Future Journal of Pharmaceutical Sciences

RESEARCH

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Antifungal activity of *Ocimum gratissimum* L., *Lantana camara* L. & *Pteleopsis suberosa* Engl. & Dies used in the treatment of vulvovaginal candidiasis in Benin

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Abstract

Background: Vulvovaginal candidiasis is a widespread mycotic infection that affects a large proportion of women of childbearing age. Its management in traditional medicine is based on the use of medicinal plants. This study aimed to evaluate the antifungal activity of *Ocimum gratissimum* L., *Lantana camara* L. and *Pteleopsis suberosa* Engl. & Diels used in the treatment of vulvovaginal candidiasis in Benin.

Results: The data obtained from the in vitro antifungal test show that the strains tested (ATCC 90028 and two clinical strains: 1MA and 3MA) were more sensitive to aqueous extracts with a better effect for *Pteleopsis suberosa*. This potential of the tested extracts correlated with their richness in total polyphenols. The extract of the *Pteleopsis suberosa* was very active on the inhibition of the reference strain ATCC 90028. On the clinical strains (1MA and 3MA) the aqueous extract of *Pteleopsis suberosa* showed a better MIC on the 1MA strain. In vivo model, inoculation of 100 µL of the concentrated *Candida albicans* suspension 1.5×10^5 UFC/mL induced the candidiasis of the female Wistar rat. The treatment with the aqueous extract of *Pteleopsis suberosa*, like fluconazole (reference drug), significantly reduced *Candida albicans* infection at a dose of 100 mg/kg after 1, 7 and 13 days of treatment.

Conclusion: This study revealed the potential antifungal of the *Ocimum gratissimum*, *Lantana camara* and *Pteleopsis suberosa*. *Pteleopsis suberosa* has better antifungal activity in vitro and in vivo. These observations justify the use of their medicinal plant in the traditional treatment of vulvovaginal candidiasis in Benin.

Keywords: Antifungal activity, *Ocimum gratissimum* L., *Lantana camara* L., *Pteleopsis suberosa* Engl. & Diels, Vulvovaginal candidiasis, Benin

Background

Vulvovaginal candidiasis (VVC) is a widespread mycotic infection that affects a large proportion of women of childbearing age [1]. It is a frequent reason for consultation in gynecology. It ranks second after bacterial vaginosis [2]. It is an infection often caused mainly by *Candida albicans*, a yeast of the Ascomycetes family. Vulvovaginal candidiasis is characterized by the emission of whitish, creamy leucorrhoea accompanied by vulvar pruritus, burning sensations and dyspareunia which, if left untreated, tends to spread to the inguinal folds and the perineum [3]. According to Gonçalves et al. [3], 75–85% of vulvovaginal candidiasis cases are due to *Candida albicans* and 10–20% of women who have had

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an episode of vulvovaginitis will have a recurrence. Other authors report that three quarters (75%) of women experience an episode of vulvovaginal candidiasis in their lifetime and 50% of them will experience at least a second episode [4–6]. Studies in Africa show that this prevalence is 32.6% in Senegal [7] and 38.9% in Benin [8].

The management of patients with vulvovaginal candidiasis (VVC) has been an issue of concern in recent years. Indeed, clinicians are increasingly confronted with cases of refractory vulvovaginal candidiasis caused by azole-resistant *Candida* species [9]. Other authors had detected a few years earlier cross-resistance of *Candida albicans* to itraconazole and fluconazole, 67.4% of the cases of resistance to fluconazole during vulvovaginal candidiasis were due to *Candida albicans*, although they are the antifungal agents of choice for the treatment of this pathology [10, 11].

Antifungal resistance is a growing threat. Everyone, from researchers to health professionals to the public, has a role to play in preventing and treating fungal infections and reducing antifungal resistance. Faced with this public health problem, it is imperative to find new solutions through a constant renewal of active ingredients [12]. Medicinal plants remain the most important source of molecules used in the composition of pharmaceutical drugs. Benin's floristic arsenal is full of impressive plant species [13]. The antibacterial properties of many of these medicinal plants widely used empirically to prevent or cure several types of diseases have been proven. In Benin, *Ocimum gratissimum* L., *Lantana camara* L. and *Pteleopsis suberosa* Engl. et Diels are three medicinal plants used in traditional treatment of candidiasis [14]

Ocimum gratissimum L. is a plant of the family Lamiaceae. The species appears as a perennial terrestrial herb that can reach 2 m high. Its leaves are simple, opposite, with an oval, coarsely serrated blade and an attenuated, decurrent base. The flowers of *Ocimum gratissimum* are white with a small calyx. The fruits consist of 4 spherical capsules and are 2 millimeters long [15]. *Lantana camara* L. is a thorny, rounded or spreading, bushy shrub. It reaches a height of 0.5–3 m. The stem is quadrangular, with numerous downward-pointing hooked spines. Its leaves of dark green color and toothed are composed and opposite. Its inflorescence is in panicles of multiple colors. Its fruits are small drupes and are grouped, but not fused together [16]. *Pteleopsis suberosa* Engl. et Diels (Combretaceae) is an arborescent plant of the family Combretaceae. It is a shrub or a small tree from 6 to 7 m high, with a more or less slender and cylindrical erect stem. The bark is very characteristic, coarse and densely covered with corky warts [17].

In the scientific literature, studies showed the in vitro antifungal activity, *Ocimum gratissimum* [18], *Lantana camara* and *Pteleopsis suberosa* [19]. However, data on the efficacy of these plants in vivo are almost non-existent. This justifies the present study, which aimed to evaluate the antifungal activities of *Ocimum gratissimum*, *Lantana camara* and *Pteleopsis suberosa*, three plants often recommended in the traditional management of candidiasis in Benin.

Methods

Plant material

Leafy stems of *Ocimum gratissimum* L. and *Lantana camara* L. and bark of *Pteleopsis suberosa* Engl. & Diels were used as plant material. The samples of these plants were collected in their natural habitat in June 2020 and then identified and certified at the National Herbarium of Benin of the University of Abomey-Calavi under the numbers AA6749/HNB for *Lantana camara* L. YH391/HNB for *Ocimum gratissimum* L. and AA6753/HNB for *Pteleopsis suberosa* Engl. & Diels.

Biological materials

Three strains of *Candida* were used in this study. These were the reference strain ATCC 90028 and two vaginal clinical strains 1MA and 3MA identified as causing vaginal candidiasis. Tables 1 and 2 present the characteristics of these clinical strains.

Animal material

Female albino rats of Wistar strain weighing between 180 and 200 g and three 12 weeks old from the animal house of the Institute of Applied Biomedical Sciences (ISBA) of the University of Abomey-Calavi were used for the animal experiment. Upon receipt, the rats were randomly assigned to standard cages for an acclimatization period of 2 weeks prior to the experiment. During this period, the animals had free access to food and water. They were maintained in the animal house of the Research Unit in Applied Microbiology and Pharmacology of natural substances (URMAPha) at a constant temperature of 22 ± 2 °C and subjected to a 12/12-h light/dark cycle. For animal experimentation, the study was authorized by the Ethics Committee of the University of Abomey-Calavi under number N0 012-2020/URMAPHA/EPAC/UAC.

Table 1 Susceptibility profiles of the clinical strains studied

Clinical strains	Sensibility profiles
3MA	$AmB^{S}Nys^{I}Flu^{S}Itr^{I}Ket^{I}Clt^{I}$
1MA	$AmB^{S}Nys^{S}Flu^{S}Itr^{S}Ket^{I}Clt^{I}$

AmB, amoxicillin B; Nys, nystatin; Flu, fluconazole; Ket, ketoconazole; Clt, ceftriaxone

Table 2 Virulence profiles of the clinical strains studied

Clinical strains	Hemo	Gel	Leci	Ad	CSH	Biof
1MA	+	−	+	+	+	−
3MA	+	−	+	+	+	−

(+): productrice; (−): no productrice

Hemo, hemolysin; Gel, gelatinase; Leci, lecithinase; Adh, adhesion; CSH, hydrophobicity; Biof, biofilm

Preparation of extracts

Three types of extracts (aqueous, hydroethanolic and ethanolic) were prepared from the plant organs used for each of the plants studied [20]. The parts of the plants used were carefully washed with distilled water and dried at laboratory temperature. Then, they were reduced to powder. The powders obtained were sieved with a 0.2-mm mesh and then stored in containers in the laboratory. Fifty (50) grams of the powder was macerated in 500 mL of solvent (distilled water for the aqueous extract, ethanol for ethanolic extract and the equal volume mixture of water and ethanol for the hydroethanolic extract) on a "Stuart Bioblock scientific Fisher" shaker for 72 h at room temperature. The homogenate obtained was filtered three times on absorbent cotton and once on Wattman No 2 paper. This filtrate obtained was then dried at 45 °C in the oven until a dry mass was obtained which represented the extract. This extract was then weighed for the evaluation of the extraction yield.

Phytochemical screening and total polyphenols content of the studied plants

Qualitative screening of the powder of the studied plants

A qualitative screening of the three plants studied was carried on their powder sample out to assess the major chemical groups present according to the precipitation and coloring reactions [21]. The main groups investigated were polyphenols, polyphenolic compounds (flavonoids, tannins, anthocyanins, leuco-anthocyanins) saponosides, reducing compounds, mucilages, sterols/terpenes and alkaloids.

Research of polyphenols: The reaction with ferric chloride ($FeCl_3$) was used to characterize the polyphenols. Five grams of powder was added to 100 mL. To five (5) mL of this solution, an alcoholic drop of 2% ferric chloride was added. The appearance of a more or less dark blue-black or green coloration indicated the presence of polyphenols.

Research of polyphenolic compounds: In Erlenmeyer, we put 5 g of powder to which we added 100 mL of boiling water. The infused mixture is left for 15 min under continuous stirring and then filtered. This filtrate divided into several portions will be used to research tannins, flavonoids, anthocyanins and leuco-anthocyanins.

- Tannins: To one portion of the filtrate, a few drops of 1% ferric chloride were added. The observation of a dark blue, green or black coloration indicates the presence of tannins. To 30 mL of this filtrate, 15 mL of STIASNY reagent is added. The whole is heated in a water bath at 90 °C for 15 min. The appearance of a pink precipitate indicates the presence of catechic tannins. For gallic tannins, after recovery of the filtrate, it was saturated with sodium acetate and a few drops of 1% ferric chloride. A blue or black tint indicates the presence of gallic tannins.
- Flavonoids: To 5 mL of a portion of the filtrate, 5 mL of hydrochloric alcohol (SHINODA reagent) and a pinch of magnesium powder were added: This is the cyanidin reaction, called SHINODA reaction. The appearance of a coloration: Orange indicates flavones, red indicates flavonols, violet indicates flavones, pink-orange or purplish indicates the presence of flavonoids.
- Anthocyanins: A few drops of 5% hydrochloric acid were added to 1 mL of a portion of the filtrate. This mixture was then alkalized by adding a few drops of ammonia diluted by half. A red color that becomes more pronounced and turns purplish blue or greenish indicates the presence of anthocyanins.
- Leuco-anthocyanin: To 5 mL of the second portion of the filtrate, 5 mL of SHINODA reagent was added. The mixture was then heated for 15 min in a water bath at 90 °C. The observation of a cherry red or purplish coloration indicates the presence of leuco-anthocyanin.

Saponosides: 10 mL of the sample solution (1 g of powder dissolved in 100 mL of distilled water) was poured into a test tube. The tube was shaken for 15 s and then left to stand for 15 min. A persistent foam height greater than 1 cm indicates the presence of saponosides.

Reducing compounds: The 10% decoctate was obtained by moderate boiling for 3 min of a mixture of 50 mL of distilled water and 5 g of powder. After cooling, the filtrate was adjusted to 50 mL with distilled water. Into a test tube, 5 mL of filtrate is introduced. After heating in a water bath at 90 °C for a few minutes, 1 mL of Fehling's reagent (Fehling's liquor A + Fehling's liquor B in equal volume) is added. The filtrate is reheated a few minutes

later. The observation of a bright red precipitate indicates the presence of reducing compounds.

Mucilage: 5 mL of absolute alcohol was added to 1 mL of 10% decoction. The appearance of a flaky precipitate indicates the presence of mucilage after about 10 min.

Alkaloids: This research was done in acid medium. Indeed, 5 g of the powder was mixed with 25 mL of hydrochloric acid diluted to 5%. The mixture was macerated for 24 h. To 1 mL of the filtrate is added 5 drops of MAYER reagent. If alkaloids are present, a yellow or squinty precipitate is observed in the tube.

Sterols and triterpenes: Sterols and terpenes were investigated by the Liebermann reaction. Five (5) milliliters of the prepared solution (dissolution of 1 g of the sample powder in 100 mL of distilled water) was evaporated on a sand bath. The residue was dissolved hot in 1 mL of acetic anhydride; we added 0.5 mL of concentrated sulfuric acid to the triturate. The appearance, at interphase, of a purple or violet ring, turning blue and then green, indicated a positive reaction.

Determination of the total polyphenols content of the extracts

In the extracts prepared, total polyphenols were quantified using the commercial Folin–Ciocalteu reagent [22]. This Folin–Ciocalteu reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$), which is reduced during the oxidation of phenols to a mixture of blue oxides of tungsten and molybdenum. The blue coloration produced, whose maximum absorption is at 760 nm, is proportional to the quantity of polyphenols present in the different extracts. Briefly, a volume of 50 µL of the diluted solution (1/100 in distilled water) of concentrated extract at 25 mg/mL was added to 250 µL of Folin–Ciocalteu 10% reagent (diluted to the 10th in distilled water) and 750 µL of an aqueous solution of sodium carbonate (Na_2CO_3) at 75 g/L. After 8 min of incubation, 950 µL of distilled water was added and vortexed and incubated for 2 h in the dark at room temperature. After incubation, the optical densities (OD) were read at 760 nm using a CECIL CE 2041 spectrophotometer. The reading was taken against a blank consisting of a mixture of 250 µL of Folin–Ciocalteu Reagent (FCR) and 750 µL of Na_2CO_3 and 1 mL of distilled water. Total polyphenol levels were determined using the equation from the calibration curve of gallic acid (0–200 µg/mL) taken as reference standard. Samples were prepared in triplicate for each analysis. Total polyphenol content was determined in mg of gallic acid equivalent/g extract (mg GAE/g) by the formula:

$$\mathrm{TPT} = (X \times V)/m$$

with TPT, the total polyphenol content, X the gallic acid concentration in mg/mL; V the volume of extract used in mL; and m the mass of the extract in grams [23].

DPPH antioxidant activity of the extracts

The antioxidant activity of the extracts was evaluated by the DPPH free radical scavenging assay. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable purplish-colored free radical that absorbs at 517 nm. In the presence of anti-free radical compounds, the DPPH radical is reduced and changes its color to yellow. Thus, 100 µL of different concentrations of each extract is added to 1900 µL of the ethanolic solution of DPPH (0.4 mg/mL) [20]. The blank is prepared by mixing 100 µL of the extraction solvent with 1900 µL of the DPPH solution. After incubation in the dark for 1 h at room temperature, absorbance readings were taken at 517 nm using a MINDRAY spectrophotometer (BA-88-A). The recorded optical densities were used to calculate the percentage of DPPH radical scavenging which is proportional to the antioxidant power of the sample. The percentage of DPPH radical scavenging was determined by the formula:

$$P = \frac{\mathrm{Ab} - \mathrm{Ae}}{\mathrm{Ab}} \times 100$$

P, percentage of trapping; Ab, absorbance of control; Ae, absorbance of sample.

Evaluation of the antifungal power of extracts

Identification of active extracts was done by solid-state diffusion method [24] and determination of minimum inhibitory concentration (MIC) by liquid dilution method [25].

- Preparation of culture media

  Mueller Hinton agar supplemented with 2% glucose and 0.5% methylene blue (MHGB) and yeast extract peptone dextrose (YPD or YEPD) broth were prepared and sterilized ("Appendix").
- Preparation of fungal suspensions
  For this preparation, two averages 24-h colonies of the test strain, grown on SDA + Chloramphenicol agar was picked and emulsified in 5 mL of 9‰ saline. The fungal suspensions thus prepared were adjusted to the 0.5 Mc Farland density corresponding to 1.5×10^5 UFC/mL.
- Preparation of the parent solution of extract
  The parent solution of the extract was prepared by dissolution of the 200 mg of each extract in 1 mL of sterile distilled water to obtain a stock solution of final concentration 200 mg/mL. The prepared suspension was then vortexed well before each use.

- Antifungal activity in vitro
  Two Mueller Hinton agar plates supplemented with 2% glucose and 0.5% methylene blue (MHGB) were inoculated with the fungal suspension prepared for each type of extract. Six wells of 7 mm diameter were then sterilely made in the agar media using Pasteur pipette. The five peripheral wells were used as test wells, and the central well was used for the negative control. Thus, 100 µL of each extract (aqueous or hydroethanolic extracts) of *Ocimum gratissimum* L., *Lantana camara* L. and *Pteleopsis suberosa* Engl. and Dies prepared at 200 mg/mL was, respectively, transferred in a sterile way in three consecutive wells and 100 µL of fluconazole (100 µg/mL) in the two following ones, then 100 µL of distilled water in the one (negative control well). The treated plates were covered, left for 2 h on the bench and then incubated at 37 °C for 24 h. The inhibition diameters observed around the wells after incubation were then measured with a double decimeter for each type of extract studied. The most active extract corresponded to the one with the largest inhibition diameter. Determination of minimum inhibitory concentrations (MICs) was done in liquid medium [25] and modified (by replacing RPMI 1680 medium with YEPB medium). The tests were performed on successive dilutions (1/2; 1/4; 1/8; 1/16; and 1/32) of the parent preparations of extracts corresponding to the concentrations (100; 50; 25; 12.5; and 6.25) expressed in mg/mL of extract, respectively. The extracts were then mixed with 100 µL of fungal suspensions with concentrations ranging from 8×10^4 to 1.2×10^5/µL in the microplate and incubated at 37 °C for 24 h. The yeasts were counted using a Malassez cell, and the number of cells per µL was noted. Thus, the MIC corresponds to the concentration of extract that inhibited 50% of the yeasts.

Evaluation of the in vivo antifungal activity of the extract of *Pteleopsis suberosa* (most active extract)

The methodology adopted in this study is inspired from the work of the several authors [26, 27]. Four groups of 3 rats each were randomly constituted. These were one group treated with fluconazole (10 mg/kg/BW), one control (untreated) group and two test groups treated with aqueous extract of *Pteleopsis suberosa* at doses of 50 mg/kg and 100 mg/kg of body weight (BW). Prior to induction of infection, a vaginal swab was taken and streaked on sabouraud agar with chloramphenicol and incubated in an oven at 37° for 48 h. At the end of the incubation, the observation of the Petri dishes showed an absence of *Candida albicans* colony, thus indicating that the rats included in the animal experiment did not present candidiasis. Rats were pre-treated with estradiol valerate by esophageal gavage (2 mg/kg of BW) to induce a pseudoestrus situation (dilatation of the vulva of the rats) for 72 h. Then an inoculation of 100 uL of concentrated *Candida albicans* suspension 1.5×10^5 UFC/mL was performed over 48 h for candidiasis induction. On the third day, a new inoculation of the *Candida albicans* suspension was followed by a swabbing performed for a culture on Sabouraud agar + chloramphenicol. After 24 h of incubation, the observation of positive culture counting 7–8 colonies confirmed the induction of candidiasis [27]. The treatment with the various substances (extract and fluconazole) lasted 13 days. The dose of 50 mg/kg at 100 mg/kg of the tested extract was selected based on the MIC after in vitro antifungal tests. The dose of fluconazole (10 mg/kg) was chosen on the basis of the literature data [26].

- Lot A ($n=3$): Rats infected and treated with 50 mg/kg of BW of the active extract
- Lot B ($n=3$): Rats infected and treated with 100 mg/kg of BW of the active extract
- Lot C ($n=3$): Rats infected and treated with fluconazole 10 mg/kg
- Lot D ($n=3$): Infected and untreated rats

During the treatment, a daily vaginal swab was performed on all animals followed by the counting of *Candida albicans* germs with the Malassez hematimeter. On the 13th day, a swabbing and a plating were performed.

Statistical analysis of data

The obtained data were subjected to statistical analysis using SPSS 26.0 software and Microsoft Excel 2016 spreadsheet. Quantitative variables are presented as mean and standard deviation. Categorical variables are presented as percentages. One-factor analysis of variance (ANOVA) and Tukey's post hoc tests and Kruskal–Wallis nonparametric test were used to assess the influence of the solvent factor on total polyphenol content and extraction yield. The probit analysis was used for the determination of the IC_{50} and MIC of the most active extract. The multiple comparison test was used to assess the effect of treatments on the regression of *Candida albicans* infection. The significance level was set at 5%.

Results

Extraction yield

The yield obtained from the extraction process of the studied plants is summarized in Fig. 1. From this figure we note a variation in yield depending on the type of extract and the plant. The aqueous extract of *Ocimum gratissimum* and *Pteleopsis suberosa* plants showed

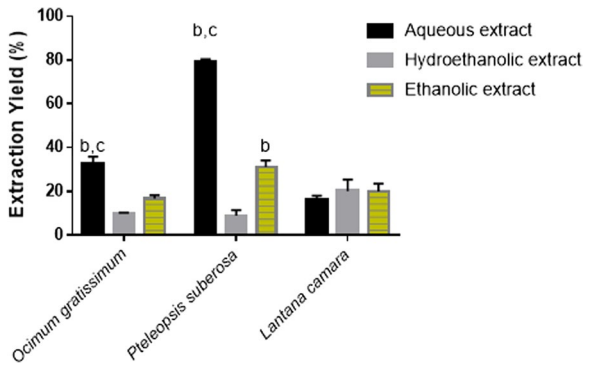

Fig. 1 Extraction yield of the studied plants. For the comparison between the extracts of each plant: a: significant different from aqueous extract, b: significant different from hydroethanolic extract and c: significant different from ethanolic

Table 3 Results of the qualitative screening of the powder of studied plants

	Pteleopsis suberosa	*Ocimum gratissimum*	*Lantana camara*
Tannins	+	+	+
Catechic Tannins	−	+	+
Gallic Tannins	+	+	+
Flavonoids	+	−	−
Anthocyanins	−	−	−
Leuco-anthocyanins	+	+	+
Saponosides	+	+	+
Reducing compound	+	−	−
Sterols/Terpenes	+	−	−
Mucilages	−	−	−
Alkaloids	−	−	−

(−): Absence; (+): Presence

significantly better yield than the hydroethanolic and ethanolic extracts ($P < 0.05$). However, for *Lantana camara* plant no significant difference ($P > 0.05$) was noted between the extraction yield of the three extracts.

Phytochemical screening of the studied plants

- Qualitative screening of the studied plants

  The results of the qualitative screening of the studied plants are presented in Table 3. From this table it is noted that compounds such as gallic tannins, leuco-anthocyanins and saponosides are identified in the studied plants. Flavonoids, reducing compounds and sterol/terpenes are present only in *Pteleopsis suberosa*, while catechic tannins are present only in *Ocimum gratissimum* and *Lantana camara*.

Table 4 Total polyphenol content of the plant extracts studied

Plants	Extract	Polyphenols Content mgEAG/gMS	Ecart type
Ocimum gratissimum	Aqueous	18890,67*	565,68542
	Hydroethanolic	9710	775,29208
	Ethanolic	13247,14286	63,976328
Pteleopsis suberosa	Aqueous	21103,17*	1443,6763
	Hydroethanolic	10892,14	330,82496
	Ethanolic	12947,14286	703,73961
Lantana camara	Aqueous	23824*	282,84271
	Hydroethanolic	10474,28	118,69292
	Ethanolic	14270,95238	407,42819

*Significantly different when the comparison between the extracts of each plant

Table 5 Antioxidant power of the extracts expressed in IC_{50}

Plants studied	Extract	IC_{50} (mg/mL)
Ocimum gratissimum	Aqueous	0.05
	Hydroethanolic	0.01
	Ethanolic	0.02
Pteleopsis suberosa	Aqueous	0.01
	Hydroethanolic	0.01
	Ethanolic	0.07
Lantana camara	Aqueous	0.07
	Hydroethanolic	0.01
	Ethanolic	0.06

- Total polyphenol content of the extracts studied
  The results of the quantification of total polyphenols in the extracts produced are presented in Table 4. From this table, it is noticed that the aqueous extracts of the three studied plants presented a significantly high content of total polyphenols compared to the hydroethanolic and ethanolic extracts ($P < 0.05$).

Antioxidant activity of the extracts of the studied plants

All the extracts tested reduced the DPPH radical in variable proportions. The DPPH radical inhibitory power of the different extracts and reference standards is expressed in IC_{50} (Table 5). Since the IC_{50} is inversely proportional to the antioxidant potential of the extract, the lower the IC_{50} value, the better the antioxidant power of the extract. A comparative analysis of the antioxidant powers of the different extracts of the studied plants indicates a variation of the antioxidant activity according to the plant and the type of extract. The

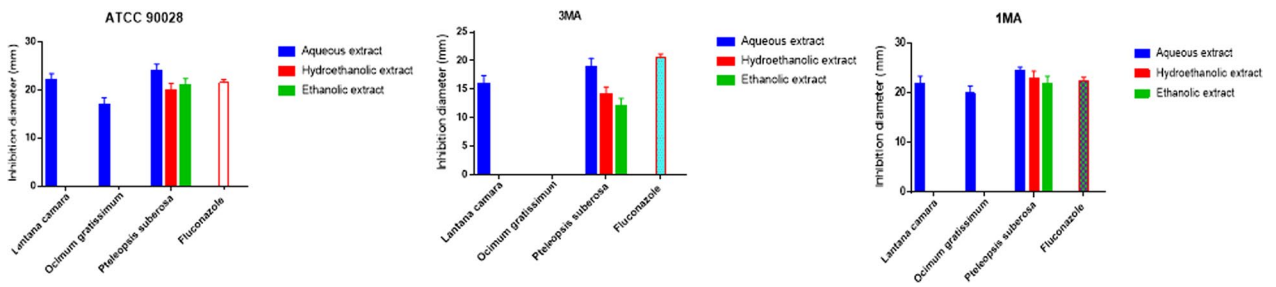

Fig. 2 In vitro antifungal activity of plant extracts on the tested strains

Table 6 MIC of the aqueous extract of *Pteleopsis suberosa* on the tested strains

Candida albicans strains	MIC (mg/mL)	Ecart type
ATCC 90028	33.52	0.34
1MA	43.49	0.36
3MA	80.11	1.34

hydroethanolic extracts of *Ocimum gratissimum* and *Lantana camara* plants are reducing more the DPPH radical than the aqueous and ethanolic extracts. For *Pteleopsis suberosa,* the aqueous and hydroethanolic extracts showed the best antioxidant activity.

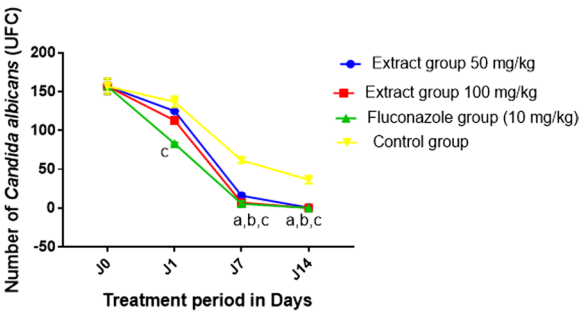

Fig. 3 Evolution of the number of *Candida albicans* germs in rats during of the treatment. The letters (a: extract group 50 mg/kg; b: extract group 100 mg/kg; and c: fluconazole) marked following the period of the treatment reflect a significant different between these groups and the control group

In vitro antifungal activity

The results of the in vitro antifungal tests carried out are presented in Fig. 2. From the analysis of this figure it appears that the plants studied presented a variable antifungal activity according to the tested strain with a better activity for the *Pteleopsis suberosa* plant. On the reference strain (ATCC 90028), the three extracts of *Pteleopsis suberosa* exerted antifungal activity with a better effect for the aqueous extract. Regarding *Lantana camara* and *Ocimum gratissimum*, only the aqueous extract was active on the inhibition of the reference strain. The same trend was obtained for the 1MA strain. On the 3MA strain, it was not sensitive to the *Ocimum gratissimum* extracts contrary to the *Pteleopsis suberosa* extracts. Concerning *Lantana camara*, only the aqueous extract exerted an antifungal effect on the 1MA strain. The minimum inhibitory concentrations of 50% of the *Candida albicans* germs tested for the aqueous extract of *Pteleopsis suberosa* are presented in Table 6. From this table, it comes out that the tested extract was very active on the inhibition of the reference strain ATCC 90028. On the clinical strains (1MA and 3MA) the tested extract showed a better MIC on the 1MA strain.

In vivo antifungal activity

Figure 3 shows the number of *Candida albicans* germs counted in rats of the different groups during the experimental period. The figure shows a general decrease in the number of *Candida albicans* germs with the duration of the treatment. After one day of treatment, a reduction in the number of *Candida albicans* germs was noted in all groups with a significant effect for the groups treated with fluconazole and the extract at a dose of 100 mg/kg compared to the control group. This regression of *Candida albicans* infection continued in all groups after 7 days of treatment with a significant effect for the groups treated with extract and fluconazole compared to the control group ($P < 0.05$). After 14 days of treatment, both extract and fluconazole significantly inhibited the growth of *Candida albicans* germs compared to the control group ($P < 0.05$). No significant effect was noted between the effects of fluconazole and the extract obtained after 7 and 14 days of treatment ($P > 0.05$). On the other hand, after 1 day of treatment, fluconazole induced a significant regression of induced candidiasis in comparison with the control and test groups ($P < 0.05$).

Discussion

This study aimed to evaluate the antifungal activity of *Ocimum gratissimum*, *Lantana camara* and *Pteleopsis suberosa* used in the treatment of vulvovaginal candidiasis in Benin. The qualitative phytochemical screening of these plants studied revealed the presence of tannins, catechic tannins, leuco-anthocyanins and saponosides. The presence of polyphenolic compounds (tannins, catechic tannins, leuco-anthocyanins) in these plants could explain the high content of total polyphenols obtained for the extracts. In the literature, scientific studies have reported the presence of secondary metabolites identified in these plants [28–30]. Some of them have even attributed their antimicrobial potential to these bioactive molecules [31–33]. Furthermore, the extraction yields obtained, varied from one medicinal plant to another and from one extract to another. The best yield was obtained for aqueous extractions for *Pteleopsis suberosa* and *Ocimum gratissimum* plants and hydroethanol extraction for *Lantana camara*. These observations show that water and the mixture (water–ethanol) offer a better quantitative bioavailability of the active ingredients of the studied plants. In the literature, several studies have reported that the mixed solvent (water–ethanol) improves the extraction yield more than pure solvents [34–36]. In addition, the secondary metabolites of *Pteleopsis suberosa* and *Ocimum gratissimum* were more soluble in water than in ethanol and the mixed solvent. These observations could justify the choice of water and alcohol as solvents for the preparation of medicinal recipes in traditional Beninese medicine.

The results of the present study revealed that the extracts of the studied plants showed variable content of total polyphenols. The content of total polyphenols was significantly influenced by the solvents used for the extractions. Thus, the high contents of total polyphenols were obtained for the aqueous extracts. These results are in agreement with those obtained for the extraction yield. Regarding the antioxidant activity, the data obtained established that the plants studied are provided with antioxidant properties. Similar conclusions have been reported by several authors [37–39]. From all the extracts tested, the aqueous extract of *Pteleopsis suberosa* shows the better antioxidant activity. This strong antioxidant power could be explained by the richness of this extract in total polyphenols.

The antifungal activity evaluated in vitro indicated a variation of the antifungal power depending on the plant and the extracts tested with a better effect for the aqueous extract of *Pteleopsis suberosa*. In the literature the antifungal potential on candida strains has been reported for *Lantana camara* [40] and for *Ocimum gratissimum* [41]. However, few studies have provided information on the antifungal potential of *Pteleopsis suberosa*, which proved to be the most active plant in our study. The potential of this plant could be related to its richness in bioactive compounds, particularly in total polyphenols for its most active extract (aqueous extract). On the in vivo model experimented using this extract, the data obtained inform that the aqueous extract of *Pteleopsis suberosa* ensured a regression of the *Candida albicans* infection with a better effect at the dose of 100 mg/kg. In the light of literature data, this study confirms that medicinal plants of the Combretaceae family (botanical family of *Pteleopsis suberosa*) are very active on candida strains in in vivo model [42]. These authors maintain that polyphenols are responsible for these antifungal effects of medicinal plants. From these observations it appears that the effectiveness of the aqueous extract of *Pteleopsis suberosa* studied would be due to its richness in polyphenols and antioxidant revealed by the present study. Certain authors have supported this idea and report that bioactive substances extracted from plant species are responsible for the anti-candida potential in vivo [43]. Other authors have underlined the beneficial role of flavonoids in the anti-candida activity of medicinal plants [44]. However, it should be noted that the lower sensitivity of the 3MA strain to *Ocimum gratissimum* and *Lantana camara* extracts can be explained by the resistance profile of this strain.

Moreover, the fungal isolate 1MA used in this study was a virulent strain (Table 2) since it is adherent, hydrophobic (HSC positive), hydrolase (lecithinase) and toxin (hemolysin) producing. The aqueous extract of *Pteleopsis suberosa* would have favored the regression of the fungal infection in vivo by preventing the yeasts not only from colonizing the vaginal flora of the animals but also by inhibiting the expression of one or the other of these virulence factors in the pathogen by various mechanisms. According to some authors, hydrophobicity is a virulence factor controlled by genes and generally positively correlated with the metabolic activity of the biofilm since hydrophobic interactions seem crucial to promote tissue invasion [3, 45]. Thus, this expressed trait for the studied strain should allow it to adhere and colonize the vaginal flora. Other authors indicate that adhesion to tissue cells or surfaces is the first step in biofilm formation and colonization of the flora by the Candida strain [45]. Thus, the aqueous extract of *Pteleopsis suberosa* was therefore able to prevent this stage of fungal colonization from occurring even before the yeast had time to excrete lecithinase to destroy the vaginal flora and contribute to the installation of the infection. Some data in the literature indicate that the production of hydrolytic enzymes would promote yeast adhesion [46]. Lecithinase being a phospholipase, its production by *Candida albicans*, could destroy cell lipids, one of the main components of the membrane [47] and be the cause of oxidative stress [48]. In addition, the extract, by preventing adhesion with the vaginal flora, prevents

the possible formation of biofilm, a powerful resistance structure that is installed by many pathogens such as *Candida albicans* to resist against antimicrobials [49].

In view of the above, it should be noted that *Pteleopsis suberosa* is endowed with antioxidant and antifungal properties in vitro and in vivo.

Conclusions

This study aimed at evaluating the antifungal properties of *Ocimum gratissimum*, *Lantana camara* and *Pteleopsis suberosa* plants. The in vitro antifungal tests revealed a variation in antifungal power depending on the plant and the type of extract.

The aqueous extract of the studied plants and particularly that of *Pteleopsis suberosa* were more active on the inhibition of the tested *Candida strains*. In in vivo model, the aqueous extract of *Pteleopsis suberosa* induced regression of candidiasis in Wistar rats within 13 days of treatment. This activity is supported by the high total polyphenol content and antioxidant power of the extract revealed by the present study. Further studies will elucidate its mechanism of action for improved conventional drug formulation.

Appendix: Preparation of YEPD and MHGB media culture

YEPD agar

Ingredients	g/L
Peptone	20.000
Yeast extract	10.000
Dextrose	20.000
Agar	15.000

Final pH (at 25 °C): 6.5 ± 0.1A suspension of 65 g of this YEPD agar powder was made in 1 L of distilled water. Mix well and bring to a boil with continuous stirring for 1 min. Autoclave the agar and broth media at 121 °C for 15 min. After cooling down (45–50 °C), portion equally into Petri plates.

Mueller Hinton agar, 2% glucose with methylene blue (MHMB)

Ingredients	g/L
Beef infusion from	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Glucose	20.000
Methylene blue	0.0005
Agar	17.000

Final pH (at 25 °C) 7.3 ± 0.1Suspend 58 g of this power in 1000 mL distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min. Mix well before pouring.

Abbreviations

Ab: Absorbance of control; Ae: Absorbance of sample; ATCC: American Type Culture Collection; CFU: Colony Format Unit; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FCR: Folin–Ciocalteu Reagent; GAE: Gallic Acid Equivalent; $H_3PMO_{12}O_{40}$: Phosphomolybdic acid; $H_3PW_{12}O_{40}$: Phosphotungstic acid; HNB: National Herbarium of Benin; MHGB: Methylene Blue; MIC: Minimum Inhibitory Concentration; Na_2CO_3: Sodium carbonate; OD: Optical density; SDA: Sabouraud Dextrose Agar; VVC: Vulvovaginal candidiasis; YEPD: Yeast Extract Peptone Dextrose.

Acknowledgements

Thanks to Professor Tossou Jacques DOUGNON (*in memoriam*) for authorizing the progress of the work and for scientific coaching.

Authors' contributions

JRK, VD and FL were the designers of the research project and supervised the work. BAF, EA, AH participated in study implementation and data collection. PA ensured the statistical processing of the data. All the authors participated in the reading and validation of the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

This study did not take into account information or samples obtained from humans. So no consent was required. For animal experimentation, the study was authorized by the Ethics Committee of the University of Abomey-Calavi under number N0 012-2020/URMAPHA/EPAC/UAC.

Consent for publication

Not applicable.

Competing interests

Authors declare that they have no competing interest.

Plant authentication

The plants were used in this study were authenticated at the National Herbarium of Benin of the University of Abomey-Calavi under the numbers AA6749/HNB for ***Lantana camara***, YH391/HNB for ***Ocimum gratissimum*** and AA6753/HNB for ***Pteleopsis suberosa***.

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Received: 11 August 2021 Accepted: 19 November 2021
Published online: 11 December 2021

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