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Title:
Effect of Gel from Erythrina Subumbrans And Plectranthus Amboinicus Leaf Extracts as Anti-Inflammatory Agent

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Principal Author:
Putu Indah Budiapsari1*, Ida Bagus Bramantya Dwinanta Kartha2, Putu Ayu Jaya Switi2, Komang Trinia Amanda Riadi2, Evellyne Natania2, Anak Agung Raka Dyah Nindyaswari2

Corresponding Author:
Putu Indah Budiapsari

Corresponding Author's Email:
putuindah51@yahoo.com

Corresponding Author's Mobile Number:
+6282236602539

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Effect of Gel from Erythrina Subumbrans And Plectranthus Amboinicus Leaf Extracts as Anti-Inflammatory Agent

Putu Indah Budiapsari[1*], Ida Bagus Bramantya Dwinanta Kartha[2], Putu Ayu Jaya Switi[2], Komang Trinia Amanda Riadi[2], Evellyne Natania[2], Anak Agung Raka Dyah Nindyaswari[2]
[1*]Department of Parasitology Faculty of Medicine and Health Sciences, Warmadewa University, Denpasar, Indonesia, 80235, https://orcid.org/0000-0003-0572-9581
[2]Faculty of Medicine and Health Sciences, Warmadewa University, Denpasar, Indonesia, 80235
Ida Bagus Bramantya Dwinanta Kartha: https://orcid.org/0009-0005-6777-0098
Evellyne Natania: https://orcid.org/0009-0002-8227-2220
Anak Agung Raka Dyah Nindyaswari: https://orcid.org/0009-0007-8485-7990
Putu Ayu Jaya Switi: https://orcid.org/0009-0005-6830-7249
Komang Trinia Amanda Riadi: https://orcid.org/0009-0001-8779-6560

Corresponding Author E-mail: putuindah51@yahoo.com
Orcid ID: 0000-0003-0572-9581

Abstract

Background: Various types of nutritious plants owned by Indonesia can be used as traditional medicine by the community because they are considered more economical and cause fewer side effects than chemical drugs. For example, the dadap plant (Erythrina subumbrans) and the cumin plant (Plectranthus amboinicus) which have potential as anti-inflammatory drugs. Dadap leaves

contain active ingredients, such as alkaloids, flavonoids, and tannins. Meanwhile, cumin leaves contain saponins, flavonoids, polyvenols, and essential oils. Flavonoids work by inhibiting the action of COX-2 which causes inflammation. Objective: This study aimed to determine the effectiveness of the extract gel of dadap leaves and cumin leaves as anti-inflammatory. Methods: The research was carried out through the stages of making extracts, making gel formulas, and testing male mice based on literature, such as journals, articles, and books. The data analysis method used was the ANOVA test which was obtained by observing the tumor, calor, rubor, and dolor of male mice. Conclusion: Dadap leaves and cumin leaves can be used as anti-inflammatory gels on the extremities. The three gel formulations of dadap leaves and cumin leaves can relieve inflammation. Results: From the test on mice, the most effective gel formulation was the formulation of 7.5% dadap leaf extract and 17.5% cumin leaf extract.

Keywords: *dadap, cumin, flavonoids, gel, inflammation.*

Introduction

Indonesia has various types of plants that are efficacious to be used as traditional medicines. Indonesian people are known for their traditional medicine from the past until now.[1] Traditional medicine is more in demand by the people of Indonesia because it is more economical, easy to obtain, and causes fewer side effects than chemical drugs. For example, the dadap plant (Erythrina subumbrans) and the cumin plant (Plectranthus amboinicus). Plants as traditional medicines are usually used by pounding and then affixing them to places that are sick.[2]

Inflammation is the body's response to infection and tissue damage caused by trauma or infection.[3] This response aims to eliminate the cause of tissue damage.[4] Inflammation can be acute or chronic inflammation.[5] Acute inflammation occurs within minutes to days and is characterized by increased blood flow and edema.[6] While chronic inflammation occurs in the long term that triggers an increase in cell type turnover.[7] Inflammation begins with an increase in capillary permeability, then continues with the recruitment of leukocytes in the injured area for phagocytosis that causes tissue damage, and ends with termination.[8] Inflammation is characterized by calor (heat), dolor (pain), tumor (swelling), and rubor (redness).[9] Rubor and calor occur simultaneously, where rubor is caused by dilatation of the arteries that supply blood to the injured area, resulting in increased blood flow.[10] Then, the presence of a tumor is caused by an increase in capillary permeability so that plasma leaves the blood vessels.[11] The release of chemicals or inflammatory mediators, such as histamine, prostaglandins, and serotonin can cause pain.[12] Dadap leaves are one of the plants that contain active ingredients, such as alkaloids, flavonoids, and tannins.[13] While cumin leaves contain saponins, flavonoids, polyphenols, and essential oils.[14] Both plants have the potential as anti-inflammatory drugs. Several countries in Southeast Asia use the stems and leaves of dadap as a medicine for infections and joint pain.[15] From this description, dadap leaves and cumin leaves have the potential as a traditional alternative in anti-inflammatory in the extremities which are formulated in the form of a gel.[16] This research is expected to be able to provide scientific information related to the effectiveness of dadap leaves and cumin leaves as anti-inflammatories in further research activities and provide benefits to the community to overcome inflammation in the extremities.[17] In addition, increasing the use value

of natural resources in Indonesia.[18] The purpose of this study was to determine the effectiveness of the extracted gel of dadap leaves and cumin leaves as anti-inflammatory.

Material And Methods

The research activity was carried out at the Biomedical Laboratory and the Animal Care Laboratory, Faculty of Medicine, Warmadewa University for 2 months starting in July 2021. Research was conducted by using several tools and materials. The tools used are a chopper, filter, oven, analytical balance, beaker, glass jar, evaporator, water bath, hot plate, glass stirrer, stone pulverizer, plethismometer, measuring cup, and thermometer.[19] Meanwhile, the ingredients used were dadap leaves, cumin leaves, 90% ethanol, aluminum foil, filter paper, dadap leaf extract, cumin leaf extract, Na-CMC, glycerin, propylene glycol, aqua dest, 20 male mice, dadap leaf extract gel. and cumin, syringes, alcohol swabs, 0.9% NaCl, and carrageenan.[20]

Research procedure

This experimental study used mice as animal model that modified to imitate the inflammation sign. The method used was collected by literature study, such as journals, books, and scientific articles. Primary data was obtained from the results of trials and observations of male mice species Mus musculus.[21] The first step of study was making extracts and making gel formulas from extracts of dadap leaves and cumin leaves. Furthermore, the experiment was conducted on 20 male mice. The test animals were divided into 4 groups and each group consisted of 5 male mice. The groups were given different treatments, namely the negative control group, treatment group 1, treatment group 2, and treatment group 3.[22] The data analysis method used was the ANOVA test which was obtained by observing the tumor and heat of male mice.[22] Furthermore, data analysis was carried out using the SPSS 27 program to determine the effectiveness of gel from dadap leaves and cumin leaves as an anti-inflammatory in the extremities.

Preparation of Dadap Leaf Extract and Cumin Leaves

Extracts were made by the maceration method. The first step is to clean the dadap and cumin leaves using clean water and dry them in the oven for 3 days. Then, each leaf is crushed using a chopper.[17] The crushed leaves will be sieved first. After being sifted, the dadap leaves and cumin leaves were put into different beakers and then ethanol was added up to 1 knuckle above the leaves. The leaves were placed in a glass jar covered with aluminium foil and allowed to stand for 3 days. After 3 days, it was filtered using filter paper to obtain the filtrate. The filtrate was evaporated and thickened using an evaporator and a water bath at a temperature of 70°C.[22]

Preparation of Dadap and Cumin Leaf Extract Gel

Starting with weighing the ingredients for making the gel according to the formula based on the standard gel based on Sodium Carboxymethyl Cellulose (Na-CMC), composed with Na-CMC 5%, glycerine 10%, propylene glycol 5%, 100% aqua dest. Gel formulation 1 was consist of Dadap leaf extracts 12.5% and cumin leaf 12.5%, Dadap leaf extract 0.3125 g, Cumin leaf extract 0.3125 g. Gel formulation 2 was consist of Dadap leaf extracts 17.5% and cumin leaf 7.5%, Dadap leaf extract 0.4375 g, Cumin leaf extract 0.1875 g. Gel formulation 3 was consist of Dadap leaf extract 7.5% and cumin leaf 17.5%, Dadap leaf extract 0.1875 g, Cumin leaf extract 0.4375 g. Each formula was dissolved with distilled water, then heated at a temperature of 50°C. After that, add 0.125 grams

of Na-CMC, 0.25 grams of glycerine, and 0.125 grams of propylene glycol, then stir regularly until a gel is formed.[1]

Gel Test Method on Mice

It was carried out using 20 healthy male (Mus musculus) mice that had been adapted to their environment for 1-2 weeks. After that, one leg of each mouse was injected intraplantar using 1% carrageenan which had been homogenized with 0.9% NaCl. Then the 20 male mice were divided into 4 groups and each group consisted of 5 male mice. Once every 30 minutes for 2 hours, each group of mice was given a different treatment, namely: Negative control group: the feet of mice were not treated.[5] Treatment group 1: mice's feet were treated with gel formula 1. Treatment group 2: mice's feet were treated with gel formula 2. Treatment group 3: mice's feet were treated with gel formula 3. Measurements of leg volume, and feet temperature observations in each group of mice to test the effectiveness of dadap and cumin leaf gel extracts as anti-inflammatories were carried out before and after the treatment.[11] Tumor measurements were examined by the volume of swelling feet of mice by dipping the feet to the ankles into the water on a plethysmometer. The temperature of feet also examined by using a thermometer. After induction of inflammation, then each group was given treatment for second time according to the formula. [13]

Results and Discussion

The results show combination of dadap and cumin in the formula I produced 2.25 grams gel, formula II produced 2.12 grams gel, and formula III produced 2.09 grams gel. The physical stability of each gel was evaluated by organoleptic analysis. Organoleptic analysis was carried out through observations in terms of clarity, colour, odour, and amount of gel extracts. The result of observation was describe in table 1. We found the formula 1 was less odour compared with formula 2 and 3. The strong smell of dadap found in formula 2, and the strong smell of cumin found in formula 3. The consistency of each formula appear to be same solid and minimal liquid.

Table 1. Result of Gel Analyses

Formula	Observation		
	Color	**Smell**	**Form**
Formula1	Dark green	Sharp, like extract	Solid, minimal liquid
Formula 2	Green	Sharp, like extract, leaves, strong smell of Erithrina	Solid, minimal liquid
Formula 3	Black green	Sharp, like extract, leaves, strong smell of Cumin	Solid, minimal liquid

Twenty male mice aged 7-8 weeks weight 25-30 gram was used as experimented animal. Each mouse was adapted for 7 days to environmental conditions. The condition of male mice before intervention was healthy, there were no abnormalities in behavior and clinical appearance. The cage was maintained to be clean so the mice were not contaminated. At the first day inflammation induction, we measure the leg volume and temperature of feet before treated by gel formula. The result was described in table 2.

Table 2. Mean temperature and Volume of mouse leg before treatment

Variable	Temperature (°C) mean	Leg Volume (ml) mean
Negative control	36,58	0,14

Treatment 1	36,6	0,13
Treatment 2	36,61	0,12
Treatment 3	36,7	0,12

Table 3. Mean temperature and Volume of mouse leg after induction of inflammation

Variable	First 30 minute temperature (°C)	First 30 minute Volume (ml)	After 60 minute temperature (°C)	After 60 minute Volume (ml)
Negative control	37,3	0,21	37,5	0,22
Treatment 1	37,4	0,19	37,5	0,24
Treatment 2	37,3	0,23	37,2	0,23
Treatment 3	37,2	0,15	37,6	0,24

Table 4. Mean temperature and Volume of mouse leg after gel application

Variable	After 30 minute temperature (°C)	After 30 minute Volume (ml)	After 60 minute temperature (°C)	After 60 minute Volume (ml)	After 90 minute temperature (°C)	After 90 minute Volume (ml)
Negative control	37,2	0,18	37,1	0,19	37,3	0,19
Treatment 1	36,9	0,13	36,9	0,12	36,5	0,12
Treatment 2	37,3	0,13	37	0,13	36,8	0,13
Treatment 3	37	0,12	36,6	0,13	36,5	0,13

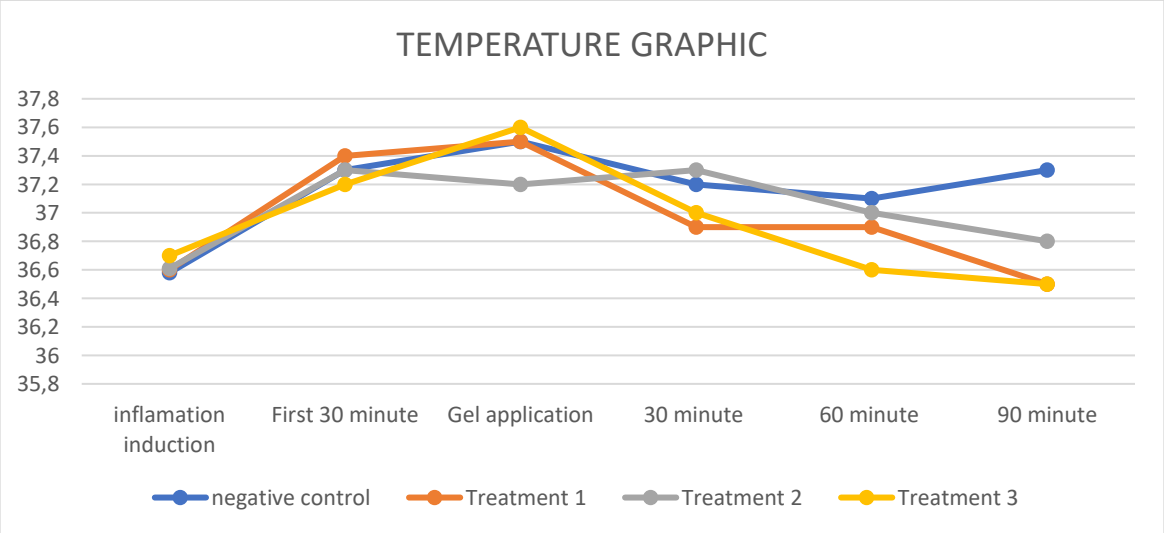

Figure 1. Temperature change of any time and treatment

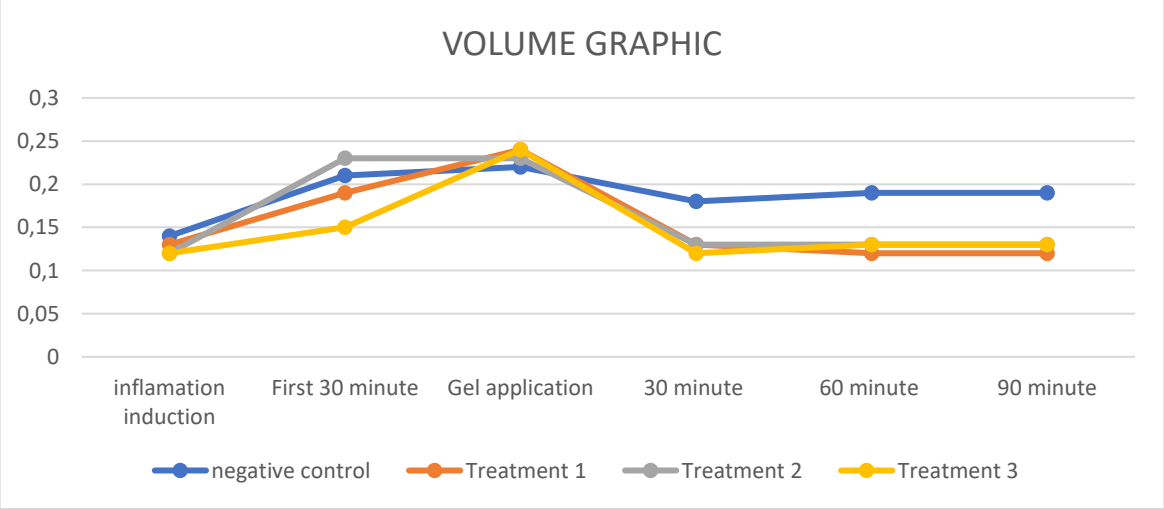

Figure 1. Volume change of any time and treatment

Table 5. The Difference of leg temperature and volume before intervention, after inflammation and after gel application.

Variable	Time Observation	Mean	SD	P	95% *Confidence Interval*	
					Lower	Upper
Temperature	Before Intervention	36.6	0.10	0.00	36.66	36.58
	After Inflammation	37.3	0.15		37.42	37.34
	After Gel application	36.9	0.33		36.86	37.012
Leg Volume	Before Intervention	0.12	0.01	0.00	0.13	0.12
	After Inflammation	0.21	0.03		0.22	0.20
	After Gel application	0.13	0.02		0.14	0.13

*Significance at p<0.05, Temperature: p 0.00, Volume: p 0.00

One-way Anova test was using to analyze the mean difference of mice temperature before intervention, after inflammation induction and after gel application. The result show there was significant statistically (p<0.05). The leg volume before intervention, after inflammation induction and after gel application also significant statistically (p<0.05). Although the mean difference are not represented the fever.

Discussion

Dadap leaves and cumin leaves contain active compounds that can act as anti-inflammatories. Dadap leaves contain alkaloids, flavonoids, and tannins.[4] Meanwhile, cumin leaves contain saponins, flavonoids, polyphenols, and essential oils.[5] Flavonoids are a typical compound that is usually found in green plants. Flavonoid bio-actives are considered the most important phytochemicals that have broad biological benefits for humans such as anti-inflammatory, antioxidant, and antimicrobial (Arifin and Ibrahim 2018). Flavonoids are phenolic compounds that can inhibit the work of COX-2, causing a decrease in the production of prostaglandins and inhibiting of the release of arachidonic acid (Putra, 2015). Arachidonic acid and prostaglandins are mediators of inflammation. Saponins work through interactions with membrane lipids, such as phospholipids which are precursors of prostaglandins and other inflammatory mediators.[12]

Conclusion

From the results of this study, we can conclude that dadap leaves and cumin leaves can be used as anti-inflammatory gels on the extremities. The three formulations of dadap leaf and cumin leaf gel formulations can relieve inflammation. The results of the test on mice showed that the most effective gel formulation was the formulation of 7.5% dadap leaf extract and 17.5% cumin leaf extract. Scientific conjectures for future research, namely: Finding the right level of flavonoid and alkaloid test so that it is effectively used as an anti-inflammatory in the extremities. Applying the formulation of dadap leaves and cumin leaves in other forms that have a higher probability of effectiveness than the gel form. Testing the effectiveness of the gel from extracts of dadap leaves and cumin leaves on other areas of the body other than the extremities.

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Conflict of Interest

None declared

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Review report BPJ_6086

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Note: This review report is divided into four sections to evaluate manuscript, kindly explain your

observation in a precise manner in each section.

Manuscript Title: ***Effect of Gel from Erythrina Subumbrans and Plectranthus Amboinicus Leaf Extracts as Anti-Inflammatory Agent***

Section I: Evaluation

1. **Scope**: Is the work fits better in the scope of the journal?
Comment: Yes, the article deals with animal study models which slightly fits the scope of our journal.
2. **Title**: Is the title appropriate, informative, concise, and clear? Does the title clearly and sufficiently reflect the content?
Comment: There is absence of mention of animal study model in title. It is too concise. The study is in-vivo or in-vitro, it is not clear by title. It is a general trend in Scopus level articles to mention it and is expected.

3. **Abstract**: Is it comprehensive by itself? Is the important and essential information of the article included?
Comment: Yes, it mentions all the aspects i.e., Background, Objectives, Method and Conclusion.

4. References: Are appropriate and adequate references to related works covered sufficiently in the list?
Comment: Given references are related to work covered but can add more for animal study perspective.

5. Structure and length: Is the overall structure of the article well organized and well balanced? Is the article written with the minimum length necessary for all relevant information?
Comment: Yes, overall article structure is well organised and balanced.
6. Logic: Is the article written clearly and correctly? Is it logically consistent?
Comment: Yes, it is written clearly and correctly. logical consistent is there in article.
7. Figures and tables: Are they essential and clearly presented?
Comment: Mostly the figures and tables are clearly presented but the pictures of animal study are missing. In all tables 1,2,3 and 4 commas have been used instead of decimal point. Which leads to calculation error.
Images pertaining to organoleptic properties are missing which tells it colour and form.
8. Discussion: Are all possible interpretations of the data considered or are there alternative hypotheses that are consistent with the available data?
Comment: ANOVA table is not mention here. Pharmacological mechanism of action of gel formulation is very short, it should be explained in more detail.

9. Conclusion: Are the conclusions of the study supported by appropriate evidence or are the claims exaggerated?
Comment: Yes, conclusion is supported by evidences but there are few points missing

a) Name of gel formulation best working (formula 1 or 2 or 3) is missing
b) Reason as to why a particular formulation is successful is not mentioned clearly.

10. Language: Is the English used in the article readable and good enough to convey the scientific meaning correctly? Comment: English used in the article is very understanding but some spellings and grammatical errors are present, highlighted with yellow colour in article.

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Section II: Reviewer's remarks to author

Please recommend specific changes below in detail (if any). Review comments may also be listed by page and line number, or marked separately in the manuscript.
Comments:

There is no mention of feed given to animals during the course of study as feed provided always has influence.

The duration or time span of study is too small. Study indicated that whole animal model study was conducted in just 90 minutes. Which is too short a study for any conclusion irrespective of how many groups were taken for study.

In table 3, the effect of inflammation seems very low in case of treatment 3 after 30 minutes. indicating that group is flawed in some sense, as all groups should have inflammation in same range as formulation as not been applied yet. The reason for such abnormality should be mentioned.

There is no mention of approval from Animal Ethics committee of the institution and neither the reference number is present.

Source and time of procurement of leaves is missing. Identification/authentication of plants is also missing

Phytochemical screening of leaf extracts is missing, which indicates what constituents are present in it.

Some of the references are irrelevant e.g. 3 and 6.

☐ Accept unconditionally
☐ Accept with Minor Revisions

✓ Major Revision, reject in current form; can be accepted after recommended changes.

☐ Reject outright

Section V- Referee's Suggestions to Editor-in-Chief

Please add your suggestion(s) that will be communicated to Editor-in-Chief only.

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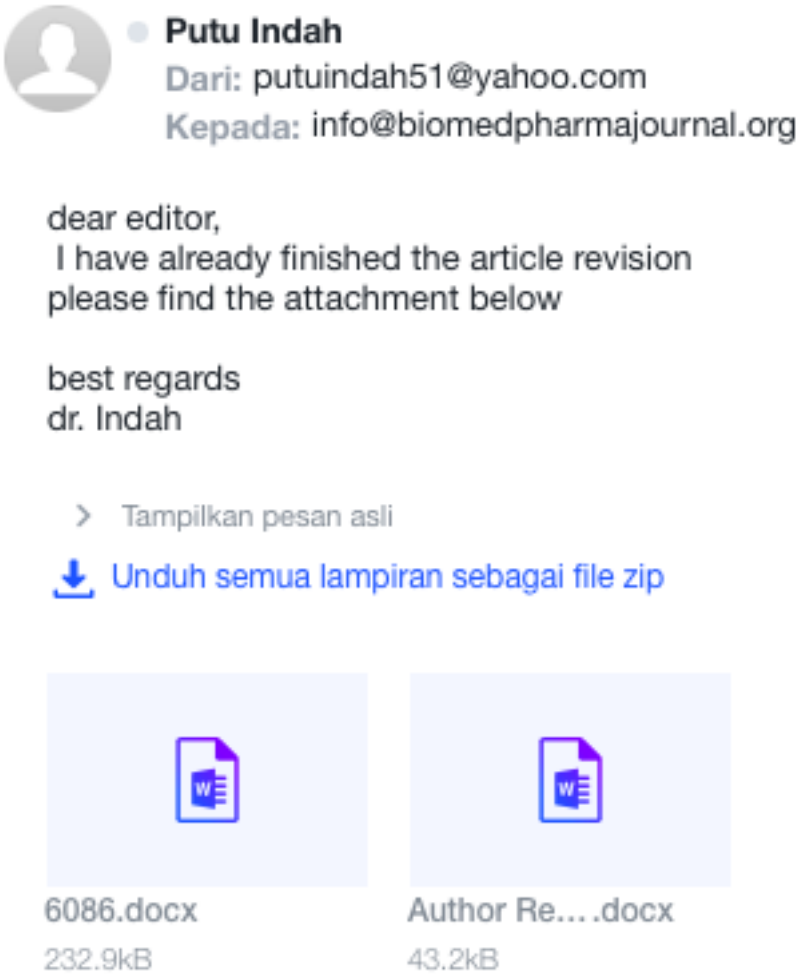

Effect of Gel from Erythrina Subumbrans And Plectranthus Amboinicus Leaf Extracts as Anti-Inflammatory Agent

Abstract

Background: Various types of nutritious plants owned by Indonesia can be used as traditional medicine by the community because they are considered more economical and cause fewer side effects than chemical drugs. For example, the dadap plant (Erythrina subumbrans) and the cumin plant (Plectranthus amboinicus) which have potential as anti-inflammatory drugs. Dadap leaves contain active ingredients, such as alkaloids, flavonoids, and tannins. Meanwhile, cumin leaves contain saponins, flavonoids, polyvenols, and essential oils. Flavonoids work by inhibiting the action of COX-2 which causes inflammation. Objective: This study aimed to determine the effectiveness of the extract gel of dadap leaves and cumin leaves as anti-inflammatory. Methods: The research was carried out through the stages of making extracts, making gel formulas, and testing male mice based on literature, such as journals, articles, and books. The data analysis method used was the ANOVA test which was obtained by observing the tumor, calor, rubor, and dolor of male mice. Conclusion: Dadap leaves and cumin leaves can be used as anti-inflammatory gels on the extremities. The three gel formulations of dadap leaves and cumin leaves can relieve inflammation. Results: From the test on mice, the most effective gel formulation was the formulation of 7.5% dadap leaf extract and 17.5% cumin leaf extract.

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Keywords: *dadap, cumin, flavonoids, gel, inflammation.*

Introduction

Indonesia has various types of plants that are efficacious to be used as traditional medicines. Indonesian people are known for their traditional medicine from the past until now.[1] Traditional medicine is more in demand by the people of Indonesia because it is more economical, easy to obtain, and causes fewer side effects than chemical drugs. For example, the dadap plant (Erythrina subumbrans) and the cumin plant (Plectranthus amboinicus). Plants as traditional medicines are usually used by pounding and then affixing them to places that are sick.[2]

Inflammation is the body's response to infection and tissue damage caused by trauma or infection.[3] This response aims to eliminate the cause of tissue damage.[4] Inflammation can be acute or chronic inflammation.[5] Acute inflammation occurs within minutes to days and is characterized by increased blood flow and edema.[6] While chronic inflammation occurs in the long term that triggers an increase in cell type turnover.[7] Inflammation begins with an increase in capillary permeability, then continues with the recruitment of leukocytes in the injured area for phagocytosis that causes tissue damage, and ends with termination.[8] Inflammation is characterized by calor (heat), dolor (pain), tumor (swelling), and rubor (redness).[9] Rubor and calor occur simultaneously, where rubor is caused by dilatation of the arteries that supply blood to the injured area, resulting in increased blood flow.[10] Then, the presence of a tumor is caused by an increase in capillary permeability so that plasma leaves the blood vessels.[11] The release of chemicals or inflammatory mediators, such as histamine, prostaglandins, and serotonin can cause pain.[12] Dadap leaves are one of the plants that contain active ingredients, such as alkaloids, flavonoids, and tannins.[13] While cumin leaves contain saponins, flavonoids, polyphenols, and essential oils.[14] Both plants have the potential as anti-inflammatory drugs. Several countries in Southeast Asia use the stems and leaves of dadap as a medicine for infections and joint pain.[15] From this description, dadap leaves and cumin leaves have the potential as a traditional alternative in anti-inflammatory in the extremities which are formulated in the form of a gel.[16] This research is expected to be able to provide scientific information related to the effectiveness of dadap leaves and cumin leaves as anti-inflammatories in further research activities and provide benefits to the community to overcome inflammation in the extremities.[17] In addition, increasing the use value of natural resources in Indonesia.[18] The purpose of this study was to determine the effectiveness of the extracted gel of dadap leaves and cumin leaves as anti-inflammatory.

Material And Methods

The research activity was carried out at the Biomedical Laboratory and the Animal Care Laboratory, Faculty of Medicine, Warmadewa University for 2 months starting in July 2021. Research was conducted by using several tools and materials. The tools used are a chopper, filter, oven, analytical balance, beaker, glass jar, evaporator, water bath, hot plate, glass stirrer, stone pulverizer, plethismometer, measuring cup, and thermometer.[19] Meanwhile, the ingredients used were dadap leaves, cumin leaves, 90% ethanol, aluminum foil, filter paper, dadap leaf extract, cumin leaf

extract, Na-CMC, glycerin, propylene glycol, aqua dest, 20 male mice, dadap leaf extract gel. and cumin, syringes, alcohol swabs, 0.9% NaCl, and carrageenan.[20]

Research procedure

This experimental study used mice as animal model that modified to imitate the inflammation sign. The method used was collected by literature study, such as journals, books, and scientific articles. Primary data was obtained from the results of trials and observations of male mice species Mus musculus.[21] The first step of study was making extracts and making gel formulas from extracts of dadap leaves and cumin leaves. Furthermore, the experiment was conducted on 20 male mice. The test animals were divided into 4 groups and each group consisted of 5 male mice. The groups were given different treatments, namely the negative control group, treatment group 1, treatment group 2, and treatment group 3.[22] The data analysis method used was the ANOVA test which was obtained by observing the tumor and heat of male mice.[22] Furthermore, data analysis was carried out using the SPSS 27 program to determine the effectiveness of gel from dadap leaves and cumin leaves as an anti-inflammatory in the extremities.

Preparation of Dadap Leaf Extract and Cumin Leaves

Extracts were made by the maceration method. The first step is to clean the dadap and cumin leaves using clean water and dry them in the oven for 3 days. Then, each leaf is crushed using a chopper.[17] The crushed leaves will be sieved first. After being sifted, the dadap leaves and cumin leaves were put into different beakers and then ethanol was added up to 1 knuckle above the leaves. The leaves were placed in a glass jar covered with aluminium foil and allowed to stand for 3 days. After 3 days, it was filtered using filter paper to obtain the filtrate. The filtrate was evaporated and thickened using an evaporator and a water bath at a temperature of 70°C.[22]

Preparation of Dadap and Cumin Leaf Extract Gel

Starting with weighing the ingredients for making the gel according to the formula based on the standard gel based on Sodium Carboxymethyl Cellulose (Na-CMC), composed with Na-CMC 5%, glycerine 10%, propylene glycol 5%, 100% aqua dest. Gel formulation 1 was consist of Dadap leaf extracts 12.5% and cumin leaf 12.5%, Dadap leaf extract 0.3125 g, Cumin leaf extract 0.3125 g. Gel formulation 2 was consist of Dadap leaf extracts 17.5% and cumin leaf 7.5%, Dadap leaf extract 0.4375 g, Cumin leaf extract 0.1875 g. Gel formulation 3 was consist of Dadap leaf extract 7.5% and cumin leaf 17.5%, Dadap leaf extract 0.1875 g, Cumin leaf extract 0.4375 g. Each formula was dissolved with distilled water, then heated at a temperature of 50°C. After that, add 0.125 grams of Na-CMC, 0.25 grams of glycerine, and 0.125 grams of propylene glycol, then stir regularly until a gel is formed.[1]

Gel Test Method on Mice

It was carried out using 20 healthy male (Mus musculus) mice that had been adapted to their environment for 1-2 weeks. After that, one leg of each mouse was injected intraplantar using 1% carrageenan which had been homogenized with 0.9% NaCl. Then the 20 male mice were divided into 4 groups and each group consisted of 5 male mice. Once every 30 minutes for 2 hours, each group of mice was given a different treatment, namely: Negative control group: the feet of mice were not treated.[5] Treatment group 1: mice's feet were treated with gel formula 1. Treatment group 2: mice's feet were treated with gel formula 2. Treatment group 3: mice's feet were treated

with gel formula 3. Measurements of leg volume, and feet temperature observations in each group of mice to test the effectiveness of dadap and cumin leaf gel extracts as anti-inflammatories were carried out before and after the treatment.[11] Tumor measurements were examined by the volume of swelling feet of mice by dipping the feet to the ankles into the water on a plethysmometer. The temperature of feet also examined by using a thermometer. After induction of inflammation, then each group was given treatment for second time according to the formula. [13]

Results and Discussion

The results show combination of dadap and cumin in the formula I produced 2.25 grams gel, formula II produced 2.12 grams gel, and formula III produced 2.09 grams gel. The physical stability of each gel was evaluated by organoleptic analysis. Organoleptic analysis was carried out through observations in terms of clarity, colour, odour, and amount of gel extracts. The result of observation was describe in table 1. We found the formula 1 was less odour compared with formula 2 and 3. The strong smell of dadap found in formula 2, and the strong smell of cumin found in formula 3. The consistency of each formula appear to be same solid and minimal liquid.

Table 1. Result of Gel Analyses

Formula	Observation		
	Color	**Smell**	**Form**
Formula1	Dark green	Sharp, like extract	Solid, minimal liquid
Formula 2	Green	Sharp, like extract, leaves, strong smell of Erithrina	Solid, minimal liquid
Formula 3	Black green	Sharp, like extract, leaves, strong smell of Cumin	Solid, minimal liquid

Twenty male mice aged 7-8 weeks weight 25-30 gram was used as experimented animal. Each mouse was adapted for 7 days to environmental conditions. The condition of male mice before intervention was healthy, there were no abnormalities in behavior and clinical appearance. The cage was maintained to be clean so the mice were not contaminated. At the first day inflammation induction, we measure the leg volume and temperature of feet before treated by gel formula. The result was described in table 2.

Table 2. Mean temperature and Volume of mouse leg before treatment

Variable	Temperature (°C) mean	Leg Volume (ml) mean
Negative control	36,58	0,14
Treatment 1	36,6	0,13
Treatment 2	36,61	0,12
Treatment 3	36,7	0,12

Table 3. Mean temperature and Volume of mouse leg after induction of inflammation

Variable	First 30 minute temperature (°C)	First 30 minute Volume (ml)	After 60 minute temperature (°C)	After 60 minute Volume (ml)
Negative control	37,3	0,21	37,5	0,22

Treatment 1	37,4	0,19	37,5	0,24
Treatment 2	37,3	0,23	37,2	0,23
Treatment 3	37,2	0,15	37,6	0,24

Table 4. Mean temperature and Volume of mouse leg after gel application

Variable	After 30 minute temperature (°C)	After 30 minute Volume (ml)	After 60 minute temperature (°C)	After 60 minute Volume (ml)	After 90 minute temperature (°C)	After 90 minute Volume (ml)
Negative control	37,2	0,18	37,1	0,19	37,3	0,19
Treatment 1	36,9	0,13	36,9	0,12	36,5	0,12
Treatment 2	37,3	0,13	37	0,13	36,8	0,13
Treatment 3	37	0,12	36,6	0,13	36,5	0,13

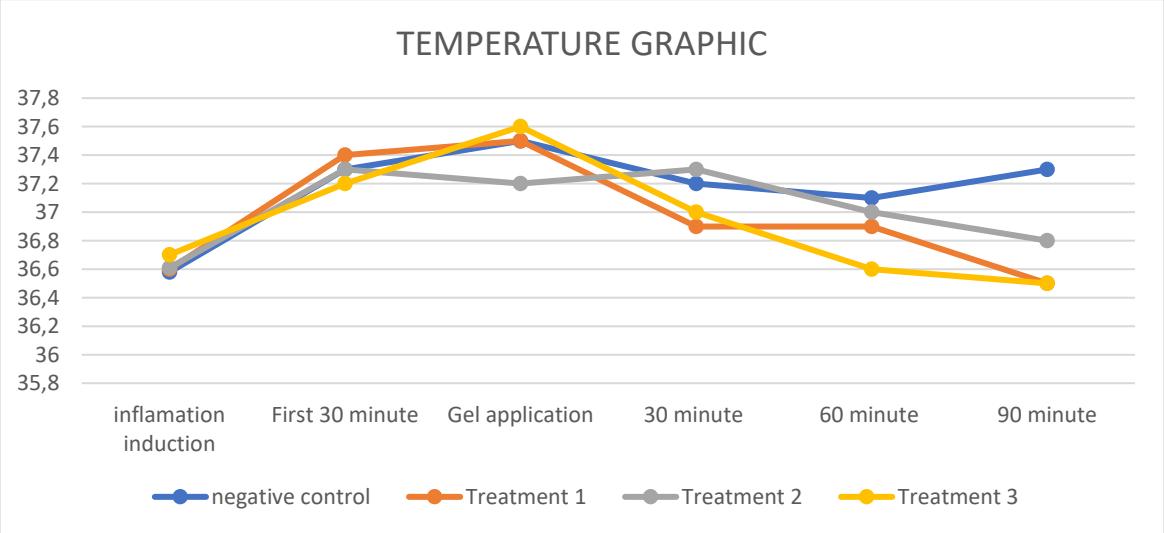

Figure 1. Temperature change of any time and treatment

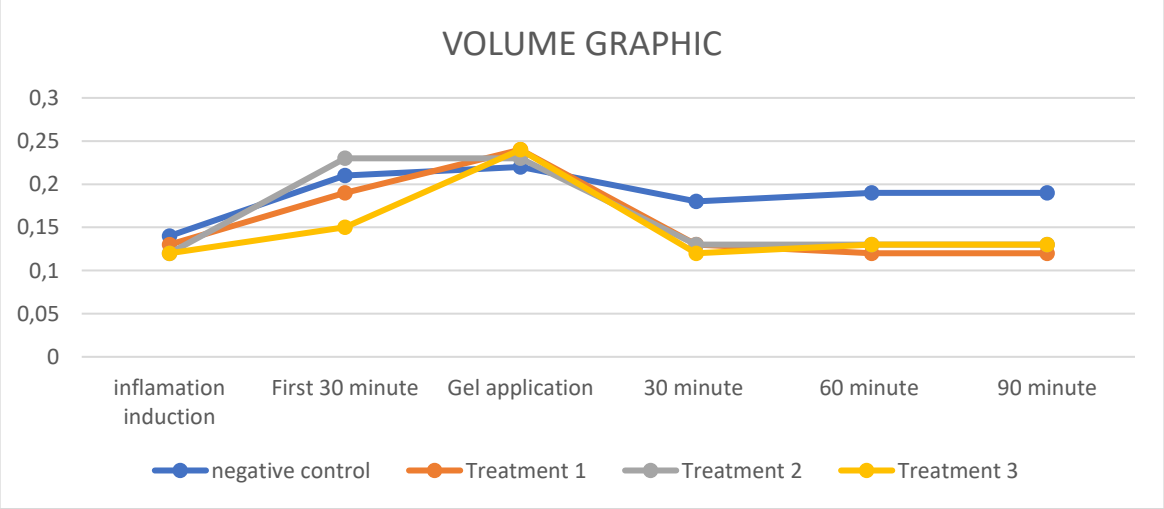

Figure 1. Volume change of any time and treatment

Table 5. The Difference of leg temperature and volume before intervention, after inflammation and after gel application.

Variable	Time Observation	Mean	SD	P	95% *Confidence Interval*	
					Lower	Upper
Temperature	Before Intervention	36.6	0.10	0.00	36.66	36.58
	After Inflammation	37.3	0.15		37.42	37.34
	After Gel application	36.9	0.33		36.86	37.012
Leg Volume	Before Intervention	0.12	0.01	0.00	0.13	0.12
	After Inflammation	0.21	0.03		0.22	0.20
	After Gel application	0.13	0.02		0.14	0.13

*Significance at p<0.05, Temperature: p 0.00, Volume: p 0.00

One-way Anova test was using to analyze the mean difference of mice temperature before intervention, after inflammation induction and after gel application. The result show there was significant statistically (p<0.05). The leg volume before intervention, after inflammation induction and after gel application also significant statistically (p<0.05). Although the mean difference are not represented the fever.

Discussion

Dadap leaves and cumin leaves contain active compounds that can act as anti-inflammatories. Dadap leaves contain alkaloids, flavonoids, and tannins.[4] Meanwhile, cumin leaves contain saponins, flavonoids, polyphenols, and essential oils.[5] Flavonoids are a typical compound that is usually found in green plants. Flavonoid bio-actives are considered the most important phytochemicals that have broad biological benefits for humans such as anti-inflammatory, antioxidant, and antimicrobial (Arifin and Ibrahim 2018). Flavonoids are phenolic compounds that can inhibit the work of COX-2, causing a decrease in the production of prostaglandins and inhibiting of the release of arachidonic acid (Putra, 2015). Arachidonic acid and prostaglandins are mediators of inflammation. Saponins work through interactions with membrane lipids, such as phospholipids which are precursors of prostaglandins and other inflammatory mediators.[12]

Conclusion

From the results of this study, we can conclude that dadap leaves and cumin leaves can be used as anti-inflammatory gels on the extremities. The three formulations of dadap leaf and cumin leaf gel formulations can relieve inflammation. The results of the test on mice showed that the most effective gel formulation was the formulation of 7.5% dadap leaf extract and 17.5% cumin leaf extract. Scientific conjectures for future research, namely: Finding the right level of flavonoid and alkaloid test so that it is effectively used as an anti-inflammatory in the extremities. Applying the formulation of dadap leaves and cumin leaves in other forms that have a higher probability of effectiveness than the gel form. Testing the effectiveness of the gel from extracts of dadap leaves and cumin leaves on other areas of the body other than the extremities.

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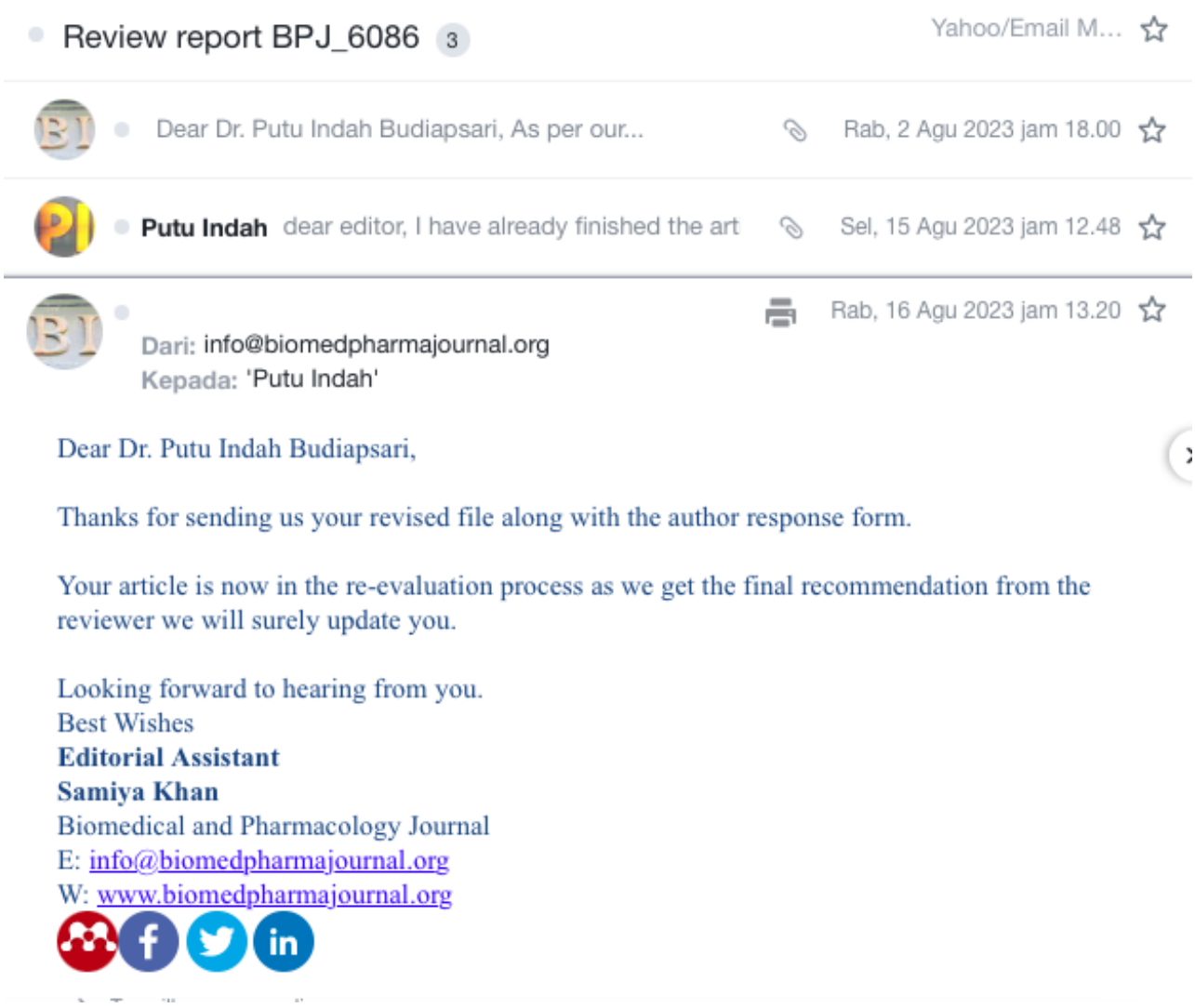

Review report BPJ_6086 3

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This experimental study used mice as animal model that modified to imitate the inflammation sign. The method used was collected by literature study, such as journals, books, and scientific articles. Primary data was obtained from the results of trials and observations of male mice species Mus musculus.[21] The first step of study was making extracts and making gel formulas from extracts of dadap leaves and cumin leaves. Furthermore, the experiment was conducted on 20 male mice. The test animals were divided into 4 groups and each group consisted of 5 male mice. The groups were given different treatments, namely the negative control group, treatment group 1, treatment group 2, and treatment group 3.[22] The data analysis method used was the ANOVA test which was obtained by observing the tumor and heat of male mice.[22] Furthermore, data analysis was carried out using the SPSS 27 program to determine the effectiveness of gel from dadap leaves and cumin leaves as an anti-inflammatory in the extremities.

Identification/Authentication of plants

Erythrina Subumbrans characterized by medium-sized tree, reaching 15–20 m high and 50–60 cm tall. The young, smooth bark has green, gray, light brown or whitish vertical stripes; Stems usually with small (1–2 mm) black sticky spines. The canopy is like an umbrella or loosely rounded, dropping leaves in the dry season. The compound leaves have three leaves, green to light green, leaf axils with 10–40 cm long stalks. Child leaves round egg upside down, triangular, to a rhombus shape with a blunt tip; the largest leaflets in size, 9–25 × 10–30 cm. The flowers are arranged in conical bunches, beside or at the ends of bare branches, usually appearing when the leaves fall, attracting many birds to pollinate them. The crown is orange-red to dark red; flag 5.5–8 × 8 cm, with short nails, no white stripe.[3] Thick, dark pods, narrow between the seeds, 15–20 cm × 1.5–2 cm, containing 5–10 ovoid, brown, red, or purple glossy seeds.[1,2]

Figure 1. Erythrina Subumbrans (Dadap leaves)

Plectranthus Amboinicus (Cumin leaf) is an annual herb with an often woody, ascending base and reaching 1 m in height. The trunk is jointed, and what touches the ground will come out of it. The leaves are single, fleshy, oval in shape, the tip and base are pointed with jagged/ringgitated edges, except at the base. The leaf bones are pinnate, and the branches form a

net-like shape. The surface is thick hairy, like white velvet with a length of 5-7 cm, and a width of 4-6 cm and the color is light green, smells good when squeezed. The inflorescence is compound in the form of bunches with a length of 20 cm, coming out of the ends of the branches, and the axils of the leaves with a purplish-blue color. The seeds are hard, flattened, and light brown in color.[15]

Figure 2. Plectranthus Amboinicus

Preparation of Erythrina Subumbrans and Cumin Extract

Extracts were made by the maceration method. The first step is to clean the dadap and cumin leaves using clean water and dry them in the oven for 3 days. Then, each leaf is crushed using a chopper.[17] The crushed leaves will be sieved first. After being sifted, the dadap leaves and cumin leaves were put into different beakers and then ethanol was added up to 1 knuckle above the leaves. The leaves were placed in a glass jar covered with aluminium foil and allowed to stand for 3 days. After 3 days, it was filtered using filter paper to obtain the filtrate. The filtrate was evaporated and thickened using an evaporator and a water bath at a temperature of 70°C.[22]

Preparation of Dadap and Cumin Leaf Extract Gel

Starting with weighing the ingredients for making the gel according to the formula based on the standard gel based on Sodium Carboxymethyl Cellulose (Na-CMC), composed with Na-CMC 5%, glycerine 10%, propylene glycol 5%, 100% aqua dest. Gel formulation 1 was consist of Dadap leaf extracts 12.5% and cumin leaf 12.5%, Dadap leaf extract 0.3125 g, Cumin leaf extract 0.3125 g. Gel formulation 2 was consist of Dadap leaf extracts 17.5% and cumin leaf 7.5%, Dadap leaf extract 0.4375 g, Cumin leaf extract 0.1875 g. Gel formulation 3 was consist of Dadap leaf extract 7.5% and cumin leaf 17.5%, Dadap leaf extract 0.1875 g, Cumin leaf extract 0.4375 g. Each formula was dissolved with distilled water, then heated at a temperature of 50°C. After that, add 0.125 grams of Na-CMC, 0.25 grams of glycerine, and 0.125 grams of propylene glycol, then stir regularly until a gel is formed.[1]

Gel Test Method on Mice

It was carried out using 20 healthy male (Mus musculus) mice that had been adapted to their environment for 1-2 weeks. After that, one leg of each mouse was injected intraplantar using 1% carrageenan which had been homogenized with 0.9% NaCl. Then the 20 male mice were divided into 4 groups and each group consisted of 5 male mice. Once every 30 minutes for 2 hours, each group of mice was given a different treatment, namely: Negative control group: the feet of mice were not treated.[5] Treatment group 1: mice's feet were treated with gel formula 1. Treatment group 2: mice's feet were treated with gel formula 2. Treatment group 3: mice's feet were treated with gel formula 3. Measurements of leg volume, and feet temperature observations in each group of mice to test the effectiveness of dadap and cumin leaf gel extracts as anti-inflammatories were carried out before and after the treatment.[11] Tumor measurements were examined by the volume

of swelling feet of mice by dipping the feet to the ankles into the water on a plethysmometer. The temperature of feet also examined by using a thermometer. After induction of inflammation, then each group was given treatment for second time according to the formula. [13]

Ethical Approval

This research has been approved by Ethical Committee of Medical faculty of Udayana University by number: 2177/UN14.2.2.VII.14/LT/2021

Results and Discussion

The results show combination of dadap and cumin in the formula I produced 2.25 grams gel, formula II produced 2.12 grams gel, and formula III produced 2.09 grams gel. The physical stability of each gel was evaluated by organoleptic analysis. Organoleptic analysis was carried out through observations in terms of clarity, colour, odour, and amount of gel extracts. The result of observation was described in table 1. We found the formula 1 was less odour compared with formula 2 and 3. The strong smell of Erythrina Subumbrans was found in formula 2, and the strong smell of cumin found in formula 3. The consistency of each formula appear to be same solid and minimal liquid.

Table 1. Result of Gel Analyses

Formula	Observation		
	Color	**Smell**	**Form**
Formula1	Dark green	Sharp, like extract	Solid, minimal liquid
Formula 2	Green	Sharp, like extract, leaves, strong smell of Erithrina	Solid, minimal liquid
Formula 3	Black green	Sharp, like extract, leaves, strong smell of Cumin	Solid, minimal liquid

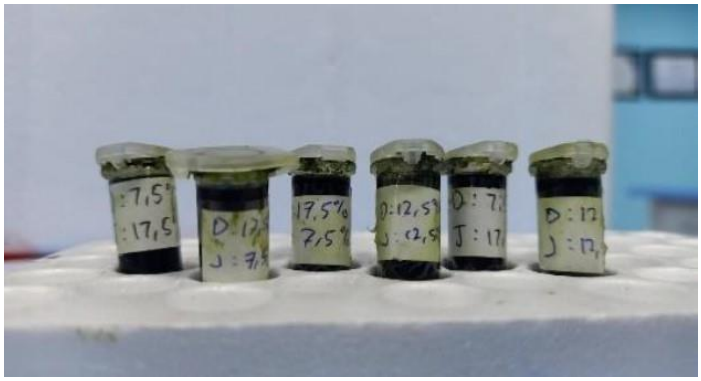

Figure 3. Quality of Each Extract Concentration

The quality of each extract was described as smell, color and concistency. There were no difference concistency of all formula, furthermore formula 2 which more dominantly composed by eritryna colored greener than formula 3 composed dominantly by Cumin darker. Twenty male mice aged 7-8 weeks weight 25-30 gram was used as experimented animal. Each mouse was adapted for 7 days to environmental conditions. The condition of male mice before intervention was healthy, there were no abnormalities in behavior and clinical appearance. The cage was maintained to be clean so the mice were not contaminated. At the first day inflammation induction, we measure the leg volume and temperature of feet before treated by gel formula. The result was described in table 2.

Table 2. Mean temperature and Volume of mouse leg before treatment

Variable	Temperature (°C) mean	Leg Volume (ml) mean
Negative control	36.58	0.14
Treatment 1	36.6	0.13
Treatment 2	36.61	0.12
Treatment 3	36.7	0.12

Table 3. Mean temperature and Volume of mouse leg after induction of inflammation

Variable	First 30-minute temperature (°C)	First 30-minute Volume (ml)	After 60-minute temperature (°C)	After 60-minute Volume (ml)
Negative control	37.3	0.21	37.5	0.22
Treatment 1	37.4	0.19	37.5	0.24
Treatment 2	37.3	0.23	37.2	0.23
Treatment 3	37.2	0.15	37.6	0.24

The mean of leg temperature before and after treatment shown to be same range, this result may be caused by two possible reason, first the dose of carrageenan must be low so could not induce massive increasing of temperature, second time observation too short, so increasing of temperature not reached optimum level.

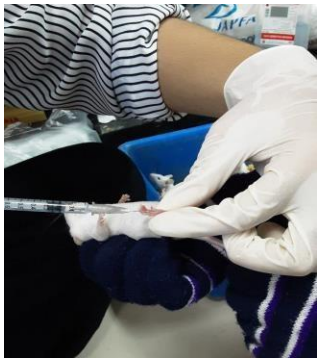

Figure 4. Inflamation induction

Figure 5. Gel application in mice

Figure 6. Temperature examination

Figure 7. Measurement of Leg volume

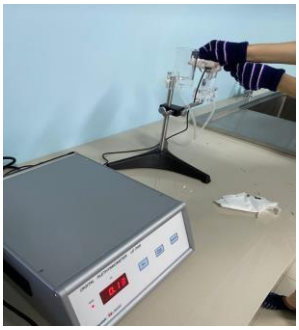

Table 4. Mean temperature and Volume of mouse leg after gel application

Variable	After 30-minute temperature (°C)	After 30-minute Volume (ml)	After 60-minute temperature (°C)	After 60-minute Volume (ml)	After 90-minute temperature (°C)	After 90-minute Volume (ml)
Negative control	37.2	0.18	37.1	0.19	37.3	0.19
Treatment 1	36.9	0.13	36.9	0.12	36.5	0.12
Treatment 2	37.3	0.13	37	0.13	36.8	0.13
Treatment 3	37	0.12	36.6	0.13	36.5	0.13

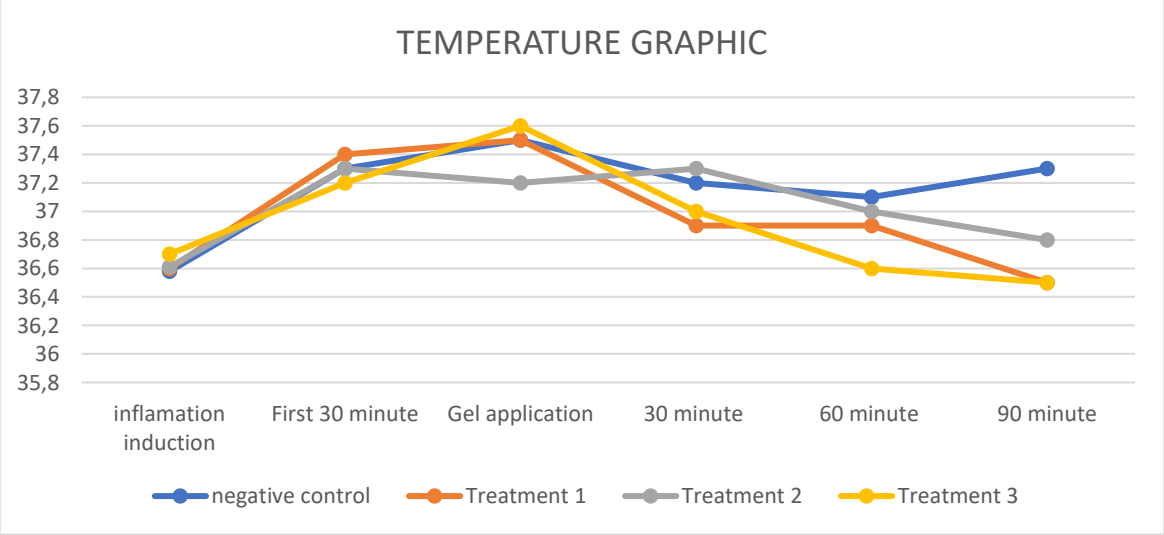

Figure 8. Temperature change of any time and treatment

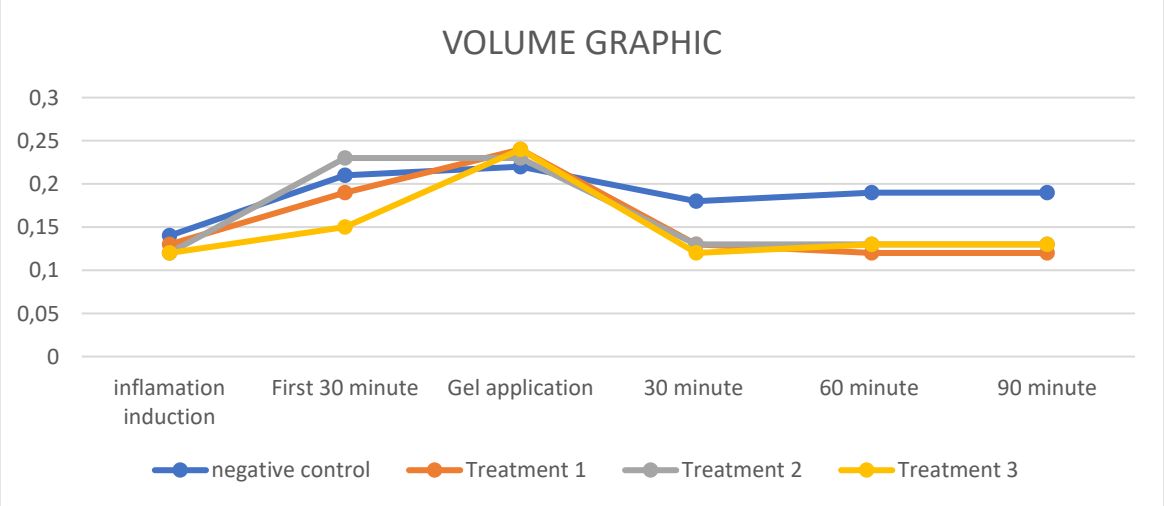

Figure 9. Volume change of any time and treatment

Table 5. The Difference of leg temperature and volume before intervention, after inflammation and after gel application.

Variable	Time Observation	Mean	SD	P	95% *Confidence Interval*	
					Lower	Upper

Temperature	Before Intervention	36.6	0.10	0.01	36.66	36.58
	After Inflammation	37.3	0.15		37.42	37.34
	After Gel application	36.9	0.33		36.86	37.012
Leg Volume	Before Intervention	0.12	0.01	0.01	0.13	0.12
	After Inflammation	0.21	0.03		0.22	0.20
	After Gel application	0.13	0.02		0.14	0.13

*Significance at p<0.05, Temperature: p 0.01, Volume: p 0.01

One-way Anova test was using to analyze the mean difference of mice temperature before intervention, after inflammation induction and after gel application. The result show there was significant statistically (p<0.05). The leg volume before intervention, after inflammation induction and after gel application also significant statistically (p<0.05). Although the mean difference is not represented the fever.

Table 6. Result of Anova Effect of Temperature and Leg Volume change after Gel Application.

Observation After Gel Application	Group	N	Mean	SD	95% Confidence Interval		Min	Max	P
					Lower	Upper			
30' Temperature	Neg	6	37,20	0,12	37,0	37,3	37,0	37,4	0.01
	1	7	36,90	0,14	36,7	37,0	36,7	37,1	
	2	8	37,30	0,17	37,1	37,4	37,0	37,6	
	3	8	37,00	0,16	36,8	37,1	36,8	37,2	
30' Leg Volume	Neg	6	0,18	0,013	0,17	0,19	0,17	0,20	0.01
	1	7	0,13	0,013	0,12	0,14	0,12	0,15	
	2	8	0,12	0,007	0,11	0,13	0,11	0,13	
	3	8	0,12	0,010	0,11	0,13	0,11	0,14	
60' Temperature	Neg	6	37,10	0,08	37,0	37,1	37,0	37,2	0.01
	1	7	36,98	0,30	36,7	37,2	36,7	37,4	
	2	8	37,00	0,16	36,8	37,1	36,7	37,3	
	3	8	36,60	0,13	36,4	36,7	36,4	36,8	
60' Leg Volume	Neg	6	0,19	0,010	0,18	0,20	0,18	0,21	0.01
	1	7	0,12	0,01	0,11	0,13	0,11	0,14	
	2	8	0,12	0,00	0,12	0,13	0,12	0,14	
	3	8	0,13	0,00	0,12	0,13	0,12	0,14	
90' Temperature	Neg	6	37,30	0,15	37,1	37,4	37,1	37,5	0.01
	1	7	36,50	0,08	36,4	36,5	36,4	36,6	
	2	8	36,80	0,22	36,6	36,9	36,5	37,1	
	3	8	36,75	0,59	36,2	37,2	36,3	37,7	
90' Leg Volume	Neg	6	0,19	0,01	0,18	0,20	0,18	0,21	0.01
	1	7	0,12	0,01	0,11	0,13	0,11	0,14	
	2	8	0,12	0,00	0,12	0,13	0,12	0,14	
	3	8	0,13	0,00	0,12	0,13	0,12	0,14	

*Significance level at p< 0.05

Discussion

The result of one-way Anova test show there were significance difference of mice temperature before intervention, after inflammation and after gel application. Formula 1 was slightly most effective in decreasing temperature compared with formula 2 and 3. This result accordance with

previous research, Erytrina gel application had cooling down effect that work as same as ice compression after inflammation or injury. Dadap leaves and cumin leaves contain active compounds that can act as anti-inflammatories. Dadap leaves contain alkaloids, flavonoids, and tannins.[4] Meanwhile, cumin leaves contain saponins, flavonoids, polyphenols, and essential oils.[5] Flavonoids are a typical compound that is usually found in green plants. Flavonoid bio-actives are considered the most important phytochemicals that have broad biological benefits for humans such as anti-inflammatory, antioxidant, and antimicrobial. Flavonoids are phenolic compounds that can inhibit the work of COX-2, causing a decrease in the production of prostaglandins and inhibiting of the release of arachidonic acid. Arachidonic acid and prostaglandins are mediators of inflammation. Saponins work through interactions with membrane lipids, such as phospholipids which are precursors of prostaglandins and other inflammatory mediators.[12] Flavonoids are well-known phytochemicals for their anti-inflammatory activity by inhibitingcyclooxygenase and lipoxygenase.

Short observation 30’ 60’ 90’ was conducted to evaluate fast action of gel extract that could be apllicated in real case of acute injury. The anti-inflammatory assay of Dadap leaves extract was carried out using the paw edema method by observing the test substance's ability to prevent swelling due to carrageenan induction. Inflammation volume changes was analysed by Anova test and shown to be significantly difference with negative control group, each formula was proven to be effective as antiinflmatory agent but the most effective seems in formula 2.[21]

Previous research showed that cumin leaf extract contains phenols, flavonoids, alkaloids, and saponins which have been shown to be anti-convulsants in experimental animals and contain carene, terpinene, camphor, and carvacrol compounds which function as anti-rheumatic arthritis. Phytochemical analysis of cumin leaf extract contained isopropyl phenol, squalene, caryophelen, and phytol. Phytopharmacological analysis of the cumin plant has active ingredients in the form of caryophyllene, cavacrol, and forskolin which have antinephropathy and antioxidant activity.[19] Another study stated that cumin leaf extract containing rosmarinic acid (CHM9102) has been shown to be anti-inflammatory and inhibits activator protein-1 (AP-1) binding which is responsible for cellular processes of inflammation, stress response, cell differentiation, and tumor formation. Several studies have found that the content of the active compounds in cumin leaf extract is quite varied and consistent with other studies, this shows the level of uniformity of the active compounds present in cumin leaves and different geographical conditions determine the composition of the compounds contained in the cumin plant. Wei et al also mentioned anti-Inflammatory Effects of Cumin Essential Oil by Blocking JNK, ERK, and NF-κB Signaling Pathways in LPS-Stimulated RAW 264.7 Cells. According to this study, other research also found at doses of 250, 500, 1000 mg / Kgbw it has an anti-inflammatory effect by significantly reducing the edema volume of 90-330 minutes ($p < 0.05$). Phytochemical compounds of black cumin seed extract are flavonoids, tannins, saponins, triterpenes, and steroids.[23]

Conclusion

From the results of this study, we can conclude that dadap leaves and cumin leaves can be used as anti-inflammatory gels on the extremities. The three formulations of dadap leaf and cumin leaf gel formulations can relieve inflammation. The results of the test on mice showed that the most effective gel formulation was the formulation of 7.5% dadap leaf extract and 17.5% cumin leaf extract. Scientific conjectures for future research, namely: Finding the right level of flavonoid and alkaloid test so that it is effectively used as an anti-inflammatory in the extremities. Applying the formulation of dadap leaves and cumin leaves in other forms that have a higher probability of effectiveness than the gel form. Testing the effectiveness of the gel from extracts of dadap leaves and cumin leaves on other areas of the body other than the extremities.

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