Health Sciences & Disease
The Journal of Medicine and Biomedical Sciences

Original Research

Antibacterial Potential of Hydro-Ethanolic Extracts from *Syzygium Aromaticum* (*Myrtaceae*) on Periodontal Bacteria

Évaluation du potentiel antibactérien des extraits hydro-éthanoliques de Syzygium aromaticum (Myrtaceae) sur les bactéries parodontales

Fotsing Kwetché PR [1,2,3], Essama Eno Belinga L [4], Yawat Djogang AM [2,3], Chamdjeu VT [1], Agbor MA [1], Tchoukoua SH [1,2,3], Tekam JM [2]

[1] School of Dentistry, Higher Institute of Health Sciences, Université des Montagnes; Bangangté, Cameroon
[2] School of Pharmacy, Higher Institute of Health Sciences, Université des Montagnes; Bangangté, Cameroon è
[3] Laboratory of Microbiology, Université des Montagnes Teaching Hospital; Bangangté, Cameroon
[4] Department of Surgery and Specialities, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon

Correspondant author :
Dr Yawat Djogang Anselme Michel
Tel: +237 697149412 / +237 675247865
Mail : ydamanselme@gmail.com

Keywords: Antibacterial activity, Hydro-ethanolic extract, Syzygium aromaticum (Myrtaceae), periodontal bacteria, Penja
Mots-clés : Activité antibactérienne, Extrait hydro-éthanolique, *Syzygium aromaticum* (*Myrtaceae*) , bactéries parodontales, Penja

ABSTRACT

Introduction. The emergence of bacterial resistance is one of the main current major public health threats in controlling infectious diseases, including periodontal diseases. The objective of our study was to assess potentiel antibacterial activity of hydro-ethanolic extracts of Syzygium aromaticum (Myrtaceae) on periodontal bacteria. **Materials and methods.**:This was an experimental study conducted in 2019 over a period of 06 months at the Université des Montagnes, (Cameroon). The plant material was harvested in Penja (Littoral Cameroon). Periodontal germs were isolated from the periodontal pockets of patients with periodontitis. The phytochemical screening was carried out according to the technique of Bruneton. The antibacterial activity was studied using the disk diffusion method on agar medium and the macro dilution technique. **Results.** Phytochemical tests revealed at varied concentrations, the presence of polyphenols, flavonoids, gallic tannins, alkaloids, coumarins triterpenes and sterols in the maceration product. For the antibacterial potential, the MIC values for the tested organisms ranged from 6.25 through 25 mg/mL. The dry non-delipidated extract was the most effective with a bactericidal effect on all bacteria subjected. Bactericidal property was also recorded with the delipidated extract on *F. nucleatum* and the fresh extract on *A. actinomycetemcomitans.* **Conclusion.** The hydro-ethanolic extracts of Syzygium aromaticum (Myrtaceae) have antibacterial activity on periodontal bacteria.

RÉSUMÉ

Introduction. L'émergence des résistances bactériennes est l'une des principales menaces actuelles pour la santé publique en matière de lutte contre les maladies infectieuses dont les maladies parodontales. L'objectif de notre étude était d'évaluer l'activité antibactérienne potentielle des extraits hydro-éthanoliques de *Syzygium aromaticum* (*Myrtaceae*) sur les bactéries parodontopathogènes. **Matériels et méthodes.** Il s'est agi d'une étude expérimentale menée en 2019 sur une période de 06 mois à l'Université des Montagnes au Cameroun. Le matériel végétal a été récolté à Penja (Littoral Cameroun). Les germes parodontaux ont été isolés à partir des poches parodontales des patients souffrant de parodontite. Le criblage phytochimique a été mené selon la technique de Bruneton. L'activité antibactérienne a été étudiée selon la méthode de diffusion des disques sur le milieu gélosé et la technique de macro dilution. **Résultats.** Des tests phytochimiques ont révélé, à des concentrations variées, la présence de polyphénols, de flavonoïdes, de tanins galliques, d'alcaloïdes, de coumarines, de triterpènes et de stérols dans le produit de macération. Les valeurs de CMI pour des organismes testés se situaient entre 6,25 et 25 mg/ml. L'extrait sec non délipidé était le plus efficace, avec un effet bactéricide sur tous les isolats. La propriété bactéricide a également été enregistrée avec l'extrait délipidé sur *F. nucleatum* et l'extrait frais sur *A. actinomycetemcomitans*. **Conclusion.** Les extraits hydro-éthanoliques de *Syzygium aromaticum* (*Myrtaceae*) ont une activité antibactérienne sur les bactéries parodontales.

INTRODUCTION

Oral diseases such like dental caries, periodontal diseases, oral mucosal lesions, oropharyngeal cancers, immunodeficiency syndrome (HIV/AIDS)-related oral diseases and orodental trauma are major public health issues in underprivileged and poor communities in developed and developing countries [1]. Periodontitis is defined as a progressive inflammation of tooth attachment systems, caused by bacteria. Bacterial aetiologies of periodontitis include *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*), *Fusobacterium nucleatum* (*F. nucleatum*) and *Prevotella intermedia* (*P. intermedia*), with could engender irreversible damage of attachment system of the tooth

(gum, cementum, alveolar bone and alveolar ligament) and eventually lead to dental mobility and tooth loss [2]. Owing to the complexity of the oral environment in which a very large number of microorganisms coexist and interact, periodontal health appears as a fragile outcome of the balance between the aggressiveness of some components of this ecosystem, the responses from the host and associated risk factors (genetic, diabetes, tobacco) [2]. Any disruption of this balance could result into inflammatory process and clinical manifestations (loss of attachment system of the periodontium and bone resorption) involving certain bacterial agents (particularly anaerobic Gram-positive bacteria) and invigorated by other related niche-related conducive factors [2].

Depending on the sources, it is estimated that periodontitis affect to varying degrees, 20 to 50% of adults in most countries throughout the world [3]. Investigations conducted in Cameroon in 2018 revealed that 15% of the population actually developed periodontitis [4]. Treatment options encompass mechanical therapy like scaling, root planning based on the removal of bacterial plaque, calculus and toxins [5]; chemical procedures which include the use of antiseptic like chlorhexidine [6] and the use of antibacterial agents like penicillin's, the cyclin's and imidazole's families of drugs [7]. In addition, most of the aetiologies of these diseases have developed resistance to several antibacterial agents [8]. Constraints like the high cost of oral healthcare, limited financial resources, scarcity of adequate health facilities, insufficient skilled health personnel, and trends of resistance to common antibacterial drugs reduce the rate of attendance at dental facilities then and the likelihood for disease control. These constraints encourage the common use herbal drugs in traditional medicines (TM) [9].

Based on the fact that close to 80% of people in resource-limited countries rely on traditional medicine for their daily healthcare, the WHO encouraged investigations which aim at identifying and developing new available and affordable drugs to most populations throughout the world [10]. The present survey falls in line with this WHO's option and focuses on *Syzygium aromaticum* (*S. aromaticum*), commonly called "cloves". *S. aromaticum* is a plant from the *Myrtaceae* family. Throughout generations it had being used as anaesthetic, analgesic, anti-inflammatory and antibacterial. The presence of secondary metabolites such as alkaloids, phenols, flavonoids, coumarins in this plant could be anticipated to justify its antibacterial potential [11]. In order to attest its efficacy on bacterial aetiologies of periodontitis, an *in vitro* assessment was conducted on its extracts. More specifically, the parameters investigated were the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and the inhibition zone diameters on some bacterial isolates. Intermediate and long term benefits in connection with the test results will be oriented towards developing new scientifically proven alternatives drugs (improved traditional medicines) from the Cameroon biodiversity inheritance.

MATERIALS AND METHODS

Plant material and working sites

The flower buds of *S. Aromaticum* used in the present investigation were harvested in Penja, Moungo Division (Littoral–Cameroon) in January 2019. Authentication and identification were subsequently conducted at the National Herbarium of Cameroon (reference N°: 66995/HNC).

For laboratory works, extract preparation, fractionation and chemical screening were performed in the Laboratory of Chemistry and the Laboratory of Pharmacognosy at the Université des Montagnes and the antibacterial potential tests conducted in the Laboratory of Microbiology of the Université des Montagnes (UdM) Teaching Hospital.

Type of study, sample collection and subjected bacteria

This experimental study was conducted from January 1st to June 30th 2019. Isolates were collected from patients with periodontitis who visited dental Clinic of the Université des Montagnes (UdM) Teaching Hospital, Banekane and who accepted to participated to the study. These isolates collection was conducted according to the following protocol.

Supragingival plaque was removed with a sterile cotton ball impregnated with sterile physiological saline (0.9%, NaCl). The sampling sites were then isolated with salivary cotton rolls. With a tweezer, 4 points of paper were inserted one after the other, down to the bottom of the periodontal pocket. At that position, it was kept for 20 seconds, then removed and transferred into Brain Heart Infusion contained in test tubes and conveyed immediately the laboratory for microbial screening. All isolations and identifications were performed according to the reference guides (REMIC, 2019).

Extraction procedure

With regards to the floral buds of *S. aromaticum*, a part of this raw material was washed with distilled water in order to remove dust and debris, reducing thereby the microbial load. Another one was allowed to dry for 30 days at room temperature (≈ 23°C) to preserve the integrity of inherent product properties. All were thereafter crushed with a Royalty Line® brand crusher until a fine powder was obtained. A part of the resulting powder was delipidated while the other one was not. Delipidation was conducted by Soxhlet.

Preparation of hydro-ethanolic extracts

The procedure began with the non-delipidated hydro-ethanolic extract. In this, 200 g of powder were put into the maceration flask. Into that flask, 4 L of a mixture made up of distilled water and ethanol (30% and 70%, respectively) was added. With the flask closed, the preparation was stirred, then, left to macerate for 48 hours. Upon completion of maceration, the macerated product was filtered through a Whatman paper N° 3 adjusted to a vacuum pump until a liquid filtrate free of brown particles was obtained. This filtrate was then passed to the rotavapor at 39°C and the resulting

concentrate was transferred into an oven (60°C) for 72 h to get the extracts used in the experiment.

The preparation of the delipidated hydro-ethanolic extracts underwent the same protocol except that before maceration, the powder was delipidated by the Soxhlet method [10]. Briefly, 200 g of powder from dry flower buds and 55 g of powder from fresh buds were subjected to solid-liquid extraction by maceration in hydro-ethanol solution (30% and 70%) for 48 hours. The macerates of the fresh floral buds, and that of dry floral buds (non-delipidated and delipidated) underwent vacuum filtration, then concentrated in the rotary evaporator at 39°C, dried in the oven at 60°C for 48 hours.

Phytochemical screening

Investigations through the target chemical groups in the plant extracts were conducted according to Brunneton 1999 [20], and Ngoupayo *et al.*, 2015 [13]. This was done for eight secondary metabolites namely Alkaloids, Polyphenols, Flavonoids, Reducing sugars, Tannins, Saponins, Coumarins and Terpenes [14].

Antibacterial Activity

Bacterial inoculum preparation and MIC assessment

All isolates in pure culture were streaked on Mueller Hinton agar (Liofilchem ®) supplemented with 5% sheep blood and incubated overnight under 5% CO_2. From the resulting bacterial population, a suspension equal to 0.5 McFarland (˜10^6 -10^8 CFU/mL) was prepared and adjusted to the final density recommended for susceptibility tests by the " Comité de l'Antibiogramme de la Société Française de Microbiologie, CA-SFM (2014) ".

To assess the MIC, the macro-dilution technique in liquid medium was used with slight modifications. The stock solutions were made in Mueller Hinton broth with DMSO (5 % v/v). They were prepared at 400 mg/mL.[12] Two millilitres of Muller Hinton broth was dispensed into each of the series of ten tubes of the dilution range as well as into the three control tubes. Then a volume of 2 mL of the extract at a concentration of 5 g per 12.5 mL was dispensed into the first tube of the dilution range. From this initial preparation, serial dilutions were conducted in Muller Hinton broth. Upon dilution completion, 15 µL of the above prepared bacterial inoculum was dispensed into each tube in the dilution range and into the positive control tube. Three drops of mineral oil were subsequently added to each tube for anaerobiosis and the set underwent incubation at 37°C for 18 to 24 hours. The MIC for each extract was determined from the first tube of the range in which no visible bacterial growth was observed (attested by absence of turbidity). All tests were conducted in triplicate

Determination of minimal bactericidal concentration (MBC)

About 5 µL of the dilutions of the extract in which no bacterial growth was recorded were streaked on MHA supplemented with 5% sheep blood. The streaked preparations were incubated anaerobically under 5% CO_2 at 37°C for 18 to 24 h. The MBC for each extract was recorded from the lowest concentration at which no culture was observed on MHA upon incubation completion. These experiments were also conducted in triplicate.

Evaluation of MBC/MIC ratios

This ratio made it possible to attest the bacteriostatic or bactericidal property of each extract. When it was larger than or equal to 4, the extract was referred to as bacteriostatic. It was regarded as bactericidal when the value was less than 4. When it was equal to 1, the extract was said exert absolute bactericidal effect on the tested organisms.

Inhibition zone diameter

The aqueous solution was dissolved into a convenient MHB volume and concentration. The original solution was used to prepare a serial dilution (d/2 step) in the appropriate broth as to, eventually obtain extract concentrations ranging from 400 mg/mL through 0.39 mg/mL, as for the MIC test. About 15 µL of each of these solutions at the MIC and MBC concentrations was used to inoculate five paper disks (Whatman N°2) of six millimetre's diameter previously adjusted on the MH agar supplemented with 5% sheep blood on which the bacterial inoculum were lawn to confer a monolayer confluent growth upon overnight incubation (0.5 McFarland) under 5% CO_2 at 37°C, according to the CA-SFM (2014).

The results were read upon incubation completion by measuring the inhibition zone diameters (in mm) that developed around the impregnated disks. The negative control consisted of a disk impregnated with sterile distilled water. Each of the above experiments was conducted three times. In this essay, Amoxicillin was used to guide activity comparison.

RESULTS

Extraction yield of the hydro-alcoholic extracts from *Syzygium aromaticum*

The extraction of the flower buds by maceration in the water/ethanol mixture (30/70) resulted in the products and yields summarized as indicated in table 1.

Table 1: Yield of the various extracts

Extract	Mass of the flower buds used (g)	Mass of the dry extract obtained (g)	Yield (%)
Fresh floral buds	55	4.75	8.6
Dry floral buds not delipidated	200	77.628	38.8
Delipidated dry floral buds	200	59.99	30

This table reveals that the highest yield was recorded with the non delipidated dry floral buds and the lowest with the fresh floral buds.

Phytochemical screening of total extracts

The semi-quantitative phytochemical screening of the various products from *S. aromaticum* indicated key facts from which major characteristics were summarized and presented in table 2.

Table 2: Phytochemical categories in the extracts

Phytochemical Categories	Fresh Extracts	Dry non delipidated Extracts	Delipidated Extracts
Alkaloids	-	++	+
Alkaloids	+	+	+
Saponins	-	-	-
Tri-terpenes and sterols	+	++	++
Flavonoids	-	++	-
Polyphenols	+++	+++	+++
Catechic tannins	-	-	-
Gallic tannin	+++	+	+++
Coumarins	+	+	+

+++: Intensively positive reaction, ++: Positive response; +: Suspicious reaction - : Negative reaction

The overall picture indicated specific richness in polyphenols for the three products under study. Gallic tannins, tri-terpens, sterols, alkaloids and coumarins were detected in all of these products as well; while saponins and catechic tannins were found in none.

Polyphenol content of the various extracts

With the gallic acid as standard, the quantification of polyphenols revealed the total polyphenol content in hydroalcoholic extracts. In these, the values were expressed in grams of gallic acid equivalent per gram of extract (mg EqAG /g of extract). The calibration associated was presented as y = ax + b. This line was used to estimate the polyphenol content in each of the extracts. Finding from calculations were shown as presented in figure 1.

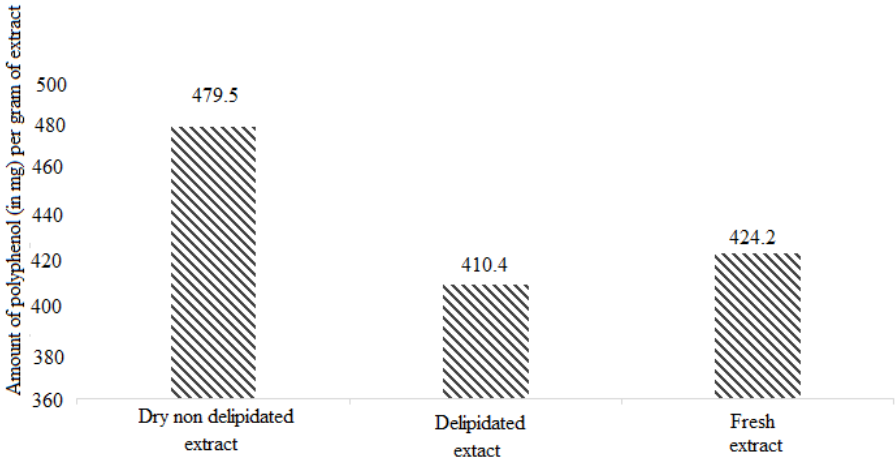

Figure 1 : Comparative diagram of the polyphenol contents of crude extracts

Polyphenol content was highest in the dry non delipidated extract, while the values were closer in the delipidated and the fresh extract.

Table 3 further provides details on the optical density during the assessment.

Table 3: Repeatability of the Folin-ciocalteu method

Concentration (g/l)	Mean OD (nm)
25	0.055 ± 0.011
50	0.129 ± 0.015
75	0.202 ± 0.006
100	0.261 ± 0.008

OD: Optical density

Typically, the optical density increased with the product concentration in the raw material.

Isolated bacteria

Microbiological screening of the specimens resulted in identification of three aetiologies of periodontitis. Namely, they were: *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Fusobacterium nucleatum* (*F. nucleatum*) and *Prevotella intermedia* (*P. intermedia*).

***In vitro* investigation through the antibacterial potential of the extracts**

The MICs, MBCs and the MBC/MIC values recorded for each extract were summarized as displayed as shown in table 4.

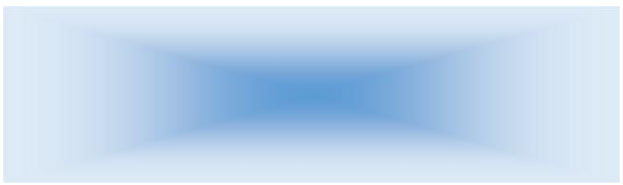

Table 4: CMI, CMB and CMB/CMI values of the hydro-alcoholic extracts of *Syzygium aromaticum*

Bacteria	Extract								
	Dry, non delipidated			Dry delipidated			Fresh		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
A. actinomycetemcomitan	6.25	12.5	2	12.5	50	4	25	50	**2**
F. nucleatum	6.25	12.5	2	25	50	**2**	6.25	25	4
P. intermedia	6.25	12.5	2	25	100	4	6.25	25	4

MIC : minimal inhibitory concentration , MBC: minimal bactericidal concentration

To varying degrees, hydro-alcoholic extracts exerted antibacterial potential on bacteria subjected to the study. Overall with the three extracts in fact, the MIC values ranged from 6.25 mg/mL through 25 mg/mL. The same MIC value was recorded for *A. actinomycetemcomitans*, *F. nucleatum* and *P. intermedia* with the non-delipidated dry extract. The highest MIC was observed with the fresh extract on *A. actinomycetemcomitans*. Further details indicated highest MBC's with the dry delipidated extract on all isolates, and the MBC value for *P. intermedia*, twice larger than the ones recorded with *A. actinomycetemcomitans* and *F. nucleatum*. Based on the MBC/MIC, bacteriostatic and bactericidal potentials were recorded.

Ethical Considerations

The study was conducted in accordance with the Helsinki Declaration and approved by the Ethical Committee of the Université des Montagnes (Bangangté, Cameroun).

DISCUSSION

To contribute to valorising herbal resources used in traditional medicine, the present investigation focussed on assessing the antibacterial potential of hydro-ethanolic extracts from *Syzygium aromaticum* on bacterial aetiologies of periodontal diseases. The findings highlighted that extraction of polyphenols could be efficiently conducted by maceration. Though ultrasounds and Soxhlet's could provide like or better results, maceration was chosen because it is easier to implement. According to Bourgou in 2016 in fact, it offers a better yield than the Soxhlet. [13].

Hydro-alcoholic solvents are generally used for the extraction of phenolic compounds. [14] Thus, the ethanol-water mixture (70:30) offers a high concentration of polyphenols, particularly in flavonoids, although this yield is less important than the one obtained when methanol is used. With hydro-alcoholic solvent, the yields were 8.6%; 30% and 38.8% for the fresh extract, dry fatty extract and fat-free dry extract, respectively. Otherwise, the highest yield was recorded with the dry non-delipidated's. This finding agrees with the one reported by Chikere and *al*. in Nigeria (37.67%) when methanol was used as extraction solvent [15], but the value recorded is lower than the one documented with the use of ethanol by Houngbèmè and al. [16] The lowest yield was found with the fresh extract. This could be explained, at least in part, on the one hand by the fact that the fresh extract is richer in water which, otherwise, probably diluted the extraction solvent concentration along the process and, on the other, that the extraction solvent played an important role in the solubilisation of the active compounds present in the plant.[17]

A glance throughout data pointed out that gallic tannins, coumarins, tri-terpenes, sterols, alkaloids and polyphenols were present in all three extracts while catechetical tannins and saponins were not detected in anyone. Further, not detected in the fresh extract, alkaloids were found in dry non-delipidated and the dry delipidated extracts. Otherwise and in line with above discussion, higher water content likely reduced the detection threshold of alkaloids in the fresh extract. Flavonoids were absent in the fresh and the dry delipidated extracts, and detected in the dry non-delipidated's. Arguments about the role of water on detection threshold could be anticipated once again as done above, but also, delipidation might have depleted the extract from this secondary metabolite. Also, both might have come in combination during the process. This difference could be due to the fact that the composition of a plant might change according to several factors such as climate, location, type of soil, time and period of harvest or even species differences. Other stochastic environmental stresses might have also influenced the plant material content in specific secondary metabolites. In fact, the compound concentrations which depend upon the ecological niche characteristics generally influence the threshold-dependent detection.

Quantifying polyphenols revealed that their content varied according with extract types; that is 478.48 mgEqAG/g, 410.32 mgEqAG/g and 422.16 mgEqAG/g, respectively in the dry non-delipidated extract, in the dry extract delipidated and in the fresh extract. Once again, these finding are consistent with above development concerning the role of water and delipidation. In fact, the lower content in polyphenol is likely due or related to depletion in flavonoids. In 2018, Medfouni reported similar findings with the dry extract (439.88 mgEqAG/g).[18] This slight difference recorded could be connected to the solvent used for extraction. However, not all arguments are readily clear for accurate comparison with those found in literature because the use of different extraction methods reduces the essay reliability. Several factors could therefore impact the content of phenolic compounds. Recent studies found that extrinsic factors (geographical and climatic factors), genetic factors, but also the degree of spice maturation and storage time could play significant roles on the polyphenol content [19].

Phytochemical compounds found in hydro-alcoholic extracts of *Syzygium aromaticum* could be endowed with several biological potentials including antibacterial activity. Susceptibility tests carried out with the extracts

revealed that for all the non-volatile extracts tested, the minimum inhibitory concentrations (MICs) globally varied between 6.25 and 25 mg/mL. Among these extracts, those with MICs at 6.25 mg/mL were regarded as more active against the tested bacteria. According to Awa Wade who worked on *Shigella*, the MIC values were 45 µg/mL with methanolic extract, 94 µg/mL with aqueous extract, 370 µg/mL with chloroform fraction and 187 µg/mL for acetone fraction, in line with allegations about the extract potential which could be strongly related to the extraction solvent [20].

Aligiannis *et al*. suggested à MIC value-dependent classification of crude extract activity as highly inhibitory (MIC<0.500 mg/mL); moderately inhibitory (MIC ranges from 0.600 - 1.500 mg/mL); weakly inhibition (MIC >1.600 mg/mL).[21] According to this scheme the extracts used in the present survey would be regarded as weakly inhibitory on the bacterial subjected to the study.Also the MBC/CMI ratios recorded for the delipidated dry extract in the present research appeared to be bacteriostatic on *A. actinomycetemcomitans* and *P. intermedia.* This property was also documented with the fresh extract when it was tested on *F. nucleatum* and *P. intermedia.* Likely discussed, the non-delipidated dry extract displayed a bactericidal potential on all bacteria subjected, while similar potential was observed for the dry delipidated on *F. nucleatum* and fresh extract on *A. actinomycetemcomitans.* These findings are consistent with those reported in Nigeria by Ugwu *et al*. in 2017.[22] An overall glance on the potential clearly indicated that the dry non-delipidated extract was most potent. Otherwise, removal of lipids probably reduced the inhibitory potential, just as the water in the fresh extract as discussed earlier. Accordingly, amongst polyphenols, flavonoids could play a significant role in to antibacterial potential recorded. On the other hand, the fact that, polyphenols contents were, in general, not influenced by delipidation might imply that depletion of flavonoids could parallel the increase concentration in other member(s) of this large group of chemical compounds. Based on data from the present survey, however, it would be difficult to identify and accurately appreciated these hypothetical influences in their types and magnitudes.

As observed earlier in this discussion, delipidation depleted the extracts from flavonoids. Known for their potentials on bacteria, this depletion could explain, at least in part the lower potential observed with the delipidated dry extract. The flavonoids and other phenolic compounds in plants have also been reported to exert several biological effects including antioxidant, free radical scavenging, anti-inflammatory and anti-carcinogenic.[23] Moreover, they are shown to inhibit the initiation step, promotion and progression of tumours.[24] attach to extra-cellular and membrane-borne proteins. Collectively, these effects result in microbial inactivation [25].

Tri-terpens and other secondary metabolites not screened for in the present study could have also played, as individual entity or in combination (synergetic or antagonistic with the others) significant role in the potential recorded. The antimicrobial activity of the extracts could also be explained by the presence of tannins. The mechanism of action of tannins is based on their ability to bind proteins and inhibit cell protein synthesis.[26]. Alone, members could act and increase the permeability of the cellular envelope and cause bacterial death by lysis (like beta-lactams and glycopeptides), impair the supercoiling process of the bacterial ADN (like quinolones) or act as anti-metabolites (like sulfonamides) [27].

The values of the inhibition diameters recorded were proportional to the CMI's and MBC's. Globally they varied from one bacterial type to the other, probably in connection with the mode of action of the secondary metabolites on the bacterial cells. According to another classification scheme (Moreira *et al.*, 2005), *A. actinomycetemcomitan* and *P. intermedia* (20 mm) could be said to be extremely susceptible (25 mm), and *F. nucleatum* (16 mm) highly susceptible. It should, however, be pointed out that for these conclusions to be widely accepted, specific standards for each extract should be investigated, and commonly accepted. That should be done in comparison with known current conventional antibacterial agents, acknowledging that investigations into plant extracts are in line with efforts to developing alternative to conventional drugs, and particularly with the evergreen trends of microbial resistance and growing infectious disease threats.

CONCLUSION

Findings from the present work proved that extracts used exerted, to varying degrees, bactericidal activity on all bacteria although the most potent was the dry non delipidated's. Bacteriostatic potential of the delipidated dry extract was recorded on *A. actinomycetemcomitans* and *P. intermedia; and* the fresh extract on *F. nucleatum* and *P. intermedia*. Delipidation and water content seemed to reduce the antibacterial potential in connection with flavonoids, while the most susceptible bacteria was *A. actinomycetemcomitan*. Overall, the hydro-ethanolic extracts of *S. aromaticum* could be used to control periodontal infections. However, other parameters like the inherent standard and side effects should be carefully investigated for sustainability.

AUTHOR CONTRIBUTIONS

Design: Pr Tekam J M, Dr Fotsing Kwetché P R , Dr Essama Eno Belinga L., Dr Yawat Djogang AM , Dr Chamdjeu V T.

Writing of the article: Dr Fotsing Kwetché P R , Dr Essama Eno Belinga L., Yawat. Djogang AM

Review of the article: Dr Agbor MA, Dr Tchoukoua. Serge Honoré.

Critical analysis and supervision: Pr Tekam JM

Acknowledgments

The authors are thankful to Professor Lazare Kaptué, Head of the University Teaching Hospital for the invaluable support provided during this survey. They are also highly indebted to the "Association pour l' Education et le Développement" (AED) for the literature resources and laboratory equipment.

REFERENCES

1. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ. 2005;83:661–9.
2. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000. 1997;14(1):216–48.
3. Dye BA, Tan S, Smith V, Barker LK, Thornton-Evans G, Eke PI and al. Trends in oral health status; United States, 1988-1994 and 1999-2004. 2007;
4. Belinga LEE, Ngan WB, Lemougoum D, Nlo'o ASPE, Bongue B, Ngono A and al. Association between periodontal diseases and cardiovascular diseases in Cameroon. J Public Health Africa. 2018;9(1).
5. Santos A. Evidence-based control of plaque and gingivitis. J Clin Periodontol. 2003;30:13–6.
6. Zaura-Arite E, Van Marle J, Ten Cate JM. Confocal microscopy study of undisturbed and chlorhexidine-treated dental biofilm. J Dent Res. 2001;80(5):1436–40.
7. Loesche WJ, Syed SA, Morrison EC, Kerry GA, Higgins T, Stoll J. Metronidazole in periodontitis: I. clinical and bacteriological results after 15 to 30 Weeks. J Periodontol. 1984;55(6):325–35.
8. World Health Organization. Programme on Traditional Medicine. (1998). Réglementation des médicaments à base de plantes : la situation dans le monde. Genève : Organisation mondiale de la Santé. https://apps.who.int/iris/handle/10665/67060
9. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. *Syzygium aromaticum L.* (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020 Jan 30;10(2):202.
10. Agbizounon A. Tests d'efficacité d'extraits d'huiles végétales sur Anophèles gambiae Giles et Culex quinquefasciatus Say, résistants aux pyréthrinoîdes.[Mémoire]. Abomey-Calavi. Université Abomey-Calavi;2010.
11. Jean Bruneton. Pharmacognosie, phytochimie, plantes médicinales (4e ed.). Lavoisier :Technique et Documentation ; 2009
12. Kuaté TCR, Fotsing KPR, Ngoupayo J, Ndelo J, Kouamouo J, Gamwo DS. Phytochemical screening, antibacterial and antifungal activity of crude aqueous and methanol extracts of stem bark of Garcinia brevipedicellata. Int J Pharm Biomed Res. 2013;4(4):221–6.
13. Bourgou S, Beji RS, Medini F, Ksouri R. Effet du solvant et de la méthode d' extraction sur la teneur en composés phénoliques et les potentialités antioxydantes d'*Euphorbia helioscopia.* J New Sci.2016;28(12):1649–55.
14. Nnanga N, Ngolsou F, Lobe VS, Maniépie N, Ndongo MN, Meva'a FE, et al. Identification des Composés Bioactifs Pouvant Justifier l'Usage des Feuilles de *Psychotria Calceata* en Médecine Traditionnelle au Cameroun. Heal Sci Dis. 2020;21(9):1–4.
15. Chikere OU, Liman FL, Nnabuihe ED, Uchechi EE, Jesse AC. The Effects of Methanolic Extract of Syzygium aromaticum (Clove bud) on the Histology of Testis in Adult Male Wistar Rats. J.Nat.Sci.Res. 2015;5(12):1–7.
16. Houngbeme AG, Gandonou C, Yehouenou B, Kpoviessi SDS, Sohounhloue D, Moudachirou M, et al. Phytochemical analysis, toxicity and antibacterial activity of Benin medicinal plants extracts used in the treatment of sexually transmitted infections associated with HIV/AIDS. Int J Pharm Sci Res. 2014;5(5):1739.
17. Kouadio NJ, Guessennd NK, Kone MW, Moussa B, Koffi YM, Guede KB et al. Evaluation de l'activité des feuilles de Mallotus oppositifolius (Geisel .) Müll . -Arg (Euphorbiaceae) sur des bactéries multirésistantes et criblage phytochimique. IJBCS.2015;9:1252–62.
18. Medfouni. Contribution à l ' étude phytochimique et les activités biologiques d ' une plante médicinale Syzygium aromaticum.[Mémoire]. Oum El-Bouaghi. Université Larbi Ben Mhidi Oum El Bouaghi. 2018;
19. Bouzid W. Etude de l'Activité Biologique des Extraits du Fruit de Crataegus monogyna Jacq . 2009;1–88.
20. Awa Wade. Composition phytochimique et activité antibacterienne du clou de girofle(syzygium aromaticum).[Thèse]. Dakar. Universite Cheikh Anta Diop. 2018;
21. Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem. 2001 ;49(9):4168-70
22. Ugwu MC, Edeani GI, Ejikeugwu CP, Okezie U, Ejiofor SO. Antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* causing childhood diarrhoea in Awka Municipality, South-Eastern Nigeria. Clin Microbiol. 2017;6(277):2.
23. Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S. Herbs as an antioxidant arsenal for periodontal diseases. J Intercult Ethnopharmacol. 2016;5(1):92-6
24. Lata N, Dubey V. Preliminary phytochemical screening of Eichhornia crassipes: the world's worst aquatic weed. J Pharm Res. 2010;3(6):1240–2.
25. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res. 2008;7(3):1019–24.
26. Labed B, Gherraf N, Hameurlaine S, Ladjel S, Zellagui A. The antibacterial activity of water extracts of Traganum nudatum Del (Chenopodiaceae) growing in Algeria. Der Pharm Lett. 2010;2(6):142–5.
27. Das S, Tanwar J, Hameed S, Fatima Z, Manesar G. Antimicrobial potential of epigallocatechin-3-gallate (EGCG): a green tea polyphenol. J Biochem Pharmacol Res. 2014;2(3):167–74.