Original Article

Formulation of Salam (Syzygium polyanthum (wight) walp) Leaf Ethanolic Extract Matrix Patch and Its Evaluation

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Abstract: The total flavonoid content in salam leaf has an anti-inflammatory activity at a dose of 2.1% w/w. For topical anti-inflammatory agents, matrix patches have a delivery mechanism in which drugs pass through the skin in a controlled manner for an extended period. Polymers were the patch's main component to control the drug release. Polyvinyl alcohol (PVA) and alpha-cellulose (AC) are polymers that can increase the rate of drug diffusion and form a strong film layer. Salam leaves were extracted by maceration using 96% ethanol. Matrix patches were made with a combination of PVA and AC in ratios of 1:1; 3:1; and 1:3. The patches were tested for physicochemical properties. The stability test was conducted at 4, 25, and 40 °C temperatures for 8 h, respectively. Statistical analysis of physicochemical properties test data used One-way ANOVA with a confidence level of 95%. The results showed that a high concentration of PVA significantly affected the weight and moisture content but did not affect the organoleptic thickness, folding endurance, and pH of the matrix patch. The best matrix patch of salam leaf ethanolic extract was PVA and AC of 3:1 because it met the requirements for the matrix patch, including a weight of 1.96-2.06 grams, thickness of 1.15-1.18 mm, folding endurance >300 times, humidity 25.75 – 30.17% and pH 6.3 – 7.1. The patch contains flavonoids with Rf values for extract and patch of 0.95 and 0.96, respectively. Further study, release, and in vivo anti-inflammatory tests are necessary.

Keywords: polyvinyl alcohol; alpha-cellulose; patch; physicochemical properties

1. INTRODUCTION

Salam leaves are used as food seasoning by Indonesian people [1]. Wientarsih et al. (2007) reported that salam leaves are anti-inflammatory because they contain active compounds such as tannins, flavonoids, and essential oils such as citric acid and eugenol [2]. One of the main components in salam leaves is quercetin which is included in a flavonoid group. According to The Indonesian Herbal Pharmacopoeia (2017), salam leaves contain total flavonoids of 0.40%, calculated as quercetin [3]. Quercetin in salam leaf has anti-inflammatory activity by inhibiting cyclooxygenase and lipoxygenase enzymes [4]. The anti-inflammatory mechanisms of action of salam leaf extract involved inflammatory mediators, such as interleukins (ILs), nuclear factor kappa B (NF-κB), prostaglandin E2 (PGE2), cyclooxygenase (COX) and reactive oxygen species (ROS) [32]. Research conducted by Yusuf et al. (2020) reported that the salam leaf ethanolic extract dose used for patch preparation was 2.1% [5]. The dose has an anti-hyperlipidemic activity also acts as an anti-
inflammatory. The delivery system for the active compound on the matrix patch has a delivery mechanism in which drug molecules pass through the stratum corneum in the skin in a controlled manner for an extended period [6]. This differs from topical preparations such as gels, creams, and ointments given 2-3 times a day but disappears more quickly and absorbs, so it wears off faster. Therefore, to overcome this, it is necessary to develop new formulas for other modern topical preparations, to deliver the active compound by patch delivery system. The matrix patch is one of the topical preparations with adhesive (coating adhesive), which contains a specific dose of a drug to be delivered to the action [7].

Polymers such as hydroxypropyl methylcellulose, carboxymethyl cellulose, and polyvinyl alcohol were the primary constituent components in formulating patches with a matrix system [8]. In this study, the polymers used were polyvinyl alcohol and alpha-cellulose because polyvinyl alcohol adheres well to the skin and forms a film layer that is transparent, strong, and plastic [9]. At the same time, alpha-cellulose can thicken the matrix produced and has the characteristics of being inert and suitable biocompatibility for topical preparations [10]. Previous studies on the formulation of matrix patches have been carried out using a combination of HPMC and PVP polymer ratios at a ratio of 1:1 to produce good physical characteristics [5]. A combination of PVA polymer ratios higher than ethyl cellulose at a ratio of 7:3 can provide good film characteristics and deliver the active substance through a semipermeable membrane in the skin [11]. Alpha-cellulose polymer is soluble in water but will expand if formulated in large quantities of patch preparations. Therefore, the different variations in the concentration of polymers will affect the characteristics of the patch preparation. The ratio of PVA and AC of 1:2 is the chosen formula because it meets the patch requirements. The results showed the combination of PVA and AC patch matrix of *Moringa oleifera* leaves extract had a significant effect on the physicochemical properties there are thickness, patch weight, and moisture content, but they did not affect folding endurance and pH. The percentage of total flavonoid that released from patch was 37.23% for 5 hours. The release kinetics followed the Higuchi kinetics model with a diffusion mechanism [33]. Alpha cellulose has different properties from ethyl cellulose, where AC is hydrophilic while EC is hydrophobic. Based on this, this research is expected to determine the effect of the combination of PVA polymer and alpha-cellulose on the physicochemical properties of salam leaf ethanolic extract patches and to find the combination polymer ratio of PVA and alpha-cellulose that gave the best physicochemical evaluation. Using the TLC method, the chosen formula was then used for was then used for stability test and detection of quercetin content.

## 2. MATERIALS AND METHODS

**Materials:** Salam leaves (Ngawi, East Java, Indonesia); 96% Ethanol (repackaged by PT. Bratachem); NaOH (Merck KGaA, Germany); silica gel 60 F254 (Merck KGaA, Germany); n-butanol (Merck KGaA, Germany); acetic acid (Merck KGaA, Germany); Aquadest (CV Nitra Kimia, Yogyakarta); quercetin standard (Sigma Aldrich Production, USA); polyvinyl alcohol (repackaged by PT. Bratachem, Surakarta); alpha-cellulose (Sigma Aldrich Production, USA); phenoxyethanol (repackaged by Cipta Kimia, Surakarta, batch. 028/SE/0817); 70% ethanol solvent; PEG 400 (DOW, Singapore); and propylene glycol (DOW, Singapore). **Instruments:** Digital balance (Precisa), water bath, stirring rod, sieve No.40, petri dish diameter 5 cm (normax), pH meter (OHAUS), caliper (TOKI), moisture analyzer (OHAUS, Japan), magnetic stirrer (IKA C-MAG HS 7), and oven (Memmert).
2.1. Sample Preparation

Determination of salam plants was conducted at the Biology Laboratory, Mathematics and Natural Science Faculty, Universitas Sebelas Maret, Surakarta, Indonesia. Salam leaves were dried in an oven at 50 °C, powdered using a blender, and then sieved using a sieve of 40 mesh sleeve. Salam leaf powder was weighed at 1.0 grams and placed on a the moisture analyzer to analyze the moisture content of salam leaves powder. Salam leaf powder of 500 grams was put into a maceration jar, and then soaked with 2.5 liters of 96% ethanol solvent until the powder was completely submerged. The maceration jar was closed and protected from light for three days and stirred twice daily to homogenize the solvent. The obtained macerate was separated by filtration (filter) using filter fabric. The macerate was evaporated using a rotary evaporator at a temperature of 40 ℃ and followed by a water bath at 50 ℃ for 4 hours until a thick extract was obtained [3]. The extract obtained was weighed, and the yield, moisture content, and detection of active constituents were determined by the TLC method.

2.2. Salam Leaf Ethanolic Extract Tests

The extract yield was the percentage weight (w/w) of the division between the weight of the extract and the salam leaf powder. The yield must reach at least the requirement of the extract monograph [3]. The higher the yield value, the greater the extract that can be obtained from a salam leaves powder. The organoleptic examination was carried out to describe the extract’s consistency, color, and odor. The purpose of this examination was for the simple initial identification of extracts. Organoleptic activity was a specific parameter of an extract. Determining moisture content in the extract aims to provide a minimum limit or range of the concentration of the moisture content in the extract. The higher moisture content makes contaminating the extract easier with fungus or mold. It can reduce the biological activity of the extract during storage. Generally, the required moisture content was less than 10%. Qualitative analysis of the extract was conducted to detect the flavonoid content in the extract by weighing 1.0 grams, then adding 5 drops of a NaOH solution. Identification by TLC method using standard quercitin because it includes the flavonoid group. The stationary phase was a silica gel 60 F254 plate, which was activated by heating in an oven at 105 ℃ for 10 minutes. The mobile phase was n-butanol: acetic acid: water (4:1:5) in a saturated chamber glass saturated. After being completely eluted, the TLC plate was evaluated for spot appearance formed by a UV lamp at wavelengths of 254 nm and 366 nm [12].

2.3. Formula of Salam Leaf Ethanolic Extract Matrix Patch

Table 1. The patch formula of the Salam leaf ethanolic extract with a variation ratio of polymer

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Function</th>
<th>Grams</th>
<th>Formula 1 with PVA: Alpha-cellulose 1:1</th>
<th>Formula 2 with PVA: Alpha-cellulose 3:1</th>
<th>Formula 3 with PVA: Alpha-cellulose 1:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>Active substance</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>Hydrophilic polymer</td>
<td>0.50</td>
<td>0.75</td>
<td>0.25</td>
<td>0.75</td>
</tr>
<tr>
<td>Alpha-cellulose</td>
<td>Hydrophobic polymer</td>
<td>0.50</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Polyethylene-glycol 400</td>
<td>Plastizizers</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>Solvent</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Penetration enhancer</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Phenoxyethanol</td>
<td>Preservatives</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Aquadest</td>
<td>Solvent</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

*each formula was replicated three times

All ingredients in Table 1 were weighted according to the matrix patch formula. Alpha-cellulose was dispersed in 70% ethanol solvent, and PVA was dissolved in water at 50 ℃. The mixed
solution was stirred using a magnetic stirrer at 500 rpm. Alpha-cellulose was added to the PVA solution and stirred until homogeneous. The salam leaf ethanolic extract was dissolved in 70% ethanol solvent and then put into the mixture. The mixture was added PEG 400, propylene glycol, and phenoxyethanol were added to the mixture. The mixture was stirred until homogeneous and poured into a glass disk mold. The patch preparation was dried in an oven at 40 °C for 3 hours, and then stored at room temperature for 4 days. The patch preparation was tested on the 14th day. The data from day 0, the first week, and the second week were then processed [13].

2.4. Evaluation of physicochemical Properties of Salam Leaf Ethanolic Extract Matrix Patch

The pH test of the matrix patch was carried out by soaking the patch in 10 mL of distilled water for ± 20 minutes at room temperature. The pH meter was immersed in the patch solution. The pH meter’s value was the patch’s pH value at each [13]. The thickness test of the patch use a caliper with an accuracy of 0.1 mm, and than the measurement results were averaged. The weight test of a patch was carried out using three patches that weighed respectively, and the measurement results were averaged [13]. The folding endurance test was carried out manually by folding the patch repeatedly on the same line until it tears. The patch meets the requirement if it can be folded up to 300 times [13]. The moisture content test of patch preparations was carried out using a moisture analyzer at a temperature of 105 °C to a constant weight. Moisture analyzers utilize infrared or halogen lamps as heat sources. The percentage of moisture content of the patch can be seen on the display monitor [13].

2.5. Stability Test

A stability test of the patch was carried out with variations in temperature at time intervals to determine the effect of temperature on the physicochemical properties of the patch [14]. The patch matrix was saved at a low temperature (4±2°C) for 8 hours and then stored at room temperature (28±2°C) for 16 hours. The test was continued in the oven at a high temperature (40±2°C) for 8 hours and then stored at room temperature (28±2°C) for 16 hours. The test counts as one cycle. This test was carried out in 6 cycles or for 12 days. Evaluation of the physicochemical stability of the patch included organoleptic, moisture content, folding endurance, and pH [13].

2.5. Data Analysis

The data was analyzed using the SPSS 21 program. One-way ANOVA test followed if the data were normally distributed to determine the presence of a significant difference between the three formulas in the physicochemical properties test. One-way ANOVA test data results with a significant difference continued with the Post-hoc test with a confidence level of 95%. The patch’s physiological stability test result data were analyzed using the paired samples t-test.

3. RESULTS AND DISCUSSION

The determination result showed that the salam plant species used was Syzygium polyanthum (Wight) Walp. with the document number UN27.9.6.4/Lab/2021. The results of the organoleptic test of the extract showed that the salam leaf ethanolic extract produced a thick consistency, dark-brown color, and smelled salam leaf. The yield value of the extract was 7.49%; in previous research conducted by Hidayati et al. [15], the yield value of salam leaf maceration with 96% ethanol solvent was 10.21%. The yield value result of this research was lower than that of previous research. This may be because of differences in the maceration process without remaceration and soil nutrient contents that affects the content of the active compounds in salam leaves. In addition, the presence of climatic factors and rainfall can affect secondary metabolites [17]. According to the Herbal Pharmacopoeia Indonesia Edition II (2017) [3], the standard yield value of salam leaves ethanolic extract was at least 18.2%. The moisture content of salam leaf ethanolic extract
was 8.40%. This met the required moisture content of Indonesian Pharmocopeia Edition IV [16] was less than 10%.

3.1. Detection of Active Compounds in Salam Leaf Ethanolic Extract

The salam leaves extract solution was yellowish after the NaOH solution was added. This indicates that the salam leaf ethanolic extract contains flavonoids. This was due to the decomposition by bases into acetophenone molecules, which were yellow due to the cleavage of the bond to the isoprene structure [17]. The principle of UV light 254 nm detection was that the plate provided fluorescence while the sample was dark. The stain appears raised because of the interaction between UV light and the fluorescence indicator on the TLC plate. In contrast, the principle of detection by UV light 366 nm was that the stain gives fluorescence and dark-colored plates, visible stains due to the interaction between UV light and the bound chromophore group (auxochrome) on stains [18] (Figure 1).

Figure 1. The Result of TLC analysis were the quercetin standard (Q), salam leaf ethanolic extract (Ex), and salam leaf ethanolic extract patch (Ptc). The stationary phase was silica gel F254, and the mobile phase was n-butanol: acetic acid: water (4:1:5). Detection of spots under the UV light at 256 and 366 nm.

The Rf values obtained from standard quercetin (Q), salam leaf ethanolic extract (Ex), and salam leaf patches were 0.95; 0.96 and 0.96, respectively. Previous research by Fitri et al. (2020) [19] obtained and the Rf value of 0.925 for the quercetin standard. The standard Rf value for quercetin was 0.80 [20]. The thin layer chromatography for phenols using a methanolic extract of Centella in the solvent system gave a retention factor (Rf) value of 0.83, similar to that of standard gallic acid [21]. Flavanoids were presence in all extracts with one spot in each (Rf 0.8 for acetone, 0.918 for methanol, 0.816 for chloroform, and 0.737 for aqueous extract) [22]. The results show that the salam leaf ethanolic extract may contain a total flavonoid compound counted as quercetin, because it was close to the Rf value of the quercetin standard.

3.2. Matrix Patch Formula of Salam Leaf Ethanolic Extract

The results of an organoleptic test on the three formulation of salam leaf ethanolic extract patches showed a dark brown appearance, smooth surface but not even, it may because the saponin compound in salam leaf that appearedance foam when it was stirred, and a salam leaves an odor. Organoleptic observations on patch preparations on day-0, days 7, and days 14 did not change color or odor during storage. These results indicate that the variation in the ratio of PVA and alpha-cellulose polymer does not affect the organoleptic properties of the three formulas, so it can be concluded that they are quite resistant to environmental changes during storage (Figure 2).
Table 2. The Results of physicochemical test of salam leaf ethanolic extract patches for 14 days in room temperature

<table>
<thead>
<tr>
<th>Formula</th>
<th>Patch weight (mg)</th>
<th>Patch thickness (mm)</th>
<th>Moisture content (%)</th>
<th>pH value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-0</td>
<td>Day-14</td>
<td>Day-0</td>
<td>Day-14</td>
</tr>
<tr>
<td>Formula 1 with PVA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-cellulose 1:1</td>
<td>1.66±0.10</td>
<td>1.52±0.10</td>
<td>1.15±0.01</td>
<td>1.11±0.01</td>
</tr>
<tr>
<td>Formula 2 with PVA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-cellulose 3:1</td>
<td>2.06±0.03</td>
<td>1.96±0.05</td>
<td>1.18±0.01</td>
<td>1.15±0.02</td>
</tr>
<tr>
<td>Formula 3 with PVA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-cellulose 1:3</td>
<td>1.89±0.09</td>
<td>1.76±0.03</td>
<td>1.19±0.01</td>
<td>1.13±0.02</td>
</tr>
</tbody>
</table>

*a,b,c significantly different (p<0.05); mean±SE

The weight uniformity test of the salam leaf ethanolic extract patch aims to determine the uniformity of the weight of the patch to evaluate the consistency of the manufacturing process. An exemplary process of manufacture would produce uniformity of active substance content in every patch. A slim patch matrix would be preferred because it was convenient to use. The increase in the patch’s weight was related to its constituents’ polymer properties. Formulas with a higher PVA ratio gained weight after 14 days compared to other formulas. PVA polymer is a hygroscopic polymer. Hygroscopic properties could bind moisture during the manufacturing process and storage patch so that the weight of the patch increases [23]. In contrast, alpha-cellulose is also hydrophilic, which accelerates the diffusion process of active substances [24]. The formula with a ratio of PVA-alpha cellulose of 1: 1 and 3: 1 for 14 days of storage showed no significant difference (p<0.05) compared to the formula with a ratio of 1: 3. This is because 70% ethanol used as a solvent will evaporate during the drying process. Ethanol has a lower relative boiling point, so it evaporates quickly. The polymer ratio of PVA and alpha-cellulose significantly affected the increase in patch weight (p<0.05).

The thick patch affects the release of the active substance, which is made longer than a gel or cream [5]. The thicker patch film matrix was also less desirable because it lacked comfort when used [13]. Polymer properties influence increased patch thickness. The formula with alpha-cellulose has different properties from ethyl cellulose, where AC is hydrophilic while EC is hydrophobic. The highest PVA ratio was retained and bound in the patch preparation, thus affecting the thickness of the patch [25]. Variations in the PVA and alpha-cellulose polymer concentrations significantly affected increasing patch thickness (p<0.05). Due to the use of PVA polymer, this matter could improve the characteristics of elastic and strong films [9]. While the alpha-cellulose is hydrophilic, it may cause the active substance to penetrate dissolution media easily [26]. The research by Ermawati 2022 reported that The ratio of PVA and AC of 1:2 in Moringa patch released total flavonoid from patch was 37.23% for 5 hours [33]. There was a significant difference in the formula with a ratio of
PVA and alpha-cellulose of 1: 1 and 1: 3 for 14 days of storage (p<0.05). This was because the PVA polymer could absorb the solvent in the patch during storage [25].

The increase in patch moisture affected the polymer properties. The high PVA polymer ratio in the formulas greatly increases the moisture value. This was due to PVA being easily soluble in water, so water would be retained in the patch for drying at the time of formulation [27]. This could retain moisture in the patch. The variation in the PVA polymer and alpha-cellulose ratio affected patch moisture (p<0.05). The high concentration of PVA polymer used, the higher the value of the moisture of the patch preparation. In addition, propylene glycol in the patch could attract water to affect the skin’s hydration by softening the keratin layer in the stratum corneum, thereby increasing the amount of active substance that penetrates [28]. The formula with a ratio of PVA-alpha cellulose of 1:3 for 14 days of storage had a significant difference (p>0.05) compared to other formulas because of the effect of the added PVA concentration, which causes fluid to be retained in the patch.

The three formulas meet the pH requirements of preparations that do not irritate the skin, namely 5-9 [29]. However, the variation in the ratio of PVA polymer and alpha-cellulose did not affect the pH of the patch (p>0.05). The three formulas for 14 days of storage showed no significant difference (p>0.05). This was because oxygen oxidized the water as a solvent due to the testing process at room temperature.

The folding endurance of the three salam leaf Ethanolic extract patch formulations met the requirement because it obtained a folding endurance value of more than 300 times during storage for 14 days. The results obtained have good integrity when applied to the skin. The variation ratio of PVA and alpha-cellulose polymer in each formula does not affect patch folding endurance. However, adding PEG 400 as a plasticizer may affect the folding endurance of the patch because it could increase elasticity and reduce the risk of tearing [30].

### 3.3. Stability Test of Salam Leaf Ethanolic Extract Matrix Patch

<table>
<thead>
<tr>
<th>Formula</th>
<th>pH 4°C</th>
<th>Moisture Content 4°C</th>
<th>Folding Endurance</th>
<th>pH 28°C</th>
<th>Moisture Content 28°C</th>
<th>Folding Endurance</th>
<th>pH 40°C</th>
<th>Moisture Content 40°C</th>
<th>Folding Endurance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series 1</td>
<td>1</td>
<td>6.5</td>
<td>26.13</td>
<td>350</td>
<td></td>
<td></td>
<td>6.3</td>
<td>24.5</td>
<td>350</td>
</tr>
<tr>
<td>Series 2</td>
<td>2</td>
<td>6.7</td>
<td>27.23</td>
<td>350</td>
<td></td>
<td></td>
<td>6.5</td>
<td>28.15</td>
<td>350</td>
</tr>
<tr>
<td>Series 3</td>
<td>3</td>
<td>6.4</td>
<td>25.85</td>
<td>350</td>
<td></td>
<td></td>
<td>6.2</td>
<td>24.36</td>
<td>350</td>
</tr>
</tbody>
</table>

**Figure 3.** The results of the stability test of salam leaf Ethanolic patches at various temperatures

Factors affecting physical stability were radiation, heat, light, and humidity [31]. Tests were carried out for each formula to determine the stability of patches stored at cool (4 °C), room (28 °C), and hot temperatures (40 °C). Furthermore, the instability of a product can be observed through physical changes, including color, surface appearance, and odor by organoleptic analysis. Preparations can be
stable when they do not change during the specified storage limits. This is because the elasticity of patch storage at cool, room, and hot temperatures can be changed. The patch becomes moist and the color after being stored at cool temperatures changes to pale due to the cooling process. In contrast, the color darken when stored at room and hot temperatures. The patches stored at higher temperatures and lost water and showed a dark color at the condition of 40 °C. The patches stored at room temperature (28 °C) had no difference in color was observed. This indicated that room temperature did not cause a color change.

4. CONCLUSION

An increased ratio of PVA polymers in a combination of PVA and alpha-cellulose polymer on the physicochemical properties of the salam leaf ethanolic extract patch can increase the patch’s weight, thickness, and moisture content but does not affect folding endurance and pH. The selected formula is a formula with a ratio of PVA and alpha-cellulose of 3:1 because it showed the best physicochemical properties of the patch, such as weight of 1.96 – 2.06 grams; patch thickness of 1.15-1.18 mm; patch folding endurance > 300 times; the patch moisture content of 25.75 – 30.17% and pH value of 6.3 – 7.1. The selected formula was identified using TLC analysis containing a quercetin compound with an Rf value of 0.96. Temperature variations significantly affected organoleptic (color and surface texture) and moisture content but did not affect folding endurance and pH. Further study, release and in vivo anti-inflammatory test is necessary.

Conflicts of interest: The authors declare no conflicts of interest.

References


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