

Physical-chemical stability test on spray gel with active compound ethyl p-methoxycinnamate (*Kaempferia galanga* Linn.) And menthol, using combination gelling agent na cmc and copovidone

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ABSTRACT: The largest component in galangal rhizome (*Kaempferia galanga* Linn) is ethyl p-methoxycinnamate (EPMS), which amounts to 80.05%. Significant anti-inflammatory activity is exhibited by EPMS from galangal rhizome through the inhibition of carrageenan-induced mouse edema and the inhibition of IL-6 production. Various useful activities are attributed to EPMS, especially as a wound healer due to its anti-inflammatory activity. The choice of spray was made because it meets the characteristics of anti-inflammatory preparations in the wound healing process. The preparation must be moist, easily adjusted to the shape of the wound (flexible), sterile, and evenly distributed, covering the tissue, and can be easily removed from the tissue, including the wound (Holland et al., 2002). A spray gel dosage form was formulated with varying concentrations of the gelling agent Na CMC, F1 (0.5%), F2 (1.0%), and F3 (1.5%). Physical stability was evaluated at room temperature storage on days 0, 7, 14, and 21. Organoleptics, homogeneity, viscosity, pH, spray pattern, adhesive spreadability, centrifugation test, and cycling test were evaluated. Chemical stability evaluation was carried out using UV-Vis spectrophotometry to assess the EPMS levels in the preparation. The results of the physical evaluation showed that all formulas were stable in terms of organoleptics, homogeneity, and pH, which was in the range of 6.6–7.1; the weight per spray was uniform and relatively stable in centrifugation and cycling tests. The use of a combination of sodium carboxymethylcellulose and copovidone can produce a spray gel preparation of a good aromatic ginger (*Kaempferia galanga* Linn) crystal with a 1%.

KEYWORDS: Copovidone, ethyl p-methoxycinnamate, menthol, sodium carboxymethylcellulose, spectrophotometry UV-Vis, spray gel.

INTRODUCTION

Aromatic ginger (*Kaempferia galanga* L.) is a Zingiberaceae family plant with an essential oil content that has the potential to be developed as an anti-inflammatory (Lisdawati, et al., 2007). Empirically, aromatic ginger is used as a cough medicine, itching in the throat, abdominal bloating, nausea, colds, aches, compression swelling or inflammation, tetanus, and appetite enhancer (Hasanah, 2011). The aromatic ginger rhizome contains essential oils composed of various compounds (e.g., ethyl p-methoxycinnamate 58.47%, isobutyl β -2-furyl acrylate 30.90%); oxygenated monoterpenes derivatives (eg 0.03% borneol) as well as monoterpenes of hydrocarbons (eg 0.02% terpinolene) (Sukari, et al., 2008). The largest component contained in aromatic ginger rhizome is ethyl p-methoxycinnamate (EPMC) (Umar et al., 2012).

Aromatic ginger extract has been reported to have activity as a wound healer (Tara, 2006), antibacterial and antifungal (Tewtrakul, et al., 2005), to treat hypertension, rheumatism, and asthma (Sulaiman, et al., 2007). EPMC compounds have also been reported to have anti-inflammatory activity by inhibiting edema of induced carrageenan rats (Umar, et al., 2012) and also inhibiting IL-6 production (Aroonrerk, et al., 2009).

According to earlier research, gel compositions containing EPMC of the gum rhizome's most physically stable stem outperformed cream and ointment (Wardyah, 2015). The chemical stability of EPMC from the gum

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rhizome in the gel during storage is also reported to have better stability than the cream and ointment preparations so that preparations can be considered for further development (Robbani, 2015).

The advantages of a topical delivery system, especially gel preparation, provide a convenient active ingredient effect as it gives the skin a sense of coldness, easily dries and forms a film coating that is easy to wash and use (Lukman, et al., 2012). For the application of anti-inflammatory preparations in the wound healing process necessary preparations that can protect the wound and help improve the wound healing process. In general, the preparation should be moist, easily adjustable to the wound shape (flexible), sterile, and spread evenly over the tissue and can be easily removed from the tissues including the wound (Holland et al., 2002). In this case, selected menthol as enhancer penetration of the dosage that has been investigated when applied to the skin of menthol will dilate the blood vessels, causing a cold sensation followed by an analgesic effect. It is appropriate to apply to antiinflammatory preparations in wound healing processes (Rowe, et al., 2009).

Based on numerous studies, the most powerful aromatic ginger component is EPMC, which exhibit a variety of useful activities, particularly in wound healing anti-inflammatory activity. Thus, it is advantageous to be formulated into basis of topical dosage formulation, namely spray gel. Spray gel meets the characteristics of anti-inflammatory preparations for the wound healing process.

The presence of polymer combinations between sodium carboxymethylcellulose and copovidone can produce spray gel preparations with viscosities ranging from 371 to 376 cPs, but when sprayed is still flowing and the power is poorly distributed (Sheilayanti, 2015). Subsequent studies reported that when using a combination of sodium carboxymethylcellulose as gelling and copovidone forming film layers, compared to only using sodium carboxymethylcellulose alone, the results of all gel spray preparations were stable at the time of storage, did not occur syneresis and spray gel preparations which only using sodium carboxymethylcellulose is seen to result in a film coating after drying, but the film layer formed is not elastic when compared to the film coating on the preparation using a combination of sodium carboxymethyl cellulose with copovidone (Dewi, 2016).

Therefore, based on the above description the authors conducted a study on the formulation of EPMC spray gel from rhizome aromatic ginger as an anti-inflammatory on the wound healing process with variations of sodium carboxymethylcellulose as polymers forming gels and copovidone as filmmakers and test the physical stability and chemistry of the preparations.

▪ MATERIALS AND METHODS

MATERIALS

The fresh rhizome of ker, Na CMC, copovidone, propylene glycol, menthol, triethanolamine, methylparaben, propylparaben, aquadest, ethanol 96% (Technical), as well as solvents and other foaming agents such as n-hexane (Technical), ethyl acetate, methanol Technical), and methanol pro analysis.

Equipment

A grinder (Philip), analytical scales (AND GH-202), aluminium foil, filter paper, cotton, knives, maceration bottles, a vacuum rotary evaporator (Eyela), pH meter (Horiba F-52), viscometer (Brookfield LV) oven (Eyela NDO-400), refrigerator (Sanyo Medicool), centrifuge 5417R (Eppendorf), homogenizer (IKA RW 20 digital), dry vacuum pump/compressor (Welch), apparatus melting point (Stuart), Gas Chromatography - Mass Spectrometry (GCMS), UV-Vis spectrophotometer (U-2900, Hitachi, USA), glass object, hot plate (Cimarec), F254 silica gel plate (Merck Millipore, Germany), thermometer, tip and micropipette, spray bottle, capillary pipe, rulers, glass containers, mica plastics, labeled paper, and glassware in laboratories such as, glasses, measuring glass, measuring flask, funnel, watch glass, spatula, spatula, stirrer, dropper.

METHODS

Sample processing

The aromatic ginger rhizomes used as plant samples were obtained from Balitro Bogor in December 2016 and were further determined by LIPI Botanical Gardens Conservation Center, Bogor. A total of 4 kg of rhizome

samples were wet and washed with clean running water. The samples were dried and protected from direct sunlight, and drying was done until the samples were completely dry. The dried samples were then reduced to a particle size with a grinder to a powder. The 800g powder was stored in a tightly sealed container and was protected from direct sunlight for the extraction process.

Sample extraction

The aromatic ginger rhizome was extracted by the maceration method using n-hexane. The replacement of the n-hexane solvent is carried out for 1-3 days, with occasional stirring or by shaking the container. After the extraction process is complete, the extract obtained is filtered using cotton and filter paper. Then it is concentrated with a vacuum rotary evaporator at a temperature of 48-50 °C to obtain a viscous extract.

Isolation of EPMC

EPMC isolation is performed by precipitating the viscous extract at room temperature to form crystals. Then the crystals formed in the filtrate are separated by storage and purified using n-hexane, which is subsequently recrystallized by dissolving the crystals in n-hexane and a few drops of methanol, and then left at room temperature until crystalline structures are formed again. The crystals formed are separated by filtration. The EPMC crystals are then dissolved in ethyl acetate and tested for purity using TLC with an eluent n-hexane: ethyl acetate ratio of 9: 1, and the percentage of yield is calculated using the formula

percentage of yield with the formula:

$$\% \text{Rendement of n-Hexane extract of aromatic ginger rhizome} = \frac{\text{Weight of obtained extract (g)}}{\text{Initial simplicia weight (g)}} \times 100\%$$

$$\% \text{ EPMC Crystalline Rendement} = \frac{\text{Weight of obtained crystal (g)}}{\text{Weight of n-hexane extract (g)}} \times 100 \%$$

Identification of EPMC

Organoleptic

The obtained crystals are identified in color, shape, and smell (Robbani, 2015).

Melting point

The obtained crystals are identified melting point by using the melting point apparatus (Rohmah, et al., 2009).

Identification using GC-MS

a. Sample solution making

The parent solution of EPMC crystals is made with a concentration of 5000 ppm. A total of 50 mg of EPMC crystals were dissolved in 10 mL methanol pro analysis. Dilution was performed with a concentration of 1000 ppm by plucking 1 mL of a aliquots into a 5 mL measuring flask and added with methanol pro analysis on a measuring flask up to the 5 mL boundary line (Robbani, 2015).

b. Identification of EPMC compounds using GC-MS

The analysis was performed by 1 µL prepared sample injected into the GCMS tool (Umar, et al., 2012), The columns used were HP-5MS (30 m x 0.25mm ID x 0.25 µm) with an initial temperature of 70 °C for 2 min, then the temperature was raised 285 °C. Temperature MSD 285 °C. The flow rate was 1.2 mL/min with a split of 1: 100. The scanning parameters were performed from the lowest mass of 35 to 550. The retention time is 32.07 minutes (Umar, et al., 2012).

c. Preparation of EPMC spray gel

Table 2.1 Formulation of EPMC Spray Gel.

Materials	Concentration (%)		
	F1	F2	F3
EPMC	1	1	1
Menthol	0.05	0.05	0.05
Na CMC	0,5*	1*	1,5*
Copovidone	0,5*	1*	1,5*
Propylene glycol	2.7	2.7	2.7
Methylparaben	0.18	0.18	0.18
Propylparaben	0.02	0.02	0.02
Ethanol 96%	20.00	20.00	20.00
Ad Aquadest	100.00	100.00	100.00

Copovidone was dispersed in 96% ethanol, then stirred with a homogenizer at ± 200 rpm to achieve full dispersion and low viscosity (A). The sodium carboxymethylcellulose was dispersed in aquadest at 60 °C, then stirred for 30 minutes until complete dispersion was achieved, and stirred with a homogenizer at ± 350 rpm to form a clear gel (B). Mixture A was introduced bit by bit into mixture B, then stirred with a homogenizer at ± 350 rpm until homogeneous. Methylparaben and propylparaben were dispersed in 96% ethanol and introduced into mixtures A and B. The crystal was dispersed in 96% ethanol and added to the mixture. Menthol was dissolved in propylene glycol, then added to the mixture. Propylene glycol and the mixture were dispersed using a homogenizer until complete dispersion was achieved. Homogeneous preparations were then obtained, and the remaining aquadest that had been weighed was added until a predetermined weight was reached (Dewi, 2016). The resulting spray gel was then placed in a sealed container and stored at room temperature for 21 days for the evaluation of the physical and chemical properties of the preparation.

Physical evaluation of EPMC spray gel

Organoleptic

The preparations were observed organoleptically, including shape, color, and odor on days 0, 7, 14, and 21 at room temperature (Depkes RI, 1995).

Homogeneity

The preparation were examined visually by applying a preparation to a glass preparation, flattened by attaching to other glass preparations and observed. Observations were made by looking at the presence or absence of particles that have not been mixed homogeneously. Homogeneity observation were performed on days 0, 7, 14, and 21 (Aponno, et al., 2014; Depkes RI, 1995).

pH

pH of the preparation was measured using calibrated pH meter. pH measurements were performed on days 0, 7, 14, and 21 (Depkes RI, 1995).

Viscosity

The viscosity test was performed using a Brookfield LV viscometer. The preparations are prepared in a 250 mL beaker, then the spindle is lowered into the preparation to the specified limit. Measurements are carried out at a set rate of 0.3; 0.6; 1.5; 3; 6; 12; 30 rpm then reversed 30; 12; 6; 3; 1.5; 0.6; 0.3 rpm. At each measurement, the rpm difference is read when the moving red needle has stabilized. The viscosity value were then calculated. Viscosity measurements were performed on days 0, 7, 14, and 21 (Kuncari, et al., 2014; Depkes RI, 1995).

Examination of spray patterns

The Examination were done by spraying the preparation of the bottle with a distance of 3, 5, 10, and 15 cm on a sheet of mica plastic. The test was performed three times and observed the spray forming pattern, the diameter of the spray pattern formed and weight per spray (Dewi, 2016).

Spreading screening power check

The preparation was sprayed once on the upper arm of the skin from 3 cm. Once sprayed, it calculated for 10 seconds to see if the dye was attached or drops from the spray dripped down (Suyudi, 2014).

Testing weight per spray

The test can be done by weighing the initial weights of the dosage, spraying the dosage five times, then weighing the weight of the preparation in the container after spraying (Suyudi & Rajab, 2014 ; Nawal A,2013 ; Dewi, 2016) . Thereafter, the volume of delivery of each spray preparation was calculated using the equation

$$AL \frac{Wt-Wo}{Da}$$

AL is the weight of dosage delivered per spray. Wt is the weight of the preparation after spraying. Wo is the initial weight of dosage before spraying and Da is the number of sprays (Suyudi & Rajab, 2014 ; Nawal A,2013 ; Dewi, 2016).

Centrifugation test

The preparations were inserted into the Eppendorf tube, then fed into a centrifuge at a rate of 5000 rpm for 30 minutes. After centrifugation physical condition of the preparation was observed, such as whether there is syneresis after testing (Sheilayanti, 2015).

Cycling test

The preparation was stored at a temperature (4±2 °C) for 48 hours and continued by storing the dosage at (40 ± 2 °C) for 48 hours (1 cycle). The test was done 3 cycles and physical changes of the preparation was observed at the beginning and end of the test which includes organoleptic, homogeneity, viscosity and pH (Djajadisastra, et al., 2009).

Measurement of EPMC concentrations in spray gel during storage creation of calibration curves

10 mg of ethyl p-methoxycinnamate crystals were dissolved in 100 mL of n-hexane to make 100 ml of aliquots. The aliquots were extracted by vortex for 10 minutes and centrifuged at 3000 rpm for 10 minutes (Reddy, et al., 2015) with modification. The aliquots were diluted and a series of concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, 6 ppm, 7 ppm and 8 ppm were prepared. Then determined maximum wavelength at one concentration, a concentration of 7 ppm. The uptake in each series of concentrations was measured at the wavelengths that were obtained at λ max, and the calibration was made. The linear regression equation obtained from the calibration curve was then used to calculate the sample concentration on the determination of ethyl p-methoxycinnamate in the preparation (Charinna, 2015) with modification.

Percent recovery (Accuracy)

Measurements were performed by UV-Vis spectrophotometry with standard addition methods including the preparation of standard EPMC solutions and the determination of EPMC levels in the preparation. The spray gel preparations extracted as much as 0.5 g were dissolved in 50 mL of distilled n-hexane. Then the dissolved preparation was transferred into centrifugation tube for vortex for 10 minutes and centrifuged at 3000 rpm for 10 minutes (Reddy, et al., 2015 with modification). Thereafter, dilution of the extracted solution was prepared with a concentration of 10 ppm. The absorption was performed using a UV-VIS spectrophotometer. The resultant absorption was subtracted by the blank uptake (base of the preparation) and substituted to the linear equation obtained from the calibration curve to obtain the concentration value and calculated average (Celeber and Altnoz, 2007) with modification.

Preparation of standard EPMC solution was done with EPMC weighed 7 mg, then dissolved in a 10 mL measuring flask and added n-hexane to a concentration of 700 ppm. The solution will then be used as a standard solution in the standard method of addition. Taken as much as 100 μ l of standard solution and feed

into a 10 mL measuring flask, then the solution was satisfied with the pre-made sample solution to the limit. The Solution in a vortex and its absorption test using UV-vis spectrophotometer. The resultant uptake was then substituted into the linear equation obtained from the calibration curve to obtain the concentration value and calculated percent recovery of the formula:

$$\% \text{ Recovery} = \frac{[C]_{\text{Sample+Standard}} - [C]_{\text{Sample}}}{[C]_{\text{Standard}}} \times 100\%$$

Workmanship as much as three times (triple) (Wardani, 2012 with modifications). The permissible error range at 1% analytical concentration is 97-103% (Yuwono and Indrayanto, 2005).

Precision

Precision was expressed by calculating the standard deviation (SD) value to produce the Relative Standard Deviation (RSD). From the absorbance data obtained on the determination of the ethyl *p*-methoxycinnamate content of the preparation was calculated the standard deviation and Relative Standard Deviation with the formula:

$$SD = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n-1)}}$$

Information : x_i = a single measurement
 \bar{x} = average
 n = number of measurements

Relative Standard Deviation (RSD) based on Sumardi (2005) is calculated by the formula :

$$\% \text{ RSD} = \frac{SD}{\bar{x}} \times 100\%$$

Linearity and range

Measurement of linearity was done with a standard solution consisting of 8 concentrations and then the absorption obtained from each concentration made linear regression so that linear response can be obtained to the concentration of a standard solution with correlation coefficient value close to 1 or above 0.995 for a good analytical method. linear regression analysis $y = bx \pm$ the curve between absorbance and concentration, the relationship must be positive, and the requirement for linearity is based on the R^2 value. A value indicates the sensitivity of the instrument analysis used (Harmita, 2004).

Determination of EPMC content in stock

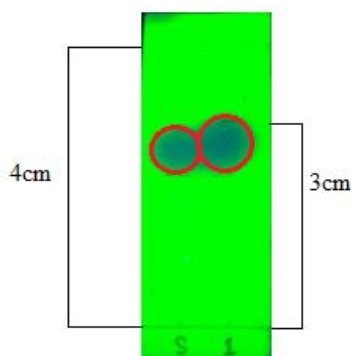
The determination of ethyl *p*-methoxycinnamate was performed by UV-Vis spectrophotometry. The spray gel preparations extracted as much as 0.5 g were dissolved in 50 mL of distilled *n*-hexane. Then the dissolved preparation was transferred into a centrifugation tube for vortex for 10 minutes and centrifuged at 3000 rpm for 10 minutes (Reddy, et al., 2015 with modification). Thereafter, a dilution of the extracted solution with a concentration of 7 ppm was prepared. For each formula, the absorption test was determined using a UV-VIS spectrophotometer. The resultant absorption was then subtracted by the blank uptake (base of the preparation) and substituted to the linear equation obtained from the calibration curve to obtain the concentration value. The ethyl *p*-methoxycinnamate content in each formula is determined in percent by dividing the actual concentration results by the theoretical concentration multiplied by one hundred percent. Workmanship three times (triple) and measurement of levels performed on days 0, 7, 14 and 21.

RESULTS AND DISCUSSION

Organoleptic

An organoleptic examination of the crystalline ethyl *p*-methoxycinnamate isolate was conducted to confirm its identity. The crystals shape appeared like thin needles with a distinctive weak and white aromatic odor.

Result of isolation EPMC



(S) standard EPMC, (1) EPMC Isolate
Figure 1. The isolated EPMC with TLC

Measurement of melting point

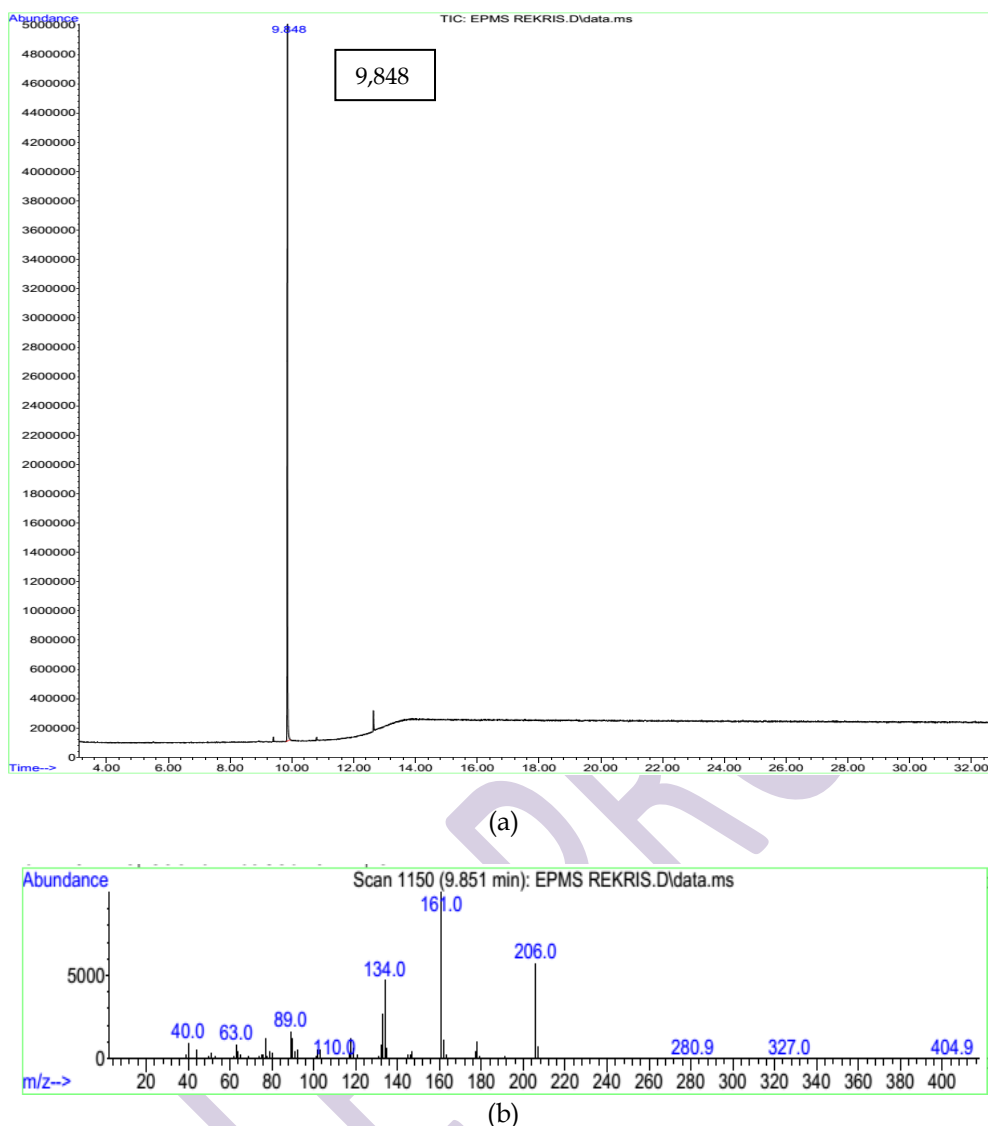
Examination of melting point using melting point apparatus showed that the result of EPMC melting point which has been recrystallized with the triple test is 49 °C. The results are by the pure melting point EPMC value of 49-50 °C (Umar, et al., 2012).

Table 3.1 Melting point.

Testing	Melting Point Results (°C)
1	49
2	49
3	49
Average	49

EPMC Analysis using GC-MS

The EPMC used GC-MS to identify and test the purity of EPMC crystals from isolation. The obtained GC-MS interpretation results showed that EPMC crystals had a retention time of 9.848 minutes, with a molecular weight of 206.1 and a mass fragment of 161, 134, 110, 89, 63, and 40. Meanwhile, GC-MS interpretation of standard EPMC crystals appeared at a retention time of 9.913 minutes, with a molecular weight of 206.0 and a mass fragmentation of 161, 134, 118, 89, 77, 63, and 51.



(a) retention time, (b) mass fragmentation and molecular weight

Figure 2. The isolated EPMC chromatogram (a = GC -Chromatogram of, b = MS Spectrum of EPMS peak)

Based on the interpretation and Umar et al. (2012) reported that EPMC compounds emerged (appeared) at the retention time of 9.9 minutes with the molecular weight Ion molecule at m/e of 206.4 and mass fragmentation fragments at m/e 161, 134, 118, 89, 77, 63, and 51. The isolated EPMC crystals were concluded to be pure, with the retention time, fragmentation, and percent value of EPMC crystalline areas of isolation equal to the retention time, fragmentation, and percent value of the standard EPMC crystal area of 100%.

Physical evaluation results of the EPMC spray gel

Organoleptic, turbidity, and air bubbles examination

The results of the organoleptic examination showed that the formulas of 1,2 and 3 resulted in the preparation of spray gel with the active substance of ethyl p-methoxycinnamate, having a distinctive odor of alcoholic odor, and the dosage form of a slightly viscous (F1 and F2), and viscous liquid gel (F3). The three dosage formulations produced an organoleptically stable gel at room temperature (27°-28°C) both on days 0, 7, 14, and 21. This is because the preparation did not change the smell, color, shape, and growth of the fungus.

The result of turbidity inspection of third gel spray gel formulas at room temperature (27-28 °C) both on days 0, 7, 14, and 21 there was no turbidity, it showed that the components in the formula can be mixed into one phase so that the spray gel preparation looks clear and transparent. Meanwhile, the air bubble examination results showed the three formulas from day 0 to day 21 did not see air bubbles trapped in the

preparation. This is because of the treatment of silent preparations for 24 hours at room temperature after manufacture. The longer the storage period, the number of trapped air bubbles will decrease (Dewi, 2016).

Homogeneity examination

Homogeneity checks were performed from day 0, 7, 14, to day 21. Aims to see the particle distribution of the preparations (Mumtihanah, 2015). In the homogeneous examination results of the three formulas at room temperature (27-28°C) on days 0, 7, 14, and 21 indicated that each preparation remained homogeneous and had evenly distributed particles. This is because in all dosage formulas there is no coarse grain and shows a homogeneous arrangement or there is no gel former still clot in the preparation.

pH examination

Table 3.2 Examination pH results

Day	pH ± SD		
	Formula 1	Formula 2	Formula 3
0	6.992±0.107	6.979±0.044	6.870±0.171
7	6.965±0.918	7.181±0.064	6.686±0.006
14	6.815±0.500	7.145±0.113	6.762±0.100
21	6.725±0.052	6.633±0.097	6.655±0.026

*Formula 1 (Gel preparation with 0.5% Na CMC)

*Formula 2 (Gel preparation with 1.0% Na CMC)

*Formula 3 (Gel preparation with 1.5% Na CMC)

Examination of pH spray gel during storage was done to find out whether the pH of the gel preparation is consistent with the pH of the skin (Mumtihanah, 2015). The results showed a comparison of the pH values of the three dosage formulations during storage and observations made on days 0, 7, 14 and 21. The pH values of F1 and F2 decreased on day 21, while F3 decreased on day to day 7 and 21. The decrease in pH may be due to environmental factors such as temperature and poor storage but the decrease is not significant, so it is not too influential. The pH measurements in each of the formulas show an inverse relationship between base concentration and pH. The higher the concentration of the base used, the pH of the preparation will be lower (Wijayanto, 2013).

The pH values of the preparations ranged from 6.63 to 7.14 and skin pH values were ranged from 4.5 to 7.00 (Lukman et al., 2012). Sustainability of skin pH with topical pH dosage affects skin acceptance of the preparation. Based on Table 3 data indicates that the EPMC gel spray preparation has pH values that fall within the normal range of skin pH, so it is expected to be accepted by the skin and not cause irritation.

Viscosity

Measurement of the viscosity of the preparation is done to determine whether or not the dosage is applied on the skin surface. In this case, the viscosity examination on the spray gel preparation aims to find out whether or not the spray gel preparation can be delivered through a spray applicator. The spray gel preparation has a low viscosity value with the aim of facilitating the application by spraying (Shafira, et al., 2015).

Table 3.3 Viscosity measurement results

Day	Viscosity(cPs)		
	Formula 1	Formula 2	Formula 3
0	347.14	1.868	10.866
7	168.43	1.644	9.833
14	160.71	1.444	8.150
21	151.428	1.440	7.100

*Formula 1 (Gel preparation with 0,5% Na CMC)

*Formula 2 (Gel preparation with 1,0% Na CMC)

*Formula 3 (Gel preparation with 1,5% Na CMC)

At day 0, F1 exhibited a low viscosity of 347.14 cps, which provides a consistency that is easy to dilute and drip when sprayed. This is due to the low Na CMC concentration of 0.5%. On F3 day 0, the viscosity of F2

was 1,868 cps, which met the criteria of viscosity range of 500-5000 cps for spray gel preparation (Nisak, 2016). Meanwhile, on day 0, F3 viscosity was found to be 10.866 Cps, had thick consistency and was difficult to spread when sprayed. This is due to the excessively high Na CMC viscosity, when Na CMC is introduced into water, Na⁺ releases and is replaced with H⁺ ions and forms HCMC which increases the viscosity (Mappa, 2013). During the 21 days viscosity of the dosage stored was decreased, especially F1.

Study of spraying patterns and weight per spray

Examination of the spray pattern aims to evaluate the quality of the applicable spray applicator. The close spray will give a more uniform spraying result with finer drops (Ngudi, 2014).

The result of the spray pattern examination of formulas 1, 2, and 3 varies, the spraying distance is directly proportional to the diameter of the spraying pattern of the preparation, the greater the spraying distance the greater the spraying pattern is produced. The spraying pattern of formulas 1 and 2 tended to produce an elongated and diffuse pattern. While the spraying pattern in Formula 3 tended not to spread and only to be on a single point straight from a small, shaped spray with an average diameter of 0.6 - 1 cm. This is because of formula 3 with a concentration of 1.5% Na CMC gelling agent, having the viscosity cPs, where the viscosity of the preparation is too high for the spray gel preparation. The increase of Na CMC concentration affects viscosity and pressure required to spray the gel, thus high concentration of Na CMC causes more difficult to spray (Kamishita, et al., 1992).

Table 3.4 Average weight per spray

Formula	Average Weight / Spray \pm SD (g)
1	0,1345 \pm 0,0010
2	0,1341 \pm 0,0080
3	0,1446 \pm 0,0007

*Formula 1 (Gel preparation with 0,5% Na CMC)

*Formula 2 (Gel preparation with 1,0% Na CMC)

*Formula 3 (Gel preparation with 1,5% Na CMC)

The results of the delivery weighting for each spray showed no significant differences between the formulas. This shows the effectiveness of the applicator used in delivering a reproducible amount of the gel in each spraying formula (Rajab, Nawal., 2013).

Examination of spreading pattern of pattern

The scattering test aims to determine the rate of dissemination and to ensure even distribution of the dosage when applied to the skin (Mappa, et al., 2013). On the results of the prolonged scattering test results of the three formulas showed the dosage less adhered after being sprayed on the upper arm for 10 seconds, but can form a strong layer attached to the non-flowing skin. Formula 1 and formula 2 showed more evenly spread than formula 3 shows that the preparations do not spread but only accumulate at one spray point. This is because the formula 3 has the highest viscosity so it is less suitable to be intended as a spray gel preparation.

Good scattering causes the contact between the drug and the skin to become widespread, so that drug absorption to the skin is rapid. The viscosity of a preparation greatly affects the extent of its spreadability (Aryani, 2015).

Centrifugation test result

A centrifugation test or mechanical test is performed to determine the separation of the phases of the preparation. The centrifugal force effect given at a speed of 5000 rpm for 30 minutes equals the gravitational force the spray gel receives on storage for a year. The results of the centrifugation tests of formulas 1, 2, and 3 showed that the spray gel preparation did not undergo phase separation. Based on the above, it showed the three formulations of stable gel spray preparations so that syneresis does not occur.

Cycling Test Results

The cycling test is a stability test as a simulation with the change of temperature every year even every day. A cycling test is also conducted to compare the physical condition of the dosage form from the previous (Anwar, et al., 2014). Testing with this method is done at temperature and or humidity at certain time interval so that the product in its packaging will experience varying stress.

The organoleptic examination of the three formulas did not show any phase separation phenomenon. This is because the active substance and base of the spray gel are mixed homogeneously (Buang, 2013). The organoleptic examination of the cycling test method with the addition of EPMC from the aromatic ginger rhizome in the three formulas yield a clear gel preparation, has a distinctive odor of alcohol, and has a variation of the dosage form of slightly thicker, less viscous and viscous liquors respectively in F1, F2 and F3.

The results of turbidity and air bubbles showed turbidity and air bubbles trapped in dosage in all three formulas. Organoleptically there were also no change in the form of clear viscous or transparent viscosity and the absence of air bubbles trapped in the preparation. But on F3 the 21st day showed the color change from the clear to the yellow. It shows that different temperatures cause organoleptic changes. Meanwhile, different temperature conditions at 4°C and 40°C result in fixed air bubbles that did not decrease during testing.

pH testing was conducted on all the three of spray gel formulas. The results showed that the pH values of each of the formulas in the preparation were in the range of 6.5-7 both before and after the cycling test. the physiological pH range of human skin ranges from 4.0 -7.0 and the results of this test was in the range of physiological pH required of human skin (Anief, 2007). The viscosity test in Table 5 showed that the three dosage formulations have decreased viscosity after being treated with different temperature displacements i.e. temperature 4 °C and temperature 40 °C. However, formula 2 still showed the viscosity value corresponding to the spray gel viscosity value range of 500-5000 cPs. Meanwhile, the formula 1 and 3 does not meet the range of viscosity values required which refers to the literature.

Overall testing of the cycling test method, organoleptic examination, homogeneity examination, pH testing, and viscosity testing showed that physically the formula 1, 2, and 3 spray gel did not change when in the refrigerator with 4°C temperature and while in oven with a temperature of 40°C. However, in formula 3 there is an organoleptic change that is the color of the preparation from the nodes to yellowish and also the pH test results change although the change is still within the range of literature. Formulas 1 and 3 on the viscosity test also show results that do not meet the range of the literature range.

Table 3.5 Result of pH of cycling test method

Formula	pH		Average pH ± SD
	Early	End	
1	6.982	6.851	6.917 ± 0.092
2	6.946	6.914	6.930 ± 0.023
3	6.829	6.740	6.785 ± 0.063

*Formula 1 (Gel preparation with 0.5% Na CMC)

*Formula 2 (Gel preparation with 1.0% Na CMC)

*Formula 3 (Gel preparation with 1.5% Na CMC)

Table 3.6 Result of viscosity of cycling test method

Formula	Viscosity (cps)		Average Viscosity (cPs) ± SD
	Early	End	
1	294.64	209.28	251.96±60.36
2	1.844.00	1.684.00	1764.00±113.14
3	10.333.00	9.112.00	9722.50±863.38

*Formula 1 (Gel preparation with 0.5% Na CMC)

*Formula 2 (Gel preparation with 1.0% Na CMC)

*Formula 3 (Gel preparation with 1.5% Na CMC)

Result

EPMC calibration curve in n-Hexane

The calibration curve was made from EPMS absorbance measurements in n-hexane with various concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, 6 ppm, 7 ppm and 8 ppm at wavelength 318.2 nm. The use of n-hexane as a solvent for the extraction process is due to EPMS polarity closer to n-hexane, whereas EPMC has two clusters supporting the non-polar properties of ether and benzene, whereas the polar supportive group is only one that is the presence of carbonyl in the cluster ether (Taufikurohmah, et al., 2008). Then the absorbance value obtained is made into a calibration curve as in Appendix 15. The curve has regression value $R^2 = 0,9997$ and linear equation $y = 0,094x + 0,006$. The linear equation is used in the calculation of EPMC in spray gel preparation.

Percent recovery percentage (accuracy)

The percent measurement of recovery was done by standard addition method. Carried out by adding a certain amount of concentration of known EPMS standard into the sample and treating it the same as the sample test. Here are the results of the percent recovery measurements.

Table 3.7 Percent recovery result

Test	% Recovery
1	99,3
2	98,6
3	98,6
Average	98.8

The average yield of percent recovery showed that the measured values are within the required range of 98.8%. Meanwhile, the analytical content of ≥ 1 the average percentage of allowable re-entry is 97-103% (Yuwono & Indrayanto, 2005). The results of the percent recovery measurements showed the UV-Vis spectrophotometry method using n-hexane solvent has good accuracy for determination of EPMC content in the preparation.

Precision measurement

Table 3.8 Precision calculation result (cons. 7 ppm)

Formula	Average of Absorbance	SD	%RSD
1	0.67	0.026	0.379
2	7.01	0.002	0.040
3	0.67	0.001	0.015

Good precision is expressed by a smaller percentage of RSD and also provides a standard deviation of 2% or less of variation or coefficient of variation and $RSD \leq 15\%$. Based on the results of the table above showed that the value of Standard Deviation (SD) and Relative Standard Deviation (RSD) meet the range of conditions specified. The RSD value of $\leq 1\%$ indicates that the percentage of RSD is very through (Sumardi, 2005). Standard Deviation (SD) results also show a value of $\leq 2\%$.

Linearity and range measurement

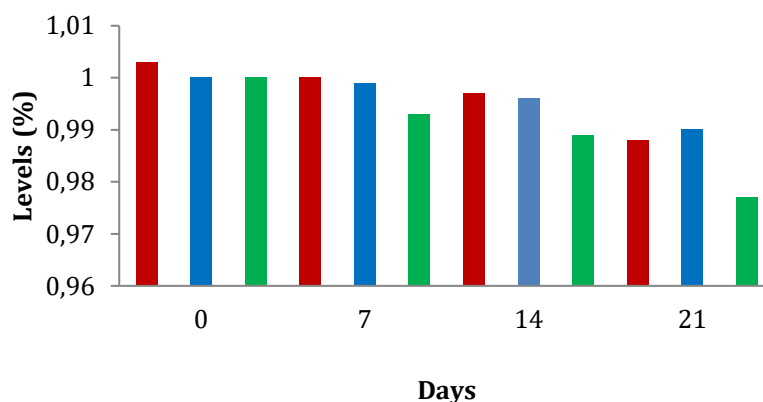
Linearity measurement is done to know the standard capability, so can prove the existence of a linear correlation between analyte concentration with detector response. As the parameter of the linear relationship, using the relation coefficient (r) in linear regression analysis $y = bx \pm a$ (Harmita, 2004). Appendix 16 showed the result of the linear regression equation $y = 0,094x + 0,006$ with R^2 that is 0,997 indicating that the resulting linearity relationship is good. A good analytical method obtained a linear response to the concentration of the standard solution with a correlation coefficient value close to 1 or above 0.995 (Harmita, 2004).

Assay of EPMS

The following is the result of measuring EPMS levels in spray gel preparations for 21 days.

Table 3.9 Result of EPMC on Days 0, 7, 14, and 21

Day	Formula	EPMS Levels \pm SD(%)
0	1	1.001 \pm 0,002
	2	1.000 \pm 0,010
	3	1.000 \pm 0,003
7	1	1.000 \pm 0.005
	2	0.999 \pm 0.002
	3	0.994 \pm 0.002
14	1	0.997 \pm 0,013
	2	0.996 \pm 0,007
	3	0.989 \pm 0,002
21	1	0.988 \pm 0.001
	2	0.990 \pm 0.001
	3	0.976 \pm 0.010

**Figure 3.** The EPMC Levels

Results of EPMS measurements in the spray gel preparations on days 0, 7, 14 and 21 both formula 1, 2, and 3 appear to have decreased stability marked by the decrease in graph and EPMC levels (Table 7). On the 21st day, there was a significant decrease compared to the decrease on the 7th and 14th days, i.e. in formula 1 of 0.015%, formula 2 of 0.01% and formula 3 of 0.023%. Meanwhile, for chemical stability of the preparations for more than 21 days of storage needs further study.

Based on the calculation of levels (Table 9) and Graph 1. shows the lower viscosity of the spray gel preparation, the components of the chemical compounds contained therein are more stable. In this study showed formula 1 had the lowest viscosity, while formula 3 had the highest viscosity. However, when viewed from the amount of decrease in the formula 2 has a more stable decline.

CONCLUSION

The results showed that all formulas are stable in terms of organoleptic, and homogeneity, have a pH range of 6,0-7,1, have similar weight per spray, and are relatively stable in the centrifuge test and a cycling test. The use of a combination of sodium carboxymethylcellulose and copovidone can produce a spray gel preparation of a good aromatic ginger (*Kaempferia galanga* Linn) crystal with a 1%.

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