



Evaluation of the *in vitro* antibacterial activity of *Azadirachta indica* used for the treatment of alveolitis

Nokam Abena Marie Elvire^{1,4}
Gonsu Kamga Hortense³
Nnanga Nga Emmanuel⁶
Ngono Mballa Rose⁵
Messina Nomo Christelle
Francine¹
Fokunang Charles⁷

¹Dentistry Department, cite verte District Hospital of Yaoundé, Cameroon

²Scientific and Industrial Research Centre (CSIR) of Bruneton in Pretoria

³D Department of Microbiology, Parasitology, Haematology and Infectious Diseases (FMSB)

⁴Department of Oral, Maxillofacial and Periodontology Surgery of the Faculty of Medicine and Biomedical Sciences (FMBS)

⁵Department of Traditional African Medicine and Traditional Pharmacopoeia (FMBS)

⁶Department of Galenic Pharmacy and Pharmaceutical Legislation (FMSB)

⁷Department of Pharmacotoxicology and Pharmacokinetics (FMSB)

Summary

Introduction: Alveolitis is a circumscribed peripheral osteitis occurring 2-3 days after teeth extraction. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* are among the incriminated bacteria. However, ill-adapted antibiotic therapy generates multi-resistance. Research is therefore focusing on the use of medicinal plants. **The aim** of the study was to evaluate the *in vitro* antibacterial activity of the essential oil of *Azadirachta indica* (Neem) on the said bacteria.

Methodology: This was an experimental study carried out at the FMBS in Yaoundé and the CSIR of Bruneton in Pretoria for 7 months in 2019. Chemical screening was done by CPG/SM. The antibacterial activity was studied according to the method of disc diffusion on this agar medium and the macro dilution technique. Statistical analysis was carried out using Graphpad Instat version 5.1 software.

Results: Chemical screening of Neem oil revealed the compounds responsible for its antibacterial activity, such as fatty acids including oleic acid (42.5%) and several volatile compounds including Lineoleoyl chloride (10.24%) and Methyl petroselinic acid (10.23%). Susceptible bacterial strains had diameters of 14.9 mm for *A. actinomycetemcomitans*, 11.3 mm *P. gingivalis* and 12.6 mm *F. nucleatum*. The MIC and MBC were 125 mg /ml and 500 mg /ml respectively; the MBC / MIC ratio was 4.

Conclusion: Neem oil has bacteriostatic activity on the germs *Aggregatibacter actinomycetemcomitans*, *porphyromonas gingivalis* and *fusobacterium nucleatum*; and would be an alternative for the management of alveolitis.

Keywords

Neem essential oil; *Azadirachta indica*; Alveolite; Antibacterial activity

Introduction

Osteitis is an inflammatory bone disease of bacterial infectious origin [1]. It can occur a few days after a tooth extraction, following a fracture with bone clearance or a severe periodontal infection leading to loss of attachment [2,3]. Alveolitis is a micro osteitis of the alveolar rim that can spread, characterised by severe, acute, radiating pain that is resistant to painkillers and requires appropriate and effective treatment [2]. Clinically, two forms of alveolitis are described: dry and suppurated [1,2]. The microorganisms incriminated are Gram+ (*Streptococcus*, *staphylococcus*, *enterobacilli*), Gram-pycyanic (*Pseudomonas aeruginosa*) and anaerobic (*Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*) [2-4]. The diagnosis of alveolitis is clinical and the therapeutic means used are medico-surgical [2,3]. The most commonly used antibiotics are betalactam antibiotics, macrolides, cyclines and imidazoles [2,3]. However, the massive and repeated use of antibiotics generates an increase in bacterial resistance over time [5]. Today, this multi-microbial resistance poses major problems in a context such as ours where conventional antibiotic therapy used after exodontics may prove ineffective. In fact, very few antimicrobial agents remain effective against some multi-resistant microbes. Scientists are therefore

Article Information

DOI: 10.31021/ijbs.20203119
Article Type: Research
Journal Type: Open Access
Volume: 2 Issue: 2
Manuscript ID: IJBS-2-118
Publisher: Boffin Access Limited
Received Date: 22 August 2020
Accepted Date: 03 September 2020
Published Date: 05 September 2020

*Corresponding author:

Dr. Nokam Taguenné Epse Abéna Marie Elvire

Department of Dentistry
Cite verte District Hospital of Yaoundé
Cameroon
Tel: (237)699975202
E-mail: nokamabena@yahoo.fr

Citation: Marie Elvire NA, Hortense GK, Emmanuel NN, Rose NM, Francine MN. Evaluation of the *in vitro* antibacterial activity of *Azadirachta indica* used for the treatment of alveolitis. Int J Biopharm Sci. 2020 Nov;2(2) :119.

Copyright: © 2020 Marie Elvire NA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 international License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.



Figure 1: Trees, leaves and fruits of Neem in Faro (Photograph of the study).

looking for new antimicrobial products, including phytochemical compounds isolated from plants used in traditional medicine. Among them, *Azadirachta Indica*, commonly known as “Neem”, is known as a traditional medicine plant in many rural areas of Asia and Africa for its antiseptic, anti-inflammatory, hypoglycaemic, anti-ulcer, antibacterial, anti-malarial, and antiviral properties [8,9]. Used for aesthetic needs in the north of Cameroon, Neem oil can cause undesirable reactions [10]. Few studies have been conducted on the antibacterial activity of Neem in Cameroon. This work has made it possible to highlight its antibacterial effect in vitro on the periodontopathogenic bacteria of the essential oil of *Azadirachta indica*.

Material and Methods

This was an experimental study conducted over a 7-month period from November 2018 to May 2019. The chemical screening of Neem oil from Faro in northern Cameroon (Figure 1) was carried out at the bio-analytical laboratory of the Scientific and Industrial Research Centre (CSIR) in Pretoria, South Africa. Patient samples were taken at the stomatology Department of the Centre Yaoundé University teaching hospital (CHUY). The evaluation of the antibacterial activity of Neem oil was carried out at the microbiology laboratory of the CHUY.

Included in the study were all consenting patients 18 years and above with aggressive periodontitis with periodontal pockets greater than 5 mm, located on a tooth with a degree 2 mobility. The patient had to have been free of periodontal treatment and topical or systemic antibiotics for at least six months.

The plant material was *Azadirachta indica* oil obtained by first cold-pressing seeds collected in the locality of Faro in northern Cameroon (Figure 2). The material for oil extraction

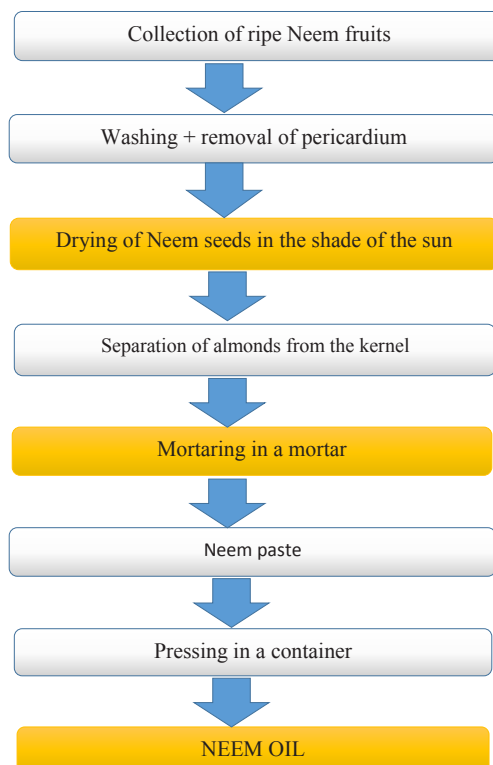


Figure 2: Diagram of oil production by cold mechanical pressure.

was the oil press, model NF 500. One hundred millilitres (100 ml) of Neem oil was conditioned (dried on anhydrous sodium sulphate and stored at 4°C) and sent to the bioanalysis laboratory of CSIR for chemical characterization.

The **microbiological material** consisted of **bacterial strains** from the different cultures taken from the samples taken from patients meeting the inclusion criteria; and **material for use in the microbiology laboratory**. The **culture media** were chocolate + polyvitex agar, blood + vancomycin + Amicacyn agar, methylene blue eosin agar, chocolate + polyvitex + vancomycin + Amicacyn agar.

Procedure for the evaluation of antimicrobial activity

Samples were taken two hours before feeding and brushing the patient's teeth. The choice of sampling site was made after evaluation of the clinical parameters of periodontal disease (bleeding on probing, gingival oedema, loss of attachments, pocket depth) [11]. Samples were taken quadrant by quadrant and pool by pool and placed in the same transport medium. The technique used was paper-tip sampling according to WOLF 2005 [11]. The supragingival plaque was first removed using a sterile curette, compress and sterile saline; then the specimen sites were isolated from the saliva with saliva cotton rolls and air-dried; afterwards, a sterile paper spike, grasped with a sterile tweezers, was inserted into the pouch until resistance was felt ; 10 to 20 seconds after the tip was removed, making sure that it was not in contact with the mucous membrane or saliva; finally, the tip was immediately inserted into a sterile bottle containing 1ml of heart-brain broth and transported directly to the bacteriology laboratory (Figure 3).

Bacteria culture

The samples were homogenised using a vortex at maximum power and then incubated for 15 minutes. The culture media were previously prepared according to the standards recommended by the manufacturers. After incubation, the bacterial inoculum was



Figure 3: Removal of subgingival plaque.



Figure 4: Oil obtained for study.

taken with a propette and spread using a sterile platinum handle according to the quadrant method and exhausted. The Petri dishes were placed in a transparent jar; a Genbox (anaerobiosis generators) bag was introduced into it to create anaerobic conditions at 37°C. Then, all this was incubated for 5 to 10 days.

Method for evaluating the antibacterial activity of Neem essential oil

Preparation of the bacterial inoculum: From a pure culture of the isolated and transplanted bacteria with a maximum of 24 hours, three to five colonies of the same morphological type and perfectly identical were scraped off using a platinum loop; the contents of this loop were then discharged into 5ml of sterile physiological saline and then homogenised in order to obtain a turbidity equivalent to that of the 0.5 standard of the Mc Farland range corresponding to $1 - 2 \times 10^8$ CFU/ml then diluted to 1/100th. The inoculum was sown in less than 15 min after its preparation.

Sensitivity test: Agar disc diffusion method: To test the sensitivity of the bacteria, the principle used was that of the antibiogram [13]. A volume of 15 µL of Neem oil supplemented with 1% Tween 80 was introduced into a tube and homogenised by vortexing. Sterile discs of 6 mm diameter cut from Whatman No. 1 paper were then inserted. Once the discs were impregnated with Neem oil, they were removed and gently placed on the surface of a Mueller Hinton agar previously swabbed with the bacterial inoculum. At the same time, positive control antibiotic discs (Amoxicillin + Clavulanic Acid 30 µg, metronidazol 4 µg) were also deposited on the surface of the agar. The plates were then incubated for 24 hours under strict anaerobiosis. On leaving the incubator, the discs were surrounded by circular inhibition zones corresponding to an absence of culture, which allowed

No.	Name of Compounds	t_R (min)	Percentage (%)
1	Levoglucosenone	30.831	2.115
2	Benzaldehyde, 2-methyl	28.540	2.251
3	3, 7, 11,15-Tetramethyl-2-hexadecen-1-ol	34.202	3.236
4	Methyl 14-methylpentadecanoate	39.812	3.151
5	Petroselinat	40.234	10.023
6	Lineoleoyl chloride	50.222	10.243
7	Phytol	47.421	09.600
8	Methylisoheptadecanoate	39.812	3.151
9	Butyl palmitate	39.443	2.008
10	Heptacosane	51.436	2.822
11	Eicosane 7-hexyl	45.122	8.111
12	Heptacosane, 7-hexyl	43.319	3.131
13	Nonacosane	44.227	2.146
14	Hentriacontane	46.555	8.890
15	(Z, E)- α -Farnesene	3.131	2.901
16	Hexahydrofarnesyl acetone	23.623	2.358
17	2-Methyl-5-ethylfuran	20.008	3.664

t_R = Retention time in minutes; % =percentage

Table 1: Volatile composition of *Azadirachta indica* oil

us to measure the inhibition diameters using a calliper. This was done for each bacterial strain three times during the three experiments.

The determination of the diameter of the inhibition zone allowed an estimation of a clinical categorisation (S, I, R) of the strain, based on the correlation between the results obtained by the diffusion method and the reference method [13]. Oil sensitivity is classified according to the diameter of the inhibition zones as follows: Non-sensitive (-) for diameter less than 8 mm; Sensitive (+) for diameter between 8-13.9 mm; Very sensitive (++) for diameter between 14-19 mm; Extremely sensitive (+++) for diameter greater than 19 mm. This sensitivity test provides qualitative results. The experiment was repeated three times.

Determination of minimum bactericidal concentrations (MBC): The Minimum Bactericidal Concentration (MBC) is the lowest concentration of substance capable of killing more than 99.9% of initial bacterial inoculum (i.e. less than 0.11% survivors) after 24 hours incubation at 37°C [14]. Thus, their determination is based on subculture from the MIC on an agar medium.

For each of the tubes devoid of bacterial pellets (visible growth not observed) and positive control of the concentration range achieved for the MIC determination, we performed streak plating on Mueller Hinton agar in Petri dishes. The Petri dishes were incubated for 24 hours at 37°C. The BMC of Neem oil is inferred from the lowest concentration at which no culture is observed on Mueller Hinton agar [13]. This procedure was repeated three times for each bacterium.

CMB/CMI ratio: The CMB/CMI ratio made it possible to define the bacteriostatic or bactericidal character of our Neem oil. An essential oil is said to be bacteriostatic when this ratio is greater than or equal to 4 and bactericidal when this ratio is less than 4 [13].

Results

The mechanical pressure of 1000g of *Azadirachta indica* seeds allowed us to obtain 186g of oil, a yield of 18.6%.

Macroscopic evaluation of Neem oil showed the brown

Strains	Settings	Strain 1	Strain 2	Strain 3
Morphological characteristics	Form	Round/starred	Round	Round
	Mobility	Immobile	Immobile	Immobile
	Colour	White	Black	Violette
Microscopic characters	Gram	-	-	-
	Form	Coccobacillus	Coccobacillus	Lactobacille
	Morphology	Short chain	Chainette	Isolated
Biochemical tests	Catalase	+	-	-
	Oxydase	-	-	-
	Indole	-	+	+
		V	Sensitive	Resistant
		A	Resistant	Sensitive
Bacteria	Bacteria	<i>Aggregatibacter actinomycetemcomitans</i>	<i>Porphyromonas gingivalis</i>	<i>Fusobacterium nucleatum</i>

Table 2: General results of the bacteriological study of the different isolates studied.

Bacteria	Amoxicillin clavulanic acid	Metronidazol	Neem Oil
<i>Actinobacillus</i>	28,33 ± 2,52	8,70 ± 0,75	14,90 ± 0,26
<i>Fusobacterium nucleatum</i>	32,67 ± 1,53	26,73 ± 2,61	12,63 ± 0,81
<i>Porphyromonas gingivalis</i>	24,00 ± 2,65	17,1 ± 2,61	11,3 ± 0,51

Table 3: Inhibition diameter of Neem oil, Amoxicillin clavulanic acid and metronidazole on isolated bacteria.

colour, characteristic pungent garlic-repellent odour, viscous consistency, with a density of 37.30-0.85 at 20°C and a refractive index of 30.6621-1.6331 at 40°C. Its iodine value was 447.0 -81.0, its Azadirachtine content by HPLC (A+B) was 110-247 PPM and its sap content 168-221 (Figure 4).

Chemical characteristics of the essential oil of *Azadirachta indica*

The centesimal fatty acid composition of *Azadirachta indica* oil has highlighted its richness in acids, of which oleic acid is the predominant one with an average content of 42.5%, followed respectively by palmitic acid with a value of 22-36%, stearic acid of 13-22%, linoleic acid of 18% and palmitoleic acid of 13%.

The volatile compound composition of *Azadirachta indica* oil by Gas Chromatography coupled with Mass Spectrometry revealed the presence of 17 compounds. The results show that the most important compounds are respectively Lineoleoyl chloride 10.24%, Methyl petroselinat 10.23%, Phytol 9.6%, Hentriacontane 8.8%, 7-hexyl Eicosane 8.1% (Table I)

Bacterial strain identifications [12]

After 7 days, a morphological identification oriented by the appearance of the colonies was made as well as the staining of control Gram, then a selective transplanting with the isolated bacteria to obtain only pure cultures. The Gram test allowed us to differentiate Gram positive from Gram negative. The transplanted bacteria were used for biochemical and enzymatic identification tests.

✓ *Actinobacillus actinomycetemcomitans*

It is a Gram-negative, capnophilic, non-mobile coccobacillus that adheres strongly to the agar. Biochemical tests: positive catalysis, negative oxidase, negative indole.

✓ *Porphyromonas gingivalis*

Small size bacilli, gram-negative, strict anaerobic non-sporing and immobile colony of 1 to 3mm, round, creamy, irregular edges, smooth, hydrophobic, haemolytic and brown; catalase-negative and oxidase-negative.

✓ *Fusobacterium nucleatum*

Long bacillus, Gram negative, strict anaerobic. Catalase negative, oxidase negative, indole positive.

Biochemical tests were done, strain 1 had the enzyme catalase positive, there was no colour shift to purple, so all isolates are oxidase-free. Indole production has been demonstrated for some bacteria, notably strains 2 and 3. Strain 2 was sensitive to vancomycin unlike strain 3. All these characteristics enabled us to identify three bacterial strains, namely *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (Table 2).

In vitro evaluation of the antibacterial activity of *Azadirachta indica* oil

Azadirachta indica oil inhibition zone diameters: The three identified bacterial strains were tested for sensitivity to control antibiotics and Neem oil. All bacterial strains tested were found to be sensitive to our Neem oil. We obtained diameters of 14.9 ± 0.26mm *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* 12.3±0.81mm and *Porphyromonas gingivalis* 11.3 ± 0.51mm.

All these bacterial strains were also sensitive to amoxicillin clavulanic acid. However, apart from *Fusobacterium nucleatum* which was sensitive to metronidazole with a diameter of 26.7mm the other bacterial strains showed resistance to metronidazole (Table 3).

Minimum inhibitory and bactericidal concentration of Neem oil: The minimum inhibitory and minimum bactericidal concentrations and their MBC/MIC ratio of Neem oil are shown in Table 3. The MIC for all three strains was 125mg/ml and the MBC 500mg/ml. The MIC/MBC ratio gave a value of 4.

Discussion

The present work has made it possible to evaluate the antibacterial activity of *Azadirachta indica* oil on bacteria responsible for periodontal pathologies. The aim of this study was to investigate whether Neem essential oil could be an alternative for the effective treatment of alveolitis. Volatile compounds such as Lineoleoyl chloride and many others, saturated and unsaturated fatty acids were identified by the GPC of the oil. Three bacteria

were identified, namely *Aggregatibacter actinomycetemcomitans*, *porphyromonas gingivalis* and *fusobacterium nucleatum* with diameters of 14.9 mm; 12.3 mm and 11.3 mm sensitivity respectively. The ratio CMB to CMI was 4, which showed that Neem oil had antibacterial bacteriostatic properties.

An extraction yield of 18.6% of *Azadirachta indica* oil was obtained by cold mechanical pressing without using chemicals that could alter the oil. Extraction by cold mechanical pressure avoids any alteration of the oil. This result is similar to that obtained by Sagoua et al. in 2009 [15] in Senegal, which had a yield of 18.3%. Chemical screening of *Azadirachta indica* oil revealed a light brown colour, a pungent garlic smell, the presence of fatty acids with a level of 42.5% oleic acid, 13% for palmitoleic acid. This result differs from that obtained in Senegal in 2009 by Sagoua et al. [15] which had a light green oil. The GPC also showed many volatile compounds with high content of Lineoleoyl chloride 10.24%, Methyl petroselinat 10.23%, Phytol 9.6%, Hentriacontane 8.8%, 7-hexyl Eicosane 8.1% and many other compounds that were poorly represented with percentages below 3%. The presence of volatile compounds would be at the origin of the antibacterial activity of this Neem oil. However, the volatile compounds in our oil are different from those found by Fokunang Charles et al, in 2019 in Cameroon who found a predominance of methyl14methylpentadecanoate (38.12%) followed by m-toluyaldehyde (22.76%), and hentriacontane (13.98%) [16]. This could be explained by the difference in the location where the Neem seeds are harvested. The Neem oil used in this study was harvested in the locality of Faro in the northern region of Cameroon while the Neem oil used by Fokunang et al. [16] in 2019 came from the commercial centre of Maroua [16].

The search for bacteria after successive cultures and transplanting has made it possible to identify three bacterial strains. This result is different from that found by Lakhssassi et al. [17] in 2005 in Toulouse who found more than ten bacterial strains. This difference could be justified by the use of culture media specific to certain bacteria. The bacteria identified on the basis of cultural and biochemical characteristics corresponded to *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* and *porphyromonas gingivalis*. This result is in agreement with the one found by Criton in 2007 at the Nancy academy [12].

All these isolated bacterial strains were susceptible to Neem oil to varying degrees. Thus, the determination of the inhibition diameter gave us a result of 12.3 mm for *Fusobacterium nucleatum*. This diameter is smaller than that found by Packia Lekshmi et al. [18] in 2012 in India who had obtained a diameter of 16mm. This difference could be explained by the fact that they had used the Soxhlet extraction method using chloroform and ethanol as solvent. However, the resulting diameter shows that *Fusobacterium nucleatum* is sensitive to Neem oil. *Actinobacillus actinomycetemcomitans* showed an inhibition diameter of 14.9mm and *Porphyromonas gingivalis* showed 11.3mm. According to Moreira's classification [19] both strains are susceptible to Faro Neem oil.

Conclusion

In a nutshell, neem oil contains volatile compounds such as Lineoleoyl chloride, Methyl petroselinat, Phytol, Hentriacontane, 7-hexyl Eicosane and fatty acids which are believed to be responsible for its bacterial growth inhibiting properties of the strains *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* tested. Neem du Faro oil showed bacteriostatic antibacterial activity on periodontal bacteria. It could therefore be a good alternative in the treatment of post-extraction alveolitis in order to reduce bacterial resistance due to antibiotics. However, it seems important to evaluate the acute and sub-acute toxicities of

Neem essential oil in order to secure its use and to formulate an Improved Traditional Medicine.

Acknowledment

Bruneton's Centre for Scientific and Industrial Research (CSIR) in Pretoria.

The Yaounde University Teaching Hospital (CHUY).

The FMBS Multidisciplinary Galenic Laboratory.

The Organic Chemistry Laboratory of the Faculty of Sciences of the University of Yaounde I.

Source of Funding

This research has not received any grant from any international or public organisation.

References

1. Seguin P, Breton P. Ostéites des os de la face. Encycl. Med Chir. 5 Elsevier, Paris], Stomatologie Odontologie I. 1996;22-62.
2. Piette E, Rechler H. Traité des pathologies buccales et Maxillo-faciales. De Boeck Université 1991;1345-1355.
3. Haute Autorité de Santé - Parodontopathies : diagnostic et traitements. France; 2002.
4. Dufour T, Svoboda JM. Pathogénie bactérienne des parodontolyses. EMC - Odontol. 1 2005 Mar;1(1):46-57.
5. Résistances antibiotiques. Comité de Lutte Contre les Infections Nosocomiales Sud -Est. France; 2010.
6. Alzohairy MA. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evid-Based Complement Altern Med. 2016;7382506.
7. Bourrain JL. Allergies aux huiles essentielles : aspects pratiques. Revue Française d'Allergologie. 2013;53: 30-32.
8. Vandal J, Abou-Zaid MM, Ferroni G, Leduc LG. Antimicrobial activity of natural products from the flora of Northern Ontario, Canada. Pharm Biol. 2015 Jun;53(6):800-806.
9. Faye M. Nouveau procédé de fractionnement de la graine de Neem (*Azadirachta Indica* A. Juss.) sénégalais : production d'un bio-pesticide d'huile et de tourteau [phd]. Sénégal; 2012 Nov.
10. Poirot T. Bon usage des huiles essentielles, effets indésirables et toxicologie. Thèse de Doctorat. France; 2016.[en ligne].
11. Houle MA, Grenier D. Maladies parodontales : connaissances actuelles. Médecine Mal Infect. 2003 Jul;33(7): 331-340.
12. Criton M. Diagnostic microbiologique en parodontologies: méthodes et intérêts cliniques. Thèse de Doctorat. Université Henri Poincare Nancy. France; 2007.
13. Bouguerra M. al. Etude des activités biologiques de l'huile essentielle extraite des graines de *Foeniculum vulgare* Mill. en vue de son utilisation comme conservateur alimentaire. République démocratique du Congo Thèse; 2012.
14. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrobial Chemotherapy. Oxford Academic. 2001 Jul;48:5-16.
15. Sagoua W. Etude synergique du couplage du Système Lactoperoxydase avec d'autres molécules naturelles actives ayant des propriétés antifongiques pour l'amélioration de la conservation en frais des bananes [thesis]. [Montpellier]: Cirad; 2009.
16. Fokunang C, Ngum WL, Gonssu H, Ngameni B, Tembe E. Phytochemical characterization, in vitro antibacterial activity, in vitro acute toxicity studies of the sees oil of *Azadirachta*

- indica (neem oil) in Wistar rats. Cameroun; 2019. [en ligne].
17. Lakhssassi N, Sixou M. Variabilité de l'efficacité de l'érythromycine et de la spiramycine sur les pathogènes parodontaux dans les parodontites agressives. Étude in vitro comparative. Pathol Biol. 2005;53(8-9):527-535.
 18. Packia Lekshmi NCJ, Sowmia N, Viveka S, Raja Brindha J, Jeeva S. The inhibiting effect of *Azadirachta indica* against dental pathogens. India; 2012.
 19. Bouguerra M. Al. Etude des activités biologiques de l'huile essentielle extraite des graines de *Foeniculum vulgare* Mill. en vue de son utilisation comme conservateur alimentaire. République démocratique du Congo Thèse; 2012.
 20. Alvaro M, Alejandra H, Antonio D. *In vitro* Antibacterial Activity of *Maclura tinctoria* and *Azadirachta indica* against *Streptococcus mutans* and *Porphyromonas gingivalis*. Br J Pharm Res. Grèce; 2015;7(4):291-298.