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Research Article

Therapeutic properties of aqueous extracts of leaves and stems bark of *Prosopis africana* (Guill. & Perr.) Taub. (Fabaceae) used in the management of dental caries

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Abstract

Prosopis africana (Guill. & Perr.) Taub. (Fabaceae) is used in the herbal medicine of Burkina Faso to treat dental caries. This study aims to contribute to the valorization of the said plant by investigating the antioxidant, anti-inflammatory and antibacterial properties of aqueous leaves and stems extracts.

The inhibitory activity on lipoxygenase was used to evaluate the anti-inflammatory effect of the extracts. The antioxidant activity of both extracts of the plant was assessed using DPPH radical scavenging, ABTS+ radical cation decolorization. The anti-biofilm effect of the extracts was evaluated on *Streptococcus mutans* ATCC 25175, *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* PAOI and the anti-Quorum sensing effect on *Chromobacterium CV026*.

Aqueous extracts of *Prosopis africana* stems show the highest content of phenolic compounds (30,04± 0,59 mgAGE/100 mg extract) while those of the leaves show the highest content of total flavonoids (3.29 ± 0.53 mgQE/100mg extract). The aqueous extract of stem bark show the strongest antioxidant activity (IC₅₀ = 4.58±0.07µg/ml for the ABTS), a best Inhibitory action on activity of lipoxygenase (IC₅₀ = 13.42 ± 1.26 µg/mL), a highest anti-biofilm activity (63.6%; at the concentration of 100µg/ml) without affecting the bacterial growth. In addition, this extract has the strongest anti-quorum sensing activity with an percentage of inhibition 53,5%.

These findings suggested that the aqueous extracts of stem bark and leaves of *Prosopis africana* contain promoted phytochemicals to combat dental caries infections.

Keywords : Anti-biofilm, Anti-quorum sensing, Lipoxygenase, *Prosopis africana*

INTRODUCTION

Despite the advances in public policies so far, dental caries remains the most prevalent and costly oral infectious disease worldwide ¹, representing a global public health problem to be managed by authorities and dental professionals ^{2,3}. Effective caries-preventive methods have been developed and amended in the last decades. It is well known that the chemical control of plaque is an effective strategy to prevent dental caries development ⁴. The main chemical agents currently available are fluoride ⁵, chlorhexidine ⁶, triclosan, cetylpyridinium chloride, and natural products ^{4,6}. However, like most antimicrobials, these drugs suffer from resistance phenomena towards cariogenic bacteria due to their union in biofilm⁷. Still, their regular use can also cause other inconveniences such as vomiting, tooth discoloration, and oral cancer ^{8,9}. Also, the problem of timely accessibility of these drugs in all places for the poorest segments of the

population, the lack of access to health services is a painful reality ^{10,11}.

Consequently, the association of traditional medicine with this struggle constitutes a less expensive and more accessible community alternative, provided that the therapeutic effectiveness and safety of the plants used are scientifically proven. Hence, scientists are shifting their attention to folk medicine to find new leads for better against microbial infections. Plant materials are known as a source of new antimicrobial agents; as a result, a search has been made to discover new antibacterial drugs of plant origin ¹².

Numerous medicinal plant extracts or phytochemicals have been shown to inhibit the formation of dental biofilms by reducing the adhesion of microbial pathogens to the tooth surface, a primary event in the initiation and the progression to dental decay^{13,14}.

Prosopis africana (Guill. & Perr.) Taub (Fabaceae) is a plant used in traditional medicine for the treatment of oral diseases. An ethnobotanical survey conducted by ¹⁵ reported using the plant leaves and stems in rural areas to treat dental caries and the bark in treating green diarrhea in infants. Its antibacterial activity against several strains such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* has also been reported ^{15,16}. However, the literature has little investigated the anti-biofilm anti quorum sensing properties of leaves and stem bark extracts. This study investigated the antioxidant, anti-inflammatory, and anti-biofilm activity of the aqueous leaves and stem bark extracts of *Prosopis africana*.

MATERIALS AND METHODS

Chemicals

All chemicals used were analytical grade. FCR 2N (Folin-ciocalteu reagent); AlCl₃ (aluminium trichloride (75 L, 2% in methanol)) Prolabo (Paris, France); ABTS (2, 2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic acid]) Sigma (Steinheim, Germany); DPPH (2, 2-diphényl-1-picrylhydrazyl) Sigma (Steinheim, Germany); potassium persulfate (K₂S₂O₈) (Fluke, France), Trolox (Fluke, France), Zileuton, lipoxygenase (type I-B) Sigma® (St Louis, USA); boric acid; linoleic acid; quercetin Sigma (Steinheim, Germany); gallic acid Sigma (Steinheim, Germany).

Bacterial Strains

Streptococcus mutans ATCC 25175 and *Staphylococcus aureus* ATCC 43300 (methicillin-resistant) strains were provided by the bacteriology laboratory of the Muraz Center in Bobo Dioulasso and the wild type *Pseudomonas aeruginosa* PAO1 and *Chromobacterium violaceum* CV026 strain by the plant biotechnology laboratory (L.B.V.) of the Université Libre de Bruxelles (Belgium). All strains were stored at -80°C in B.H.I. (Brain Heart Infusion) liquid medium for *Staphylococcus aureus* and *Streptococcus mutans*; and L.B. (Luria-Bertani) for *Pseudomonas aeruginosa*, *Chromobacterium violaceum* CV026 supplemented with 50% glycerol until use.

Plant material and extraction

P. africana stem bark and leaves were collected from the province of Zounweogo under traditional healer assistance. The plant material was taxonomically identified under the voucher N° 6852 by a botanist, and a specimen was deposited at the herbarium of University Joseph KI-ZERBO. The fresh material collected was dried in a greenhouse with air circulation and then powdered until use.

For the extraction, one hundred grams (100 g) of leaf and stem bark powders of *Prosopis africana* (Guill. & Perr.) Taub obtained are boiled in 1000 mL distilled water for 30 min. After cooling, the extract was first filtered through a nylon cloth and then cotton. The filtrates thus obtained were immediately concentrated in the oven after centrifugation at 2000rpm for 5 min. The obtained supernatant was recovered and concentrated, then lyophilized and recovered in white hermetically sealed vials and stored at + 4° C until use.

Total polyphenol and Flavonoid Content determination

Total polyphenol was determined according to the colorimetric method of Folin-Ciocalteu ¹⁷.

Plant extract (25 L, 100 g/mL in methanol) was mixed with Folin-Ciocalteu Reagent (125 L, 0.2 N) and, 5 min later, with sodium bicarbonate (100 L, 75 g/L). After incubation (1 h, room temperature), absorbance was measured at 760 nm against a methanol blank. Gallic acid (0–100 mg/L) was used

to generate a standard calibration curve ($Y = 0.005X + 0.00968$; $R^2 = 0.99$), and total phenolic content was expressed as mg gallic acid equivalent to 100 mg of plant extract (mg GAE/100 mg). Total flavonoid was estimated according to the Dowd method ¹⁷. Plant extract (75 L, 100 g/mL in methanol) was mixed with aluminum trichloride (75 L, 2% in methanol). Absorbance was subsequently read at 415 nm after incubation (10 min, room temperature) against a methanol blank. Quercetin (0–100 mg/L) was used to plot a standard calibration curve ($Y = 0.02891X + 0.0036$; $R^2 = 0.99$), and total flavonoid content was expressed as mg of quercetin equivalent to 100 mg of plant extract (mg QE/100 mg).

Anti-oxidant Assays

ABTS (2, 2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic acid]) test

The capacity of extracts to scavenge the ABTS radical cation was determined according to the procedure described previously¹⁸. A stock solution of ABTS (7 mM) was mixed with 2.45 mL of potassium persulfate (K₂S₂O₈). The mixture was stored out of the light at room temperature for 12-16 h before use. A cascade dilution range of the extracts and Trolox (reference substance) was performed from a 1mg/mL concentration to determine the inhibitory concentration at 50% (IC₅₀). So, 20 µL of each dilution was mixed with 200 µL of the ABTS solution diluted in ethanol in the 96-wells microplate. The absorbances were read against blank (ethanol) at 734 nm on a spectrophotometer (Epoch Biotek Instruments, U.S.A.) after 30 min of incubation in the dark at room temperature. The test was performed in triplicate, and the percentage inhibition was determined by the following formula:

$$\% \text{ Inhibition} = (A_b - A_s) / A_b \times 100$$

A_b: Absorbance of blank; A_s: Absorbance of sample/reference compound.

DPPH (2, 2-diphényl-1-picrylhydrazyl) test

DPPH radical scavenging ability of extracts was assayed as described previously¹⁹. A cascade dilution of the extract and Trolox (reference substance) was performed from a concentration of 1 mg/mL.

For this purpose, 200 µL of DPPH solution freshly prepared in methanol (4 mg/100 mL) was mixed with 100 µL of each dilution in the 96-wells microplate. The mixture was incubated for 30 min at ambient temperature, and the absorbances were measured at 517 nm against a blank (methanol) with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). The DPPH radical inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A_b - A_s) / A_b \times 100.$$

A_b: absorbance of blank; A_s absorbance of sample/reference compound.

Anti-inflammatory activity *in vitro*

The inhibitory activity of lipoxygenase was determined according to the spectrophotometric method described by ²⁰ with slight modifications. 146.25 µL of lipoxygenase solution (820.51 U/ml) prepared in boric acid buffer (0.2 M, pH 9.0) was mixed with 3.75 µL of extracts (8mg/ml) in the 96-wells microplate and then incubated at room temperature for 3 min. The reaction was initiated by adding 150 µL of the substrate (1.25 mm of linoleic acid). The absorbances were recorded for 3 min at 234 nm with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). All tests were performed in triplicate, and Zileuton was used as a reference compound.

The inhibitory percentages were calculated using the formula:

$$\% \text{ Inhibition} = (V_b - V_s) / V_b \times 100$$

V_b: Enzymatic activity without inhibitor; V_s: Sample: Enzymatic activity with sample/reference compound.

Anti-biofilm activity

The anti-biofilm activity of the extracts was evaluated according to the method described by²¹. *Streptococcus mutans* ATCC 25175, *Staphylococcus aureus* ATCC 43300, and *Pseudomonas aeruginosa* PAO1 were used as bacterial strains. The extracts and the reference (salicylic acid) were prepared at the 1 mg/mL concentration in DMSO (1%) to have a final concentration in each well of 100 µg/mL. After incubating the plates for 24 h at 37 °C containing each bacterial strain and extract, bacterial turbidity was measured at 600 nm to ensure that the extracts did not affect bacterial growth compared to the negative control (1% DMSO). For the anti-biofilm activity, the supernatant from each well was then removed, and the biofilms were washed with distilled water, fixed with methanol for 15 min, and dried. Crystal violet 0.1% (in water) was added to each well, and the plates were incubated for 30 min at room temperature. After removal of the crystal violet, the wells were rinsed with distilled water, and 200 µL of acetic acid (33% in water) was added to dissolve the crystal violet. The absorbances of the solution were read at 590 nm, and the biofilm/bacterial growth ratio (OD 590 nm/DO 600 nm) was determined.

Anti-quorum sensing activity

Inhibition test of violacein production in *C. violaceum* CV026

C. violaceum was grown for 24 h at 30°C under agitation at 175 rpm. CV026 was diluted and introduced into 12-well plates. Then extracts/reference (salicylic acid) dissolved in DMSO (1%) were added (final concentration of 100 µg/mL) in the presence of 10 µM of C6-HSL. The plates were incubated for 24 h at 30°C, 175 rpm. Bacterial turbidity (OD 600 nm) was measured to assess bacterial growth, and then 1 mL of each well was collected and introduced into tubes to quantify violacein. The solution was vortexed vigorously for 30 s to solubilize the violacein, and then the supernatant was removed, and 1 mL of DMSO was added to the pellet. After centrifugation at 7000 rpm for 10 min, 200 µL of the supernatant containing violacein was introduced into 96-well microplate wells. The violacein production was quantified by measuring the absorbance at 585 nm, and the ratio of OD 585 nm to OD 600 nm was also determined.

Statistical analysis

The experiments were performed in triplicate (n = 3), and the results were expressed as mean ± standard deviation. Graph Pad Prism version 6 software was used for statistical analysis, graphing. The results obtained were expressed as mean ± Standard Error Mean (m ± S.E.M.). One-way analysis of variance (ANOVA) followed by Dunnett's test was used to measure the statistical significance of the results.

RESULTS

Total phenolics and total flavonoid contents

Table 1 presents the phenolic and total flavonoid contents obtained in the extracts according to the type of plant organ. Indeed, the highest content of phenolic compounds was obtained with the aqueous extracts of stems (30.04 ± 0.59

mgAGE/100 mg extract), and the aqueous extract of leaves show the highest quantity of total flavonoids (mgQE/100mg extract).

Table 1: Total phenolics and flavonoids contents of aqueous extracts of leaves and stem of *P. africana*

Extracts	Total flavonoids (mgQE/100mg extract)	Total phenolics (mgAGE/100 mg extract)
LDE	3.29 ± 0.53 ^{ns}	14.42 ± 0.25 ^{****}
SDE	1.03 ± 2.69 ^{ns}	30.04 ± 0.59 ^{****}

Mean values ± standard deviation were presented (n = 3) (****) = p < 0.0001 is considered significant ; ns: no significance P > 0.05 ; LDE: Leaves Decocted Extract; S.D.E.: Stems decocted extract; G.A.E.: Gallic acid equivalent; Q.E.: quercetin equivalent

Antioxidant activity

Data of the antioxidant activities of extracts through two methods (ABTS, DPPH) are indicated in Table 2. Both extracts exhibited good antioxidant activities. The stem bark extract exhibited the best anti- DPPH activity, while the leaves extract showed the best anti- ABTS activity. The anti-DPPH activity of plant extracts was less than the reference compound (Trolox). However, the plant extracts and Trolox showed similar anti-ABTS activity (p>0.05).

Table 2: Antioxidant activity (ABTS, DPPH) of plant extracts

Extracts	DPPH	ABTS
	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
LDE	20,32 ± 1,89 ^{****}	4.58 ± 0.07 ^{ns}
SDE	16,39 ± 0,26 ^{****}	7.57 ± 0.04 ^{ns}
Trolox	7,17 ± 0,11 ^{ns}	3.82 ± 0.018 ^{ns}

Values are expressed as mean ± SEM, n = 3 (****) = p < 0.0001 is considered significant compared to the control, ns: no significance P > 0.05. Plant extracts vs. Trolox for ABTS, DPPH (one-way ANOVA analysis followed by Dunnett multiple comparisons). LDE: Leaves decocted extract; S.D.E.: Stem decocted extract

Anti-inflammatory activity

The inhibitory effect of *P. africana* extracts on lipoxygenase activity is presented in Table 3 below. The aqueous stem bark extract showed the best inhibitory concentration (IC₅₀ = 13.42 ± 1.26 µg/mL) compared to the aqueous leaf extract

Table 3 : Lipoxygenase (L.O.X.) inhibitory concentrations (IC₅₀) of extracts

Extracts	Anti-lipoxygenase activity (IC ₅₀) µg/mL
LDE	35.11 ± 1.05 ^{****}
SDE	13.42 ± 1.26 ^{**}
Zileuton	4.08 ± 0.80

p<0.01, *p<0.001, Zileuton vs extracts. LDE: Leaves decocted extract; S.D.E.: Stem decocted extract

Anti-biofilm effect of extracts

As shown in figure 1, the aqueous leaves and stems bark extract at the 100 µg/ mL concentration did not affect the bacterial growth of *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus* ATCC 43300, *Streptococcus mutans* ATCC 25175, and *Chromobacterium violaceum* 026. Nevertheless, compared to DMSO, the extracts significantly reduced the biofilm formation. For all strains, the inhibition of biofilm by the extracts was more significant on *Streptococcus mutans* ATCC 25175. Plant extracts were more active than the reference compound (salicylic acid) in the *Streptococcus mutans* biofilm inhibition.

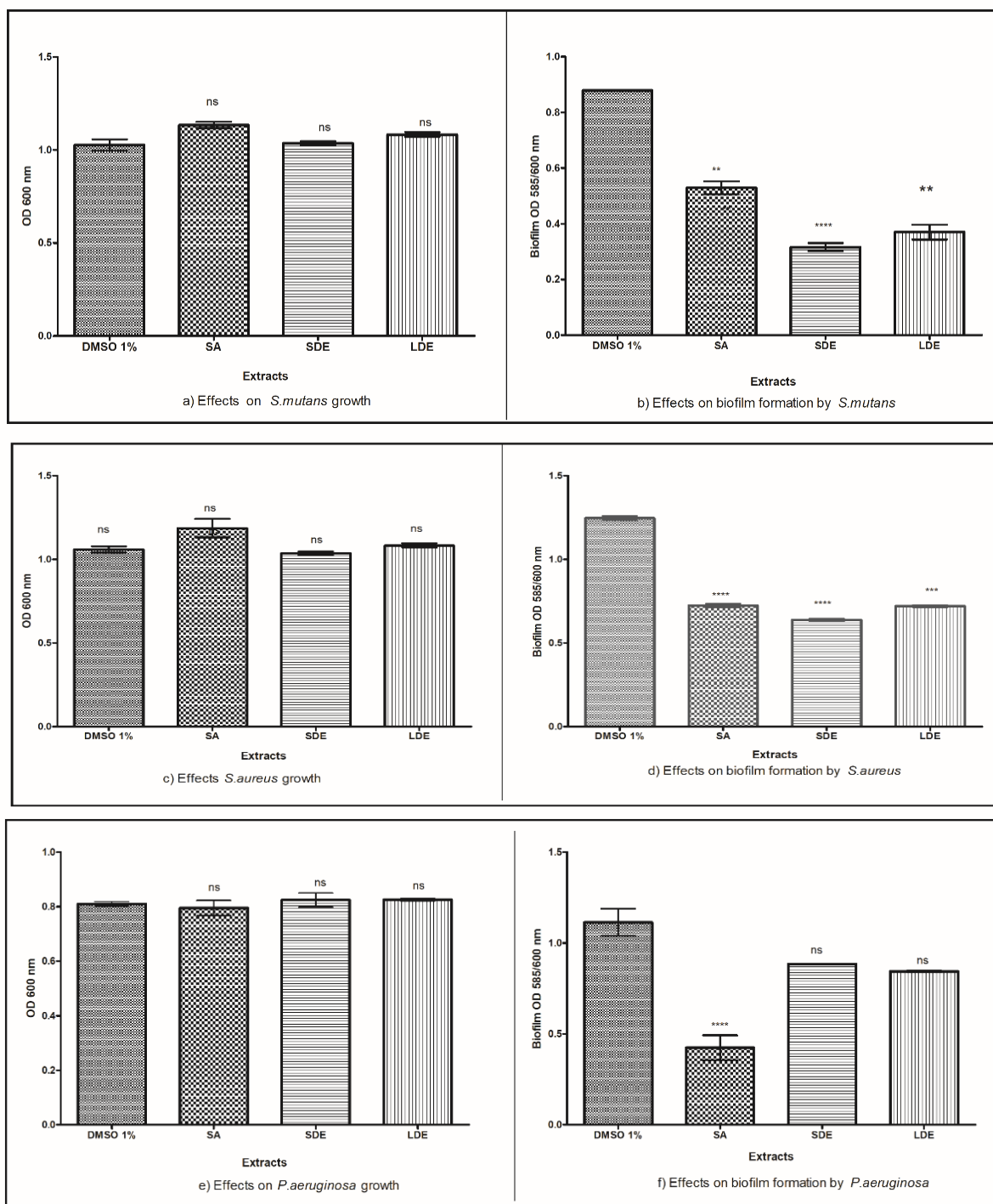


Figure 1: Effect of *P. africana* extracts on biofilm formation of cariogenic bacteria

ns: not significant compared to DMSO control (P< 0.05). SA : Salicylique Acid ; SDE : Stem Decoated extract ; LDE: Leaves Decoated Extract

Effect of extracts on violacein production in *Chromobacterium violaceum* CV026

At the final concentration of 100µg/ml, the extracts did not affect the bacterial growth of *Chromobacterium violaceum* CV026 figure 2a. The *C. violaceum* CV026 strain, deficient in the homoserine-lactone synthase gene *civil*, was used to

evaluate the ability of the extracts to interfere with the Quorum Sensing mechanism. As shown in figure 2b, the extracts significantly reduced the production of violacein compared to DMSO. The stem bark extract showed higher anti-quorum sensing activity than the leave extract and the reference compound.

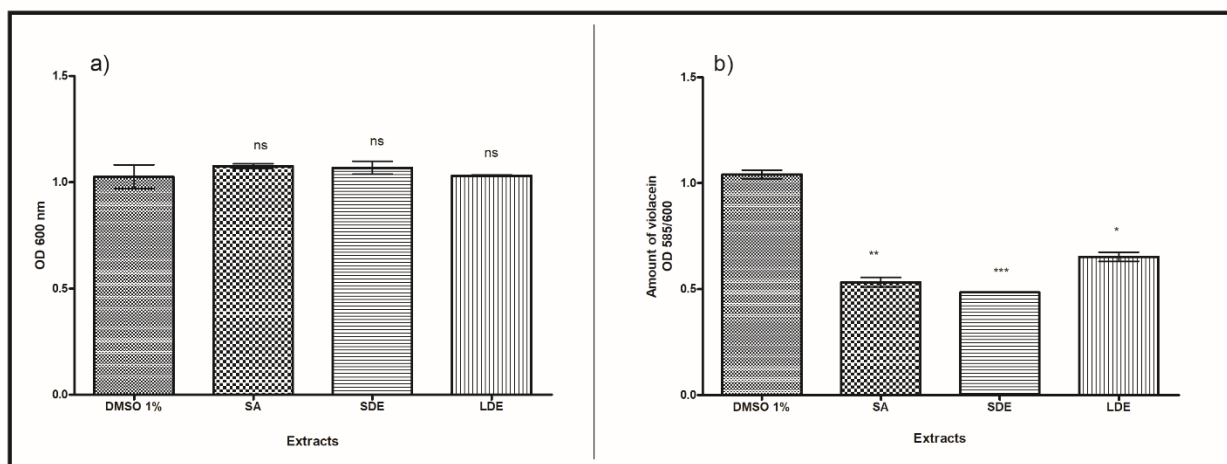


Figure 2: Effect of *P. africana* extracts on violacein production in *C. violaceum* CV026

ns: not significant compared with DMSO control ($P < 0.05$)*, ($P < 0.0001$)***significant difference compared to DMSO.SA : Salicylique Acid ; SDE : Stem decocted extract ; LDE: Leaves decocted extract

DISCUSSION

Prosopis africana is used in traditional medicine to treat microbial infections, particularly bacterial ones. This plant is thus a potential source for the research of new compounds targeting the inhibition of biofilm and the production of virulence factors in a dental infection.

The results of the ABTS antiradical activity show that the IC_{50} of the leaf and stem extracts of *Prosopis africana* is comparable to that of the reference substance (Trolox). For DPPH, the best activity was observed by the stem decoctate with an IC_{50} . In general, the extracts presented an excellent antioxidant activity. The antioxidant capacity of the extracts of stems bark and leaves would be due to the polyphenols. This activity would be mainly due to their redox properties, which may play an important role in absorbing and neutralizing free radicals¹⁶. The antioxidant activity of *Prosopis africana* extracts thus observed is an argument that can justify its traditional use, especially for its anti-inflammatory and antibacterial properties. Indeed, the literature has often noted the close link between these properties and the antioxidant potential of the plant, and several authors^{22,23} explain the anti dental biofilm effect of many plants by their ability to relieve cells of oxidative stress induced by dental biofilm and quorum sensing. Similarly, it is now recognized that oxidative stress is an essential underlying factor in immune system dysfunction and plays a crucial role in inflammatory processes²⁴⁻²⁶. The oxidative stress caused by dental inflammation could be treated with *Prosopis africana* extracts.

The lipoxygenase inhibition test allowed us to appreciate a possible anti-inflammatory mode of action of *P. africana* extracts. Indeed, the extracts inhibited the activity of lipoxygenase. The best inhibition was obtained with the stem decoctate (SDE) ($IC_{50} = 13.42 \pm 1.26 \mu\text{g} / \text{mL}$), the leaf decoctate (LDE) ($IC_{50} = 35.11 \pm 1.05 \mu\text{g} / \text{mL}$).

Indeed, the lipoxygenase inhibition would be done by synergizing the actions of phytochemical groups, such as polyphenols that compose the extracts. Polyphenols are known for their inhibitory properties of pro-inflammatory enzymes^{27,28}. Lipoxygenase (L.O.X.) is an enzyme used in leukotriene biosynthesis^{29,30}. These leukotrienes play a crucial role in inflammatory diseases such as dental caries, periodontitis, asthma, rheumatoid arthritis, cardiovascular diseases, and cancer.³¹. Inhibiting L.O.X. by the extracts

could help decrease the increased release of pro-inflammatory cytokines (IL-1 β , IL-6, IL-10, TNF α , INF γ , PGE2...), and several metalloproteinases due to dental inflammation³².

Dental biofilm confers antibiotic resistance to cariogenic bacteria³³. The factor attributed to resistance in dental biofilm is the presence of extracellular polymeric substances (EPS), also known as glycocalyx, that surround the cells and act as a physical barrier that prevents molecules from entering the cells^{34,35}. The results obtained from this study reflect an inhibitory effect of *P. africana* leave and stem decocts on the biofilm of *S. mutans* ATCC, *S. aureus* ATCC, *P. aeruginosa* PAO1, on the production of violacein, without affecting the bacterial growth. Indeed biofilm inhibition, violacein inhibition is not associated with a bactericidal or bacteriostatic effect but with an interference with the quorum sensing system³⁶. Polyphenols and flavonoids are known for their anti-biofilm and anti-Quorum sensing potential^{37,38}. The anti-biofilm and anti-Quorum sensing activity of *Prosopis africana* extracts could be due to these compounds quorum sensing and biofilm are, therefore, new targets for the development of new antimicrobial strategies to inhibit the production of virulence factors by pathogenic bacteria³⁹. The inhibitory effect of *P. africana* extracts on dental biofilm and virulence factors controlled by quorum sensing could explain its use in traditional medicine against oral diseases¹⁴.

CONCLUSION

This study showed that aqueous extracts of *Prosopis africana* leaves and stem barks are potent sources of anti-biofilm, anti-quorum, anti-inflammatory and anti-oxidant phytochemicals. The results justify the use of *Prosopis africana* in Burkina Faso herbal medicine by traditional healers to treat dental caries. Further phytochemical studies are needed to characterize the bioactive compounds responsible for these biological properties.

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Conflict of Interest : The authors declare that they have no conflicts of interest

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