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In vitro evaluation of the antifungal activity of extracts of *Baillonella toxisperma* (Pierre), a Sapotaceae, on the growth of some human pathogenic yeasts

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An ethnopharmacological survey concerning medicinal properties of *Baillonella toxisperma* (Pierre) was carried out by interview with the indigenous population of Dimako village situated in the East region of Cameroon. The result showed that the plant is implicated in the treatment of many infections among which is fungal infections. To confirm the antifungal property of *B. toxisperma* (Pierre), the barks and leaves of the plant were collected and serial extractions in water, hydro-ethanol mixture (3:7), ethanol, methanol and ethyl acetate were performed *in vitro*. One part of the hydro-ethanol (3:7) extract was degreased by mixing in water-hexane mixture (1:1). The extracts were then tested *in vitro* against *Candida albicans, Candida parasilopsis, Candida* sp. responsible for superficial, deep or systemic mycosis and against *Cryptococcus neoformans* responsible for sub-acute meningitis in immunodeficient individuals. The susceptibility of yeasts to plant extracts was evaluated using the wells diffusion method and yeasts growth inhibition parameters were evaluated according to the proposed National Committee for Clinical Laboratory Standards (NCCLS) M27-A2 standard guidelines (2002). The minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) determined were between 0.93 and 30.0 mg/ml. The extracts were fungicidal on clinical yeasts tested with MFC/MIC ratio of 1 or 2. The hexane phase HT2 from the hydro-ethanol crude extract of the barks gave the best antifungal activity on *C. neoformans,* with a MIC of 0.93 mg/ml and a MFC of 1.87 mg/ml. This activity was similar to the one obtained with fluconazole. Phytochemical screening revealed the presence of polyphenols, phenols, tannins, flavonoids, steroids, alkaloids, saponins, phlobatannins, triterpenes, anthocyanins, cardiac glycosides, leucoanthocyanins and fats, which are bioactive substances. The results could explain scientific validation to the traditional medical uses of *B. toxisperma* (Pierre) to treat fungal infections.

Key words: Cameroon, *Baillonella toxisperma* (Pierre), ethnopharmacology survey, fungal infections, bioactive compounds.

INTRODUCTION

Fungal infections are global public health problems in Africa and particularly in Cameroon. The prevalence of

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resistance to antifungal agents has significantly increased in the past decade (Tasleem et al., 2011). Furthermore, the problem of fairly higher toxicity and limited numbers of effective drugs now available underline the necessity to discover new antifungal compounds. Medicinal plants have been used for centuries as remedy for the treatment of various diseases and are the major sources of drugs by virtue of their high secondary metabolites content (Nostro et al., 2000). The World Health Organization had recognized traditional herbal medicine as a building block of primary health care. Many investigators have evaluated the bioactivity of plant extracts and the isolated constituents against the serious infectious organisms (Parekh and Sumitra, 2006). History shows that plants have been an important source of medicines against microbial infections. The compounds isolated from plants such as 2-decanone, hydroxydihydrocornin-aglycones, various indole derivatives and isoflavanones are reported to have interesting antifungal activities (Tasleem et al., 2011).

Baillonella toxisperma (Pierre) is a plant used in traditional medicine by many communities in Cameroon. This plant is used in the treatment of over 50 diseases among which are microbial infections. *B. toxisperma* (Pierre) grows in primary tropical rain forest in hot and humid climates (Louppe, 2005; Angerand, 2006). In Cameroon, it is found abundantly in the East and South regions. Several studies have already been conducted on *B. toxisperma* (Pierre) but to the best of our knowledge the antimicrobial property of this plant has never been studied before. Nothing has been published on its antifungal activity. Debroux (1998) and Ngueguim et al. (2009) reported, respectively that this plant is traditionally used to cure microbial infections like rheumatism, toothache, hemorrhoids, injury, sexually transmitted infections, diarrhea and malaria. Decoction of barks or leaves is traditionally drunk to treat systemic candidiasis or is rubbed on the skin to prevent and to treat vaginal and oral mycoses (Bouopda, 2013). Almond oil of fruits of *B. toxisperma* (Pierre) is used to cure superficial infections like ringworms (Laird, 2000).

To validate this ethnomedicinal information, we have performed an ethnopharmacological survey on the antimicrobial properties of *B. toxisperma* (Pierre) in Dimako village situated in the east region of Cameroon. This village was chosen according to the socio-cultural importance that the indigenous population of this locality confers to this plant and according to the frequency of its utilization to treat microbial infections by the indigenous population.

Candida species and *Cryptococcus neoformans* remain the most common causes of invasive opportunistic fungal infections (Kao et al., 1999). Among *Candida* species, *Candida albicans* and *Candida parasilopsis* are responsible for extremely varied clinical manifestations, ranging from superficial to deep or systemic mycosis and *C. neoformans* (Pierre) is the causative agent of sub-acute meningitis in immune depressed persons. These infections can lead in certain cases to the death of the patient. In this framework, the antifungal activity of *B. toxisperma* (Pierre) extracts was evaluated *in vitro* against some clinical yeast among which *C. albicans, C. parasilopsis, Candida* sp. and against *C. neoformans*.

MATERIALS AND METHODS

Ethnopharmacology survey

The ethnopharmacological survey was carried out from the 11th to the 29th August, 2012. One hundred dwellers in Dimako village among whom 70 men, 21 women and 9 children were submitted to an interview concerning local names of *B. toxisperma* (Pierre), description of the part of the plant used as a remedy (for example, barks, leaves, roots or fruits), mode of preparation (for example, decoctions or infusions) and mode of administration of the recipes. They were equally questioned because of their equal access to natural medicines. The provided information was collected using a questionnaire approved by Department of Biochemistry of University of Yaoundé I. The data collected permits us to determine number of citations for the medicinal uses and to calculate percentage of citations of each part of *B. toxisperma* (Pierre) in the treatment of ailments.

$$\text{Percentage of citations of each part} = \frac{\text{Number of citations of each part}}{\text{Total number of citations}} \times 100$$

Plant

Barks and leaves of *B. toxisperma* (Pierre) were harvested in Dimako on September 15th, 2012. The botanical identification was confirmed at the National Herbarium of Yaoundé-Cameroon, by comparison with specimen No. 54060/HNC.

Fungal strains

Fungi strains were gratefully given by “Centre Pasteur du Cameroun” (Yaoundé) and were made up of four yeasts which are *C. albicans, C. parasilopsis, Candida* sp and *C. neoformans*. These yeasts were clinically isolated in patient, identified and stored at ± 4°C in a refrigerator. *Candida* sp was identified like *Candida* genre but her specie has not been determined.

Methodology for extraction and antifungal screening

Plant extracts

About 2000 g of leaves and barks each was dried in the shade and away from moisture and then crushed using an electric grinder. The extraction was performed in ethanol, methanol, water-ethanol mixture (3:7), ethyl acetate and water. Samples of 200 g of bark powder and 120 g of leaf powder were weighed and macerated in 1000 ml of solvent for every system for the bark and 600 ml in each solvent system for the leaves, for a period of 3 days at room temperature. The macerate obtained was filtered through Watman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator. The extractions were repeated three times. A portion of 10 g of the ethanol extract of leaves or barks was subjected to liquid-liquid partition in 400 ml of water-hexane mixture (1:1) by emulsion. The aqueous and hexane phases obtained from this

partition were separated and concentrated. Four hundred milligrams of each extract were weighed out exactly and dissolved in 4 ml of dimethyl sulphoxide (DMSO) 10% to give a stock solution at 100 mg/ml used for the biological tests.

Phytochemical screening

Phytochemical tests were carried out using standard procedures described by Harborne (1998) and Edeoga et al. (2005).

Antifungal screening

Preliminary susceptibility test to plant extracts: From a 48 h culture of each clinical yeast, the inoculum was prepared by suspending in 1 ml of 0.85% NaCl saline solution, a pure colony of clinical yeast (National Committee for Clinical Laboratory Standards (NCCLS), 2002). Seeding of 100 µl of each inoculum was realized on the surface of Sabouraud dextrose agar (SDA) medium previously cast to a thickness of 4 mm, in Petri dishes of 90 mm in diameter. The inoculated dishes were dried at room temperature under a fumes cupboard. After 15 min, vertical cylindrical wells of 6 mm of diameters were made on these media using pasteur pipettes. To limit the spread of extracts in the agar, the bottom of the wells were blocked with a few drops of SDA, then the fixed volumes of 50 ml stock solutions of the extracts (100 mg/ml) or of fluconazole (40 mg/ml) were distributed in each well separately. Influence of DMSO 10% on the fungal growth was evaluated (negative control). After a pre-diffusion of the test substances for 20 min at room temperature, the plates were incubated at 35°C for 48 h. The diameters of halos of inhibition around the wells were measured using a caliper. Each test was performed three times (Singh et al., 2009).

Evaluation of the inhibition parameters: The inhibition parameters of yeast growth were evaluated according to the modified M27-A2 broth microdilution method described by NCCLS (2002). This involved preparing double dilutions of tested substances in 100 µl of SDB medium into the wells of a microtiter. The range of final concentrations tested were 0.46 to 30.0 mg/ml for each plant extract and 0.03 to 3.75 mg/ml for fluconazole. Each serial dilution was performed in triplicate. The fungal inoculum was prepared in 0.85% NaCl saline solution from a 48 h of clinical yeast culture on SDA and adjusted to 2.5×10^5 CFU/ml. Volumes of 30 µl of this inoculum were distributed to all the wells of the microtiter. A line of the plate without plant extract served as a control for the growth of the organism (negative control) and another (without plant extract and without inoculum) served as sterility testing medium (positive control). The plates were sealed with parafilm paper and the preparations were incubated at 35°C for 48 h. Phenol red was used as a color indicator and after incubation; the minimum inhibitory concentration (MIC) was retained as the lowest concentration for which no color change of the medium was observed. MFC were determined by subcultures. 50 µl of the contents of wells with concentration greater than or equal to MIC were introduced in 150 µl of new SDB medium. After 72 h of incubation at 35°C, subcultures for which there was no resumption of growth corresponded to MFC.

Statistical analysis of data

Statistical package for social sciences (SPSS) 16.0 software for Windows was used for statistical analysis. The inhibition diameters of the growth of yeasts by extracts during susceptibility tests were analyzed using one-way analysis of variance (ANOVA). P values < 0.05 were considered as significant. The results were expressed as means ± standard deviations.

RESULTS

Ethnopharmacological information

The inquiry about local names and the indigenous medicinal properties of *B. toxisperma* (Pierre) was performed in Dimako village. Commonly called Moabi, Ayap and Adjap by indigenous population of this village, *B. toxisperma* (Pierre) is traditional medicinal plant implicated in the treatment of many diseases among which microbial infections like Fungal infections, malaria, rheumatism, gastro-intestinal infections, ringworm, toothache, pulmonary infections and hemorrhoids. Parts of the plant commonly used are barks, leaves, latex and almond oil. Percentage of citations of medicinal uses of different parts has been calculated and the result show that barks and almond oil are the most used parts with proportions of 42.91 and 22.38%, respectively. Table 1 reports ailments, parts of the plant used to prepare medicinal potions, mode of administration and number of citations of parts of the plant in the treatment of theses ailments. Figure 1 reports percentage of citations of each part of *B. toxisperma* (Pierre) in the treatment of ailments.

Extraction and antifungal screening

The barks and leaves of *B. toxisperma* (Pierre) were extracted and their antifungal activities were tested. Table 2 reports the results obtained from phytochemical screening of the different extracts. The results obtained from preliminary susceptibility tests of yeast to extracts and from antifungal screening tests showed that the yeasts were sensitive to all plant extracts and fluconazole. The inhibition diameters obtained from barks crude extracts varied from 7.11 ± 0.54 to 17.65 ± 0.54 mm and those obtained from leaves crude extracts varied from 7.33 ± 0.12 to 15.76 ± 0.17 mm. The solvent of dissolution of the extracts DMSO 10% has not influenced the yeasts growth (absence of inhibition zone around of well). The results obtained from inhibition parameters showed that MIC_s determined ranged from 0.93 and 40 mg/ml and MFC from 1.87 to 40 mg/ml. The antifungal activities of different extracts and fluconazole are reported in Tables 3 and 4, respectively. The MFC/MIC ratio was calculated. According the classification of Fauchère and Avril (2002) adapted to fungi by Nyegue (2006), an antifungal is fungicidal when MFC/MIC = 1 or 2, fungistatic when 4 < MFC/MIC < 16 and tolerant when MFC/MIC > 32. The extracts of *B. toxisperma* (Pierre) are fungicidal on tested strains with MFC/MIC = 1 or 2.

DISCUSSION

Ethnopharmacological survey showed that *B. toxisperma*

Table 1. Traditional medicinal property of *B. toxisperma* (Pierre).

Ailment	Part used	Preparation/mode of administration	Number of citations
Fungal infections	Barks or leaves	Decoction/oral or rub	17
Pulmonary infections	Latex	Inhalation	05
Malaria and fever	Leaves	Decoction/oral	20
	Barks	Steam bath and bath	20
Backache	Latex	Rub	14
Wounds			09
Rheumatism	Almond oil	Rub	07
	Barks	Decoction or infusion/oral	16
	Latex	Inhalation	10
Gastro-intestinal infections	Latex		02
	Barks	Decoction/oral	21
	Leaves		21
Ringworm	Almond oil	Rub	13
Itches			23
Scabies			17
Toothache	Barks	Decoction or infusion/oral	15
Leucorrhea			11
Hemorrhoids			15
Sinusitis	Latex	Inhalation	12
Total number of citations			268

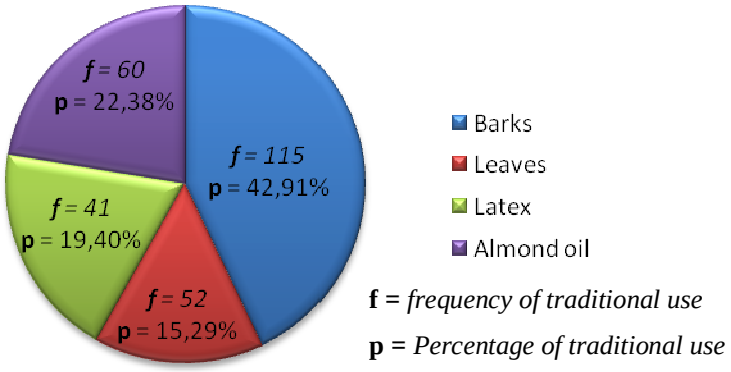


Figure 1. Frequency and percentage of traditional use of each part of *Baillonella toxisperma* (Pierre).

(Pierre) is of great importance in the treatment of various ailments. Ailments recorded in the interviews with dwellers in Dimako village were in majority infectious diseases like fungal infections, malaria, pulmonary infections, rheumatism, itches, scabies, toothache, hemorrhoids, leucorrhea, wounds, gastro-intestinal infections and ringworm. According to the number of citations for medicinal uses (Table 1), fungal infections appear to be one of the most important group of ailment to be treated using *B. toxisperma* (Pierre) extracts. Decoction or infusion of barks and leaves has been cited in the treatment of fungal diseases. This potion is

Table 2. Phytochemical screening of *Baillonella toxisperma* (Pierre) extracts.

Phytomolecules tested	Vegetal extracts													
	Barks							Leafs						
	T1	T2	ET2	HT2	T3	T4	T5	S1	S2	ES2	HS2	S3	S4	S5
Saponosides	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Polyphenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	-	+	+	+	+	+	+	-	+	-	+
Phlobatannins	+	+	-	-	+	+	+	-	-	-	-	-	-	-
Flavonoids	+	+	+	-	+	+	-	-	+	+	-	-	+	-
Anthocyanins	+	+	-	+	+	+	+	-	-	-	-	-	+	+
Leucoanthocyans	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	+	+	+	-	+	+	+	-	-	-	-	-	+	-
Steroids	+	+	+	+	+	-	-	-	+	-	+	-	+	+
Cardiac glycosides	+	+	+	-	+	+	-	-	-	-	-	-	-	-
Lipids	+	+	-	+	+	+	+	-	+	-	+	-	+	+

T1 and S1 = aqueous extracts, T2 and S2 = hydro-ethanol extracts, T3 and S3 = ethanol extracts, T4 and S4 = methanol extracts, T5 and S5 = ethyl acetate extracts, ET2 and ES2 = aqueous phase of water-hexane partition, HT2 and HS2 = hexane phase of water-hexane partition, T = barks, S = leaves, (+) = presence and (-) = absence.

traditionally drink to treat systemic candidiasis or rub on the skin to prevent or to treat superficial mycosis such as vaginal, oral or cutaneous candidiasis. Almond oil of fruits has been too cited in treatment of skin diseases like ringworms due to filamentous fungi. This suggests that, *Baillonella toxisperma* (Pierre) contains antifungal substances which could be used for therapeutic purposes. According the data collected, barks was the part of plant most cited in the preparation of traditional remedies with a percentage of citations of 42.91%, follow by almond oil (22.38%), latex (19.40%) and leaves (15.29%) (Figure 1).

The results obtained from chemical composition (Table 2) of extracts of *B. toxisperma* (Pierre) revealed that *B. toxisperma* (Pierre) contains metabolic groups like polyphenols, phenols, saponins, tannins, phlobatannins, flavonoids, anthocyanins, leucoanthocyanins, triterpenes, steroids, cardiac glycosides and lipids. These bioactive compound groups are cited in many studies for theirs antifungal properties. Several phenolic compounds such as 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, vismiaquinone, bivismiaquinone and chlorogenic acid, isolated from plants, demonstrated significant antifungal activities (Kuete et al., 2007a; Jassim and Naji, 2003). Terpenoids like cymbopogonol and 3-oxo-(20S, 24S)-epoxydammarane-19,25-diacetate isolated from the barks of *Caesalpinia pulcherrina* exhibited a prominent antifungal activity (Nasimul et al., 2003). They have the property of precipitating fungal proteins (Sher, 2009; Kansole, 2009). Flavonoids like angusticornin B and bartericin A were reported to be very active against yeasts such as *C. albicans*, *C. glabrata* and *C. krusei* (Kuete et al., 2007b). They have the ability to complex with the polypeptides of microbial cell wall, leading to the loss of function and consequently to the death of the pathogens (Boris, 1996). Steroids, tannins, phlobatannins, anthocyanins, leucoanthocyanins, Saponins and cardiac glycosides have been also reported by several authors for their antifungal activity.

Preliminary susceptibility tests (Table 3) showed effective inhibition of micro-organisms growth. At 100 mg/ml, the inhibition diameters obtained from barks crude extracts varied from 7.11 ± 0.54 mm (aqueous crude extract of barks on *C. albicans*) to 17.65 ± 0.54 mm (hydro-ethanol extract of barks on *C. parasilopsis*) and those obtained from leaves crude extracts varied from 7.33 ± 0.12 mm (aqueous crude extract of leaves on *Candida* sp) to 15.76 ± 0.17 mm (hydro-ethanol crude extract of leaves on *Candida* sp). The analysis of these results shows that hydro-ethanol crude extracts of the barks and leaves exhibited on the whole the better inhibition activities on the yeasts tested, comparing to those obtained from aqueous, ethanol, methanol and ethyl acetate extracts. However, theses inhibition diameters obtained from crude extracts remain lower compared to those obtained with fluconazole (17.84 ± 0.42 to 21.44 ± 0.54 mm). Difference of antifungal efficiency of crude extracts could be explained by the fact that the solvent system used for the extraction plays a significant role in the solubility of the active principles of plant materials which in turn influences the antimicrobial activities of the extracts and can also be explained by the composition of each extract which could influences her diffusion in the gel (Bouopda, 2013).

Table 3. Preliminary susceptibility of yeasts against *Baillonella toxisperma* (Pierre) extracts, fluconazole and DMSO 10%

Yeasts	Vegetal extracts										Ref	Negative control
	Barks					Leafs						
	T1	T2	T3	T4	T5	S1	S2	S3	S4	S5	Fluc	DMSO 10%
C. albicans	7.11^{a}±0.54	16.48^{b}±0.71	11.17^{ab}±0.31	10.84^{a}±0.25	9.54^{a}±0.54	9.14^{a}±0.11	15.38^{b}±0.31	11.22^{a}±0.41	11.24^{a}±0.24	9.84^{a}±0.21	17.84^{a}±0.42	6.00^{a}±0.00
C. parasilopsis	10.85^{b}±042	17.65^{b}±0.54	12.11^{b}±0.62	11.45^{ab}±0.38	10.57^{a}±0.47	9.52^{a}±0.58	12.54^{a}±0.32	10.41^{a}±0.78	11.29^{a}±0.23	11.25^{a}±0.17	18.63^{a}±0.11	6.00^{a}±0.00
C. neoformans	7.41^{a}±0.45	12.54^{a}±0.38	9.55^{a}±0.47	13.20^{b}±0.34	9.66^{a}±0.84	9.65^{a}±0.75	12.68^{a}±0.74	9.83^{a}±0.62	10.58^{a}±0.24	10.84^{a}±0.42	21.44^{b}±0.54	6.00^{a}±0.00
Candida sp.	12.34^{b}±0.34	11.47^{a}±0.29	10.33^{ab}±0.27	13.29^{b}±0.19	11.33^{a}±0.18	7.33^{a}±0.12	15.76^{b}±0.17	9.16^{a}±0.62	11.42^{a}±0.18	10.88^{a}±0.14	18.82^{a}±0.32	6.00^{a}±0.00

T1 and S1 = aqueous extracts, T2 and S2 = hydro-ethanol extracts, T3 and S3 = ethanol extracts, T4 and S4 = methanol extracts, T5 and S5 = ethyl acetate extracts, T = barks, S= leaves, Ref = reference, Fluc = fluconazole and DMSO = dimethylsulfoxide. Means of inhibition diameter ± standard deviations.

Table 4. MIC, MFC and MFC/MIC values of *Baillonella toxisperma* (Pierre) extracts and fluconazole.

Yeasts	Inhibition parameters (mg/ml)	Vegetal extracts														Ref
		Barks							Leafs							
		T1	T2	ET2	HT2	T3	T4	T5	S1	S2	ES2	HS2	S3	S4	S5	Fluc
C. albicans	MIC	30	7.50	30	3.75	30	15	15	30	15	15	7.50	30	30	30	3.75
	MFC	N/D	7.50	30	7.50	30	30	30	30	30	15	7.50	30	30	N/D	3.75
	MFC/MIC	N/D	1	1	2	1	2	2	1	2	1	1	1	1	N/D	1
C. parasilopsis	MIC	30	7.50	30	3.75	30	15	30	30	15	30	3.75	15	15	30	1.87
	MFC	30	15	N/D	7.50	30	30	30	30	15	30	3.75	30	30	30	1.87
	MFC/MIC	1	2	N/D	2	1	2	1	1	1	1	1	2	2	1	1
C. neoformans	MIC	N/D	15	30	0.93	30	15	30	15	7.50	30	7.50	15	15	30	0.93
	MFC	N/D	15	N/D	1.87	30	30	30	30	15	30	7.50	15	30	N/D	1.83
	MFC/MIC	N/D	1	N/D	2	1	2	1	2	2	1	1	1	2	N/D	2
Candida sp	MIC	15	7.50	15	3.75	15	15	30	30	30	30	15	30	30	15	3.75
	MFC	30	7.50	30	7.50	30	30	30	N/D	30	N/D	15	N/D	30	30	3.75
	MFC/MIC	2	1	2	2	2	2	1	N/D	1	N/D	1	N/D	1	2	1

T1 and S1 = aqueous extracts, T2 and S2 = hydro-ethanol, T3 and S3 = ethanol extracts, T4 and S4 = methanol extracts, T5 and S5 = ethyl acetate extracts, ET2 and ES2 = aqueous phase of water-hexane partition, HT2 and HS2 = hexane phase of water-hexane partition, T = barks, S = leaves, Ref = reference and Fluc = fluconazole, N/D = undetermined.

The results obtained from inhibition parameters (Table 4) showed that after 48 h of incubation and at 35°C, MIC_s determined varied from 0.93 to 30 mg/ml and MFC_s determined varied from 1.87 to 30 mg/ml. Hydro-ethanol crude extracts of barks and of leaves were the most active crude extracts on the yeasts tested with 7.50 mg/ml < MFC_s < 15 mg/ml for the barks and 15 mg/ml < MFC_s < 30 mg/ml for the leaves. These results are in accordance with those obtained in preliminary susceptibility tests. The hexane phase HT2 of the hydro-ethanol barks crude extract gave the most significant antifungal activity obtained in this work on *C. neoformans* with inhibition parameters similar to those of fluconazole (MIC = 0.93 mg/ml and CMF = 1.87 mg/ml). The effectiveness based on the MFC (MFC of the ethanol crude extract/MFC of hexane phase from water-hexane

partition of ethanol crude extract) illustrates that the hexane phase HT2 of the hydro-ethanol barks crude extract was 4 times more active than this latter one on *C. albicans*, 2 times more active against *C. parasilopsis*, 8 times more active against *Candida* sp and *C. neoformans*. The hexane phase of the HS2 hexane-water partition of the basic ethanol leaves extract was 4 times more active on *Candida* sp, and 2 times more active against *C. albicans* and *C. parasilopsis*. This amelioration of antifungal activity would be due to concentration of non-polar substances into hexane phase. Hexane is a non-polar solvent which extracts non-polar substances (Boulenouar et al., 2009). The hydro-ethanol extraction followed by the hexane-water partition extracts proved therefore to be a promising way for concentrating the active principle of *B. toxisperma* (Pierre).

According the classification of Fauchère and Avril (2002) adapted to fungi by Nyegue (2006), extracts of *B. toxisperma* (Pierre) were fungicidal on yeasts tested with MFC/MIC ratio of 1 or 2; this justifies the use of this plant in traditional medicine. The activity of a plant substance depends on several factors including the concentration of active ingredients (Achraf, 2012). According to the results obtained in this study, *B. toxisperma* (Pierre) contain many useful substances some of which are biologically active and can be used for therapeutic purposes or to serve as precursors for the synthesis of new drugs. Further isolation and characterization of metabolic compounds of *B. toxisperma* (Pierre) could lead to discovery of new antimicrobial substance.

Conclusion

The numerous adverse side effects reported with the use of many current antifungal substances calls for an urgent need to search for new therapeutic agents. Present preliminary *in vitro* antifungal properties of *B. toxisperma* (Pierre), a plant of the Cameroon traditional pharmacopoeia, revealed that the extracts of this plant were found to be effective for the growth control of test fungi. The phytochemical screening showed that the plant extracts contain bioactive metabolic groups cited for their antimicrobial activities by many authors. These extracts could be then used for further isolation and purification of active compounds. This study is an important step towards clinical evaluation in order to produce improved phytomedicine in the treatment of microbial infections or to produce new therapeutic drugs.

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Conflict of interest

There is no conflict of interest as regard this study.

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