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Formulation and Production of Ethanol Extract Derived from Black Turmeric (*Curcuma caesia Roxb*) for use as an Antibacterial Hand Sanitizer Spray

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ABSTRACT
Formulation and production of an ethanol extract derived from black turmeric (*Curcuma caesia roxb*) for use as an antibacterial hand sanitizer spray were successfully performed. The method used is the maceration method with 96% ethanol solvent. Formulation of rimpang kunyit hitam (*Curcuma caesia roxb*) ethanol extract was formulated into four formulas: without extract, 10%, 8%, and 6% ethanol extract. The results showed that the ethanol extract of black turmeric rhizome with the phytochemical screening method contained flavonoids and alkaloids, which had antibacterial activity. Formulation of rimpang kunyit hitam ethanol extract in hand sanitizer spray with a concentration of 10% has a more significant inhibition zone compared to other concentrations, namely 9.9 mm in *Escherichia coli* and 9.8 mm in *Staphylococcus aureus* bacteria.

Keyword : Antibacterial, Black Tumeric, Ethanol Extract, Hand Sanitizer Spray

ABSTRAK
Formulasi dan pembuatan ekstrak etanol yang berasal dari rimpang kunyit hitam (*Curcuma caesia Roxb*) pada penggunaan sebagai hand sanitizer spray antibakteri. Metode yang digunakan yaitu metode maserasi dengan pelarut etanol 96%. Ekstrak etanol rimpang kunyit hitam diformulasi menjadi 4 formula yaitu tanpa ekstrak, ekstrak etanol 10%, 8%, dan 6%. Hasil penelitian menyatakan bahwa ekstrak etanol rimpang kunyit hitam dengan metode skrining fitokimia mengandung senyawa flavonoid dan alkaloid yang mempunyai aktivitas antibakteri. Formulasi ekstrak etanol rimpang kunyit hitam pada hand sanitzer spray dengan konsentrasi 10 % memiliki zona hambat yang lebih besar dibandingkan dengan konsentrasi yang lain yaitu 9,9 mm pada bakteri *Escherichia coli* dan *Staphylococcus aureus* yaitu 9,8 mm.

Kata Kunci : Antibakteri, Ekstrak Etanol, Rimpang Kunyit Hitam, Hand Santizer Spray.

1 Introduction

Bacteria of various kinds can accumulate on our hands daily. We must wash our hands with soap and clean water to minimise their presence. However, the scarcity of clean water in many regions has led researchers to explore alternatives, such as antibacterial-based hand sanitizers. One such product is hand sanitizer [1].

Hand sanitizers are formulated with antimicrobial agents to promote hand hygiene and prevent the spread of germs. These portable and convenient products are designed to eliminate bacteria and other microorganisms from the hands [2]. While gel-based sanitizers are commonly used, their viscous consistency can make it difficult to dispense the recommended amount [3]. The viscous nature of gel-based hand sanitizers often makes it difficult for users to accurately determine the appropriate amount to apply [4]. Developing spray-based sanitizers with a lower viscosity to address this challenge can enhance user experience and ensure practical application.

Black turmeric is one of the medicinal plants used as a crucial substance in the manufacture of hand-sanitizer spray, which is part of traditional medicine. Black turmeric is one of the findings we often encounter around us. Usually, turmeric can be used as a spice and ingredient for conventional medicines. Black turmeric

is a new variant that has emerged as a newcomer in herbal medicine, especially in India, Pakistan and Turkey. In Indonesia, black turmeric comes from the Curcuma species, which is still less well-known [5].

According to Yadav's research (2019), black turmeric contains flavonoids by phytochemical screening method and uv-vis spectrophotometer analysis. The flavonoid compounds in black turmeric are 2,752 (mg/100 mg of dry extract) [6]. The solvent can draw the content of active ingredients in the extracted compound through extraction by maceration. Maceration is a simple extraction method that involves soaking the sample in a solvent for several days at room temperature, protected by sunlight. This process aims to extract black turmeric rhizome samples that produce a low oil yield. The advantage of this method is that the equipment used is simple and does not require expensive costs [7].

The maceration method for turmeric involves using various solvents such as water, acetone, methanol, ethanol, and petroleum ether. This study chose ethanol as the solvent due to its ability to dissolve curcumin efficiently. Curcumin, a key compound in turmeric, is highly soluble in ethanol. Ethanol was also selected for its favourable safety profile, having lower toxicity than solvents like acetone and petroleum ether [8]. Based on these considerations, the researchers are interested in exploring black turmeric as a critical component in the production of hand-sanitizer spray.

2 Materials and Methods

2.1 Materials

Black turmeric rhizome (*Curcuma caesia Roxb*) (s), aquadest (aq), glycerin (l), triethanolamine (l), Methylparaben (s), Mueller Hinton Agar (MHA) Merck (s), Nutrient agar merck (s), DMSO (Dimethylsulfoxide) Merck (l), Ethanol 96% food grade (l), $FeCl_3$ Merck 5% (l), H_2SO_4 98% Merck (l), Meyer (l), Dragendorf (l), Salkowsky (l), H_2SO_4 10% Merck (l), $CeSO_4$ 1% (s), HCL 2N merck (l), Ethanol 70% food grade (l), *Staphylococcus aureus*, *Eschercia coli* and Antis (l), rotary evaporator, autoclave

2.2 Research Procedures

2.2.1Black Tumeric Preparation

Black turmeric was washed using clean water, drained, and then cut into thin strips. The cut black turmeric rhizomes were dried by aerating. Then, it is mashed with a blender until it becomes powder and sifted, and the sieve results are stored in a closed container.

2.2.2Black Tumeric Extraction

The extraction process of black turmeric rhizome was first carried out by weighing 500 grams of black turmeric rhizome simplisia powder. Weighed black turmeric rhizome simplisia powder was put into a 5-litre beaker glass, and 96% ethanol solvent was added as much as 3000 mL. After that, it was tightly closed and extracted by maceration for 24 hours. The simplisia macerated was filtered in a rotary evaporator to remove the remaining solvent. The extract in the rotary evaporator is concentrated in a water bath.

2.2.3 Phytochemical Screening of Ethanol Extract Derived Black Turmeric

The extracts obtained were subjected to screening tests, including tannin, terpenoid, alkaloid, saponin, and flavonoid testing. A tanning test was conducted, and ethanol extract was placed into a test tube. Then, $FeCl_3$ 5% was added and shaken to obtain the blackish green. Secondly, a terpenoid test was performed in which ethanol extract was put into a test tube, and then Liebermann-Burchad was added to reach a red-brown colour. Furthermore, the obtained extract was introduced into 10 mL of aquadest and shaken to form a foam. It would confirm the saponin. In addition, magnesium powder and concentrated HCl were added to the extract to evaluate the flavonoid test. If a reddish-yellow colour solution is formed, it is positive for flavonoids.

2.2.4Making of Hand Sanitizer Spray

Making hand sanitizer spray, the first thing to do is dissolve methylparaben in glycerin, then add ethanol extract of black turmeric rhizome into a 50 mL volumetric flask. Triethanolamine is added gradually into a 50 mL volumetric flask, after which 70% ethanol is added to the limit line.

2.2.5Physical and Antibacterial Evaluation of Black Turmeric Ethanol Extract Hand Sanitizer Spray Formulation

The physical and antibacterial evaluation of black turmeric ethanol extract hand sanitiser spray formulation was conducted with some tests, such as density, viscosity, homogeneity, pH, stability, and favorability tests. In addition, an antibacterial test was performed on *Escherichia coli* and *Staphylococcus aureus* bacteria.

3 Results and Discussion

3.1 Yield of Ethanol Extract of Black Turmeric

The yield of black turmeric powder obtained from fresh black turmeric with a wet weight of 500 g using 96% ethanol solvent is 15.06%—black turmeric powder obtained from fresh black turmeric with a wet weight of 500 grams. Wet black turmeric rhizomes were dried at room temperature at 37 ºC for 4 days. The dried black turmeric rhizomes were pollinated using a blender. Making simplisia in powder form expands the surface, making it easier for simplisia to dissolve in filter substances. The powder that has been ground is sieved to obtain a uniform powder size so that the release of the active substance is evenly distributed.

Ethanol extracts of black turmeric rhizomes are made using black turmeric rhizome powder that has been mashed. Making ethanol extracts using the maceration method is quite simple. The equipment used is also simple and easy to use, and this method is suitable for compounds easily damaged by heating. In this study, the solvent used was 96% ethanol because ethanol is a universal solvent. Ethanol is also cheaper than other solvents, easy to obtain and high selectivity.

Maceration is done by soaking the fine powder of black turmeric rhizome using 96% ethanol in a glass beaker covered with aluminium foil to avoid oxidation by sunlight. The maceration process was carried out for 1 day; after 1 day, the maceration was filtered and evaporated using a rotary evaporator. Then, the concentrated extract obtained was weighed to determine the resulting yield. Yield is an essential value in product manufacturing. Yield is the product's dry weight ratio to the raw material's weight. The yield result obtained is 15.06%. The greater the yield produced, the more efficient the treatment applied by not ruling out other properties, and high yields indicate the number of bioactive components contained therein [9].

3.2 Chemical Content Identification of Ethanol Extract of Black Turmeric

Identification of the chemical content of ethanol extracts of black turmeric rhizomes was carried out by phytochemical screening methods using chemical reagents or what is often called tube reactions. The chemical content identified in this study are flavonoids, alkaloids, terpenoids, tannins, and saponins. The identification of the chemical content of ethanol extracts of black turmeric is displayed in Table 1.

3.3 Hand Sanitizer Spray Manufacture

The screening results are the first step for making hand sanitizer spray to determine the presence or absence of chemical compounds. Therefore, the next step is to make hand sanitizer spray with various concentrations, namely 6%, 8% and 10%. In addition to black turmeric as an active compound contained in the manufacture of hand sanitizer spray, there are also additional chemical compounds in the form of glycerin which functions as a gel emollient that helps the preparation of hand sanitizer spray when used on hands, not too dry, triethanolamine functions to stabilize pH and methylparaben functions as a preservative [10]. Additional ingredients should be added to each formulation as an SNI 06-2588- 1992 requirement. The prepared black turmeric ethanol extract spray hand sanitizer is shown in Figure 1.

Figure 1. The pictures of prepared black turmeric ethanol extract spray hand sanitizer

Description:
F1 : without ethanol extract
F2 : with ethanol extract of black turmeric 10%
F3 : with ethanol extract of black turmeric 8%

F4 : with ethanol extract of black turmeric 6%

After being made in the form of a hand sanitizer spray, several formulations are carried out organoleptic observations first, which are to assess the quality of the hand sanitizer spray ethanol extract of black turmeric rhizome by observing the physical appearance, which has several colors, aroma, and texture parameters.

Table 2. Organoleptical Observation Results of Each Formula

Formula	Color	Smell	Texture
1	No color	No smell	Liquid
2	Dark yellow	typical turmeric	Liquid
3	Medium yellow	typical turmeric	Liquid
4	Light yellow	typical turmeric	Liquid

Based on the observations in Table 2. It can be seen that the addition of ethanol extract of black turmeric rhizome greatly affects the colour, odour and texture of the hand sanitizer spray. The colour intensity of the hand sanitizer spray preparation increases or becomes more intense due to the increase in the addition of ethanol extract of black turmeric rhizome. The smell contained in the hand sanitizer spray preparation is a distinctive smell of turmeric which, the more the addition of ethanol extract of black turmeric rhizome, the more unique the scent of turmeric and the shape of the hand sanitizer spray preparation becomes thinner due to a decrease in viscosity.

3.4 Favorability Test

The favorability test is a test of a product by asking for responses from panellists regarding their likes or dislikes. In addition to being asked for responses about whether they liked it or not, panellists were also asked to express their level of preference using the questionnaire method. Whether or not the product is liked is influenced by aroma, texture, colour and non-sticky impression. Below are the results of the favorability test with 25 panellists.

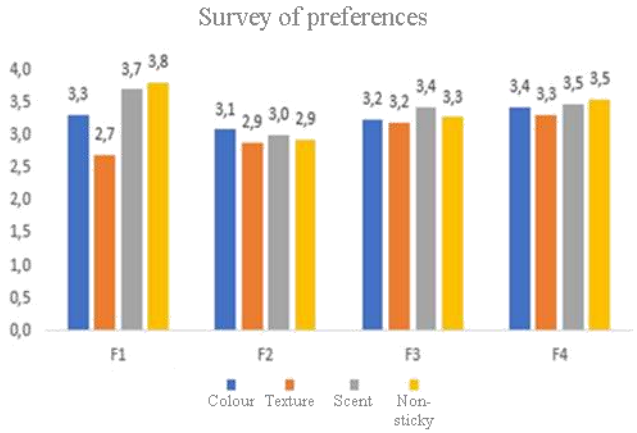

Figure 2. The graph of organoleptic test

Description:
F1 : Strongly-disliked
F2 : Disliked
F3 : Liked
F4 : Highly-favored

The results obtained from the favorability test with the questionnaire method, which was administered to 25 untrained panelists to assess 4 test parameters, showed that formulation 4 had a good impression compared to formulations 2 and 3 because the concentration of formulation 4 is smaller than that of formulations 2 and 3. Therefore, if the concentration is too high, it will affect the comfort of the panelists.

3.5 Density Test

A specific gravity test is a comparison between the weight of the substance compared to the volume of the substance. Measurement of specific gravity using a pycnometer. Mass density will affect the flow properties of the granule, where the more significant the mass density will increase its flow properties and vice versa [11]. Below are the results obtained from the specific gravity test.

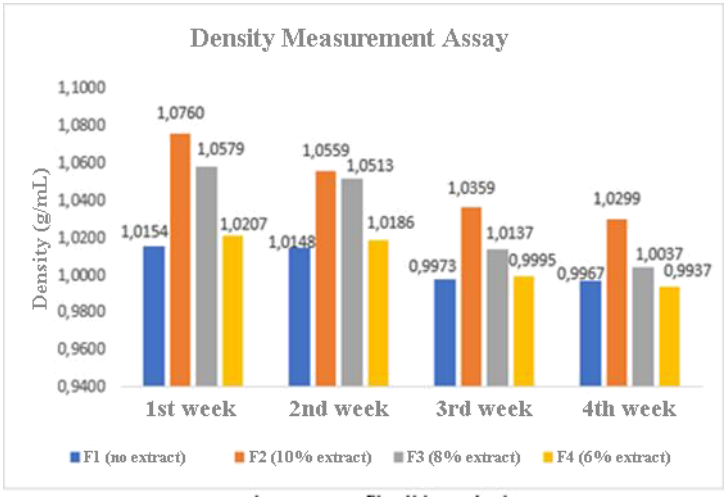

Figure 3. The graph of density test

Description:
F1 : without ethanol extract
F2 : with ethanol extract of black turmeric 10%
F3 : with ethanol extract of black turmeric 8%
F4 : with ethanol extract of black turmeric 6%

From the curve graph in Figure 3 in the viscosity test, it is known that the viscosity of the hand sanitizer spray has decreased each week, which may be due to the evaporation of several components of the hand sanitizer spray preparation. The existence of storage influences can also cause viscosity to decrease [12].

3.6 Viscosity Test

The viscosity test is a number that expresses the amount of resistance of a liquid material to flow or a measure of the shear resistance of the liquid. If a liquid's viscosity is higher, its properties will be thicker and more difficult to flow. In this study, the viscosity of the hand sanitizer preparation was tested using a tool that complies with national standards, namely the Ostwald Viscometer. The results of the viscosity test are below.

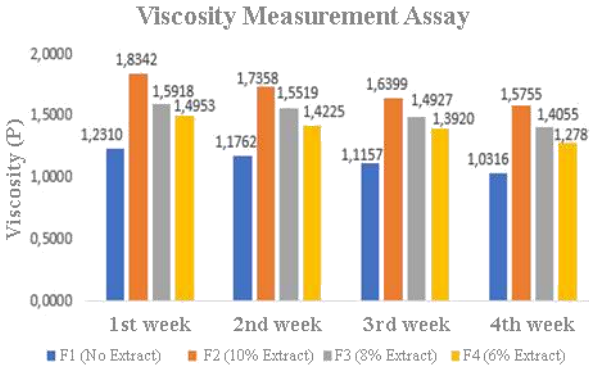

Figure 4. The graph of viscosity test.

From the curve graph in Figure 4 in the viscosity test, it is known that the viscosity of the hand sanitizer spray has decreased each week, which may be due to the evaporation of several components of the hand sanitizer spray preparation [13]. The existence of storage influences can also cause viscosity to decrease [12].

3.7 pH Test

The next test is pH using a pH meter. The pH standard based on SNI No-2588-1992 is 4.5-8.0. Based on the pH data obtained, it meets the standard. So the concentration does not affect the pH value of the hand sanitizer spray. The pH test data of hand sanitizer preparations from each formula can be seen below.

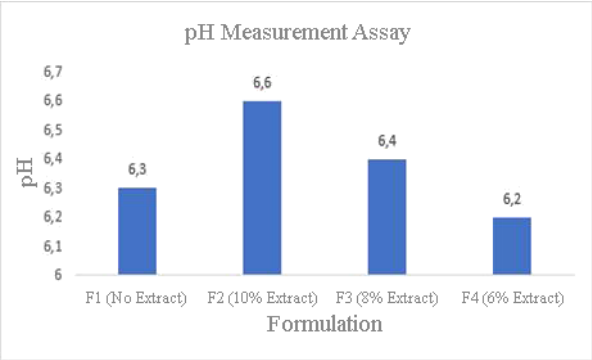

Figure 5. The graph of pH test

pH or degree of acidity is a chemical parameter used to determine the nature of the hand sanitizer spray that produces acidic or alkaline and can also be used as an indicator of irritation to the skin, which is the target of its application. Based on the table above, it can be seen that the pH of the preparation produced has a pH range of 6. The pH range of the four formulations meets SNI, which is 4.5-8.0. The presence of active substances, such as saponin compounds, can increase the pH to be alkaline; it can be seen from the graph above that the higher the concentration, the higher the pH obtained or closer to the alkaline pH. If the hand sanitizer spray is too acidic, it is feared that it will irritate the skin, but if it is too alkaline, it is feared that the skin will dry.

3.8 Homogenity Test

The preparation's homogeneity test is intended to determine whether the ethanol extract of black turmeric rhizome in the preparation is homogeneous. This result is important because homogeneity significantly affects the preparation's therapeutic effectiveness. If the preparation is homogeneous, the concentration of the active substance is assumed to be the same or uniform at the time of use or collection.

For 4 weeks, the formulation was in liquid form and tended to be homogeneous (no separation of two phases). That there were no found from formulation 1-4, the presence of small fine particles (granules) indicates that the formulation is homogeneous.

3.9 Stability Test

The stability test at room temperature includes organoleptical observations to observe changes in color and aroma. The data in Table 3 shows that the variation in concentration affects the organoleptic test; the resulting preparation should have an attractive color, pleasant smell, and good consistency to be comfortable in use.

The results obtained from the stability test are in Table 3. The spray was kept at room temperature for 4 weeks, with observations made once a week. Hand sanitizer spray is declared stable because there is no change in color and aroma. This is due to the presence of methylparaben compounds, which function as additives that function as preservatives [10].

3.10 Antibacterial Activity of Black Tumeric Extract in Hand Sanitizer Spray

Testing the antibacterial activity of black turmeric rhizome ethanol extract in Hand Sanitizer Spray preparations was carried out against E.Coli and Staphylococous aureusi bacteria by diffusion method on MHA (Mueller Hilton Agar) media, especially for the pitting method. Tests were carried out on the test samples of the four formulas (formula 1, formula 2 and formula 3 and formula 4), positive control, namely Hand Sanitizer Spray branded Antis and negative control, namely DMSO. The following is table 3.6 of the antibacterial activity test results from each formula:

Table 3 results of the antibacterial activity test show the difference in inhibition of the four formulations. This is due to the influence of differences in the content of ethanol extract of black turmeric rhizome from the

four-hand sanitizer spray formulations. The negative control in DMSO did not show any inhibition zone, meaning it did not have antibacterial activity. The order of the zone of inhibition of bacterial growth starts from the largest to the smallest, namely Formula 2, Formula 3, Formula 4 and Formula 1. The effect of the difference in the size of the inhibition zone is from the content of black turmeric rhizome ethanol extract in the hand sanitizer spray. The greater the ethanol extract of black turmeric rhizome contained in the hand sanitizer spray, the greater the inhibition zone. The criteria for antibacterial strength, according to Davis (2015), that the diameter of the inhibition zone of 10-20 mm has potent inhibition, the diameter of the inhibition zone of 5-10 mm has moderate inhibition, and the diameter of the inhibition zone less than 5 mm has weak inhibition [14].

Based on the activity test results, the formulation with high inhibitory power is at a concentration of 10% compared to other formulation concentrations. Black turmeric rhizome has active compounds in the form of alkaloids and flavonoids based on the results of phytochemical screening tests that have been carried out.

The results of the inhibition zone formed in the pits of the Hand sanitizer spray ethanol extract of black turmeric rhizomes are due to the content of active compounds in black turmeric rhizomes with antibacterial activity that can inhibit the growth of *E.Coli* and *Staphylococcus aureus* bacteria. The mechanism of action of alkaloids as antibacterials is through inhibition of cell wall synthesis, which causes the lysis of the bacterial cell wall so that bacterial growth is inhibited. The mechanism of antibacterial action of flavonoids in black turmeric rhizome extract is by binding to proteins, thus disrupting the metabolic process of bacteria; it is because the hydroxyl group contained in the structure of flavonoid compounds causes changes in organic components and nutrient transport, which will eventually lead to the emergence of toxic effects on bacteria [15].

The antibacterial activity index of *E.coli* is greater than that of *S. aureus*. *S. aureus* is a gram-positive bacterium whose cell wall consists of thick peptidoglycan, and *E.coli* is a gram-negative bacterium with a thin peptidoglycan. Peptidoglycan, contained in gram-negative bacteria, has a thinner structure than gram-positive bacteria. Peptidoglycan functions to break cell lysis, cause cell stiffness and give shape to the cell [16]. The magnitude of the antibacterial activity index on E.coli is due to the alkaloid content in the ethanol extract of black turmeric rhizome. The suspected mechanism of alkaloids is interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact, causing the cell's death [17].

4 Conclusion

Based on the results of the research that has been done, it can be concluded that the four formulations of hand sanitizer spray preparations with the concentration of adding 6%, 8%, and 10% black turmeric rhizome ethanol extract have met the SNI 06-2588-1992 standard, which contains more than 5% active substance content. The hand sanitizer spray does not change/stabilize during the stability test and is homogeneous, with a pH value of around six and with various concentrations. It has the antibacterial activity of *E.Coli* and *S. aureus* and is the most active at a concentration of 10%. The diameter of the inhibition area produced has moderate inhibition.

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6 Conflict of Interest

Authors declare no conflicts of interest

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