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Physico-chemical and microbiological characterizations of an herbal toothpaste formulated with extracts of some selected medicinal plant harvested in Benin Republic

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Abstract

Beninese flora is endowed with effective medicinal plants used locally for hygiene and oral care but very little valued. This work therefore aims to promote these medicinal plants through the formulation of an herbal toothpaste. For this purpose, the chemical composition and anti-radical activity of extracts of four plants (*Syzygium aromaticum, Zingiber officinale, Vernonia amygdalina, Thevetia peruviana*) were determined. Then an antimicrobial toothpaste was formulated by incorporating bioactive extracts from the plants studied. The physico-chemical and antimicrobial properties of the toothpaste formulated were determined. The results reveal the presence of a large number of secondary metabolites and a significant quantity of phenolic compounds in the four plants studied. *Syzygium aromaticum* and *Thevetia peruviana* presented the highest contents of total phenolic compounds, respectively 1258.85 and 616.12 µg EGA/g. Plant extracts show strong anti-radical activity with IC_{50} ranging from 0.1 to 45 µg/µL. Only the essential oils studied showed antioxidant activity close to that of the reference compounds, i.e. 0.029 µg/µL and 0.022 µg/µL respectively for quercetin and gallic acid. The formulated toothpaste has good physicochemical characteristics and acceptable hygienic quality. The antimicrobial activity shown by the toothpaste is interesting with a minimum inhibitory concentration of the toothpaste being 0.100g/mL on most strains tested. Subject to the toxicity results, the formulated product can be recommended for good oral hygiene.

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Introduction

The burden of oral diseases on people of every age in the world is still a great challenge because it represents the third global scourge after cardiovascular diseases and cancer (Petersen, 2003; Rehab, 2020). They are among the most common Non-Communicable Diseases (NCDs) in Africa and can manifest throughout a lifetime, causing pain, facial deformities, social isolation, profound distress which can, in some extreme cases, lead to suicide according to the World Organization of Health (WHO). They constitute a public health problem due to their high prevalence and incidence in all regions of the world, particularly in Africa (Petersen, 2003; WHO Regional Committee for Africa, 2016). In Benin, a clinical examination carried out by Djossou *et al.* (2016) in a university of Abomey-Calavi setting showed a prevalence of dental caries of 59.16 % and a dental plaque accumulation rate greater than 50 %. Oral diseases occurs through food remnants and saliva which are used by oral bacteria such as Lactobacillus acidophilus, Eschrichia coli, Staphylococcus aureus to produce dental plaque which is able damage the oral cavity (Anyiam and Ariyo, 2021; Imam *et al.*, 2024). The management of these oral and dental conditions often proves difficult for low-income populations (Petersen, 2003).

Toothbrushing is a daily practice around the world that helps improve oral hygiene and remove dental plaque. In traditional societies, the stems of therapeutic plants commonly called “chewing stick” were used to clean the surface of the teeth and to reduce the bacterial load in the mouth (Gbenou, 2014; Diatta, 2021). Recent studies have revealed the presence of essential oils and non-volatile extracts with therapeutic properties in these plant resources (Girard, 2010).

Chewing sticks are still used by many low income populations for oral care and hygiene instead of toothpastes which mostly contain chemical therapeutic agents such as fluorides with multiple side effects (Guimard, 2002; Ahouanse, 2015). So, because of these side effects, there is a tendency to promote the incorporation of natural products such as plants traditionally used in the prevention of oral ailments which contain bioactive compounds in the formulation of oral hygiene products (Lachance, 2015). Among these plants, Syzygium aromaticum and Zingiber officinale are well known to be used in the treatment of dental diseases (Barbelet, 2015; Olayé *et al.*, 2020). The anti-inflammatory properties of Thevetia peruviana and Vernonia amygdalina are also used in the prevention of oral and dental conditions (Habtamu and Melaku, 2018; Mathuravalli and Lakshmi, 2012). However, none of the previous work has focused on the valorization of these plants in cosmetic formulation for effective treatment of oral and dental conditions. That is why the present study was initiated and aims to formulate and characterize toothpaste enriched with bioactive extracts of the four medicinal plants mention above.

Materials and methods

Plant material

Medical plants used in this study are composed of the leaves of *Thevetia peruviana* and *Vernonia amygdalina,* rhizomes of *Zingiber officinale* and flower buds of *Syzygium aromaticum*. There were collected in the south region of Benin Republic

Microorganisms

The bacteria strains used in this study were constituted of *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* were used in this study.

Preparing the extracts

Volatile extract

The essential oils from the flower buds of *Syzygium aromaticum* and the rhizomes of *Zingiber officinale* were extracted by hydrodistillation using a Clevenger-type apparatus (Koudoro, 2014 ; Kokutse, 2016).

Non-volatile extract

After harvest, the plant material was dried in the laboratory until their mass stabilized before being reduced to powder. Then 100 g of powder from each sample were subjected to exhaustive extraction using

ethanol-water 70/30 as extraction solvent for 72 hours with continuous stirring. After filtration, the extracts were concentrated in an oven at 60°C then evaporated to dryness using a rotary evaporator (Koudoro *et al*., 2022).

Chemical characterization of plant material

Phytochemical screening

Secondary metabolites were identified in the four medical plants used in this study by coloring and precipitation reactions specific to each family of metabolites. The different metabolites investigated are flavonoids, coumarins, saponosides, sterols and terpenes, alkaloids, mucilages, catechins and galls, and cyanogenetic compounds following the protocols presented by Agbangnan *et al.* (2012) and Koudoro *et al.* (2018).

Determination of total phenolic compound content

Total polyphenols content was determined using the Folin-Ciocalteu reagent according to the method (Ainsworth and Gillespie, 2007; Olayé *et al.,* 2018 ; Koudoro *et al.*, 2018). This method consists of mixing in a test tube, 200 µL of Folin-ciocalteu (10%) with 20 µL of each ethanolic extract obtained after maceration of plants powder in ethanol 70% for 24 hours and filtration. After 5 minutes, 800 µL of sodium carbonate Na_2CO_3 (7%) was added to the mixture was the vigorously vortexed and kept in dark for 2 hours. The absorbance is read at 765 nm after 2 hours against a blank (ethanol) using gallic acid 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 THIS).

Determination of condensed tannins content

The determination of condensed tannins content was carried out using the Butanol-HCl method described by Makkar (2000) with some modifications. This method consists of extracting the tannins by adding 10 mL of 70% ethanol to 200 mg of powder from each plant then subjecting the mixture to stirring on a shaker for 20 minutes then centrifugation at 3000 rpm for 10 minutes. The mixture is filtered and stored at 4°C for subsequent analyses. After that, 3.0 mL of the butanol-HCl reagent (95:5) and 0.1 mL of the ferric reagent were added to 0.50 mL of the tannin extract previously prepared and then vortexed. The resulting mixture was placed in a boiling water bath for 60 minutes. After cooling, the absorbances were read at 550 nm. The content of condensed tannins (TC), expressed in g-eq leucocyanidin/g DM, is given by the following formula:

$$TC = \frac{A_{550nm} * 78{,}26 * 0.5}{\%\ \text{Dry matter}}$$

Determination of total flavonoids content

The estimation of the total flavonoid content was carried out by the method used by Koudoro *et al.* (2022) and Olayé *et al.* (2018). The preparation of the extracts used for this test was done by taking 2 g of plant powder in 25 mL of ethanol-distilled water (70:30). The mixture was macerated for 24h, filtered and kept at 4°C for father use. After that, 100 µL of the each plant extract was mixed with 0.4 mL of distilled water and 0.03 mL of sodium nitrite solution (5%) and 5 minutes later, 0.02 mL of a 10% solution of $AlCl_3$ was added. After 5 minutes again, 0.2 mL of $Na_2\ CO_3$ 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).

Anti-radical activity of the plants studied

The anti-radical activity of the targeted plant extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method based on their ability to trap free radicals in solution and results in the disappearance of the purple color of that -this. 100 µL of each extract are added to 1900 µL of the DPPH solution then left in the dark for one hour and the absorbance measured at 517 nm. The percentage of DPPH inhibition reflecting the anti-radical activity of the extracts was determined according to the equation below (Koudoro *et al*., 2021):

$$P(\%) = \frac{Ab - As}{Ab} * 100$$

P (%): percentage of inhibition; Ab: absorbance of blank; As the absorbance of the sample.

Toothpaste formulation

Several formulas were tested and the proportions of ingredients for the toothpaste formulation are recorded in Table 1.

Table 1. Different tests carried out for the formulation of toothpaste

Composition	Function	Percentage
Phase A		
Xanthan gum	Thickening	1-5%
Sorbitol	Humectant	10-30%
Phase B		
Distilled water	Solvent	20-30%
Plant extracts	Antimicrobial	<4%
Phase C		
Silica	Abrasive	10-60%
Phase D		
Menthol	Aroma	1-3%
Essential oils	Antimicrobial	1%
Phase E		
Sodium dodecyl sulfate	Surfactant	1-2%

The formulation consisted of preparing the different phases before mixing them according to the method proposed by Brandy and Robert (2006) and Subramanian *et al.* (2017) with some modifications. Thus, phase A was prepared by dissolving Xanthan gum in sorbitol with stirring. Phase B was prepared by dissolving the crude extracts in a small volume of the extraction solvent and then diluted in water. Phase B thus prepared was added to phase A followed by vigorous stirring until a stable homogeneous gel was obtained. After 45 minutes of stirring, phase C is gradually added to the mixture (A+B) followed by further stirring for 30 minutes. Finally, successively add phase D (liquid menthol + essential oils) and phase E (surfactant) with 5 minutes of stirring each time. The paste thus formulated is packaged while awaiting subsequent analyses.

Physico-chemical characterization of formulated toothpaste

The physicochemical properties of the formulations were studied using the Indian Standard Bureau standards for toothpastes (Sharma *et al.*, 2014).

Abrasiveness

The method consists of extruding a 15-20 cm long contents onto the butter paper and repeating the same process for at least ten collapsible tubes. After that, the contents were squeezed along the entire length with the fingertip to detect the presence of sharp and hard-edged abrasive particles (Sharma *et al.*, 2014).

Determination of spreading ability

It was determined with the method described by Deshmukh *et al.* (2017) in which 2g of the toothpaste is sandwiched between a slide and covered with another glass slide for 5 minutes in order to expel air and obtain a uniform film of paste between the slides. The excess dough was scraped off the edges. The upper plate was then subjected to a traction of 80 g for 10 minutes. The diameter of the dough spread on the plate is measured.

Determination of pH

The pH was determined by introducing 10 g of the formulated toothpaste into a 150 mL beaker containing 10 mL of boiled water cooled to 37°C then stirred until a complete suspension was obtained. The pH was measured using an automatic pH meter (HANNA HI 2211 pH/ORP Métier).

Homogeneity

Good homogeneity is observed if the toothpaste extrudes a homogeneous mass from the collapsible tube or other suitable container by applying normal force at 27±20°C. In addition, most of the contents should extrude from the crimp of the container and then roll out gradually (Sekar and Noor Jasmin, 2016).

Foaming

The foaming ability of the formulated toothpaste was evaluated by taking a small amount of the formulation with water in a measuring cylinder; the initial volume was noted and then shaken for 10 times. The final volume of the foam was noted (Sekar and Noor Jasmin, 2016).

Foaming power = V_1-V_2

V_1 : Volume in mL of the foam with water.

V_2 : Volume in mL of water alone.

Moisture content

Moisture content is determined by drying 10g of the formulated toothpaste in an oven at 105°C then cool it in the desiccator. Weight loss is recorded as a percentage of humidity and calculated by the following formula:

$\%\ \text{humidity} = \frac{(m_{wet} - m_{dry}}{m_{wet}} * 100$ (Sharma *et al.*, 2014)

m_{wet}: wet weight ; m_{dry} : Weight after drying

Antibacterial activity of toothpaste

The evaluation of the antimicrobial activity consisted of determining the Minimum Inhibition Concentration (MIC) or the Minimum Bactericidal Concentration (MBC) by the diffusion technique by incorporation of the extract into agar as described by Kpatinvoh *et al.* (2017). Different masses of toothpaste (0; 0.5; 1; 1.5; 2 and 2.5 g) were introduced into 20 mL of culture medium poured at a temperature of 45°C in Petri dishes. A few drops of Tween 20 served as an emulsifier. Controls without toothpaste were carried out (0g/20mL). The incubation was carried out according to the time/temperature pair of each microbial strain. The development of the microorganisms is evaluated and compared to that of the control. The MIC corresponds to the lowest concentration above which no microbial growth is observed. From the MIC, the wells which have shown no microbial growth visible to the naked eye are re-isolated on the specific agar plates and incubated in order to obtain the CMB.

Evaluation of hygienic quality and saftinness of the formulated toothpaste

The preparation of the stock dilution and decimal dilutions was done according to the method described by (Quenum *et al.*, 2020). 1 mL of sample to be analyzed was taken aseptically to which 9 mL of sterile salt peptone water (EPT) was added. The mixture was homogenized by vortexing for 2 min, serving as a stock solution. Then 1 mL of the stock solution, was taken, using a sterile pipette and introduced into a sterilized tube containing 9 mL of Salted Peptone Water (EPS) to make the 10 -1 dilution. The 10 -2 dilution was obtained by taking 1 mL of the 10^{-1} dilution and introducing it into a sterilized tube containing 9 mL of Salted Peptone Water and so on until achieving the suitable dilution for the seeding of Petri dishes (Quenum *et al.*, 2020).

The total aerobic mesophilic flora was counted using PCA (Plate Count Agar) agar at 30°C for 72 hours. 1 mL of each of the dilutions as well as the mother suspension was introduced into different sterile Petri dishes. Then, 20 mL of the agar was poured into the contents of the Petri dishes. The whole was gently homogenized so as to incorporate the inoculum into the agar. After solidification, the agar is poured again to make a second layer. The germ count was carried out according to the ISO 6222 1999 standard (Kere, 2017).

Yeasts and molds were enumerated following incubation of 1 mL of each dilution on Sabouraud Chloramphenicol agar at 25°C for 72 hours. The enumeration of germs in Colony Format Unit per milliliter (CFU/ mL) of sample analyzed was carried out according to standard ISO 21527-1: 2008. The colonies of yeasts have a milky appearance while those of molds are filamentous (Sanou *and al.*, 2017). Coliform enumeration was carried out using VRBA (Violet Red Bile Agar) medium. 1 mL of each dilution was inoculated into a double layer of 10 to 15 mL of VRBA agar then incubated after solidification of the medium at 37°C for 72 hours according to standard ISO 21528-2: 2008. The characteristic colonies of coliforms on this culture medium are wine red in color (Sanou *et al.*, 2017)

Staphylococci were counted using Baird Parker (BP) medium. Seeding was done on the surface with 0.1 mL of each of the chosen dilutions and the stock suspension on BP agar. The incubation was carried out at 37°C for 24 hours according to the NF EN ISO 6888 1999 standards. The black, shiny, rounded colonies surrounded by a white precipitate and a flashing halo were taken into account (Kere, 2017).

Sensory analysis

The evolution of the sensory characteristics of the formulated toothpaste was analyzed using a panel of tasters according to the method of Watts *et al.* (1991). The panel of tasters is made up of people of different sexes and from all social categories, chosen from among regular toothpaste users. The descriptors or attributes taken into account are: color, flavor, aroma, odor, texture, viscosity, taste and general impression of the toothpaste.

Hedonic analysis

The hedonic test is a consumer test aimed at measuring the pleasure and/or satisfaction experienced when seeing or consuming a product. The evaluation of the product was carried out according to an assessment scale which was put in place.

Test description

The descriptive test made it possible to determine the sensory profile of the formulated toothpaste. The testers determined a total sensory description of the sample.

Results and discussion

Phytochemical screening

Secondary metabolites identified in the plant material are presented in Table 2. From the analysis of the results in Table 2, we see that catechic tannins, flavonoids and antraquinones are present in all four plants. On the other hand, none of the plants contain gallic tannins, mucilages and cyanogenic derivatives. The metabolites identified in the flower buds of *S. aromaticum* are in agreement with the results obtained by Kokutse in 2016. The work of Rajhans *et al.* (2019) on *Thevetia peruviana*, revealed the presence of alkaloids unlike our study. Similarly, Agbankpe *et al.* (2015) identified gallic tannins and mucilages in *Vernonia amygdalina leaves* which are absent in our sample. Regarding the rhizomes of *Zingiber officinale*, Osabor *et al.* (2015) identified anthraquinones in Nigeria unlike our study. The variation in secondary metabolites noted in our samples compared to previous work could be due to the harvest period, the nature of the soil or climatic hazards.

Phenolic compound content of plant extracts

The contents of total phenolic compounds, flavonoids and condensed tannins of the hydroethanolic extracts of the plants investigated are expressed in Table 3. From the results of the determination of phenolic compounds, the hydroethanolic extracts richest in total phenolic compounds are *Syzygium aromaticum* followed by *Vernonia amygdalina* . The lowest content is obtained for the hydroethanolic extract of Zingiber officinale. The highest content (1258.85 µgEq/g) of total flavonoids is found in the hydroethanolic extract of the flower buds of *Syzygium aromaticum* while the lowest content (71.19 µgEq/g) is observed in the extract hydroethanolic agent of *Zingiber officinale*. As previously, *Syzygium aromaticum* presents the highest total tannin content followed by the hydroethanolic extract of *Zingiber officinale* and Thevetia peruviana.

Results from the quantification of total phenolic compounds and total flavonoids of the hydroethanolic extract of *Syzygium aromaticum* at the end of this work are respectively higher than those obtained (15.174 mg EAG/g; 503µgEq/g) by Ramzi and Kamouche (2021) in Algeria. Likewise, the hydroethanolic extract of *Zingiber officinale* has a total flavonoid content higher than that obtained (35.2 µgEq/g) by Dehina (2019). On the other hand, the content of total phenolic compounds obtained by Bourai and Azzouk (2018) in this same species (8.41 mg EAG/g) is higher than that obtained in the present study. Concerning the hydroethanolic extract of the leaves of *Vernonia amygdalina*, the content of total phenolic compounds obtained during our work is 4 times higher than that obtained (3.61 mgEAG/g E) by Lubis (2022). Otherwise, the total flavonoid contents (94.08 mgEq/g) obtained by Alara *et al.* (2018) in Nigeria are higher than that obtained in the present study. As for *Thevetia peruviana*, its total flavonoid content is close to that reported (750 µgEq/g) by Srivastava *et al.* (2012) in India. On the other hand, the content of total phenolic compounds obtained (41mgEAG/g) by the same author is 4 times greater than that obtained during our work.

Table 2. Secondary metabolites identified

Metabolites	*Thevetia peruviana*	*Syzygium aromaticum*	*Vernonia amygdalina*	*Zingiber officinale*
Catechical tannins	Present	Present	Present	Present
Gallic tannins	Absent	Absent	Absent	Absent
Flavonoids	Present	Present	Present	Present
Anthocyanins	Present	Present	Present	Absent
Leuco anthocyanins	Absent	Absent	Present	Present
Reducing compounds	Present	Present	Absent	Absent
Mucilages	Absent	Absent	Absent	Absent
Coumarins	Present	Absent	Absent	Present
Absent Alkaloids	Absent	Absent	Absent	Absent
Cyanogenic derivatives	Absent	Absent	Absent	Absent
Saponosides	Absent	Present	Present	Absent
Anthraquinones	Present	Present	Present	Present
Sterol and terpenes	Present	Present	Absent	Absent

Table 3. Phenolic compound contents of the four plants studied

Plant	Total phenolics (mg EAG/g DM)	Flavonoids (µgEq/g DM)	Condensed tannins (EgL/g DM)
Thevetia peruviana	9.600 ± 0.100	616.120 ± 5.333	2.060 ± 0.020
Vernonia amygdalina	12.180 ± 0.128	391.99 ± 2.333	1.280 ± 0.020
Syzygium aromaticum	16.600 ± 0.210	1258.850 ± 13.660	12.760 ± 0.333
Zingiber officinale	5.220 ± 0.150	71.190 ± 1.721	5.060 ± 0.0120

Table 4. IC_{50} of the different plant extracts studied

Excerpts	Tp (EtOH)	Va (EtOH)	Her. EtOH)	Zo (EtOH)	Her. (HEY)	Zo (ET)	Gallic acid	Quercetin
IC_{50} (µg/µL)	N / A	3,000	45,000	25,000	0.380	0.100	0.022	0.029

Tp: *Thevetia peruviana;* Va: *Vernonia amygdalina;* Sa: *Syzygium aromaticum;* Zo: *Zingiber officinale* ; EtOH extract: Hydroethanolic extract; HE: Essential Oil; NA: Not Active

Anti-radical activity

Table 4 presents the result of the anti-radical activity of the hydroethanolic extracts and essential oils of the plants investigated. The antiradical capacity was determined from the concentration necessary to reduce 50% of the DPPH radical (IC_{50}). From the analysis of this table, it appears that the essential oils of *S. aromaticum* and *Z. officinale* have respectively an inhibitory capacity of 0.38 and 0.1 µg/µL while those of the hydroethanolic extracts are respectively 45 µg /µL and 25 µg/µL. *V. amygdalina* has an IC_{50} of 3 µg/µL. Only the essential oils of *Syzygium aromaticum* and *Zingiber officinale* had a performance close to that of the reference compounds (gallic acid and quercetin) which presented a respective median inhibitory concentration of 0.022 and 0.029 µg/µL.

From the evaluation of the reducing capacity of plant extracts, the work of Qing *et al.* (2014) relating to the hydroethanolic extract of *V. amygdalina*, showed an inhibitory concentration of 3.19µg/µL similar to that obtained during the present study. As for the essential oil of *Z. officinale*, Nesrine (2019) found an IC_{50} of 1.29 µg/µL which is 10 times less interesting than that obtained during our work. The work of Marrelli *et al.* (2015) reports an IC_{50} of 207.5 mg/mL for the hydroethanolic extract of *Z. officinale*, lower than that observed in our samples. The use of *Z. officinale extracts* would give the toothpaste effective antioxidant activity to combat free radical attacks suffered by the oral cavity. Regarding the essential oil of *S. aromaticum*, the work of Selles *et al.* (2020) revealed an inhibitory concentration of 0.00482 µg/µL more pronounced than that obtained during our work. As for the ethanolic extract of the latter, the work of Taroq (2020) also showed an IC_{50} of 0.12µg/µL, better than that found during our work. The antioxidant activity of the essential oil of the flower buds of *S. aromaticum* is due to the contribution of compounds such as eugenol and eugenyl acetate (Nurdjannah and Bermawie, 2012).

Toothpaste formulation

After documentation, several formulas were tested and the physical characteristics of the toothpastes obtained were compared to some existing toothpastes on the market. The best formula was chosen for the formulation of the toothpaste. The proportions of ingredients for the formulation are presented in Table 5.

Table 5. Proportions of ingredients for the formulation

Composition	Percentage (%)
Xanthan gum	0.8
Water	22
Sorbitol	47
Plant extracts	2
Silica	24
Mint crystals	1.5
Sodium dodecyl sulfate	2
Dye	0.5

Physico-chemical properties of the formulated toothpaste

The physicochemical properties of the formulated product compared to those of other toothpastes present on the Beninese market are recorded in Table 6 below. From the physicochemical analysis of the toothpaste, we observe a pH which meets the normative requirements (Madagascar Standards Bureau, 2018). The foamability is higher than the minimum quantity (50 mL) of foam set by the Bureau of Indian Standards (BIS, 2006) while the spreading capacity is close to that found by Sharma *et al.* (2014). The water and volatile matter content obtained is close to commercial Colgate and Maxam pastes. We note good homogeneity of the formulated product and an almost absence of abrasive particles.

Study of the Stability of toothpaste

The product is exposed to room temperature for a period of 28 days. Table 7 presents the results of the study of the stability of the formulated product. The result reveals that the formulated paste remained intact for 28 days. Its organoleptic characteristics have not undergone any modification.

Antibacterial activity of the formulated toothpaste

The Minimum Inhibitory Concentrations (MIC) of toothpaste formulated with plant extracts are presented in Table 8. The antimicrobial tests carried out reveal that the Minimum Inhibitory Concentration of the toothpaste with respect to the microbial strains tested is 0.1 g/mL with the exception of *S. aureus* where the MIC is 0.125 g/mL. The MICs obtained reveal the effectiveness of the formulated toothpaste with respect to the microbial strains tested. Among these strains tested, *Candida albicans* is directly involved in oral diseases known as candidiasis while other microorganisms take advantage of the presence of an oral condition to act (Olayé *et al.*, 2020; Kolenbrander *et al.*, 1993; London, 1993).

Regarding the evaluation of the antibacterial activity of formulated toothpaste enriched with plant extracts, previous work has reported the inhibitory concentrations of these extracts against strains and bacteria. The work of Pundir *et al.* (2010) relating to the determination of the antibacterial activity of the non-volatile extract of *S. aromaticum*, reports a respective inhibitory concentration of 0.010 g/mL and 0.005g/mL against *Escherichia coli* and *Staphylococcus aureus*. The essential oil of *Zingiber officinale* against *E. coli* and *S. aureus* showed a respective minimum inhibitory concentration of 0.002g/mL and 0.001g/mL and a minimum bactericidal concentration of 0.004g/mL and 0.002g/mL according to the work of Wang *et al.* (2020). The work of Kokutse (2016) in Benin reveals an MIC of 0.091 g/mL for the essential oil of *Syzygium aromaticum* against *E. coli* and *S. aureus*. The extracts of *T. peruviana* were tested against *C. albicans, S. aureus* and *P. aeruginosa* by Alhashimi *et al.*, (2013), this work reported a respective MIC of 0.200, 0.150 and 0.200 g/mL. Thus, the results of previous work have clearly shown that plant extracts are effective in restoring the balance of the microbial flora. The richness of plants in secondary metabolites could be at the origin of their antimicrobial activities and the effectiveness of the formulated toothpaste with respect to microbial strains would therefore be due to the effectiveness of these plant extracts which are used in its formulation.

Table 6. Physico-chemical characteristics of toothpaste

Physico-chemical parameters	Toothpastes				
	Formulated	Colgate	Close-up	Charlie	Maxam
Foaming power (mL)	100	100	100	100	100
pH	7.20	9.92	7.92	8.70	9.72
Moisture content and volatile matter	36.58%	36.30%	33.81%	29.88%	36.70%
Spreading capacity (cm)	4.5	6.0	5.5	4.8	5.5
Homogeneity	Good	Good	Good	Good	Good
Abrasive particle	Absent	Absent	Absent	Absent	Absent

Table 7. Study of toothpaste stability

Attributes	Smell	Color	Texture	Flavor	Homogeneity
1st week	Mint	Lime green	Dough	Fresh sweet	Homogeneous
2nd week	Mint	Lime green	Dough	Fresh sweet	Homogeneous
3rd week	Mint	Lime green	Dough	Fresh sweet	Homogeneous
4th week	Mint	Lime green	Dough	Fresh sweet	Homogeneous

Table 8. Minimum inhibitory concentration, MIC of the formulated toothpaste

Microbial strain	MIC (g/mL)
Candida albicans	0.100
Staphylococcus aureus	0.125
Escherichia coli	0.100
Pseudomonas aeruginosa	0.100

MIC: minimum inhibitory concentration

Hygienic quality and saftinness of the formulated toothpaste

The results from the enumeration of microorganisms associated with the product are recorded in Table 9. The bacterial loads are expressed in colony format units per gram of toothpaste (CFU/g). The formulated product is contamination-free and meets the normative requirements. Quantitative data from the counting of germs, total and fecal coliforms, yeasts and molds meet normative requirements (AFNOR, 2016).

Table 9. Microbiological analysis of formulated toothpaste

Sprouts	Load (cfu/g)	AFNOR standards (2016)
Total aerobic mesophilic flora	3.10^{2}	10^{5}
Total and fecal coliforms	<1	10^{2}
Yeasts	5	10^{3}
Mold	<1	10^{3}
Staphylococcus spp.	2	10

Sensory evaluation

The sensory analysis carried out on the formulated toothpaste reveals that overall, the tasters especially appreciated the fresh sensation, the color and the smell. Sensory tests were carried out at the end of the formulation. The attributes considered are: color, taste, smell and texture.

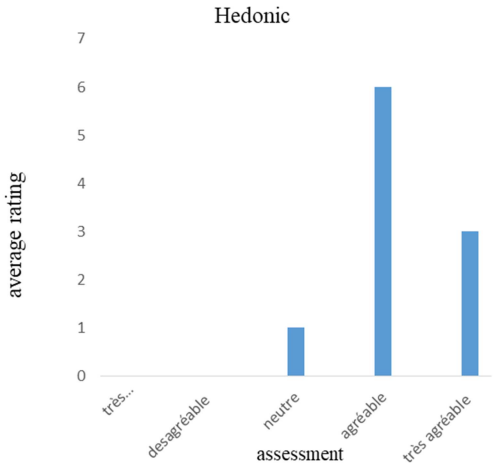

Fig. 1. Formulated toothpaste acceptability diagram

Fig. 2. Sensory profile of the formulated product

Hedonic analysis

The results of the hedonic analysis are characterized by the evaluation of consumé acceptability. The 5-level

scale (very unpleasant, unpleasant, neutral, pleasant, very pleasant) was adopted for product evaluation. The results of the hedonic evaluation are illustrated by the diagram in Fig. 1. Overall, consumers appreciated the formulated product. 60% of consumers found the toothpaste pleasant while 30% found it very pleasant.

Table 10. Organoleptic properties

Organoleptic properties	Global appreciation
Texture	Dough
Taste	Sugar
Smell	Fresh minty
Color	Light green

Descriptive test

Fig. 2 describes the results of the sensory analyzes carried out. The solid lines in bold represent the average values of consumé assessments. From these values, we were able to establish the sensory profile of the toothpaste formulated using Excel software. The herbal-formulated toothpaste is light green in color, has a minty odor and a slightly sweet taste.The average of the results from the panel of tasters is recorded in Table 10.

Conclusion

Beninese flora contains a wide variety of plants used in traditional medicine to treat illnesses. Our work focused on the formulation, physicochemical and microbiological characterization of a toothpaste formulated based on four medicinal plants used to treat oral and dental conditions. The product obtained at the end of this study meets the normative requirements with regard to physicochemical characteristics and microbiological quality. Organoleptically, it is appreciated by a panel of tasters formed for this purpose. It retained its stability after 28 days of exposure to room temperature. The evaluation of toxicity will make it possible to formulate other dosage forms of toothpastes (gels and powder) and to move from laboratory scale production to pilot scale.

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