

Phytochemical and biological studies of the hydroethanolic extract of *Pteleopsis suberosa* and *Piliostigma thonningii* used in herbal medicine in Togo

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**Phytochemical and biological studies of the hydroethanolic extract of
Pteleopsis suberosa and *Piliostigma thonningii* used in herbal medicine in
Togo.**

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Abstract

Background: The emergence of multidrug-resistant isolates to conventional antibiotics calls for the search for other active molecules. The aim of this study was to evaluate the antimicrobial

activity of *Pteleopsis suberosa* and *Piliostigma thonningii*, two Togolese medicinal plants used to treat vaginal, pulmonary, gastric and skin infections.

Methods: The total hydroethanolic extracts of the various organs were obtained by maceration. Qualitative phytochemistry of the extracts was performed using staining methods to identify the main chemical groups. The total flavonoid content was quantified using a spectrophotometric assay. Two methods were used to assess antioxidant activity: the phosphomolybdate reduction method and the FRAP test. The solid-well diffusion method and the microplate microdilution method were used to evaluate the antimicrobial activity of the study extracts.

Results: The highest hydroethanol extraction yield was obtained with *P. thonningii* trunk bark extract (22.67%). The results revealed the presence of tannins, saponosides, alkaloids, phenols, triterpenes and reducing sugars. The highest total flavonoid content was found in *P. thonningii* leaves, with 313.82 µg Eq routine/mg ES. *P. suberosa* leaves showed high *in vitro* antioxidant activity with 90.70 EAA/g ES and 430.50 µmol Eq FeSO₄/mg ES. The largest inhibition zone diameter was observed with the *P. thonningii* trunk bark extract against the *C. albicans* strain (20 mm). *P. suberosa* trunk bark and both organs (leaves and trunk bark) of *P. thonningii* showed a low MIC of 1.562 mg/ml on *S. aureus* strains.

Conclusion: The present study supports the local use of both plants for pathologies caused by the microbial strains studied. However, further research is needed to isolate and identify the chemical molecules responsible for the observed activities, and to study the toxicity of the two plants.

Key words: *Pteleopsis suberosa*, *Piliostigma thonningii*, phytochemistry, activity, antioxidant, antimicrobial.

Introduction

Antibiotic resistance occurs when bacteria develop the ability to evade therapeutic molecules [1], [2]. The evolution of bacterial resistance to conventional antibiotics is a problem of increasing concern [3]. This development, which was only a marginal phenomenon a few years ago, has become a public health issue. In 2019, more than 1.2 $\bar{M}$ deaths due to bacterial resistance worldwide were recorded with a frequency of 27.3 deaths/100 million inhabitants in West Africa [4]. According to the WHO, "if no further action is taken, antimicrobial resistance will kill up to 10 $\bar{M}$ people per year by 2050" [5]. Indeed, the indiscriminate use of antibiotics in a large number of cases is an essential factor for the increase in resistances [3]. This resistance is a current reality

and threatens the future through its impact on morbidity and mortality[6]. In West Africa, the endemicity of respiratory infections, bacterial meningitis, diarrhoea and other infectious diseases has increased the use of antibiotics for both symptomatic treatment and prophylaxis [7].

Faced with the outbreak of multidrug-resistant strains and the therapeutic failures observed here and there, the search for new series of antibiotics is becoming necessary [8], [9]. In addition, the high cost of health services and medicines, as well as socio-economic factors, push a large part of the population to use medicinal plants for treatment [10]. Natural products, whether pure compounds or standardized plant extracts, offer limitless possibilities for the development of new drugs due to their unmatched chemical diversity [11]. Currently, 80% of the population has had at least one use of herbal medicine [12]. An attitude that can also be linked to ancestral culture and civilization, which are based entirely or partially on phytotherapy due to the effectiveness, accessibility and availability of medicinal plants [13].

Medicinal plants contain several molecules with antioxidant and antimicrobial properties [14]. In this context, we were interested in the biological and phytochemical study of hydroethanolic extracts from the leaves and bark of [14] *Pteleopsis suberosa* (Combretaceae) and *Piliostigma thonningii* (Caesalpiniaceae). Both plants are shrubs 6 to 8 m high and are present in West Africa, from Senegal eastwards to Nigeria [15]. The antimicrobial activity of plants such as *P. suberosa* and *P. thonningii* has been proven in Togo [10], [16]. However, for the reliable use of antibacterial agents, it is advisable to have as much information as possible on the sensitivity of bacteria isolates to antibacterial agents and their effectiveness.

Some biological properties have been identified in both plants, but scientific data that can justify their use in traditional medicine are insufficient. Therefore, this study aimed to explore and evaluate other biological properties of these two plants to valid their use in traditional medicine.

Materials and methods

Collection of plant material

The leaves and trunk bark of *Pteleopsis suberosa* (Figure 1) and *Piliostigma thonningii* (Figure 2) were collected in Agamadè and Adabaworé (Kara, a region of Togo) in October 2021 (Figure 3). The material plants have been botanically authenticated at the Herbarium of the Department of Botany, Faculty of Sciences, University of Lome.



Figure 1: Leaves and trunk of *P. suberosa* (Source : Nikon Coolpix P300, 2022)



Figure 2: Leaves of *P. thonningii* (Source : Nikon Coolpix P300, 2022)

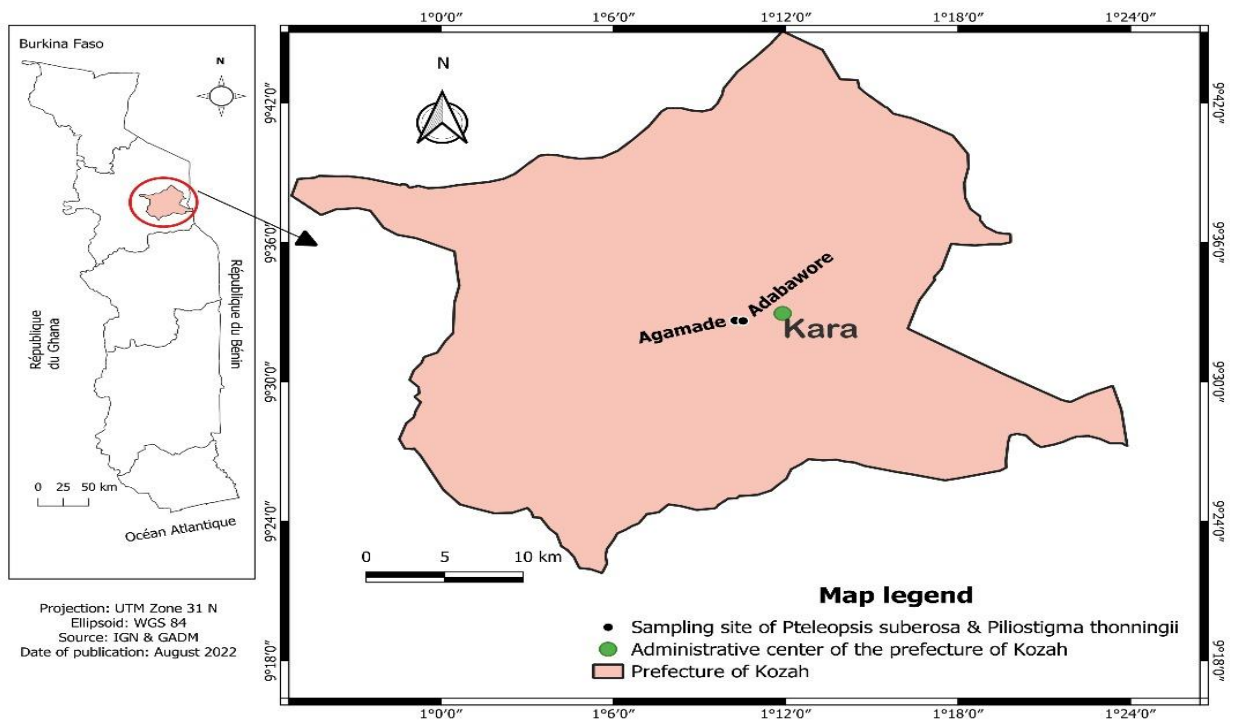


Figure 3: Map showing the locations of organ collection from the two plants in the study

Bacterial strains

The microorganisms used in the test were clinical and reference strains of *Streptococcus pneumoniae* (ATCC 49619 and n₀₃₄), *Escherichia coli* (ATCC 25922 and n₁₆₂₈), *Staphylococcus aureus* (ATCC 29213 and n₀₆₈₉) and *Candida albicans* (ATCC 10231 and n₁₁₃₄) all isolated at the National Institute of Hygiene of Lomé (INH-Lomé).

Extraction

This extraction was carried out in accordance with [17] and taken over by [18], [19]. The samples of each plant were carefully washed under running water and dried in open air at room temperature for two weeks. After drying, the plant materials were reduced to powder using the grinding machine. The ethanolic extraction was carried out by macerating 100 g of powder in 1,000 ml of 70% ethanol (v/v), under continuous stirring for 48 h at room temperature. The extract was filtered through Whatman No.1 filter paper and evaporated at 45°C using a rotary evaporator until dryness under reduced pressure. The extracts were stored at 4°C until further use. The physical characteristics of the extracts were observed with the naked eye (aspect, colour and solubility) and pH using the ph-2005 digital electronic pH meter. Extraction yield is determined by the following formula:

$$\text{Yield(\%)} = \frac{\text{Mass of extract}}{\text{Mass of plant material}} * 100$$

Phytochemical analysis

Phytochemical screening was performed based on staining characteristic tests to identify major chemical groups. This study focused on the hydroethanolic extracts of the plants studied. The chemical groups have been identified by reference to the methods described by [20] and taken up by [18], [21]

Alkaloids

The dissolved extracts were divided into two tubes (2 mL each). In the first tube (test tube), 4 mL of sulfuric acid (H₂SO₄) diluted to 10% and a few drops of Dragendorff's reagent were added. The appearance of an orange-red precipitate indicated the presence of alkaloids in the hydroethanolic extract. The second tube served as the control.

Flavonoids

The dissolved extracts were divided into two tubes (2 mL each). In the first tube (test tube), 4 mL of methanol and a few drops of magnesium turns were added, followed by 1 mL of concentrated HCl at 1%. The appearance of a red coloration indicates the presence of flavonoids in the hydroethanolic extract. The second tube served as a control.

Saponines

The dissolved extracts were divided into two tubes (2 mL each). A few drops of distilled water were added to the first tube (test tube). The appearance and persistence of foam after stirring indicated the presence of saponosides. The second tube served as a control.

Tannins

The dissolved extracts were divided into two tubes (4 mL each). In a first tube (test tube), a few drops of FeCl_3 were added. The appearance of a blackish-brown color after homogenization of the mixture indicated the presence of tannins in the hydroethanol extract. The second tube served as a control.

Phenols

The dissolved extracts were divided into two tubes (4 mL each). In a first tube (test tube), a few drops of FeCl_3 were added. The appearance of a blackish-brown color after homogenization of the mixture indicated the presence of phenolic compounds in the hydroethanolic extract.

Triterpenes and sterols

The dissolved extracts were divided into two tubes (4 mL each). In a first tube (test tube), 1.6 mL of chloroform and 2.6 mL of sulfuric acid (H_2SO_4) were added. The appearance of a reddish-brown ring between the two phases, one clear at the bottom and the other green at the top, indicates the presence of triterpenes and sterols in the hydroethanolic extract. The second tube served as a control.

Coumarins

The dissolved extracts were divided into two tubes (4 mL each). In the first tube (test tube), 2 mL of distilled water was added and a 100-200 μL of ammonia was added to the mixture. The

appearance of fluorescence after ultraviolet illumination indicates the presence of coumarins in the hydroethanolic extract. The second tube served as a control.

Reducing sugars

The dissolved extracts were divided into two tubes (2 mL each). In the first tube (test tube), 2 mL of Fehling's Liquor (1 mL of solution A, also known as 7% copper sulphate II + 1 mL of solution B, consisting of the mixed solution of 35% sodium potassium tartrate and 10% soda) were added and boiled in a boiling sea bath for 2 to 3 min. The appearance of a brick-red precipitate indicated the presence of reducing sugars in the hydroethanolic extract. The second tube served as a control.

Flavonoid content

The flavonoid content was estimated according to the method described by [22] and taken up by [18], [21]. Plant extracts were prepared at 1mg/mL in distilled water, 2% aluminium chloride (AlCl₃) in distilled water, and rutin at different concentrations (0, 5, 25, 50, 75, 100, 150, 200 µg/mL) in methanol. The operation consisted of mixing 1 mL of the extract solution at 1 mg/ml or 1 mL of each rutin concentration with 1 mL of 2% aluminum chloride (AlCl₃) using a vortex. After 10 min of incubation, the absorbance was directly measured using a UV-visible spectrophotometer (METASH UV-5200PC UV/VIS Spectrophotometer) at 415 nm against a blank. Rutin was used as the standard. The total flavonoid content of the extracts was inferred from the calibration curve established with rutin (0 - 200 µg/mL), and the results were expressed in microgram equivalent of rutin per milligram of dry matter (µg ER/mg DE). Three tests were performed for each extract. The total flavonoid content (**XFlav**) was calculated using the following formula:

$$\mathbf{XFlav} = \left[\frac{1}{0,0078} (\mathbf{DO} + 0,0428) \right]$$

XFlav: Total flavonoid content (µg ER/mg DE)

OD: Optical Density read at 415 nm

Antioxidant activity

Phosphomobyldate reduction test

Determination of antioxidant activity by the phosphomobyldate reduction assay was performed using the method described by Phosphomobyldate Reduction Test [23]. The phosphomolybdate

reagent was prepared (100 mL of reagent) from a mixture of 90 mL of 0.6 M sulfuric acid, 5 mL of 0.1% sodium phosphate and 5 mL of 1% ammonium molybdate. For this test, 1 mL of each extract was added to 9 mL of the above reagent. The mixture was heated to 95°C in a water bath for 90 min. The mixture was then cooled to room temperature. Absorbances were measured at a wavelength of 820 nm against a blank consisting of the reagent and distilled water. Ascorbic acid was used as the standard antioxidant under the same operating conditions and the results were expressed in milligrams of ascorbic acid equivalent per gram of dry matter (mg EAA/g DE). Three tests were performed for each tested product concentration. The antioxidant activity related to the reducing power of the extracts was expressed in Antioxidant Content (XMob) using the following formula:

$$\text{XMob} = \left[\frac{1}{0.0057} (\text{DO} + 0.0599) \right]$$

XMob: Antioxidant Content (mg EAA/g DE)

OD: Optical Density read at 820 nm

Test FRAP (Ferric Reducing antioxidant Power)

The determination of antioxidant activity by the FRAP assay was carried out using the method described [24] by and taken up by [18], [21], [25]. Thus, 3 mL of the freshly prepared FRAP test reagent in a test tube, 100 µL of the various solutions of iron II sulphate with concentrations between 0 and 2000 µmol. L⁻¹ were added. The mixture was vigorously shaken by vortexing, and the optical density was read after 5 min with a spectrophotometer at 593 nm. The absorbance of the TPTZ-Fe²⁺ complex allowed us to draw a calibration curve from the concentration range (0 - 2000 µM) of the iron sulfate solution (FeSO₄, 7H₂O) dissolved in methanol. For the samples of the extracts to be tested, the FRAP reagent (3 mL) and the solution of the extract to be tested (100 µL) with a strength of 1 mg/mL were mixed in the same proportions as for the standard curve plotting. The optical density was measured after 5 min at 593 nm. The antioxidant capacity of the extracts was measured using a calibration curve based on the color change related to the formation of the complex (Fe²⁺ TPTZ) and expressed in micromole equivalents of iron sulfate per milligram of dry matter (µmol Eq FeSO₄/mg DE). The tests were repeated 3 times. The antioxidant activity related to the reducing power of the extracts was expressed in Reducing Power (XFRAP) using the following formula:

$$\text{XFRAP} = \left[\frac{1}{0,0002} (\text{DO} + 0,0331) \right]$$

XFRAP: Reducing power ($\mu\text{mol Eq FeSO}_4/\text{mg DE}$)

OD: optical density read at 593 nm

Evaluation of the antimicrobial potency of extracts

Identification of the active extracts was conducted by the solid-state diffusion method, and the Minimum Inhibitory Concentration (MIC) was determined by the liquid dilution method [26].

Preparation of microbial strains

After collection, the strains were transplanted to their appropriate media (Chapman for *S. aureus*, Sabouraud + Chloramphenicol for *C. albicans*, Mac Conkey for *E. coli* and Fresh Blood Agar (FSG) for *S. pneumoniae*) and stored. To obtain the 24-hour colonies that were used to prepare the inoculum, the strains were transplanted onto agar medium without inhibitors [27].

Preparation of the inoculum

From an 18-24 h culture on agar medium, a suspension was prepared in saline solution (0.9% NaCl) equivalent to the Mac Farland standard 0.5 ($\sim 10^8$ CFU/ml). Inoculation was performed by flooding with the inoculum suspension diluted to 1/100 ($\sim 10^6$ CFU/ml) in accordance with the necessary safety measures [27].

Preparation and sterilization of the stock solution of extracts

The extracts were dissolved in sterile distilled water to an initial concentration of 200 mg/mL (200 mg of each extract in 1 mL of distilled water). To verify their sterility, an aliquot of each stock solution was inoculated onto Muller Hinton agar. After incubation at 37°C for 24 h, the colonies were filtered through a millipore membrane of 0.45 μm in diameter. After sterilization, each stock solution was diluted to 1/4 with distilled water to obtain 50 mg/mL solutions of each extract.

***In vitro* antimicrobial activity**

Mueller Hinton (MH) agar plates for bacteria and Sabouraud dextrose + Chloramphenicol for yeasts were inoculated by flooding following the recommendations of the AntibioGram Committee of the French Society of Microbiology [28]. After the dishes have dried, (approximately 5 min), the agar was perforated into a maximum of six wells (90 mm Petri dishes) with a sterile tip

previously cut so that the diameter was approximately 6 mm. The cavities formed were filled with 50 μ L of the 50 mg/mL extract solution (50 μ L/ well). The boxes were kept at room temperature 25°C for 15 min for the pre-diffusion phase. The Petri dishes were incubated at 37°C for 24 h for bacteria and 48 h for yeasts. Ciprofloxacin (bacteria), and Nystatin (yeast) were used as positive controls, and distilled water as negative control. After 24 h of incubation, the diameters of inhibition zones were measured using a graduated ruler. The tests were performed three times, and the average was obtained [26]. The Minimum Inhibitory Concentration (MIC) and the minimal bactericidal concentration (MBC) were determined for extracts with inhibition diameters greater than or equal to 11 mm. The minimum inhibitory concentration (MIC) was determined using the microplate microdilution method [27]. Mueller Hinton Broth was used to prepare serial dilutions at half concentrations ranging from 50 to 0.097 mg/mL. Inoculum (10 μ L) was added to each cup containing 50 μ L of the extract. Cupules without inoculum were used as negative controls. All the cups were incubated at 37°C for 24 h. The first cup in the series showing no sign of culture visible to the naked eye (To the naked eye the middle is clear, absence of turbidity, significant absence of bacterial growth) was considered as the MIC. An aliquot of the contents of each cupule with no visible growth was taken and seeded by spreading on Mueller Hinton medium [27]. The dishes were incubated at 37°C for 24 to 48 h at the end of which the number of colonies was determined. After incubation, the smallest concentration of the extract that did not produce colonies was considered as the MBC. To determine whether the observed antimicrobial effect was bactericidal or bacteriostatic, the MBC/MIC ratio was used to assess the activity of the extracts (bactericidal/bacteriostatic) as follows: MBC/MIC ratio > 1 indicated a bacteriostatic activity, whereas MBC/MIC ratio of 1 indicated a bactericidal activity [29].

Statistical analysis of the data

The data obtained were subjected to statistical analysis using R Studio 4.1.3 software from 10/03/2022 and Microsoft-Excel® version 2408 Build: 16.0.17932.20428 of the year 2021. Quantitative variables (flavonoid content, FRAP test, and phosphomolybdate reduction method) are presented as a mean and standard deviation. A One-Factor Analysis of Variance (ANOVA) was used to assess the diameters of the zones of inhibition. The significance level was set at 5%.

Results

In this study, the highest yield was obtained by extracting the trunk bark of *P. thonningii* (22.67%). The lowest yield was obtained with the extract of *P. suberosa* leaves (7.33%). In addition, all extracts had an acidic pH (pH < 7) and ranged from 4.81 to 5.88. From the point of view of

solubility, it can be said that all the extracts studied have a fairly good solubility with respect to the solvent used. The color of the extracts is sometimes a function of the part of the plant used. For example, extracts from trunk bark are brown or coffee, and extracts from the leaves are black (Table 1).

The extracts were analyzed for alkaloids, tannins, flavonoids, saponins, reducing sugars, triterpenoids, and sterols. Table 2 presents the results. Hydroethanolic extracts from trunk leaves and bark, *P. thonningii*, and *P. suberosa*, contained all secondary metabolites tested to varying degrees, except coumarins (Table 2).

Quantitative tests confirmed the presence of flavonoids in all the extracts at various doses (Table 3). The highest flavonoid content was measured with the hydroethanolic leaf extract *P. thonningii* (313.82 µg ER/mg ES) and the lowest with the hydroethanolic extract of the trunk bark of *P. suberosa* (114.33 µg ER/mg DE).

The evaluation of the antioxidant activity revealed that all the extracts have antioxidant properties. The best antioxidant activity was observed with *P. suberosa* leaf extract with 90.70 mg EAA/g ES by the phosphomolybdate reduction assay and 430.50 µmol FeSO₄/mg ES by the FRAP assay (Table 5).

Regarding antimicrobial activity, at the initial concentration of 50 mg/ml, the four hydroethanolic extracts tested in this study significantly inhibited the growth of microorganisms to varying degrees ($p = 2.e-16$, CV = 3.862% to 5.562%) with the solid media diffusion method.

The hydroethanolic extract from the trunk bark of *P. thonningii* yielded the largest inhibition diameter of 20 mm (Table 6) on *C. albicans* ATCC 10231 and 16 mm on the clinical strain of *S. pneumoniae* (n₀₃₄).

All the extracts tested had MICs ranging from 1.562 to 25 mg/ml. The smallest MIC (1.562 mg/ml) was obtained from hydroethanolic extracts from the trunk bark of *P. suberosa*, *P. thonningii* and *P. thonningii* leaves on *S. aureus* (n₀₆₈₉). Hydroethanolic extract of *P. suberosa* (leaves and trunk bark) and *P. thonningii* (trunk bark) had bactericidal activity on *S. aureus* (ATCC 29213 and n₀₆₈₉), *S. pneumoniae* (ATCC 49619 and n₀₃₄), *C. albicans* (ATCC 10231) and *E. coli* (ATCC 25922).

Tableau 1: Some characteristics of the extracts obtained

Plants	Organs	Aspect	Colour	Solubility	pH	Yield
<i>P. suberosa</i>	Leaves	Crystals	Crochet	Pg	5,00	7,33%
	Trunk bark	Powder	Brown	Pg	4,81	11,34%

<i>P. thonningii</i>	Leaves	Powder	Crotchet	Pg	5,88	9,88%
	Trunk bark	Gum	Coffee	Pg	5.22	22,67%

pH: Hydrogen potential; Pg: Pretty good

Tableau 2: Phytochemical study of the studied extracts

Secondary metabolites	Extracts from essential oils from <i>P. suberosa</i>		HE extracts from <i>P. thonningii</i>	
	Leaves	Trunk bark	Leaves	Trunk bark
Alkaloids	+++	+++	++	++
Flavonoids	+	+	++	+++
Tannins	+++	+++	+++	++
Coumarins	-	-	-	-
Saponosides	+++	+	++	+
Triterpenes	+	+	++	++
Phenol	+++	+++	++	+++
Sugar Reducers	+	+	+	+

Legend : - : Absence; +: Less important; ++: Important; +++: More Important; HE: Hydroethanolic

Tableau 3: Flavonoid content of the extracts studied

HE Extracts	<i>P. suberosa</i>		<i>P. thonningii</i>	
	Leaves	Trunk bark	Leaves	Trunk bark
Total flavonoid content (µg ER/mg DE)	262.66 ± 0.97	114.33 ± 0.91	313.82 ± 0.88	146.51 ± 1.28

DE: DE: Dry extract, ER: Equivalent of Rutine.

Tableau 4: Antioxidant activities in vitro Excerpt studied

Hydroethanolic extracts	Phosphomolybdate Reduction Test (mg EAA/g DE)	FRAP Testing (µmol FeS04/mg DE)
Leaves of <i>P. suberosa</i>	90.70 ± 0.19	430.50 ± 5.00
Trunk bark of <i>P. suberosa</i>	81.98 ± 0.74	408.83 ± 2.89
Leaves of <i>P. thonningii</i>	87.62 ± 0.16	337.17 ± 5.77
Trunk bark of <i>P. thonningii</i>	77.41 ± 0.34	268.53 ± 5.00

EAA: Equivalent of Ascorbic Acid ; FRAP: Ferric Reducing antioxidant Power ; DE: Dry extract

Antimicrobial activities

Table 5: Antimicrobial testing result of extracts on germs tested by the well method

Microbial strains	Extracts from essential oils from <i>P. suberosa</i>		EO extracts from <i>P. thonningii</i>		Water- Ethanol CN	Cipro. /Nyst. CP (50 µg/mL)	P-value	CV (%)
	Leaves (50 mg/mL)	Trunk bark (50 mg/mL)	Leaves (50 mg/mL)	Trunk bark (50 mg/mL)				
<i>S. aureus</i> ATCC 29213	12.00 ± 1.00d	17.33 ± 0.58b	12.67 ± 0.58d	15.33 ± 0.58c	6.00 ± 0.00e	37.67 ± 0.57a	2 ^{e-16} ***	3.856
<i>S. aureus</i> n ₀₆₈₉	13.67 ± 0.58c	13.67 ± 0.58c	10.67 ± 0.58d	14.67 ± 0.58b	6.00 ± 0.00e	25.67 ± 0.57a	2 ^{e-16} ***	3.833
<i>E. coli</i> ATCC 25922	11.67 ± 0.58e	12.67 ± 0.58d	13.67 ± 0.58c	15.67 ± 0.56b	6.00 ± 0.00f	44.00 ± 1.00a	2 ^{e-16} ***	4.225
<i>E. coli</i> n ₁₆₂₈	8.67 ± 0.58d	12.67 ± 0.58c	8.67 ± 0.58d	13.67 ± 0.58b	6.00 ± 0.00e	17.00 ± 1.00a	2 ^{e-16} ***	4.037
<i>S. pneumonia</i> ATCC 49619	15.33 ± 0.58d	16.67 ± 0.58c	12.67 ± 0.58e	18.67 ± 1.15b	6.00 ± 0.00f	42.33 ± 0.57a	2 ^{e-16} ***	3.922
<i>S. pneumonia</i> n ₀₃₄	15.67 ± 0.58c	12.67 ± 0.58d	10.67 ± 0.58e	16.33 ± 0.58b	6.00 ± 0.00f	33.33 ± 0.57a	2 ^{e-16} ***	3.362
<i>C. albicans</i> ATCC 10231	18.67 ± 0.58c	14.67 ± 0.58d	13.00 ± 1.00e	20.00 ± 1.00b	6.00 ± 0.00f	32.00 ± 0.57a	2 ^{e-16} ***	4.495
<i>C. albicans</i> n ₁₁₃₄	12.33 ± 1.15c	10.67 ± 0.58d	9.67 ± 0.56e	14.00 ± 1.00b	6.00 ± 0.00f	29.33 ± 0.57a	2 ^{e-16} ***	5.562

The values are expressed as the Mean ± Standard Error. Values in the same column followed by the same lowercase letter are statistically identical (Duncan, p < 0.05)

EO: Hydroethanolic; CN: Negative control; CP: positive control; CV: Coefficient of Variance

Table 6: Results of the MIC, MBC or MFC of the active extracts

HE Extracts	Microbial strains	MBC	MIC	MBC/MIC	Interpretation
Leaves of <i>P. suberosa</i>	<i>S. aureus</i> ATCC 29213	12.5	12.5	1	Bactericidal
	<i>S. aureus</i> n ₀₆₈₉	6.25	6.25	1	Bactericidal
	<i>E. coli</i> ATCC 25922	50	25	2	Bacteriostatic
	<i>S. pneumoniae</i> ATCC 49619	50	25	2	Bacteriostatic
	<i>S. pneumoniae</i> n ₀₃₄	12.5	12.5	1	Bactericidal
	<i>C. albicans</i> ATCC 10231	25	12.5	2	Bacteriostatic
	<i>C. albicans</i> n ₁₁₃₄	12.5	6.25	2	Bacteriostatic
Trunk bark of <i>P. suberosa</i>	<i>S. aureus</i> ATCC 29213	12.5	6.25	2	Bacteriostatic
	<i>S. aureus</i> n ₀₆₈₉	12.5	1.562	8	Bacteriostatic
	<i>E. coli</i> ATCC 25922	25	6.25	4	Bacteriostatic
	<i>E. coli</i> n ₁₆₂₈	50	12.5	4	Bacteriostatic
	<i>S. pneumoniae</i> ATCC 49619	50	12.5	4	Bacteriostatic
	<i>S. pneumoniae</i> n ₀₃₄	12.5	6.25	2	Bacteriostatic
	<i>C. albicans</i> ATCC 10231	12.5	12.5	1	Bactericidal
Leaves of <i>P. thonningii</i>	<i>C. albicans</i> n ₁₁₃₄	12.5	6.25	2	Bacteriostatic
	<i>S. aureus</i> ATCC 29213	25	3.125	8	Bacteriostatic
	<i>S. aureus</i> n ₀₆₈₉	50	1.562	32	Bacteriostatic
	<i>E. coli</i> ATCC 25922	50	25	2	Bacteriostatic
	<i>S. pneumoniae</i> ATCC 49619	50	25	2	Bacteriostatic
	<i>S. pneumoniae</i> n ₀₃₄	50	3.125	16	Bacteriostatic
	<i>C. albicans</i> ATCC 10231	50	25	2	Bacteriostatic
Trunk bark of <i>P. thonningii</i>	<i>S. aureus</i> ATCC 29213	12.5	6.25	2	Bacteriostatic
	<i>S. aureus</i> n ₀₆₈₉	6.25	1.562	4	Bacteriostatic
	<i>E. coli</i> ATCC 25922	12.5	12.5	1	Bactericidal
	<i>E. coli</i> n ₁₆₂₈	50	25	2	Bacteriostatic
	<i>S. pneumoniae</i> ATCC 49619	12.5	12.5	1	Bactericidal
	<i>S. pneumoniae</i> n ₀₃₄	12.5	6.25	2	Bacteriostatic
	<i>C. albicans</i> ATCC 10231	25	12.5	2	Bacteriostatic
	<i>C. albicans</i> n ₁₁₃₄	12.5	6.25	2	Bacteriostatic

HE: Hydroethanolic; MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, MFC: Minimum Fongicid Concentration

Discussion

The study evaluated the antimicrobial activities of *P. suberosa* and *P. thonningii* on clinical strains of *E. coli*, *S. aureus*, *S. pneumoniae* and *C. albicans*. The choice of these plants as study materials is guided by their common use in African traditional medicine to cure various diseases [30].

- Yield

Our results showed that *P. thonningii* trunk bark extract had the best extraction yield (21.44%), followed by *P. suberosa* trunk bark (11.34%). The work of Klotoe et al. [31] obtained a similar

yield of 8.93% for the trunk bark of *P. suberosa* with the same solvent. The choice of extraction solvent was based on the fact that 70% ethanol gives a better yield [32]. Hydroethanolic extraction is valued because of its ability to extract a wide range of compounds, relative safety of use, and low cost compared to other extraction methods [33]. However, it is important to note that the composition of the hydroethanolic mixture, temperature, extraction time, and other parameters must be carefully controlled to achieve the desired results.

- Qualitative and quantitative phytochemical study

Qualitative phytochemical studies of extracts from the trunk bark and leaves of *P. suberosa* and *P. thonningii* have revealed the presence of alkaloids, tannins, triterpenoids, saponosides, reducing sugars, phenols and flavonoids. These results corroborate those described by [34], [35] in their work at Benin for the hydroethanolic extract of *P. suberosa* leaves and those of [36] for the hydroethanolic extract of *P. thonningii* leaves.

Our results showed that *P. thonningii* leaves contained a high flavonoid content of 313.82 µg ER/mg DE. Previous works The work [37], [38] shows that the leaves of *P. thonningii* have a significant flavonoid content, using quercetin as a reference molecule.

- Antioxidant activity

With regard to antioxidant activity, the FRAP test and the phosphomolybdate reduction test gave a better reducing power of *P. suberosa* leaves with 430.5 µmol FeSO₄/mg ES and 90.7 mg EAA/g ES, respectively. A previous study [31] obtained similar results using the DPPH reduction test. The remarkable presence of flavonoids could explain the strong antioxidant activity in the leaves of *P. suberosa* [39].

- Antimicrobial activity

Presumptive testing using the agar diffusion method revealed that the four extracts significantly inhibited the growth of microorganisms to varying degrees ($p = 2.e-16$, $cv = 3.862\%$ to 5.562%). The leaf and trunk bark extracts of *P. suberosa* and *P. thonningii* significantly inhibited ($P < 0.05$) the growth of the sprouts tested.

The hydroethanolic extract from the trunk bark of *P. thonningii* yielded the largest inhibition diameters of 20 mm (Table 6) on *C. albicans* ATCC 10231 and 16 mm on the clinical strain of *S. pneumoniae* (n₀₃₄). The work [40] obtained a similar inhibition zone diameter with hydroethanolic extract of *P. thonningii* on *C. albicans*.

The smallest MIC (1.562 mg/ml) was obtained from *P. suberosa* trunk bark extracts and *P. thonningii* leaf and stem bark extracts from *S. aureus*. These values are higher than those obtained by who obtained a [41] MIC of 0.15 mg/ml on *S. aureus* with the methanolic extract of the trunk bark of *P. suberosa* using the microdilution method with a growth indicator, purple p-iodonitrotetrazolium.

In terms of effect, *P. suberosa* leaf and trunk bark extracts and *P. thonningii* stem bark extract had bactericidal effects on *S. aureus* strains and bacteriostatic effects on other germs.

The presence of phytochemicals is thought to be the basis for the antimicrobial activity observed in both plants (*P. suberosa* and *P. thonningii*). Most of the biological properties of flavonoids are related to their ability to form complexes with macromolecules, especially fungal, bacterial or viral proteins and are therefore antibacterial, antifungal, antiviral and anti-inflammatory [42], [43]

Conclusion

The objective of our study was to evaluate the biological and phytochemical activities of *P. suberosa* and *P. thonningii* on strains of *E. coli*, *S. aureus*, *S. pneumoniae* and *C. albicans*. Qualitative phytochemicals have shown that extracts of *P. thonningii* and *P. suberosa* (leaves and trunk bark), contain flavonoids, alkaloids, tannins, reducing sugars, phenols, triterpenes and saponosides. Quantitative phytochemistry gave a higher total flavonoid content on *P. thonningii* leaf extract (313.82 µg Eq routine/mg ES). *P. suberosa* leaves showed high antioxidant activity *in vitro* with 430.50 µmol Eq FeSO₄/mg ES. The trunk bark of *P. suberosa* and the two organs (leaves and trunk bark) of *P. thonningii* showed the lowest MIC (1.562 mg/ml) on *S. aureus* strains. The trunk bark of *P. suberosa* showed a bactericidal effect on *C. albicans* and the trunk bark of *P. thonningii* on *E. coli* and *S. pneumoniae*.

In conclusion, the extracts of *P. suberosa* leaves and *P. thonningii* stem bark were the most active on all the sprouts tested.

A subsequent study with the aim of isolating and identifying molecules with antioxidant and antimicrobial activity could provide a better understanding of the nature of the active ingredients.

Abbreviations

ATCC: American Typographic Culture Collection

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

MFC: Minimum Fungicidal Concentration

CS NDE: Sisters of Our Lady of the Church Health Center

INH: National Institute of Hygiene of Lomé

MH: Mueller-Hinton

WHO: World Health Organization

Ethics Approval and Consent to Participate

Not Applicable

Consent to Publication

Not Applicable

Availability of Data and Materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

Tchilabalo Bouyo, Sandrine Tènè Salifou, Jules Koffi Kpatagnon, Passimna Pissang and Komi Koukoura Komi harvested and identified the plant parts used. Tchilabalo Bouyo, Sandrine Tènè Salifou, Passimna Pissang, Yao Hoekou, Holaly Efui Gbékley and Komi Koukoura Komi participated in the extraction and flavonoid assay. Antioxidant activity tests were carried out by Tchilabalo Bouyo, Bawimodom Bidjada, Sandrine Tènè Salifou, Passimna Pissang, Jules Koffi Kpatagnon, Samadou Tchakondo, Yao Hoekou and Holaly Efui Gbékley. The results were analyzed statistically by Tchilabalo Bouyo, Holaly Efui Gbékley, Blaise Etienne M'BOUMBA and Passimna Pissang. Finally, Tchilabalo Bouyo, Komla Mawunyo Dossouvi, Passimna Pissang, Komi Koukoura Komi wrote the maniscrit and Komi Koukoura Komi and Tchadjobo Tchacondo approved and supervised the work.

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