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Transdermal Film-Forming Hydrogel Loaded with Nigella Sativa and Trigonella foenum-graecum Extracts for Enhanced Wound Healing

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Topical wound treatment using conventional hydrogels is easily lost due to friction so that treatment is less effective, this can inhibit wound healing which causes chronic wounds. Convensional hydrogel containing a combination of N. sativa and T. foenum-graecum alcohol extracts at concentrations of 10% and 5% showed that the preparation was able to healing process of burns in diabetic rats better than single extracts. Compared to conventional preparations, topical Film Forming Hydrogel (FFH) preparation is capable of forming thin sheets some time after applications. A good FFH preparation has strong adhesion and can be adjusted to the shape of the wound so that it is expected to increase the effectiveness of wound healing, including wounds in patients with diabetes mellitus. This study aimed to formulate FFH preparation containing active ingredient combination of Nigella sativa L. and Trigonella foenum-graecum L. extract. The combination of polyvinyl alcohol (PVA) and carboxymethyl chitosan (CMCh) was used as base, while 96% ethanol and a variation of propylene glycol (PG) content served as solvent and plasticizer, respectively. The experiment was carried out to obtain FFH preparation with desired physical, chemical, and mechanical characteristics, including pH, viscosity, swelling ratio, tensile strength, and elongation. Furthermore, homogeneity of the preparation was assessed through the analysis of Fourier Transform Infrared (FITR) Spectroscopy functional groups, and the stability of physicochemical characters was determined during a 30-day storage period. The results showed that all variations of PG levels of 10%, 16%, and 22% in the test formulations met the physicochemical, and mechanical requirements of FFH preparation. Only F1-Extract meets the minimum standard of 70% for the elongation parameter, and could release the drug gradually. The FFH formulation with active ingredients of N. sativa extract of 10%, T. foenum-graecum of 5%, PG content of 10% (F2-Extract), with a combination of PVA-CMCh at a ratio of 1:1 of 43% each, and 96% ethanol of 4% produced an optimal FFH preparation. Stability test results showed that F2-E and F3-E also had stable pH, viscosity, and swelling ratio at 30 days of storage.

Keywords: CMCh (carboxymethyl chitosan); FFH (Film-forming hydrogel); Nigella sativa L.; PVA (polyvinyl alcohol); Trigonella foenum-graecum L.

Wound healing process is associated with sequential occurrence in 4 phases, namely hemostasis, inflammation, proliferation (angiogenesis and epithelialization), and remodeling (changes in type III collagen to type I). In diabetic patients, wound often experiences disturbances in the healing process comprising various complex pathophysiological conditions. These include

hypoxia inhibiting angiogenesis, increased free radicals that prolong the inflammatory phase, and risk of infection. Therefore, a combination of herbal ingredients with different competencies in healing process is needed to improve treatment success.

Herbal ingredients such as *Nigella sativa* L. oil are a gram-positive antibacterial agent against *Methicillin-Resistant Staphylococcus aureus* (MRSA).[1] Thymoquinone content in *N. sativa* has been shown to have functions as an anti-inflammatory, antimicrobial, and antioxidant,[2] accelerating the inflammatory process of wound healing without affecting the granulation and proliferation process[3]. Another herbal ingredient, *Trigonella foenum-graecum* L. extract, also has activity as an antibacterial *Propionibacterium-acne* and *Staphylococcus aureus,*[3] which are often found in diabetic wound. The methanol extract of *T. foenum-graecum* seeds inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* better than the acetone extract, while the aqueous extract showed no antibacterial activity.[4] Hydroalcoholic extract of *T. foenum-graecum* seeds at concentrations of 5% and 10% can accelerate the proliferation process in diabetic rat wound healing[5] and increase the excision re-epithelialization wound of Wistar rats.[6] Based on these studies, *N. sativa* and *T. foenum-graecum* extracts have shown complementing effects in fulfilling their potential for wound healing. Thymoquinone in *N. sativa* extract can accelerate the inflammatory process, serving as anti-MRSA antimicrobial and strong antioxidant. *T. foenum-graecum* extract also possesses anti-MRSA alongside the ability to increase proliferation and re-epithelialization process which is not found in *N. sativa* activity. Previous studies have reported topical preparations containing a combination of *N. sativa* and *T. foenum-graecum* alcohol extracts at concentrations of 10% and 5% showed that the preparation was able to accelerate the healing process of burns in diabetic rats better than single extracts of both *N. sativa* and *T. foenum-graecum* as well as the drug poviodin.[7]

Conventional semisolid preparation such as ointments, creams, and gels topically have many disadvantages and limitations when used. These include a sticky feeling and often irritating to the skin, causing discomfort. Additionally, conventional topical preparation has a short contact time with the skin because of the ability to be easily erased by friction with the patient's clothing, requiring frequent treatment. One of the latest developments to become an option for transdermal dosage forms is the Film Forming System (FFS) dosage form.[8] Transdermal drug delivery is capable of avoiding first-pass metabolism, easy to use, and provides comfort to patients, with a large surface area and absorption for topical and systemic delivery.[9] Film Forming Hydrogel (FFH) is the second-generation formulation development of FFS. The advantages of FFH include stronger adhesion and easy application, which can be used to follow the irregular wound shape often found in chronic diabetic patients.

FFH comprises active ingredients, film-forming hydrophilic polymers, plasticizers, and skin-tolerant volatile solvents. The hydrogel dosage will contact the skin by forming a semi-occlusive film to concentrate the active ingredients in the polymer matrix.[10] Occlusive and semi-occlusive FFH can isolate the wound and retain moisture to help the healing process.[11] In theory, moist conditions in wound healing process will accelerate fibrilinosis, angiogenesis, and growth factor formation to reduce the risk of infection.[12] The results of FFH development studies using polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) film-forming polymers with a mixture of propylene glycol (PG), ethanol, and water produced a hydrogel dosage form that can adhere to the skin surface and protect the wound from external influences.[13]

The physicochemical properties of drug, type, concentration of polymer and plasticizer, with other additives in the formulation, solvent evaporation, and determine the drug release rate through the skin.[14] Previous optimization tests on adding PG, glycerol, and polyethylene glycol (PEG) 600 plasticizers to PVA hydrogels showed high film flexibility and low water resistance of PVA. However, PEG 600 showed incompatibility with PVA hydrogels, forming a precipitate during the manufacturing process that did not dissolve under moderate heat conditions.[15] As plasticizer, glycerol has good efficiency, large availability, and low exudation.[16] Permeability studies of the combination of chitosan, PVA, and PG showed that the permease rate increased with decreasing PVA concentration.[17]

In topical or transdermal preparation, active ingredients must be able to penetrate the stratum corneum. Lipophilic active ingredients can penetrate the stratum corneum better than hydrophilic active ingredients. As excipients, polymers as in FFH can affect wound healing. Generally, a good polymer will form a thin and bright film on the skin surface with a temperature of 28–32°C. The resulting film should have high flexibility and affinity to the skin to avoid excessive plasticizers [8]. Polymers and other excipients have the potential to affect drug permeation due to complex interactions between components. These interactions affect the preparation's physicochemical properties, including the drug's charge and lipophilicity.[18]

A good FFH can form a film resistant to physiological stresses caused by skin movement and maintain contact between the film as well as the skin for a long time. The optimal composition of FFH preparations as hydrogels is generally required to be easy to apply, dry quickly on the skin, have the right hardness and stickiness, including good flexibility and elasticity. Therefore, this study aimed to formulate FFH preparation containing active ingredient combination of *Nigella sativa* L. and *Trigonella foenum-graecum* L. extract. The combination of polyvinyl alcohol (PVA) and carboxymethyl chitosan (CMCh) was used as base, while 96% ethanol and a variation of propylene glycol (PG) content served as solvent and plasticizer, respectively. The FFH preparation was expected to have good physicochemical characteristics and stability based on predetermined parameters. These included namely physicochemical parameters of hydrogel such as pH, viscosity, and swelling ratio, as well as mechanical properties comprising tensile strength and elongation, test, and characterization of the preparation.[19]

MATERIAL AND METHODS

The materials used in the study had pharmaceutical-grade purity, including polyvinyl alcohol (PVA) (Merck), carboxymethyl chitosan (CMCh) with molecular weight of 543.5 g/mol (Merck), propylene glycol (PG) with molecular weight of 70.09 g/mol (Merck), and ethanol 96%. The tools used were a vacuum rotary evaporator, magnetic stirrer, pH meter, freezer, analytical balance, cone and plate viscometer (Brookfield DVII, TF spindle), Texture Analyzer (TA.XT2), and Scanning Electron Microscopy (SEM).

Preparation of *N. sativa* and *T. foenum-graecum* extracts

The extraction process of both herbal seeds was carried out using the maceration method with 70% alcohol solvent. The filtrate was evaporated using a vacuum rotary evaporator at 40°C.

FFH Formulation of *N. sativa* extract and *T. foenum-graecum* extracts

A total of 6 FFH formulations were made, consisting of 3 without extracts as well as 3 with *N. sativa* and *T. foenum graecum* extracts (**Table 1**).

FFH preparation using *N. sativa* and *T. foenum-graecum* extracts

FFH preparation and test were carried out in 4 stages. (1) Preparation of base: PVA (weight according to formula) was dissolved using 96 mL of distilled water and stirred with a magnetic stirrer for 4 hours at 90°C. This was followed by dissolving CMCh (weight according to formula) into 96 mL of distilled water and stirring with a magnetic stirrer for 1 hour at 40°C. Subsequently, PVA and CMCh solutions were mixed (1:1) and stirred with a magnetic stirrer for 2 hours at 40°C. (2) Extract dissolution: The extract was dissolved using 96% ethanol at 4%. (3) FFH-extract blending: PG and extract solution were added to FFH PVA-CMCh preparation base gradually, stirred with a magnetic stirrer for 1 hour at 40°C, and incubated for 2 hours at room temperature. (4) Molding of test preparation: FFH preparation was poured into a petri dish with a diameter of 90 mm and stored in the freezer at -20°C for 12 hours and at room temperature for 12 hours.[20]

Evaluation of physical and chemical characteristics of FFH-extract

Physical and chemical parameter measurements were carried out 3 times.

pH assay

FFH and FFH-extract were weighed at 1 g, added 10 mL of CO_2-free distilled water, and measured using a pH meter. Subsequently, FFH preparations were made in an acidic pH range of approximately 5.5±0.5.

Viscosity assay

FFH and FFH-extract preparations were measured using a cone and plate viscometer (Brookfield DVII, TF spindle). Viscosity showed the resistance of FFH preparation to flow. Based on Indonesian National Standard (SNI) 16– 4399–1996, the standard value of good viscosity for gel preparation was 6000–50000 cP or 6–50 Pa.S.

Assay of film formation time

FFH and FFH-extract samples were weighed at 0.2 g, applied to an acrylic board with a size of 2 x 2 cm and allowed to stand at 25±2°C until film formation. Observation of film formation was performed visually and time taken was measured from when the hydrogel was applied until formation. Film formation time was divided into 3, where the first group was classified as fast (<5 minutes), the second as medium (5–7 minutes), and the third as slow (>7 minutes).

Swelling ratio assay

FFH and FFH-extract samples with a size of 2 x 2 cm were dried at 60°C for 12 hours, weighed (W initial), and soaked in phosphate buffer solution (PBS, pH 7.4) at 37°C (W soaked) for 24 hours. Based on some literature, the swelling ability of FFH preparation with PVA-CMCh combination was made in the 200–600% range. The percentage of swelling ratio was calculated from the formula:

$$\%RS = \frac{W\ soaked}{W\ initial} x100$$

Mechanical strength assay

FFH and FFH-extract were left at room temperature for 10 minutes until a film formed. A piece of film was placed in a cell with a diameter of 5.6 cm and perforated to determine the breaking point. Mechanical properties were determined using a Texture Analyzer (TA.XT2). The breaking strength was expressed by ä (MPa) and modified by å (%). The tensile strength and elongation values of the hydrogel films formed were 5–32 MPa and 30–115%, respectively.[21]

Characterization of the functional groups of preparation using Fourier Transform Infrared (FTIR) spectroscopy

Characterization was carried out to determine the functional groups in a preparation using Infrared Absorption Spectrum analysis with FTIR. Approximately 1 mg of substance was taken, crushed with 100 mg of dry KBr powder, and pressed or compressed using a hydraulic press equipped with a water vapor pulling device to obtain a thin translucent pellet. The pellet was observed on an FTIR spectrophotometer and the results obtained were compared with previous reports.[22]

Assay for short-term physical stability

The short-term stability test was conducted on optimized FFH and FFH-extract preparations stored at 25±2°C for 1 month. This was followed by observation of changes in physical characteristics.[13]

Drug release assay

To prove the potential release of extracts from FFH preparation that has optimal quality produced, extract release power was measured by determining the cumulative release using the Franz Diffusion Cell method used mouse skin as membrane diffusion. This method started with defining the wavelength using the maximum absorption of the 500 ppm extract. Determination of the maximum wavelength was conducted to prepare the standard curve and measure the released extract. Subsequently, extract release assay from FFH was carried out twice at each observation time for 240 minutes with an interval of 20 minutes. For the measurement of extract content, dapar phosphate pH 7.4 was used as solvent.[23]

Statistical analysis

The data were observed to fulfill the quality requirements by comparing with standard values of SNI 16–4399–1996 transdermal FFH preparation. These included pH 5.5±0.5, viscosity 6000–50000 cP or 6–50 Pa.S, film formation time categorized as fast (<5 minutes), medium (5–7 minutes), and slow (> 7 minutes), with swelling ratio 200–600%, tensile strength value 5–32 MPa, and elongation 30%-115%.

RESULTS AND DISCUSSION

The observation of physical and chemical properties in Table 2 shows that FFH preparation containing *N. sativa* and *T. foenum-graecum* extract from the 3 tested formulations fulfills the standard requirements as stated in SNI 16-4399–1996. The FFH preparation produced had pH 5.12±0.1 to 5.21±0.05, viscosity 23546.3±1.15 to 23547.67±0.58 cps, film formation time 6.24±0.01 min -6.33±0.07 min, swelling ratio 472±0.10

%-531±0.01%, tensile strength 11.00±0.00 MPa -14.00±0.00 MPa, and elongation 60.00±0.00%-70.33±0.00%.

FTIR characterization and SEM morphology

The identification of functional groups of *N. sativa* extract and *T. foenum-graecum* extract was carried out on the components of FFH, extract, and FFH-extract using FTIR (Figures 1-3). The infrared spectra results (Table 1) indicated that *N. sativa* extract contained molecules with 6 functional groups, while *T. foenum-graecum* extract contained 8 functional groups. When combined, the 2 extracts showed a total of 9 distinct functional groups. The 5 functional groups found in both extracts with absorption at different wave numbers were O-H, C-H, C=O, C=N, and C-O. Only the C=C group in *N. sativa* was not found in *T. foenum-graecum*, while Ca"C, -CH_3, and C-N were identified in the *T. foenum-graecum* extract, namely Ca"C, -CH_3, and C-N. In FFH preparations containing extracts, 7 functional groups indicated the content of *N. sativa* and *T. foenum-graecum* extracts, namely O-H, C-H, C=O, C- O, Ca"C, -CH_3, and C-N.

Assay for short-term physical stability

The physical and chemical stability test of the FFH-extract preparations was conducted to determine potential change during storage. This stability test was performed for 1 month at 25°C±2°C with 60%±5% humidity by comparing the pH, viscosity, and swelling ratio parameters.

Table 1. Components of FFH using *N. sativa* and *T. foenum graecum* extracts

Ingredient	Function	Concentration (%)					
		FFH without extract			FFH with *N. sativa* extract and *T. foenum-graecum* Extract		
		F1-0	F2-0	F3-0	F1-E	F2-E	F3-E
N. sativa extract	Active ingredient	-	-	-	10	10	10
T. foenum-graecum extract	Active ingredient	-	-	-	5	5	5
Polyvinyl alcohol	Base	43	40	37	43	40	37
CMCh	Base	43	40	37	43	40	37
Propylene glycol Ethanol 96%	PlasticizerSolvent	104	164	224	104	164	224

Notes:
The formulation comprised 100 grams and replicated 3 times.
F1-0= FFH formulation without active ingredients with 10% plasticizer concentration
F2-0= FFH formulation without active ingredients with 16% plasticizer concentration
F3-0= FFH formulation without active ingredients with 22% plasticizer concentration
F1-E= FFH formulation with active ingredient and 10% plasticizer concentration
F2-E= FFH formulation with active ingredient and 16% plasticizer concentration
F3-E= FFH formulation with active ingredient and 22% plasticizer concentration

Table 2. Results of physical and chemical characterization evaluation of FFH preparation

Assay	Standard	FFH *N. sativa* extract and *T. foenum-graecum* extract		
		F1-Extract	F2-Extract	F3-Extract
pH	5,5±0,5	5,12±0,10	5,15±0,02	5,21±0,05
Viscosity (cps)	6000–50000	23546,33±1,15	23547±1,73	23547,67±0,58
Film Formation Time (minute)	5–7	6.33±0.07	6.25±0.01	6.24±0.01
Swelling Ratio(%)	200–600	472±0.10	531±0.01	526±0.00
Tensile strength (MPa)	5–32	11,00±0,00	14,00±0,00	13,00±0,00
Elongation (%)	30–115	73,33±0,00	66,67±0,00	60,00±0,00

The observation of physical and chemical properties in Table 4 shows that FFH preparation containing active ingredients of *N. sativa* and *T. foenum-graecum* extracts after storage for 30 days from 3 tested formulations was stable. These formulations still meet the requirements of hydrogel preparation contained in SNI 16–4399–1996. The resulting FFH preparation has a pH of 5.13±0.1 to 5.19±0.06, Viscosity 23542, 00±0.00 cps, and swelling ratio 471.00±6.08%-535.67±2.89%.

Drug release assay

Determination of the wavelength of light with maximum absorption was carried out on extract combination with 500 ppm. The results obtained maximum absorption of the extract at a wavelength of 340 nm. Furthermore, a standard

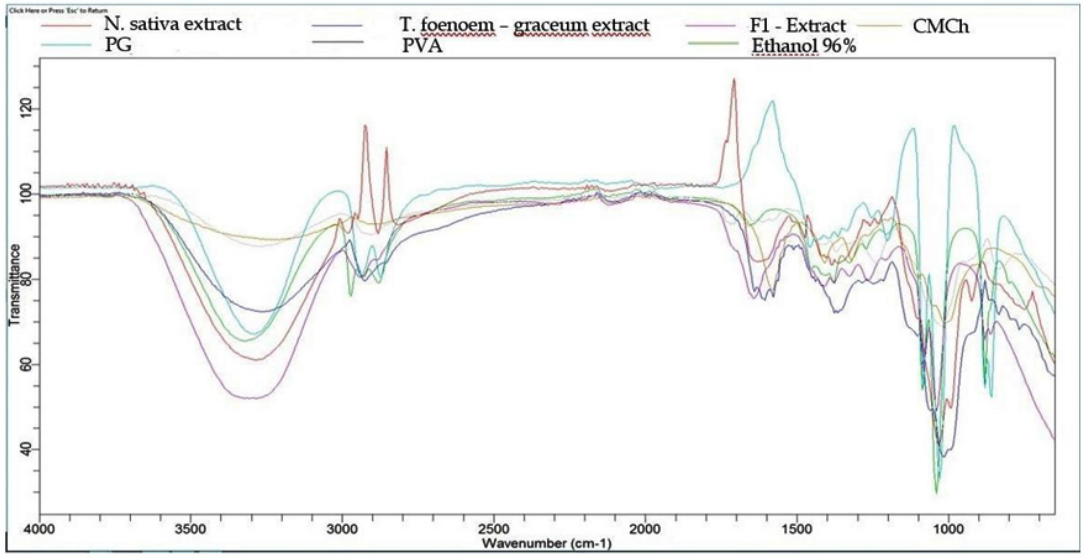

Fig. 1. Infrared spectrum of FFH-extract formulation 1 (F1-Extract). Notes: CMCh (Carboxymethyl Chitosan); PG (Propylene glycol); PVA (Polyvinyl alcohol)

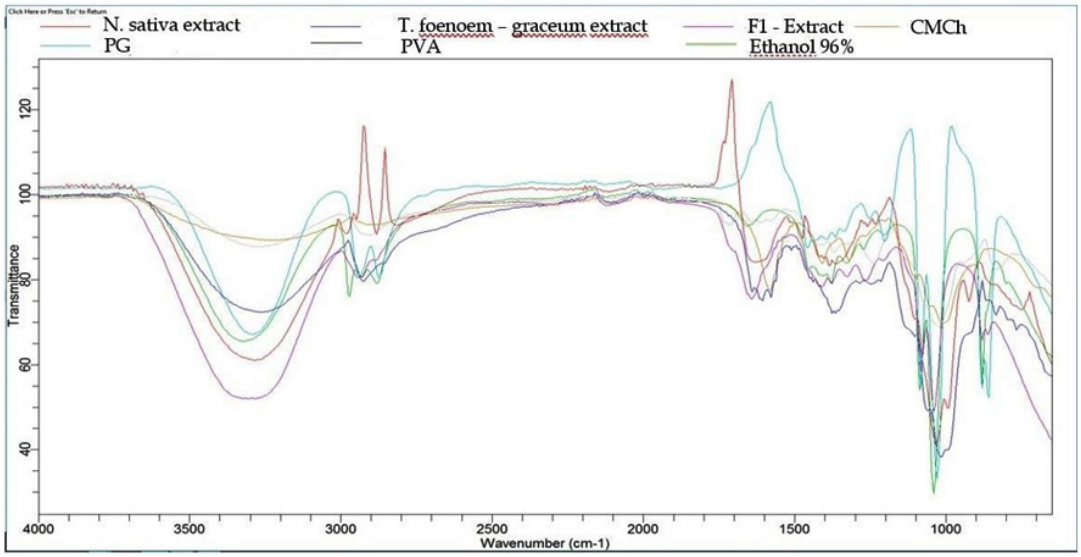

Fig. 2. Infrared spectrum of FFH-extract formulation 2 (F2-Extract). Notes: CMCh (Carboxymethyl Chitosan); PG (Propylene glycol); PVA (Polyvinyl alcohol)

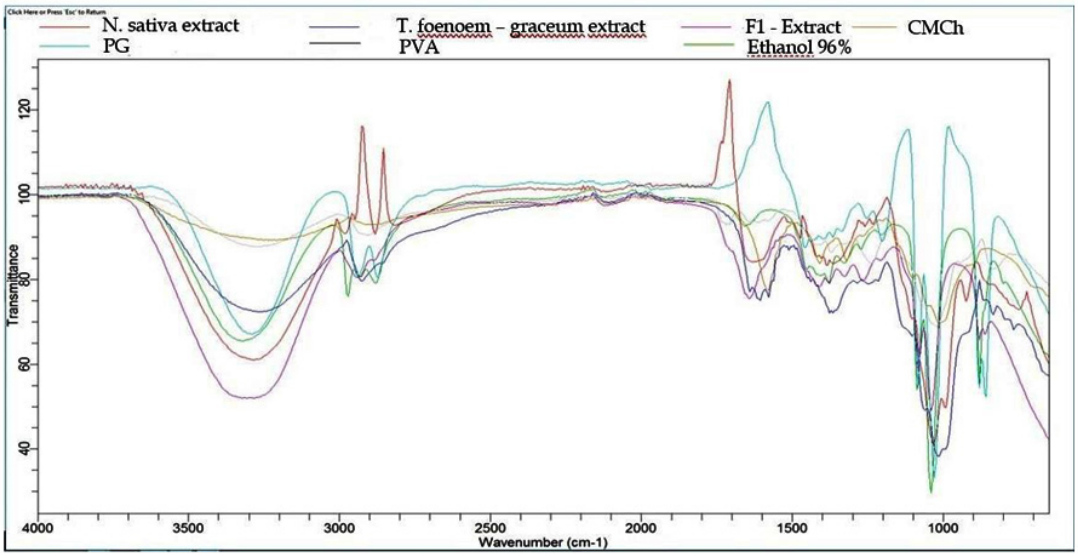

Fig. 3. Infrared spectrum of FFH-extract formulation 3 (F3-Extract). Notes: CMCh (Carboxymethyl Chitosan); PG (Propylene glycol); PVA (Polyvinyl Alcohol)

curve of extract content was obtained with the equation Y = 0.0066x-0.00006 (x = absorbance). The results of the extract release test from FFH showed that within 240 minutes, F1-Extract was able to release 136612.728 µg/cm2 with the amount of extract dissolved per unit area transdermal diffusion membrane of 5.864 ± 0.23 %/cm2 (Table 5).

The identification of functional groups using Infrared Absorption Spectra showed the presence of O-H, C-H, C=O, C=C, and C-O in *N. sativa* extract and *T. foenum- graecum* extract [22]. The results of the infrared spectral of FFH-extracts in this study showed that absorption at wave number 1647 cm-1 of the C=C (from *N. sativa* extract) and at wave number 1449 cm-1 of the C=N (*N. sativa* and *T. foenoem-graecum* extracts) were not visible. Meanwhile, the functional groups in the components that make up FFH preparation have no significant difference from those produced by the microsphere delivery system. In F2-Extract and F3-Extract with 16% and 22% PG content, there was a slight decrease in transmittance value at 1200 cm-1. This showed that the preparation was homogeneously mixed and allowed interaction between the extract in the PVA-CMCh base.

Transdermal Drug Delivery System (TDDS) is a drug preparation that belongs to a controlled delivery system. It is used topically in the form of patches and semisolids such as gels, ointments, or creams. TDDS delivers drugs systemically in a controlled manner, as the molecules diffuse in the transdermal system and penetrate through skin cells.[24]

The fresh FFH preparation produced had a pH of 5.12±0.1 to 5.21±0.05, viscosity of 23546.3±1.15 to 23547.67±0.58 cps, film formation time 6.24±0.01 min -6.33±0.07 min, swelling ratio of 472±0.10 %-531±0.01 %, and remained stable on observation after 30 days storage. The pH value of the preparation determines the comfort of use and ensures stability. Under normal conditions, human skin has a pH between 4–6 which becomes alkaline (7–8) when there is a wound. Since alkaline conditions will inhibit the healing process, FFH preparation must be able to normalize the pH of the skin. Normal wound pH occurs typically in the slightly acidic range of approximately 5.5±0.5.[25] Therefore, FFH preparation in this study has a pH of 5.12–5.21, which is safe from skin irritation, stinging, or itching. This pH range ensures that FFH maintains good stability in short-term and long-term storage.

The viscosity of preparation is very influential on the application and spread of FFH-extract formulation. Low viscosity can

affect the retention of extract on the skin surface. The viscosity value of FFH meets the standard requirements, ensuring that *N. sativa* and *T. foenum-graecum* formulation are easily applied to the skin or wound due to easy flow. The film formation time was measured from when the hydrogel was used until the film was formed. Generally, the topical preparation should dry to create an invisible thin film on the surface within 5–7 minutes, minimizing discomfort for patients.

Swelling ratio testing was carried out to determine the ability to absorb liquid from the FFH-extract formulation. This parameter could specify the hydrogel's ability to absorb wound exudates. The results of the swelling ratio test showed stability in storage time for 1 month. As presented in **Table 3**, the swelling ratio showed the difference between FFH and FFH-extract. The swelling ratio of FFH without extract is higher than FFH with extract, but the difference is insignificant.

Table 3. Analysis results with overlay Fourier transform infrared extract of *N. sativa.*

Sample	Wave number	Function group
N. sativa extract	3285 cm-1	O-H
	2927 cm-1	C-H
	1647 cm-1	C=O
	1550 cm-1	C=N
	1449 cm-1	C=C
	1013 cm-1	C-O
T. foenum-graecum extract	3352 cm-1	O-H
	2871 cm-1	C-H
	2104 cm-1	Ca”C
	1647 cm-1	C=O
	1578 cm-1 and 1550 cm-1	C=N
	1375 cm-1	-CH3
	1319 cm-1 and 1149 cm-1	C-N
	1060 cm-1 and 1023 cm-1	C-O
FFH Formula 1-extract (F1-E)	3352 cm-1	O-H
	2922 cm-1 and 2853 cm-1	C-H
	2117 cm-1	Ca”C
	1647 cm-1	C=O
	1375 cm-1	-CH3
	1149 cm-1	C-N
	1060 cm-1 and 1023 cm-1	C-O
FFH Formula 2-extract (F2-E)	3358 cm-1	O-H
	2922 cm-1 and 2853 cm-1	C-H
	2105 cm-1	Ca”C
	1647 cm-1	**C=O**
	1377 cm-1	-CH3
	1149 cm-1	C-N
	1060 cm-1 and 1023 cm-1	C-O
	3358 cm-1	O-H
FFH Formula 3-extract (F3-E)	2922 cm-1 and 2853 cm-1	C-H
	2105 cm-1	Ca”C
	1647 cm-1	**C=O**
	1377 cm-1	-CH3
	1149 cm-1	C-N
	1060 cm-1 and 1023 cm-1	C-O

Table 4. 30-day short-term testing results

Assay	FFH without extract			FFH *N. sativa* extract and *T. foenum-graecum* extract		
	F1–0	F2–0	F3–0	F1-Extract	F2-Extract	F3-Extract
Assay on Day-15						
pH	5,05±0,05	5,15±0,06	5,22±0,05	5,18±0,01	5,13±0,01	5,19±0,06
Viscocity (cps)	20443,67±1,15	20439,33±5,69	20438,33±6,35	23542,00±0,00	23542,00±0,00	23542,00±0,00
Swelling Ratio (%)	543,00±5,77	538,33±2,08	539,00±4,00	471,00±6,08	535,67±2,89	523,00±0,00
Day-30						
pH	5,04±0,06	5,17±0,06	5,21±0,01	5,20±0,01	5,16±0,05	5,22±0,01
Viscocity (cps)	20442,33±6,43	20444,33±5,51	20439,33±5,51	23543,00±0,00	23543,33±0,58	23543,00±1,00
Swelling Ratio (%)	543,00±5,77	538,33±2,08	539,00±4,00	471,00±6,08	535,67±2,89	523,00±0,00

Mean value±standard deviation (n=3)

Therefore, both formulations remain within range of hydrogel limits that meet the standard requirements.

FFH preparation showed tensile strength of 11.00±0.00 MPa -14.00±0.00 MPa and elongation of 60.00±0.00%-70.33±0.00%. These results suggested that the films formed were soft, tough, and meet the standard requirements. Only F1-Extract meets the minimum standard of 70% for the elongation parameter, while F2-Extract and F3-Extract have less elongation of 66.67% and 60%, respectively. The differences in polymer affect the tensile strength of the film. PG is a plasticizer component with a large enough concentration, showing the potential to be hygroscopic. Therefore, water will be retained in the film and affect the film's weight, thickness, and drying time. Ethanol concentration also affects the film formation time. Since pure ethanol is volatile, it is commonly used in the development of FFH formulation as a solvent.[26]

PVA is a synthetic water-soluble hydrophilic polymer with excellent film-forming ability. It has good biodegradable, non-toxic, non-carcinogenic, and biocompatible properties, high hydrophilicity, good chemical resistance, and bioadhesive.[27] The good biocompatibility of PVA shows potential for wide application in the biomedical field as wound management, drug delivery systems, artificial organs, and

Table 5. Dissolution of extract from F1-Extract

Time (minute)	Amount of extract dissolved per unit area transdermal diffusion membrane (%/cm^2)	
	Mean	SD
0	0.008	0.00
20	0.385	0.20
40	0.650	0.25
60	0.953	0.21
80	1.330	0.13
100	1.695	0.05
120	2.085	0.06
140	2.508	0.15
160	2.955	0.30
180	3.683	0.09
200	4.395	0.05
220	5.134	0.12
240	5.864	0.23

contact lenses.[28] However, single PVA has rigid properties that need to be more elastic, which limits application in the wound healing process.[29] Other studies have shown that the combination of PVA with natural or synthetic polymers such as chitosan, CMCh, PVP, dextran, and collagen can improve the physicochemical, mechanical, and biological properties of PVA hydrogels.[28] The results of this study proved that the use of PVA and CMCh in a 1:1 ratio produced excellent outcomes.

CMC has antioxidant and antimicrobial properties, excellent biocompatibility, the ability to withstand humid conditions, and high antimicrobial effectiveness.[30,31] The addition of CMC affects the structure of the porous network on the surface texture of the hydrogel. During this process, CMC molecules will enter into PVA, causing the structure to become looser.[32] Therefore, adding CMCh affects the hydrogel's swelling ability and release rate of active ingredients. To address this challenge, a plasticizer is used in FFH to enhance flexibility and improve the tensile strength of film. The selected plasticizer must be compatible with the polymer and have low permeability. Common plasticizers are glycerol, polyethylene glycol, sorbitol, dibutyl phthalate, PG, triethyl citrate, and others.[8]

The tensile strength and elongation test results of FFH-extract in **Table 2** show an increasing value with high plasticizer concentration in F1-Extract and F2-Extract. However, a decrease is observed in F3-Extract with a plasticizer concentration of 22%. Integrating PVA and CMCh as polymers and PG as plasticizers produced excellent outcomes. Among all formulations, F2-Extract experienced an increase in tensile strength and a decrease in elongation. The increase in tensile strength of F2-Extract compared to F1-Extract was 3 MPa for the tensile strength value. There was no increase in the tensile strength of F3-Extract due to the plasticizer concentration being smaller than the polymer. Furthermore, the interaction between the polymer and plasticizer was not sufficient, showing the need for further investigation.

In FFH preparation, the solvent must be able to dissolve the polymer and active drug ingredients. The selection of solvent affects the drug flux, depending on the nature of the solvent and the presence of permeation enhancers, despite the short contact time with the skin surface. Drying speed is also a factor in selecting the type of solvent to improve patient compliance. Ethanol is a suitable solvent for FFH because it is volatile and can function as a permeation enhancer. The development of ethanol as a solvent in FFH at 8% concentration showed the fastest drying time. Increasing ethanol concentration in the formulation showed high strength and adhesive properties of the film without affecting mechanical properties.[8] Furthermore, 96% ethanol at a concentration of 4% produced FFH preparation according to SNI standards with a formation time of 6.2–6.3 minutes.

The use of PVA in the hydrogel preparation causes the formation of an occlusive film on wound surface to keep the surrounding area moist and accelerate healing time by approximately 40%. Additionally, moist conditions help the hydration process of dead tissue, prevent irritation, reduce pain, and improve patient compliance.[33] The drug release test results showed that F1-Extract could release the drug gradually, with the amount of dissolved extract increasing along with the observation time. This suggested that F1-Extract could be considered for treatment. However, in vivo tests should be conducted to confirm the efficacy of F4 F1-Extract on wound healing process.

CONCLUSION

In conclusion, the results showed that the most effective formulation for creating FFH-extract using *N. sativa* and *T. foenum-graecum* consisted of PVA- CMCh in a 1:1 ratio as the polymer, 10% PG as the plasticizer, and 96% ethanol as the solvent. This formulation produced FFH-extract with excellent uniformity and stability.

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Conflict of Interest

All the authors declare that there are no conflicts of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author contributions

Retno Susilowati: Conceptualization, methodology, supervised the project, writing – original draft, review and editing; Rahmi Annisa: Conceptualization, methodology, writing – original draft, review and editing; Maharani Retna Duhita: Data collection, analysis, review & editing; Evika Sandi Savitri: visualization, validation, review & editing.

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