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Research Article

Antibacterial Activity of *Aloe schweinfurthii* Gel in the Preservation of Periodontal Ligament Cells of an Erupted Immature Permanent Tooth

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Abstract

Effective management of an expelled tooth requires its rapid transport to the dental office, in a liquid adapted to cell survival. For this, some preservation media have been evaluated in tropical areas with regenerative and antibacterial properties. These inaccessible and/or non-regenerative media have pushed our research towards alternative media including *Aloe schunfurthii*. Our general objective was to evaluate the antibacterial activity of *Aloe schweinfurthii* gel on the survival of periodontal ligament cells of an expelled immature permanent tooth. To achieve this objective, we conducted an experimental study. It was carried out at the Yaound é University Hospital on extracted immature permanent premolars that were preserved in *Aloe schweinfurthii* gel at different dilutions and pH. Cell vitality was read using optical microscopy after aqueous eosin staining at three-hour intervals. The antibacterial activity was determined from bacterial samples of the periodontal ligaments of the premolars according to three successive steps ranging from the culture of the bacterial strains to the determination of the diameters and the inhibition parameters. The Chi 2 test was used to compare the vitality of the different media. The methodology used allowed us to obtain results which showed that the *Aloe schweinfurthii* gel had a CMB/MIC ratio lower than 4, revealing a bactericidal activity on all the bacteria tested. The results obtained allowed us to conclude that the *Aloe schweinfurthii* gel is bactericidal in the conservation of the cells of the periodontal ligaments of an expelled immature permanent tooth.

Keywords

Aloe schweinfurthii, Antibacterial Activity, Conservation Medium, Expelled Immature Permanent Tooth, Cell Vitality

1. Introduction

Dental trauma diseases continue to be a major oral health problem and various oral microbes are associated with the development of various infectious complications during dental expulsions. The use of antibiotics is systematic in cases

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of trauma with tooth expulsion. Faced with the resistance observed with marketed products, the use of plant products for pharmaceutical purposes has gradually increased; according to the World Health Organization, medicinal plants are the best source for obtaining a variety of medicines [1]. Authors have looked into the antibacterial properties of plants [2]. *Aloe schweinfurthii* is a plant from the Anteriasae family known for having various properties including antibacterial properties [1]. Ekwenye and Elegalam were able to demonstrate the antibacterial activity of *Aloe vera* extract [2]. Dental trauma with expulsion almost always occurs in a context of psychological, functional and above all aesthetic tension which leads to ridicule and stigmatization of the child; it is then imperative to re-establish it [3, 4]. Reimplantation of the tooth must be undertaken to restore aesthetics and comfort to the patient. It is imperative that the preservation liquid used to arrive at the dental office is antibacterial to maintain good vitality of the periodontal tissues and avoid infectious complications. The most used storage media have always been whole skimmed milk, HBSS solutions (Hanks balanced salt solution), and physiological serum [5, 6]. However, the latter have shown limits linked to reimplantation times which are two to six hours [3-6], to non-bactericidal effects and some for their: cost and unavailability particularly in our country. To overcome these limitations, we considered studying the antibacterial activity of *Aloe schweinfurthii* and observing its capacity for preserving cellular vitality as an alternative conservation media. *Aloe schweinfurthii* (Aloe vera) is a plant renowned for its multiple virtues, including antibacterial activity and cell regeneration [7, 8]. Aloe gel is said to be particularly rich and contains 98% water and 2% active ingredients! In addition, there are 75 active agents, including aloin, aloe-emodin, anthraquinones and phenolic acids; These compounds have been shown to exhibit significant antibacterial properties against various pathogens [9]. Its pulp contains amino acids which promote the regeneration of skin cells. The general objective was to evaluate the antibacterial activity of *Aloe schweinfurthii* gel in the survival of periodontal ligament cells of an expelled immature permanent tooth.

2. Methods

This was an experimental, transversal, descriptive and analytical study which took place over two years in Yaoundé (Cameroon). It received the approval of the Ethics Committee of the Yaoundé University Hospital in 2015.

2.1. Material

The materials used for this study included:

1. Plant material: *Aloe schweinfurthii* gel identified by the national herbarium of Cameroon under reference No: 26842/HNC;
2. Solvents: Aqueous eosin and physiological serum;
3. Human material: Periodontal ligaments of teeth extracted atraumatically;
4. Bacteriological study materials: reagents and culture media; bacteria (Klebsiella pneumoniae ATCC 700603, Escherichia coli ATCC 35218, Pseudomonas aeruginosa Cip 7610, Staphylococcus aureus ATCC 25923, Streptococcus Mutants.
5. Production equipment: consisting of laboratory, clinical examination and other equipment.

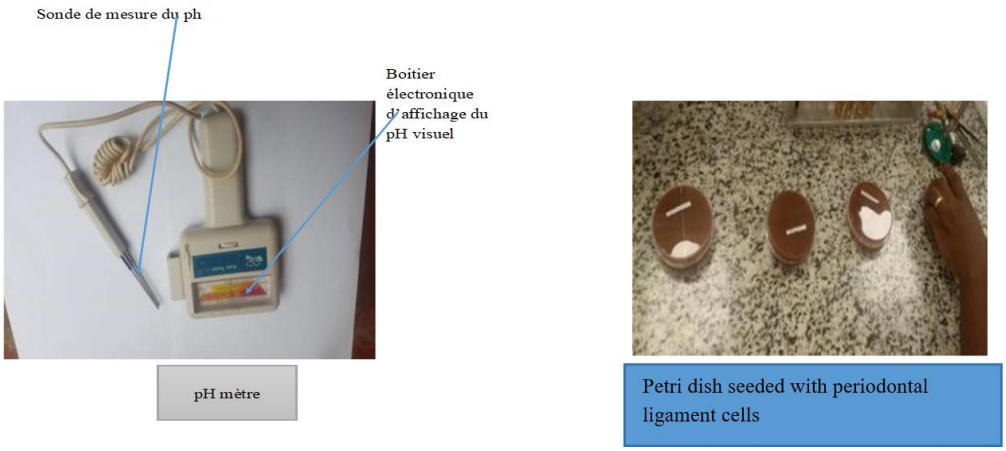

Figure 1. *Some materials used during the study.*

Table 1. *Summary of some materials used.*

Devices	Roles	Brands
Precision balance	Weighing of the collected gel	METLAB

Devices	Roles	Brands
Dental chair	Setting up the patient for treatment	Siemens
Fridge	Storage of A.S gel before use	BIOBASE
Magnetic barometer	Homogenization	RS
pH meter	Measurement of the pH of solutions	pH metre auto testeur pH-CL Logrono
Photon microscope	Observation of periodontal cell vitality	Olympia
Oven	Drying of extracts	BIOBASE
Magnetic stirrer	Liquefy, homogenize the solutions	Vortex
Mueller Hinton Broth	study of bacterial sensitivity	Biolab

2.2. Procedures

2.2.1. Preparation of Solutions and Obtaining Concentrations

The leaves of *Aloe schweinfurthii* were harvested with pruning shears, cleaned with filtered tap water and rinsed with physiological serum. The gel was obtained by cutting the leaf longitudinally with secateurs and scraping the central pulp. The pulp was crushed, sieved and stored in test tubes in the refrigerator at 5 ℃; the ambient working temperature was 27 ℃. The concentrations of *Aloe schweinfurthii* gel were obtained by dilution in 1% aqueous eosin at a fixed volume of 100ml (0.1 l). The method of calculating the concentrations was as follows: an empty 10 cc syringe of *A. schweinfurthii* was weighed p then successive samples of the mass of *A. schweinfurthii* (A.S) were taken so as to obtain large masses P of the syringe and *A. schweinfurthii* set of 5g, 10… up to 50g). The mass of *A. schweinfurthii* was calculated by the difference m As=P-p the concentration C was obtained by the ratio =mass of AS on the flight of 100ml of aqueous eosin which allowed us to obtain the concentrations of 5 to 50%.

2.2.2. Experimental Conditions

The pH is used to evaluate the pH of the solutions used. The ambient temperature was 27 ℃, the pH of each medium was measured at the start of the experiment.

2.2.3. Obtaining Periodontal Ligaments

The biological material consisting of periodontal ligaments came from immature premolars extracted atraumatically using the technique described by Thomas and Gopikrishna [5]; immature permanent teeth were extracted for orthodontic reasons. These teeth were rinsed with physiological serum, then preserved in different environments.

Figure 2. *Obtaining Periodontal Ligament Cells.*

2.2.4. Staining Technique: Eosin Staining

A 10cc syringe without a needle was used every 3 hours to collect the concentration mixture of *A. schweinfurthii* gel - 1% aqueous eosin and ligaments; Three drops of the solution were placed on the slide then covered by the coverslip before reading under an objective 40 optical microscope.

2.2.5. Effect of Different Concentrations of *A. Schweinfurthii* Gel in the Survival of LP Cells from a DPI Expelled over Time

This study consisted of finding the smallest effective concentrations of *A. schuweinfurthii* for which cell vitality is still observed over time. The reading was done according to the storage times in the different concentrations of *A. schuweinfurthii* according to the chronology below: 1H, 3H, 6H, 9H, 24 and 48H and 72 hours respectively. The percentage of viable cells was noted by counting a reading field then multiplied by four. Each medium was tested three times. Cell viability was determined using aqueous eosin-based chemical assays. The existence of viable periodontal ligament cells was identified by a red coloring of cells having retained their activity in the preservation solution diluted with aqueous eosin. Therefore,

the browner the shade, the more indication there is of the presence of more viable cells.

2.2.6. Procedure for Determining Antibacterial Activity

Culture of bacterial strains

For each strain, three tubes were used. The cells previously preserved in a liquid medium at 40 ℃ were removed from the freezer and homogenized in Mueller Hinton broth and seeding was done in a petrie dish by the streaking method using a loop. plastic. Each seeded inoculum was incubated at 37 ℃ for 24 hours. Some steps in testing for streptococci are found in Figure 3.

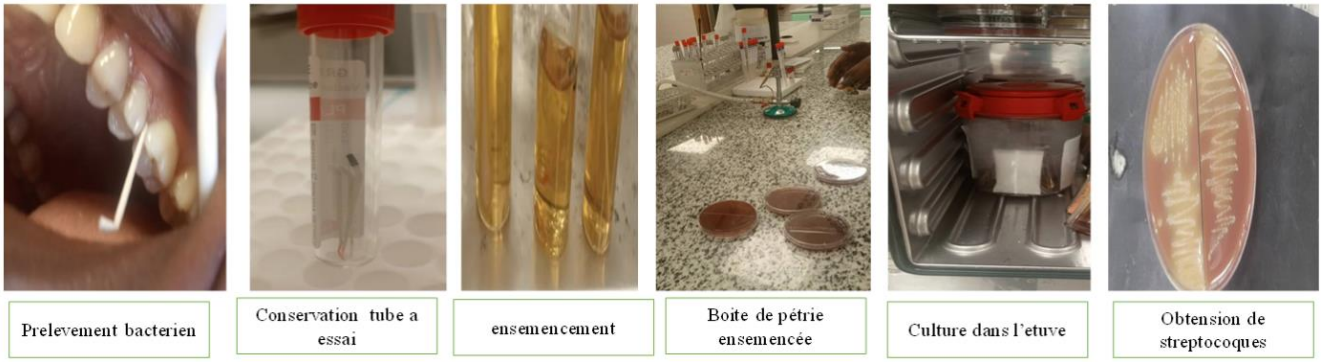

Figure 3. *Some steps for obtaining mutant Streptococcus.*

Preparation of inocula

To prepare the inoculum, each of the pure colonies of Klebsiella pneumoniae ATCC 700603, Escherichia coli ATCC 35218, Pseudomonas aeruginosa Cip 7610, Staphylococcus aureus ATCC 25923, Streptoccocus Mutans were collected using a plastic loop on an agar medium. Mueller Hinton cast in box Kneaded and inoculated in Mueller Hinton broth. The optical density (OD) was read at 625 nm and between 0.08 and 0.1, making it possible to standardize the concentrated bacterial inoculum at 1.5×108 CFU/mL [10].

Determination of the diameters of bacterial inhibition zones

The determination of the diameters of the inhibition zone using the diffusion method on Wattman No. 1 filter paper discs soaked in antibacterial substances was used to carry out these tests [11]. The diameters of the inhibition zone around the discs expressed in millimeters (mm) were measured using the caliper. Sensitivity to different gel concentrations was classified according to the diameter of the inhibition zones as follows: not sensitive: less than 8 mm; sensitive (+) between 9-14 mm; very sensitive (++): between 15-19 mm and extremely sensitive (+++) for diameters over 20 mm [12].

Determination of inhibition parameters Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC).

We used the colorimetric method described by Collins and Franzblau in 1997 [12] followed by some modifications. 100 µL of Mueller Hinton broth were introduced into the wells of the second to the last row and 200 µL of extract into the first well of the plate. From the wells of the second row, a series of 10 decreasing dilutions obeying a geometric progression of reason 2 was carried out for each solution. Subsequently 100 µL of solution from the first well were taken and introduced into the 100 µL of the second well. After homogenization, 100 µL of this new solution was introduced into the third well and so on until 100 µL of solution was obtained in all the wells except those in the first row which still contained 200 µL of solution. 100 µL of the solution from the last well was taken and discarded. An inoculum volume of 106 cells/mL [12] was subsequently seeded and the volume completed to 200 µL with Mueller Hinton broth. Controls containing no gel (negative control) and those containing only microorganisms (positive control) were made and the microplates were incubated for 24 hours at 37 ℃. After incubation, 40 µL of 9 0.01% w/v Blue Alamar prepared in sterile distilled water was added to each microwell and the microplate was covered and incubated at 37 ℃ for 30 min. The absence of a change in the Blue Alamar color to red indicated the antibacterial effect of the test substances. The MIC was considered to be the lowest concentration that inhibited any visible growth of microorganisms under appropriate culture conditions. For the determination of MBC, 50 µL of the wells of the third test column with a MIC concentration ≥ were subcultured into 150 µL of MHB medium and the whole was incubated at 37 Ce for 24 hours, then bacterial growth was demonstrated by adding 40 µL of a solution of Blue Alamar to each well of the three test columns. The whole was re-incubated at 37 ℃ for one hour. The MBC was defined as the lowest concentration of the tested substance at which no visible growth of the germ was observed [12, 13]. The MBC/MIC ratio was used to determine the bacteriological profile of the gel. The bactericidal effect was observed if the MBC/MIC ratio was less than 4, bacteriostatic if the MBC/MIC was between 4 and 16, and tolerant to the microorganism in question, if the ratio was greater than 16 [12, 13].

Statistical analysis of data

The qualitative variables were described by their number and percentage. The associations between variables were highlighted using the odds ratio. Each experiment was repeated three times. The graphical representations were obtained using the Microsoft Excel 2013 Windows spreadsheet and the GraphPad/Prism 7 software. The statistical analyses were performed using the GraphPad/Prism 7 software and ANOVA Windows SPSS 23, Student t-test. Chi-square tests for comparison of different results. Differences were considered significant for $p < 0.05$.

3. Results

3.1. Concentrations Obtained

The concentrations obtained varied from 50 to 5%.

Table 2. *Concentrations of Aloe schweinfurthii obtained.*

Dilution tube	T1	T2	T3	T4	T5	T6
Volume of aqueous eosin	100 ML	100 ML	100 ML	100 ML	100 ML	100 ML
Mass of Aloe schweinfurthi (mg)	50	25	20	15	10	5
Concentration of *Aloe schweinfurthii* (mg/100ml)	50	25	20	15	10	5

3.2. pH of the Solutions

The pH of the solutions obtained varied from 7 to 7.2.

3.3. Antibacterial Activity of *A. schweinfurthii* Gel

3.3.1. Diameters of the Inhibition Zones of *A. schweinfurthii* Gel

The diffusion of *A. schweinfurthii* gel in the selected culture media showed the presence of inhibition zones on almost all the bacteria tested after incubation with the exception of P. aeruginosa Cip 7610 which had an inhibition diameter of 7.31 ± 0.25 mm.

Table 3. *Diameters of the inhibition zones of A.S gel and Ciprofloxacin.*

Samples	Diameters of inhibition zones (mm)	
	AS Gel	Ciprofloxacine
K. pneumoniae ATCC 700603	8,67 ±0,52	17,76 ±0,03
E. coli ATCC 35218	13,59 ±0,07	20,07 ±1,03
P. aeruginosa Cip 7610	7,31 ±0,25	9,91 ±0,16
S. aureus ATCC 25923	10,05 ±0,84	14,20 ±0,63
S Mutans. ATCC 49619	9,4 ±0,25	24,25 ±0,15

3.3.2. Minimal Inhibitory Concentrations (MIC) and Bactericidal Concentrations (MBC) of *A. schweinfurthii* Gel and Ciprofloxacin

MIC of A. schweinfurthii gel and Ciprofloxacin

It is found that *A. schweinfurthii* gel inhibits the growth of all bacteria tested with S. aureus ATCC 25923 and S. Mutans. ATCC were the most sensitive bacteria with a MIC of 750 and 800 µg/ml. The activities of *A. schweinfurthii* gel are lower than those of Ciprofloxacin with a MIC of 2 µg/ml for the most sensitive bacteria.

Table 4. Minimal inhibitory concentration (MIC).

Samples	Inhibition parameters (µg/mL)	
	AS Gel	Ciprofloxacine
	CMI	CMI
K. pneumoniae ATCC 700603	1500	8
E. coli ATCC 35218	3000	16
P. aeruginosa Cip 7610	1500	16
S. aureus ATCC 25923	750	2
S Mutans. ATCC 49619	800	8

MBC of *A. schweinfurthi* gel and Ciprofloxacin

A. schweinfurthii gel inhibited the growth of all bacteria tested with S. aureus ATCC 25923 and S. Mutans. ATCC were the most sensitive bacteria at MBCs of 1500 and 1600 µg/mL.

Table 5. MBC of A.S. gel and Ciprofloxacin.

Samples	Inhibition parameters (µg/mL)	
	AS Gel	Ciprofloxacine
	CMB	CMB
K. pneumoniae ATCC 700603	1500	32
E. coli ATCC 35218	>3000	16
P. aeruginosa Cip 7610	3000	>32
S. aureus ATCC 25923	1500	8
S Mutans. ATCC 49619	1600	1644

3.3.3. Ratio of MICs and MBC of *A. schweinfurthii* Gel

The ratio between MBC and MIC is less than 4 which shows that *A. schweinfurthii* is bactericidal for the strains tested according to Kamanzi's theory [14]. While ciprofloxacin is bactericidal only on 2 strains out of the 4 tested.

Table 6. Report on MIC/CMB of A. schweinfurthii gel and ciprofloxacin.

AS Gel (µg/ml)	Parameters	*K. pneumoniae* ATCC 700603	*E. coli* ATCC 35218	*P. aeruginosa* Cip 7610	*S. aureus* ATCC 25923	S Mutans. ATCC 49619
Aloes schureefurthii	CMI	1500	3000	1500	750	800
	CMB	1500	>3000	3000	1500	1600
	CMB/CMI	1	1	2	2	2
Ciproflo xacine	CMI	8	8	16	16	8
	CMB	32	16	>32	8	4
	CMB/CMI	4	2	4	2	2

3.4. Live Periodontal Cells at Different Concentrations of *A. schweinfurthii* over Time

All concentrations from 5 to 50% of *Aloe schweinfurthii* maintain a cell vitality of at least 88% after 24 hours. The cell viability of 99% of periodontal ligament cells after 24 hours is observed with the concentration of 15%. The cell viability rate after 72 hours is 67% of live cells compared to the control of the first hour for the concentration of 50%.

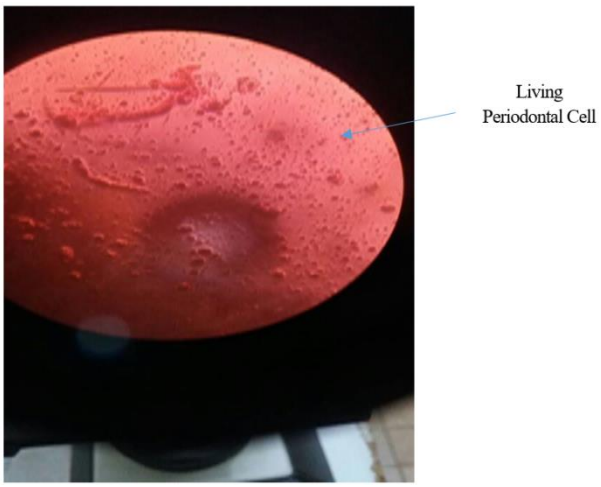

Figure 4. *Microscopic observations of the periodontal cell at the concentration of A. schweinfurthii after 24 hours.*

Aloe schweinfurthii maintains the survival of periodontal ligament cells of immature permanent teeth expelled at the concentration of 5% with an optimal time of preservation of periodontal ligament cells of 24 hours; This time is 72 hours for the concentration of 50%.

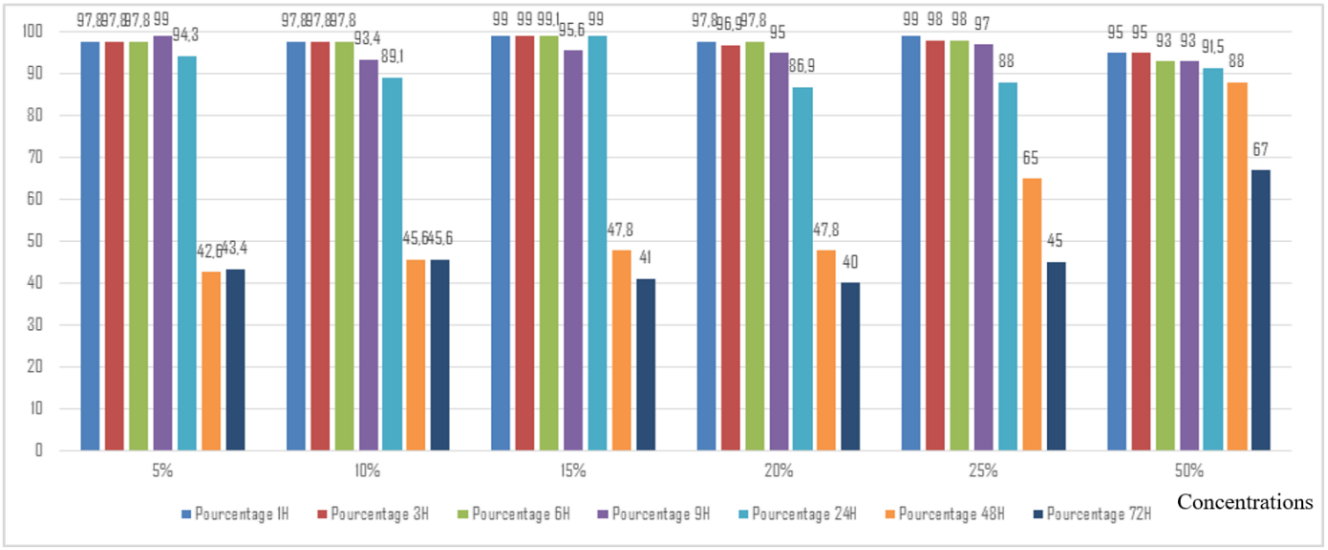

Figure 5. *CLP viability and AS usage at different concentrations over time.*

4. Discussions

4.1. Determination of Antibacterial Activity

The expulsion of a tooth can be accompanied by microorganisms at the site of loss and a risk of superinfection of the reimplantation area. Thus Ram D et al in 2004 [15] and auther autors [16] recommend local antibiotic therapy of the expelled tooth and the reimplantation site. The antibacterial activity of *Aloe schweinfurthii* was tested through the parameters of bacterial inhibition.

Determination of the diameters of the bacterial inhibition zones

The activity of *Aloe schweinfurthii* gel was evaluated on the in vitro growth of 4 bacterial strains. The tests showed that the gel is active to varying degrees on the bacterial strains. This is demonstrated by the differences noted in the inhibition diameters according to the Moreira et al 2005 scale [17]. The lowest diffusion was observed with Pseudomonas aeruginosa Cip 7610 whose diameter was less than 8mm.

This low diffusion can be explained by the structure of its bacterial wall, free diffusion can be limited by the outer membrane of the wall or a natural resistance [18]. In addition, the mechanisms of action of the biomolecules present in the plant extract can vary from one species to another and are a function of the genome of each microorganism [14].

The largest inhibition diameter (13.59 ± 0.07mm) is obtained on E. coli ATCC 35218 at a concentration of 3000 µg/ml. Our results are different from those of Wafa who found a lower inhibition diameter, not allowing the neutralization of Escherichia coli [19]. The inhibition diameters of 10.05 ± 0.84mm were obtained on Staphylococcus aureus ATCC 25923 or golden staphylococcus. These values are different from those of Abubaker Ali's and L. Tamilarasi et al in 2014 who respectively found the values of 19 and 14mm in diameter with Aloe sinkatanain gel [20]. The inhibition of staphylococcus aureus and streptococcus Mutans was observed which will help in the management of periodontal disease of expelled teeth. This sensitivity on all bacteria is important for an expelled immature permanent tooth. Indeed, oral diseases are multibacterial, mixed type with aerobic and anaerobic bacteria.

Determination of inhibition parameters MIC and MBC

The inhibition parameters Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. schweinfurthii* gel were lower than those of Ciprofloxacin ($2 \leq MIC \leq 16$ µg/ml). These results are different from those found in 2011 by Lamiae A et all who found that Aloe arborescent had inhibition parameters higher than those of tetracycline hydrochloride, taken as a control [7]. This difference would be linked to the fact of the entire use of the leaf and the extraction method used. *A. schweinfurthii* gel inhibits the growth of all bacteria tested with MICs ranging from 750 µg/ml to 3000 ug/ml. The MIC/MBC ratio lower than 4 shows its bactericidal power on the strains tested. This study provides scientific basis for the use of *A. schweinfurthii* gel in the treatment of infectious diseases of the oral cavity in general and in the process of treatment of expelled PID in particular. Another study supported these results: the antimicrobial effect of the ethanolic extract of Aloe vera was observed in vitro on several bacteria such as Enterococcus bovis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Morganella Morganii and Klebsiella pneumoniae but also on Streptococcus pyogenes [11].

4.2. Viable CLP Using *A. schweinfurthii* at Differents Concentrations

The pH of the different solutions ranged from 7 to 7.2, approaching the physiological pH. For Souza et al. [21], the pH of a good preservation medium must be physiological for the survival of periodontal ligament cells. The ambient working temperature was 27 ℃, it plays an important role in the composition of nutrients in a plant; Indeed, according to Chen et al [22], the concentration of chemical compounds can vary depending on the ambient temperature. Our study took place in an equatorial zone with a hot and rainy climate, moreover the pH of our different concentrations was identical to that of HBSS, which is an effective solution used for the preservation of organs to be transplanted. This solution is recommended by the American Society for the preservation of expelled teeth, [23] which could contribute to a good composition of the constituent elements of our gel and consequently a good preservation of the vitality of the cells of the periodontal ligament. Indeed, patients often present at the dental office sometimes after many hours or the next day. To recover an expelled tooth that cannot be reimplanted immediately, it is essential to have an adequate, available and accessible preservation medium.

At all concentrations, *Aloe schweinfurthii* appears to be a good medium for preserving cell viability because the minimum of vital cells found even after 48 hours was still greater than 50%. Concentrations of 25, 20, 15, 10, 5% still maintained approximately 90% of living cells after 24 hours. These results are identical to those of Badakhsh S et al in 2014 [24] who found 95% rates of preservation of vitality of periodontal ligament cells for a concentration of 50%. These results of preservation of cellular vitality are explained by the fact that *Aloe schweinfurthii* has antibacterial properties; was bactericidal on all bacteria tested. Which is a considerable asset for preserving cellular vitality. The gel is made up of a wide variety of nutrients giving it regeneration and tissue repair capacities. This preservation of cellular vitality can also be explained by the oxygenation resulting from the dilution of *A. schweinfurthii*. Indeed, the pure or weakly diluted gel is heavy and viscous, its use makes it difficult to circulate oxygen and nutrients, thus causing the phenomenon of apoptosis more quickly. Highly diluted concentrations such as 10 or 5% are relatively fluid, facilitating the passage of oxygen and nutrients which promotes the high cell survival observed in the first 24 hours. There would be a reduction in oxygen consumption and cell mortality despite its significant nutritional potential at high concentrations. Our study corroborates with the study of Davis [25]. He reported that wound healing by Aloe Vera is due to the increased supply of oxygenated blood which promotes fibroblast activity and collagen proliferation in concentrations of 10 and 30%.

5. Conclusion

From the results obtained we can conclude that:

1) The antibacterial activity of *Aloe schweinfurthii* gel allows the preservation of living periodontal cells.
2) The concentration of 5% have an optimal periodontal ligament cells conservation time of 24 hours; This time can reach 72 hours for a concentration of 50%.
3) *Aloe schweinfurthii* could be the ideal solution for maintaining the survival of periodontal ligament cells of the expelled DPI do to is antibactérial activity contribution.

Abbreviations

AS	*Aloe schweinfurthii*
ATCC	American Type Culture Collection
CLP	Periodontal Ligament Cells
CHU	University Hospital Center
MII	Minimum Initiating Concentration
MBC	Minimum Bactericidal Concentration
DPI	Immature Permanent Tooth
ML	Milliliter
pH	Hydrogen Potential
SPSS	Statistical Package for the Social Sciences

Conflicts of Interest

The authors declare no conflicts of interest.

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