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Bio-guided anti-cariogenic and phytochemical valorization of *Guiera senegalensis* and *Pseudocedrela kotschyi* stem extracts

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The objective of the present study is to carry out bio-guided phytochemical investigation of *Guiera senegalensis* and *Pseudocedrela kotschyi* stem extracts. The two plants are used as toothpicks for oral hygiene. The inhibition test revealed a bacteriostatic effect of hexane extract PK1 of *P. kotschyi* against *Streptococcus mutans* ATCC 25175 and *Streptococcus salivarius* ATCC 20560, two bacterial cariogenic strains, with a decrease in the number of bacterial colonies of 1 Log/control. The aqueous extract GS5 obtained from the stems of *G. senegalensis* is bactericidal, with total inhibition of *S. salivarius* ATCC 20560. The antimicrobial effect of the stem extracts from the two plants studied varies according to the plant species and the type of bacterial strain. The phytocompounds 8-Hydroxy-6,7-dimethoxy-3-methylisochroman-4-one, 1-(4-hydroxy-3-methoxyphe-nyl) propane-1,2-dione and (4E, 15E) -Nonadeca-4,15- dien-10-one were isolated, respectively from GS5A extracts of *G. senegalensis* and PK1 of *P. kotschyi* by normal column chromatography.

Key words: *Guiera senegalensis*, *Pseudocedrela kotschyi*, stem extracts, *Streptococcus mutans*, *Streptococcus salivarius*, spectroscopic characterization.

INTRODUCTION

Plants have always been an indispensable source for human beings as regards health and food, especially in sub-Saharan Africa. In general, plant stems are used in oral care. Such plant stems include *Guiera senegalensis* (Combretaceae) and *Pseudocedrela kotschyi* (Meliaceae), which are commonly used as toothbrush by the populations. *G. senegalensis* and *P. kotschyi* are often used in sub-Saharan regions to cure various diseases (Kerharo et al., 1948; Faye et al., 1980; Sanogo et al., 1998; Ancolio et al., 2002; Adamu et al., 2005; Alex et al., 2005; Hadissa and Deschamps, 2006; Ahua et al., 2007; Dieye et al., 2008; Somboro et al., 2011; Ohemu et al., 2014; Traore et al., 2014; Diarra et al., 2015; Sonibare et al., 2015; Kantati et al., 2016; Kpodar et al., 2016).

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The African continent is endowed with an impressive floristic biodiversity, with a large variety of plants for food and therapeutic needs. This natural floristic richness is only slightly valued chemically and pharmacologically. The bacterial resistance towards synthetic antibiotics is one of the major concerns of the medical research today (Ouelhadj et al., 2017). Yet medicinal plants are interesting alternatives to explore alongside synthetic drugs. That is why, the objective of this study consists of a phytochemical investigation of *G. senegalensis* and *P. kotschyi* stem extracts by a bio-guided anti-cariogenic way to find out more new and effective antibacterials.

MATERIALS AND METHODS

Plant

The plant parts (leaves, stems) were harvested in July 2016 from Longorola in Sikasso region (11° 11 ' 59 " North, 7° 05 ' 49" West) in Southern Mali. The leaves of each plant were identified at Abidjan National Floral Center (CNF) according to herbariums N°^{s} 7569 and 8664, respectively for *G. senegalensis* and *P. kotschyi*. The *G. senegalensis* (GS) and *P. kotschyi* (PK) stems used for phytochemical and antibacterial analyses were dried under permanent air conditioning (16°C) for 10 days. Then, they were reduced to powders with an electric grinder to obtain the analysis extracts.

Pathogenic bacteria

Two international cariogenic bacterial strains from the American type culture collection, namely *Streptococcus mutans* (*S. mutans* ATCC 25175) and *Streptococcus salivarius* (*S. salivarius* ATCC 20560) were used for antibacterial tests.

Preparation of extracts

The powder maceration of each plant (200 g) in hexane (600 mL) under agitation at ambient temperature during 48 h, gave dry hexanic extracts from *G. senegalensis* (GS1) and *P. kotschyi* (PK1) after filtration and solvent elimination, using a rotary evaporator (Büchi R-210 Rotavapor TM).

The marc (10 g) from GS and PK after delipidation with hexane, were brought into contact in different solvents at room temperature for 48 h to provide the dry extracts: GS2, PK2 (acetone, 50 mL); GS3, PK3 (dichloromethane-methanol, 50:50 mL); GS4, PK4 (methanol-water, 70:30 mL); GS5, PK5 (water, 50 mL) after filtration and concentration with the rotary evaporator.

Antibacterial power evaluation

The antibacterial potency of the different extracts GS1-GS5 and PK1-PK5 were evaluated according to Yew (2015). The bacterial strains to be tested were cultured for 24 h in culture media specific for streptococci. The initial absorbance (650 nm) was first measured for each tube. Then, 300 µL of the overnight culture was added to each tube, and the mixture was adjusted to an absorbance of 1.0 +/- 0.05 at the initial wavelength. After 24 h of culture, the absorbance was determined for each tube to estimate bacterial growth. A series of dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} for the seeded tubes were performed. An aliquot of 50 µL of the dilutions obtained were subsequently spread on the specific agar media. Colony counting was performed between 24 and 72 h after seeding, at which time the averages of the triplicates of each series were calculated. The results obtained were converted into Colony Forming Units per mL of medium (CFU/mL) and expressed in logarithmic decimals (CFU Log/mL).

Aglyconic extracts preparation

The aglyconic extracts were obtained from plant extracts according to Alilou et al. (2014) method, and it showed a better antibacterial profile.

GS5 and PK5 (15 g) were put in touch with HCl (328 mL, 2N) in a flask. The reactional mass was refluxed for 150 min. After filtration and cooling at room temperature, the hydrolyzate was treated with diethyl ether (3 × 100 mL). After decantation, the organic phase was washed with water (3 × 100 mL), then recovered and dried on anhydrous $MgSO_4$ for 60 min. After filtration on Whatman paper and elimination of the solvent with a rotary evaporator to dryness (40°C), GS5A and PK5A aglyconic extracts were provided.

Secondary phytoconstituants fractionation, purification and separation

PK1, GS5A and PK5A extracts were retained for chromatographic fractionation and purification with regard to their better antibacterial profile. Isolation and purification were achieved through an elution series on silica gel in the normal phase (Figures 1 and 2).

Isolated phytoconstituants spectroscopic characterization

Structural elucidation of secondary metabolites has been performed on purified native phytoconstituants. The ^{1}H and ^{13}C NMR spectra were obtained on BRÜKER Avance 400 MHz. IR spectra were recorded on Perkin Elmer FT-IR 2000 between 4000 and 500 cm^{-1}.

RESULTS AND DISCUSSION

Extraction yields

The extraction yield of each plant extract is shown in Table 1.

In Table 1, maceration in water supplied the best extracts yields; which seems to accredit the recurring use of this extraction process in endogenous phytotherapy. However, the latter also shows that the yield may differ from a botanical species in another one. The aglyconic extracts yields from *G. senegalensis* and *P. kotschyi* are 0.78 and 0.67%, respectively.

Plant extracts antibacterial profile

The antibacterial profile of ten raw extracts (GS1-GS5, PK1-PK5) has been estimated towards the two pathogenic bacterial strains directly involved in tooth decay. The results are shown in Tables 2 and 3.

Extracts effect was appreciated according to the

Table 1. Extraction yields.

Extract	*G. senegalensis*					*P. kotschyi*				
	GS1	**GS2**	**GS3**	**GS4**	**GS5**	**PK1**	**PK2**	**PK3**	**PK4**	**PK5**
Yield (%)	1.34	1.79	1.58	0.96	**3.89**	0.53	3.1	6.72	4.4	**8.21**

GS1, PK1: Hexanic extracts; GS2, PK2: acetone extracts; GS3, PK3: dichloromethane-methanol extracts; GS4, PK4: hydromethanolic extracts; GS5, PK5: aqueous extracts.

Table 2. Antibacterial profile of plant extracts (in CFU Log/mL) by inhibition of *S. mutans* ATCC 25175 growth.

Extract	GS1	GS2	GS3	GS4	GS5	PK1	PK2	PK3	PK4	PK5
Average triplicate (Log CFU/mL) (0.1 g/L)	8.56	8.60	8.43	8.75	8.74	8.22	8.73	8.69	8.71	8.56
Average triplicate (Log CFU/mL) (0.2 g/L)	8.51	8.62	8.45	8.85	8.62	7.92	8.48	8.56	8.85	8.38
Average triplicate (Log CFU/mL) (0.5 g/L)	8.38	8.41	8.48	9.03	-	7.64	8.26	8.46	8.58	8.58
Witnesses: Average triplicate (CFU Log/mL)	8.79	8.62	8.33	8.64	8.71	8.80	8.76	8.40	8.86	8.58

Table 3. Antibacterial profile of plant extracts (in log CFU/mL) by inhibition of *S. salivarius* ATCC 20560 growth.

Extract	GS1	GS2	GS3	GS4	GS5	PK1	PK2	PK3	PK4	PK5
Average triplicate (Log CFU/mL) (0.1 g/L)	6.87	7.52	6.66	7.14	5.78	6.67	7.04	7.30	6.86	6.38
Average triplicate (Log CFU/mL) (0.2 g/L)	6.90	7.35	6.79	6.91	0	6.28	6.83	7.42	6.73	6.97
Average triplicate (Log CFU/mL) (0.5 g/L)	6.20	7.06	7.06	6.64	-	6.05	6.07	6.81	6.34	6.41
Witnesses: Average triplicate (CFU Log/mL)	6.99	7.74	6.3	7.22	7.15	6.81	6.92	6.9	6.9	6.26

classification of Yew (2015). Indeed, an extract is bacteriostatic with regard to a decrease in the number of 1 Log/control colonies. On the other hand, it is bactericidal if the total inhibition of bacterial culture is observed.

The results of the antibacterial tests reveal that, GS1, GS2, GS3, GS4, PK2, PK3, PK4 and PK5 do not exhibit any significant inhibition effect on the growth of *S. mutans* and *S. salivarius* at the tested concentrations. PK1 is bacteriostatic towards *S. mutans* (Table 2). Indeed, this extract inhibits the growth of this bacterial strain by stopping the number of colonies of more than 1 Log/control at concentrations of 0.2 and 0.5 g/L. GS5 inhibits the growth of *S. salivarius* because no bacterial colony is found on the agar in its presence. GS5 is therefore bactericidal at 0.2 g/L (Table 3).

On the other hand, the latter showed no inhibitive activity against *S. mutans* (Table 2). GS1 and PK1 extracts caused a decrease in the number of *S. salivarius* colonies from about 0.75 Log/control to 0.5 g/L. This value close to 1 Log/control could be improved either by increasing the concentration of these extracts, or by isolating the active phytocompounds which they contain.

Phytochemical screening of *G. senegalensis* and *P. kotschyi* stems has already been performed (Kadja, 2014). The author reports that these organs contain phytophenols (coumarins, flavonoids, etc.) and terpenes among other identified active second principles. Besides, the beneficial effects of terpenes and polyphenols on oral health are known (Bitty, 1982; Kadja et al., 2011; Atsain et al., 2016). Thus, the antibacterial activities exhibited by hexanic extracts GS1 and PK1 suggest a synergic action due to the existence of terpenic phytoconstituents. As for the aqueous extracts of GS5 and PK5, their manifest antibacterial profile seems to have a correlation with the aforementioned extracts of water-soluble secondary metabolites with antibacterial potential.

Isolated phytocompound structural elucidation

PK1, GS5A and PK5A extracts were chromatographically fractionated on a normal phase silica gel column. Fractionation and purification of GS5A led to the isolation of phytoconstituents A and B, with yields of 43.30 and 2.62%, respectively, with regard to the mass fractions (Figure 1). As for PK1, its fractionation and purification allowed the isolation of phytocompound C, with a yield of 4.92% (Figure 2). The interpretation of IR spectra was done according to the method of Brown et al. (1992) and Robert et al. (2005).

Structure of isolated phytocompound A from GS5A extract

Analysis of ^{13}C NMR, JMOD and DEPT 135 spectra of

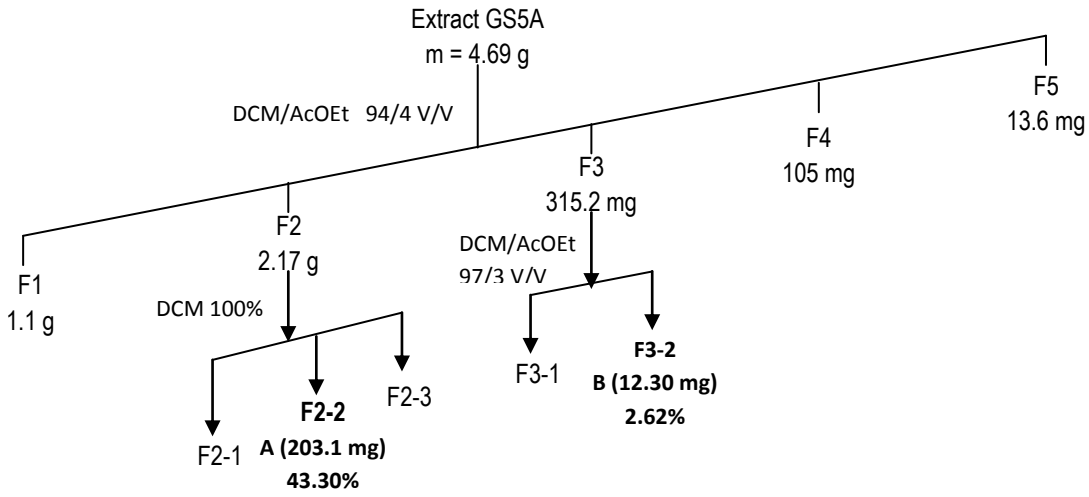

Figure 1. Fractionation and purification of GS5A. P: Petroleum ether, DCM: dichloromethane; AcOEt: Ethyl acetate. Column: length 45 cm, diameter 4 cm, height of the layer of normal silica gel 15 cm.

Table 4. NMR spectral data of compound A.

N°C	Type	^{13}C δ (ppm)	HSQC δ (m, J Hz)	HMBC δ (ppm)	COSY δ (m, J Hz)
1	C	190.59	-	-	-
2	C	201.54	-	-	-
3	CH_3	26.98	2.58 (s)	190.59; 201.54	-
1’	CH	127.19	7.60 (dd; 8.3; 1.9)	152.57	6.98 (d; 8.3)
2’	CH	111.35	7.58 (d ; 1.9)	127.19; 190.59; 147.25; 152.59	-
3’	C	147.25	-	-	-
4’	C	152.57	-	-	-
5’	CH	114.69	6.98 (d; 8.3)	124.68; 147.25; 152.57	7.60 (dd; 8.3; 1.9)
6’	C	124.68	-	-	-
7’	CH_3	56.40	3.96 (s)	147.25	-

compound A (Table 4) reveals the presence of 5 C quaternary sp^2 hybridization, of which 2 C=O at 190.59 and 201.54 ppm; 2 C primary at 2.98 and 56.48 ppm; C3 secondary ethylenic 111.35, 114.49 and 127.19 ppm. Thus, 10 C is the carbon skeleton of the compound. The 1 H NMR spectrum ($CDCl_3$) shows the presence of 3 H (CH_3O-) at 3.96 ppm; 3 H aromatics at 6.97, 7.57 and 7.61 ppm; 1 H at 6.23 ppm; and 3 H (CH 3) at δ 2.51 ppm. The 2D spectrum, COSY, shows the correlation of proton signals resonating at 7.61 and 6.97 ppm; confirmed by the HSQC and HMBC spectra in the sense that these 2H are carried by adjacent carbons whose signals resonate at 114.69 and 127.19 ppm. IR spectrum analysis shows 3408 cm^{-1} absorption bands (C4'-OH, valence vibration); 1658 cm^{-1} (C1=O, deformation vibration), 1710 cm^{-1} (C2=O, valence vibration), 3080 cm^{-1} (aromatic C-H, valence vibration), 1294 cm^{-1} (aromatic C-H, deformation vibration), 1588 and 1513 cm^{-1} (cyclic C=C, valence vibrations), 1267 cm^{-1} (asymmetric C-O-C, valence vibration), 2940 and 2840 cm^{-1} (aliphatic C-H). All the spectral data allowed identification of the phytocompound A (Figure 3).

Structure of isolated phytocompound B from GS5A extract

The ^{1}H NMR spectrum of compound B ($CDCl_3$) shows the presence of 14 H including 1 H aromatic at 7.34 ppm; 6 H of 2 (CH_3O) at 3.91 and 3.94 ppm; 3 H (CH_3) at 1.50 ppm; 2 H appearing at 4.77 and 5.06 ppm as doublets each; 1 H at 4.20 ppm as a quartet; 1 H (OH) at 6.20 ppm wide signal. 13C NMR, DEPT 135 and JMOD show the presence of 12 C including 6 C quaternary (C1; C2; C3; C4; C5; C6), 1 aromatic CH (C7) resonant at 103.96 ppm; 2 CH_3 methoxy (C10; C11) appearing at 56.78 and 60.89 ppm; 1 CH_3 at 16.11 ppm; 1 C secondary (C9) at 62.48 ppm. The HSQC, HMBC and COSY data is shown

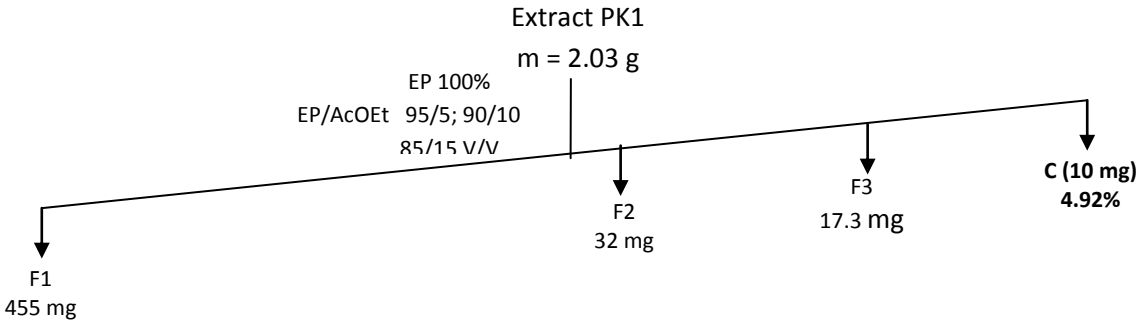

Figure 2. Fractionation and purification of PK1. EP: Petroleum ether, DCM: dichloromethane, AcOEt: ethyl acetate. Column: length 30 cm, diameter 3 cm, height of the layer of normal silica gel 15 cm.

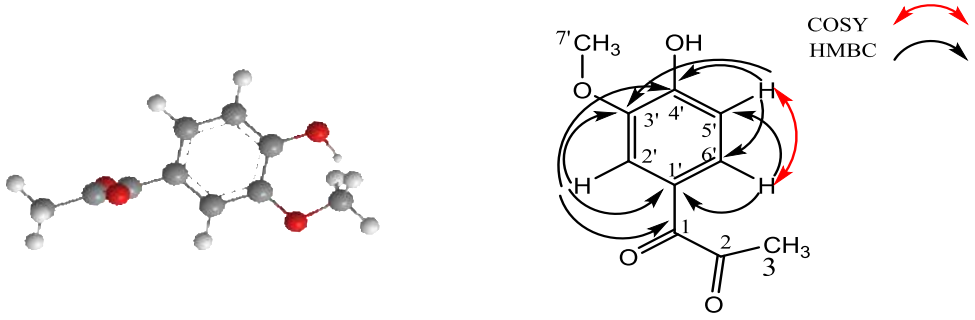

Figure 3. 1-(4'-hydroxy-3'-methoxyphenyl) propane-1, 2-dione with different COSY and HMBC correlations

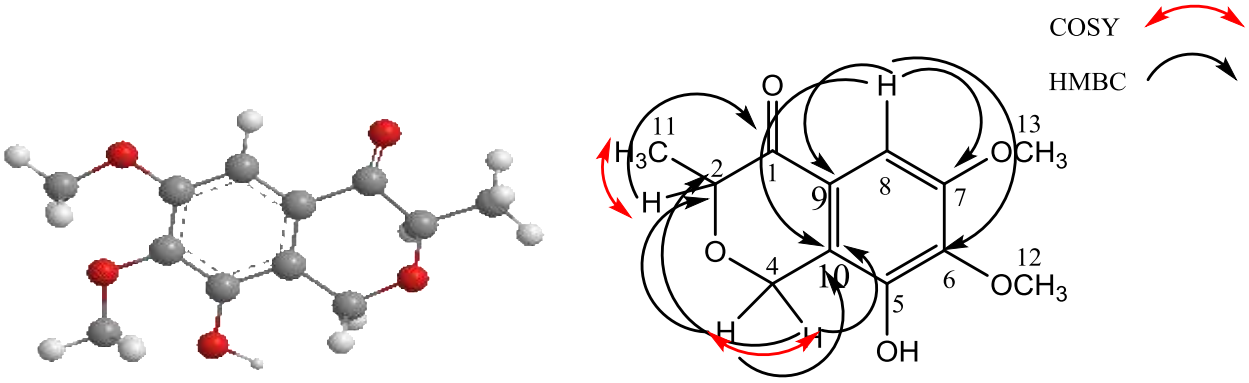

Figure 4. 5-Hydroxy-6,7-dimethoxy-2-methylisochroman-1-one with different COSY and HMBC correlations.

in Table 5. The IR spectrum reveals the presence of an absorption band at 3504 cm^{-1} (C5-OH, valence vibration in dilute solution); 3054 cm^{-1} (aromatic C-H, valence vibration); 1683 cm^{-1} (C1=O, valence vibration); 1016 cm^{-1} (C2-O-C4, valence vibration); and 2986.74 cm^{-1} (aromatic C-H, deformation vibration). All the spectral data allowed identification of the phytocompound B (Figure 4).

Structure of isolated phytocompound C from PK1 extract

NMR analysis shows that compound C has molecular symmetry. The ^{13}C NMR and HMBC correlation spectra revealed the presence of 2 primary C which resound in 14.08 ppm; 16 secondary C among which 4 C equivalent type C=C (C4=C5, C15=C16) appearing at 128.07 and

Table 5. NMR spectral data of compound B.

N°C	Type	^{13}C δ (ppm)	HSQC δ (m, J Hz)	HMBC δ (ppm)	COSY δ (ppm)
1	C	195.21	-	-	-
2	C	78.06	4.20 (q; 6.7)	195.21	1.50
4	CH_2	62.85	5.06 (d; 15.7)	78.05; 121.64; 141.73; 195.21	4.77
			4.77 (d; 14.4)	78.05; 121.63; 195.21	5.06
5	C	144.10	-	-	-
6	C	141.73	-	-	-
7	C	147.54	-	-	-
8	CH	103.96	7.30(s)	121.63; 144.10; 147.54; 195.21	
9	C	130.51	-	-	-
10	C	121.63	-	-	-
11	CH_3	16.11	1.50 (s)	78.05; 195.21	4.20
12	CH_3	56.78	3.94 (s)	147.54	-
13	CH_3	60.89	3.91 (s)	141.73	-

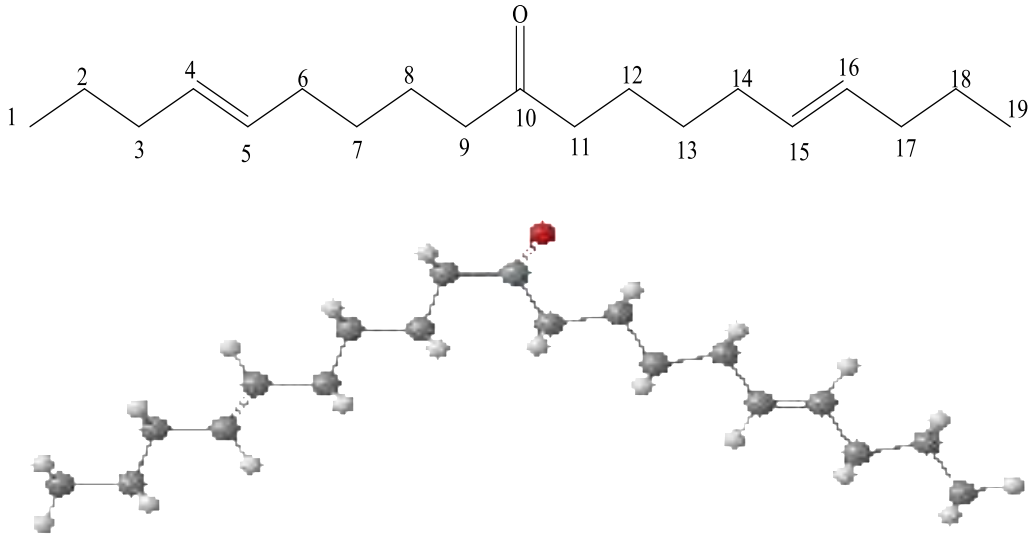

Figure 5. (E, E) -Nonadeca-4,15-dien-10-one.

130.03 ppm, and 12 C (CH_2) equivalents (C2-C18, C3-C17; C6-C11, C7-C13, C8-C12 and C9-C11), resounding at 29.71, respectively; 27.19, 22, 70, 25.63, 24.67, and 34.06 ppm; 1 quaternary C in 180 ppm. ^{1}H NMR spectra, HSQC direct correlations and COSY correlations allowed to assign the different values of protons and carbons (Table 6). Analysis of the IR spectrum showed an absorption band at 3054 cm^{-1} (C-H asymmetric, valence vibration in CH=CH); 1680 cm^{-1} (C=C, E configuration, valence vibration); 1709 cm^{-1} (C=O, valence vibration); and 738 cm^{-1} (C-H, deformation vibration). The spectral data set confirm the molecular structure of phytocompound C (Figure 5).

Conclusion

In this work, valorization of the extracts of stems for *G. senegalensis* and *P. kotschyi* has been undertaken, for two plant species used by the populations in sub-Saharan Africa used like toothbrush for the maintenance of oral health. Biologically, the stem extracts of these plants have exhibited inhibitory effects on both tested cariogenic bacterial strains. These antibacterial effects were owed to the synergic combination of the active secondary metabolites, which they contain. On the one hand, these results support the first intention, the utility of popular use of both plants as toothpicks; on the other

Table 6. NMR spectral data of compound C.

N°C	Type	^{13}C δ (ppm)	HSQC δ (m, J Hz)	HMBC δ (ppm)	COSY δ (m, J Hz)
1=19	CH_3	14.13	0.84 (m)	22.61; 31.90	1.25
2=18	CH_2	29.71	1.25 (t, 7.1 Hz)	-	-
3=17	CH_2	27.19	2,00 (d, 4.6 Hz)	127.89; 128.07; 130.01, 130.19	2.73 (t, 6.5 Hz)
4=16	C	130.03	5.30 (s)	25.60	-
5=15	C	128.07	5.30 (s)	25.60	-
6=14	CH_2	22.70	1.25 (t, 7.1 Hz)	-	-
7=13	CH_2	25.63	2.73 (t, 6.5 Hz)	127.89; 128.07; 130.01, 130.19	2,00 (d, 4.6 Hz)
8=12	CH_2	24.67	1.59 (dd, 14; 6.9 Hz)	29.14; 34.06; 180.01	2.30 (t, 7.5 Hz)
9=11	CH_2	34.06	2.30 (t, 7.5 Hz)	24.66 ; 29.14 ; 34.06; 180.01	1.59 (dd, 14; 6.9 Hz)
10	C	180.01	-	-	-

hand, it recommends the possibility of the use of these plants in the prevention of dental caries. In the phytochemical plan, three isolated phytoconstituants have been characterized. These are 1-(4'-Hydroxy-3'-methoxyphenyl) propane-1, 2-dione, 5-hydroxy-6, 7-dimethoxy-2-methylisochroman-1-one and (E, E)-nonadeca-4, 15-dien-10-one. The study of their anticariogenic potential is in progress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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