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Bioactive compounds and antimicrobial potential of the roots extract of *Anogeissus leiocarpa,* a chewing stick used for oral care in Benin Republic

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Chewing stick are still used in developing countries for oral hygiene in other to prevent oral diseases. But still, few is known about their phytochemical potential and antimicrobial activity. The present work was devoted to one of these plants used in the Republic of Benin, namely the root of *Anogeissus leiocarpa*. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods were used for the antioxidant activity of three crude extracts (aqueous, decoction and ethanolic). Antimicrobial activity of the crude extracts as well as three fractions namely the chloroform fraction, the ethyl acetate fraction and the butanol fraction was carried out by the diffusion method. High performance liquid chromatography (HPLC) analysis of the three fractions of *A. leiocarpa* was performed to identify the active fraction as well as bioactive compounds. The results show that the crude extracts exhibited a good ability to inhibit the DPPH radical and a good ability to reduce ferric Fe^{3+} ions to ferrous Fe^{2+} ion and this could be explained by their good content in phenolic compounds. The ethanolic extract of *A. leiocarpa* was the most active against all microorganisms used in this study. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) ranged from 0.195 to 12,500 mg/mL. The butanolic fraction was the most active with an inhibition diameter of 20.666 ± 0.577 and 22.333 ± 2.081 mm, respectively at the concentration of 50 and 100 mg/mL. HPLC analysis revealed the presence of phenolic acids such as chlorogenic, ferulic and gallic acids as well as tannins including tannic acid and ellagic acid and from these results, *A. leiocarpa* is a good plant candidate for the production of herbal toothpaste.

Key words: Phenolic compound, antimicrobial, antioxidant, chewing stick, oral care.

INTRODUCTION

Human oral cavity plays important roles like nutrition and defense against microbial infections. Due to that, it almost suffer histopathological and inflammatory injuries (Koohi-Hosseinabadi et al., 2015). In fact, oral cavity is one of the part of the human organism harboring the most diverse microbial flora, consisting of more than 700 bacterial species but only a few of them are known to be true dental pathogens or odontopathogenic agents (Henley-Smith et al., 2013; Azelmat and al., 2015). A lack of good oral hygiene is an open door for the accumulation and multiplication of pathogenic bacteria in oral biofilms which can lead to common oral diseases: tooth decay, periodontitis and oral mucositis. Oral diseases are considered as a major public health problem due to their relationship with general health and the fact that their treatment is extremely expensive (OMS, 2016). In addition, the antibiotics and synthetic chemicals used for the treatment and prevention of oral diseases have some side effects and are also subjected to oral microbial resistance (Chinsembu, 2016; Sintim and Gürsoy, 2016). For that reason, it is very important to find out alternative solutions that would be more effective and affordable for the prevention and treatment of oral diseases. Plants have played a very effective roles in the protection against microbial infections throughout mankind history and for that, many studies have attempted to explore them as a potential source of new remedies (Showraki et al., 2016 ; Mardani et al., 2016). Basing on their tast or color, the stems or roots of some plants are shaped into plant brushes call chewing stick for oral hygiene (Muhammad and Lawal, 2010). Chewing sticks are still commonly used in many developing countries due to their traditional value and their effectiveness in the prevention and treatment of oral diseases. More interestingly, many scientific studies have demonstrated the antimicrobial activity of chewing stick against dental pathogens and odontopathogenic agents (Rotimi et al., 1988; Cai et al., 2000; Adekunle and Odukoya, 2006; Akande and Ajao, 2011).

According to Yédomonhan et al. (2017) about 163 species of plants are used as chewing stick in Benin Republic. One of the most used of these plants is *Anogeissus leiocarpa* belonging to Combretaceae family. Both the roots and stem are used as chewing stick but the root is particularly used for its medicinal property. To the best of our knowledge, no previous study has attempted to determine the chemical characterization and biological activity of the root of this plant in Benin Republic. This is why the present study was initiated to evaluate the antibacterial activities and the phytochemical potential of the roots of *A. leiocarpa* use as chewing stick in the central region of Benin Republic.

MATERIALS AND METHODS

Plant materiel

The roots of *A. leiocarpa* were collected in a rural zone of the Central Benin Republic (latitude/longitude: 8° 52' 60 '' North/2° 36' 0 '' east) in September, 2017.

Microorganisms

The bacteria strains used in this study were obtained from the Bacteriology section of the National Laboratory of the Ministry of Health (LNMSP). They were constituted of reference strains, namely: Gram positive cocci: *Enterococcus faecalis* ATCC 10240, *Staphylococcus aureus* ATCC 29223; Bacillus negative Gram: *Proteus mirabilis* ATCC 24974, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922; Fungus: *Candida albicans* IP 4872.

Plant extracts preparation

The plant, once collected, was dried in laboratory under air-conditioned (20°C) and was then ground to a fine powder using an electric grinder to make three types of crude extracts, namely semi ethanolic 50% macerate (eth), aqueous macerate (aq) and aqueous decoction (de). The ethanolic and aqueous macerates were prepared by maceration for three successive days, while the decoction was done by boiling the plant powder for 30 min in distilled water. The extracts were filtered through a Whatman No. 1 paper filter and the filtrates were concentrated using a rotary evaporator (BUCHI Rotavapor RII) and then stored at 4°C for assay (Kazemipour et al., 2015). Three types of fractions were also prepared by liquid-liquid extraction successively with chloroform ($CHCl_3$ extract), ethyl acetate (EtOAc extract) and butanol (BuOH extract) for phenolic compound analysis by HPLC.

Antioxidant activity by DPPH method

The DPPH method was carried out by adding 50 µl of the diluted extracts to 1950 µL of DPPH at 130 µM. Discoloration of DPPH was measured at 516 nm against the blank (1950 µl of DPPH at 130 µM and 50 µl of ethanol) after 45 min (Haddadi et al., 2011). The scavenging percentage was calculated by the following formula

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Table 1. Standard values used to interpret the results of the susceptibility tests of the plant extracts.

Inhibition zone diameter (Δ)	Degree of susceptibility of the germ	Symbol
Δ < 7 mm	Resistant	−
7 mm ≤ Δ < 8 mm	Susceptible	+
8 mm ≤ Δ < 9 mm	Fairly Susceptible	++
Δ ≥ 9 mm	Very Susceptible	+++

Source: OMS (2002); Tsirinirindravo and Andrianarisoa (2009).

(Haddadi et al., 2011):

$P = [(Ab - As) / Ab] \times 100$

where P: percentage of trapping; Ab: absorbance of the blank; As: Absorbance of the sample.

The extract inhibitory concentration necessary for trapping 50% of free radicals of DPPH (IC_{50}) is graphically determined by linear regression of the plotted graphs of DPPH free radical scavenging percentages as a function of the concentrations of extracts tested. Catechin and gallic acid were used as positive control.

Ferric reducing antioxidant power (FRAP) method

FRAP method is based on the ability of extracts to reduce ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) ion. The total antioxidant capacity of each plant extract and reference compounds was determined by the method used by Bangou (2012) and Chung et al. (2002) with a slight modification. Thus 2 ml of an aqueous solution of each extract was mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After 20 min incubation at 50°C; 2 ml of trichloroacetic acid (10%) was added. The mixture was then centrifuged at 3000 rpm for 10 min. Then 2 ml of the supernatant was mixed with the same volume of water and 20 µl of a freshly prepared aqueous solution of $FeCl_3$ (0.1%) was added. Absorbances were measured at 700 nm against a calibration curve obtained from gallic acid and catechin. The reducing power was expressed as function of gallic acid equivalent per gram of crude extract (mg eq AG/g CE) and also as function of catechin equivalent per gram of crude extract (mg eq EC/g CE).

Antimicrobial activity

Preliminary antimicrobial screening of the extracts

It was carried out by the diffusion method of the extracts in wells dug in Mueller Hinton agar plates. Thus bacteria mentioned earlier were suspended according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2017). A swab of each inoculum was cultured onto Mueller-Hinton II agar plates. The three crude extracts of plant previously prepared at a concentration of 100 mg/ml in DMSO were filtered using 0.4 µm multi-pore membranes in order to obtain sterile extract solutions. 16 wells of about 6 mm were dug in the agar plates as described by Agbankpe et al. (2016) and 50 µl of each of the sterile extract solutions were transferred in each well. DMSO solution was used as the negative control. The positive control was conventional Vancomycin (30 µg) antibiotic discs for Gram-positive cocci and Imipenem (10 µg) and Colistin (10 µg) discs for Gram-negative bacilli. The different Petri dishes were left at room temperature for 1 h for pre-diffusion and then incubated at 37°C for 18 h as described by Oke et al. (2013). Each test was conducted three times for quality control purposes. Inhibition diameters were measured and compared to the standards indicated in the Table 1.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The determination of the MIC was performed according to the microwell methodology described by Lagnika et al. (2011). Different successive dilutions of 180 µl of the extract at initial concentrations 50 mg/mL prepared in Mueller Hinton broth were distributed in the wells. Then 20 µl of a 10% dilution of a suspension of 0.5 Mc Farland strains in Mueller Hinton broth were distributed in all wells. On each plate, bacterial suspension + Mueller Hinton broth served as a positive control and Negative control was DMSO + Mueller Hinton broth. The plates were then stirred for 5 min and placed in an oven at 37°C for 18 h. After that, 40 µl of a solution of 0.2% p-iodonitrotetrazolium (INT) prepared in distilled water was added to each well. The plates were then deposited for 20 min in the dark. The presence of a red color in a well indicates the presence of viable bacteria. The MIC is the first concentration for which viable bacteria are present. Wells that did not show a red color are seeded on Mueller Hinton agar. CMB is the first concentration for which there is a present surviving bacteria.

Fractions preparation and antimicrobial analysis

The phenolic compounds were extracted from 150 g of dry plant in ethanol/water (70/30: V / V) for 3 × 24 h. The extracts were concentrated and then taken up with 500 ml of boiling water. After filtration, the aqueous solution was defatted using petroleum ether and the defatted extract was successively exhausted with chloroform ($CHCl_3$), ethyl acetate (EtOAc) and finally n-butanol (BuOH). The same methodology previously described for preliminary antimicrobial screening of the crude extracts was applied for the antimicrobial analysis of the fractions with a slate modification. The only difference is that crude extracts were initially

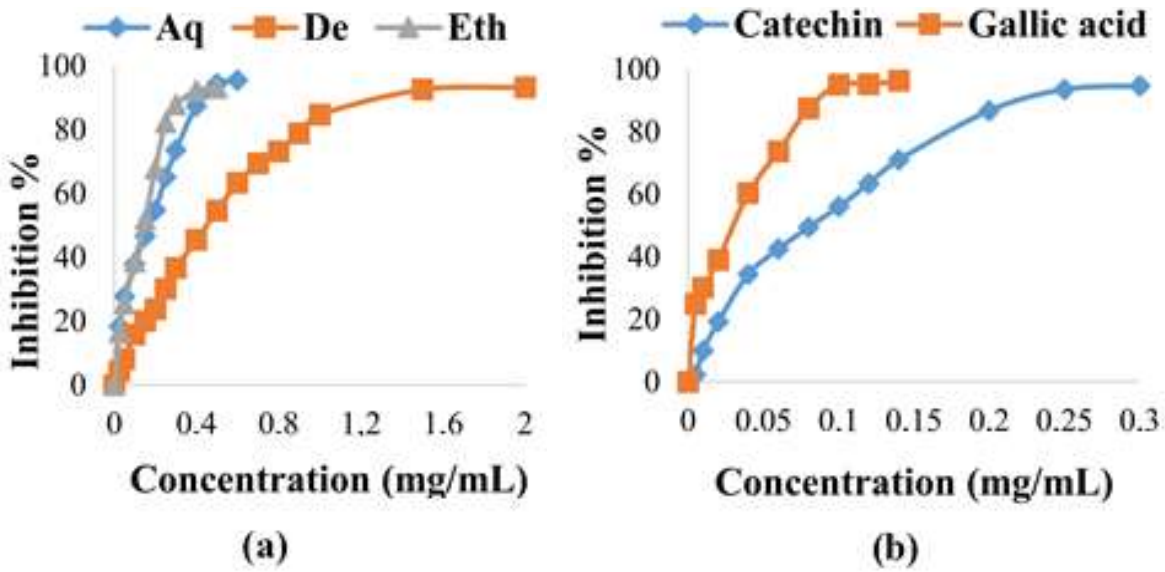

Figure 1. Radical DPPH scavenging activities of extracts of *A. leiocarpa*, catechin and gallic acid (Aq: aqueous; De: decoction; eth: ethanolic).

prepared only at 100 mg/mL whereas fractions were prepared at 50 and 100 mg/mL.

HPLC assay of phenolic compounds

The CECIL Wagtech HPLC EN 91-500 system equipped with a CE 4102 double piston pump connected to an EN 91-502 degasser was used for the identification and quantification of phenolic compounds. The extracts were prepared at 1 mg/ml with methanol/acetonitrile according to their solubility and then filtered using a 0.22 µm millipore filter. Detection was performed with a variable wavelength detector (200, 254, 272 and 365 nm), UV-Visible Adept CE 4201. Chromatographic analyses were performed with a C18 120 Å column (4.6 mm × 100 mm, 5 µ), AcclaimTM, by a binary program with solvent systems such as water (1% phosphoric acid), methanol (1% phosphoric acid) and/or acetonitrile (1% phosphoric acid). The program used is described as follows: 0-20 min, 20-50% B; 20-25 min, 50-70% B; 25-30 min, 70-80% B; 30 to 35 min, 80-20% B; 35-50 min, 20% B. The flow rate was 1 ml/min and the injection volume was 20 mL. The content and qualitative analysis of phenolic compounds in fractions were achieved by comparing their retention times and UV-Vis spectra with those of standard phenolic compounds.

Statistical analysis

All experiments were conducted in triplicate, and results analyzed using SPSS Statistics 17.0 software, were reported with means ± standard deviation (SD). An analysis of variance (ANOVA single factor) was used to compare the means of the inhibition zone diameters of the same plant on different strains, and also the inhibition zone diameters of plants extracts with reference antibiotic. The level of significance was defined at 5%.

RESULTS AND DISCUSSION

Antioxidant activity by DPPH method

The inhibitory concentration of each crude extract of plant and positive control necessary for trapping 50% of free radicals of DPPH (IC_{50}) were graphically calculated using the curve describing the percentage of inhibition as a function of the extract concentration as shown in Figure 1a and b. The IC_{50} were 0.13 ± 0.014, 0.189 ± 0.012 and 0.48 ± 0.042 mg/mL, respectively for the ethanolic extract, aqueous extract and decoction. According to these results, the ethanolic extract was more effective against free radicals of DPPH. Catechin and gallic acid which were used as positive control exhibited IC_{50} value of 0.071 ± 0.012 and 0.028 ± 0.001 mg/mL, respectively. The ethanolic extract was about 4.62 times less active than gallic acid and 1.83 times less active than catechin. A comprehensive analysis of the two curves show that 50 µl of the diluted ethanolic and aqueous extracts at a concentration 0.6 mg/mL was needed to completely inhibit a volume of 1950 µL of DPPH at 130 µM whereas 0.15 and 0.3 mg/mL of gallic acid and catechin were respectively needed for the same complete inhibition. The results suggest that the roots of *A. leiocarpa* could

Table 2. Ferric reducing power of the extracts.

Plant	Extract	Reducing power	
		(mg E AG/g CE)	(mg EC/g CE)
A. leiocarpa	Aqueous	137.281 ± 2.828	321.701 ± 3.412
	Decoction	46.540 ± 1.152	77.409 ± 4.549
	Ethanolic	189.822 ± 4.399	415.397 ± 6.255

increase the antioxidant capacity of saliva when used as chewing stick and this could explain the fact that they are used by rural populations of Benin republic. Salau et al. (2015a) confirm the antioxidant potential of the aqueous extract of the root bark of *A. leiocarpa*. The antioxidant activity of this chewing stick may be attributed to its phenolic content as stated; Lee et al. (2004) who showed that tea polyphenols could increase the antioxidant capacity of saliva. In the same logic, Chinsembu (2016) reported that green tea polyphenol is a good inhibitor of oral oxidative stress and inflammation. Thus, plants with good antioxidant activity may contribute significantly to the prevention of oral diseases by increasing the antioxidant capacity of saliva (Petti and Scully, 2009).

DPPH method has some advantages like allowing a total reaction of DPPH with the whole sample even with weak antioxidants due to the time it requires (Prakask, 2001). However, this method is limited because DPPH radical can interact with other radicals and it is not a suitable method for evaluating the antioxidant activity of plasma because of precipitation of proteins in the alcoholic medium (Kedare and Singh, 2011). That is why the reducing power method was also used to confirm the antioxidant activity of the plant studied.

Ferric reducing antioxidant power method

The reducing power of Fe^{3+} iron by the plant extracts was evaluated using the FRAP method described in the experimental section. Gallic acid and catechin were used as reference compounds and the reducing power of the various extracts was determined and expressed in milligram equivalent of gallic acid per gram of crude extract (mg EAG/g CE) and in milligram equivalent of catechin per gram of crude extract (mg EC/g CE). The results of this study are shown in Table 2. According to these results, the ethanolic extracts have the best reducing power presented as 189.822 ± 4.399 mg E AG/g CE and 415.397 ± 6.255 mg EC/g CE due probably to their phenolic content. Results interpretation simply means that 1 g of the ethanolic extract of *A. leiocarpa* might react as 189.822 mg of gallic acid and 415.397 mg of catechin approximately. It could then be concluded that this plant has a relatively good reducing power towards ion Fe (III) as compared to pure reference compounds and this reducing power could be improved by farther purifications of this extracts. The aqueous extract also exhibited a relatively good reducing power with 137.281 ± 2.828 mg E AG/g CE and 321.701 ± 3.412 mg EC/g CE whereas the reducing power of the decoction was very low. Barku and Abban (2013) tested the Ferric Reducing Antioxidant Power (FRAP) antiradical activity on methanol extracts and ethyl acetate of *A. leiocarpa* leaves (400 and 800 ppm) and the results were promising. Percentage of inhibition was better at 800 ppm and was 94.19 and 92.43%, respectively. This test is a very simple one and can be applied to both organic and aqueous plant extracts and plasmas (Li et al., 2008). The FRAP method makes it possible to measure the ability of phenolic compounds to reduce Fe (III) ions to Fe (II) and during this process there is an electron donation which is able to stabilize free radical (Hinneburg et al., 2006).

Antimicrobial activity of the crude extracts

Preliminary screening

Inhibition zones diameter of the extracts of the selected plant on the strains tested were determined and the results are recorded in Table 3. These results reveal that all the microorganisms were sensitive to all the crude extracts of *A. leiocarpa.* The ethanolic extract was the most effective against all of the microorganisms ($p<0.05$). All the extracts of *A. leiocarpa* were able to inhibit *C. albicans*, known to be implicated in oral infection, with remarkable inhibition zone diameters.

The ethanolic extract of *A. leiocarpa* was interestingly more effective against *S. aureus* and *E. faecalis* than vancomycin ($p <0.05$) (Table 4) comparatively to synthetic antibiotics. *A. leiocarpa* was also more sensitive than colistin against *E. coli* and *P. aeruginosa* ($p < 0.05$). Okunade et al. (2007) found that the ethanolic extract of

Table 3. Inhibition zone diameters of plant extracts.

Stain	Inhibition Zones Diameter (mm)		
	Aqueous	Decoction	Ethanolic
S. aureus	14666 ± 0.577[a]	11.000 ± 1.000[a]	21.666 ± 1.527[a]
E. coli	16.333 ± 0.577[b]	12.333 ± 2.081[ab]	14.333 ± 0.577[b]
E. faecalis	12.666 ± 1.154[c]	11.000 ± 1.732[a]	22.000 ± 1.000[a]
P. mirabilis	12333 ± 0.577[c]	11.000 ± 1.000[a]	14.333 ± 1.572[b]
C. albicans	13.000 ± 1.000[c]	15.000 ± 1.000[b]	15.333 ± 0.577[b]
P. aeruginosa	11.666 ± 0.577[c]	13.000 ± 1.000[ab]	22.000 ± 1.000[a]

Means of the inhibition zone diameters followed by the same letters in the same row are not significant different ($p > 0.05$).

Table 4. Inhibition zone diameters of antibiotics.

Strain	Inhibition zone Diameters (mm)		
	Vancomycin (30 µg)	Imipenem (10 µg)	Colistin (10 µg)
S. aureus	17.666 ± 0.577	ND	ND
E. coli	ND	26.666 ± 1.154	9.333 ± 0.577
E. faecalis	17.666 ± 0.577	ND	ND
P. mirabilis	ND	26.666 ± 1.154	19.333 ± 0.577
C. albicans	ND	ND	ND
P. aeruginosa	ND	26.666 ± 1.154	9.333 ± 0.577

ND: Not done.

the roots of *A. leiocarpa* was not active against *C. albicans*, which is contrary to our findings. The difference in the current study and the one of Okunade et al. (2007) may be related to the season of collection, the climate or the quality of the soil. Nevertheless, this extract showed appreciable bioactivities against *S. aureus*, in accordance with ours findings. From these results, *A. leiocarpa* could be regarded as a potential source of antibiotics to prevent and to treat oral infections as stated by Okunade et al. (2007). All plant extracts were submitted to the determination of MICs and MBCs to better highlight their antimicrobial activity.

MIC and MBC

MICs and CMBs were determined to better appreciate the antimicrobial activity and the results are recorded in Table 5. From the analysis of this table, the aqueous extract and the aqueous decoction of *A. leiocarpa* were not very active. However, the ethanolic extract of *A. leiocarpa* was very active against all the microorganisms used in the present study. MICs and MBCs range from 0.195 to 12.500 mg/mL. Generally, it should be emphasized that the ethanolic extract of *A. leiocarpa* was the most active on Gram +, Gram - and *C. albicans*. This could justify the use of this plant in traditional medicine for the oral hygiene (Akpona et al., 2009). At this stage, it can only be supposed that natural compounds, especially phenolic compounds in the chewing sticks are responsible for the observed antimicrobial activities of the chewing sticks studied. This hypothesis seems to be confirmed by Matsumoto et al. (1999), who showed that administration of the oolong tea extract and its chromatographically isolated polyphenol compound into diet and drinking water resulted in significant reductions in caries development and plaque accumulation in rats infected with *Mutans streptococci*.

HPLC analysis of the fractions

Three fractions of *A. leiocarpa* were prepared for the extraction of the phenolic compounds contained in this plant, since these compounds are often endowed with an obvious antimicrobial activity (Matsumoto et al., 1999).

Table 5. MICs and MBCs of different plant extracts.

Extract		Strains					
		S. aureus	***E. coli***	***E. faecalis***	***P. mirabilis***	***C. albicans***	***P. aeruginosa***
Aq	MIC	12.500	12.500	50.000	50.000	25.000	25.000
	MBC	25.000	50.000	> 50.000	> 50.000	> 50.000	> 50.000
De	MIC	50.000	50.000	50.000	50.000	25.000	50.000
	MBC	> 50.000	> 50.000	> 50.000	> 50.000	> 50.000	> 50.000
Eth	MIC	6.250	12.500	12.500	0.195	6.250	0.781
	MBC	6.250	12.500	12.500	1.562	12.500	0.781

Aq: aqueous extract; De: decoction; Eth: ethanolic extract

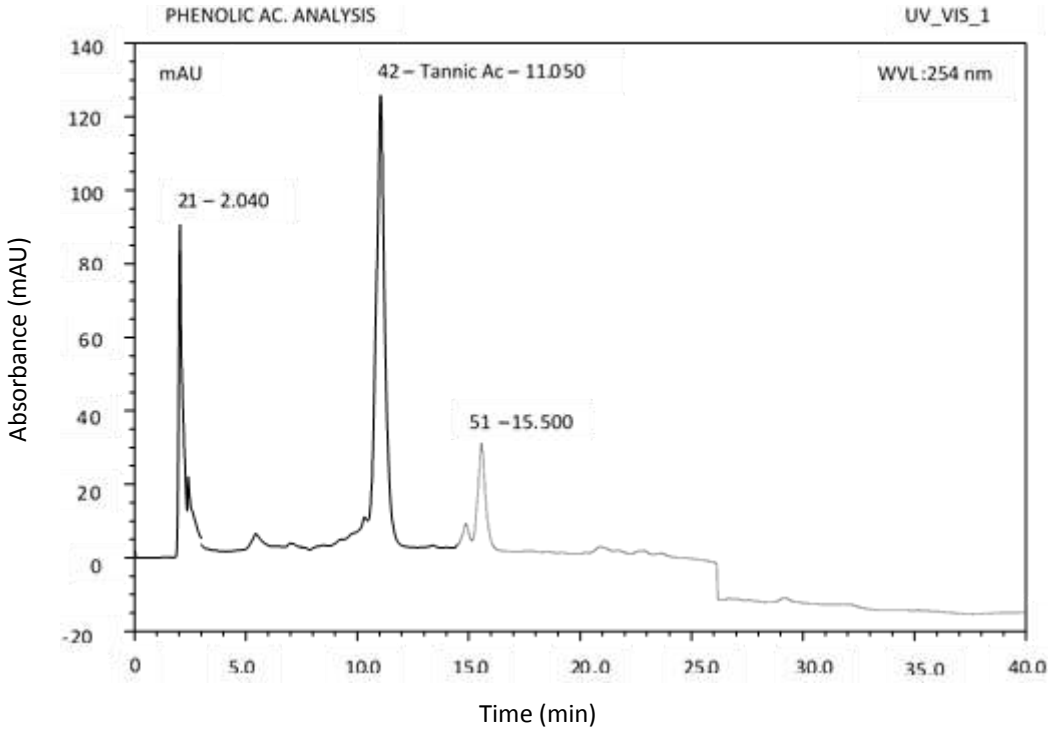

Figure 2. HPLC / UV-Vis Chromatogram of the $CHCl_3$ extract of *A. leiocarpa*.

HPLC analysis revealed the presence of phenolic acids such as caffeic, ferulic, chlorogenic, ferulic and gallic acids. Tannins, including tannic and ellagic acids are also present in this plant. Ellagic acid appeared to be present in all extracts of this plant. The most remarkable compound, which appeared with a retention time of about 2 min in all chromatograms as shown in Figures 2 to 4 should undoubtedly be the major compound of this plant. Its structure is not known but its position on the chromatogram shows that it is of the family of phenolic acids. Tannic acid and its derivatives were also strongly represented in view of the high levels recorded in this compound. In fact, *A. leiocarpa* is known to be rich in tannin, up to 17% and in phenolic acids, and the largest

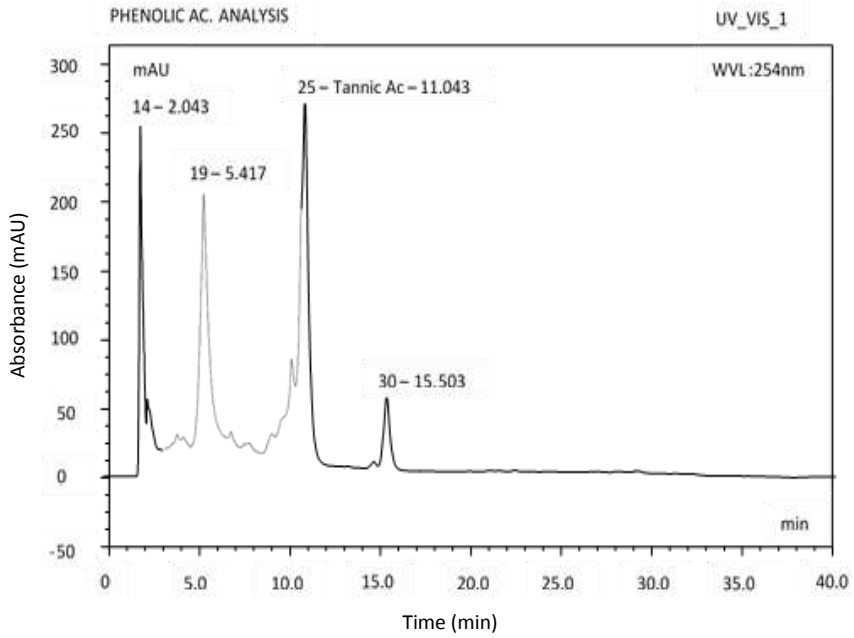

Figure 3. HPLC/UV-Vis Chromatogram of the EtOAc extract of *A. leiocarpa*.

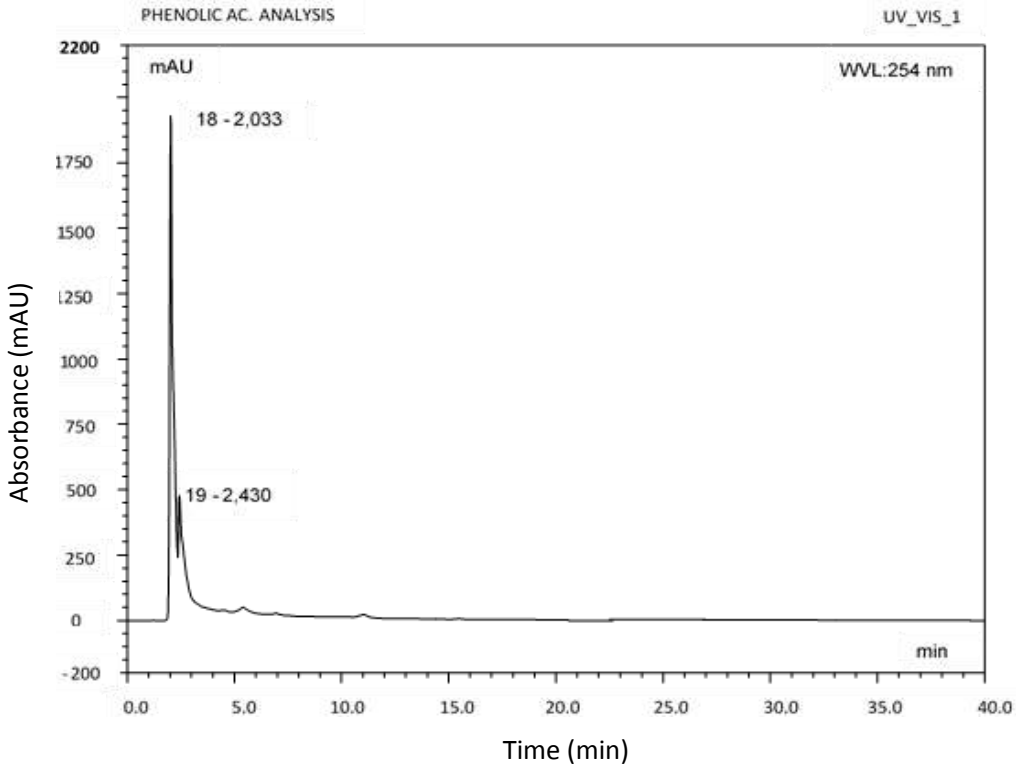

Figure 4. HPLC/UV-Vis Chromatogram of the BuOH extract of *A. leiocarpa*.

Table 6. Inhibition Zone Diameters of fractions.

Fraction	Inhibition zone diameter (mm)	
	50 mg/mL	100 mg/mL
$CHCl_3$	15.666 ± 0.577[a]	17.666 ± 1.154[a]
EtOAc	16.666 ± 0.577[a]	17.666 ± 1.527[a]
BuOH	20.666 ± 0.577[b]	22.333 ± 2.081[b]

Means followed by the same letters in the same line and row are not significant different (p > 0.05).

unidentified peaks in our chromatogram could be attributed to tannins. Further study is needed to elucidate that fact. The most cited compounds in the literature, both in the leaf and trunk bark, are: 3,3,4-tri-O-methylflavellagic acid, 3,3,4-tri-O-methylflavellagic acid D-glucoside, gentisic, protocatechic, gallic acid, chebulagic acid, chebulinic acid, ellagic acid, chlorogenic acid, flavogallonic acid, etc. (Waterman, 2010; Adigun et al., 2000; Chaabi et al., 2008).

Antimicrobial activity of fractions

Finally, the antimicrobial activity of the three different fractions ($CHCl_3$, EtOAc and BuOH) was analyzed and the results are presented in Table 6. The butanolic fraction had the best antimicrobial activity with 20.666 ± 0.577 and 22.333 ± 2.081 mm inhibitory diameter, respectively at the concentration of 50 and 100 mg/mL. The $CHCl_3$ and EtOAc fractions exhibited the same inhibition diameters ($P > 0.05$) but the EtOAc fraction appears to be slightly more active. The analysis of the results shows that the fractions presented better inhibition diameters, even at 50 mg/mL than crude extracts previously prepared at 100 mg/mL. Moreover, there was no significant difference ($P > 0.05$) between the inhibition diameters obtained with the 50 and 100 mg/mL fractions.

Analysis of the HPLC chromatograms indicated that the bioactive compound would be a phenolic acid mentioned previously. Indeed, this compound is found at 84% in the butanolic fraction which has the best activity. In addition the observed activity is likely to be dose-dependent of this compound because the peaks which represent this compound have heights of 100, 250 and 2000 mAU, respectively in the fractions $CHCl_3$, EtOAc and BuOH whose antimicrobial activities are increasing in the same order. There could also be a synergistic action between this compound and tannic acid or one of its derivatives.

Conclusion

Throughout this study, it clearly appears that *A. leiocarpa* root is very rich in phenolic compounds. As consequence, this plant exhibited a relative good antioxidant activity and was very effective against all the microorganisms used in this study. Phenolic acid were found to be bioactive compounds in this chewing stick through HPLC analysis. Further chemical characterization is needed to determine the exact structures of the bioactive compounds. Toxicity of this plants extract should also be studied in order to consider its likely incorporation into toothpastes or mouth bath.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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