weighed to obtain a final weight of 56 g and percentage yield of 11.2 % and stored for further use.

Preliminary phytochemical screening of leaf ethanol extract

The methods described by Evans ¹³, and Sofowora ¹⁴, were employed to determine the presence of the following secondary metabolites: alkaloids, tannins, saponins, anthraquinones, cardiac glycosides, flavonoids, and carbohydrates.

Test organism

Adult earthworms (*Lumbricus terrestris*) were collected from loamy soil by pouring water from cassava retting on the soil at suspected site as indicated by the presence worm cast on the soil, and used for anthelmintic evaluation because of morphological and anatomical similarities between the earthworm and the intestinal worm. The earthworms were maintained under normal vermi form and supplied with adequate humus as nourishment as well as water for two weeks before the experiment. The earthworm were washed with distilled water prior to study. Adult earthworms of approximately 4 cm length and 0.3 cm in width were used for the experiment.

These organisms were selected as model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal round worm parasite of humans.

Preparation of extract for *in vitro* study

Various concentrations of ethanol leaf extract of *D. senegalense* were prepared into 10, 20, 30, and 40 mg/mL while a standard anthelmintic drug piperazine citrate B.P was used as positive control. These were dissolved in 0.5 % DMSO in 10 mL distilled water, and used for *in vitro* anthelmintic activity of the plant.

In vitro anthelmintic evaluation of *D. senegalense* crude extract

The anthelmintic activity of leaf extract of *D. senegalense* as far as the methods reported by ¹². Briefly, five groups of earthworm *Lumbricus terrestris* with four worms in each group. Each worm was separated in to 10 mL of desired formulation in distilled water. The earthworms were used because of their similar physiology and body anatomy with intestinal parasitic worm. The experiment was designed as showed below:

Group I: Earthworms were released into *D. senegalense* extract (DSE) containing 20 mg/mL standard drug piperazine citrate (B.P Elixir) as positive control.

Group II: Earthworms were released into 10 mL distilled water in a clean petri dish and were maintained as negative control.

Groups III, IV and V: Earthworms were released in 20 mg/mL, 30 mg/ mL and 40 mg/ mL DSE respectively.

Earthworms were observed for time of paralysis and time of death. Paralysis was observed based on the behavior of the worm with no revival of body state. Death was observed based on the total loss of motility with faded body colour. The experiment was repeated for each group three times and mean values were calculated.

Statistical analysis

The data obtained were express as mean \pm SD for three consecutive readings. The differences

between the groups at $p \leq 0.05$ was considered as statistically significant by analysis of variance (one-way ANOVA).

Results and Discussion

Detarium senegalense is a well-known medicinal plant and is widely used in traditional medicine. In the present study, distilled water was used orderly for crude extraction of *D. senegalense*, leaf. To justify ethno-medicinal claim of *D. senegalense*, an efficient attempt in evaluating the anthelmintic property of *D. senegalense* was made. Earthworm (*Lumbricus terrestris*) belongs to the Class Oligochaetae and Phylum Annelida has similar anatomical structure with the intestinal helminthes, hence it was used an experimental model to evaluate the potency of this plant on helminthes^{14, 15}. Preliminary phytochemical screening of the

ethanol leaf extract showed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides secondary metabolites. It is no doubt that these metabolites are responsible for the observed anthelmintic activity shown in Table 2. Anthelmintic activity of the plant showed a dose-dependent time of paralysis (TOP) and time of death (TOD) in the *in vitro* experiment. These results were comparable to that observed in a standard anthelmintic drug piperazine citrate USP at $p \leq 0.05$ (one-way ANOVA). Ukwubile *et al.*¹⁶, reported that aqueous extract of *Pseudocedrella kotchsyii* showed anthelmintic activity in *Lumbricus terrestris* in dose-dependent fashion by creating higher degree of haemolysis in the worm through osmotic shock. This result was not different from that of the present study in all the five groups of earthworms investigated.

Table 1: Phytochemical profile of *Detarium senegalense* leaf methanol extract

Constituent	Test	Observation	Inference
Carbohydrates	Molisch	No colour change	-
	Fehling	No colour change	-
Glycosides	Fehling A & B	Brick red ppt	++
	Ferric chloride	Green black ppt	+
Anthraquinone	Borntrager	No colour change	-
	Modified Borntrager	No colour change	-
Cardiac gly	Kella-killiani	Purple ring colour	+
	Kadde	Purple blue colour	+
Saponins	Frothing	Foam formed	+
Flavonoids	Shinoda	Orange colour	+
	NaOH	Yellow colour	+
Tannins	Lead sub-acetate	Brown ppt	++
	Ferric chloride	Green ppt	++
Alkaloids	Dragendorff	Rose red ppt	++
	Wagner's	White ppt	++
	Picric acid	Yellow solution	+
	Tannic acid	Dark- black	++

++ (strongly detected), + (moderately detected), - (not detected)

Table 2: Anthelmintic activity of *Detarium senegalense* leaf ethanol extract

Test sample	Concentration (mg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Piperazine citrate	20	12 ± 1.01	16 ± 1.02
<i>D. senegalense</i> extract:			
	10	20 ± 5.02	24 ± 2.03
	20	13 ± 1.02	21 ± 2.02
	30	8.0 ± 0.5	14 ± 1.04
	40	5.0 ± 0.3	10 ± 1.02

Results are mean ± SD, P ≤ 0.05 (One-way ANOVA)

Conclusion

The present study is an assessment of the effectiveness of Tallow tree (*Detarium senegalense*) plant which is used locally against helminthes diseases using *Lumbricus terrestris* as experimental model. Ethanol extract of *D. senegalense* demonstrated a significant ($p \leq 0.05$) anthelmintic effect. The result of this study showed that the plant has anthelmintic property in dose dependent fashion. It thus, represent a sure source of anthelmintic drug discovering and an immediate remedy for many helminthes infections in traditional medicine. However, further research is suggested to know the specific compound responsible for the observed biological activity, as well as ethno-medicinal preparations from the leaves of this plant is highly recommended for easy access-to by local dwellers in Nigeria.

Acknowledgements

The authors are thankful to Benrock chemicals Ltd who supplied us with chemicals used in this study, and also to Mr. Dalhatu who helped in getting the earthworms used for the study.

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