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# Challenge in Propolis Biocompatibility as a Potential Medicament in Dental Medicine

## *A Literature Review*

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**ABSTRACT**
The application of natural materials in biomedicine has developed quite rapidly. Nowadays, such materials' therapeutic properties become a diminishing topic, including the use of bee products. Bees produce several useful products, one of which is propolis. Propolis or "bee glue" is a honeybee product in the form of a mixed resin from the mixture of certain enzymes in bee saliva and beeswax. Research shows that more than 300 active compounds are found in propolis. The active compounds in propolis are useful as antioxidants, anti-inflammatory, anticancer, antibacterial, antibiofilm, and others. The content and benefits of propolis make it an attractive material to be developed in several dental applications. Some dental literature states that propolis can inhibit oral bacteria activity, a mixture in improving dental materials quality, alternative endodontic regenerative materials, medicament ingredients, post-surgical wound healing, and other uses. By identifying that propolis is a natural ingredient widely used in dentistry, the biocompatibility problem in using propolis must be confirmed before going to the clinical stage. The complex conditions of the oral cavity make the application of propolis have to consider the biocompatibility aspect. Besides that, the regulation for the application of a new material must consider standards guided by the Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare (JMHW), and the International Organization for Standardization (ISO), requiring producers to carry out adequate testing of their ingredients following biocompatibility evaluation phases as part of the biosafety process and protocols. This study aims to review the biocompatibility of propolis in dentistry. These studies suggested that propolis was sufficiently biocompatible in several dental applications as an alternative medicament at specific concentrations.

***Keywords:*** *Biocompatibility, Dentistry, Propolis*

## 1. INTRODUCTION

Nowadays, many biomedical applications use various types of products from natural ingredients. This condition produces biologically active substances derived from significant natural concern, including propolis as a bee product with therapeutic properties [1]. Propolis is a resinous substance consisting of a mixture of various plant parts and molecules secreted by bees [2]. Bees produce propolis using a combination of beeswax and their saliva [3]. Propolis contains 80% resin-wax substances, 5% pollen, and around 15% essential oils, including other organic compounds [4,18]. Variations in composition, smell, colour, and possibly the propolis's therapeutic properties are influenced by the type, season, and food sources available to bees [5]. Based on several current reports, most compounds such as polyphenols, steroids, sugars, terpenoids, amino acids, and others have been reported to be provided by the propolis [6]. Propolis can be used as an antibacterial, anti-inflammatory, aesthetic, anti-tumour, anticancer, antifungal, antimutagenic, and antihepatotoxic agent [7,19].

Studies on propolis use have concluded that propolis is a natural product in great demand to be developed in medicine and dentistry [8]. In dentistry, propolis can be used for surgical wound healing, caries prevention, hypersensitive tooth treatment, aphthous ulcer treatment, avulsed tooth storage media, root canal irrigation solutions, mouthwash, reduction of oral mucositis due to chemotherapy, oral cancer, treatment of gingivitis and periodontitis. It also can inhibit plaque formation, control the oral microbiota, direct pulp capping and analgesic agents, and delay the growth and development of early stages of herpes simplex infection [9,10]. Propolis has a bulk composition, which is an appropriate solvent frequently essential for enhancing the targeted

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compound. One of the commonly used solvents is ethanol from existing solvents such as chloroform, methanol, acetone, etc. [11]. The maceration technique with ethanol is frequently used since this method is considered to have the advantage of a simple, effective, and suitable process for obtaining propolis extract with low wax content and rich in biologically active compounds [12].

The medical application of propolis has increased due to its chemical composition, which has the potential for clinical use in humans [13]. However, the biocompatibility of propolis should be confirmed and resolved before clinical application [14]. The selection and evaluation of a substance for clinical utilization require a serial controlled testing procedure to examine its biosafety and biocompatibility concerning human ethics. Current regulations are guided by the FDA, JMHW, and ISO, requiring manufacturers to conduct appropriate safety concerns via pre-clinical and clinical phases following the regulatory licensing protocol. Considering the oral cavity's complex and heterogeneous environments, biocompatibility (or tissue compatibility) concerns the potential adverse reactions from a particular substance toward the body responses during the application [15]. The suitability of the dental material with living tissue is essential to avoid risks to the patient [16]. The purpose of writing this literature review is to determine the biocompatibility of propolis extract used in several dental applications.

## 2. REVIEW

### *2.1. Propolis*

**Fig 1**. Propolis *Apis Trigona* from Nglipar, Gunung Kidul, Yogyakarta

The word ‘Propolis’ comes from the Greek, where pro means "at the entrance to" while polis is close to "community" or "city", which means that it can be functioned for nest protection. Propolis is a mixture of natural resins earned by the bees from several collected ingredients from plants, shoots, and some exudates [17]. It is initially used by the bees to seal holes and cracks, as a sealant and disinfectant, smooth the inner surfaces, maintain the internal hive’s temperature, and prevent hive weathering. It is also known as the third most important bee’s products since it contains some essential oils, bee pollen, and some other bioactive organic compounds [68].

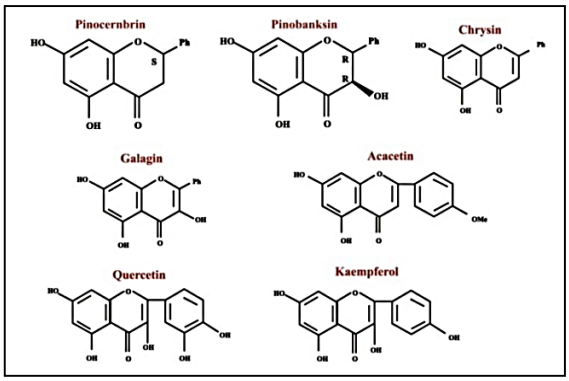


**Fig 2.** Nomenclature and Chemical Structure of Propolis [52]

Flavonoids, aldehydes, steroids, and a phenolic compound in propolis are the essential macro-structured bioactive organic component’s origin. Flavonoid is the most attractive one, previously observed in having several derivatives such as chrysin, luteolin, kaempferol, galanin, quercetin, apigenin, myricetin, resveratrol, pinocembrin, and others by capillary zone electrophoresis analysis of the extracted propolis. In addition to the bioactive organic substances, propolis also harbours several essentials, including thiamine, riboflavin, pyridoxine, ascorbic acid, tocopherol as the vitamins, and some minerals as potassium, magnesium, iron, zinc, copper, calcium, and manganese. Furthermore, it is also reported that propolis may have several enzymes, such as adenosine triphosphate, succinic dehydrogenase, acid phosphatase, and glucose phosphatase [4,18]. Propolis has also been studied to have potential antibacterial capacity [7,14,20,21,22], antifungal [23,24,25], antiviral [7,19], antiparasitic [7,19], antioxidant [7,19,27,28,29,30], anti-cancer [7,19,32], anti-inflammatory [7,19,26], antiulcer and antidiabetic effects [7,19].

**Table 1.** Biological Activity of Propolis Extract

| Types of Propolis Extract | Bees Species | Origin of Propolis | Biological Action | References |
|---|---|---|---|---|
| EEP | *Apis Trigona* | Indonesia | Antibacterial | 14 |
| EEP | *Apis mellifera* | Taiwan | Antibacterial | 20 |
| Dichloromethane Extract Propolis (DEP) | *Apis cerana* | China | Antibacterial | 21 |
| EEP | *Tetragonula laeviceps Tetrigona melanoleuca* | Thailand | Antibacterial | 22 |
| EEP | *Unconfirmed* | Turkey | Antifungal | 23 |
| EEP | *Apis mellifera* | Brazil | Antifungal | 24 |
| Chloroform Extract Propolis (CEP) | *Unconfirmed* | Northern Jordanian | Antifungal | 25 |

| | | | | |
|---|---|---|---|---|
| EEP | Unconfirmed | Cameroon | Anti-inflammatory | 26 |
| EEP | Unconfirmed | China | Antioxidant | 27 |
| EEP | Unconfirmed | Malaysia | Antioxidant | 28 |
| EEP | *Apis mellifica* intermissa | Algeria | Antioxidant | 29 |
| WEP | *Apis Mellifera* | Brazil | Antioxidant | 30 |
| EEP | Unconfirmed | Italia | Antibiofilm | 31 |
| EEP | *Trigona* | India | Anti-cancer | 32 |

## *2.2. Propolis Extraction*

Propolis has a complex and bulk structure; thus, it is difficult to apply directly and may require being extracted prior to its utilization. Extraction is the separation of medicinally active components from the source using a selective solvent through an appropriate standard procedure [33]. The extraction procedure is essential in phytochemical analysis. Several conventional methods, such as maceration and percolation extraction methods, are frequently used for screening studies in the bioactive compound analysis. Several advanced extraction methodologies have also been used, including supercritical fluid extraction (SFE), Ultrasound-based extraction, and Microwave-based extraction [34].

**Table 2.** Various Extraction Methods

| Method | Description | References |
|---|---|---|
| Maceration | All powdered material is allowed to contact the solvent in the closed container for a period of time with frequent agitation. This extraction method is considered simple but has the disadvantage of long extraction time and low extraction efficiency. | [35, 36] |
| Percolation | Perlocators are the tools used for this technique. The plant material is moistened with solvent and allowed to be placed in the percolation chamber. Then the plant material is rinsed with solvent several times until the active ingredient is extracted. The solvent is used to the point of saturation | [37, 64] |
| Soxhlet | This method is widely used when the desired compound has limited solubility in a particular solvent. Finely ground samples are placed in porous bags or "thimbles" made of filter paper or cellulose. | [34] |
| Supercritical fluid extraction | The extraction method is carried out with supercritical gases such as carbon dioxide, nitrogen, methane, ethane, ethylene, nitrous oxide, sulfur dioxide, propane, propylene, ammonia, and sulfur hexafluoride to extract the active ingredients. The plant material is stored in vessels filled with gas under controlled conditions such as temperature and pressure. | [38] |
| Microwave-assisted extraction | In this method, microwave energy facilitates the separation of the active ingredient from the plant material into the solvent. In contrast to the classical method, microwave-assisted extraction heats the entire sample simultaneously. During extraction, heat interferes with weak hydrogen bonds as the rotation of molecular dipoles and dissolved ions' migration increases the solvent's penetration into the sample or matrix. | [65] |
| Ultrasound-assisted extraction | This technique is an advanced technique that has the ability to extract a large number of bioactive compounds in it with a shorter extraction time, which could be the main advantage of this technique | [66] |

One of the most important factors affecting the efficiency of extraction of bioactive compounds from natural materials and their health benefits is solvent extraction [39]. Some of the frequently used extracting solvents are water, ethanol, methanol, and dichloromethane [40].

### *2.2.1. Extract Ethanol Propolis (EEP)*

Ethanol is the most widely used solvent as it can produce propolis extract with low wax content and rich in biologically active compounds [41]. Ethanol is an efficient solvent for extracting natural substances, especially when it is used at 60% (v / v) and in a ratio of 35 ml / g dry matter, for 30 minutes at 60-65°C [42]. A mixture of ethanol and water with the proportion of 60:40 (v/v) is most suitable for obtaining extracts with high phenolic compounds and antioxidant activity; therefore, ethanol is a material that is quite in demand as a solvent [43].

### *2.2.2. Water Extract Propolis (WEP)*

Research by Rocha et al. [30] conducted a study by chemically characterizing WEP and EEP and evaluating in vitro antioxidant/antimicrobial activity extracts and the safety of WEP through the determination of its genotoxic potential. The results of this study indicated that both extracts exhibited antioxidant and antimicrobial activity. WEP proved to be quite safe, marked by the XTT colorimetric assay WEP with a 25% concentration or lower and the percentage of cell viability V79> 80%.

### *2.2.3. Methanol Extract Propolis (MEP)*

Lawal et al. [44] conducted a study revealing that 200 grams of Nigerian propolis were extracted in 1600 mL of absolute methanol. Their research showed the existence of various important phytochemicals in the methanol extract of Nigerian bee propolis, such as alkaloids,

flavonoids, saponins, anthraquinone, tannins, glycosides, phlobatnins. The presence of many chemical components is an indication that the methanol extract of Nigerian bee propolis, when properly filtered, has great potential as a medicament material.

### *2.2.4. Dichloromethane Extract Propolis (DEP)*

The research conducted by Yan et al. [21] with the propolis method was carried out by stirring in the dichloromethane solvent. The result was that the propolis extract with dichloromethane had the highest methylene content and the maximum type of propolis component was effective compared to the 90% ethanol extraction method, 70% ethanol, and ethanol-ligarin extraction method. Research related to DEP biocompatibility conducted by Utispan et al. [45] showed that cytotoxic activity in HN30 cells with viability decreased significantly with 200 µg / ml DEP, HN30 cell viability <75%, and HN31 cell viability <50%.

## *2.3. Applications of Propolis in Dentistry*

Along with the increasing interest in using natural ingredients as medicament alternatives, propolis's therapeutics are widely used in dentistry. Propolis in dental materials is used in the modification of GICs as EEP is proven to be able to provide good antibacterial properties in the modification [46]. Besides, EEP incorporated in ceramic-reinforced glass ionomer (Amalgomer CR) can improve its mechanical properties [47].

Propolis in orthodontics effectively reduces the marginal bleeding index in patients during orthodontic treatment [48]. Mouthwash containing propolis can reduce plaque accumulation in patients with fixed orthodontic treatment [49]. Propolis in endodontics showed that propolis could be compared with Triple Antibiotic Paste (TAP) as a disinfection treatment option for regenerative endodontics. Furthermore, propolis paste was able to induce a progressive increase in root length and dentin thickness, as well as a decrease in apical diameter similar to MTA [5]. Another use of propolis in endodontics is that propolis can be used as an alternative for proper root canal irrigation. Propolis also can reduce the development of *Enterococcus faecalis* and *Candida albicans* [11]. The addition of EEP to the calcium hydroxide paste increases its antibacterial activity. It has been shown that the flavonoid content of propolis provides slightly better antimicrobial activity than calcium hydroxide without propolis [9].

Propolis in oral medicine in patients with recurrent aphthous ulcers provides a personal report that propolis reduces recurrent aphthous ulcers [50]. In oral surgery, studies showed that mouthwash with propolis could improve wound healing characterized by epithelial repair [51]. Propolis in periodontics can be used in subgingival irrigation. This propolis extract during periodontal treatment gives better results than root planning and scaling. Besides, propolis extract, when it is used in gingival pockets, is beneficial for healing periodontal disease [52]. Propolis in pedodontics is used as the age group of children is a group that is susceptible to a drug. Complications/side effects due to the use of artificial drugs have paved the way for natural products such as propolis for pharmacotherapy purposes. The potential of propolis for dental treatment in children is as a mouthwash, anti-cariogenic, direct pulp, pulpotomy, endodontic therapy, avulsion tooth storage media, new bone formation in children, and wound healing. Propolis can be used as an alternative to pedodontics medicaments that are easy to use, patient-friendly, and easy to reach in the future [53].

## *2.4. Propolis Biocompatibility*

In dentistry, propolis' therapeutic properties as natural ingredients are mostly carried out in the form of test ingredients or extracts. Biocompatibility challenges various types of biological models with test materials or extracts. Researchers must follow FDA guidelines and ISO 10993 standards. Biocompatibility analysis or biological testing methods should be carried out in stages from initial screening of new substances to product release, adhering to periodic clinical, nonclinical, preclinical, and post clinical audit testing to meet FDA and international standards [54].

TABLE I. ISO STANDARDS FOR BIOCOMPATIBILITY TESTING

| ISO Standard | Title |
|---|---|
| ISO 10993 | Biocompatibility |
| ISO 10993-1:2018 | Evaluation and testing in the risk management process |
| ISO 10993-3:2014 | Genotoxicity, carcinogenicity, and reproductive toxicity tests |
| ISO 10993-4:2016 | Selection of tests for interaction with blood |
| ISO 10993-5:2015 | In vitro cytotoxicity test |
| ISO 10993-10:2017 | Skin irritation and sensitization testing |
| ISO 10993-11:2016 | Systemic toxicity test |
| ISO 10993-12:2017 | Preparation of samples and reference materials |
| ISO 15223-1:2015 | Symbols used on labels, markings, and medical device information |

Biocompatibility is the ability of a material to obtain an appropriate biological response after application. When a material is placed into living tissue, it interacts with complex biological systems and produces a biological response [55]. Biocompatibility is considered biologically compatible with no toxic, adverse, or immunological response in living tissue. When an object is introduced into the body without an immune response, it can be considered biocompatible. The biocompatibility test was carried out in stages starting from *in vitro*, *in vivo* to entering clinical trials [56].

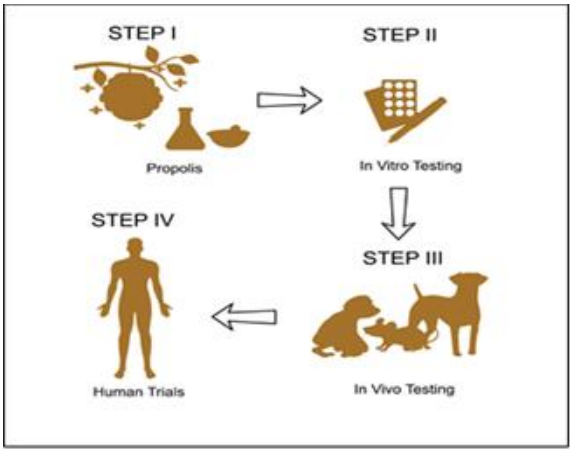


**Fig 3**. Summary of the systematic approach to the biological evaluation of medical devices as part of a risk management process [67].

### *2.4.1. In Vitro Propolis Biocompatibility*

a) Biocompatibility of Extract Ethanol Propolis was investigated by Fauzi et al. [14]. Propolis Apis trigona from Nglipar, Yogyakarta, was selected in this study. In this study, adult human fibroblast cells as an in vitro model were closely related to cell populations in dental pulp, root canals, lamina propria gingiva, and oral mucosa with open wounds. MTT test with ELISA reader at a wavelength of 550 nm was carried out for cytotoxic analysis, and the results showed that the lower the EEP concentration was, the higher the cell viability would be. EEP Apis Trigona is a compound with a concentration of 0.0025% viability> 75%.

b) Research conducted by Al-Shaher, et al. [57] tested the tolerance of fibroblasts from the periodontal ligament (PDL) and dental pulp to propolis in vitro. Cells from human dental pulp and PDL were obtained from healthy third molars and then treated with various propolis' concentration from 0 to 20 mg/ml. Cell viability after propolis treatment was analysed by crystal violet staining of cells followed by spectrophotometric analysis. Outcome data revealed that concentrations of 4 mg/ml or lower resulted in> 75% viability of PDL cells or pulp fibroblasts.

c) The study of Shi et al. [58] evaluated the effect of mineral trioxide aggregate (MTA) and Brazilian propolis on the viability of human dental pulp cells (hDPC). EEP as a test material was diluted with culture media (DMEM) to 10, 20, 40, 80, and 160 µg / mL; MTA was also diluted with 1: 1, 1: 2, 1: 4, and 1: 8. Cell viability was evaluated with the CCK-8 Kit (CCK-8; Dojindo, Kumamoto, Japan) on days 1, 5, 7, and 9. Absorbance was conducted with a wavelength of 450nm. The viability of hDPC was slightly higher at the 10µg / mL EEP dilution than the other dilutions. The results of this study indicated that EEP and MTA significantly increased the viability of hDPC compared to the control group at days 7 and 9. This in vitro study showed that Brazilian propolis showed similar cell viability to MTA.

d) Grenho et al. [59] conducted a study on the biocompatibility of nanohydroxyapatite containing propolis. The propolis' red and green ethanol extracts were diluted in a hydroalcoholic solution (50% (v / v)) to make 6, 12, and 25µg ml-1 solutions. Furthermore, the ceramic samples were immersed overnight, at room temperature, in this solution. NanoHA samples were also immersed in hydroalcoholic solution without propolis and used as control and referred to nanoHA without treatment. The results of this study showed that nanohydroxyapatite containing propolis was quite compatible.

### *2.4.2. In Vivo Propolis Biocompatibility*

a) Raheem et al. [60] conducted a study on Egyptian propolis nanoparticles' in vivo biocompatibility as root canal nano sealers. The EEP nano sealer can be used as an innovative root canal sealant, with improved sealing capabilities, and in vivo biocompatibility. This research revealed that propolis nanoparticle sealer could be applied as a substitute for the current sealer, which had a toxic effect.

b) Research by Almeida et al. [61] analysed the toxicity of propolis ethanol extract using Artemia franciscana (A. franciscana) as a model. In the plates, ten Artemia franciscana nauplii were given 20 mL of ethanol extract of propolis at a concentration of 8% and incubated for 24 hours at room temperature in the dark. This procedure was carried out in a triplicate manner. The mortality rate was defined as% mortality = (number of individuals who died × 100) / total number of individuals, and the degree of toxicity was classified according to observed mortality: 0-9% = non-toxic (NT); 10-49 = slightly toxic (ST); 50-89% = toxic (T); 90-100% = highly toxic (HT).The toxicity test result showed that the extract tested concentration was classified as HT (100%) based on the A. franciscana mortality rate, or it could be concluded that the concentration tested was very toxic.

c) The research by Eskandarinia et al. [62] evaluated the biocompatibility of Bilayer Wound Dressings from polycaprolactone/gelatine (PCL/Gel), polyurethane and ethanol extracts of propolis (PU/EEP) and polyurethane and ethanol extracts of propolis-polycaprolactone/gelatine (PU / EEP-PCL/Gel) with fibroblast (normal) cells from the L929 mouse. A total of 104 L929 cells were inserted in each well, and 400 µL of RPMI culture medium was supplemented with 10% fetal bovine serum (Sigma, USA). Furthermore, 1% penicillin/streptomycin (Sigma, USA) was added to each well. There were 3 control wells. 24-well cell culture plates were incubated at 37C in a 5% CO2 atmosphere, and the culture media refreshed every 48 hours. Cell viability was evaluated on days 1, 4, and 7 of culture

using the MTT assay following the kit manufacturer's protocols (Sigma-Aldrich, USA). Optical density was recorded at 590 nm by a microplate reader (Bio-RAD 680, USA). PBS solution was used to remove non-adherent cells through several washes. Furthermore, incubation with 2 vol% glutaraldehyde solution for 1 hour was used to fix the sample. After several PBS was washed, the samples were still dehydrated in ethanol concentration (30, 50, 70, 80, 90, 95, 100%) and dried. In our previous study, 0.5 wt% EEP showed the most suitable biocompatibility for cells. L929 fibroblasts were compared to 0.25 wt% and 1 wt% concentrations. Therefore, an EEP concentration of 0.5 wt% was used in this study. The results of this study indicated that PU/EEP and PU/EEP-PCL/Gel with these concentrations were quite biocompatible. The viability was investigated after incubation of 1, 4, and 7 days with samples. After 7 days of incubation with PU / EEP, cell viability was 140%, and PU/EEP-PCL/Gel showed a cell viability of 175%. PCL / Gel and PCL/Gel-PUEEP samples showed more pro-proliferative effects on fibroblast cells compared to PU / EEP, which became significant from the 4th day of incubation.

d) Mori et al. [16] conducted a study to evaluate calcium hydroxide and propolis's biocompatibility in rat subcutaneous tissue. The study was conducted on 15 male Wistar rats by creating an incision in the back of each test animal to insert 4 tubes: one empty tube; one containing zinc oxide-eugenol cement, and the other two tubes were filled with an experimental paste prepared with 1g CH (Biodinamica) powder and 2mL of 11% non-alcoholic propolis. After 7, 14, and 30 days, the test animals were euthanized, and the specimens were prepared for histochemical technics with haematoxylin, and eosin staining was analysed by light microscopy. The analysis was carried out by taking into account the presence and type of inflammatory processes, proliferation of connective tissue, or the occurrence of destructive processes, such as abscesses or tissue necrosis. The inflammatory process was present in several parts, with a large number of neutrophils. However, on the 14th and 30th day, the inflammatory process occurred to be mild or insignificant, characterized by several parts showing lymphocytes and macrophages, which characterized moderate inflammation. The experimental paste (test paste) was assessed as biocompatible with tissues after 14 days. By taking into account the results of this study, it was concluded that 1gram of calcium hydroxide with 2 mL of 11% non-alcoholic propolis as the material was tested for biocompatibility with connective tissue.

e) Meneses et al. [46] conducted research related to the modification of GICs with ethanol propolis extract (EEP), where GIC was as cement while EEP was 10%, 25%, and 50% as a liquid. These modifications' biocompatibility was evaluated by analysing Wistar rat tissue under an optical microscope by observing cellular changes at different time intervals. The results of this study indicated the intensity of the histological changes. During the study, tissue biocompatibility was investigated by qualitative-quantitative analysis based on the intensity of tissue aggression caused by the treatment. In this study, initial intense inflammation was observed when 10 and 25% EEP were added at 7 and 15-day intervals. The histocompatibility analysis results showed that the intensity of histological changes in the test material proved to be inversely related to the concentration of propolis addition. In other words, the lower the concentration of the test material was, the more compatible the material would be. Semen Ketac test material with 50% EEP was the one that showed the smallest inflammatory process. The results in this study indicated the compatibility of the test material within the parameters considered safe, which was attributed to the fact that EEP was not used in its liquid form or paste form but as an intrinsic part of the polymerized GIC and was slowly released into living tissue.

### *2.4.3. Propolis Biocompatibility in Clinical Studies*

a) Research by Kripal et al. [63] assessed the effectiveness of propolis 5% mouthwash in chronic generalized gingivitis and compared the effectiveness of 5% propolis mouthwash to chlorhexidine mouthwash. This study was conducted on a sample of 45 patients randomly selected in the 18-70 years age group, divided into 3 groups: Group I with 15 patients treated with 5% propolis mouthwash, group II with 15 patients treated with the control group chlorhexidine gargle, and group III with 15 patients treated with normal saline (placebo). After scaling and root planning, subjects were advised to rinse their mouth with the instructions to rinse their mouths in the morning after brushing their teeth and after breakfast, and evening after dinner and before going to bed consistently for 5 days. This study showed a decrease in the gingival index (GI) and plaque index (PI) in group 1 treated after scaling and root planning with propolis when it was compared to the other 2 groups treated after scaling and root planning. The composition of 5% propolis mouthwash as a mouthwash after scaling and root planning in the treatment of chronic generalized gingivitis showed positive results. This study's data indicated that propolis mouthwash was more effective than other mouthwashes in accumulating plaque and gingival inflammation. The study showed that propolis could be used as an alternative to mouthwash. However, in this study, the propolis ingredients and extraction method were not included.

b) Research by Dehghani et al. [49] evaluated the effect of propolis and chlorhexidine mouthwash on plaque and gingival index in patients undergoing orthodontic treatment. 30 g Propolis material was mixed with 100 ml distilled water, then mixed with a mixer at

30°C for 2 hours. After the resulting mixture, the propolis extract was centrifuged from purified water at 30% as the base concentration. The propolis mixing solution was 1% with a saline concentration of 0.25%, and together with the essential oil of turmeric and flavour, a mouthwash solution was produced. The prepared solution was poured into 60 equal bottles. In this triple-blind study in total, 37 patients aged 15 to 35 years who had undergone fixed orthodontic treatment were studied. After that, one mouth rinse containing either Propolis or Chlorhexidine was randomly prescribed to the patient. Patients were asked to use mouthwash twice a day after brushing their teeth for three consecutive weeks. Plaque, gingival, and periodontal status indicators (PI, GI, CPI) were determined at the beginning and the end of the treatment (after three weeks) for each patient. This study indicated that the PI GI and CPI of patients with propolis mouthwash were higher than patients with chlorhexidine mouthwash. However, propolis mouthwash was considered more compatible. This condition is in line with the statement of Kubiliene et al. [12] that *aquades* was less effective in producing extracts with high bioactive compounds.

**Table 3.** Results of the propolis biocompatibility

| Test | Types of Propolis | Concentration | Sample | Results | References |
|---|---|---|---|---|---|
| In Vitro | EEP | 0; 0,0003125; 0,000625; 0,00125; 0,0025; 0,005; 0,01; 0,05%; 0,1% | Fibroblast cells | in concentration of 0.0025% produces a viability> 75% | [14] |
| | EEP | 0-20mg/ml | PDL fibroblasts | ≤4mg / ml results in viability of PDL cells or pulp fibroblasts> 75% | [57] |
| | EEP | 10, 20, 40, 80, dan 160 µg /mL | hDPC | 10 µg / mL yields viability> 60% | [58] |
| | EEP | nanoHA impregnated with 12µgml − 1 of red (nanoHA RP) and green propolis (nanoHA-GP) | 3T3-L1 mouse fibroblast | On day 7 nanoHA-EP 12 µgml-1 showed higher viability (125%) than nanoHA-GP (110%) | [59] |
| In Vivo | EEP | EEP 0.5% sealer (group I) and nano sealer (group II) | 20 adult male Wistar albino rats | - The sealer group shows a higher absorbance value than the noanosealer.<br>Group II (nano sealer), there is a dense fibrous band of tissue rich in fibroblasts and collagen fibers that appear thicker and more regular than group I. Ep Nanosealer promotes higher healing rates than PE sealer | [60] |
| | EEP | EEP 8% | *Artemia franciscana (A. franciscana)* | After incubation of 24 hours, the extract concentrations tested were classified as highly toxic HT (100%) based on the A. franciscana mortality rate | [61] |
| | EEP | EEP wt%<br>ethanol extract of propolis (PU / EEP) and polyurethane and ethanol extract of propolis-polycaprolactone / gelatin (PU / EEP-PCL / Gel) | the subcutaneous connective tissue of mouse | EEP 0.5 wt%<br>ethanol extract of propolis (PU / EEP) and polyurethane and ethanol extract of propolis-polycaprolactone / gelatin (PU / EEP-PCL / Gel) | [62] |
| | WEP | 1g CH (Biodinâmica) powder and 2 mL of 11% non-alcoholic propolis | 15 male Wistar rats | 1 gram of calcium hydroxide with 2 mL of 11% non-alcoholic propolis as material tested for biocompatibility with connective tissue | [16] |
| | EEP | EEP 10%, 25%, dan 50% sebagai liqiud | Wistar mouse | Ketac cement with 50% EEP is the one that shows the least inflammatory process. | [46] |
| Clinic | unconfirmed | 5% propolis mouthwash | 45 patients in the 18-70 year age group had scaling and root planning | Indicators of plaque status, gingiva in the mouthwash group 5% propolis mouthwash lower patients with chlorhexidine mouthwash | [63] |
| | WEP | 1% propolis mouthwash | 37 patients aged 15 to 35 who underwent fixed orthodontic treatment | Indicators of plaque, gingival, and periodontal status (PI, GI, CPI) in the sample group with 1% propolis mouthwash were higher than patients with chlorhexidine mouthwash. | [49] |

## 3. CONCLUSION

Based on the existing reports, propolis as a natural compound was considered to be used in dentistry. Most of the propolis extract was produced by extraction method using ethanol as it was considered adequate to be able to produce extracts with high bioactive compounds. Besides, ethanol extract seemed to be more biocompatible than extracts with other solvents. Ethanol extract of propolis was the material most frequently used in dental applications. Propolis ethanol extract could be used effectively and compatible with the host by using the right concentration based on the study reports' reference. Evaluation of propolis' biocompatibility in dentistry was generally carried out in vitro and in vivo. However, reports on clinical biocompatibility studies are still very limited.

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