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Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Test of Gambir Extract (*Uncaria gambir Roxb.*) and NaOCl Against *Candida albicans* as Root Canal Irrigation Material

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Abstract

Background: Root canal treatment failure that causes re-infection can occur due to various causes, one of which is by *Candida albicans* fungus in the root canal. Gambir extract is a natural ingredient that can be developed as a root canal irrigation solution, this plant has many benefits and contents, such as catechin and flavonoid compounds as antibacterial and antifungal. ***Purpose:*** This study aims to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of gambir extract (*Uncaria gambir Roxb.*) and NaOCl on the growth of *Candida albicans* as a root canal irrigation material. ***Methods:*** The type of experimental laboratory research on the extraction of gambir plants (*Uncaria gambir Roxb.*) using the maceration method, fractionation to separate active compounds in gambir (*Uncaria gambir Roxb.*), then the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test of gambir extract and 5% NaOCl using the broth microdilution method on 96 well plates observed with a microplate reader and the minimum bactericidal concentration (MBC) test using the spread method on agar calculated using a colony counter. ***Results:*** MIC of gambir extract (*Uncaria gambir Roxb.*) is 10% and MBC is 40% and MIC of NaOCl is 0,010% and MBC is 0,020%. ***Conclusion:*** Gambir extract (*Uncaria gambir Roxb.*) and NaOCl have effective minimum inhibitory concentration and minimum bactericidal concentration eagainst *Candida albicans*.

Keywords: ***Candida albicans, Minimum Bactericidal Concentration, Minimum Inhibitory Concentration, NaOCl, Uncaria gambir Roxb.***

Introduction

Oral and dental health issues have become a global concern, as expressed by the World Health Organization (WHO) in 2022 [1]. Data shows that around 60-90% of school-aged children and almost all adults have experienced dental decay. In Indonesia, the prevalence of oral and dental health problems reaches 57.6%, with the highest cases caused by dental caries. As age increases, dental health problems tend to worsen, emphasizing the importance of prevention and treatment efforts for dental damage [2].

Root canal treatment is a crucial procedure to clean and disinfect the root canal system to eliminate microorganisms, although failure still occurs due to aerobic and anaerobic microbes, including Candida [3]. The root canal treatment process consists of three main stages: mechanical preparation of the root canal, chemical cleaning with irrigating solutions, and root canal filling. This combination of three stages, known as the endodontic triad, aims to clean and prepare the root canal to be filled with materials that effectively prevent recurrent infections and maintain the original tooth [4].

The success of root canal treatment heavily depends on the ability to eliminate microorganisms, necrotic tissue, and debris from the root canal. Effective cleaning is achieved through a combination of mechanical and chemical methods, known as chemomechanical preparation [5]. Microorganisms that remain in the root canal, even after cleaning, can trigger periradicular infections through toxin production. Failure to completely eliminate them is a major cause of unsuccessful root canal therapy, making it necessary to have proper coronal and apical restoration after endodontic treatment to prevent the supply of nutrients to remaining microorganisms [6]. One microorganism found in the root canal is *Candida albicans*, a fungus frequently found in infected root canals [7].

Irrigation is a process that facilitates the mechanical preparation of the root canal using specific solutions that support the success of this treatment procedure [8]. The use of irrigating solutions aims to clean debris, dissolve infected tissue, and support the instrumentation process [9]. Commonly used irrigants include sodium hypochlorite (NaOCl), ethylene diamine tetraacetic acid (EDTA) solution, chlorhexidine (CHX), NaCl, and Qmix [10]. NaOCl is an effective irrigant in killing infection-causing microorganisms,

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but it has several drawbacks, such as an unpleasant odor and ineffectiveness in removing the smear layer [11]. In addition to synthetic chemicals, natural substances like gambir extract also show potential as an alternative irrigant due to their polyphenol and catechin content, which have antibacterial and antifungal properties that can help address infections in the root canal [12].

Materials and Method

This research method uses a laboratory experimental approach with a post-test only group design. The study was conducted at the Chemical Application and Service Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, from February to April 2025. The research population consisted of *Candida albicans* fungal cultures, while the sample was obtained using a simple random sampling technique. The inclusion criteria included *Candida albicans* cultures, gambir extract from maceration, and NaOCl solution, while the exclusion criteria covered contaminated samples or media and degraded test solutions.

The tools used in this study were a rotary evaporator (Buchi Rotavapor R-100, Switzerland), separatory funnel, 96-well microwell plate, micropipette, laminar air flow, multichannel micropipette (Research Plus, Eppendorf), spreader, microplate reader (EZ Read 400, Biochrom), and incubator. The materials used included gambir (*Uncaria gambir Roxb.*) from a plantation in Padang, West Sumatra, Indonesia, which was processed into powder in the Chemical Application and Service Laboratory, Padjadjaran University. NaOCl 5%, *Candida albicans* culture, 96% methanol (Merck, Germany), 10% ethyl acetate, aquadest, Sabouraud Dextrose Broth (Merck, Germany), and Sabouraud Dextrose Agar (Merck, Germany).

The research process was conducted in a structured manner through several stages. First, gambir extraction was carried out using 96% methanol as a solvent, followed by evaporation using a rotary evaporator to obtain a concentrated extract. Second, fractionation was performed using a separatory funnel with ethyl acetate solvent to obtain pure fractions, repeated twice using the rotary evaporator [13]. Third, *Candida albicans* rejuvenation was carried out on SDA media and incubated at 37°C for 24 hours [14]. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MBC) tests were then performed using the liquid microdilution method with a microplate reader. The MIC was determined from the wells that remained clear without turbidity, while the MBC was determined by the absence of colony growth after inoculation on SDA media [15,16]. Therefore, this study was designed to assess the effectiveness of gambir extract and NaOCl as antifungal agents against *Candida albicans*.

Results

Results of the MIC and MBC Test of Gambir Extract (*Uncaria gambir Roxb.*) and NaOCl Against *Candida albicans*

The results of the minimum inhibitory concentration (MIC) test showed that the gambir extract (*Uncaria gambir Roxb.*) at a concentration of 20% still exhibited growth of *Candida albicans*.

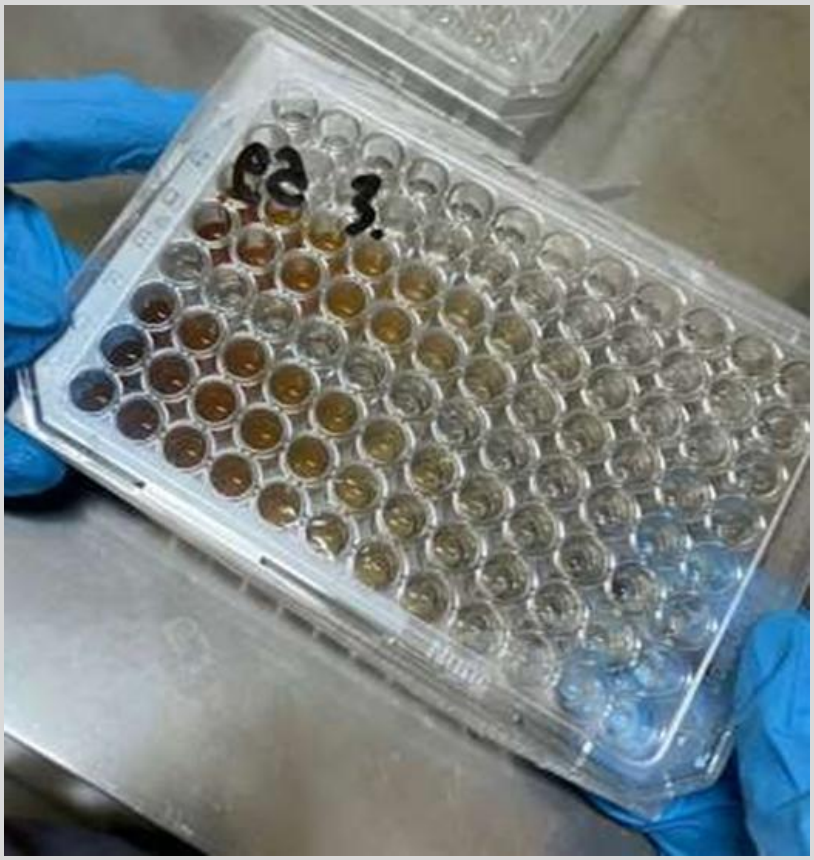

Figure 1 Microwell plate Minimum Inhibitory Concentration Test Results.

Table 1: Absorbance or Optical Density of Gambir Extract (*Uncaria gambir Roxb.*) Against *Candida albicans*.

	80%	40%	20%	10%	5%	2,5%	1,25%	0,625%	0,313%	0,156%	0,078%	0,039%
P	0,004	-0,001	0,002	-0,002	0,004	0,016	0,201	0,288	0,354	0,467	0,583	0,659
S	0,000	0,043	0,002	-0,198	-0,159	-0,082	0,090	0,308	0,448	0,703	0,647	0,657

Description: P: Solvent, S: Sample

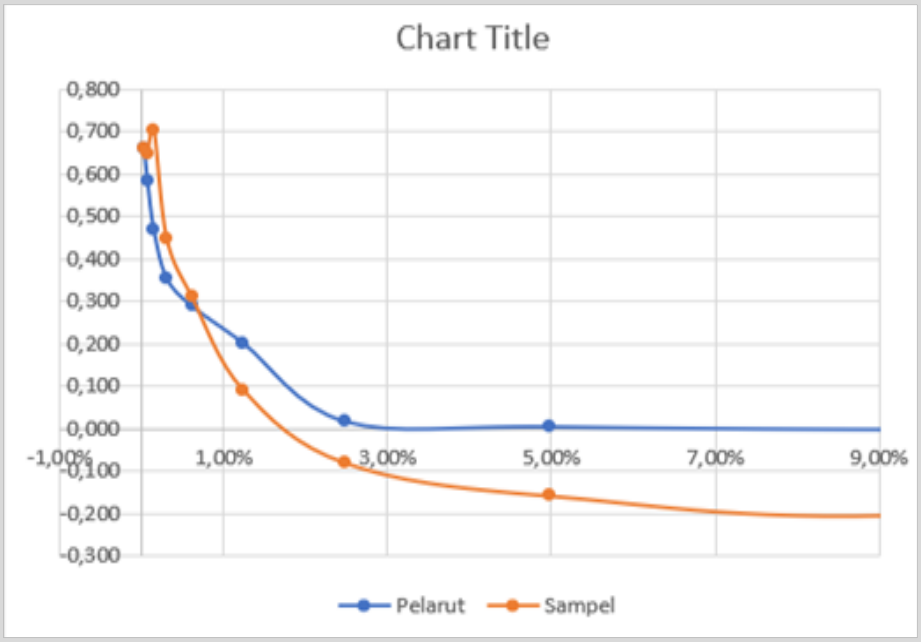

Figure 2: Chart of Minimum Inhibitory Concentration of Gambir Extract Against *Candida albicans*.

"The determination of the Minimum Inhibitory Concentration (MIC) was carried out by calculating the percentage of cell death based on the absorbance values obtained using an ELISA reader. The testing was conducted using a 96-well microwell plate, with a dilution method at 12 different concentrations to obtain absorbance or optical density data, which measures how much light is absorbed by the solution to determine the concentration of a substance in the sample at a specific wavelength. Based on the test results, the MIC of gambir extract (*Uncaria gambir Roxb.*) against *Candida albicans* was found at a concentration of 10%, as indicated by the absorbance data and the first intersection graph of the sample, which was lower compared to the solvent. Further testing on solid media showed that at a concentration of 10%, the growth of *Candida albicans* started to decrease, and at a concentration of 40%, no fungal growth was detected, indicating the Minimum Fungicidal Concentration (MBC). Therefore, the MIC of gambir extract is 10%, and the MBC is 40%. The MIC and MBC testing against *Candida albicans* for the positive control, NaOCl, is presented in Table 2.

Table 2. Absorbance of NaOCl against *Candida albicans*.

	2,5%	1,25%	0,63%	0,31%	0,16%	0,08%	0,04%	0,020%	0,010%	0,005%	0,002%	0,001%
P	0,374	0552	0,704	0,674	0,633	0,592	0,744	0,758	0,712	0,744	0,819	0,821
S	0,025	0,007	0,004	0,003	0,003	0,005	0,006	0,005	-0,008	0,011	0,025	0,217

Description: P: Solvent, S: Sample

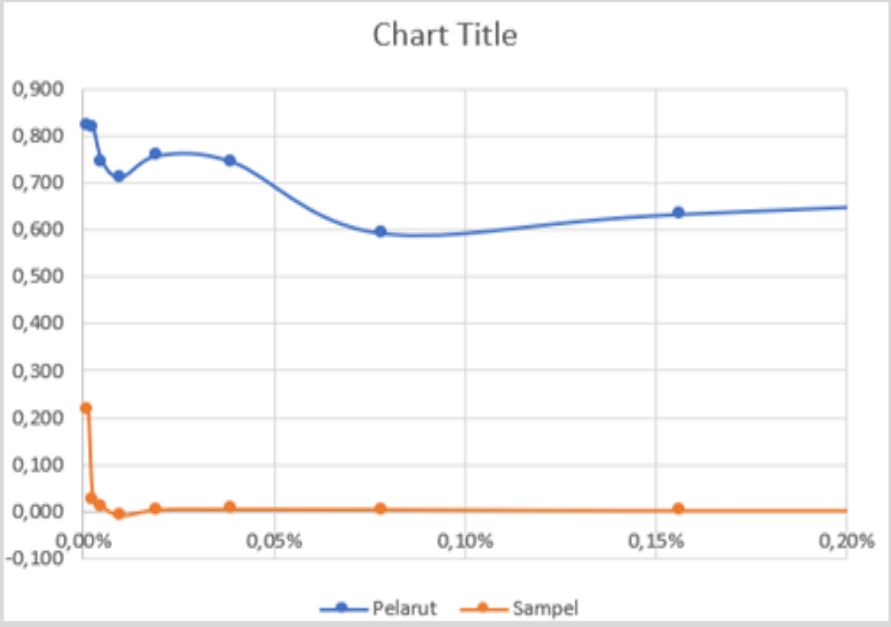

Figure 3. Chart of Minimum Inhibitory Concentration of NaOCl Against *Candida albicans*.

The determination of the Minimum Inhibitory Concentration (MIC) was carried out by calculating the percentage of cell death based on the absorbance values obtained through readings using an ELISA reader. In this study, a 96-well microwell plate was used with a dilution method performed at 12 different concentrations to obtain absorbance or optical density data, which measures how much light

is absorbed by the solution to determine the concentration of a substance in the sample at a specific wavelength. Based on the test results, the MIC for NaOCl against *Candida albicans* was found at a concentration of 0.010%, as indicated by the graph showing a decrease in fungal growth at this concentration. At a concentration of 0.020%, no growth of *Candida albicans* was observed on solid media, as measured using a colony counter, making the Minimum Bactericidal Concentration (MBC) for NaOCl 0.020%. A similar test was conducted for gambir extract, which also showed significant results in inhibiting and killing fungal growth at certain concentrations. The results can be observed in Figures 4 and 5.

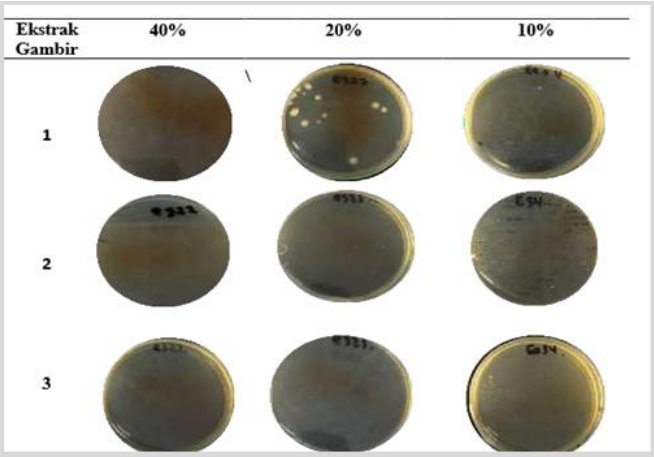

Figure 4. Results of MIC and MBC of the Number of *Candida albicans* Colonies on Gambir (*Uncaria gambir Roxb.*)

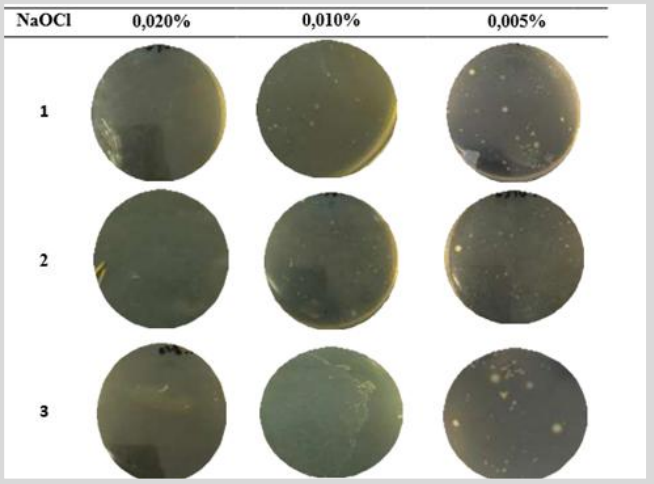

Figure 5: MIC and MBC Results of the Number of *Candida albicans* Colonies Against NaOCl.

Discussion

Root canal treatment is a therapeutic procedure that has long been recognized and widely used as a preferred method for preserving teeth with inflammation in the root canal, a pathological condition characterized by inflammatory processes and infections in the root apex area [17]. The success of this therapy heavily depends on the elimination of pathogenic microorganisms from the root canal system, including bacteria and fungi that play a role in the pathogenesis of persistent infections. One of the microorganisms frequently found is *Candida albicans*, which is known to have high resistance to several conventional irrigating agents [18]. This has led to the exploration of alternative herbal-based materials with potential as additional antimicrobial agents in root canal treatment. Gambir is known to possess various properties, such as the presence of catechins, tannins, alkaloids, flavonoids, terpenoids, and saponins, which function as antibacterial and antifungal agents, particularly against *Candida albicans* [19]. The first process of extraction used a maceration method, followed by fractionation using an ethyl acetate fraction to enhance the purity and antimicrobial activity of the gambir extract.

The testing was conducted using the serial microdilution method on a 96-well microwell plate, where gambir extract and NaOCl were tested against *Candida albicans*. The concentrations of gambir extract used ranged from 160% diluted stepwise to 0.039%, and NaOCl concentrations ranged from 5% to 0.001%. This testing process aimed to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of both substances. The results showed that gambir extract had an MIC at 10% and an MBC at 40%, while NaOCl had an MIC at 0.010% and an MBC at 0.020%.

This study confirms that both gambir extract and NaOCl are effective in inhibiting and killing *Candida albicans* growth. The findings suggest that gambir extract has the potential to be used as an alternative root canal irrigating material, with proven effectiveness at its MIC and MBC. Previous studies have shown that gambir extract has significant antifungal potential, providing a scientific basis for its use in dental care, especially in root canal therapy [20].

This research aims to test the effectiveness of gambir extract (*Uncaria gambir Roxb.*) and NaOCl as root canal irrigants against *Candida albicans* growth. These findings are consistent with previous studies showing that gambir extract has antifungal potential, supported by active compounds like catechins and flavonoids. This study reinforces the evidence that natural materials, such as gambir extract, can be an effective alternative in treating root canal infections, previously dominated by chemical materials like NaOCl [21].

As described in previous studies conducted by Suraini et al. (2015), gambir is known to have significant antifungal activity, especially against *Candida albicans*. Suraini et al. found that gambir extract could kill fungal growth at higher concentrations, such as 100%, while this study shows its effectiveness remains significant at lower concentrations, such as 40%. This indicates the potential of gambir to be used as a safer, more environmentally friendly natural substance compared to chemicals.

This study also discusses NaOCl as a commonly used irrigant in root canal treatment. The NaOCl test results support previous findings that show the effectiveness of this material in inhibiting *Candida albicans* fungi. A study by Anumedha Sharma, Nena Naorem, Binita Srivastava, Nindhi Gupta, Bidya Konsam, and Khustar Haider in 2024 stated that the inhibition zone of 3% NaOCl showed the largest average inhibition of 13.4429 mm, while the inhibition zone of 2% chlorhexidine against *Candida albicans* showed an average inhibition of 5.1714 mm [23]. According to Soniya R, Savithri S, Sangeetha R, and Sridevi G in 2024, the average inhibition zone diameter against *Candida albicans* was 21 mm at 3% NaOCl and 19 mm at chlorhexidine. The inhibition zone diameter for Enterococcus faecalis was 17 mm at 0.5% NaOCl and 18 mm at chlorhexidine. The inhibition zone diameter for Staphylococcus aureus was 18 mm at chlorhexidine and 19 mm at 3% NaOCl [24].

Based on the results of the research conducted on the minimum inhibitory concentration and minimum fungicidal concentration of gambir extract (*Uncaria gambir Roxb.*) and NaOCl against the growth of *Candida albicans* as root canal irrigants, it can be concluded that gambir extract is effective in inhibiting and killing *Candida albicans*, making it a natural alternative with added benefits from its polyphenol content and other bioactive compounds. This

opens the opportunity for further research in developing more biocompatible natural irrigating materials for root canal treatment by studying gambir extract (*Uncaria gambir Roxb.*) against *Candida albicans* not only with NaOCl but also with other irrigating materials, and further phytochemical testing using LC-MS methods is needed to identify the specific active compounds involved in the antifungal activity against *Candida albicans*.

Declarations

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

Sari Dewiyani conceptualized and designed the study, performed data interpretation,anqlysis and wrote the first draft and approved the final manuscript

Bani Imran contributed to literature review, manuscript editing and revision.

R Risky Nurainunisah participated in data verification, literature review, designed the study and final manuscript editing.All authors read

Gading Prsmeswari participated in data verification, literature review, and final manuscript editing.All authors read

Ethical approval

Granted by the University of Prof. Dr. Moestopo (B) Research Ethics Committee (Ethical Clearance Reference: KEPK/FKGUPDMB/VI/2025)

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