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Formulation and Evaluation of Spray Gel Fraction of Ethyl Acetate of Purslane Leaves (Portualaca Olercea.L) as an Anti-inflammatory in Wistar Rats

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ABSTRACT

The Pharmacy Study Program is an academic program that prepares students to become pharmacists, focusing on the study of medications, their effects, and the safe and effective practice of dispensing them. This program includes courses in Pharmaceutical Sciences, where students learn about drug development, formulation, and delivery; Pharmacology, which studies how drugs interact with the body; Pharmacy Practice, training students in patient interactions, medication counseling, and pharmacy management; Clinical Pharmacy, where students work with healthcare teams to optimize medication therapy; and Pharmaceutical Chemistry, focusing on the chemical properties and synthesis of drugs. Upon graduation, typically with a Bachelor of Pharmacy or PharmD degree, students are qualified to work in hospitals, clinics, and community pharmacies, ensuring the safe and effective use of medications.

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1. INTRODUCTION

Inflammation is a disease characterized by swelling, redness, pain, and heat (Harvey and Pamela, 2013). Inflammation can be classified into acute inflammation, which occurs relatively quickly within minutes to days, and chronic inflammation, which lasts for a long period. Commonly used anti-inflammatory drugs are divided into two classes: steroids and non-steroidal anti-inflammatory drugs (NSAIDs). Both classes of drugs have significant side effects, such as skin rashes. The most serious side effect of NSAIDs is gastrointestinal bleeding, with a prevalence of 19.6%. The main drugs causing these side effects include Ibuprofen, Potassium, Diclofenac, and Piroxicam. The study also reports that 7.8% of deaths are caused by Adverse Drug Responses (ADR). Therefore, there is a need for the development of alternative medications using natural materials.

One of the plants that can act as an anti-inflammatory is purslane (Portulaca oleracea L.). According to a study purslane has various pharmacological effects, including antibacterial, anti-inflammatory, antioxidant, and wound healing properties. Research shows that ethyl acetate extract of purslane leaves contains compounds such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, vitamins, proteins, and minerals, including omega-3 fatty acids. The dose of ethyl acetate extract of purslane leaves at 400 mg can reduce 30.2% edema in male Wistar rats. The mechanism of flavonoids in preventing

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inflammation involves inhibiting the release of arachidonic acid and the secretion of lysosomal enzymes from neutrophil and endothelial cell. Purslane extract contains phenolic compounds and flavonoids, which are present in different concentrations in various parts of the plant, with the highest concentration found in the leaves at 113.26 + 3.85 mg GAE/g.

The method used for extracting the ethyl acetate fraction from purslane leaves is maceration. Maceration is a simple extraction process where the plant material is soaked in a suitable solvent for 3-5 days. The advantage of this method is its simplicity and versatility, as it can be used for both wet and dry samples, and it is thermolabile. The process is followed by fractionation, which is the second step in the separation of compounds. Fractionation is a technique for separating and grouping chemical contents of the extract based on polarity. Two immiscible solvents with different polarity levels are used for this process. The compounds in the extract are separated according to their polarity. Then, thin-layer chromatography (TLC) is used to identify the types of compounds in the fractionated extracts by separating spots and selecting the appropriate eluents. The choice of solvent in fractionation depends on the analytical properties, where the solvent and analyte should have similar properties because fractionation separates compounds based on their polarity. In this study, hexane (non-polar) and ethyl acetate were used as solvents.

One formulation that can be developed as an anti-inflammatory treatment is spray gel. Gel is a semi-solid system composed of small particles or large organic molecules suspended in liquid. Spray gel formulations are easy to make, portable, and the ingredients are easily accessible. A spray is a component that is aerosolized, similar to small or large liquids used in spray pumps. The flavonoid compounds found in purslane are semi-polar and soluble in semi-polar solvents like ethyl acetate. Despite the active compounds, purslane has not yet been utilized in a spray gel formulation. This study will formulate ethyl acetate fraction of purslane leaves into a topical spray gel as an anti-inflammatory, aiming to make its use more practical and effective. Spray gels offer comfort in anti-inflammatory treatment due to their high water content, which helps alleviate inflammation. Additionally, spray gels spread well on the skin, provide a cooling effect, are easy to clean, transparent, and comfortable to use. Given that herbal use for anti-inflammatory purposes is still underdeveloped, this research seeks to explore alternatives with fewer side effects. The use of HPMC and carbopol is based on their availability and previous successful use in research.

2. RESEARCH METHOD

This research employs an experimental laboratory method, focusing on the formulation of Portulaca oleracea L. (purslane) herbal extract in a spray gel for testing its anti-inflammatory properties in Wistar rats. The study utilizes several variables: the independent variables include the ethyl acetate extract of purslane and the HPMC base, while the dependent variables are the physical properties of the spray gel and edema thickness in the rats' feet. Controlled variables include rat species, age, sex, and the conditions of the spray gel and rats. The study defines purslane as a simple herb known for its medicinal properties, including flavonoids, and explains the processes of maceration (a simple extraction method) and fractionation (to separate compounds based on polarity).

The study location includes the Pharmacy Biology Laboratory for plant extraction, the Pharmaceutical Technology Laboratory for gel formulation, and the Pharmacology Laboratory for animal testing. The sampling technique used is total sampling, selecting 20 tuberculosis patients (cases) and 20 controls from Puskesmas Dayeuhluhur 1's registry. Data collection includes primary data (observations, interviews, measurements) and secondary data from health records. The extraction process used 300 grams of purslane leaves, which were macerated and then fractionated using ethyl acetate.

For physical testing of the spray gel, several tests were conducted, such as organoleptic, homogeneity, pH, viscosity, and spreading power. The anti-inflammatory testing was carried out using Wistar rats, divided into four groups: a negative control, and three experimental groups receiving different concentrations of the ethyl acetate extract (0.02%, 0.03%, 0.04%). Edema was induced with 1% carrageenan, and the edema percentage was measured at different time intervals, using the trapezoidal

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method to calculate the Area Under the Curve (AUC). Tables and formulas for analysis include various spray gel formulations and the calculation of edema percentage:

Table 1. Composition of Ethyl Acetate Purslane Spray Gel

Formula (%)	F1	F2	F3
Ethyl Acetate Extract	0.02	0.03	0.04
Carbopol 940	0.1	0.1	0.1
HPMC	0.3	0.3	0.3
Triethanolamine	0.2	0.2	0.2
Propylene Glycol	15	15	15
DMDM Hydantoin	0.6	0.6	0.6
Aquadest	Add to 100	Add to 100	Add to 100

This study aims to develop a spray gel formulation for anti-inflammatory use based on purslane herbal extract, making it more practical and effective for everyday use with fewer side effects. The use of HPMC and carbopol ensures compatibility and ease of preparation, offering a promising alternative to traditional topical treatments.

3. RESULT AND DISCUSSIONS

Identification of Purslane Leaf Plant

3.1 Preparation of Purslane Leaf Powder

Purslane plants were obtained from Limpakuwus Village, Banyumas Regency. The collected plants were washed thoroughly to remove dirt and ensure cleanliness. The leaves were then separated from the stems and dried under direct sunlight for one week until completely dry.

3.2 Preparation of Ethyl Acetate Extract of Purslane Leaves

The preparation of Purslane leaf extract involved several stages, including collection, cleaning, drying, grinding, sieving, and extraction. Fresh leaves were collected from Limpakuwus Village, washed, and drained to obtain simplicia. The drying process was carried out under direct sunlight to remove moisture and prevent degradation of active compounds caused by fungi and bacteria.

The dried simplicia were ground into powder to reduce particle size and increase surface area, then sieved using a 40-mesh sieve to remove impurities. The powder was extracted using ethyl acetate, chosen for its ability to dissolve polar compounds such as flavonoids. Prior to this, n-hexane was used to remove non-polar compounds, ensuring the extract was rich in polar substances like flavonoids, alkaloids, and saponins.

Extraction was performed using the maceration method for three days, followed by remaceration for two hours to maximize the yield of active compounds. The extract was then concentrated using a water bath at 70°C and stored at 2–8°C to maintain its stability and prevent contamination. The final extract weighed 70.0 grams with a rendement of 14% (w/w), indicating that ethyl acetate was fairly effective in extracting semi-polar compounds from purslane leaves.

3.3 Phytochemical Screening

Table 2. Phytochemical Screening Results of the Ethyl Acetate Fraction of Purslane Leaves

No	Test	Color/Observation in Extract	Result
1	Alkaloid (+ Pereaksi Dragendrof)	Endapan Merah Jingga	+
2	Alkaloid (+ Pereaksi Wagner)	Endapan coklat	+
3	Flavanoid	Warna Merah Jingga	+
4	Saponin	Berbusa	+

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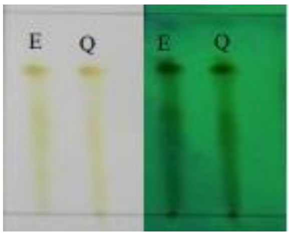
In Table 2, the alkaloid content test was carried out to identify the presence of alkaloid compounds in the Purslane leaf extract. This test was performed using Dragendorff's and Wagner's reagents. The results obtained from the alkaloid test with the addition of Dragendorff's reagent showed a positive indication of alkaloids, marked by the formation of an orange-red precipitate. This precipitate is a potassium-alkaloid complex. Alkaloid compounds, when reacted with Dragendorff's reagent, form an orange-red tetraiodobismuth salt.

Furthermore, the alkaloid test using Wagner's reagent also showed a positive result, indicated by the formation of a brown precipitate. The research reported that purslane leaf extract contains alkaloid compounds, as evidenced by the formation of a brown precipitate. The brown precipitate formed with Wagner's reagent is presumed to occur due to the interaction between potassium ions (K^+) from potassium iodide and nitrogen atoms in alkaloids, forming a precipitated complex. This precipitate is identified as a potassium-alkaloid complex.

Flavonoid testing was conducted to identify the presence of flavonoid compounds in the purslane leaf extract. This test was performed by taking 2 ml of the extract and heating it, followed by the addition of ethyl acetate. Magnesium powder was then added into the solution along with concentrated HCl. The addition of concentrated HCl is intended to hydrolyze flavonoids into their aglycone forms by breaking O-glycosidic bonds. The glycosyl group is replaced by H^+ ions from the acid due to its electrophilic nature. Reduction using Mg and concentrated HCl can produce a colored complex with an orange-red color. The formation of an orange-red solution indicates the presence of flavonoids.

The results of the flavonoid test showed the formation of an orange-red solution, which proves that the thick purslane leaf extract contains flavonoid compounds. The results obtained are consistent with previous studies; reported that purslane leaf extract contains flavonoid compounds, as indicated by the formation of an orange-red solution.

Saponins are compounds that contain both hydrophilic and hydrophobic groups. When shaken, the hydrophilic groups bind to water while the hydrophobic groups bind to air, resulting in foam formation. The saponin content test was conducted to identify the presence of saponin compounds in the purslane leaf extract. This test was carried out by diluting the extract with 5 ml of demineralized water, then shaking it. The results of the saponin test showed the presence of stable foam lasting for 30 minutes, which proves that the thick purslane leaf extract contains saponin Purslane leaf extract also tested positive for saponins, indicated by the formation of stable foam, thus the results of this study are consistent with previous research findings.



. Visual UV 254

Figure 1. Qualitative Test Results of Flavonoids Using TLC Method

Description:
E : Ethyl acetate extract of Purslane leaves
Q : Quercetin

Phytochemical screening was conducted to determine the compounds present in the Purslane leaf extract, focusing on flavonoids. The analysis used Thin Layer Chromatography (TLC), a separation method based on the distribution of compounds between a stationary phase and a mobile phase. The stationary phase used was polar silica gel GF254 plates (5 × 10 cm, Merck), while the mobile phase consisted of chloroform:ethyl acetate:water (25:1:0.5), which has semi-polar characteristics.

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Before use, the TLC plates were activated in an oven at 100°C for 1 hour to remove moisture[2]. A good eluent is indicated by clear, well-separated spots without tailing. Separation in TLC occurs through adsorption and partition mechanisms. This method was chosen because it is simple, efficient, and suitable for both separation and identification of compounds.

Flavonoid testing was performed using the TLC method with the same mobile phase composition. The results showed fluorescence on the TLC plate under UV light at 254 nm, appearing yellow. Further visualization was enhanced by spraying ammonia solution, which increased sensitivity and made the spots visible as pale yellow under visible light, confirming the presence of flavonoids. The Rf value of the ethyl acetate extract was 0.87, while the standard (quercetin) showed an Rf value of 0.88. The similarity of these values indicates that the extract contains flavonoid compounds. The Rf value is calculated as the ratio of the distance traveled by the analyte to the distance traveled by the eluent.

However, the spots showed tailing, indicating that the mobile phase was not fully optimal and the spotting technique was not precise, causing the spots to spread and overlap. Despite this limitation, the results confirm that the ethyl acetate extract of purslane leaves contains flavonoids, which are known to have anti-inflammatory activity.

3.4 Preparation of Spray Gel Formulation

Table 3. Composition of Ethyl Acetate Purslane Leaf Spray Gel

Composition	**Formula (% b/b)**		
	F1	***F2***	***F3***
Ethyl Acetate Extract of Purslane Leaves	0,02	0,03	0,04
Carbopol 940	0.1	0.	0.1
HPMC	0,3	0,3	0,3
Triethanolamine		0.2	0.2
Propylene Glycol	15	15	15
DMDM Hydantoin	0.6	0.6	0.6
Distilled Water (Aquadest)	Add 100	Add 100	Add 100

The spray gel formulation of the ethyl acetate fraction of Purslane leaves was prepared using Carbopol 940 variation. First, Carbopol was dispersed in hot water using a water bath and stirred until homogeneous, then triethanolamine was added to form a transparent gel (Mixture 1).

Separately, HPMC was dissolved in hot water until fully dispersed, forming a clear and slightly viscous solution (Mixture 2). After cooling, Mixture 2 was combined with Mixture 1 and mixed until homogeneous. Propylene glycol and DMDM hydantoin were then added, followed by the ethyl acetate fraction of purslane leaves.

Finally, distilled water was added gradually until the volume reached 100 mL, and the mixture was stirred until homogeneous before being transferred into a spray bottle

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F1 : Concentration 0.02
F2 : Concentration 0.03
F3 : Concentration 0.04

Figure 2. Formulation of Purslane Leaf Preparation

The ethyl acetate extract of Purslane leaves was formulated into a spray gel to allow rapid spreading on the skin. This dosage form also provides a cooling effect due to Carbopol and HPMC, caused by slow water evaporation and good skin penetration. HPMC was used as the gelling agent because it is water-soluble, produces a clear gel, is stable at pH 3–11, has good microbial resistance, low toxicity, neutral properties, good drug release, wide spreadability, and stable viscosity during long-term storage. The formulations consisted of four types: a negative control (without extract), and F1, F2, and F3.

Distilled water (aquadest) was used as the dispersion medium. Due to its high water content, a preservative was needed to inhibit microbial growth. Carbopol 940 functions as a suspending agent and stabilizer, preventing phase separation and controlling consistency. Triethanolamine acts as an emulsifier and gelling agent, producing a homogeneous and stable system. Propylene glycol serves as a solvent and carrier, characterized as a clear, viscous, nearly odorless liquid. DMDM hydantoin is used as a preservative that releases small amounts of formaldehyde, providing broad-spectrum antimicrobial activity and stability across a wide pH and temperature range.

3.5 Preparation Evaluation

a. Organoleptic Test

Organoleptic evaluation was conducted through visual and olfactory observations to assess the form, color, and odor of the spray gel. The results showed that all formulations had acceptable characteristics, although differences in texture consistency were observed due to variations in gelling agent and extract concentrations. Formulations F1, F2, and F3 exhibited a semi-solid form with a characteristic odor due to the presence of the extract. These findings are consistent with the present study. Additionally, differences in the concentration of Purslane leaf extract influenced the color and odor of the formulations.

b. pH Test

Table 4. pH Test Results

Replication	F1	F2	F3
1	8	8	7
2	9	8	8
Average	8.5	8	7.5
SD	0.5	0	0.5

Description:
F1: Formula with 0.2 extract concentration
F2: Formula with 0.3 extract concentration
F3: Formula with 0.4 extract concentration

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The pH test was conducted to determine whether the formulation has a pH suitable for skin to avoid irritation during use. Based on the results, the average pH values were 8.5 for F1, 8 for F2, and 7.5 for F3, showing a slight decrease influenced by storage conditions and ingredients.

c. Viscosity Test

Table 5. Viscosity Test Results

Replication	F1 (cps)	F2 (cps)	F3 (cps)
1	4,50	268,0	720
2	5,20	258,0	684
3	5,00	218,0	698
Average	4,9	248	700,66
SD	0,36	26,45	18,14

Description:

F1: Formula with 0.2 extract concentration

F2: Formula with 0.3 extract concentration

F3: Formula with 0.4 extract concentration

The viscosity test was performed using a viscometer to determine the thickness of the spray gel. Viscosity affects the ease of the formulation to flow out of the container, release active substances, and be applied to the skin. Triethanolamine (TEA) can increase viscosity when combined with Carbopol.

Storage conditions also influence viscosity due to polymer behavior. At high temperatures, polymer chains expand, causing decreased viscosity (more liquid). At low temperatures, polymer chains contract and aggregate, increasing viscosity. Additionally, trapped air bubbles during preparation can affect viscosity values, where more bubbles may increase viscosity. Based on the results, only F3 falls within the acceptable viscosity range for semi-solid spray gels (500–5000 cps).

d. Spreadability Test

Table 6. Spreadability Test Results

Replication	F1 (cm)	F2 (cm)	F3 (cm)
1	12	15	8,3
2	12	17,5	7,2
3	14,5	19	7,6
Average	12,833	17,166	7,7
SD	1,443	2,020	0,556

Description:

F1: Formula with 0.2 extract concentration

F2: Formula with 0.3 extract concentration

F3: Formula with 0.4 extract concentration

Spreadability is an important characteristic that determines the ease of application on the skin. The results showed that F1, F2, and F3 met good spreadability criteria, as they formed a stable layer on the skin within 10 seconds.

An increase in gelling agent concentration leads to decreased spreadability. In this study, the reduction in spreadability was influenced by differences in HPMC concentration in each formula. Higher gelling agent concentration increases resistance, making the spray gel more difficult to spread.

3.6 Anti-inflammatory Activity Test

This study evaluated the anti-inflammatory effect of the ethyl acetate extract of Purslane leaves using an induced edema model in male Wistar rats. Acute inflammation was induced by injecting 1%

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carrageenan into the plantar region of the left hind paw. This method assesses the ability of a substance to inhibit swelling. Wistar rats were chosen due to their physiological similarity to humans in inflammatory responses, particularly involving arachidonic acid metabolism. Edema thickness was measured using a caliper, and data were analyzed using SPSS with MANOVA at a 95% confidence level.

a. AUC of Edema Thickness

The results showed that all treatment groups (F1, F2, F3) significantly differed from the negative control. However, there was no significant difference among F1, F2, and F3, indicating comparable anti-inflammatory effects. Among them, F3 showed the greatest reduction in edema thickness. The anti-inflammatory mechanism is attributed mainly to flavonoids, which inhibit arachidonic acid pathways, enzyme secretion, and inflammatory mediators. Other compounds such as saponins and alkaloids also contribute by reducing vascular permeability, exudate formation, histamine release, and inflammatory mediators.

b. Percentage of Anti-inflammatory Activity

The percentage of inflammation inhibition showed that F2 (0.03%) had the highest activity (41.09%), followed by F3 and F1. Higher extract concentrations generally resulted in stronger anti-inflammatory effects, likely due to increased levels of active compounds. A higher percentage indicates stronger inhibition of edema.

Although AUC and percentage results showed some differences, this may be due to experimental limitations such as measurement inaccuracies or animal movement during testing. Overall, the anti-inflammatory effect is attributed to secondary metabolites such as flavonoids, alkaloids, and saponins. Flavonoids act by inhibiting COX and lipoxygenase enzymes, preventing prostaglandin formation, while saponins and alkaloids reduce inflammation through vascular and immune modulation.

5. CONCLUSION AND RECOMMENDATION

Based on the results of the research, it can be concluded that the ethyl acetate extract of purslane leaf spray gel at the minimal dose has shown an anti-inflammatory effect on the edema of the feet of male Wistar rats induced by carrageenan. Additionally, the variation of ethyl acetate extract of purslane leaf and the HPMC base influenced the physical properties of the spray gel, with noticeable differences in color and odor across the formulations.

It is recommended that further testing be conducted to evaluate the adhesion, stability, and cycling test of the purslane leaf spray gel formulation. Optimization of the formulation is also suggested to improve its effectiveness. Furthermore, it is advised to conduct additional research using alternative methods for testing the anti-inflammatory effects of the purslane leaf extract to enhance the resulting anti-inflammatory properties. Lastly, optimization of the mobile phase for qualitative flavonoid testing using Thin Layer Chromatography (TLC) should be performed to achieve more accurate results.

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