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Development, Evaluation, and Characterization of a Novel Mucoadhesive Film Containing *Pterodon pubescens* Benth Ethanolic Extract for Dental Use

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ABSTRACT

Periodontal diseases involve infectious and inflammatory processes that affect oral tissues. Extracts from *Pterodon pubescens* Benth (sucupira) have demonstrated antinociceptive, anti-inflammatory, anti-edematogenic, and antiplatelet effects, highlighting their therapeutic potential for periodontal treatment. However, an effective pharmaceutical delivery system is necessary for targeted administration. This study aimed to develop and characterize a mucoadhesive oral film loaded with sucupira extract for dental applications. Films were prepared using polyvinyl alcohol (PVA) and characterized for nanoparticle size, mechanical properties, permeation, and cytotoxicity. Nanoparticles ranged from 200 to 500 nm with crystalline structures observed. Increased extract concentration enhanced zeta potential and reduced nanoparticle count with a polydispersity index of 0.5. Mechanical tests showed strong films with low mucoadhesive properties. Permeation studies indicated that films with 3% (w/w) extract released approximately 41% of methyl 6α-acetoxy-7β-hydroxy-vouacapan-17β-oate ester after 8 h, outperforming films with 1% extract. Cytotoxicity assays with human gingival fibroblasts (HGF-1) revealed higher cell viability for the film-incorporated extract compared to free extract. Furthermore, the sucupira extract loaded-PVA film was able to mitigate the pro-inflammatory pathway exhibited by reducing neutrophil infiltration. These findings suggest that the mucoadhesive film with 3% (w/w) *P. pubescens* extract is a promising candidate for the development of a new oral formulation for dental use.

1 | Introduction

Periodontal disease encompasses different pathological conditions ranging from chronic inflammation to erosion of alveolar bone and gingival tissue. Gingivitis, characterized by localized inflammation of the gums, is triggered by the presence of bacteria within the dental plaque, forming a microbial biofilm on the teeth and gums, increasing the risk of developing and progressing periodontal diseases [1].

Effective management and treatment of periodontal diseases at an early stage, as disease progression often leads to irreversible

loss of periodontal bone and soft tissue. However, accurate diagnosis is necessary for treatment, which can be both time-consuming and technically challenging. Early onset of gingivitis is typically painless and usually involves slight bleeding during brushing, which some patients may perceive as harmless. Therefore, periodontal disease often advances to a more severe stage before detection and intervention occur [1].

Treatment of periodontal disease primarily involves the removal of bacterial plaque, achieved through mechanical cleaning of the tooth surface associated with the elimination of dental calculus [2]. In addition, certain antimicrobial agents such as tetracycline, minocycline, clindamycin, metronidazole, and chlorhexidine, as well as anti-inflammatory drugs like flurbiprofen, have been used particularly in the treatment of refractory disease and early-onset cases [3]. The host's immune response also plays a critical role in both the development and progression of periodontal disease, influencing treatment outcomes [4].

An example of a plant extract with therapeutic potential for managing periodontal disease is derived from a Brazilian flora known as *Pterodon pubescens* Benth. This plant, popularly known as faveiro or sucupira, belongs to the genus *Pterodon* within the Leguminosae–Papilionoideaefamily. Numerous studies have documented the efficacy of sucupira-based extracts, which exhibit antinociceptive, anti-inflammatory, anti-edematogenic, anti-proliferative, and anti-platelet properties [5–7].

Building on the advanced stage of our group's non-clinical research, which established the efficacy, standardization, and safety parameters of sucupira-derived products and given the beneficial properties of sucupira extract, the formulation of mucoadhesive pharmaceutical films for oral delivery presents a promising application. Oral drug administration offers an attractive route due to the local dental environment, with rich vascularization and potential for systemic absorption of therapeutic agents [8]. The buccal route has better acceptability by patients when compared to other medication delivery methods [9]. However, the significant challenge with this route is the short residence time of conventional formulations at the application site, which limits drug absorption through the oral mucosa [10].

Compared to buccal tablets, mucoadhesive films offer superior flexibility, comfort, and adaptability to the oral mucosa, making them more suitable for buccal drug delivery. Their thin, uniform structure ensures better retention, reduces discomfort, and allows for easier application, especially in patients with swallowing difficulties. Additionally, these films enhance drug bioavailability by bypassing first-pass metabolism and enabling controlled release, benefits that are less achievable with rigid dosage forms. Recent studies confirm their morphological and mechanical advantages, reinforcing their potential as a patient-friendly and efficient alternative for oral administration [11, 12].

In this study, mucoadhesive films for buccal administration were developed using a combination of polyvinyl alcohol and crude *Pterodon pubescens* Benth ethanolic extract. The films were evaluated for their physical, chemical, microbiological, and mucoadhesive properties. Furthermore, their therapeutic effectiveness was demonstrated in vivo using a rodent model of periodontal disease. If this system is translated into clinical practice, this drug delivery system could offer a minimally invasive approach for the management and treatment of periodontal disease. To the best of our knowledge, this is the first report on the application of such a formulation for the administration of a mucoadhesive plant-based extract.

2 | Materials and Methods

Polyvinyl alcohol (Dinamica, São Paulo, Brazil), Viscosity (20°C, 4% CP) 40–48, hidrolyse level (% mol) 87–89. All other reagents were of pharmaceutical grade. Mucosa porcine was obtained from a certified local slaughterhouse (Piracicaba, São Paulo, Brazil).

2.1 | Phytochemical Study

2.1.1 | Plant Material and Extraction

Pterodon pubescens Benth. fruits from Ponto Chique, Minas Gerais, Brazil (16° 37′51″ S, 45° 3′57″ W) were collected in September 2013 and cataloged by Prof. Dr. Jorge Yoshio Tamashiro of the Department of Botany at IB-UNICAMP. A voucher specimen was deposited in the Herbarium of the (UNICAMP), under no. 1398–1402. Authorization for access to genetic heritage—National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under process A0FC528.

The extraction process involved grinding the fruits with dry ice, followed by adding ethanol as an extraction solvent. Extraction was carried out in a stainless-steel tank with mechanical agitation using a plant material (kg) to solvent (L) ratio of 1:3. Three consecutive extractions were carried out, each lasting 1.5 h at room temperature, totaling 4.5 h, renewing the solvent at each step. Partial extracts were filtered, combined, and concentrated under vacuum by a rotary evaporation system at 40°C (Büchi RE 120). The extract was stored in an amber bottle and stored at room temperature. Analysis and quantifications of the extracts revealed the presence of major compounds methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate ester (m/z 404) and 6α,7β-dihydroxyvouacapane-17β-oate ester of methyl (m/z 362).

2.1.2 | Performance Analyses the Extract

Performance analyses were carried out in different equipment to quantify the different compositions present in the *Pterodon pubescens* Benth extract. Gas chromatography–mass spectrometry (GC–MS), GC (Gas chromatography) and HPLC-DAD system equipment were used. The conditions used are detailed in Table 1.

2.2 | Pharmacotechnical Study

2.2.1 | Development of Mucoadhesive Films

A 3% polyvinyl alcohol (PVA) solution was prepared by dispersing the polymer in distilled water, kept under stirring with heating at 100°C for 48 h. The resulting solution was filtered through a porous plate funnel and poured into Teflon molds for water

evaporation at room temperature, leading to film formation (Figure 1). *Pterodon pubescens* Benth extracts, at 1% and 3% concentrations, were added to the PVA solution prior to film casting.

2.2.2 | Dynamic Light Scattering—Nanosizer

After incorporating the mixture, the dynamic light scattering technique (DLS) to measure the intensity fluctuations of light scattered by particles in suspension at a fixed angle. Particle size, polydispersity index (PDI) and zeta potential (ZP) were determined with the ZetaSizer, model Nano ZS (Malvern Instruments, UK). Samples of different concentrations were diluted 10-fold with ultrapure water to reach the working concentration for analysis. All measurements were performed at 25°C ± 2°C [13].

2.2.3 | Transmission Electronic Microscopy (TEM)

The morphology of the nanoparticles was characterized at the University of Dentistry of Piracicaba (UNICAMP), with a transmission electron microscope (JEOL, JEM 1400, Tokyo, Japan). Samples were diluted in deionized water (1:500, MNs: deionized water, w/w) and then transferred to a copper grid (Sigma, grid size 600 mesh × 42 μm pitch) and dried at room temperature. The nanoparticle measurement was analyzed using ImageJ software.

2.2.4 | Mechanical Properties Analysis

The mechanical properties of the films were evaluated in a texture analyzer model TA-XT2 (Stable Micro Systems). A puncture probe with a ball tip (5 mm) was used, and the probe was moved down to 1 mm s^{-1}, with the firing force 0.005 kg. Force versus displacement curves were recorded until the film broke and measured to determine the puncture resistance (Ps) and puncture energy (Ep) parameters [14].

2.2.5 | Mucoadhesion Measurement

Mucoadhesion of the control and mucoadhesive films was evaluated on a TA-XT2 texture analyzer (Stable Micro Systems).

TABLE 1 | Performance analyses.

Conditions	GC–MS	GC	HPLC
Operating conditions	Column used was HP5 (30 m × 0.25 mm, film thickness of 0.25 μm)	Column used was HP5 (30 m × 0.25 mm, film thickness of 0.25 μm)	C18 reverse phase column (Gemini-C18 250 mm × 4.6 mm × 5 μm) with 10 μL sample inject
Carrier gas	Helium gas (1.0 mL/min)	Helium gas (1.0 mL/min)	Mobile phase was a mixture of 70:30 Acetronitrile: water with 0.1% acetic acid (v: v)
Temperature	Programmed to 240°C at a rate of 3°C/min	FID (Flame ionization detector) 250°C	—
Mass range m/z	100 to 600	α-Humulene, *trans*-caryophyllene and geranylgeraniol	The flow rate and UV wavelength were set at 1 mL/min and 220 nm
Equipment	Agilent 5975 GC–MS system	Agilent 6890 N GC system	HPLC-DAD system (Shimadzu)

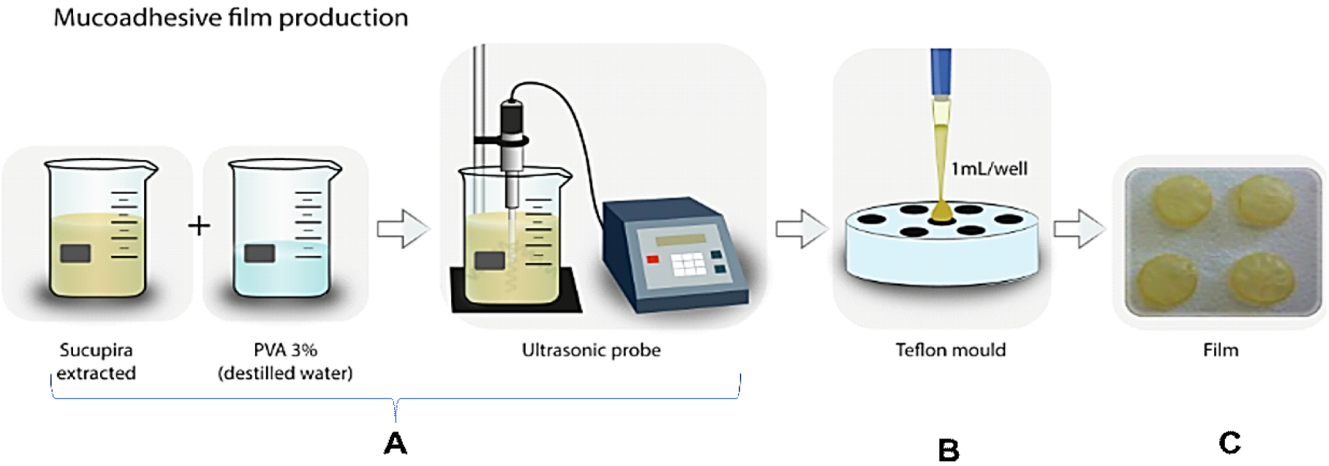

FIGURE 1 | Schematic illustration of the mucoadhesive films preparation technique (A) nanodispersion added on Teflon plates before drying (B) and after drying (C). [Color figure can be viewed at wileyonlinelibrary.com]

Fresh porcine mucosa (4 cm^2) samples were used, and the films were carefully fixed to a cylindrical probe (10 mm). Prior to testing, films were prehydrated with phosphate buffer at colonic pH (pH 6.8), at 37°C for 1 min. The probe was then lowered at a constant speed of 10 mm min^{-1} until reaching the predetermined compression force (0.5 N). Subsequently, the probe was retracted at a speed of 20 mm min^{-1} and the mucoadhesion force (MAF) defined as the maximum detachment force (N) was measured for each sample [14].

2.3 | In Vitro Cytotoxicity Studies

Human gingival fibroblasts HGF-1 (ATCC CRL-2014) were cultured in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin and100 μg/mL streptomycin (Invitrogen Life Technologies, CA, USA). Cells were maintained in a humidified incubator at 37°C in 5% CO_2. Changes in cell viability following contact with membranes were assessed using indirect cytotoxicity tests of the SRB assay [15, 16]. Cell attachment to the membranes was evaluated by direct cytotoxicity tests using the same SRB assay.

2.3.1 | Cell Viability Study Indirect Method

The indirect cytotoxicity assay was performed by seeding HGF-1 cells (~6×10^4 cells/ml) into 96-well plates followed by overnight incubation. Formulations were sterilized by UV irradiation for 2 h prior to the assay. After incubation, 100 μL of the formulation extract was transferred to each well. Cells were then incubated for 48 h and assessed using the SRB assay. Absorbance was measured at 540 nm using a microplate reader (Molecular Devices, model VersaMax). At each time point, three samples were analyzed to determine cell viability, with culture medium serving as the negative control.

2.4 | In Vitro Permeation Studies

In vitro permeation studies were performed using Franz-type vertical diffusion cells with a permeation area of 0.6 cm^2 and a receptor compartment volume of 4.7 mL. Assays were performed using fresh porcine esophageal epithelium obtained from a local slaughterhouse (Frigorífico Angelelli Ltd, located in Piracicaba, SP, Brazil), according to the method described by Diaz del Consuelo [17].

The mucoadhesive film, epithelium, and membrane filter were fixed between the donor and recipient compartments. Water-ethanol solution (50:50, v/v) was used in the receiving compartment to maintain sink conditions. The experiment was carried out at 37°C for 8 h, under magnetic stirring (400 rpm) and the samples (300 μL) were periodically removed from the receiving compartment and immediately replaced by the same volume of solution, considering the effects of dilution. Samples were transferred to chromatography vials for HPLC analysis for quantification of vouacapane methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate ester as: time required for initial permeation or lag; flux; and permeability coefficient. The experiments were performed in sextuplicate. Data were expressed as mean ± standard deviation ($n = 6$).

2.5 | Microbiology Study

2.5.1 | Minimal Inhibitory Concentration (MIC)

Strains of different species of *Streptococcus* were used as described: *Streptococcus mitis* (NCTC 12261); *Streptococcus oralis* (ATCC 10557); *Streptococcus sanguinis* (SK36); *Streptococcus salivarius* (ATCC 7073); *Streptococcus gordonii* (NCTC7868); *Streptococcus mutans* (UA159). All strains were stored at the Laboratory of Microbiology and Immunology of the University of Dentistry of Piracicaba/UNICAMP. The ethanolic extract and films were tested for antimicrobial activity by determining the MIC against different *Streptococcus* spp. strains using the broth microdilution technique. The inoculum was prepared by transferring from frozen glycerol stock cultures kept at −20°C to the culture medium *Mitis Salivarius Agar* (MSA) for 24 h in a 10% CO_2 atmosphere. Cultures were then transferred to Brain Heart Infusion (BHI) broth and incubated overnight under the same conditions.

2.5.2 | Inoculum Adjusted

The inoculum was adjusted with saline solution, comparing turbidity with the Mac Farland scale (0.5), equivalent to an absorbance of 0.08 to 0.10 at 625 nm in the spectrophotometer, followed by dilutions in saline and Müller–Hinton. The inoculum was standardized at 5.0×10^5 CFU/mL, following the recommendations of protocol M2-A6 (CSLI, 2003- standardization document developed by the Clinical Laboratory Standards Institute). 100 μL of the Müller–Hinton medium, 100 μL of the extract, and films at the concentrations mentioned below were diluted in 0.05% Tween 80 + culture medium, and 100 μL of the adjusted inoculum were deposited on a sterile microplate. The plate was incubated at 37°C, 10% CO_2 for 24 h. The groups tested were: NC: negative control, containing only the Müller–Hinton culture medium; PC: positive control Clorexidine (Sigma-Aldrich) at a concentration of 2.5 mg/mL; Film blank; films with extract *P. pubescens* at the initial concentration of 25 mg/mL (1 and 3%) with incubation of the microplates at 37°C ± 1°C for 2 h. MIC was defined as the lowest concentration of extract and films capable of inhibiting visible growth of bacteria. Three independent experiments were carried out in triplicate.

2.5.3 | Method of Diffusion in Agar

The agar diffusion method was performed according to protocol M2-A6 of the Clinical Laboratory Standards Institute (CSLI). Dry, sterile paper disks were used. The mucoadhesive films were cut with a paper punch with 6 mm diameter sterilized with UV light for 30 min exposure on each side. The procedure was performed as follows: Plates containing Mueller Hinton agar (Sigma-Aldrich) pre-prepared, were removed from the refrigerator until room temperature; the

pre-incubated overnight inoculum was adjusted to 2×10^8 CFU/mL and, with a sterile swab, the bacterial inoculum (100 μL) was evenly distributed over the surface of the agar and allowed to stand at room temperature for approximately 3 min. Using sterile forceps, the paper disks were evenly distributed over the surface of the agar, and the mucoadhesive film samples were placed on top of the paper disks. Plates were incubated in an oven at 37°C in 10% CO_2 for 24 h. Growth inhibition halos were measured using a digital caliper. Three independent experiments were carried out in triplicate.

2.5.4 | Evaluation of the Formulation Action on Biofilm Formation

The formulations were tested against *Streptococcus mutans* biofilm (UA159). The culture was incubated overnight in BHI in 10% CO_2 at 37°C. The inoculum was adjusted to 0.05 O.D. and added to the plate in BHI + 1% sucrose. In a microtiter plate, 100 μL BHI medium, 100 μL of formulation in the concentration of 0.08 μL/mL, and then serial dilutions of the treatment group's concentration were deposited. Subsequently, 100 μL of adjusted inoculum was incubated for 24 h. After the incubation period, the plate was washed 3 × with distilled water, and 150 μL of Crystal violet was added to each well of the plate for 30 min. After this period, the plate was washed with 3 × distilled water, and complete drying was expected. The dye was then solubilized with 96° alcohol for 30 min, and the absorbance read at 575 nm in a microplate spectrophotometer. Three experiments were carried out independently for each test.

2.5.5 | Scanning Electron Microscopy (SEM)

After biofilm quantification, the samples were centrifuged to form pellets, then washed twice with phosphate buffer (pH 7.4) and then added to 2% glutaraldehyde for 30 min. The samples were then dehydrated using sequential ethanol rinses at concentrations of 50%, 70%, 90%, and absolute ethanol. Each wash concentration was used twice. The samples were coated with gold for 120 s (BAL-TEC SCD 050, Balzers Liechtenstein). Sample analysis was performed using a SEM operating at a 15 kV acceleration (JSM-5600 Lv; JEOL, Tokyo, Japan).

2.6 | Pharmacological Study

2.6.1 | Animals

The present study was approved by the Ethics Committee for Animal Use (CEUA) of the University of Campinas (UNICAMP) under protocol number 4378-1, in accordance with Brazilian national legislation regulated by the National Council of Animal Experimentation Control (CONCEA). Rats were housed in plastic boxes in a temperature-controlled room, under a 12-h light–dark cycle with free access to standard chow and water. The rats were divided into 4 groups: Group I (6 rats) served as an untreated control group, Group II (8 rats) served as an untreated periodontal disease by placement of a ligature around molar teeth, Group III (8 rats) served as an untreated periodontal disease with treatment of crude ethanolic *Pterodon pubescens* Benth., Group IV (8 rats) served as an untreated periodontal disease with film mucoadhesives *Pterodon pubescens* Benth.

To induce periodontitis, rats were anesthetized with an intraperitoneal injection of Ketamine (Francotar, Virbac do Brasil Ind Com Ltda., São Paulo, Brazil) at a 0.08 mL/100 g of body weight dose and Xylazine at a 0.04 mL/100 g of body weight dose (Virbaxyl 2%, Virbac do Brasil Ind Com Ltda., São Paulo, Brazil). The animals were placed on an appropriate surgical (Anexo 3) pad to facilitate access to the mandibular posterior teeth. Periodontal disease was induced following the method described by Crawford et al. [18] and Koide et al. [19] by placing a nylon 4.0 (Shalon) ligature around the lower first molars of each animal. Rats were given a normal food and water ad libitum daily beginning on day 0 (Baseline). Eight rats of each group were euthanized 7 days after baseline. Gingival tissues were collected for myeloperoxidase (MPO) analysis via ELISA, and jaws were harvested for bone loss analysis.

2.6.2 | MPO Kinetic-Colorimetric Assay

Neutrophil infiltration in the gingival tissues of rats was evaluated by the MPO kinetic-colorimetric assay, as a quantitative marker of neutrophil infiltration in inflammatory processes in multiple tissues. For this, 50 mg of gingival tissue were placed in a buffer with potassium bromide 0.5% hexadecyltrimethylammonium (pH 6.0), subsequently homogenized in an IKA crusher (Disperser T 10 basic, Staufen, Germany) and centrifuged for 10 min at 12,000 rpm at 4°C, and the supernatant was collected. MPO activity per mg of tissue was measured using 0.0005% hydrogen peroxide as substrate for MPO, where MPO activity was defined as being able to convert 1 μmol of hydrogen peroxide into water for 1 min at 22°W. Results were expressed as MPO activity/mg of tissue [20].

2.6.3 | Morphometric Analysis

The hemimandibles were immersed in hydrogen peroxide solution (3%) for 3 h to remove the excess of tissue. After this procedure, the hemimandibles were stained with methylene blue 1% (30 min) to demarcate the cement-enamel junction (CEJ). Photographic documentation was then performed using a Nikon digital camera (D40, Melville, NY, USA). Bone loss measurements were taken along both roots of the lower first molar. The analyzed areas were quantified using ImageJ software (ImageJ 1.32j, National Institutes of Health, USA), as previously described by Lima [21].

3 | Statistical Analysis

Results were expressed as mean ± SD (standard deviation). For permeation studies of porcine esophageal epithelium, the Mann Whitney *U* test was performed for comparison with pure drug and film, respectively. For in vivo data, statistical comparisons between groups were made using ANOVA analysis of variance

followed by the Bonferroni test. The statistical program used was GraphPad Prism 5. Significance was accepted when the p value was ≤ 0.05.

4 | Results and Discussion

4.1 | Phytochemical Study

4.1.1 | Phytochemical Analysis

Two analytical methods—HPLC-UV and GC-MS—were employed to ensure accurate quantification of the extracted compounds. Quantifications were performed using analytical standards a-Humulene, *trans*-Caryophyllene, and geranylgeraniol (Sigma Aldrich Saint Louis USA). The peak area calculated from HPLC chromatograms was correlated linearly with the compound methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate ester concentrations and the compound 6α,7β-dihydroxyvouacapane-17β-oate ester of methyl. The percentage amounts of the separated compounds are shown in Table 2.

In the GC analyses, volatile compounds such as *trans*-caryophyllene, α-humulene and diterpene geranylgeraniol were Detected. In contrast, HPLC analysis provided better separation of major vouacapane compounds. In this study, the vouacapane compound extracted as one of the major constituents of sucupira fruits, methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate ester (m/z 404) was specifically monitored. Previous studies by our group demonstrated that this compound, together with 6α,7β-dihydroxyvouacapan-17β-oate methyl ester (m/z 362), exhibits antinociceptive and anti-inflammatory activities relevant to the development of mucoadhesive films for treating periodontal diseases. Although the amount of geranylgeraniol in these samples is very low, this small percentage does not compromise the pharmacological activity of the plant extract [22].

4.2 | Pharmacotechnical Study

4.2.1 | Development Mucoadhesive Films

An aqueous solution of 3% w/w of PVA was prepared under constant stirring and heating (100°C) followed by filtration to remove any undissolved PVA residues. The study determined that 12 mL of the 3% w/v of PVA was required to be cast into the Teflon molds to obtain a polymeric film with the desired thickness, 0.05–0.15 mm, and a diameter of 10 cm. *P. pubescens* Benth. extracts were incorporated via nano dispersion, with different concentrations of the extract (1%–3%) being mixed with the PVA solution under sonication for approximately 1 min.

4.2.2 | Dynamic Light Scattering—Nanosizer

The data presented are the sample size (nm), polydispersity index (PDI) and zeta potential (ZP) of nano dispersion formulated at room temperature at 25°C for *P. pubescens* Benth (Table 3). The measurements were carried out on the same day as formulated. As shown in Table 3, both particle size and PDI decreased with increasing nanoparticle concentration in the nano dispersion.

TABLE 2 | Percentage amounts of active compounds present at crude ethanolic *Pterodon pubescens* fruit extract, methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate ester (m/z 404) and 6α,7β-dihydroxyvouacapane-17β-oate ester of methyl (m/z 362).

Extracts	*trans*-Caryophyllene (% w/w)	α-Humulene (% w/w)	Geranylgeraniol (% w/w)	Voucapane 362 (% w/w)	Voucapane 404 (% w/w)
Ethanolic *Pterodon pubescens*	1.16 ± 0.04	0.31 ± 0.003	0.23 ± 0.02	5.70 ± 0.50	12.58 ± 1.16

On the other hand, we observed that ZP fluctuates with the increase in sucupira extract concentration, ranging from −19.8 to −9.26 mV. The negative zeta potential can be attributed to the presence of ionized hydroxyl groups on the surface of the PVA-based nanoparticle. This finding corroborates with studies where other PVA concentrations showed electrical potential values around −12 mV like those obtained in this study [23]. The zeta potential is a critical parameter that is often used to predict the colloidal stability of nanoparticles and the potential interaction between nanoparticles and mucous membranes. Zeta potential values of 30 mV or higher of nanoparticles are considered to have good colloidal stability with less propensity to agglomerate during storage [24]. However, previous studies have shown that a colloidal system consisting of nanoparticles with zeta potential as low as 5 mV is still sufficient to prevent a considerable degree of agglomeration [25]. Therefore, the zeta potential exhibited by nanoparticles loaded with *P. pubescens* Benth. may provide sufficient colloidal stability to ensure that the particles remain mono dispersedly loaded onto the PVA film after drying.

4.2.3 | TEM

Transmission Electron Microscopy was employed to evaluate the structure and morphology of the PVA nanoparticles formed. The TEM images confirmed the presence of nanometric size of the particles (Figures 2 and 3), which demonstrate the spherical morphology of PVA-containing particles. Additionally, the images suggest the presence of crystal characteristics of compound methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate.

Figure 2 shows the newly prepared nano dispersion. Nanoparticles smaller than 200 nanometers and agglomerated with both oval and spherical shapes are observed in Figure 2A,C. Crystals are visible in Figure 2B,D images in both samples. The

TABLE 3 | Size, polydispersity, and zeta potential with different proportions of extract, evaluated by dynamic light scattering (Malvern Instruments). (mean ± standard deviation, $n = 3$).

Proportion of *P. pubescens* Benth.	Size (nm)	Polydispersity (PDI)	Zeta potential (mV)
Blank	27.87 ± 4.94	0.7 ± 0.07	−9.26 ± 4.04
1.0%	379.1 ± 29.95	0.36 ± 0.05	−17.9 ± 2.18
3.0%	359.0 ± 23.33	0.34 ± 0.007	−19.8 ± 0.46

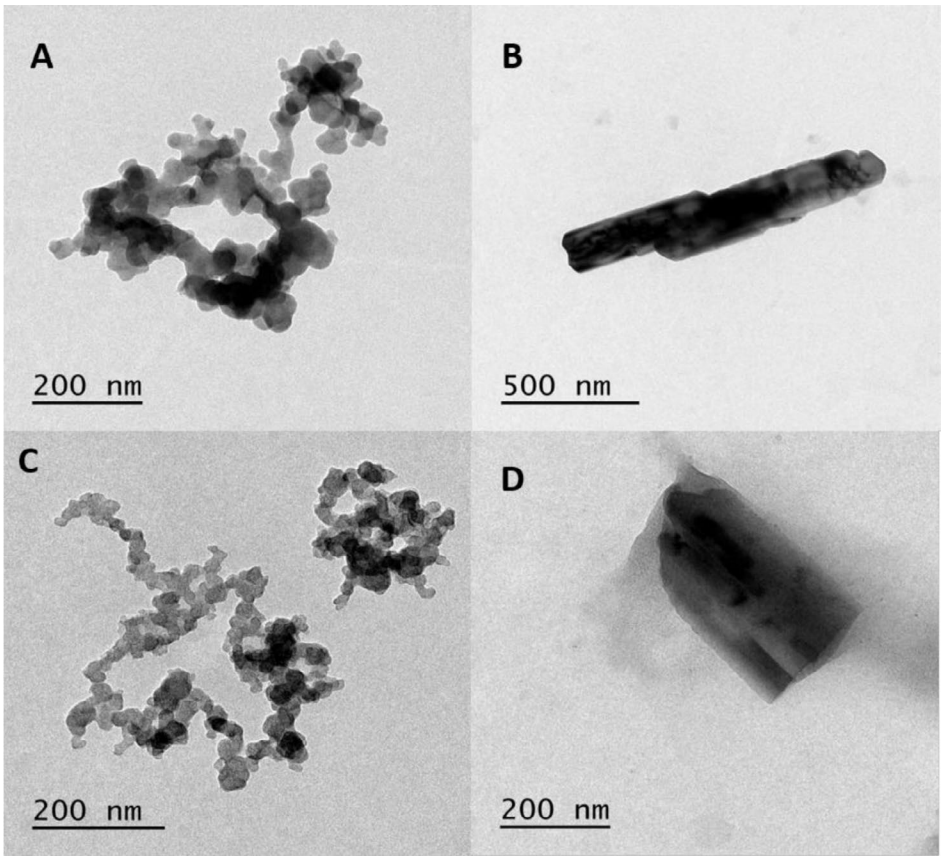

FIGURE 2 | Images of PVA nanoparticles—*P. pubescens* Benth. obtained from transmission electron microscopy. (A) 1% film nanoparticles; (B) crystals of the film 1%; (C) 3% film nanoparticles; (D) 3% film crystal.

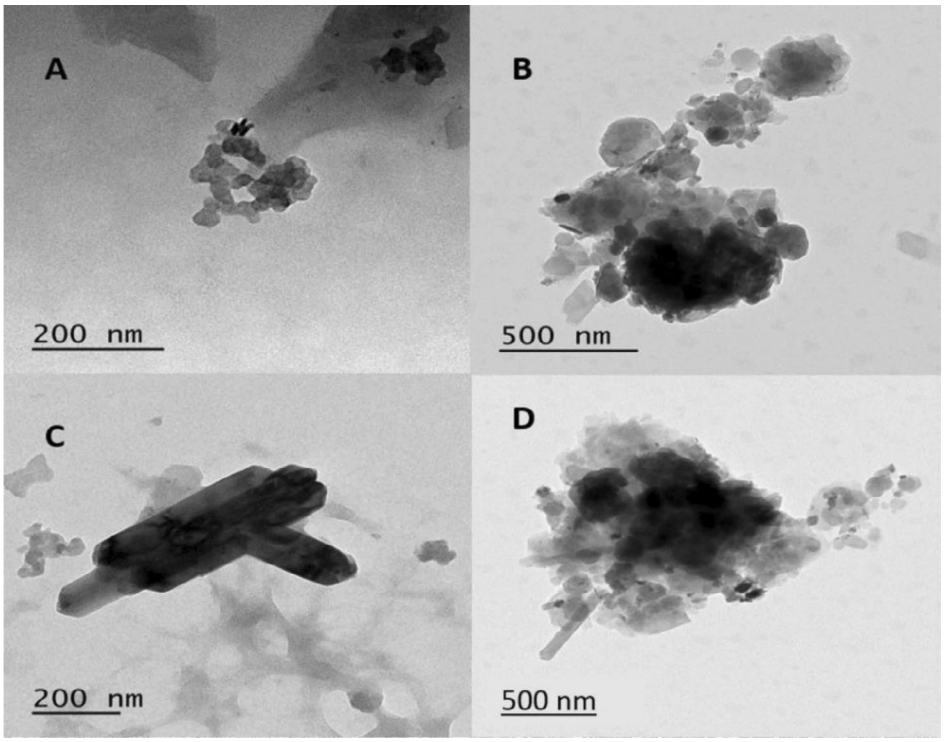

FIGURE 3 | Images from the 3% film with *P. pubescens* Benth. diluted obtained from transmission electron microscopy. (A) nanoparticles; (B) clustered nanoparticles; (C) vouacapane crystal 404 (m/z) (D) nanoparticles and crystal.

particle agglomeration may be due to the PVA polymer, which promotes film formation. For this analysis, the nano dispersion was 1:100 of the original sample.

In Figure 3, the 3% concentration mucoadhesive film, which was dried, diluted in distilled water (1:100), and added to Formvar, is shown. In Figure 3A,B, nanoparticles approximately 200 nm in size are observed, along with larger nanoparticles around 500 nm. These particles remain agglomerated, exhibiting both oval and spherical shapes. Crystals are also visible in Figure 3C,D.

4.2.4 | Mechanical Properties Analysis

When selecting a coating film, it is essential that it possesses adequate mechanical strength to withstand manufacturing, packaging, storage, and transportation processes. This ensures the effectiveness of coating procedures and prevents the formation of pores and cracks on the surface, which could accelerate drug degradation and premature release, thereby promoting physicochemical instability [26]. Furthermore, film thickness is a critical parameter; recent studies have shown that thicker films can significantly delay drug release kinetics compared to thinner ones, directly affecting pharmacological activity [27].

The impact of different concentrations of *Pterodon pubescens* extract on the mechanical properties of mucoadhesive films is shown in Table 4, characterized by high strength and low perforation energy.

Observations showed that adding *Pterodon pubescens* extract at different concentrations during the manufacture of PVA films gradually increased the overall tensile strength of the mucoadhesive film, while the elongation at break initially decreased compared to the blank film. The blank film (no extract) exhibited moderate tensile strength (~10.49 MPa) and high puncture energy (195.14 kJ m^{-3}), indicating a balanced structure with significant elasticity. The 1% extract film showed a decrease in tensile strength (to 5.19 MPa) and a sharp drop in puncture energy (88.41 kJ m^{-3}). This may result from minor polymer–extract compatibility issues or phase discontinuities at low loading. The 3% extract film, however, demonstrated a notable increase in tensile strength (14.07 MPa) and thickness (0.152 mm), though puncture energy remained low (93.56 kJ m^{-3}).

These initial weakening effects at low concentration (1%) are reminiscent of observations in PVA films loaded with plant extracts, where tensile strength decreased with low-level incorporation due to poor dispersion or subtle structural defects. For instance, grape peel extracts reduced tensile strength by ≈12% relative to pure PVA films (~56 MPa → ~50 MPa) [28].

TABLE 4 | Mechanical properties of mucoadhesive films, thickness, tensile strength, and drilling energy of the formulations (mean ± standard deviation, $n = 3$).

Sample	Thickness (mm)	Tensile strength, Ps (Mpa)	Drilling energy, Ep (Kj m^{-3})
Blank	0.057 ± 0.02	10.49 ± 2.66	195.14 ± 59.60
Film 1%	0.064 ± 0.03	5.19 ± 1.31	88.41 ± 34.68
Film 3%	0.152 ± 0.01	14.07 ± 1.31	93.56 ± 17.60

Note: Puncture resistance (Ps) and puncture energy (Ep) parameters.

At higher extract loading (3%), a rearrangement of the polymer matrix occurs. Similar studies (chitosan/PVA films with cashew nut extract) show that increasing extract concentration increases tensile strength and Young's modulus but reduces elongation at break, indicating a denser, more rigid film network [29]. The increase in tensile strength at 3% concentration can be attributed to chemical interactions—mainly hydrogen bonding—between hydroxyl groups of PVA and phenolic compounds in *P. pubescens* extract. This reinforces the film matrix and enhances thickness and strength, as seen in studies on anthocyanin-PVA composite films (*Piper betle* extract) that similarly showed tensile strength increases with concentration while elongation decreased [30].

Although tensile strength improves substantially at 3%, the puncture energy remains low compared to the blank. This indicates that rigidity increased, but flexibility (the ability to dissipate energy under puncture/loading) remained compromised. Based on the evaluated parameters, an adequate balance is essential. The ideal film for treating periodontal disease must be malleable enough to be handled without breaking, especially during oral application. However, the flexibility of the film must be taken into account, as excessive flexibility can result in unwanted extension and deformation during the preparation and packaging processes [31]. Future optimization should aim to balance tensile strength and elasticity, possibly by modifying extract ratios, incorporating plasticizers, or microstructural tuning.

4.2.5 | Mucoadhesion Measurement

Oral films made from buccal polymers should possess at least medium mucoadhesive strength; therefore, one or more polymeric film-forming excipients can be selected. Anionic polymers bind to mucin proteins by hydrogen bonds through hydroxyl groups [32, 33].

The properties of an ideal film are to include flexible, elastic, and smooth, and the film must be strong enough to withstand recasting due to the stress of mouth movements. The film must also have good mucoadhesive strength to be retained in the mouth for the desired duration of action. One way to quantify this mucoadhesive force is to verify the detachment after the interaction of a mucoadhesive dosage form with the mucosal tissue, thus quantifying the mucoadhesive performance of the film. The mucoadhesive strength of a polymer increases with molecular weights above 100.000 and the study used PVA with a molecular weight of approximately 45.000. The mucoadhesive properties of films manufactured in porcine mucosa are shown in Table 5.

This parameter refers to the maximum force required to separate the film from the mucosa of the porcine esophagus.

TABLE 5 | Mucoadhesive force (mN), mucoadhesive films (control) and incorporated extract *Pterodon pubescens* (mean ± SD, $n = 5$).

Sample	Mucoadhesive force (mN)
Blank	0.0226 ± 0.015
Film 1%	0.0139 ± 0.008
Film 3%	0.0155 ± 0.014

The comparison among the samples prepared with PVA showed that the increase in the concentration from extract 1.0%–3.0% do not significantly impact ($p > 0.05$) the overall mucoadhesive force of the film.

4.3 | Cytotoxicity Study *Using* Human Gingival Fibroblast (HGF-1)

An in vitro cytotoxicity test was conducted to establish the baseline toxicity of PVA film containing the sucupira extract. The cytotoxicity of the developed formulations was evaluated against human gingival fibroblasts, HGF-1 (ATCC CRL-2014) cultures. This assay employed sulforhodamine B, a colorimetric dye that binds to the basic amino acid residues of viable cellular proteins at the time of fixation, producing a purplish color. The intensity of the staining correlates with the amount of SRB dye bound, which is proportional to the total protein content and, consequently, to the number of viable cells [15].

Figure 4 shows the cell viability of the pure extract, the control film (Blank), and the formulations in mucoadhesive films with concentrations of 1% and 3%. The results demonstrated that free extract resulted in lower cell viability (73%) at the concentration of 15.62. However, this cytotoxic effect was mitigated when the extract was incorporated into PVA films. Films loaded with 1% w/v extract maintained 100% cell viability across all concentrations tested. This finding aligns with the study by Pabst et al. [34] which showed the biocompatibility of PVA, promoting cell growth without cytotoxicity. In their work, Patil et al. analyzed films containing SF-PVA composite that were cytocompatible and did not show cytotoxicity when evaluated in culture of L929 fibroblast cells. However, when sucupira extract concentration in the films was increased to 3%, cell viability of the HGF-1 cell line decreased to 70% viability. This suggests that incorporating the extract into the film is an effective strategy to mitigate the cytotoxic effect at higher concentrations.

The in vivo study conducted by Souza et al. [35] showed the safety of using the crude dichloromethane *Pterodon pubescens* fruit extract as well as the mixture of diterpene furan isomers from vouacapan (1:1) isolated from the crude extract (ester isomers) of 6a-hydroxy-7b-acetoxy-vouacapan-17b-oate and 6a-acetoxy-7b-hydroxy-vouacapan-17b-oate methyl ester at lower doses. These findings support further clinical studies and the development of herbal medicines.

4.4 | In Vitro Permeation Studies

An in vitro permeation study using porcine esophageal epithelium was conducted using a Franz-type diffusion cell. This was done to evaluate the release profile of the methyl compound 6α-acetoxy7β-hydroxy-vouacapan-17β-oate, present in *Pterodon pubescens* Benth extract from mucoadhesive films (1 and 3%). Samples were collected from the receptor compartment for vouacapane quantification. Figure 5 shows the permeation profiles of methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate from mucoadhesive films through porcine esophageal mucosa.

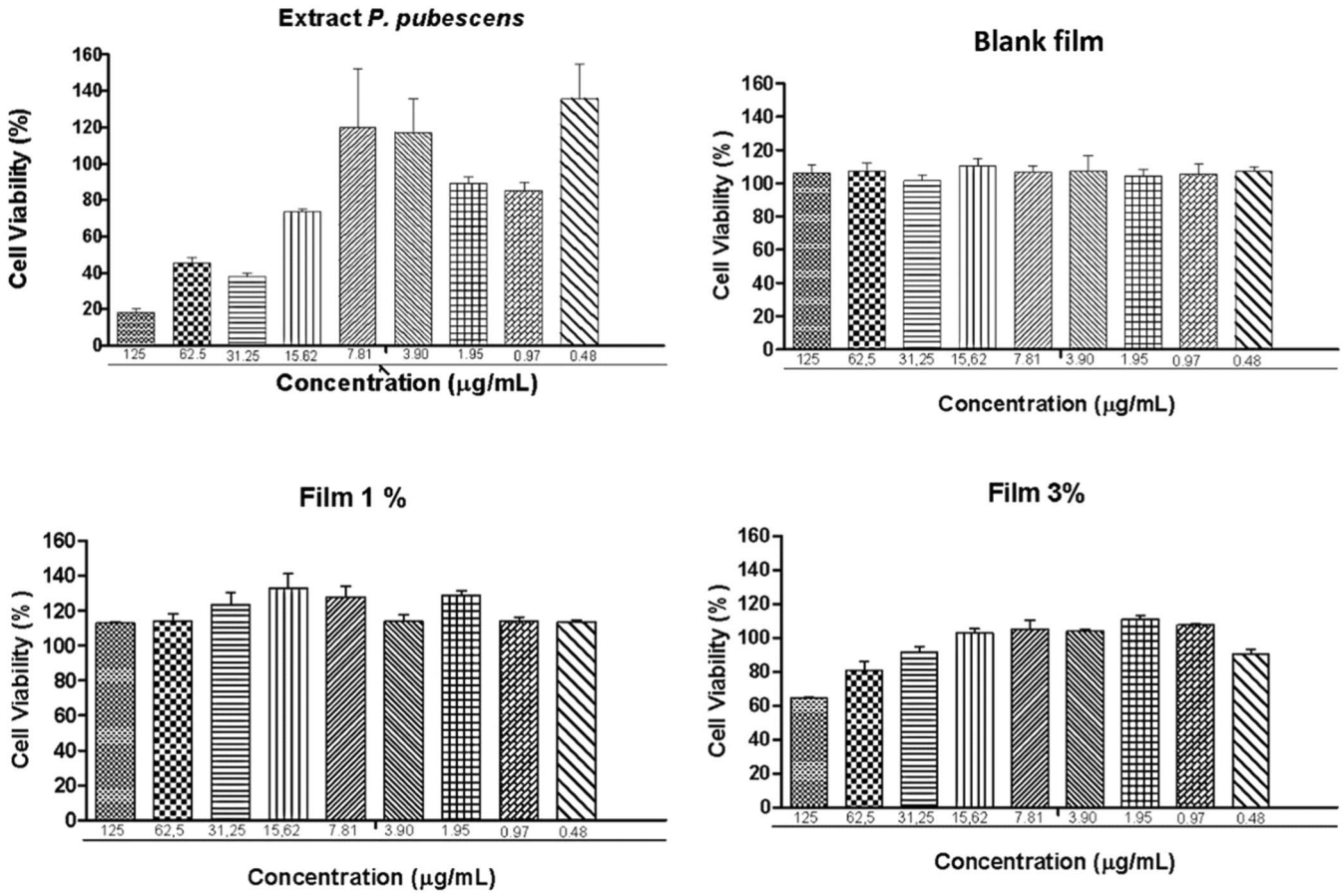

FIGURE 4 | Cell viability indirect method in HGF-1 mucoadhesive films and extract of *P. pubescens* Benth at concentrations of 1% and 3%. Blank film with PVA; (Film 1%): Formulation containing extract of *Pterodon pubescens* 1%; (Film 3%): Formulation containing extract of *Pterodon pubescens* 3%. Data reported as mean ± SD $*p < 0.05$ one-way ANOVA with the Bonferroni post hoc.

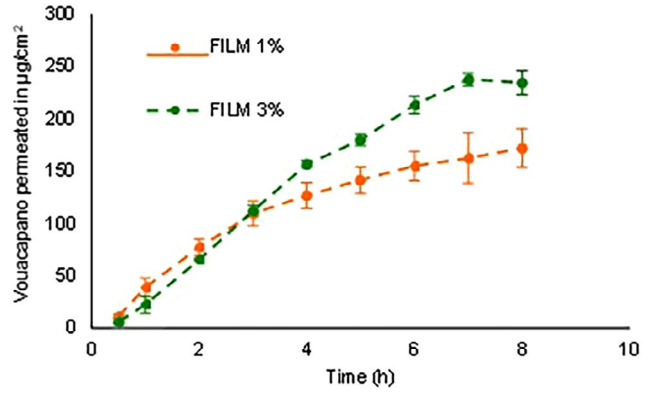

FIGURE 5 | Permeation profile of film 1% and film 3%. The amount of drug loading was 0.71 mg (1%) and 1.04 mg (3%) of methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate for each formulation. Data are expressed as mean ± standard deviation of $n = 6$ samples. [Color figure can be viewed at wileyonlinelibrary.com]

For quality control and standardization of *Pterodon*-derived products, vouacapanes were used as phytochemical markers due to their pharmacological activities and permeation studies.

Figure 5 shows the permeation values, revealing that the mucoadhesive film containing 3% *P. pubescens* extract (33.84 ± 3.14 µg/cm²/h) produced a higher flux of vouacapane. The greater flux and release observed in films loaded with 3% w/w of sucupira extract can be attributed to the higher concentration gradient of vouacapane present in the films. This led to a faster release of the compound from the film and through the mucosa. Moreover, the rapid release and flux of vouacapane from the 3% w/w film may also serve as a plausible explanation for the greater cytotoxicity exhibited by the 3% w/w film shown in the cytotoxicity assay. The swift release of a high concentration of the compound over a short period in the 3% film, compared to the 1% film, may cause unwanted cytotoxic effects on the HGF-1 cell line at the highest tested concentration (125 µg/mL), as shown in Figure 6. However, the more gradual flow and the lower concentration of vouacapane released from the 1% film allow for better controlled delivery, avoiding cellular toxicity. This makes the 1% concentration a promising candidate for future studies focusing on exposure time and pharmacological effects (Table 6).

4.5 | Antimicrobial Analysis of the Buccal Film

4.5.1 | MIC

An antimicrobial study was conducted against different *Streptococcus* strains, which are known to disrupt homeostatic and interfere with metabolic processes in humans. Different species were plated after 24 h of bacterial contact with the

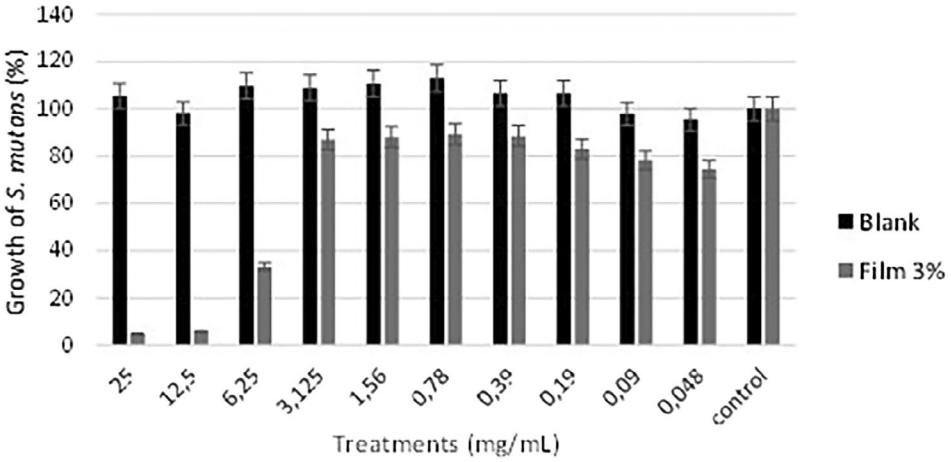

FIGURE 6 | Effect of mucoadhesive films on *Streptococcus mutans* adherence biofilm. Bacterial growth profile, presented by absorbance at 595 nm. Data are presented as mean ± standard deviation ($n = 3$).

TABLE 6 | Permeation parameter values obtained of methyl 6α-acetoxy7β-hydroxy-vouacapan-17β-oate vouacapane over an 8-h period.

	Vouacapans	
	Flux (μg/cm²/h)	**Permeability coefficient ($\times 10^{-2}$ cm h^{-1})**
Film 1%	21.14 ± 7.27	3.09 ± 0.01
Film 3%	33.84 ± 3.14	3.28 ± 0.30

Note: Data are expressed as mean ± standard deviation of $n = 6$ samples.

extract and the mucoadhesive films. Table 7 shows that the free *P. pubescens* extract exhibited stronger antimicrobial activity compared to the mucoadhesive formulations against buccal *Streptococcus* strains. Contrary to expectations, the films containing 1 and 3% extract were more effective in comparison to the free extract and the white film. Furthermore, antimicrobial activity was observed against several *Streptococcus* species with the extract-loaded films, where the free extract alone showed no activity (including *S. sanguinis* SK 36, *S. salivarius* ATCC 7073, *S. gordonii* NCTC 7868, and *S. mutans* UA 159). These results suggest that incorporating the extract into the PVA film may provide a synergistic effect that enhances the antibacterial properties compared to the free extract.

The study by Silva et al. [36] evaluated the compounds geranylgeraniol, 6α ester, 7β-dihydroxyivouacapane-17β-oate methyl ester and methyl iso-6α-hydroxy-7β-acetoxy-valeric-17β-oate and acetoxy-7β-hydroxy-valeric-17β- methyl ester against four species of *Candida* spp., and on the bacteria *Streptococcus mutans* and *Staphylococcus aureus*. In their work, it was found that these compounds exhibited antimicrobial effect against the four *Candida* spp. strains but showed no activity against the bacteria strains. This finding aligns with our current results, which show that the free extract was unable to elicit an antibacterial effect against certain bacteria strains. This lack of activity may be related to the presence of secondary metabolites, such as terpenes, which are likely associated with the inhibitory effect on specific microorganisms. Supporting this, the work of Duarte et al. reports microbial inhibition by various agents, including different extracts, essential oils, and fractions [37].

Furthermore, the diffusion study, presented in Table 8, shows the antibacterial activity of the mucoadhesive films indicating correlation with the MIC methodology, as the films inhibited bacterial strain growth in a manner that mirrors the MIC results.

TABLE 7 | Result of visual reading of extract and mucoadhesive films formulation, in mg/mL in standard *Streptococcus* strains.

Microorganisms	Clorexidine (mg/mL)	Film blank	Extract (mg/mL)	Film 1% (mg/mL)	Film 3% (mg/mL)
Streptococcus mitis NCTC 12261	0.0037	[a]	2.5	1.56	0.78
Streptococcus oralis ATCC 10557	0.015	[a]	2.5	3.12	1.56
Streptococcus sanguinis SK 36	0.0075	[a]	[a]	[a]	[a]
Streptococcus salivarius ATCC 7073	0,0037	[a]	[a]	2.5	1.25
Streptococcus gordonii NCTC 7868	0.0075	[a]	[a]	2.5	6.25
Streptococcus mutans UA 159	0.0037	[a]	[a]	2.5	1.25

[a]Growth of bacteria.

TABLE 8 | Activity antibacterial mucoadhesive film 3% formulation, in mm[a] in standard *Streptococcus* strains using the disk diffusion method.

Microorganisms	Inhibition halo (mm)[a]
Streptococcus mitis NCTC 12261	4
Streptococcus oralis ATCC 10557	2
Streptococcus sanguinis SK 36	—
Streptococcus salivarius ATCC 7073	—
Streptococcus gordonii NCTC 7868	2
Streptococcus mutans UA 159	1,5

[a]Mean values of the experiment in triplicate.

4.5.2 | Evaluation of the Formulation Action on Biofilm Formation

The presence of microbial biofilm adhering to the dental surface represents a bacterial deposit composed of numerous species predominantly gram-positive, facultative anaerobic cocci, mainly of the genus *Streptococcus* [38]. These biofilms create a protective environment that enhances bacterial resistance against antimicrobial agents. Among the *Streptococcus* strains commonly found in these biofilms, *S. mutans* is considered the most relevant, as this strain plays a key role in the development of dental caries. This is attributed to the ability of the bacteria to produce acids and extracellular polysaccharides (PEC) capable of initiating the onset of periodontal disease. A study by Michielin and collaborators [39] observed the inhibitory potential of *Cordia verbenacea* extract against gram-positive bacteria, with alpha-humulene as the compound exhibiting the strongest antimicrobial activity.

The crude ethanolic *Pterodon pubescens* fruits contain essential oils such as a-humulene and *trans*-Caryophyllene, aromatic compounds produced through the plant's secondary metabolism that serve as a defense mechanism against insects. These essential oils, composed of terpenes and derivatives, are commonly found in various plants and are responsible for the aroma but also for several pharmacological properties, including insecticidal activity and as food flavoring, among other properties [40].

In the biofilm inhibition experiment, dilution of the control formulations (blank) and 3% *P. pubescens* extract film were tested. As shown in Figure 6, the control formulation did not show toxicity on the *Streptococcus mutans* biofilm, but when compared with the 3% extract film formulation, there was a reduction in the number of bacterial cells at the highest concentrations (25, 12.5 and 6.25 mg/mL), showing an inhibitory effect on biofilm formation (4.75%, 5.90% and 33.15%).

4.5.3 | SEM

An SEM analysis was performed on the biofilm samples to elucidate the structure and morphology of biofilm formation in the absence and presence of sucupira-loaded PVA films.

In the dental field, natural products have gained increasing attention for therapeutic use, particularly due to their anti-inflammatory properties, analgesics for example [41]. Oral biofilm formation is a multi-step process consisting of bacterial adhesion, EPS production, and biomass accumulation. EPS plays a crucial role in bacterial adhesion, biofilm formation, and structural integrity, whose presence enhances bacterial cell accumulation on surfaces, increases the volume and stability of the biofilm, contributing to virulence [41]. When fermentable carbohydrates, such as sucrose, fructose, and glucose, are present, the EPS structure may dissolve, promoting acid production by *Streptococcus mutans* and further contributing to biofilm development and pathogenicity [42].

The present study comprehensively demonstrated that the investigated treatments were effective against *S. mutans* biofilms over a 24 h period. The findings are corroborated by previous investigations, Firmino et al. [43], that evaluated diterpenes isolated from *Croton blanchetianus* Baill, exhibiting antibiofilm activity by inhibiting both the formation of biofilms and established biofilms of *S. mutans* and *S. sanguinis*.

The images in Figure 7 demonstrate the effect of Blank films at three different tested concentrations (3, 6, and 12 μg). The formation of some cell agglomerates in the division process, many monotypic cells, and disruption of the extracellular matrix, a key feature in streptococci in biofilm formation, are observed.

Changes in cell morphology, shown in Figure 8, suggest damage to the cell membrane and integrity, which could lead to cell death and consequently reduce biofilm development. The presence of terpenes is believed to contribute to this inhibition of *S. mutans* biofilm formation. This reduction in homogeneity can be attributed to secondary compounds (terpenes—sesquiterpenes, diterpenes) found in the extract that enhance this inhibitory effect of *P. pubescens* by interfering with *S. mutans* adhesion and thus preventing the formation of a thick biofilm layer [44].

The presence of several active constituents in plant extracts is explained by the fact that plants normally develop a series of metabolites with complementary defensive functions against pests and diseases. These compounds, known as secondary metabolites, play a role in plant defense by exhibiting cytotoxic activity against pathogens. They have been proven to be valuable antimicrobial agents in medicine. Such compounds can be found in teas and medicines, either as natural products or as prototypes for the development of new semi-synthetic or synthetic drugs [44–46].

The result of this study corroborates the study by Gartika [47], who found that extracts from *Myrmecodia pendans* containing terpenoid were able to destroy approximately 40% of preformed *S. mutans* biofilms. Other studies have shown that terpenoids can disrupt membrane integrity in various organisms and promote the release of planktonic cells from biofilms, effectively eradicating a significant portion of biofilm cells [48]. In our study, treatment with *P. pubescens* extract resulted in uneven arrangements, reduced cell density, asymmetrical projections, and loss of structural integrity on

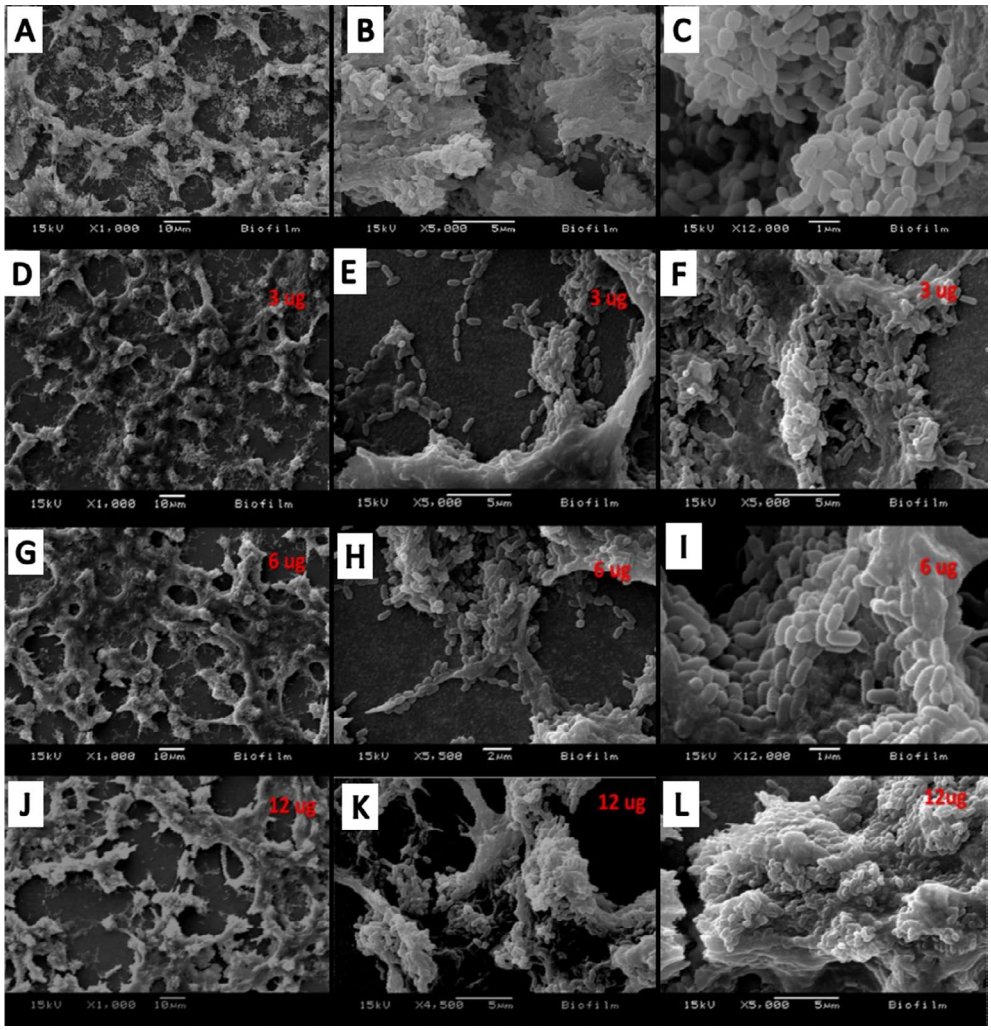

FIGURE 7 | SEM images of *Streptococcus mutans* biofilm after treatment with mucoadhesive film (control). Images (A–C) represent the control of *S. mutans* growth at different magnifications (1000; 5000 and 12,000 magnification X 15 KV); The other images (D–F) refer to the tested concentration of 3 μg of blank film; (G–I) at the tested concentration of 6 μg and (J–L) at the tested concentration of 12 μg. [Color figure can be viewed at wileyonlinelibrary.com]

the surface when compared to the control (blank). Bacterial eradication can be achieved through several mechanisms that interfere with biofilm formation. One mechanism involves the redistribution of cells attached to the surface, which may consist of one or multiple bacterial species depending on the microorganism and environmental physicochemical conditions. Another mechanism is the immobilization of microorganisms on the substrate, promoting bacterial multiplication due to the presence of surface adhesins and extracellular polymers that aid adhesion. A third mechanism is the development of a mature biofilm, where bacteria exhibit specific adaptations, such as the production of exopolysaccharides (EPS) that enhance adhesiveness. The final mechanism is biofilm maturation, which during the microbial community produces various saccharolytic enzymes that degrade the biofilm matrix, facilitating the dispersion of bacteria to colonize new areas [42].

The terpenoids class of compounds can promote the release of planktonic cells, disrupting membrane integrity of various organisms, contributing to the elimination of cells from the biofilm [46].

Therefore, examining the components that play a role in the process of formation of EPS that is closely linked to dental biofilm development is essential. Further studies are needed to isolate the most active molecules in the *Pterodon pubescens* Benth ethanolic extract against these and other oral bacteria and to elucidate their mechanisms of action, with a perspective for their potential use either alone or as an adjuvant in the treatment of oral infections.

4.6 | Pharmacologic Study

4.6.1 | MPO—Kinetic-Colorimetric Assay

This study is the first to evaluate the action of the *Pterodon pubescens* Benth ethanolic extract in an experimental rat model of periodontal disease. The extract demonstrated a significant reduction ($^*p < 0.0416$) in neutrophils infiltration within gingival tissue, suggesting a potential anti-inflammatory effect. As shown in Figure 10, the extract also produced a reduction in neutrophil presence comparable to that observed with the

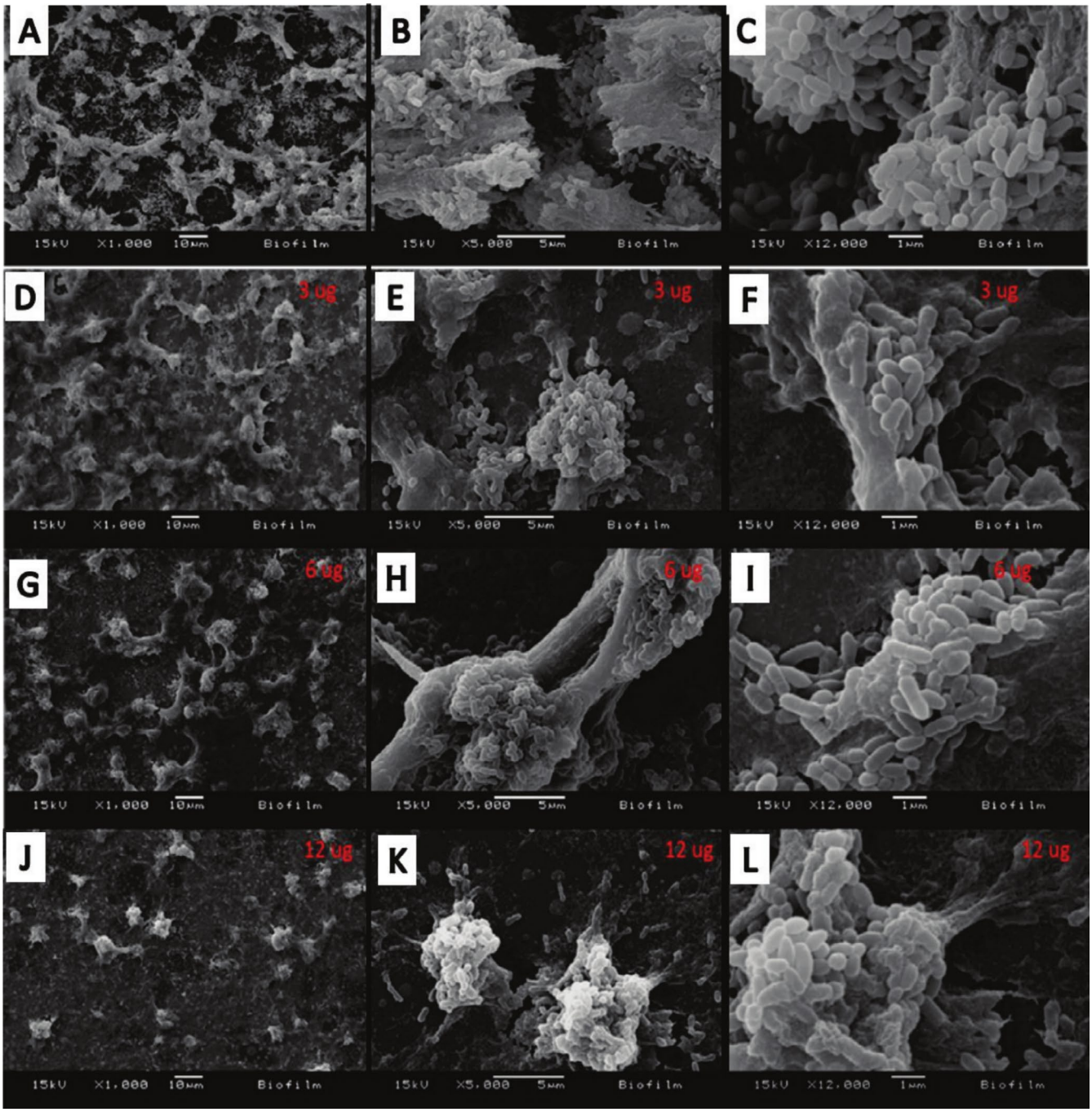

FIGURE 8 | SEM images of the *Streptococcus mutans* biofilm after treatments with mucoadhesive film containing sucupira ethanolic extract. Images (A–C) represent the control of *S. mutans* growth at different magnifications (1000; 5000 and 12,000 magnification X 15 KV); The other images (D–F) refer to the tested concentration of 3 µg of the film containing *Pterodon pubescens* crude extract; (G–I) at the tested concentration of 6 µg and (J–L) at the tested concentration of 12 µg. [Color figure can be viewed at wileyonlinelibrary.com]

film formulation. When compared to the satellite group, both treatments led to a noticeable decrease in neutrophil infiltrates. Furthermore, as shown in Figure 9, the PVA film exhibited strong mucoadhesion to the buccal tissue, as the film remained in place throughout the study and allowed for sustained delivery of the extract at the target site of inflammation.

Among the commercially available agents used in managing periodontal disease, nonsteroidal anti-inflammatory drugs (NSAIDs) are sometimes utilized due to their ability to antagonize the pro-inflammatory pathways. This leads to a reduction in the production of arachidonic acid metabolites that are pro-inflammatory mediators implicated in the progression of periodontal disease [49].

The terpene class, identified as major components in the genus *Pterodons*, includes sesquiterpenes, which exhibit potential antioxidant activities and anti-inflammatory properties. Volatile compounds such as *trans*-Caryophyllene, α-Humulene, and diterpene geranylgeraniol present in the extract of this study

in the GC analysis are associated with anti-nociceptive, anti-inflammatory, and antispasmodic effects [35].

In the current study, we demonstrated that the PVA film containing sucupira extract could mitigate the pro-inflammatory pathway as exhibited by the reduction in neutrophil infiltration as shown in Figure 9. Such reduction in neutrophil infiltration may help reduce buccal inflammation, which is typically associated with the progression of dental tissue degeneration.

As shown in Figure 10, the animals that received only the ligature (Figure 10A) exhibited intense gingival swelling and dental mobility. Whereas the animals that received the treatments buccally (Figure 10B,C) once daily presented gingival regeneration along with a decrease in gingival tissue edema, corroborating with a result presented above MPO kinetic colorimetric assay, which further supports the inflammation of sucupira extract.

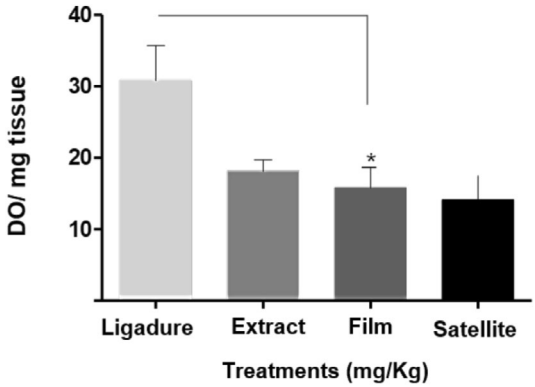

FIGURE 9 | Myeloperoxidase dosage of gingival homogenates from rats induced with periodontal disease over a 7-days treatment period with 3% extract and 3% mucoadhesive films. The vertical lines indicate the SEM, the asterisk indicates the level of significance, $*p < 0.05$, when compared to ligature. Statistical significance was determined by ANOVA one way followed by Bonferroni test, $*p < 0.0416$.

4.6.2 | Morphometric Analysis

Periodontal diseases are characterized by the presence of bacterial plaque that can trigger a local inflammatory reaction in susceptible individuals. Natural products have been investigated more deeply as promising agents in the prevention of oral diseases, especially diseases related to bacterial plaque, such as gingivitis and dental caries and due to having powerful anti-inflammatory properties. In addition, the incorporation of bioactive natural compounds into periodontal therapy represents a promising strategy to complement conventional approaches, with the potential to mitigate challenges such as antimicrobial resistance and microbiota dysbiosis, thereby contributing to improved clinical outcomes [50–52].

A study by Toker et al. [53] reported the use of natural products in bone resorption and reported a reduction in alveolar bone loss with propolis treatment after 11 days. Propolis significantly reduced alveolar bone loss in experimental periodontitis in a ligature-induced experimental periodontitis rat model that contains flavonoids (Ex: quercetin), aromatic acids, terpenoids, and phenylpropanoids fatty acids, as well as several other compounds. The synergistic effects of these components contribute to the extracts well-known properties, including antibacterial, antifungal, antiviral, anti-inflammatory action, hepatoprotective, antioxidant, antitumor, immunomodulatory, among others [44].

Other studies using a methodology like ours have also demonstrated a protective effect against periodontal bone loss of two Brazilian plants, rosemary pepper (*Lippia sidoides*) and mastic (*Myracrodruon urundeuva*) [45, 46]. The antimicrobial properties of *L. sidoides* are involved with the presence of essential oils rich in carvacrol and thymol and are used against important periodontal pathogens [46].

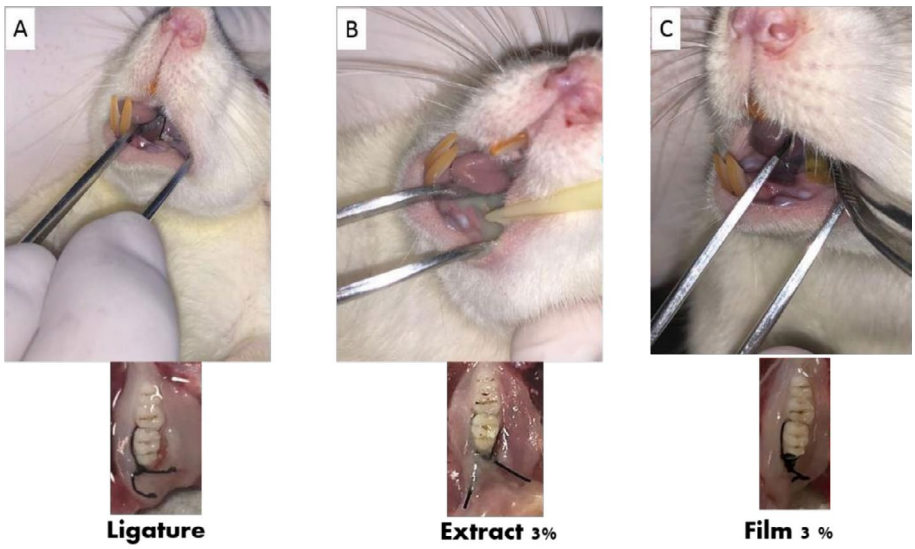

FIGURE 10 | Image of animals receiving buccal treatments. (A) only ligature, (B) extract 3%, (C) film 3%. [Color figure can be viewed at wileyonlinelibrary.com]

Regarding the evaluation of alveolar bone loss, the study demonstrated a significant decrease ($p < 0.05$) in the group treated with herbal gel containing carvacrol when compared to rats treated with the vehicle. This result by Botelho and collaborators [46] corroborates ours because, after 11 days of treatment with the gel, it was possible to observe a reduction in the injured tissue, a decrease in the MPO enzyme, which is abundant in neutrophils and very present in the gingival tissue, and also observed a reduction in the growth of oral pathogenic microorganisms.

Several studies have examined the effects of geranylgeraniol, which is a diterpenic alcohol compound that has been shown to enhance viability and migration in different cell types, including fibroblasts and oral keratinocytes, especially following treatment with zoledronic acid—an inhibitor of osteoclast-mediated bone resorption [34, 48, 49].

Another approach by Koneski et al. [50] investigated the effects of the compound geranylgeraniol, reporting positive activity on clinical signs during the healing of oral wounds, including reduced inflammation, infection, and exposed bone. Scores notably, histological analysis showed a decrease in osteonecrosis and improved new tissue formation. The sucupira extract used in our study contains approximately six times less geranylgeraniol compared to the pure geranylgeraniol (Sigma) used in Koneski's research. In the present study, morphometric analysis showed that treatment with *Pterodon pubescens* Benth extract loaded into mucoadhesive films did not result in a significant difference ($p > 0.05$) in alveolar bone loss among the treated groups as illustrated in Figure 11. The experimental rat model of periodontal disease induced by ligatures is widely used, favoring a large accumulation of biofilm and thereby promoting epithelial ulceration [37].

In summary, our PVA-based film enables controlled, localized release directly at the periodontal site. In contrast, conventional formulations (mouthwashes, gels) suffer from poor retention due to salivary wash-out and mechanical disturbances. Similar mucoadhesive PVA films (e.g., with resveratrol) have demonstrated prolonged release profiles, adequate mechanical strength, and consistent mucosal adhesion under dynamic in vitro conditions [51, 52, 54–56]. In vitro cytotoxicity assays using HGF-1 cells showed less cellular stress when the extract is embedded in the film versus the free form, indicating the carrier's protective effect during prolonged exposure. This aligns with previous findings where encapsulation of bioactives within PVA matrices reduced cytotoxicity and improved cellular compatibility. The use of *P. pubescens* offers multi-modal therapeutic activity—anti-inflammatory, antinociceptive, and antimicrobial—particularly relevant to periodontal pathophysiology. *P. pubescens* extract-modified biomaterials have shown enhanced hydrophilicity and fibroblast adhesion, indicative of improved integration within oral tissues. Furthermore, preclinical studies have not reported acute toxicity or mutagenicity at extract doses far exceeding those applied in local formulations [35]. Our films display sufficient tensile strength and integrity for handling, placement, and application in the oral cavity—superior to conventional gel-based dosage forms that may fail to localize or suffer displacement. Reports on plant-extract-loaded polymer films

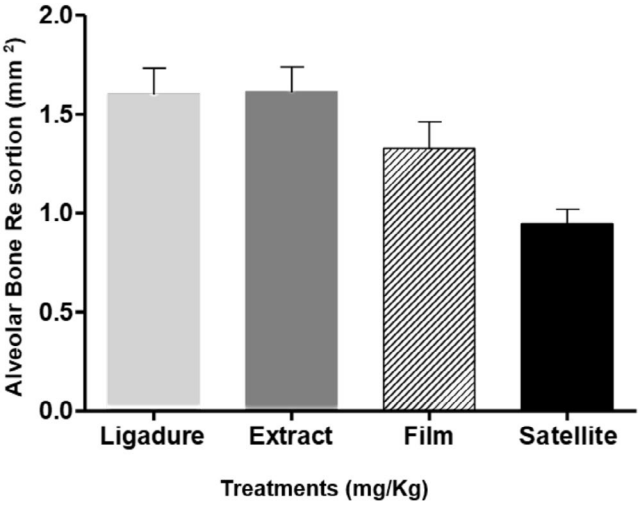

FIGURE 11 | Effect of treatments buccal with extract and mucoadhesive Film 3% *P. pubescens* Benth on alveolar bone resorption of rats submitted to periodontal disease. Periodontal disease was induced by ligature around the second right upper molars. The hemimaxillae were dissected, photographed, and measured for alveolar bone resorption (mm^2). Barrs represent the mean value ± standard error of the mean (SEM).

(including PVA/chitosan composites) confirm increased tensile strength and stiffness with higher extract incorporation, albeit accompanied by decreased flexibility, matching our observations in the 3% sucupira extract film.

5 | Conclusion

In conclusion, the current work highlights the preparation, characterization, and evaluation of a polyvinyl alcohol (PVA) film loaded with sucupira extract for the treatment of periodontal disease. The extraction process employed mechanical stirring under ethanolic extraction, minimizing the use of harmful organic solvent in extracting the active agent from sucupira fruits. The sucupira extract-loaded PVA film demonstrated adequate tensile strength with acceptable flexibility suitable for buccal application. Moreover, the films displayed mucoadhesive properties, which are pertinent in enhancing the residence time of the film within the mouth upon application. Biocompatibility assessments using the HGF-1 cell line confirmed that the sucupira-loaded films are well tolerated. In vitro permeation studies with porcine mucosal tissue showed efficient delivery of the active compound, vouacapane, across the mucosa. Additionally, the films exhibited enhanced antibacterial activity against five different *Streptococcus* strains commonly implicated in periodontal disease progression. SEM analysis further revealed that the sucupira-loaded films effectively inhibited biofilm formation compared to controls. An in vivo rodent model of periodontal disease demonstrated the formulation's efficacy, as exemplified by reductions in gingival tissue edema and dental mobility. Furthermore, myeloperoxidase kinetic-colorimetric assay demonstrated that the buccal film exhibited an anti-inflammatory effect at the application site, which is crucial for mitigating periodontal disease progression. Should this mucoadhesive polymeric film be translated into clinical practice, the delivery of sucupira

extract may be a promising therapeutic strategy to manage and treat periodontal disease.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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