Research Square

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Anti-biofilm, anti-quorum sensing potential, cytotoxicity, and UPLC-UV/DAD-MS/MS/QTOF profiling of Prosopis africana (Guill. & Perr.) Taub. leaves and stems: benefits of a traditional medicine in dental care

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Research Article

Keywords: Prosopis africana, UPLC-UV/DAD-ESI-MS/MS, anti-biofilm, anti-quorum sensing, dental care, polyphenols, alkaloids

Posted Date: October 8th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7348474/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Prosopis africana (Guill. & Perr.) Taub. is traditionally used in folk medicine in Burkina Faso for oral diseases. Leaves and stems are used in rural areas to treat dental caries, and the bark is used to treat green diarrhea in infants. In the context of a better understanding of the herbal drug and its bioactivity or toxicity, the present study deals with the chemical profiling of the different botanical parts used in phytomedicine. The impact of herbal medicine on various factors contributing to oral infections and caries, and specifically as anti-biofilm and anti-quorum sensing properties has also been little investigated.

Methods

The anti-biofilm effect of methanolic extracts of leaves and stems of *P. africana* was evaluated on *Streptococcus mutans, Staphylococcus aureus, and Pseudomonas aeruginosa*, as well as the anti-quorum sensing effect on *Chromobacterium* CV026 and *Pseudomonas aeruginosa*. The cytotoxicity of the leaves and stems extracts was also evaluated. The chemical composition of the extracts was characterized by UPLC-UV/DAD-MS^2/ESI-QTOF analysis.

Results

The extracts (100µg/ml), without affecting cells viability, significantly reduced the biofilm formation of *S. mutans* with the best inhibition rates of 56.7% and 47.6% for stem and leaf extracts respectively. Inhibition rates of 49.03%, 40.2%, and 46.7% were obtained for pyocyanin, elastase, and rhamnolipids respectively, with leaf extracts. No cytotoxic effect on gingival cells was observed for stem extract. UPLC-UV-MS^2 analysis identified sixteen compounds among which mainly polyphenols and alkaloids. They could be related to the activities.

Conclusion

The present study provides evidence of efficacy and basic scientific justification for the use of *P. africana* in the treatment of dental caries.

Introduction

Microbial communities living in an extracellular polymeric matrix that they produce themselves, called bacterial biofilms, are responsible for over 60% of microbial infections in humans. The transformations in gene expression and the activation of numerous extracellular communication pathways during biofilm formation often result in increased pathogenicity and bacterial virulence. [1]. Surprisingly, the antimicrobial resistance of a biofilm becomes thousand times higher than a free planktonic biota.

Quorum Sensing (QS) is a mode of bacterial communication between species and intra-species, based on the production of small mediator molecules called autoinducers, which are produced during bacterial growth. QS is the mechanism used by cariogenic Gram-negative and Gram-positive bacteria to synchronize and regulate the expression of several genes depending on cell density [2]. Physiological processes that affect the production of virulence factors [3], can be characterized by the emission of bioluminescence [4], the production of pigments [5], the formation of biofilm and motility [6, 7]. In Gram-negative bacteria, QS involves the production of small signaling molecules called N-acyl homoserine lactones (AHLs) [8]. AHLs are generally produced by an enzyme, a synthase, and diffuse passively across the cell membrane [9]. When their concentration in the external environment reaches a critical threshold, generally at high cell density, AHLs invade the cell, bind and activate specific intracellular receptors [10]. Activated receptors bind to promoters of targeted genes and regulate their transcription [11]. QS and biofilm are therefore linked because QS controls the formation and maturation of biofilm in several types of bacteria like *Pseudomonas aeruginosa* PAO1 [12]. A buccal biofilm contains hundreds of different oral bacteria responsible of serious diseases in the oral cavity. In addition, virulence in response to drastic changes in the biofilm's microenvironment can be spread systemically and cause significant infections in other organs. [13]. The presence of *Streptococcus mutans, Staphylococcus aureus*, and *Pseudomonas aeruginosa* in supragingival and subgingival biofilm can cause dental caries. Dental plaque, composed of Gram-positive and Gram-negative bacteria, can adhere to the surface of teeth, proliferate, and produce lactic acid, leading to the demineralization of enamel and dentin. The limitations of conventional antibiotic therapy, as well as the increasing drug resistance, have led to an urgent need to find other approaches to treat infections related to oral biofilm.

Previous studies have shown that plant drugs or molecules isolated from medicinal plants have properties that inhibit dental biofilm formation. These properties include a reduction in the adhesion of bacteria to dental plaque, a key step in the initiation and progression of dental caries. *Prosopis africana* (Guill. & Perr.) Taub (fabaceae) is a tree widely used in Burkina Faso to treat traditionally oral and dental infections. Ethnomedicinal surveys conducted in the south-central region reported the use of stems and leaves of *P. africana* in the treatment of dental caries. Also, the barks are used to treat diarrhea in young children. Antibacterial properties against *Escherichia coli, Staphylococcus aureus, Streptococcus mutans, Klebsiella* have also been reported [14, 15]. However, its anti-biofilm and anti-quorum sensing properties have been poorly investigated. We report here the anti-biofilm, the anti-quorum sensing activity, and the cytotoxicity of the methanolic leaves and stem bark extracts of *Prosopis africana.*

Material and Methods

Plant material and extraction

A collection guided by traditional practitioners in the province of Zoundweogo (11° 34'60"N; 1°0'00") allowed to harvest bark and leaves of *Prosopis africana*. The identification of the plant material was carried out by Dr. Souleymane COMPAORE junior researcher of botany from the plant biology and ecology laboratory of the Life and Earth Sciences Training and Research Unit (UFR/SVT) of the Université Joseph KI-ZERBO. The wild plant was harvested on public land. A herbarium was deposited at the Herbarium of the UFR/SVT under the identification code No. 6852. The barks and stems were dried under laboratory conditions before to pulverize. The powders were kept cool at 4°C for the subsequent extraction of the plant drug. One hundred grams (100 g) of the leaves and stems powders were macerated in one liter of methanol (MeOH) during 24 hours. The methanolic maceration was filtered using Whatman No. 1 paper and concentrated under reduced pressure. The concentrate was placed in the Speedvac (SpeedVac ThermoFischer®) until complete dryness to yield two crude methanolic extracts (Leaves Methanolic Extract (LME), Stems Methanolic Extract (SME).

Bacterial Strains

The bacteriology laboratory of the Muraz Center in Bobo Dioulasso (Burkina Faso) and the plant biotechnology laboratory of the Université Libre de Bruxelle (Belgium) respectively provided the standard strains (*Streptococcus mutans* ATCC 25175 and *Staphylococcus aureus* ATCC 43300 methicillin-resistant) and the wild strains (*Pseudomonas aeruginosa* PAO1 and *Chromobacterium vio-laceum* CV026). *S. aureus* and *S. mutans* strains were stored at -80°C in Brain Heart Infusion liquid medium while *P. aeruginosa* and *C. violaceum* strains were inoculated in Luria Bertani medium containing 50% glycerol until use.

. **Determination of the minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration of crude methanolic extracts (Leaves Methanolic extract (LME), Stems Methanolic extract (SME)) was determined using the 96-well microplate method [14]. A range of extracts was carried out on a sterile microplate from a stock concentration of 4 mg/mL dissolved in 5% DMSO to get a final volume of 100 µL for each well. Then, 100 µL of the prepared inoculum in the corresponding culture medium were added to each well. DMSO medium (2.5%) was used as DMSO growth control and negative medium control, and DMSO-free medium as a bacterial growth control and negative medium control. The microplates were then incubated at 37° C for 24 h. At the final point 30 µL of iodonitrotetrazolium (INT) (2 mg/mL) were added to each well. 30 min after wereition of INT, MIC of the extracts was deduced regarding the color changes observation. Pink staining of INT indicates the presence of microbial growth in the wells. Indeed, the MIC is defined as the concentration of the first well of the range of dilutions devoid of bacterial growth [16]

Determination of the minimum bactericidal concentration (MBC)

The minimum bactericidal concentration of an extract or a pure compound corresponds to the lowest concentration capable of killing more than 99.9% of the initial bacterial inoculum (*i.e.* less than 0.01% of survivors). The range of concentrations produced to determine the MIC in a liquid medium was used to determine the MBC of the extracts. Samples in the control tube (2.5% DMSO) and each of the tubes devoid of bacterial growth were deposited in a "streak" on LB-agar/BHI-agar medium previously prepared and poured into Petri dishes. Plates were incubated for 24 h at 37°C. The MBC was deduced as being the first bacteria-free dish. The tests were carried out in triplicate [16].

Inhibition of biofilm formation

The anti-biofilm activity of the crude methanolic extracts (LME, SME) was evaluated according to the method previously described [17]. For this purpose, sterile 96-well microplates were used. Extracts and reference (salicylic acid) were prepared at the concentration of 1 mg/mL in DMSO (1%) to have a final concentration of 100 µg/mL in each well. After incubating the plates during 24 h at 37°C, absorbances at λ600 nm were read to ensure that the extracts did not affect bacterial growth compared to the negative control (1% DMSO). Planktonic bacteria were removed with the supernatant, and the biofilms were thoroughly washed three times with distilled water and 2ml of methanol were used to fixed the adhered bacteria. After 15 min of incubation at 37°C, the methanol was removed, and the formed biofilm was quantified with crystal violet. Crystal violet (0.1% in water) was added to each well (200 µL/well) for 30 min at room temperature. The excess of crystal violet was removed, and 1 ml of distilled water was used to wash the stained biofilms. The crystal violet fixed by the biofilm bacteria was solubilized with acetic acid (33%) and the absorbance was measured at λ590 nm. The biofilm/bacterial growth ratio (OD 590 nm/OD 600 nm) was determined. The percentage of inhibition was calculated using the following formula:

Inhibition (%) = [(Abs control – Abs extract)/Abs control] x100

Anti-quorum sensing activity

Violacein production inhibition test by Chromobacterium violaceum CV026

Inhibition of violacein production in *Chromobacterium violaceum* CV026 by all crude extracts (LME, SME) was tested by direct assay of culture medium according to protocols reported Choo *et al.* (2006). Violacein production was induced in *C. violaceum* CV026 by adding N-hexanoyl-L-homoserine lactone (HHL) (5 µM final concentration) in the presence or absence of the extracts (100µg/mL final concentration). *C. violaceum* CV026 was first grown in the Luria Bertani medium for 24 h (30°C ;175 rpm) and the diluted CV026 was next introduced into 12-well plates. The extracts or the reference salicylic acid dissolved in DMSO 1% were added to have a final concentration of 100 µg/mL, in the presence of C6-HSL (final concentration 10 µM). Plates were incubated for 24 h at 30°C with shaking at 175 rpm.

Bacterial growth was first evaluated by measuring bacterial turbidity at 600 nm and the produced violacein was then quantified using a spectrophotometer. Briefly, the solution was vigorously vortexed for 30 S to solubilize the violacein and the supernatant was removed. The pellet was dissolved using 100%

DMSO. The violacein contained in the supernatant was recovered by centrifugation (7000 rpm; 10 min) and 200 µL were introduced into microplate wells for measuring the absorbance at 585 nm. The percentage of inhibition was calculated against the negative control (DMSO 1%) (18).

The ratio of OD 585 nm to OD 600 nm was determined as:

Inhibition (%) = [(Abs control – Abs Extract)/Abs control] x100

Pyocyanin production inhibition test in Pseudomonas aeruginosa PAOl

The pyocyanin production inhibition assay was performed for the crude methanolic extracts (LME, SME). After 18 h incubation in a 24-well plate at 37°C with shaking at 175 rpm, the bacterial suspension was gently homogenized and 800 µl was transferred to a 1.8 mL Eppendorf tube, were then centrifuged at 16000 × g for 1 min. 200 µL of the remaining suspension was directly transferred to a flat-bottom microtiter plate to measure absorbance at 600 nm, corresponding to the turbidity of the suspension. Then, 700 µL of supernatant from each sample after centrifugation was taken and transferred to 2 mL Eppendorf tubes; 600 µL of chloroform was added to each tube and the mixture was vortexed for a few seconds to extract pyocyanin. After centrifugation, two phases were formed and 500 µL of the lower phase, which contains pyocyanin, was transferred to a new 1.8 mL Eppendorf tube. Then, 250 µL of a 0.2 N hydrochloric acid solution was added to each tube to extract again the pyocyanin. The use of an acidic phase results in a color shift of pyocyanin from turquoise blue to pink. In nature, pyocyanin was blue, but it has been shown that in an acidic medium, it changes its structural configuration and turns pink [19]. After centrifugation, two phases appear again with pyocyanin in the upper phase. Finally, 200 µL of this upper phase was recovered and the absorbance of this solution was measured at 360 nm to quantify the pyocyanin produced by the bacteria [18]. The experiments were repeated three times (n = 3). The percentage of inhibition was calculated by the following formula:

Inhibition (%) = [(Abs control – Abs Extract)/Abs control] x100

Elastase inhibition test in Pseudomonas aeruginosa PAO1

The elastase inhibition test was performed with the methanolic crude extracts (LME, SME) and fractions. Elastase activity performed on *P. aeruginosa* PAO1 in the absence or presence of extract was measured by the Elastin-Congo Red method [20]. This method was based on the properties of the elastase produced by the bacteria to degrade the elastin. *P. aeruginosa* PAO1 was incubated in LB medium with extracts (final concentration of 100 µg/mL) for 18 hours (37°C; 175 rpm) and the tubes were centrifuged for 5 min (3000 ; 24°C). Two hundred and fifty microliters (250 µL) of congo red elastin at 5 mg/mL dissolved in Tris-HCl buffer (0.1 M Tris-HCl pH 8;1 mM $CaCl_2$) was added to 750 µL of supernatant contained in each Eppendorf tube. The mixture was incubated at 37°C for 16 h at 200 rpm. During this incubation phase, elastin degradation results in solubilization of congo red, yielding a red solution while the undegraded Elastin-Congo Red complex was insoluble in water [18]. The reaction mixture was centrifuged at 3000 *g* for 10 min to separate the non-soluble portion and the absorbance of 200 µL of supernatant was measured at λ495 nm to estimate the elastin activity [21]. The percentage of inhibition was calculated as follows:

Inhibition (%) = [(Abs control – Abs Extract) / Abs control] x100

Rhamnolipid inhibition test in Pseudomonas aeruginosa PAO1

The rhamnolipid inhibition test was performed with crude methanolic extracts (LME, SME). For rhamnolipid extraction, *P. aeruginosa* PAO1 grew at 37°C with shaking at 175 rpm for 18 h in a 5 mL LB medium in the presence of extract (100 µg/mL) or DMSO (1%). Bacterial cultures were then collected in Eppendorf tubes and centrifuged (3000 *g* at room temperature for 5 min). One milliliter of bacteria-free supernatant was recovered and filtered through an Ultrafree® CL-Millipore filter, and the pH of the supernatant was adjusted to 2.3 ± 0.2 with HCl 1N. The rhamnolipids were extracted from the supernatant with 4 mL of ethyl acetate (EtOAc) on the vortex for a few seconds. After centrifugation, the upper phase, which contains the rhamnolipids, was transferred to a new 30 mL tube. This extraction procedure was repeated three times. All the collected organic solvents were finally removed by evaporation under vacuum centrifugation. Quantification of rhamnolipids was performed by the methylene blue complexation method according to the literature with some modifications [22]. Briefly, 4 ml of chloroform were used to dissolve the rhamnolipid produced by the bacteria in each extract treatment and contacted with a methylene blue solution consisting of 200 µL of 1 g/L methylene blue reagent and 4.9 mL of distilled water that was adjusted to pH 8.6 ± 0.2 by adding 50 mM borax buffer (15 µL). After vigorous shaking for 4 min, the mixture was allowed to stand for 15 min to allow complexation of methylene blue to rhamnolipids. One milliliter of the lower chloroform phase turning blue was transferred to a new Eppendorf tube and vortexed with 500 µL of 0.2 N HCl, allowing the transfer of the complex methylene blue into the acid phase. Finally, 200 µL of the acidic phase was transferred to a 96-well microplate and the absorbance measured at λ638 nm [18]. The percentage of inhibition was calculated by the following formula:

Inhibition (%) = [(Abs control – Abs sample) / Abs control] x100

Cytotoxicity Assay

The cytotoxicity study was performed for LME and SME. Gingival cells were grown and maintained in a humidified incubator with 5% CO_2 at 37°C in Petri dishes in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum, 1% penicillin-streptomycin, and 1% L-Glutamine [23]. Cultures were subjected to medium changes and passages or transplants when cells were at the confluence. All manipulations were performed under highly sterile conditions in a laminar flow hood.

After observation under a reverse-phase microscope (Jenco USA), gingival cells were seeded on sterile 96-well microplates in 100 µL of complete culture medium at 2.104 cells/well. The microplates were then incubated for 24 h to ensure adequate cell adhesion. The crude methanolic extracts from the leaves

and stems were dissolved in DMSO and diluted in the culture medium to a concentration of 1% DMSO. A range of dilutions was performed for concentrations from 500 µg/mL to 15.625 µg/mL. 100 µL of each extract was then contacted with the cells in the wells and the plates returned to incubation for 72 h. The blank contained only the culture medium and the control contained cells with the culture medium, without extract. The cell suspension in the presence of hydrogen peroxide (H_2O_2) (50 µL, 300 mM) served as the positive control, and 1% DMSO served as the negative control.

Phytochemical Analysis: Ultra-High-Performance Liquid Chromatography Coupled with High-Resolution Tandem Mass Spectrometry (UHPLC/MS-MS)

Extracts were analyzed using Agilent Technologies Accurate-Mass Q-TOF LCMS 6530, with LC 1290 Infinity system. The separation was carried out at 40°C using a 120 EC-C18 column (3.0*100 mm*2.7 µm; Agilent Poroshell). Each sample (10 mg. mL^{-1}) was injected (3 µL), and the column was eluted at 0.7 $mL.min^{-1}$ with a solvent gradient using solvent A (water with formic acid 0.4% (v/v)) and solvent B (acetonitrile). The proportion of solvent B increased, step by step, from 10 to 36% during 4.5 min; 36% to 100% during 4 min, followed by 2 min isocratic phase with 100% solvent B; back from 100% to 10% in 0.5 min and equilibration at 10% solvent B during 3 min until the end of the run at 14 min. Mass analyses were made in positive and negative modes, with the nebulization gas (Nitrogen) at a flow of 10 $L.min^{-1}$ and 40 psg pressure. The capillary tension was 3000 V and gaz ionization energy of 100 eV. Analyses included QC (Qualitative Control) and blank samples, which were regularly injected between runs, giving a total of 8 blanks and 8 QCs. QC was prepared by mixing 100 µL of each sample prepared at 10 mg. mL^{-1}. Moreover, to facilitate the identification of specific compounds samples were analyzed with tandem mass spectrometry (MS/MS). MS/MS analyses were performed by collision-induced dissociation (CID) with a collision energy of 20 eV. Chromatograms were explored with MassHunter Qualitative Analysis B.07.00 software (Agilent® Technologies), and the SemetI1.0.2020.11.10 version 2 in-house database containing the analyzed by UHPLC-MS/MS of 400 commercial standards in positive and negative mode. Compounds identifications were performed by comparison of experimental data to that of SemetI database (in house database) and to the existing literature.

Statistical analysis

The statistical analysis was performed by using Graph Pad Prism version 6 software. The results were expressed as mean of three independent repeated experiments ± Mean Standard Error. One-way analysis of variance (ANOVA) followed by the post-test Dunnett's was used to measure the degree significance of the results. The extracts were compared with the negative control and the value of $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered significant, very significant. The experiments were carried out in triplicate (n = 3) and the results were expressed as mean ± standard deviation.

Results

The MIC (> 0.250 mg/mL) and MBC (> 1mg/mL) values evaluated allow the selection of a sub-inhibitory concentration for the bioassay on all strains (Table 1).

Table 1
Minimum inhibitory concentration and minimal bactericidal concentration of methanolic extract of leaves and stem bark of *Prosopis Africana.*

Extracts	LME (mg/mL)		SME (mg/mL)	
Strains	MIC	MBC	MIC	MBC
Streptococcus mutans ATCC 25175	0.5	1	1	> 1
Staphylococcus aureus ATCC 43300	1	> 2	1	> 2
Pseudomonas aeruginosa PAOI	0.5	1	2	> 2
Chromobacterium violaceum CV 026	1	> 2	0.5	1
MIC: Minimum inhibitory concentration, MBC: minimal bactericidal concentration, LME: methanolic extract of leaves, SME: stem bark				

Inhibition of biofilm production

As shown in Fig. 1, the methanolic extracts from the leaves and stems of *P. africana*, at a concentration of 100 µg/mL affected neither the viability nor the growth of *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus, Streptococcus mutans.* Overall, compared to salicylic acid (SA), all extracts significantly reduced biofilm formation of *Pseudomonas aeruginosa* PAO1 with respective inhibition percentages of 32.8 ± 5.3 for LME and 36.3 ± 4.6. for SME. Concerning *Staphylococcus aureus*, biofilm formation was inhibited with respective inhibition percentages of 31.8 ± 1.9% for LME; 40.4 ± 4.6% for SME. For *Streptococcus mutans*, biofilm formation was inhibited with respective inhibition percentages of 47.6 ± 0.8% for LME and 56.7 ± 0.6% for SME.

Effect of extracts on violacein production in Chromobacterium violaceum CVO26

As shown in Fig. 2, methanolic extracts of *P. africana* significantly reduced violacein production compared to 1% DMSO. At the final concentration of 100 µg/mL, the extracts did not affect either viability or bacterial growth of *C. violaceum* CV026. For LME, the inhibition of violacein was 37.9 ± 3.7% and for SME the inhibition was 42.6 ± 1.4%.

Effect of extracts on pyocyanin, rhamnolipid, and elastase production in P. aeruginosa PAO1

The effect of *P. africana* extracts at the final concentration of 100 µg/mL was evaluated on the production of pyocyanin, elastase, and rhamnolipids (Fig. 3) which are the main virulence factors excreted by *P. aeruginosa* PAO1 and whose production was under the control of the QS in dental biofilm. As shown in Fig. 3, the extracts at the final concentration tested of 100 µg/mL significantly inhibited the production of pyocyanin, and rhamnolipids compared to the control (DMSO 1%) without affecting bacterial growth. Pyocyanin inhibition was marked by methanolic leaf extract (LME 49.03 ± 0.03%). The reduction of elastase was marked by both extracts (LME 40.2 ± 0.05%; SME 39.6 ± 0.014). For rhamnolipids, the percentage of inhibition of methanolic leaf extract (LME) was 46.70 ± 0.02% and that of methanolic stem extract (SME) was 39.60 ± 0.01%. This reduction of virulence factors by the extracts implies a probable interference with the regulatory mechanism of pyocyanin production that depends on QS in dental biofilm formation.

Cytotoxic effect of extracts on gingival cells

The cytotoxic effect of the crude methanolic extracts of leaves and stems on gingival cell viability was determined after 72 h following the MTT colorimetric cytotoxicity test. The methanolic stem extract (SME) showed no adverse effect on gingival cell viability compared to the DMSO control (Fig. 4). In contrast, crude leaf extract (LME) had a significant cytotoxic effect on gingival cells compared to DMSO starting from 15.625 µg/mL (Fig. 4).

Identification of compounds of interest by HPLC/MS-MS in methanolic extracts of leaves and stems of Prosopis africana

Tables 2 and 3 provide informations on the characterized compounds in both methanolic extract LME and SME. Comparing chromatograms in both positive- and negative modes ESI–MS experiments, a first group of forteen polar compounds were detected between 2.00 and 7.00 min. They were better ionized in negative mode than in postive one. A second group was eluted between 7.00 and 13.00 min, corresponding to less polar molecules that were better ionized in negative mode. To complete identifications the UV spectra was also used. This data combination was compared to published data and to the in-house database SemetI. As an example, compound **11** in LME was identified as follows, and the same approach was used to characterized the chemical composition of both extracts. The UV spectrum showed the following maxima of absorption: 222, 260, 300sh et 348 nm suggesting a polyhydroxylated flavonoid like a 3-*O*-substituted myricetin derivative (Mabry *et al.*, 2012). The pos-ESI-MS spectrum exhibited a pseudo-molecular ion at *m/z* 465.0998 u corresponding to $[M + H]^+$ (Fig. 5). The ions at *m/z* 487.0848 $[M + Na]^+$ and 951.1756 u $[2M + Na]^+$ in ESI^+, together with the ion in ESI^- at *m/z* 464.0899 u $[M-H]^-$ confirmed a molecular weight of 464 uma. A fragment at *m/z* 319.0425 in ESI^+ (and *m/z* 316.0243 u in ESI^-) showed out the loss of 146 u fragment, corresponding to the loss of a desoxyhexosyl unit. Altogether these data confirmed the formula of $C_{21}H_{20}O_{12}$ (with a score of 99,64%) (Fig. 5). Compared to published data, this analysis led to a presumed identification of compound **11** to myricetin 3-*O*-rhamnoside or myricitrin (Sharifi-Rad *et al.*, 2019).

The same method allowed us to identify thirteen other compounds in LME, among which 2,4,6-trihydroxyphenyl β-D-glucopyranoside, procyanidin A, catechin 7-*O*-glycoside, procyanidin dimer digallate, glucopyranosyl-*O*-tyrosine, myricetin-3-*O*-galactoside, myricitrin, phloridzin, and quercitrin. Most of the identified compounds were flavonoids.

Similarly, SME (methanolic stem extract) was analyzed and nine compounds were characterized such as galloylquinic acid, digalloylshikimic acid, 3,4-digalloylquinic acid, 1,3-digalloylquinic acid, prosopinin, pinelic acid, and spectalin.

Table 2
Identification by UHPLC-ESI-QTOF of the main compounds of the methanolic leaves extract (LME) of *Prosopis africana*

N°	Rt (min)	UV (nm)	*m/z* (M - H)⁻	ΔM ppm m/z (M - H)⁻	*m/z* MS² (fragment)	*m/z* (M + H)⁺	ΔM ppm *m/z* (M + H)⁺	*m/z* MS² (fragment)	Formule	Annotation (Semetl) or proposal	Confidence level [a]	I
1	2.733	224, 286	303.0728	-2.13	141.0187 [M-H-$C_6H_{10}O_5$]⁻	305.0672	2.48	327.0672 [M + Na]⁺ 631.1464 [2M + Na]⁺ 143.0331 [M + H-$C_6H_{10}O_5$]⁺	$C_{12}H_{16}O_9$	2,3,5-trihydroxyphenyl glucoside	2b	[
2	3.260	224, 278–279	451.1244	0.41	289.0728 [M-H-$C_6H_{10}O_5$]⁻	453.1374	3.72	475.1199 [M + Na]⁺ 291.0851 [M + H-$C_6H_{10}O_5$]⁺ 273.0747 [M + H-$C_6H_{12}O_6$]⁺	$C_{21}H_{24}O_{11}$	catechin 5-*O* or 7-*O*- glycoside	3	[
3	3.455	224, 276	593.1314			595.1433	3.67	617.1245 [M + Na]⁺ 1211.2554 [2M + Na]⁺	$C_{30}H_{26}O_{13}$	derivative of Procyanidin A	3	[
4	3.355	224, 276	593.1299	0.28	1187.2746 [2M-H]⁻ 447.0706 [M-H-146]⁻	595.1425	2.50	617.1238 [M + Na]⁺ 1211.2554 [2M + Na]⁺	$C_{30}H_{26}O_{13}$	procyanidin derivative	3	[
5	3.763	224, 278	593.1305	-0.73		595.1430	-	617.1240 [M + Na]⁺.	$C_{30}H_{26}O_{13}$	procyanidin derivative	3	[
6	3.947	223, 284	-	-	325.0923 [M-H_2O]⁻ 163.0395 [M-H_2O-$C_6H_{11}O_5$]⁻	344.1327	3.76	365.0617 [M + Na]⁺ 183.0306 [M + H-$C_6H_{11}O_5$]⁺ 165.0535 [M + H-$C_6H_{11}O_6$]⁺	$C_{15}H_{21}NO_8$	Glucopyranosyl-*O*-tyrosine	2b	[
7	4.177	223, 284	881.1936	-0.17	1764.3936 [2M]⁻ 603.1352 ; 577.1352 451.1245 ; 440.0914 289.0714	883.2047	3.74	1788.3812 [2M + H + Na]⁺	$C_{45}H_{38}O_{19}$	dimer procyanidin digallate – type B	3	[
8	4.367	224, 278	577.1351	0.09	498.1257 ; 435.1299	579.1472	4.32		$C_{30}H_{26}O_{12}$	procyanidin derivative	3	[
9	5.053	224, 262, 302 sh, 357	479.0827	0.86	-	481.0965	2.43	503.0782 [M + Na]⁺ 983.1650 [2M + Na]⁺ 319.0436 [M + H-$C_6H_{10}O_5$]⁺	$C_{21}H_{20}O_{13}$	myricetin 3-*O*-hexoside 1	2a	[

N°	Rt (min)	UV (nm)	*m/z* $(M - H)^-$	ΔM ppm m/z $(M - H)^-$	*m/z* MS^2 (fragment)	*m/z* $(M + H)^+$	ΔM ppm *m/z* $(M + H)^+$	*m/z* MS^2 (fragment)	Formule	Annotation (Semetl) or proposal	Confidence level [a]	I
10	5.132	224, 258, 304 sh, 354	479.0825	1.28	316.0221 [M-H-$C_6H_{10}O_5$]$^-$	481.0979	-0.49	503.0796 [M + Na]+ 983.1667 [2M + Na]$^+$ 319.0445 [M + H-$C_6H_{10}O_5$]$^+$	$C_{21}H_{20}O_{13}$	myricetin-3-*O*-galactoside 2 (isomer)	2a	[
11	5.627	222, 260, 300sh, 348	463.0872	1.95	317.0636 [M-H-$C_6H_{10}O_4$]$^-$	465.1023	1.08	487.0834 [M + Na]+ 651.1789 [2M + Na]+ 319.0427 [M + H-$C_6H_{10}O_4$]$^+$	$C_{21}H_{20}O_{12}$	myricitrin	2b	[
12	6.05	224, 280	435.1305	-1.73	313.0931 [M-H-C_8H_9O]$^-$ 273.0774 [M-H-$C_6H_{10}O_5$]$^-$151.0401 ; 121.0292	-	-	-	$C_{21}H_{24}O_{10}$	phloridzin	2a	[
13	6.427	256, 346	447.0949	-3.93	301.0357 [M-H-$C_6H_{10}O_4$]$^+$	449.1066	2.4	919.1845 [2M + Na]+ 303.0490 [M + H-$C_6H_{10}O_4$]$^+$	$C_{21}H_{20}O_{11}$	quercitrin	2b	[

[a] according to Schymansky et al., 2014.

Table 3
Identification by UHPLC-ESI-QTOF of the main compounds of the methanolic stems extract (SME) of *Prosopis africana*.

N°	$t_{R\ (min)}$	UV (nm)	*m/z* $(M - H)^-$	ΔM ppm *m/z* $(M - H)^-$	*m/z* MS^2 (Fragment)	*m/z* $(M + H)^+$	ΔM ppm *m/z* $(M + H)^+$	*m/z* MS^2 (fragment)	Formula	Annotation or proposal	Confidence level[a]	Reference
1	1.905	222. 272	343.0677	-1.87	687.1442 $[2M-H]^-$	345.0810	0.98	153.,0171 $[M + H-C_7H_{12}O_6]^+$ 327.0683 122.0222	$C_{14}H_{16}O_{10}$	galloylquinic acid	3	[31]
3	3.360	222, 274	495.0810	-3.5	343.0683 $[M-H-C_7H_5O_4]^-$ 191.0564 $[M-H-2(C_7H_5O_4)]^-$ 169.0144	497.0907	-2.52	519.0732 $[M + Na]+$ 479.0808 $[M + H-H_2O]+$ 309.0583 153.0167	$C_{21}H_{20}O_{14}$	digalloylshikimic acid isomer 1	3	(32)
4	3.458	222, 276	495.0782	-0.82	343.0683 $[M-H-C_7H_5O_4]^-$ 191.0556 $[M-H-2(C_7H_5O_4)]^-$ 169.0138	497.0905	4.19	479.0790 $[M + H-H_2O]^+$ 309.0590 $[M + H-C_7H_{12}O_6]^+$ 153.0169 $[M + H-C_7H_{12}O_6-C_7H_{10}O_4]^+$	$C_{21}H_{20}O_{14}$	digalloylquinic acid isomer 1	3	[33]
4'	3.557	222, 275	495.0795	-	343.0676 $[M-H-C_7H_{12}O_6]^-$ 191.0545 $[M-H-2(C_7H_{12}O_6)]^-$ 169.0130	497.0939	-2.7	519.0733 $[M + Na]^+$ 479.0809 $[M + H-H_2O]^+$	$C_{21}H_{20}O_{14}$	digalloylquinic acid isomer 2	3	[33]
9	9.045	-	-	-	-	314.2693	-1.58	298.2743 278.2460	$C_{18}H_{35}NO_3$	prosopinine isomeric isoprosopinin	3	[34]
10	9.2	-	-	-	-	314.2693	-1.61	298.2742	$C_{18}H_{35}NO_3$	prosopinine isomeric isoprosopinin	3	[34]
11	9.327	-	-	-	-	314.2698	-2.55	296.2561 278.2460 262.2521	$C_{18}H_{35}NO_3$	prosopinine isomeric isoprosopinin	3	[34]
12	10.242	-	329.2340	-2.13	-	-	-	-	$C_{18}H_{34}O_5$	pinellic acid	2b	(34)
13	11.223	-	-	-		342.2994	1.42	324.2883 $[M + H-18]+$ 306.2780 $[M + H-2*18]+$	$C_{20}H_{39}NO_3$	7-hydroxy-spectaline	2b	[34]

[a] according to Schymansky et al., 2014

Discussion

Prosopis africana was a plant used in traditional medicine and known in the treatment of dental caries and dental hygiene (35). It was also used in the treatment of childhood diarrhea, ulcer, wounds, dermatoses, and gonococcal disease [36]. All these diseases were influenced by bacterial biofilm [37]. The antibacterial activity of the plant against several strains such as *E. coli, S. aureus, S. mutans, K. pneumoniae, P. aeruginosa* has also been reported [36].

Bacteria biofilm formation is responsible for the development of antibiotic resistance [38]. Indeed, bacteria surround themselves with extracellular polymers (glycocalyx) which constitute a physical barrier, thus limiting the access of antibiotics to the interior of the cells. The methanolic extracts of leaves and stems of *P. africana* showed in this study a potential inhibitor of biofilm formation by *S. mutans ATCC, S. aureus ATCC, P. aeruginosa* PAO1 without inhibiting bacterial growth. These finding showed that the inhibition of biofilm formation by the extracts is in no way associated with a bactericidal or bacteriostatic effect, but rather with a interference of the QS system [38]. The effect of extracts could be explained by the fact that they act on the mechanism of dental biofilm either by preventing bacteria from adhering to dental surfaces, or they would prevent cell divisions that lead to the formation of a truly three-dimensional, structurally and functionally well-organized bacterial network that ensure the integrity, resistance, and nutrition of the biofilm or on the formation of the exogenous pellicle that can create favorable micro-niches for stable adhesion of bacteria [39]. Extracts could act on extracellular polymeric substances (EPS). According to previous studies, EPS can be considered "the home" of biofilm cells within which they organize their lives [40]. It consists mostly of water but also polysaccharides, nucleic acids, and proteins, of which extracellular enzymes retained in the form of consolidated polysaccharide complexes, participate in its formation and structuration [41, 42].

The properties of *P. africana* extracts to quench the dental biofilm controlled by the quorum sensing could justify the use of *P. Africana* in the folklore medicine to treat oral infections [37]. The reduction of violacein production was therefore a consequence of interference of the extracts with the Quorum Sensing mechanisms of *C. violaceum* CV026. The effect of *P. africana* extracts at the final concentration of 100 µg/ml was evaluated on the production of pyocyanin, rhamnolipids, and elastases, which are the main virulence factors excreted by *P. aeruginosa PAO1* and whose production was under the control of the QS in dental biofilm. The reduction of virulence factors by the extracts implies a probable interference with the regulatory mechanism of pyocyanin production which depends on the QS in the formation of the dental biofilm.

The results of the cytotoxic effect of the methanolic crude extracts of stems and leaves showed that the methanolic extract of stems under our experimental conditions did not induce any cytotoxicity on gingival cells. The inhibitory effect of the crude methanolic leaf extract could be explained by the abundant presence of alkaloids in the leaves and whose cytotoxicity was demonstrated previously [43].

UHPLC/UV/MS-MS analysis characterized in the methanolic extract of leaves and stems 2,4,6-trihydroxyphenyl β-D-glucoside, procyanidin A, catechin 7-*O*-glycoside, procyanidin dimer digallate, glucosyl-*O*-tyrosine, myricetin-3-*O*-galactoside, myricitrin, phloridzin and quercitrin, galloylquinic acid, digalloylshikimic acid, 3,4-digalloylquinic acid, 1,3-digalloyl quinic acid, prosopinin, pinelic acid, and spectalin. Most of these compounds were known to have anti-biofilm and anti-quorum sensing properties such as reported for catechin, galloyl shikimic acid, galloyl quinic acid, procyanidin, and quercitrin [44]. Indeed, these compounds could support the medical applications of *Prosopis africana* since the formation and structure of biofilm contribute greatly to the establishment of oral infections by increasing the properties of resistance to antibacterials [45]. They are therefore important building blocks for the development of new therapeutic products against biofilm encapsulated pathogens by disrupting the biofilm structure, thereby increasing the exposure of the pathogen to antibiotics. QS systems and biofilm are therefore new targets for the development of novel antibacterial strategies to inhibit the production of virulence factors by pathogenic bacteria [46].

Conclusion

The present study whose general objective was to evaluate the anti-biofilm and anti-quorum sensing properties of methanolic extracts of leaves and stems of *Prosopis africana*, also allowed for evaluating the cytotoxicity of these extracts after phytochemical analyses. The evaluation of the anti-biofilm and anti-quorum sensing properties showed very appreciable effects of the extracts. The cytotoxicity study showed that the methanolic extracts of *P. africana* do not induce any cytotoxicity on gingival cells. Phytochemical analysis by HPLC/UV/MS^2 allowed the characterization of flavonoid glycosides, tannin derivatives (catechin, procyanidin, gallic acid derivatives) and alkaloids (prosopinin, pinelic acid, and spectalin).

The biological effects of the extracts demonstrated in this study, as well as their chemical composition, may justify the use of *Prosopis africana* in the treatment of oral affections, as a mouthwash, especially since they are of low toxicity.

Abbreviations

Abs Absorbance

AHLs N-acyl homoserine lactones

B.H.I. Brain Heart Infusion broth

DMEM Dulbecco's Modified Eagle Medium

DMSO DiMethyl SulfOxide

ESI-QTOF Electrospray Ionization-Quadrupole Time Of Flight

INT IodoNitroTetrazolium

L.B. Luria-Bertani broth

LME Leaves Methanolic Extract

MBC Minimum Bactericidal Concentration

SME Stems Methanolic Extract

MIC Minimum Inhibition Concentration

MS Mass Spectrometry

OD Optical Density

QC Qualitative Control

QS Quorum Sensing

UPLC-UV/DAD Ultrahigh Performance Liquid Chromatography coupled to DAD UV-detector

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data were presented in the present manuscript

Competing interests

The authors declare no conflicts of interest.

Funding

Not applicable

Authors' contributions

B.A. Writing- Original draft, data curation, methodology, investigation; **R.A**. data curation, methodology, supervision and validation; **C.E**. data curation, methodology; **C.M.** data curation, methodology; **B.W.** data curation, methodology; **O.N.** data curation, methodology, supervision and validation; **H.E** data curation, supervision, investigation; methodology, writing - review & editing; **K.M**. data curation, supervision, investigation, methodology; **D.M**. data curation, supervision, investigation, methodology, writing - review & editing

Acknowledgements

The authors are thankful to Mrs. Marijolène. Rey, Dr. Serge Michalet, Dr Isabelle Kerzaon, members of the CESN, for their support in processing the LC/MSMS experiment.

References

1. Szafrański SP, Winkel A, Stiesch M. The use of bacteriophages to biocontrol oral biofilms. J Biotechnol. 2017;250:29–44.
2. Hasan S, Danishuddin M, Adil M, Singh K, Verma PK, Khan AU. Efficacy of E. officinalis on the cariogenic properties of streptococcus mutans: A novel and alternative approach to suppress Quorum-sensing mechanism. PLoS One. 2012;7(7):1–12.
3. Teresa R, Iglewski BH. MINIREVIEW Bacterial Quorum Sensing in Pathogenic Relationships. Society. 2000;68(9):4839–49.
4. Visick KL, Foster J, Doino J, McFall-Ngai M, Ruby EG. Vibrio fischeri lux genes play an important role in colonization and development of the host light organ. J Bacteriol. 2000;182(16):4578–86.
5. Mcclean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, et al. Quorum sensing and Chromobacterium violaceum : exploitation of violacein production and inhibition for the detection of N -acylhomoserine lactones. 1997;3703–11.
6. Labbate M, Queck SY, Koh KS, Rice SA, Givskov M, Kjelleberg S. Quorum Sensing-Controlled Biofilm Development in Serratia liquefaciens MG1. 2004;186(3):692–8.

7. Riedel K, Hentzer M, Geisenberger O, Huber B, Steidle A, Wu H, et al. N -Acylhomoserine-lactone-mediated communication between Pseudomonas aeruginosa and Burkholderia cepacia in mixed biofilms. 2017;(2001):3249–62.
8. Papenfort K, Bassler B, Chase C. HHS Public Access. 2017;14(9):576–88.
9. Czajkowski R, Jafra S. Quenching of acyl-homoserine lactone-dependent quorum sensing by enzymatic disruption of signal molecules. Acta Biochim Pol. 2009;56(1):1–16.
10. Reading NC, Sperandio V. Quorum sensing: The many languages of bacteria. FEMS Microbiol Lett. 2006;254(1):1–11.
11. Lu L, Hu W, Tian Z, Yuan D, Yi G, Zhou Y, et al. Developing natural products as potential anti-biofilm agents. Chinese Med (United Kingdom) [Internet]. 2019;14(1):1–17. Available from: https://doi.org/10.1186/s13020-019-0232-2
12. Parsek MR, Greenberg EP. Sociomicrobiology: The connections between quorum sensing and biofilms. Trends Microbiol. 2005;13(1):27–33.
13. Yu OY, Zhao IS, Mei ML, Lo EC man, Chu C hung. Models for Cariology Research. 2017;
14. Alimata B, Dofini MR, Souleymane C, Eli C, Noufou O, Seydou SD, et al. The Ethnobotanical Survey, Antibacterial Activity and Phytochemical Screening of Extracts of Prosopis africana (Guill. & Perr.) Taub. European J Med Plants. 2020;31(3):39–47.
15. Karou SD, Tchacondo T, Micheline AT. Screening Togolese medicinal plants for few pharmacological properties. 2012;(April).
16. Guinoiseau E. Molécules antibactériennes issues d ' huiles essentielles : séparation , identification et mode d'action To cite this version : HAL Id : tel-00595051 Faculté des Sciences et Techniques Mention : Biochimie - Biologie moléculaire. THESE Dr Univ Corse. 2011;(f):149.
17. Vandeputte OM, Kiendrebeogo M, Rajaonson S, Diallo B, Mol A, Jaziri M El, et al. Identification of catechin as one of the flavonoids from combretum albiflorum bark extract that reduces the production of quorum-sensing-controlled virulence factors in pseudomonas aeruginosa PAQ1. Appl Environ Microbiol. 2010;76(1):243–53.
18. Rasamiravaka T. Inhibition du mécanisme de quorum sensing et de la formation de biofilm chez Pseudomonas aeruginosa par des composés bioactifs de Dalbergia trichocarpa (Fabaceae). 2014.
19. Walker TS, Bais HP, Déziel E, Schweizer HP, Rahme LG, Fall R, et al. Pseudomonas aeruginosa-Plant Root Interactions. Pathogenicity, Biofilm Formation, and Root Exudation. Plant Physiol. 2004;134(1):320–31.
20. Ishida T, Ikeda T, Takiguchi N, Kuroda A, Ohtake H, Kato J. Inhibition of quorum sensing in Pseudomonas aeruginosa by N-acyl cyclopentylamides. Appl Environ Microbiol. 2007;73(10):3183–8.
21. Sarabhai S, Sharma P, Capalash N. Ellagic Acid Derivatives from Terminalia chebula Retz. Downregulate the Expression of Quorum Sensing Genes to Attenuate Pseudomonas aeruginosa PAO1 Virulence. PLoS One. 2013;8(1):1–11.
22. Ju NMPÆL kwang. Improved detection of rhamnolipid production using agar plates containing methylene blue and cetyl trimethylammonium bromide. 2009;1583–8.
23. Bationo R, Rouamba A, Diarra A, Beugré-Kouassi MLA, Beugré JB, Jordana F. Cytotoxicity evaluation of dental and orthodontic light-cured composite resins. Clin Exp Dent Res. 2020;(August 2020):40–8.
24. Guo L xiu, Li R, Liu K, Yang J, Li H jun, Li S lin, et al. Structural characterization and discrimination of Chinese medicinal materials with multiple botanical origins based on metabolite profiling and chemometrics analysis : Clematidis Radix et Rhizoma as a case study. J Chromatogr A. 2015;1425:129–40.
25. Zheng MHM shan, Son M hee WJ keun, Lee SK seon. (2)-Catechin glycosides from Ulmus davidiana. 2013;
26. Xu J, Zheng T, Huang X, Wang Y, Yin G, Du W. International Journal of Biological Macromolecules Procyanidine resists the fi bril formation of human islet amyloid polypeptide. Int J Biol Macromol. 2021;183.
27. Ni J cheng. Derivatives from the Fruit of Ailanthus altissima. 2017;1–12.
28. Granica S, Hadzik J, Matkowski A. Phytochemical Diversity in Rhizomes of Three Reynoutria Species and their Antioxidant Activity Correlations Elucidated by LC-ESI-MS / MS Analysis. Molecules. 2019;1–38.
29. Shari J, Dey A, Koirala N, Shaheen S. Cinnamomum Species : Bridging Phytochemistry Knowledge , Pharmacological Properties and Toxicological Safety for Health Bene fi ts. 2021;12(May):1–27.
30. Vihakas M, Gómez I, Karonen M, Tähtinen P, Sääksjärvi I, Salminen J pekka. Phenolic Compounds and Their Fates In Tropical Lepidopteran Larvae : Modifications In Alkaline Conditions. 2015;822–36.
31. Moore JP, Westall KL, Ravenscroft N, Farrant JM, Lindsey GG, Brandt WF. The predominant polyphenol in the leaves of the resurrection plant Myrothamnus flabellifolius, 3,4,5 tri-O-galloylquinic acid, protects membranes against desiccation and free radical-induced oxidation. Biochem J. 2005;385(1):301–8.
32. Singh A, Bajpai V, Kumar S, Sharma KR, Kumar B. Profiling of gallic and ellagic acid derivatives in different plant parts of Terminalia arjuna by HPLC-ESI-QTOF-MS/MS. Nat Prod Commun. 2016;11(2):239–44.
33. Wallis CM, Chen J. Grapevine Phenolic Compounds in Xylem Sap and Tissues Are Significantly Altered During Infection by Xylella fastidiosa. 2012;
34. Ingh SS, Erma SK V. Study of the distribution profile of piperidine alkaloids in various parts of Prosopis juliflora by the application of Direct Analysis in Real Time Mass Spectrometry (DART-MS). 2012;206–9.
35. Nacoulma OG. Plantes médicinales et Pratiques médicales Traditionnelles au BURKINA: cas du plateau central. Université de Ouagadougou; 1996.
36. Ajiboye AA, Agboola DA, Fadimu OY, Afolabi AO. Antibacterial, Phytochemical and Proximate Analysis of Prosopis Africana (Linn) Seed and Pod Extract. FUTA J Res Sci. 2013;9(1):101–9.
37. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evidence-based Complement Altern Med. 2011;2011.

38. Ouedraogo V, Kiendrebeogo M. Methanol Extract from Anogeissus leiocarpus (DC) Guill. et Perr. (Combretaceae) Stem Bark Quenches the Quorum Sensing of Pseudomonas aeruginosa PAO1. Medicines. 2016;3(4):26.
39. Larsen T, Fiehn N erik. Dental biofilm infections – an update. 2017;376–84.
40. Wingender J, Flemming HC. Biofilms in drinking water and their role as reservoir for pathogens. Int J Hyg Environ Health. 2011;214(6):417–23.
41. Jain K, Parida S, Mangwani N, Dash HR, Das S. Isolation and characterization of biofilm-forming bacteria and associated extracellular polymeric substances from oral cavity. Ann Microbiol. 2013;63(4):1553–62.
42. Seviour T, Derlon N, Dueholm MS, Flemming HC, Girbal-Neuhauser E, Horn H, et al. Extracellular polymeric substances of biofilms: Suffering from an identity crisis. Water Res. 2019;151:1–7.
43. Somda GD, Ouedraogo GG, Ilboudo S, Ouedraogo N. Phytochemical Screening , Acute Toxicity and Anti- Diarrhoeal Effect of Extracts from Prosopis africana (Guill . & Perr .) Taub (Mimosaceae). Int J Sci Res. 2019;8(8):1361–6.
44. Mombeshora M, Chi GF, Mukanganyama S. Antibiofilm Activity of Extract and a Compound Isolated from Triumfetta welwitschii against Pseudomonas aeruginosa. B. 2021;2021.
45. Landry RM, An D, Hupp JT, Singh PK, Parsek MR. Mucin-Pseudomonas aeruginosa interactions promote biofilm formation and antibiotic resistance. Mol Microbiol. 2006;59(1):142–51.
46. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents. 2010;35(4):322–32.

Figures

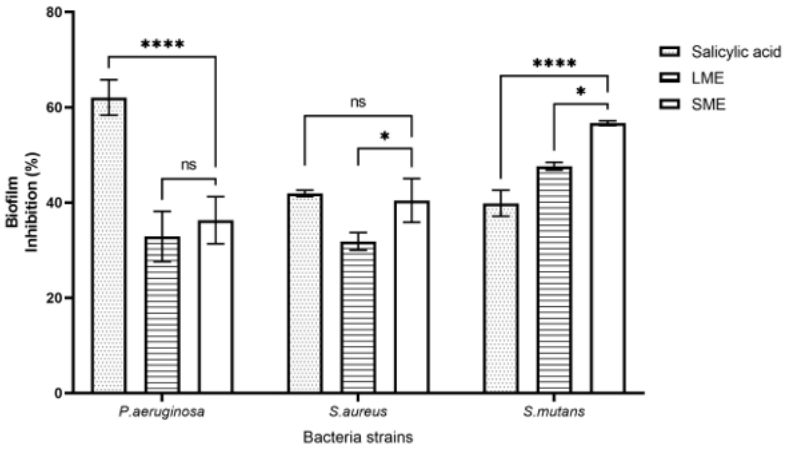


Figure 1

Effect of *P. africana* extracts on biofilm formation of cariogenic bacteria. ns: not significant compared to the positive control. *P<0.05; **P<0.01; *** P<0.001; **** P <0.0001 versus Salicylic acid. LME: Leaves methanolic extract; SME: Stem Methanolic Extract

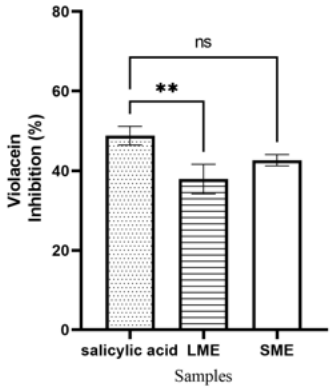


Figure 2

Effect of extracts on violacein production in *Chromobacterium violaceum* CVO26. ns: not significant compared to the positive control. *P<0.05; **P<0.01; *** P<0.001; **** P <0.0001 *versus* Salicylic acid. LME: Leaves methanolic extract; SME: Stem Methanolic Extract

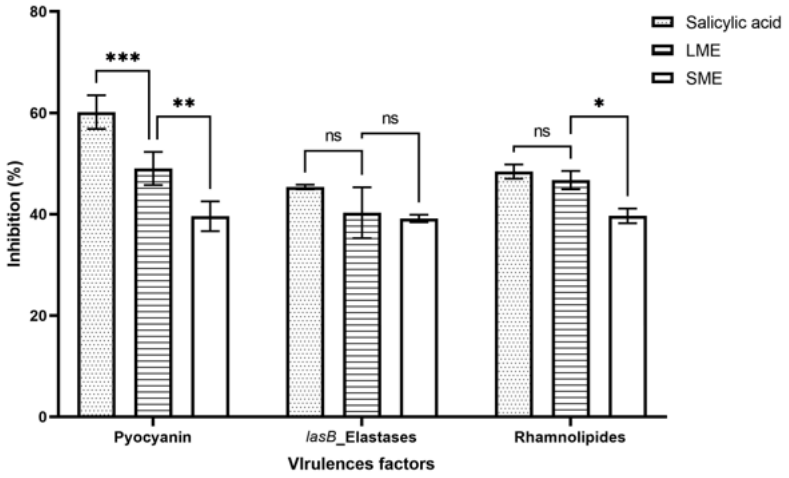


Figure 3

Effect of extracts on virulence factors. ns: not significant compared to the positive control. *P<0.05; **P<0.01; *** P<0.001; **** P <0.0001 *versus* Salicylic acid. LME: Leaves methanolic extract; SME: Stem Methanolic Extract

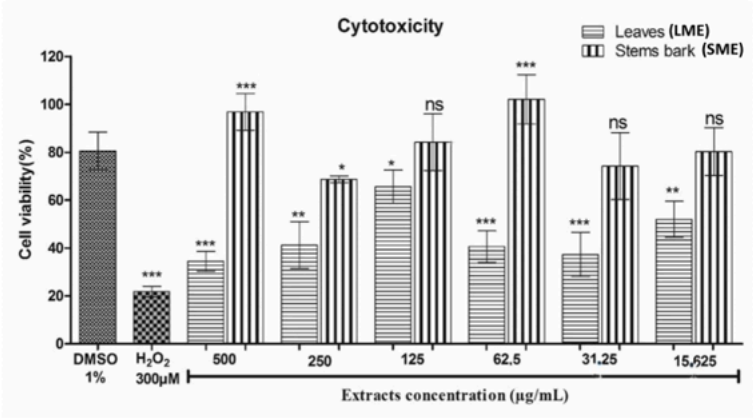


Figure 4

Cytotoxicity of the methanol extracts of leaves (LME) and stems bark (SME) of *P. africana.* ns: not significant, *P<0.05, **P<0.01, ***P<0.0001 significant difference compared to the vehicle (DMSO 1%)

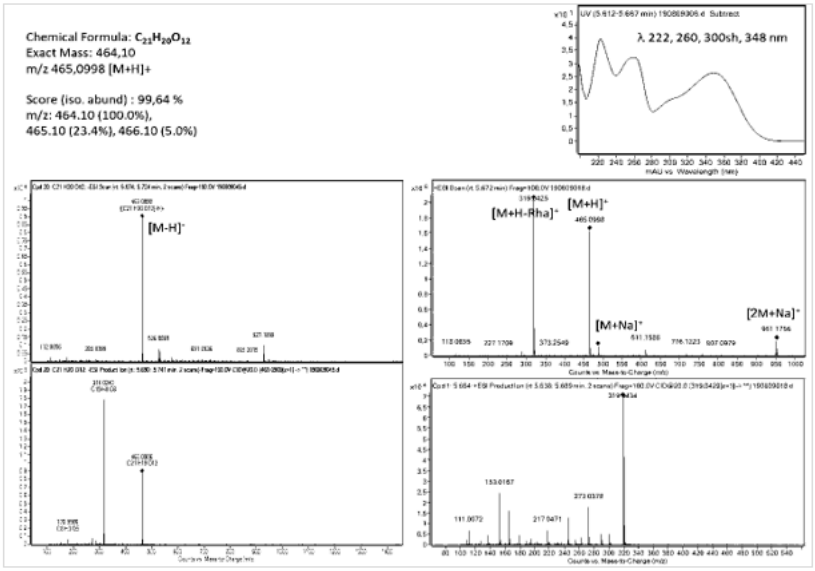


Figure 5

ESI^+ and ESI^- mass spectra and UV spectrum of compound **11**.