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Phytochemical test and optimization of transdermal patches of *Carica papaya* extract: Formulation design of candidate drug for wound healing

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Abstract. *Santi TD, Siregar TN, Sutriana A, Andini R, Candra A. 2022. Phytochemical test and optimization of transdermal patches of* Carica papaya *extract: Formulation design of transdermal patch as candidate drug for wound healing. Biodiversitas 23: 2904-2913.* Papaya leaves contain metabolite compounds that have anti-inflammatory and wound-healing properties. This leaf extract as a transdermal patch allows easy application for wound healing. This study aimed to examine the phytochemicals of papaya leaves and determine the formulation of the transdermal patch. This study employed qualitative methods with color testing and quantitative methods with Gas Chromatography-Mass Spectrophotometry (GC-MS) to determine the phytochemical profiles of papaya leaves. The optimization test of patches used Response Surface Methodology with Box-Behnken design on Expert Version 12 software. There were three factors (x) tested in the study, namely PVP, ethyl cellulose, and Ethanol Extract of Papaya Leaves (EECP). There were also three responses (y) investigated, namely thickness, weight, and moisture content. The phytochemical color testing results revealed the presence of alkaloids, steroid, terpenoid, saponins, flavonoids, phenolic compounds and the GC-MS analysis showed six compounds with the widest areas. From an ANOVA analysist, the p-value was significant (<0.5) while the match value was less significant (p-value >5%). The optimal patch found in this study was the one containing PVP (0.3 mg), ethyl cellulose (0.4 mg), and EECP (0.3 mg). In terms of response values (y), the patch had a thickness of 0.25 mm, a weight of 82 mg, and a moisture content of 4.39%.

Keywords: Box-Behnken, formulation, GC-MS, transdermal patch

INTRODUCTION

Transdermal patch is a patch containing active substances for medication. It is attached directly to the skin to allow the drugs in it to be absorbed slowly and more protectively into the skin (Kováčik et al. 2020). Manufacturing transdermal patches require a penetration enhancer with no therapeutic effect. Transdermal patches can increase skin permeability, penetrate the penetration barrier (stratum corneum), and transport drugs into the skin (Seah and Teo 2018; Szunerits and Boukherroub 2018).

The use of a transdermal patch can minimize drug interactions, avoid the first-pass effects, and cause a systemic reaction. Plasma drug levels are more stable for a long period due to good bioavailability. They can also be applied to patients who have difficulty swallowing drugs and can improve patients' compliance. The patch use can be discontinued immediately if side effects occur (Khatoon 2017; Saghazadeh et al. 2018; Sharma et al. 2021). Preparing traditional medicine as a new drug candidate will increase its therapeutic effects, convenience, and dosage accuracy. One plant that can be formulated as a transdermal patch is *Carica papaya* L. In Aceh, papaya leaves have been traditionally used by the locals as an antioxidant, anti-inflammatory and wound healing (Candra and Santi 2017).

Papaya leaves have a number of bioactive compounds (Nandini et al. 2020), such as flavonoids (Jimenez et al. 2020), alkaloids (Adedayo et al. 2020), phenolic (Santi 2015), saponins (Nafiu et al. 2019), and other active components (Sankarganesh et al. 2018). Secondary metabolites from active *C. papaya* leaves can be utilized in the form of transdermal patches for anti-inflammatory, and wound healing. To obtain an optimal wound healing effect, it is necessary to develop an optimization formula for transdermal patches using the Response Surface Methodology. This method is a mathematical-statistical technique to analyze response models related to several factors to optimize responses (Oramahi 2016). One of the designs in the Response Surface Methodology (RSM) is

Box-Behnken Design on Expert Version 12 software which tests three factorial levels (Tekindal et al. 2012).

This study aimed to examine the phytochemicals of papaya leaves using color testing method and Gas Chromatography-Mass Spectrophotometry (GC-MS) and to optimize the formulation of transdermal patches of *Carica papaya* leaves for wound healing using Box-Behnken design with the quadratic model on Design Expert Version 12 with three factors (x) and three responses (y).

MATERIALS AND METHODS

Materials and tools

Papaya (*Carica papaya* L) leaves were collected from Lambhuk, Banda Aceh City, Indonesia. Taxonomic identification was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia.

The other materials used in the study were polyvinyl pyrrolidone (PVP), ethyl cellulose, Dimethyl Sulfoxide (DMSO), Papaya Leaf Ethanol Extract (EECP), propylene glycol, and 50% ethanol. The following tools were utilized: GC-MS (Agilent Technologies 7890 Gas Chromatograph, California, USA), Whatman No.1 filter paper, and a vacuum rotary evaporator (BUCHI R-300, Switzerland).

Research methods

Maceration

Papaya leaves were harvested at 10.00 WIB. The leaves were purposively selected based on the following criteria: green and not too old. Then, 1 kg leaves were cleaned using running water. After being drained, the leaves were cleaned from any dirt that might still attach to them. Next, they were dried in the shade and protected from direct sunlight for 7 days. Dried leaves were then re-sorted and turned into powder using a blender.

The preparation of *C. papaya* ethanol extract (EECP) consisted of several steps. Firstly, five hundred grams of papaya leaf powder were put into a bottle and mixed with 96% ethanol solvent to produce an extract. Secondly, the extract was soaked for 72 hours with occasional stirring to make it completely submerged. Next, a green liquid filtrate was obtained after the extract was filtered using Whatman No.1 filter paper. Evaporation was also carried out using a vacuum rotary evaporator (T: 50ºC) to obtain a thick extract (16 g).

GC-MS method

EECP analysis was carried out at Labkesda Jakarta using GC-MS (Agilent 5975 with 7890 GC). The mass spectrum was recorded at 70 eV with fragments of 40,650 Da. GC-MS works by separating the components in the mixture through gas chromatography. Each of the components can be made into a mass spectrum with higher accuracy. The separation by gas chromatography produces chromatograms, whereas mass spectrometry examination of each compound results in a spectrum (Nurhaen et al. 2016).

Transdermal patch formulation

Transdermal patch formulation comprised several steps. Firstly, a polymer base was created by dissolving ethyl cellulose in 2 mL of 50% ethanol with the addition of PVP until they were homogeneous. Next, DMSO was added to it. After that, the ethanol extract of *C. papaya* leaves was made by dissolving propylene glycol and 3 mL of ethanol. Then, the solvent was added to the polymer base. After they were homogenized, 10 mL of 50% ethanol was added to the mixed formula which was then poured into a petri dish with an area of 19.94 cm and evaporated to dryness at room temperature.

Characteristics of transdermal patch

The response characteristics (y) of transdermal patches in the study included the thickness, weight, and moisture content.

Patch thickness

Patch thickness was measured using a micrometer at three different points (David et al. 2015), namely the edge part, middle part, and the part in between. The values of those three parts were then added and divided by 3 to achieve the mean of the thickness.

Weight

The transdermal patch weight was obtained by weighing each patch using an analytical balance. Then, the average weight was determined.

Moisture content

The transdermal patches that had been shrunk in a desiccator were weighed and put into a climatic chamber at 40ºC for 24 hours and re-weighed. The percentages of their moisture content were obtained by reducing the final weight with the initial weight and dividing it by the initial weight (Patel et al. 2009).

$$\% \text{ Moisture content} = \frac{W2 - W1}{W1} \times 100$$

In the above formula, W1 represents the initial weight while W2 is the final weight.

Organoleptic

The EECP transdermal patch organoleptic test was assessed from the shape, color, odor, and consistency of the patch surface.

Statistical analysis

The optimization test of transdermal patch formulation used the Box Behnken design on Design Expert 12 software. Three factors (x) were tested here, namely Polyvinyl Pyrrolidone (PVP), ethyl cellulose, and EECP. Three responses (y) were also tested, namely thickness (mm), weight (mg), and moisture content (%). The polynomial equation was obtained based on (Joshi and Garud 2021):

$$y = \beta 0 + \beta 1A + \beta 2B + \beta 3C + \beta 4AB + \beta 5AC + \beta 6BC + \beta 7A^2 + \beta 8B^2 + \beta 9C^2$$

In this equation, y represents the responses to factor A (polyvinyl pyrrolidone (PVP)), factor B (ethylcellulose), and factor C (EECP).

Optimum formula prediction

The optimum transdermal patch formulation was obtained by analyzing 17 runs, as shown in Table 3. The research used a quadratic model by looking at the R2 value and PRESS value, which were then compared with the responses of the linear model and 2FI model. The value of R2 shows the regression factors (x) and responses (y), which is adequate if it is above 70% (Tekindal et al. 2012).

RESULTS AND DISCUSSION

Taxonomy and morphology of *C. papaya*

The result of taxonomy identification carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, is *Carica papaya* L. The taxonomy identification was deemed necessary to ensure the correctness of the plant characteristics being studied. The results of leaf morphology showed large leaf blades, fingered, single, and serrated with hollow petioles. The leaves had a length of 37 cm, a diameter of 25-47 cm, a green color, and were located at the top of the stem (Figure 1).

The color test results showed the presence of secondary metabolites of flavonoids, steroid, alkaloids, terpenoid, saponins, and phenolic, as presented in Table 1. The analysis of metabolite compounds used several polar-specific reagents that interacted with EECP with the principle of like dissolves like. The reagents were Mayer's reagent, Dragendoffs reagent, and Wagner's reagent. Mayer's reagent contains potassium mercury iodide which reacts with nitrogen alkaloids to form a precipitate. Dragendoffs reagent contains bismuth-potassium iodide which reacts with nitrogen alkaloids to form sediments, whereas Wagner's reagent consists of iodide-potassium iodide which reacts with alkaloids to form colored salt complexes that are difficult to dissolve in water (Raal et al. 2020). The formation of precipitates in the Mayer, Wagner, and Dragendoff tests proved that EECP contains alkaloids.

Alkaloids have anti-inflammatory activity (Abbiw 1990), which inhibits cytokine production (Hess 2011), accelerates wound healing, increases cell motility and proliferation of dermal fibroblasts, increases the rate of epithelial cell formation, which thereby accelerates the process of wound regeneration. In addition, alkaloids accelerate angiogenesis (Zahra et al. 2011), increasing blood supply to new epithelial cells (Fetse et al. 2014).

The flavonoid content in EECP was tested using a Liebermann-Burchard reagent, resulting in a bluish-green color which indicated the presence of flavonoids. Flavonoids have broad biological activity as anti-inflammatory and antioxidant (Gutiérrez et al. 2014). Antioxidants play a role in accelerating wound healing by preventing reactive oxygen species (ROS) from penetrating the wound area (Barku 2019). Flavonoids inhibit the activity of cyclooxygenase (COX), lipoxygenase enzymes, leukocyte accumulation, neutrophil degranulation, and histamine release (Nijveldt et al. 2001). The presence of these secondary metabolites indicates that papaya leaves have a pharmacological effect and potential for wound healing.

The saponins in EECP have wound healing activity that increases the number of fibroblast cells, macrophages, and growth factors (FGF-2, GF, TGF-β) for proliferation. TGF-β is associated with fibroblast activation and collagen deposition that make up the epithelial layer (Minutti et al. 2017). Saponins can kill or prevent the growth of microorganisms and form collagen which is essential for the wound healing process (Hassan et al. 2012).

The results of the analysis in Table 1 showed that EECP contains phenolics that have antioxidant effects that inhibit lipid peroxidation and accelerate wound healing (Miladiyah and Prabowo 2012). Tannins compounds in papaya leaves, on the other hand, were unidentifiable in the current study which might be due to several factors, such as environmental factors that affect the number of secondary metabolites in plants, including altitude, soil pH, the intensity of sunlight, and humidity. The results of the current study differed from those of the phytochemical test conducted by Shubham et al. (2019) which succeeded in identifying the presence of tannins. Tannins can also be identified by (Nandini et al. 2020) and (Nugroho et al. 2017) in a green-black color when tested with ferric chloride (FeCl3).

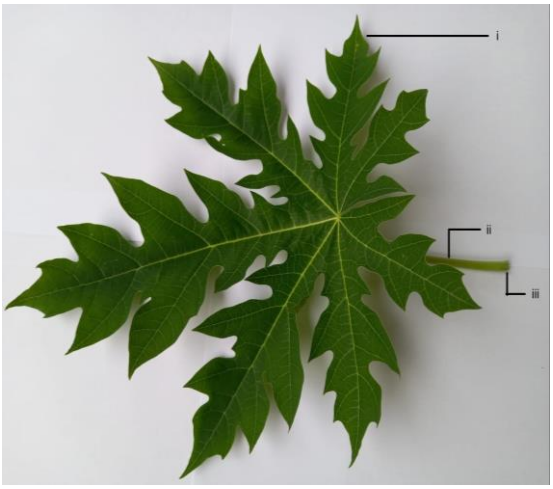


Figure 1. Papaya leaf. i: lobes of the lamina, ii: petiole, iii: leaf base

Table 1. EECP phytochemical test results

Secondary metabolite	Reagen	Result
Alkaloids	Mayer	+
	Wagner	+
	Dragendorff	+
Steroid	Liebermann- Burchard	+
Terpenoid	Liebermann- Burchard	+
Saponins	Shaken	+
Flavonoids	HCl and Mg	+
Phenolic	$FeCl_3$	+
Tannins	$FeCl_3$	-

The property of EECP can be attributed to wound healing and anti-inflammatory. In study by Santi (2015), *C. papaya* leaves have been investigated for anti-inflammatory activity in rat using carrageenan-induced paw edema. The result of study showed papaya leaves extract can inhibit prostaglandins mediated inflammation. Similarly, in another study conducted by Owoyele et al. (2008), and Osuki et al. (2010) and the ethanolic extract of *C. papaya* significantly reduced paw edema, which might resemble the effect of a COX selective inhibitor.

Phytochemical compounds are secondary metabolites in bioactive compounds that play a crucial role in the research of drugs from plants. In this study, testing of phytochemical compounds was carried out qualitatively to prove that the secondary metabolites act as an anti-inflammatory and wound healing agent. Wound healing is a series of biochemical reaction to repair the damage in steps, namely, hemostasis, inflammation, proliferation and maturation.

EECP phytochemical test with GC-MS

The results of the EECP analysis with GC-MS showed the presence of several active compounds with different areas in EECP. Meanwhile, Figure 2 indicated that there were six compounds with the widest areas, namely n-Hexadecanoic acid, phytol, methyl linolenate, linolenic acid, oleoyl chloride, and carpaine (Table 2).

GC-MS analysis can identify various components of compounds in plant cells, unlike ordinary phytochemical screening which is only able to identify compound groups (Hamuel 2012). The chromatogram revealed that there were six compounds with the largest areas and the largest component of EECP from Lambhuk, Banda Aceh, Aceh Province, namely linolenic acid with an area of 16.79%, methyl linolenate 14.4%, carpaine 11.78%, n-Hexadecanoid acid 8.77% and phytol 8.51%. The retention time of each compound was determined by the boiling point of the compound. The difference in retention time of the compounds can be caused by the interaction of the compounds with the stationary phase, which in this case, is the column used in the gas chromatography system. The resulting chromatogram was formed based on the total number of ions formed in each component of the chemical compound in a sample. The greater the percentage of a component in the sample, the higher the peak produced, and vice versa.

The GC-MS mass spectra of EECP with retention time of 28.079 minutes are identical to mass spectra of n-hexadecanoid acid. This compound belongs to saturated long-chain fatty acid with the other name Hexadecanoic acid, palmitic acid. This compound has been reported to have anti-inflammatory, antioxidant, reduce high-fat diet, inhibit arthritis, decreased the secretion of exosomes in the prostate cancer cell, alpha-reductase inhibitor activities, hypocholesterolemic and hemolytic agents (Souza et al. 2018).

Phytol is a diterpene with other name 2-hexadecen-1-ol, 3,7, 11, 15- tetramethyl-, [R-[R*,R*-{E}]; trans-phytol; 3,7,11,15- tetramethyl-2-hexadecen-1-ol. This compound obtained in our extract had retention time of 28.603 minutes which is identical to the GC-MS mass spectra. Phytol has been evident to have anti-inflammatory, antioxidant, antimicrobial, antitumorous, antidiabetic, dan antidepressant (Pejin et al. 2014). Similarly, study in silico by Islam et al. (2015) showed that phytol efficiently with COX-1 and 2, IL-1β.

The methyl linolenate has other name of methyl (9E, 12E, 15e)-9,12,15-octadecatrienoate; 9,12,15-octadecatrienoic acid, methyl ester; methyl 9,12,15-octadecatrienoate which is commonly used as anti-melanogenic activity, antioxidant, antifungal, anti-inflammatory (Huh et al. 2010). This compound is a fatty methyl ester derived and has retention time of 28.472 minutes is identical to mass spectra of methyl linolenate.

The GC-MS mass spectra of the papaya extract with retention time of 29.155 minutes are identical to mass spectra of linolenic acid. This compound is an essential fatty acid that belongs to the omega-3. The other name 9,12,15-Octadecatrienoic acid, (z,z,z); alpha-linolenic acid; all-cis-9,12,15-octadecatrienoic acid. This compound has been reported to inhibit the synthesis of prostaglandin, reduced inflammation, prevention of chronic diseases, against oxidative stress (Pal and Ghosh 2012).

The oleoyl chloride compound is a fatty acid derivative, which applications in industries and cosmetic products. (Prakash et al. 2019) The peak has a retention time of 30.513 minutes and having a relative peak area of 6.93%. In study by Cardoso (2011), the lesions closure in the 5-day experimenetal (faster healing of the injured skin). Furthermore, oleoyl chloride concomitant elevated expression of IL-17 and TNF-α in angiogenesis (Sainson et al. 2008).

Table 2. The main EECP compounds identified by GC-MS and biological activities

Phytochemical compounds	RT (menit)	Area (%)	Activity
n-Hexadecanoic acid	28.079	8.77	Wound healing, anti-inflammatory, antioxidant, nematicide, pesticide, hemolytic, 5-alpha reductase inhibitor, potent mosquito larvicide, antiandrogenic, hypocholesterolemic (Thi et al. 2011; Devi et al. 2018)
Phytol	28.472	8.51	Wound healing, antioxidant, antinociceptive, antimicrobial, anti-inflammatory, diuretic and anticancer (Pejin et al. 2014)
Methyl linolenate	28.603	14.4	Antioxidant, antibacterial, antifungal (Jalalvand et al. 2019)
Linolenic acid	29.155	16.79	Anti-inflammatory, cancer preventive, hepatoprotective, antiarthritic, insectifuge, antiacne (Kumar et al. 2010)
Oleoyl chloride	30.513	6.93	Antioxidant (Kannaian et al. 2019), antibacterial (Ghosh et al. 2021)
Carpaine	39.905	11.78	Antimicrobial, antithrombocytopaenic, antiplasmodial, anti-inflammatory (Priyadarshi and Ram 2018)

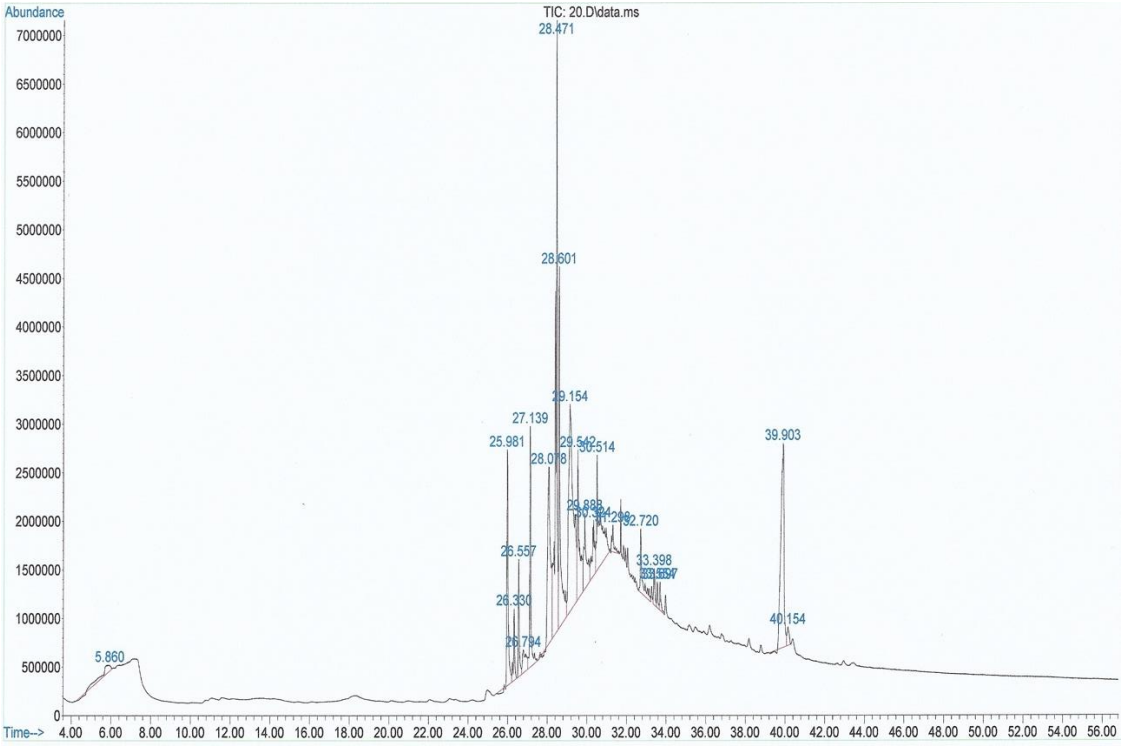


Figure 2. GC Chromatogram of EECP

Carpain is an alkaloid group and have been shown to antithrombocytopenic activity, exhibit cardiovascular effects. The peak of these compounds has a high retention time of 39.905 minutes. Carpaine contributes to anti-thrombocytopenic activity and has antioxidant properties. (Zunjar et al. 2016; Yap et al. 2021). The result study by Sudi et al. (2022) showed carpaine by improving the wound healing through the proliferation and improved the recovery of the MMP

The characteristics of the transdermal patch were identified based on several parameters: organoleptic, thickness, weight, and moisture content.

Organoleptic

Overall, an EECP transdermal patch had a film shape, a brownish-green color, a characteristic odor. It was also slightly wet, flat, and rather sticky. The organoleptic results showed that the patch can be applied to the skin.

Optimum formula prediction

The formulation design and responses of the EECP transdermal patches are shown in Table 3. Analysis of variance (ANOVA) of the transdermal patch design model is shown in Table 4.

The results of the formulation of the transdermal patch preparation of *C. papaya* leaves are shown in Figure 3.

Thickness

Patch thickness is an important parameter in transdermal patch preparations. The thickness of the transdermal patches in the study varied from 0.1 to 0.25 mm. In line with the research (Suryani et al. 2019), the variation of patch thickness is 0.15-0.21mm. The thickness of the EECP patches in the current study met the criteria of an ideal patch thickness, which are less than 1 mm, flexible, compact, and not brittle (Singh and Bali 2016).

The thickness factor of the quadratic model design had a significant value (p-value 0.0004<0.05), as shown in Table 5. Significant thickness factors A, AB, and BC were 0.0405, 0.0120, and 0.0773, respectively. The relationship between the thickness of the transdermal patch and factors (x) based on the coefficient value is stated in equation (1).

$$y = 0.14 - 1.27A + 0.60B - 1.07C + 4.42AB – 1.02AC + 2.09BC + 0.14A^2 - 2.09B^2 + 2.21C^2 \quad (1)$$

Patch thickness increased with the increasing concentration of PVP and ethyl cellulose following the value of p<0.0004, as shown by the 3D plot in Figure 3. Ethyl cellulose acts as a polymer forming a thin, matrix-forming film that coats the EECP and controls its release.

Patch weight

Based on the EECP patch weight evaluation, the patches weighed between 78.4 to 82 mg. The variation in weight was due to the different weights of formulas A, B, and C. The increase in the number of polymers A and B resulted in an increase in the weight of the patch matrix formed. Polymer A (PVP) is hydrophilic, and its solubility depends on its molecular weight. Low solubility increases molecular weight. On the other hand, Polymer B (ethyl cellulose) is hydrophobic, forming a thin film with a strong barrier. To facilitate drug release, a combination of hydrophobic and hydrophilic polymers with certain weights and ratios should be used (Mukherjee et al. 2005).

Table 3. Formulation Design and Responses of the EECP Transdermal Patches

Run	Factor 1 A:PVP mg	Factor 2 B: ethyl cellulose mg	Factor 3 C:EECP mg	Respone 1 Thickness mm	Response 2 Weight mg	Response 3 Moisture content %
1	0.2	0.3	0.3	0.16	80.1	3.87
2	0.2	0.3	0.1	0.14	78.4	1.91
3	0.3	0.5	0.2	0.24	81	4.07
4	0.2	0.4	0.1	0.13	79.5	2.64
5	0.3	0.4	0.1	0.2	81	4.19
6	0.3	0.4	0.3	0.25	82	4.39
7	0.1	0.4	0.1	0.1	79	2.15
8	0.2	0.4	0.1	0.13	79.5	2.51
9	0.3	0.3	0.1	0.1	78.6	1.78
10	0.2	0.4	0.1	0.13	79.5	2.38
11	0.1	0.3	0.1	0.1	78.5	1.65
12	0.2	0.4	0.1	0.13	79.5	2.51
13	0.2	0.4	0.1	0.13	79.5	2.52
14	0.2	0.5	0.3	0.25	81	4.07
15	0.1	0.4	0.3	0.2	80.2	3.99
16	0.2	0.4	0.2	0.16	80	3.87
17	0.1	0.4	0.1	0.1	79	2.53

Table 4. Characterization of the EECP statistical transdermal patch design model

Response	Source	Std.Dev	R-Square	Adj R-square	Pred R-square	Adeq precisior	PRESS
Thickness	Linear	0.0205	0.8776	0.8494	0.7581	17.0586	0.0108
	2FI	0.0179	0.9282	0.8852	0.5583	13.8857	0.0198
	Quadratic	0.0159	0.9607	0.9103	0.4095	13.0128	0.0265
Weight	Linear	0.4419	0.8439	0.8079	0.6729	15.0797	5.32
	2FI	0.4329	0.8848	0.8157	0.2005	12.081	13
	Quadratic	0.2516	0.9728	0.9377	-1.8834	19.0194	46.9
Moisture content	Linear	0.4720	0.8042	0.7591	0.6239	12.5731	5.56
	2FI	0.4245	0.8782	0.8051	0.5587	10.5740	6.53
	Quadratic	0.4269	0.9138	0.8029	-5.3958	8.7053	94.61

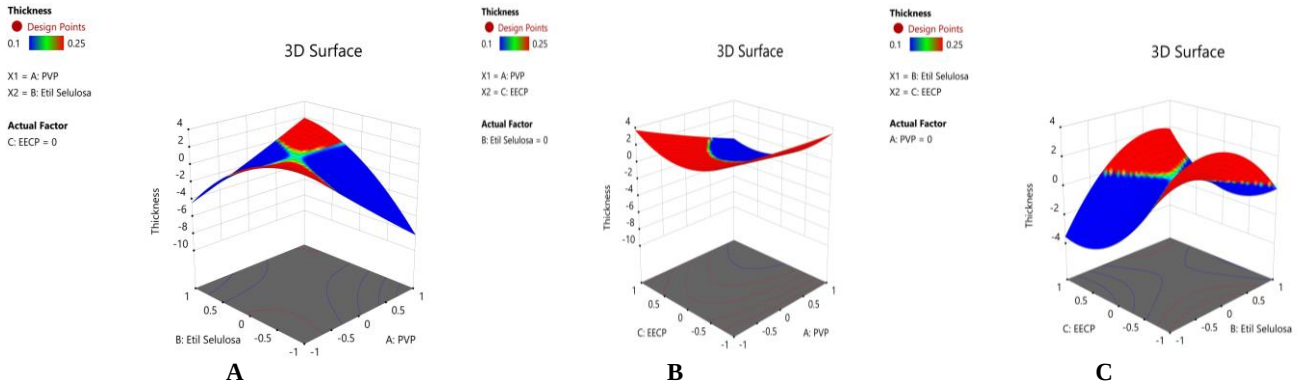


Figure 3. 3D plot of the relationship. A. PVP and ethyl cellulose and thickness. B. PVP, EECP, and thickness. C. Ethyl cellulose, EECP, and thickness

The results of the ANOVA analysis of the quadratic model of transdermal patch EECP weights are shown in Table 6. The model showed significant results where the p-value was 0.0001. Significant weight values were also influenced by the values of factor A, B, AB, and B^2, which were 0.0176, 0.0068, 0.0152, and 0.0107, respectively. The relationship between the weight of the transdermal patch and factors (x) based on the coefficient value is stated in equation (2).

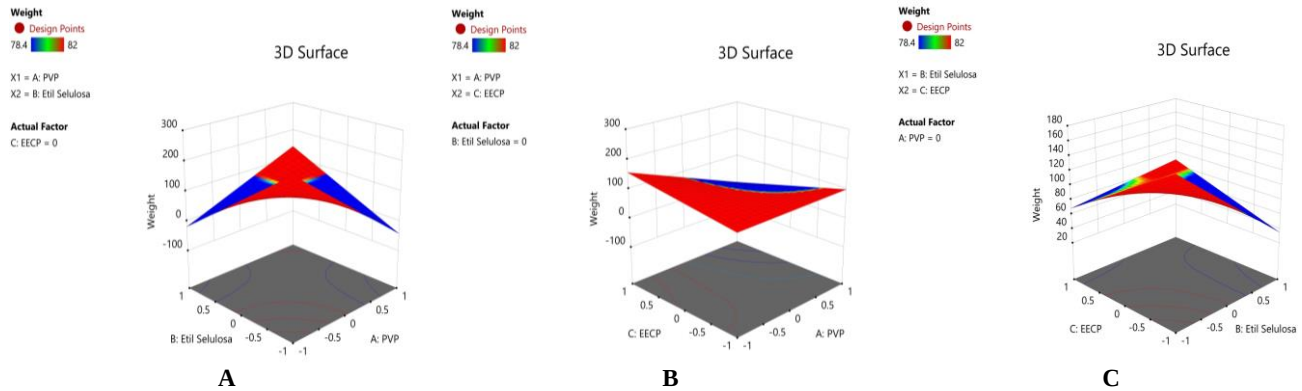
$$y = 71.7 - 24.9A + 47.1B - 13.1C + 66.7\,AB + 1.66AC - 3.54BC + 15.5A^2 - 69.3B^2 + 54.3C^2 \quad (2)$$

Table 5. ANOVA analysis for the quadratic model of EECP transdermal patch thickness

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F	Characterization
Model	0.0431	9	0.0048	19.03	0.0004	significant
A-PVP	0.0016	1	0.0016	6.29	0.0405	
B-Ethyl cellulose	0.0002	1	0.0002	0.6035	0.4627	
C-EECP	0.0007	1	0.0007	2.65	0.1473	
AB	0.0028	1	0.0028	11.33	0.0120	
AC	0.0005	1	0.0005	2.00	0.2001	
BC	0.0011	1	0.0011	4.28	0.0773	
A^2	7.665E-06	1	7.665E-06	0.0305	0.8663	
B^2	0.0007	1	0.0007	2.74	0.1417	
C^2	0.0006	1	0.0006	2.24	0.1784	
Residual	0.0018	7	0.0003			
Lack of Fit	0.0018	2	0.0009			
Pure Error	0.0000	5	0.0000			
Cor Total	0.0448	16				

Table 6. ANOVA Analysis for the EECP Transdermal Patch Weight of the Quadratic Model

Source	Sum of squares	df	Mean Square	F-value	p-value	
Model	15.82	9	1.76	27.77	0.0001	significant
A-PVP	0.6041	1	0.6041	9.54	0.0176	
B-Ethyl cellulose	0.9113	1	0.9113	14.39	0.0068	
C-EECP	0.0993	1	0.0993	1.57	0.2506	
AB	0.6459	1	0.6459	10.20	0.0152	
AC	0.0013	1	0.0013	0.0210	0.8887	
BC	0.0031	1	0.0031	0.0484	0.8321	
A^2	0.0945	1	0.0945	1.49	0.2613	
B^2	0.7527	1	0.7527	11.89	0.0107	
C^2	0.3390	1	0.3390	5.36	0.0539	
Residual	0.4431	7	0.0633			
Lack of Fit	0.4431	2	0.2216			
Pure Error	0.0000	5	0.0000			
Cor Total	16.26	16				



A **B** **C**

Figure 4. 3D plot of the relationship. A. PVP and Ethylcellulose and weights. B. PVP and EECP and weights. C. Ethyl cellulose and EECP and weights

The combination of hydrophobic polymer (ethyl cellulose) and hydrophilic polymer (PVP) with different concentrations resulted in weight variation. The weight of the formulation was visible on the 3D plot in Figure 4. From the image, it can be seen that PVP and ethyl cellulose play an important role in determining the weight of a transdermal patch. The higher the PVP, the higher the patch weight.

Moisture content

The results of the moisture content analysis showed the percentages of the minimum moisture content, ranging

from 1.7% to 4.4%. This means the patches have stability and are protected from microbial contamination. These moisture content values continued to increase along with the increasing levels of polyvinylpyrrolidone (PVP) in Formula 3, Formula 5, and Formula 6. However, the highest moisture content value was found in Formula 6, accounting for 4.4%, because it had the highest amount of PVP. This is due to the fact that PVP has hygroscopic properties and is able to attract moisture from its surrounding environment during the patch formulation process. Another cause was the release of other volatile materials from the formula, which were presumably attracted by the desiccator containing silica gel, causing the density of the air in the desiccator to become more tenuous than the density of the outside air. The relationship between the moisture content of the transdermal patch and factors (x) based on the coefficient value is stated in equation (3).

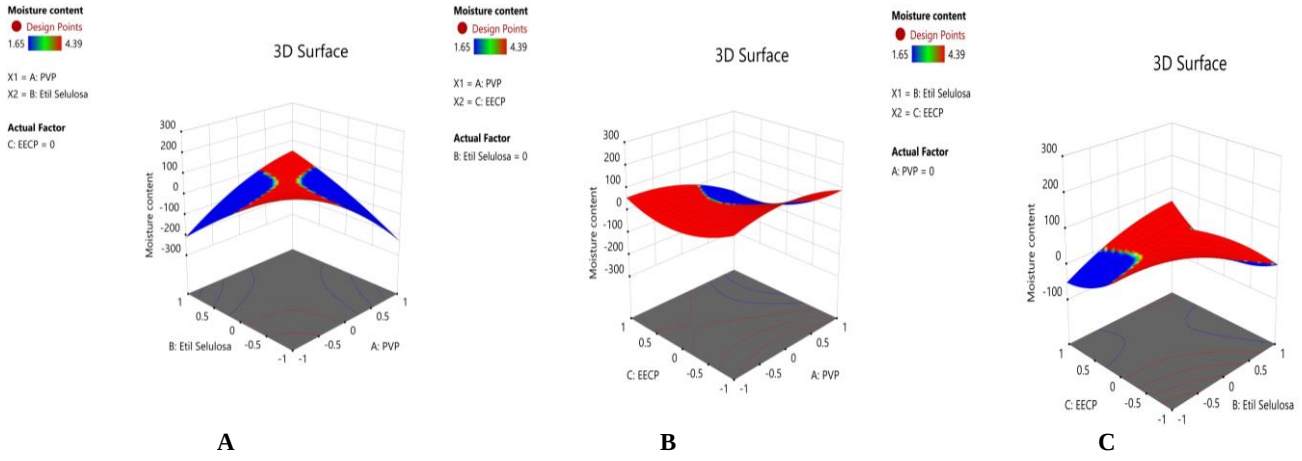
$$y = 16.956 – 38.376A -29.8807B – 55.0799C + 168.006AB - 36.578AC + 86.4921BC -44.8112A^2 –22.6121B^2 + 66.5631C^2 \quad (3)$$

The values of moisture content patches varied among formulas. PVP has an important role in increasing the values of moisture content as shown in Figure 5, in which an increase in PVP resulted in an increase in the moisture content. The hygroscopic nature of PVP can attract water vapor in the formula.

The results of the ANOVA analysis for quadratic moisture content of EECP patch are shown in Table 7. The model F-value of 8.29 implies the model is significant. There is only a 0.54% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. Model reduction may improve the model. The lack of fit F-value of 27.42 implies the lack of fit is significant. There is only a 0.20% chance that a lack of fit F-value this large could occur due to noise.

Table 7. ANOVA Analysis for Quadratic Moisture content Transdermal Patch EECP Model

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	13.52	9	1.50	8.24	0.0055	significant
A-PVP	0.2104	1	0.2104	1.15	0.3182	
B-Ethyl cellulose	0.5544	1	0.5544	3.04	0.1246	
C-EECP	0.4177	1	0.4177	2.29	0.1738	
AB	0.2878	1	0.2878	1.58	0.2492	
AC	0.3177	1	0.3177	1.74	0.2282	
BC	0.1195	1	0.1195	0.6555	0.4448	
A^2	0.0856	1	0.0856	0.4698	0.5151	
B^2	0.3673	1	0.3673	2.02	0.1986	
C^2	0.0191	1	0.0191	0.1048	0.7556	
Residual	1.28	7	0.1822			
Lack of Fit	1.17	2	0.5859	28.24	0.0019	significant
Pure Error	0.1037	5	0.0207			
Cor Total	14.79	16				



A **B** **C**

Figure 5. 3D plot of the relationship. A. PVP and Ethyl cellulose and moisture content. B. PVP, EECP, and moisture content. C. Ethyl cellulose, EECP and moisture content

EECP from Lambhuk, Banda Aceh, Aceh Province, Indonesia, contains several active compounds. Six of the compounds have an area above 6%, namely n-Hexadecanoic acid, phytol, methyl linolenate, linolenic acid, oleoyl chloride, and carpaine. These compounds have potential to be studied further in the future. EECP has an active component that functions as an anti-inflammatory and wound healer, which can be formulated as a transdermal patch that is easy and flexible to use. Based on the quadratic model in the Box-Behnken design, optimum formulation was obtained, with 0.3 mg of PVP, 0.4 mg of ethyl cellulose, and 0.3 mg of EECP. Meanwhile, the response values were 0.25 mm for thickness, 82 mg for weight, and 4.39% for moisture content. Therefore, EECP patch have opportunities arising as agent of formal herbal medicine product for wound healing.

REFERENCES

Abbiw DK. 1990. Useful Plants of Ghana: West African uses of wild and cultivated plants, intermediate technology publications. The Royal Botanical Garden, Kew, London.

Adedayo BC, Oyeleye SI, Okeke BM, Oboh G. 2020. Anti-cholinesterase and antioxidant properties of alkaloid and phenolic-rich extracts from pawpaw (*Carica papaya*) leaf: A comparative study. Flavour Fragr J 36: 47-54. DOI: 10.1002/ffj.3615.

Barku VYA. 2019. Wound healing: contributions from plant secondary metabolite antioxidants. In: Dogan KH (eds). Intechopen 63675. DOI: 10.5772/intechopen.81208.

Candra A, Santi TD. 2017. Efektivitas ekstrak daun pepaya (*Carica papaya* L) sebagai anti inflamasi. Jurnal Aceh Medika 1 (2): 63-66. [Indonesian]

David SRN, Rajabalaya R, Zhia ES. 2015. Development and in vitro evaluation of self-adhesive matrix-type transdermal delivery system of ondansetron hydrochloride. Trop J Pharm Res 14 (2): 211-218. DOI: 10.4314/tjpr.v14i2.4.

Devi DR, Lakshna S, Parvathi S, Hari BNV. 2018. Investigation of wound healing effect of topical gel of *Albizia amara* leaves extract. S Afr J Bot 119: 400-409. DOI: 10.1016/j.sajb.2018.10.005.

Ghosh A, Majumder S, Saha S, Sarkar S, Bhattacharya M. 2021. Gas Chromatography-Mass Spectrometry profiling, and evaluation of antioxidant and antibacterial activity of *Albizia* spp. Nusantara Biosci 13 (2): 177-184. DOI: 10.13057/nusbiosci/n130207.

Gutiérrez A DM, Bah M, Garduño R ML, Mendoza D SO, Serrano CV. 2014. Anti-inflammatory and antioxidant activities of methanol extracts and alkaloid fractions of four Mexican medicinal plants of Solanaceae. Afr J Tradit Complement Altern Med 11 (3): 259-267. DOI: 10.4314/ajtcam.v11i3.36.

Hamuel JD. 2012. Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: Phytochemicals - A Global Perspective of Their Role in Nutrition and Health. DOI: 10.5772/26052.

Hassan HS, Sule MI, Musa AM, Musa KY, Abubakar MS, Hassan AS. 2011. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. Afr J Tradit Complement Altern Med 9 (2): 250-255. DOI: 10.4314/ajtcam.v9i2.10.

Hess CT. 2011. Checklist for factors affecting wound healing. Adv Skin Wound Care 24 (4): 192. DOI: 10.1097/01.ASW.0000396300.04173.ec.

Huh SH, Kim Y-S, Jung E, Lim J, Jung KS,Kim M, Lee J, Park D. 2010. Melanogenesis inhibitory effect of fatty acid alkyl esters isolated from *Oxalis triangularis*. Biol Pharm Bull 33 (7): 1242-1245. DOI: 10.1248/bpb.33.1242.

Islam MT, de Alencar MVOB, Machado KdaC, Machado KdaC, Melo-Cavalcante AAdeC, de Sousa DP, de Freitas RM. 2015. Phytol in a pharma-medico-stance. Chem Biol Interac 240: 60-73. DOI: 10.1016/j.cbi.2015.07.010.

Jalalvand AR, Zhaleh M, Goorani S, Zangeneh MM, Seydi N, Zangeneh A, Moradi R. 2019. Chemical characterization and antioxidant, cytotoxic, antibacterial, and antifungal properties of ethanolic extract of *Allium saralicum* R.M. fritsch leaves rich in linolenic acid, methyl ester. J Photochem Photobiol. 192: 103-112. DOI: 10.1016/j.jphotobiol.2019.01.017.

Joshi R, Garud N. 2021. Development, optimization and characterization of flurbiprofen matrix transdermal drug delivery system using Box–Behnken statistical design. Future J Pharm Sci 7: 57. DOI: 10.1186/s43094-021-00199-2.

Kannaian N, Sripriya NS, Raj DD, Deepa S, Bhuvaneswari S. 2019. Antioxidant potency and gc-ms composition of *Origanum majorana* Linn. Pak J Pharm Sci 32 (5): 2117-2122.

Khatoon M, Shah KU, Din FU, Shah SU, Rehman AU, Dilawar N, Khan AN. 2017. Proniosomes derived niosomes: Recent advancements in drug delivery and targeting. Drug Deliv 24 (1): 56-69. DOI: 10.1080/10717544.2017.1384520.

Kováčik A, Kopečná M, Vávrová K. 2020. Permeation enhancers in transdermal drug delivery: Benefits and limitations. Expert Opin Drug Deliv 17 (2): 145-155. DOI: 10.1080/17425247.2020.1713087.

Kumar PP, Kumaravel S, Lalitha C. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res 4 (7): 191-195.

Jimenez VM, Gutierrez-Soto MV, Barboza-Barquero L, Guevara E. 2020. Taxonomy, Botany, and Plant Development. In: Mitra S. The Papaya: Botany, Production and Uses (eds). CABI, Boston MA.

Miladiyah I, Prabowo BR. 2012. Ethanolic extract of *Anredera cordifolia* (Ten.) steenis leaves improved wound healing in guinea pigs. Univ Med 31 (1): 4-11. DOI: 10.18051/UnivMed.2012.v31.4-11.

Minutti CM, Knipper JA, Allen JE, Zaiss DM. 2017. Tissue-specific contribution of macrophages to wound healing. Semin Cell Dev Biol 61: 3-11. DOI: 10.1016/j.semcdb.2016.08006.

Mukherjee B, Kanupriya, Mahapatra S, Das S, Patra B. 2005. Sorbitan monolaurate 20 as a potensial skin permeation enhancer in transdermal patches. J Appl Res 5 (1): 96-108.

Nafiu BA, Alli-Oluwafuyi AM, Halimat A. 2019. Papaya (*Carica papaya* L., pawpaw). Nonvitamin and Nonmineral Nutritional Supplements 2019: 335-359. DOI: 10.1016/B978-0-12-812491-8.00048-5.

Nandini G, Gopenath T, Nagalambia P, Kanthesh B. 2020. Phytochemical analysis and antioxidant properties of leaf extracts of *Carica papaya*. Asian J Pharm Clin Res 13 (11): 58-62. DOI: 10.22159/ajpcr.2020.v13i11.38956.

Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. 2001. Flavonoids: A review of probable mechanisms of action and potential applications. Am J Clin Nutr 74 (4): 418-425. DOI: 10.1093/ajcn/74.4.418.

Nugroho A, Heryani H, Choi JS, Park HJ. 2017. Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity. Asian Pac J Trop Biomed 7 (3): 208-213. DOI: 10.1016/j.apjtb.2016.12.009.

Nurhaen, Winarsi D, Ridhay A. 2016. Isolation and identification of chemical components of essential oils from leaves, stems, and flowers of salembangu plants (*Melissa* sp.). J Nat Sci 5 (2): 149-157.

Oramahi HA. 2016. Optimasi dengan RSM dan Rancangan (Aplikasi dengan SPSS dan SAS). Gava Media, Yogyakarta. [Indonesian]

Pal M, Ghosh M. 2012. Prophylactic effect of α-linolenic acid and α-eleostearic acid against mehg induced oxidative stress, DNA damage and structural changes in RBC membrane. Food Chemi Toxicol 50 (8): 2811-2818. DOI: 10.1016/j.fct.2012.05.038.

Patel DP, Setty CM, Mistry GN, patel SL, Patel TJ, Mistry PC, Rana AK, Patel PK, Mishra RS. 2009. Development and evaluation of ethyl cellulose-based transdermal films of furosemide for improved in vitro skin permeation. AAPS PharmSciTech 10 (2): 437-442. DOI: 10.1208/s12249-009-9224-3.

Pejin B, Savic A, Sokovic M, Glamoclija J, Ciric A, Nikolic M, Radotic K, Mojovic M. 2014. Further in vitro evaluation of antiradical and antimicrobial activities of phytol. Nat Prod Res 28 (6): 372. DOI: 10.1080/14786419.2013.869692.

Priyadarshi A, Ram B. 2018. A review on pharmacognocy, phytochemistry and pharmacological activity of *Carica papaya* (Linn) Leaf. Intl J Pharm Sci Re. 9 (10): 4071-4078.

Saghazadeh S, Rinoldi C, Schot M, Kashaf SS, Sharifi F, Jalilian E, Nuutila K, Giatsidis G, Mostafalu P, Derakhshandeh H, Yue K, Swieszkowki W, Memic A, Tamayol A, Khademhosseini A. 2018. Drug delivery systems and materials for wound healing applications.

Adv Drug Deliv Rev 1 (127): 138-166. DOI: 10.1016/j.addr.2018.04.008.

Sainson RCA, Johnston DA, Chu HC, Holderfield MT, Nakatsu MN, Crampton SP, Davis J, Conn E, Hughes CCW. 2008. TNF primes endothelial cells for angiogenic sprouting by inducing a tip cell phenotype. Blood 111 (10): 4997-5007. DOI: 10.1182/blood-2007-08-108597.

Sankarganesh P, Joseph B, Kumar AG, Illanjiam S, Srinivasan T. 2018. Phytomedicinal chemistry and pharmacognostic value of *Carica papaya* l. leaf. J Pure Appl Microbiol 12 (2): 751-756. DOI: 10.22207/JPAM.12.2.35.

Santi TD. 2015. Uji toksisitas akut dan efek antiinflamasi ekstrak metanol dan ekstrak n-heksana daun pepaya (*Carica papaya* L). Pharm Sci Res 2 (2): 101-114. DOI: 10.7454/psr.v2i2.3341.

Seah BC, Teo BM. 2018. Recent advances in ultrasound-based transdermal drug delivery. Intl J Nanomed 13: 7749-7763. DOI: 10.2147/IJN.S174759.

Sharma A, Puri V, Kumar P, Singh I. 2021. Rifampicin-loaded alginate-gelatin fibers incorporated within transdermal films as a fiber-in-film system for wound healing applications. Membranes 11: 7. DOI: 10.3390/membranes11010007.

Shubham S, Mishra R, Gautam N, Nepal M, Kashyap N, Dutta K. 2019. Phytochemical analysis of papaya leaf extract: Screening test. Ec Dental Sci 18 (3): 485-490.

Singh A, Bali A. 2016. Formulation and characterization of transdermal patches for controlled delivery of duloxetine hydrochloride. J Anal Sci Technol 7: 250. DOI: 10.1186/S40543-016-0105-6.

Suryani, Musnina WAOS, Ruslin, Nisa M, Aprianti R, Hasanah M, Putri FR, Adjeng ANT, Yuniar N, Sahumen MH, Aswan M. 2019. Formulation and physical characterization of Curcumin nanoparticle transdermal patch. Intl J Appl Pharm 11 (6): 217-221. DOI: 10.22159/ijap.2019v11i6.34780.

Szunerits S, Rabah B. 2018. Heat: A highly efficient skin enhancer for transdermal drug delivery. Front Bioeng Biotechnol 6: 15. DOI: 10.3389/fbioe.2018.00015.

Tekindal MA, Bayrak H, Ozkaya B, Genc Y. 2012. Box-Behnken experimental design in factorial experiments: The importance of bread for nutrition and health running head. Turkish J Field Crop 17 (2): 422-430.

Thi T, Hang DTT, Minh CV, Dat NT. 2011. Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*. Asian Pac J Trop Med 4 (10): 760-763. DOI: 10.1016/S1995-7645(11)60189-2.

Yap JY, Hii CL, Ong SP, Lim KH, Abas F, Pin KY. 2021. Quantification of carpaine and antioxidant properties of extracts from *Carica papaya* plant leaves and stalks. J Bioresourand Bioprods 6 (4): 350-358. DOI: 10.1016/j.jobab.2021.03.002.

Zahra A, Kadir F, Abdula M, Mahmood H, Fanous S, Sabri S, Latif I, Ketuly K. 2011. Acute toxicity study and wound healing potential of *Gynura procumbens* leaf extract in rats. J Med Plant Res 5 (12): 2551-2558. DOI: 10.5897/JMPR.9000042.

Zunjar V, Dash RP, Jivrajani M, Trivedi B, Nivsarkar M. 2016. Antithrombocytopenic activity of carpaine and alkaloidal extract of *Carica papaya* Linn. leaves in busulfan induced thrombocytopenic wistar Rats. J Ethnopharmacol 181 (2): 20-25. DOI: 10.1016/j.jep.2016.01.035.