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Transdermal Patch of Gnetum gnemon L. Leaf Extract as Drug Delivery System and Anti-Inflammatory Activity Agains Wistar Mice

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Abstract

The melinjo leaf (*Gnetum gnemon L.*) is a plant of the *Gnetaceae family* that contains compounds including flavonoids, which have the potential to act as anti-inflammatories. Formulating melinjo leaf extract into a transdermal patch aims to deliver active compounds to the systemic circulation via the skin. The research aimed to develop a melinjo leaf extract into a transdermal patch its anti-inflammatory activity. The extraction of melinjo leaf using the maceration method with 70% ethanol. The transdermal patch containing melinjo leaf extract is formulated in three versions, each containing 20%, 25%, or 30% propylene glycol. The formulation was characterised by organoleptic, pH, moisture content, uniformity of thickness and weight, and elongation. The best formula is characterised by stability, uniformity of drug content, and anti-inflammatory activity in Wistar mice. The results indicated that transdermal application and oral administration of the melinjo leaf extract produced anti-inflammatory effects comparable to those of the positive control group. There were no significant differences between the transdermal treatment group and the positive control in terms of percentage inflammation (48.35%) and inflammation inhibition (45.75%). Similarly, the oral treatment group exhibited an inflammatory percentage of 63.44% and an inflammatory inhibition of 52.21%. It can be concluded that the transdermal patch formulation of melinjo leaf extract is effective as an anti-inflammatory treatment.

Keywords: transdermal, patch, anti-inflammatory, melinjo leaf, propylene glycol

Introduction

The body has a natural response to defend against the entry of organisms or infection into its cells, called inflammation.[1] The inflammatory response occurs due to the emergence of mediators such as histamine, bradykinin, serotonin, leukotrienes, and prostaglandins. One mediator that plays a significant role in the inflammatory process is prostaglandin, which is derived from arachidonic acid metabolism. Prostaglandins and leukotrienes are important mediators in inflammatory symptoms.[2] Eliminating inflammation and pain can be achieved with anti-inflammatory drugs. However, using anti-inflammatory drugs can have side effects on the digestive system, such as stomach pain and kidney disorders, and can cause stomach ulcers.[3] To minimize unwanted side effects, anti-inflammatory drugs from natural ingredients, such as plants, are developed. One plant anti-inflammatory properties is melinjo.

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Melinjo (*Gnetum gnemon L.*) is a plant native to Southeast Asia. This plant belongs to the genus Gnemon (*Gnetaceae family*).[4] Flavonoid compounds in melinjo leaves have anti-inflammatory activity by inhibiting cyclooxygenase or lipoxygenase. They will inhibit the production of prostaglandins by arachidonic acid, thereby reducing pain.[3] Research conducted by Widarto *et al*. showed that melinjo leaf extract at a 563 mg/kg dose has anti-inflammatory activity against white Wistar rats.[5]

Flavonoid compounds have many health benefits, but have low bioactivity.[6] Therefore, it is necessary to develop drug delivery that can increase the bioactivity of these compounds. One preparation option is to deliver the transdermal system as a patch. Administering transdermal patches can maintain plasma drug bioavailability during use, compared with oral administration. Besides that, transdermal drug delivery delivers drugs directly to the systemic circulation by penetrating the deepest layers of the skin.[7]

Transdermal delivery of anti-inflammatory drugs offers advantages over topical preparations, which typically provide only local effects. As a result of the inflammation and pain suffered, it will give the sufferer a feeling of discomfort, so it is necessary to deliver drugs with an immediate therapeutic effect. To get drug delivery through the transdermal patch properly, it is essential to know the patch's basic components. The components of the transdermal patch include polymers, penetration enhancers, and plasticisers.[8] The use of PVP will cause pore formation and prevent crystallisation of the drug in the matrix, so that it can increase drug release. Whereas Hydroxypropyl Methylcellulose (HPMC) exhibits better swelling characteristics than other polymers, this property also causes rapid drug release from the matrix.[9]

Many enhancers can be used to formulate transdermal patch preparations, including propylene glycol (PG). In the literature, propylene glycol is also reported to be used as a plasticiser.[10] Adding a plasticiser can provide elastic properties to the patch while increasing the flexibility of the transdermal patch so that the resulting patch will have flexible and adaptable properties that can facilitate use without causing trauma during replacement.[11]

Based on the description above, in this study, melinjo leaf extract (*Gnetum gnemon L.*) will be used as an active substance in transdermal formulations using three different concentration variations of propylene glycol as a plasticizer, which aims to determine the characterization and stability of transdermal patch preparations, and effectiveness of transdermal patches of melinjo leaf extract (*Gnetum gnemon L.*) as anti-inflammatory in white male rats.

Method

Tool

The tools used in this study are glassware (Pyrex®), analytical balance (Ohaus®), magnetic stirrer (IKA® C-MAG HS 4), vernier callipers (Krisbow Digital), oven (IMU55L), plethysmometer, UV-Vis spectrophotometer (UV-1700 Shimadzu®), pH meter (Lutron® pH Electrode PE-03), and rotary evaporator (Yamato®RE 301).

Material

The plant used in the study was melinjo leaf (*Gnetum gnemon L.*) obtained from Indralaya, South Sumatra, Indonesia. 70% ethanol (Dira Sonita Group), PVP (Merck), HPMC (Merck), propylene glycol (Bratacem, Indonesia), carragenan (Sigma Aldrich), quercetin (Sigma Aldrich), methanol p.a (Merck).

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Procedure

Extraction of Melinjo Leaves

Melinjo leaf simplicia powder was macerated in 70% ethanol at a 1:10 ratio for 3 × 24 hours in a place protected from sunlight, with occasional stirring. The macerate obtained was then filtered using filter paper. The rotary evaporator applies gentle heat (50°C) to evaporate the solvent (ethanol) and concentrate the desired compounds present in the extract. The process continues until a thick extract is obtained. Extract yield can be calculated using the following formula:

$$\% \text{ Extract yield} = \frac{\text{extract weight}}{\text{simplisia weight}} \text{ x } 100\%$$

Characterization of Melinjo Leaf Extract

The parameters of the characteristic extract tests carried out in this study included organoleptic, water content, drying shrinkage, and phytochemical screening.[12]

Preparation of Transdermal Patch

The formula of the transdermal patch of melinjo leaf extract is in Table 1. In the formulas, propylene glycol variants are used at concentrations of 20%, 25%, and 30%. Dissolve HPMC in cold water and mix with 96% ethanol. Then, homogenize the mixture using a magnetic stirrer at 300 rpm until it is homogeneous. Dissolve PVP in 96% ethanol using a magnetic stirrer at 300 rpm until dissolved. Add the HPMC solution to the PVP solution, stir until homogeneous, then add the melinjo leaf extract and propylene glycol, and homogenize with a magnetic stirrer at 300 rpm. The solution was poured into a petri dish and dried in an oven at 40 °C for 18 hours.

Table 1. Formula Transdermal Patch Melinjo Leaf Ethanol Extract

Composition	Formula (mg)		
	F1	F2	F3
Ethanol extract of melinjo leaves	112,6	112,6	112,6
HPMC	300	300	300
Polivinil Pirolidon	150	150	150
Propylene glycol	90	112,5	135
Ethanol 96%	ad 50 mL	ad 50 mL	ad 50 mL

Characterization of a Transdermal Patch

Organoleptic

The finished patch was visually observed for its organoleptic characteristics, including shape, color, smell, and surface consistency.

Uniformity of Transdermal Patch Weight and Thickness

The weight of the transdermal patches was measured by weighing 10 patches individually with an analytical balance. The thickness of the transdermal patches was measured by taking 10 patches using a digital micrometer at nine points on each patch.[13]

Moisture Content Test

The transdermal patches were weighed initially and stored in a desiccator containing silica gel at room temperature for 24 hours. Then, the patch was weighed again to determine the final weight.[13]

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Moisture content = $\frac{\text{Initial weight} - \text{Final weight})}{\text{Initial weight}}$ x 100%

Elongation Test

Patches were prepared from up to five samples and tested using the Clipping test tool to quantify the elongation resulting from the patch preparations. The elongation test is performed by comparing the increase in the patch's length with its original length. Elongation can be calculated using the formula:

$\sigma = \frac{\Delta L}{L0}$ x 100%

Noted:

σ = elongation (%)
$\Delta L = (L - L_0)$ increase in length (mm)
L_0 = starting length (mm)

Folding Resistance Test

Folding resistance is the number of folds required to break the patch. The patch's folding endurance is assessed by repeatedly folding it at the exact location until failure. Folding is done a maximum of 300 times. The number of times a patch can be folded at the precise area without stopping is the patch's folding durability.[14]

Transdermal pH Test

The patches were measured using a pH meter, with a transdermal patch placed in distilled water and allowed to swell for two hours at room temperature.[15]

Characterization of the Best Formula

Stability of Preparation

The stability of the preparation was assessed by monitoring changes over 24 hours at 4°C (cold) and 40°C (hot) for 12 days (6 cycles). Observation of physical stability includes organoleptic content, humidity, elongation, and pH.

Content Uniformity

A uniformity test for transdermal content was conducted by preparing a 2 x 2 cm film and placing it in a container containing phosphate buffer at pH 7.4 (100 mL). The sample is stirred using an incubator shaker. The same preparation was carried out on the non-extracted patch to use as a blank. The sample is then measured at a predetermined wavelength. The uniformity of flavonoid levels was assessed by quantifying the flavonoids in the patch preparations. The specified conditions are 85-115% at a predetermined length. The sample was placed in a quartz cuvette and then analyzed using a UV-Vis spectrophotometer. The regression equation and regression coefficients were calculated using y = mx + c (y = µg/mL concentration, x = absorbance, m & c = constant).[16] Content uniformity testing was conducted on the best formula obtained.

In Vivo Anti-inflammatory Testing

Rats were fasted for approximately 18 hours before the experiment, with access to drinking water. The back of each rat was shaved to create a 4 cm x 4 cm area. Rats were randomly assigned to four treatment groups, with five rats per group. Each rat was weighed and assigned a specific code for identification. The initial volume of each rat's feet (baseline volume) was measured before any treatment. This measurement provided

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the essential leg volume (V0) for each rat. The transdermal patch preparations were applied to the backs of the rats, corresponding to their respective treatment groups. One hour after patch administration, each rat received an injection of 1% carrageenan (0.1 mL) into the foot. The carrageenan injection was performed subplantar, meaning it was injected below the plantar surface of the foot. Before injection, rats' paws were cleaned with 70% ethanol to ensure proper hygiene. The volume of each rat's feet was measured using a plethysmometer. Measurements were taken at 1, 2, 3, 4, 5, and 6 hours after carrageenan induction. The volume measurements obtained at each time point were summed to yield the final volume (Vt). All data, including the initial leg volume (V0) and the final volumes (Vt) at different time points, were tabulated for each treatment group. The results for each treatment group were averaged and analyzed to assess the transdermal patch's anti-inflammatory effect. All study participants provide informed consent for their inclusion in the study. The study was conducted in accordance with the protocol, which was approved by the Ethics Committee of Ahmad Dahlan University.[17]

Table 2. Anti-Inflammatory Test Animal Design

Group	Treatment
Positive Group	Karagenan 1% + Na-CMC
Negative Group	Karagenan 1% + Na Diclofenac
Testing Group I	Karagenan 1% + TPEDM (*Transdermal Patch* Melinjo leaves extract)
Testing Group 2	Karagenan 1% + EEDM (Melinjo leaves extract)

Data Analysis

Edema percentage data were analyzed using the normality test with the Shapiro-Wilk test to determine whether the data obtained had a normal distribution. If the data are normally distributed ($P > 0.05$), a one-way ANOVA will be conducted at the 95% significance level. Suppose there is a significant difference between the treatments. In that case, it will proceed with the Duncan multiple-range test (DMRT) at the 0.05 significance level to detect fundamental differences among groups. For data that is not normally distributed, data processing is done with the non-parametric Kruskal-Wallis test and followed by the Mann-Whitney test.

Result

Table 3. Result of Extract Characterization

Parameter	Test Results ± SD	Requirement (Depkes RI, 2017)
Organoleptic	Viscous, sticky, and blackish-green, it has a distinctive odor of melinjo leaves	-
Water Content	4,67±0,58	< 10%
Drying Loss	4,8±0,6	< 10%

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Table 4. Result of Preparation Evaluation

Parameter	Results and Observations		
	F1	F2	F3
Organoleptic	Greenish, elastic, slightly sticky	Greenish, elastic, sticky	Greenish, elastic, very sticky
Thickness (mm)	0,0543 ± 0,001*	0,0570 ± 0,001*	0,0593 ± 0,001*
Content Uniformity (mg)	0,0342 ± 0,002*	0,0366 ± 0,001*	0,0552 ± 0,003*
Moisture (%)	6,533 ± 0,452*	7,646 ± 0,918*	8,586 ± 0,293*
Elongation (%)	131,447 ± 0,200*	140,609 ± 0,989*	151,350 ± 1,390*
Folding Endurance	>300	>300	>300
pH	5,755 ± 0,012	5,747 ± 0,027	5,723 ± 0,017
Permeation test (min.180)	25,55 ± 0,6*	42,53 ± 0,3*	45,99 ± 0,3*

Note: * indicates that the parameter has a significant effect on the variation of propylene glycol as a plasticizer ($p<0.05$)

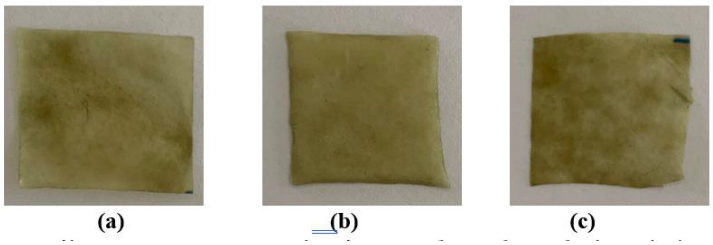

Figure 1. (a). F1, (b). F2, (c). F3.

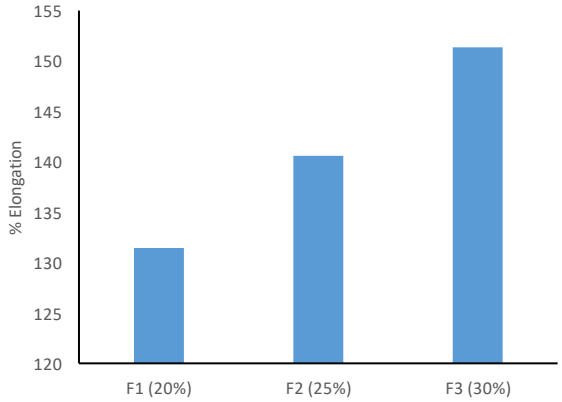

Figure 2. Transdermal elongation diagram of melinjo leaf extract patch

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Table 5. Result of Permeation Test

Time (Min)	Concentration (%)		
	F1	F2	F3
15	13,84 ± 0,04	26,34 ± 0,55	22,79 ± 0,27
30	17,16 ± 0,10	28, 93 ± 0,26	28,77 ± 0,33
45	19,49 ± 0,08	33, 44 ± 0,20	30,86 ± 0,35
60	20,51 ± 0,28	37,40 ± 0,43	29,35 ± 0,20
90	21,53 ± 0,10	39,21 ± 0,41	35,46 ± 0,31
120	22,41 ± 0,32	43, 29 ± 0,41	38,15 ± 0,67
150	23,63 ± 0,14	41,95 ± 0,48	42,79 ± 0,07
180	25, 55 ± 0,6	42,53 ± 0,3	45,99 ± 0,3

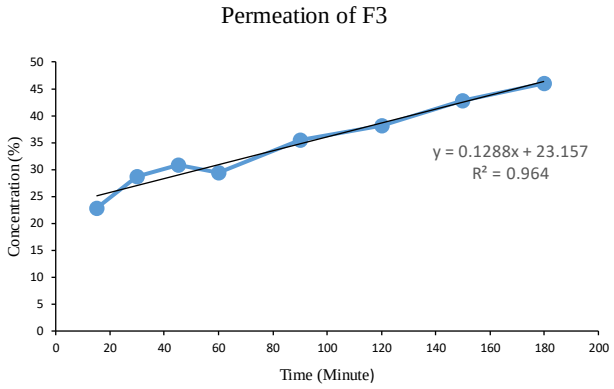

Figure 3. Curve of the relationship between the permeation rate and time in the F3 rat membrane

Table 6. Result of Preparation Stability

Parameter	Observation Results	
	Before	After
Organoleptic	Greenish, elastic, very sticky	Greenish, elastic, sticky
Content (mg)	16,937 ± 0,748 %recovery: 100,15%	14,249 ± 0,379 %recovery: 100,053%
Moisture (%)	8,586 ± 0,293	5,603 ± 0,446
Elongation (%)	151,350 ± 1,390	132,467 ± 0,719
pH	5,732 ± 0,017	5,685 ± 0,012

Table 7. Average Percent Inflammation in Rats

Group Treatment	Percent Inflammation (%)						Inflammation Average ± SD
	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours	
Negative Control	64,28 ± 4,38	90,47 ± 4,67	106,67 ± 5,27	116,19 ± 2,44	135,71 ± 2,01	148,57 ± 2,66	110,31 ± 9,55[b]
Positive Control	34,00 ± 1,64	55,33 ± 4,06	59,33 ± 4,54	38,00 ± 8,19	24,67 ± 1,20	20,67 ± 7,22	38,67 ± 8,79[a]
Group 1 (TPEDM)	32,66 ± 9,54	50,66 ± 9,90	76,00 ± 4,60	57,47 ± 3,40	44,00 ± 4,60	29,33 ± 8,87	48,35 ± 7,20[a]

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Table 7. (Extension)

Group Treatment	Percent Inflammation (%)						Inflammation Average ± SD
	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours	
Group 2 (EEDM)	36,66 ± 5,63	58,66 ± 8,62	76,66 ± 9,36	90,66 ± 7,19	66,00 ± 5,02	52,00 ± 6,56	63,44 ± 8,93[a]

Desc: values followed by lowercase letters (a/b) in the row indicate significant differences between groups in Duncan's post hoc test.

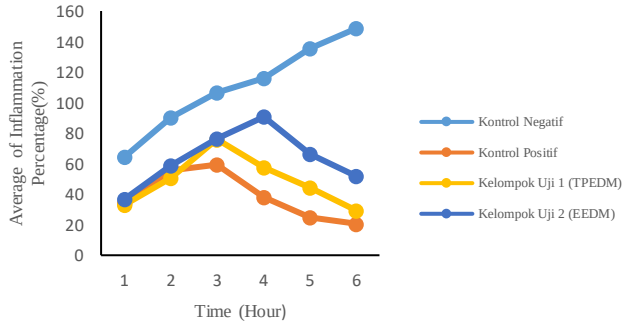

Figure 4. Graphic of the relationship between the average percent inflammation of the treatment group

Table 8. Average Percent Inflammation Inhibition in Mice

Group Treatment	Percent Inflammation (%)						Average Inflammation Inhibition ± SD
	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours	
Positive control	47,11	38,84	44,37	67,29	81,82	86,08	52,21 ± 9,56[a]
Group 1 (TPEDM)	49,18	44,00	28,75	50,53	67,57	80,25	45,75 ± 6,09[a]
Group 2 (EEDM)	50,96	43,15	36,12	29,96	59,36	73,00	41,79 ± 5,10[a]

Desc: values followed by lowercase letters (a/b) in the row indicate significant differences between groups in Duncan's post hoc test.

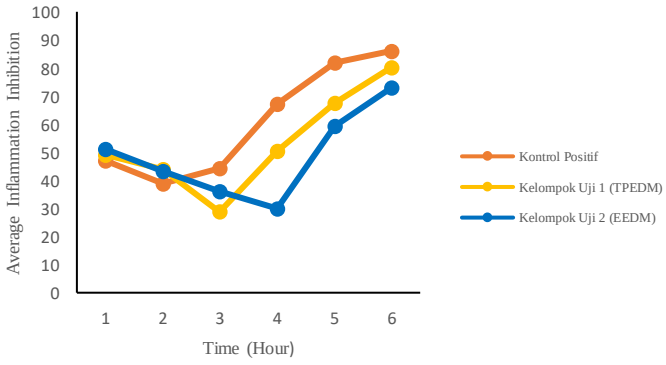

Figure 5. Graph of the relationship between the average percent inflammation of the treatment group

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Discussion

Melinjo Leaves Extract

Extraction of Melinjo leaves with 70% ethanol yielded a viscous extract weighing 191.5 g (19.15% yield). The use of 70% ethanol as the solvent was based on the polar properties of flavonoid compounds present in Melinjo leaves.[18] These compounds are in the form of glycosides and are better dissolved in 70% ethanol due to its higher polarity compared to 96% ethanol.[19]

To ensure that the extract meets the requirements of the Indonesian Herbal Pharmacopoeia, characterization tests are performed. The specific characterization tests may vary depending on the particular requirements set by the pharmacopoeia or the desired quality standards.

Evaluation of Transdermal Patch

Patch preparations were evaluated for organoleptic properties, thickness, weight uniformity, moisture content, elongation, folding resistance, and pH. Based on observations, the evaluation results for patch preparations are presented in Table 4.

Organoleptic

Organoleptic testing is carried out to know the preparation's physical form using the five senses, which includes observing the patch preparation's color, texture, and smell. The resulting preparations are shown in Figure 1.

Thickness

The results of the patch thickness measurements were compared against the patch thickness requirements, which in this case were specified as 0.05-0.2 mm.[16] If the average thickness and standard deviation fall within this range, it indicates that the patches meet the specified patch thickness requirements.[13] By conducting the patch thickness testing and analyzing the results, researchers can assess the uniformity and consistency of the patch solution and ensure that the resulting patches have the desired thickness for their intended application.

Weight Uniformity

To perform the weight uniformity test, five samples from each formula were randomly selected and weighed individually. The weights of these samples were then used to calculate the average weight and standard deviation.[20] Based on the test results data, it was observed that the patch with the highest weight was found in F3. This can be attributed to the fact that Formula 3 had the highest propylene glycol concentration (30%), compared with 20% and 25% in F1 and F2, respectively. It's important to note that the observed differences in weight among the formulas may be attributable to variations in propylene glycol concentration. This information can help elucidate the effect of propylene glycol concentration on patch weight.

Moisture

Moisture testing is conducted to determine the water content of the patch preparation, as excessive moisture can adversely affect its stability. High water content can lead to microbial contamination and reduce the overall stability of the patch.[21] The moisture testing procedure involves placing the patch in a desiccator containing silica gel for 24 hours. The initial weight of the patch is measured before placement in the desiccator, and the final weight is measured after 24 hours of storage. This process is

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repeated three times for each formula to ensure reliability, and the average percent water content and standard deviation are calculated.

Based on the moisture testing results, Formula 3 (F3) had the highest moisture content at 8.58%. Formula 2 (F2) had a slightly lower moisture content at 7.64%, and Formula 1 (F1) had the lowest moisture content at 6.53%. It is worth noting that a good patch typically contains a low water content, preferably below 10%. In this case, all three formulas meet the water content requirements as their respective moisture contents fall within the acceptable range. This indicates that the patch preparations have an appropriate moisture level, which is essential for maintaining stability and minimizing the risk of microbial contamination.

Elongation

The elasticity test is conducted to assess the patch preparation's ability to stretch and conform to various body contours. This is important to ensure that when the patch is applied, it does not cause trauma or restrict limb movement. The elongation test is specifically carried out to measure the stretchability or elasticity of the transdermal patch. Based on the information provided, Figure 2 shows the relationship between plasticizer concentration and elongation at break for the patch preparations. The results indicate that increasing the plasticizer concentration results in higher elongation values. The highest elongation value, 151.350, was observed in the patch prepared with a 30% plasticizer concentration. The elongation values for the patch preparations with concentrations of 25% and 20% plasticizer were slightly lower at 140.609 and 131.447, respectively.

This suggests that increasing the plasticizer concentration enhances the flexibility of the patch preparations. The plasticizer molecules can form hydrogen bonds with the polymer during patch preparation. This interaction weakens the bonds within the polymer structure, thereby increasing the patch's flexibility and stretchability. As a result, the patch preparations become more elastic, soft, and flexible. This is advantageous because it allows the patches to conform more closely to the body's contours and ensures greater comfort during application. The higher elongation values observed with increasing plasticizer concentration indicate improved flexibility and elasticity of the patch preparations.

Folding Endurance

The folding endurance test is conducted to evaluate the folding capacity or flexibility of the polymer and plasticizer used in the patch preparation. The test involves folding the patch multiple times along a defined line or axis. The number of times the patch can be folded before exhibiting signs of damage or failure is recorded as the folding endurance.[20] In the case of the patch preparations using HPMC and PVP polymers with propylene glycol as the plasticizer, the plasticizer plays a crucial role in enhancing the flexibility of the patch. Propylene glycol reduces the hydrogen bonding within the polymer structure, resulting in a more flexible and pliable patch. By weakening hydrogen bonding, the plasticizer helps to loosen the polymer chains and increase their mobility.[16] This contributes to the patch's ability to undergo repeated folding without breaking or experiencing structural damage. The folding endurance test provides valuable information on the patch's strength and durability. A higher folding endurance value indicates a greater ability of the patch to withstand folding and indicates its flexibility and resistance to damage.

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pH

The pH test is conducted to assess the safety of the patch preparation when applied to the skin. The pH level of the patch surface is an essential factor as it can affect skin compatibility. An excessively acidic pH may cause skin irritation, whereas an overly alkaline pH can cause dryness and flakiness. In the context of patch preparations, it is crucial to ensure that the pH falls within a safe range. The recommended preparation pH range for applying patch preparations to the skin is typically 8.[15] The pH value can be influenced by the choice of solvents used in the formulation. In this case, the patch formulation employs ethanol as a solvent, which has a pH value of 7.33. Additionally, aquadest (distilled water) with a pH value of 6-7 is also used. Furthermore, propylene glycol, a plasticizer in the patch formulation, has a pH range of 3-6. The addition of acidic propylene glycol can potentially lower the pH of the patch preparation. Upon testing the pH value of the patch preparation, the results indicated a pH range of 5-6. This falls within the safe pH range of 4-8 for skin application. Therefore, based on the pH test results, the patch preparation falls within the pH safety range, ensuring it is suitable for skin application without causing excessive acidity or alkalinity.

Permeation Test

The permeation ability of the patch preparation was evaluated using the Franz diffusion cell. In this test, a rat skin membrane with a surface area of 3 cm2 was cut to fit the diffusion cell. The liquid compartment of the diffusion cell was filled with 50 ml of phosphate buffer at pH 7.4. The patch preparation was affixed to the rat skin membrane, which was then placed on the Franz diffusion cell apparatus. The donor compartment of the cell contained samples taken from the patch, representing the condition when the transdermal preparation is applied to the skin. The same volume of solution was replaced in the receptor compartment, simulating the condition within the body after the drug had permeated the skin. Samples from the receptor compartment were collected at various time points and analyzed by spectrophotometry to quantify the amount of aspirin that permeated the compartment. Based on the results of the rat skin permeation test, the highest amount of aspirin permeated was observed for formula F3. This indicates that the higher the propylene glycol concentration in the patch preparation, the greater the permeation of the active substance (aspirin). The presence of propylene glycol, a plasticizer in the patch, can increase the permeability of the active substance through the skin. Propylene glycol can disrupt the structure of the stratum corneum, the outermost layer of the skin, and increase its permeability, thereby facilitating greater drug absorption.[10] Therefore, the results suggest that the formulation containing a higher concentration of propylene glycol (F3) exhibits improved permeation ability compared to the formulations with lower propylene glycol concentrations (F1 and F2).

Determination of the Best Formula

In this study, the optimal formula for transdermal patch preparations, with varying propylene glycol concentrations as a plasticizer, was 30% propylene glycol (F3). This determination is based on the results of the evaluation of the transdermal patch preparation, which included organoleptic properties, thickness, weight uniformity, moisture content, elongation, folding resistance, and pH. The use of propylene glycol as a plasticizer has been shown to significantly affect organoleptic properties, thickness, weight uniformity, moisture content, and elongation, but not the pH of the transdermal patch preparation.

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Evaluation of the Best Formula for Transdermal Patch Preparations Content Uniformity

The uniformity of active substance levels in patch preparations is crucial for ensuring a consistent and effective therapeutic effect. In this study, the uniformity of active-substance levels in the patch preparations was evaluated, and the results were compared with the required range of 85-115%. To determine the levels of the active substance, quercetin, the maximum wavelength of 435 nm was used. A calibration curve was prepared at this wavelength, enabling quantification of melinjo leaf extract levels. The percent recovery was calculated to assess the accuracy of the analysis in determining the concentration of the active substance.[21] The percent recovery values obtained in the content uniformity test were 100.74%, 97.715%, 94.314%, 102.00%, and 105.99%.

The average percent recovery for the transdermal patch preparations was 100.15%. These results indicate that the analysis accurately determined the concentration of the active substance in the patch preparations. Furthermore, the average percent recovery results met the required range of 85-110%,[16] indicating that the uniformity of the active substance levels in the patch preparations was within acceptable limits. This ensures that the patch preparations deliver a consistent and effective dose of the active substance, quercetin, which is derived from the melinjo leaf extract. It is important to note that although the percent recovery values meet the requirements, further analysis and evaluation may be necessary to fully assess the uniformity of active substance levels and their impact on the therapeutic efficacy of the patch preparations.

Stability of Preparation

The stability of transdermal patch preparations is critical to ensuring that the formulation remains effective and safe throughout its shelf life. In this study, a stability test was conducted using the cycling method, in which the patch preparations were subjected to alternating storage conditions at 4°C (cold) and 40°C (hot) for a total of 12 days, corresponding to 6 cycles. The purpose of this test is to evaluate whether the patch preparations undergo significant changes or degradation during storage, which could affect their quality, efficacy, and safety. Based on observations and test results, the best formula preparations demonstrated good stability throughout the six cycles of the cycling test. This indicates that the patch formulations maintained their integrity, physical characteristics, and active substance content under the specified storage conditions. The results of the preparation stability test are presented in Table 6.

In Vivo Anti-inflammatory Test

The rat paw edema assay is a well-established and widely used method for evaluating the anti-inflammatory activity of compounds. In this study, the technique was employed to assess the effectiveness of transdermal patch preparations containing melinjo leaf extract in reducing carrageenan-induced paw edema in rats. The procedure involved inducing inflammation in the rat paw by injecting a 1% carrageenan solution subplantarly into the right hind paw. The injection site was carefully cleaned with 70% alcohol before to the injection to minimize the risk of contamination. The volume of the rat paw edema was then measured at regular intervals over a period of 6 hours. Measurements were taken to assess the degree of inflammation and to evaluate the anti-inflammatory effect of the transdermal patch preparations. The percentage of inflammation was calculated as the change in paw volume relative to the initial volume.

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The rat paw edema method is widely used and relatively simple to perform, care must be taken during the data collection process to minimize potential errors. Attention to detail, proper handling of animals, and adherence to ethical guidelines are crucial for ensuring accurate and reliable results. Overall, the rat paw edema method provides valuable insights into the anti-inflammatory activity of transdermal patch preparations and enables assessment of their effectiveness in reducing inflammation without the need for invasive procedures or sacrificing experimental animals.

Based on the average percentage of inflammation over time, the negative control group showed an increase in inflammation throughout the 6-hour period. This indicates that no treatment or intervention was applied to reduce the inflammation in this group. Both test group 1 (TPEDM) and test group 2 (EEDM) showed a decrease in inflammation at the 4th and 5th hours, similar to that observed in the positive control group. This suggests that transdermal patch preparations containing melinjo leaf extract (TPEDM) and the extract alone (EEDM) exhibited anti-inflammatory effects, as they reduced inflammation relative to the negative control group. The positive control group, which likely received a known anti-inflammatory agent, also demonstrated a decrease in inflammation at the 4th hour. This indicates that the selected positive control effectively reduced inflammation, thereby validating the reliability of the experimental setup.

Based on the graph and the provided information, it is evident that the negative control group experienced an increase in inflammation over time, as expected with carrageenan-induced edema. This indicates that the negative control group did not receive any effective anti-inflammatory treatment or compound to inhibit the inflammation. In contrast, both the positive control group and test group 1 (TPEDM) demonstrated a decrease in inflammation at the 4th hour. This reduction in inflammation suggests the presence of compounds that inhibit COX-2, thereby decreasing histamine and bradykinin synthesis.[6] The fact that the TPEDM group showed a similar time frame of action as the positive control group indicates that the active substances released from the transdermal patch formulation were able to penetrate the skin and exert a therapeutic effect. To assess the anti-inflammatory potential of a substance, the mean percentage inhibition of inflammation at each measurement time point is considered. A higher percentage of inhibition indicates a greater potential for anti-inflammatory activity. Therefore, the positive control group and test group 1 (TPEDM) with a decrease in inflammation and higher inhibition percentages suggest their potential as anti-inflammatory agents.[22]

Based on the results, the positive control group exhibited the highest average percentage of inflammation inhibition, followed by test group 1 (TPEDM) and test group 2 (EEDM). This indicates that the positive control group, which received diclofenac sodium, showed the most potent anti-inflammatory action among the tested groups. Diclofenac sodium is a nonselective nonsteroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase (COX), thereby reducing the availability of arachidonic acid. It is known for its potent, relatively nonselective inhibition compared with other drugs in its class, such as piroxicam and indomethacin.[23] The different values of inflammation inhibition among the treatment groups suggest that the formulations used in test group 1 (TPEDM) and test group 2 (EEDM) also exhibited anti-inflammatory effects. However, they may not be as potent as diclofenac sodium. Further analysis and comparison would

be needed to determine the significance and efficacy of the anti-inflammatory activity of each treatment group.

Based on the graph and the provided information, both the positive control and the test groups exhibited anti-inflammatory effects. This effect is attributed to the flavonoids present in the extract, which inhibit cyclooxygenase. Flavonoids are known to inhibit both lipooxygenase and cyclooxygenase, primarily acting on the microvascular endothelium to reduce hyperpermeability and edema. By block the synthesis of prostaglandins, leukotrienes, histamine, bradykinin, and thromboxane, thereby reducing edema volume.[24] In the specific case of test group 1 (TPEDM) and test group 2 (EEDM), the percentage of inflammation inhibition increased at different time points. TPEDM exhibited an increase in inflammation inhibition at 4 hours, while EEDM showed an increase at 5 hours. This difference could be attributed to differences in the release profiles and bioavailability of the active substances from the transdermal patches. Transdermal drug delivery increases drug bioavailability by bypassing gastrointestinal absorption. The active substance in the patch matrix is released and penetrates the skin, diffusing through the stratum corneum and epidermal layers. It then reaches the dermis and gets absorbed into the systemic circulation through blood vessels.[6] On the other hand, oral administration of drugs undergoes absorption in the digestive system, where the bioavailability of the drug can be affected. In this study, the active substances are flavonoids, which have low bioactivity. Depending on their structure, flavonoids can be absorbed in the small intestine or the large intestine. Following absorption, flavonoids undergo phase II metabolism in the liver through glucuronidation and are later excreted in urine.[25] However, only 50% of the dose of the active substance enters the systemic circulation due to various factors.[26] The bioavailability and absorption of drugs can vary based on several factors, including the specific drug, formulation, route of administration, and individual variability. Further research and investigation would be required to fully understand the pharmacokinetics and pharmacodynamics of the tested transdermal patches and oral administration to optimize their therapeutic efficacy.

Conclusion

Increasing the concentration of propylene glycol as a plasticizer had a significant effect on organoleptic properties, thickness, weight uniformity, moisture content, and elongation ($p<0.05$), but did not affect the pH of the transdermal patch preparation ($p>0.05$). The use of 30% propylene glycol as a plasticizer produces good and physically stable preparations from an organoleptic point of view, with good % recovery in the range of 85-110%, patch moisture <10%, elongation in the range of 10-15%, and a stable pH of the preparation. The transdermal patch of melinjo leaf extract (*Gnetum gnemon L.*) is effective as an anti-inflammatory compared to the ethanol extract of melinjo leaves orally in white male rats.

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