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Reserach Article

IN VITRO ANTHELMINTIC ACTIVITY AND ASSESSMENT OF FUNGICIDAL AND FUNGISTATIC EFFECTS OF POLYPHENOL-RICH FRACTIONS OF STEM BARK FROM *LANNEA ACIDA* A. RICH (FAM. ANARCADIACEAE)

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A B S T R A C T

Introduction: Based on the results of ethno medical on the one hand and the other hand the resistance of some medico-veterinary parasite and *candida* strains as well as the unavailability and toxicity of synthetic anthelminthics and antifungal on humans, animals have pushed scientists to turn to plants with anthelminthic and antifungal properties. Hence, the aim of this work was to contribute to the fight against infectious and parasitic diseases. **Materials and Methods:** The anthelmintic activity of polyphenol-rich fraction of stem bark from *Lannea acida* a. Rich., has been evaluated using earthworms as parasites. Various concentrations of the formulation (25 mg/ml, 50 mg/ml and 100 mg/ml) were tested which involved determination of paralysis time and death time of the worm. Albendazole (25 mg/ml) was used as the reference standard. The antifungal activity was investigated using the microplate dilution method. **Results:** The polyphenol-rch fractions of stem bark from *lannea acida* was showed the anthelmintic activity. The polyphenol-rch fractions at a concentration of 100mg/ml showed paralysis at 2.82 minutes and death of earth worm at 26.91 minutes. As for the antifungal activity, result varied according to microorganism. **Conclusions:** These findings obtained from this work confirmed the using of *Lannea acida* to treat intestinal worm infections and antifungal activity. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed anthelmintic and antifungal activities.

INTRODUCTION

Infection by gastrointestinal helminths constitutes aserious public health problem especially in developing countries where climatic factors, poverty and the poor hygienic condition influence the proliferation of disease [1]. In tropical areas, human helminthosis is counted among the first seven parasitic diseases registered as Neglected Tropical Diseases [2], whereas they shackle the health of over two million of people worldwide.

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In Burkina Faso, the endoparasitisis caused by polyparasitism constitute a major problem which compromise the survival and performances of traditional poultry farming. In these conditions gastrointestinal nematode infections are of considerable economic importance, causing clinical disease with mortalities but more importantly by causing chronic production losses as a result of reduced weight gain, weight loss, and reduced milk production[3]. However, several methods of helminthosis control exist and the most frequently used are chemotherapy and phytotherapy. This is often done through the use of synthetic anthelminthic combined with the management of pasture [4] in developed countries while it is done by medicinal plants with anthelminthic properties in developing countries [5]. Natural products have also been

considered an important source of bioactive compounds against infectious diseases [6]. Fungal infections have increased gradually over the last 30 years, becoming one of the more relevant public health problems. Candida yeasts are among the main etiological agents of invasive fungal infections, which are responsible for high mortality and morbidity rates throughout the world [7]. These fungi have developed resistance mechanisms against antibiotics, favoring the persistence and progression of the infection even when antifungal therapy is adequately performed [8]. *Candida albicans, Candida krusei, Candida parapsilosis* and *Candida glabrata* are among the most prevalent causers of candidiasis [9].Several plants have been reported for prevention and treatment of infectious diseases of animals and humans in Burkina Faso [10]. In this context, investigations on medicinal plants might contribute to develop alternative and sustainable methods readily adapted to rural communities. One of those plant species commonly used in the treatment of infectious diseases is *lannea acida* A. Rich. This plant is known for its medicinal properties and has been reported to possess antimicrobial and antidiarrhoeal activities [11].Its stem bark protects against snake bites and are very active also against all intestinal parasites and possess antifungal properties [11]. However, scientific validation of these practices has been lacking. This study aimed to test the *in vitro* anthelmintic and antifungal properties of polyphenol-rich fractions of stem bark from *lannea acida* A. Rich.

MATERIAL AND METHODS

Plants material

The vegetable materials (Fresh stem bark)of *lannea acida* A. Rich (Fam. Anarcadiaceae)were collected in August 2014 in Dedougou, 230 Km West of Ouagadougou, capital of Burkina Faso. This plant was botanically identified by Dr. TraoréLassina from the plants Biology Department of the University of Koudougou.

Bacterial strains

The studies microorganisms included reference strains of *Candida albicans* ATCC 9002, *Candida albicans* ATCC 2091, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 750, *Candida Krusei* ATCC 6258.Fungal strains were maintained on agar slant at 4°C and sub-cultured on a fresh appropriate agar plates 24h prior to any antifungal activity. Sabouraud Glucose Agar was used for the activation of fungi. The Mueller Hinton Broth (MHB) was used for the MIC and MFC determinations.

Polyphenols extraction

The harvested plant materials fresh (Fresh stem bark) were dried in the laboratory at room temperature (20-25°C), afterwards samples were ground to pass a sieve of 0.3 mm. Polyphenols were extracted with aqueous acetone (80%, v/v). The extract was then washed with hexane to remove chlorophyll and other low molecular weight compounds. Acetone was evaporated and the extract was lyophilized and stored at 22°C prior to biological tests. For the tests, lyophilized sample was dissolved with 10% DMSO in water at the desired concentration [11].

In vitro anthelmintic activity

The anthelmintic activity of polyphenol-rch fractions of stem bark from *lannea acida* A. Rich of different concentrations (25, 50 and 100 mg/ml in double distilled water) was performed on earthworm as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Earthworms 3-5 cm in length were used for the experiment. All the earthworms were washed properly with normal saline to remove dirt and earthy materials. In the experiment Albendazole was used as a standard drug and 5% Tween 80 in normal saline used as control. All the necessary solutions were prepared freshly before starting the experiment. The earthworms were divided into five groups in the following manner. Anthelmintic activity was evaluated as per method with minor modifications [12].

Group 1: Control (normal saline + tween 80)
Group 2: Standard (albendazole - 25mg/ml)
Group 3: Test group (25 mg/ml)
Group 4: Test group (50 mg/ml)
Group 5: Test group (100 mg/ml).

Earthworms of each group were released into 20 ml solutions containing test and standard drug and observed for the time taken for paralyze and death. Time taken for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time taken for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C followed by their fading of body colour [13].

In vitro antifungal activity

Preparation of inocula

The fungal strains grown on nutrient agar (MullerHinton broth) at 35°C for 72 h were suspended in a saline solution (0.9%, w/v) NaCl and adjusted to a turbidity of 0.5 Mac Farland standard (5×10^5 CFU/ml) [14].

Preparation of polyphenol-rich fractions substances

The stock solutions of polyphenol-rch fractions of stem bark from *lannea acida* A. Rich were dissolved in 10%dimethylsulfoxide (DMSO) in water [14, 15]at a final concentration of 800 µg/ml. The stock solutions were sterilized by filtration through 0.22 µm sterilizing Millipore express filter.

Minimum inhibitory concentration (MIC) assay

Minimum inhibitory concentration (MIC) was determined by the microdilution method in culture broth as recommended by [14, 16] with low modifications. 12 serial two-fold dilutions of EAF solutions or convention alantibiotic were prepared as described before, to obtain final concentration ranges of 800-0.78125µg/ml and 50-0.0488 µg/ml for fraction and reference substances respectively. The last wells (n°12) served as sterility controls (contained broth only) or negative control (broth + inoculums). The 96-well micro-plates (NUNC, Danemark) containing 100 µL of Mueller Hinton (MH) broth were used. For each fungi strain, three columns of eleven wells to the micro-plate were used. Each well has getting: the culture medium + fraction solution or Nystatin or the combination of fraction solution with Nystatin + inoculum standardized at 5×10^5 CFU/ml (10 µl of inocula) and INT (50 µl; 0.2 mg/ml for 30 min). The plates were sealed with parafilm, then

agitated with a plate shaker to mix their contents and incubated at 35°C for 48 h. All tests were performed in triplicate and the fungi activity was expressed as the mean of inhibitions produced. Viable microorganisms reduced the yellow dye to a pink colour. The MIC was defined as the lowest concentration of fraction substance at which no colony was observed after incubation. So, the MIC was defined as the lowest concentration where no change was observed, indicating no growth of microorganism.

Minimum fungicidal concentration (MFC)

Minimum fungicidal concentration (MFC) was determined by the microdilution method in culture broth as recommended by [14, 16] with low modifications. Minimum fungicidal concentration (MFC) was determined by adding 50 µl aliquots of the clear wells to 150 µl of freshly prepared broth medium and incubating at 35°Cfor 48 h. The MFC was regarded as the lowest concentration of test sample which did not produced a colour. All tests were performed in triplicates.

Evaluation of fungicidal and fungistatic capacity

The MFC/MIC ratio was calculated to determine whether fraction has a fungistatic (MFC/MIC ≥ 4) or fungicidal activity (MFC/MIC <4) [17].

Statistical analysis

The data were expressed as Mean±Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.05$ and linear regression) was carried out with XLSTAT 7.1.

RESULTATS

In vitro anthmintic activity

From the above experimental data, the polyphenol-rch fractions of stem bark from *lannea acida* was showed the anthelmintic activity. We noticed that, 100 mg/ml concentration showed the better activity than the other concentrations and it is almost comparable to the standard drug albendazole. The polyphenol-rch fractions at a concentration of 100mg/ml showed paralysis at 2.82 minutes and death of earth worm at 26.91 minutes. Results are summarize in Tables 1.

Table 1 Time taken for paralysis and death of earthworms after treating with various treatment groups

Group	Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Control	-	-	-
2	Standard	25	3.23±0.062	28.18±0.09
3	Fraction	25	8.10±1.54	75.46±0.58
4	Fraction	50	6.38±0.001	62.18±0.03
5	Fraction	100	2.82±0.003	26.91±0.54

Values are Mean ±SD (n=3).

Minimum inhibitory concentration (MIC) assay and Minimum fungicidal concentration (MFC)

As for the Minimum inhibitory concentration assay (MIC) and Minimum fungicidal concentration (MFC) of polyphenol-rich fractions, result varied according to microorganism and results are summarize in Tables 2 and 3.

Table 2 Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of polyphenol-rch fractions of stem bark from *lannea acida* A. Rich

Microorganisms	MIC (µg/ml)	MFC (µg/ml)
Candida parapsilosis ATCC 22019	$8.33±3.61^{c}$	$20.83±7.22^{d}$
Candida albicans ATCC 2091	$7.29±4.77^{b}$	$14.58±9.55^{b}$
Candida albicans ATCC 9002	$6.25±0.00^{a}$	$12.50±0.00^{a}$
Candida tropicalis ATCC 750	$9.28±5.41^{d}$	$17.71±12.63^{c}$
Candida krusei ATCC 6258	$6.25±0.00^{a}$	$12.50±0.00^{a}$

Values are Mean ±SD (n=3). Different letters in the same column indicate significant difference ($P<0.05$) for fraction.

Table 3 Fungicidal effect and Fungistatic effect of polyphenol-rch fractions of stem bark from *lannea acida* A. Rich

Microorganisms	MIC (µg/ml)	MFC (µg/ml)	Effect
Candida parapsilosis ATCC 22019	$8.33±3.61^{c}$	$41.67±14.43^{d}$	-
Candida albicans ATCC 2091	$7.29±4.77^{b}$	$14.58±9.55^{b}$	+
Candida albicans ATCC 9002	$6.25±0.00^{a}$	$12.50±0.00^{a}$	+
Candida tropicalis ATCC 750	$9.28±5.41^{d}$	$50.00±0.00^{c}$	-
Candida krusei ATCC 6258	$6.25±0.00^{a}$	$12.50±0.00^{a}$	+

The results are the means of number of the colonies ± standard deviations.
+: fungicidaleffect, **- :** fungistaticeffect

The MIC values of polyphenol-rich fraction were ranged from 6.25 to 9.28 g/ml and the MFC values were ranged from 12.50 to 50 g/ml. Fungicidal effect was noticed which candida species.

DISCUSSION

In the present study we evaluated *in vitro* at various concentration levels the efficacy of the polyphenol-rch fractions of stem bark from *Lannea acida* against earthworms. From the above experimental data, the polyphenol-rch fractions of stem bark from *lannea acida* was showed the anthelmintic activity. We noticed that, 100 mg/ml concentration showed the better activity than the other concentrations and it is almost comparable to the standard drug albendazole. The polyphenol-rch fractions at a concentration of 100mg/ml showed paralysis at 2.82 minutes and death of earthworms at 26.91 minutes. The complete cessation of motility and mortality of earth worm is a concentration-dependent effect. The polyphenol-rch fractions of stem bark from *lannea acida* have shown promising *in vitro* anthelmintic activity against earthworms. Several groups of plant secondary metabolites are considered the sources of chemicals responsible for wide anthelmintic activity [18]. Polyphenol compounds could be responsible for enhancing the anthelmintic action of the fraction. Therefore, the presence of secondary metabolites in this Anarcadiaceae might justify anthelmintic properties observed [11]. Anthelmintic effect of secondary metabolites would act directly on the earthworms. These secondary metabolites could disrupt the integrity of the cuticle of the parasite [19] The parasite tegument has been ascertained as the principal target site of different classes of synthetic drugs and natural anthelmintic products [20] Drugs like levamisole and its related compound are known to bind to nicotinic acetylcholine receptors by mimicking the action of acetylcholine. This binding induces a change in the postsynaptic membrane permeability causing muscle contraction, spastic paralysis and eventual death of earthworms [21]. Tannins are found to bind to free proteins in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite and cause death and this might be the reason for the anthelmintic activity of the fraction

[22].Concerning antifungal activity, we could be say that natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. The high proportion of active extracts in the assayed species, selected according to available ethnobotanical data, corroborates the validity of this approach for the selection of plant species in the search for a specific activity. In effect, we noticed that the polyphenol-rich fractions showed an interesting and demonstrated an antifungal properties against Candida species. These results could be explained by the presence of secondary metabolites in this Anarcadiaceae such phenolics and flavonoids which exhibited antifungal property [11].

CONCLUSIONS

The results obtained from this work confirmed the using of *Lannea acida* to treat intestinal worm infections and antifungal activity. The *in vitro* provided a rationale for the traditional use of this plant as anthelmintic and antifungal.This report confirmed the presence of the rich variety of bioactive compounds in the plant of the study and it leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. Therefore, further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed anthelmintic and antifungal activities.

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