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Antifungal Activity of *Pericopsis* (*Afrormosia*) *laxiflora* (Benth.) Bark on Ringworm Germs

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OA, OK and CA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors BGEK and YH managed the analyses of the study. Author KAE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Dermatophytes are responsible for ringworms that are very often found on the heads of children in Africa. In Côte d'Ivoire, ringworms have been the subject of several studies revealing fairly high frequencies.
Aims: The present work consisted essentially in studying the antifungal activity of the barks of *Pericopsis laxiflora*, a plant from the Ivorian pharmacopoeia on germs responsible for ringworm.
Methodology: The 70% hydroethanolic extract of the bark of *Pericopsis laxiflora* was prepared and tested on *Trichophyton mentagrophytes* and *Trichophyton rubrum*. In addition, by staining and precipitation tests, phytochemical sorting was carried out on this extract.

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Results: Faced with the hydroethanolic extract, *Trichophyton mentagrophytes* recorded a Minimun Inhibitory Concentration (MIC) which is equal to the Minimun Fungicidal Concentration(MFC) (MIC = MFC = 6.25 mg/mL). For the fungal strain of *T. rubrum*, the MFC obtained (100 mg/mL) was twice the MIC (50 mg/mL). The phytochemical study of this extract revealed the presence of sterols and polyterpenes, flavonoids and catechic tannins.
Conclusion: The results suggest that *P. laxiflora* extract could therefore be useful in the fight against dermatophytes.

Keywords: Pericopsis laxiflora; ringworm; Trichophyton mentagrophytes; Trichophyton rubrum; antifungal effect.

1. INTRODUCTION

Ringworms are conditions caused by the invasion of the hair by keratinophilic fungi called dermatophytes [1,2]. They are often contagious and due to lack of hygiene [3,4]. Despite the improvement in the hygiene level of African populations, ringworms of the scalp still constitute a frequent reason for consultation in dermatology [5].

In Côte d'Ivoire, ringworms remain relatively common where they have been the subject of several studies revealing fairly high frequencies [6,1]. Statistics continue to show an upsurge in fungal conditions and an increase in the resistance of many pathogens to current treatments [7,8].

Faced with this data and despite significant progress, the high cost of antifungal drugs and the fact that they are quickly ineffective remains a major problem for society. Indeed, certain dermatophytes are endowed with formidable faculties of adaptation and it is constantly necessary to find new drugs or new therapeutic combinations to fight against the emergence of resistant species [9,10]. An effective alternative to these chemical therapies is the development of phytotherapy, a large reservoir of active ingredients. Moreover, the multiple use of plants in traditional medicine continues to encourage researchers to give a scientific basis to several plant extracts and to purify the active molecules.

In traditional Ivorian medicine, *P. laxiflora* is used for the treatment of many infections: headaches, stomach ulcers, ringworms, snake bites, stomach aches, gastritis enteritis, heart pain, abdominal pain [11,12].

This plant is also used almost everywhere in the dry forests and Sudanese savannas of Africa. In Guinea, it is used against shigellosis, eczema, mycosis and colibacillosis [13]. In Ghana *P. laxiflora* is used in the treatment of malaria [14]. In Nigeria, this plant is used as an ancestral antiulcer in the Benoue region [15].

The present work consisted in evaluating the antifungal activity of the barks of *Pericopsis laxiflora* on germs responsible for ringworm. In addition, a chemical screening was done in order to know the main families of secondary metabolites to which we can attach the pharmacodynamic properties attributed to the barks of this plant.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consisted of the bark of *Pericopsis laxiflora*. The bark of this plant were collected in Lataha, a village located 10 km from Korhogo (Côte Ivoire), in July 2019 and identified at the National Floristic Center of Cocody (Abidjan, Côte d'Ivoire). Once collected, they have been thoroughly cleaned and then cut into small pieces to facilitate drying. Then, they were dried in the dark and at room temperature for two weeks. At the end of the drying, the bark was pulverized using an electric grinder (RETSCH brand, Type AS 200) to obtain fine powders.

2.2 Fungal Strains

The fungi used were *Trichophyton mentagrophytes* and *Trichophyton rubrum*. These fungal strains were supplied by the Microbiology Laboratory at the Félix Houphouet Boigny University of Cocody (Abidjan).

2.3 Preparation of the 70% Hydroethanolic Extract

The crude 70% hydroethanolic extract (codified E.heth) was prepared according to the method described by Zirihi et *al.*[16]: 100 g of bark powder of *P. laxiflora* were dissolved in one liter

(1 L) of a solution of cold water and ethanol (300 mL of cold distilled water for 700 mL of ethanol) then were homogenized in a blender. After homogenization at room temperature, the homogenate obtained was first wrung in a square of white fabric. Then, doubly filtered on cotton wool and once on 3 mm Whatman paper. The filtrate obtained was concentrated in an oven at 50 ° C for 24 hours. The extract obtained was weighed and stored in a sterile bottle and then stored in the refrigerator for the study of antifungal activity. The extraction yield was also determined.

2.4 Preparation of the Fungal Inoculums

The inoculum of each Trichophytona was prepared from young colonies of 4 to 5 days old. One (1) colony of each Trichophytona was taken and homogenized in 10 mL of sterile distilled water to obtain a concentration of fungal germs of 10^0. For the 1/10 dilution, 1 mL of this suspension was transferred and homogenized in 9 mL of sterile distilled water to have a final volume of 10 mL at a concentration of 10^{-1}. This suspension, the charge of which is estimated at 10^3Trichophyton cells, was used for seeding at a rate of 10 µL per tube.

2.5 Determination of the Antifungal Activity of the 70% Hydroethanolic Extract

One thousand (1000) Trichophyton cells contained in 10 µL of inoculum were inoculated by transverse streaks on the Sabouraud agar prepared from a concentration range of the plant extract. The cultures were incubated at 30°C for 5 days. After this incubation time, the Trichophyton colonies were first counted. Then, the percentage of survival was evaluated, compared to 100% of survival in the growth control tube which did not contain the plant extract [17,18]. The different antifungal parameters that are the Minimum Inhibitory Concentrations (MIC) and Minimum Fungicide Concentration (MFC) have been determined. The MIC is the minimum concentration of *P. laxiflora* extract that inhibits the growth of the two germs with the naked eye while MFC is the extract concentration which gives 99.99% inhibition compared to the growth control tube. The MIC was determined after five (5) days of incubation and the tubes were reincubated for an additional 24 hours to determine the MFC [19].

The IC_{50} was determined graphically from the sensitivity curve of each fungal strain against the different concentrations of the extract of *P. laxiflora*.

2.6 Phytochemical Test of the 70% Hydroethanolic Extract

This study was based on staining and precipitation tests in tubes and mainly targeted alkaloids, total polyphenols, flavonoids, saponins, tannins, sterols and polyterpenes because of their great importance for the health sector [20,21].

3. RESULTS

The 70% hydroethanolic extract of the bark of *Pericopsis laxiflora* gave a yield of 10.4%.Table 1 presents the different percentages of living

Table 1. Percentages of live colonies of *T. mentagrophytes* and *T. rubrum* facing 70% hydroethanolic extract

Fungal strains	Concentrations of 70% hydroethanolic extract (mg/mL)								
	100	50	25	12.5	6.25	3.12	1.56	0.78	TC
TM	-	0%	0%	0%	0%	40%	60%	90%	100%
TR	0%	0%	5%	20%	40%	70%	90%	-	100%

TM: T. mentagrophytes; TR: T. rubrum, TC: growth witness; -: not tested

Table 2. Antifungal parameters of the 70% hydroethanolic extract of the barks of *Pericopsis laxiflora* on the germs

Fungal strains	Extract	Antifungal parameters			MFC/MIC	Effect
		MIC (mg/mL)	MFC (mg/mL)	IC_{50} (mg/mL)		
Trichophyton mentagrophytes	Heth	6.25	6.25	5.00	1	Fungicide
Trichophyton rubrum	Heth	50	100	7.50	2	Fungicide

Heth: 70% Hydroethanolic extract; MIC: Minimun Inhibitory Concentration; MFC: Minimum Fungicidal Concentration

Table 3. Phytochemical study of the 70% hydroethanolic extract of the bark of *Pericopsis laxiflora*

E. heth	Chemical groups								
	Sterols and polyterpenes	Polyphenols	Flavonoids	Tannins		Quinones	Alkaloids		Saponins
				Gal	Cat		B	D	
	+	+	+	-	+	-	-	-	-

E.heth: 70% hydroethanolic extract; +: presence; -: absence; Gal: gallic; Cat: catechic; D: Dragendorff; B: Bouchardat

colonies facing the 70% hydroethanolic extract. It is noted that the 70% hydroethanolic extract of *P. laxiflora* completely inhibited the growth of *T. mentagrophytes* with concentrations above 6.25 mg/mL. As for the *T. rubrum* strain, it was totally inhibited only from the concentration of 50 mg/mL of the 70% hydroethanolic extract studied.

The fungal strains that were the subject of this study were sensitive to the 70% hydroethanolic extract tested according to a dose-response relationship. Faced with this extract, *T. mentagrophytes* recorded a Minimun Inhibitory Concentration (MIC) that is equal to the Minimun Fungicidal Concentration (MFC) (MIC = MFC = 6.25 mg/mL). For the fungal strain of *T. rubrum*, the MFC obtained (100 mg/mL) is twice the MIC (50 mg/mL).

The efficacy ratio (MFC/MIC) of the 70% hydroethanolic extract tested is equal to 1 against *T. mentagrophytes* whereas it was 2 against *T. rubrum*. Furthermore, the IC_{50} of this extract was 5.00 mg/mL for *T. mentagrophytes* while it was 7.50 mg/mL for *T. rubrum*. It is retained from this study that *T. mentagrophytes* is more sensitive to the crude 70% hydroethanolic extract of the bark of *P. laxiflora* than *Trichophyton rubrum*.

The value of the antifungal parameters (MIC, MFC, IC_{50}), as well as that of the efficacy ratio (MFC/MIC) for each germ are mentioned in Table 2.

The phytochemical study carried out from the hydroethanolic extract of the barks of *Pericopsis laxiflora* revealed the presence of sterols and polyterpenes, total polyphenols, flavonoids and catechic tannins. However, gallic tannins, alkaloids, quinones and saponins were absent in the same extract in the present study (Table 3).

4. DISCUSSION

The preparation of the hydroethanolic extract 70% of the bark of *Pericopsis laxiflora* gave a yield of 10.4%. The yield obtained is linked to the affinity that the secondary metabolites contained in the vegetable powder of *Pericopsis laxiflora* have for the binary solvent used (Water-ethanol, 30: 70, V/V). Several authors have also used this same type of solvent in identical proportions in the preparation of 70% hydroethanolic extracts in order to test their biological activities. Thus the 70% hydroethanolic extract prepared from *Parkia biglobosa* bark for antibacterial activity gave a yield of 12.35% [22].

As for N'Guessan [23], he had found a yield of 8.50% with the hydroethanolic extract 70% from the barks of *Combretum racemosum* during a study carried out on strains of *Staphylococcus aureus* resistant to methicillin (*S. aureus* Meti-R) .

Konate [24] also found different extraction yields of 11.56% and 13.50 respectively with *Funtumia elastica* and *Caesalpinia bonduc* with the same type of solvent (Water-ethanol, 30: 70, V/V).

The differences in yield observed with the same type of solvent could be explained by the chemical (intrinsic) nature of each plant used, the organ, the type of extraction and especially the size of the particles in the vegetable powder as well as the coefficient of solvent diffusion [25].

Furthermore, the choice of composite solvents would be to easily and quickly extract the active ingredients which could be responsible for the various biological activities. This idea is supported by Djahra [25] which states that the method of Zirihi*et al* [16] makes it possible to speed up the extraction process and minimize the time of contact of the solvent with the extract while preserving the bioactivity of the chemical constituents. Likewise, the course of this extraction at ambient temperature as well as the exhaustion of the solvent at reduced pressure makes it possible to obtain the maximum of the compounds and to prevent their denaturation or probable modification due to the high temperatures used in other extraction methods. Also, the current use of water in composite solvents for various extractions is linked to the fact that it is very polar and thus allows the extraction of several metabolites at once, in particular those which especially have in their formulas groupings of ketones and enolics [26].

The phytochemical tests carried out in this study on the 70% hydroethanolic extract of *Pericopsis laxiflora*, plant used for the treatment of ringworms report the presence of important secondary metabolites which are the sterols and polyterpenes, the tannins catechiques, the total polyphenols and flavonoids while alkaloids, quinones and saponins are absent. However, the work carried out by Kubmarawa*et al* [27] on the different families of molecules contained in the bark of *Pericopsis laxiflora* revealed the presence of saponins, glycosides and the absence of

tannins, flavonoids and alkaloids. Furthermore, the work of Ouattara*et al* [28] has also shown an absence of saponins in the hydroethanolic extract 70% of the plant studied. Several factors can explain these findings. In fact, the composition of a plant in secondary metabolites varies depending on the geographic location, the organ removed, the sampling period, the sampling time, storage conditions and the extraction solvent [29].

Analysis of the results of the antifungal effect shows that *Trichophyton mentagrophytes* is more sensitive than *Trichophyton rubrum* to the 70% hydroethanolic extract tested. In fact, faced with the studied extract, *T. mentagrophytes* recorded the smallest values of the antifungal parameters (MIC = CMF = 6.25 mg/mL) unlike *T. rubrum* (MIC = 50 mg/mL; MFC = 100 mg/mL). Furthermore, the extracts efficacy ratio (MFC/CMI) was 1 (*T. mentagrophytes*) and 2 (*T. rubrum*). However, the 70% hydroethanolic extract shows a fungicidal effect on the two fungal strains tested and responsible for ringworms in the population. This fungicidal power is explained by the fact that the efficacy ratios of the extract on the strains studied remain below 4 [30].

The fungicidal effect of the 70% hydroethanolic extract of the bark of *P. laxiflora* in this study could be attributed to the action of the sterols and polyterpenes, the catechic tannins and the flavonoids it contains. This action can be synergistic within the same group of secondary metabolites (several chemical groups). It can also be synergistic including two or more other families of secondary metabolites. Indeed, several studies have already shown that plant extracts containing secondary metabolites found in the extract which was the subject of this study had interesting biological activities.

Scalbert*et al* [31] reported that the antioxidant and antibacterial activities of plant extracts were probably linked to phenolic compounds, particularly phenols, flavonoids and tannins. Also N'guessan *et al* [32], reported an excellent correlation between the amounts of phenolic compounds and various anti-radical activities of plant extracts. Studies have also shown that the phenolic compounds (flavonoids, catechic tannins) and sterols and terpenes contained in the hydroethanol extract 70% of the leaves of *Ecliptaprostrata* have fungicidal activities [33].

In the work carried out by Yapi *et al* [33], the 70% hydroethanolic extract from the leaves *of Eclipta prostrata* showed a dose-dependent antifungal activity with better fungal potential on *T. mentagrophytes* (MFC = 6.25 mg/mL and IC_{50} = 0.54 mg/mL) than on *C. neoformans* (MFC = 25 mg/mL and IC_{50} = 0.75 mg/mL) and *C. albicans* (MFC > 50 mg/mL and IC_{50} = 6.25 mg/mL). Our 70% hydroethanolic extract presented a fungicidal power identical to that of these authors on the *Trichophyton mentagrophytes* strain (MFC = 6.25 mg/mL).

The antifungal effects of the ethanol and distilled water extracts of *Azadirachta indica*, *Jatropha curcas*, *Jatropha gossypifolia*, *Cassia alata, Anacardium occidentale* and *Aloe vera* at different concentrations (2.5-10 mg/mL) have also been proven against *Trichophyton mentagrophytes* and *Trichophyton rubrum* isolated from the skin of ringworm infected patients [34]. The results of these authors show that the extracts from these six plants are more active on *T. rubrum* compared to our 70% hydroethanolic extract (MIC = 50 mg/mL; MFC = 100 mg/mL).

The 95% hydroethanolic extract of the leaves of *Tetradenia riparia* was tested against three dermatophytes: *Trichophyton tonsurans, T. mentagrophytes and Microsporum audouinii*. The MICs of this crude extract on the fungal germs tested were between 62.5 and 250 mg/mL while the MFCs varied from 125 to 500 mg/mL [35]. Our 70% hydroethanolic extract showed an excellent fungicidal effect on *T. mentagrophytes* (CMF = 6.25 mg/mL) compared to the 95% hydroethanolic extract of the latter authors.

The various observations recorded in terms of the antifungal powers of these plant extracts would depend on the nature of the extract each plant, the concentration of chemical constituents, the methodology, the mode of action and especially the nature of the germ tested (sensitive or resistant). These intrinsic and extrinsic factors have already been reported by several authors [36,37].

The sensitivity of *Trichophyton mentagrophytes* and *Trichophyton rubrum* to the 70% hydroethanolic extract of *Pericopsis laxiflora* is of great importance because these strains are strongly implicated in infections of the skin and scalp. Therefore, any antifungal agent to which they are sensitive deserves special attention. In addition, the concentrations at which this extract remains active leads us to affirm that this plant

could be used against various pathologies linked to these fungal germs. This work justifies the use in a traditional environment of this plant as an antifungal especially in the fight against moths.

5. CONCLUSION

The work carried out has shown that the 70% hydroethanolic extract of *Pericopsis laxiflora*has a fungicidal effect on *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Minimun Fungicidal Concentration of hydroethanolic extract was 6.25 mg/mL on *Trichophytonmentagrophytes* against 100 mg/mL for *T. rubrum.*

Furthermore, the phytochemical sorting of the plant extract studied shows that the bark of *Pericopsis laxiflora* contains various secondary metabolites, including phenols, sterols and polyterpenes, gallic tannins and flavonoids.

Given the results obtained in the present work, the studied extract could, after toxicological studies including skin irritation test, be used as a phytomedicine to combat ringworms.

In our future work, we will focus on the toxicological study of the hydroethanolic extract 70% of the barks of *Pericopsis laxiflora* on laboratory animals.

Also, the future formulation of an ointment based on hydroethanolic extract of *Pericopsis laxiflora* would be a real hope and a big step in the fight against ringworms in order to be able to eradicate certain antifungal infections from where the valorization of this medicinal plant in Côte Ivoire.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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