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Habibou Hama Hamadou
Laboratory of Natural Substances, School of Science and Technology, Abdou Moumouni University, Niamey, Niger., BP 10662, Niamey, Niger

Abdoulahi Mahamane Idi Issa
Laboratory of Natural Substances, School of Science and Technology, Abdou Moumouni University, Niamey, Niger., BP 10662, Niamey, Niger

Idrissa Moussa
Laboratory of Natural Substances, School of Science and Technology, Abdou Moumouni University, Niamey, Niger., BP 10662, Niamey, Niger

Khalid Ikhiri
Laboratory of Natural Substances, School of Science and Technology, Abdou Moumouni University, Niamey, Niger., BP 10662, Niamey, Niger

Corresponding Author:
Habibou Hama Hamadou
Laboratory of Natural Substances, School of Science and Technology, Abdou Moumouni University, Niamey, Niger., BP 10662, Niamey, Niger

Ethnobotanical study and anthelmintic investigation of *Detarium microcarpum* (Fabaceae)

Habibou Hama Hamadou, Abdoulahi Mahamane Idi Issa, Idrissa Moussa and Khalid Ikhiri

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Abstract
African flora in general, and Nigerian flora in particular, is rich in medicinal plants. The aim of this study is to contribute to a better understanding of *Detarium microcarpum* and its evaluation in terms of the anthelmintic activity of its organs. Ethnobotanical surveys were carried out using questionnaires with herbalists and traditional practitioners, and extracts of the various organs were evaluated *in vitro* for their anthelmintic activity. The results enabled us to list the medicinal uses of *D. microcarpum*, particularly for gastrointestinal diseases. Extracts of trunk bark, roots and leaves showed a positive effect on earthworms during the trial (1.50 mg/mL < LD_{50} < 2.70 mg/mL). The results obtained may justify the traditional use of *Detarium microcarpum* in the treatment of certain diseases of parasitic origin.

Keywords: Ethnobotany, anthelmintic, *Detarium microcarpum*

1. Introduction
One third of humanity is said to be parasitized by worms [1]. The number of worms parasitizing humans is estimated to be at least 400 billion, representing a considerable biomass and à significant loss of proteins, vitamins and other nutrients for the individuals concerned [2-3]. Years of life lost through premature death or disability due to helminthiasis rank first among children aged 5 to 14 in developing countries, ahead of the usual infectious diseases [4]. Helminths are macroscopically visible, multicellular worms with separate sexes [5]. Adult worms have no locomotor organs and move around thanks to their plasticity. They are characterized by their host attachment organs (suckers, hooks), by a simple digestive tract, sometimes partially or totally atrophied, and by considerable hypertrophy of the genital tract with very high egg production [6].
K. Ikhiri *et al.* (1984) reported on the use of plants in traditional pharmacopoeia in Niger [7]. *D. microcarpum* Guill. & Perr. is one of 186 medicinal species identified for the treatment of intestinal parasitosis. *D. microcarpum* is a plant belonging to the Fabaceae family, widely used in traditional pharmacopoeia for the treatment of several human diseases in several regions of Africa [8]. The natural range of *D. microcarpum* covers the whole of arid sub-Saharan Africa, from Senegal to Sudan. The species is found in Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea Bissau, Mali, Niger, Nigeria, Central African Republic, Senegal, Sudan and Chad [9].
The fruits available to the inhabitants of sub-Saharan Africa play an important nutritional role, and several studies have been carried out on their nutritional and therapeutic composition [10-14]. The decoction of trunk bark is used as a drink to cure simple or bloody diarrhea. It is said to be antihemorrhoidal and antiblennorrhagic [15]; antimicrobial [16-17]; and anti-edematous [18]. Root macerate is used to relieve stomach aches, especially dysenteric diarrhea. Root decoction is said to give off a pleasant fragrance; the resulting liquid is used as a drink to treat syphilis [8]. The roots have anti-diabetic [19] and antifungal properties [20]. The decoction of *D. microcarpum* leaves is used to treat stomach ache, malaria and diarrhoea. The leaves are used to treat chest pain, mental disorders and kwashiorkor, while others use them to treat tooth decay, chest pain and childbirth complications [8]; anti-diarrheal and anti-asthenic [8]; antimicrobial [21]; larvicidal [22].
It is within this framework that this study is undertaken for an ethnobotanical study and *in vitro* evaluation of the anthelmintic activity of *D. microcarpum* Guill. & Perr. used in the treatment of parasitic diseases in Niger.

2. Materials and Methods

2.1 Plant matériels

The organs (leaves, fruits, flowers and trunk and root barks) of *D. microcarpum* were freshly harvested in March 2018 in the Dosso region (Niger). They were identified in comparison with an authentic sample of the herbarium specimens deposited under number 756 at the Department of Biology (Faculty of Sciences and Technology of Abdou Moumouni University). The plant material was air-dried away from the sun at room temperature (37 °C), then ground to a powder.

2.2 Animal material

The animal material consisted of earthworms (*lombricus terrestris*) with a body mass of 1 ± 0.1 g. Earthworms belong to the oligochaete family and the annelid phylum. They were collected at the Niger River dam. Earthworms are used in this study because their biology is similar to that of helminths, which are generally digestive parasites, and because they are easily accessible.

2.3 Preparation of the extract

30 g of plant powder from each *D. microcarpum* organ was macerated in 300 mL (×2) of methanol. After continuous maceration extraction for 24h at room temperature under magnetic stirring, the macerate was filtered and the methanol removed by rotavapor evaporation. The residue obtained is transferred to distilled water for biological testing.

2.4 Ethnobotanical survey on *D. microcarpum*

An ethnobotanical survey is a field study that involves meeting traditional practitioners to find out about their methods of treating disease. This survey was carried out in the city of Niamey over a two-month period (July and September 2016). The study population consisted of all herbalists and tradithérapeutes during the course of the survey. The ethnobotanical survey was carried out using a form completed by oral questioning on the uses of the plant in traditional pharmacopoeia.

The questionnaire focused on therapeutic habits, enabling us to assess knowledge of the plant, diseases treated, organs of the plant used, therapeutic indications, preparation methods, modes of administration, etc. The sample size was 60. The aim of the survey was to find out about the use of *Detarium microcarpum* in traditional Nigerian medicine.

2.5 Test to assess anthelmintic activity

The anthelmintic activity evaluation test was carried out using the techniques described by Guissou *et al.*, 1998 [23] and Ongoka *et al.*, 2012 [24].

Ordinary sand was first washed with distilled water, then placed in an oven for 72 hours. 75 g of sand were placed in a petri dish used as a survival tank. 25 mL of solution were added. After homogenization by shaking, 5 earthworms were placed in the dish and their behavior observed for 14 hours.

Three batches were tested:

- Batch 1 or control batch of earthworms treated with distilled water.
- Batch 2, comprising 8 sub-batches corresponding to 8 concentrations of extract of each plant part: 1, 2, 3, 4, 5, 6, 7 and 8 mg/mL. Each sub-batch contained 5 earthworms.
- Batch 3 or reference batch with 5 earthworms treated with the reference product, Levamisole, at concentrations of 2, 4, 6 and 8 mg/mL. For each extract concentration, the experiment is repeated 2 times.

The evolution of the earthworms, i.e. their behavior and lethality, was observed for 14 hours. The intoxication syndrome was manifested by hyper-mobility of the earthworms, whose death was marked by parasitic lysis after short- or long-term starvation. For each concentration of extract, the time after which the dose of extract caused the death of all the earthworms placed in the petri dish was recorded; this time is still referred to as the 100% lethality time.

The anthelmintic effect was considered effective when the time to onset of hypermobility was short: 1 to 6 hour(s) [24]. The shorter the time to onset of hypermobility and 100% lethality, the greater the anthelmintic activity of the extract. The relationship between the percentage of earthworm deaths and the concentration of extracts from different organs was analyzed after the onset of 100% mortality in a study batch, using a concentration-dependent percentage death curve (%) after six hours' exposure. The LD_{50} of the extracts were determined.

3. Results and Discussion

3.1 Results of the ethnobotanical survey

The ethnobotanical survey is a link between traditional and conventional medicine.

Distribution of respondents by gender: in the city of Niamey, sixty (60) people with ethnobotanical knowledge (herbalists and tradithérapeutes) were surveyed. These included both men (80%) and women (20%). This profile of herbalists and tradithérapeutes is generally that observed in most similar studies [25], also confirming that the practice of traditional medicine is the preserve of men.

Age distribution: The age groups recorded are between 20 and 50, and over 50. In Niamey, the majority of herbalists and tradithérapeutes are over 50. Older people are expected to provide more reliable information, as they hold much of the ancestral knowledge that is passed on orally. The transmission of this knowledge is currently in danger, as it is not always fully guaranteed [26].

***D. microcarpum* use:** Various plant organs are used to meet therapeutic needs, from leaves, stems, fruit and roots to bark and flowers. All *D. microcarpum* organs, except flowers, are used in traditional medicine. *D. microcarpum* is a medicinal plant with proven therapeutic value in many parts of Africa, where traditional therapists use the plant's recipes to treat a variety of tropical pathologies. Leaves, trunk bark and root bark have proved to be the most widely used to treat various pathologies.

In the course of this survey, we identified a dozen illnesses or infections that herbalists and traditherapists treat with the various organs of *D. microcarpum*, including: malaria; stomach ache; infantile cough; haemorrhoids; constipation; bilharzia; rheumatism; tooth decay; sexual weakness; genital infection; skin infections; intestinal worms.

Most of these observations corroborate those reported in the literature by other researchers [7-8, 10, 27]. Of all the pathologies surveyed, two were the most frequently cited: malaria and stomach ache. Moreover, herbalists and traditional practitioners treated these pathologies differently, either with root or trunk barks, or with leaves.

In general, bark is used as a febrifuge, which is why maceration of the bark is recommended for the fever that accompanies malaria and for stomach aches. Root bark is

used to treat malaria and digestive disorders [7-8, 10, 17].

Method of preparation: All these organs are mainly prepared in macerated or decocted form. This is because decoction allows the most active principles to be collected, and attenuates or cancels out the toxic effect of certain recipes [28].

Method of administration: According to our survey, all medicinal preparations are prescribed orally and as a drink. This prescription may be explained by the fact that the pathology is linked to deep-seated organs. To reach them, any compound must pass through the digestive tract to facilitate its assimilation [29].

3.2 Results of the antihelminthic activity of *D. microcarpum* organs

Table I shows the lethal dose 50 (LD_{50}) of methanolic extracts from *D. microcarpum* organs after six hours' exposure time.

Table 1: LD50 *of D. microcarpum* organs

Organes	Levamisol	Root barks	Trunk barks	Leaves	Fruits	Flowers
LD_{50} (mg/mL)	<1	1,50	1,70	2,70	>8	>8
LD_{100} (mg/mL)	<1	2	3	6	>8	>8

- For the effect of *D. microcarpum* trunk bark, a positive effect on earthworms was observed during the trial. Trunk bark produced an LD_{50} of 1.7 mg/mL. The disruption caused by anthelmintic plants on the survival or prolificacy of worms constituting the pathogenic stage would be an important element in the fight against these parasites. The results of the trunk bark extract show that after 6 hours' exposure of earthworms to the extract, the mortality rate was 100% at concentrations of 3 mg/mL and above.
- As for *D. microcarpum* root bark, a positive activity on earthworms was observed, similar to that of trunk bark with an LD_{50} of 1.5 mg/mL. The mortality rate was 100% at concentrations above 2 mg/mL after 6 hours' exposure of earthworms to the extract.
- For *D. microcarpum* leaves, the LD_{50} is 2.7 mg/mL with 100% mortality from a concentration of 6 mg/mL.
- *D. microcarpum* fruit and flower extracts did not show anthelmintic effects on earthworms.
- The reference product (Levamisol) has also shown an anthelmintic effect on earthworms. This drug acts at lower doses than the *D. microcarpum* organs, and has a shorter onset of action. Better cell permeability could explain this phenomenon. Levamisol significantly reduced the motility of adult worms within 6 hours of contact with *Haemonchus contortus*, a gastrointestinal nematode parasite of small ruminants [30-31].

This study revealed that extracts of the various organs of *D. microcarpum* resulted in 100% lethality of earthworms after a period of time (1 to 6 h), compared with the results of other researchers who had worked on other earthworm species and other plants (lethality times ranging from 3 h to 72 h) [24, 32-34]. The doses of extracts used to obtain the 100% lethality time would explain the differences observed. The results obtained with trunk, root and leaf barks support the use of this plant as a parasiticide.

Chemical profiles carried out by our previous studies and many others on the organs studied have revealed the presence of flavonoids and tannins [17, 21, 35-37]. Studies have shown that flavonoids and tannins are involved in anthelmintic activity [32, 38]. Thus, the anthelmintic activity of *D. microcarpum* organ extracts observed in this work is probably due to flavonoids and/or tannins. The latter have the capacity to inhibit the oxidative phosphorylation of helminths [24]. In addition, they can bind to a glycoprotein, collagen, which plays a protective role in the parasite's cuticle. This binding causes damage to the cuticle, leading to the death of the helminth [32]. Sainfoin extract, a plant rich in tannins, affects the unsheathing kinetics of *H. contortus* larvae *in vitro* and *in vivo* [39]. Similarly, Olounladé *et al.* (2011) reported tannin-induced migration inhibition of *H. contortus* larvae for *Z. zanthoxyloides* and *N. laevis* [40]. Tannin-rich extracts from eight plants have shown anthelmintic activity on two stages (larvae and adult worms) of *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*, with strong variations depending on the parasite stage and the tannin content of the plants [32, 41].

In addition to tannins, flavonoids play an essential role in the anthelmintic activity of plants [42-43]. Ayers *et al.* (2008) reported the contribution of phenols and flavonoids to the anthelmintic activity of *Struthiola argentea* [44].

4. Conclusion

The work presented in this study concerns the ethnobotanical and anthelmintic study of *Detarium microcarpum* Guill. & Perr. The ethnobotanical survey revealed that all *D. microcarpum* organs, except the flowers, are used in traditional West African medicine. The diseases concerned are of diverse origins, but mainly concern bacterial or parasitic infections of the gastrointestinal sphere. The anthelmintic activity of *D. microcarpum* organs used to treat parasitic diseases was tested *in vitro* on earthworms. From the present work, it appears that extracts of trunk bark, roots and leaves have anthelmintic activity on earthworms. The traditional use of these three organs as anthelmintics therefore seems justified. However, it would be necessary to evaluate this activity using parasites commonly found in humans and animals.

5. Authors' Contribution

HHH: designed and performed the laboratory experiments and drafted the manuscript; HHH, MIIA, MI and IK: designed the study and revised and approved the manuscript; HHH and MIIA: analyzed the data.

6. Conflicts of Interest Disclosure

The authors declare that they have no conflicts of interest.

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