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Antibacterial, Antibiofilm and Antioxidant Activities of some Medicinal Plants from Pharmacopoeia of Tassili N'ajjer

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Abstract

Three plants adopted by nomads at Tassili n'ajjer (south Algeria) in traditional medicine namely, *Cymbopogon schoenanthus*, *Anabasis articulata* and *Salvia chudaei*, were analysed for their antibacterial, antibiofilm and antioxidant properties. Total flavonoid and phenolic contents were measured with 2% AlCl_3 and Folin-Ciocalteu's reagent method, respectively. The antibacterial propriete was investigated by measurement of MIC of plants extract inhibing bacterial proliferation. The antibiofilm propriete was calculated by fluorescent quantization of the DAPI labeled bacterial biomass fixed on the surface and by COMSTAT analysis of confocal scanning laser microscopy (CSLM) images. DPPH radical scavenging and β -carotene/linoleate bleaching methods were used to determine the antioxidant activities of the plants. Total phenolic content was ranged from 21.98 to 2.51 (gallic acid equivalents mg/g methanolic extracts) and the total flavonoid content ranged from 19.27 to 1.65 (catechin equivalent mg/g methanolic extracts). Antibacterial activity against four Gram positive bacteria was shown with plants extracts. The biofilm inhibition concentration of extracts decreasing 50% of biofilm cell density (BIC_{50}) for *Salvia chudaei* and *Cymbopogon schoenanthus* extracts ranges from 1 to 10 $\mu\text{g/mL}$. CSLM images analysis revealed that both surface covering by germ and three dimensional development of the biofilm were reduced with plants extracts. For antioxidant activities, the methanol extracts of the plants evaluated showed low antioxidant activity, with a IC_{50} between 1.94 and 6.16 mg/ml. Thus, Our systematic research showed that this three common plants of Sahara desert has diversified phytochemicals possessing satisfying extent of antimicrobial, antibiofilm and antioxidant activities.

Keywords: Saharian plants, methanolic extracts, antibacterial, antibiofilm, antioxidant

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INTRODUCTION

Resistance to antibacterial molecules is a growing significant public health problem¹. Even if a more accurate use of these molecules is required, discovery of new antibacterial compounds seems to be unavoidable. It is also true that, germs can generate biofilms whatever can form a sort of sticky gel, that forms its passage into various situations ranging from clinical to forming firms, and habitats where water and food are prepared and opported. On the other hand, these biofilms have learned activity to oppose antibiotics².

Several ways of research must be taken to isolate active substances such as synthetic chemistry, or extraction of molecules isolated from microorganisms, fungi, algae, invertebrates or plants (peptides, phenolic compounds...). One of them is to identify the antimicrobial active substances of plants known for their use in traditional medicine³⁻⁵.

The flora of Algerian central Sahara (Tassili n'ajjer) presents a remarkably diversified vegetation of hundreds of endemic species that are valued by the local population in the field of traditional medicine⁶. *In vitro* and *in vivo* phytochemical researches have provided scientific justification and validation for their traditional uses⁷. The therapeutic properties of these plants are related to the existence of secondary metabolites which are actually biologically active compounds. They are mainly phenolic compounds⁸ that exert their biological activities as antioxidant or antimicrobial⁹. *Anabasis articulata* (noted *Aa*,

Chenopodiaceae), *Cymbopogon schoenanthus* (noted *Cs*, *Poaceae*) and *Salvia chudaei* (noted *Sc*, *Lamiaceae*) are three Saharian desert plants, used for their medicinal properties by the local population and known as "ajrem"; lemmad "and" tagrouft ", respectively¹⁰.

Analyzes carried out on *A. articulata* species revealed the presence of various compounds that can explain its medicinal potential, including phenol acids¹¹. Several studies report the biological properties of essential oil of *C. schoenanthus*¹². Aerial parts uses of *S. Chudaei* in local traditional medicine for the therapy of gonorrhoea, spasms, abdominal pain and dysmenorrhoea is well documented¹⁰.

In this work, phenolic compounds from these three plants were extracted using solubilization in methanol. The antibacterial, including antibiofilm development and antioxidant potentials of these extracts were determined thus validating their ancestral medicinal uses.

MATERIALS AND METHODS

Plant materials

Anabasis articulata (Forssk) Moq. (*Chenopodiaceae*), *Cymbopogon schoenanthus* (L.) Spreng. (*Poaceae*) and *Salvia Chudaei* Batt. & Trab. (*Lamiaceae*) were collected from the Hoggar region (southern Algeria) during the flowering period, in 2019 (Table 1). Botanical identification was carried out by Dr. Rabea Sahki, in the National Forest Research Institute (INRF), Tamanrasset, Algeria. "*A. articulata*, *C. schoenanthus* and *S. chudaei* were deposited at Abdelhamid Ibn-Badis

Table 1. Locations, name and folkloric uses of plants subject of our study⁶.

Scientific name	Tamahaq name	Arabic name	Part used	Traditional used	Altitude (m)	Latitude/ longitude
(<i>Chenopodiaceae</i>) <i>Anabasis articulata</i>	bender	ajrem	Air part	Skin diseases	1459	N22°48.205'E05°36.949'
(<i>Poaceae</i>) <i>Cymbopogon schoenanthus</i>	tiberimt	lemmad	Whole plant	Respiratory infections, Urinary tract infections	1459	N22°48.205'E05°36.949'
(<i>Lamiaceae</i>) <i>Salvia Chudaei</i>	aouit	tagrouft	Whole plant	Hepathic Liver diseases, palpitations, anxiety	1439	N22°48.190'E05°36.940'

University and INRF herbarium with the voucher specimen number MPS2019/42, MPS2019/45 and MPS2019/43, respectively."

Prior to extraction, the plant material was dried and then ground.

Qualitative phytochemical analysis

The presence or absence of biologically active secondary metabolites was carried out on plants powder homogenate according to the standard methods described by Harbone¹³.

Methanolic extraction

Methanolic extraction was performed for 24 hours under stirring. The extract was purified and then was concentrated using a rotary evaporator until complete drying and stored at +4°C.

Quantitative phytochemical analysis

Total phenols content (TPC) determination

The quantity of total phenols was measured with Folin-Ciocalteu reagent¹⁴ and gallic acid like a standard. Data were reported as milligram of gallic acid equivalent/gram methanolic extract (GAE mg/g).

Determination of the total flavonoids content

Colorimetric method with $AlCl_3$ was adopted to quantify total flavonoids¹⁵ using catechin like a standard. Total flavonoid content was reported as milligram of catechin equivalent/gram methanolic extract (CE mg/g).

Determination of antimicrobial activity

Minimal inhibitory concentration (MIC) evaluation

The antibacterial power of plant extracts against different bacteria was first estimated by the disk diffusion method as described by¹⁶ paralleling to the CLSI M02-A10 standard and clinical laboratory guidelines¹⁷. Strains of the succeeding bacterial species were used: *Pseudomonas aeruginosa* (kind gift from Reuben Ramphal) (Florida, USA), *Escherichia coli*, *Proteus mirabilis*,

Dickeya dadantii, *Bacillus subtilis*, *Micrococcus luteus* (kinds gift from Michel Simonet from National Institute of Health and Medical Research (INSERM) U801 France), *Staphylococcus aureus* (ATCC® 25923™) was obtained from American Type Culture Collection (ATCC), and *Streptococcus gordonii* DSM20568, species were obtained from Structural and Functional Glycobiology Unit (UGSF) laboratory collection, Lille, France.

The MIC of plant extracts with antibacterial activity was examined by micro-dilution technique^{18,19} in 96-well microplates. Wells were filled with 0.1 ml of bacteria corresponding to $5 \cdot 10^7$ CFU/mL obtained from an overnight culture grown at 37°C in MHB (Muller Hinton broth). Test wells were finally filled-up with 20 µL of dilutions of extracts while control wells were filled-up with or without 20 µL of methanol used as solvent for the plant extract dilutions. Each analysis was replicated three times²⁰.

Microbial growth was measured with a microplate reader (Clariostar fluorescence plate reader, BMG-Labtek) at 600 nm.

Biofilm inhibition evaluation

S. aureus (ATCC® 25923™) or *M. luteus* (kind gift from Michel Simonet from National Institute of Health and Medical Research (INSERM) U801 France) were grown at 37°C/16h in LB (lysogeny broth) medium and diluted to 10^6 CFU/mL in the same medium added with 10 µg/mL of 4',6'-diamidino-2-phenylindole (DAPI, Sigma). 12-wells cell culture plates (Greiner Bio-One, Black, FB) were inoculated with 1mL of this bacterial suspension then filled-up with 100 µL of dilutions of extracts ranging from 0,1 to 100 µg/ml while control wells were filled-up with or without 100 µL of methanol used as solvent for the plant extract dilutions. Biofilms were incubated at 37°C in CO₂ incubator Heracell™ 150i (Thermo Scientific) during 24h²¹. DAPI display no

Table 2. Phytochemical screening of different plant powder homogenate

Plant name	Alcaloids	Saponins	Tanins	Flavonoids	Steroids	Terpenoids
<i>A. articulata</i>	+	+	+	+	+	-
<i>C. schoenanthus</i>	-	+	+	+	-	+
<i>S. Chudaei</i>	-	+	+	+	-	+

"+" means presence, while "-" means absence of each compound in the plant

fluorescence in solution and no effect towards germ cell. Biofilms were washed four times with Dulbecco's phosphate buffered saline (DPBS) to quantify fluorescence. Surface colonization in each well was evaluated by quantification of DAPI associated fluorescence (excitation 350 nm/emission 460 nm) with Clariostar fluorescence plate reader (BMG-Labtek).

Biofilms Three-dimensional organization

Biofilm three-dimensional organization was examined by confocal laser scanning microscopy (CLSM) and imaged with ZEN 2009 Light Edition program. Biofilm were developed as described just before except that incubation was performed in Permanox Chamber Slide (Nunc® Lab-Tek® Chamber Slide™ system) and after washing, biofilms were fixed (PFA 4%) and mounted with Mowiol® 4-88.

Zeiss confocal microscope (LSM780) with 40x objective 1.3NA Oil Plan-Apochromat DIC was used to observe biofilms. The DAPI die was stimulated using a 405 nm laser diode and the emission was accumulated between 410-500 nm on GaAsP detector.

Architectural analysis was performed using the COMSTAT program²². The image stacks captured for each CSLM analysis were analysed for the four succeeding architectural characteristics: size of the biofilm dissociated by substratum area ($\mu\text{m}^3/\mu\text{m}^2$) indicating total biomass of germ, biofilm average thickness (μm) as well as maximum thickness (μm), roughness coefficient (adimensional) measuring biofilm surface diversity reflecting variations of biofilm thickness, substratum coverage (%) indicating the capability with which germ developed on the surface.

Antioxidant activity

The antioxidant activity of the methanolic extracts was measured paralleling to two methods.

DPPH scavenging radical activity

The capacity to catch 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) by methanolic extracts was determined by the method of Khaled-Khodja²³.

The IC_{50} concentration which inhibits 50% of the DPPH groups present in the mixture was calculated using the graph $I(\%) = f(C)$ where (C) is the concentration of standard/methanolic extract. These same data were also reported in AEAC (Ascorbic acid Equivalent Antioxidant Capacity) according to the method of Bassolé²⁴. Butylated hydroxyanisole and ascorbic acid (BHA) were employed as witness.

β -carotene/ linoleate model system

The rate of antioxidant activity (AA %) was measured according to the method described by Abdolhamid²⁵.

Statistical analysis

All results are expressed as the average \pm SD of three tests. The statistical analysis of the

Table 3. Total phenolic and flavonoid content of plant extracts

Plant/standard	TPC (GAE mg/g)	TFC (CE mg/g)
<i>A. articulata</i>	02,51 \pm 0,06	01,65 \pm 0,04
<i>C. schoenanthus</i>	03,59 \pm 0,10	02,96 \pm 0,07
<i>S. Chudaei</i>	21,98 \pm 0,82	19,27 \pm 0,61

TPC: Total phenolic content (mg GAE/g methanolic extract);
TFC: Total flavonoid content (mg CE/g methanolic extract)

Table 4. Antibacterial activity of methanolic plant extracts against four Gram positive bacteria

Plant	Bacterial species					
	<i>B. subtilis</i>	<i>S. gordonii</i>	<i>S. aureus</i>		<i>M. luteus</i>	
	MIC (mg/ml)	MIC (mg/ml)	MIC (mg/ml)	BIC50 ($\mu\text{g/ml}$)	MIC (mg/ml)	BIC50 ($\mu\text{g/ml}$)
<i>A. articulata</i>	2	1	2	>100	0.1	>10
<i>C. schoenanthus</i>	2	2	0.2	10	0.5	1
<i>S. chudaei</i>	0.5	0.2	0.5	10	0.5	1

MIC: Minimal Inhibitory Concentration; BIC50: Biofilm Inhibition Concentration of extracts able to reduce more than 50% of biofilm cell density

data was carried out by the one-way ANOVA. To complete the statistical analysis multi-comparison Dunnett's test was performed.

RESULTS

The methanolic extracts yields of *A. articulata*, *C. schoenanthus* and *S. chudaei*, expressed in relation to dry weight plant were 6.2 %, 7.3 % and, 22.6%, respectively.

Qualitative phytochemical analysis

Phytochemical tests of *Anabasis articulata* (*Aa*) *Cymbopogon schoenanthus* (*Cs*) and *Salvia Chudaei* (*Sc*) extracts showed the existence of saponins, flavonoids and tannins (Table 2).

Quantitative phytochemical analysis

Total phenol content (TPC) of all extract was measured. Values were expressed as mg gallic acid equivalent/g methanolic extract (GAE mg/g) (Table 3). *Sc* extract has a seven-fold higher TPC value (21.98 ± 0.82 GAE mg/g) as compared to *Cs* extract (3.59 ± 0.10 GAE mg/g) and eight-fold higher as compared to *Aa* extract containing (2.51 ± 0.06 GAE mg/g).

Total flavonoid content (TFC) of all extract was also measured. Results were represented as mg catechin equivalent/g methanolic extract (CE mg/g) (Table 3). The same kind of results were observed since *Sc* extract contains a six-fold higher amount of flavonoid, with (19.27 ± 0.61 CE mg/g) as compared to *Cs* extract (2.96 ± 0.07 CE mg/g) and more than a eleven-fold higher amount as compared to *Aa* extract (1.65 ± 0.04 EC mg/g).

These data suggest that *Sc* display the best activity potential.

Antibacterial activity

Antibacterial activity is also often associated with molecules extracted from plants. Antibacterial activities of *Sc*, *Cs* and *Aa* extracts on four Gram-positive bacteria species and four Gram-negative bacteria species was first tested by the disk diffusion method. The four Gram-positive bacteria species examined were sensitive to all extracts. Unfortunately, no antibacterial activity was observed against Gram-negative bacteria (data not shown). Antibacterial inhibition of growth was then analyzed in liquid condition for the Gram-positive bacteria species in medium containing various plant extract concentration allowing calculation of minimal inhibition concentration (MIC). All the plant extracts displayed inhibition of bacterial growth with a MIC classifying from 0.1 to 2.0 mg/mL (Table 4). Thus each methanolic extract contained active antibacterial compounds of the plants. *Sc* extract displayed the best MIC with 0.2 to 0.5 mg/mL against the four bacterial species, while *Aa* presented the lower (2 mg/mL for three of the four tested germs). MIC of each plant extract reflects directly the total concentration of phenolic compounds and particularly flavonoids (Table 3), as expected for plant biological compound containing such families of molecules.

Antibiofilm activity

Antibiofilm activity of each extract was tested against two Gram positive bacteria,

Table 5. COMSTAT analysis of CSLM images of biofilm of *M. luteus* and *S. aureus* in presence of 10 µg/mL of *Sc* or of *Cs* extracts

	Total biomass (µm ³ /µm ²)	Roughness coefficient	Maximum thickness (µm)	Average thickness (µm)	Surface coverage (%)
<i>M. luteus</i>	4.21 ± 0.89	1.05 ± 0.39	35.15 ± 5.26	10.57 ± 2.52	19.33 ± 5.76
<i>Sc</i> 10 (µg/mL)	0.06 ± 0.05	1.93 ± 0.07	5.21 ± 0.43	0.06 ± 0.06	2.90 ± 2.83
<i>Cs</i> 10 (µg/mL)	0.07 ± 0.12	1.92 ± 0.14	5.79 ± 3.08	0.06 ± 0.10	0.10 ± 0.09
<i>S. aureus</i>	2.81 ± 0.68	0.59 ± 0.04	14.90 ± 3.05	4.24 ± 1.11	59.01 ± 7.82
<i>Sc</i> 10 (µg/mL)	0.52 ± 0.46	1.65 ± 0.25	9.84 ± 1.75	0.59 ± 0.48	13.69 ± 11.06
<i>Cs</i> 10 (µg/mL)	0.19 ± 0.03	1.85 ± 0.04	11.54 ± 1.57	0.25 ± 0.04	5.91 ± 1.68

Sc: *Salvia chudaei* extract, *Cs*: *Cymbopogon schoenanthus* extract. Values represent means ± SD.

M. luteus and the pathogen *S. aureus*. Biofilm formation occurred on abiotic surface during 24h in LB medium with or without various concentration of plant extract. Biofilm development was assessed by fluorescent quantization of the DAPI defined bacterial biomass deposited on the surface (Table 4) and by COMSTAT analysis of confocal scanning laser microscopy (CSLM) images (Table 5). Plant extract concentrations inhibiting bacterial growth (i.e. around the MIC) will also prevent biofilm formation. Thus only subinhibitory concentrations (below 10 µg/mL for *M. luteus* and below 100 µg/mL for *S. aureus*) allowing bacterial growth were tested. The biofilm elimination concentration of extracts decreasing 50% of biofilm cell density (BIC₅₀) was measured (Table 4). *Cs* and *Sc* extracts displayed antibiofilm activity at concentration as low as 1 µg/mL for *M. luteus* and 10 µg/mL for *S. aureus*, while extracts of *Aa* display no antibiofilm activity at all the concentrations tested for *S. aureus* and for *M. luteus* (Table 4).

Reduction of biofilm formation can result from lower bacterial density but may also reflect disorganization of the overall structure. Thus,

we decided to analyze the three-dimensional configuration of the biofilm elaborated with *M. luteus* and *S. aureus* in the existence of 10 µg/mL of *Cs* and *Sc* extracts. COMSTAT analysis confirmed the efficiency of both plant extracts to inhibit biofilm formation. Image analyses showed that *M. luteus* biofilm and *S. aureus* biofilm grown in presence of 10 µg/mL of each plant extract contained less biomass (Table 5), with a large decrease (between 81.5 and 98.6%) of the biomass as compared to the controls. In addition, biofilms developed in presence of plant extracts displayed a higher roughness coefficient (mostly twice higher, Table 5) than control indicating that biofilm structure was less homogeneous. The biofilm average and maximum thickness were also strongly reduced for both *M. luteus* and *S. aureus* when grown in presence of plant extracts as compared to the control (Table 5). Plant extracts led finally to the development of a thin and sparse biofilm containing only 1.4% to 18.5% of the biomass of the untreated samples. Finally, biofilms grown in the presence of both plant extracts displayed a lower surface coverage than the

Table 6. Scavenging power of plant extracts

Plant/standard	DPPH IC ₅₀ (mg/ml)	AEAC (mg AA/100 g)	β-carotene (AA%)
<i>A. articulata</i>	6.16	2435.06	8.47 ± 0.77
<i>C. schoenanthus</i>	4.36	3440.37	10.13 ± 1.15
<i>S. Chudaei</i>	1.94	7731.96	15.61 ± 1.29
Ascorbic Acid	0.15	ND	40.98 ± 2.51
Gallic Acid	NA	NA	47.07 ± 2.73
BHA	0.16	93750	37.69 ± 2.62

IC50: Inhibition Concentration 50%; AEAC: Ascorbic acid Equivalent Antioxidant Capacity (mg AA/100 g); NA: Not Applicable; ND: Not Done; Values are average of three independent replicates ± SD.

Table 7. Correlation matrix between phenolic compounds and antioxidant activities of methanolic plant extracts

Tests	Correlation coefficient (r)			
	TPC	TFC	IC ₅₀	AA
IC ₅₀	0.91	0.95	1	0.94
AA	0.97	0.98	0.93	1

TPC: Total phenolic content (mg GAE/g), TF: Total flavonoid content (mg CE/g); IC50: Inhibition Concentration 50%; AA: Antioxidant Activity.

control (Table 5) and showed an increased number of holes in overall structure. Consequently, while growth of *S. aureus* and *M. luteus* in presence of subinhibitory concentrations of *Sc* and *Cs* plant extracts is unaffected, their ability to build a biofilm is severely reduced. Taken together, these data demonstrate that both plant extracts can reduce the surface colonization by bacteria and prevent biofilm development.

Antioxidant activity

The scavenging power of the three methanolic extracts was examined with two free

radical scavenging tests. *Aa*, *Cs* and *Sc* methanolic extracts activities were first evaluated against scavenging DPPH radical (Table 6). The results showed that each extract has a free radical scavenging power. As expected, *Sc* extract was the best free radical inhibitor with a calculated IC_{50} of 1.94 mg/mL succeeded by *Cs* extract with an IC_{50} of 4.36 mg/mL and finally *Aa* extract with an IC_{50} of 6.16 mg/mL compared with ascorbic acid and BHA having IC_{50} of 0.15 and 0.16 mg/mL, respectively. The methanolic extracts showed little antioxidant activity with IC_{50} values between 10 and 60 times higher than some flavonoids, renowned DPPH free radical scavengers.

For each extract, DPPH radical scavenging power was directly correlated with total phenolic and flavonoid content measured ($R^2=0.91$) and ($R^2=0.95$), respectively (Table 7).

To confirm these results, a second free radical scavenging test was used with β -carotene / linoleate model system (Table 6). After 2 hours, the elimination percentage of bleaching β -carotene of *Cs*, *Sc* and *Aa* extracts were between $08.47 \pm 0.77\%$, $10.13 \pm 1.15\%$ and $15.61 \pm 1.29\%$ against $47.07 \pm 2.73\%$, $40.98 \pm 2.51\%$ and $37.69 \pm 2.62\%$, for standards gallic acid, ascorbic acid and BHA, respectively. The whitening inhibition hierarchy of the extracts (i. e. *Sc* extract > *Cs* extract > *Aa* extract) was correlated with total phenolic and flavonoid concentration contained in each extract ($R^2=0.97$) and ($R^2=0.98$), respectively (Table 7).

DISCUSSION

Most plants contain numerous phytochemicals compounds having biological activity with medicinal properties, like antibacterial and/or antioxidant activities. For some of them, our ability to extract these phytochemicals within essential oils allowed their use in alternative medicine and subsequently their detailed characterization²⁶. In this work, we demonstrated the antibacterial, antibiofilm and antioxidant properties of some local medicinal plants from the central desert (Tassili n'ajjer) that are frequently used in local traditional medicine, thus demonstrating their potential for use in human therapy on a larger scale.

Phytochemical analysis

Previous study on *Cs* has endeavored to validate its traditional use. It has been reported

the isolation of limonene, β -phellandrene, δ -terpinene and α -terpineol in essential oil extract. These compounds were found to possess *in vitro*, significant antiacetylcholinesterase, antimicrobial and antioxidant properties^{11,27}. Phytochemical investigations of *Aa* have shown the existence of several constituents, like phenolic compounds⁸. Several studies have highlighted the biological properties of phytochemicals constituents extracted from various plants like antibacterial, antifungal and antioxidant activity²⁸. The amount of extractable secondary metabolites, such as TPC, often present a great variability in the literature, depending on the environmental conditions of plant growth (salinity, temperature...), biological factors (mainly plant organ and genotype) as well as extraction method (namely the type of solvent used)²⁹.

Antibacterial activity

Many plant extracts are known for their antibacterial properties, particularly effective against pathogenic species³⁰. This overall antibacterial activity is usually correlated with the synthesis of secondary metabolites, especially phenolic compounds, exhibiting this antibacterial effect³⁰. All Methanolic extracts of *Aa*, *Cs* and *Sc* display antibacterial effect on four Gram-positive germs species while no effect was detected on Gram negative species As observed for the antioxidative activity, the antibacterial activity is also the best for *Sc* extract, but the two other extracts, differing largely in TPC, are quite equivalent for antibacterial activity even if *Cs* is particularly effective against *S. aureus* while *Aa* is the more efficient against *M. luteus*. For the two other plant extracts, explanation of one specific antibacterial activity has to be dig deeper by complete characterization by Gas Chromatography-Mass Spectrometry Analysis.

It is difficult to explain why all plant extracts show no antibacterial effect on Gram negative germs. Despite the large difference in envelope composition between Gram negative and Gram positive germs, it is now appropriately known that antibacterial effect of phenolic compounds is mostly due to membrane disruption with, to a less extend, intracellular enzymes activity inhibition (topoisomerases, kinases...)³⁰. Before the present article no other work described antibacterial activity for plants extracts of *Aa* and

Sc and the only antibacterial activity reported in the literature concerns *Cs* acetone/water extracts demonstrating *Streptococci* inhibition of growth at concentration ranging from 4 to 8 mg/mL⁸ which is in agreement with the *Cs* antibacterial activity (1 mg/mL) reported here against *S. gordonii*. Indeed, the medicinal potential of each plant extract is the consequence of the extraction of the phenolic compounds by simple methanolic extraction.

Antibiofilm activity.

Skin infection caused by bacteria such as *S. aureus*, results mostly from penetration of wounded tissue by bacteria already present at skin surface. The persistence of these bacteria is the result of its ability to colonize skin surface³¹ and develop biofilm structure on any kind of biotic or abiotic surface (catheter, implants, prosthesis, etc...)³². When established in a biofilm structure, bacteria are more protected against host defenses, antibiotics and most of the known antibacterial compounds used in medicine. It is of great importance in therapeutic strategies to prevent biofilm formation, particularly the initial colonization events.

In the literature, antibiofilm activity of plant extracts is mostly measured using concentration above or equal to the MIC mostly because it is believed that biofilm development allows stronger protection against antibacterial compounds. In the present work, subinhibitory concentrations of each plant extracts were used to prevent biofilm development of two bacterial species usually associated with human skin, the non-pathogen *M. luteus* and the pathogen *S. aureus*. Interestingly, the biofilm inhibition concentration of *Cs* and *Sc* plant extracts able to reduce more than 50% of the biofilm cell density (BIC_{50}) display values as low as 2% to 10% of the MIC calculated, while no inhibition of biofilm development was observed for *Aa* plant extract at the concentration used. At subinhibitory concentrations, while they are unable to prevent bacterial growth in culture medium, methanolic plant extracts exert a strong inhibitory effect on the bacterial ability to form biofilms.

COMSTAT analyses of CSLM images of biofilms with plant extracts show that the biofilm architecture is strongly reduced. This reduction of biofilm structure is probably the result, in a large part, of reduction of the surface colonization

determinant step required for normal biofilm formation. It is largely described in the literature that surface colonization by bacteria depends on the presence of a conditioning film of organic compounds coming from the medium and covering the immersed surface attracting bacteria and making this local environment suitable to biofilm development. One can imagine in our experiments that phenolic compounds of the plant extract displaying antibacterial activity may have adsorb on the surface generating a non-suitable surface to colonize for the bacteria. Plant extracts leads finally to the development of a thin and sparse biofilm containing only 1.4% to 18.5% of the biomass of the untreated samples. Since methanol can quickly evaporate at temperature above 30°C, methanolic extract containing phenolic compounds might be of interest for surface treatment against bacterial colonization.

Antioxidant activity

Over phenolic compounds, flavonoids have the best antioxidant and are the most useful in human health¹¹. Recently, research of new sources of natural antioxidants has attracted increasing attention of the scientific community to these compounds since they are inexpensive and safe³³. Our systematic research exposed that the medicinal plants selected *Aa*, *Cs* and *Sc* are rich in polyphenols. A positive relationship was found between the TPC and diverse scavenging actions evaluated *in vitro*, signifying that polyphenols could be the principal contributors to the elimination of free radicals and to the capacity to reduce extracts²³. The three plant extracts tested displayed an important antioxidant activity as demonstrated by free radical scavenging and inhibition of lipid peroxidation experiments. These results are directly correlated with the TPC and TFC quantization of each plant extract ascertaining a direct rapport between Scavenging power and concentration of polyphenols inside of plants used in traditional medicine. These results indicated that each methanolic extract obtained from the three plants during this work has an antioxidant activity in proportions corresponding to what has been reported in the literature for *Aa*¹¹ and *Cs*^{12,34} respectively.

CONCLUSION

The high antibacterial and antibiofilm

activity associated with the high antioxidant activity of the plant extracts justifies their use for medicinal purposes and suggests that they can be used as a potential source of metabolites, nutritional supplement and alternative preservatives of food or in the pharmaceutical industry.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors have made direct contribution on idea creation, research work and editing of the manuscript.

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ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and are available with the corresponding author.

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