



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article


September 2020 Vol.:19, Issue:2

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Antifungal Potential Activity of Extracts from the Stem Bark of *Harungana madagascariensis* Lam. Ex Poir (Hypericaceae)



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals



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Submission: 20 August 2020
Accepted: 26 August 2020
Published: 30 September 2020



HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: *Harungana madagascariensis*, *Trichophyton soudanense*, *Trichophyton interdigitalis*, 70% ethanolic extract, antifungal activity

ABSTRACT

Natural plant extracts could contain a variety of biologically active molecules. In this context, we evaluated the antifungal activity of ethanol and aqueous extracts prepared from the stem bark of *Harungana madagascariensis*. The double dilution method in tubes leaning over Sabouraud agar was used to evaluate the antifungal activity of the extracts of this plant. The 70% ethanolic extract of the stem bark of *Harungana madagascariensis* tested has antifungal activity on *Trichophyton Soudanense* and *Trichophyton interdigitalis*. The minimum fungicidal concentration (MFC) of the 70% ethanolic extract was 0.39 mg/mL on *Trichophyton soudanense* and 0.58 mg/mL on *Trichophyton interdigitalis*. The phytochemical screening carried out revealed the presence of large groups of chemical molecules such as alkaloids, flavonoids, polyphenols, sterols and terpenes, and saponins in the most active extract. The stem bark of *Harungana madagascariensis* therefore has antifungal activity on *Trichophyton soudanense* and *Trichophyton interdigitalis*. This result therefore justifies the use of the bark of this plant in the traditional treatment of superficial mycosis.

INTRODUCTION

Traditotherapy is a practice that our ancestors have used since ancient times for the treatment of various ailments. It still holds an important place today despite the progress of modern medicine. It is therefore up to researchers to work to explore this natural wealth of plants. This would make it possible not only to understand this medicine but also to enhance and rationalize it in the light of modern scientific techniques. Thus, new molecules could be isolated for the development of new drugs to combat germs that have become increasingly resistant to current antibiotics. This state of affairs is reinforced not only by the resurgence of certain infectious diseases but also by the difficulties encountered in their treatment^[1]. Although antifungal drugs are now available, the treatment of dermatophytes remains difficult, partly because of the limited number of really effective principles and partly because of the emergence of strains resistant to certain common antimycotics^[2-5]. Indeed, dermatophytes are mycoses responsible for diseases of the skin and its appendages known as dermatophytoses^[6,7]. They are keratinophilic fungi that secrete antigenic substances called trichophyton or epidermophyton depending on the genus^[8]. In traditional environments, *Harungana madagascariensis* is well known to have antimicrobial properties^[9]. The development of medicinal plants from the Ivorian flora has led us to focus on the antifungal activity of extracts from the stem bark of *Harungana madagascariensis*.

MATERIAL AND METHODS

MATERIAL

Plant material

The plant material used consisted of trunk bark of *Harungana madagascariensis* (figure 1) harvested in Abidjan (Ivory Coast) and identified by the National Floristic Centre of Ivory Coast.



A- Leafy fruiting branch

B- Leaves

Figure No. 1: *Harungana madagascariensis*

Microbial material

It consists of two clinical strains of *Trichophyton* provided by the Laboratory of Mycology of Pasteur Institute of Ivory Coast (*Trichophyton soudanense* and *Trichophyton interdigitalis*).

METHODS

Preparation of the plant extract

Aqueous extract

The stem bark of *Harungana madagascariensis* was harvested, sorted, washed and dried at room temperature at the Microbiology Laboratory of the Higher Teacher Training School (Ecole Normale Supérieure, ENS) and then crushed to obtain a vegetable powder which was used to prepare the various extracts. The aqueous extract was prepared according to the following method: One hundred grams (100g) of plant powder was macerated in one litre of distilled water by homogenisation in a blender. The homogenate obtained was successively filtered twice on cotton wool and then on Whatman 3 mm filter paper. The filtrate obtained was dehydrated using a "Prolabo" type oven at a temperature of 50°C^[10].

Ethanolic extract

The method of ^[11] was used to obtain the various 70% hydroethanol extracts of *Harungana madagascariensis*. A 70% hydroethanol solution (ethanol/water 70:30) was used for the preparation of hydroethanol extracts of *Harungana madagascariensis* in vials. One litre of the hydroethanolic solution and 100 g of *Harungana madagascariensis* powder were used for this purpose. The resulting mixtures were homogenised using a magnetic stirrer for 24 hours.

The homogenates were filtered on Whatman paper 3 mm in diameter and then under vacuum for 1 hour. The collected filtrates were concentrated in a rotary evaporator and then heated in an oven at 40°C for a complete drying for one week.

Preparation of culture media

The cultures of the two germs were made on Sabouraud medium. The incorporation of the various plant extracts into Sabouraud agar was done using the double dilution method, in inclined tubes ^[10]. All extracts were tested separately. For each plant extract, each set contains 10 test tubes containing the plant extracts and 2 control tubes, one of which is without plant extract, serving as a germ growth control, the other without germ and without an extract to control the sterility of the culture medium. For the 10 test tubes, the concentrations range from 50 to 0.098 mg/mL according to a geometric linkage of reason $\frac{1}{2}$. After incorporation of the extracts, all 12 tubes of each series are autoclaved at 121°C for 15 minutes and then tilted with a small cap at room temperature to allow cooling and solidification of the agar ^[10].

Antimicrobial testing

The inoculum is prepared from young cultures of *Trichophyton soudanense* and *Trichophyton interdigitalis*. The stock suspension (called 100) concentrated to 10⁶ cells/mL is first prepared by homogenizing one colony of each strain in 10 mL of sterilized distilled water. From suspension 100, a second suspension (10⁻¹) is prepared by diluting 1/10th of the first. The latter is concentrated to 10⁵ cells/mL. For each of the test tubes in each set of eight plant extracts, the culture of the germs was done on media previously prepared by the cross streaking (until exhaustion) of 10 µL of the 10⁻¹ suspension. This corresponds to 1,000 seeded cells. The cultures thus produced were incubated at 30°C. After 5 to 10 days of incubation at 30°C, the colonies of *Trichophyton soudanense* and *Trichophyton interdigitalis* were counted by direct counting with a colony counting pen (Bel-Art Scinceware serial number 23382). Growth in the 10 experimental tubes of each series was assessed as percentage survival, compared to 100% survival in the growth control tube ^[12].

The following antifungal parameters were determined by processing the experimental data: Minimum fungicidal concentration (MFC).

It is the concentration of extract in the tube that gives 99.99% inhibition compared to the growth control tube. Conversely, it is the concentration of extract in the tube that gives 0.01%

survival compared to the growth control. Concentration for fifty percent inhibition (IC_{50}). This is the concentration that gives 50% inhibition, estimated relative to the number of colonies counted in the growth control tube. It is a graphically determined parameter.

Phytochemical characterization

The extracts obtained were subjected to phytochemical sorting using reagents suitable for the search for alkaloids (solution of Dragendorff, Bouchardat and Valsen-Mayer), sterols and triterpenes (Lieberman reaction), phenolic compounds (2% ferric chloride), tannins (Stiasny and sodium acetate + $FeCl_3$ reaction), flavonoids (Cyanidin reaction), saponins (foam index) ^[13].

RESULTS

Antimicrobial testing

After 10 days of incubation at 30°C, a progressive decrease in the number of colonies of *Trichophyton sudanense* and *Trichophyton interdigitalis* was observed compared to the control as the concentrations of plant extracts increased in the experimental tubes. This is observed for all series of extracts. Clear and effective inhibitions were obtained at different concentrations in different extracts. MFC (minimum fungicidal concentration) values for both extracts are reported in Table 1. The experimental data translated into sensitivity curves are summarized in Figures 2 and 3. In general, all the curves for both extracts have a decreasing trend, with varying degrees of slope.

Table No. 1: Values of the antifungal parameters of the different extracts of *Harungana madagascariensis*

Different extracts from <i>Harungana madagascariensis</i>	Antifungal Parameters			
	<i>T. soudanense</i>		<i>T. interdigitalis</i>	
	MFC (mg/mL)	IC ₅₀ (mg/mL)	MFC (mg/mL)	IC ₅₀ (mg/mL)
Aqueous extract	> 50	> 50	> 50	> 50
70 % Ethanolic extract	0,39	0,57	0,39	0,58

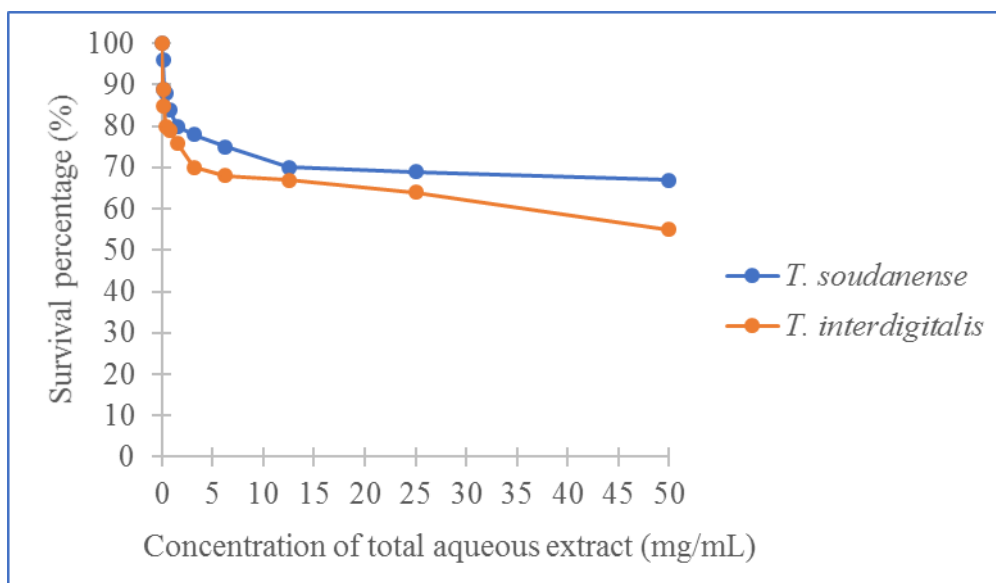


Figure No. 2: Dose-response action of the aqueous extract of *Harungana madagascariensis*

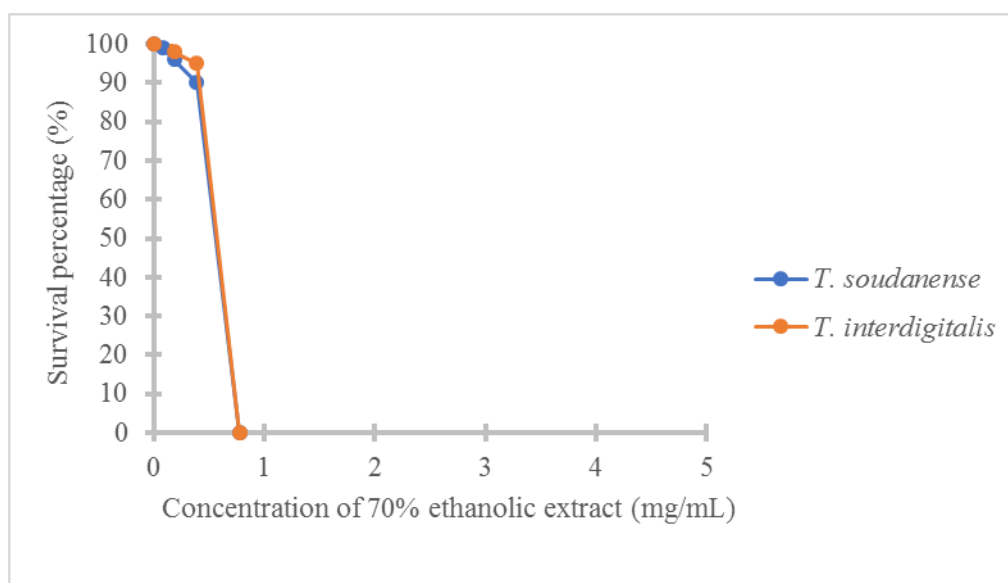


Figure No. 3: Dose-response action of the 70% ethanolic extract of *Harungana madagascariensis*

Phytochemical screening

Table 2 gives the results obtained during the phytochemical screening of the ethanolic and aqueous extract of *Harungana madagascariensis*. The tests carried out reveal the presence of various secondary metabolites.

Table No. 2: Chemical compound found in the 70% ethanolic extract and aqueous extract of *Harungana madagascariensis*

Species	Extract	Chemical groups							
		Sap	Flav	Terp/ster	Tannins		Quin	Alc	Poly
					Gall	Cathé			
<i>Harungana madagascariensis</i>	ETA	++	++	+	-	+	-	+	++
	EE 70 %	+	++	++	-	-	-	+	++

TAE : total aqueous extract; EE 70 %: 70 % ethanolic extract.

+: presence of the chemical group

- : absence of the chemical group

+++ : abundant presence of the chemical group

Sap: saponins; Flav: flavonoids; Terp / Ster: Terpenes / Sterols; Gall: gallic ;

Cathé: catechic; Quin: quinones; Alc: alkaloids; Poly: polyphenol

DISCUSSION

In order to verify the anti-infectious virtues granted to *Harungana madagascariensis*, we first prepared the aqueous extract since water is the most used solvent for the preparation of traditional recipes. Then, we wanted to know if we could improve the activity of the aqueous extracts by using another solvent for the extraction. Based on the work of ^[10], we chose 70/30(v/v) ethanol-water mixture as the extraction solvent. It is from this solvent that we prepared the 70 % ethanolic extract. L analysis of the results of antifungal tests with extracts of *Harungana madagascariensis* shows that *Trichophyton soudanense* and *Trichophyton interdigitalis* are more sensitive to the 70% ethanolic extract tested. This could be justified by the difference in chemical composition between the aqueous extract and the ethanolic extract. This leads us to deduce that from the point of view of their mass, the active ingredients might not be macromolecules. They are certainly molecules of medium size, soluble in an organic solvent. The presence of terpenes and sterols in large quantities, alkaloids, polyphenols, flavonoids in the ethanolic extract would certainly be responsible for the activity observed in

vitro ^[14,15]. Our results are comparable to those of ^[9]. Indeed this author showed that the aqueous extract of the stem bark of *Harungana madagascariensis* had a pronounced antibacterial activity.

CONCLUSION

This study situates us on the real anti-infectious potential of *Harungana madagascariensis*. The results of this investigation allowed us to understand that the strains of *Trichophyton soudanense* and *Trichophyton interdigitalis* are sensitive to the 70% ethanolic extract tested and that this sensitivity is dose-dependent.

REFERENCES

- [1] Mubié M., Bonga G.M., Vangba -Manda M., De Souza C. Action antifongique d'une huile végétale sur *Trichophyton rubrum*. Rewe méd. Pharm. Afr, 1998 ; 11 : 185-195.
- [2] Dzoïem J.P., Tangmouo J.G., Manfouo J.R., Lontsi D., Etoa F. X, Lohouc P.J. Activité antifongique des extraits de quelques plantes médicinales camerounaises Nig. J. Nat. Prod. And Med, 2002 ; 10 : 94-96.
- [3] Sheehan D.J., Hitchcock, C.A., Sibley C.M. Current and emerging azole antifungal agents Clin. Microbiol. Rev, 1999 ; 12(1), 40–79.
- [4] Young L.Y., Hull C.M., Heitman J. Distribution of ergosterol biosynthesis confers resistance to amphotericin B in *Candida lusitanae* Antimicrob. Agents Chemother, 2003 ; 47 : 2717–2724.
- [5] Rex J.H., Rinaldi M.G., Pfaller M.A. Resistance of *Candida* species to Fluconazole Antimicrob. Agents Chemother (1995) 39, 1–8.
- [6] Basex J, Loche F. Infection à dermatophytes de la peau glabre et des plis : Diagnostic et traitement. Revue du praticien. Paris : 1996 : Vol. 46. 1135-1140.
- [7] Geotilini M., Danis M., Briicker G., Duo B., Richard L. D. Diagnostic en parasitologie. Paris : Masson, 1983 : 153 P.
- [8] Bourée P. Maladies tropicales. Paris : Masson. 1987 : 396 P.
- [9] Toty A A., Guessennd N., Bahi C ., Kra A. M ., Otokore D. A., Dosso M. Évaluation *in-vitro* de l'activité antibactérienne de l'extrait aqueux de l'écorce de tronc de *Harungana madagascariensis* sur la croissance de souches multi-résistantes, Bulletin de la Société Royale des Sciences de Liège, 2013 ; 82 :12 – 21.
- [10] Zirihi G. N., Kra A. M., Guede-Guina F. "Evaluation de l'activité antifongique de *Microglossapyrifolia* (LAMARCK) O. KUNZE (ASTERACEAE) << PYMI>> sur la croissance *in vitro* de *Candida albicans*". Revue de Med. et Pharm. Afr, 2003 ; 17 :11-18.
- [11] Guédé-Guina F., Kra A. K. M., Vangah-Manda M., Bonga G. M. F. Inhibition par MISCA-F1 de la croissance de *Aspergillus fumigatus*, *Candida albicans* et *Cryptococcus neoformans* opportunistes du SIDA. Afr. Biomédicale, 1997 ; 2 (1) : 11 – 16.
- [12] J. A. ACKAH, "Spectre anti-infectieux de MISCA-F3 sur la croissance *in- vitro* de *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* et *Trichophyton rubrum*". Mémoire de DEA de biotechnologies, option pharmacologie-microbiologie. Université de Cocody, (Côte d'Ivoire), 2004 ; 34p.
- [13] Latifou L., Étude phytochimique et Activité Antipaludique de substances Naturelles issues de plantes Béninoises. Thèse de Doctorat. Univ. Louis Pasteur de Strasbourg/ Univ. d'Abomey-calavi, (Bénin), 2005 ; 280 p
- [14] Bagré I., Bahi C., Ouattara K., Zirihi G.N., Djaman A.J., Coulibaly A., N'Guessan J.D. Étude botanique et exploration de l'activité antifongique de *Morinda morindoides* (Baker) Milne-Redh. sur la croissance *in vitro* de *Cryptococcus neoformans*. Phytothérapie, 2011 ; 9 : 136-141.

[15] Gnahoué G., Béné K., Coulibaly K. Etude botanique, screening phytochimique et activité anticandidosique *in vitro* de *pyncanthusangolensis* (welw.) warb. (Myristicaceae). European Scientific Journal, 2015 ; 11 (36): 1857-7881.

