Full Length Research Paper

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-methoxyphene-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 (Ouelhad 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° of each plant extractation, purification and “serial strains directly involved in tooth decay. The results obtained were converted into Colony Forming Units per mL of medium (CFU/mL) and expressed in logarithmic decimale (CFU Log/mL).

Aglyconic extracts preparation

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

Secondary phytoconstituents 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 phytoconstituents spectroscopic characterization

Structural elucidation of secondary metabolites has been performed on purified native phytoconstituents. 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
ions of 0.2 and 0.5 g/L.

...ening of number of colonies of more than 1...hyd polyphenols on oral health are known (Bitty; 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 phyto compound 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 phyto compound 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 phyto compound A from GS5A extract**

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

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**Table 1.** Extraction yields.

<table>
<thead>
<tr>
<th>Extract</th>
<th><strong>G. senegalensis</strong></th>
<th><strong>P. kotschyi</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GS1</td>
<td>GS2</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>1.34</td>
<td>1.79</td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Extract</th>
<th>GS1</th>
<th>GS2</th>
<th>GS3</th>
<th>GS4</th>
<th>GS5</th>
<th>PK1</th>
<th>PK2</th>
<th>PK3</th>
<th>PK4</th>
<th>PK5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average triplicate (Log CFU/mL) (0.1 g/L)</td>
<td>8.56</td>
<td>8.60</td>
<td>8.43</td>
<td>8.75</td>
<td>8.74</td>
<td>8.22</td>
<td>8.73</td>
<td>8.69</td>
<td>8.71</td>
<td>8.56</td>
</tr>
<tr>
<td>Average triplicate (Log CFU/mL) (0.2 g/L)</td>
<td>8.51</td>
<td>8.62</td>
<td>8.45</td>
<td>8.85</td>
<td>8.62</td>
<td>7.92</td>
<td>7.94</td>
<td>8.48</td>
<td>8.56</td>
<td>8.85</td>
</tr>
<tr>
<td>Average triplicate (Log CFU/mL) (0.5 g/L)</td>
<td>8.38</td>
<td>8.41</td>
<td>8.48</td>
<td>9.03</td>
<td>-</td>
<td>7.64</td>
<td>8.26</td>
<td>8.46</td>
<td>8.58</td>
<td>8.58</td>
</tr>
<tr>
<td>Witnesses: Average triplicate (CFU Log/mL)</td>
<td>8.79</td>
<td>8.62</td>
<td>8.33</td>
<td>8.64</td>
<td>8.71</td>
<td>8.80</td>
<td>8.76</td>
<td>8.40</td>
<td>8.86</td>
<td>8.58</td>
</tr>
</tbody>
</table>

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

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

The beneficial effects of terpenes and polyphenols on oral health are known (Bitty; 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.

The results of the antibacterial tests reveal that, GS1, GS2, GS3, GS4, PK2, PK3, PK4 and PK5 do not exhibit any significant inhibitory 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).

### 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 inhibitory 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 inhibitory 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 phyto compounds which they contain.

### Phytochemical screening of *G. senegalensis* and *P. kotschyi* stem has already been performed (Kadja, 2014). The author reports that these organs contain phyto phenols (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; 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 phyto compound 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 phyto compound 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 phyto compound A from GS5A extract

Analysis of $^{13}$C NMR, JMOD and DEPT 135 spectra of
compound A (Table 4) reveals the presence of 5 C quaternary sp² 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 1H NMR spectrum (CDCl₃) shows the presence of 3 H (CH₃-O-) 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 (CH3) 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 (C=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 1H NMR spectrum of compound B (CDCl₃) shows the presence of 14 H including 1 H aromatic at 7.34 ppm; 6 H of 2 (CH₃O) at 3.91 and 3.94 ppm; 3 H (CH₃) 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 CH₃ methoxy (C10; C11) appearing at 56.78 and 60.89 ppm; 1 CH3 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.

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

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).
Table 5. NMR spectral data of compound B.

<table>
<thead>
<tr>
<th>No</th>
<th>Type</th>
<th>$^{13}$C δ (ppm)</th>
<th>HSQC δ (m, J Hz)</th>
<th>HMBC δ (ppm)</th>
<th>COSY δ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>195.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>78.06</td>
<td>4.20 (q; 6.7)</td>
<td>195.21</td>
<td>1.50</td>
</tr>
<tr>
<td>3</td>
<td>CH$_2$</td>
<td>62.85</td>
<td>5.06 (d; 15.7)</td>
<td>78.05; 121.64; 141.73; 195.21</td>
<td>4.77</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>144.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>141.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>147.54</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>141.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>CH</td>
<td>103.96</td>
<td>7.30 (s)</td>
<td>121.63; 144.10; 147.54; 195.21</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>C</td>
<td>130.51</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>C</td>
<td>121.63</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11</td>
<td>CH$_3$</td>
<td>16.11</td>
<td>1.50 (s)</td>
<td>78.05; 195.21</td>
<td>4.20</td>
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<tr>
<td>12</td>
<td>CH$_3$</td>
<td>56.78</td>
<td>3.94 (s)</td>
<td>147.54</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>CH$_3$</td>
<td>60.89</td>
<td>3.91 (s)</td>
<td>141.73</td>
<td>-</td>
</tr>
</tbody>
</table>

![Figure 5](image_url) (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
hand, it recommends the possibility of the use of these plants in the prevention of dental caries. In the phytochemical plan, three isolated phytoconstituents 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.

REFERENCES


