Sains Malaysiana 51(5)(2022): 1411-1424
*http://doi.org/10.17576/jsm-2022-5105-12*

# Anti-Inflammatory Activity of *Alpinia malaccensis* (Burm. f.) Roscoe and *Kaempferia galanga* L. Rhizome Essential Oil Gel Formulations by Carrageenan Induction Method

(Aktiviti Anti-Radang *Alpinia malaccensis* (Burm. f.) Roscoe dan *Kaempferia galanga* L. Rizom Formulasi Gel Minyak Pati melalui Kaedah Aruhan Karaginan)

MUCHTARIDI MUCHTARIDI[1,*], DERIF A. ABDULLAH[1], CECEP SUHANDI[1], SRI A. SUMIWI[2] & NUR KUSAIRA KHAIRUL IKRAM[3,4]

[1]*Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy Universitas Padjadjaran, Bandung, Indonesia*

[2]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy Universitas Padjadjaran, Bandung, Indonesia*

[3]*Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Federal Territory, Malaysia*

[4]*Centre for Research in Biotechnology for Agriculture (CEBAR), 50603 Kuala Lumpur, Federal Territory, Malaysia*

*Received: 6 September 2021/Accepted: 14 October 2021*

ABSTRACT

Bioactive compound from essential oils of *Alpinia malaccensis* (AM) and *Kaempferia galanga* (KG) rhizomes, respectively, such as methyl cinnamate and ethyl-p-methoxycinnamate (EPMS) have been reported to have anti-inflammatory activity. The present study aimed to determine the topical anti-inflammatory activity of 3 gel formulations (Formula 1, 2 and 3) having different concentrations of essential oil from AM and KG rhizomes. The gelling agent used in this study was Carbopol 934. The physicochemical parameters of the gel formulations such as organoleptic, pH, viscosity, and spreadability as well as hedonic test were also examined. The anti-inflammatory activity assay was carried out using the carrageenan induced rat paws edema method. The chemical composition of AM and KG rhizomes was identified by GC-MS with LRI index. The major essential oils content in AM and KG rhizome was methyl cinnamate (58.80%) and ethyl-p-methoxy cinnamate (59.68%), respectively. Based on the results of physical evaluation, hedonic test and anti-inflammatory activity, Formula 1 with 5% AM and 2.5% KG essential oils is the best formula with % inflammation inhibition of 36.32 ± 6.32%.

Keywords: *Alpinia malaccensis*; anti-inflammatory; essential oil; gel preparations; *Kaempferia galanga*

ABSTRAK

Sebatian bioaktif daripada minyak pati rizom *Alpinia malaccensis* (AM) dan *Kaempferia galanga* (KG) seperti metil sinamat dan etil-p-metoksisinamat (EPMS) telah dilaporkan mempunyai aktiviti anti-radang. Kajian ini bertujuan untuk menentukan aktiviti anti-radang topikal daripada 3 formulasi gel (Formula 1, 2 dan 3) yang mempunyai kepekatan minyak pati yang berbeza daripada rizom AM dan KG. Agen gel yang digunakan dalam kajian ini adalah Carbopol 934. Parameter fizikokimia daripada formulasi gel seperti organoleptik, pH, kelikatan dan daya sebaran serta ujian hedonik juga diperiksa. Ujian aktiviti anti-radang dilakukan menggunakan kaedah karaginan yang menyebabkan kebengkakan pada tapak kaki tikus. Komposisi kimia daripada rizom AM dan KG dikenal pasti oleh GC-MS dengan indeks LRI. Kandungan minyak pati utama dalam rizom AM dan KG adalah metil sinnamat (58.80%) dan etil-p-metoksisinnamat (59.68%). Berdasarkan hasil penilaian fizikal, ujian hedonik dan aktiviti anti-radang, Formula 1 dengan minyak pati 5% AM dan 2.5% KG adalah formula terbaik dengan % perencatan keradangan sebanyak 36.32 ± 6.32%.

Kata kunci: *Alpinia malaccensis*; anti-radang; *Kaempferia galanga*; minyak pati; sediaan gel

## Introduction

Inflammation is part of the body's immune response towards tissue injuries caused by physical trauma, chemical substances, or microbial infection (Chen et al. 2017). Such response is via deactivation of invading organisms in the tissues, elimination of irritants and regulation of tissue repair (Woodell-May & Sommerfeld 2020). The most common inflammation treatment is the NSAIDs (Non-Steroid Anti-Inflammatory) drugs (Barkin 2015). However, NSAIDs cause side effects such as gastric mucosal damage (Matsui et al. 2011). To overcome this shortcoming, the topical formulation was a great alternative that could minimize gastric side effects. However, this strategy still has disadvantages because topical NSAID exhibits poor bioavailability and it could lead to drug accumulation in the area of inflammation even after remission (Rannou et al. 2016). Therefore, the discovery of new potent anti-inflammatory active compounds with fewer side effects is important in anti-inflammatory drug discovery. The rhizomes of *Alpinia malaccensis* [Burm. F.] (AM) has been reported to have antipyretic, analgesic, and anti-inflammatory effects (Sethi et al. 2017). Besides AM, *Kaempferia galanga* L. (KG) has also been reported to have anti-inflammatory effects (Jagadish et al. 2016; Sulaiman et al. 2008; Umar et al. 2012).

Methyl cinnamate and ethyl-p-methoxycinnamate in the rhizomes of AM and KG, respectively, have anti-inflammatory activity (Gui et al. 2018). Ethyl-p-methoxycinnamate of KG inhibits inflammation by suppressing interleukin-1β, tumor necrosis factor-α, and angiogenesis hence blocking the endothelial functions (Umar et al. 2014). While, methyl cinnamate inhibits the cytokine interleukin-1β that plays role as a mediator of the inflammatory response (Lima et al. 2013; Lopez-Castejon & Brough 2011). Since both ethyl-p-methoxycinnamate and methyl cinnamate inhibit interleukin-1β, the combination of both in one preparation could exhibit potent anti-inflammatory activity.

The combination of both essential oils; ethyl-p-methoxycinnamate and methyl cinnamate for topical dosage as an anti-inflammatory agent has yet been reported. Study on the combined effect of both essential oils is necessary to produce topical gel products containing essential oils as aromatherapy that can be absorbed through the skin and olfactory system (Muchtaridi et al. 2011).

In previous studies, physical evaluations have been performed including organoleptic, homogeneity, pH, viscosity, and dispersibility of 6 formulas based on variations in a gel base for 28 days of storage. The gel base used included Hydroxypropyl Methyl Cellulose (HPMC) with concentration of 4, 6, and 8% and Carbopol 934 with a concentration of 0.5, 0.75, and 1%. From this evaluation, the best gel base formulation based on the optimization results was carbopol 934. Further physical evaluation was performed using gel base carbopol 934 at a concentration of 0.5, 0.75, and 1% in combination with 1% AM and 0.5% KG essential oil and the best formula selected for anti-inflammatory activity was 0.75% Carbopol gel base (Fitriani 2018).

The anti-inflammatory activity was performed by preparing the gel containing AM and KG essential oils into 3 dosages using the carrageenan induction method on male white rats (wistar strain). Sethi et al. (2017) reported that oral dose of AM rhizome extract reduced inflammation up to 32.69% at a dose of 100 mg/kgBW with the carrageenan induction method. The oral dose using KG rhizome extract also shows a reduction of inflammation at a dose of 18 mg/kgBW with 42.24 ± 6.19% reduction; 36 mg/kgBW, 40.08 ± 4.65% reduction and 45 mg/kgBW with 32.62 ± 3.1% reduction (Hasanah et al. 2011). According to Soeratri et al. (2014), the topical anti-inflammatory dose of KG essential oil was determined by comparing the anti-inflammatory activity of 1% Na-Diclofenac solution with 5.59% ethyl-p-methoxycinnamic acid (EPMS). Ethyl-p-methoxycinnamic (EPMS) is the main ingredient in KG's essential oil, thus the effective dose for KG's essential oil in gel preparations is 5% (Soeratri et al. 2014). In this study, an anti-inflammatory topical dosage form was prepared to determine a systemic effect that is absorbed into the blood vessels of the skin. This is similar to the oral administration of anti-inflammatory drugs that are absorbed through the intestinal mucosa (Hua 2020).

The composition of AM rhizome essential oils was determined by comparing the oral dose of 100 and 45 mg/kgBW with ratio 2:1 for the oral dose of AM:KG. Therefore, the topical dose of KG and AM's essential oil is 5 and 10%, respectively. Furthermore, a gel formulation with 3 variations of dosage for the essential oil of AM:KG was prepared based on a ratio of 2 :1 consisting of Formula I (concentration of essential oil of AM 5% and KG 2.5%); Formula II (concentration of essential oils of AM 10% and KG 5%) and dose III (concentration of essential oils of AM 20% and KG 10 %).

## Materials and Methods

### Plant material

The rhizome of AM (*Alpinia malaccensis* (Burm. f.)) Roscoe and KG (*Kaempferia galanga* L.) were

obtained from the Pangandaran and Sumedang, West Java, respectively. Both plants were identified in Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran by Joko Kusmoro with registration numbers 063/HB/II/2019 and 064/HB/II/2019, respectively. The two rhizomes were sorted, washed, and dried in indirect sunlight. Distillation of essential oils from the rhizomes of AM and KG was carried out by the steam distillation method. First, a pot with a porous cover was placed on the heating stove and filled with 5 L of water until it reached just below the porous cover. Then, AM and KG were placed onto the porous cover. The pot was heated at 25 °C and distillation was carried out for 7 h. The resulting distillate was collected and kept in a vial, then stored at 4 °C.

### CHEMICALS

The chemicals used in this study were aquadest, glycerin (Brataco, Indonesia), carbomer 934 (Merck, USA), methyl paraben (Brataco, Indonesia), diclofenac sodium (Sigma, USA), propyl paraben (Brataco, Indonesia), carrageenan (Sigma, USA) and triethanolamine (Sigma, USA).

### EXPERIMENTAL ANIMALS

Ethical clearance for experimental animals has been registered with the ethics committee of the Faculty of Medicine Unpad with no. 1461/UN6.KEP/EC/2018. This study used male white rats Wistar strain weighing 180-250 g, obtained from the Pharmacology and Therapy Laboratory of the Central Hospital Building, Jl. Eyckman No. 38 Bandung. The rats need to be acclimatized for at least 1 week before use and the weight was observed every day to ensure the change in weight did not exceed 10%.

### EXAMINATION OF ESSENTIAL OIL CHARACTERISTICS
### ORGANOLEPTIC EXAMINATION

Organoleptic examination was carried out by observing the shape, colour and smell of the essential oils obtained from dried AM and KG rhizomes and compared with the literature.

### DETERMINATION OF THE WEIGHT OF ESSENTIAL OIL

Determination of the specific gravity of essential oils was carried out using a pycnometer. The essential oil was put into a pycnometer which has been weighed and the volume is equalized, then the weight is measured with an analytical balance. Specific gravity (BJ) of essential oils was calculated using the following (1):

$$\text{Specific Gravity} = \frac{(\text{Berat picnometer weight} + \text{oil}) - \text{empty picnometer weight}}{\text{Volume of sample}} \quad (1)$$

### CONTENT ANALYSIS OF ESSENTIAL OILS

Essential oils were analyzed using QP5050A GC/MS-(Shimadzu) equipped with a fused silica capillary DB-5ms 30 mm × 0.25 nm, 0.25 μm, carrier gas helium 95.3 kPa, flow rate 1.7 mL/min. The temperature was set as follows: 60 °C for 5 min and then 250 to 10 °C/min, ending with 5 min at 300 °C. Port Injector and detector temperature was 250 and 280 °C, respectively. The sample was injected by split and split ratio 1:20. MS operating conditions were interface temperature 240 °C; electron impact ionization at 70 eV by scanning the mass range (m/z) of 40 - 350 Daltons with a sampling rate of 1.0 scans/s.

Identification of compounds was done by screening in digital library of mass spectral data by a Class-5000 software and by comparison of retention indices and mass spectra library authentic (Adams 1995; Ausloos et al. 1999; Babushok et al. 2007; Strehmel et al. 2008), relative to the C8-C20 and C21-C40 n-alkane series (Sigma, USA) (Mijin et al. 1999) in the temperature-programmed run.

### GEL PREPARATION FORMULA

Carbopol 934 (0.75 g) was dissolved in 15 mL distilled water (aquadest that had been heated at 70 °C for ± 30 min) and allowed to swell in a mortar. Glycerin (15 g) was divided into 2 parts in an evaporating dish. Some of the glycerin was used to dissolve 0.18 g of methyl paraben and 0.02 g of propyl paraben (part 1) as well as dissolving the essential oils of AM and KG according to their concentration ratios.

Part 1 was mixed in carbomer 934 which had been expanded and ground until homogeneous. The second part was added and grind again until homogeneous. Next, 1 g triethanolamine (TEA) was added as an alkalizing agent and ground until there is an increase in gel consistency. Lastly, the gel mixture was added with aquadest up to 100 g (add up to 100%) in a glass beaker followed by stirring with a mechanical stirrer at a speed of ± 400 rpm for ± 25 min to form a homogeneous gel preparation (Wijayanto et al. 2013).

The gel formulation was made with 3 doses of AM and KG's essential oils based on a 2:1 ratio for the essential oil content of AM:KG. This 2:1 comparison is based on a reference from the comparison of AM and KG rhizomes extracts oral doses of 100 mg/kgBW with

32.69% inflammation reduction (Sethi et al. 2017) and at a dose of 45 mg/kgBW with up to 32.62 ± 3.1% inflammation reduction respectively (Hasanah et al. 2011).

## PHYSICAL EVALUATION OF GEL PREPARATIONS

Physical evaluation of the gel preparation includes organoleptic examination, homogeneity, pH, viscosity measurement, and dispersion test. Evaluation of the preparation was carried out on days 0, 1, 4, 7, 14, 21, and 28 days. The following is a series of procedures for evaluating gel preparations:

*Organoleptic Examination and Homogeneity*

The organoleptic test was carried out by visually observing the gel formulation, which included consistency, colour, and odor during storage (Nurman et al. 2019). Homogeneity examination was carried out by applying 3 parts of the gel; top, middle and bottom on the glass slide. In this test, substances or particles that are not yet homogeneous can be observed (Tadros 1992).

*pH Measurement*

The pH examination was carried out using a calibrated pH meter. 1 g of gel sample was dissolved in 10 mL distilled water free $CO_2$, and then the sample solution was measured using a pH meter (Ballance 1996).

*Viscosity Measurement*

Viscosity measurement was done by placing the gel preparation into the viscometer container until the spindle was submerged. The spindle used was spindle number six with a speed of 100 rpm.

*Spreadability Test*

The spreadability test was carried out to observe and ensure the even distribution of the gel preparation when applied to the skin. One gram of gel preparation was placed in the middle of glass measuring 10 × 10 cm and covered with another glass whose weight has been taken. A 125 g weight was placed onto the glass cover, and let it stand for 1 min, before measuring the diameter of the spread gel (Shawesh et al. 2003).

## TOPICAL ANTI-INFLAMMATORY ACTIVITY TEST

The topical anti-inflammatory activity test was carried out using the Winter method (Induction of inflammation) including the stages of preparing test animals, grouping test animals and testing topical anti-inflammatory activity.

*Experimental Animal Setup*

Male white rats from the Wistar strain, the weight of 180 - 250 g were used as the test candidate. The rats were acclimatized for ± 1 week and their body weight was observed every 3 days. Rats are said to be healthy if their body weight increases or decreases by no more than 10% and their activities are normal.

*Experimental Animal Grouping*

The research was conducted in a laboratory and the sampling was done randomly. The experiment was carried out 3 times according to Frederer's formula with 5 rats per treatment, thus a total of 15 rats were used (Federer 1963). Rats were grouped into 5 treatment groups as follows: (1) Positives Control: 1% Na-Diclofenac gel was applied; (2) Negatives Control: applied gel base; (3) Formula I: smeared with 5% AM's essential oil gel and 2.5% KG; (4) Formula II: AM's essential oil gel is smeared with 10% and 5% KG; (5) Formula III: smeared with 20% AM's essential oil gel preparation and 10% KG.

*Topical Anti-Inflammatory Activity Assay*

### Initial Volume Measurement of Rat Feet

The initial volume of mercury from the plethysmometer was recorded, followed by dipping the right leg of the rat into the plethysmometer. The final volume of mercury was recorded and the volume of the rat's foot was calculated by the following formula:

$Vn = V_1 – Vo$

where Vn is the volume of normal mouse paw; $V_1$ is the volume of final Mercury; Vo = Volume of initial mercury.

### Administration of Test Preparations and Induction of Inflammation

After the initial volume of the rat's paws was measured, the rats were anesthetized with diethyl ether thus the rats would not feel pain when invasive measures were given in the form of carrageenan induction hence facilitate the administration of the test preparation and the induction of inflammation (Soeratri et al. 2014). The test preparation was given topically on the right leg of the rat according to the group and body weight and left for 1 h. The rats were placed in cages with copographic snares for 1 h. Next, an injection of carrageenan 1% w/w was injected into the rat's foot that had been smeared to induce edema.

### Anti-Inflammatory Activity Assay

Paw edema volume was measured using a plethysmometer every 1, 2, 3, 4, 5, and 6 h after

carrageenan induction. The percentage increase in rat paw volume was calculated using the following (2):

$$\% \text{ inflammation} = \left(\frac{Vc - Vn}{Vn}\right) \times 100\% \qquad (2)$$

### GEL PREPARATION HEDONIC TEST

In the hedonic test, the panelists expressed their opinion about the likes or dislikes of the gel preparations. The hedonic test was carried out on 21 panelists to see the level of favorites based on five assessment parameters which include aroma, colour, consistency, appearance, and convenience in use. The hedonic scale was transformed into a numerical scale through the scaling in Table 1.

TABLE 1. Numerical l of AM and KG's essential oil gel preparations

| Hedonic Scale | Numeric Scale |
|---|---|
| Extremely very like | 5 |
| Very like | 4 |
| Slightly like | 3 |
| Neutral | 2 |
| Slightly dislike | 1 |
| Very dislike | 0 |

### STATISTICAL ANALYSIS

Data analysis was performed using One Way ANOVA for normally distributed data ($P>0.05$) and Kruscall–Wallis for data that were not normally distributed ($P<0.05$). Especially for the advanced test stage, where the post Hoc Dunnett T3 follow-up test is for analysis of topical anti-inflammatory activity test data and multiple comparisons follow-up test for hedonic test data analysis. The experimental units analyzed included physical evaluation tests, topical anti-inflammatory activity tests and hedonic tests of essential oil gel preparations.

## Results and Discussion

### DRIED HERBAL PREPARATION AND ESSENTIAL OIL DISTILLATION

The rhizomes of AM and KG were washed, cut into small pieces and dried. 6.88 kg of dried AM and 3.445 kg of dried KG were obtained. The dried samples were further distilled to obtain the essential oils. The essential oils from the distillation of AM yield 0.54% whereas the essential oil from KG yields 0.377%. The essential oil content is still within the range of the previous studies. Huong et al. (2017) reported that the yield of essential oils from AM rhizome is 0.32%, while (Azah et al. 2005) reported the yield of AM essential oil reach 1%. Another study has reported AM essential oils within the range of 0.1 - 1.22% (Bhuiyan et al. 2010; Muchtaridi et al. 2011, 2004; Sahoo et al. 2014).

### EXAMINATION OF ESSENTIAL OIL CHARACTERISTICS

*Organoleptic Examination Results*

The results of organoleptic examination of AM and KG essential oils are shown in Table 2.

*Specific Gravitation Analysis*

Specific gravity analysis was carried out to determine the quality of essential oils. Specific gravity is associated with the weight fraction of a component contained in the essential oil (Susetyo & Reny 2004). Calculation of AM and KG essential oils specific gravity, were 0.996 and 1.003 gram/mL, respectively. This met the quality standards of essential oils (ISO 279:1998).

*Analysis of AM and KG Rhizome Essential Oil Content*

Analysis of GC-MS (Gas Chromatography Mass Spectrometry) using Linear Retention Index (LRI) of the AM and KG rhizome essential oil are shown in Table 4. According to previous research study, the content of AM rhizome was dominated by methyl cinnamate (58.8%) (Table 3) (Azah et al. 2005). However, the content of this compound in AM rhizome from Pangandaran is not greater than AM rhizome taken from

Sumedang (>60%). Bhuiyan et al. (2010) and Azah et al. (2005) reported that the essential oils composition of the AM rhizome varies according to its countries of origin. The volatile oil content of KG rhizome is dominated by ethyl-p-methoxycinnamate followed by ethyl cinnamate as studied by Raina et al. (2015), Bhuiyan et al. (2010), and Li et al. (2017).

TABLE 2. Results of organoleptic examination of AM and KG's essential oils

| No | Sample | Organoleptic test | | |
|---|---|---|---|---|
| | | Form | Colour | Odor |
| 1. | KG | Liquid | yellowish | Typical odor |
| 2. | AM | Liquid | Cloudy yellow | Typical odor |

TABLE 3. Constituents of stem essential oil from *AM*

| No | LRI[1] | LRI[2] | Compounds name | Percentage (%) in rhizomes | |
|---|---|---|---|---|---|
| | | | | AM | KG |
| 1. | 779 | 781 | n-hexanal | 0.03 | 0.06 |
| 2. | 930 | 936 | α-pinene | 14.90 | 0.01 |
| 3. | 982 | 985 | β-pinene | 12.44 | 0.1 |
| 4. | 944 | 947 | camphene | - | 0.05 |
| 5. | 1031 | 1039 | 1,8-cineole | 9.89 | 0.03 |
| 6. | 1063 | 1069 | γ-terpinene | 3.78 | - |
| 7. | 1074 | 1077 | terpinolene | 0.45 | 0.03 |
| 8. | 1077 | 1080 | fenchone | 0.43 | - |
| 9. | 1089 | 1092 | linalool | 0.75 | 0.01 |
| 10. | 1097 | 1095 | sabinene | 0.28 | - |
| 11. | 1141 | 1144 | camphene hydrate | - | 0.70 |
| 12. | 1144 | 1146 | camphor | 6.22 | 0.01 |
| 13. | 1151 | 1153 | borneol | - | 0.03 |
| 14. | 1187 | 1190 | α-terpineol | 6.00 | - |
| 15. | 1380 | 1383 | methyl cinnamate | 58.8 | - |
| 16. | 1395 | 1390 | isoeugeunol | - | 0.03 |
| 17. | 1465 | 1470 | trans-ethyl cinnamate | 2.3 | 12.8 |
| 18. | 1518 | 1520 | γ-cadinene | - | 0.10 |
| 19. | 1525 | 1523 | nerolidol | 0.01 | - |
| 20. | 1555 | 1557 | γ-caryophyllene | 0.25 | 0.03 |
| 21. | 1537 | 1540 | viridiflorol | 0.22 | - |
| 22. | 1583 | 1580 | hexadecane | 0.17 | 0.03 |
| 23. | 1604 | 1606 | carotol | 0.21 | - |
| 24. | 1635 | 1632 | α-cadinol | 0.58 | - |
| 25. | 1624 | 1626 | patchouli alcohol | 0.30 | 0.01 |
| 26. | 1667 | 1668 | α-bisabolol | 1.03 | - |
| 27. | 1659 | 1660 | juniper camphor | 0.38 | - |
| 28. | 1683 | 1688 | tetradecanal | 0.03 | - |
| 29. | 1722 | 1727 | ethyl p-methoxy cinnamate | 0.23 | 59.68 |
| 30. | 1832 | 1835 | geranyl-n- heptanoate | 0.03 | - |

1 : LRI experiment with DB5-MS column, 2 : LRI reference in Adams with DB5 column

TABLE 4. Formulation of gel preparations with variations in dosage of AM and KG's essential oils

| Ingredients | FI (%) | FII (%) | FIII (%) |
|---|---|---|---|
| AM essential oil | 5 | 10 | 20 |
| KG essential oil | 2,5 | 5 | 10 |
| Carbopol 934 | 0,75 | 0,75 | 0,75 |
| TEA (Triethanolamine) | 1 | 1 | 1 |
| Glycerin | 15 | 15 | 15 |
| Methyl Paraben | 0,18 | 0,18 | 0,18 |
| Propyl Paraben | 0,02 | 0,02 | 0,02 |
| Aquadest add until | 100 | 100 | 100 |

## GEL PREPARATIONS WITH AM AND KG'S ESSENTIAL OILS

The formulation process of gel preparation was done in 3 variations of dose:

FI = Formula with 5% AM and 2.5% KG essential oil;
FII = Formula with 10% AM's and 5% KG essential oil;
FIII = Formula with 20% AM's and 10% KG essential oil
The results of the gel preparation formulation are shown in Table 1.

## PHYSICAL EVALUATION OF GEL PREPARATIONS

*Organoleptic Observations and Homogeneity*

Organoleptic and homogeneity observations were carried out to determine the physical appearance of the gel formulation such as colour, odor, shape, and homogeneity. The physical appearance of the gel is important for usage comfort. The results of organoleptic observations after 28 days of storage only changed the gel colour from white to clear white. Based on Table 5, the different levels of essential oils of AM and KG affects the physical appearance of the gel preparations such as colour and odor.

*pH Measurement*

The graph in Figure 1 shows that the pH of the gel preparation is in the range of 4.7-6.3. The pH of topical gel preparations should ideally be the same as the skin's pH, of 4.5 - 6.5 (Ali & Yosipov 2013). The level of stability and average pH of gel containing AM and KG essential oils is: F3>F2>F1. Based on the normality test, the data were normally distributed with ANOVA significant values of 0.000 ($p<0.05$). From this, it is evident that the storage times affect the pH of the gel preparation.

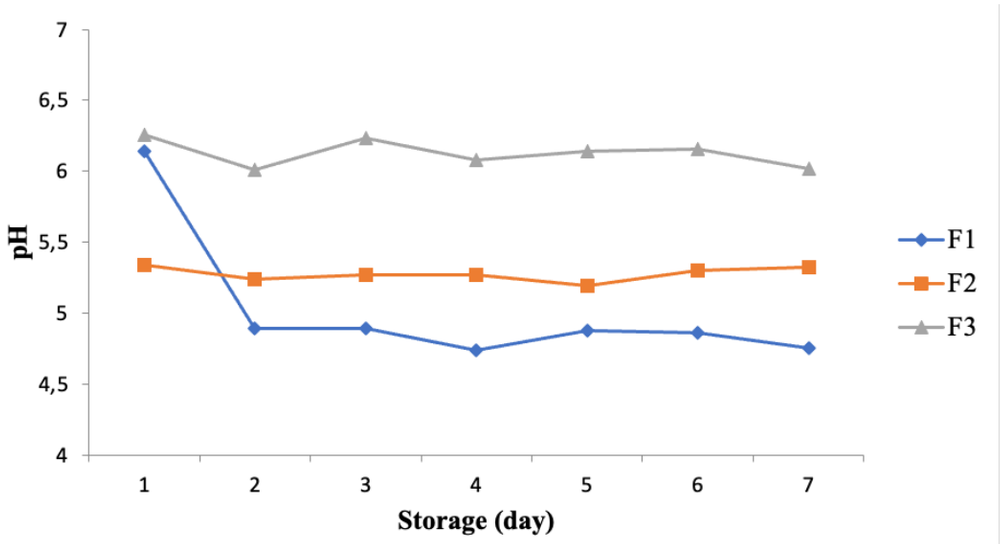


FIGURE 1. The pH of different formula gel preparation (F1, F2, and F3)

*Viscosity Measurement*

From the observations (Figure 2), the viscosity of the gel preparation increased up to the 14th day then decreases to 28th day. The average value of viscosity was F3>F1>F2, thus the levels of AM and KG essential oils in the preparation did not affect the gel consistency. Based on the normality test, the data were not normally distributed with Kruskal–Wallis significant value of 0.006 ($p < 0.05$) indicating that there are viscosity differences in the gel preparation formula with variation dose of AM and KG essential oils.

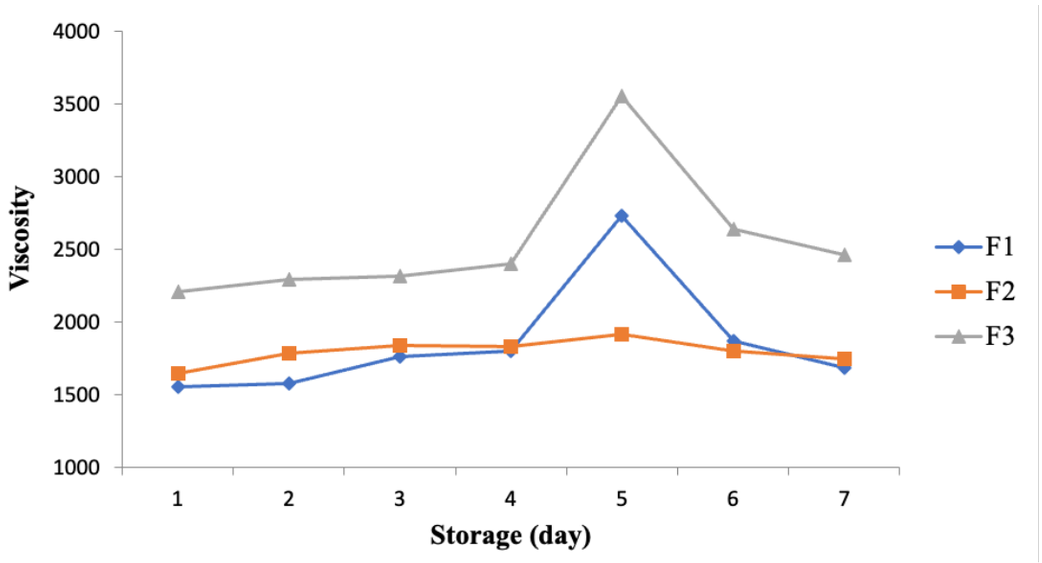


FIGURE 2. The viscosity of different formula gel preparation (F1, F2 and F3)

TABLE 5. Results of organoleptic observations and homogeneity of gel preparations

| Characteristics | Formula | Day Storage- | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 4 | 7 | 14 | 21 | 28 |
| Colour | F1 | P | P | P | PB | PB | PB | PB |
| | F2 | P | P | P | PB | PB | PB | PB |
| | F3 | P | P | PB | PB | PB | PB | PB |
| Odor | F1 | BK | BK | BK | BK | BK | BK | BK |
| | F2 | BK | BK | BK | BK | BK | BK | BK |
| | F3 | BK | BK | BK | BK | BK | BK | BK |
| Shape/Consistency | F1 | K | K | K | K | K | K | K |
| | F2 | K | K | K | K | K | K | K |
| | F3 | K | K | K | K | K | K | K |
| Homogeneity | F1 | H | H | H | H | H | H | H |
| | F2 | H | H | H | H | H | H | H |
| | F3 | H | H | H | H | H | H | H |

Description : P (White); PB (Clear white); BK (Typical odor); K (Viscous); H (homogeneous)

SPREAD POWER MEASUREMENT

Dispersion results of the 3 formulas were in the range of 5-6.5 cm, which was within the accepted range for topical preparations. Garg et al. (2002) reported that a good distribution of semisolid preparations for topical application ranges from 3-5 cm in diameter.

Based on Figure 3, there was a decrease in dispersion power despite fluctuations in the 3 gel formulations throughout the 28-day storage period. The decrease in dispersion occurs due to the presence of liquid solvent that was retained by the gel preparation base (Wijayanti et al. 2015).

Based on the normality test, the data were normally distributed with ANOVA significant values of 0.984 ($p>0.05$). This indicates that there were no differences in the dispersion power of the 3 gel formulations.

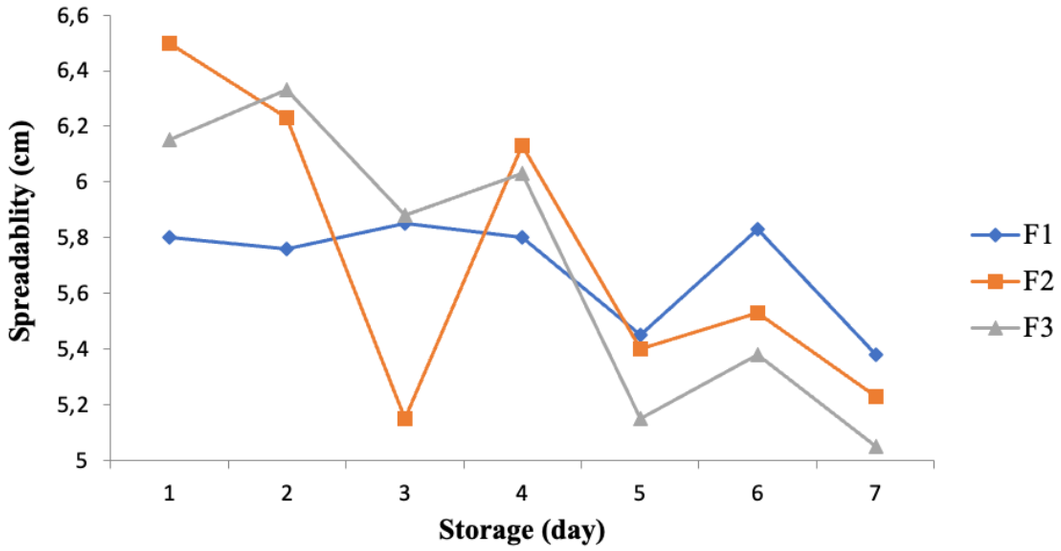


FIGURE 3. The spread observation of different formula gel preparations (F1, F2 and F3)

TOPICAL ANTI-INFLAMMATORY ACTIVITY TEST

The results of the anti-inflammatory activity test were expressed in percentage value of inflammation produced, where % inflammation indicates the amount of inflammation or edema formed in the rat's feet. The smaller the % inflammation, the smaller the inflammation formed. The percentage value of inflammation over a time interval of 6 h is shown in Figure 4.

Figure 4 shows that the value of % inflammation increases from the 1st to 3rd h, this is because the inflammatory process (acute phase) occurs during the first 3 h (Chen et al. 2017). In this acute phase, inflammation or edema formed due to carrageenan induction which occurs in two phases. The first phase (early phase) occurs in the 2 h after carrageenan induction which causes trauma due to inflammation (Abdulkhaleq et al. 2018). The trauma is caused by the release of serotonin and histamine to the site of inflammation and an increase in prostaglandin synthesis in the damaged tissue (Barung et al. 2021).

Based on Figure 4, the % of inflammation starts to decrease at the 3rd h for all 3 dosages. This indicates that the anti-inflammatory activity was activated after the second phase (late phase) (Saini & Singhal 2012). Anti-inflammatory activity testing was carried out 3 times and the % inflammation is the average value from each test group (Table 6).

Formula I (Dosage I) shows the highest reduction of % inflammation (edema) as compared to the other 2 formula/doses; Formula I > Formula II > Formula III. Based on the normality test, the data was normally distributed with ANOVA significant value of 0.000. It was mean that the dose distinction of resulting % inflammation.

Furthermore, test analysis Post hoc Dunnett T3 of all five treatment groups showed that the most significant % inflammation reduction compared to the

negative control. Meanwhile, the comparation of dose II and dose III has a significant different. Meanwhile, the third dose group had a $p$ value > 0.05, meaning that the third dose % inflammation was close to the negative control. The negative control is the gel base that has no anti-inflammatory activity. Since the difference in % inflammation by the negative control and dose III was close, it could be that the anti-inflammatory activity in dose III is not effective in reducing edema. Furthermore, the 3 doses when compared to the positive control were not significant ($p$>0.05), with sig value of 0.502; 0.903 and 0.550 for Formula I, II and III, respectively. Therefore, Formula I, II and III has good anti-inflammatory activity; with Formula 1 having the highest activity (Table 7). Besides, Formula I also showed the highest percentage of inhibition with an average value of 36.32 ± 6.32% (Figure 5 & Table 7). Meanwhile for Formula II and III, the average % inhibition was 30.01 ± 4.42% and 17.16 ± 6.20%, respectively (Figure 5).

The assay results showed that the first dose group having the lowest essential oil content of 5% AM and 2.5% KG had the highest anti-inflammatory activity as compared to the second and third doses with higher essential oil content. This could be due to several factors, one of which is influenced by the chemical stability of the active compounds in the essential oils (Figueiredo et al. 2008). Based on previous study, the stability of essential oils is generally influenced by oxidation reactions and deterioration reactions, which can also cause changes in sensory and pharmacological activities (Turek & Stintzing 2013). Changes in chemical components in essential oils can be caused by heat, catalytic quantities of redox-reactive metals, exposure to light, and alkyl radicals (Iyer et al. 2021; Neuenschwander & Hermans 2010). However, the activity cannot be identified to only one of the major constituents (Dhifi et al. 2016). Compound such as methyl cinnamate from AM and ethyl $p$ -methoxycinnamate (EPMS) from KG can undergo hydrolysis process which cause dissociation of the methyl and ethyl group in methyl cinnamate and EPMS (Fahelbum & James 1977). The higher the volatile oil content in the gel preparation, the lower the anti-inflammatory activity produced. Other factors that affect the anti-inflammatory activity can be due to the homogeneity of the test animals, including the differences in body weight, blood volume and body tissue area, especially on the size of the rat's paw used in the study (Hasanah et al. 2011).

From the study of topical anti-inflammatory activity, it can be concluded that the essential oils of AM and KG have good potential as anti-inflammatory in the form of gel preparations. In several studies, the compound that has the potential to have anti-inflammatory activity from KG's essential oil is ethyl- $p$- methoxycinnamate (EPMS), while from AM's essential oil is methyl cinnamate.

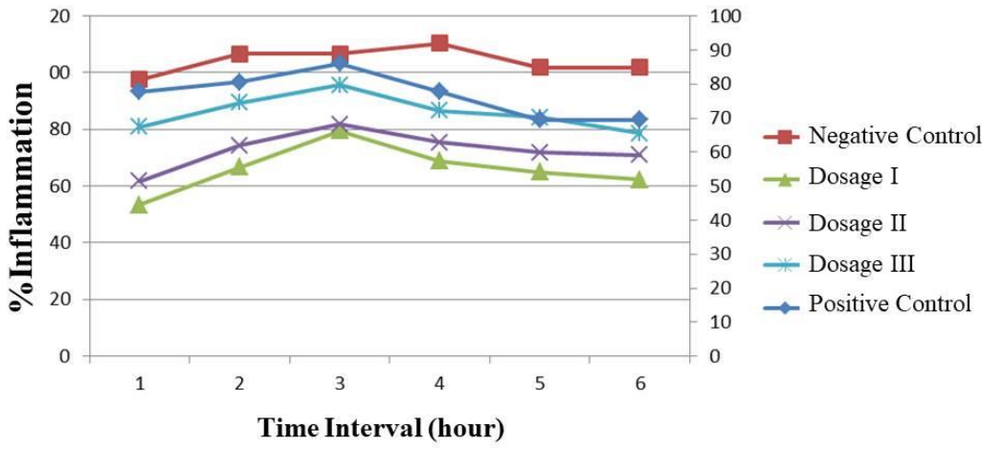


FIGURE 4. The percentage of inflammation in each treatment group over time interval (6-h)

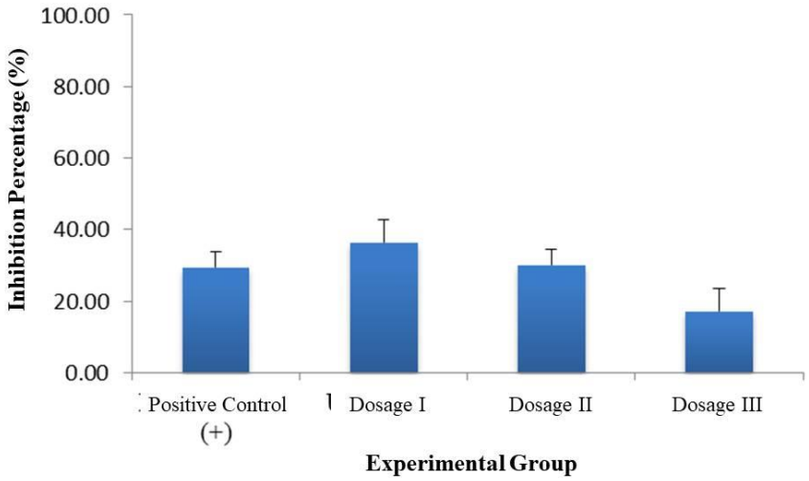


FIGURE 5. The average inhibition percentage of each test group

TABLE 6. Average inflammation percentage of each test group

| Group | Inflammatory percentage (%) | | | |
|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Average |
| Positive Control (+) | 80.56 ± 4.82 | 77.78 ± 3.74 | 72.22 ± 8.67 | 76.85 ± 3.71 |
| Negative Control (-) | 110.67 ± 9.56 | 100.00 ± 3.43 | 101.82 ± 9.42 | 104.16 ± 1.01 |
| Formula I | 57.50 ± 7.41 | 73.61 ± 6.65 | 66.67 ± 2.48 | 65.93 ± 8.08 |
| Formula II | 69.76 ± 12.83 | 78.20 ± 8.42 | 70.00 ± 6.44 | 72.65 ± 5.79 |
| Formula III | 79.29 ± 3.54 | 93.06 ± 12.42 | 85.34 ± 9.43 | 85.90 ± 6.90 |

TABLE 7. Percentage of inflammation inhibition average of each test group

| Group | Inflammatory percentage (%) | Inhibitory percentage (%) |
|---|---|---|
| Positive Control (+) | 76.85 ± 2.45 | 29.56 ± 4.38 |
| Formula I | 65.93 ± 4.67 | 36.32 ± 6.32 |
| Formula II | 72.65 ± 2.77 | 30.01 ± 4.42 |
| Formula III | 85.90 ± 3.98 | 17.16 ± 6.20 |

### GEL PREPARATION HEDONIC TEST RESULTS

The hedonic test (preference) was conducted to determine the panelists' preference for the gel formulation with 3 variations of the test dose, namely F1 (Formula I), F2 (Formula II) and F3 (Formula III). The assessment parameters in the hedonic test include aroma, colour, appearance (shape), viscosity and comfort in the use of gel preparations. The following is a graph of the results of the hedonic test assessment from 21 panelists (Figure 6). Statistical analysis was carried out using the Kruskal–Wallis test, and all test parameters including aroma, colour, appearance, viscosity, and comfort generally have sig values of $p < 0.05$. This indicates that there is a difference in preference between aroma, colour, appearance, viscosity, and comfort of the gel formulation with three doses of AM and KG essential oils.

Further test on multiple comparisons was carried out and the results show that the appearance and viscosity of formula I is different than the second and third dose formulas. For aroma and comfort, dose I formula only show difference with dose II, while dose III the results are the same. Furthermore, on the colour parameter, it shows that the formula for dose I, dose II and dose III have the same average colour.

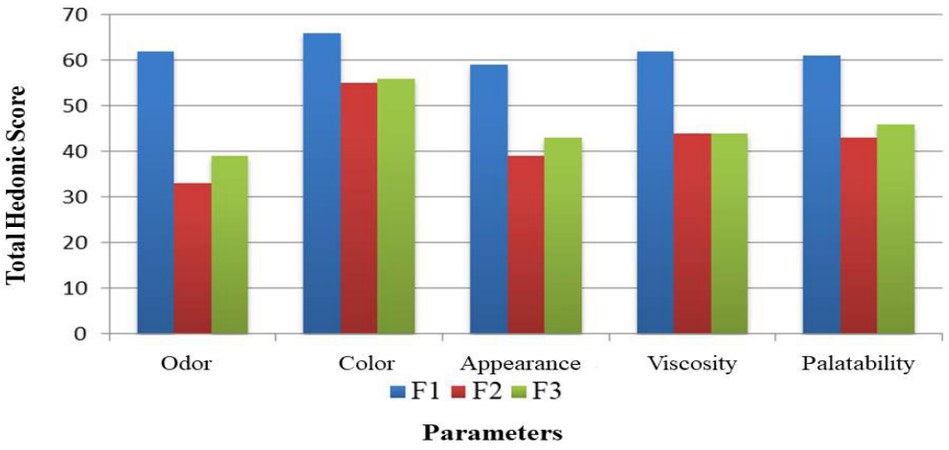


FIGURE 6. Hedonic test for the 3 different formula of gel preparations

## CONCLUSION

In conclusion, Formula 1 with essential oil content of 5% AM and 2.5% KG shows the best topical anti-inflammatory activity with inhibition percentage of 36.32 ± 6.32%. In addition, formula I shows better anti-inflammatory activity than positive control (1% Na-diclofenac gel) with the inhibition percentage of 29.56 ± 4.38%. Similarly, formula 1 is also the best formulation in the physical and hedonic test evaluation. AM and KG essential oils in the forms of gel demonstrate a significant topical anti-inflammatory activity; hence can be considered for topical inflammatory treatments.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the Rector of Universitas Padjadjaran and the Indonesian Ministry of National Education for funding this work through the DIPA research grant.

## REFERENCES

Abdulkhaleq, L.A., Assi, M.A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y.H. & Hezmee, M.N.M. 2018. The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary World* 11(5): 627.

Adams, R.P. 1995. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Illinois: Allured Publishing Corporation. p. 804.

Ali, S.M. & Yosipovitch, G. 2013. Skin pH: From basic science to basic skin care. *Acta Dermato-Venereologica* 93(3): 261-269.

Ausloos, P., Clifton, C.L., Lias, S.G., Mikaya, A.I., Stein, S.E., Tchekhovskoi, D.V., Sparkman, O.D., Zaikin, V. & Zhu, D. 1999. The critical evaluation of a comprehensive mass spectral library. *Journal of the American Society for Mass Spectrometry* 10(4): 287-299.

Azah, M.N., Sam, Y.Y., Mailina, J. & Chua, L.S.L. 2005. (E)-Methyl cinnamate: The major component of essential oils of *Alpinia malaccensis* var. nobilis. *Journal of Tropical Forest Science* 17(4): 631-633.

Babushok, V.I., Linstrom, P.J., Reed, J.J., Zenkevich, I.G., Brown, R.L., Mallard, W.G. & Stein, S.E. 2007. Development of a database of gas chromatographic retention properties of organic compounds. *Journal of Chromatography A* 1157(1-2): 414-421.

Ballance, R. 1996. Physical and chemical analysis. In *Water Quality Monitoring*, edited by Bartram, J. & Balance, R. Florida: CRC Press. pp. 1-90.

Barkin, R.L. 2015. Topical nonsteroidal anti-inflammatory drugs: The importance of drug, delivery, and therapeutic outcome. *American Journal of Therapeutics* 22(5): 388-407.

Barung, E.N., Dumanauw, J.M., Duri, M.F. & Kalonio, D.E. 2021. Egg white-induced inflammation models: A study of edema profile and histological change of rat's paw. *Journal of Advanced Pharmaceutical Technology & Research* 12(2): 109.

Bhuiyan, M.N.I., Chowdhury, J.U., Begum, J. & Ch, N. 2010. Essential oils analysis of the rhizomes of *Alpinia conchiger*a Griff. and leaves of *Alpinia malaccensis* (Burm. f.) Roscoe from Bangladesh. *African Journal of Plant Science* 4(6): 197-201.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X. & Zhao, L. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9(6): 7204.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. & Mnif, W. 2016. Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines* 3(4): 25.

Fahelbum, I.M. & James, S.P. 1977. The absorption and metabolism of methyl cinnamate. *Toxicology* 7(1): 123-132.

Federer, W.T. 1963. *Experimental Design: Theory and Application.* New Delhi: IBH Publishing Co. pp. 1-591.

Figueiredo, A.C., Barroso, J.G., Pedro, L.G. & Scheffer, J.J. 2008. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour and Fragrance Journal* 23(4): 213-226.

Fitriani, B.A. 2018. Massage gel preparations formulation of *Alpinia malaccensis* [Burm. F.] and *Kaempferia galanga* L. rhizome essential oils. Universityas Padjadjaran. Bachelor Research Thesis (Unpublished).

Garg, A., Aggarwal, D., Garg, S. & Singla, A.K. 2002. Spreading of semisolid formulations: An update. *Pharmaceutical Technology North America* 26(9): 84.

Gui, Y., Chen, L., Duan, S., Li, G., Tang, J. & Li, A. 2018. Methyl cinnamate alleviated CCI-induced upregualtion of spinal AMPA receptors and pain hypersensitivity by targeting AMPK. *European Journal of Pharmacology* 833: 183-189.

Hasanah, A., Nazaruddin, F., Febrina, E. & Zuhrotun, A. 2011. Analysis of essential oil content and anti-inflammatory activity test of rhizome extract of *Kaempferia galanga* L. *Jurnal Matematika dan Sains* 6(1): 147-153.

Hua, S. 2020. Advances in oral drug delivery for regional targeting in the gastrointestinal tract - Influence of physiological, pathophysiological and pharmaceutical factors. *Frontiers in Pharmacology* 11: 524.

Huong, L.T., Dai, D., Van Chung, M., Doan, D. & Ogunwande, I. 2017. Constituents of essential oils from the leaf, stem, root, fruit and flower of *Alpinia macroura* K. Schum. *Bol. Latinoam Caribe Plant Med. Aromat*. 16(1): 26-33.

Iyer, S., Rissanen, M.P., Valiev, R., Barua, S., Krechmer, J.E., Thornton, J., Ehn, M. & Kurtén, T. 2021. Molecular mechanism for rapid autoxidation in α-pinene ozonolysis. *Nature Communications* 12(1): 1-6.

Jagadish, P.C., Latha, K.P., Mudgal, J. & Nampurath, G.K. 2016. Extraction, characterization and evaluation of *Kaempferia galanga* L. (Zingiberaceae) rhizome extracts against acute and chronic inflammation in rats. *Journal of Ethnopharmacology* 194: 434-439.

Li, Y., Ji, H., Li, X., Zhang, H. & Li, H. 2017. Isolation of nematicidal constituents from essential oil of *Kaempferia galanga* L. rhizome and their activity against *Heterodera avenae* Wollenweber. *Tropical Journal of Pharmaceutical Research* 16(1): 59-65 https://doi.org/10.4314/tjpr.v16i1.8

Lima, F.J., Brito, T.S., Aragão, K.S., Ferreira, L.C., Lahlou, S., Santos, A.A. & Magalhães, P.J. 2013. Anti-inflammatory effects of methyl cinnamate, the major constituent of the essential oil of *Ocimum micranthum*, on the gastrointestinal tract of rats submitted to acetic acid-induced colitis. *Planta Medica* 79(13): PF5.

Lopez-Castejon, G. & Brough, D. 2011. Understanding the mechanism of IL-1β secretion. *Cytokine & Growth Factor Reviews* 22(4): 189-195.

Matsui, H., Shimokawa, O., Kaneko, T., Nagano, Y., Rai, K. & Hyodo, I. 2011. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. *Journal of Clinical Biochemistry and Nutrition* 48(2): 107-111.

Mijin, D.Z., Petrovic, S.D. & Antonovic, D.G. 1999. Gas chromatography retention indices of 2-phenyl- 2 alkylacetonitriles on packed coloumns. *The Sci. J. Fact. Univ.* 2(1): 1-8.

Muchtaridi, Adjeng Diantini & Anas Subarnas. 2011. Analysis of Indonesian spice essential oil compounds that inhibit locomotor activity in mice. *Pharmaceuticals* 4(4): 590-602. https://doi.org/10.3390/ph4040590

Muchtaridi, M., Arifin Sutasli, Nurdjannah Azinar & Ida Musfiroh. 2004. Chemical characteristics of laja gowah rhizome essential oil (*Alpinia malaccensis* (Burm f.)). Bionatura: *Journal of the Physical and Life Sciences* 6(3): 272-282.

Neuenschwander, U. & Hermans, I. 2010. Autoxidation of α-pinene at high oxygen pressure. *Physical Chemistry Chemical Physics* 12(35): 10542-10549.

Nurman, S., Yulia, R., Irmayanti, Noor, E. & Candra Sunarti, T. 2019. The optimization of gel preparations using the active compounds of arabica coffee ground nanoparticles. *Scientia Pharmaceutica* 87(4): 32.

Raina, A.P., Abraham, Z. & Sivaraj, N. 2015. Diversity analysis of Kaempferia galanga L. germplasm from South India using DIVA-GIS approach. *Industrial Crops and Products* 69(C): 433-439. https://doi.org/10.1016/j.indcrop.2015.02.052

Rannou, F., Pelletier, J.P. & Martel-Pelletier, J. 2016. Efficacy and safety of topical NSAIDs in the management of osteoarthritis: Evidence from real-life setting trials and surveys. *Seminars in Arthritis and Rheumatism* 45(4): S18-S21.

Sahoo, S., Singh, S. & Nayak, S. 2014. Chemical composition, antioxidant and antimicrobial activity of essential oil and extract of *Alpinia malccensis* Roscoe (Zingiberaceae). *International Journal of Pharmacy and Pharmaceutical Sciences* 6(7): 183-188.

Saini, N.K. & Singhal, M. 2012. Anti-inflammatory, analgesic and antipyretic activity of methanolic *Tecomaria capensis* leaves extract. *Asian Pacific Journal of Tropical Biomedicine* 2(11): 870-874.

Sethi, S., Prakash, O., Pant, A.K. & Kumar, M. 2017. Phytochemical analysis and pharmacological activities of methanolic extract and essential oil from rhizomes of *Alpinia malaccensis* (Burm. f.) Roscoe. *Journal of Essential Oil Bearing Plants* 20(4): 1018-1029.

Shawesh, A.M., Kaukonen, A., Kallioinen, S., Antikainen, O. & Yliruusi, J. 2003. Development of indomethacin Carbopol ETD 2001 gels and the influence of storage time and temperature on their stability. *Pharmazie* 58(2): 130-135.

Soeratri, W., Erawati, T., Rahmatika, D. & Noorma, R. 2014. Determination of p-methoxycinnamic acid (APMS) as a topical anti-inflammatory and penetration study of APMS through rat skin with and without stratum corneum. *Jurnal Farmasi dan Ilmu Kefarmasian* 1(1): 28-30.

Strehmel, N., Hummel, J., Erban, A., Strassburg, K. & Kopka, J. 2008. Retention index thresholds for compound matching in GC-MS metabolite profiling. *Journal of Chromatography B* 871(2): 182-190.

Sulaiman, M.R., Zakaria, Z.A., Daud, I.A., Ng, F.N., Ng, Y.C. & Hidayat, M.T. 2008. Antinociceptive and anti-inflammatory activities of the aqueous extract of *Kaempferia galanga* leaves in animal models. *Journal of Natural Medicines* 62(2): 221-227.

Susetyo, R. & Reny, H. 2004. *Tips to Produce Citronella Oil.* Jakarta: Penebar Swadaya Press.

Tadros, T.F. 1992. Future developments in cosmetic formulations. *International Journal of Cosmetic Science* 14(3): 93-111.

Turek, C. & Stintzing, F. 2013. Stability of essential oils: A review. *Comprehensive Reviews in Food Science and Food Safety* 12(1): 40-53.

Umar, M.I., Asmawi, M.Z., Sadikun, A., Atangwho, I.J., Yam, M.F., Altaf, R. & Ahmed, A. 2012. Bioactivity-guided isolation of ethyl-p-methoxycinnamate, an anti-inflammatory constituent, from *Kaempferia galanga* L. extracts. *Molecules* 17(7): 8720-8734.

Umar, M.I., Asmawi, M.Z., Sadikun, A., Majid, A.M.S.A., Al-Suede, F.S.R., Hassan, L.E.A., Altaf, R. & Ahamed, M.B.K. 2014. Ethyl-p-methoxycinnamate isolated from *Kaempferia galanga* inhibits inflammation by suppressing interleukin-1, tumor necrosis factor-α, and angiogenesis by blocking endothelial functions. *Clinics* 69: 134-144.

Wijayanti, N., Astuti, K., Prasetia, I., Darayanthi, M., Nesa, P. & Wedarini, L. 2015. Physical chemical stability profile peel-off gel mask mangosteen peel extract (*Garcinia mangostana* L.). *J. Univ. Udayana* 9(1): 99-103.

Wijayanto, B.A., Kurniawan, D.W. & Sobri, I. 2013. formulation and effectiveness of antiseptic hand gel preparations containing galanga essential oils (*Alpinia galanga* (L.) willd). *Jurnal Ilmu Kefarmasian Indonesia* 11(2): 102-107.

Woodell-May, J.E. & Sommerfeld, S.D. 2020. Role of inflammation and the immune system in the progression of osteoarthritis. *Journal of Orthopaedic Research®* 38(2): 253-257.

*Corresponding author; email: muchtaridi@unpad.ac.id