

**PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITIES
OF *COMBRETUM RACEMOSUM* (P. BEAUV.) (COMBRETACEAE)
EXTRACTS AGAINST FIVE CLINICALS FUNGALS STRAINS.**

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ABSTRACT

In an effort to help people get real benefit of the effectiveness of medicinal plants which are less expensive and affordable, aqueous and ethanolic extract of *Combretum racemosum* has been tested on the *in vitro* growth of *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans*. The antifungal tests were performed on Sabouraud medium in which the plant extract were incorporated according to the method of inclined tube double dilution. Concentrations were ranging from 200 to 0.78 mg /mL. The results showed that all the extracts tested have led to a significant inhibition of *in vitro* growth of all the strains. Ethanolic extract showed best antifungal activity.

KEYWORDS: *Combretum racemosum*, extraction, antifungal, phytochemical screening.

INTRODUCTION

Due to the increase of the number of immunocompromised individuals, fungal infections have increased in the last two decades.^[1] Among them, opportunistic systemic mycoses are associated with high mortality rates.^[2] This is essential for systemic mycoses that are typically in immunocompromised patients as toxicities are induced by commercial antifungal drugs. The side effects are often observed in these patients because of the dosage and prolonged therapy.^[3]

The inefficiency of current treatments led populations stripped to direct itself towards pharmacopeia plants for their cure.^[4; 5; 6; 7; 8] In fact, medicinal plants use by populations exists since old times. More than 80% of populations use plants for their primary health care.^[9] However the badly using of medicinal plant could have health accidents (renal insufficiency, cardiopathies and intoxications).

To help populations from medicinal plants use, our team had work to extract active principles from medicinal plants by checking their therapeutic virtues and in order to give them scientific basis. Among many plants requested by faith healers, *Combretum racemosum* (Combretaceae), a straggling shrub widespread across Africa is traditionally reputed to be anthelmintic and antimicrobial for genito-urinary and gastrointestinal infections.^[10] To check these anti-infectious virtues, antifungal activities of extracts of *Combretum racemosum* is improved on the *in vitro* growth of *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans*.

MATERIAL AND METHODS

Plant Material

The leaves of *Combretum racemosum* were collected in May, 2014 from Daloa, Côte d'Ivoire Western Africa and identified by Herbarium of the floristic national center, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire, western Africa.

Microbial isolates used

Strains of *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans* (ref 22590-5777; 22589-5778) have been provided by the Department of Mycology of the Faculty of Medical Sciences, University of Félix Houphouët Boigny-Abidjan. These strains were isolated from people living with HIV (PLHIV) in the Department of infectious diseases at University Teaching Hospital Treichville in Côte d'Ivoire.

Preparation of plant extracts

The leaves were collected, washed, dried under shade at a temperature between 25 and 27°C and made into a fine powder using an electric grinder type IKA-MAG. The total aqueous and ethanolic (70%) extracts were prepared as follows: One hundred (100) grams of powder were extracted by homogenization in blender with one liter (1L) of distilled water (mixer). After six cycles of crushing, the homogenate obtained was first centrifuged in a square of fabric

and then filtered successively twice on absorbent cotton and once on Whatman filter paper 3 mm. The obtained filtrate was concentrated using under vacuum at 60°C. The powder obtained is the crude aqueous extract. The ethanolic (70%) extract was prepared using the same method. Both extracts were tested separately.^[11, 12]

Preparation of culture medium

We used Sabouraud agar (Bio-RAD/Réf: 64449, Lot: 8B2212) buffered to pH 5.7 for this test. The medium was prepared according to the instructions of the manufacturer's protocol. The incorporation of plant extracts in Sabouraud agar was done by using the double dilution method agar slopes.^[13, 14] For each plant extract, each series consists of 10 test tubes. Eight (8) of these test tubes containing plant extract. And the other 2 tubes are considered as control tubes. Among these 2 tubes, one without extract was used as witness of germs growth control while the other without germs and extract was used as witness of culture medium sterility control. For 8 test tubes concentrations ranging from 100 to 0.78 mg/mL binding by a geometrical reason of 1/2. All tubes were autoclaved (121°C for 15 min.), then inclined at room temperature to allow cooling and solidification of the medium.^[11, 12, 13, 14] Ketoconazole is used as drug control.

Antimicrobial test

Culture of germs on previously prepared medium were made by seeding 1000 cells of each strain. The cultures were incubated at 30°C for 48 hours for *Aspergillus fumigatus*, *Aspergillus flavus* and *Cryptococcus neoformans* and at 30°C for five days for *Trichophyton rubrum* and *Trichophyton mentagrophytes*. After this incubation time, colonies were counted by direct counting with a colony counting pen (CEINCEWARE serial number 23382) and the growth in the 10 experimental tubes was evaluated on percentage survival, compared to 100% of survival in the pilot tube of growth control. Data processing permit to see fungicidal concentration minimal (FMC) values and determine 50% of inhibition concentration (IC₅₀) values graphically (on extracts activity curves).^[15;16] All the tests were made in triplicates.

Phytochemical analysis of the plant extract

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids, flavonoids, quinones and glycosides in accordance with Trease and Evans (1989) and Harborne (1998) with little modification.^[17; 18]

RESULTS AND DISCUSSION

Antifungal essay

All the extracts of *Combretum racemosum* were active against all the fungal strains. However the report/ratio of CMF showed that the Ethanolic extract has been more active than the aqueous extract (Table 1; 2 and 3).

Combretum racemosum commonly known as Christmas rose belongs to the family Combretaceae. The plant has been used for several years in African traditional medical practices and as a condiment in soups. It is a shrub indigenous to the tropical and pan tropical regions. In addition to its anthelmintic and antimicrobial properties, the plant is also used for the treatment of haemorrhoids, convulsive coughing, tuberculosis, toothache and male sterility.^[19, 20]

In the present study *C. racemosum* leaves extracted with water and hydroalcohol were investigated for its antifungal potentiality against *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans* clinically important fungal strains.

Both extracts were active against all the strains. However the report/ratio of CMF and IC₅₀ showed that the hydroalcoholic extract has been more active than the aqueous extract. In a previous study Onocha *and al* (2005)^[10] showed that ethylacetate and methanol extracts of *C. racemosum* inhibited 5 clinical bacteria to different degrees.

Table 1: Antifungal Parameter Values of Aqueous extract

		<i>A. fumigatus</i>	<i>A. flavus</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>C. neoformans</i>
Aqueous extract	CMF (mg/mL)	50	50	50	200	100
	CI ₅₀ (mg/mL)	4 ± 1.1	11 ± 2.1	5.5 ± 0.6	22 ± 2.7	12.5 ± 1.5

Table 2: Antifungal Parameter Values of Ethanolic extract

		<i>A. fumigatus</i>	<i>A. flavus</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>C. neoformans</i>
Ethanolic extract	CMF (mg/mL)	0.048	25	12.5	200	100
	CI ₅₀ (mg/mL)	0.01 ± 0.002	1.56 ± 0.4	0.24 ± 0.09	21 ± 2.01	4.5 ± 1.3

Table 1: Antifungal Parameter Values of Ketoconazole

		<i>A. fumigatus</i>	<i>A. flavus</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>C. neoformans</i>
Ketoconazole	CMF (mg/mL)	0.8 .10 ⁻³	48. 10 ⁻³	1.5.10 ⁻³	195. 10 ⁻³	97.5. 10 ⁻³
	CI ₅₀ (mg/mL)	0.042. 10 ⁻³ ± 0.01. 10 ⁻³	0.07. 10 ⁻³ ± 0.009. 10 ⁻³	0.043. 10 ⁻³ ± 0.008. 10 ⁻³	0.2. 10 ⁻³ ± 0.07. 10 ⁻³	0.08. 10 ⁻³ ± 0.012. 10 ⁻³

Phytochemical screening

Result of preliminary screening are presented in Table 4.

Investigations on the phytochemical screening of *Combretum racemosum* leaves extracts revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *Combretum racemosum*. These secondary metabolites exert antimicrobial activity through different mechanisms. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with prolinerich protein^[21] resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007)^[22] reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery.^[23]

Table 4: Phytochemical composition of *Combretum racemosum* extracts

<i>Combretum racemosum</i>		
	Aqueous extract	Ethanolic extract
Flavonoids	+	-
Saponins	+	++
Tanins Catéchics	+	++
Tanins gallics	+	++
Phenolics Compounds	+	-
Quinones	-	-
Steroids	+	++
Cardiac glycosides	+	++
Alkaloids	+	++

-Absence + Presence.

CONCLUSION

This study has allowed us to highlight the anti-infectious property attributed to this plant. Both extracts of *Combretum racemosum* tested showed good antifungal activity and this activity was more pronounced with the ethanolic extract. Both crude extract acts on germs in a dose response relationship.

We suggest to perform *in vivo* investigations to find out more information about the treatment of fungal infections.

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