

Potential of avocado seed extract mouthwash in preventing dental caries: A literature review

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Abstract

Background: Dental caries is an oral disease caused by low awareness of oral health with high prevalence in Indonesia varying from children to adults. Chlorhexidine has been used as mouthwash to kill dental caries bacteria such as *Streptococcus mutans* and *Prevotella intermedia* in the oral cavity but it has many side effects in a long term use. Extraction of avocado seeds by maceration method in dilution with ethanol 70% could present bioactive compounds which have a similar role as conventional chlorhexidine mouthwash.

Purpose: To explain potencies of avocado seed extracts as mouthwash for dental caries prevention.

Method: Study based on past literature with publication age under or at least five years ago conducted from ResearchGate, ScienceDirect, PubMed, and Google Scholar according to related keywords and being selected so that the information collected could be relevant to the topic of the article.

Discussion: Avocado seed extracts that have been manipulated to be mouthwash sample could present inhibition activity to bacteria colony in petri dish by the same level as chlorhexidine as well with a minimum extract concentration of under 35%, thus cytotoxicity value of avocado seed extracts in parameter of IC₅₀ is 77.298 ug/mL which is non-toxic so that the extracts give a good potency as therapeutic mouthwash to dental caries by causing no systemic side effects.

Conclusion: Avocado seed extracts-based mouthwash has potency as prevention to dental caries without other systemic side effects.

Keywords: Bacteria; Chlorhexidine; Cytotoxicity; Dental caries; Extract

1. Introduction

Persea americana Mill., commonly known as avocado, is a tropical–subtropical dicot plant from the *Lauraceae* family, widely favored as a bio-commodity across almost all parts of the world. This fruit is typically found in the Americas, Africa, New Zealand, and Indonesia.^[1] Avocados have a diverse genetic makeup, either through natural processes or genetic engineering. The Hass variety of avocado is the most widely distributed globally, with the flesh making up 65%, the seed 20%, and the outer skin occupying the smallest proportion of 15%.^[2] Avocados are not only valued for their

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use as food but also for their medical applications. These include treatments for cardiovascular diseases, antiviral, antidiarrheal, antifungal, anti-inflammatory purposes, and in dermatology to prevent skin aging.^[3,4,5]

The seed of the avocado is often discarded as waste, though it offers numerous medical benefits if properly processed. Avocado seeds constitute about 13–17% of the fruit's components and are rich in bioactive elements such as carbohydrates, proteins, lipids, multivitamins, and minerals.^[6] They also contain phenolic compounds, flavonoids, and condensed tannins, making them effective as antioxidants, antimicrobials, and agents that prevent nerve cell degeneration.^[7,8,9] Therefore, avocado seeds hold great potential for managing dental caries and can be used as a preventive measure against more serious conditions like gingivitis and its chronic form, periodontitis. Avocado seeds also contain essential fatty acids such as linoleic, palmitic, and oleic acids, which have antioxidant properties when extracted and dissolved in ethanol.^[10]

Dental caries is a highly prevalent oral disease in Indonesia, caused by plaque from food residues that are not properly cleaned. This leads to the growth of various microbes, such as *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*.^[11,12,13] Dental caries affects people of all ages due to a lack of attention to dental and oral health. If untreated, caries can progress to total tooth decay and damage the surrounding soft tissues, such as the gums, leading to gingivitis. If gingivitis goes unnoticed, it can develop into a more severe form known as periodontitis.^[14]

Streptococcus mutans, the primary cause of dental caries, has virulence factors that activate in the host's oral cavity, including acid production (acidogenic), heat tolerance (acidophilic), and the production of glucosyltransferase (GTF), which catalyzes the conversion of sucrose into glucan.^[15,16] Other bacteria, like *Prevotella intermedia*, a Gram-negative anaerobic bacterium, can be found in subgingival plaques and produce beta-lactamase enzymes that degrade beta-lactam antibiotics, such as penicillin, commonly used to treat oral infections.^[17]

Effective, affordable, and accessible treatment methods for dental caries must be developed using bio-commodities that are easy to obtain, making avocado a promising alternative for managing dental caries and even advanced periodontal tissue damage. Avocado seeds can be processed into extracts that can be applied in various ways while maintaining good pharmacological activity. The bioactive compounds in avocado seeds—flavonoids, saponins, tannins, phenols, and alkaloids—can be maximized through proper extraction. Flavonoids can cause bacterial cell necrosis by inhibiting nucleic acid synthesis, disrupting cell membrane function, and inhibiting cell metabolism, leading to the destruction of bacterial cell walls and vital compartments like microsomes and vesicles.^[15,18] Saponins degrade bacterial cells by disrupting membrane permeability, causing cytoplasmic leakage and cell death.^[15] Tannins inhibit DNA topoisomerase and reverse transcriptase enzymes, preventing the formation of new bacterial cells while inactivating microbial adhesion, enzymes, and protein transport processes.^[15] Alkaloids disrupt the peptidoglycan in bacterial cell walls, causing them to become fragile and easily lysed.^[19] In addition to selecting the right materials, ease of use must also be considered to ensure regular application at the appropriate dosage, achieving the desired pharmacological effects. Mouthwash is a recommended application method as it is commonly used to maintain oral hygiene and freshen breath.

Mouthwash is a form of chemical plaque control that helps clean areas of the teeth that cannot be reached by brushing, such as the interproximal surfaces. The most commonly used ingredient in mouthwash is chlorhexidine at a concentration of 0.2%, but long-term use can cause undesirable side effects such as tooth discoloration, calculus buildup, and taste alterations.^[18] Thus, alternative mouthwash ingredients with fewer side effects are needed, such as avocado seed extract. Using organic ingredients like this is expected to reduce the potential side effects associated with non-organic compounds. Herbal or bio-based ingredients, like avocado seeds, are rich in phenolic compounds, alkaloids, flavonoids, and similar substances, making them effective for use in extracts, especially those dissolved in ethanol.^[19]

2. Material and Methods

A literature review exploring the potential of herbal remedies, specifically avocado seeds, as a therapeutic method for dental caries, which can be an early symptom of more severe manifestations, was conducted by searching and compiling scientific articles and data. These form the foundation and provide supporting evidence for arguments favouring the use of avocado seeds in caries treatment. Articles and data were sourced from reputable scientific databases, such as PubMed, ScienceDirect, ResearchGate, and supplementary databases like Google Scholar, to gather research findings relevant to the situation in Indonesia, which aligns with the objectives of this literature review.

Several key phrases were used to aid the search process, including terms related to avocado plants, dental caries, conventional caries treatments, avocado seed content, avocado seed extraction processes, and the pharmacological effects of avocado seed components. Another aspect considered during the collection process was ensuring that the

articles and data were up-to-date. Only articles published within the last five years were selected, with the oldest articles being from 2018.

A total of 392 articles were retrieved through the search process and were gradually filtered based on their relevance to the objectives of the literature review. Ultimately, 31 related articles were used in the preparation of this review.

3. Result and Discussion

After understanding the advantages of extracted avocado seeds, the proper extraction procedure and subsequent *in vitro*, *in vivo*, and clinical testing are essential to ensure that a herbal or phytopharmaceutical product can be applied to humans with minimal side effects and deliver the desired results in treating dental caries. Herbal medicines or phytopharmaceuticals intended for extraction are usually dissolved in solvents that match the properties of the bioactive components within the plant. Solvents used for extraction include ethyl acetate and 70% ethanol.

A study by Rachmawati et al. (2022) demonstrated the extraction procedure and provided evidence through avocado seed extract obtained from their experiments.^[20] Initially, avocado seeds were ground into a fine brown simplicia powder with a strong avocado seed smell and a bitter taste due to its saponin content. During the grinding process, the dried avocado seeds are first oven-dried at 60°C and then sieved using a 60-mesh filter to minimize air gaps.^[20]

Considering that flavonoids and alkaloids are sensitive to heat and degrade easily due to overheating, the extraction process should use maceration or cold conditions to preserve the chemical compounds in the avocado seed sample.^[21] Ethanol at 70% concentration is recommended as the solvent because it is non-toxic, has a high absorption capacity for active compounds in simplicia, and is volatile enough to speed up the extraction process, yielding optimal results.^[21,22]

Next, the formulation of mouthwash can be entirely performed using cold methods, avoiding any heating due to the risk of oxidation. The different solubility of the components in the mouthwash formulation results in the formation of two phases—an oil phase and a water phase. Since these phases are made of opposing properties, surfactants are added as wetting agents, defoamers, emulsifiers, and to dissolve and normalize the insoluble materials in water.^[21] The mouthwash formulation still has a bitter taste due to the strong saponin content in the avocado seed extract. Therefore, the addition of a dual-effect sweetener such as sodium saccharin is necessary, which can inhibit bacterial growth and metabolism, making it a potential agent for preventing caries caused by bacteria. Sodium saccharin can be added to the mouthwash at concentrations ranging from 0.05% to 0.25%, which is the maximum safe usage limit for this compound.^[23]

Next, testing the mouthwash formulation containing 70% ethanol avocado seed extract can be conducted by evaluating various concentrations of the avocado seed extract in comparison to a 0.2% chlorhexidine gluconate mouthwash as a positive control and distilled water (aquadest) as a negative control. This allows for the determination of the effect and efficacy of the avocado seed extract in treating dental caries.^[18] Different concentrations of the avocado seed extract in the mouthwash can be achieved by diluting the 70% ethanol avocado seed extract with distilled water to create varying concentrations, ranging from 10%, 15%, 20%, and 25%.^[17,18]

The mouthwash formulations containing different concentrations of avocado seed extract will then be tested on bacterial colonies suspended in petri dishes. The bacteria used in this study are *Streptococcus mutans* and *Prevotella intermedia*, both known to cause dental caries. The bacterial suspension is prepared by using an inoculating loop to transfer bacterial colonies into petri dishes prefilled with 0.9% NaCl solution, which is then spread over the surface of Mueller-Hinton Agar (MHA) plates using the spreading technique.^[17] Sterile cotton swabs and paper discs soaked in the mouthwash formulation containing the purified extract and control solutions can assist in preparing the bacterial suspensions.

The inoculated petri dishes are incubated for 24 hours at 37°C.^[18] After 24 hours of incubation, clear zones around the paper discs will form. These zones indicate the inhibition of bacterial growth, and the diameter of the inhibition zones can be measured using a sliding caliper to assess the antibacterial effect.^[18] The size of the clear zones will be used to determine the Minimum Inhibitory Concentration 90 (MIC 90) and Minimum Bactericidal Concentration (MBC) of the avocado seed extract.

Table 1 Average Inhibition Zone Diameter Data of Each Test Agent Against Bacterial Activity on Test Petri Dishes

Kelompok	n	Mean diameter zona hambat	SD	Nilai P
<i>Aquadest</i>	5	0,00	0,00	0,00
<i>Chlorhexidine gluconate 0,2%</i>	5	13,18	1,08	0,37
Ekstrak biji alpukat konsentrasi 10%	5	10,48	1,25	0,77
Ekstrak biji alpukat konsentrasi 15%	5	11,78	0,89	0,23
Ekstrak biji alpukat konsentrasi 20%	5	11,98	1,07	0,68
Ekstrak biji alpukat konsentrasi 25%	5	12,25	0,89	0,07

Based on the data obtained from Table 1, avocado seed extract at increasing concentrations shows a directly proportional effect on the average diameter of the inhibition zone against bacteria in the petri dish. According to the data on the average inhibition zone diameter, with 0.2% chlorhexidine gluconate as a positive control comparison, the MIC 90 is estimated to be within the 15-20% concentration range of avocado seed extract. This estimation assumes that the inhibition by 0.2% chlorhexidine gluconate in conventional mouthwash is perfect, as seen in the statistical data in Table 2, which was independently processed from the data in Table 1.

MIC 90 refers to the minimum concentration of an antibacterial agent that inhibits 90% of bacterial colony activity under in vitro conditions, preventing any visible bacterial growth in the test petri dish.^[24] The absolute concentration value of the effective avocado seed extract based on the minimum inhibitory concentration variable can be specifically determined through linear regression analysis using an interpolation technique via a linear equation derived from the average inhibition zone diameter data of avocado seed extract alone, disregarding the negative and positive control data, as seen in Table 3. Based on the linear equation from Table 3 and using perfect inhibition data from chlorhexidine gluconate as a positive control, it is determined that the average inhibition zone diameter with 90% inhibition is 11.862 units. Therefore, the MIC 90 value for avocado seed extract is found to be at a concentration (rounded to two decimal places) of 19.67%.

Table 2 Data on the Percentage of Bacterial Activity Inhibition from Aquadest, 0.2% Chlorhexidine Gluconate Solution, and Avocado Seed Extract in the Concentration Range of 10% to 25%

Kelompok Percobaan	Mean Diameter Zona Hambat	Persentase Penghambatan
Akuadest	0	0
Ekstrak biji alpukat 10%	10,48	0,795144158
Ekstrak biji alpukat 15%	11,78	0,893778452
Ekstrak biji alpukat 20%	11,98	0,908952959
Ekstrak biji alpukat 25%	12,25	0,929438543
Klorheksidin Glukonat 0.2%	13,18	1

In addition to determining the MIC 90 value, the linear equation in Table 3 derived from the data processing in Table 1 can also be used to determine the MBC as a measurement parameter for the ability of an antibacterial agent to completely kill all bacterial colonies in the petri dish. The MBC value can be obtained by assuming that a drug has an average inhibition zone diameter equal to that of 0.2% chlorhexidine gluconate, indicating that the concentration of avocado seed extract in the mouthwash formulation should ideally be between 30% and 32%.

Table 3 Linear Equation of Avocado Seed Extract in the Concentration Range of 10% to 25% Based on Average Inhibition Zone Diameter

Kelompok Ekstrak Biji Alpukat	Mean Diameter Zona Hambat	Persamaan Linier
Ekstrak biji alpukat 10%	10,48	$y = 0.1102x + 9.694; r = 0.9054$
Ekstrak biji alpukat 15%	11,78	
Ekstrak biji alpukat 20%	11,98	
Ekstrak biji alpukat 25%	12,25	

After the mouthwash preparation containing avocado seed extract has been tested successfully in vitro, further in vivo testing is also necessary to ensure that this medicine is biocompatible and has good bioavailability when interacting with living cells. This is because some mouthwash fluids may remain and can be swallowed, potentially causing reactions with other systemic functions. In this in vivo testing, the Brine Shrimp Lethality Assay method can be used to evaluate the pharmacological effects of the components of the avocado seed extract. The Brine Shrimp Lethality Assay is essentially a cytotoxicity test, serving as a basis for in vivo testing on the simple species *Artemia salina*, by evaluating the toxicity of several biological products marked by the mortality of brine shrimp, thereby also examining the principles of toxicology as pharmacology at overdose levels. The content of flavonoids, saponins, tannins, and alkaloids does not adversely affect the cytotoxicity on brine shrimp, resulting in lethality only when the effective dose is within the minimum levels.

Another study conducted by Munthe et al. (2023)^[27] showed that the bioactive component content of avocado seed extract is very safe, as seen in Table 4. The IC₅₀ value of the 70% ethanol extract from avocado seeds using the maceration method is 77.298 µg/mL, which is still within the safe threshold and not toxic in terms of cytotoxicity. Furthermore, the avocado seed extract dissolved in 70% ethanol and extracted using the maceration method exhibits increased antioxidant activity as the concentration in the mouthwash preparation rises. The ability to act as an antioxidant targeting reactive oxygen species is supported by the phenolic and flavonoid compounds contained in this 70% ethanol-extracted avocado seed extract.

Table 4 Data on absorbance, percentage of bacterial inhibition, and cytotoxicity values in the IC₅₀ parameter for the 70% ethanol extract of avocado seeds using the maceration method

Sample	Concentration (µg/mL)	Absorbance	Inhibition (%)	IC ₅₀ (µg/mL)
ME	5	1.134 ± 0.020	6.667	77.298
	10	1.079 ± 0.030	11.221	
	25	0.927 ± 0.021	23.704	
	50	0.753 ± 0.007	38.025	
	125	0.305 ± 0.028	74.925	
	Control	1.215 ± 0.001		

Polyphenols, which are composed of multiple phenolic benzene groups, have the potential to act as proton and electron donors, thereby participating in redox (reduction-oxidation) processes by donating protons and electrons to DPPH free radicals, neutralizing them into stable diamagnetic molecules.^[28,29] The flavonoid components present in avocado seed extract also function by stabilizing reactive oxygen species through reactions with free radical compounds and inactivating free radicals via the high reactivity of hydroxyl groups on flavonoids.^[27]

Utilizing waste from avocado fruit, such as seeds that contribute significant biomass, can be effectively transformed into mouthwash through proper procedures and suitable combinations as supporting agents to prevent dental caries. The application of biological agents in this mouthwash is grounded in the common human practice of using mouthwash to freshen breath and reduce yellowing of teeth. Traditionally, chemical agents like chlorhexidine gluconate at a 0.2%

concentration are more familiar than natural herbal ingredients. This is due to the assumption that herbal remedies are less effective in combating bad breath compared to chlorhexidine. However, people are often unaware that prolonged use of chlorhexidine, which can lead to dependency, has severe negative health impacts on teeth and oral cavities, such as demineralization and incompatibility with the oral immune system due to strong interactions with cellular and chemical components in the oral cavity.

The paradigm that underestimates the use of herbal ingredients in addressing oral cavity issues needs to be eradicated because herbs contain compounds like phenolics, flavonoids, and alkaloids, which possess pharmacological properties capable of killing bacteria responsible for dental caries while also preventing the proliferation of these bacteria, which can lead to gingivitis and eventually periodontitis, a chronic phase of the disease.^[14,15] Through proper extraction processes and careful manipulation in the formulation of mouthwash, avocado seed extract can be utilized to address dental caries.

In summary, the extraction process begins with the creation of dry *simplicia* through drying and grinding before extraction using the cold maceration method and dissolving in a universal solvent, namely 70% ethanol, which is more compatible with the bioactive content of the avocado seed itself. This 70% ethanol extract will be packaged into two phases: oil and water, before being transformed into a mouthwash formulation aimed at treating dental caries. This formulation must also undergo *in vitro* testing by counting colonies of caries-causing bacteria, such as *Streptococcus mutans* and *Prevotella intermedia*, on petri dishes manipulated with sterile mouthwash formulations containing negative controls (distilled water), positive controls (the conventionally used chemical compound chlorhexidine gluconate 0.2%), and test variables (70% ethanol extract of avocado seeds at various concentrations.^[17,18,21,22]

Once the effectiveness of the mouthwash formulation containing the 70% ethanol extract of avocado seeds in inhibiting caries-causing bacterial colonies is validated in petri dishes, the next step is to conduct *in vivo* testing. This testing occurs at a simpler cellular level on individuals with basic complexity before trials on humans, who have more complex systemic integration. *In vivo* testing focuses more on the systemic conditions of the human body, considering that mouthwash is a liquid formulation that can easily be swallowed and interact with the systemic conditions of humans. Therefore, the bioactive compounds in avocado seeds that are extracted must also be considered in terms of their interaction with cellular components related to systemic functions.

Based on *in vivo* tests using the Brine Shrimp Lethality Assay and other supporting methods, the bioactive components found in avocado seeds, such as flavonoids, phenols, and alkaloids, do not exhibit significant cytotoxicity compared to chlorhexidine; in fact, they demonstrate antioxidant effects and prevent the spread of free radicals in individuals, such as reactive oxygen species.^[26-29] The flavonoid, alkaloid, and phenolic content in avocado seed extract also exhibits a slightly basic pH, making it effective in killing pathogenic bacteria that cause caries in the oral cavity. The titration results of flavonoids, which are the primary components of avocado seeds, show a moderately basic pH that is acceptable for the oral cavity environment, ensuring it is not caustic and does not cause damage to the oral cavity, as depicted in the results from the study by Meganintyas et al. (2021).^[30]

The mouthwash formulation itself should maintain a basic pH because the acidic conditions of the oral cavity create a favourable environment for pathogenic bacteria responsible for caries to thrive and reproduce, potentially leading to more severe infections in the oral cavity.^[31] Additionally, excessive acidity can cause demineralization of teeth and corrosive damage to the hard structures of the oral cavity. The addition of effective artificial sweeteners, such as sodium saccharin, is also necessary to mask the bitter taste of avocado seed extract, given its good inhibitory capacity against bacterial activity. Therefore, herbal mouthwash formulations can be widely recommended for use and may be well-received by many due to their palatable taste.

4. Conclusion

The utilization of avocado seed extract as a mouthwash has significant potential in the prevention of dental caries. This avocado seed extract is safe for administration in the human body, making it a viable option as a mouthwash ingredient. However, clinical testing of the mouthwash formulation with avocado seed extract must be conducted on study groups of humans who meet medical requirements for such trials, taking into account ethical considerations and health regulations. Nevertheless, this article serves as a recent reference, compiled from previous literature, to guide the development of future research designs related to mouthwash formulations containing avocado seed extract. Several other considerations should also be taken into account in future clinical testing phases of this mouthwash formulation.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

As this article is a review of previously published research, no new human or animal studies were conducted by the authors. Therefore, ethical approval was not required.

Statement of informed consent

This review article is based on previously published studies, and no new human participants were involved. Therefore, informed consent was not required. All cited studies obtained informed consent from their participants as per their respective ethical guidelines.

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