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In-vitro effects of almond oil, barks and leaves extracts of *Baillonella toxisperma* (Pierre) against *Trichophyton soudanense* and *Trichophyton rubrum*

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The purpose of this study was to evaluate anti-trichophytic effects of *Baillonella toxisperma* (Pierre) extracts against *Trichophyton rubrum* and *Trichophyton soudanense*. The tests were performed by the agar dilution method and the results have showed the effective inhibition of the mycelia growth of theses strains by *B. toxisperma* (Pierre) extracts. After 7 days of incubation at 28°C, the percentages of mycelia growth inhibition PI (%) by plant extracts and fluconazole were range between 20.81 and 100% on *T. soudanense*, and between 19.92 and 100% on *T. rubrum*. Some *B. toxisperma* (Pierre) extracts were exhibited similar anti-trichophyton activities compared to those obtained with fluconazole which was used like reference antifungal drug ($p < 0.05$). The best anti-trichophyton activities of *B. toxisperma* (Pierre) extracts were obtained at 5 mg/ml on *T. Rubrum* with almond oil and hexane phase of water-hexane partition of hydro-ethanol crude extract of barks bromobentseeni (HBb), and at 10 mg/ml on *T. soudanense* with (HBb). This anti-trichophyton activity of *B. toxisperma* (Pierre) extracts was attributed to secondary metabolites. The phytochemical analysis has showed that the plant contain bioactive metabolites groups such as phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. The metabolic groups have been mentioned in several studies for their antifungal activities. The results from this study provide a scientific validation for the ethno-medicinal uses of *B. toxisperma* (Pierre) in the treatment of fungal infections.

Key words: Trichophytic infections, *Baillonella toxisperma* (Pierre), bioactive metabolites, anti-trichophyton drugs.

INTRODUCTION

Trichophytons species are highly prevalent in adults and represents a serious clinical problem (Kwon et al., 2004). Among these species, *T. soudanense*, is a common causes of tineacapitis in parts of Africa (Menan et al., 2002) and *T. rubrum* is the most frequently isolated dermatophyte, accounting for 80 to 90% of the

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infectious strains (Elewski et al., 2002).These infectious agents are responsible to superficial fungal infections of the skin, nails and hair of humans and animals (Kobylak et al., 2015). These strains colonize the upper layers of dead skin and are the most common cause of athlete's foot, jock itch and ringworm worldwide (Zaugg et al., 2009). The prevalence of trichophytic infections, dues to *T. soudanense* and *T. rubrum,* has considerable increased all over the world especially in low and middle income countries like Cameroon. According to the development of fungal resistance and toxic side-effects problems correlated to the use of several current anti-trichophytic drugs, there is a need to search for news actives principles.

In the last few decades, the traditional system of medicine has become the interest of many scientists as a tool to improve the global health issues (Radfar et al., 2012). Several antifungal compounds isolated from medicinal plants have been reported in many studies for their capacities to inhibit the fungal growth. For example, the plants metabolites such as benzoic acid, thymol, citronellol, geranioland 1,8-cineol have been reported by Shin and Lim (2004) for their interesting antifungal activities against *T. rubrum*, *T. soudanense*, *T. schoenleinii*and *T. tonsurans*. Lupeol, a significant lupine-triterpene represented in plant, has been reported by Gallo and Sarachine (2009) to have remarkable antifungal properties against yeasts and filamentous strains like *Candida albicans*, *Cryptococcus neoformans*, *Microsporum canis* and *Aspergillus fumigates*. Medicinal plants have been used for centuries as remedy for the treatment of various diseases and are the major sources of drugs (Nostro et al., 2000). Many bioactive molecules isolated in plants are in long time served to develop modern antifungal drugs.

The Cameroonian traditional medicine is a reservoir of several medicinal plants species used to cure a lot of infectious diseases. Among these plants, *Baillonella toxisperma* (Pierre) is a Sapotaceae family plant very reputed traditionally for her antimicrobial potential. This plant is used to cure infectious diseases like fungal infections, diarrhea, sexually transmitted infections, rheumatism and toothache. Almond oil of this plant is used in divers by the inhabitants of Cameroonrural zones to cure skin affections like scabies, itch, dermatitis and many others superficial infections dues to microbes. The antifungal activity of *B. toxisperma* (Pierre) was studied for the first time by Riwom et al. (2015) against five yeasts among *C. albicans*, *Candida sp*., *Candida parasilopsis* and *Cryptococcus neoformans*.

The results showed remarkable ability of *B. toxisperma* (Pierre) extracts to inhibit the *in vitro* growth of these organisms. The main objective of this study is to provide a scientific validation for the ethno-medicinal uses of *B. toxisperma* (Pierre) extracts in the treatment of infectious diseases.

This study evaluates anti-trichophyton activity of barks and leaves extracts of *B. toxisperma* (Pierre) against *T. soudanense* and *T. rubrum*.

MATERIALS AND METHODS

Fungal strains

Fungal material consist of two filamentous strains, *T. soudanense* and *T. rubrum*. These fungal strains were gratefully given by “Centre Pasteur du Cameroun” and were conserved at +4°C on Sabouraud Dextrose Agar (SDA). Before biological tests, these strains have been first reactivated.

Plant material

The plant material was collected at East-Cameroon region in Dimako on the 21st October, 2011. The botanic identification was performed at National Herbarium of Cameroon where a specimen has been deposed on number 54060/HNC.

Extraction of almond oil

A weighed quantity of 300 g of dried almond of *B. toxisperma* (Pierre) was crushed into a mortar. Pastry obtained was carried at ebullition into a pot containing 250 ml of distilled water. The mixture was constantly moved until total evaporation of water and appearance of oil. After cooling at 25°C during 30 min, the cooked pastry was pressed to extract the oil. Oil thus obtained has been warmed on wood fire, decanted and filtered using a sieve to eliminate the lather and impurities (Bouopda, 2013). The pH of oil was determined at 25°C using a pH meter and the density has been determined using scales.

Extraction of barks and leaves

The barks and leaves of *B. toxisperma* (Pierre) were shade-dried and crushed using electric grinder. A weighed quantity (100 g) of the any powder was then subjected to extraction in water, ethanol, water-ethanol mixture (3:7), methanol and ethyl acetate. After 3 days of maceration, extract was filtered through Watman No 1 filter piper and evaporated using a rotary evaporator. Extractions were repeated tree times. Aliquots of 10 g of water/ethanol powders were fractioned by liquid-liquid partition in 400 ml of water-hexane mixture (1:1) by emulsification. After decantation, the aqueous and hexane phases obtained where filtered and concentrated (Riwom et al., 2015).

Determination of extraction yield

The extraction yield EY (%) was determined for extraction of oil and for extraction of barks and leaves of *B. toxisperma* (Pierre) according the following formula:

$$EY(\%) = (Mo/Me) \times 100$$

Mo: Mass of extract obtained (oil, barks or leaves extract)
Me: Mass of powder extracted (almonds, barks or leaves powder)

Phytochemical screening

The standard procedures described into literature by Harbone

Table 1. Extraction yield and physicochemical properties of almond oil of *Baillonella toxisperma*(Pierre)

Extraction of almond oil	Mass of almonds extracted	Volume of oil obtained	Mass of Oil obtained	Extraction yield	Physicochemical properties of oil obtained
Almond oil of *Baillonella toxisperma* (Pierre)	300g	12.7 ml	10.78g	3.59 %	pH : 6.98, Density (mg/mL) : 849 Color : Yellowish, Appearance at room temperature: fluid, Consistence : fatty at low temperature

(1998) and Edeoga et al. (2005) have been used for phytochemical tests.

Evaluation of mycelial growth inhibitionon agar medium

A weighed quantity of 400 mg of powder of each extract or of reference antifungal (Fluconazol) was introduced into atest tube containing initially 10 ml of Sabouraud Dextrose Agar (SDA) medium maintained in fusion at 45°C. After homogenization by vigorous agitation of the mixture, a double dilution of the tested substance was carried out. In fact, 5 ml of mixture previously prepared was deducted and introduced into Petri dishes of 55 cm of diameter and the remaining solution in test tube (5 ml) was completed with 5 ml of sterile SDA medium in fusion. And after homogenization the operation was repeated to obtain the following concentrations. The obtained concentrations of vegetal extracts and Fluconazol in the SDA medium were ranged between 40 and 2.5 mg/ml. The Petri dishes were left at room temperature until solidification of different preparations. Then, a mycelium disk of 6 mm of diameter, deducted to pre-culture of 7 days of *T. soudanense*or of *T. rubrum* was deposed in the center of each dishes. The positives (sterility of the medium controls) and the negatives (growth controls) controls were realized. The Petri dishes were sealed using a parafilm paper and incubated in reverse position during 7 days at 28°C. After incubation, the mycelium growth diameters were measured using a caliper. Other test was performed in triplicate. Fungitoxicity was expressed in terms of percentage of mycelial growth inhibition [PI (%)] determined according the formula of Pander et al. (1982).

$$PI\ (\%) = [(DT - D)/DT] \times 100$$

DT: Diameter of mycelial growth of negative control

D: Diameter of mycelial growth of test

Statistical analysis

The statistical analysis was done using Graph Pad Prism 5. Other test was performed in triplicate. Data were reported as the mean ± standard deviation from replicate experiments and one-way analysis of variance (ANOVA) was applied via Dunnett's post hoc multiple comparison tests for determining the statistical significance between *B. toxisperma* (Pierre) extracts (Dependantgroups) and Fluconazole (Control group). The values were considered significantly different in the level of $*p < 0.05$. Microsoft Excel has been used to drawn diagrams.

RESULTS

Extraction

The yields of extraction and the physico-chemical characteristics of extracts obtained from almond, barks and leaves of *B. toxisperma*(Pierre) are mentioned in Tables 1, 2 and 3. These results show that, the extraction yields of barks range between 6.84 (aqueous extract) and 14.52% (methanol extract) respectively. The extraction yields of leaves are range between 5.44 (aqueous extract) and 16.76% (ethanol extract). Alcohol solvents have proved to be the best solvents for the extraction of barks and leaves crude extracts of *B. toxisperma* (Pierre). From the liquid-liquid partition of hydro-ethanol extracts in water-hexane system, the best extraction yields have been obtained with hexane phases, with a yield of 52.2 % for hexane phase of hydro-ethanol extract of barks and 39.5 % for hexane phases of hydro-ethanol extracts of leaves. Almond oil extraction has shown a yield of 3.59 %.

Phytochemical screening

The qualitative detection of secondary metabolites groups in *B. toxisperma* (Pierre) extracts has been carried out according to the standard procedures described in literature. This screening released that *B. toxisperma* (Pierre) contains bioactive metabolites groups like phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. The results obtained from phytochemical analysis of each extract are summarized in Table 4.

Evaluation of the mycelia growth inhibition

The mycelial growth inhibition has been evaluated on agar medium by agar dilution method. Diameters of mycelia growth inhibition of fungi strains on agar medium by *B. toxisperma* (Pierre) extracts and fluconazole, after 7 days of incubation at 28°C are ranged between 0.00 ± 0.00 and 21.8 ± 0.89 mm on *T. soudanense*, and between 0.00 ± 0.00 and 22.2 ± 0.74 mmon *T. rubrum* (Tables 5 and 6). The percentage of mycelial growth inhibition [PI (%)] obtained from

Table 2. Extraction yield and physicochemical properties of barks and leaves extracts of *B. Toxisperma* (Pierre).

Extraction solvents	Part of vegetal extracted	Mass of vegetal powder extracted (g)	Mass of extract obtained (g)	Extraction yield (%)	Physicochemical properties of extracts obtained	
					Color	Appearance
Water	Barks	100	6.84	6.84	Brown	Powdery
	Leaves	100	5.44	5.44	Dark-green	Powdery
Water/ethanol (3:7)	Barks	100	12.66	12.66	Brown	Pasty
	Leaves	100	12.58	12.58	Green	Pasty
Ethanol	Barks	100	13.70	13.70	Brown	Pasty
	Leaves	100	16.28	16.28	Green	Pasty
Methanol	Barks	100	14.52	14.52	Brown	Powdery
	Leaves	100	16.76	16.76	Dark-green	Powdery
Ethyl acetate	Barks	100	8.16	8.16	Brown	Pasty
	Leaves	100	6.34	6.34	Green	Pasty

Table 3. Extraction yield and physicochemical properties of extracts obtained from water-hexane partition of hydro-ethanol crude extracts of barks and leaves *B. toxisperma* (Pierre).

Extract partitioned in water-hexane mixture	Mass of extract (g)	Solvent phase	Yield (%)	Physicochemical properties of extracts obtained	
				Color	Appearance
Hydro-ethanol extract of barks	10	Water	16	Blackish	Powdery
		Hexane	52.2	Brown	Fatty
Hydro-ethanol extract of leaves	10	Water	26	Green	Powdery
		Hexane	39.5	Green	Fatty

B. toxisperma (Pierre) extracts and Fluconazole are ranged between 20.81 and 100 % on *T. soudanense*, and between 19.92 and 100 % on *T. rubrum*. According to Adejumo and Bamidele (2009) a mycelial growth inhibition higher than 60 % is considered effective, and an inhibition of 100 % indicates no mycelial growth on agar plate (0.00 ± 0.00 mm). The results (Figures 1 and 2) show that:

1. At 2.5 mg/ml, the mycelial growth inhibition was effective with HBb (60.82 %) and Fluconazole (72.48 %) on *T. soudanense*, and with almond oil (65.65 %) and HBb (64.66 %) on *T. rubrum*.
2. At 5.0 mg/ml, the mycelial growth inhibition was effective ($60 < PI (\%) < 100$) with HBb (73.38 %), Lb (67.01 %) and HLb (68.10 %) on *T. soudanense*, and with extract Bb (73.31 %) on *T. rubrum*. A total inhibition (100 %) was observed on *T. soudanense* with Fluconazole, and on *T. rubrum* with HBb, almond oil and Fluconazole.
3. At 10 mg/ml, the inhibition was effective ($60< PI (\%)< 100$) on *T. soudanense* with Ba (60.53 %), Bb (63.35 %), Bc (61.07 %), Bd (68.50 %), and with Bc (61.07 %), Bd (70.27 %), Lb (65.53 %), HLb (68.08 %) on *T. rubrum*. A total inhibition (100 %) was observed on *T. soudanense* with HBb and fluconazole, and with almond oil, Bb, HBb and Fluconazole on *T. rubrum*.
4. At 20 mg/ml, the mycelial growth inhibition was effective ($60 < PI (\%) < 100$) with Ba (68.24 %), Be (60.46 %), La (66.94 %), WLb (73.42 %), Lc (70.49 %) and Le (72.59 %) on *T. soudanense*, and with Ba (62.35 %), WBb (63.78 %), Bc (68.72 %), Be (64.54 %), Lb (71.87 %), WLb (60.35 %), Lc (67.49 %), Ld (67.05 %) and Le (67.05 %) on *T. rubrum*. A total inhibition (100 %) was observed with almond oil, Bb, HBb, Bc, Bd, Lb, HLb and Fluconazoleon *T. soudanense*, and with almond oil, Bb, HBb, Bd, HLb and Fluconazole on *T. rubrum*.
5. At 40 mg/ml, a total inhibition (100 %) was observed on *T. soudanense* with almond oil, Ba, Bb, HBb, Bc, Bd, Lb, WLb, HLb, Lc, Le and Fluconazole, and with almond oil, Bb, HBb, Bc, Bd, Lb, HLband Fluconazole on *T. rubrum*. The inhibition was effective ($60 < PI (\%) < 100$) with WBb (66.87 %), Be (72.12 %), La (73.93 %) and Ld (65.60 %) on *T. soudanense*, and with Ba (73.31 %),

Table 4. Phytochemical screening.

Metabolic groups Tested	*Baillonellatoxisperma* (Pierre) extracts													
	Barks							Leaves						
	Ba	Bb	WBb	HBb	Bc	Bd	Be	La	Lb	WLb	HLb	Lc	Ld	Le
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoïds	+	+	+	+	+	+	-	-	-	+	-	-	+	-
Saponins	+	+	+	+	+	+	-	+	+	-	-	-	-	-
Coumarins	+	+	+	-	+	+	+	+	+	+	-	+	+	-
Tanins	+	+	+	-	+	+	+	+	+	+	-	-	-	+
Anthocyanins	+	+	-	+	+	+	+	-	-	-	-	-	+	+
Lipids	-	+	-	+	+	+	+	-	+	-	+	-	+	+
Alcaloïds	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	+	+	+	-	+	+	+	-	-	-	-	-	+	-

WBb (72.31 %), Be (68.60 %), La (64.78 %), WLb (68.72 %), Lc (72.07 %), Ld (66.49 %) and Le (72.03 %) on *T. rubrum*.

DISCUSSION

The results obtained to the antifungal sensitivity (Tables 5 and 6) reveal that *B. toxisperma* (Pierre) extracts managed to inhibit the mycelium development of *T. soudanense* and *T.rubrum*. Almond oil and hexane phases of water-hexane partition of hydro-ethanol extracts of barks and leaves of *B. toxisperma* (Pierre) were globally the most effective extracts on tested strains. According to Adejumo and Bamidele (2009) a mycelial growth inhibition high than 60 % is considered like effective in mycelial growth inhibition. The comparison of percentage of mycelial growth inhibition (Figures 1 and 2) show that at 2.5 mg/Ml, the mycelial growth inhibition began to be effective on *T. soudanense* with hexane phase of water-hexane partition of hydro-ethanol extract of barks HBb (60.82 %), and with almond oil (65.65 %) and HBb (64.66 %) on *T. rubrum*.

Total inhibition (100 %) was observed at 5.0 mg/ml on *T. rubrum*with extracts HBb and almond oil, and at 10 mg/ml on *T. soudanense* with extract HBb. At different concentrations, almond oil and hexane phases of water-hexane partition of hydro-ethanol extracts of barks, and leaves of *B. toxisperma* (Pierre) extracts exhibited similar anti-trichophyton activities compared to those obtained with fluconazole which was used like reference antifungal drug ($P < 0.05$). The antifungal activity of almond oil would be due to the residual lipids. Many greasy substances were examined for their antimicrobial properties; it is the case of the oleic acid and the linoleic acid which have been reported to have antibacterial and antifungal activities (Skalicka et al., 2010). In their study, Chifu et al. (2010) have mentioned the fatty acids of the ω-6, ω-7 and ω-9 families and their esters to have a strong antimicrobial activity. The antifungal activity of hexane phases could be attributed to the non polar molecules which have migrated in hexane during the water-hexane partition. Hexane is known to be a non polar extracting solvent which extract non polar molecules.

At 10 mg/ml, 100 % of mycelia growth inhibition was obtained with extracts of HBb, and on *T. rubrum* with extracts of Ao and HBb on *T. soudanense*. At 20 mg/ml, 100 % of mycelia growth inhibition was also obtained with extracts Ao, Bb, HBb, Bc, Bd, Lb and HLb on *T. soudanense*, and with extracts Ao, Bb, HBb, Bd, HLb on *T. rubrum*. The same results were also observed at 40 mg/ml with extracts Ao, Ba, Bb, HBb, Bc, Bd, Lb, WLb, HLb and Bc on*T. soudanense*, and with extracts Ao, Bb, HBb, Bc, Bd, Lb, HLb on *T. rubrum*.

Globally, the majority of *B. toxisperma* (Pierre) extracts exhibited their capacity to inhibit the radial growth of *T. soudanense* and *T. rubrum*on agar medium. This antifungal activity has been attributed to their chemical composition. The results obtained from phytochemical screening (Table 4) reveal that *B. toxisperma* (Pierre) contains several bioactive metabolites groups among which phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. In studies done in recent years, several authors have indicated theses metabolites

Table 5. Diameters (mm) of mycelia growth inhibition of *T. soudanense* on agar medium by *B. toxisperma* (Pierre) extracts and fluconazole after 7 days of incubation.

Concentrations of extracts in gel (mg /ml)	*Baillonella toxisperma*(Pierre) extracts															
	Almond oil	Barks							Leaves							Ref.
	Ao	Ba	Bb	WBb	HBb	Bc	Bd	Be	La	Lb	WLb	HLb	Lc	Ld	Le	Fluc
40	0.00 NS±0.00	0.00 NS±0.00	0.00 NS±0.00	9.15*±1.70	0.00±0.00	0.00 NS±0.00	0.00 NS±0.00	7.70*±0.42	7.20*±0.30	0.00 NS±0.00	0.00 NS±0.00	0.00 NS±0.00	0.00 NS±0.00	9.50*±1.29	0.00 NS±0.00	0.00±0.00
20	0.00 NS±0.00	8.77*±1.52	0.00 NS±0.00	14.0 NS±0.81	0.00*±0.00	0.00 NS±0.00	0.00 NS±0.00	10.9*±0.69	9.13*±0.71	0.00 NS±0.00	7.11*±0.21	0.00 NS±0.00	8.15*±0.67	12.2*±1.02	7.57*±0.61	0.00±0.00
10	11.7*±1.60	10.9*±1.44	10.1*±1.03	17.2*±1.70	0.00 NS±0.00	10.7*±2.10	8.70*±0.52	15.3*±0.87	13.0*±0.20	7.55*±0.11	10.5*±0.31	7.11*±0.17	11.8*±0.43	13.3*±0.45	10.5*±0.57	0.00±0.00
5.0	13.6*±0.86	13.0*±1.13	12.2*±0.50	18.9*±0.94	9.35*±0.78	13.0*±0.40	15.8*±0.62	16.7*±0.60	16.5*±0.68	9.11*±0.47	12.4*±0.24	8.80*±0.32	14.3*±0.55	15.3*±0.45	17.1*±1.86	0.00±0.00
2.5	16.6*±0.75	15.0*±0.82	19.3*±2.28	21.8*±0.89	12.8*±0.92	18.8*±2.25	18.9*±0.90	21.4*±0.67	19.6*±0.45	13.5*±0.24	16.1*±0.18	10.1*±0.28	19.9*±0.45	19.8*±0.85	21.2*±1.70	7.60±0.30

Ao = Almond oil extract, Ba and La = aqueous extracts, Bb and Lb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb = hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole. Diameter of mycelial growth of negative control = 28.5 ± 0.12 mm. Data are means of three replicates. Compared to those obtained from Fluconazole at the same concentration, diameters (mm) of mycelia growth inhibition obtained from *Baillonella toxisperma*(Pierre) extracts followed by NS, are not significantly different by Dunnett's Multiple Comparison Test. Those followed by * are significantly different at $P < 0.05$.

Table 6. Diameters (mm) of mycelia growth inhibition of *T. rubrum* on agar medium by *B. toxisperma* (Pierre) extracts and fluconazole after 7 days of incubation.

Concentrations of extracts in gel (mg /ml)	*B. toxisperma* (Pierre) extracts															
	Almond oil	Barks						Leaves								Ref.
	Ao	Ba	Bb	WBb	HBb	Bc	Bd	Be	La	Lb	WLb	HLb	Lc	Ld	Le	Fluc
40	0.00NS±0.00	7.20*±0.31	0.00 NS±0.00	6.95*±0.50	0.00 NS±0.00	0.00 NS±0.00	0.00 NS±0.00	7.88*±0.75	8.84*±1.32	0.00 NS±0.00	7.85*±1.07	0.00 NS±0.00	7.01*±0.33	8.41*±0.24	7.02*±0.42	0.00±0.00
20	0.00 NS±0.00	9.45*±0.54	0.00 NS±0.00	9.09*±0.47	0.00 NS±0.00	7.85*±0.46	0.00 NS±0.00	8.90*±1.34	11.44*±0.34	7.06*±1.05	9.95*±0.42	0.00 NS±0.00	8.16*±0.48	11.1*±0.12	8.27*±0.41	0.00±0.00
10	0.00 NS±0.00	10.8*±1.38	0.00 NS±0.00	14.7*±1.22	0.00 NS±0.00	9.77*±1.44	7.46*±0.33	13.5*±0.94	13.6*±1.52	8.65*±0.41	11.7*±0.76	8.01*±0.18	10.64*±0.94	14.2*±0.44	11.0*±0.47	0.00±0.00
5.0	0.00 NS±0.00	12.7*±1.03	7.20*±0.33	17.4*±0.66	0.00 NS±0.00	12.3*±0.77	9.81*±0.54	15.3*±1.30	15.2*±0.26	10.8*±1.47	13.8*±1.63	8.94*±0.35	12.2*±0.64	14.9*±0.74	15.4*±1.34	0.00±0.00
2.5	8.62*±0.75	14.1*±0.67	15.3*±1.38	20.1*±0.37	8.83*±0.42	16.9*±1.85	14.3*±1.52	19.8*±1.34	18.3*±0.75	15.0*±0.14	15.2*±0.13	11.7*±0.95	17.2*±1.55	17.9*±1.84	22.2*±0.74	0.00±0.00

Ao = Almond oil extract, Ba and La = aqueous extracts, Bb and Lb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb =hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole. Diameter of mycelial growth of negative control= 25.1 ± 0.39 mm. Data are means of three replicates. Compared to those obtained from Fluconazole at the same concentration, diameters (mm) of mycelia growth inhibition obtained from *Baillonella toxisperma*(Pierre) extracts followed by NS, are not significantly different by Dunnett's Multiple Comparison Test. Those followed by * are significantly different at $P < 0.05$.

to be effective against fungal growth. Phenolic compound like 2-acetylfuro-1, 4-naphthoquinone, angusticornin Bandbartericin A, isolated from medicinal plants, have been reported for their fungitoxic activity (Kuete, 2010).

Saponins like 6α-O-[β-D-xylopyranosyl-(1→3)-β-Dquinovopyranosyl]-(25,S)-5α-spirostan-3β-ol, isolated from the leaves of *Solanumhispidum* Pers. (Solanaceae), have been reported for their fungitoxic activity against *T. mentagrophytes* and *T. rubrum* (Gonzalez et al., 2004; Abad et al., 2007). 6-cinnamoyloxy-1-hydroxyeudesm-4-en-3-one, a sesquiterpene isolated from the roots of *Vernonanthura tweedieana* (Baker) H. Rob. (Asteraceae) by Portillo et al. (2005), has showed a significant antifungal against *T. mentagrophytes* (Abad et al., 2007). Flavonoids have been reported for their ability to complex with the polypeptides of microbial cell wall (Boris, 1996). Terpenoids have the property of precipitating fungal proteins (Sher, 2009; Kansole, 2009). Theses bioactive metabolites groups in *B. toxisperma* (Pierre) extracts are also known for their various physiological effects like anti-Candida, ant-bacterial, anti-oxidant, anti-inflammatory and anti-parasitic (Riwom et al., 2015; Kuete,2010; Gonzalez, 2004).

The prevalence of fungal infections dues to *Trichophyton* species has become very alarming these last years. Among these fungal species, *T. soudanense* and *T. rubrum*are cited in many investigations to be most virulent. These infections are typically treated by topical or systemic administration of polyene or azole antibiotics, which are efficacious but problematic (McMichael, 2001), due to their inefficacity against

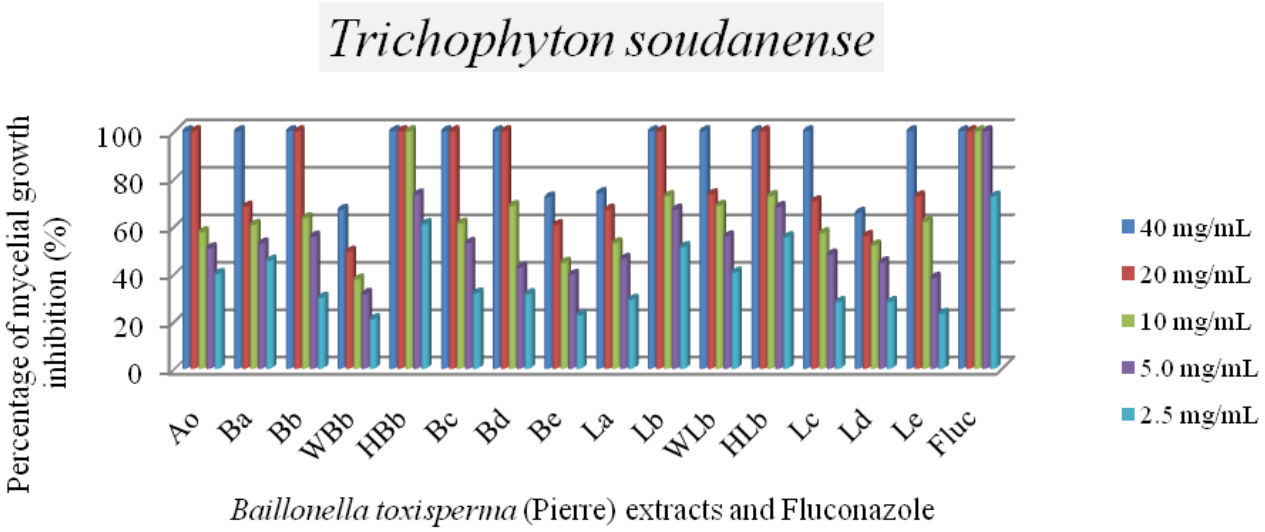


Figure 1. Percentage of mycelia growth inhibition of *Trichophyton soudanense*. Ao = Almond oil extract, Ba and La = aqueous extracts, Bb and Lb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb =hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole.

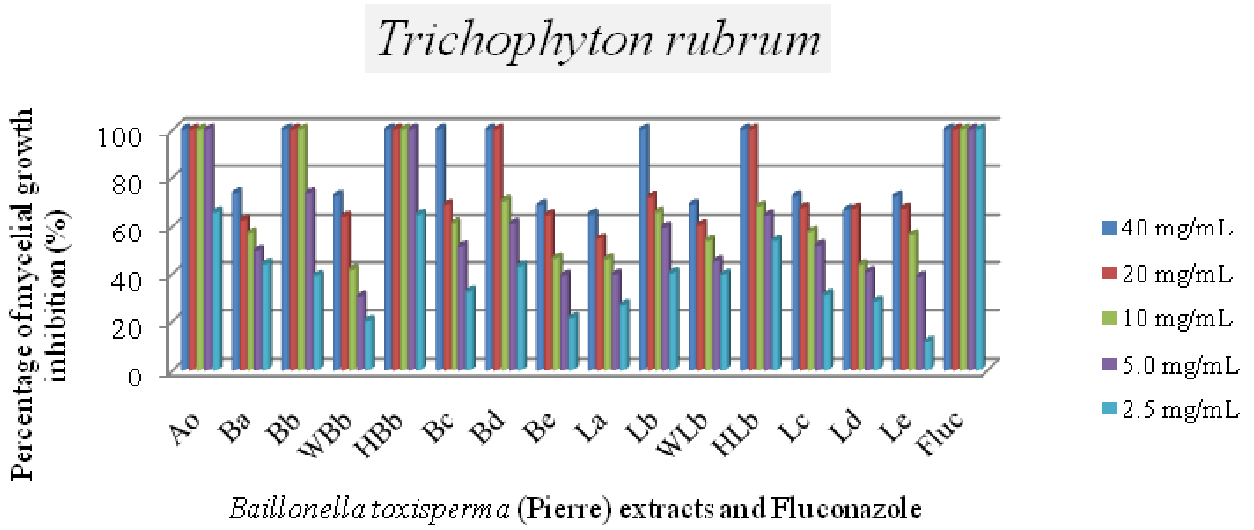


Figure 2. Percentage of mycelia growth inhibition of *T. rubrum.* Ao = Almond oil extract, Ba and La = aqueous extracts, Bb and Lb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb =hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole.

some trichophytic infectious and the toxicity of some of them. There is an urgency to search for new actives principles. These results obtained from this study provide a scientific validation for the ethno-medicinal uses of *B. toxisperma* (Pierre) extracts to treat fungal infections and prove that *B. toxisperma* (Pierre) contain active principles against *Trichophyton sp.* which could be isolate, and serve to develop the new antifungal therapeutic drugs.

Conclusion

During the last few years, development of drug

resistance to antifungal agents in human dermatophytic fungi has increased. According to inefficacity and toxicity problems that present several current antifungal drugs, there is an urgency to search and discover new actives principles. In this study, the *B. toxisperma* (Pierre) extracts have showed promising antifungal activity against *T. soudanense* and *T. rubrum*. Phytochemical analysis has released that this plant contain biologically active metabolic groups such as; phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. These metabolic groups have been reported in several studies to be used for therapeutic purposes or to serve as precursors for the synthesis of many antifungal drugs.

Conflict of Interest

The authors have not declared any conflict of interest.

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