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Inhibition of Garlic Ethanol Extracts (*Allium Sativum* L.) as a Solvent in Alginate Impression Materials in the Growth of *Candida Albicans*

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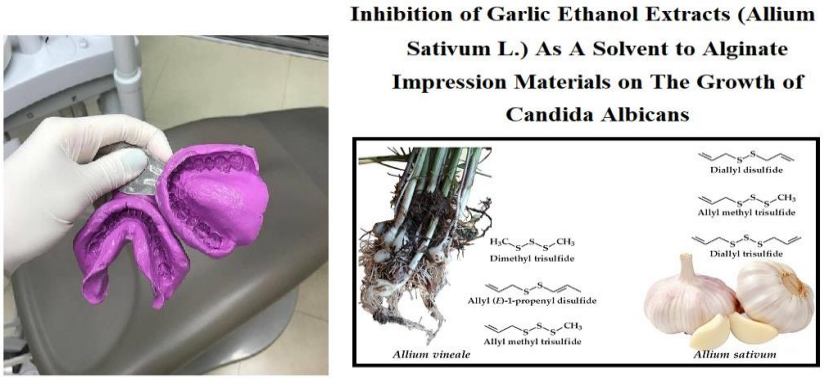
ABSTRACT

Alginate is an impression material currently used in dentistry because it has numerous advantages. However, alginate will come into direct contact with blood, saliva, and plaque in its use. Hence, it has the potential to contain pathogenic micro-organisms. One of the micro-organisms that can be pathogenic is *Candida Albicans*. It can infect the oral cavity and cause candidiasis. Therefore, it is necessary to inhibit *Candida Albicans*' growth in the form of a natural ingredient from garlic (*Allium sativum* L.). Garlic (*Allium sativum* L.) has benefits as an antifungal since it has an active ingredient, namely allicin. The objective is examining the effect of garlic (*Allium sativum* L.) ethanol extract as a solvent in alginate impression materials on the growth inhibition of *Candida Albicans*. The research method was disc diffusion with alginate manipulation using a solvent in distilled water and with ethanol extract of garlic concentrations of 10%, 20%, 40%, and 80%. After that, the clear zone around the disc was measured with a caliper. One-way ANOVA test results indicated that the value was p=0.000 (p<0.05). It means that there were significant differences in the entire treatment group. The results of the Post Hoc LSD test showed that there were significant differences among the sample groups. Therefore, the ethanol extract of garlic (*Allium sativum* L.) as a solvent in the alginate impression material has an effect on the growth inhibition of *Candida Albicans (in vitro*).

GRAPHICAL ABSTRACT



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Introduction

Alginate is one of the irreversible impression materials that is produced from a chemical reaction so that the sol phase changes to the gel phase, which is identified as the gelation process [1-5]. When the gelation process is complete, the alginate cannot return to the sol phase. Therefore, alginate is classified to be an irreversible impression material and distinguished from reversible impression materials [6-12]. Alginates are found in both marine brown algae (Phaeophyceae) and bacteria as capsular polysaccharides, although all commercial alginates are currently derived from algal sources alone. In high-tech specialist applications such as pharmacy and biotechnology, microbial fermentation and postpolymerization of alginates will be critical. Alginates' capacity to retain water, as well as its gelling, viscosifying, and stabilizing characteristics, are quantitatively important industrial uses [13].

Alginate has advantages, such as affordable price, easy manipulation, good taste, hydrophilicity, and being easy to be filled in by casts. However, alginate also has disadvantages; it can be easily torn, must be drained with water after being removed from the oral cavity, has details limited, has unstable dimensions, and can only be used for one impression [14–18]. When performing alginate manipulation, the ratio of powder to water as a solvent must be precise to achieve the desired consistency of results. Changes in the ratio of powder and water will affect the consistency and setting time and the impression material's strength and quality [19].

Alginate will come into direct contact with saliva, plaque, and blood in the oral cavity. It will potentially contain pathogenic micro-organisms [20]. There is a possibility that dentists, assistants, staff, and laboratory technicians can be contaminated with these micro-organisms [21]. Therefore, it is necessary to have a control to prevent cross-infection through impression materials in dentistry. One of the pathogenic micro-organisms that can infect the alginate impression material is a fungus [22].

Fungi of the genus *Candida* are fungal pathogens that are often discovered in the oral cavity and partly in the intestinal mucosa [23, 24]. Infection of *Candida sp.* on the mucosa can also occur in the oropharynx, esophagus, and vagina. *Candida sp.* can cause the incidence of disease and incidence of death in a healthy environment. *Candida sp.* can also cause superficial or systemic candidiasis. One *Candida sp.* that can cause disease in humans is *Candida Albicans* [25]. *Candida Albicans* infection of the oral mucosa can cause candidiasis. Candidiasis can also occur from cross-infection of alginate impression materials in which *Candida Albicans* have previously left contamination. Thus, a chemical solvent that can inhibit *Candida Albicans*' growth on alginate impression materials is needed [26].

The Centers for Disease Control and Prevention (CDC) in 2008 explained that these chemical solvents have disadvantages: They have unstable chemical reactions, are toxic at high concentrations, and in some people can have side effects such as irritation in the oral mucosa, asthma, and rhinitis [27].

Therefore, the empiric use of drugs as antifungals has been widely used as an alternative treatment. One of the drugs that function as an antifungal is garlic (*Allium sativum* L.). One of the active ingredients in garlic (*Allium sativum* L.) is allicin [28].

Anti-microbial effects

The main chemicals responsible for garlic's antibacterial activity are considered to be allicin and other sulfur compounds. Garlic kills gram-negative, gram-positive, and acid-fast bacteria such as Staphylococcus, Salmonella, Vibrio, Mycobacteria, and Proteus [29]. The pathogenic bacteria were suppressed by aqueous, ethanol, and chloroform extracts of garlic, but to variable degrees of susceptibility. The toxic effects of garlic were more severe in gram positive Staphylococcus aureus than in gram negative Staphylococcus aureus. It has been demonstrated that the aqueous extract of garlic may be used in conjunction with conventional antibiotics to combat nosocomial infections, which are common in hospitals [30].

In vitro testing of the effects of aqueous and ethanolic extracts of garlic against bacteria such as Escherichia coli and Salmonella typhi has revealed that the aqueous extract has little or no inhibitory impact, but the ethanolic extract has a greater inhibitory effect [31]. Allicin has been discovered to have antibacterial action against multidrug-resistant enterotoxicogenic E. coli strains in its pure form. In another investigation, the aqueous extract was shown to have antibacterial action against both Gram positive and Gram-negative bacteria, whereas the methanol extract has been found to have antibacterial activity against all species except Staph aureus [32].

Material and methods

This type of research was a laboratory experimental research design with a post-test-only control group design with *Candida Albicans* as the research object. The treatment in this study was alginate manipulated with garlic ethanol extract solvent with a concentration of 10%, 20%, 40%, and 80%, which then affected a mold with a diameter of 10 mm and a thickness of 1 mm.

The number of samples in this study was 25 samples divided into five petri dishes containing *Candida Albicans* cultures. These samples were then divided into five groups, including the negative control group with alginate dissolved in distilled water, the alginate group dissolved with 10% garlic ethanol extract, 20 %, 40%, and 80%. After that, the alginate in the petri dish containing *Candida Albicans* was incubated 24 hours at 370C. The method used in this study was the disk diffusion method. Hence, after incubation, a clear zone appeared around the disc. This clear zone was the zone of inhibition, which was then measured with a caliper to the accuracy of 0.05 mm horizontally, vertically, and diagonally.

Results and Discussion

Table 1 presents the results of the mean and standard deviation of the inhibition zone diameter.

Table 1: The inhibition zone's mean diameter, the mean, and standard deviation of the inhibition zone around the disk

Replication	Treatment				
	K (-)	10%	20%	40%	80%
I	0	2.80	3.67	4.33	5.23
II	0	3.00	3.00	3.46	3.10
III	0	2.46	3.53	3.85	4.73
IV	0	2.38	3.70	4.31	4.28
V	0	2.03	2.38	2.85	4.31
Mean±SD	0,00±0,00	2,53±0,37	3,26±0,56	3,76±0,62	4,33±0,78

Table 1 shows an increase in the inhibition zone's diameter around the disk after 24 hours of incubation in each treatment group. The negative control did not have a diameter of the inhibition zone around the disk than the other treatment groups. The inhibition zone diameter began to appear at a concentration of 10% and increased, followed by an increase in concentration (Figure 1).

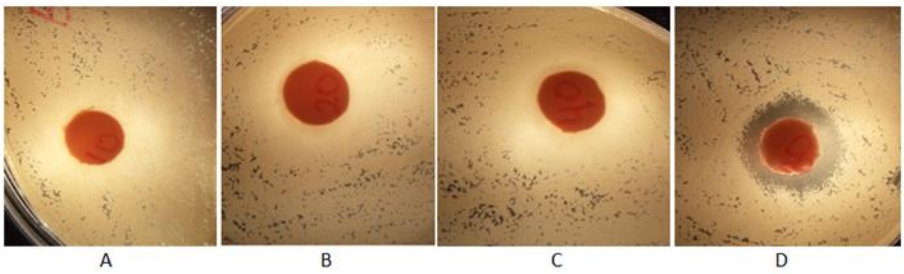

Figure 1: Zones of the inhibition of alginate with ethanol extract of garlic at various concentrations. A. 10%, B. 20%, C. 40%, D. 80%

The next step was to test the data's normality, which showed that each treatment group had a p-value <0.05. Thus, the data were normally distributed. The negative control treatment group

was ignored because the inhibition zone's diameter showed a zero value. Furthermore, the test was continued with the homogeneity test using Levene's test, which showed that the data was homogeneous or had the same variance between treatment groups (p-value <0.05). Based on the normality and homogeneity tests, one-way ANOVA test was applied. Table 2 demonstrates the results of one-way ANOVA.

Table 2: One-way ANOVA test

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	56,892	4	14,223	48,319	0,000
Withing Groups	5,887	20	0,294		
Total	62,779	24			

One-way ANOVA test results in Table 2 indicate p = 0.000 (p < 0.05). It can be concluded that the data showed a significant difference between the effects of garlic ethanol extract as a solvent at several concentrations of alginate impression material on the growth of Candida Albicans. Then, a Post Hoc test was conducted. The Post Hoc test implemented was LSD (Least Significant Different) to identify the differences between treatment groups with a confidence level of 95%. Table 3 shows the results of the Post Hoc Test.

Table 3: LSD Post Hoc Test

Treatment group	**Control -**	**10%**	**20%**	**40%**	**80%**
Kontrol -		0,000*	0,000*	0,000*	0,000*
10%	0,000*		0,048*	0,002*	0,000*
20%	0,000*	0,048*		0,157*	0,005*
40%	0,000*	0,002*	0,157*		0,112*
80%	0,000*	0,000*	0,005*	0,112*	

The Post Hoc test results from Table 3 indicate a significant difference among the groups with a value of p = 0.000 (p < 0.05). However, in the treatment group, 20%, 40%, and 80%, p value> 0.05 indicated that there is no significant difference at these concentrations.

Alginate is an irreversible impression material that results from a chemical reaction from changing the sol phase to the gel phase, which is identified as the gelation process. When the gelation process is complete, the alginate cannot return to the sol phase. Therefore, alginate is classified to be an irreversible impression material and distinguishes it from impression materials to make it reversible [12, 19].

Alginate as an impression material has supportive characteristics such as being easy to mix and manipulate, requiring minimum tools, having flexibility as an impression material, having good accuracy if it is adequately manipulated, and consuming a low price. These characteristics trigger alginate an impression material that has been widely used in dentistry today. Positive casts from alginate impression materials were used as a model study to make treatment plans, monitor changes, create temporary restorations, and construct removable denture casts [19].

Alginate impression materials are susceptible to infection transmission to dentists, according to a study [33]. Microorganisms can adhere to impression materials and turn into infectious agents, posing a disease transmission risk. The medium for the transmission of infection can be blood, saliva, pus, and debris. Preventive measures that can be done against the occurrence of cross-infection are the use of disinfectants on alginate impression materials, such as sodium hypochlorite and glutaraldehyde.

One of the disinfection techniques for alginate impression materials is mixing powder and disinfectant solvent during the manipulation process. This technique is quite effective in the alginate impression materials' disinfection

process and will not affect the alginate impression material's dimensional stability [34].

Infection by *Candida sp.* has increased since the 1980s based on its incidence and prevalence, especially in individuals with low immunity or in individuals who are hospitalized with severe illnesses. *Candida sp.* is generally harmless in the gastrointestinal and genitourinary tract [35]. Meanwhile, *Candida Albicans* can be a pathogen due to various conditions such as superficial infections. *Candida Albicans* has two types of infection in humans, namely superficial infections such as in the oral cavity and vaginal candidiasis and systemic infections that attack the vagina and body system to be life-threatening [36, 37].

Candida Albicans and another *Candida sp.* are revealed in about 75% of the population's oral cavity. In healthy individuals, this colonization remains generally benign. Conversely, individuals with decreased immunity, such as individuals exposed to HIV, transplant recipients, chemotherapy patients, and low birth weight babies (LBW), are susceptible to infection with *Candida Albicans* or *Candida sp.* [37].

Garlic (*Allium sativum L.)* is an herb with a height of approximately 60 cm. The trunk is a pseudo-stem and is green. Garlic (*Allium sativum L.*) is a layered tuber with a scalloped bottom and merges into large white tubers. Each clove is covered in thin, paper-like skin. It smells very sharp when it is sliced. It has long, flat roots and leaves [38].

The main ingredients of garlic (*Allium sativum* L.) are *ajoene, allicin, alliin, allyl disulfides, allyl trisulfides, cycloalliin, cysteine sulfoxides, cysteine, diallyl sulfides, dimethyl sulfides, disulfides, glutathione, methionine, methyl sulfide, pseudocordinanes, scordinine, sulfanes, tetrathiol, thiosulfinates,* and *trisulfides.* Garlic *(Allium sativum L.)* is also identified to contain phosphorus, calcium, and iron. Garlic has vitamins like riboflavin, thiamine, nicotinic acid, and vitamin C. Garlic (*Allium sativum L.)* also contains linalool, citral, alpha-phellandrene, geraniol, propionic aldehyde, and valeraldehyde. These active compounds' content is useful in the prevention and treatment of several diseases such as cancer, coronary heart disease, obesity, type 2 diabetes, high blood pressure, cataracts, and digestive disease [39].

The active compound that is the focus of this research is allicin. Allicin, also known as an organosulfur compound, is classified as thiosulfinate, which gives garlic its distinctive odor. Thiosulfinate contains two bonded sulfur atoms, and one of them binds to an oxygen atom. Allicin has various activities, including an antioxidant that causes apoptosis of cancer cells and antibacterial activity. Allicin is also effective in inhibiting fungi, especially *Candida, Cryptococcus, Trichophyton,* and *Microsporum* species [39].

Maceration comes from the Latin word "*macere*", which means watering, softening. This method is suitable for both small and large scales. The maceration method is done by softening the material by cutting it or creating a coarse powder, followed by soaking the powder and suitable solvent in a tightly closed inert container to prevent light catalyzed reactions or discoloration at room temperature and shake them again. The solvent will penetrate the cell wall and enter the cell cavity containing the active substance. The active substance will dissolve due to the difference in concentration between the active substance solvent inside and outside the cell. Thus, the most concentrated solvent will be pushed out. The maceration duration is 4-10 days. However, it generally takes five days. After obtaining equilibrium between the solvent compound concentration and the concentration inside, the extraction process is stopped. Furthermore, the solvent is separated from the sample by filtering [40].

The advantage of the maceration method is that it can avoid the destruction of thermolabile compounds. It is also an easy procedure and requires simple tools, so that this maceration method is most widely used. However, it also has disadvantages: It takes a long time, the solvent is used quite a lot, some compounds may be lost, and some compounds are difficult to extract at room temperature [40].

The garlic consists of 60% water, 32% carbohydrates, 6.5% in the form of dietary fiber, and 0.25-1.15% alliin. A 100gram garlic can

contain 31 mg of vitamin C, 0.2 mg of vitamin B1, vitamin B2, vitamin B3, provitamin A, minerals, and other elements [41]. One of the active ingredients in garlic is allicin, which is produced by changes in the enzyme alliin *[(+)-(S)-allyl-L-cysteine-sulfoxide].* In the presence of alliinase, an unstable alliin-alliinase complex is formed and then dehydrated by pyridoxal phosphate and transformed into allyl sulfenic acid, pyruvic acid, and ammonia. Allyl sulfenic acid is unstable and highly reactive to room temperature so that it condenses and forms allicin [42]. The content of allicin and its derivatives can damage candida cell membranes' integrity, inhibit growth, and produce oxidative stress, which can damage cell oxidative in *Candida Albicans*. Ajoene, a derivative of garlic ethanol extract, can inhibit the synthesis of phosphatidylcholine (PC) in the cytosolic membrane and block changes in the morphological form of dimorphic fungi as *Paracoccidioides brasiliensis*. The high concentration of garlic extract can suppress hyphae formation [43, 44].

This study indicated a clear zone after incubation for 24 hours with a temperature of 37^{o}C around the alginate disk. However, after the gelation process of the alginate ended, a liquid exudation phenomenon can be discovered, called syneresis. Syneresis describes a contraction of the material caused by the reorganization of polymer molecules in the alginate gel [45].

The Scanning Electron Microscope (SEM) results in Figure 2 present a microscopic image of the surface of the alginate impression material manipulated with distilled water that shows a strong bond. Hence, a dense surface and a little cavity (porosity) can be observed. However, Figure 3 demonstrates the bond that is not too strong. Therefore, the surface was slightly stretched and had a lot of cavities (porosity). It was because the amount of water when it reacted with the alginate impression material in the manipulation process that occurred only in the small portion.

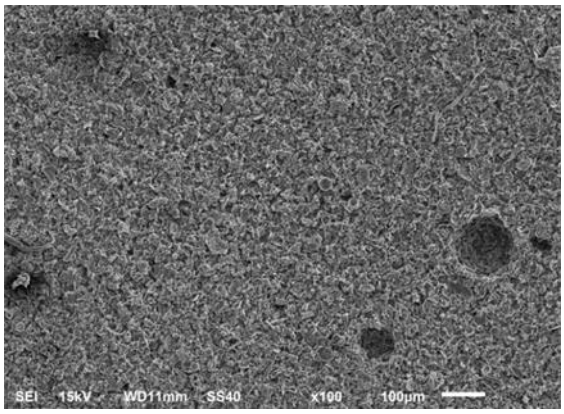

Figure 2: Scanning Electron Microscope (SEM) results on the surface of the alginate impression material manipulated with distilled water

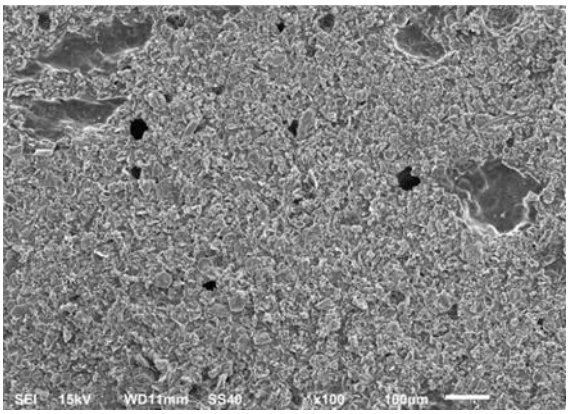

Figure 3: Scanning Electron Microscope (SEM) results on the alginate impression materials surface manipulated with garlic ethanol extract

The results showed that the higher the concentration of garlic (*Allium sativum L.)* ethanol extract as a solvent in the alginate impression material, the higher the growth inhibition of *Candida Albicans*. This study statistically showed that a concentration of 20% to a concentration of 40% had no significant difference. There was no significant difference between the 40% concentration and the 80% concentration. However, if you observed the real data (Table 1), there was an increase in the concentrations of 10%, 20%, 40%, and 80%. Therefore, at a concentration of 20% to a concentration of 40% and a concentration of 40% to a concentration of 80%, it had almost the similar effect in inhibiting *Candida Albicans'* growth. Nevertheless, based on the measurement of the mean inhibition zone, there was an increase in the concentration of 10%, 20%, 40%, and 80%. It is in line with past research [43], reporting that high concentrations of garlic extract could suppress hyphae formation.

Conclusion

In deciduous teeth, dental caries is a serious issue. Caries was shown to be widespread in deciduous teeth as a consequence of the findings. Extensive caries can cause pulp exposure, which can result in pulp necrosis. Necrotic deciduous teeth should be treated to keep teeth for mastication so that children can get the best nutrition possible for their growth and development. The endodontic or root canal therapy was conducted to eliminate germs and prevent reinfection. This procedure will preserve the function of teeth in the jaw for a long time, allowing periodontal tissues to remain healthy and pain-free. Root canal irrigation, antimicrobial agents, or root canal dressing are used in endodontic or root canal therapy. Calcium hydroxide and cresophene are two popular dressing materials used in juvenile dentistry; however, they must be evaluated both clinically and *in vitro*, as well as how they influence oral periapical tissues.

The ethanol extract of garlic *(Allium sativum L.)* as a solvent in the alginate impression material has an effect on the growth inhibition of *Candida Albicans* (*in vitro*).

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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