CHAPTER 4

RESULTS AND DISCUSSION

4.1 Quality control of longan seed extract by HPLC

Longan seed extract was identified by matching their retention times with standard gallic acid. The calibration curve of standard gallic acid was linear with r^2 value of 0.990 (**Figure 4.1**). The HPLC fingerprint of phenolic compounds from the longan seed extract were shown in **Figure 4.3**. The peak of the standard gallic acid was at the retention time of 11.007 min (**Figure 4.2)**. The peak presented in the chromatogram of the longan seed extract (500 µg/ml) was at the retention time of 11.035 min which represented gallic acid. The percentages of the gallic acid contents containing in the longan seed extract were determined by the calculating of peak area as shown in **Table 4.1.**

Table 4.1 Percentages of gallic acid contents from longan seed extract

Samples	Retention time (min)	Percentages of gallic acid content
Standard gallic acid	11.007	100.00 ± 0.85 %
Longan seed extract	11.035	8.89 ± 0.71%

The data from the extract supplier (Prima Herb Thailand Co., Ltd) mentioned the gallic acid content of the longan seed extract should be presented 9.06%. The content of gallic acid from the extract was 8.89 % ± 0.71 (The value varies not less than 5-10%) which approximate to the requirement data.

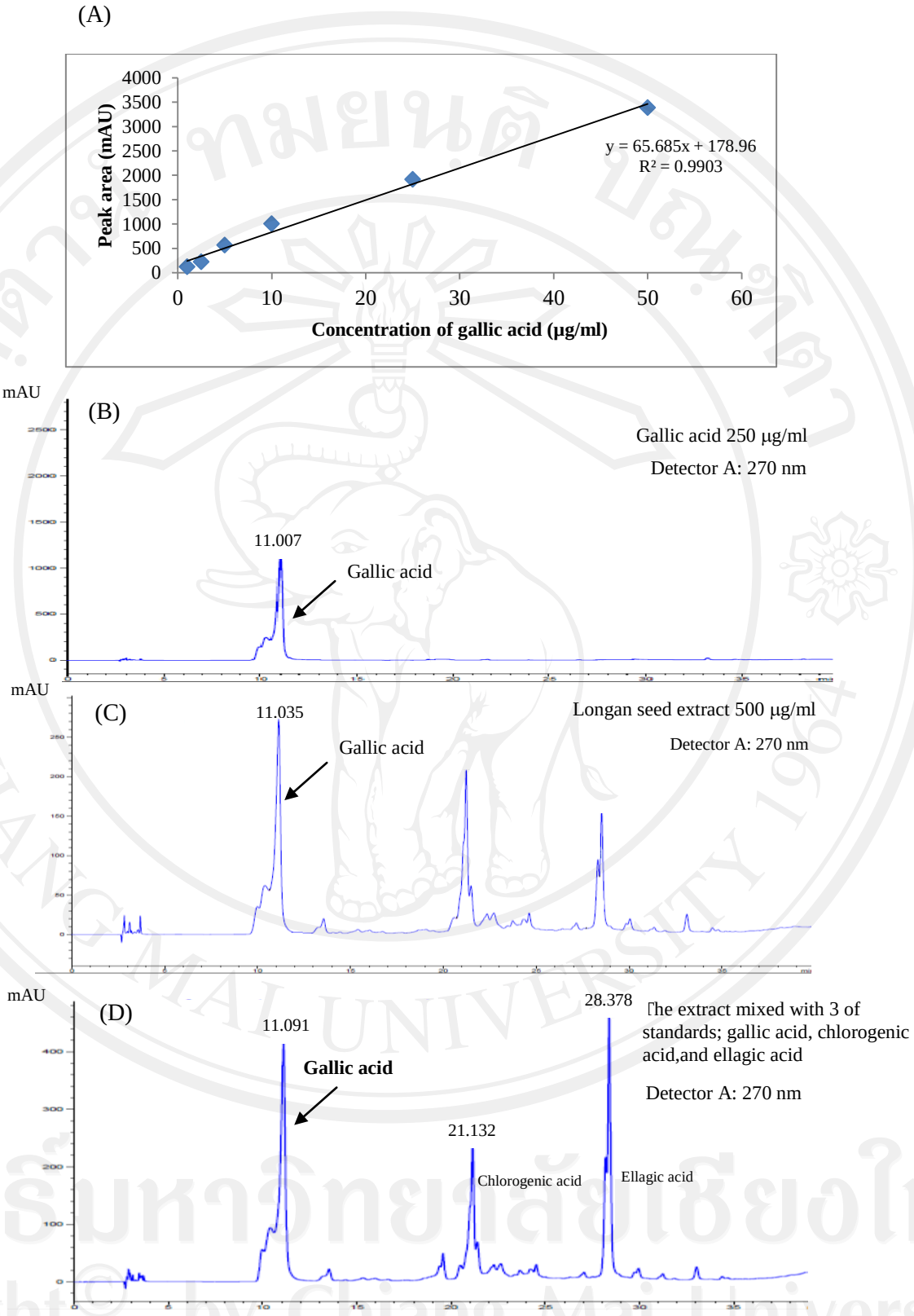


Figure 4.1 Chromatogram of standard gallic acid and longan seed extract (A) The standard curve of gallic acid, (B) Chromatogram of standard gallic acid (250 µg/ml) at the retention time of 11.007 min. (C) Chromatogram of longan seed extract (500 µg/ml) that gave the gallic acid peak at the retention time of 11.035 min. (D) Chromatogram of the extract mixed with 3 of standards; gallic acid, chlorogenic acid,and ellagic acid.

4.2 Preformulation studies of longan seed extract

Physicochemical characterization

Solubility test of longan seed extract

The solubility of longan seed extract was determined in various solvents and ratios (**Table 4.2**). The results showed that the extract was completely soluble in propylene glycol and DMSO in the ratio of 1:10, 1:20 and 1:30 (w/v) and completely soluble in PEG 400 in the ratio of 1:30 (w/v) as shown in **Figure 4.2**. Otherwise the extract was sparingly soluble (1:20 and 1:30) in DI water, glycerin, 10% tween 80 and acetic acid (**Figure 4.3**).

Table 4.2 Solubility of longan seed extract

Solvent	**Solubility of longan seed extract in various ratios of solvent**			
	1:1	**1:10**	**1:20**	**1:30**
DI water	Insoluble	Insoluble	Sparingly soluble	Sparingly soluble
Glycerin	Insoluble	Insoluble	Sparingly soluble	Sparingly soluble
Propylene glycol	Sparingly soluble	Soluble	Soluble	Sparingly soluble
10% tween 80	Insoluble	Insoluble	Sparingly soluble	Sparingly soluble
DMSO	Sparingly soluble	Soluble	Soluble	Soluble
PEG 400	Insoluble	Insoluble	Sparingly soluble	Soluble
Acetic acid	Insoluble	Insoluble	Sparingly soluble	Sparingly soluble

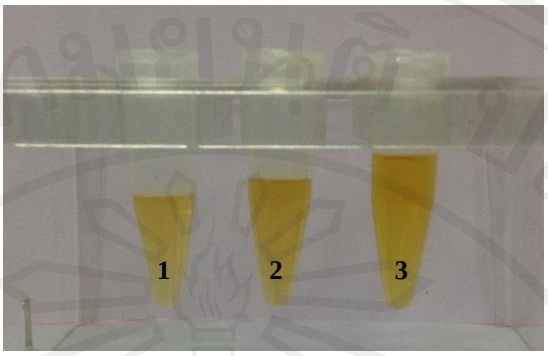


Figure 4.2 The solubility of longan seed extract in solvents (1= DMSO, 2 = propylene glycol and 3=PEG 400)

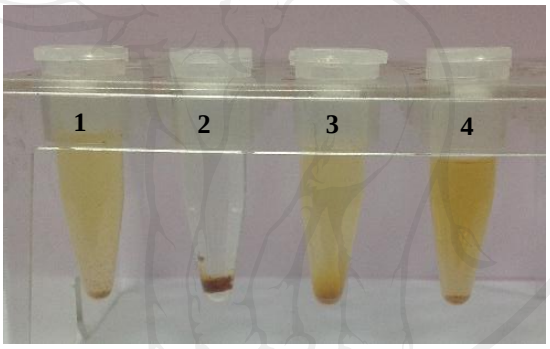


Figure 4.3 The solubility of longan seed extract in solvents (1= DI water, 2 = glycerin, 3 = 10% tween 80, 4 = acetic acid)

Acid/base stability test of longan seed extract

The stability of longan seed extract solution was determined by dissolving the extract in soluble solvents; propylene glycol, DMSO and PEG 400 at various pH (3-8). These were determined under room temperature, room temperature no light, 4°C and 45°C for 1 month and at accelerated test: heating/cooling cycling method for 6 cycles. From this study, it was found that all of the extract solutions were stable at pH 3-6, but exhibited a dark brown color at pH 7-8. All of the extract was stable under room

temperature, room temperature no light, 4°C and heating/cooling cycling for 6 cycles. For 45°C, the extract solution presented dark brown in all solvents so it was indicated that logan seed extract was unstable at 45°C. These results were demonstrated in **Table 4.3-4.5**. According to these results, the appropriate storage temperature for the extract should not be at high temperature (≥45°C) and it should be suitable kept at pH 3-6.

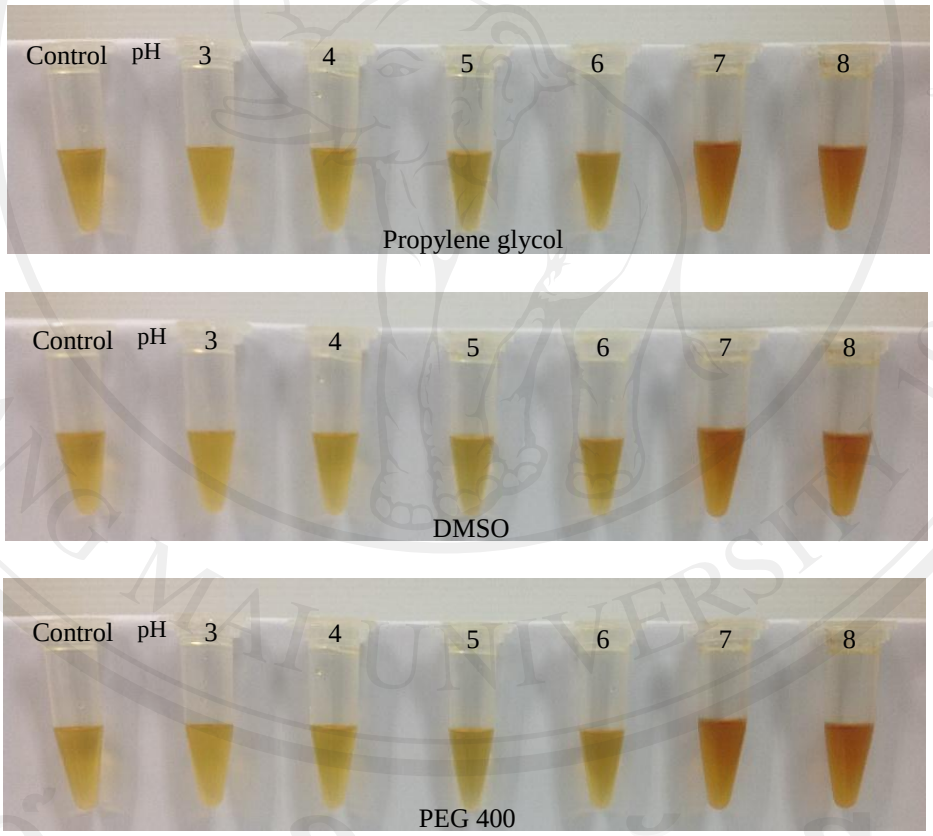


Figure 4.4 Longan seed extract dissolved in various solvents at pH 3-8

Table 4.3 Stability test of longan seed extract dissolved in propylene glycol at pH 3-8

Solvent	Test condition	Physical characteristics					
		pH 3	**pH 4**	**pH 5***	**pH 6**	**pH 7**	**pH 8**
Propylene glycol	**Day 0**	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Room temp.	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Room temp. (no light)	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	4°C	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	45°C	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Heating-Cooling	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate

* Control extract solution pH = 5

Table 4.4 Stability test of longan seed extract dissolved in DMSO at pH 3-8

Solvent	Test condition	Physical characteristics					
		pH 3	**pH 4**	**pH 5***	**pH 6**	**pH 7**	**pH 8**
DMSO	**Day 0**	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Room temp.	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Room temp. (no light)	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	4°C	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	45°C	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Heating-Cooling	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate

* Control extract solution pH = 5

Table 4.5 Stability test of longan seed extract dissolved in PEG 400 at pH 3-8

Solvent	Test condition	Physical characteristics					
		pH 3	**pH 4**	**pH 5***	**pH 6**	**pH 7**	**pH 8**
PEG 400	**Day 0**	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Room temp.	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Room temp. (no light)	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	4°C	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	45°C	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate
	Heating-Cooling	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Brown - No precipitate	- Dark brown - No precipitate	- Dark brown - No precipitate

Note: Control extract solution pH = 5

4.3 Evaluation of preliminary study of transdermal patch bases properties

The transdermal patches were prepared by incorporating HPMC, squid chitosan and PVP K90 as polymers along with PEG 400, propylene glycol or glycerin as plasticizer. Physical characteristics of the prepared patches are shown in **Table 4.6**. All the formulations were homogeneous and smooth films. For the films without plasticizer, the formulation F4 has shown higher flexibility than others. This could be due to the increasing of cross-linked structure in HPMC that caused the increase in their flexibility. Formulation F1, F2, F3 and F5 presented the brittleness of the films. Therefore formulation F4 was selected by adding plasticizers for better flexibility. The formulation F4-PG (containing HPMC, squid chitosan and PVP K-90 in the ratio of 12:3:1 with propylene glycol as plasticizer) revealed the appropriate physical properties, being colorless, good flexibility, smooth and ease to remove.

The transdermal patch bases with good physical characteristics were determined for their tensile strength, percentage elongation. Formulations containing HPMC, squid chitosan and PVP K-90 (12:3:1) with different type of plasticizers and penetration enhancers were selected to determine their mechanical properties. Comparing among the plasticizers, the result revealed that the tensile strength and percentage elongation at break of the film containing propylene glycol was higher than the films containing PEG 400 and glycerin (data not shown). It is obviously showed that propylene glycol was the best plasticizer for the combination of HPMC, PVP K-90 and squid chitosan. However, the preliminary stability test of formulation F4-PG showed good appearances only 1 month and its adhesiveness expressed in term of poor after the stability test (data not shown). Therefore, the transdermal patch base was going to develop for further study.

Table 4.6 Physical appearances of the transdermal patch bases

Code	Physical appearances	
	Color	Flexibility
F1	Yellowish	-
F2	Yellowish	+ 1
F3	Yellowish	+ 1
F4	Colorless	+ 2
F5	Yellowish	+ 1
F4-PEG	Colorless	+ 4
F4-PG	Colorless	+ 3
F4-GC	Colorless	+ 2
F4-PG-D	Colorless	+ 4
F4-PG-L	Colorless	+ 4
F4-PG-O	Colorless	+ 4

Note: The symbols (-) and (+) represent no appearance and appearance, respectively. The number of (+) symbols indicates the degree of the appearance; 1 is very slightly, 2 is slightly, 3 is moderate, 4 is strong and 5 is very strong.

4.4 Evaluation of transdermal patch bases properties

4.4.1 Physical appearances

4.4.1.1 Screening of polymer types

According to the poor adhesiveness and short stability of the transdermal patch base (F4-PG), the new polymer types screening was resolved again. The first screening of suitable formulation, a selection of polymers from different sources was tested in the formulation experiments. Various types of polymers were combined to determine the best composition based on physical appearances. The suitable formulation consisted of Eudragit® NE 30 D, squid chitosan and HEC in the

ratio of 1:1:1. The blended polymer solutions did not show any sign of incompatibility. **Table 4.7** showed the physical appearances of the transdermal patch bases. The polymer solution of Eudragit® NE 30 D, squid chitosan and HEC presented higher viscosity and more flexible film than others. In contrast, the formulations that consist of Eudragit® NE 30 D combined with shrimp chitosan and HEC presented hard and brittle films as well as the combination of Eudragit® NE 30 D with shrimp chitosan and HPMC. Moreover, the combination of Eudragit® E 100 with other polymers exhibited too stiff films and left more residues after removal.

Table 4.7 Physical appearances of the transdermal patch bases

Types of polymer Syn: Nat: Semi-syn (1:1:1)*	**Physical appearances**		
	Color	Flexibility	Residues after removal
Eu NE: Sq: HPMC	Yellowish	+1	+3
Eu NE: Sq: HEC	Yellowish	+2	+2
Eu NE: Sh: HPMC	Colorless	+1	+3
Eu NE: Sh: HEC	Colorless	+1	+3
Eu E100: Sq: HPMC	Yellowish	+1	+4
Eu E100: Sq: HEC	Yellowish	+1	+4
Eu E100: Sh: HPMC	Colorless	+1	+4
Eu E100: Sh: HEC	Colorless	+1	+4

Note: The symbols (-) and (+) represent no appearance and appearance, respectively. The number of (+) symbols indicates the degree of the appearance; 1 is very slightly, 2 is slightly, 3 is moderate, 4 is strong and 5 is very strong.

Eudragit® NE 30 D and E 100 have been extensively used in transdermal drug delivery system because of their controlling drug delivery which is independent on pH medium [44]. Eudragit® NE 30 D is an aqueous dispersion which is environment-friendly but Eudragit® E100 is dissolved in organic solvent. Moreover, in previous study, the film of Eudragit® NE 30 D was sufficiently flexibility without adding more plasticizers whereas E 100 film was hard and brittle. Moreover, Eudragit® NE 30 D offers the optimizing adhesion property [45].

Chitosan has received an interest in medical and pharmaceutical applications due to its beneficial intrinsic properties. Chitosan has excellent film-forming properties as well as a potential for controlling drug release [46]. According to the polymer solution of squid chitosan was highly viscous than shrimp chitosan resulting in high flexibility, squid chitosan was then selected to develop transdermal patches. The pacth obtained from squid chitosan was very flexible whereas that obtained from shrimp chitosan was hard and brittle. This may cause a difference in the arrangement of the polymer chain within the chitosan structure. The structure of chitosan is related to the structure of chitin. According to chitin, it was classified into three different forms as α-, β- and γ-chitin. The α-form exhibits an anti-parallel orientation that found in shrimp and crab chitin. The β-form is arranged in a parallel manner that found in squid chitin whereas the γ-form sets of two parallel strands alternating with single anti-parallel strand (mushroom and fungal mycelia). The parallel arrangement reduces the packing tightness and the numbers of inter-chain hydrogen bonds, resulting in an increased number of hydrogen bond with water [47]. This leads to a more flexible and soft polymeric structure of squid chitosan patch. In addition, HEC is recognized as materials supporting chitosan to obtain material with sufficient mechanical strength of

the films prepared. Therefore, optimal properties cannot be achieved for a single polymer. It necessary to used the combination between synthetic polymer and natural polymer to improve physical appearances. The blending of Eudragit® NE 30 D, squid chitosan and HEC was done in optimizes performances. The physical appearances of the films were depended on types and quantity of polymers in the formulations. The chemical structure and chain alignment of each component affected the physical cross-linking of the films.

4.4.1.2 Screening of polymer ratios

From the formulation that obtained in Part 4.3.1.1, the patch was then developed by using the selected polymers in different ratios. The selected polymers (Eudragit® NE 30 D, squid chitosan and HEC) were varied in the ratio of three combined polymers into 5 formulations (P1-P5). The physical appearances of the prepared patches which evaluated by visualization are shown in **Table 4.8**. Formulation P2 which consists of Eudragit® NE 30 D: squid chitosan: HEC (4:2:4) has a higher flexibility compared to other formulations with only slightly residues after removal.

Table 4.8 Physical appearances of the transdermal patch Formulation P1-P5

Formulation No.	Ratio of polymers Eu NE: Sq: HEC*	Physical appearances		
		Color	Flexibility	Residue after removal
P1	4: 1: 5	Yellowish	+2	+2
P2	4: 2: 4	Yellowish	+3	+1
P3	4: 3: 3	Yellowish	+2	+2
P4	4: 4: 2	Yellowish	+1	+2
P5	4: 5: 1	Yellowish	+1	+2

* Eu NE = Eudragit® NE 30 D, Sq = Squid chitosan and HEC = Hydroxyethylcellulose

4.4.1.3 Effect of plasticizers

As the varying polymer ratios, the suitable formulation was selected to add plasticizers (Propylene glycol and triethyl citrate). The physical appearances of the formulation 1A-3C were shown in **Table 4.9**. Formulation 3C which containing 25% of propylene glycol together with 15% of triethyl citrate (**Figure 4.5**) exhibited the best all physical appearances. The result indicted that the increasing in concentration of two plasticizers yielded an increasing in flexibility. However, the formulation that containing high amount of both plasticizers (propylene glycol > 25% and triethyl citrate > 15%) became too weak film (data not shown).

Figure 4.5 Transderamal patch Formulation 3C

Table 4.9 Physical appearances of the transdermal patch Formulation 1A-3C

Formulation No.	**Physical appearances**		
	Color	**Flexibility**	**Residue after removal**
1A	Yellowish	+1	-
2A	Yellowish	+3	-
3A	Yellowish	+4	-
1B	Yellowish	+2	-
2B	Yellowish	+3	-
3B	Yellowish	+4	-
1C	Yellowish	+2	-
2C	Yellowish	+3	-
3C	Yellowish	+5	-

4.4.2 Thickness uniformity

The thickness of all the patches was varied from 0.4516 ± 0.0024 mm to 0.6164 ± 0.0022 mm. The minimum standard deviation values in the film thickness ensured uniformity of the patches.

4.4.3 Mechanical properties

4.4.3.1 Screening of polymer ratios

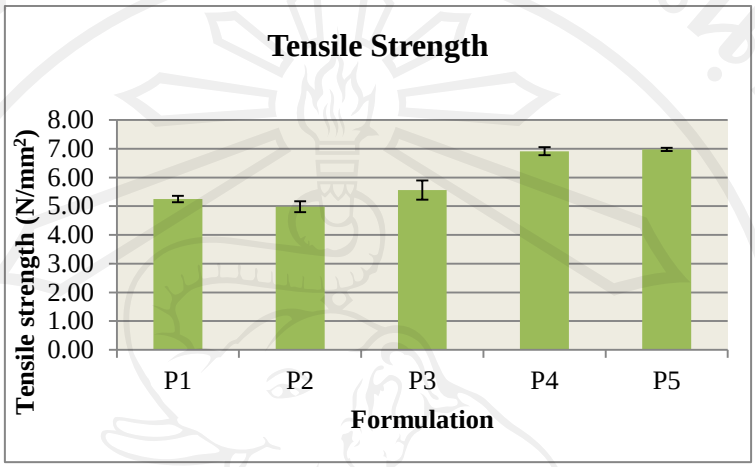
The strength and elasticity of the prepared patches are usually determined by the tensile strength and percentage elongation at break values, respectively. The suitable films for application as transdermal patch should be flexible enough to follow the movements of the skin without breaking. However, the films must show an increased strength to prevent abrasion of the films during contact with clothing [48]. The results of tensile strength and percentage elongation at break of the test patches are shown in **Figure 4.6 A** and **4.6 B,** respectively.

Table 4.10 Mechanical properties of the transdermal patches Formulation P1-P5

Formulation No.	Ratio of Eu NE: Sq: HEC	Tensile strength (N/mm^2) ± SD	% Elongation ± SD
P1	4: 1: 5	5.25 ± 0.11	30.73 ± 0.67
P2	4: 2: 4	4.98 ± 0.19	37.62 ± 0.34
P3	4: 3: 3	5.56 ± 0.33	25.40 ± 0.76
P4	4: 4: 2	6.92 ± 0.14	13.27 ± 0.54
P5	4: 5: 1	6.98 ± 0.05	10.50 ± 0.66

Note: Eu NE = Eudragit® NE 30 D, Sq = Squid chitosan and HEC = hydroxyethylcellulose

A



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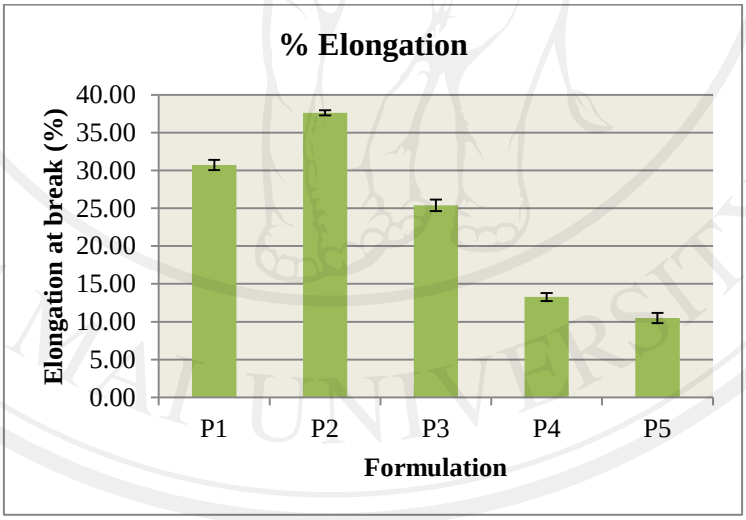


Figure 4.6 Mechanical properties of the transdermal patches Formulation P1-P5

(A) Tensile strength; (B) Percent elongation at break

Comparing among the polymer ratios, the results revealed that the patch containing Eudragit® NE 30 D, squid chitosan and HEC in the ratio of 4: 2: 4 (Formulation P2) showed the highest percentage elongation at break of 37.62 ± 0.34% with the tensile strength of 4.98 ± 0.19 Kg/mm^2 (**Table 4.9**). The blending Eudragit® NE 30 D together with increasing HEC and decreasing squid chitosan in the formulations were able to soften the brittle patches into flexible patches (Formulation P1-P2). The formulations containing too high content of chitosan (P4-P5) probably resulted in the compactness of the polymer macromolecule, consequently difficulty in moving the polymer molecules and decreasing in their flexibility [43]. However, the high ratios of HEC trended to increase the weakness of the patch and decreased tensile strength value. The addition of HEC into the formulations tends to decrease the compactness of the polymeric network. Unfortunately, a high ratio of HEC (Formulation P1) resulted in too weak polymeric network. Therefore, the optimum concentration ratio of blended Eudragit® NE 30 D- squid chitosan-HEC will result in a stronger intermolecular bonding with the polymer network [43].

4.4.3.2 Effect of plasticizers

According to the good physical appearances including flexibility, strength and residue after removal of the optimal transdermal patch was selected to determine their mechanical properties. Plasticizers are strong influence on their mechanical properties, resulting in higher flexibility and reduced brittleness. As unsuitable concentration of the plasticizers in the patch formulations, relatively brittle films and need to be handled very carefully. Therefore, the combination between propylene glycol and triethyl citrate were used as plasticizers at different

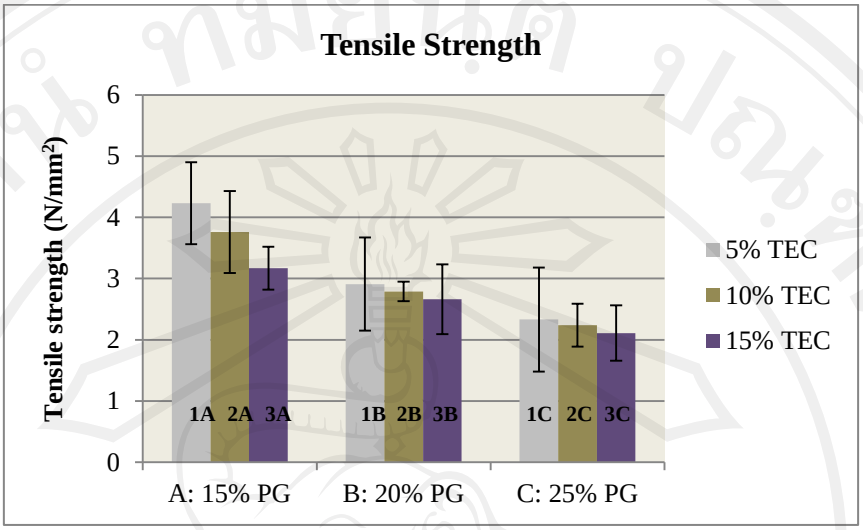
concentrations. **Figure 4.7 and Table 4.10** showed the tensile strength and percentage elongation at break of the test patches.

Table 4.11 Mechanical properties of the transdermal patches Formulation 1A-3C

Formulation No.	Tensile strength (N/mm^2) ± SD	% Elongation ± SD
1A	4.23 ± 0.67	40.43 ± 0.34
2A	3.76 ± 0.87	50.17 ± 0.66
3A	3.17 ± 0.35	55.33 ± 0.66
1B	2.91 ± 0.76	52.56 ± 0.44
2B	2.79 ± 0.67	58.88 ± 0.87
3B	2.66 ±0.57	64.43 ± 0.78
1C	2.33 ± 0.85	60.45 ± 0.65
2C	2.24 ± 0.16	63.43 ± 0.29
3C	2.11 ± 0.45	69.88 ± 0.85

The results showed that with an increasing in the concentration of plasticizers, decreasing in tensile strength but increased percentage elongation at break of the films were obtained. The tensile strength values decreased from 4.23 ± 0.67 to 2.11 ± 0.45 N/mm^2, while percentage elongation values increased from 40.43 ± 0.34 % to 69.88 ± 0.45 %. The patch containing 25% of propylene glycol along with 15% of triethyl citrate presented the highest percentage elongation at break of 69.88 ± 0.45 % with the tensile strength of 2.11 ± 0.45 Kg/mm^2. The changes in mechanical properties were characterized by the plasticizers weakening the intermolecular forces between the chains of adjacent macromolecule allowed loosening of tightness of the intermolecular forces. Therefore, the increasing in plasticizer concentration causes a reduction of the tensile strength because the intermolecular interactions were decreased [49].

A



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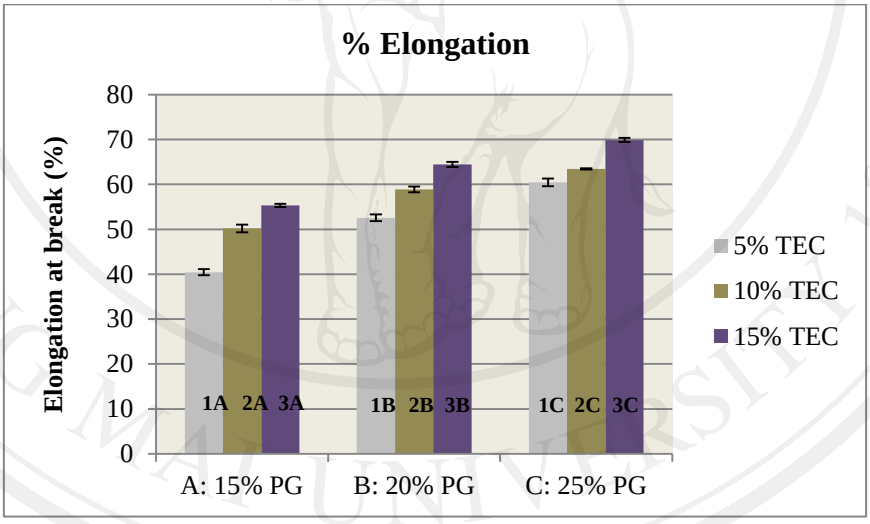


Figure 4.7 Mechanical properties of the transdermal patches Formulation 1A-3C

(A) Tensile strength; (B) Percent elongation at break

4.4.4 Adhesive test (Modified Rolling Ball Tack Tester)

4.4.4.1 Screening of polymer ratios

The tack adhesion values of the patches formulation P1-P5 compared to commercial patch are shown in **Figure 4.8** and **Table 4.11**. In comparison between the adhesive properties of the patches that different in polymer ratio, it was found that the highest to the lowest tack values were as follow: P5 > P4 > P1 > P3 > P2. The tack value tells about the immediate adhesion on a sample. If the tack value is very high, then the adhesiveness is normally rather low. In other words, highly adhesiveness of the sample has a lower tack value. When comparing among the Formulations P1-P5, it was indicated that Formulation P2 was the greatest adhesive property (7.98 ± 0.69 cm).

Although chitosan has an adhesive property result from the interaction between positive charges of chitosan and negative charges of skin, the formulations containing too high content of chitosan had lower flexibility resulted in decreasing their adhesion [44]. It is refered that the flexibility of the transdermal patch affected the adhesive property of the skin. However, the transdermal patch Formulation P1 had about 2 times higher tack value comparing with the commercial patch test.

Table 4.12 Tack values of the transdermal patch Formulation P1-P5

Formulation No.	Ratio of Eu NE: Sq: HEC	Tack (cm) ± SD
P1	4: 1: 5	8.88 ± 0.55
P2	4: 2: 4	7.98 ± 0.69
P3	4: 3: 3	8.44 ± 0.98
P4	4: 4: 2	10.34 ± 0.47
P5	4: 5: 1	10.48 ± 0.66
Commercial patch	-	3.50 ± 0.23

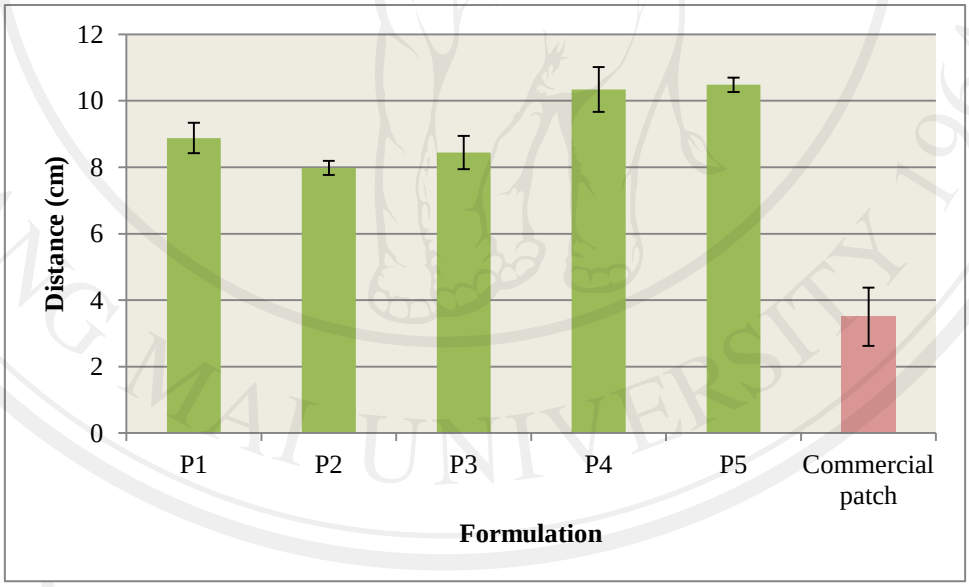


Figure 4.8 Tack values of the transdermal patch Formulation P1-P5

4.4.4.2 Effect of plasticizers

The adhesiveness evaluation of the transdermal patches consisted of two plasticizers; propylene glycol and triethyl citrate in various concentrations. The tack adhesion values of the patches formulation 1A-3C compared to commercial patch are displayed in **Figure 4.9**. The binary blending of selected plasticizers along with their increasing content in the formulations resulted in increase of the adhesion ability. In general, triethyl citrate not only acted as the plasiticizer, but also acted as the tackifier in the formulation. Without triethyl citrate, the patch could not adhere or remain on the skin.

Additionally, the flexibility of the transdermal patch related the adhesive property of the skin. The increasing flexibility of the transdermal patch can improve the contact between the patch and the skin. Adding plasticizers also decreased tack value leading to better adhesiveness as shown **Table 4.12**.

Table 4.13 Tack values of the transdermal patch Formulation 1A-3C

Formulation No.	**Tack (cm) ± SD**
1A	8.13 ± 0.78
2A	8.02 ± 0.65
3A	7.86 ± 0.78
1B	6.11 ± 0.90
2B	5.18 ± 0.78
3B	4.88 ± 0.66
1C	6.14 ± 0.77
2C	5.10 ± 0.89
3C	4.54 ± 0.88

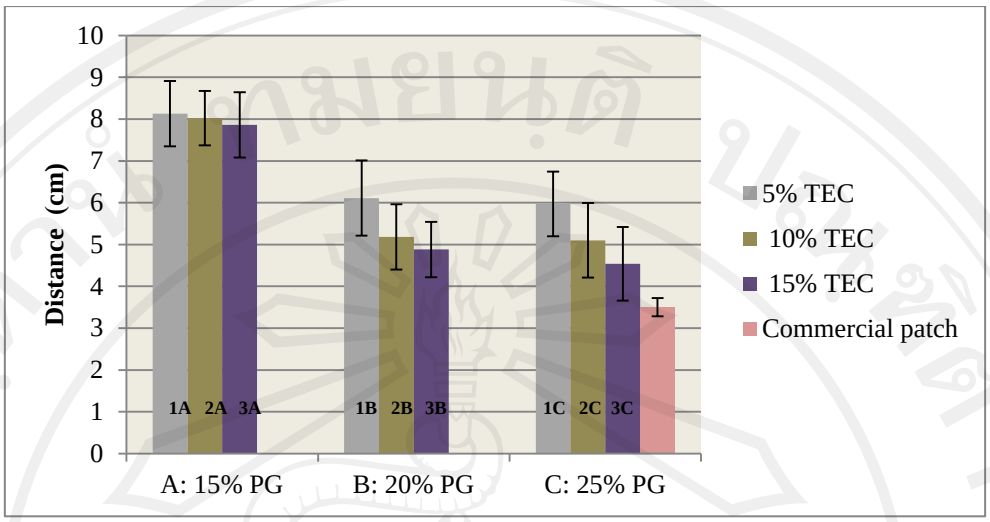


Figure 4.9 Tack values of the transdermal patch Formulation 1A-3C

4.5 Evaluation of transdermal patches containing longan seed extracts properties

4.5.1 Thickness uniformity

Thickness of patches containing longan seed extracts were varied from 0.423 ± 0.0056 mm to 0.426 ± 0.0028 mm. The minimum standard deviation values in the film thickness ensured uniformity of the patches.

4.5.2 Physical appearances

Physical appearances of transdermal patches containing longan seed extract along with DMSO (3C-D-E), oleic acid (3C-O-E) or lemon oil (3C-L-E) as penetration enhancer were shown in **Table 4.13**. Any sign of incompatibility including separation or precipitation from the formulations did not appear. The color of all formulations presented brown after longan seed extract incorporation. The flexibility of the longan transdermal patches was slightly decreased comparing with the transdermal patch formulation 3C.

Table 4.14 Physical appearances of the transdermal patches containing longan seed extract

Formulation No.	Physical appearances			
	Picture	Color	Flexibility	Residues after removal
3C-D-E		Brown	+ 4	+ 1
3C-L-E		Brown	+ 4	-
3C-O-E		Brown	+ 4	+ 1

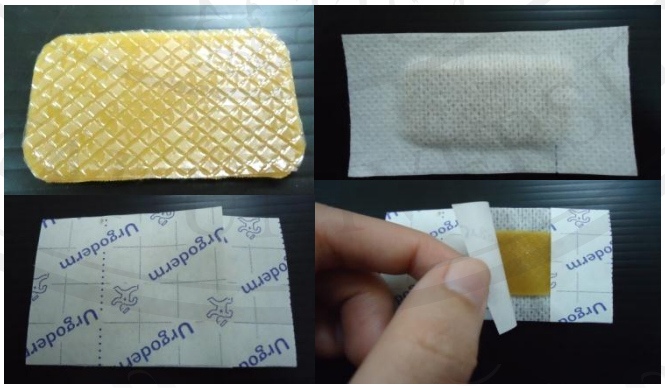
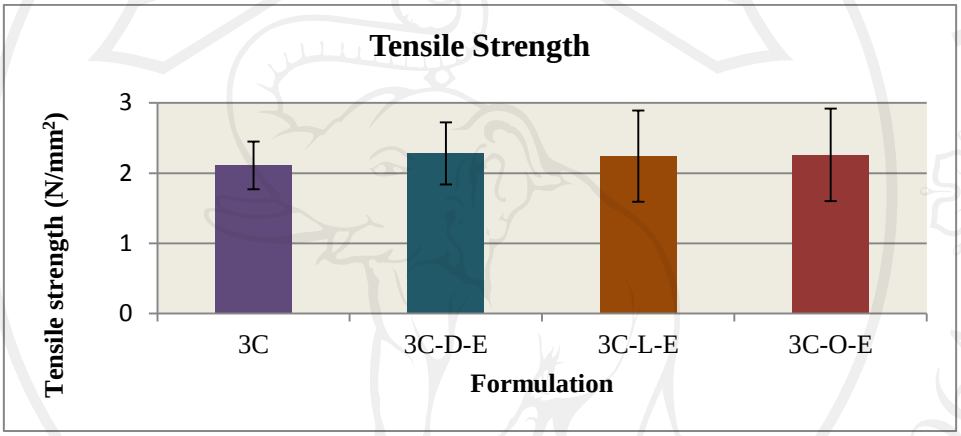


Figure 4.10 Transdermal patch containing longan seed extract

4.5.3 Mechanical properties

As the formulation 3C exhibited preferable strength and flexibility, it was selected to develop as the transdermal patch containing longan seed extract. The results of tensile strength and percentage elongation at break of the transdermal patches containing longan seed extract were shown in **Figure**.**4.11**.

A



B

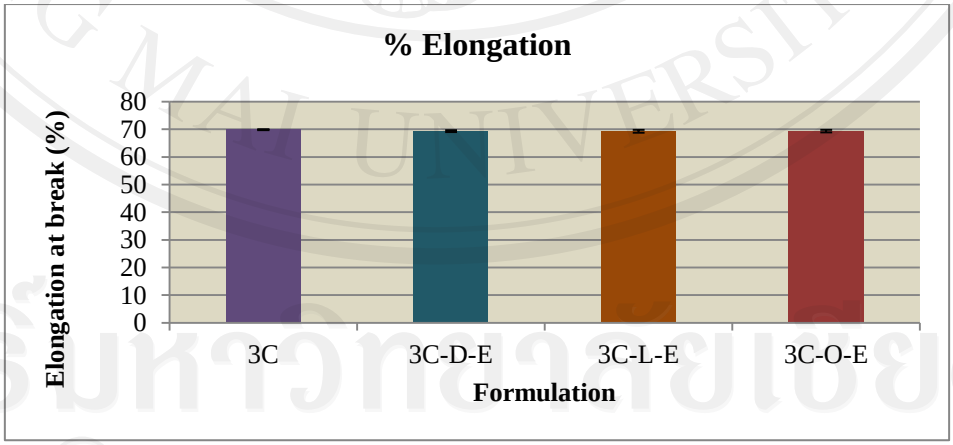


Figure 4.11 Comparison of mechanical properties from transdermal patches without longan seed extract and transdermal patches containing the extract. (A) Tensile strength; (B) Percent elongation at break.

The results revealed that the formulations after loading 0.5% of the extract (3C-D-E, 3C-L-E and 3C-O-E), their tensile strength and percentage elongation at break were not changed comparing the transdermal patch base (Formulation 3C). Therefore, it was implied that the penetration enhancers (DMSO, oleic acid or lemon oil) and the extract were not affected the expansion of the polymer network resulting in changeless flexibility and strength of the transdermal patches containing longan seed extract. There were no significant different between transdermal patch base and all transdermal patch containing longan seed extract ($p > 0.05$).

4.5.4 Adhesive test (Modified Rolling Ball Tack Tester)

The adhesiveness of transdermal patches containing longan seed extract was determined comparing the transdermal patch base. The distance of the ball rolled gives an inverse scale of tack; the greater the distance, the less tacky adhesive. All the patches containing longan seed extract showed closely tack values thus no different in their adhesiveness. This result referred that the penetration enhancers (DMSO, oleic acid or lemon oil) and the extract were not affected adhesive properties. However, there were significant different between transdermal patch containing longan seed extract and comercial patch. The adhesiveness of the transdermal patch containing longan seed extract was weak comparing comercial patch.

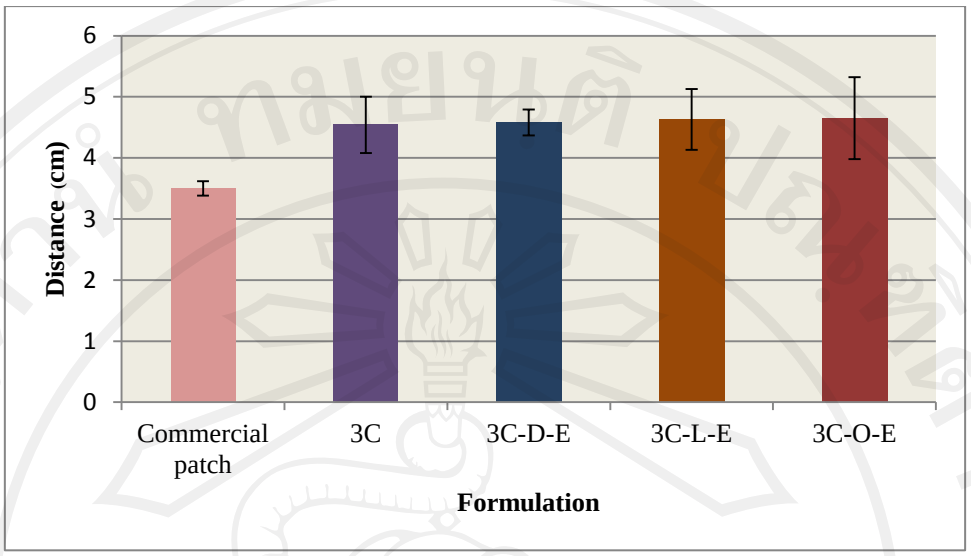


Figure 4.12 Tack values of commercial patch, transdermal patches without longan seed extract and transdermal patches containing the extract.

4.5.5 Gallic acid content determination

The actual content of gallic acid in the transdermal patches containing longan seed extract (3C-L-E) was determined by HPLC at wavelength 270 nm. The amount of gallic acid content was calculated from standard gallic acid curve. The average content of gallic acid in the transdermal patches was 81.69 ± 0.92% w/w.

4.6 Stability test of transdermal patches containing longan seed extract

4.6.1 Physical appearances

Tarnsdermal patches containing longan seed extract (Formulation: 3C-D-E, 3C-L-E and 3C-O-E) were investigated for their long term stability by three-month storage in room temperature (RT), cool place (4°C) and hot place (45°C). The accelerated stability test in cool place and hot place (6 cycles) could predict their physical stability.

After the tarnsdermal patches containing longan seed extract were incubated in all test conditions. The physical appearances including color, flexibility and residue after removal of the transdermal patch Formulation 3C-L-E were not different compared with the freshly prepared patch. In contrast, at 45°C its flexibility decreased and became dark brown as shown in **Figure 4.13**. Transdermal patches of Formulation 3C-D-E and 3C-O-E were not stable at all test conditions. These patches became dark brown in color and decreased in flexibility along with more residues after removal. The results were showed in **Table 4.14**.

From these results, Formulation 3C-L-E exhibited the most optimal physical appearances after storage at room temperature and 4°C for 3 months whereas unstable at 45°C. Therefore, the appropriate storage temperature for the transdermal patch containing longan seed extract should not be at high temperature (≥ 45°C).

Table 4.15 Physical properties of transdermal patches containing longan seed extract after stability test

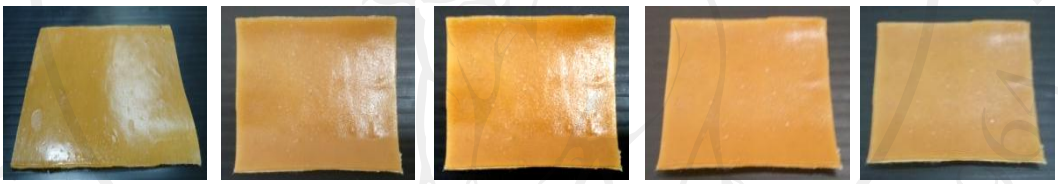
Formulation	**Physical appearances**	**Stability test**				
		Day 0	**RT**	**4 ºC**	**45 ºC**	**H/C**
3C-D-E	Color	Brown	Dark brown	Dark brown	Dark brown	Dark brown
	Flexibility	+ 4	+ 3	+ 3	+ 3	+ 3
	Residue after removal	+ 1	-	-	+ 2	+ 2
3C-L-E	Color	Brown	-	-	Dark brown	-
	Flexibility	+ 4	-	-	+ 3	-
	Residue after removal	-	-	-	-	-
3C-O-E	Color	Brown	Dark brown	Dark brown	Dark brown	Dark brown
	Flexibility	+ 4	+ 3	+ 3	+ 3	+ 3
	Residue after removal	+ 1	+ 3	+ 3	+ 3	+ 3

Freshly prepared RT Cool place Hot place H/C cycles

Formulation 3C-D-E

Freshly prepared RT Cool place Hot place H/C cycles

Formulation 3C-L-E



Freshly prepared RT Cool place Hot place H/C cycles

Formulation 3C-O-E

Figure 4.13 Appearances of transdermal patches containing longan seed extract in any conditions of stability test; at room temperature (RT), cool place = 4ºC, hot place = 45ºC, and H/C cycles = 6 cycles of heating-cooling cycling

4.6.2 Gallic acid content determination

To determine the gallic acid content from the transdermal patch containing longan seed extract after stability test (storage in room temperature, cool place, hot place for three months and six cycles of heating-cooling cycling). From the results as shown in **Table 4.15**, the content of gallic acid was slightly decreased and significantly ($p < 0.05$) at the temperature stress condition – heating-cooling cycling. Otherwise, at room temperature, cool place and hot place, gallic acid contents from the transdermal patch showed not significantly decrease comparing freshly prepared patch. Although at high temperature (≥ 45°C) cause unstable physical appearances of the transdermal patch containing longan seed extract, the gallic acid content rather remained in the transdermal patch.

Table 4.16 Gallic acid content from transdermal patches containing longan seed extract after stability test

Stability test	**Gallic acid content (% w/w)**
Day 0	81.69 ± 0.92
Room temperature	80.53 ± 0.86
4 ºC	80.29 ± 0.81
45 ºC	81.64 ± 0.78
Heating-Cooling	80.14 ± 0.91*

Note: * Significant difference ($p < 0.05$) compared to Day 0

4.6.3 Mechanical properties

Figure 4.14 showed the tensile strength and percentage elongation at break of all transdermal patches containing longan seed extract formulation after storage in all test conditions. The results revealed that the tensile strength values and percentage elongation of Formulation 3C-L-E were not changed and there are not significant between freshly prepared patch and the tested patches at all test conditions. On the other hand, Formulation 3C-D-E and 3C-O-E showed significantly ($p < 0.05$) decreasing in percentage elongation and increasing in tensile strength values resulting low flexibility and hard films. These changing were appeared at all test condition particularly, at hot place (45°C). As high temperature may cause the crystallization of the longan seed extract, and/or cross-links formation between the extract and polymer molecules.

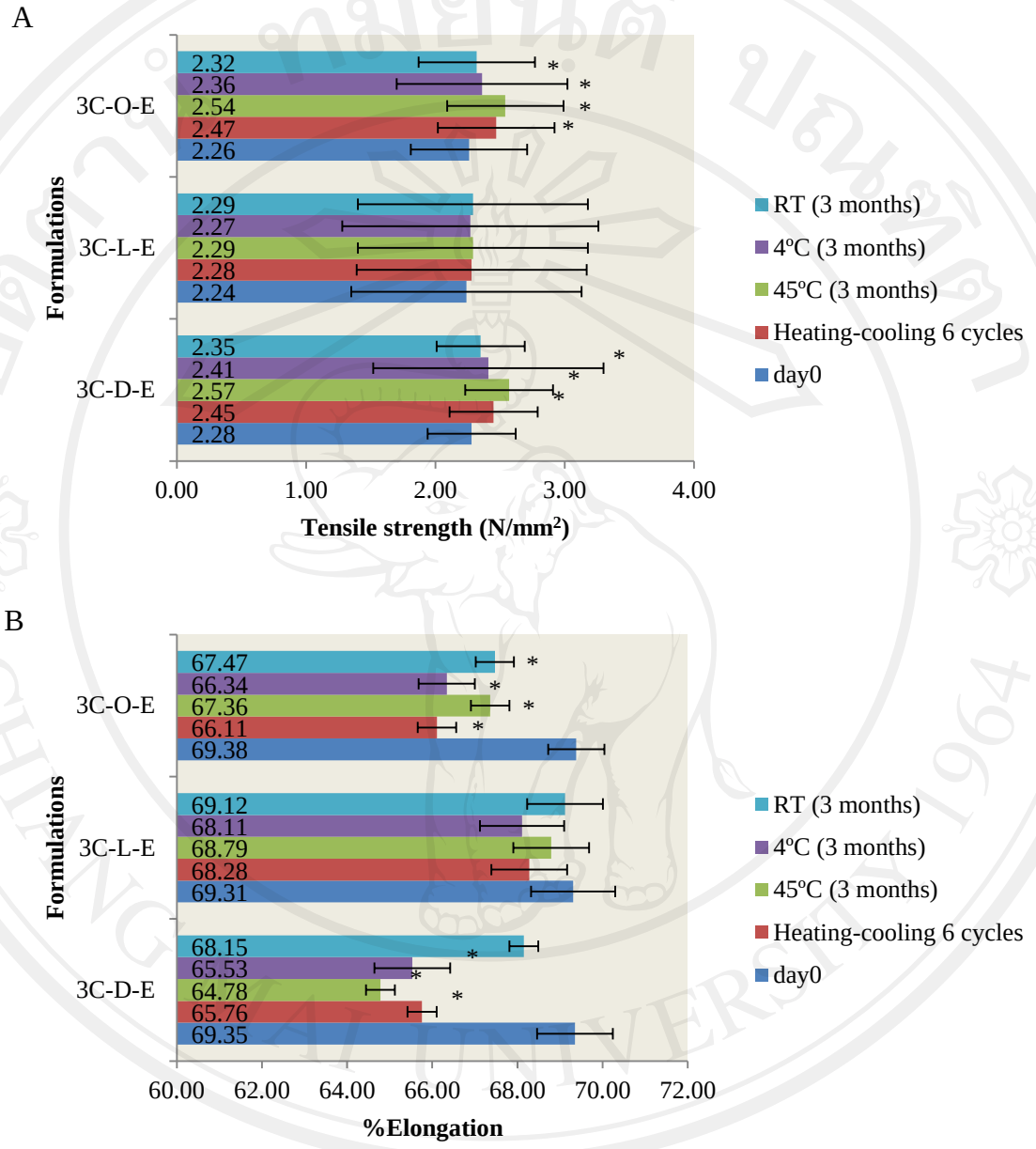


Figure 4.14 Mechanical properties of the transdermal patches containing longan seed extract after stability test (A) Tensile strength; (B) Percent elongation at break; * Significant difference (p <0.05) compared to Day 0.

4.6.4 Adhesive test

To determine the adhesive properties after stability test (storage in room temperature, 4°C, 45°C for three months and six cycles of heating-cooling cycling) by modified rolling ball tack tester. From the results, (**Figure 4.15**) the tack adhesion values of the patch Formulation 3C-L-E were not different significantly compared with the freshly prepared patch at room temperature, 4°C and 45°C. However, significantly ($p < 0.05$) decreased its adhesiveness at temperature stress condition – heating-cooling cycling. Formulation 3C-D-E and 3C-O-E showed significantly ($p < 0.05$) increasing tack values at all test conditions. This consequently results in poor adhesive properties of the patch.

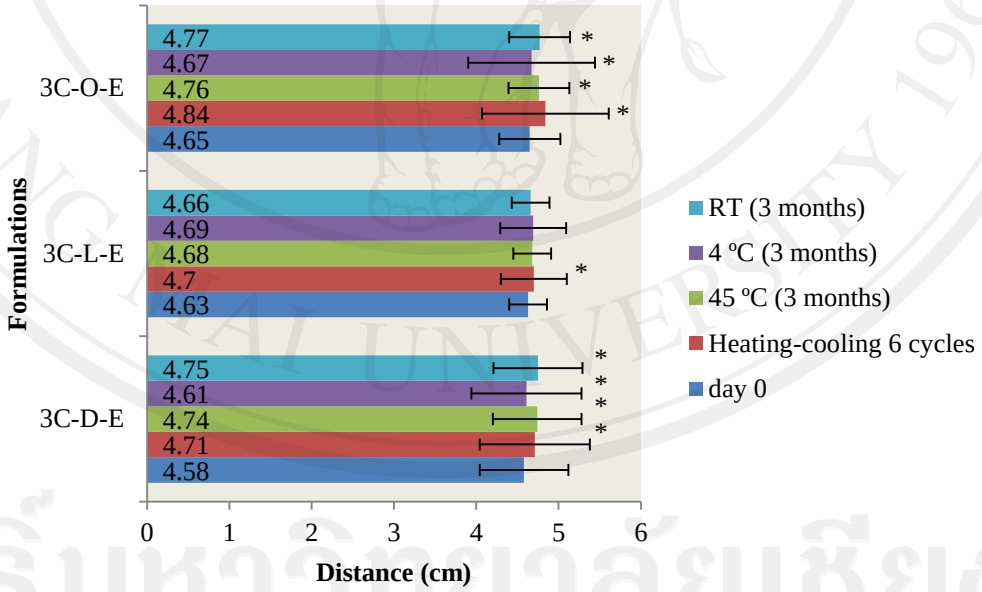


Figure 4.15 Tack values of the transdermal patches containing longan seed extract after stability test. * Significant difference (p <0.05) compared to Day 0.

4.7 In *vitro* release study of transdermal patches containing longan seed extract

Table 4.17 Comparative in releasing profiles of transdermal patch containing longan seed extract

Time (hours)	% Cumulative gallic acid released			
	3C-E	3C-L-E	3C-O-E	3C-D-E
0	0	0	0	0
1	3.11 ± 1.34	15.35 ± 1.06	16.26 ± 1.45	9.22 ± 1.56
2	10.27 ± 0.43	31.35 ± 0.33	36.61 ± 1.02	19.39 ± 0.34
3	16.76 ± 1.22	48.56 ± 1.56	50.42 ± 2.31	27.10 ± 0.78
6	27.98 ± 2.34	71.15 ± 1.34	73.42 ± 1.74	50.66 ± 1.09
12	37.94 ± 0.56	85.79 ± 1.29	89.15 ± 1.42	75.95 ± 1.45
24	50.73 ± 0.34	98.52 ± 0.88	98.61 ± 1.23	95.82 ± 1.56

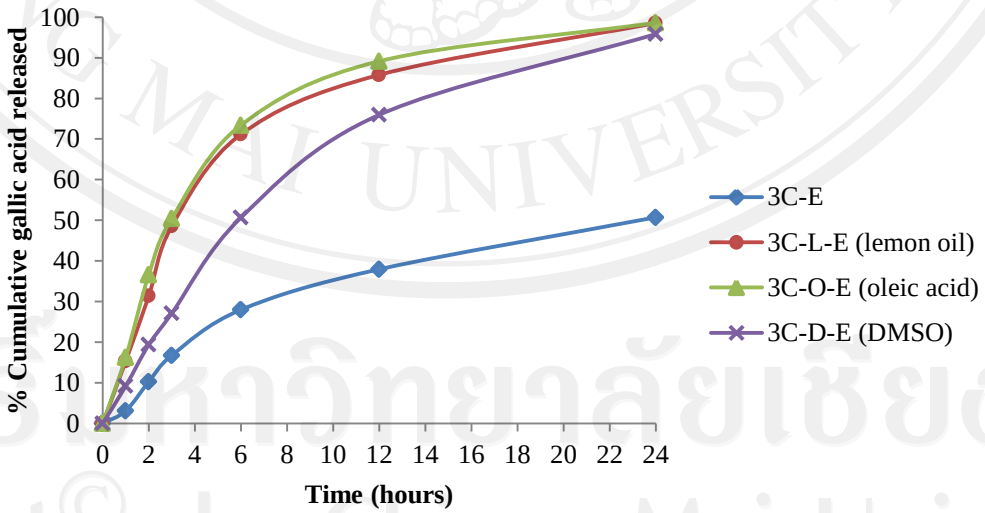


Figure 4.16 Comparative in release profiles of transdermal patch containing longan seed extract

The effect of penetration enhancers on release profiles was investigated by using modified Franz-diffusion cells. Gallic acid release profiles from formulation 3C-D-E, 3C-L-E and 3C-O-E comparing control formulation 3C-E were shown in **Figure 4.16** and their data were shown in **Table 4.16**. The cumulative percent of gallic acid release in 24 hours from all formulations could be ranked as follows: 3C-O-E > 3C-L-E > 3C-D-E > 3C-E. The release of gallic acid from the formulation 3C-O-E, containing oleic acid as penetration enhancer, showed the highest release of 98.61% at 24 hours. Furthermore, the transdermal patch containing lemon oil and DMSO provided the gallic acid released of 98.52 and 95.82% at 24 hours, respectively. There was not significant different between Formulation 3C-O-E and 3C-L-E (p>0.05). On the other hand formulation 3C-E, without the penetration enhancer, increased slowly and reached maximum of 50.73% at 24 hours. The transdermal patch without penetration enhancer showed the lowest release of gallic acid comparing other formulations. The percents of gallic acid released increased significantly when present of penetration enhancer. According to the enhancing effect of chemical enhancers could be dependent on the physicochemical properties of drugs and the combination with vehicle or ingredients in preparations, resulting difference in the percents of drug released [50].

The profiles releasing plots of all formulations have shown that the drug release followed the Higuchi's model which was evidenced from the regression value of the mentioned plot (**Figure 4.17**). This model is based on the hypotheses; initial drug concentration in the matrix is much higher than drug solubility, drug diffusivity is constant, and perfect sink conditions are always attained in the release environment [51]. According to the cumulative released gallic acid percentage was constantly

maintainable over 24 h and the release rate reached to over 90% of gallic acid content, the gallic aid release could be sufficient for a therapeutic effect.

The selection of transdermal patch was considered both good stability and drug release. Therefore, Formulation 3C-L-E was the most suitable formula and was selected for further development of transdermal patch containing longan seed extract.

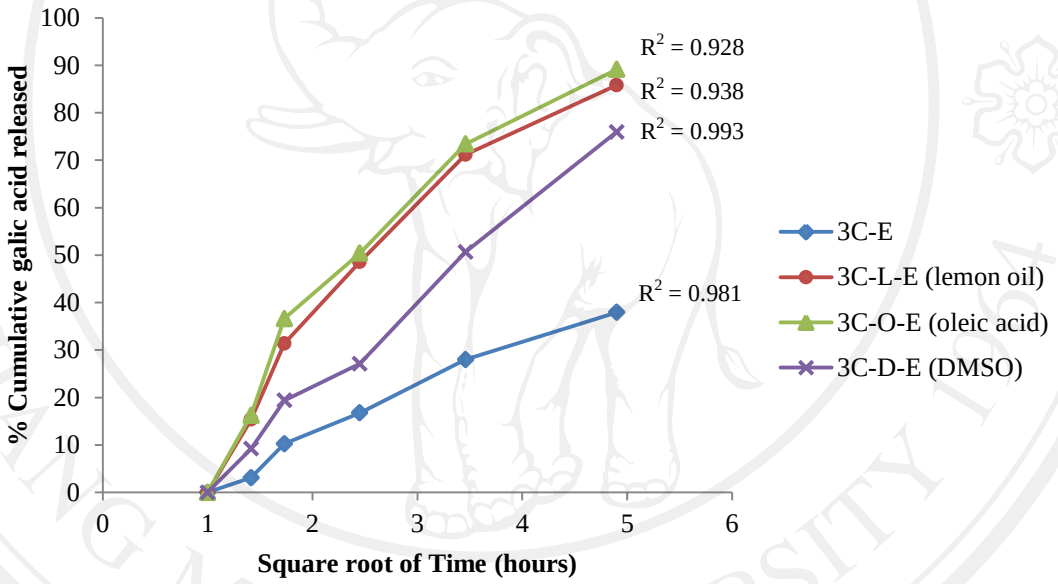


Figure 4.17 Higuchi releasing model of transdermal patch containing longan seed extract

4.8 Rabbit skin primary irritation test

The formulations (3C-D-E, 3C-L-E and 3C-O-E) were tested for skin irritation on three male albino rabbits (NZW rabbits). Erythema and edema reactions were scored based on Draize scoring system, and calculated in term of PII values (**Table 4.17**). The skin irritation study indicated that no evidence of erythema or edema in or

around all the patch area either during the period of study or after patch removal (**Figure 4.18**). However, 1 % w/v SLS (positive control) revealed slightly irritation.

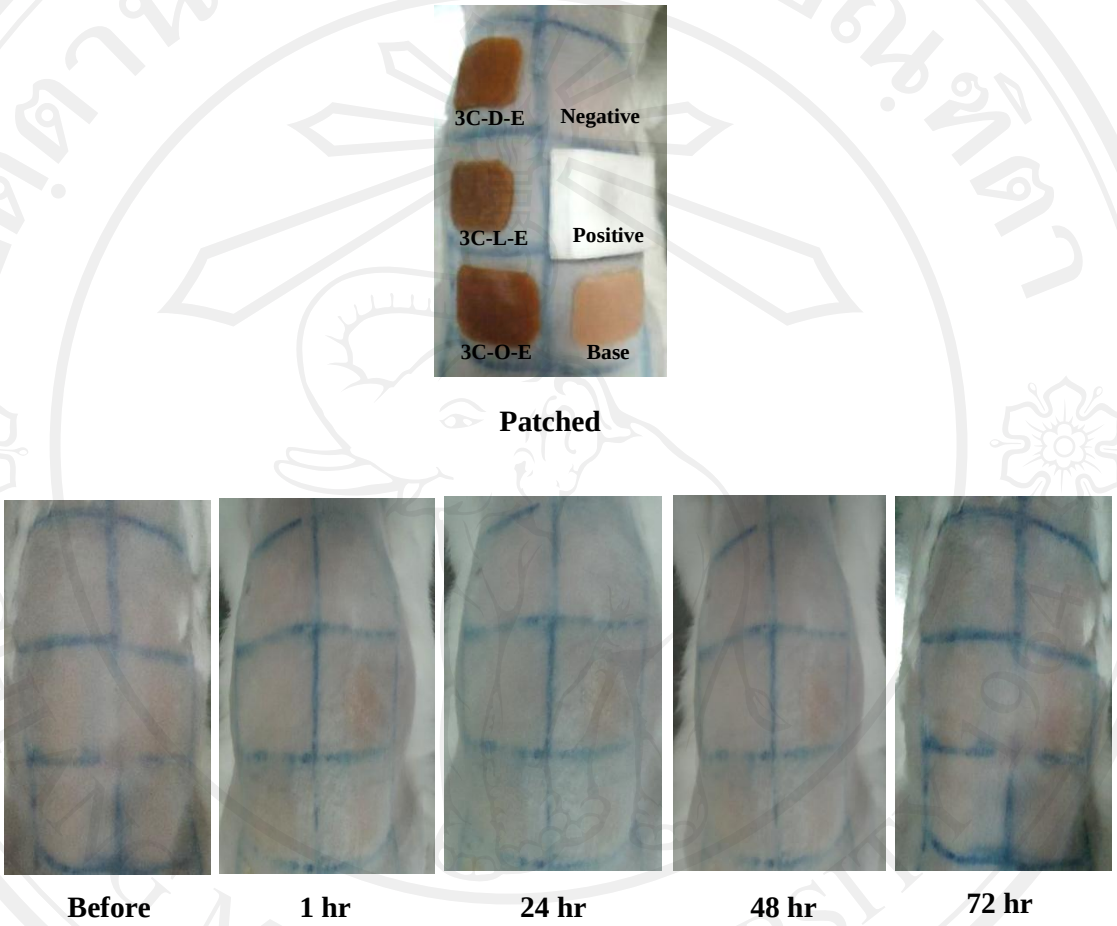


Figure 4.18 Rabbit skin primary irritation reaction of transdermal patches containing longan seed extract

Table 4.18 Primary irritation index (PII) and skin irritation reaction in rabbits

Test substances	PII value	Classification of skin reaction
Patch base	0.00	No irritation
3C-D-E	0.00	No irritation
3C-L-E	0.00	No irritation
3C-O-E	0.00	No irritation
Positive (1 % w/v SLS)	1.17	Slightly irritation
Negative (DI water)	0.00	No irritation

4.9 Skin compatibility test of transdermal patch containing longan seed extract in human volunteers

Skin compatibility was studied in 30 Thai healthy volunteers by transdermal patch containing longan seed extract for skin primary irritation evaluation. The results revealed that negative control (DI water), transdermal patch base and transdermal patch containing longan seed extract (3C-L-E) showed no irritation (PII < 0.5) whereas positive control (1 % w/v SLS) exhibited slightly irritation (PII = 0.58) as showed in **Table 4.17** and **Figure 4.19**.

Table 4.19 Primary irritation index (PII) and skin irritation reaction in 30 volunteers

Test substances	PII value	Classification of skin reaction
Transdermal patch containing longan seed extract (3C-L-E)	0.15	No irritation
Transdermal patch base	0.15	No irritation
Positive (1 % w/v SLS)	0.58	Slightly irritation
Negative (DI water)	0.00	No irritation

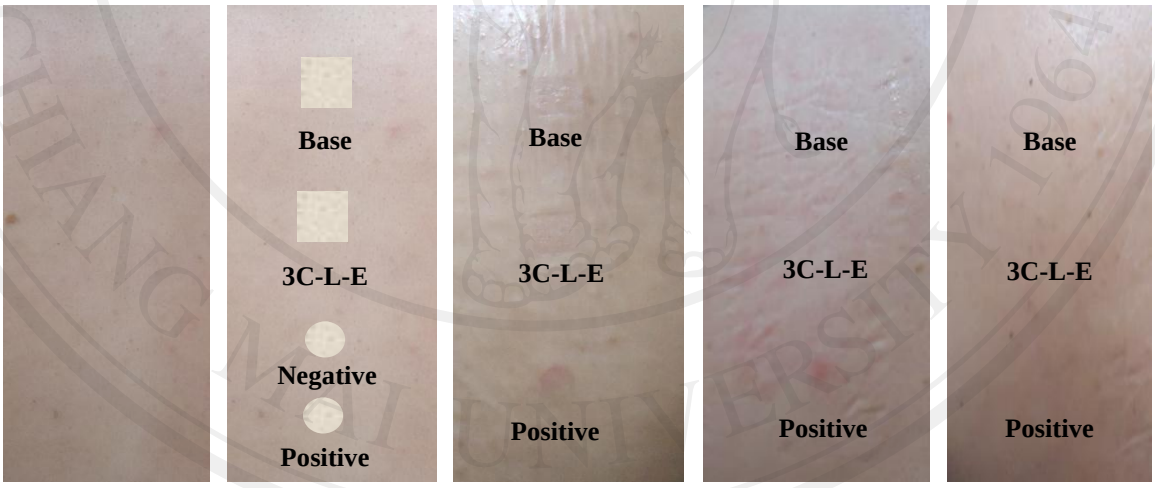


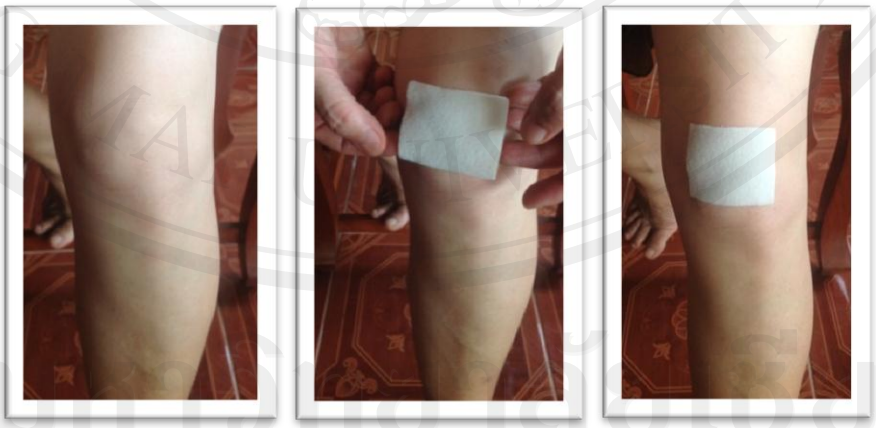
Figure 4.19 Skin compatibility test of transdermal patch containing longan seed extract and transdermal patch base in 30 volunteers

In agreement with skin compatibility test, transdermal patch containing longan seed extract (3C-L-E) and transdermal patch base were compatible to human skin (absence of skin irritation) and could be use for the performance test.

4.10 Satisfaction test of volunteers by questionnaire

The satisfaction of transdermal patch containing longan seed extract of thirty volunteers was completed by the questionnaire. After all volunteers held a test patch on one side of the knee (**Figure 4.20**), questioned on the product satisfaction using a five-point like scale. The transdermal patch performed the satisfaction as showed in **Table 4.18** and **Figure 4.21**.

The satisfaction results ranged from like excellent to very good like exhibited more than 90% for appearances, flexibility, easiness in use (easy to remove and easy to adhere), feeling on use (stickiness and irritation) as well as easy to carry and overall of the product showed more than 80%. For the odor, adhesiveness (on use) and residue after removal (after use) showed more than 90%, 80% and 70% ranged from very good to good, respectively. The color of the product presented more than 80% ranged from good to fair.



Before **Patched** **After**

Figure 4.20 Satisfaction test of volunteers by questionnaire

Table 4.20 The percentage of satisfaction on transdermal patch containing longan seed extract

Topic	**Satisfaction of product (%)**				
	Excellent	**Very good**	**Good**	**Fair**	**Poor**
1. Appearance	34.24	65.76	0	0	0
2. Flexibility	88.32	11.68	0	0	0
3. Color	0	11.91	45.12	39.43	3.54
4. odor	5.32	66.78	24.91	2.99	0
Topic	**Satisfaction of easiness in use (%)**				
	Excellent	**Very good**	**Good**	**Fair**	**Poor**
1. Easy to adhere	7.66	88.45	3.89	0	0
2. Easy to remove	91.97	8.03	0	0	0
3. Easy to carry	30.76	45.43	23.81	0	0
Topic	**Satisfaction of feeling on use (%)**				
	Excellent	**Very good**	**Good**	**Fair**	**Poor**
1. Stickiness	77.81	22.19	0	0	0
2. Irritation	93.64	6.39	0	0	0
3. Adhesiveness	10.43	15.22	69.82	4.53	0
Topic	**Satisfaction of feeling after use (%)**				
	Excellent	**Very good**	**Good**	**Fair**	**Poor**
1. Residue after removal	21.32	55.23	23.45	0	0
2. Stickiness	22.14	69.17	8.69	0	0
Topic	**Overall satisfaction (%)**				
	Excellent	**Very good**	**Good**	**Fair**	**Poor**
1. Overall satisfaction	41.56	47.11	11.33	0	0

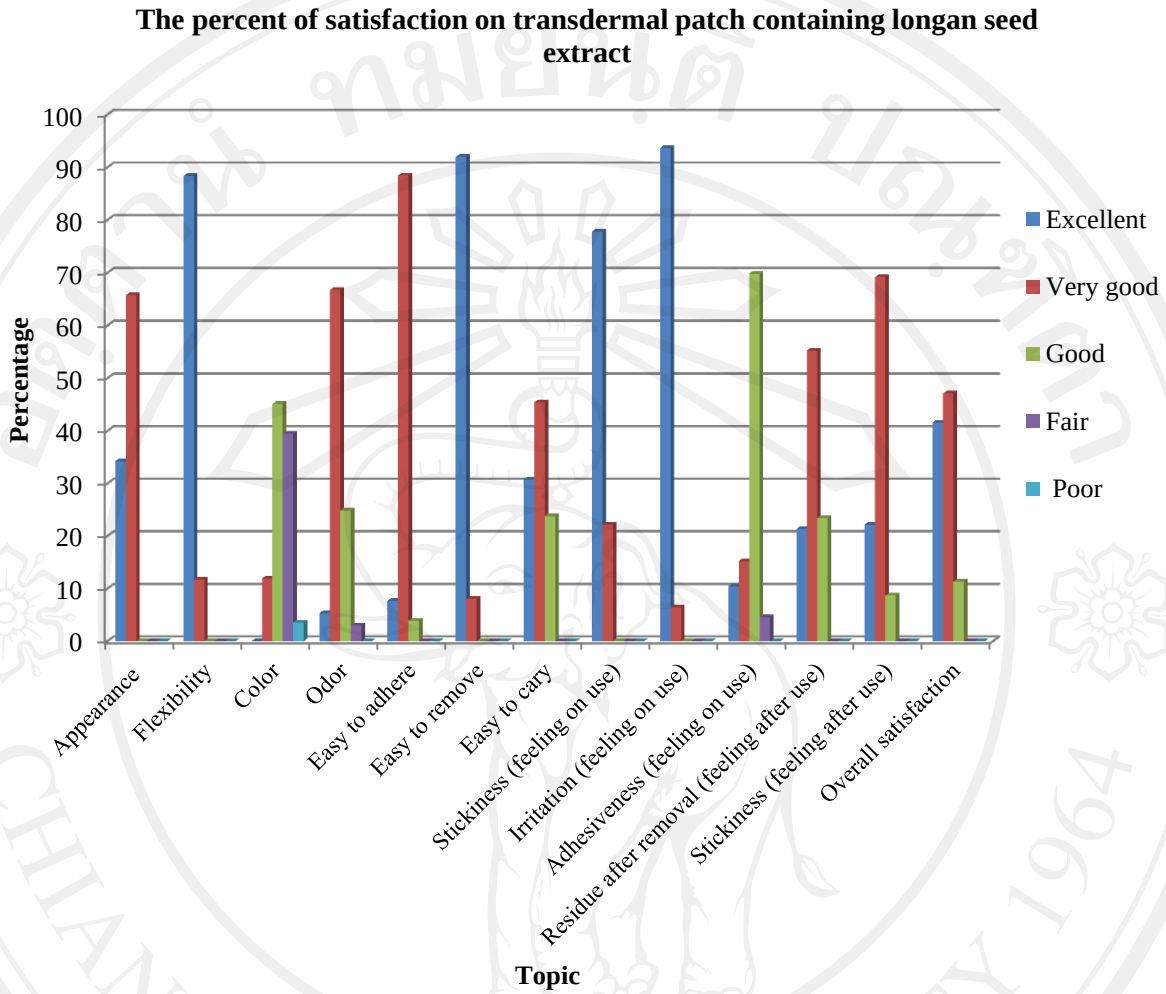


Figure 4.21 The percentage of satisfaction on transdermal patch containing longan seed extract