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Mixture of Garlic (*Allium sativum*) Extract 50% and Red Dragon Fruit (*Hylocereus polyrhizus*) peel 50% Effectiveness Against *Enterococcus faecalis* Bacteria

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Abstract: *Enterococcus faecalis* may infiltrate dentinal tubules in the cementum and dentin of the root, causing endodontic failure. A key stage in root canal therapy is irrigation. NaOCl is an efficient irrigation at higher concentrations, although its toxicity causes periapical tissue injury. Garlic and red dragon fruit peel suppress *Enterococcus faecalis*. To test the antibacterial activity of garlic (*Allium sativum*) extract and red dragon fruit (*Hylocereus polyrhizus*) peel extract at 50% against *Enterococcus faecalis* bacterium. A post-test only control-group design was used in this in vitro study. A 50% blend of garlic (*Allium sativum*) extract and red dragon fruit (*Hylocereus polyrhizus*) peel extract was compared to 2.5% NaOCl against *Enterococcus faecali*. ANOVA test results revealed significant differences ($p < 0.05$) in *Enterococcus faecalis* inhibition between garlic extract (*Allium sativum*), red dragon fruit peel extract, a mixture of both, and NaOCl: 9.30 ± 0.89, 15.88 ± 2.65, 12.30 ± 2.53, and 20.39 ± 2.41 mm. Red dragon fruit (*Hylocereus polyrhizus*) peel extract killed *Enterococcus faecalis* better than garlic (*Allium sativum*) and garlic-red dragon fruit peel combo.

Keywords: *Allium sativum*; Antibacterial; *Enterococcus faecalis*; *Hylocereus polyrhizus*; Root canal irrigation

Introduction

WHO states that dental caries is a destruction of the enamel and dentin layers caused by acids that are produced when bacteria consume sugar (WHO, 2017). Dental caries is the most common chronic condition and is a widespread public health problem worldwide that adversely affects quality of life (Shitie et al., 2021). Untreated tooth decay may lead to variety of severe infections and other issues such as periodontal disease and inflammation of the pulp tissue that could lead to root canal infection and pulp necrosis (Rathee et al., 2023; Sulistyo et al., 2023).

Root canal treatment is a type of endodontic treatment that includes removal of necrotic pulp tissue, irrigation, disinfection, and obturation (Sari et al., 2020). Eliminating or reducing bacteria from the root canal system is the main focus in endodontic treatment (Asnaashari et al., 2019). Endodontic failure has been associated with *Enterococcus faecalis* because of its capacity to penetrate dentinal tubules and form tight bonds with collagen, which is abundantly present in the cementum and dentin of the tooth root (Colaco, 2018).

Irrigation is an important step in root canal treatment. During root canal preparation, irrigation helps to clear the root canal from debris and facilitate instrument movement (Nirvana et al., 2018). Sodium

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hypochlorite (NaOCl) from 0.5% to 5.25% is the most popular endodontic material (Yu et al., 2019). NaOCl is commonly used because it has many benefits including antibacterial activity, tissue dissolving ability and acceptable biological compatibility at low concentrations (Estrela et al., 2018).

According to Cai et al. (2018), and Moliz et al. (2009) NaOCl is an effective irrigation solution as its concentration increases, but its toxicity also increases the possibility of periapical tissue damage. However, the lower the concentration, the more time it takes to dissolve organic tissue. Therefore, a concentration of 2.5% is the most suitable concentration (Armanda et al., 2017; Parisay et al., 2021). Some research have examined whether herbal components may inhibit *Enterococcus faecalis* development in infected root canals (Soraya et al., 2019).

Indonesia is a country rich in herbal plants (Lestari et al., 2019). The awareness of people's quality of life is increasing and plants that have benefits as antimicrobials or drugs are increasingly being used (Moulia et al., 2018; Nirwana et al., 2018). In Indonesia, garlic is a commonly used spice (Mardiyah, 2018). Garlic extract has been shown to have antimicrobial activity that shows antibacterial effects against various bacterial species in the oral cavity, especially gram-negative bacteria (Indrayati & Diana, 2020; Kshirsagar et al., 2019; Abgo, 2021; Batiha et al., 2020; Soraya et al., 2018; Purwantiningsih et al., 2019; Prihandini, 2015). The antibacterial properties of phytochemicals include flavonoids, ajoene, allicin, and essential oils. Allicin and essential oils block bacterial DNA, RNA, and protein creation, ajoene inhibits growth, and flavonoids denaturate protein to reduce growth (Octavia et al. (2019) and Mehta et al. (2020) found that garlic extract (*Allium Sativum*) inhibited *Enterococcus faecalis* bacteria.

In addition, red dragon fruit is one of the plants that is widely consumed by Indonesians because it is believed to have antibacterial properties (Setiani, 2020). The antioxidant quality of red dragon fruit has made it one of the natural sources of food additives (Zakaria et al., 2022). Because red dragon fruit peel is frequently wasted as food, its usage has not been optimized (Hendra et al., 2019). Due to its greater antioxidant activity than fruit meat, dragon fruit peel might be exploited as a natural antioxidant source (Mahargyani, 2018). *Hylocereus Polyrhizus* fruit peel contains alkaloids, terpenoids, tannins, and saponins (Wijayanti et al., 2022). Sari et al. (2021) found that red dragon fruit peel extract (*Hylocereus Polyrhizus*) inhibits *Enterococcus faecalis* growth.

Although various research have evaluated the antibacterial properties of garlic extract and red dragon fruit peel extract individually, none have directly compared their efficiency against *Enterococcus faecalis* bacterium. Soraya et al. (2019) found that a 50% concentration of garlic extract (*Allium Sativum*) reduces *Enterococcus faecalis* bacteria viability, but Aryanto et al. (2023), and Kristiananda et al. (2022) found that it does not. Another research by Sari et al. (2021) demonstrated that 50% red dragon fruit peel extract (*Hylocereus Polyrhizus*) destroyed *Enterococcus faecalis* bacteria.

Referring to the previous description, the researcher felt compelled to conduct research in the form of "Mixture of Garlic (*Allium Sativum*) Extract 50% and Red Dragon Fruit (*Hylocereus Polyrhizus*) peel 50% Effectiveness Against *Enterococcus faecalis* Bacteria".

Method

A post-test only control-group design was used in this in vitro study. This study was conducted at FKKGIK Universitas Prima Indonesia Laboratory from August to September 2024.

Tools and Materials

This study uses blender, digital scales, maceration container, rotary evaporator, beaker glass, measuring cup, autoclave, petri dish, ose, digital caliper, and test tube.

This research used 50% garlic extract, 50% red dragon fruit peel, 50% garlic-red dragon fruit extract mixture, 70% ethanol, 10% DMSO, 2.5% NaOCl, NaCl, aquadest, *Enterococcus faecalis* bacteria, paper disc, sterile cotton swabs, nutrient agar (NA), and plastic wrap.

Methods

To prepare extracts, 2 kg of garlic and 2 kg of dragon fruit peel are blended, macerated with 6 L of ethanol, and sealed to prevent evaporation. For 5 days, the maceration solution is agitated for 15 minutes every 24 hours and filtered. A rotating evaporator and water bath condense the extract (Farida et al., 2021; Saridewi et al., 2017). For a 50% concentration, dilute 5 g garlic extract with 10 mL of 10% DMSO (Panden et al., 2019). Weigh 5 g of each extract and dissolve in 10 mL of 10% DMSO to 50%. Mixing 2 mL garlic extract and 2 mL red dragon fruit peel extract yields a 2:2 ratio (Rifda et al., 2022; Handayani, 2015).

After thorough washing and rinsing, glass instruments were sterilized using an autoclave set to 121°C for a duration of 15 minutes (Sari et al., 2021). 3 g of nutrient agar media was placed in a beaker glass and 120 mL of aquadest was added. The media was then heated to boiling and mixed well, then autoclaved for 15 minutes at 121˚C. The nutrient agar (NA) media was then transferred to a sterile petri dish, cover with plastic wrap and allow it to solidify (Isnaeni et al., 2021; Susanti

et al., 2022). *Enterococcus faecalis* bacteria are taken using a sterile cotton swab, then streaked on nutrient agar media and then incubated at 37°C for 1x24 hours.

Paper discs soaked in 50% garlic extract, 50% red dragon fruit peel extract, 50% garlic-red dragon fruit peel extract combination, and 2.5% NaOCl were put in sterile petri dishes with bacteria-cultured nutritional agar. They're incubated at 37°C for 24 hours and monitored. The clear zone surrounding the disk is measured using a digital caliper to determine the inhibition zone (Guntur et al., 2021).

Result and Discussion

Plant Determination

The results of the determination at Sistematika Tumbuhan Herbarium Medanese (MEDA) Laboratory showed that the sample studied:

Garlic

Kingdom : Plantae
Division : Spermatophyta
Class : Monocotyledoneae
Order : Asparagales
Family : Amaryllidaceae
Genus : Allium
Species : *Allium Sativum* L.
Local Name : Garlic

Red Dragon Fruit Peel

Kingdom : Plantae
Division : Spermatophyta
Class : Dicotyledoneae
Order : Caryophyllales
Family : Cactaceae
Genus : Hylocereus
Species : *Hylocereus Polyrhizus*
Local Name : Red dragon fruit peel

Phytochemical Screening Test Results

Table 1 and table 2 indicate active compound components in garlic (*Allium Sativum*) and red dragon fruit (*Hylocereus Polyrhizus*) peels after phytochemical screening.

Table 1. Results of the Phytochemical Screening of Garlic (*Allium Sativum*)

Active Compound Content	Inhibition Zone Reagent (mm)	Test Results	Observation Results
Flavonoids	Alkaline (NaOH)	+	Yellow color - colorless
Tannins	$FeCl_3$	+	Blackish green color
Saponins	Foam Test	+	Formed foam
Triterpenoids & Steroid	Salkowski	+	Brown color
Alkaloids	Ethanol + dilute HCl + *Dragendroff*	+	Red Color

Table 2. Results of the Phytochemical Screening of Red Dragon Fruit (*Hylocereus Polyrhizus*) Peel

Active Compound Content	Inhibition Zone Reagent (mm)	Test Results	Observation Results
Flavonoids	Alkaline (NaOH)	+	Yellow color - colorless
Tannins	$FeCl_3$	+	Blackish green color
Saponins	Foam Test	+	Formed foam
Triterpenoids & Steroids	Salkowski	+	Green color
Alkaloids	Etanol + HCl encer + *Dragendroff*	-	Black color

Antibacterial Test Results

Garlic extract (*Allium Sativum*), red dragon fruit peel extract (*Hylocereus Polyrhizus*), and the combination of 50% garlic extract, red dragon fruit peel extract, and 2.5% sodium hypochlorite (NaOCl) (positive control) against *Enterococcus faecalis* were measured by measuring the clear zone around the paper disc with a digital.

Table 3. The Average Inhibition Zone Width of Garlic Extract (*Allium Sativum*), Red Dragon Fruit (*Hylocereus Polyrhizus*) Peel Extract, and a 50% Combination of the Two against *Enterococcus faecalis* Bacteria

Group	Inhibition Zone Diameter (mm) Repetition						Mean ± SD
	1	2	3	4	5	6	
GE 50%	7.98	9.11	9.61	8.94	9.46	10.70	9.30±0.89
RDF 50%	15.60	19.21	15.86	12.62	13.40	18.55	15.88±2.65
GE + RDF 50%	11.25	10.41	13.28	8.93	15.60	14.35	12.30±2.53
(+)	21.99	17.60	23.25	17.47	21.83	20.17	20.39±2.41

Description: GE 50%= 50% of garlic extract; RDF 50%= 50% of red dragon fruit peel; GE + RDF 50%= 50% of mixture of garlic extract and red dragon fruit peel extract; (+)= 2.5 % NaOCl.

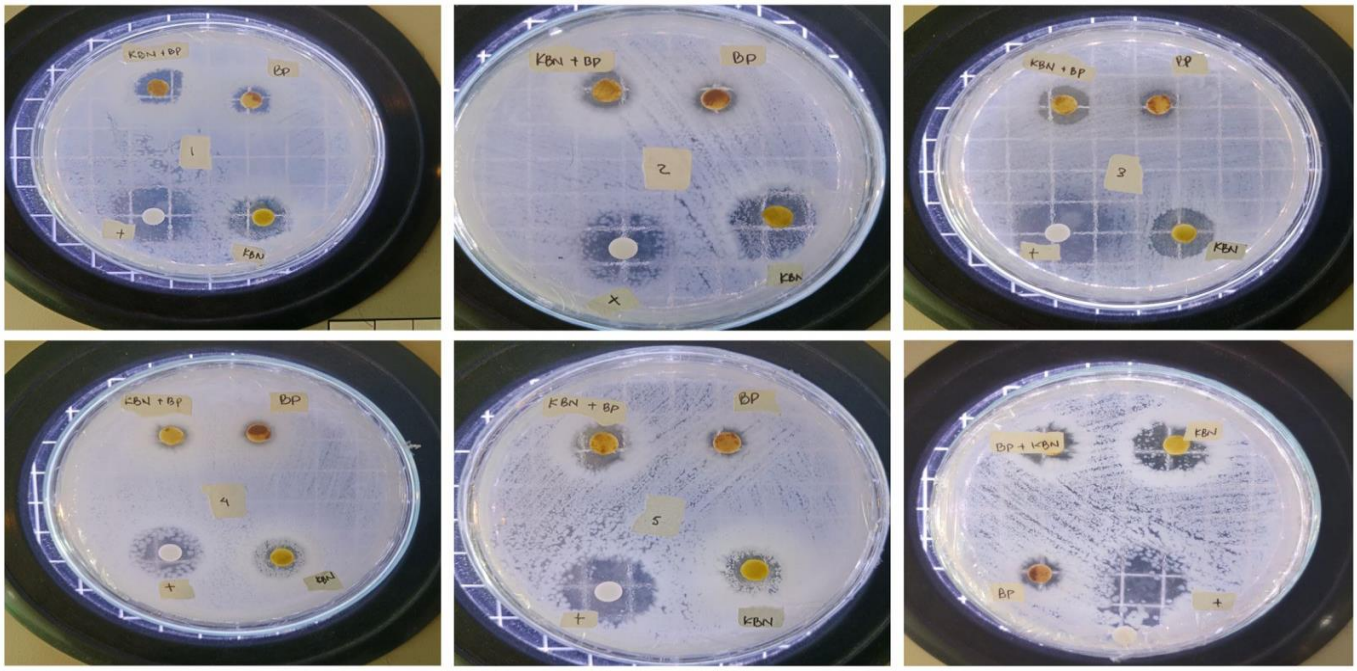

Figure 1. Inhibition zone width of garlic extract (*Allium Sativum*), red dragon fruit (*Hylocereus Polyrhizus*) peel extract, and a 50% combination of the two against *Enterococcus faecalis* bacteria

Garlic extract (*Allium Sativum*) inhibited *Enterococcus faecalis* bacteria with an average of 9.30 ± 0.89, red dragon fruit peel extract (15.88 ± 2.65), and a mixture of both with an average of 12.30 ± 2.53. The study found that 2.5% NaOCl had the greatest average value (20.39 ± 2.41) against *Enterococcus faecalis* bacteria.

Statistical Test Results

One-Way ANOVA showed p=0.000, indicating that garlic extract (*Allium Sativum*), red dragon fruit (*Hylocereus Polyrhizus*) peel extract, and a mixture of both against *Enterococcus faecalis* bacteria differ significantly. The post-hoc test revealed a significant difference between 50% garlic extract and 50% red dragon fruit peel extract against *Enterococcus faecalis* bacteria ($p < 0.05$), but no significant difference was found between the two concentrations ($p > 0.05$). Tables 4 and 5.

Table 4. The One-way ANOVA Statistical Test of Garlic Extract (*Allium Sativum*), Red Dragon Fruit (*Hylocereus Polyrhizus*) Peel Extract, 50% Combination of the Two, and NaOCl 2.5% (Positive Control) against *Enterococcus faecalis* was Performed

Group	*Enterococcus faecalis*	
	Mean±SD	p *value*
GE 50%	9.30±0.89	0.000
RDF 50%	15.88±2.65	
GE + RDF 50%	12.30±2.53	
(+)	20.39±2.41	

Description: GE 50%= 50% of garlic extract; RDF 50%= 50% of red dragon fruit peel; GE + RDF 50%= 50% of mixture of garlic extract and red dragon fruit peel extract; (+)= 2.5 % NaOCl.

Table 5. Statistical Studies Employing Post Hoc of Garlic Extract (*Allium Sativum*), Red Dragon Fruit (*Hylocereus Polyrhizus*) Peel Extract, 50% Combination of Garlic and Red Dragon Fruit Peel Extract, and NaOCl 2.5% (Positive Control) against *Enterococcus faecalis* Bacterium

Group		p *value* *Enterococcus faecalis*
GE 50%	RDF 50%	0.000
	GE+ RDF 50%	0.126
	(+)	0.000
RDF 50%	GE 50%	0.000
	GE + RDF 50%	0.054
	(+)	0.011
GE + RDF 50%	GE 50%	0.126
	RDF 50%	0.054
	(+)	0.000
(+)	GE 50%	0.000
	RDF 50%	0.011
	GE + RDF 50%	0.000

Description: GE 50%= 50% of garlic extract; RDF 50%= 50% of red dragon fruit peel; GE + RDF 50%= 50% of mixture of garlic extract and red dragon fruit peel extract; (+)= 2.5 % NaOCl

Conclusion

From the results of the research conducted, the following conclusions were obtained: Garlic extract (*Allium Sativum*) at 50% concentration inhibits *Enterococcus faecalis* bacteria with an average of 9.30 ± 0.89; Red dragon fruit (*Hylocereus polyrhizus*) peel extract at 50% concentration inhibits *Enterococcus faecalis* bacteria most, with an average of 15.88 ± 2.65; The 50%

garlic extract and red dragon fruit peel combo inhibits *Enterococcus faecalis* bacteria with an average of 12.30 ± 2.53.

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Conflicts of Interest
All author declares that there is no conflict of interest.

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