Trop J Nat Prod Res, September 2025; 9(9): 4297 - 4303 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Biocompatibility of *Acemannan* Paste as A Root Canal Filler and Its Antibacterial Activity against *Enterococcus faecalis* in Rat Teeth Model

Yuli Nugraeni[1]*, Rachmavidianti[1], Wibi Riawan[2] , Kristina Puspo[3], Regitha D. Bestari[3], Nathanael F. Nuralim[3]

[1]*Depertement of Conservative and Endodontic Dentistry, Dental Faculty of Brawijaya University, Malang, Indonesia*
[2]*Laboratory of Molecular Biochemistry, Medical Faculty of Brawijaya University, Malang, Indonesia*
[3]*Undergraduate Study Program, Dental Faculty of Brawidjaya University, Malang, Indonesia*

ARTICLE INFO

Article history:
Received 16 March 2025
Revised 25 July 2025
Accepted 28 July 2025
Published online 01 October 2025

ABSTRACT

Aloe vera together with its *Acemannan* component has been shown to possess antimicrobial activity against resistant microorganisms such as Enterococcus faecalis found in pulp chambers. This study aimed to determine the biocompatibility of *Acemannan* as a root canal filler and its antibacterial activity against Enterococcus faecalis. The study employed an experimental design with a post-test-only control design. *Acemannan* paste was prepared from a mixture of *Acemannan* powder and saline. The antibacterial activity of Acemannan paste was evaluated using the tube dilution and streak plate methods. The biocampactibility of *Acemannan* paste as a root canal filler was determined by evaluating its anti-inflammatory and cytotoxic activity *in vivo* by measuring tumour necrosis factor-α (TNF-α) and Caspase-9 expressions in periapical tissues of the incisor of Wistar rats following the administration of *Acemannan* paste for 7 days. Results showed that *Acemannan* possess antibacterial activity against Enterococcus faecalis with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 300 µg/mL and 400 µg/mL, respectively. *Acemannan* paste exhibited potent anti-inflammatory and cytotoxic activity against pathological cells by significantly ($p < 0.05$) decreasing TNF-α expression, and increasing caspase-9 expression in periapical tissues of incisor teeth of Wistar rats. Findings from this study indicate that *Acemannan* paste with its potent antibacterial, anti-inflammatory activities has potential to be used as a root canal filler.

Keywords: *Aloe vera*, Acemannan, *Enterococcus faecalis*, Tumor Necrosis Factor-α, Caspase-9.

Introduction

The *Aloe vera* plant is commonly utilized for medicinal purposes and as an ingredient in a variety of cosmetic products. The gel derived from *Aloe vera* (a mucilaginous substance) is is widely available in the market today. The pharmacological benefits of *Aloe vera* encompass anti-inflammatory, anti-rheumatic, antibacterial, and hypoglycemic properties. *Aloe vera* demonstrates antimicrobial activity against resistant pathogens such as *Enterococcus faecalis* and *Candida albicans*, which are present in the pulp chambers. Different extracts of *Aloe vera*, including those made with water, alcohol, and chloroform, also display antimicrobial activity and can serve as an intracanal treatment. *Aloe vera* is composed of various active substances, including anthraquinones, phenols, acemannan, and saponins, which are recognized for their antibacterial properties.[1,2] As a gel form, *Aloe vera* can promote periodontal ligament tissue regeneration in post-replantation avulsion teeth *in vivo*.[3]

*Corresponding author.Email: yulinugraeni@ub.ac.id
Tel.: +62811304803

Citation: Nugraeni Y, Rachmavidianti, Riawan W, Puspo K, Bestari RD, Nuralim NF. Biocompatibility of *Acemannan* Paste as A Root Canal Filler and Its Antibacterial Activity against *Enterococcus faecalis* in Rat Teeth Model. Trop J Nat Prod Res. 2025; 9(9): 4297 – 4303
https://doi.org/10.26538/tjnpr/v9i9.27

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Acemannan, a key component of *Aloe vera*, may support the formation of dentin by promoting the proliferation and differentiation of primary pulp cells, as well as facilitate the formation of extracellular matrix and mineralization.[4] *Aloe vera* gel can enhance the levels of active ingredients it contains, including sugars, which is made up of monosaccharides and glucomannan, known as Acemannan.[5] Acemannan represents the primary polysaccharides fraction derived from *Aloe vera* gel. It can accelerate the wound healing process by mobilizing macrophages, and promoting collagen synthesis.[6] Acemannan stimulates the expression of *bone morphogenetic protein-2* (BMP-2) in periodontal tissue and pulp fibroblasts.[7] Acemannan acts as an immunomodulator by increasing the level of antibodies, it also act as a special antiviral agent against tumor causing viruses.[5] *Acemannan* also exerts antibacterial effect indirectly by enhancing the phagocytic activity of leukocytes.[1] Studies have investigated the antibacterial properties of *Aloe vera* extract against resistant antimicrobial strains in endodontics *in vitro*.[8] The findings indicated that *Aloe vera* extracts, particularly the chloroform, and methanol extracts demonstrated notable antimicrobial activity against resistant microorganisms, such as *Enterococcus faecalis* present in pulp chambers.[9] Findings from other studies have indicated that *Aloe vera* polysaccharides (Acemannan) with low levels of deacetylation possess strong antibiofilm properties.[10] The primary factors contributing to diseases of the pulp and periradicular tissues are closely linked to microorganisms.[11] Root canal therapy is the most frequently performed endodontic procedure for addressing root canal infections resulting from persistent caries. The primary objective of root canal therapy is to extract as many bacteria as possible from the root canal and to establish an environment that is unfavorable for any remaining microorganisms. Key phases of root canal therapy include root canal preparation, sterilization, and obturation. Effective root canal treatment relies on achieving a tight seal through proper canal shaping and thorough irrigation.[12] *Enterococcus faecalis* a facultative anaerobe, is commonly associated with persistent and symptom-free endodontic infections. In fact, this bacterium is found in recurrent root canal infections up to nine times more often than

in initial infections. The most reliable way to eliminate *Enterococcus faecalis* from root canals and dentinal tubules is the use of sodium hypochlorite and 2% chlorhexidine, either in gel or solution form. Sodium hypochlorite is especially favored as an irrigant due to its strong antibacterial action against canal microbes. However, it does have drawbacks, including its toxicity and its potential to compromise tooth structure by reducing the hardness and integrity of root dentin.[10] Root canal medicaments made from natural ingredients have been widely developed due to the perception of their lower side effects compared to synthetic agents.[13] Acemannan has great potential as an alternative antibacterial agent in root canal infection, owing to it safety, and affordability. Acemannan exhibit a broad spectrum antibacterial activity against both gram-positive and gram-negative bacteria.[1] The application of acemannan in paste form aims to increase the penetration of its active ingredient into the dentin tubules, thereby increasing its antimicrobial capacity, radiopacity, flow, and consistency.[14] Based on the above, there is the need for further research into the biocompatibility of Acemannan paste as a root canal filling material against the progression of *Enterococcus faecalis* bacteria *in vivo*.[10]

Materials and Methods

Collection and preparation of Acemannan sample

Acemannan powder was obtained from the Pharmacy Laboratory of Universitas Brawijaya, Malang, Indonesia. *Acemannan* paste was prepared by mixing 0.5 g *Acemannan* powder (Oligotech Elicytil Co. Ltd) and 0.75 mL of saline.

Animals

Wistar rats were obtained from the Animal House, Faculty of Dentistry, University of Brawijaya, Malang, Indonesia. The animals were kept in well-ventilated plastic cages (50 cm x 40 cm x 15 cm), and acclimatized to the laboratory conditions for 3 days. They were maintained under stable environmental conditions of 12 hours of light and 12 hours of dark cycle, room temperature (27 - 28℃). The rats were fed with standard rodent pellets and had access to drinking water drunk *ad libitum*. The animal beddings were changed every two days.

Ethical approval

Ethical clearance with reference number: 110-KEP-UB.2020 was granted by the Ethic Committee of Medical School Brawijaya University. The experiment was performed at the Pharmacy Laboratory, Pharmacology laboratory and Biomolecular laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

Study design

This study used a post-test randomized controlled group design in rat model. Twenty-four (24) Wistar rats were divided into three groups of 8 rats per group, consisting of a control group (K-), and two treatments groups: MTA group (K(M), and Acemannan paste group (K(A)). MTA is a mixture of paste with sterile water containing tricalcium silicate, usually used as a filling material for dental root canals. The sample size and replication (n) per treatment (t = 3) was determined using the Federer's formula as shown below:

$t(n-1) > 15$
$3n-1 > 15$
$3n > 15+1$
$3n \geq 16$
$n \geq 16/3 = 5.33 \Rightarrow 5$ sample replications

Where:
t = Number of treatment groups
n = Number of repetitions required
To give room for attrition that would likely occur in the course of the research, 8 replicates were used in each treatment group.

Determination of antibacterial activity
Microorganism

Typed culture of *Enterococcus faecalis* was obtained from the Microbiology Research Laboratory, Faculty of Dentistry, Universitas Airlangga. The organism was characterized as *Enterococcus faecalis* ATCC 29212 PK by gram staining and biochemical test (catalase and oxidase tests).

Determination of antibacterial activity

The antibacterial activity of *Acemannan* paste against *Enterococcus faecalis* was determined *in vitro* using the tube dilution and the streak plate methods.[10]

In the preliminary *susceptibility* test, sterile tubes containing brain heart infusion agar (BHIA) were inoculated with *Enterococcus faecalis*, and the organism was treated with various concentrations (2000, 1000, 500, 250, and 125 µg/mL) of *Acemannan* paste. The tubes were incubated at 37ºC for 24 h. The positive control used was 5.25% NaOCl and distilled water was used as the negative control. Bacterial growth was characterized by turbidity in the tube. In addition, inoculum in each tube containing the organism and *Acemannan* at the various concentrations were streaked on BHIA plates using a sterile loop. The plates were cultured at 37ºC for 24 h, after which the number of colony forming unit were counted.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC were determined by tube dilution and the streak plate methods, respectively. Based on the results obtained from the preliminary microbial susceptibility test, *Acemannan* paste was further prepared at concentrations of 200 µg/mL, 250 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, and evaluated against the test bacterial *Enterococcus faecalis*. Sodium hypochlorite (5.25%) and distilled water were used as the positive and negative controls, respectively. The MIC was determined qualitatively by observing the turbidity of each bacterial culture tube containing the various concentrations of *Acemannan* paste.

Furthermore, the minimum bactericidal concentration (MBC) was determined by sub-culturing each tube on BHIA media. The concentration that produced less than 0.1% of the number of colonies contained in the original inoculum where no bacterial growth was observed was regarded as the MBC.

Enterococcus faecalis induction of the root canal

Enterococcus faecalis was induced into the incisor tooth root canal of Wistar rats by injecting 10 µL of BHIA containing *E. faecalis* ATCC212 (10^6 CFU) with a micropipette. Induction was carried out on the first day, after which the root canal was filled with cavity and left for 3 weeks. During the 3 weeks, periodic checks were carried out to ensure that the cavity was still intact in the incisors. If the cavity was loose, the bacteria were induced again and the canal closed with cavity.

Determination of anti-inflammatory activity

The anti-inflammatory activity of *Acemannan* paste was determined *in vivo* by assessing the expression of tumour necrosis factor-α (TNF-α) in periapical tissues of the incisor teeth of Wistar rats. Wistar rats were randomly divided into three groups; treatment (*Acemannan* paste), positive (+) control (MTA), and negative (-) control (distilled water) groups. Prior to treatment, the root canals of the incisor teeth of the rats were infected with *Enterococcus faecalis*. After three weeks post-infection (i.e., on the 22nd day), treatments (*Acemannan* paste and MTA) were applied into the root canal of the infected teeth up to the length of the incisor tooth canal. The experiment was divided into two phases: Phase 1 (treatments were applied only on the first day (day 1 corresponding to 22nd day post-infection) and phase 2 (treatments were applied for 7 days (from 22nd to 28th day post infection). On the 22nd and 28th days, the rats were decapitated under anesthesia (Diazepam 10 mg/kg BW administered intraperitoneally). Subsequently, the mandibular bones were removed, and prepared for immunohistochemical staining for the determination of TNF-α expression.

Immunohistochemistry

Histological sections of the periapical tissues of the incisor teeth were washed with phosphate buffered saline (PBS) three times for 5 minutes each. Peroxidase blocking solution was added and left for 40 minutes,

followed by 30 µL of serum and triton-100 detergent to keep the tissue tension low, and the preparation was left for 30 minutes. Thereafter, the tissue preparation was washed with PBS (3x for 5 minutes each). The primary antibody was added to the preparation and incubated for 1 hour. The preparation was washed with PBS (3x, 5 minutes), followed by addition of the secondary antibody and incubation for 40 minutes. The preparation was washed again with PBS (3x), then SA-HRP (Streptavidin Conjugated with Horseradish Peroxidase) was added, followed by incubation for another 40 minutes. The preparation was washed with PBS (1x), then with distilled water until the SA-HRP was completely removed. The preparation was incubated for 30 minutes, then washed 3 times with PBS, followed by thorough washing with distilled water until the tissue preparation was clean, after which it was counter stained with Mayer Hematoxylin at pH 8. The preparation was rinsed with distilled water, dried, and then covered with a stinging cover. TNF-α expression marker characterized by brownish-red flourescence were then observed under a microscope (OLYMPUS digital microscope) at 40x, 100x, and 400x magnifications. The expression level was assessed by manual counting of the brownish-red flourescence for five field of view. Photographs were taken at the 400x magnification.

Cytotoxicity test

The cytotoxicity of *Acemannan* paste was determined *in vivo* by assessing the expression of caspase-9 (a protein that initiates the process of intrinsic apoptosis) in periapical tissues of the incisor teeth of Wistar rats. Caspase-9 expression level was evaluated following the same protocol as explained above for TNF-α, but in this case using caspase-9 antibodies.

Dental X-ray of lower incisor of Wistar rats

Three weeks post-bacterial induction of the root canal of incisor of Wistar rats, a periapical radiographic X-ray was performed on one of the experimental animals to ensure that the periapical lesion had formed. Prior to periapical radiography, Wistar rats were anesthetized by intraperitoneal injection of diazepam (10 mg/kg bw).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 27. Data were presented as the mean ± standard deviation (mean ± SD). A normality test was conducted using the Shapiro-Wilk test. Levene's test was used to assess data homogeneity. Differences between mean values were determined using the one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The Pearson correlation test was used to determine the strength of the relationship between the duration of administration of *Acemannan* paste and TNF-α expression in periapical tissue of rat teeth.

Results and Discussion

Antibacterial activity of Acemannan paste

Preliminary antibacterial activity test was conducted to determine the concentration range of *Acemannan* paste that effectively inhibits the growth of *Enterococcus faecalis*. The results as presented in Table 1 and Figure 1 indicated that *Acemannan* paste effectively inhibited the growth of *Enterococcus faecalis* in a concentration-dependent manner, with no growth observed at 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, while bacterial growth was observed at 250 µg/mL and 125 µg/mL.

For the MIC determination, the number of colony forming unit in each tube were obtained as follows; 169.67 CFU/mL for the negative control, 72.00 CFU/mL at 200 µg/mL, 33.33 CFU/mL at 250 µg/mL, 10.67 CFU/mL at 300 µg/mL, and 0 CFU/mL at 400 µg/mL, 500 µg/mL, and positive control (5.25% NaOCl). The 300 µg/mL Acemannan was found to be the lowest concentration where no turbidity was observed in the tube. Based on these results, 300 µg/mL of *Acemannan* paste was regarded as the MIC (Figure 2). Subsequently, the minimum bactericidal concentration (MBC) determination found 400 µg/mL of *Acemannan* paste as the MBC (Figure 3).

The antimicrobial effect of *Acemannan* – a major polysaccharide of Aloe vera has been confirmed in many studies. For example, findings from a previous study showed that *Acemannan* has antibiofilm potential, especially against gram-positive and gram-negative bacteria.[15] AVB5 an *Acemannan* polymer was found to kill biomass and reduces microbial viability very effectively.[15] In a previous study, it was also found that *Acemannan* has a good effect as an antimicrobial through its ability to stimulate phagocytosis and therefore suitable for use as a therapeutic agent.[16,17]

Table 1: Antibacterial activity of *Acemannan* paste against *Enterococcus faecalis*

Concentration (µg/mL)	Number of colonies			Total	Average
	I	II	III		
200	66	72	78	216	72.00
250	31	33	36	100	33.33
300	10	10	12	32	10.67
400	0	0	0	0	0
500	0	0	0	0	0
Control (+)	0	0	0	0	0
Control (-)	174	164	171	509	169.67

Control (+) = NaOCl (5.25%), Control (-) = Distilled water

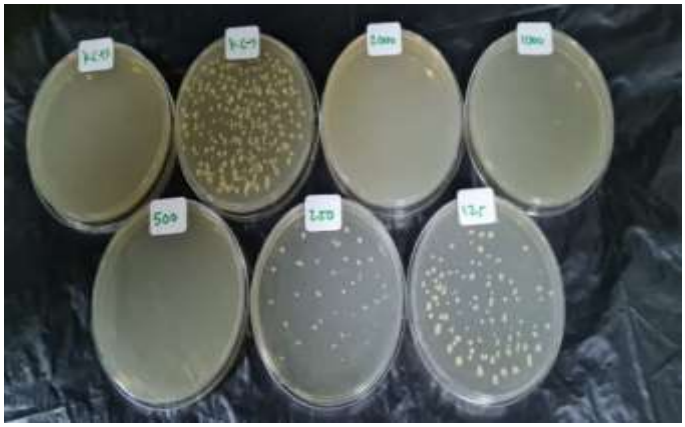

Figure 1: Bacterial colony growth inhibition with *Acemannan* paste

In the present study, *Acemannan* paste was found suitable for use as a root canal filling paste due to its bactericidal effect against*Enterococcus faecalis*, which is the main cause of root canal treatment failure.[18]

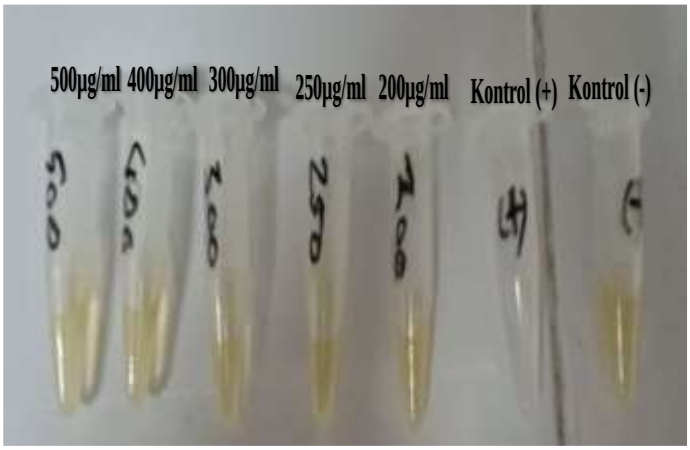

Figure 2: Minimum inhibitory concentration (MIC) of *Acemannan* paste from tube dilution

Anti-inflammatory activity of Acemannan paste

The mean TNF-α expression in the negative control, positive control (MTA), and *Acemannan* treatment groups are shown in Figure 4. From the results, there were significant differences ($p < 0.05$) in the expression levels of TNF-α among the various groups on day 1 and day 7 after the insertion into the root canal of the incisor teeth of rats. The lowest average amount of TNF-α on day 1 was found in the *Acemannan* paste treatment group K(A)1 with a mean TNF-α expression level of 8.27. The MTA group K(M)1 showed a mean TNF-α expression level of 8.85, while the negative control group K(-)1 exhibited the highest TNF-α expression with a mean expression level of 10.40. Similarly, the average amount of TNF-α expression on day 7 was found to be lowest in the *Acemannan* paste treatment group with a mean expression of 6.80, this was closely followed by the MTA paste group with a mean expression of 7.47, and finally the negative control group exhibited the highest TNF-α expression with expression level of 17.60.

The above results were supported by histological findings, where significant differences were observed in the number of blood vessels count on days 1 and 7 between the negative control group and the intervention groups (*Acemannan* and MTA treatment groups) (Figure 5). Histological examination revealed that all treatment groups given *Acemannan* paste and MTA experienced a decrease in TNF-α expressions on days 1 and 7 compared to the negative control group. In this study, the choice of treatment for 3 weeks was from the knowledge that it takes approximately 3 weeks for periapical abnormalities to manifest after pulp infection.[19] After the histological examination, periapical radiographic photos were taken, but the expected x-ray results were not obtained because of the difficulty associated with taking radiographic photos of the jaws of small animals like rats. The results of this study are supported by the findings from a previous study that showed that paste treatment gradually decreased the release of lymphocytes and macrophages from day 1 to day 7 post treatment.[20] Lymphocytes and macrophages released the most inflammatory cytokines from infected or injured cells.[21] The release of cytokines by injured cells triggers an acute inflammatory reaction.[21] Key cytokines produced by the innate immune system during this response include TNF-α, IL-1, and IL-6, all of which are involved in promoting acute inflammation. Additionally, TNF-α plays the most significant role in this process due to its constitutive nature.[22] TNF-α produced by lymphocytes in an inflammatory reaction serves as a crucial signalling molecule between cells forming a communication network in an immune response. These cytokines affect inflammation and immunity through the regulation of growth, mobility and differentiation of leukocytes and other types of cells.[22] TNF-α can increase levels of inducible Nitric Oxide Synthase (iNOS), an increase in iNOS is followed by the formation of Nitric Oxide (NO), so that an excessive levels of TNF-α will results in excessive NO secretion as well, resulting in septic shock, and damage to various organs. Thus, TNF-α levels indicate the degree of inflammation, higher TNF-α levels correspond to more severe inflammation, while lower levels suggest milder inflammation and a faster healing process.[15] Based on the results of the present study, TNF-α expression on the 1st and 7th day after treatment was lowest in the *Acemannan* paste group on day 7 and highest in the negative control (untreated) group on day 7.

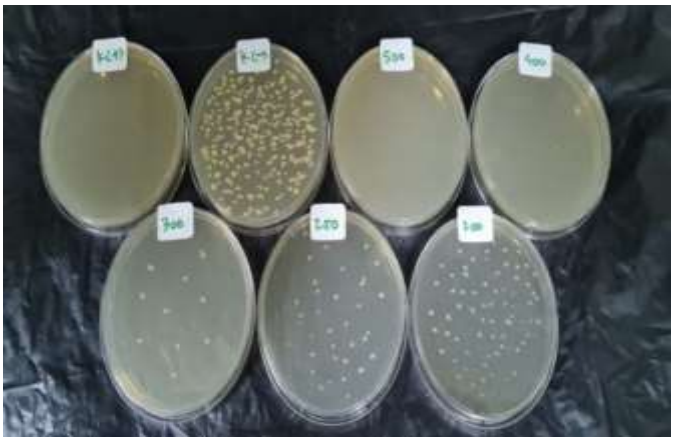

Figure 3: Minimum bactericidal concentration (MBC) of *Acemannan* paste from streak plate method

The reduced expression of TNF-α by *Acemannan* paste treatment can be attributed to its anti-inflammatory properties.[23] *Acemannan* as an active anti-inflammatory substance in aloe vera play a role in wound healing by inhibiting the activity of macrophages in the inflammatory phase, causing a decrease in TNF-α expression. The group treated with MTA paste also showed a low average expression of TNF-α, this is because MTA containing hydroxyapatite has a good immune suppressive response during the inflammatory process by decreasing the expression of TNF-α.[20]

The inflammatory response remains essential in the healing process, as it represents a complex biological reaction of blood vessel tissues to harmful stimuli, including pathogens, damaged cells, or irritants. Without the healing process, wounds and infections can result in continued tissue damage.[24] Inflammation is a manifestation of an immune response to eliminate antigens from the body.[25] From the results of the present study, it can be concluded that MTA paste and *Acemannan* paste are potent ingredients that can be used to accelerate wound healing.

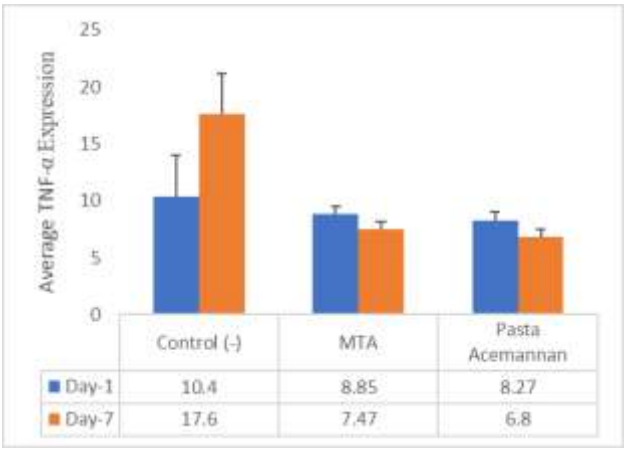

Figure 4: Effect of *Acemannan* paste on TNF-α expression in mandibular tissues in Wistar rats

Cytotoxicity of Acemannan paste

The *cytotoxicity of Acemannan paste* was evaluated by assessing caspase-9 expressing levels in rats' mandibular tissues. The purpose of this study was to determine the effect of *Acemannan* paste on caspase-9 expression in the incisor teeth of Wistar rats that had been infected with *Enterococcus faecalis* bacteria. Figure 6 shows the mean expression of caspase-9 in the negative control, positive control, and *Acemannan* treatment groups. The lowest average amount of caspase-9 on day 1 was found in the negative control group with a mean caspase-9 expression of 10.8 followed by the MTA paste group with a mean caspase-9 expression of 52.2, while the highest average amount of caspase-9 was found in the *Acemannan* treatment group with an expression value of 77.07. The expression levels of caspase-9 on day 7 followed a similar trend as that observed on day 1, with the lowest average caspase-9 expression found in the negative control group with a mean value of 8.4, followed by the MTA paste group, with a mean value of 45.07, and the highest expression level was found in the *Acemannan* treatment group with an average expression value of 135.93.

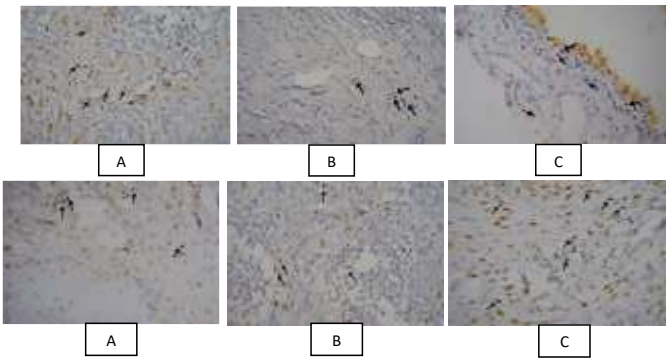

Figure 5: Photomicrographs of pulp and periapical tissue of rat teeth showing TNF-α expression on days 1 and 7 in one field of view at 400x magnification **(A)** *Acemannan* treated group; **(B)** MTA treated group (positive control); **(C)** Negative control group

The above results were corroborated by the histological findings, where significant differences were observed in the of stained cells showing caspase-9 expression on days 1 and 7 between the negative control group and the treatment (MTA and *Acemannan*) groups (Figure 7).

Acemannan is a compound with many benefits. According to Yuli *et al*.,[3] active substances from aloe vera can attack pathological cells such as cancer cells without affecting normal cells. This is supported by work of Songsiripradubboon *et al*.[26] which found that *Acemannan*, a polymer extracted from the aloe vera plant, can kill colon cancer cells without attacking normal cells by releasing cytochrome c that initiates the caspase reaction.[26] Therefore, polysaccharides such as *Acemannan* are being widely researched in the medical world for their ability to kill pathological cells without serious side effects as well as having low toxicity to normal cells. In dentistry, *Enterococcus faecalis* is one of the main causes of root canal failure, due to several virulence factors that make the bacteria resistant in root canals.[18] It has been found that *Enterococcus faecalis* can prevent the process of apoptosis that occurs in host cells (mouse macrophages) by inhibiting the activation process of caspase-9.[27]

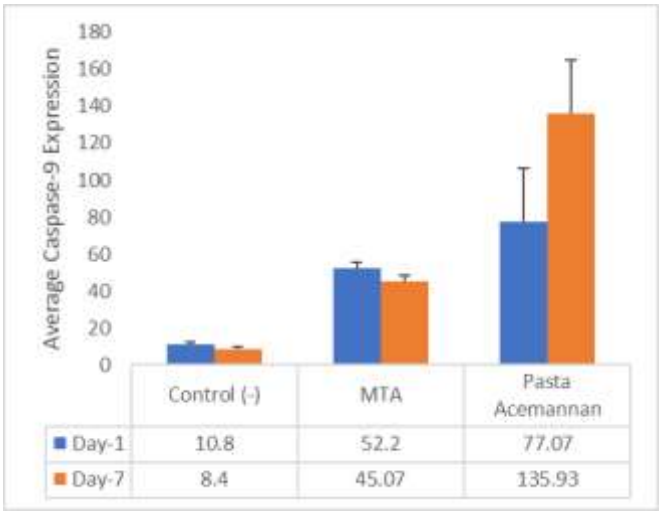

Figure 6: Effect of *Acemannan* paste on Caspase-9 expression in mandibular tissues in Wistar rats

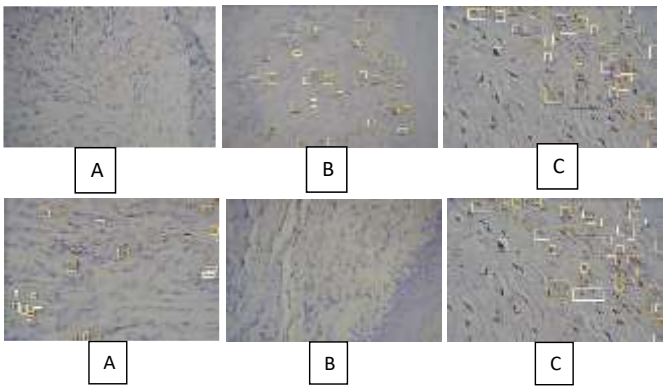

Figure 7: Photomicrographs of pulp and periapical tissue of rat teeth showing Caspase-9 expression on days 1 and 7 in one field of view at 400x magnification **(A)** *Acemannan* treated group; **(B)** MTA treated group (positive control); **(C)** Negative control group

Caspase-9 itself is a biomarker that indicates that cells have gone into the apoptotic pathway. When caspase-9 is activated, it is an indication that the process of cell damage or apoptosis has occurred. Therefore, higher number of caspase-9 indicates that more cells infected with *Enterococcus faecalis* are undergoing apoptosis.[27] These results are in line with the findings from the study of Chou *et al*.[28] that states that *Acemannan* can cause the release of cytochrome-c, which initiates the caspase reaction.

Conclusion

The findings from the present study have shown *Acemannan* from aloe vera possess antibacterial activity against *Enterococcus faecalis* a causative agent of root canal infection. *Acemannan* paste as a root canal filler was shown to exhibit anti-inflammatory activity and induce apoptosis of pathological cells by decreasing TNF-α expression, and stimulating caspase-9 expression, respectively in the periapical tissue of the incisor in Wistar rats.

Conflict of Interest

The author`s declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowlegments

This study was supported by a grant from non-tax revenue (PNBP) Faculty of Dentistry, Brawijaya University.

References

1. Nonong YH, Patawulandari S, Satari M, Indriyanti R. Antibacterial Test Between *Aloe vera* and Chlorhexidine based on The Number of Colony of *Streptococcus mutans* ATCC25175 *In Vitro*. Int J Sci Res. 2016; 5(1):1379–1385. Doi: 10.21275/V5I1.NOV153049.
2. Sadgrove NJ and Simmonds MSJ. Pharmacodynamics of *Aloe vera* and *Acemannan* in therapeutic applications for skin, digestion, and immunomodulation. Phytother Res. 2021; 35(12):6572–6584. Doi: 10.1002/ptr.7242.
3. Yuli N, Wibi R, Nur P, Edy W, Dradjat RS. Effect of *Aloe vera* gel on the expression of FGF-2, TGF-β, and Smad3 in the root surface of rat teeth after traumatic avulsion. Res J Pharm Technol. 2019; 12(9):4405–4409. Doi: 10.5958/0974-360X.2019.00758.
4. Mangaiyarkarasi SP, Manigandan T, Elumalai M, Cholan P, Kaur R. Benefits of *Aloe vera* in dentistry. J Pharm Bioallied Sci. 2015; 7(1):S255-259. Doi: 10.4103/0975-7406.155943.
5. Sangur R, Bajwa W, Mahajan T, Banerjea A. *Aloe vera*: An Ancient Option for Modern Day Dental Problems-A Review. Int J Contemp Med Res. 2016; 3(8):2351-2354.
6. Mehrabani D, Sholehvar F, Yaghmaei P, Zare S, Razeqhian-Jahromi I, Jalli R, Hamzavai M, Mehrabani G, Zamiri B, Karimi-Busheri F. The impact of acemannan, an extracted product from *Aloe vera*, on the proliferation of dental pulp stem cells and healing of mandibular defects in rabbits. Am J Stem Cells. 2024; 13(2):75-86. Doi: 10.62347/UAFC3719.
7. Chantarawaratit P, Sangvanich P, Banlunara W, Soontornvipart K, Thunyakitpisal P. *Acemannan* sponges stimulate the alveolar bone, cementum and periodontal ligament regeneration in a canine class II furcation defect model. J Periodontal Res. 2014; 49(2):164–178.Doi: 10.1111/JRE.12090.
8. Le VC, Thi THP, Sangvanich P, Chuenchompoonut V, Thunyakitpisal P. *Acemannan* induces rapid early osseous defect healing after apical surgery: A 12-month follow-up of a randomized controlled trial. J Dent Sci. 2020; 15(3):302–309. Doi: 10.1016/J.JDS.2019.09.012.
9. Pal S, Raj M, Singh M, Saurav K, Paliwal C, Saha S, Sharma AK, Singh M. The Effect of *Aloe vera* on Skin and Its Commensals: Contribution of *Acemannan* in Curing Acne Caused by *Propionibacterium acnes*. Microorg. 2024; 12(10):1-11. Doi: 10.3390/MICROORGANISMS12102070.
10. Matsuo T, Nakao K, Hara K. Inhibitory Effects of the Heat-Killed Lactic Acid Bacterium *Enterococcus faecalis* on the Growth of *Porphyromonas gingivalis*. Curr Ther Res. 2024; 100:100731
11. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological Update Properties of *Aloe vera* and its Major Active Constituents. Molecules. 2020; 25(6):1-37. Doi: 10.3390/MOLECULES25061324.
12. Rizqi J and Amestiasih T. Effect of Honey and *Aloe vera* Combination on Proliferation in NIH3T3 Fibroblast Cell Line: An *In Vitro* Study. Penelitian. 2021; 1-7.
13. Ravishankar and Kumar A. Ethno-Botanical Approach for Root Canal Treatment –An Update. J Pharm Sci Res. 2011; 3(10):1511-1519.
14. Bai Y, Niu Y, Qin S, Ma G. A New Biomaterial Derived from *Aloe vera—Acemannan* from Basic Studies to Clinical Application. Pharmaceutics. 2023; 15(7):1-43. Doi: 10.3390/PHARMACEUTICS15071913.
15. Salah F, Ghoul YE, Mahdhi A, Majdoub H, Jarroux N, Sakli F. Effect of the deacetylation degree on the antibacterial and antibiofilm activity of *Acemannan* from *Aloe vera*. Ind Crops Prod. 2017; 103:13–18. Doi: 10.1016/J.INDCROP.2017.03.031.
16. Jansisyanont P, Tiyapongprapan S, Chuenchompoonut V, Sangvanich P, Thunyakitpisal P. The effect of *Acemannan* sponges in post-extraction socket healing: A randomized trial. J Oral Maxillofac Surg Med Pathol. 2016; 28:105–110.
17. Vu NB, Chuenchompoonut V, Jansisyanont P, Sangvanich P, Pham TH, Thunyakitpisal P. *Acemannan*-induced tooth socket healing: A 12-month randomized controlled trial. J Dent Sci. 2021; 16(2):643–653.
18. Alghamdi F and Shakir M. The Influence of *Enterococcus faecalis* as a Dental Root Canal Pathogen on Endodontic Treatment: A Systematic Review. Cureus 2020; 12:1–10.
19. Fan X, Li X, Wan K, Zhao X, Deng Y, Chen Z, Luan X, Lu S, Liu H. Construction and immunogenicity of a T cell epitope-based subunit vaccine candidate against *Mycobacterium tuberculosis*. Vaccine. 2021; 39(47):6860–6865. Doi: 10.1016/j.vaccine.2021.10.034.
20. Liu C, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: From Clinical Significance to Quantification. Adv Sci. 2021; 8(15):1-29. Doi: 10.1002/ADVS.202004433.
21. Liu BM, Martins TB, Peterson LK, Hill HR. Clinical significance of measuring serum cytokine levels as inflammatory biomarkers in adult and pediatric COVID-19 cases: A review. Cytokine. 2021; 142:1043-4666. Doi: 10.1016/J.CYTO.2021.155478.
22. Liu C, Cui Y, Pi F, Cheng Y, Guo Y, Qian H. Extraction, purification, structural characteristics, biological activities and pharmacological applications of *Acemannan*, a polysaccharide from aloe vera: A review. Molecules. 2019; 24(8):1-22. Doi: 10.3390/MOLECULES24081554.
23. Jayawardena TU, Asanka SKK, Nagahawatta D. Anti-Inflammatory Effects of Sulfated Polysaccharide from Sargassum Swartzii in Macrophages via Blocking TLR/NF-Kb Signal Transduction. Mar Drugs. 2020; 18(12):601. Doi: 10.3390/MD18120601.
24. Chen L, Deng H, Cui H, Feng J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018; 9(6):7204-7218. Doi: 10.18632/ONCOTARGET.23208.
25. Martelli D, Farmer DGS, Yao ST. The splanchnic anti-inflammatory pathway: could it be the efferent arm of the inflammatory reflex? Exp. Physiol. 2016; 101(10):1245–1252. Doi: 10.1113/EP085559.
26. Songsiripradubboon S, Kladkaew S, Trairatvorakul C, Sangvanich P, Soontornvipart K, Banlunara W,

Thunyakitpisal P. Stimulation of Dentin Regeneration by Using *Acemannan* in Teeth with Lipopolysaccharide-induced Pulp Inflammation. J Endod. 2017; 43(7):1097–1103. Doi: 10.1016/J.JOEN.2017.01.037.

27. Zou J, Shankar N. Enterococcus faecalis infection activates phosphatidylinositol 3-kinase signaling to block apoptotic cell death in macrophages. Infect Immun. 2014; 82(12):5132-5142. doi: 10.1128/IAI.02426-14.
28. Chou MH, Chen YH, Cheng MT, Chiang HC, Chen HW, Wang CW. Potential of methacrylated *Acemannan* for exerting antioxidant-, cell proliferation-, and cell migration-inducing activities *in vitro*. BMC Complement. Med Ther. 2023; 23(1):1–10. Doi: 10.1186/S12906-023-04022-8/FIGURES/6.