Formulation And Evaluation Of Hand Sanitizer Gel From Clove Flower Extract (*Eugenia Aromatica L.)*

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Abstract

Clove flower (Eugenia aromatica L.) is a plant from the Myrtaceae family, the compounds contained in clove flowers are flavonoids, tannins, alkaloids, and saponins that can function as antibacterial. In addition, clove flowers also function as analgesic, anti-inflammatory, antimicrobial, antiviral, antifungal, antiseptic, antispasmodic, antiemetic, stimulant, and local anesthetic. The purpose of this study was to determine does clove flower extract (Eugenia aromatica L.) could be used as a hand sanitizer gel preparation. Clove flower simplicia powder was extracted with ethanol 96% by maceration. The extract obtained was then screened for phytochemicals, then formulated in the form of a hand sanitizer gel, by adding clove flower extract to each formula with different concentrations. The clove flower extract concentrations used were 5%, 10%, and 15%. After the hand sanitizer gel preparation was formed, the clove flower extract was then tested for the stability of the gel preparation. The clove flower extract obtained was 116.4 grams of thick extract. The results of phytochemical screening of clove flower extract showed the presence of secondary metabolites, namely flavonoids, alkaloids, saponins and tannins. Based on the evaluation of the hand sanitizer gel preparation, clove extract showed that it was physically stable during storage. Based on the acceptability or liking test conducted on 20 respondents using a questionnaire sheet, the results showed that the respondents really liked the aroma of formula F3.

Keywords: *Hand sanitizer, clove flower extract and phytochemical screening.*

I. INTRODUCTION

The clove plant (Syzigium aromaticum) is a spice plant that can be found in Indonesia and is used in the cigarette, food and medicine industries (Sidabutar, 2016). Until now, it is recorded that 7000 species of plants have known their efficacy, but less than 300 plants are used as raw materials for the pharmaceutical industry on a regular basis (Saifudin et al., 2011). The clove plant has a distinctive aroma, a distinctive clove aroma is produced by the compound eugenol, which is the main compound (72-90%). Eugenol also has antiseptic and anesthetic properties (Razafimamonjison et al., 2015). Cloves contain eugenol with a composition of eugenol (81.20%), trans-β-caryophyllene (3.92%), -humulene (0.45%), eugenol acetate (12.43%), caryophyllene oxide (0 .25%) and trimetoxy acetophenone (0.53%) (Prianto et al., 2013).

According to research by Bhuiyan et al, 2010 that the analysis of clove leaf compounds using the GC-MS method obtained 74.28% eugenol compounds, 5.78% eucalyptol, 3.85% caryophyllene, 2.43% -cardinol, 2.08% limonene. . Clove flower eugenol compounds are antibacterial compounds that are able to inhibit the growth of pathogenic bacteria both gram-positive and gram-negative. The ability to inhibit gram-positive bacteria is due to the clove flower extract which has the property of eugenol which is a weak acid.

Based on research (Huda et al., 2018) showed that clove flower extract concentrations of 10% to 100% were able to inhibit the growth of Staphylococcus aureus bacteria. Based on the effectiveness of the clove flower, a hand sanitizer gel formulation was developed. Hand sanitizer is a hand sanitizer that has

antibacterial ability to inhibit and kill bacteria (Retnosari and Isdiartuti, 2006). According to Diana (2012) there are two hand sanitizers, namely hand sanitizer gel and hand sanitizer spray. Hand sanitizer gel is a gel-shaped hand sanitizer that is useful for cleaning or eliminating germs on hands, containing 60% alcohol as an active ingredient.

II. METHOD

2.1 Sampling

Samples were taken from the Medan Market Center, North Sumatra Province (Sambu) in the form of dried clove flowers.

2.2 Materials

The materials used in this study were clove flower extract, aquadest, 96% ethanol, magnesium powder, concentrated HCl, 10% iron (III) chloride, 2N HCl, Libermann-Bourchard reagent, Bouchardat reagent, Mayer's reagent,Dragendorff's reagent, amyl alcohol , CMC Na, Glycerin, and Propylene glycol.

2.3 Extraction

Clove flower powder (Syzygium aromaticum) is weighed as much as 500 grams, Put the clove powder into a vessel add 96% ethanol solvent, then the vessel is closed and then allowed to stand for 5 days in a place protected from light and moisture, the pulp is washed again with 96% ethanol for 5 days. 2 days later filtered (Depkes RI, 1979). The extract was concentrated using a rotary evaporator at 70°C and evaporated in a water bath at 60°C until a thick extract was obtained (Marianne et al., 2014).

2.4 Phytochemical Screening Test

2.4.1 Alkaloid Examination

Clove flower ethanol extract was weighed 0.5 g added 1 ml HCl 2 N added 9 ml distilled water, then heated on a water bath for 2 minutes, cooled and filtered the filtrate was used for the examination of alkaloids:

1. 3 drops of filtrate are added with 2 drops of Mayer reagent, a white or yellow lumpy residue will be formed.
2. 3 drops of filtrate are added with 2 drops of Bouchardat reagent, a brown to black residue will be formed.
3. 3 drops of filtrate are added with 2 drops of Dragendroff reagent to form brown or orange.

If there is a residue or turbidity in at least 2 test tubes in the above experiment, the alkaloid is positive (Ditjen POM, 1979).

2.4.2 Flavonoid Examination

As much as 10 g of clove flower ethanol extract was weighed and then added 100 ml of hot distilled water, boiled for 5 minutes and filtered in a hot state, into 5 ml of the filtrate added magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, shaken vigorously and allowed to separate. The presence of flavonoids is indicated by the presence of a red, yellow or orange color on the amyl alcohol layer (Ditjen POM, 1979).

2.4.3 Saponin Examination

As much as 0.5 g of clove flower ethanol extract was put into a test tube, then added 10 ml of hot distilled water and cooled, then shaken vigorously for 10 minutes. If foam is formed with a height of 1-10 cm which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl, it indicates the presence of saponins (Ditjen POM, 1979).

2.4.4 Tannin Examination

As much as 1 g of clove flower ethanol extract with 10 ml of distilled water and then filtered the filtrate was diluted with distilled water until it was colorless. 2 ml of the solution was taken and 1-2 drops of 1% iron (III) chloride reagent were added. If a blue-black or green-black color occurs, it indicates the presence of tannins (Ditjen POM, 1979).

2.5 Formulation and Procedure for Making Gel Hand Sanitizer

2.5.1 Clove Flower Extract Gel Formula

In this study, gel preparations were made with variations in different extract concentrations, namely concentrations of 5%, 10%, and 15%. The standard formula of CMC-Na gel base according to Maswadeh, et al (2006). Based on the standard gel base, a 30 gram gel formulation with three concentrations was made, namely as follows:

Table 1. The formulation of 30 gram gel with three concentrations

Ingredient	F1 0 %	F2 5 %	F3 10 %	F4 15 %
Clove flower extract (g)	0	1,5	3	4,5
CMC-Na (g)	0,5	0,5	0,5	0,5
Propylene glycol (ml)	1,5	1,5	1,5	1,5
Glycerin (ml)	3	3	3	3
Aquadest ad (ml)	30	30	30	30

2.5.2 Making clove flower extract gel

How to make a gel formulation, the ingredients are weighed first according to the formulation. The extract with a concentration of 5% was dissolved in some water which had been heated while stirring, then added to the mortar with heated aquadest and then gradually added Na-CMC while stirring continuously so that it did not clot. Furthermore, glycerin, propylene glycol and aquadest was added and then stirred until a homogeneous gel is formed. After formation, the gel was stored in a dark and cool place for 1 night at room temperature (Titaley et al., 2014). The same procedure was also carried out on extracts with concentrations of 5%, 10% and 15%.

2.6 Evaluation of Gel Preparations

2.6.1 Organoleptic Test

Organoleptic test or sensory test is a test method using the human senses as the main tool for measuring product acceptance. Organoleptic testing has an important role in the application of quality. The gel preparation was evaluated physically including odor, color, consistency for 4 weeks with observations every 1 week. This observation was carried out on hand antiseptic gel (hand sanitizer) stored at room temperature (Ansel, 2008).

The organoleptic test was carried out visually and seen directly, the shape, color, and smell of the gel made. Gels are usually clear with semisolid concentrations (Ansel, 2008).

2.6.2 Homogeneity Test

Homogeneity test was carried out by visual observation of the preparation. The gel homogeneity test is carried out by applying a thin layer of gel on the object glass, observing whether there are coarse particles or not, if there are no coarse particles, the gel preparation is declared homogeneous (Segara, 2019).

2.6.3 pH test

Measurement of pH was carried out on gel preparations that had been made before and after storage conditions. Measurement of pH is carried out with a pH meter by dipping the pH meter into the preparation. The pH value should ideally be the same as the pH of the skin or the site of application. This is to avoid irritation. The normal pH of human skin ranges from 4.5–6.5 (Draelos & Lauren, 2006).

2.6.4 Spreadability Test

The gel dispersion test used 0.5 grams of gel sample placed on a round glass with a diameter of 15 cm, placed another glass on top of the gel and left for 1 minute. The diameter of the spread of the gel was measured by taking the average diameter from several sides, then adding a load of 50 g, 100 g, 150 g, 200 g on the glass as an additional load. Each additional load was allowed to stand for one minute and the spreading diameter was measured as before. (Segara, 2019).

2.6.5 Viscosity Test.

The instrument used to measure viscosity is the Brookfield viscometer. The gel is put into the container, then a spindle size 4 is attached to the viscometer and the rotor is run at a speed of 30 rpm (Wasiaturrahmah and Jannah, 2018).

III. RESULT

In this study, the extraction method used is the maceration method. This method was chosen because the process is easy, the equipment used is simple, and it does not damage the compounds contained in the test sample. The solvent used in this maceration process is ethanol. The choice of ethanol as a solvent is because ethanol can dissolve almost all secondary metabolites in the polar test sample (Prima, 2012). Extraction results obtained 116.4 grams of thick extract.

3.1 Phytochemical Screening Test

Phytochemical screening in this study was carried out on clove flower extracts with the aim of knowing the class of secondary metabolites contained in clove flowers. The results of the phytochemical screening of clove flower extract can be seen in the table below:

Table 2. Results of Examination of the Simplicia Characterization of Clove Flower Extract

No.	Group of Chemical Compounds	Identification
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+

Description:
(+) = contains the substance being examined
(-) = does not contain the substance examined

Based on the results of the phytochemical screening examination above, it shows that clove flower extract contains secondary metabolite chemical compounds, namely flavonoids, alkaloids, saponins, and tannins. Based on the results of research conducted by Chairawati (2019), clove flower extract (Eugenia aromatica L.) was positive to contain chemical compounds such as alkaloids, flavonoids, saponins and tannins.

On examination of alkaloid compounds, it was indicated by the presence of a blackish brown precipitate on the addition of Bouchardat reagent, and a reddish brown precipitate on the addition of Dragendorff's reagent. Alkaloids have antibacterial activity with the mechanism of action of interfering with the peptidoglycan constituent components of bacterial cells, so that the cell wall layer is not fully formed and causes bacterial cell death. In the alkaloid compound there is also a nitrogen-containing base group that will react with the amino acid compounds that make up the bacterial cell wall. This reaction results in changes in the structure and composition of amino acids which will cause a genetic imbalance in the DNA chain so that it will be damaged and encourage bacterial lysis which will cause cell death in bacteria (Arlofa, 2015).

On examination of the flavonoid group compounds, it was indicated by the presence of an orange color on the separating amyl alcohol layer, proving that the clove flower extract positively contained chemical compound flavonoids. Flavonoids are a group of phenolic compounds that have a tendency to bind to proteins, thereby disrupting bacterial metabolic processes. In addition, flavonoids also function as antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane. Flavonoids have a chemical structure in the form of a beta ring and -OH group which are thought to be responsible for antibacterial activity (Nugraha et al., 2017).

Saponin compounds, the presence of saponin compounds is indicated by the height of the foam obtained from the clove flower extract, which is 2 cm, which proves that the minimum limit for saponin foam is 1 cm. Saponins have activity as an antibacterial with a working mechanism that causes leakage of proteins and enzymes from the cell. This can happen because the active substances found on the surface of saponins are similar to detergents, as a result, saponins will reduce the surface tension of the bacterial cell wall and damage the membrane permeability. Then the saponins diffuse through the outer membrane and cell wall, thereby disrupting and reducing the stability of the cell membrane. This causes leakage and cytoplasm exit from the cell resulting in death in both gram-negative and gram-positive bacterial cells (Suresh et al., 2013).

In the tannin test, the clove flower extract was indicated by the presence of a blackish green color with the addition of FeCl3 reagent, which means that the clove flower extract was positive for tannin compounds. Tannins have antibacterial activity with a mechanism of action, namely interfering with cell permeability. This causes the cells to be unable to carry out life activities so that their growth is inhibited and died. Tannin compounds can induce the formation of tannin complexes to metal ions which can increase the toxicity of tannins (Arlofa, 2015).

3.2 Evaluation of Gel Hand Sanitizer

3.2.1 Organoleptic

Pig 1. Hand sanitizer gel preparation (F1, F2, F3)

Organoleptic test is a test carried out by naked eye or direct observation to describe the preparation. The organoleptic test includes the shape, color, and odor of the resulting preparation. This clove flower extract shows a thick / concentrated form, a brownish color and a characteristic smell of cloves. The purpose of the organoleptic test is to find out whether there are organoleptic changes in the hand sanitizer gel preparation of clove flower extract. The dosage forms of the three formulas show that all the dosage formulas have a thick form.

Clove flower extract has a very strong clove flower characteristic aroma. The color in the hand sanitizer gel formulation is brown, the higher the concentration of clove flower extract, the more concentrated the color of the hand sanitizer gel preparation. The addition of active substances can affect the color and odor of the dosage formula. The results of the organoleptic test above did not show any change in color, odor, and shape because the preparation was completely mixed and was stable in storage.

3.2.2 Homogeneity

This homogeneity test was carried out with the aim of knowing the homogeneity of the clove flower extract hand sanitizer gel by looking at the uniformity of the particles in the preparation. The three formulations of the clove flower extract hand sanitizer gel have a homogeneous composition, which is characterized by no parts that are not mixed well. From the results of research on all hand sanitizer gel formulations, it shows that the dosage formula has good homogeneity, homogeneous results and the absence of coarse grains. The homogeneity of all dosage formulas until the fourth week is still good, because the active substances are distributed evenly. The hand sanitizer gel formula shows that it has met the homogeneity requirements because there are no coarse grains and gel clumps (Ningsih et al., 2017).

3.2.3 pH

pH examination is one of the chemical tests in determining the stability of gel preparations during storage, pH stability must be considered. The pH value of the preparation that can be accepted by the skin is between 6-8 (Emma, et al., 2014). pH testing is carried out to determine the pH stability of each gel formula made according to the pH of the skin, because if it is not in accordance with the pH of the skin, it can cause irritation if it is too acidic, and can cause scaly skin if it is too alkaline. From the pH test results, it is known that the pH values obtained from the three formulas are still in the pH range of preparations

that can be accepted by the skin, so it can be ascertained that the gel produced has a pH range that is classified as safe because it is close to neutral pH and meets the specified requirements (Table 3).

Table 3. pH test results

Formulation	Concentration (%)	pH
F0	0	6,7
F1	5	5,7
F2	10	5,5
F3	15	5,2

3.2.4 Spreadability Evaluation

The dispersion test was carried out to determine the ability of the clove flower extract hand sanitizer gel to spread on the skin surface. Semi-solid preparations are expected to be able to spread easily at the site of distribution, without any significant pressure. The easier it is to be applied to the skin, the greater the contact surface area of the nutritious substance with the skin and the optimal absorption of the drug. The results of the tests carried out show that the increase in the spread area is accompanied by an increase in the given load. Semisolid preparations that are convenient to use have a spread of 5-7 cm (Garg, A, et al., 2002).

The results of the formulas made, each formula meets the specified dispersion requirements (Table 4). However, all formulas can be said to have good stability. Good dispersion causes the drug to be in contact with the skin quickly. The increase and decrease in spreadability is strongly influenced by the consistency of the gel, which is related to the viscosity value of the preparation. If the viscosity value of the preparation is high, the resulting dispersion area is low, and vice versa. This happens because the high viscosity makes it difficult for the gel to flow so that the resulting dispersion area is small (Arista, et al., 2013).

Table 4. The results of the observation of dispersion

Formulation	Concentration (%)	Average Diameter of Scatter (cm)
F0	0	5,5
F1	5	5,7
F2	10	5,9
F3	15	6

3.2.5 Viscosity Evaluation

Viscosity evaluation was carried out using a Brookfield viscometer, which is a rotary type viscometer with a cylindrical rotor (spindle) immersed in the sample. Viscosity evaluation aims to observe the viscosity of the preparation. The hand sanitizer gel preparation showed that the higher the concentration of clove flower extract added to the preparation, the lower the viscosity of the preparation. This is due to the concentration of extracts contained in the preparation. In addition to the effect of the extract gel viscosity is influenced by the concentration of CMC-Na, in the gel system CMC-Na is responsible for the formation of the gel matrix. During storage, CMC-Na can be damaged which causes changes in gel viscosity (Titaley et al., 2014). The addition of other ingredients such as propylene glycol and glycerin, which are liquid in consistency, can reduce the viscosity of the gel preparation. Viscosity observation results can be seen in the table. 5 gel preparations showed that they met the requirements in the range of 2000 – 50000 cP (centipoise) (Wasiaturrahman and Jannah, 2018).

Table 5. The results Viscosity

Formulation	Concentration (%)	Viscosity
F0	0	5640
F1	5	5300
F2	10	5020
F3	15	4803

3.3.5 Preference Test on Preparations

The preference test on the respondents was carried out through the distribution of questionnaires. The acceptability or preference test on the hand sanitizer gel preparation of clove flower extract was carried out by measuring the level of preference or hedonicity on the physical appearance of the gel prepared including color, aroma, texture and skin sensation. This research was conducted on 20 respondents to be asked to rate the aroma, shape, color filled through the questionnaire that has been provided.

Based on the acceptability or liking test conducted on 20 respondents using a questionnaire sheet, the results showed that the respondents really liked the aroma of the F2 and F3 formulas because of the distinctive aroma of cloves, then the texture of the respondents preferred because the gel texture was not too sticky, then the respondent's color parameter was less I like it because the color of the clove flower extract is less attractive, respondents only know that the hand sanitizer gel preparation is colorless or clear like the F0 preparation, so it can be concluded that the F3 preparation is preferred by the respondents because of the aroma and texture.

IV. CONCLUSION

The results of this study showed that the clove flower extract (Eugenia aromatica L.) obtained was 116.4 g and contained flavonoids, alkaloids, saponins and tannins. Clove flower extract (Eugenia aromatica L.) can be formulated into hand sanitizer gel preparations. The hand sanitizer gel preparation of clove flower extract (Eugenia aromatica L.) has a thick concentration and shows physical stability during storage. The preparation preferred by volunteers was F3 with a concentration of 15%.

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