**Pandanus amaryllifolius Roxb.** leaf extract prepared by nanoemulsion technique as a natural mouthwash

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**Abstract.** *Pandanus amaryllifolius Roxb.* leaf has a natural mouthwash used as an alternative to commercial mouthwash. The purpose of this study was to determine the potential of giving scented Pandan leaf extract (*Pandanus amaryllifolius* Roxb.) using nanoemulsion technique as a natural mouthwash. This study used an experimental method with a trial of Completely Randomized Design (CRD) with five treatments and five replications. Data were analysed using Analysis of variance Test and Duncan at α=0.01 (99% confidence level). The result of giving 21-day natural mouthwash showed that P3 (4% extract) has the average salivary pH higher than the value of P1 (1% extract) and P2 (2 % extract), and the average salivary pH value of P3 (4% extract) approached the average salivary pH of P0+ (commercial mouthwash). Besides, there was a significant effect of 21-day of treatment to the respondents that is showed by F (4.20) = 20.57, p (0.01) = 4.43. The conclusion of this study is the administration of *P amaryllifolius* extract with nanoemulsion technique can be used as a natural mouthwash which is to maintain the salivary pH.

1. **Introduction**

Oral and dental health is an essential part of overall health which can affect a person's quality of life and is an important concern in the healthy development of the Indonesian population. The value of dental and oral hygiene is essential for every individual to know [1]. In its growth and development, adolescents often experience health problems, one of which is dental and oral hygiene problems [2].

Salivary acidity is one of the crucial factors that can affect oral hygiene. Saliva is a combination of various fluids and components that are excreted into the oral cavity. Saliva consists of dilute liquid containing enzymes and thick liquid containing mucus [3]. Saliva is one component that contributes to acidity (pH) in the oral cavity. Saliva has a pH between 6.0-7.4, a range that is advantageous for digestion from ptyalin [4]. Decreasing oral pH below 5.5-5.0 will cause the demineralisation process in the teeth, resulting in tooth and mouth disease [5]. The pH of the saliva that is too alkaline or about 8.0-9.5 in the oral cavity can stimulate deposition and accumulation of calcium and phosphate salts to facilitate the occurrence of calculus or the formation of tartar [6].

One effort to maintain oral hygiene is to use a mouthwash. Marketed mouthwash contains chemicals that have a detrimental effect if used for a long time. Examples of hazardous chemicals contained in mouthwash are alcohol and chlorhexidine gluconate. Alcohol can make the mouth dry, increasing the risk of cancer in the mouth and chlorhexidine gluconate causes stains on the teeth and bad breath. Gargles are environmentally friendly, easy to obtain, and have minimal side effects,
namely mouthwash made from natural ingredients [7]. One of the natural ingredients that can be used is P. amaryllifolius Roxb.

P. amaryllifolius is a plant that is often found so that it is easily accessible to the public. P. amaryllifolius Roxb. leaf extract contained several compounds such as essential oils, tocopherols, tocochromanes, alkaloid fatty acids, esters of non-specific lipid transfer proteins, and carotenoids and flavonoids (Nor et al., 2008). Pandanus species (P. amaryllifolius, P. utilis, and P. dubius) have been investigated and found to contain new alkaloids possessing a pyrrolidinyl-α, β-unsaturated γ-lactone, a γ-butylidene-α-methyl-α, β -unsaturated γ-lactam, and/or indolizidine residues (Tan and Takayama, 2019). Pandanus amaryllifolius extracts have content of epicatechin, naringin, catechin, kaempferol, gallic acid, cinnamic acid [8].

P. amaryllifolius leaves contain flavonoids, alkaloids, saponins, tannins, polyphenols, and dyes, thought to have contributed to antibacterial activity [9]. The bioavailability of flavonoid compounds tends to be low under high particle size conditions. Therefore, to improve the bioavailability of plant extracts can be done by modifying the ethanol extract of plants in the form of nanoemulsion [10]. Nanoemulsion is non-toxic and does not irritate [11]. Therefore, it can be applied easily through the skin and mucous membranes, so that by consuming nanoemulsion solution of fragrant Pandan leaf extract mouthwash it is expected to maintain salivary pH under normal conditions so that health the oral cavity is maintained. This study aimed to determine the potentials of P.amaryllifolius Roxb. leaf extract using nanoemulsion technique as a natural mouthwash. If you don’t wish to use the Word template provided, please set the margins of your Word document as follows.

2. Methods
This study used an experimental approach with an experimental design using a Completely Randomized Design (CRD) consisting of 5 treatments and each of 5 replications, while the treatments were:
P0⁻: Control negative with distilled water
P0⁺: Control positive with commercial mouthwash
P1: Giving of P amaryllifolius extract leaf with nanoemulsion technique 1%
P2: Giving of P amaryllifolius extract leaf with nanoemulsion technique 2%
P3: Giving of P amaryllifolius extract leaf with nanoemulsion technique 4%

2.1. Research Location
This research was carried out at the Babun Najah Modern Islamic Boarding School in the District of Ulee Kareng, Banda Aceh City, Laboratory of the Department of Biology and laboratory Department of Chemical Education, Faculty of Teacher Training and Education, Syiah Kuala Banda Aceh.

2.2. Population and Sample
The study population consisted of students aged 12-16 years. The research population was 260 students from Babun Najah Modern Islamic Boarding School. The research sample was taken by purposive sampling. The number of samples used 25 male students. The sample criteria used referred to the research conducted by Azmi [12] including:
1. Complete and regular dental arrangement
2. Not using orthodontic devices
3. No oral disease
4. Approaches and Types of Research
The approach used in this study was a quantitative approach that focuses on measuring and analysing causal relationships between various variables. This type of research was experimental research which intended to find the effect of specific treatments on others in controlled conditions.
2.3. Procedure
The research applied several methods of extracting scented Pandan leaves using nano-emulsion and of the sample testing.

2.3.1. The procedure of getting the extract of scented Pandan leaves
*P. amaryllifolius* leaves were separated by the right parts and parts that are damaged or contaminated with foreign material. After washing, the leaves are then chopped and dried by aerating in the room and avoiding exposure to direct sunlight. *P. amaryllifolius* leaves were mashed using a blender, then sifted with a sieve. Fragrant Pandan leaf powder was ready to be macerated. *P. amaryllifolius* leaf powder was macerated with 70% ethanol until it was completely submerged. Then after one day (24 hours) the solvent was replaced with a new one so that all active substances are thoroughly extracted. The solvent replacement was done three times. The filtrate was filtered and concentrated with a rotary evaporator at a heating temperature of 40 °C until the solvent did not evaporate again. Fragrant *P. amaryllifolius* extract leaf was ready to use [13].

The procedure of getting the extract of scented Pandan leaves with Nanoemulsion technique
Nanoemulsion formula consists of *P. amaryllifolius* extract leaf, tween 80, maltodextrin and pH seven buffer solution. Nanoemulsion composition: *P. amaryllifolius* extract leaf 30 mL, Maltodextrin 30 g, Tween 80 3 mL, 67 mL Phosphate Buffer Solution.

The process of making *P. amaryllifolius* extract leaf nanoemulsion used two solutions which differentiate into oil phase solutions and aqueous phase solution. Then the two solutions were homogenised using a centrifuge. The flow of the manufacturing process for *P. amaryllifolius* extract leaf nanoemulsion can be seen in Appendix A1.

2.3.2. The pH test of respondents’ saliva
The pH measurement was carried out by taking 10 mL nanoemulsion of fragrant Pandan leaf extract and pH test using a digital pH meter. Eighty (n=80) students were selected according to the criteria of the sample for equalisation of the initial pH. After their salivary were collected and measured by the salivary pH value using a digital pH meter, a sample of 25 students was chosen based on pH value close to 7 (neutral) criteria. Next, they were given the same toothpaste and were instructed to brush their teeth for 30 days (2 times daily: after breakfast and before sleep at night).

2.3.3. The treatment of scented Pandan leaves extract on respondents
The respondents selected were divided into five groups, namely, two groups for control and three groups for treatment. All selected samples were instructed to do mouthwash every evening at 17.00 WIB for 21 days on a regular basis, salivary collection was carried out four times before giving the test solution on April 17, 2018, on the first day of treatment on April 24, 2018, mid-study on May 1, 2018, and the final treatment was given on May 8, 2018. Samples were instructed to rinse with a nanoemulsion solution of *P. amaryllifolius* extract leaf for 30 seconds. Then the sample was asked to dispose of the water gargled and spit out saliva into each of the saliva storage pots provided, then measured using a digital pH meter.

2.4. Data Analysis
Data that has been tabulated are then analysed using a Variant of Analysis (ANOVA) with a value of p <0.01.

3. Result and Discussion
3.1. Results: Saliva pH after treating for seven days, 14 days, and 21 days of scented Pandan leaves extract
The results of the analysis of the average pH of saliva on the first seven days of treatment can be seen in Table 1 The average pH of saliva on the seventh day of treatment ranged from 6.18-7.2. In addition,
there is significant difference in the mean saliva pH of the 7th day after treatment, F (4,20) = 8.0, \( p (.01) = 4.43 \). Therefore, based on the results of the DMRT test at a significant level of 0.01, it indicates that P0- has the lowest average saliva pH when compared to P0 +, P1, P2 and P3. The average P3 saliva pH is equal to P0 +, and P3 has a higher average salivary pH compared to P1 and P2. The average Saliva pH P1 and P2 is higher than P0-. From the results above, it can be seen that P3 is the best treatment for increasing the pH of saliva.

**Table 1.** Test Results for saliva pH after giving treatment for seven days

| Treatment | The Average of Saliva pH | DMRT \( \alpha : 0,01 \) |
|-----------|--------------------------|--------------------------|
| P0-       | 6.12                     | A                        |
| P0+       | 6.98                     | B cde                    |
| P1        | 6.24                     | Ab                       |
| P2        | 6.34                     | Abc                      |
| P3        | 6.92                     | C d                      |

The average salivary pH after being given treatment for 14 days showed a massive increase (Table 2). Based on statistical tests showed that there were no significant differences in the mean saliva pH of the 14th day after treatment. In the analysis of variance on the pH of saliva on day 14 after being given treatment, it was known that F count < F table or F (4,20) = 3.38, \( p (.01) = 4.43 \).

**Table 2.** The comparison of the average of salivary pH after giving treatment for seven days and 14 days

| Treatment | The Average of Saliva pH (7 days) | The Average of Saliva pH (14 days) |
|-----------|----------------------------------|----------------------------------|
| P0-       | 6.12                             | 6.28                             |
| P0+       | 6.98                             | 7.34                             |
| P1        | 6.24                             | 6.44                             |
| P2        | 6.34                             | 6.56                             |
| P3        | 6.92                             | 7.2                              |

Moreover, the average salivary pH after being given treatment after 21 days shows a significant increase (Table 3). Based on statistical tests showed that the average salivary pH on the 21-day after being given treatment had a very significant effect on the treatment. An analysis of variance on the pH of saliva on the 21-day after being given treatment, it was known that F count > F table or F (4,20) = 20.57, \( p (.01) = 4.43 \).

**Table 3.** Test results of saliva after giving treatment for 21 days.

| Treatment | The Average of saliva pH on 21 days | DMRT \( \alpha : 0,01 \) |
|-----------|----------------------------------|--------------------------|
| P0-       | 6.46                             | A                        |
| P0+       | 7.66                             | De                       |
| P1        | 6.7                              | Ab                       |
| P2        | 6.88                             | Abc                      |
| P3        | 7.58                             | D                        |

Based on the results of the BNJ test in table 3.1.3 at a significant level of 0.01 it indicates that P0- has the lowest average saliva pH when compared to P0 +, P1, P2 and P3. The average salivary pH of
P1 and P2 is close to pH 7 (neutral pH) and higher than P0, while P3 has a higher average salivary pH than P1 and P2. The average salivary pH value of P3 approaches P0 +. From the results above, it can be seen that P3 is the best treatment in increasing the best salivary pH.

3.2. Discussion
Administration *P. amaryllifolius* extract leaf influences increasing the pH of saliva. The average pH value of saliva that can improve the condition of the pH in the oral cavity can be best seen in treatment 5 (4% fragrant Pandan leaf extract). This finding shows that nanoemulsion fragrance of pandan leaves extract has the same ability as a branded mouthwash to increase the pH of saliva, but it is also more effective in maintaining the pH conditions of saliva in the oral cavity. The results obtained under the initial hypothesis, namely nanoemulsion mouth extract of fragrant pandan leaf extract can increase the pH of saliva.

The ability of *P. amaryllifolius* extract leaf with emulsion technique in increasing salivary pH is shown through the chemical components it contains. The content of fragrant pandan leaves, including flavonoids, alkaloids, saponins, tannins, polyphenols and dyes and with a distinctive aroma, is thought to have a contribution to antibacterial activity [14]. The bioavailability of flavonoid compounds tends to be low under significant particle size conditions. Therefore, to improve the bioavailability of plant extracts can be done by modifying the ethanol extract of plants in the form of nanoemulsion [10].

Nanoemulsion of extract from *P. amaryllifolius* extract leaf shows an influence on increasing salivary pH, as a drug delivery system, nanoemulsion can penetrate the membrane surface, and this is what causes increased penetration of active substances. The tiny size of nanoemulsion droplets allows the absorption of drugs to be more efficient, besides being able to provide good dispersion so that it can work directly in the oral cavity and not cause a sharp odour [15,16].

The acidity of saliva can be measured in units of pH (Potential of Hydrogen). The pH scale ranges from 0-14, with an inverse ratio, where the lower the pH value, the more acid in the solution. Conversely, increasing the pH value means increasing base in solution. At pH 7, there is no acidity or alkalinity of the solution and is called neutral. If there is a decrease in one pH unit, it will cause the rate of release of calcium from tooth enamel, the hardness of the enamel will become soft, pH less than seven or acidic can reduce the surface hardness of the enamel [17].

Saliva has a pH between 6.0-7.4, a range that is advantageous for digestion from the ptyalin enzyme [18]. Ptyalin enzymes are included in the amylase enzyme which plays a role in accelerating the process of amylase reform which is contained in food into simple sugars, namely maltose, which will be utilised further by the body to produce energy. The ability of fragrant pandan nanoemulsion extract in increasing salivary pH is also able to maintain the pH of saliva in the oral cavity under normal conditions so that the rate of action of digestive enzymes remains normal.

Compounds in *P. amaryllifolius* extract leaf, which play an essential role in influencing the pH of saliva in the oral cavity are flavonoids which are antibacterial compounds. The antibacterial mechanism of action includes the activation of certain enzymes, protein denaturation, changing permeability of bacterial cell membranes, intercalation into deoxynucleic acid (DNA) and chelating formation [19]. Flavonoids inhibit bacterial growth by preventing the composition of new phospholipids, in addition to damaging the outer membrane of flavonoids, also breaking the bonds found in the peptidoglycan layer of bacterial cell membranes, resulting in uncontrolled compound permeability from the cells so that bacteria die [20], with reduced bacterial activity in the oral cavity it can affect the increase in salivary pH.

Increased salivary flow rate is caused by mechanical stimulation (gargling movements) and chemistry (bitter taste of fragrant pandan leaf extract). With this stimulation, salivary levels increase. Saliva helps maintain the integrity of the teeth, tongue and oral mucous membranes. Saliva can also reduce the accumulation of plaque on the surface of the tooth and also increase the level of the cleansing of leftover food from the oral cavity. Besides, with increasing salivary secretion, it can neutralise the acidic pH of the plaque and maintain the pH balance of the oral cavity to maintain dental and oral health from various diseases [20].
4. Conclusion
The administration of *P amaryllifolius* extract with nanoemulsion technique can be used as a natural mouthwash which is to maintain the salivary pH of the respondents.

5. Appendix

![Diagram of nanoemulsion process](image)

**Figure 1.** The plot of nanoemulsion extract process of scented Pandan leaves
6. References

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