

Effect of lemon on saliva and *Staphylococcus aureus*

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ABSTRACT

Introduction: Lemon juice contains citric acid. It is a highly antibacterial organic acid by gargling with, will be able to decrease the spreading of *Staphylococcus aureus* colony in saliva. The acid stimulation of lemon juice able to increase salivary secretion. The purpose of this study was to see the effect of gargling lemon juice of 100%, 50%, 25%, and 12.5% concentrations towards the salivary flow rate, salivary pH, the number of *Staphylococcus aureus* colony, and also, correlation between salivary flow rate and pH, salivary flow rate and number of *Staphylococcus aureus* colony, and salivary pH and number of *Staphylococcus aureus* colony. **Methods:** This research conducted with a pre-and-post-test group design. Saliva was taken from 24 subjects before and after the subjects gargled with lemon juice. Measurements performed were salivary flow rate per minute, and salivary pH (using pH Hanna Instrument). The *Staphylococcus aureus* bacteria were placed in a Mannitol Salt Agar for 24 hours at 37°C temperature, then the number of the colonies were counted ($\times 10^3$ CFU). **Results:** The Wilcoxon test results showed that lemon juice with 100%, 50%, 25%, and 12.5% concentrations significantly increased the salivary flow rate and decreased the number of *Staphylococcus aureus* colony ($p < 0.05$). The result of the paired t-test showed that lemon juice with 25% and 12.5% concentration decreased the salivary pH significantly ($p < 0.05$). The Pearson's correlation results showed no significant correlation between salivary flow rate and salivary pH, flow rate and the number of *Staphylococcus aureus* colony, and salivary pH and number of *Staphylococcus aureus* colony. **Conclusion:** Lemon juice can decrease the salivary pH and the number of *Staphylococcus aureus* colony, but increases the salivary flow rate. It is considered to be good enough as one of the ingredients of mouthwash.

Keywords: Flow rate, lemon, pH, saliva, *Staphylococcus aureus*

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INTRODUCTION

Citrus limon, known as lemons, belongs to *Rutaceae* family, is commonly cultivated in South Asian countries. Lemon is rich in nutritional ingredients containing carbohydrates, fats, proteins, vitamins such as thiamine, riboflavin, niacin, pantothenic

acid, folate, choline and rich sources of vitamin C. Lemon also contains elements such as calcium, magnesium, potassium and phytochemicals such as tannins and flavonoids.¹

Lemon juice contains about 5% citric acid, which gives a lemonic acid flavour and pH 2 to 3.² One of the mechanisms of salivary secretion is a

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reflex activity with stimuli from within the oral cavity.³ The acidic chemical stimuli are the most potent in increasing salivary secretion. The sour taste generated by lemon juice can stimulate the gustatory-salivary reflex which found in the taste buds.⁴ Lemon juice can act as an antibacterial agent because it contains tannin and flavonoid compounds. Tannin acts like a mild acid based on many phenolic -OH groups. One of the essential properties of tannins and tannic acids is their ability to form chelate complexes with metal ions. Also, flavonoids disrupt the cell wall that functions as a giver of cell shape and protects cells from osmotic lysis. Disruption of cell walls will cause lysis of bacterial cells.^{5,6}

The oral cavity is the gateway of various microorganisms into the body. The mouth with its warm temperatures, moisture, and nutrient-rich environment can increase the number of microorganisms in the oral cavity.² *Staphylococcus aureus* is a gram-positive bacteria that can be found in the oral cavity as a normal flora, but in certain circumstances may turn into pathogens in case of imbalance in the oral cavity.⁷ *Staphylococcus aureus* grows well in a variety of bacteriological media in aerobic conditions.⁸ *Staphylococcus aureus* causes infection in the oral cavity such as white sponge nevus and angular cheilitis.⁹

Therefore, the authors are interested in researching the effect of lemon juice with 100, 50, 25 and 12.5% concentration on salivary flow rate, salivary pH and number of *Staphylococcus aureus* colonies. The objective of this research was to examine the effect of lemon juice with different concentrations on the salivary flow rate, salivary pH, and the number of *Staphylococcus aureus* colony.

METHODS

This study was an experimental laboratory, with pre and post group design, conducted from March to December 2017. Samples of this study consisted of 24 healthy subjects and fulfilled the inclusion criteria (aged 18-25 years old, in good health, and willing to participate in the study) and exclusion criteria (systemic illness, using orthodontic devices, using dentures, smoking, craving, alcohol drinkers, women during menstruation, pregnancy, breastfeeding, and taking antibiotics,

antifungal, steroids or drugs that affect salivary secretions). Ethical clearance for the studies had been obtained from the Ethical Committee of the Faculty of Medicine University of North Sumatra with registry number 612/TGL/KEPK FK USU-RSUP HAM/2017.

The whole salivary sample was taken with spitting method from students of the Faculty of Dentistry University of North Sumatra and was conducted at the Oral Biology Laboratory of the Faculty of Dentistry University of North Sumatra. The culture and count of the *Staphylococcus aureus* colony had performed at the Pharmacy Laboratory University of North Sumatra. The researcher asked the subject's participation agreement before salivary sampling. Subjects were asked to provide saliva using saliva pot for 5 minutes, then gargled with lemon juice for 1 minute, the subject returned to provide saliva for 5 minutes in different saliva pot. After that, the salivary flow rate was measured using digital scales and salivary pH measurements using pH Hanna Instrument. The saliva samples were diluted 3 times (10^3 CFU) and then were cultured on Mannitol Salt Agar medium at 37°C. After 24 hours, the number of *Staphylococcus aureus* colony was counted.

Data obtained in this study were processed with software, including flow rate, pH value and the number of *Staphylococcus aureus* colony. A dependent t-test was used to determine the significant influence between observed and expected frequency. The significant relationship used was $p \leq 0.05$.

RESULTS

Salivary flow rate data and number of *Staphylococcus aureus* colony were tabulated and analysed statistically using the Wilcoxon test ($p < 0.05$); the salivary pH data were analysed by the paired t-test ($p < 0.05$) to see the salivary flow rate, salivary pH and number of *Staphylococcus aureus* colony before and after gargling lemon juice with concentrations of 100%, 50%, 25%, and 12.5%. Correlation between salivary flow rate and salivary pH, salivary flow rate and the number of *Staphylococcus aureus* colony, and also salivary pH and the number of *Staphylococcus aureus* colony, were analysed by the Pearson correlation test with the significance value $p < 0.05$.

Table 1. Average of salivary flow rate before and after stimulation of lemon juice based on concentration differences

Concentration (%)	Mean \pm SD		$\Delta \bar{X}$	p
	Pre	Post		
100	0.61 \pm 0.28	1.44 \pm 0.92	0.83	0.046*
50	0.38 \pm 0.19	0.76 \pm 0.29	0.38	0.046*
25	0.40 \pm 0.22	0.65 \pm 0.27	0.25	0.043*
12.5	0.63 \pm 0.35	0.84 \pm 0.33	0.21	0.046*

*Significance $p < 0.05$

Table 1 shows a significant increase in salivary flow rate ($p < 0.05$) after gargling with lemon juice in all concentrations (100, 50, 25 and 12.5%).

Table 2. Average of salivary pH before and after stimulation of lemon juice based on concentration differences

Concentration (%)	Mean \pm SD		$\Delta \bar{X}$	p
	Pre	Post		
100	6.97 \pm 0.31	6.73 \pm 0.35	0.24	0.328
50	6.77 \pm 0.28	6.55 \pm 0.20	0.22	0.411
25	6.87 \pm 0.24	6.32 \pm 0.26	0.55	0.001**
12.5	7.02 \pm 0.23	6.67 \pm 0.14	0.35	0.013**

*Significance $p < 0.05$

Table 2 shows a significant decrease in the salivary pH ($p < 0.05$) after gargling with lemon juice with concentrations of 25 and 12.5%.

Table 3. Average of *Staphylococcus aureus* colony before and after stimulation of lemon juice based on concentration differences

Concentration (%)	Lemon juice's pH	Mean \pm SD ($\times 10^3$)		$\Delta \bar{X}$	p
		Pre	Post		
100	3.2	65.67 \pm 7.94	0.67 \pm 0.82	65	0.028*
50	4.8	15.33 \pm 4.88	1.67 \pm 0.52	13.66	0.027*
25	5.2	9.00 \pm 1.79	3.67 \pm 0.82	5.33	0.027*
12.5	6.1	7.00 \pm 0.89	4.67 \pm 0.52	2.33	0.026*

*Significance $p < 0.05$

Table 4. Correlation between salivary flow rate to salivary pH

Concentration (%)	$\Delta \bar{X}$ Salivary Flow Rate	$\Delta \bar{X}$ Salivary pH	r	p
100	0.83	0.24	-0.056	0.706
50	0.38	0.22		
25	0.25	0.55		
12.5	0.21	0.35		

Table 5. Correlation between salivary flow rate to the number of *Staphylococcus aureus* colonies

Concentration (%)	$\Delta \bar{X}$ Salivary Flow Rate	$\Delta \bar{X}$ Colonies	R	p
100	0,83	65	-0,233	0,111
50	0,38	13,66		
25	0,25	5,33		
12,5	0,21	2,33		

Table 6. Correlation between salivary pH to the number of *Staphylococcus aureus* colonies

Concentration (%)	$\Delta \bar{X}$ Salivary pH	$\Delta \bar{X}$ Colonies	R	p
100	0,24	65	0,311	0,032*
50	0,22	13,66		
25	0,55	5,33		
12,5	0,35	2,33		

*Significance $p < 0.05$

Table 3 shows that there was a significant decrease in the number of *Staphylococcus aureus* colony ($p < 0.05$) after gargling with lemon juice with concentrations of 100%, 50%, 25%, and 12.5%, while significant correlation ($p > 0.05$) between salivary flow rate and salivary pH is shown at Table 4. Based on data in Table 5, there was a significant correlation ($p < 0.05$) between salivary flow rates and the number of *Staphylococcus aureus* colony. Table 6 shows a significant correlation ($p < 0.05$) between salivary pH and the number of *Staphylococcus aureus* colony.

DISCUSSION

Saliva is an exocrine liquid composed of various complex, colourless components, and has a role in the digestion process of food, regulation of water balance, maintaining tooth integrity, antibacterial activity, buffer, and an important role in oral hygiene.¹⁰ The acidic chemical stimulus is the most powerful in increasing salivary secretion, which may change with reflex activity involving the central nervous system.⁴

Table 1 shows the highest average flow rate after gargling with lemon juice with a concentration of 100% (1.44 ml/min) compared with concentrations of 50%, 25%, and 12.5% (0.76 ml/min, 0.65 ml/min, and 0.84 ml/min) so that lemon juice with 100% concentration was effective in increasing salivary flow rate. This result was consistent with the research conducted by Indriana³ who conducted the study by using citric acid obtained from apples to observe the significance of salivary volume increase before and after the acid was introduced at the base of the tongue.³

Chemical stimulation of the tongue can activate the autonomous nervous system indirectly through the central nervous system so that the salivary glands are stimulated for secretion. This stimulation primarily produces salivary secretions such as water, which implies a cholinergic stimulus. Cholinergic stimuli will deliver the stimulus to the parasympathetic nerves, thereby activating the autonomic nervous system, causing increased salivary secretion. The rate of secretion of the parotid gland is five times higher by 1% citric acid. Cholinergic antagonists can not completely block secretion, and there is also no cessation of the

cholinergic nerve pathway. This condition suggests that sympathetic nerve pathways are also involved in salivary secretions indicated by citric acid. It is proven that citric acid can activate sympathetic nerve pathways reflexively.¹¹

Table 2 shows that the mean of salivary pH decreased significantly at lemon juice concentrations of 25% and 12.5%. This result was consistent with the research conducted by Indriana³ which stated that there was a decrease in pH when chemically stimulated (acid), where food intakes containing acid were proven to reduce pH values.³

This result was probably caused by salivary pH values before and after gargling with lemon juice with 100% and 50% concentrations which were not significantly different, so the mean of salivary pH value did not decrease significantly. Also, at 50% and 100% concentrations, the rate of saliva flow rate increased significantly so that the production of ions in saliva also increased. The increased salivary volume will increase bicarbonate ions that act as salivary buffer capacity to neutralise the pH that falls due to carbohydrate fermentation.¹²

The average number of *Staphylococcus aureus* colony was decreasing significantly at concentrations of 100%, 50%, 25%, and 12.5%, while in saliva samples from two subjects applied with 100% concentration, no more *Staphylococcus aureus* bacteria were found. This result was similar to the study conducted by Hindi et al¹³ who reported that 100% lemon juice concentration zone in *Staphylococcus aureus* was 26 mm.¹³

Gram-positive bacteria showed higher susceptibility values than gram-negative bacteria because the cell wall of gram-positive bacteria contains a polysaccharide which is a water-soluble polymer, which acts as incoming positive ions. This soluble nature shows that the gram-positive cell wall is more polar. The flavonoid compounds contained in lemon juice are non-polar, causing disruption of the cell wall function as a giver of cell shape and protects the cells from osmotic lysis. Disruption of the cell wall will cause lysis of the cell.¹⁴

Table 4 shows that the salivary flow rate has no significant relationship with salivary pH with negative relations coefficient value ($r = -0.056$). From this research, it is shown that when

saliva flow rate increases, salivary pH value will decrease. This result, however, was not consistent with the research conducted by Wu et al¹⁵, which stated that the higher the salivary flow rate, the saliva pH would increase. The different sample used might cause this difference, while the sample used in the previous study was 18 girls and 26 boys with an average age of 7 years old, this study used a sample of dentistry students aged between 18-25 years old. This difference was might also occur because the saliva component of children is different from adults. Variations in the constituent components of saliva can reflect hormonal factors, external influences and systemic conditions.¹⁵

Table 5 shows that the association of salivary flow rate with the number of *Staphylococcus aureus* colonies has an inverse relationship, with negative relations coefficient ($r = -0.233$), which means that when salivary flow rate increases, the number of *Staphylococcus aureus* colony will decrease. This result was consistent with the research conducted by Booy¹⁶, which stated that there was no significant relationship between salivary volume and the number of bacterial colonies on body condition during fasting ($p = 0.628$). Saliva samples of both studies obtained from research subjects that tested on testing media for gram-positive bacteria. The difference was that this study used special media for the growth of *Staphylococcus aureus*, namely Mannitol Salt Agar, while the prior research used Sodium Agar so that the bacteria that grown are all gram-positive bacteria. Also, the prior research subjects underwent the Ramadan fasting for approximately 16 hours, while in this study, all subjects only 2 hours before the study was conducted.¹⁶

Table 6 shows that the relationship of salivary pH to the number of *Staphylococcus aureus* colony has a direct relationship, with the value of positive relationship coefficient ($r = 0.311$). It shows that when the decrease in salivary pH value, the number of *Staphylococcus aureus* colony will decrease as well. The decreasing number of *Staphylococcus aureus* colonies after gargling with lemon juice may be caused by the presence of citric acid and phenol derivatives which give a sour taste to the lemon. Citric acid can denature bacterial cell proteins by damaging bacterial cell walls and entering into the cell

nucleus of bacteria, disrupting the process of cell respiration, inhibiting bacterial enzyme activity, and suppressing the translation of regulation of certain gene products.^{2,7}

CONCLUSION

Lemon juice can decrease the salivary pH and the number of *Staphylococcus aureus* colony, but increases the salivary flow rate. It is considered to be good enough as one of the ingredients of mouthwash.

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