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Submission date: 31-May-2021 10:30AM (UTC+0700)
by Admin Admin

Submission ID: 1092505056

File name: veness_of_Red_Ginger_Extract_Zingiber_Officinale_Var_Rubrum.pdf (348.64K)

Word count: 3562

Character count: 19709

Analysis of Antibacterial Effectiveness of Red Ginger Extract (*Zingiber Officinale Var Rubrum*) Compared to White Ginger Extract (*Zingiber Officinale Var. Amarum*) In Mouth Cavity Bacterial *Streptococcus Mutans* (In-Vitro)

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Abstract

Ginger has an active compound of phenol, flavonoids, terpenoids and essential oils that can inhibit microbial growth. *Streptococcus mutans* bacteria play an important role in the development of dental caries.

To know the Antibacterial Effectiveness Analysis of Red Ginger Extract (*Zingiber Officinale Var. Rubrum*) Compared to White Ginger Extract (*Zingiber Officinale Var. Amarum*) In Mouth Cavity Bacterial *Streptococcus Mutans* (In-Vitro).

Type of research used is laboratory experimental. The sample is a red Ginger extract (*Zingiber officinale var. Rubrum*) and white ginger extract (*Zingiber officinale var. Amarum*) and Bacteria used are *Streptococcus mutans* using the inhibitory test method.

The highest average value of the highest group is shown in red ginger extract 60% of 15.90 mm, 40% by 14.73 mm and 20% by 12.70 mm. For White Ginger extract 60% by 11.90 mm, 40% by 11.15 mm, and 20% by 10.08 mm. Based on the normality test, Mann-Whitney test and T-test obtained p value <0.05 which means there is significant difference between inhibition between treatment group and overall measurement.

Red Ginger Extract (*Zingiber officinale var. Rubrum*) and White Ginger Extract (*Zingiber officinale var. Amarum*) has an antibacterial effect on *Streptococcus mutans*. Red ginger extract at concentration of 60% has greater antibacterial effect inhibiting *Streptococcus mutans* compared to white ginger extract. Based on the results of the study the higher concentration of red ginger extract and white ginger the greater the inhibitory power diameter against *Streptococcus mutans*.

Clinical article (J Int Dent Med Res 2018; 11(2): pp. 676-681)

Keywords: Red Ginger, White Ginger, *Streptococcus mutans*.

Received date: 06 February 2018

Accept date: 26 March 2018

Introduction

Dental caries or known as cavities are one of the most common human diseases. This disease is a dental tissue disease characterized by tissue damage, starting from enamel surface of the

tooth and extending towards the pulp. The prevalence of caries in Indonesia based on Basic Health Research (Riskesdas) in 2007 and 2013 increased from 23.2% to 25.9%. This shows that caries prevalence in Indonesia is still high. *Streptococcus mutans* are the most common cause of dental caries from all other oral *Streptococcus*.^{1,2}

Streptococcus mutans organisms have been classified into four serotypes (c, e, f, and k) based on the chemical composition of specific serotype polysaccharide on its cell surface.³ *Streptococcus mutans* is a major cause of caries in early childhood. Oral problems in these

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children are the same as in the general population. However, they differ in prevalence and clinical condition. Dental caries are health problems that need to be taken into consideration. Dental caries involves an infectious pathological process in which local destruction of hard tissue is caused by microorganisms due to the interaction of several factors in the oral cavity. The cariogenic bacteria will ferment sucrose into a very strong lactic acid that is capable of causing demineralization. The mechanism of caries occurrence consists of three theories, namely theorethrolysis, proteolytic-helation and chemoparasitic or also called asidogenic theory. The asidogenic theory explains that the formation of dental caries is caused by the acid produced by the action of microorganisms on carbohydrates. This reaction is characterized by the decalcification of inorganic components followed by the disintegration of organic substances derived from the teeth.^{4,5,6,7} Dental caries is caused by the direct and indirect factors. Direct factors are host, agents or microorganisms, substrates or diet, and time.^{8,9}

Plants are the main source of medicinal compounds. Not only that more than 1000 species of plants used as raw materials of medicine. The plant produces secondary metabolites with molecular structures and diverse biological activities, has excellent potential to be developed into various disease cures. According to estimates by the World Health Organization (WHO) 80% of the world's population still rely on traditional medicine including the use of medicines derived from plants.¹⁰ One of the many herbs and easily available by the community is ginger (*Zingiber officinale*). According to data from the Central Bureau of Statistics Indonesia in 2013, ginger production reached 232.616.356 kg in industry.¹¹

Ginger (*Zingiber officinale*) has a variety of uses, such as spices, essential oils, or as a medicine. Traditionally, its purpose is to treat toothache, diabetes, hypertension, fever and infection. Based on the shape, color, and size of the rhizomes, there are 3 known types of ginger, namely large white ginger or rhinoceros ginger, small ginger or empirit and ginger sunti or red ginger. In general these three types of ginger contains starch, essential oils, fiber, small amounts of proteins, vitamins, minerals and proteolytic enzymes called *zingibain*.

When viewed from the water content, large white ginger has a water content of 82%, small white ginger 50.2%, and red ginger 81%. Meanwhile, when viewed from the oil content of atsiri, large white ginger contains oil around 1.18% -1.68%, small white ginger around 1.7-3.8% and red ginger about 2.58% -2.72%.

Based on research conducted by Prasetyo Hendrianto (2016), red ginger (*Zingiber officinale* var *Rubrum*) has an inhibitory area against *S.aureus* and *E. coli* because red ginger contains antioxidant, antibacterial, anti-inflammatory, anticariogenic, antimulagenic, antitumor.¹²

Based on the description that has been described above, this time the researcher wanted to carry out a study on the antibacterial effects of red ginger extract and white ginger in inhibiting *Streptococcus mutans* bacteria. Thus this can be considered as one of the herbal ingredients that can inhibit the bacterium *Streptococcus mutans*.

Materials and methods

Laboratory experimental research with post test control group design was conducted in Microbiology Laboratory of Faculty of Medicine (FK) and Phytochemistry Laboratory of Hasanuddin University Faculty of Pharmacy. Testing conducted in this research is antibacterial test using diffusion method.

The variables of this study include independent ariabel of red ginger extract (*Zingiber officinale* rosc var *rubrum*) and white ginger extract (*Zingiber officinale* var. *Amarum*), dependent variable is cultured pure bacteria *Streptococcus mutans* that have been updated for within 24 hours, taken each 1 ose and inoculated in sterile aquades until obtained equal turbidity with Mc. Farland'0.5.

Tools and materials

The tools used in this study is Handschoen (*Maxter*), Masker (*Masker 3ply*), Petri dish (*Crystalgrade polystyrene sterilized*), Round Ose (*Sam medical*), Buchner funnel, autoclave (*All american®*) and incubator (*Memmert®*), Microscope, Micropipet, Pipette suction, Vital Bottle, Evaporator Tubes, Evaporator (*Heidolph*), Balance (*Excellent®*), Aluminium foil (*Klinpak*), Calipers, Oven, Spoit 10ml (*One med*),

The test materials in this study were red ginger (*Zingiber officinale* rosc var *rubrum*) and

white ginger (*Zingiber officinale* var. *Amarum*), ethanol, 70% of alcohol, sterile aquades, paper dish, Muller Hinton Agar (MHA), filter paper, spirtus, label paper, and bacteria culture (*Streptococcus mutans*) were obtained from the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University.

Results

From the results of the study of inhibition of red ginger extract and white ginger on *Streptococcus mutans* bacteria showed the results of the study in the following table:

Table 1 shows that red and white ginger extract at concentrations of 20%, 40% and 60% have significant value in inhibiting bacteria *Streptococcus mutans*.

In Table 2. Based on the results of each comparison of red ginger extract concentration showed insignificant and significant results. Non-significant results were found at concentrations of 20% and 40% with a p value of 108 and visible $p > 0.05$. Significant results were found at concentrations of 20% and 60% with p values of 0.020, and concentrations of 40% and 60% with a p value of 0.019, showing results $p < 0.05$.

In Table 3. Based on the results of each comparison of white ginger extract concentration showed insignificant results on three comparison concentrations: 20% and 40% with p value 0.149, 20% and 60% with values of 0.083, 40% and 60% with p value namely 0.149, 0.083 and 0.386 that is seen $p > 0.05$.

In Table 4. Comparison of the concentration of white ginger extract and red ginger extract showed no significant results overall due to the p value at the concentration ratio $p > 0.05$.

This Research is Using Statistical Analysis: *Normality Test, Mann-Whitney Test, and T Test*

Ginger	Concentration	Mean±SD	Value P
Red	20%	12.7000±1.43759	0.004
	40%	14.7250±0.15000	0.004
	60%	15.9250±0.63966	0.004
White	20%	10.0750±0.97082	0.013
	40%	11.1500±1.11505	0.013
	60%	11.9000±1.55134	0.013

Table 1. Standard Value Table Deviation of Concentration Deviation Extract of Red Ginger and White Ginger on *Streptococcus mutans*

Bacteria. *Paired sample t-test* $p < 0.05$; *Significants* (normality test).

Ginger	Concentration	Mean±	Nilai P
Merah	20%	3.13	0.108
	40%	5.88	
Merah	20%	2.50	0.020
	60%	6.50	
Merah	40%	2.50	0.019
	60%	6.50	

Table 2. Table comparison of each concentration of red ginger extract on *Streptococcus mutans* bacteria. *Paired sampel t-test* $p < 0.05$; *Significants* (Mann-Whitney test).

Ginger	Concentration	Mean±	Nilai P
Putih	20%	3.00	0.149
	40%	6.00	
Putih	20%	3.00	0.083
	60%	6.00	
Putih	40%	3.75	0.386
	60%	5.25	

Table 3. Table Comparison of Every Concentration White Ginger Extract Against *Streptococcus mutans* Bacteria. *Paired sampel t-test* $p < 0.05$; *Significants* (uji Mann-Whitney)

Ginger	Concentration	Mean±SD	Nilai P
Red	20%	12.7000±1.43759	0.504
White	20%	10.0750±0.97082	
Red	40%	14.7250±0.15000	0.074
White	40%	11.1500±1.11505	
Red	60%	15.9250±0.63966	0.113
White	60%	11.9000±1.55134	

Table 4 Table Comparison of Each Concentration of Red Ginger Extract and White Ginger on *Streptococcus mutant* bacterias. *Paired sampel t-test* $p < 0.05$; *Significants* (uji- T)

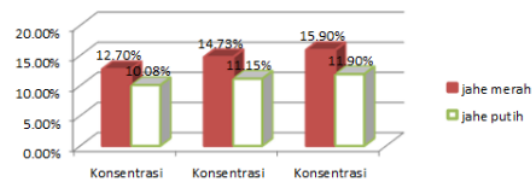


Table 5. The average concentration of red ginger extract and white ginger bacteria has been tested on bacteria *Streptococcus mutans*.

Based on the above diagram shows that red ginger extract has higher resistor test results compared with white ginger extract.

Discussion

Red Ginger (*Zingiber officinale* var *rubrum*) is a type of red ginger that has a small rhizome, reddish yellow, rough fibrous, very spicy and flavorful taste. Small white ginger (*Zingiber officinale* var *Amarum*) is a type of ginger that has a small rhizome compared to large white ginger, yellowish-white, flat-shaped, soft fibrous, and sharp. Ginger rhizome contains volatile components (oil evaporates) and non volatile oil that does not evaporate. Essential oil, is a component of smell (ginger) peculiar to ginger. While oil does not evaporate or oleoresin, is a component of spicy and bitter taste on ginger. Essential oils are odorous and present in some plants, because they are volatile when left open at room temperature so-called oils evaporate, etheric oils or essential oils.¹³

Other ingredients contained in the ginger is Flavanoid which is one of the groups of secondary metabolite compounds most widely found in plant tissues.¹⁴ Flavanoid proved as a compound and pharmacological effects are quite high for example as antibacterial, antioxidant, anti-inflammatory and antifungal on one secondary metabolite (mbadianya et al., 2013; Rahimah et al., 203).¹⁵

The results of Haluanry et al (2014), on the test of inhibiting ginger extract zone and Chlorhexidine gluconate on *Candida albicans* showed that 30% white ginger extract had the same antifungal activity as 0.2% Chlorhexidine gluconate to *Candida albicans*. However Chlorhexidine gluconate 0.2% has greater effectiveness than small white ginger extract.

The antifungal effect of the treatment of small white ginger ethanol extract, caused by the essential oil content of the active compounds of gingerol, shogaol, zingeron and zingiberen. Gingerol, shogaol, zingeron are included in phenol compounds, which are known to denature the cell membranes of *Candida albicans*, so the cell membrane becomes lysic and phenol can penetrate into the cell nucleus, causing the fungus *Candida albicans* can not develop.¹⁶

Another study was also conducted by Prasetyo (2016) who tested the antibacterial

activity of red ginger extract on *Staphylococcus aureus* and *Escherichia coli* where in the study of fresh extract of red ginger rhizome on test microbes showed different effect. This is due to the ability of microbial defense testing. The difference is that it is only composed of a thick layer of peptidoglycan and teapixic acid. The layers consist of water-soluble polymers that facilitate polar compounds of bacteria, such as phenolic compounds (flavonoids and tannins) to penetrate into cells. Gram negative bacterial cell wall (*E. coli*) is more complex and consists of substances such as lipid (non polar), ie phospholipids, polypeptides, and liposaccharides (LPS), making it difficult for polar compounds contained in the extract to penetrate. The high concentrations of extracts required to produce antibacterial effects against *E.coli* were compared to *S. aureus* is assumed to be due to the small amount of compound non polar with antibacteria activity, such as terpenoid contained in extract (Fadillah, 2014).

According to Nursal et al. (2006), rhizome ginger contains antimicrobial compounds class of phenol, flavonoids, terpenoids and essential oils contained in ginger extract is a class of bioactive compounds that can inhibit microbial growth. Inhibition of microbial growth by fresh extract of rhizome ginger (*Z. officinale*) can be seen from microbial free area formed around disc paper containing fresh extract of rhizome ginger caused by bioactive compound contained in extract. The occurrence of microbial inhibition of bacterial colony growth is also due to damage that occurs in the structural components of bacterial cell membranes. Cell membranes composed of proteins and lipids are particularly susceptible to chemicals that can reduce surface tension. Cell membrane damage causes a disruption of nutrient transport (compounds and ions) thus bacterial cells are deprived of nutrients necessary for their growth.¹⁷

Mechanism of flavonoid work as antibacterial is to form complex compounds with extracellular and dissolved proteins that can damage the bacterial cell membrane and followed by the discharge of intracellular compounds (IndoBIC, 2005 in Nuria, et al., 2009). The cytoplasm in the cells is all alive limited by the cytoplasmic membrane, which acts as a selective barrier permeability, carries the active transport function and then controls the internal composition of the cell. If the cell membrane integrity function of the

cytoplasm is destroyed, the macromolecules and ions are out of the cell, then the cell is damaged or death may occur (Brooks, 2005). Mechanism of essential oil compounds. Based on the explanation of Siswandono (1995) on the essential oil of red ginger rhizome there are the main active substances that have antimicrobial activity that is linalool, geraniol and sitral. Linalool and geraniol are alcohol groups, linalool is a tertiary alcohol class whereas geraniol is primary alcohol. The mechanism of alcohol classes in inhibiting microbial growth is by means of protein denaturation. The molecular weight of alcohol is related to antimicrobial work, ie when the molecular weight of alcohol increases then the antimicrobial action increases¹⁸

In this study it shows that each concentration of red ginger extract and white ginger has an antibacterial effect in inhibiting the growth of *Streptococcus mutans*. This is due to the active compound content of essential oils and flavanoids that have activity as an antibacterial. This study used red and white ginger extract with concentrations of 20%, 40%, and 60%, respectively. The results of each concentration in this study showed different results that the higher the concentration the higher also results from the diameter of the inhibitory power caused in *Streptococcus mutans* bacteria.

Red ginger has a greater oil content around 2.58% -2.72% compared to the content of large white ginger atsiri oil ranges

0.82% -1.68% and in small white ginger 1.5% -3.3%. So that the ability of its resistance to *Streptococcus mutans* is greater than the white ginger.

This is consistent with the Mulyani (2010) study suggesting that fresh extracts of ginger-jahean rhizome contain several essential oil components composed of a-pinena, kamophena, caryophilene, β -pinene, a-farnesena, sineol, dl-chamate, isocariophylene, caryophyloene oxide, and gercmacon that can produce antimicrobials to inhibit microbial growth.¹⁹

Conclusions

Red ginger extract is more effective as an antibacterial compared with white ginger extract. The higher concentration of ginger extract, the greater the antibacterial effect of ginger in inhibiting *Streptococcus mutans*.

Interest Conflict

There was no interest conflict in this study. This study obtained a label of ethics escaped by the number: 207/H4.8.4.5.3.1/PP 36-KOMETIK/2017 and register number UH17030185 on April 10, 2017.

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