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In-vivo study of oleic acid and tween-80 on patch transdermal *A.paniculata* as anti-diabetic

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ABSTRACT

Sambiloto (*A.paniculata*) is known empirically as a plant that could effectively treat diabetes mellitus. The substance of andrographolide in *A.paniculata* is able to increase insulin secretion, therefore, inhibiting alpha-glucosidase and alpha-amylase. The substances themselves have the ability to reduce blood glucose levels. Poor bioavailability due to pharmacokinetic interactions in the form of metabolism by the enzyme p-glycoprotein and CYP3A4 as well as poor physicochemical properties of water reduces bioavailability in oral administration which results in a decrease in pharmacological activity. The transdermal patch dosage form is chosen since it is considered capable of increasing the effectiveness of andrographolide in ethanol extract of sambiloto (EES). However, the challenge in transdermal preparations is the stratum corneum, which is the main barrier in transdermal drug delivery. Enhancers become a critical point in transdermal delivery. Oleic acid and tween-80 are enhancers that are widely used in transdermal patch preparations. The aim of this study was to determine the comparison of concentrations of oleic acid and tween-80 on the characteristics of transdermal patches. The transdermal patches were made using the solvent casting method with various concentration ratios of oleic acid enhancer and tween F1 (1:1), F2 (1:3), and F3 (1:2). Then, all of the formulas were tested for patch characteristics while also being tested via *in-vivo* antidiabetic activity using diabetic male rat. All of the formulas meet the requirements of patch characteristics. Differences in the concentration of enhancer combinations affect patch characteristics in terms of patch weight and thickness, where F2 has a greater thickness and weight than other formulas. F2 is a formula that has a greater activity than F1 and F3. F2 blood glucose level value on the fifteenth day was 105 ±2.42 mg/dL. Although subcutaneous administration of insulin is quicker in diminishing blood glucose levels, the EES F2 transdermal fix can be compelling in decreasing blood glucose levels.

Keywords: *A.paniculata*, oleic acid, tween-80, patch transdermal, anti-diabetic

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INTRODUCTION

Sambiloto (*Andrographis paniculata*) has been known empirically as a plant that has benefits as a traditional antidiabetic Javanese medicine known as jamu pahitan which is used to treat diabetes mellitus (DM). The main compound contained in sambiloto is andrographolide which induced a hypoglycemia effect through the mechanism of increasing insulin secretion and inhibiting alpha-glucosidase and alpha-amylase. Besides that, andrographolide contained in sambiloto has the ability to increase glucose utilization in streptozotocin-induced rat muscle through the stimulation of GLUT-4 transporters (Sari et al., 2015). According to Hidayat & Wulandari (2021), oral administration of extract ethanolic of *A. paniculata* at the dose of 200 mg/Kg BW can reduce levels SOD catalase, GSH activity in rats and reduces levels of malondialdehyde significantly in the kidney of STZ-diabetics.

Andrographolide is a diterpene lactone group compound that has a low solubility in water and poor bioavailability due to biotransformation by P-glycoprotein in the intestinal tract which causes andrographolide to metabolize more quickly in the duodenum and jejunum (Keerthana et al., 2022). In addition, andrographolide compounds are inhibitors of CYP3A4 and CYPC29 enzymes which cause pharmacokinetic interactions if given together with synthetic drugs such as glipizide, thereby reducing the bioavailability of glipizide (Sundhani et al., 2022). To overcome this, ethanol extract of sambiloto (EES) was defined within the shape of transdermal patches.

Transdermal patch is a systemic drug delivery through penetration of the stratum corneum found in the skin layers of the epidermis to the dermis (Nurahmanto, 2016). The advantages of transdermal preparations as a non-invasive therapeutic agent are significant in preventing first-pass effects (Jafri et al., 2019), chemical degradation in the gastrointestinal environment, and easy access because the skin provides a large surface area and ease of dose setting (Prasetyo et al., 2018).

The challenge in making transdermal arrangements itself is the stratum corneum which may be a boundary to drugs that are administered transdermal (Zaki et al., 2022). The formulation in transdermal preparations such as the selection of polymers, plasticizers, and enhancers can affect the characteristics of transdermal patch preparations which affect the pharmacological activity of transdermal patch preparations. The critical point of transdermal patch preparation is the selection of enhancers which is one of the compounds that increase the penetration of active substances in the skin by disrupting the permeability of the stratum corneum, through intercellular protein bond interactions (Haq et al., 2020).

Oleic acid is a fatty acid enhancer that has a mechanism of action as an enhancer through interaction by reducing the permeability of the stratum corneum. Oleic acid is an enhancer that is widely used in transdermal drug delivery (El-Say et al., 2021). The use of oleic acid in promethazine transdermal patch preparations has the ability to increase promethazine permeation better than propylene glycol and isopropyl alcohol (Nurahmanto, 2016). The use of unsaturated fatty acids such as oleic acid as an enhancer can potentially irritate the skin. Based on clinical research conducted in humans, it was found that the combination of oleic acid at a concentration of 5% and propylene glycol as an enhancer can irritate the skin. This is because oleic acid can increase the production of inflammatory cells such as interleukin-1 alpha and the production of cytokines which are mediators of inflammation that cause inflammation (Moore et al., 2020).

Tween 80 is an enhancer from the non-ionic surfactant group that works by increasing the permeation of active substances through interaction and binding to keratin filaments that interfere with corneocyte permeability (Budhathoki et al., 2016). The use of 1% tween-80 as an enhancer in diazepam transdermal preparations can increase the level of diazepam permeation in the skin. Tween-80 is a non-ionic enhancer that is inert and does not irritate the skin (Pandey, 2014). The use of a combination of oleic acid and tween-80 as an enhancer within the preparation of transdermal patches of EES is expected to increase the permeation of sambiloto extract (*A. paniculata*) so as to increase the viability of sambiloto extract as antihyperglycemic in transdermal delivery. The use of tween-80 as a co-enhancer in an EES transdermal patch is expected to increase the permeation effectiveness of andrographolide contained in the EES compared to the use of single oleic acid (Saitoh et al., 2023).

Physically good patch character must be flexible, thin, smooth, homogeneous, and have a low moisture content. Based on Saitoh et al. (2023) research on the combination of tween 80 and oleic acid in disulfiram patch transdermal demonstrates the increased permeation of disulfiram transdermal patch compared with the use of a single oleic acid. The combination of tween-80 and oleic acid is expected to obtain optimal patch characteristics. The aim of this research is to determine the effect of variations in the combination of tween 80 and oleic acid as an enhancer on the physical characteristics of EES patches (weight, thickness, moisture content, pH, and folding endurance) and the effectiveness as an antidiabetic transdermal patch in diabetic rats.

MATERIALS AND METHOD

Materials

Herba sambiloto simplicia from Merapi Farma, Yogyakarta (determined in Biology Laboratory Universitas Ahmad Dahlan, No. 447/lab.Biologi/B/XI/2023), Glassware (Iwaki), patch mold (Iwaki), aluminum foil, andrographolide (Sigma), desiccator (Pyrex), glucometer (easy touch glucometer), vernier scale, analytical digital balance (Ohaus Adventurer), oven (Memmert), pH meter (Ohaus), soxhlet extractor (Iwaki-pyrex), hotplate stirrer (IKA-CMAG7), rotary evaporator set (Heidolph), waterbath (Memmert), hair razor, silica gel F_{254} plate, ethanol 70% (Brataco), ethanol 96% (Brataco), HPMC (Sarda, Taiwan), oleic acid (AppliChem), tween-80 (Amresco), PEG 400 (Japan Bio Science Laboratory), PVP K-30 (JHNH Life Sciences), aquadest (Otsuka), and streptozotocin (Bioworld), methanol (merck), chloroform (merck), disposable syringe 1 mL (Onemed), buffer citrate solution pH 4.5 (Merck), sodium benzoic (Gloria InterChem).

Methods

Extraction of sambiloto

The extraction of sambiloto was carried out using ethanol 96% solvent with the soxhletation method. A total of 100 grams of powdered sambiloto was wrapped using filter paper and then put into the lead. The extraction process was carried out for 3-4 hours at a temperature of 40-50°C. The extract obtained is evaporated and thickened (Garg et al., 2016).

Phytochemical screening of andrographolide

Phytochemical screening of andrographolide compounds is tested with Thin Layer Chromatography (Rais, 2014). The first step is preparing the test solution of sambiloto extract in the amount of 10 mg/mL. The mobile phase used was chloroform: methanol P (9:1) with the stationary phase silica gel 60 F_{254} using a 20 µL sample and 2 µL as comparison solution (andrographolide solution). The observation was carried out at light UV_{254}.

Formulation of a base patch of EES

The transdermal patch base formulation can be seen in Table 1 of the formula based on the research of (Saitoh et al., 2023) where in this formula there are modifications. The evaluation of the patch characteristics includes patch weight uniformity, patch thickness, patch pH, patch durability value, and moisture content. The method of making patch bases uses the solvent casting method. For the formulation of the patch, preparation is listed in Table 1.

Polyvinyl Pyrrolidone K-30 (PVP-K30) was dissolved in 70% ethanol, then HPMC was dissolved in 70% ethanol. After that, the PVP-K30 and HPMC solutions were mixed and stirred using a hotplate stirrer. A total of 200 mg of herbal sambiloto extract was dissolved with ethanol 70% in 1 mL. PEG 400 was added to the polymer solution and then stirred as mixture 1. Enhancer in the form of tween-80 and oleic acid was mixed and then added to the extract as mixture 2. Mixture 2 is then added to mixture 1, then stirred for 20 minutes until homogeneous, then poured into a patch mold and allowed to stand for 15 minutes at room temperature. The patch was then placed in an oven at 40-45°C for 24-72 hours until

a film layer was formed, the film layer of the patch was then removed from the mold wrapped in aluminum foil, and stored in a desiccator.

Table 1. Formulation of patch transdermal EES with variations of oleic acid and tween 80

Name of Ingredient	Function	Oleic acid: tween 80		
		F1(1:1)	F2(1:3)	F3(1:2)
EES (mg)	Active ingredient	200	200	200
Oleic acid (gram)	Enhancer	0.15	0.15	0.075
Tween 80 (gram)	Enhancer	0.15	0.45	0.15
HPMC (gram)	Polymer	0.25	0.25	0.25
PVP-K30 (gram)	Polymer	0.5	0.5	0.5
PEG 400 (gram)	Plasticizer	0.075	0.075	0.075
Sodium Benzoic (mg)	Preservative agent	15	15	15
Ethanol 70% (mL)	Solvent	Add 15	Add 15	Add 15

Evaluation of transdermal patch EES

Visual appearance

The patches were evaluated visually for color, odor, and texture of the patches.

The thickness of the matrix patches

The thickness of the transdermal patches was measured using vernier calipers at five different places, and the mean value along with Standard Deviation (SD) was calculated (Shivalingam et al., 2021).

Weight uniformity

The weights of five patches were taken and the weight variation was calculated as mean and standard deviation (Shivalingam et al., 2021).

Surface pH

The evaluation of pH patches is tested with a pH meter. The test is carried out using 10 mL of CO_2 free water added to a beaker glass that contains the patch and then allowed to stand for 1 hour. The criteria for the pH range is 4.5-6.5 (Pratiwi et al., 2021).

Determination of folding endurance

The folding endurance test points to decide the folding capacity of the patch. The folding endurance test is carried out by more than once folding the patch at the same point until it breaks (Hartesi et al., 2021).

Percentage of moisture content

The prepared patches were marked, then weighed one by one, and stored in a desiccator containing active silica at room temperature for 24 hours. The patch was weighed more than once until a consistent weight was obtained. The percentage of water content is determined as the difference between the initial and final weight to the final weight (Francis, 2016).

In vivo evaluation of transdermal patches

Animals

The animals used for antihyperglycemic studies were male albino rats (weighing 100-250 g) provided by the animal house, Faculty of Medicine and Health Sciences University of Muhammadiyah

Yogyakarta. The animals were kept and maintained under standard laboratory conditions of temperature and humidity within 12 h day; and 12 h night cycle, and also allowed water consumption *ad libitium* (Hadebe et al., 2014). All the protocols followed for animal studies were reviewed and approved by the Research Ethics Committee of Ahmad Dahlan University, Yogyakarta (Ethical Approval number: 012311289).

Induction of diabetes

Diabetes was induced by streptozotocin-(STZ) (35 mg/kg BW, i.p) (Mostafavinia et al., 2016) in citrate buffer 0.1 M (pH 4.5) (Mostafavinia et al., 2016). The diabetic state was confirmed 72h after STZ injection by hyperglycaemia. Surviving rats with fasting blood glucose levels higher than 200 mg/ dL (Premanath & Nanjaiah, 2015).

Hypoglycaemic study of EES patch transdermal

Rats were shaved on the abdominal region 1-2 days prior to the application of EES patch transdermal (Hendriati et al., 2021). Rats were divided into six groups (n=5). The first group was given the EES Formula 1 (F1) transdermal patch, the second group was given the EES patch transdermal Formula 2 (F2), the third group was given the EES patch transdermal Formula 3 (F3) the patch was given twice a day, the negative group was given blank patches, the positive group was given injected SC insulin short acting novorapid (0.2 mL/200 g), and healthy group was not induced by STZ (Ahmed et al., 2014). Blood glucose levels (BGL) were measured after 24 hours by taking blood through a vein in the rat's tail.

Data Analysis

Data are communicated as mean ± SD and statically analyzed by one-way ANOVA taken after by Tukey's comparisons test with a 95% certainty level for the values of the characteristic fix.

RESULT AND DISCUSSION

Extraction of ethanolic sambiloto

Preparation of the extract by soxhlation with 96% ethanol solvent for four hours at 50^0C. Soxhlet extraction is a method of extraction that is able to extract more of the target compound than any other extraction method. The selection of solvents and temperatures used in the process of the extraction method is key in the secretion of the desired target compounds such as andrographolide (Liang et al., 2023). The solvent used in the herbal extraction method is ethanol with an ethanol concentration of 96% according to Yuan et al. (2017). Ethanol is a solvent that has a lower level of toxicity compared to other organic solvents. The 96% ethanol solvent was chosen based on its ability to draw a certain number of active compounds from the plant while minimizing as little as possible of the unwanted elements. The selection of extraction methods using the Soxhlet method is able to produce extract yields while noting more. The yield of the extract obtained is 220.71 grams with a yield percentage of 16.22% according to DepKes RI (2017). The yield of the extract is not less than 9.6% so the percentage of yield obtained in the extraction meets the requirements. A yield percentage value of more than 10% is a very good value so that the extract of sambiloto obtained is eligible to be continued at the patch formulation stage.

Phytochemical screening of andrographolide

The phytochemical screening of andrographolide on the EES was performed qualitatively using the thin-layer chromatography (TLC) method according to Rais (2014). The purpose of the test is to ensure that the extract contains andrographolide qualitatively. The test solution is prepared by dissolving the extract in a solution of ethanol pro-analyte at a concentration of 10 mg/mL, which is then compared with a 0.1 mg/mL andrographolide (sigma Aldrich) as a comparator. The mobile phase used is chloroform: methanol P (9:1) with the still phase of silica gel 60 F254 and observations are made in UV254. The results of TLC observations can be seen in Figure 1. The Rf values obtained from the test

and comparator solutions are respectively 0.2 and 0.2. The Rf value and the comparator test solution have the same value; this indicates that the test solution is an ethanol extract while still containing andrographolide qualitatively (Ramadhani et al., 2021).

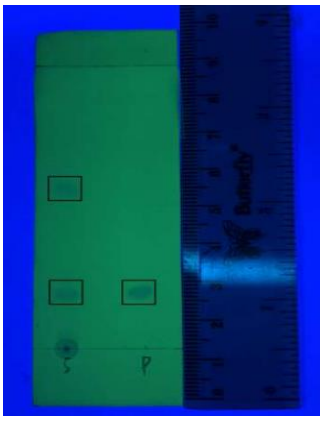

Figure 1. Result of chromatography thin layer of EES, S: sample, P: andrographolide standard solution (mobile phase used is chloroform: methanol P (9:1) in the silica gel 60 F_{254})

The Formulation and Fabrication of EES transdermal patch

The formulation of the EES transdermal patch is carried out by solvent casting technique. HPMC and PVP-K30 are selected as the polymers in the patch manufacturing of EES, which can produce patches with uniform thickness and are able to increase the permeation of the active substance (Kumar et al., 2018). According to Kemala (2016), the use of HPMC as a hydrophilic polymer can provide a clear patch layer while in addition, the use of HPMC either alone or in combination with ethyl cellulose as a polymer can provide good penetration in transdermal patch preparations. The use of a combination of HPMC and PVP-K30 patch characteristics is optimal both in terms of appearance, weight uniformity, percentage of moisture content, and folding endurance, and is able to increase the percentage of the release of pioglitazone hydrochloride (Francis, 2016).

Mixture 1 is a subsequent polymer blend, then added with a PEG 400 plasticizer. The use of PEG 400 as a plasticizer is able to increase the elasticity and folding resistance (Hartesi, et al, 2021). The use PEG 400 in transdermal patches of EES is expected to produce a smooth and elastic patch characteristic. The use of PEG 400 as a plasticizer resulted in the appearance of the EES patches transdermal having a smooth texture. According to Kemala (2016), not only does the PEG 400 increase the flexibility of the glibenclamid patch but also makes a soft patch in the transdermal texture.

Furthermore, an enhancer solution in the form of oleic acid and tween-80 is mixed in a solution of EES while being in the still phase. The use of oleic acid enhancers as promethazine transdermal patches can increase promethazine permeation to a greater extent than propylene glycol and isopropyl alcohol (Nurahmanto, 2016). Tween 80 is an enhancer classified as a class of nonionic surfactants that works by increasing the permeation of the active substance through interaction and binding through keratin filaments that disrupt the permeability of corneocytes. The use of tween-80 1% as an enhancer in diazepam transdermal preparations is able to increase the permeability of diazepam to the skin. Tween-80 is one of the nonionic enhancers that is innate and does not irritate the skin (Pandey, 2014). The herbal ethanol extract solution is then mixed with the enhancer mixture solution. The patch is then placed in an oven at 40-45^{o}C for 24-72 hours until a film is formed and kept in desiccator with aluminum foil.

Evaluation of patch transdermal EES

The visual of both the aroma and texture of the patch EES is evaluated. The visual display of the test results of the patch transdermal EES displays a dark-green-color-like with the distinctive bitter aroma

of the EES. The results of the visual display patch test are shown in Figure 2. Evaluation of the transdermal patch preparation includes testing for weight consistency, thickness uniformity, pH, folding endurance, and percentage moisture content are shown in Table 2.

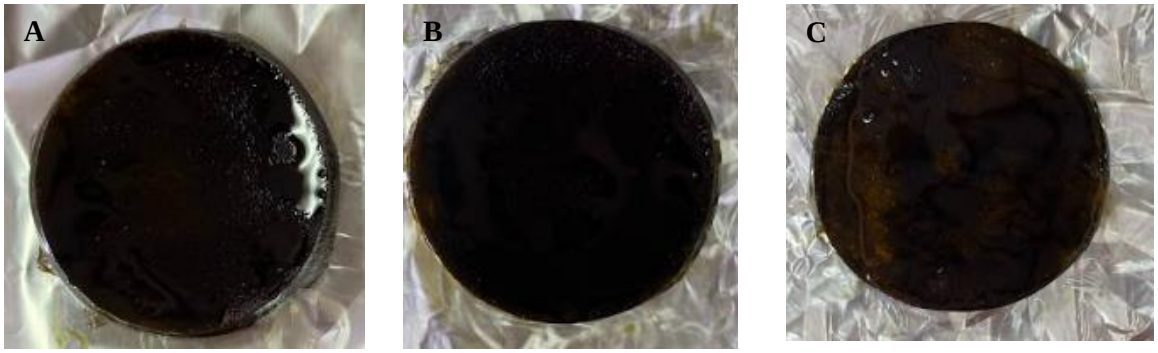

Figure 2. Visual appearance of patch transdermal EES with variations formulas of oleic acid: tween 80 (A) F1 (1:1), (B) F2 (1:3), (C)F3 (1:2)

Table 2. Result of evaluation patch transdermal of EES with variations formulas

Formula (Oleic acid: tween 80)	Thickness (mm)	Weight Uniformity (gram)	Surface pH	Folding Endurance	Moisture Content (%)
F1 (1:1)	0.34 ± 0.01	1.68 ± 0.03	5.32 ± 0.01	315 ± 1.58	2.50 ± 0.75
F2 (1:3)	0.36 ± 0.02	1.85 ± 0.04	5.32 ± 0.01	300 ± 2.70	2.52 ± 0.71
F3 (1:2)	0.32 ± 0.01	1.73 ± 0.02	5.33 ± 0.01	323 ± 1.92	2.86 ± 1. 07

Weight uniformity testing aims to affirm the weight of the patch produced by each uniform formula. Uniformity of the weights may affect the uniformity of the content of the patch. The results of the weight uniformity testing showed the average weight of the three patch formulations ranged from 1.68 to 1.85 grams. The largest average value of patch weights is derived from F2, where the average patch weight on F2 is 1.85 ± 0.04 grams. According to Kemala (2016), the difference in patch weight values in each formula is due to imperfect evaporation of the solvent and uneven dilution of the patch-based liquid in the mold. According to Shivalingam et al. (2021), the value of One-way ANOVA analysis obtained a significance value of 0.000 < 0.05 which shows a significant difference in the patch weight. This is due to the use of a large concentration of tween-80 in the F2 formula, where tween-80 is one of the non-volatile solvents of the surfactant group. In addition, tween-80 has a high viscosity in a larger patch weight (Baranauskaite et al., 2021). The comfort of using a transdermal patch is determined by the weight of the patch matrix. The lighter the patch matrix produced, the more comfortable the patch is to use. Therefore, it will not interfere with activities and provide an aesthetically beautiful patch appearance (Setyawan, 2015).

The thickness of the patches was found to be in the range of 0.32 ± 0.01 to 0.36 ± 0.02. The thickness of the patches depends on the concentration of the enhancer. The higher the concentration of the enhancer, the thicker and heavier the patch will be. Similarly, with the weight of transdermal patches. The variations in thickness may be due to different concentrations of enhancer formulations. According to Soral et al., (2021), the use PVP-K30 in large concentrations can increase the rebeprazol transdermal patch. The other reasons may be due to the need of temperature control which has influenced the controlled dissipation of solvent from the damp film surface. There's a relationship between the weight of the patch and drug substance (Francis, 2016). A suitable thickness of patch size is in the range of 0.5-1.00 mm. From the analysis by one-way ANOVA, a significance value of 0.003 < 0.05 which shows a

significant difference in the patch thickness. This is due to the fact that the heavier the patch weight, the thicker the patch is. The use of tween-80 as an enhancer and PEG 400 as a plasticizer increases the thickness of the patch preparation. The greater the concentration of tween-80 used, the thicker the patch is. EES patch thickness uniformity values of formula F1, F2, and F3 belong to the category of good patch size (Francis, 2016). The comfort of using a transdermal patch is influenced by the physical characteristics of the patch matrix, one of which is the thickness of the patch matrix. The thinner the patch matrix produced, the more comfortable the patch is to use, as it will not interfere with activities and provide an aesthetically beautiful patch appearance (Setyawan et al., 2015).

The surface pH of all the formulations was found to be in the range of 5.32 ± 0.01 to 5.33 ± 0.01, which coincides with a pH range of skin and hence can be concluded that no skin irritation should be occurring (Ravindra et al, 2022). Based on a one-way ANOVA value of sig 0.163 > 0.05, there is no difference between the value of the pH influence enhancer. The results show that the EES patch has a relatively acidic pH, where the acidic pH of the patch can effectively prevent and reduce bacterial growth (Pratiwi et al., 2021).

The folding endurance of transdermal patches was measured manually. The folding endurance of the patches was found to be in the range intended. The patches would not break and would retain their integrity with general skin folding when applied to the skin. The good patches transdermal is more 100-300 times fold resistance (Solanke et al., 2018). PEG 400 is a plasticizer that is widely used in transdermal patch preparations. PEG 400 and PVP-K30 can increase the flexibility and elasticity of the atorvastatin transdermal patch preparation, thereby preventing cracking of the transdermal patch preparation (Castañeda et al., 2017) The folding endurance values of the three formulas meet the requirements, although in F2 the value is smaller than F1 and F3, this is due to the different percentage value of enhancer content between EES transdermal patch formulas, as well as the process of making transdermal patches that should consider humidity conditions and temperature stability during the process of drying (Budhathoki et al., 2016).

The percentage of the moisture content of patches transdermal is around 2.50 ± 0.75 to 2.86 ± 1.07. The low percentage of moisture content in formulations could help them to remain stable and prevent them from being completely dried. The percentage of moisture content of the patches plays a role in maintaining the physical stability of the patch matrix because a small percentage of moisture content of patches will make the physical patch remain flexible and not brittle, resulting in the patch still being comfortable when used (Setyawan et al., 2015). In formula F3, the average %MC value is greater than in formulas F2 and F1, this is due to the drying process not being optimal so that the water content in the EES transdermal patch does not evaporate optimally, causing the water content in the patch to be greater (Ermawati & Prilantari, 2019). Several factors that can increase the %MC value in transdermal patch preparations are the use of hygroscopic transdermal patch ingredients such as the hydrophilic polymer PVP-K30 (Tripathi, 2022). Based on data from testing using one-way ANOVA, the %MC statistical test results show a sig value of 0.759 > 0.05, which means there is no significant difference in %MC between the three EES transdermal patch formulations.

The effectiveness of the patch is not only determined by the physicochemical properties of the active ingredients but is also determined by the composition of the excipients that form it. One of the excipients commonly used in transdermal patch formulations is permeation enhancer. The results of this research show that the characteristics of EES transdermal patches were affected by the ratio of tween-80 and oleic acid as the enhancers. The use of enhancers with non-volatile ingredients can increase the weight and thickness of the transdermal patch. This affects the weight and thickness of the patches produced (Francis, 2016). According to Jafri et al., (2019), The use of single oleic acid as an enhancer with a concentration of 0.5-2% does not affect the weight of the lamotrigine transdermal patch. The combination of oleic acid enhancer and tween-80 in various ratios affects the characteristics of the EES transdermal patch on the weight and thickness of the EES transdermal patch. This was caused by being influenced by the increase in tween-80 concentration in F2.

In vivo study of patch transdermal EES

The application of the EES transdermal patch to the rat was carried out by attaching the EES transdermal patch to the rat's abdomen (Hendriati et al., 2021). Rats that had been induced with STZ were then tested for blood glucose levels (BGL). After day 3 of post-induction, the rats that had been induced with STZ were then tested for BGL. The rats within the BGL range of ≥200 mg/dL were used in this study (Premanath & Nanjaiah, 2015). The experiment was carried out for 15 days. Blood samples were taken via the tail vein of the rats. Testing was carried out for 15 days, then observations were carried out on days 3, 5, 7, 9, 11, 13, and 15 (Perada & Murthy, 2022). The effect of EES patch transdermal in diabetic rats is shown in Figure 3. In the EES transdermal patch hypoglycemic activity test on the third day after STZ induction, the average BGL of the mice ranged from 230-245 mg/dL, according to the American Diabetes Association, (2024), if the BGL is ≥ 200 mg/dL then it has been declared to be in the diabetes phase.

The EES transdermal patch was administered twice a day after 24 hours of testing for BGL of 2-13 mg/dL (Hadebe et al., 2014), where positive control in the form of short-acting insulin novorapid provided a greater reduction in blood glucose levels from 225 mg/dL ± 3.94 to 202 mg/dL ± 1.85 followed, F1 245 mg/dL ± 1.72 to 240 mg/dL ± 1.72, F3 245 mg/dL ± 3.01 to 240 mg/dL ± 3.12 and F2 230 mg/dL ± 2.65. Measurement of BGL on the fifth day showed a decrease in BGL ranging from 2-13 mg/dL. The positive control group showed a greater reduction in BGL from 225 mg/dL ± 3.94 to 202 mg/dL ± 1.85 followed, by F1 245 mg/dL ± 1.72 to 240 mg/dL ± 1.72, F3 245 mg/dL ± 3.01 to 240 mg/dL ± 3.12 and F2 230 mg/dL ± 2.65. Insulin aspart is a short-acting insulin with a working mechanism of inhibiting glucose, fat, and peripheral glucose absorption, especially in muscle and fat with an onset of 3-5 hours (Haahr & Heise, 2020).

On the seventh day of observation, treatment groups F2 and F3 experienced a greater reduction in BGL than the other treatment groups by 10 and 15 mg/dL. The decrease in BGL from the F2 and F3 formula groups shows that EES is effective in the form of a transdermal patch dosage form. Andrographolide from EES was known for its action in bringing down BGL through a few mechanisms such as increased GLUT-4 protein in soleus muscle for glucose take-up, moved forward pancreatic islet, expanded beta-cell density, and expanded pancreatic insulin substance (Sari et al., 2015). An oral concentration of andrographis paniculata extract of 200 mg was able to reduce BGL in rats induced by STZ (Hidayat & Wulandari, 2021), this dose is used in all three transdermal patch formulas. Even though the three formulas have the same dosage, the difference in enhancer concentration affects the activity of reducing rat's BGL. Enhancers influence the effectiveness of the EES transdermal patch as an anti-diabetic.

Enhancers influence the effectiveness of the EES transdermal patch on the pharmacological effects of active substances made in transdermal preparations (Kumar et al., 2015). Observations on the eleventh day in the F2 group show that the average BGL in the mice showed a value of 186 mg/dL, while the treatment groups in F1 and F3 were still above 200 mg/dL. The average BGL value in the F2 group mice was almost close to the BGL value in the positive group. Observation of BGL on the 15th day of the treatment group given EES Formula 1-3 transdermal patches and the positive control group value ranged from 100-130 mg/dL, where the positive control group had a smaller BGL value of 100 mg/dL then the treatment group F2 is 105 mg/dL.

The results of testing the BGL activity of the three EES transdermal patch formulas with a combination of oleic acid enhancer and tween-80 in various comparisons showed that formula F2 was the EES transdermal patch formula which had greater BGL-reducing activity compared to the F1 and F3 groups. Based on one-way ANOVA statistical testing, the resulting significance value is 0.000 < 0.005. There were significant differences between each treatment group.

Enhancers are a critical point in the delivery of transdermal preparations with various mechanisms of action on the stratum corneum, thereby increasing the permeation of active substances that influence pharmacological effectiveness (Haq et al., 2020). The combination of oleic acid and tween-80 can provide a synergistic effect of the EES transdermal patch preparation (Bigucci et al., 2015). Oleic acid

is one of the enhancers of the unsaturated fatty acid group (Nurahmanto, 2016). In the atorvastatin polymethacrylate transdermal patch preparation, the use of an oleic acid enhancer can increase the permeation of atorvastatin as an anti-dyslipidemia (El-Say et al., 2021). Oleic acid is able to increase the permeation of ketorolac fourteen times compared to pure ketorolac (Kumar et al., 2015). At a concentration of 1-3%, the use of oleic acid in the transdermal lornoxicam preparation was able to increase the anti-inflammatory activity in rats induced by carrageenan compared to administration of the oral preparation (Hashmat et al., 2020).

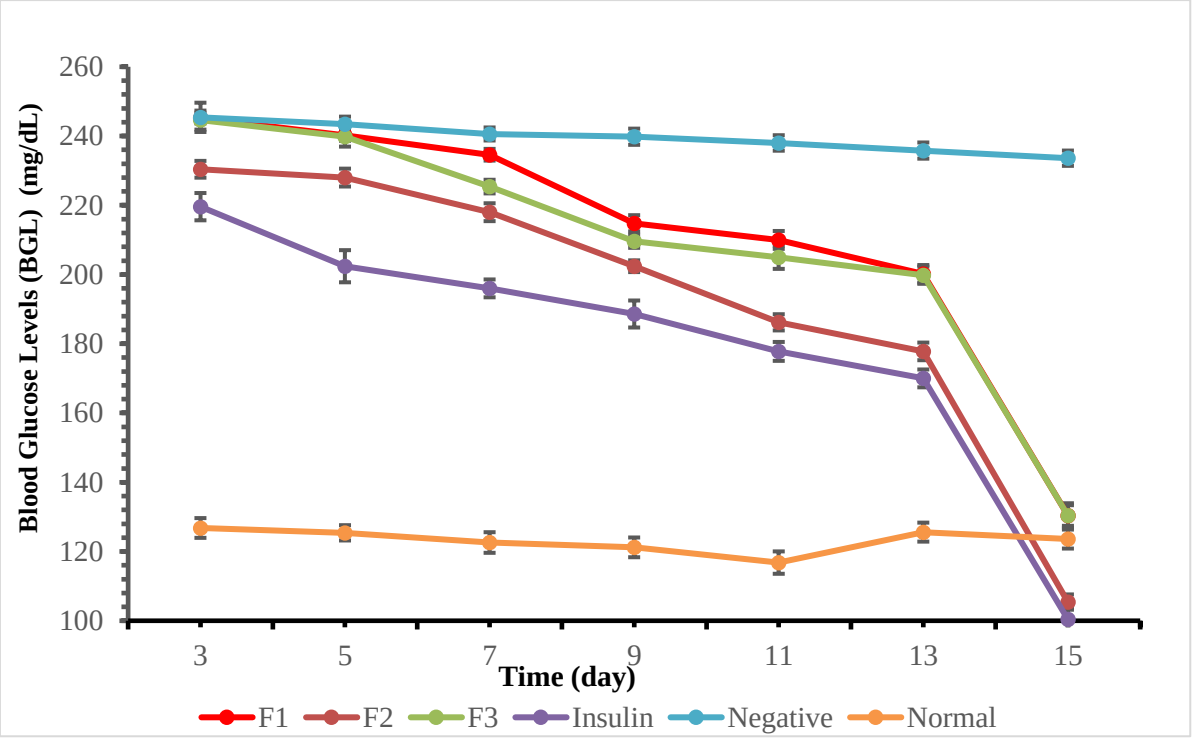

Figure 3. Effect of transdermal patch on blood glucose levels in diabetic rats treated by F1, F2, F3, positif control, negative control and normal

Tween-80 is an enhancer from the non-ionic surfactant group. The use of tween-80 at low concentrations can form lipid emulsification in the stratum corneum thereby increasing the permeability of the active substance. The choice of enhancers in the form of oleic acid and tween-80 in making EES transdermal patches is to provide a synergistic effect of enhancers in increasing the permeation of andrographolide in the bitter extract as an antidiabetic. According to Bigucci et al., (2015), using oleic acid combined with tween-80 through extensive testing in-vitro provides a better permeation effect on the propranolol HCl transdermal patch compared to the propranolol HCl transdermal patch which only uses a single enhancer of oleic acid and a single tween-80. The structure of the stratum corneum consists of parts that are lipophilic and hydrophilic, so the use of a combination of enhancers that have hydrophilic properties such as tween-80 and lipophilic properties such as oleic acid makes it easier for the active substances to penetrate optimally thereby increasing greater permeability (Saitoh et al., 2023).

Based on testing the characteristics of the patch and testing the BGL lowering activity of the EES transdermal patch, it was found that the F2 formulation was the optimal formula both in terms of characteristics and testing the blood glucose lowering activity in STZ-induced rats. The use of a combination of 1% oleic acid enhancer and 3% tween-80 is able to increase the permeation of

andrographolide contained in the EES. According to Zaki et al., (2022), a concentration of 1% oleic acid in the tacrolimus nanovesicles transdermal gel preparation is able to increase the permeation of the preparation. Tacrolimus transdermal gel is 3.6 times higher than tacrolimus oral suspension preparation.

The use of 1% oleic acid concentration in transdermal lornoxicam preparations can increase the permeation of lornoxicam (Hashmat et al., 2020). Tween-80 concentration of 3% can increase the permeation of ketoprofen by nine times compared to the standard (Mita et al., 2018). The use of oleic acid and tween-80 as enhancers in the EES transdermal patch with a ratio of 1% and 3% can increase the effectiveness of the EES transdermal patch preparation as an antidiabetic.

This is in accordance with Saitoh et al. (2023) research, the use of a combination of oleic acid and tween-80 in the disulfiram transdermal patch preparation was able to increase disulfiram permeation better than the use of oleic acid alone. The combined application of oleic acid and tween 80 significantly increased the amount of drug that permeated into the skin. Oleic acid may progress the fluidity of intercellular lipids within the stratum corneum and tween 80 may move forward the skin permeability of the drug by increasing the solubility.

CONCLUSION

The characteristics of EES transdermal patches were affected by the ratio of tween-80 and oleic acid as the enhancers. The combination of oleic acid and tween 80 as a permeation enhancer has an influence on the physical characteristics (weight and thickness) and BGL lowering activity of EES patches.

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REFERENCES

Ahmed, O. A. A., Ahmed, T. A., Abdel-Naim, A. B., Khedr, A., Banjar, Z. M., & Afouna, M. I. (2014). Enhancement of in vitro skin transport and in vivo hypoglycemic efficacy of glimepiride transdermal patches. *Tropical Journal of Pharmaceutical Research*, *13*(8), 1207–1213. https://doi.org/10.4314/tjpr.v13i8.

American Diabetes Association. (2024). 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes—2024. *Diabetes Care*, *47*(January), S20–S42. https://doi.org/10.2337/dc24-S002

Baranauskaite, J., Ockun, M. A., Uner, B., Tas, C., & Ivanauskas, L. (2021). *Effect of the amount of polysorbate 80 and oregano essential oil on the emulsion stability and characterization properties*. https://doi.org/https://doi.org/10.3390/ molecules26206304

Bigucci, F., Abruzzo, A., Cerchiara, T., Gallucci, M. C., & Luppi, B. (2015). Formulation of cellulose film containing permeation enhancers for prolonged delivery of propranolol hydrocloride. *Drug Development and Industrial Pharmacy*, *41*(6), 1017–1025. https://doi.org/10.3109/03639045.2014.925914

Budhathoki, U., Gartoulla, M. K., & Shakya, S. (2016). Formulation and evaluation of transdermal patches of atenolol. *Indonesian Journal of Pharmacy*, *27*(4), 196–202. https://doi.org/10.14499/indonesianjpharm27iss4pp196

Castañeda, P. S., Escobar-Chávez, J. J., Aguado, A. T., Cruz, I. M. R., & Melgoza Contreras, L. M. (2017). Design and evaluation of a transdermal patch with Atorvastatin. *Farmacia*, *65*(6), 908–916.

DepKes RI. (2017). Formularies. *Farmakope Herbal Indonesia Edisi II*, 213–218. https://doi.org/10.1201/b12934-13

El-Say, K. M., Ahmed, T. A., Aljefri, A. H., El-Sawy, H. S., Fassihi, R., & Abou-Gharbia, M. (2021). Oleic acid–reinforced PEGylated polymethacrylate transdermal film with enhanced antidyslipidemic activity and bioavailability of atorvastatin: A mechanistic ex-vivo/in-vivo analysis. *International Journal of Pharmaceutics*, *608*(August). https://doi.org/10.1016/j.ijpharm.2021.121057

Ermawati, D. E., & Prilantari, H. U. (2019). Pengaruh kombinasi polimer hidroksipropilmetilselulosa dan natrium karboksimetilselulosa terhadap sifat fisik sediaan matrix-based patch ibuprofen. *JPSCR : Journal of Pharmaceutical Science and Clinical Research*, *4*(2), 109. https://doi.org/10.20961/jpscr.v4i2.34525

Francis, D. J. E. (2016). Development and evaluation of matrix type transdermal patches of pioglitazone hydrochloride. *Universal Journal of Pharmaceutical Research*, *1*(1), 31–37. https://doi.org/10.22270/ujpr.v1i1.r5

Garg, C., Sharma, P., Satija, S., & Garg, M. (2016). Stability indicating studies of Andrographis paniculata extract by validate HPTLC protocol. *Journal of Pharmacognosy and Phytochemistry*, *5*(6), 337–344.

Haahr, H., & Heise, T. (2020). Fast-acting insulin aspart: a review of its pharmacokinetic and pharmacodynamic properties and the clinical consequences. *Clinical Pharmacokinetics*, *59*(2), 155–172. https://doi.org/10.1007/s40262-019-00834-5

Hadebe, S. I., Ngubane, P. S., Serumula, M. R., & Musabayane, C. T. (2014). Transdermal delivery of insulin by amidated pectin hydrogel matrix patch in streptozotocin-induced diabetic rats: Effects on some selected metabolic parameters. *PLoS ONE*, *9*(7). https://doi.org/10.1371/journal.pone.0101461

Haq, A., Chandler, M., & Michniak-Kohn, B. (2020). Solubility-physicochemical-thermodynamic theory of penetration enhancer mechanism of action. *International Journal of Pharmaceutics*, *575*(August 2019), 118920. https://doi.org/10.1016/j.ijpharm.2019.118920

Hartesi, B., Sagita, D., Andriani, L., Angke, T., & Natalia, S. (2021). Patch transdermal dari fraksi N-Heksan ekstrak ruku- ruku (Ocimum Tenuiflorum L.) sebagai antiinflamasi. *Jurnal MIPA*, *9*(2), 60. https://doi.org/10.35799/jmuo.9.2.2020.28709

Hashmat, D., Shoaib, M. H., Ali, F. R., & Siddiqui, F. (2020). Lornoxicam controlled release transdermal gel patch: Design, characterization and optimization using co-solvents as penetration enhancers. *PLoS ONE*, *15*(2), 1–23. https://doi.org/10.1371/journal.pone.0228908

Hendriati, L., Hamid, I. S., Widodo, T., Surya, R. H., Wahyudi, A. E., & Rasdianto, D. D. (2021). Analgesic activity of transdermal patch ethanol extract Piper Nigrum L Fructus with some enhancers on Mice. *Jurnal Farmasi Sains Dan Praktis*, *7*(1), 67–73. https://doi.org/10.31603/pharmacy.v7i1.4033

Hidayat, R., & Wulandari, P. (2021). Effects of Andrographis paniculata (Burm. F.) extract on diabetic Nephropathy in Rats. *Reports of Biochemistry and Molecular Biology*, *10*(3), 445–454. https://doi.org/10.52547/rbmb.10.3.445

Jafri, I., Shoaib, M. H., Yousuf, R. I., & Ali, F. R. (2019). Effect of permeation enhancers on in vitro release and transdermal delivery of lamotrigine from Eudragit®RS100 polymer matrix-type drug in adhesive patches. *Progress in Biomaterials*, *8*(2), 91–100. https://doi.org/10.1007/s40204-019-0114-9

Keerthana, A., Sunilchandra, U., Nb, S., M, V. K., B, K. R., Sheela, P., & Srinivasan, G. (2022). *Pharmacokinetic evaluation of single oral dose of meloxicam with or without co-administration of andrographolide or bromelain in rats*. *11*(5), 349–354.

Kemala, N. I. W. (2016). *Glibenklamid Menggunakan Hydroxy Propyl Methyl Cellulose (Hpmc) K15M Sebagai Polimer (Skripsi)*, Universitas Islam Indonesia, 1–121.

Kumar, M., Trivedi, V., Shukla, A. K., & Dev, S. K. (2018). Effect of polymers on the physicochemical and drug release properties of transdermal patches of atenolol. *International Journal of Applied Pharmaceutics*, *10*(4), 68–73. https://doi.org/10.22159/ijap.2018v10i4.24916

Kumar, P., Singh, S., Mishra, D., & Girotra, P. (2015). Enhancement of ketorolac tromethamine permeability through rat skin using penetration enhancers: An ex-vivo study. *International Journal of Pharmaceutical Investigation*, *5*(3), 142. https://doi.org/10.4103/2230-973x.160850

Liang, D., Zhang, W.-M., Liang, X., Tian, H.-Y., Zhang, X.-M., Li, X., & Gao, W.-Y. (2023). A review on the extraction and separation of andrographolide from Andrographis paniculata: extraction

selectivity, current challenges and strategies. *Traditional Medicine Research*, *8*(7), 38. https://doi.org/10.53388/tmr20230107001

Mita, S. R., Musfiroh, I., SK, I., & MF, D. (2018). Study in vitro of Ketoprofen patch with tween 80 as enhancer. *International Research Journal of Pharmacy*, *9*(7), 111–115. https://doi.org/10.7897/2230-8407.097134

Moore, E. M., Wagner, C., & Komarnytsky, S. (2020). The enigma of bioactivity and toxicity of botanical oils for skin care. *Frontiers in Pharmacology*, *11*(May), 1–17. https://doi.org/10.3389/fphar.2020.00785

Mostafavinia, A., Amini, A., Ghorishi, S. K., Pouriran, R., & Bayat, M. (2016). The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of type1 diabetes mellitus and mortality rate in rats. *Laboratory Animal Research*, *32*(3), 160. https://doi.org/10.5625/lar.2016.32.3.160

Nurahmanto, D. (2016). Formulasi dan evaluasi sediaan patch transdermal prometazin HCL sebagai pengobatan morning sickness. *South African Medical Journal*, *101*(2003), 16.

Pandey, A. (2014). Role of surfactants as penetration enhancer in transdermal drug delivery system. *Journal of Molecular Pharmaceutics & Organic Process Research*, *02*(02). https://doi.org/10.4172/2329-9053.1000113

Perada, S., P. N. Murthy. (2022). Design, development and evaluation of transdermal drug delivery system for treatment of diabetes. *Journal of Pharmaceutical Negative Results*, *13*(8), 3610–3617. https://doi.org/10.47750/pnr.2022.13.s08.448

Prasetyo, B. F., Wientarsih, I., Sajuthi, D., & Juniantito, V. (2018). Formation of Andrographlolide-BetaCyclodextrin Inclusion to Increase Solubility and Dissolution Rate. *Indonesian Journal of Pharmaceutical Science and Technology*, *5*(2), 49. https://doi.org/10.24198/ijpst.v5i2.14995

Pratiwi, G., Susanti, S., & Shiyan, S. (2021). *Application of factorial design for optimization of PVC-HPMC polymers in matrix film ibuprofen patch-transdermal drug delivery system*. *1*(1), 11–21.

Premanath, R., & Nanjaiah, L. (2015). Antidiabetic and antioxidant potential of Andrographis paniculata Nees. leaf ethanol extract in streptozotocin induced diabetic rats. *Journal of Applied Pharmaceutical Science*, *5*(1), 069–076. https://doi.org/10.7324/JAPS.2015.50113

Rais, I. R. (2014). Ekstraksi androgafolid dari Andrographis paniculata (Burm.f.) Nees menggunakan ekstraktor soxhlet. *Pharmaciana*, *4*(1), 85–92. https://doi.org/10.12928/pharmaciana.v4i1.402

Ramadhani, M. A., Hati, A. K., Jusman, A. H., Farmasi, P. S., Kesehatan, F., Waluyo, U. N., Insulin, N. D., & Glukosa, P. (2021). *Perbandingan aktivitas penurunan glukosa pada ekstrak dan nanoekstrak daun Insulin (Tithonia diversifolia) dengan metode in vitro*. *1*(2), 28–36.

Saitoh, H., Takami, K., Ohnari, H., Chiba, Y., Ikeuchi-Takahashi, Y., & Obata, Y. (2023). Effects and mode of action of oleic acid and tween 80 on skin permeation of disulfiram. *Chemical and Pharmaceutical Bulletin*, *71*(4), 289–298. https://doi.org/10.1248/cpb.c22-00821

Sari, K. R. P., Sudarsono, & Nugroho, A. E. (2015). Effect of herbal combination of Andrographis paniculata (Burm.f) Ness and Gynura procumbens (Lour.) Merr ethanolic extracts in alloxan-induced hyperglycemic rats. *International Food Research Journal*, *22*(4), 1332–1337.

Setyawan E.I, Warditiani N.K, D. S. . (n.d.). *Pengaruh penggunaan propilenglikol dan mentol terhadap matrik patch transdermal ekstrak air Herba Sambiloto (Andrographis paniculata (burm. F.) Nees*.

Shivalingam, M. R., Balasubramanian, A., & Ramalingam, K. (2021). Formulation and evaluation of transdermal patches of pantoprazole sodium. *International Journal of Applied Pharmaceutics*, *13*(5), 287–291. https://doi.org/10.22159/ijap.2021v13i5.42175

Solanke, P. N., Ambekar, A. W., & Chemate, S. (2018). *Formulation , development and characterization of transdermal drug delivery system for antidiabetic drug*. *4*(5), 668–672.

Soral, M., Nanjappa, S. H., & Alayadan, P. (2021). Formulation and evaluation of transdermal patch of rabeprazole sodium. *Journal of Reports in Pharmaceutical Sciences*, *10*(2), 240–246. https://doi.org/10.4103/jrptps.JRPTPS_126_20

Sundhani, E., Nugroho, A. E., Nurrochmad, A., Puspitasari, I., Prihati, D. A., & Lukitaningsih, E.

(2022). *Pharmacokinetic herb-drug interactions of glipizide with*. https://doi.org/https://doi.org/10.3390/ molecules27206901

Tripathi, R, Singh, A., & Sahu, N. (2022). *Formulation and evaluation of transdermal patches*. *11*(17), 49–68. https://doi.org/10.20959/wjpr202217-24471

Yuan, Y., Win Aung, K. K., Ran, X. K., Wang, X. T., Dou, D. Q., & Dong, F. (2017). A new sesquiterpene lactone from yacon leaves. *Natural Product Research*, *31*(1), 43–49. https://doi.org/10.1080/14786419.2016.1212028

Zaki, R. M., Ibrahim, M. A., Alshora, D. H., & Abou El Ela, A. E. S. (2022). Formulation and evaluation of transdermal gel containing tacrolimus-loaded spanlastics: in vitro, ex vivo and in vivo studies. *Polymers*, *14*(8). https://doi.org/10.3390/polym14081528

.