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PHENOLIC COMPOSITION, ANTI-BIOFILM, ANTI-QUORUM SENSING, ANTIOXIDANT AND ENZYME INHIBITORY ACTIVITIES OF *PTELEOPSIS SUBEROSA* (COMBRETACEAE) LEAVES

Alain Yaya Koudoro[1], Alfred Ngenge Tamfu[2-4]*, Aristide Mfifen Munvera[5], Selcuk Kucukaydin[4], Pascal Agbangnan Dossa Cokou[1], Félicien Avlessi[1], Dominique Sohounhloue Codjo Koko[1], Ozgur Ceylan[3]

1. ***Laboratory of Study and Research in Applied Chemistry, University of Abomey-Calavi, Polytechnic School of Abomey-Calavi, 2009 Abomey-Calavi, Benin.***
2. ***Department of Chemical Engineering, School of Chemical Engineering and Mineral Industries, University of Ngaoundere, 454 Ngaoundere, Cameroon.***
3. ***Food Quality Control and Analysis Program, Ula Ali Kocman Vocational School, Mugla Sitki Kocman University, 48147 Mugla, Turkey.***
4. ***Department of Medical Services and Techniques, Koycegiz Vocational School of Health Services, Mugla Sitki Kocman University, 48800 Mugla, Turkey.***
5. ***Department of Organic Chemistry, Faculty of Science, The University of Yaounde I, 812 Yaounde, Cameroon.***

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ABSTRACT

P. suberosa is a multipurpose medicinal plant in West and Central Africa. Fourteen phenolic compounds were identified in the *P. suberosa* leaves extract using HPLC-DAD and gallic acid (175.10±0.42 µg/g) was the most abundant. The total phenolic content was 112.16 ± 0.33 mg GAE/g DW while the total flavonoid content was 36.10±0.58 mg QE/g DW. Minimal inhibitory concentration (MIC) values for antimicrobial activity were 0.3125 mg/mL and 1.25 mg/mL on *S. aureus* and *E. faecalis* respectively and 2.5 mg/mL and 0.3125 mg/mL on *E. coli* and *S. typhi* respectively. Biofilm inhibition evaluated at sub-MIC concentrations revealed that gram-negative biofilms were more susceptible to *P. suberosa* extract than gram-positive ones and *E. coli* biofilms were the most susceptible. The extract inhibited violacein production and quorum sensing with inhibition zones varying from 17.0±0.5 mm at MIC to 12.0±0.1 mm at MIC/4. The extract showed good antioxidant capacity and was more active in the DPPH• assay than the two standards α-tocopherol and BHA used. In the ABTS•+ and CUPRAC assays, the activity of the extract was greater than that of α-Tocopherol and very close to that of BHA. The extract showed potential to alleviate Alzheimer's disease by inhibiting acetylcholinesterase and butyrylcholinesterase as well as antidiabetic activity by inhibiting α-amylase and α-glucosidase.

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Introduction

Traditional medicine is highly used in several poor countries in the treatment of various ailments. Plants develop various means to protect themselves by the production of secondary metabolites of wide structure which makes them a suitable basis for drugs and about 60% of the drugs being approved come from natural products [1]. The use of plants as medicine to cure or prevent illness in the world and particularly in Africa is very important as there is a general lack of medical facilities and trained medical practitioners in addition to high cost, unaffordability, unavailability, and inaccessibility [2, 3]. It has globally been used as an important source of traditional medicine to treat diseases since antiquity and over half of the conventional drugs are developed or discovered from plant sources [4]. The emergence of pathogens resistant to antibiotics is increasingly

Corresponding Author: Alfred Ngenge Tamfu; Department of Chemical Engineering, School of Chemical Engineering and Mineral Industries, University of Ngaoundere, 454 Ngaoundere, Cameroon. E-mail: macntamfu@yahoo.co.uk.

high and represents a serious problem for public health [5]. Resistance to antibiotics usually arises when target bacteria come up with a physiological mechanism to challenge the effects of the drug usually by changing its envelope structure which is the biofilm or by producing chemical substances that overcome the drugs or evade it [6]. Bacteria form protective extracellular matrix called biofilms through a quorum sensing (QS) regulated communication system with autoinducer signal compounds which makes them less susceptible to antibiotics and stress stimuli [7]. A suitable strategy to overcome antibiotic resistance is to target activities of bacteria that are controlled by quorum-sensing and to inhibit the establishment of biofilms. Several antibiotics produced by the pharmacological industry are usually faced with resistance over time thereby giving room for the search for natural plant-based antibiotics. Reactive nitrogen and oxygen species (RNOS) formed from cellular activities or exposure to toxic substances and adverse conditions can oxidize biomacromolecules such as lipids, proteins, and DNA in living systems, leading to oxidative stress at the cellular level and destruction of biomolecules such as lipids, DNA and various proteins [8]. Oxidative damage results from the inequality of RNOS production and the system's antioxidant defense mechanisms and this is at the origin of various pathological conditions. Natural antioxidants are gaining more interest, and phytochemicals are believed to be safer and more effective antioxidants. Enzymes are important in catalyzing biochemical reactions but their excessive activity can create unwanted conditions and lead to illnesses. Enzymes such as butyrylcholinesterase (BChE), acetylcholinesterase (AChE), tyrosinase, α-amylase, urease, and α-glucosidase are involved in pathogenesis in humans and their inhibition is an effective strategy in the management of related diseases [9, 10]. Medicinal plants and other natural products represent an immense resource, providing bioactive molecules which can act as enzyme inhibitors in the treatment of certain human diseases [11].

Pteleopsis suberosa (Combretaceae) Engl. and Diels is usually 6–10 m tall with characteristic coarse and corky warts on its barks and it is a deciduous shrub. The various parts of *P. suberosa* are used for medicinal purposes throughout West and Central Africa. Fresh root decoction of this plant is used as a remedy for dermatitis, enema, stomach ache, and gastric ulcers, as well as purgative and antidysentery. Its shoots and root extracts are consumed as an antitussive [12]. Infusions from barks or and twigs of *P. suberosa* are used in treating jaundice, hemorrhoids, toothache, filariasis, trachoma, wounds, conjunctivitis, cataract amoebic dysentery, antiulcer, skin disease, antiviral and as an aphrodisiac [12-14]. Phytochemical studies of this plant have revealed that it contains phenolics, tannins, coumarins, and triterpenoids as essential phytoconstituents [12, 13]. The different parts of *P. suberosa* possess anti-inflammatory, antifungal, and antioxidant activities [13, 14].

However, few reports exist about the medicinal uses of the leaf extract which is claimed by practitioners of natural medicine. This work aims at the valorization of *Pteleopsis suberosa* through the determination of its phenolic composition and its inhibitory potential of quorum-sensing processes, biofilm formation, and enzymes associated with some human ailments.

Materials and Methods

Plant Material and Extraction

Pteleopsis suberosa Engl. Diels leaves were collected in January 2019 in the central region of the Benin Republic. The plant identification was done by the botanist Prof. Hounnankpon YEDOMONHAN who prepared a voucher specimen YH 721/HNB and deposited it at the Benin National. The extraction was made with water: ethanol (30: 70) under ultrasonic conditions. Briefly, 5 g of leaves powder in extraction solvent (100 mL) was subjected to ultra-sound using a Bandelin (Sonorex Digitech device) bath at 50 °C for 2h. The supernatant was passed through filter paper (Whatman No.1) and evaporated using a rotary evaporator (Buchi R215) at 70 °C to afford a crude extract which was stored at 4 °C in the dark until characterization and assays.

Determination of Phenolic Profile Extract Using HPLC–DAD

RP-HPLC (reversed-phase) containing a diode-array-detector (DAD) was used to detect phenolics in the extract as described elsewhere [9, 15]. A known weight of the extract was added to H_2O/MeOH (80/20) and a 0.25 µm disk filter was used in filtration before injection. 20.0 µL injection volume in C18 column (Intersil ODS-3) at a 1 mL/min solvent flow and eluents: 0.50% CH_3COOH in H_2O (A) and 0.50% acetic acid in MeOH (B) with variable elution gradient was used. The DAD detector was set at 280 nm. Authentic samples' retention times and UV data were used for identification. Calibration plots using 0.0~1.0 ppm of twenty-six authentic samples (Gallic, Protocatechuic, Ellagic, *p*-Hydroxybenzoic, 3-Hydroxybenzoic, Chlorogenic, Syringic, *trans*-Cinnamic, Rosmarinic, Vanillic, *p*-Coumaric, Ferulic acids; then Catechin, Hesperetin, Luteolin, Apigenin, 6,7-Dihydroxycoumarin, Kaempferol, Pyrocatechol, Vanillin, Coumarin, Taxifolin, Myricetin, Rutin, Chrysin and Quercetin) were used. The detected compounds are reported as micrograms per gram of dry plant weight.

Evaluation of Total Flavonoid and Phenolic Contents of P. Suberosa Extract

The TPC (total phenolic content) of the *P. suberosa* extract was done using the reagent of Folin-Ciocalteu [16]. 0.20 mL of Folin-Ciocalteu in 0.60 mL of H_2O was added to either gallic acid or *P. suberosa* extract (0.20 mL) and stirred for 5 min. 1.0 mL of $NaHCO_3$ (8.0%) was introduced. H_2O was added to make 3.0 mL total volume followed by half an hour incubation of the mixture in the dark, then centrifugation and absorbance at 765 nm. The standard gallic acid curve was established and TPC was calculated and expressed as GAE/gDW. TFC (total flavonoid content) was measured by the $AlCl_3$ method [16]. Serial dilutions (5.0–400.0 μg/mL) were made from quercetin or extract (5 mg in 1 mL MeOH). 0.60 mL test solutions and 0.60 mL

of 2% $AlCl_3$ were added, stirred, and incubated for 1 h. Absorbances were recorded against a blank at 420 nm. The TFC of *P. suberosa* extract was deduced from a calibration plot of the standard quercetin and given as mgQE/gDW.

Microorganisms

Biofilm and antibacterial assays (*Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Salmonella* Typhi ATCC 14028, *Staphylococcus aureus* ATCC 25923); quorum-sensing and violacein (*Chromobacterium violaceum* CV026 and CV12472); swarming assay (*Pseudomonas aeruginosa* PA01).

Antimicrobial Assay (Minimal Inhibitory Concentration MIC)

MIC of *P. suberosa* extract was investigated using broth-dilution means as described elsewhere [17]. Mueller-Hinton broth and 96-well plates were used. 100.0 µL bacterial solutions (5×10^5 CFU/mL) in the presence/absence of *P. suberosa* extract (5.0 - 0.0781 mg/mL). Incubation of all microplates at 37 ºC for 24 hours was done and MIC values were deduced.

Biofilm Inhibitory Effect of P. Suberosa Extract

The effect of *P. suberosa* extract to inhibit biofilms at MIC and below MIC (sub-MIC) was evaluated using the microplate biofilm assay [17, 18]. Briefly, 200.0 µL of TSB (Tryptic-Soy-Broth) with 0.25% glucose and 1.0% of overnight cultures were incubated with or without *P. suberosa* extract at 37 ºC for 48 h. Control wells had no extract. Planktonic bacteria were removed gently and the biofilm was further stained with 200.0 µL per well of crystal violet (0.10% in H_2O). After 30 mins, the wells were rinsed and filled with 200.0 µL of 70.0% ethanol or 33.0% acetic acid. The absorbances were read at 550 nm. The calculation of percentage biofilm inhibition is shown in Equation 1.

$$\text{Biofilm inhibition (\%)} = \frac{OD550_{Control} - OD550_{Sample}}{OD550_{Control}} \times 100 \quad (1)$$

Anti-Quorum Sensing Assay Against C. Violaceum CV026

Anti-QS effect was investigated as performed previously [18]. 100 µL of CV026 overnight cultures were added to 5.0 mL of warm soft-molten-agar (agar 1.30 g, 1.00 g sodium chloride, 2.00 g tryptone, 200.0 mL H_2O) followed by 20 µL of exogenous hormone source of acyl-homoserine lactone (100 µg/mL). The mixture was poured gently over solidified sterile LBA plate. Wells with diameters of 5.0 mm were created and filled with *P. suberosa* extract (50.0 µL) at below MIC and MIC. Incubation of plates at 30°C for 3 days in upright positions was done. Diameters of cream-colored halos (QSI zones) of each well were recorded in millimeters.

Violacein Assay Against C. Violaceum CV12472

P. suberosa extract was evaluated qualitatively for its violacein inhibition [18]. To each well of a 96-well sterile plate were added 180.0 µL of LBA (Luria-Bertani-broth) and 0.40 optical density at 600 nm CV12472 (10.0 µL) overnight cultures with or without and 20.0 µL of *P. suberosa* extract at below-MIC and MIC. Control wells had no extract. Incubation of plates for 24 h at 30°C was followed by measurement of reduced violacein pigmentation at 585 nm. The violacein inhibition percentage was calculated using the formula in Equation 2 below.

$$\text{Violacein inhibition (\%)} = \frac{OD\ 585\ control - OD585\ sample}{OD\ 585\ control} \times 100 \quad (2)$$

Swarming Inhibition Against Pseudomonas Aeruginosa PA01

Anti-swarming was investigated as reported [18]. Briefly, unto swarm plates (1.0% peptone, 0.50% NaCl, 0.5% agar, 0.5% of filter-sterilized D-Glucose) centers containing *P. suberosa* extract (MIC, MIC/2, and MIC/4) was point-inoculated 5.0 µL of cultures of *P. aeruginosa* PA01. The control plates were without the extract. Incubation was done at 37°C for 1 day. Swarming migration fronts were measured and the percentage inhibition of swarming was calculated.

Antioxidant Assay

Lipid-peroxidation was done by the β-carotene-linoleic acid experiment [9, 18]. $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ activities were determined spectrophotometrically as described elsewhere [9, 18]. CUPRAC (Cupric-reducing antioxidant capacity) was evaluated as previously described [9]. Butylated hydroxyanisole (BHA) and α-Tocopherol served as standards in $DPPH^{\bullet}$, CUPRAC, $ABTS^{\bullet+}$, and lipid peroxidation assays. The Fe^{2+} metal chelating potential was evaluated spectrophotometrically with EDTA as standard [9, 15].

Anticholinesterase Assay

Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitions were investigated using the Ellman method [9, 15]. Horse serum source BChE and electric eel source AChE as enzymes and acetylthiocholine iodide and butyrylthiocholine

chloride as substrates. DTNB (5,5′-Dithio-bis(2-nitrobenzoic) acid) was used to monitor the cholinesterase assay and Galantamine served as the reference compound.

Anti-Diabetic Assay

α-glucosidase inhibitory capacity was done as described previously [9]. α-amylase inhibition was investigated with starch-iodine means [9]. The enzymes α-glucosidase (*Saccharomyces cerevisiae*) and α-amylase (porcine pancreas) were used. Acarbose served as a reference. Results were expressed as 50% inhibition concentration (IC_{50}) and percent inhibition at 100 µg/mL.

Urease Inhibition Assay

Indophenol assay was used to evaluate urease inhibition activity [9, 15]. A solution of urease from a Jack bean source (Type III) in sodium-phosphate buffer (100.0 mM, pH 8.20) was prepared. A mixture containing 10.0 µL test sample, 50.0 µL of urea (100.0 mM), and 25.0 µL urease was incubated at 30 °C for 15 mins. After incubation, 70.0 µL of 0.0050% (w/v) alkali reagent and 45.0 µL of 1.0% (w/v) phenol reagent were introduced and further incubated for 50 mins. Thiourea was the reference compound and absorbances were taken at 630 nm. Percent inhibitions at 100 µg/mL and 50% inhibitory concentration (IC_{50}) were reported.

Tyrosinase Inhibitory Assay

The anti-tyrosinase effect was investigated spectrophotometrically as described elsewhere [9, 15]. Briefly, 20.0 µL of mushroom source tyrosinase, 10.0 µL extract, and 150.0 µL sodium-phosphate buffer (100.0 mM, pH 6.80) were mixed. Incubation for 15 mins at 37°C was done. 20.0 µL substrate L-DOPA was introduced and absorbances at 475 nm were taken after 10 mins incubation. Kojic acid served as a reference compound. Percent inhibitions at 100 µg/mL and 50% inhibitory concentration (IC_{50}) were reported.

Results and Discussion

Water-ethanol mixture is a non-toxic solvent suitable for the extraction of nutraceuticals that are rich in phenolic compounds. The phenolic composition, antimicrobial, antibiofilm, anti-quorum sensing, antioxidant, anti-urease, anticholinesterase, antidiabetic and anti-tyrosinase activities of the hydro-ethanol extract of *P. suberosa* leaves are reported below.

Phenolic Composition

The following phenolic compounds were detected in the extract of *P. suberosa* using HPLC-DAD and reported in **Table 1** and the chromatograms for the analysis are provided in **Figure 1**.

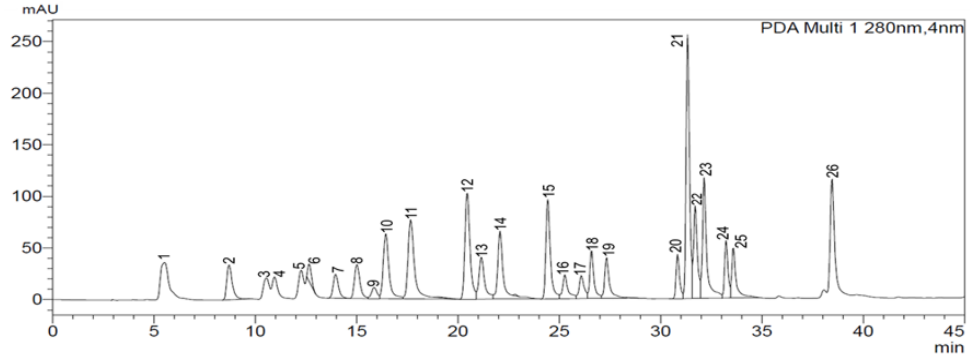
Table 1. HPLC-DAD phenolic composition of *P. suberosa* extract by (µg/g)[a]

RT (min)	Phenolic compounds	Content
5.70	Gallic acid	175.10±0.42
8.75	Protocatechuic acid	2.25±0.08
11.04	Pyrocatechol	8.71±0.14
12.35	Chlorogenic acid	4.65±0.05
15.09	Caffeic acid	6.20±0.17
20.56	*p*-Coumaric acid	7.83±0.21
22.14	Ferulic acid	9.90±0.13
24.49	Coumarin	13.54±0.36
25.30	Rutin	7.44±0.24
26.77	Rosmarinic acid	10.67±0.20
27.35	Myricetin	15.57±0.18
31.33	*trans*-Cinnamic acid	4.50±0.09
31.70	Luteolin	4.74±0.20
38.40	Chrysin	48.20±0.51

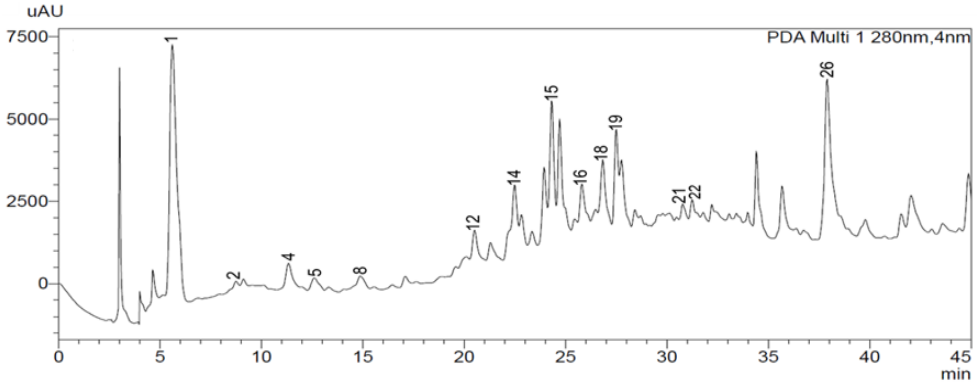
[a]Values are means ± S.E.M. ($p < 0.05$).

Gallic acid, protocatechuic acid, pyrocatechol, chlorogenic acid, *p*-coumaric acid, caffeic acid, coumarin, ferulic acid, rutin, rosmarinic acid, myricetin, trans-cinnamic acid, luteolin, and chrysin were identified in the extract and their structures are given on **Figure 2**. Gallic acid (175.10±0.42 µg/g) was the most abundant compound amongst the detected phenolic compounds. Spectrophotometric methods were used to quantify the total phenolics and flavonoids in the extract. TPC detected using the Folin-Ciocalteu reagent was 112.16 ± 0.33 mg GAE/g DW while TFC detected using the $AlCl_3$ reagent was 36.10±

0.58 mg QE/g DW.



a)



b)

Figure 1. HPLC chromatograms; a) Standards, b) Extract of *P. Suberosa*

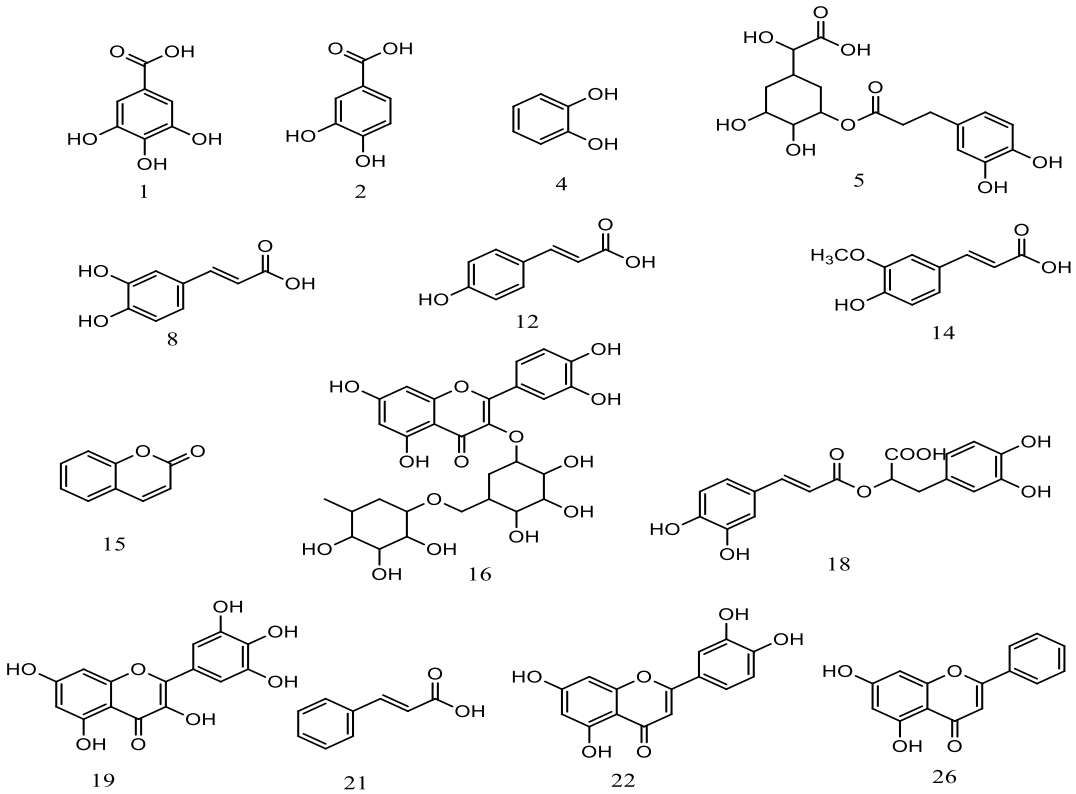


Figure 2. Structures of detected phenolic compounds in *P. Suberosa* extract

Antimicrobial and Anti-Biofilm Activities

The phenolic-rich extract of *P. suberosa* exhibited antibacterial effects on two gram-positive and gram-negative bacteria and

the minimal inhibitory concentrations are given in **Table 2**. On the gram-positive bacteria, the MIC values were 0.3125 mg/mL and 1.25 mg/mL on *S. aureus* and *E. faecalis* respectively while on gram-negative bacteria, MIC values were 2.5 mg/mL and 0.3125 mg/mL on *E. coli* and *S. typhi* respectively. Biofilm inhibition was evaluated on these bacteria at below MIC and MIC and provided in **Table 2**. For the gram-positive bacteria, the biofilm inhibition varied from 37.57±0.63% (MIC) to 8.02±0.08% (MIC/4) against *S. aureus* and from 48.46±0.72% (MIC) to 10.1±0.13 (1/4 MIC). As concerns the gram-negative bacteria, the biofilm inhibitions varied from 86.35±2.02% (MIC) to 29.1±0.35% (1/8 MIC) against *E. coli* and from 54.36±0.82% (MIC) to 15.48±0.21% (1/4 MIC) against *S. typhi*. It can be observed that gram-negative biofilms were more susceptible to *P. suberosa* extract with *E. coli* biofilms being the most susceptible.

Table 2. Antibiofilm (%inh.) and antimicrobial (MIC) activities of *P. suberosa* extract

Microorganism		**mg/mL**
S. aureus		0.3125
E. faecalis		1.2500
E. coli		2.5000
S. typhi		0.3125
Biofilm percentage inhibition (%)		
S. aureus	MIC	37.57±0.63
	1/2 MIC	19.94±0.25
	1/4 MIC	8.02±0.08
	1/8 MIC	-
E. faecalis	MIC	48.46±0.72
	1/2 MIC	28.34±0.39
	1/4 MIC	10.1±0.13
	1/8 MIC	-
E. coli	MIC	86.35±2.02
	1/2 MIC	68.74±1.62
	1/4 MIC	46.06±1.12
	1/8 MIC	29.1±0.35
S. typhi	MIC	54.36±0.82
	1/2 MIC	35.27±0.43
	1/4 MIC	15.48±0.21
	1/8 MIC	-

-: No inhibition

Violacein, Quorum-Sensing, and Swarming Inhibition Activities

Quorum sensing inhibition was measured at below MIC and MIC against two chromo-bacteria and reported in **Table 3**. The MICs of *P. suberosa* were found to be 0.625 mg/mL and 0.25 mg/mL against *C. violaceum* CV026 and *C. violaceum* CV12472 respectively. Violacein inhibition on CV12472 varied from 100% (MIC) to 12.0±0.4% (1/8 MIC) while anti-QS zones of inhibition ranged from 17.0±0.5 mm at MIC to 12.0±0.1 mm at 1/4 MIC. Swarm motility inhibition was evaluated against *P. aeruginosa* PA01 at below MIC and MIC and reported in **Table 3**. The MIC value of *P. suberosa* extract on *P. aeruginosa* PA01 was found to be 2.5 mg/mL. The percent of inhibition ranged from 75.8±1.2% at MIC to 32.6±0.5% at 1/4 MIC.

Table 3. Violacein, quorum-sensing and swarming inhibitions by *P. suberosa* extract

MIC value (mg/mL)	**% inhibition of violacein against C. violaceum CV12472**				
	MIC	**1/2 MIC**	**1/4 MIC**	**1/8 MIC**	**1/16 MIC**
0.25	100.0±0.0	58.5±1.8	23.4±0.7	12.0±0.4	-
	Diameters (mm) of quorum-sensing inhibition against *C. violaceum* CV026				
0.625	17.0±0.5	15.0±1.1	12.0±0.1	-	-
	% inhibition of swarming motility against *P. aeruginosa* PA01				
2.5	75.8±1.2	50.1±0.4	32.6±0.5	-	-

-: No inhibition

Antioxidant Activity

P. suberosa extract showed good antioxidant activity evaluated through five complementary assays as shown in **Table 4**. Since

its IC_{50} value was 21.96±0.56 µg/mL, the extract is more active in the DPPH• assay than used. In the CUPRAC and ABTS•+ assays, the activity of the extract was greater than that of α-Tocopherol and very close to that of BHA standard antioxidants. However, the extract was less active than the standards in both lipid peroxidation and chelating assays but its activity remained relatively good compared to that of the standards.

Table 4. Antioxidant activity of *P. suberosa* extract (IC_{50} in µg/mL)

Sample	β-Carotene-linoleic acid assay	DPPH˙ assay	$ABTS^{\bullet+}$ assay	CUPRAC assay	Metal chelating assay
Extract	15.80±0.71	21.96±0.56	14.24±0.78	35.48±0.60	41.25±0.90
α-Tocopherol	2.10±0.07	38.15±0.50	35.50±0.56	60.25±0.55	ND[b]
BHA	1.45±0.03	19.75±0.33	12.80±0.08	25.40±0.40	ND[b]
EDTA	ND[b]	ND[b]	ND[b]	ND[b]	5.52±0.35

[a] Values are means ± SEM ($p < 0.05$). [c] ND: not determined.

Enzyme Inhibitory Activities

The extract of *P. suberosa* inhibited some key enzymes that interfere with human illnesses as given in **Table 5**. BChE and AChE were inhibited by the extract of *P. suberosa*, but BChE exhibited an IC_{50} of 55.17±0.83 µg/mL compared to 42.20±0.48 µg/mL for galantamine used as the standard drug. Carbohydrate digestive enzymes were also inhibited by *P. suberosa* extract showing IC_{50} values of 50.63±0.58 µg/mL compared to 20.52±0.84 µg/mL for acarbose on α-glucosidase and IC_{50} value of 81.37±0.92 µg/mL compared to the standard acarbose with IC_{50} value of 32.57±0.78 µg/mL on α-amylase. The extract equally inhibited urease (38.76±0.35%) and tyrosinase (30.52±0.80%) at 100 µg/mL.

Table 5. Cholinesterase, urease, α-glucosidase, α-amylase and tyrosinase inhibitory activities of *P. suberosa* extract

	Anti-cholinesterase activity				Anti-diabetic activity							
	AChE		BChE		α-glucosidase		α-amylase		urease inhibition activity		Tyrosinase inhibition activity	
Sample	**Inh. (%)**	**IC_{50}**	**Inh. (%)**	**IC_{50}**	**Inh. (%)**	**IC_{50}**	**Inh. (%)**	**IC_{50}**	**Inh. (%)**	**IC_{50}**	**Inh. (%)**	**IC_{50}**
Extract	36.50±0.75	>100	66.25±0.54	55.17±0.83	70.85±0.91	50.63±0.58	55.72±0.69	81.37±0.92	38.76±0.35	>100	30.52±0.80	>100
Galantamine	85.50±0.57	5.50±0.25	74.65±0.21	42.20±0.48	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*
Acarbose	*NIL*	*NIL*	*NIL*	*NIL*	82.40±0.70	20.52±0.84	77.59±1.10	32.57±0.78	*NIL*	*NIL*	*NIL*	*NIL*
Thiourea	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	83.80±0.60	8.20±0.35	*NIL*	*NIL*
Kojic acid	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	*NIL*	79.50±0.32	23.75±0.30

Values are means ± SEM ($p < 0.05$). *NIL:* not determined.

Leaves of *P. suberosa* contain bioactive phenolics and our results corroborate with some studies which characterized phenolic compounds and flavonoids in the extracts of the leaves of this plant, easily extractable with alcohol [19, 20]. However, the TPC and TFC determined in this study were lower compared to those reported for a hydro-alcoholic extract of this plant but water-ethanol solvent represents a suitable extraction solvent for a phenol-rich bioactive extract of this plant [21]. Phenolic compounds and plant extracts which are rich in them possess numerous health and nutritional benefits and continue to attract research in this domain [22-25]. This plant contains several phenolic glycosides as well, notably those with myricetin, kaempferol, and quercetin as aglycones. Phenolic-rich extracts can be exploited for antioxidant, antimicrobial, and enzyme inhibition potentials.

In this study, the extract of *P. suberosa* has shown good antibacterial activity. One study, which supports our findings here,

indicates antibacterial effects of *P. suberosa* exhibited against Gram-negative and Gram-positive bacteria which ascertains its use in traditional medicine [26, 27]. Despite the antimicrobial potential of this plant, its effect on bacterial biofilms has not been evaluated previously. In this study, the extract reduced formation of biofilms by various bacteria. Biofilms are the root causes of recalcitrant chronic infectious diseases and are made up of colonies of bacteria on both abiotic and biotic surfaces covered with dense protective polymeric matrix requiring large amounts of antibiotics, almost 10 to 1000 more times to be able to kill the microbes beneath [28, 29]. Most infectious diseases and microbial resistance results from bacterial biofilm formation and this causes a serious problem in food and medical materials since biofilms create reduced penetration of antibiotics into bacterial communities, making biofilm colonies resistant to host-immune defense and antibiotics, creating persistency of infections [30-32]. It is, therefore, necessary to search for new natural treatments which may eliminate biofilm establishment and break quorum-sensing networks to decrease any development of bacterial resistance to existing antibiotics. The results of antimicrobial activity and antibiofilm potential indicate that *P. suberosa* extract can be used to inhibit bacterial growth as well as at low doses to reduce the formation of bacterial biofilms over time.

Quorum sensing consists of a cell-cell communication network within bacterial communities that involves the production, reception, and dissemination of signal compounds that help the microbes to survey their environmental conditions and population to be able to carry out their activities coordinatively and in synergy [33, 34]. Violacein pigment protects the bacteria from oxidative damage and it constitutes a simple and measurable form of signal molecule emission whose inhibition represents an anti-QS effect in bacteria [35, 36]. The fact that the extract inhibited violacein formation indicates that it can prevent the production of signal molecules that will help to promote bacteria coordination. The extract also inhibits QS in *C. violaceum* CV026 despite the AHL supplied to it and this indicates that extracts can disrupt signal reception. The quorum sensing inhibition zones corresponds to the clear halo on the violet lawn and the diameters of those zones were measured in millimeters.

Quorum-sensing breakage is a good strategy to overcome the spread of infection as well as their severity and also aids in the reduction of resistance to antibiotics, and new antimicrobial materials that fall in this domain are being increasingly studied [37-39]. Swarming movement is a phenomenon that precedes biofilm formation since it enables bacteria to get onto surfaces and subsequently colonize them [23, 36, 40]. The extract inhibited swarming movement in the flagellated *P. aeruginosa* PA01 and therefore, can aid in reducing the spread of bacteria through surfaces and biofilms as well.

The extract showed to contain reasonable amounts of flavonoids and phenolics and these compounds possess antioxidant activities. The fact that the extracts had a good antimicrobial and antioxidant activity which could be conferred by the phenolics contained in the extract [41]. The results of this research are in agreement with reported data on the antioxidant activity of *P. suberosa* [13, 42]. The hydro-ethanolic leave extract of *P. suberosa* has been shown to possess antioxidant activity attributable to the total flavonoids and total phenolic compounds they contain [43].

The enzymes AChE and BChE contribute to Alzheimer's disease, α-amylase and α-glucosidase hydrolyze starch into glucose and cause hyperglycemia and diabetes, and urease plays a role in the development of stomach ulcerations involving *Helicobacter pylori* while tyrosinase causes browning of fruits and skin pigmentation issues in animals. All of the effects above are undesirable to human health and therefore inhibitors of such enzymes especially from natural sources have been described as a suitable means of promoting human health [9, 24]. The plant extract was able to inhibit both cholinesterases (AChE and BChE) and cholinesterase inhibitors are known to help in boosting cholinergic deficit and maintaining good levels of acetylcholine for the prevention and Alzheimer's disease. The phenolic compounds present in this extract as well as other possible classes of compounds can contribute to the cholinesterase activity since phenolic units are one of the suitable natural cholinesterase inhibitors [44-46]. α-glucosidase and α-amylase break down carbohydrates and this leads to an increase in blood glucose levels therefore the inhibition of these enzymes reduces the risk of the development of diabetes [32, 47-49]. This plant has exhibited good antidiabetic potential by inhibiting both α-amylase and α-glucosidase. Our findings are in line with some studies which report that *P. suberosa* is used traditionally to remedy type-II diabetes and can inhibit carbohydrate enzymes and alleviate induced diabetes in animal model studies [20, 42]. Natural antidiabetic extracts and compounds are being developed and find applications in traditional medicines and are considered cheaper, safer, and more effective [10, 49, 50].

Conclusion

Pteleopsis suberosa is a major plant used in complementary medicine in West and Central Africa. The phenolic extract was prepared from the leaves of this plant using an ultrasonic device with a hydro-alcoholic solution as solvent. The TPC and TFC of *P. suberosa* leave extract were measured and reported while HPLC-DAD was used to establish the phenolic profile using twenty-six standard compounds. Gallic acid was the most abundant out of the fourteen phenolic compounds detected by HPLC-DAD. The extract showed good antimicrobial activity as well as antibiofilm activity at sub-MIC. QS was inhibited in *C. violaceum* CV026 as well as inhibition of production of the pigment, violacein was exhibited against *C. violaceum* CV12472. The results of antimicrobial studies indicate that the *P. suberosa* extract can slow down the emergence of bacterial resistance. The plant showed potential in reducing oxidative stress and it exhibited antioxidant potential in five complementary assays. The extract inhibited some key enzymes that interfere with human diseases including BChE, AChE, urease, α-glucosidase, tyrosinase, and α-amylase. The findings of this research ascertain the medicinal potential of this plant and its safe use in traditional medicine.

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