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RESEARCH PAPER

Physical test and irritation test of water guava leaf extract (Syzygium aqueum) as a natural spray hand sanitizer

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Abstract: This study explores the manufacture of water guava (Syzygium aqueum) leaf extract-based hand sanitizers as an effective and safe natural alternative for maintaining hand hygiene. Given the importance of hand hygiene in preventing the spread of disease, especially amid the COVID-19 pandemic, the use of hand sanitizers has increased significantly. However, alcohol-based products are often irritating to the skin. Therefore, this study aims to evaluate the potential of water guava leaf extract to improve the effectiveness and convenience of hand sanitizers. Water guava leaves contain active compounds such as flavonoids, tannins, and phenolics with antibacterial and anti-inflammatory properties. The research method includes extracting water guava leaves, hand sanitizer formulation, and organoleptic, pH, homogeneity, and irritation testing. The tests carried out were physical tests, including organoleptic tests (shape, smell, and colour), pH, homogeneity, spreadability, adhesiveness, and product irritation tests. The research was designed with a posttest-only control group design with true experimental by analyzing the test object after being given treatment. This research is expected to produce products that are useful for the community. The results showed that all hand sanitizer formulas tested had a pH within the safe range of pH 5 for the skin without irritating the skin of the test animals. Formulas with added guava extract showed improved aroma and anti-irritation, with formulas F1 and F2 gaining the highest preference in aroma and colour. This study concludes that water guava leaf extract can be effectively used in the manufacture of hand sanitizers, providing a gentler and skin-friendly alternative to alcohol-based synthetic products.

Keywords: *Syzygium aqueum, extract, hand sanitizer, flavonoids, irritation*

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INTRODUCTION

World Health Organization (WHO) data states that most diseases are transmitted through hands [1]. Even in 2019, the WHO said that the spread of COVID-19 was mostly transmitted through human-hand interaction [2]. Diseases are generally caused by bacteria and viruses that attack the human body's immune system [3]. Based on research, bacteria are the main cause of human disease [4]. Bacteria are a group of single-celled microorganisms with prokaryotic cell forms [5][6]. As living things, bacteria have genetic information in the form of DNA, but bacteria are not located in the nucleus or nuclear membrane [7]. Bacterial DNA consists only of exons and has no intron. Bacteria have long, circular cells and are called nucleoids [8][9]. Bacteria also have extrachromosomal DNA incorporated in small circular plasmids [10].

Staphylococcus aureus bacteria is one of the most common bacteria found in the human environment [11][12]. According to Juliantina, Staphylococcus aureus bacteria are arranged in groups, similar to grapes, and are round or oval [13][14]. Staphylococcus aureus bacteria are Gram-positive bacteria that do not move and do not have spores. This group is formed because daughter cells tend to be near their parent cells when dividing [15]. One of the diseases caused by Staphylococcus aureus bacteria is diarrhoea and skin diseases; this disease is experienced by many people in general [16]. One way to minimize the effects of bacteria is by using antibacterials [17]. Antibacterials are substances that can kill or suppress the growth and reproduction of bacteria [18]. Antibacterials usually come from chemical synthesis, better known as antibiotics. However, overuse of antibiotics causes resistance and side effects such as diarrhoea, allergies, and other toxic hazards [19]. Due to these side effects, treatments using natural materials are becoming more popular because natural materials have advantages that are not associated with side effects [20]. The problem in this study is whether the plant extract is effective as a stable antibacterial and anti-irritant material for use on the skin.

One of the most common ways to maintain hand hygiene is hand washing using antiseptic soap [20]. However, soap is ineffective because it requires a lot of water to wash hands, but this problem can be solved with waterless handwashing products called antiseptic hand sanitizers or hand sanitizers [21]. Hand sanitizer is useful because it kills or reduces the number of infection-causing bacteria quickly, conveniently, and practically [21]. Preparations that can be used as an alternative to reduce bacteria are also affordable and can be carried anywhere [16]. However, using hand sanitizers from excess synthetic chemicals can cause skin disorders. Therefore, it is necessary to make hand sanitizers from natural ingredients. Research on making hand sanitizers from natural ingredients is in line with Indonesia's biological wealth [10]. In Indonesia, many plants contain antimicrobial chemical compounds [16]. Water guava leaves are one of them; water guava leaves have long been used as medicine to treat skin pain, bloating, loose stools, and diarrhoea [11][12]. The classification of the water guava plant can be depicted in Figure 1.

Figure 1. Water guava plant

The classification of Syzygium aqueum is as follows: Kingdom: Plantae; Clade: Tracheophyta; Clade: Angiospermae; Clade: Eudicotyledons; Order: Myrtales; Family: Myrtaceae; Genus: Syzygium; Species: S. aqueum. Syzygium aqueum extract contains tannins that have antibacterial activity against S. aureus, E. coli, and P. aeruginosa as well as antifungal activity against A. niger, C. albicans, and gram-positive S. aureus [11]. Water guava leaf, also known as Syzygium aqueum, is one of the plants that has the potential to be used in medicine, especially as an antibacterial [23]. The secondary metabolites in the ethanol extract of water guava leaves contain flavonoids, terpenoids, and tannins [23]. Based on research on water guava leaves, the leaf content has pharmacological effects, one of which is antibacterial activity [24]. Water guava leaves or Syzygium aqueum contain various active compounds that are beneficial for health and make them an ideal choice for use in hand sanitizer formulations. Guava leaves include flavonoids, tannins, and phenolics which are known to have antibacterial and anti-inflammatory activities. Flavonoids function by forming complexes with extracellular and soluble proteins, damaging bacterial cell membranes, and inhibiting pathogenic microorganisms growth.

Conversely, tannins work by constricting bacterial cell walls, disrupting cell permeability, and inhibiting bacterial biological activities, resulting in bacterial death[18][20]. Phenolic compounds can inactivate bacterial enzymes and damage protein structures and cell membranes, contributing to antibacterial effectiveness[10]. In addition to antibacterial properties, water guava leaf extract offers anti-inflammatory and antioxidant benefits, which can help reduce inflammation and maintain

skin moisture balance. It is especially important in hand sanitizer products, as it can reduce the risk of irritation and dryness often caused by excessive alcohol use. By harnessing this wealth of compounds, water guava leaf extract not only improves the effectiveness of hand sanitizers in killing germs but also ensures that the product is gentle and does not damage skin health, making it suitable for regular use.

With the increasing awareness of the importance of hand hygiene in preventing the spread of diseases, hand sanitizer has become one of the most widely used practical solutions[14]. However, with so many products in the market, consumers are increasingly concerned about the effectiveness, safety, and impact of the ingredients used. One of the natural ingredients increasingly attracting attention in hand sanitizer formulations is water guava leaf extract. Water guava leaf, known in the herbal world for its diverse properties, offers tremendous potential as an additive in hand sanitizer products. With its antioxidant content and anti-inflammatory properties, this extract can provide additional benefits such as reducing irritation and maintaining healthy skin. This article will discuss the process of making hand sanitizer from water guava leaf extract, reveal the advantages and benefits of using this natural ingredient, and how proper formulation can improve the effectiveness and convenience of the product.

The current research focuses on making natural hand sanitizers using water guava leaves by looking at their effectiveness and safety, including phytochemical screening, physical tests, and product irritation tests. For many years, metals have been crucial to medicine. In varied amounts, many are necessary in

MATERIALS AND METHODS

Materials

Syzgium auqeum leaves were collected in Penujak village, Central Lombok Regency, West Nusa Tenggara. The plant was identified at Bumigora University. The materials required are Alcohol Denat, Glycerine, Fragrance, Triethanolamine, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Sygium Aqueum Extract, Aqua Demineralisata and three Rabbits as an animal test. The practical tools that are used are a Rotary Evaporator, Water bath, Ph meter, and extraction tools.

Methods

The research methods are scientific ways to obtain data for specific purposes and benefits [19]. Researchers used quantitative research methods to provide a researcher's perspective on the problem under study. Extraction research, physical tests, and irritation tests of Jambu air (Syzygium aqueum) leave as a natural spray hand sanitizer designed with Posttest Only Control Group Design is a true experiment by analyzing the test object after being given treatment [20].

The research begins with identifying water guava leaf plants; water guava leaves that have been obtained are then dried in the sun or dried to dry; drying is done to reduce the water content contained in water guava leaves. The next step is making simplisia, which results from grinding water guava leaves into powder. The water content test was carried out on the simplisia to determine the water content in the simplisia. Furthermore, the powdered simplisia was extracted using the maceration method with 70% ethanol solvent and obtained water guava leaf extract.

Physical Test

The physical test of water guava leaf extract as a natural hand sanitizer begins with organoleptic tests (shape, smell, and colour), pH, homogeneity, spreadability, adhesiveness, and protective power carried out on the preparation. pH testing using pH paper is a simple and effective method to determine the acidity or alkalinity of a solution. The pH paper is treated with indicators that change colour when immersed in a solution, allowing for a visual comparison against a standard pH scale. To conduct the test, a strip of pH paper is dipped into the solution for a few seconds, and the resulting colour is matched to the scale to determine the pH value. This method is widely used in laboratories, educational settings, and household applications, providing quick and reliable results for various solutions. This test aims to determine the effectiveness of the hand sanitizer preparation produced [13][14].

Irritation Test

The irritation test was carried out by the Draize test, originally developed to assess the irritancy of substances, involves testing on rabbits to evaluate the severity of irritation caused by chemicals applied to the skin or eyes. This method measures the extent of damage and irritation, providing valuable data

on the safety of various products and chemicals[15]. Despite its historical significance, the test has faced criticism for ethical concerns regarding animal welfare, leading to the exploration of alternative methods for irritation assessment. The first step taken was to divide the rabbit group in a ratio of 1: 1, namely as control animals and animal tests [15]. Treatment performed on the animal test is to shave the rabbit's back hair until clean. After that, the animal's back is divided into four square sections, each measuring 1 by 1 inch [14][24]. Before applying the test material, the rabbit's skin was cleaned with a cotton swab soaked in water. The results of the hand sanitizer product were applied to gauze and then attached to the rabbit's back for 3 x 24 hours. Then, the animal tests were returned to their cages and examined the results again the next day. After that, symptoms were observed as initial irritation in the form of neuritis and swelling at hours 24, 48, and 72 or for 3 days [14][16]. Based on the initial irritation test, irritation is calculated as swelling and erythema. The irritation test formula can be depicted in Figure 2.

$$= \frac{\sum skor\ eritema + \sum skor\ edema}{2}$$

Score erythema = 24 hours +48 hours+ 72 hours

Score edhema = 24 hours +48 hours+ 72 hours

Figure 2. Eritema and Edema Scoring Formula.

RESULTS AND DISCUSSION

This research is green economy research based on the use of natural materials in the form of plants, which are very easy to obtain and can be used to make very useful items. Based on the description of the problem and urgency obtained, research into making natural hand sanitizer from water guava leaves needs to be done to answer today's challenges. It is hoped that this research will become the basis for further research to produce natural hand sanitizer products that are effective and easy for the general public to obtain. Syzgium auqeum is known for its rich composition of active compounds that exhibit notable antibacterial properties. Among these, phenolic compounds such as flavonoids and tannins have been shown to effectively inhibit the growth of various bacteria. Additionally, vitamin C, recognized for its antioxidant effects, may also enhance antibacterial activity. Extracts from guava leaves contain essential oils with antimicrobial properties, further contributing to the fruit's protective benefits. Moreover, saponins are compounds that can disrupt bacterial membranes, leading to cell death. Quercetin, another flavonoid found in guava, has demonstrated antibacterial activity against several pathogens. Research suggests that these compounds collectively make guava effective against various bacteria, including those responsible for foodborne illnesses and infections.

The weight of syzgium aquem extract produced in this study is 45 grams, calculated with the weight of raw materials using the yield formula, it produces a 6% yield with 750 grams of raw materials. Every plant that is grown in a different location and with a different environment may contain different chemical composition. Uniformity in the production of herbal medicines is required to guarantee the quality, effectiveness, and formulation of herbal ingredients in compliance with established standards [20]. A key element in establishing the calibre and purity of the finished product is standardization, a procedure for gauging the potency of herbal remedies [21]. Standardization aims to produce herbal plant extracts that are widely utilized for formal health services and self-medication and whose quality, efficacy, and safety are ensured through scientific testing.

The antibacterial effect of ethanol extract from water guava leaves (Syzygium aqueum) against clinical isolate bacteria is caused by active compounds that are soluble in ethanol. These compounds are thought to include flavonoids, phenolics, and tannins. Flavonoids work as antibacterials by forming complexes with extracellular and soluble proteins, thereby damaging the bacterial cell membrane and causing the release of intracellular compounds [16]. Phenolic compounds, according to Singh and Bharate (2005), inhibit bacterial growth by inactivating proteins (enzymes) in the cell membrane. Phenol binds to proteins through hydrogen bonds, damaging the protein structure because most bacteria's cell walls and cytoplasmic membranes consist of proteins and fats. Damage to the cell wall and cytoplasmic membrane disrupts the function of selective permeability, active transport, and control of protein composition, which ultimately causes leakage of macromolecules and ions from the cell. As a result, bacteria lose their shape and undergo lysis. Tannins work as antibacterials by shrinking the bacterial cell wall, disrupting cell permeability, and inhibiting its life activities, resulting in bacterial

death[18]. Sari and Sari (2011) stated that tannins target cell wall polypeptides, forming imperfect cell walls and ultimately causing bacterial cell lysis due to osmotic or physical pressure so that the bacteria die.

All hand sanitizer formulas (F0, F1, F2, F3) have almost the same ingredient composition, except for Sygium Aqueum extract and Aqua Demineralisata. All formulas contain 8% Alcohol Denat, 2% Glycerine, 1% Fragrance, 2% Triethanolamine, and 1% Acrylates/C10-30 Alkyl Acrylate Crosspolymer, while Aqua Demineralisata is used as much as 60 ml in each formula. The difference lies in the Sygium Aqueum extract, which varied from 0% in F0 to 1% in F3. The hand sanitizer formula can be described in Table 1.

Table 1. Formula Handsanitizer

Ingredients	F0	F1	F2	F3
Alcohol Denat	8	8	8	8
Glycerine	2	2	2	2
Fragrance	1	1	1	1
Triethanolamine	1	1	1	1
Acrylates/C10-30 Alkyl acrylate Crosspolymer	1	1	1	1
Sygium Auqeum Extract	0	0.1	0.5	1
Aqua Demineralisata	Add up to 60 ml	Add up to 60 ml	Add up to 60 ml	Add up to 60 ml

Hand sanitizers that are made with natural ingredients tend to be gentler on the skin and have an aromatherapy effect. Natural ingredients like the extract of sygium aqeum, and another herbal extract can help keep hand skin moisturized and healthy, reducing the risk of dryness and irritation that often occurs with alcohol-based products that can reduce the risk of allergies and irritation. Natural ingredients often contain fewer additives and preservatives that can cause allergies or irritation. It makes them a better choice for individuals with sensitive skin or allergic reactions to synthetic chemicals. Sygium aqeum leaf contains antimicrobial effectiveness that can kill bacteria and viruses. While they may not be as powerful as alcohol in killing germs, combining several natural ingredients can provide effective protection.

Organoleptic test results revealed that all formulas were clear in colour, but F1 to F3 showed increasing turbidity along with the addition of Sygium Aqueum extract. The aroma in F0 was dominated by alcohol with a hint of fragrance, while F1 to F3 showed an increase in leaf extract aroma, with F3 having the most dominant aroma. All formulas had cool and non-sticky properties on the skin, consistent in user experience. From the organoleptic test results by respondents, F1 received the highest preference from 3 respondents in terms of colour, while 2 respondents preferred F2. F0 and F3 had no clear preference. For aroma, F1 and F2 were each favoured by 2 respondents, while F0 was only favoured by 1 respondent, and F3 did not get a preference. Regarding properties, F1 and F2 were also favored by 2 respondents, indicating a better user experience in terms of non-stickiness and cooling effect. The organoleptic test can be described in the table 2.

Table 2. Organoleptic Test

	F0	F1	F2	F3
Colour	Clear	Slightly cloudy clear	Slightly cloudy clear	Dark green
Aroma	The smell of fragrance mixed with alcohol	The smell of fragrance mixed with extract	The smell of fragrance mixed with extract	The smell of extract
Nature	Cold not sticky	Cold not sticky	Cold not sticky	Cold not sticky

The organoleptic test results for the natural hand sanitizer showed marked variations in some important aspects of the product. Sample F0 had a clear colour, while F1 and F2 showed a slightly cloudy but still clear colour, while F3 had a darker dark green colour. The aroma of each sample was also different: F0 had a mixed odour between fragrance and

alcohol, F1 and F2 created a mixed odour of fragrance with extract, and F3 had a more dominant extract odour. All samples, although different in colour and scent, had the same properties in terms of texture, i.e. they all felt cool and non-sticky when used. These data show that despite differences in colour and scent, all samples have consistent texture characteristics, which is important for user experience.

Maintaining the scent, shape, and spray properties of hand sanitizer made from water guava leaf extract is essential to ensure a pleasant and effective user experience. The scent of the hand sanitizer, which is influenced by water guava leaf extract, not only affects the pleasure of use but can also provide a calming effect thanks to the natural properties of the extract. In addition, the hand sanitizer's spray form affects the product's distribution and application; with guava leaf extract having certain characteristics, it is important to ensure that the spray form remains consistent to spread evenly and effectively during use. Spray properties also affect practicality and convenience, ensuring the product is easy to use without leaving unwanted residue. Maintaining these qualities helps ensure that the hand sanitizer is effective in killing germs and provides a pleasant and discomfort-free user experience, ensuring that the benefits of guava leaf extract, such as anti-irritant and antioxidant properties, are optimized.

The next test is the homogeneity test. Homogeneity testing in natural hand sanitizers is an important step in ensuring that the product has an even consistency and quality throughout the production batch. In natural hand sanitizers, which often contain ingredients such as alcohol and essential oils, a homogeneity test helps to ensure that all components are well mixed and that there is no separation or unevenness. By conducting this test, the researcher identify if any issues in the mixing or formulation process could affect the effectiveness and safety of the product. The results of the homogeneity test ensure that each application of hand sanitizer will have a consistent concentration of active ingredients, which is important for effectively killing germs and ensuring that the product meets established quality and safety standards. The homogeneity test can be described in Table 3.

Table 3. Homogeneity Test

Homogeneity	F0	F1	F2	F3
textures	soft	soft	soft	soft
grain	No coarse grains	No coarse grains	No coarse grains	No coarse grains
absorbing speed	Quick	Quick	Quick	Quick
color	Clear	Clear	Clear	Clear

The results of the Ph test are described in Table 4.

Table 4. Ph Test

Ph test	F0	F1	F2	F3
	7	5	5	5

The homogeneity test results for natural hand sanitizers showed that all samples, namely F0, F1, F2, and F3, met the same criteria in several important aspects. The texture of all samples was identified as soft, with no coarse grains that could affect the comfort of use. The absorption speed of each sample was also consistent, with all showing the ability to absorb quickly on the skin. In addition, the colour of all samples remained clear and showed no differences, ensuring a uniform appearance across production batches. Thus, these data indicate that the natural hand sanitisers tested have good homogeneity, with no significant variations in texture, absorption, and colour, supporting product quality and consistency. The pH test showed that all formulas had a pH between 5 to 7. F0 had a higher pH of 7, while F1, F2, and F3 had a lower pH, around 5, indicating that the addition of Sygium Aqueum extract slightly lowered the pH, making it more acidic than F0. pH test can be captured in Figure 3.

The composition of natural guava leaf extract in hand sanitizer plays an important role in keeping the pH of the product balanced and skin-friendly. Water guava leaf extract is known to have acidic properties that can help balance the pH of the hand sanitizer, bringing it closer to the natural pH of human skin, which ranges. Keeping the pH of hand sanitizer at the right level is essential for skin health, as an unbalanced pH can disrupt the skin's natural protective layer and cause irritation or damage. Human skin has a naturally slightly acidic pH, usually ranging from 4.5 to 5.5, which acts as a barrier to protect against infection and maintain moisture. If the pH of

the hand sanitizer is too high or too low, it can disrupt this balance, causing the skin to become dry, itchy, or even inflamed. Hand sanitizers with the right pH help ensure that the skin remains healthy and protected after use and minimize the risk of side effects such as irritation or dermatitis. Therefore, keeping the pH of hand sanitisers within a safe range and in line with the skin's natural pH is a crucial step to ensure the product effectively kills germs and gently and harmless to the skin.

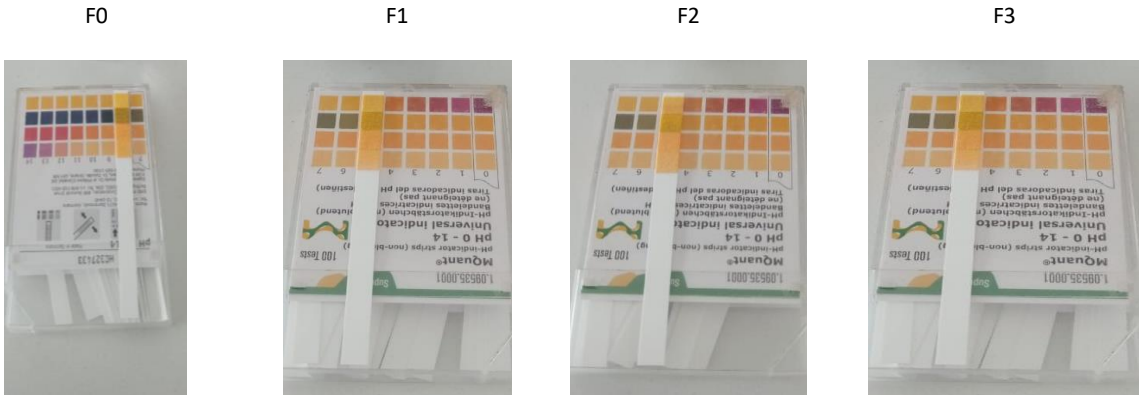

Figure 3. Ph Test

Table 5. Irritation Test 3x24 Hours

Irritation Test	F0	F1	F2	F3
Erythema	No	No	No	No
Edema	No	No	No	No
Result	No irritation	No irritation	No irritation	No irritation

The irritation test showed no signs of erythema or irritation on the skin of the animal test (rabbits), indicating that all formulas were safe to use and did not cause irritating reactions. The result of the irritation test is described in Table 5.

In conclusion, F1 and F2 appeared to be preferred over F0 and F3, especially in aroma and colour, with F1 showing the most positive results overall. The addition of Sygium Aqueum extract increased the turbidity and changed the scent of the hand sanitizer, with formulas containing higher extracts (F2 and F3) providing a more pleasant scent to some respondents. The addition of extracts affected pH and lower pH formulas may be more suitable for maintaining skin pH balance. All formulas were found to be safe and non-irritating, so they can be used without risk of skin irritation. The irritation test can be shown in Figure 4.

The use of water guava extract as an anti-irritant ingredient in hand sanitizers is very important as it can help reduce the risk of irritation and increase the comfort of using the product. Water guava extract has anti-inflammatory and antioxidant properties that can soothe the skin and reduce inflammation, making it an ideal choice to address potential irritation caused by frequent hand sanitizer use or by active ingredients such as alcohol. When skin is exposed to harsh cleansing ingredients, water guava extract works to neutralize those negative effects by helping to maintain moisture balance and reduce redness or dryness. By adding water guava extract to the hand sanitizer formula, manufacturers ensure that the product is effective in killing germs and maintaining the health of the user's skin, making it more convenient to use regularly without compromising skin condition.

CONCLUSION

The study concludes that hand-borne diseases, such as those caused by bacteria, are a significant health concern. Hand sanitizers are a practical solution to eradicate these bacteria, but synthetic chemical-based products often cause side effects. To address this issue, this study aimed to develop a natural hand sanitiser from water guava leaf extract with antimicrobial compounds such as tannins and saponins. The study involved physical and irritation testing of the product and statistical analysis to determine the effectiveness and safety of the hand sanitizer. Based on the results obtained, all tests responded well to both the physical test and the irritation test, and it can be concluded that

the hand sanitizer from water guava extract is suitable for use.

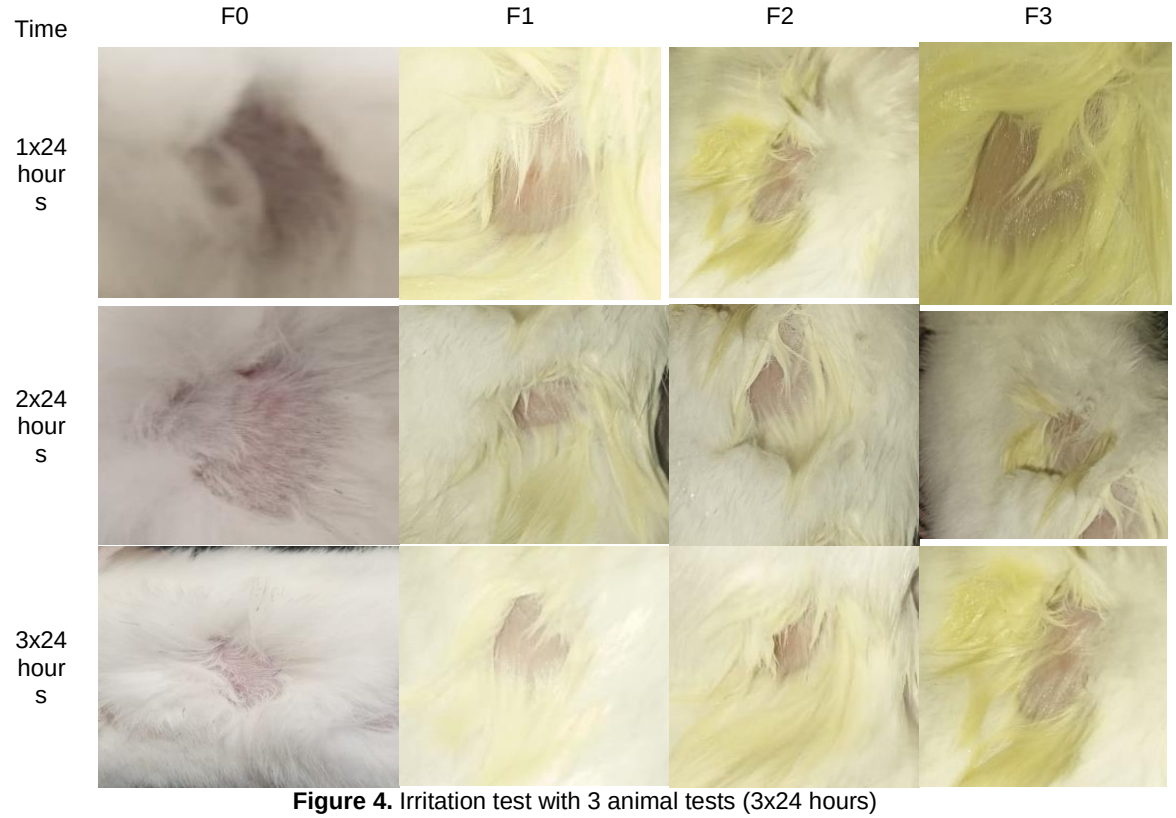

Figure 4. Irritation test with 3 animal tests (3x24 hours)

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