Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1939-1944
Received: 04-07-2018
Accepted: 06-08-2018

Théophile Olayé
Laboratoire d'Etude et de Recherches en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey-Calavi (EPAC), University of Abomey-Calavi, 01 PO Box 2009 Cotonou, Benin

Fidèle Paul Tchobo
Laboratoire d'Etude et de Recherches en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey-Calavi (EPAC), University of Abomey-Calavi, 01 PO Box 2009 Cotonou, Benin

Nicodème Worou Chabi
a) Laboratoire d'Etude et de Recherches en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey-Calavi (EPAC), University of Abomey-Calavi, 01 PO Box 2009 Cotonou, Benin.
b) Laboratoire de Biochimie et de Biologie Moléculaire, Faculté des Sciences et Techniques, University of Abomey-Calavi, 04 PO Box 0320 Cotonou, Benin

Correspondence
Fidèle Paul Tchobo
Laboratoire d'Etude et de Recherches en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey-Calavi (EPAC), University of Abomey-Calavi, 01 PO Box 2009 Cotonou, Benin

Phytochemical potential, antiradical and antimicrobial activity of crude extracts of *Caesalpinia benthamiana* roots used for oral hygiene in Benin republic

Théophile Olayé, Fidèle Paul Tchobo and Nicodème Worou Chabi

Abstract
Triclosan Oral hygiene is a determining factor in the prevention of oral diseases. In other to meet this challenge, rural populations in several developing countries including those of Benin Republic use chewing stick, whose activity are yet to be elucidated. The objective of this present work was to analyse the phytochemical, the antioxidant and antimicrobial activities of the root of *Caesalpinia benthamiana*, a chewing stick used in Benin Republic for oral hygiene using standard techniques described in the literature. The ethanolic extract exhibited a good ability to inhibit the DPPH radical and also to reduce ferric Fe^{3+} ion to ferrous Fe^{2+} ion and this could explain their rich content in phenolic compounds. The ethanolic extract also exhibited a good antimicrobial activity against *Proteus mirabilis* with MIC value of 6,250 mg/mL.

Keywords: Phytochemical, antimicrobial, antioxidant, chewing stick, oral hygiene

Introduction
Although not always life-threatening, oral diseases represent a major public health problem because of their high prevalence and significant impact on general health [1, 2]. The classical treatment of these illnesses is extremely costly, being at the fourth rank in terms of health costs in most industrialized countries [3]. Faced with this scourge, the challenge is to lead people to adopt behaviors that could help them to avoid oral diseases such as dental caries and periodontal disease, through the proper use of modern toothbrushes or plant toothbrushes. Even though many people have abandoned the traditional use of vegetable toothbrushes, and have adapted to the conventional method of brushing teeth, some peoples still use chewing stick as a daily ritual to maintain oral hygiene. This is particularly true in developing countries where the economy, customs, religion and the availability of oral hygiene tools play a role in their continued use. Chewing stick have long been used in the Greek, Roman, Jewish and Islamic empires [4].

Plants have very effective and important roles to play in oral hygiene. A number of popular plants are shaped into plant brushes, most of which contain different substances that can keep the oral cavity healthy in general [5]. Moreover, the World Health Organization (WHO) supports the use of chewing stick as an effective tool for oral hygiene because they contain many antimicrobial substances [6]. According to Oshomoh and Idu [7], Africans who use chewing stick have fewer carious lesions than those who use toothpastes. Indeed many studies have demonstrated the antimicrobial activity and phytochemical content of various chewing sticks used in Africa [8-12]. Most of these secondary metabolites have very important roles to play in maintaining oral hygiene. Thus, mucilages protect the mucous membranes by their lubricating action [13], while flavonoids strengthen the capillaries and reduce the frequency of minor hemorrhages [14]. As for the tannins, they act preventively against microbial fermentations, by precipitating the proteins from the bacterial plaque and are therefore used in mouthwashes and toothpastes. Saponosides are surfactants and this property allows them to be fluidifying secretions, emulsifiers and detergents.

The factors that determine which plants are used as sources of plant chewing stick in Africa include availability, color, taste, texture, age of user, cultural and religious customs, and family tradition [6]. In Africa, the use of vegetable toothbrushes is still widespread in Sudan, Nigeria, Namibia, Benin, etc. Around the world, 182 plant species have been used as plant brushes, of which 158 are known in Africa [15].

In Benin, the use of chewing stick is a cultural practice transmitted from generation to generation and an inexpensive option for oral hygiene because it provides substantial income and provides sanitary, medicinal and pharmaceutical benefits for communities [16]. Presently many species that are used for this purpose are highly threatened in Benin. According to Djossou [17], the functional justification of vegetable toothbrush is related to its four roles: cleaning of dental surfaces (mechanical action), gingival massage (activation of blood circulation), oral asepsis (phytotherapy) and stimulation of periodontal structures. Recently, 34 species have been identified as chewing stick across 8 communes in southern and central Benin Republic as well as assessing their market value and availability in a few habitats [18]. However, the literature review revels that there is little evidence on the subject of chewing sticks as far as their chemical characteristic and biological activity are concern, despite their wide use in Benin's Republic. This is why the present study was initiated to evaluate the antibacterial activities and the phytochemical potential of some plants used in oral hygiene in Benin's Republic. The present study is devoted to chewing stick namely, the root of *Caesalpinia benthamiana*

Materials and Methods

Chemicals

2,2-Diphenyl-2-picrylhydrazyl (DPPH), potassium hexacyanoferrate [$K_3Fe(CN)_6$], trichloroacetic acid, gallic acid, ascorbic acid, magnesium, quercetin, and ferric chloride III ($FeCl_3$) were purchased from Sigma Chemical; Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate (Na_2CO_3), aluminium chloride, potassium acetate, sodium sulfate, and solvent ethanol, formalin, hydrochloric acid, ether, ammonia, isoamyl alcohol and sulfuric acid were obtained from Merck Chemical Supplies (Darmstadt, Germany). Mueller Hinton, conventional antibiotics disc were obtained from Bio Mérieux Marcy, France. DMSO and p-iodonitrotetrazolium (INT) were purchased from Sigma Aldrich, Missouri United State. All the chemicals used were of analytical grade. All the spectrophotometric measurement was carried out using Jenway 7305 spectrophotometer.

Plant material and extracts preparation

The root of *Caesalpinia benthamiana* was collected in the southern region of Benin's Republic in September 2017. The botanical identification of the collected material was performed by the botanists of National Herbier at the University of Abomey-Calavi, Benin's Republic.

The plants, once collected, were left at room temperature (20 °C) in the laboratory for two weeks to dry. They were then grounded to a fine powder to make three types of crude extracts, namely semi ethanolic 50% macerate (eth), aqueous macerate (aq) and aqueous decoction (de). The ethanolic and aqueous macerate were obtained by maceration for three successive days, taking care to renew the solvent each day. The aqueous decoction was prepared by boiling the vegetables' powder for 30 minutes in distilled water. The filtrates obtained were concentrated on a rotary evaporator and then in an oven at 40 °C for 3 days.

Microorganisms

The used bacteria strains 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

Phytochemical Screening

The phytochemical screening was done according to the standard techniques described by Paris and Moyse [19], Harborne [20], and Noudogbessi [21] The plant extracts were screened for presence of alkaloids, tannins, flavonoids, coumarins, mucilage, saponins, terpene, triterpene, anthocyans in whole plant and in the three extracts in other in to compare the results.

Determination of total phenolic content

The determination of total polyphenols was carried out with the Folin-Ciocalteu reagent according to the method presented by Ainsworth and Gillespie [22].

This method consists in adding to 20 µL of each diluted ethanolic extract obtained by maceration of the crude extracts (eth, aq, de) in ethanol for 24 hours, 200 µL of Folin-ciocalteu (10%) in a tube. The mixture obtained is vigorously vortexed before adding 5 min after 800 µl of sodium carbonate Na_2CO_3 (7%). The absorbance is read at 765 nm after 2 hours against a blank (ethanol). Gallic acid was used as standard for the calibration curve and the content is expressed in milligram equivalent of Gallic Acid per gram of crude extract (CE) (mg eq AG / g CE)

Determination of total flavonoid content

The estimation of the total flavonoid content was carried out by the method used by Kim *et al*. [23]. The extract used were obtain from dilution of each the crude extracts (ethanolic, aqueous, and decoction) in ethanol. A volume of 100 µl of the extract of each plant was mixed with 0.4 ml of distilled water and subsequently with 0.03 ml of a solution of 5% sodium nitrite. After 5 minutes, 0.02 milliliters of a 10% solution of $AlCl_3$ was added. After 5 minutes again, 0.2 ml of Na_2CO_3 solution (1 M) and 0.25 ml of distilled water were added to the mixture. The whole was vortexed and the absorbance measured at 510 nm.

The flavonoid content is calculated from a standard curve using catechin as a standard. The results are expressed in milligram-equivalent of catechin per gram of the crude extract (mg eq EC/ g CE)

Antiradical activity by the DPPH method

50 µl of the diluted extracts were added 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 [24]. The scavenging percentage was calculated by the following formula:

$$P = [(Ab - Ae) / Ab]*100$$

With P: percentage of trapping; Ab: absorbance of the blank; Ae: Absorbance of the sample

The extract concentration necessary for trapping 50% of free radicals of DPPH (IC_{50}) is calculated graphically by linear regression of the plotted graphs of DPPH free radical scavenging percentages as a function of the concentrations of extracts tested.

Reducing power by the FRAP method

The Ferric Reducing Antioxidant Power (FRAP) method is based on the ability of extract 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 [25] 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. 2 ml of the supernatant were then 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 read 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) [25, 26].

Antimicrobial activity: susceptibility tests

It was carried out by the diffusion method of the extracts placed in wells of Mueller Hinton agar plates. Thus bacteria mentioned above were suspended according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [27]. The suspensions thus made were seeded by swabbing on Mueller Hinton square agar plates. The three extracts of plants previously prepared at a concentration of 100 mg / ml in DMSO were filtered using 0.4µm 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*. [28] and 50 µl of each of the sterile extract solutions were deposited in each well. DMSO solution was used as the negative control. The positive control was conventional Vancomycin antibiotic discs for Gram-positive cocci and Imipenem and Colistin discs for Gram-negative bacilli. The different Petri dishes were left at room temperature for one hour for diffusion and then incubated at 37 °C for 18h as described by Oke [29]. Each test was conducted three times for quality control purposes. Inhibition diameters were measured and compared to the standards indicated in the Table 1.

Table 1: Standard values of the susceptibility tests of the plant extracts [30, 31].

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	+++

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

The determination of the MIC was performed according to the microwell methodology used by Lagnika *et al*., [32]. 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. 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 minutes and placed in an oven at 37 °C. for 18 h. 40 µl of a solution of 0.2% p-iodonitrotetrazolium (INT) in distilled water was added to each well. The plates were then deposited for 20 minutes 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 (subjects are missing here) are seeded on Mueller Hinton agar. CMB is the first concentration for which there is a colony presence.

Statistical analysis

All experiments were conducted in triplicate, and results, analyzed using SPSS Statistics 17.0 software if necessary, were reported with means ± standard deviation (S.D.). An analysis of variance (ANOVA single factor) was used to compare the means when necessary. The level of significance was defined at 5%.

Results and Discussion

Phytochemical screening

Table 2 shows the results of the phytochemical screening of whole plant, aqueous extract, decoction and semi-ethanolic extract. This result shows that this plant contains saponosides, mucilages, tannins, sterols and terpenes, leuco-anthocyanins and flavonoids. All the three extracts and the whole plant contain flavonoids, saponins and tannins. Mucilages were found in the Whole plant and the aqueous macerate whereas anthocyans were found only in the ethanolic extract and sterols and terpens in the whole plant and in the whole plant and in ethanolic extract. These simply mean that aqueous macerate and the decoction which is one of the traditional way to prepare medicinal plants contain less secondary metabolites compare to the whole plant whereas most of the compounds found in this whole plant were also found in the ethanolic extract.

Table 2: Result of phytochemical screening

Phytochemicals	1	2	3	4
Saponins	+	+	+	+
Mucilages	+	+	-	-
Tannins	+	+	+	+
Anthocyans	-	-	-	+
Sterols et terpens	+	-	-	+
Coumarins	-	-	-	-
Flavonoids	+	+	+	+
Alkaloids	-	-	-	-
1: Whole plant; 2: Aqueous macerate; 3: Decoction; 4: ethanolic extract +: Present; -: Absent.				

According to Békro *et al*. [33], methanol and aqueous crude extracts of *C. benthamiana* contain sterols, polyterpenes, flavonoids, tannins, and saponins. These results are consistent with those found in this study only that sterols and terpens were not found in the aqueous extract in our study. Methanol and ethanol are known to extract the same class of compounds.

Determination of phenolic content

The total phenol content of the C. *benthamiana* extracts is in the range of 42 ± 7.071 EAG / g CE to 252.357 ± 9.091 mg EAG / g CE, the lowest level being obtained with the aqueous macerate and the highest with the ethanolic extract. The flavonoid contents of *C. benthamiana* extracts vary from 23.807 ± 3.419 mg EC / g CE to 211.650 ± 7.616 mg EC / g CE, respectively for decoction and ethanol extract. As was the

case with phenolic compound contents, ethanolic extracts have the highest flavonoid content.

Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that phenolic compounds have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Catechins, the most reduced form of the C_3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas and their antimicrobial activity against oral microbes implicated in oral infection like *Streptococcus mutans* [34].

Table 3: Phenolic content

Extraits	Total phenolic content (mg EAG / g CE)	Flavonoids content (mg EC / g CE)
Aqueous	42 ± 7.071	34.249 ± 0.989
Decoction	68.964 ± 5.303	23.807 ± 2.418
Ethanolic	252.357 ± 9.091	211.650 ± 5.385

Antiradical activity by DPPH method

The concentrations of the various extracts making it possible to inhibit 50% of the free radicals of DPPH are shown in Table 3. The analysis in this table shows that the ethanolic extract exhibits the best antiradical activity followed by the aqueous extract with respectively 0.168 ± 0.009 mg/mL and 0.189 ± 0.013 mg/mL, but there is no significant difference between these two values (p<0.05). The decoction, which has the lowest antiradical activity (p<0.05), gave an IC_{50} of 0.675 ± 0.106 mg/mL. Compared with the reference compounds, the ethanolic extract which has the best antiradical activity is about 2, 366 and 6 times less active than catechin and gallic acid respectively.

Chemical studies in order to identify metabolites with antioxidant activity in *C. benthamiana* revealed the occurrence of diterpenes deoxycaesaldekarin C and benthaminin 2. The most active compound was benthaminin 2, displaying an IC50 of 42.7 µM in the assay using DPPH, while an IC50 of 74.2 µM was determined to control thiobarbituric acid (TBA) [35]. This means that terpens may contribute significantly to the antioxydant activity of this plant.

Table 4: IC_{50} of plant extracts studied and reference compounds used

IC_{50} (mg/mL)			
Extraits	***C. benthamiana***	**catechin**	**gallic acid**
Aqueous	0.189 ± 0.013[a]	0.071 ± 0.012[c]	0.028 ± 0.001[c]
Decoction	0.675 ± 0.106[b]		
Ethanolic	0,168 ± 0.009[a]		

Means followed by the same letters are not significantly different (p<0.05)

Reducing power

In parallel with the DPPH free radical scavenging test, the reducing power of iron (Fe^{3+}) in the extracts of the studied plant was evaluated using the FRAP method. This test is a simple, rapid and reproducible test [36]; it is universal and can be applied to both plants and plasmas and to organic and aqueous extracts [37].

In the mechanism of the antioxidant activity of phenolic compounds, the reduction of Fe (III) is often used as an indicator of electron donor [38]. The FRAP method makes it possible to measure the ability of phenolic compounds to reduce Fe (III) ions to Fe (II). 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 E GA/g CE) then in milligram equivalent Catechin per gram of crude extract (mg EC/g CE). The results of this study are shown in Table 4. These results show that the ethanolic extract of *C. benthamiana* has the best reducing power, which is 82.170 ± 2.095 (mg E GA/g CE) and 175.126 ± 5.686 (mg EC/g CE).

Table 5: Ferric reducing power of the extracts

Plants	Extracts	Reducing power	
		(mg E GA/g CE)	**(mg EC/g CE)**
C. benthamiana	Aqueous	53.577 ± 1.676	96.510 ± 3.127
	Decoction	46.170 ± 1.885	78.414 ± 3.696
	Ethanolique	82.170 ± 2.095	175.126 ± 5.686

Determination of inhibition zone diameters

The inhibition zone diameters of the aqueous extracts, ethanolic and the decoction of the six plants were determined and the results are recorded in Table 5. These results reveal that, only the ethanolic extracts of *C. benthamiana* inhibited the microorganisms tested. Thus, the ethanolic extract of *C. benthamiana* induced inhibition zones varying from 20.666 ± 2.309 mm to 12.666 ± 0.577 mm respectively on *E. faecalis* and *P. aeruginosa*. Compared to reference antibiotics, the inhibition zone diameter of the ethanolic extract against *S. aueus* was not signicantely different from the one of vancomycin (P<0.05) whereas the same ethanolic extract was more effective against *E. faecalis* than vancomycin (P<0.05).

Table 6: Inhibition Zones Diameter

Inhibition Zones Diameter (mm)						
Stains	***C. benthamiana***					
	aq	**de**	**Eth**	**Vancomycin**	**Imipenem**	**Colestin**
S. au	0[a]	0[a]	16.333 ± 1.527[b]	17.666 ± 0.577[b]	Na	ND
E. co	0[a]	0[a]	15.333 ± 1.527[b]	ND	26.666 ± 1.154[c]	19.333 ± 0.577[d]
E. fa	0[a]	0[a]	20.666 ± 2.309[b]	17.666 ± 0.577[c]	ND	ND
P. mi	0[a]	0[a]	13.333 ± 1.527[b]	ND	26.666 ± 1.154[c]	ND
C. al	0[a]	0[a]	12.666 ± 1.154[b]	ND	ND	ND
P. ae	0[a]	0[a]	12.666 ± 0.577[b]	ND	26.666 ± 1.154[c]	19.333 ± 0.577[d]

Means of the inhibition zone diameters followed by the same letters in the same line are not significantly different (p<0.05). aq: aqueous; de: decoction; eth: ethanolic; *E. fa*.: *Enterococcus faecalis*; *S. au*.: *Staphylococcus aureus*; *P. mi*.: *Proteus mirabilis*; *P. ae*.: *Pseudomonas aeruginosa*; *E. co*.: *Escherichia coli*; *C. al*.: *Candida albicans*

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

The ethanolic extract that was the most effective plant extracts against the various microorganisms tested were farther inquired about their MICs and CMBs to better appreciate their activity and the results are recorded in Table 6. From the analysis of this table, it was found that *C. benthamiana* was more active on *P. mirabilis* with a MIC value of 6.250 mg/mL and a CMB of 12.500 mg/mL. For all other microorganisms tested, the MIC value is 12.500 mg/mL.

At this stage we can suppose that natural compounds, especielly 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*. [39], 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*.

Table 7: MIC et MBC of different plant extracts

	Strains					
	S. au	***E. co***	***E. fa***	***P. mi***	***C. al***	***P. ae***
MIC (mg/mL)	12.500	12.500	12.500	6.250	12.500	12.500
MBC (mg/mL)	25.000	12.500	25.000	12.500	12.500	25.000

E. fa.: *Enterococcus faecalis*; S. au.: *Staphylococcus aureus*; P. mi.: *Proteus mirabilis*; P. ae.: *Pseudomonas aeruginosa*; E. co.: *Escherichia coli*; C. al.: *Candida albicans*.

Conclusion

The present study, the first of his kind in Benin's Republic, shows that the roots of *C. benthamiana* are rich in phytochemical compound and the ethanolic extract was particularly rich in phenolic compounds. This could explain the good antimicrobial observe with this plant extract and their by justify the use of this plant in oral hygiene and against oral pathogen. Further studies should be done in other to find out new bioactive compounds against oral microbes.

Reference

1. Ndiaye CF. Oral Health in the African Region: Progress and perspectives of the Regional Strategy, African Journal of Oral Health. 2005; 2(1&2):2-9.
2. Organisation Mondiale de la Santé (OMS), comité régional de l'afrique. Soixante-sixième session Addis Abeba, République fédérale démocratique d'Éthiopie, August, 2016.
3. Baba-Moussa F, Adjanohoun A, Attakpa ES, Kpavodé L, Gbénou JD, Akpagana *et al*., Antimicrobial activity of three essential oils from Benin on five oral germs: Test of mouthbaths, Annals of Biological Research. 2012; 3(11):5192-5199.
4. Vuuren SF, Viljoen AM. The *in vitro* antimicrobial activity of toothbrush sticks used in Ethiopia. South African Journal of Botany. 2006; 72:646-648.
5. Muhammad S, Lawal MT. Oral hygiene and the use of plants (Review). Scientific Research and Essays. 2010; 5(14):1788-1795.
6. Cai L, Wu CD. Compounds from Syzygium aromaticum possessing growth inhibitory activity against oral pathogens. J Nat. Prod. 1996; 59:987-990.
7. Oshomoh EO, Idu M. Phytochemical screening and antimicrobial activities of ethanolic and aqueous root extracts of Zanthoxyllum zanthoxylloides (lam.) Waterm On selected dental caries causing microbes. Int. J Med. Arom. Plants. 2012; 2:411-419.
8. Rotimi VO, Laughon BE, Bartlett JG, Mosadomi HA. Activities of Nigerian Chewing Stick Extracts against Bacteroides gingivalis and Bacteroides melaninogenicus. Antimicrobial Agents and Chemotherapy. 32(4):598-600.
9. Cai L, Wei GX, Van Der Bijl P, Wu CD. Namibian chewing stick, Diospyros lycioides, contains antibacterial compounds against oral pathogens. Journal of Agricultural and Food Chemistry. 2000. 48:909-914.
10. Adekunle AA, Odukoya KA. Antifungal Activities of Ethanol and Aqueous Crude Extracts of Four Nigerian chewing sticks. Ethno Botanical Leaflet, 2006. http://www.siu/~ebl/leaflets, Accessed 05 April, 2017.
11. Akande TA, Ajao AT. Chemotherapeutic Values of Four Nigerian Chewing Sticks on Bacteria Isolates of Dental Infection Global Journal of Science Frontier Research. 2011; 11(8):91-95.
12. Ndukwe KC, Okeke IN, Lamikanra A, Adesina SK, Aboderin O. Antibacterial activity of Aqueous Extracts of Selected chewing Sticks. J Contemp Dent Pract. 6(3), 086-094.
13. Bitty MJ. Etude de quelques plantes utilisées comme bâtonnet frotte-dent en côte d'ivoire. Thèse pour le diplôme d'etat de docteur en chirurgie dentaire de côte d'Ivoire Dissertation, Université de Clermont 1, 1982.
14. Faussier A. Les ingrédients entrant dans la composition des dentifrices. Bilan des connaissances en matière de tolérance. Création d'une base de données. Dissertation, université de Nantes, 2013.
15. El-Desoukey MRA. Comparative Microbiological Study Between the Miswak (Salvadora persica) and the Toothpaste. International Journal of Microbiological Research. 2015. 6:47-53.
16. Akpona TJD. Ethnobotanique, commercialisation et statut écologique des espèces de brosse végétale dans les formations naturelles du Sud et centre Bénin Dissertation University of Abomey-Calavi, Benin Republic, 2007.
17. Djossou CV. La brosse végétale: Moyen traditionnel d'hygiène bucco-dentaire des Africains. Dissertation University of Abomey-Calavi, Benin Republic, 1985.
18. Akpona HA, Akpona JDT, Awokou SK, Yemoa A, Dossa LOSN. Inventory, folk classification and pharmacological properties of plant species used as chewing stick in Benin Republic. Journal of Medicinal Plants Research. 2009; 3:382-389.
19. Paris R and Moyse H. Précis De Matière Médicinale Masson, Paris, France, 1969.
20. Harborne JB. Phytochemical Methods: A Guide to Modern, Techniques of Plant Analysis Chapman and Hall, New York, NY, USA, 1973.
21. Noudogbessi JPA, Natta AK, Tchobo FP, Bogninou GS, Bothon FTD, Bossou *et al*. Phytochemical Screening of Pentadesma butyracea Sabine (Clusiaceae) Acclimated in Benin by GC/MS. Hindawi Publishing Corporation ISRN Analytical Chemistry. Article ID 172397, 2013, 8.

22. Ainsworth EA et Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature protocols. 2007; 2:875-877.
23. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric. Food Chem. 2003; 51(22):6509-6515.
24. Haddadi H, Alizadeh N, Shamsipur M. Stoichiometric and Free Radical-Scavenging Kinetic Studies of Extractable. Polyphenols from Pomegranate Husk and Pistachio Hull. Journal of the Iranian Chemical Society. 2011; 8(3):694-707.
25. Bangou MJ. Etude Phytochimique et Activites Biologiques des tiges feuillées de Lantana camara L. et de Lippia chevalieri Moldenke: deux Verbenaceae du Burkina Faso Dissertation, University of Ouagadougou, 2012.
26. Chung YC, Hang CT C, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. Journal of Agricultural and Food Chemistry 2002; 50:2454-2458.
27. Société Française de Microbilogie (CA-SFM). Recommandation V. 2.0 May, 2017.
28. Agbankpe AJ, Dougnon TV, Bankole SH, Houngbegnon O, Dah-nouvlessounon D, Baba-moussa L. *In Vitro* Antibacterial Effects of Crateva adansonii, Vernonia amygdalina and Sesamum radiatum Used for the Treatment of Infectious Diarrhoeas in Benin; Journal of Infectious Diseases and Therapy. 2016; 4(3):01-07.
29. Oke MA, Bello AB, Odebisi MB, Ahmed AM, Kazeem MO. Evaluation of antibacterial efficacy of some alcohol-based hand sanitizers sold in Ilorin (north-central Nigeria) 2013. Ife Journal of Science, 2013, 15(1).
30. OMS. L'utilisation des antimicrobiens en dehors de la médecine humaine et les résistances qui en résultent chez l'homme OMS Aide-Mémoire N°268, Genève, 2002.
31. Tsirinirindravo L, Andrianarisoa B. Activités antibactériennes de l'extrait des feuilles de Dalechampia clematidifolia (Euphorbiaceae). International Journal of Biological and Chemical Sciences. 2009; 3:01-07.
32. Lagnika L, Anago E, Atindehou M, Adjahoutonon B, Dramane K *et al*. Antimicrobial activity of Crataeva religiosa Forst against bacteria isolated from Thryonomys swinderianus Temminck. African Journal of Biotechnology. 2011; 10(49):10034-10039.
33. Békro Y-A, Békro JAM, Boua BB, Tra BFH, Éhilé EE. Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae), Sciences & Nature 2007; 4(2):217-225.
34. Cowan MM. Plant Products as Antimicrobial Agents Clinical microbiology reviews. 1999; 12(4):564-582.
35. Dickson RA, Houghton PJ, Hylands PJ. Antibacterial and antioxidant cassane diterpenoids from Caesalpinia benthamiana. Phytochemistry. 2007; 68:1436-1441.
36. Benzie IFF et Strain JJ. The ferric reducing ability of plasma as a measure of antioxidant power the FRAP assay Analytical Biochemistry. 1996; 239:70-76.
37. Li H-B, Wong CC et Cheng KW, Feng C. Antioxidant properties *in vitro* and total Phenolic contents in methanol extracts from medicinal plants. Lebensmittel-Wissenschaft and Technology. 2008; 41(3):385-390.
38. Hinneburg I, Dorman HJD, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food chemistry. 2006; 97:122-129.
39. Matsumoto M, Minami T, Sasaki H, Sobue S, Hamada S, Ooshima T. Inhibitory effects of oolong tea extract on caries-inducing properties of mutans streptococci. Caries Res. 1999; 33:441-445.