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Antioxidant Effectiveness of Guava Leaf Extract Mouthwash (*Psidium guajava* L) to Increase pH, Flow Rate, and Salivary Volume in Young Adults and Elderly Subjects

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ABSTRACT

The purpose of the research is to dissect about antioxidant effectiveness of guava leaf extract mouthwash (Psidium guajava L) to increase pH, flow rate, and salivary volume in young adult and elderly subjects. This type of research is true experimental research. The place and time of the research is carried out from June to August 2021. The sample is the subject who will do the treatment totaling 40 people. Statistical analysis is performed using repeated measure ANOVA test, unpaired t test, and paired t test. The results show that clinical trials of young adults and elderly subjects who gargled using guava leaf extract mouthwash (Psidium guajava L) extracted using the maceration method and compared with 0.2% chlorhexidine gluconate showed significant results on salivary pH, salivary flow rate, and salivary volume which are carried out for 14 days. The results of ANOVA repeated measure test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is an average difference. Unpaired t test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is some an average difference. For paired t test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is an average difference. The mean significant increase in the measurement time interval of 14 days is because the value of sig < 0.05.

Keywords: Antioxidant, Effectiveness, Guava Leaf Extract, Mouthwash

INTRODUCTION

Salivary is a complex, colorless, and oral fluid secreted from the major and minor salivary glands to maintain oral homeostasis. The nature of the salivary glands and their secretions is determined by the type of secretory, namely: serous, seromucus, and mucus (Ligtenberg et al., 2020).

Salivary has different compositions and concentrations that can affect the condition of salivary secretion so that the oral cavity environment of each individual is different. Factors that affect the composition and concentration of salivary include salivary flow rate, salivary volume, salivary pH, and salivary buffering capacity. Salivary secretion can be influenced by stimuli received by the salivary glands (Ekstorm et al., 2012).

The degree of acidity (pH) of salivary is an important factor that plays a role in the oral cavity, so that salivary can function

properly, the composition and properties of salivary must be maintained in optimal balance, especially the degree of acidity salivary (Kumar et al., 2017).

The aging process is a process of slowly disappearing the ability of tissues to repair themselves, replace and maintain their normal functions so that they cannot survive infection and repair the damage suffered. The decrease in salivary secretion in the elderly occurs physiologically, this is due to a decrease in the number of acinar cells so that salivary production decreases. Some of the elderly will show symptoms of xerostomia or dry mouth. In adults, the normal rate of salivary secretion upon stimulation is 1-2 ml/min. Changes in age are known to affect the decrease in salivary production (Primasari, 2018).

In dentistry, the use of mouthwash among the public continues to be encouraged by health workers and clinicians because in addition to brushing teeth, gargling can clean the oral cavity from debris, prevent and reduce plaque accumulation and have a direct effect on salivary (Komala et al., 2017). Alcohol-free mouthwash is also known as medicine mouthwash and tonic that can help maintain the natural balance of salivary by balancing the acidity and alkalinity of enzymes in the oral cavity (Raj et al., 2019).

One of the plants that can be used as a natural traditional mouthwash is guava leaf extract (Bijauliya et al., 2018). Psidium guajava L has properties as antidiarrheal, antioxidant, anti-inflammatory and antimicrobial (Naseer et al., 2018).

Research Handayani et al. (2017) examined the formulation and activity test of guava leaf extract mouthwash (Psidium guajava L) and concluded that 2.5%, 3%, and 3.5% guava leaf extract could be formulated in mouthwash dosage forms. The basis for determining the extract was obtained from the study of the minimum inhibitory concentration which proved that at a concentration of 3.5% guava leaf extract had antibacterial activity against streptococcus mutans. Antioxidants are compounds that can inhibit oxidation reactions, by binding free radicals and highly reactive molecules that damage cells. can be inhibited (Minic, 2019).

The purpose of the research is to dissect about antioxidant effectiveness of guava leaf extract mouthwash (Psidium guajava L) to increase pH, flow rate, and salivary volume in young adult and elderly subjects.

RESEARCH METHODS

This type of research is true experimental research. True experimental research is an experimental research design that examines the possible causes and effects between the treated group (experimental group) and the control group (not treated) and then compares the two (Mangelep, 2012).

The place and time of the research is carried out from June to August 2021.

This research is conducted in several stages. The initial stage to obtain guava leaf extract in accordance with the requirements of the Ministry of Health is to check the water content, water soluble extract content, ethanol soluble extract content, total ash content, and acid insoluble ash content at the Phytochemical Research Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Then phytochemical screening to see the content of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids. Furthermore, the antioxidant activity of guava leaf extract was tested using the DPPH method (1,1diphenyl-2-picrylhydrazil) with sample concentrations of 2, 5, 5, 10, 20, and 40 determining the concentration (ppm) on guava leaf extract mouthwash (Psidium guajava L) while in comparison vitamin c with a sample concentration of 1, 2, 3, 4, 5 determining the concentration (ppm) at the Pharmaceutical Research Laboratory, Universitas Sumatera Utara.

The characterization of mouthwash preparations used a pH meter and a viscometer, while the hedonic test was carried out by 20 panellists on color, taste, and aroma. Then a clinical trial to compare

the effectiveness of guava leaf mouthwash 3.5% and chlorhexidine gluconate 0.2%.

The sample is the subject who will do the treatment totaling 40 people. Each subject rinsed his mouth twice a day as much as 15-20 ml in the morning after brushing his teeth and at night before going to bed for 14 days. Measurements were made every day 1, 3, 7, and 14 using a pH meter. Previously, the calculation was carried out on day 1 as the base line.

Statistical analysis is performed using repeated measure ANOVA test, unpaired t test, and paired t test.

RESULT AND DISCUSSION

Antioxidant Activity Test Results of Guava Leaf Extract Mouthwash (Psidium guajava L)

The results of research conducted for 3 days at the Pharmaceutical Research Laboratory, Universitas Sumatera Utara, the results of the antioxidant activity test of guava leaf extract mouthwash (Psidium guajava L) using the DPPH method (1,1diphenyl-2-picrylhydrazil) were 20.38 determining the concentration (ppm), where can be seen through IC_{50} (inhibition concentration) with categories 0-50 (very strong), 51-100 (strong), and 101-150 (weak).

Table 1. Antioxidant Activity Test Results of Guava Leaf Extract Mouthwash (Psidium guajava L)

Solvent	Extract Type	IC_{50} (ppm)
Ethanol	Guava Leaf Extract Mouthwash (Psidium guajava L)	20,38
	Vitamin C	2,69

The results show that clinical trials of young adults and elderly subjects who gargled using guava leaf extract mouthwash (Psidium guajava L) extracted using the maceration method and compared with 0.2% chlorhexidine gluconate showed significant results on salivary pH, salivary flow rate, and salivary volume which are carried out for 14 days.

Preparation of Guava Leaf Extract Mouthwash (Psidium guajava L)

The water content in simplicia shows the amount of water contained in simplicia, from the results of the study obtained a water content of 7.31%. The water content of simplicia is related to the drying process, the water content is determined to determine the simplicia used is not overgrown with fungus and is safe to use. Drying is an attempt to reduce the water content of the material to the desired level. With a water content that meets the standard, simplicia is not easily damaged and can be stored for a long period of time. If the simplicia produced is not dry enough, there will be growth of fungi and other micro-organisms. According to the Ministry of Health of the Republic of Indonesia in 1995, simplicia was considered quite safe if it had a water content of less than 10%.

Determination of the content of simplicia extract states the amount of chemical substances extracted in water and in ethanol. Simplicia leaves Psidium guajava L has an ethanol-soluble extract of 18.57% while a water-soluble extract of 19.08%, this proves that the chemical compounds extracted in ethanol are smaller than those extracted in water.

Pre-Clinical Trials

Antioxidant Activity Test

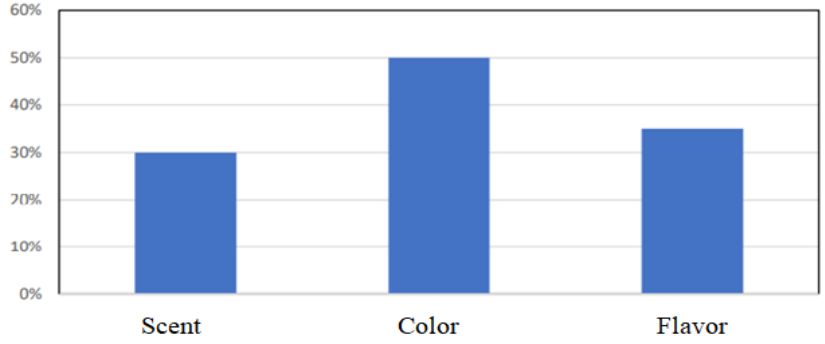
The test results of the DPPH method (1,1diphenyl-2-picrylhydrazil) found that the antioxidant content was 20.81 determining the concentration (ppm). Where, if the value ranges from 0-50, the antioxidant activity is very strong. This is in line with the research conducted. The results of the antioxidant activity test against determining the concentration (ppm) from the n-butanol extract of white guava leaves (Psidium guajava Linn) can be used as an antioxidant because it has an IC_{50} value of 37.1402 determining the concentration (ppm) (Indriana, 2011). In addition, the leaf extract of Psidium guajava L, IC_{50} value of 22.39 determining the concentration (ppm) which means that its antioxidant activity is

very strong. The antioxidant component of the extract with ethanol solvent was higher than that of the water solvent and the antioxidant level was higher in the leaves than in the fruit, namely 1,426 $mg.g^{-1}$ compared to 0.722 $mg.g^{-1}$.

Hedonic Test for Preparation of Guava Leaf Extract Mouthwash (Psidium guajava L)

From the 20 panellists who did the hedonic test, gargling with Psidium guajava L 3.5% leaf extract included aroma, color, taste, and sensation in the mouth. The results obtained are the most preferred by the panellists, namely in terms of color with a percentage of 50%.

Figure 1. Bar Chart of Hedonic Test for Preparation of Guava Leaf Extract Mouthwash (Psidium guajava L)



Clinical Trials

From the results of this study, the salivary pH of young adults in gargling treatment with Psidium guajava L leaf extract mouthwash on the first day was 6.6±0.1269 and increased on the fourth day by 7.1±0.1767. While the treatment in the gargling group with 0.2% chlorhexidine gluconate mouthwash, the salivary pH of young adults on the first day was 6.7±0.1699 and increased on the fourth day by 7.1±0.2644.

The pH of the elderly salivary in the treatment of gargling with Psidium guajava L leaf extract mouthwash on the first day was 6.7±0.1699 and increased on the fourth day by 7.1±0.994. While the treatment in the gargling group with 0.2% chlorhexidine gluconate mouthwash, the pH of young adults' salivary on the first day was 6.4±0.2424 and increased on the fourth day by 6.9±0.0994.

The salivary flow rate of young adults in gargling treatment with Psidium guajava L leaf extract mouthwash on the first day was 4.4±0.1430 and increased on the fourth day by 4.8±0.1663. While the treatment in the gargling group with 0.2% chlorhexidine gluconate mouthwash, the pH of young adults' salivary on the first day was 4.4±0.1476 and increased on the fourth day by 4.7±0.1663.

The salivary flow rate of the elderly in gargling treatment with Psidium guajava L leaf extract mouthwash on the first day was 4.8±.2366 and increased on the fourth day by 5.1±0.1595. While the treatment in the gargling group with 0.2% chlorhexidine gluconate mouthwash, the salivary pH of young adults on the first day was 4.4±0.1549 and increased on the fourth day by 4.7±0.1663.

The salivary volume of young adults in the gargling treatment with Psidium guajava L leaf extract mouthwash on the first day was 1.6±0.1588 and increased on the fourth day by 2.0±0.1663. While the treatment in the gargling group with 0.2% chlorhexidine gluconate mouthwash, the pH of young adults' salivary on the first day was 1.4±0.2108 and increased on the fourth day by 1.8±0.1663.

The volume of elderly salivary in the treatment of gargling with Psidium guajava L leaf extract mouthwash on the first day was 1.4±0.2452 and increased on the fourth

day by 2.0±0.1751. While the treatment in the gargling group with 0.2% chlorhexidine gluconate mouthwash, the salivary pH of young adults on the first day was 1.4±0.1337 and increased on the fourth day by 1.8±0.1764.

This study aims to determine the effectiveness of antioxidants possessed by guava leaf extract mouthwash (Psidium guajava L) to increase salivary pH, salivary flow rate and salivary volume. The effectiveness of antioxidants begins with determining the concentration (ppm) in guava leaf extract mouthwash (Psidium guajava L) using the DPPH method (1,1diphenyl-2-picrylhydrazil), then the minimum inhibitory level in guava leaf extract mouthwash (Psidium guajava L) has been known through previous research conducted by Handayani et al. (2017) that a concentration of 3.5% guava leaf extract mouthwash (Psidium guajava L) has antibacterial activity against streptococcus mutans and guava leaf extract mouthwash (Psidium guajava L) with a concentration of 2.5%, 3%, and 3.5% can be formulated in the form of mouthwash preparations.

Human salivary is rich in antioxidant compounds. The normal flow of unstimulated and stimulated salivary is important to ensure adequate and continuous lubrication of the teeth and mucous membranes of the oral cavity, and also helps prevent retrograde infection of the salivary glands with oral microorganisms via the salivary ducts (Minic, 2019).

Experiments conducted to analyze the effect of the MW used. After rinsing, there was a significant increase in the salivary pH of all individuals. The increase in pH reached an average maximum value of 7.24 MWb (mouthwash a) and 7.30 (mouthwash b) (Afoo et al., 2015).

This is in line with research conducted using guava leaf extract mouthwash (Psidium guajava L) after gargling there was an increase in salivary pH, salivary flow rate, and salivary volume.

ANOVA Test

Table 2. ANOVA Repeated Measures Test Results of Guava Leaf Extract Mouthwash (Psidium guajava L) 3.5% and Chlorhexidine Gluconate 0.2%

Subject	Type	Sig
Young Adult	Salivary pH	0.000
Elderly	Salivary pH	0.000
Young Adult	Salivary Flow Rate	0.000
Elderly	Salivary Flow Rate	0.000
Young Adult	Salivary Volume	0.000
Elderly	Salivary Volume	0.000

Based on Table 2, the results of ANOVA repeated measure test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is an average difference. The mean significant increase in the measurement time interval of 14 days is because the value of sig < 0.05.

Unpaired t Test

Table 3. Unpaired t Test Results of Guava Leaf Extract Mouthwash (Psidium guajava L) 3.5% and Chlorhexidine Gluconate 0.2%

Subject	Type	Sig
Young Adult	Salivary pH	0.024
Elderly	Salivary pH	0.004
Young Adult	Salivary Flow Rate	0.861
Elderly	Salivary Flow Rate	0.834
Young Adult	Salivary Volume	1.000
Elderly	Salivary Volume	0.021

Based on Table 3, the results of unpaired t test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is some an average difference.

Paired t Test

Table 4. Paired t Test Results of Guava Leaf Extract Mouthwash (Psidium guajava L) 3.5% and Chlorhexidine Gluconate 0.2%

Subject	Type	Sig
Young Adult	Salivary pH	0.000
Elderly	Salivary pH	0.001
Young Adult	Salivary Flow Rate	0.001
Elderly	Salivary Flow Rate	0.003
Young Adult	Salivary Volume	0.010
Elderly	Salivary Volume	0.001

Based on Table 4, the results of paired t test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is an average difference. The mean significant increase in the measurement time interval of 14 days is because the value of sig < 0.05.

CONCLUSION

The results show that clinical trials of young adults and elderly subjects who gargled using guava leaf extract mouthwash (Psidium guajava L) extracted using the maceration method and compared with 0.2% chlorhexidine gluconate showed significant results on salivary pH, salivary flow rate, and salivary volume which are carried out for 14 days. The results of ANOVA repeated measure test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is an average difference. Unpaired t test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is some an average difference. For paired t test of guava leaf extract mouthwash (Psidium guajava L) 3.5% and chlorhexidine gluconate 0.2% in young adult and elderly subjects with salivary pH, salivary flow rate, and salivary volume type show that there is an average difference. The mean significant increase in the measurement time interval of 14 days is because the value of sig < 0.05.

Conflict of Interest: None

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