CHAPTER IV

RESULT AND DISCUSSION

1. Purification of indicative substance in *Butea superba* Roxb.

Before the plant tuber of *Butea superba* Roxb were cutted as shown in Figure 1. The plant was identified by comparison with a herbarium specimen at the Forest herbarium, Royal Forest Department, Thailand. The plant was similar to specimen BKF 71063 as *Butea superba* Roxb. Dried powder of tuber of *Butea superba* Roxb. (18 kg) as shown in Figure 6, was macerated by 95% ethanol (5 x 20L) at room temperature. The 95% ethanol extract solution were filtered and evaporated under reduced pressure to obtain dark-red gummy residue (500 g) of 95% ethanol crude extract as shown in Figure 7.

Figure 6. Coarse powder of *Butea superba* Roxb.

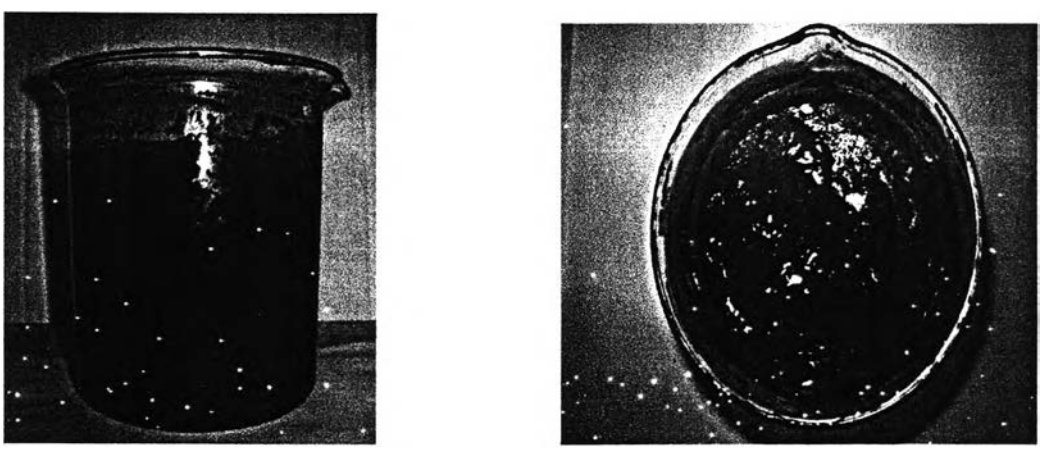


Figure 7. Dark-red gummy of *Butea superba* 95%ethanolic extract.

In a prior study, ethanol extract of grape gave more anthocyanin (flavonoid) and polyphenols than water extracts (Lapurnik et al., 2005). Flavonoids possessing a number of unsubstituted hydroxyl groups, or a sugar, are polar compounds and as the old adage "like dissolve like" suggests, are generally moderately soluble in polar solvents such as ethanol (Markham, 1982). The obtained extracts (4 spots on terminal line) displayed many spots on TLC plates when detected under UV light and addition sprayed with 10% vanillin-sulfuric acid in ethanol and heating at 110 °C (Markham, 1982) as shown in Figure 8.

Figure 8. TLC pattern of *Butea superba* 95% ethanolic extract.

The crude ethanolic extract was repeatedly partitioned with hexane and dichloromethane (CH_2Cl_2) and finally the final insoluble fraction was discarded. These dichloromethane extracts were subjected to column chromatography for purification. First, column chromatography was eluted by using gradient solvents with dichloromethane (CH_2Cl_2) and methanol (MeOH) provided ten major fractions (C2 as combined fractions of No. 5-15, 0.42g) from primary column chromatography. It was subjected to secondary column chromatography and using gradient elution with dichloromethane (CH_2Cl_2) and methanol (MeOH). The obtained ten major fractions (C2.1 as combined fractions of No.1-10, 210 mg) from secondary column chromatography, subjected to the third column chromatography. It was using gradient elution with Hexane and ethylacetate (EtOAc) in a stepwise fashion. Finally, it was

giving subfractions C2.1.4 as fraction No.15 (20.10 mg) displayed one major spot (R_f = 0.73) on TLC plate when detected under UV light and gave purple color when sprayed with 10% vanillin-sulfuric acid in ethanol and heating at 110 °C as shown in Figure 9. Purification of the compound gave a whitish amorphous powder and coded **BS 1** (20.10 mg, 0.00020 % yield) as shown in Figure 10.

Figure 9. TLC patterns of compound BS 1.

Figure 10. Whitish amorphous powder of BS 1.

The identification of compound **BS1** was based on spectroscopic evidences NMR (nuclear magnetic resonance) and mass spectra and also by comparison with previously reported data in the literatures (Herath et al., 1998; Kulesh et al., 2001). The TOF (Time-of-Flight) Mass spectra (Martin, 2005) as shown in Figure 11 showed a molecular ion at *m/z* [M^++1] 271.11, which corresponded to the molecular formula $C_{16}H_{14}O_4$ whereas other prominent ions at *m/z* 137.28 and 297.04 were also observed.

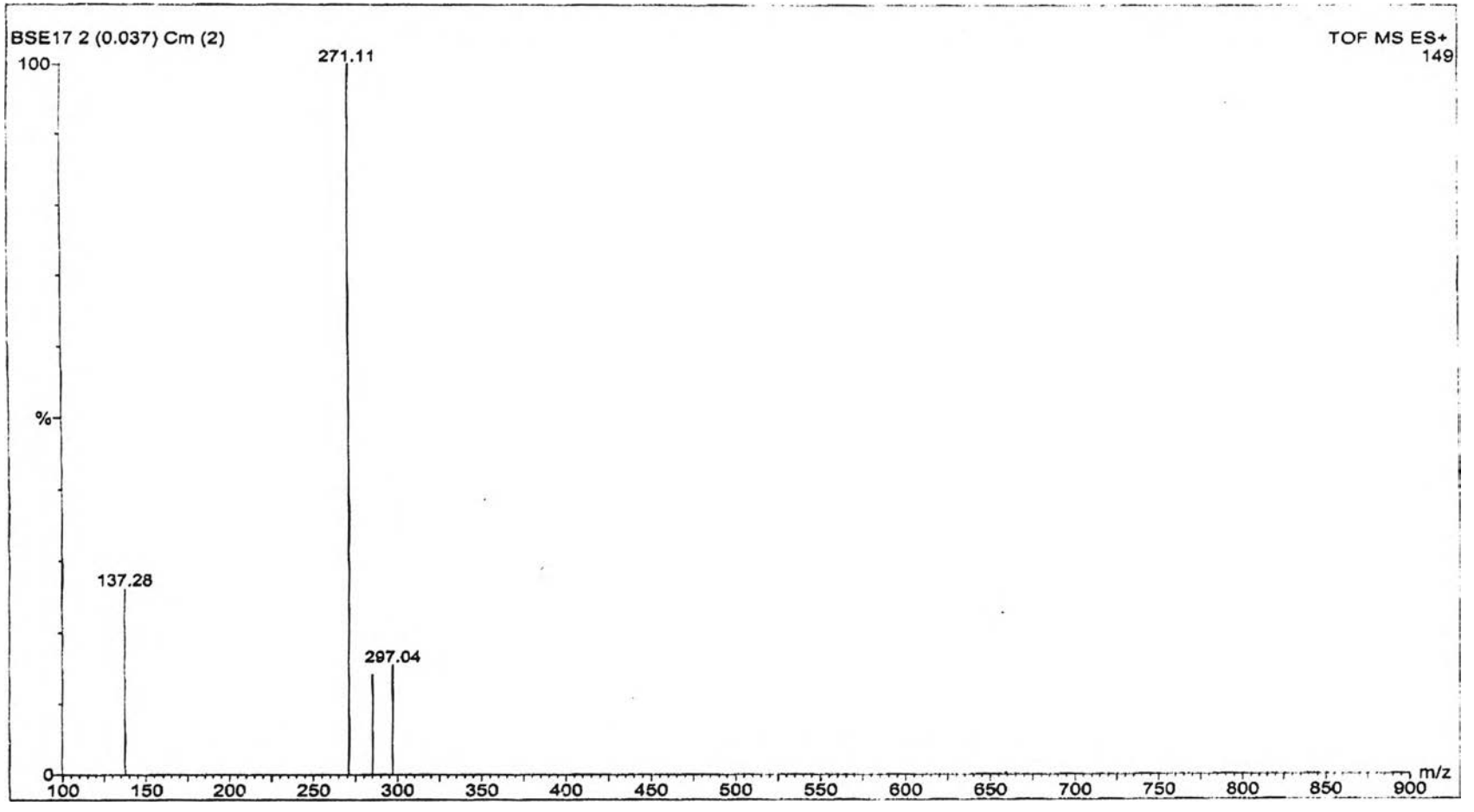
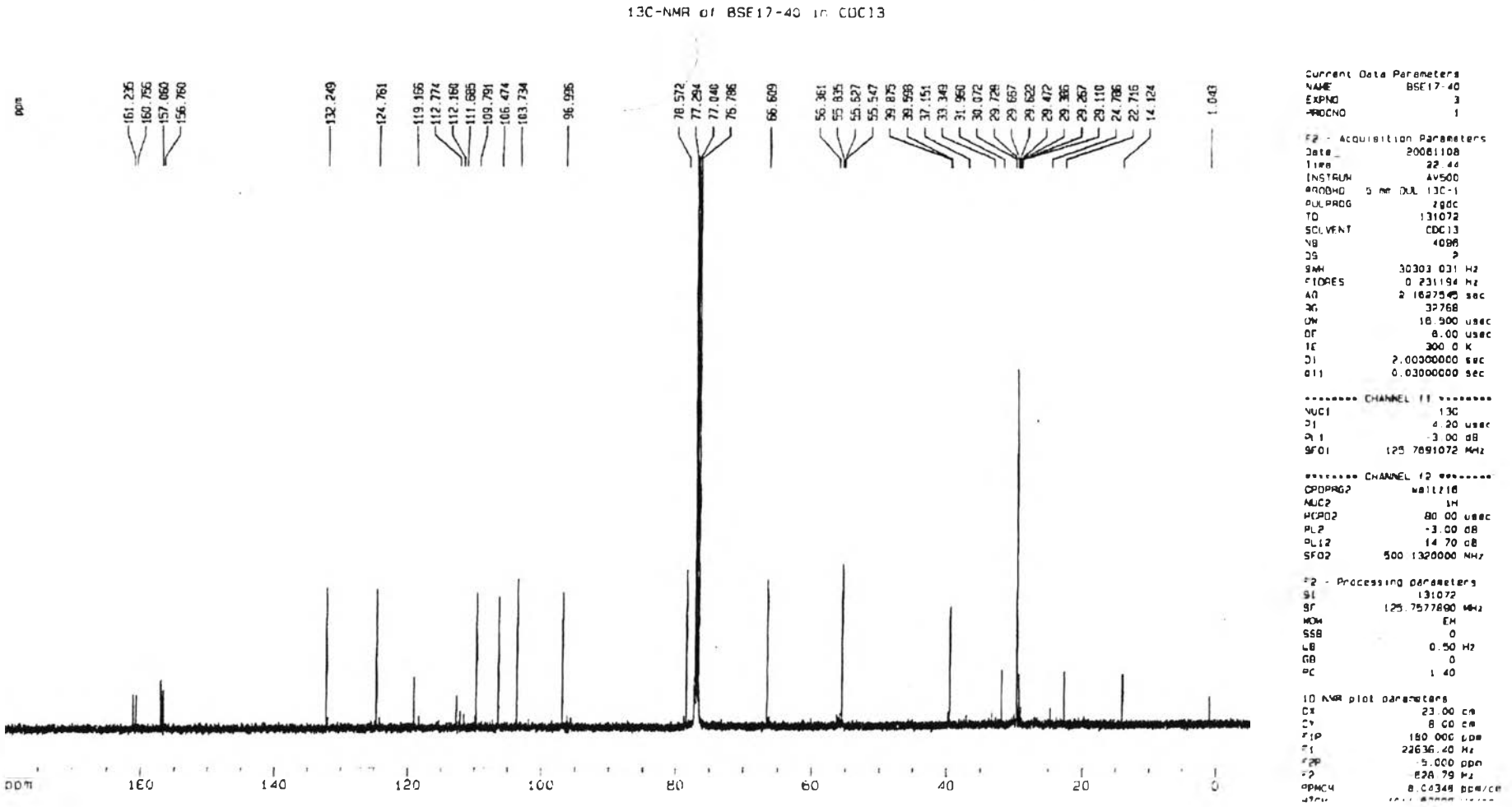


Figure 11. Mass spectrum of BS



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TE 300.0 K
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D11 0.03000000 sec

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P1 4.20 usec
PL1 -3.00 dB
SFO1 125.7691072 MHz

======== CHANNEL f2 ========
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PL12 14.70 dB
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F2 - Processing parameters
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LB 0.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
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CY 8.00 cm
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F2P 5.000 ppm
F2 629.79 Hz
PPMCM 8.04348 ppm/cm

Figure 12. ^{13}C-NMR spectrum of BS 1.

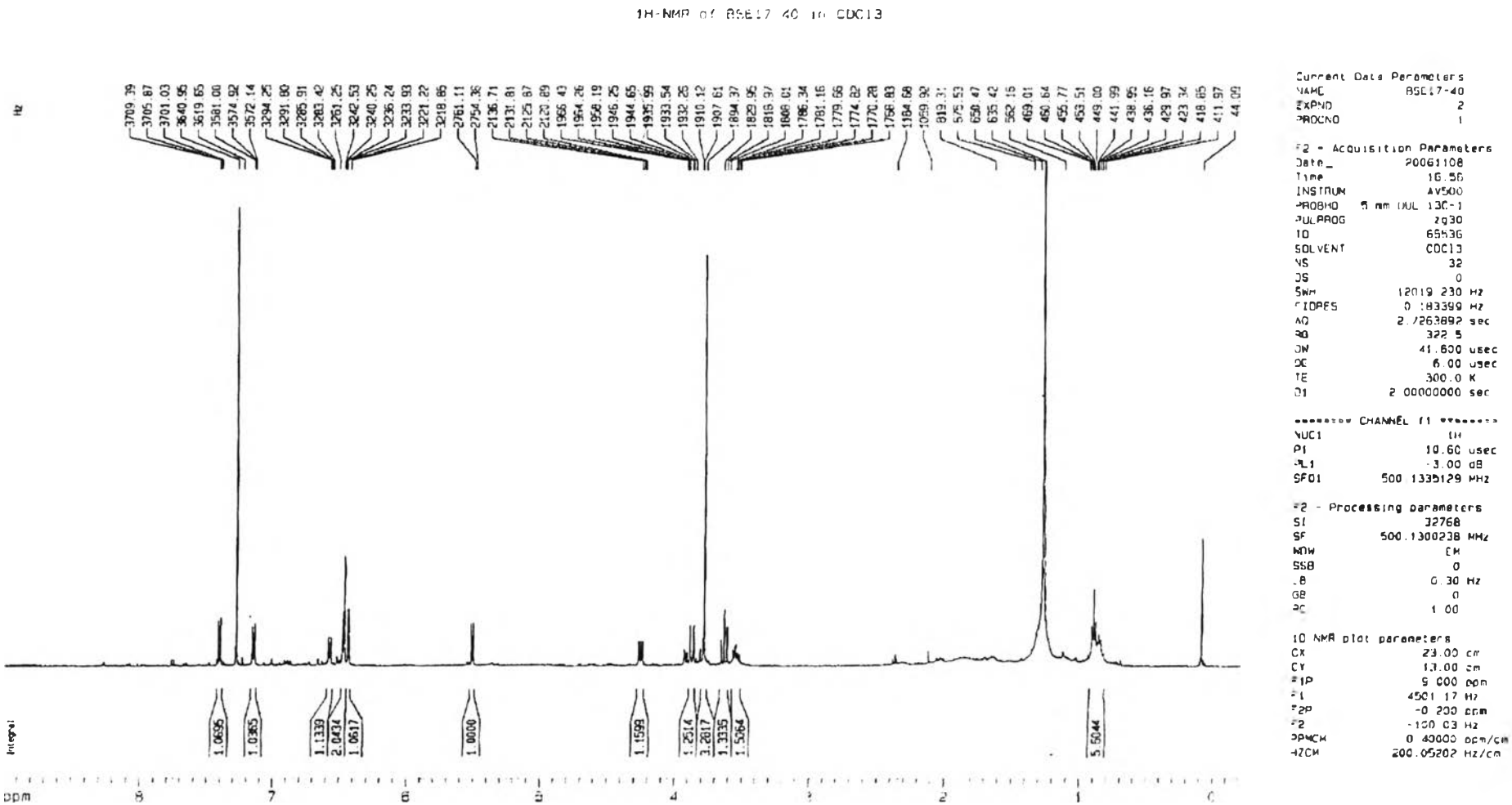


Figure 13. ^{1}H-NMR spectrum of BS 1.

The presence of 16 carbons and 12 protons were in agreement with signals observable in the ^{13}C-NMR (Figure 12) and ^{1}H-NMR (Figure 13) spectrum, respectively (Herath et al., 1998; Kulesh et al., 2001). The spectrum of DEPT (Distortionless Enhancement by Polarization Transfer) as shown in Figure 14, HMQC (^{1}H-detected Heteronuclear Multiple Quantum Coherence) as shown in Figure 15, HMBC (^{1}H-detected Heteronuclear Multiple Bond Coherence) as shown in Figure 16 and COSY (H-H Correlation spectroscopy) as shown in Figure 17 were showed appendix A.

Compound BS1 was therefore identified as the 3-hydroxy-9-methoxypterocarpan or medicarpin as showen in Figure 18. Comparison of its carbon and proton chemical shifts with those previously reported for 3-hydroxy-9-methoxypterocarpan (Herath et al., 1998) was summarized in Table 16.

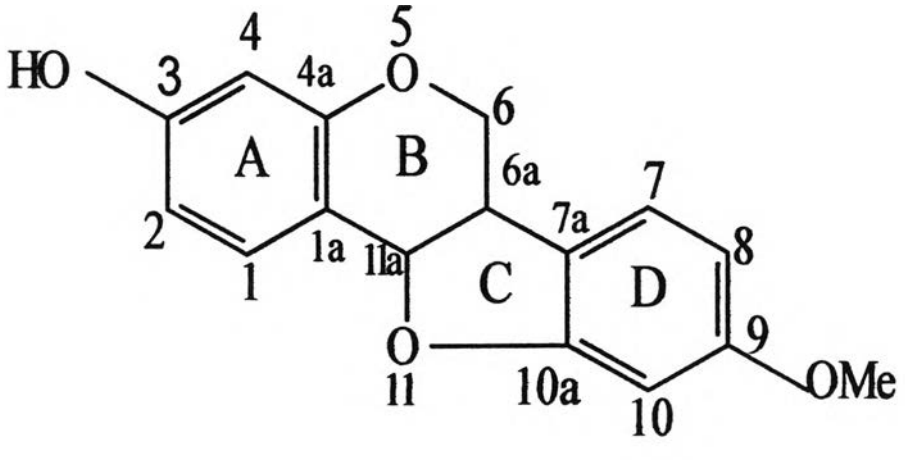


Figure 18. Numbering of chemical structure of medicarpin (Harborne, 1994).

The maximum wave length of BS1 as medicarpin in methanol by UV-spectrophotometer was detected. The result of scanning was at 287 nm as maximum wavelength and peak of 340.5 nm may be impurity from process of extraction by conventional column chromatography. Chromatogram of maximum wave length is shown in Figure 19.

Table 16. ^{13}C-NMR and ^{1}H-NMR data of BS1 and previously reported as 3-hydroxy-9-methoxypterocarpan (Herath et al., 1998)

Position	BS1		3-hydroxy-9-methoxypterocarpan	
	C-NMR	H-NMR	C-NMR	H-NMR
1	132.25	δ=7.41(1H, d, *J*=8.36)	132.6	δ=7.41(1H, d, *J*=8.4)
1a	112.77		113.0	
2		δ=6.57(1H, dd, *J*=8.37 and J=2.49)		δ=6.57(1H, dd, J=8.4 and J=2.4)
3	157.06	δ=3.80(S, OCH_3)	157.5	δ=3.79(S, OCH_3)
4	103.73	δ=6.44(1H, d, *J*=2.37)	104.1	δ=6.44(1H, d, *J*=2.4)
4a	156.76		157.1	
6	66.61	δ=4.26(H-6eq, 1H, dd, *J*=10.88 and *J*=5) δ=3.64(H-6ax, 1H, dd, *J*=10.98and *J*=10.96)	67.0	δ=4.26(H-6eq, 1H, dd, *J*=11.0 and *J*=5) δ=3.64(H-6ax, 1H, dd, *J*=11and *J*=10.9)
6a	39.87	δ=3.55(H-6a, 1H, ddd, *J*=12, *J*=6.51 and *J*=7.69)	39.9	δ=3.55(H-6a, 1H, ddd, *J*=11, *J*=5.1 and *J*=6.7)
7	124.76	δ=7.15(1H, d, *J*=8.66)	125.2	δ=7.15(1H, d, *J*=8.9)
7a	119.16		119.5	
8	106.47		106.8	
9	160.75		161.1	
10	96.99		97.3	
10a	161.23		161.5	
11a	78.57	δ=5.52(H-11a, 1H, d, *J*=6.75)	79.0	δ=5.52(H-11a, 1H, d, *J*=6.8)
OCH_3	55.83	δ=3.80(S, OCH_3)	55.9	δ=3..79(S, OCH_3)

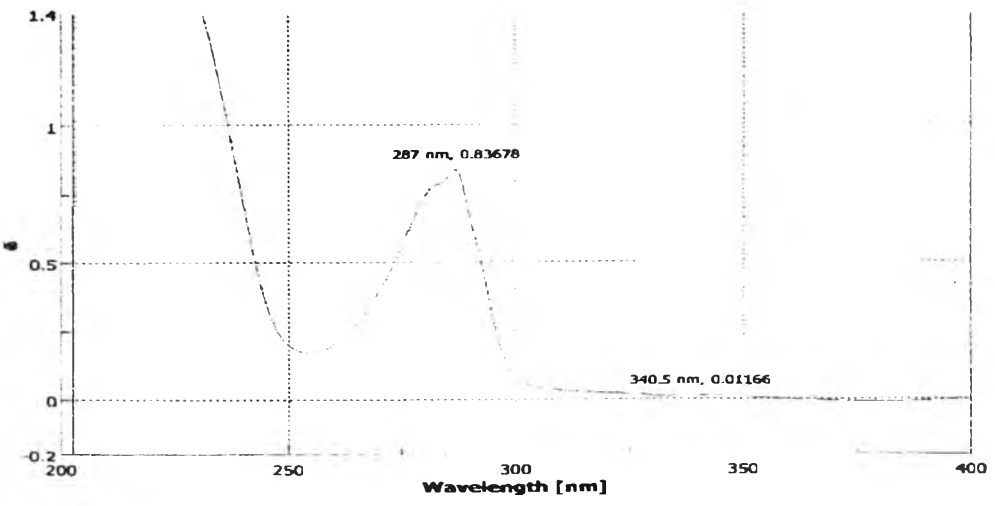


Figure 19. Maximum wave length of BS1(medicarpin).

HPLC is the most suitable to determine the compound of herbal extract both as raw material and in pharmaceutical dosage form because of its high sensitivity, specificity and convenience for the research (Handriks et al., 2005). In previous studies, HPLC isocratic method was recommended for the analysis of medicarpin from *Butea superba* because HPLC gradient method consumed quite a long time in one cycle. Medicarpin were eluted from the latter method at 16.6 min and 50.50 min, respectively and HPLC isocratic method was tried to establish but to no available (Lining et al., 1994; Rong, et al., 2005). In addition, HPLC gradient method could not selectly separate of the active components from some herbal extracts because of the more difference in polarity among the components. Consequently, the HPLC isocratic method was specified to be benefit in this investigation. The HPLC chromatogram of medicarpin was shown in Figure 20. The peak of medicarpin on retention time was about 7.629 minutes at 287 nm. Some peaks before medicarpin's peak were solvent, mobile phase and impurity from the separation in Hypersil® BDS (C18) column.

Validation of HPLC method

Analytical method validation is a process to evaluate the method. The analytical parameters considered in this validation study were specificity, linearity, accuracy and precision following USP 29/NF 24. Because of limit amount of medicarpin, the investigation performed only linearity in the experiment.

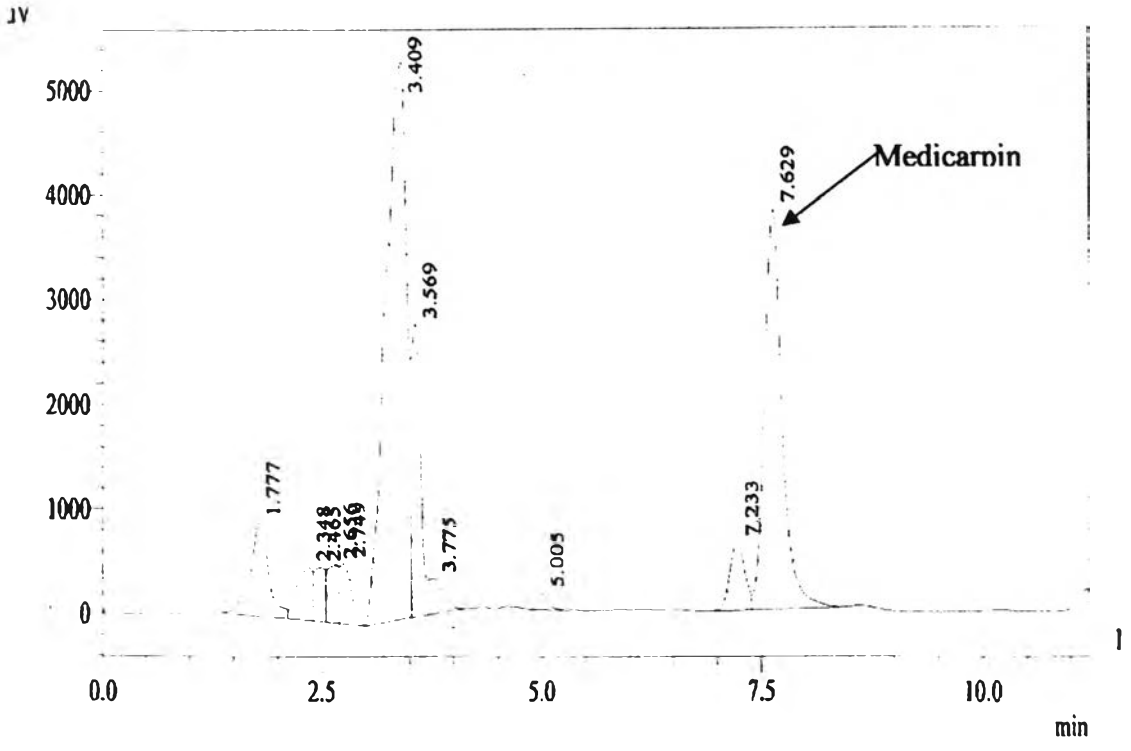


Figure 20. HPLC chromatogram of medicarpin.

Linearity

A linearity study was carried out to determine whether this method could measure accurately different concentrations of medicarpin. The linearity curve of the peak area versus the concentrations of medicarpin are shown in Figure 21.

The medicarpin concentration that gave linear standard curve was in the range of 0.12 – 4.80 µg/ml as shown in Table 16 in Appendix B. The regression coefficient (R^2) for standard curve was 0.9999 for medicarpin. This result showed a good linearity of standard concentration and peak area.

The extracts are usually complex mixtures of several chemical constituents. For a large majority of botanical extracts it is not known with the certainty which various components are responsible for the reported pharmacological effect. It is generally believed that several constituents act synergistically to provide the report effect. USP 29 defined that certain chemical constituents of botanical extracts are chosen and quantitative test procedures for determining their content are provided. The choice of such constituents, know generally as marker compounds, is based on considerations. The marker compounds are specified and may be identified in raw material. In this investigation, medicarpin was chosen as active marker because it was found in *Butea superba* Roxb. And it shown pharmacological activity contributing in

some extent to efficacy (Miller et al., 1989; Jack et al., 1992; Stadler et al., 1994; Aoki et al., 2000). However, its clinical efficacy has yet to be proven.

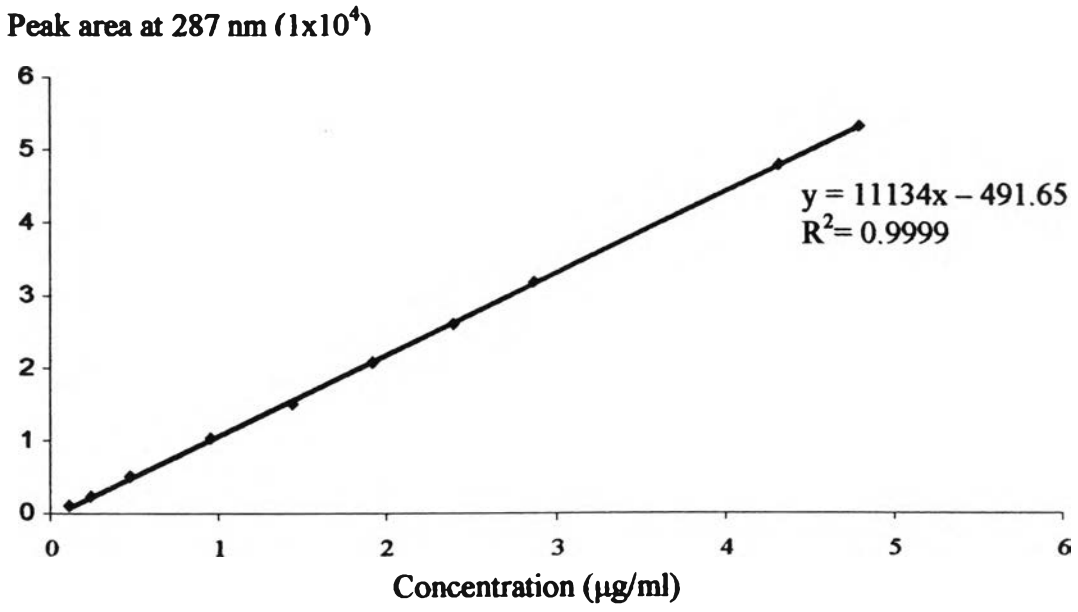


Figure 21. Linearity curve of the peak area versus the concentrations of medicarpin.

2. Formulation of dry powder of *Butea superba* extract.

2.1 Extraction of *Butea superba* Roxb in ethanol(EtOH).

2.1.1 Preparation of *Butea superba* fluidextrats.

According to USP 29 / NF 24 fluidextracts, also as liquid extracts was described, are preparations of plant matter, containing alcohol as a solvent or as a preservative, or both and are so made that each mL contains the extracted constituents of 1 g of the crude material that it presents, unless otherwise specified in the individual monograph. Pharmacopeial fluidextracts are made by percolation or maceration. The required solvent is specified in the individual monograph. The common manufacturing procedure includes concentration or distillation under vacuum at temperatures below 60°C. The time of maceration may be varied to adjust for the quantity and natural of the crude material under extraction, provided that the composition of the extract constituents of interest is not adversely affected (USP 29 / NF 24).

For the maceration technique in botanical extracts, the crude material being extracted is reduced to pieces of suitable size, mixed thoroughly with the specified

extracting solvent, and allowed to stand at room temperature in a closed container for appropriate time, with frequent agitation until soluble matter is dissolved. The mixture is filtered, the insoluble material is washed with the same solvent used for maceration, and the filtrates are combined and concentrated, usually under reduced pressure, to the desired consistency. Dried powder tuber of *Butea superba* Roxb..(18 kg) was macerated by both 50% ethanol and 95% ethanol (5 x 20L each.) at room temperature for 7 days. The 50% and 95% ethanol extract solutions were filtered and evaporated under reduced pressure to obtain dark-red gummy (as shown in Figure 7) and dark-brown residue as shown in Figure 22. (500 g and 570 g) respectively. Both extracts were identified by thin layer chromatography (TLC) method. Their TLC patterns were similarity as 95% ethanol extract shown in Figure 8. and 50% ethanol extract (4 spots on terminal line) shown in Figure 23.

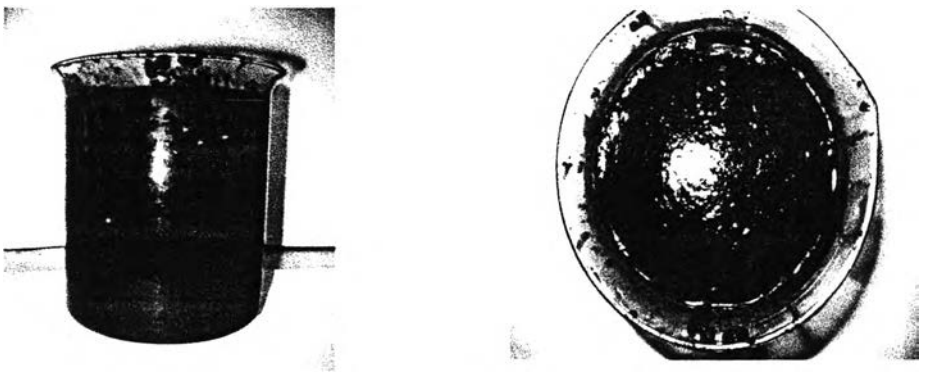


Figure 22. *Butea superba* 50% ethanolic extract.

Figure 23. TLC patterns of *Butea superba* 50% ethanolic extracts.

The texture of both extracts was like glue, because carbohydrate in the plant was soluble in water-ethanol mixture (Chu and Chow, 2000). Carbohydrate as starch

or sugar is a major component isolated from green plant. They consist of a mixture of amylopectin and amylase. Amylose is a linear polymer made of alpha-D-glucopyranose unit like through alpha (1-4) linkage. Amylopectin is a large branched molecule with side chains grafted to the linear alpha (1-4) polymer by a single alpha (1-6) junction (Sung Hyo Chough et al., 2006).

For the quality control of liquid herbal drug preparations, for example tinctures, liquid extracts, powdered herbal drug, etc, the European Pharmacopoeia 4th edition defined that alcoholic liquid extract and tinctures have to comply with test on the ethanol content (certain and prescribed) and methanol (not more than 0.05% v/v methanol) and 2-propanol (not more than 0.05% v/v 2-propanol). The ethanol content in pharmaceutical preparations is determined by means of the pycnometer or hydrometer methods after distillation. The test on methanol and 2-propanol is performed by gas chromatography using a pack glass column after distillation. The new application is performed with capillary headspace GC/MS method (Apers et al., 2003). In this study on formulation development of *Butea superba* extract tablets, ethanol content was not determined because the gummy residue obtained from ethanolic extract had dark color and viscous texture, it could not be detected by pycnometer because the viscosity of gummy may interfere the result of detection.

2.12 Determination of the water content in *Butea superba* fluidextract.

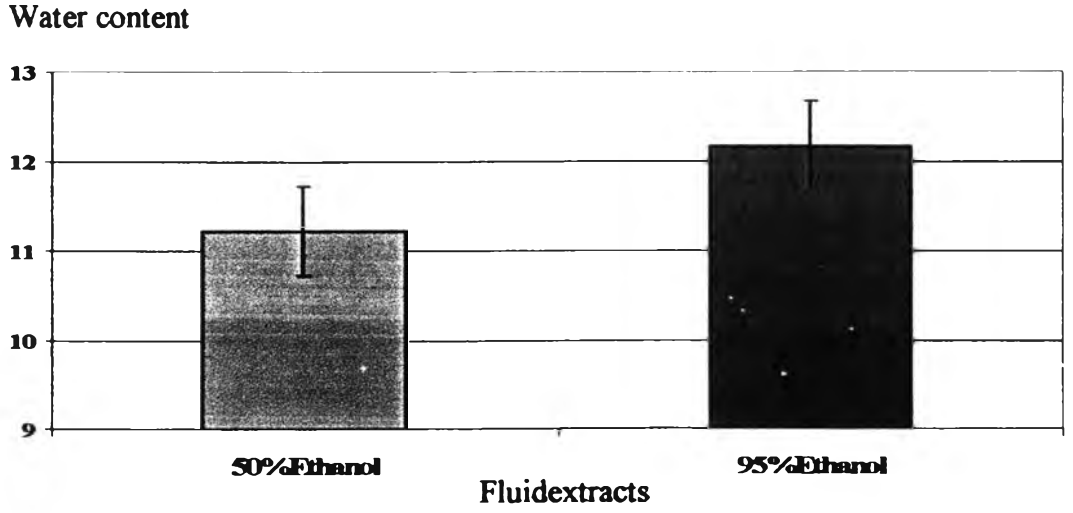


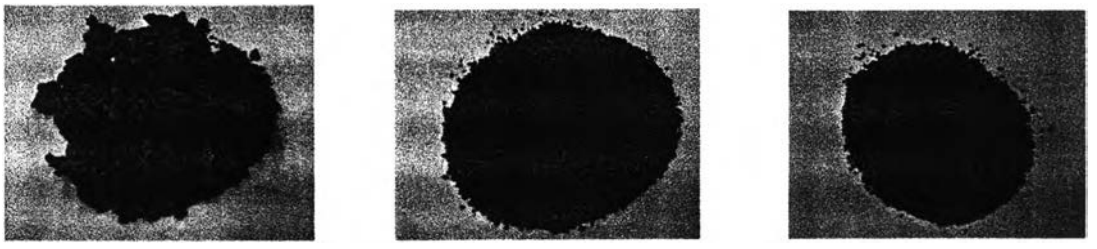
Figure 24. The water content of 50% and 95% ethanol *Butea superba* fluidextracts.

From the data obtained, 50% ethanol *Butea superba* fluidextracts have water content of 11.22% and 95% ethanol *Butea superba* fluidextracts have water content of 12.18% as shown in Figure 24 and Table 17 in Appedix B. The gravimetric method found that the physical appearance of extracts was in solid form without solvent. The texture was hard and the color of 50% ethanol fluidextracts was brownish and 95% ethanol fluidextracts was dark-red purple. Water and ethanol were exposed to heat at 105°C, and evaporated from fluidextracts because water has boiling point at 100°C and ethanol has boiling point at 78.5°C (Kibbe, 2000).

2.2 Preparation of dry powder of *Butea superba* extract.

Powdered extracts are defined following USP 29 / NF 24 as solid preparations having a powdery consistency obtained by evaporation of the solvent used for extraction. They may contain suitable added substances such as excipients, stabilizers, and preservatives. Standardized powdered extracts are adjusted to define content of constituents, using suitable inert materials or a powdered extract of the plant matter used for preparation (USP 29 / NF 24). Botanicals may have poor flow, low bulk density, variable particle size distributions and compression properties significantly different from general use excipients (Kopleman, 2001).

The inert pharmaceutical excipients were chosen to incorporate into 50% and 95% ethanolic extracts. From the preliminary study, the ratio of liquid extract and excipients (as starch or lactose) of 1:1 resulted in sticky damp mass and difficulty in the sieving process. When dried, the obtained granules were hard and brittle similar to sugar powder. The ratio of 1:2 resulted in wet damp mass and easy sieving process. When dried, the mixture was like agglomerate granules. The last ratio of 1:3 resulted in wet damp mass and also ease in sieving process. However when they were dried a lot of fine powders were obtained. Therefore, the optimum ratio of liquid extract and excipient was 1:2. The formulation with 50% ethanolic extract gave brown dry granules while 95% ethanolic extract provided mild red-purple dry granules. Figure 20 shows dry granules of different ratio of liquid extract and excipient (as starch or lactose) of 1:1, 1:2 and 1:3.



a) b) c)

Figure 25. Dry granules extract produced of the ratios of liquid extract and excipients (as starch or lactose) of a) 1:1, b) 1:2 and c) 1:3.

In addition, the diluents in all formulation were starches because of in the preliminary study, formulation in the ratio 1:2 of *Butea superba* extract with lactose and corn starch or tapioca starch gave wet sticky damp mass and difficulty to pass through the screening as shown in Figure 26. Therefore, in this study the 2 diluents were chosen as tapioca starch and corn starch as previous studies (Chen and Ramaswamy, 1999; Mishra and Rai, 2006).

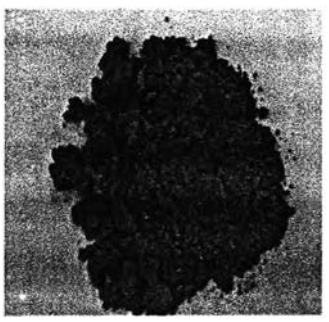


Figure 26. *Butea superba* extract with lactose and starch at the ratio 1:2.

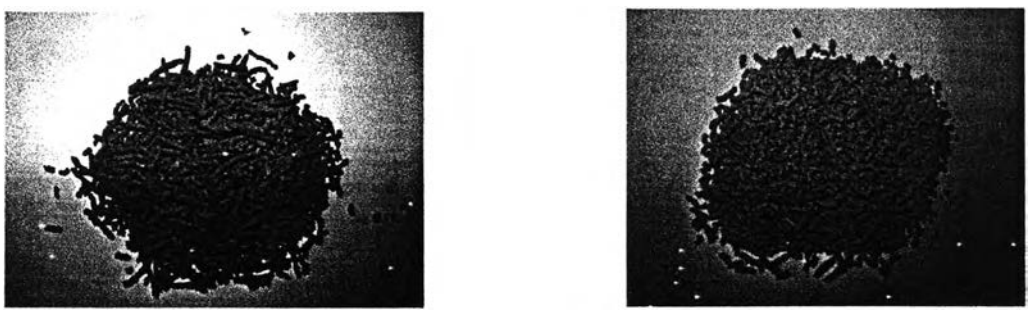
From the investigation about the storage under tropical conditions (31°C, 95% RH) on the behavior of microbial contamination of tablets as formulation contained lactose, rice starch or tapioca starch without preservative. Microbial cells were found in the starting materials (Bos et al., 1989). Responding to research contamination of aflatoxins in the herbal medicinal products in Thailand containing high amount of starch or sugar, aflatoxins is readily produced from *Aspergillus* species (Tassaneeyakul et al., 2004). Therefore, in this formulation development, microbiological stability of tablets was then to avoid the use of lactose in the dry granule *Butea superba* extract. Moreover, medicarpin in the *Butea superba* extract could be showed antimicrobial activity (Stadler et al., 1994; Aoki et al., 2000).

The binder in the formulations were microcrystalline cellulose as Avicel® PH 101, it useful in the spray-dried extract of *Maytenus ilicifolia* (Soares et al., 2005) and good binding functionality in direct compression process than starch, dicalcium phosphate (Zhang et al., 2003) as formulation of St. John's wort tablets (von Eggelkraut-Gottanaka et al., 2002) and formulation of ibuprofen (Inghelbrecht and Remon, 1998). Avicel® PH 102 has been used as binder in direct compressible diluent (Eiliazadeh et al., 2004; Zhang et al., 2003; Inghelbrecht and Remon, 1998), as vehicle carrier in fluid bed granulation (Kistensen and Hansen, 2006). Polyvinyl povidone (PVP) as polymer binder and degree were used K-30 and K-90, which have difference in the viscosity and binding property (Gibby, 2000). PVP K-30 was reported to be used in granules from the fluid bed (Kistensen and Hansen, 2006).

2.3 Evaluation of dry powder of *Butea superba* extract.

2.3.1 Organoleptic properties

Sine the original fluid extracts were different, formulations of *Butea superba* 95% ethanolic extract gave mild red-purple dry granules as shown in Figure 27a. They had odor of alcohol and astringent sweetened taste. In contrast, the formulation from *Butea superba* 50% ethanolic extract showed brown dry granules as shown in Figure 22b, odor like sugar mixed with alcohol and sweetened with bitter taste. The formulations with the same fluid extract were not different in the color, odors and taste properties. But, the texture of granules was different in some formulation as shown in Table 18.



a) b)

Figure 27. Granules of *Butea superba* extracts as a) 95% ethanolic extract and b) 50% ethanolic extract.

From the data obtained, the physical appearances were divided in 2 groups as agglomerate granules and hard granules. Agglomerate granule were made from the formulation compounding with microcrystalline cellulose (Avicel® PH101 and PH 102) and starch. Granule could be broken down with mild pressing force by fingers. It might be formulation have microcrystalline cellulose showed the binder properties (Kibbe, 2000). Hard granules were obtained from the formulation compounding with polyvinylpyrolidone as PVP K-30 and PVP K-90. Granule could be broken down upon higher pressing force by fingers than those with microcrystalline cellulose because the intrinsic property of the binders were different as microcrystalline cellulose obtained partially depolymerized cellulose but polyvinylpyrolidone were synthetic polymer (Gibby, 2000).

2.3.2. Moisture content

The moisture content of dry powder *Butea superba* extract was shown in Figure 28. Their moisture contents were in the range of 6.0-8.0% and shown in Table 19. in Appendix B . Different inert excipients showed different water absorption of granules. Moisture content is necessary in the tableting process, because it supports to inter-intragranular binding properties (Bandelin, 1989). The formulation with higher amount of starch had higher moisture content.

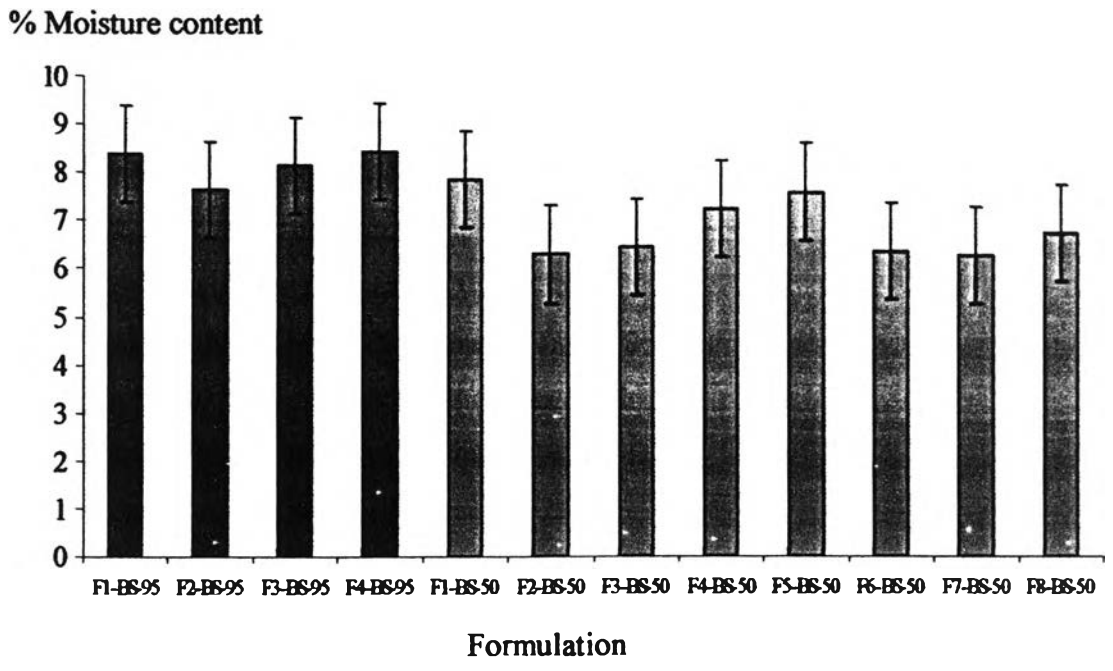


Figure 28. Moisture content of dry powder *Butea superba* extract.

Because of *Butea superba* extract contained carbohydrate, being a major constituent of plant and being soluble in solvent system (water and water-ethanol mixture) commonly used for extracting active principles from herb, are almost invariably present in crude herbal extract. Depending on their hydrophilic nature and hygroscopic character, these carbohydrates may be for the commonly observed moisture sorption tendency of herbal extract (Chu and Chow, 2000). Dry granule extract were compounding with starch. All starches are hygroscopic and rapidly absorb atmospheric moisture, for example 11% for corn starch at 50% RH (Gibby, 2000). From the result obtained, formulation were contained with high amount of tapioca starch have higher moisture content as F1-BS-95 and F1- BS-50. F4-BS-95 were contained with tapioca starch, corn starch and polyvinylpyrolidone (PVP K-30), therefore polyvinylpyrolidone as hygroscopic powder (Gibby, 2000). That resulted high moisture content. Formulation as F3-BS-50, F4-BS-50 and F5-BS-50 were high moisture content because their compounded microcrystalline cellulose as hygroscopic property (Gibby, 2000). Some formulations as F6-BS-50 and F7-BS-50 had minimum moisture content, because their without microcrystalline cellulose (Avicel® PH101 and PH 102) contained in the formulation less than the other formulation.

2.3.3 **Density and compressibility**

One important property of agglomerated products was the bulk density (Knight, 2001; Paul et al., 2001). Packing of a granular material is usually quantified by apparent (bulk) densities or poured density, that are always defined as the ratio between the sample mass and its total volume, including any internal interstice, both inter- and intraparticle. There are different ingredients compound of formulations based on the procedure used to achieve the packing (Santomaso et al, 2003). The bulk density of granule depended on the particle size distribution (Martin, et al., 1993; Heng et al, 2004). From the result obtained all of dry powder of *Butea superba* extract had similar bulk density of about range 0.26-0.31 g/ml as showed in Figure 29 and in Table 20. in Appendix B.

Table 18 The organoleptic property of dry powder of *Butea superba* 50% and 95% ethanolic extract.

Formulation	Component (%w/w)	Color of granule
F1-BS-95	*Butea superba* extract (33.33), Tapioca starch (66.66)	Mild red-purple granule, odorous of alcohol, astringent & sweeten taste, agglomerate granule.
F2- BS-95	*Butea superba* extract (33.33), Corn starch (66.66)	Mild red-purple granule, odorous of alcohol, astringent & sweeten taste, agglomerate granule.
F3- BS-95	*Butea superba* extract (33.33), Tapioca starch(33.33), corn starch (33.33)	Mild red-purple granule, odorous of alcohol, astringent & sweeten taste, agglomerate granule.
F4- BS-95	*Butea superba* extract (33.33),Tapioca starch (31.66),corn starch(31.66),PVP K-30 (3.33)	Mild red-purple granule, odorous of alcohol, astringent & sweeten taste, agglomerate granule.
F1- BS-50	*Butea superba* extract (33.33), Tapioca starch (66.66)	Brown granule, odor like sugar & alcohol, sweeten & bitter taste, agglomerate granule.
F2- BS-50	*Butea superba* extract (33.33), Corn starch (66.66)	Brown granule, odor like sugar & alcohol, sweeten & bitter taste, agglomerate granule.
F3- BS-50	*Butea superba* extract(33.33),Tapioca starch(30.55),Corn starch(30.55),AvicelPH101(5.55)	Brown granule, odor like sugar & alcohol, sweeten & bitter taste, agglomerate granule.
F4- BS-50	*Butea superba* extract(33.33),Tapioca starch(27.77),Corn starch(27.77),AvicelPH101(11.11)	Brown granule, odor like sugar & alcohol, sweeten & bitter taste, agglomerate granule.
F5- BS-50	*Butea superba* extract(33.33),Tapioca starch(30.55),Corn starch(30.55),AvicelPH102(5.55)	Brown granule, odor like sugar & alcohol, sweeten & bitter taste, agglomerate granule.
F6- BS-50	*Butea superba* extract(33.33),Tapioca starch(31.66),Corn starch(31.66), PVP K-30(3.33)	Brown granule, odorous like sugar and alcohol, sweeten and bitter taste, hard granule.
F7- BS-50	*Butea superba* extract(33.33),Tapioca starch(29.57),Corn starch(29.57),PVP K-30(7.50)	Brown granule, odorous like sugar & alcohol, sweeten & bitter taste, hard granule.
F8- BS-50	*Butea superba* extract(33.33),Tapioca starch(32.07),Corn starch(32.07), PVP K-90 (2.5)	Brown granule, odorous like sugar & alcohol, sweeten & bitter taste, hard granule.

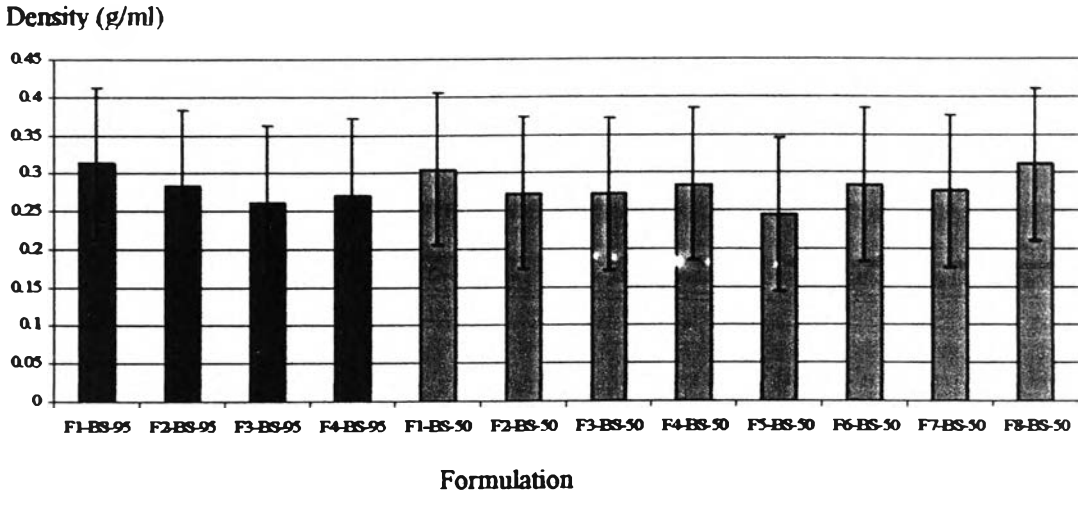


Figure 29. Bulk density of dry powder of *Butea superba* 50% and 95% ethanolic extracts.

Tap density is obtained by vibration or hitting the collector cup according to a given procedure, in an attempt to obtain the highest packing (and density then) before compaction. In practice it is difficult to prevent any compaction while hitting the powder. In the case of tap density, the condition sought is that of a dense random packing to be contrasted with the loose random packing state of the apparent density. It has been observed that the flowability is connected with the ratio of a 'high' to a 'low' density value. The 'high' density condition must be obtained by somehow forcing the powder to pack as much as possible (but not compact), while the 'low' density state is that of minimum packing that the granules can maintain naturally, without continuous addition of some force sort of force (such as fluidizing air). The higher the ratio between 'high' and 'low' density values, the more cohesive is the powder, or the less able to flow (Santomaso et al, 2003). From the result obtained, the tapped density of all dry powder of *Butea superba* extract as shown in Figure 30 and Table 20 in Appendix B ., were increased from the bulk density as defined in 3 reasons as the following.

1) For the formulations of dry powder of *Butea superba* extract without binder as F1-BS-95 (bulk density from 0.313 to tapped density of 0.385 g/ml), F2-BS-95 (bulk density from 0.283 to tapped density of 0.378 g/ml), F3-BS-95 (bulk density from 0.262 to tapped density of 0.335 g/ml), F1-BS-50 (bulk density from 0.305 to tapped density of 0.363 g/ml) and F2-BS-50 (bulk density from 0.273 to tapped density of 0.357 g/ml), the increase in density was caused from the less cohesion in

the agglomerated granules. In addition fine powder was produced and observed fine during evaluation by tapped density tester.

2) For the formulation of dry powder *Butea superba* extract with microcrystalline cellulose (Avicel® PH101 and PH 102) as binder in F3-BS-50 (bulk density from 0.272 to tapped density of 0.362 g/ml), F4-BS-50 (bulk density from 0.284 to tapped density of 0.372 g/ml) and F5-BS-50 (bulk density from 0.244 to tapped density of 0.302 g/ml), the increase in density was due to agglomerated granules could be broken to fragment and substituted in void volume. They had less fine powder when evaluated by tapped density tester.

3) For the formulation of dry powder *Butea superba* extract with polyvinylpyrolidone (PVP K-30 and PVP K-90) as binder in F4-BS-95 (bulk density from 0.271 to tapped density of 0.354 g/ml), F6-BS-50 (bulk density from 0.283 to tapped density of 0.371 g/ml), F7-BS-50 (bulk density from 0.275 to tapped density of 0.368 g/ml) and F5-BS-50 (bulk density from 0.311 to tapped density of 0.373 g/ml), granules were good agglomerates as made form polyvinylpyrolidone, carbohydrate and resin (Heng et al, 2004) in *Butea superba* extract.

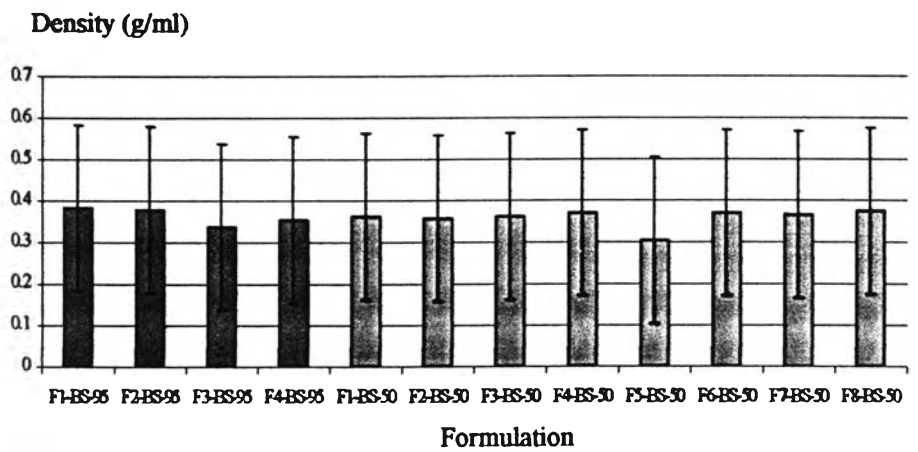


Figure 30. Tapped density of dry powder of *Butea superba* 50% and 95% ethanolic extracts.

The compressibility index was to predict the granules flow inhibition. It is a measure of activity for conical construction and useful measure of flow. The Carr's index description was shown in Table 21 (Davies, 2001).

The Carr's index of all dry powder of *Butea superba* extract formulations as shown in Figure 31 and Table 20 in Appendix B. In the 95% *Butea superba* extract formulation, F1-BS-95 gave the best value in this group (19.05%), because it contained tapioca starch which has binding property (Chen and Ramaswamy, 1999; Mishra and Rai, 2006). In addition, 95% ethanolic extract provided optimum binding property that when applied pressing force, granules could be break down to fragment and move to substitute void volume.

Table 21. Description flowability by Carr's index.

Carr's index (%)	Flow
5-12	Free flowing
12-16	Good
18-21	Faire
23-33	Poor
35-38	Very poor
>40	Extremely poor

Similarly to 50% *Butea superba* extract formulation F1-BS-50, F5-BS-50 and F8-BS-50 gave better value than other formulation. F1-BS-50 (16.18%) were compound with tapioca starch and 50% ethanolic extract made optimum binding property when applied compaction force granule could be break down to fragment granule with low compaction force (Chen and Ramaswamy, 1999; Mishra and Rai, 2006). F5-BS-50 (18.73%) were compound with tapioca starch, corn starch, Avicel® PH102 and 50% ethanolic extract, Avicel® PH102 showed binding properties to carried all excipients with compressibility (Eiliazadeh et al., 2004; Zhang et al., 2003; Inghelbrecht and Remon, 1998). F8-BS-50 was compound with tapioca starch, corn starch, PVP K-90 and 50% ethanolic extract. PVP K-90 might hold the powder to agglomerate granule with good compressibility. Result of the formulations F1-BS-95, F1-BS-50, F5-BS-50 and F8-BS-50 of dry extracts seemed to be optimal to produce good tablets.

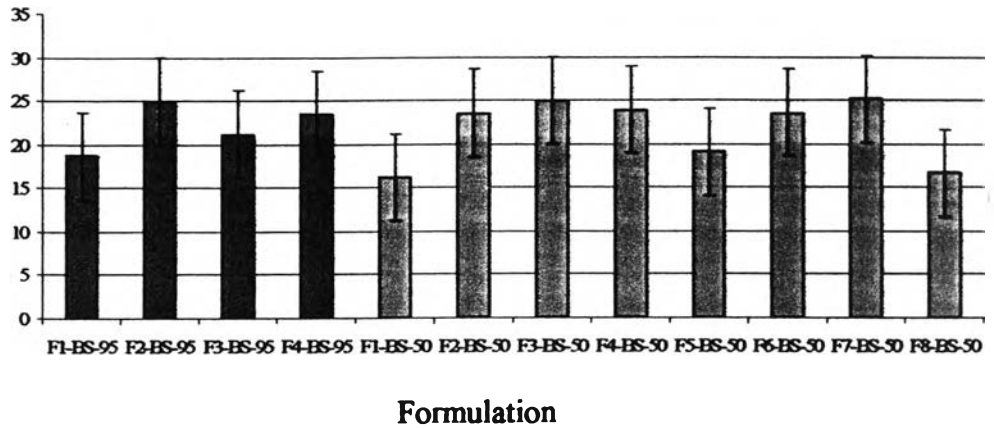


Figure 31. Carr's indices of all dry powder of *Butea superba* 50% and 95% ethanolic extract formulation.

2.3.4 Determination of flow rate

The flow rate of dry extract *Butea superba* granules can be measured by funnel method. Dry granules flowed pass the funnel orifice. Each formulation of dry extract had different flow property as shown in Figure 32 and Table 22 in Appendix B. Some granules had fast flowing property while some granules showed slow flowing. The excellent flowing was from granules with less fine particles and ease to pass through funnel orifice without obstruction. The result was shown in Figure 32. In the case of 95% *Butea superba* extract formulation, F3-BS-95 and F4-BS-95 had excellent flowing as 0.58 g/sec, 0.55 g/sec respectively. F3-BS-95 compounded with *Butea superba* extract, tapioca starch and corn starch, had less fine particle because corn starch showed good binding property (Adebayo and Itiola, 2003). Similarly, F4-BS-95 were compounding with *Butea superba* extract, corn starch, tapioca starch and PVP K-30 as binder.

From the data of 50% *Butea superba* extract formulation, the flow rate of F8-BS-50 and F5-BS-50 were 0.64 g/sec, 0.62 g/sec respectively. F8-BS-50 were compounded with tapioca starch, corn starch, *Butea superba* extract and PVP K-90 as excellent binding to produce less fine particles in the formulation. F5-BS-50 were compounded with tapioca starch, corn starch, *Butea superba* extract and Avicel®

PH102 also had less fine particle. These formulations had good flowing properties. The other formulations had similar low flow rate value that may be formulations had fine granules. Although the flowability of dry granules indicated of good tablet uniformity as expressed on tablet manufacturing process when granules filling from the hopper into die. However, it is necessary to other parameters to produce good taolet property such as friability, disintegration time, etc.

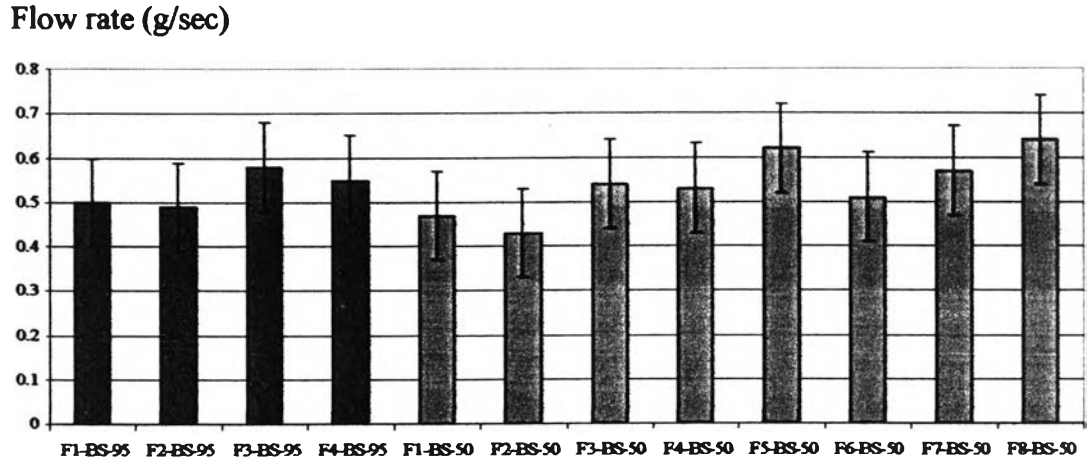


Figure 32. Flow rates of dry extract of *Butea superba* 50% and 95% ethanolic granules.

2.3.5 Determination of the amount of Medicarpin compare with fluid extract

The amount of medicarpin from dry extract *Butea superba* granule both of 50% and 95% ethanolic extract were identified by HPLC method (Eng Shi Ong, 2004; Hendrik et al, 2005). The chromatogram of powdered extracts with 50% and 95% ethanolic fluid extract were compared standard medicarpin. Their chromatograms as shown in Figure 33. were similar to that of medicarpin in Figure 20.

It was found that 50% ethanolic liquid extract had 0.04125% medicarpin as shown in Table 23. and in dry granule extract as randomized from F5-BS-50 had 0.016103% medicarpin as showed in Table 24. The 95% ethanolic liquid extract had 0.15089% medicarpin as shown in Table 25. and in dry granule extract as randomized from F1-BS-95 had 0.052467% medicarpin as shown in table 26.

Since dry granule extracts were to produce tablets following the criteria of conventional tablet such as good appearance, uniformity of thickness, ease of compression, friability of less than 1%, hardness within 5-8 kp, weight variation of

less than 5%, and disintegrate within 15 minutes, the only a conformed tablet formulations of each fluid extracts were then chosen to determine the amount of medicarpin in both fluid extract and dry granule extract.

Table 23. Content uniformity of 50% ethanolic *Butea superba* fluidextract.

N0.	Weight (mg)	Amount of medicarpin (mg)	%w/w medicarpin in fluidextract
1	122.7	0.04807	0.03917
2	122.3	0.04766	0.03896
3	124.0	0.05221	0.04211
4	123.5	0.05307	0.04297
5	123.7	0.05115	0.04135
6	123.5	0.05144	0.04165
7	123.8	0.05303	0.04283
8	122.3	0.05108	0.04177
9	123.2	0.04993	0.04053
10	123.4	0.05080	0.04117
			Mean=0.04125 SD = 0.00129 RSD = 3.13556

Table 24. Content uniformity of 50% ethanolic *Butea superba* extracts granule (F5-BS-50).

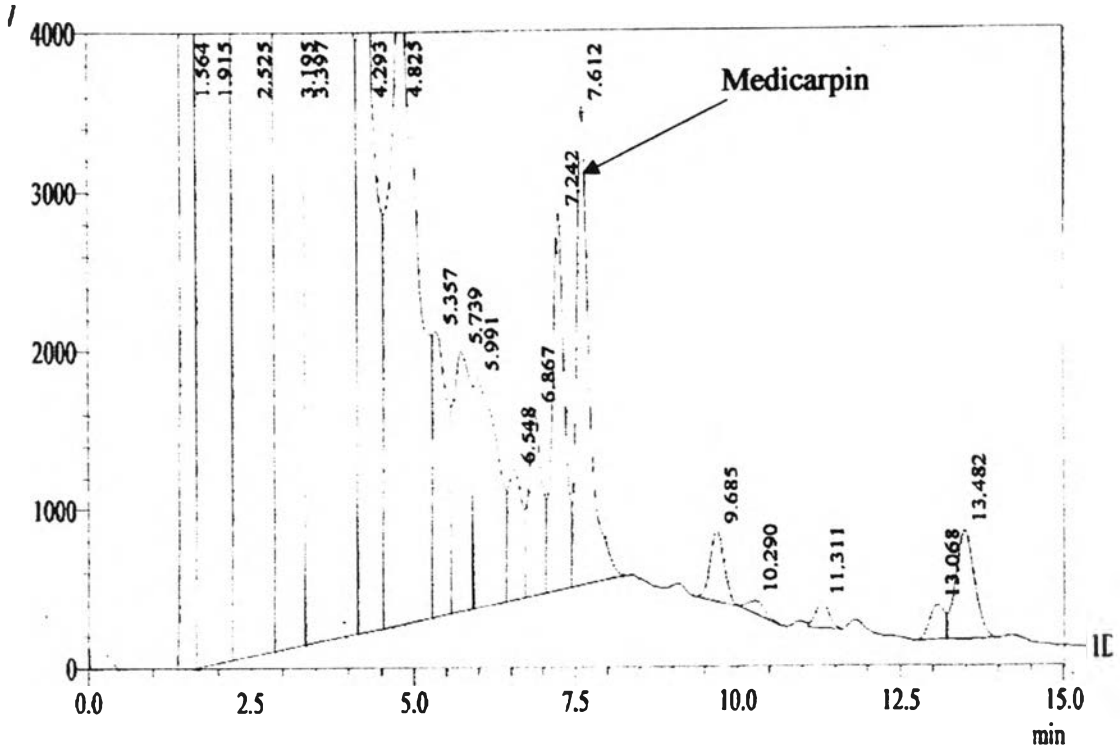
N0.	Weight (mg)	Amount of medicarpin (mg)	%w/w of medicarpin
1	400.5	0.06457	0.01608
2	400.9	0.06557	0.01635
3	401.2	0.05958	0.01485
4	400.7	0.06261	0.01562
5	400.8	0.06161	0.01537
6	401.9	0.06358	0.01935
7	401.2	0.05991	0.01637
8	400.3	0.06213	0.01455
9	400.1	0.06411	0.01523
10	400.4	0.06591	0.01668
		Mean=0.06295 SD = 0.00207 RSD = 3.29	Mean=0.016103 SD = 0.00174 RSD = 3.13

Table 25. Content uniformity of 95% ethanolic *Butea superba* fluidextracts.

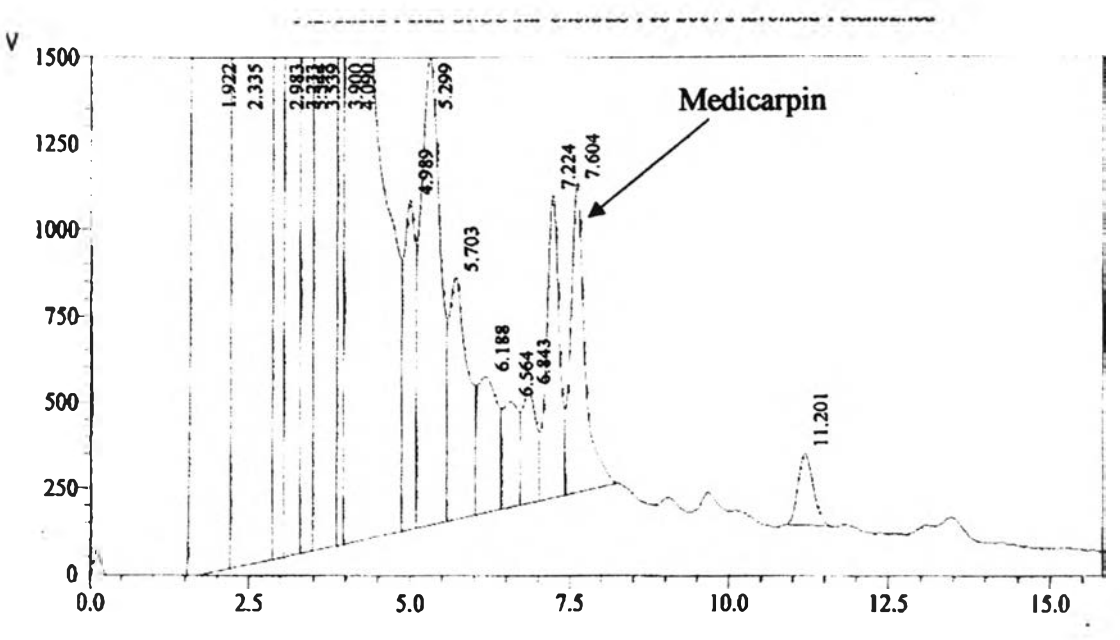
N0.	Weight (mg)	Amount of medicarpin (mg)	%w/w medicarpin in fluidextract
1	123.9	0.18643	0.15046
2	124.6	0.19199	0.15408
3	122.0	0.18357	0.15046
4	124.8	0.18890	0.15136
5	124.0	0.18848	0.15200
6	122.7	0.18346	0.14952
7	123.1	0.18502	0.15030
8	124.4	0.18649	0.15149
9	124.2	0.18618	0.14990
10	122.3	0.18266	0.14935
			Mean=0.15089 SD = 0.00133 RSD = 0.8875

Table 26. Content uniformity of 95% ethanolic *Butea superba* extracts granule (F1-BS-95).

N0.	Weight (mg)	Amount of medicarpin (mg)	%w/w of medicarpin
1	400.2	0.21451	0.05360
2	400.5	0.21750	0.05431
3	400.7	0.21915	0.05469
4	400.8	0.20291	0.05063
5	401.3	0.19978	0.04978
6	402.3	0.20581	0.05115
7	400.6	0.21116	0.05271
8	401.8	0.21018	0.05232
9	401.1	0.21315	0.05314
10	400.3	0.2958	0.05234
		Mean=0.21037 SD = 0.00585 RSD = 2.65	Mean=0.052467 SD = 0.0015 RSD = 2.862



a)



b)

Figure 33 HPLC Chromatogram as a) 95% ethanolic fluidextracts, b) 50% ethanolic fluid extracts.

3. **Formulation development of *Butea superba* extract tablets.**

Good formulation of dry extracts granules was selected for tablets formulation process. Twelve dry *Butea superba* extracts granules had satisfactory properties according to the evaluation criteria of dry extract powder. These granule properties were uniformity of color, good smell and taste compliance. The moisture content, flow rate, density and compressibility were important to predict the formulation manufacturing. Dry granules with optimized moisture content were depended on the type of active ingredients. The number of Carr's index is useful to choose the formulation, while the flow rate had benefit to present flowability of dry granules (USP 29 / NF 24).

From the twelve formulations, the investigation was further to select the ingredient for the compounding of tablet formulation. Ac-Di-Sol® (Croscamellose sodium) is a superdisintegrant and useful to tablet disintegrate in herbal extract pharmaceutical dosage form (von Eggelkraut-Gottanaka et al., 2002; Luiz Alberto Lisa Soares et al., 2005). Since formulation with herbal extract contains many substances, for example sucrose and carbohydrate (Chu and Chow, 2000; Sung et al, 2006), this causes problem in the disintegration time due to the high viscosity of extract, resulted in high tablet hardness from its binding properties when drying. Therefore Ac-Di-Sol® was selected in the formulation. Lubricant and glidant were stearic acid and Aerosil® (colloidal silicon dioxide) because medicarpin is an isoflavonoid, a phenolic compound, which is very sensitive to break aromatic ring bonding in pH range of 8-10.5 (Havsteen, 2002; Faraj and Vasanthan, 2004). Therefore the formulation should avoid substances of alkaline properties like metallic stearate (magnesium stearate). Stearic acid is also compatible microcrystalline cellulose (Rowe, 1988). Aerosil® wildely used in pharmaceutical product. Its small particle size and large specific surface area give desirable flow characteristics which exploited to improve the flow properties of dry extract granules (von Eggelkraut-Gottanaka et al., 2002; Luiz Alberto Lisa Soares et al., 2005).

4. Evaluation of *Butea superba* extract tablets.

All of the formulations were tableted by concave punches, After freshly prepared and storage in desiccators, various properties were evaluated as the following.

4.1 Physical appearance

The physical characteristics of *Butea superba* extract tablets from 2 sources of material were different. The 50% *Butea superba* ethanolic extract tablets were brown glossy concave tablets. The 95% *Butea superba* ethanolic extract tablets were mild red-purple glossy concave tablets. Both formulations had diameter about 9.66 mm and the thickness of about 4.15 mm as shown in Table 27. (Appendix B). Previous investigation found that concave and convex punch were made fundamental problem as "capping" and "laminating" of paracetamol (Eiliuzadeh et al., 2004). These did not occured in all 50% and 95% *Butea superba* ethanolic extract tablets. Dokwhan®, a commercial *Butea superba* tablets were brown, capsule-shape tablet with the diameter of 11.28 mm and the thickness about 5.55 mm as shown in Table 27. (Appendix B).. The physical characterization of both preparations and commercial tablets are shown in Figure 34 and Table 28.

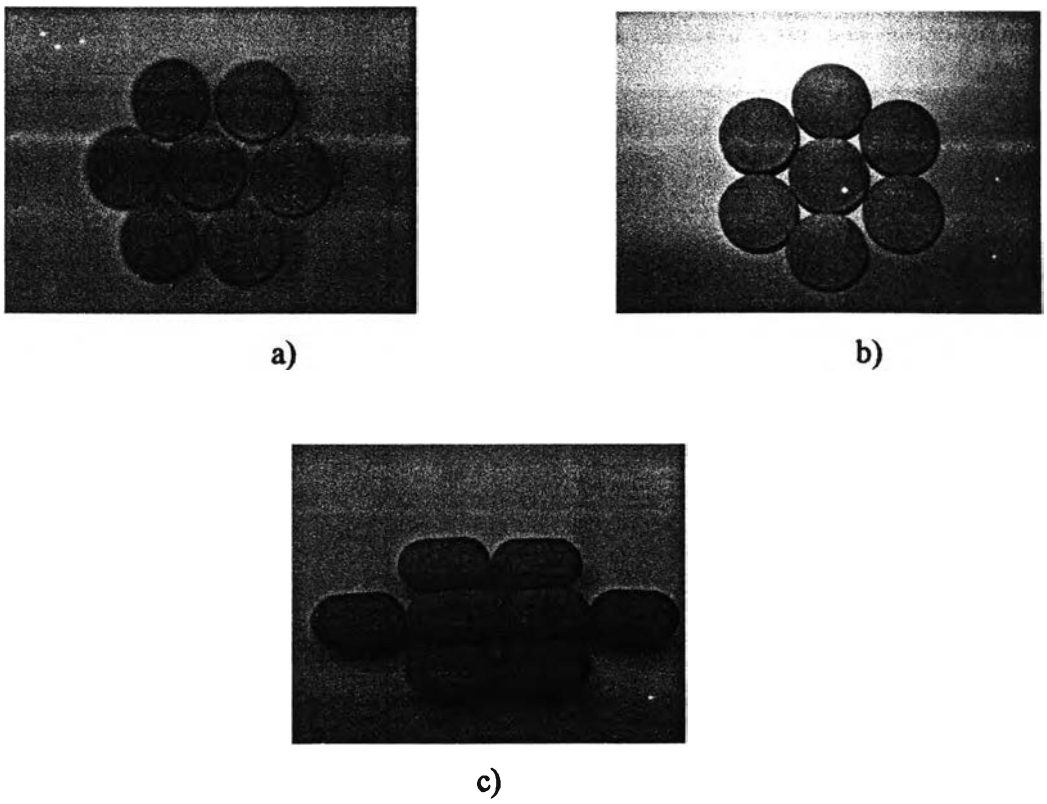


Figure 34. Physical characteristics of *Butea superba* tablets as a) 50% ethanolic extract tablets, b) 95% ethanolic extract tablets and c) Dok-wan® tablets.

Table 28 Formulation of *Butea superba* extract tablets.

Formulation	Component (%w/w)	Granule(mg)	5% Ac-Di-Sol®(mg)	0.5% Aerosil®(mg)	3% Stearic acid(mg)	Weigh/tablet
F1-BS-95	*Butea superba* extract (33.33), Tapioca starch (66.66)	400	20	2	12	434
F2-BS -95	*Butea superba* extract (33.33), Corn starch (66.66)	400	20	2	12	434
F3-BS -95	*Butea superba* extract (33.33), Tapioca starch(33.33), corn starch (33.33)	400	20	2	12	434
F4-BS -95	*Butea superba* extract (33.33),Tapioca starch (31.66),corn starch(31.66),PVP K-30 (3.33)	400	20	2	12	434
F1-BS -50	*Butea superba* extract (33.33), Tapioca starch (66.66)	400	20	2	12	434
F2-BS -50	*Butea superba* extract (33.33), Corn starch (66.66)	400	20	2	12	434
F3-BS -50	*Butea superba* extract(33.33),Tapioca starch(30.55),Corn starch(30.55),AvicelPH101(5.55)	400	20	2	12	434
F4-BS -50	*Butea superba* extract(33.33),Tapioca starch(27.77),Corn starch(27.77),AvicelPH101(11.11)	400	20	2	12	434
F5-BS -50	*Butea superba* extract(33.33),Tapioca starch(30.55),Corn starch(30.55),AvicelPH102(5.55)	400	20	2	12	434
F6-BS -50	*Butea superba* extract(33.33),Tapioca starch(31.66),Corn starch(31.66), PVP K-30(3.33)	400	20	2	12	434
F7-BS -50	*Butea superba* extract(33.33),Tapioca starch(29.57),Corn starch(29.57),PVP K-30(7.50)	400	20	2	12	434
F8-BS -50	*Butea superba* extract(33.33),Tapioca starch(32.07),Corn starch(32.07), PVP K-90 (2.5)	400	20	2	12	434
Dokwhan®	*Butea superba*	180	-	-	-	600

4.2 Friability

The percentage of friability from both extract tablet formulations after preparation are present in Figure 35 and Table 29 in Appedix B. From the data obtained, the percentage of friability of F1-BS-95 (0.49%), F2-BS-95 (0.37%) and F5-BS-50 (0.68%) were less than other formulations. Although other formulations could be compressed to tablets but percentage of friability more than 1.0% were shown inappropriate in practical manufacturer. Some Dokwhan® tablets were found to be capping and the percentage of friability was 0.68%. The friability of F1-BS-95, F2-BS-95 and F5-BS-50 formulations were conformed to the USP29 specification of less than 1.0%.

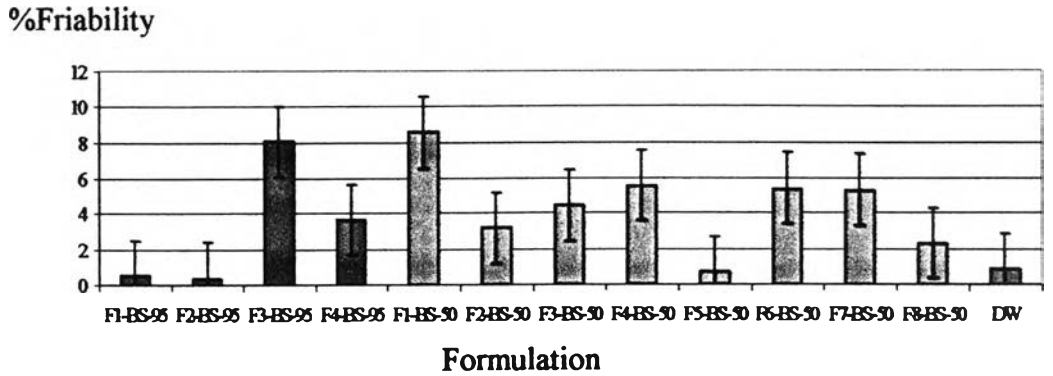


Figure 35. Friability of *Butea superba* 50% and 95% ethanolic extract tablet compared to Dokwhan® tablets (DW).

4.3 Hardness

Conventional tablet have hardness value about 4-8 kp (USP 29). The mean hardness of the prepared tablets are displayed in Figure 36 and Table 30 in Appedix B. The hardness of formulations of F1-BS-95, F2-BS-95, F4-BS-50, F5-BS-50 and F7-BS-50 were 5.82 kp, 5.48 kp, 5.94 kp, 5.72 kp and 5.52 kp, respectively. They had hardness values within specification. Both formulations tended to have satisfactory tablets properties. Dokwhan® tablets had hardness of 2.38 kp.

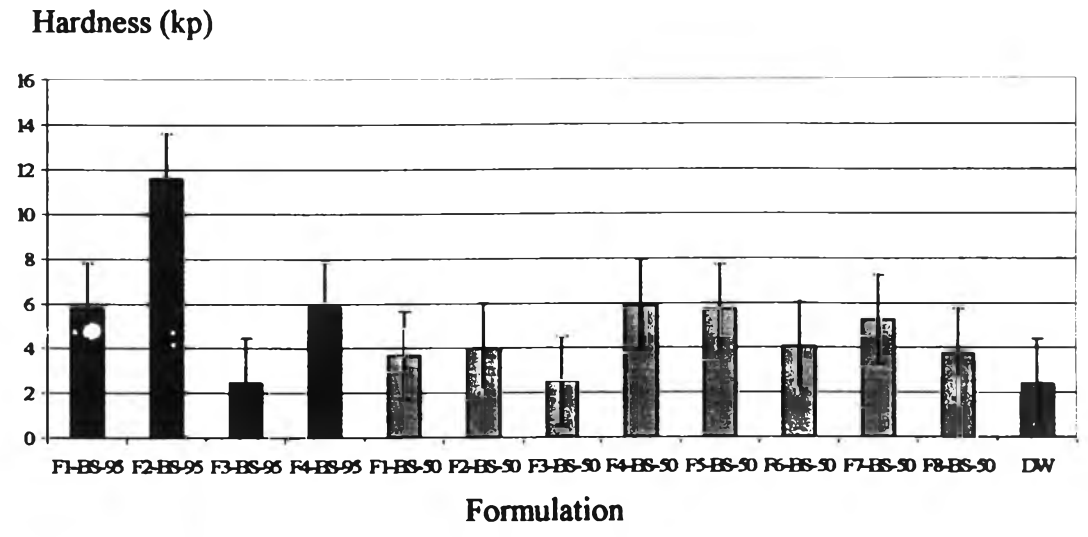


Figure 36. Hardness of *Butea superba* 50% and 95% ethanolic extract tablets compared to Dokwhan® tablets (DW).

4.4 Weight variation

The weight variations of all *Butea superba* extract tablets formulations are display in Figure 37 and Table 31. in Appedix B. The weight variations were conformed to the specification in USP27 (Average difference of less than 5%). The lower standard deviation of weight variation of extract tablets expressed to indicate that formulation had good flowability and uniformity of mixing. That was agreed with the flow rate and Carr's index of dry extract granule before compression of tablets. The weight variation formulations of F5-50, F1-95 and Dok-wan® tablets were less than 5%.

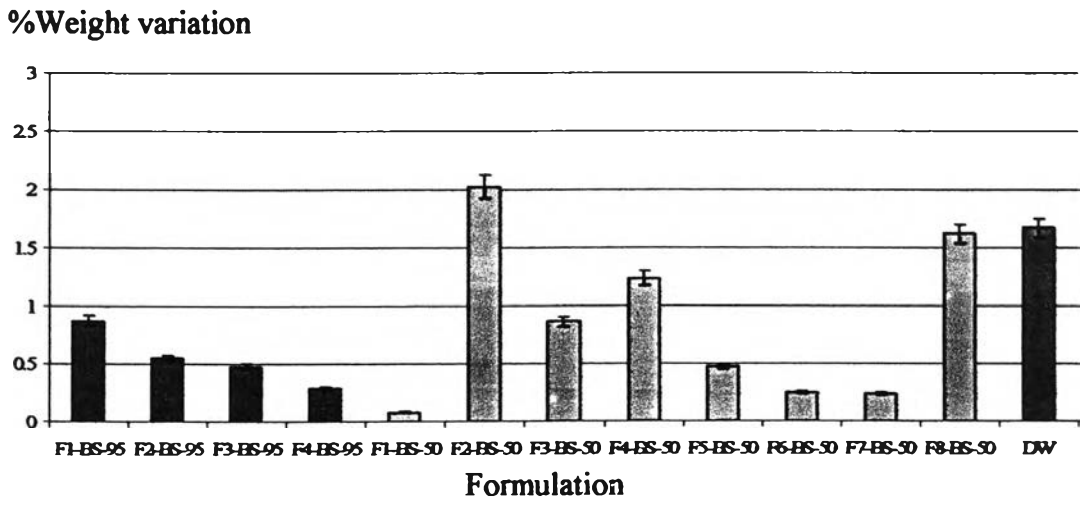


Figure 37. Weight variation of *Butea superba* 50% and 95% ethanolic extract tablets compared to Dokwhan® tablets (DW).

4.5 Disintegration time

The disintegration time of all *Butea superba* extract tablets formulations are presented in Table 32. (Appendix B) and shown in Figure 33. The disintegration time of *Butea superba* extract tablet in de-ionized water at temperature 37° C were within 15 minutes. This was probable due to the addition of Ac-Di-Sol®, a high performance disintegrant. The disintegration time of 95% *Butea superba* ethanolic extract tablet formulation in de-ionized water was longer than that of 50% *Butea superba* ethanolic extract tablet formulations. This was due to the percent of solvent in the process of *Butea superba* extraction. The 95% ethanol might dissolve many non-polarity substances such as resin and gum found in tuber root which could delay disintegration time with their binding property and non-wetability in tested medium. From the data obtained, the disintegration time of F1-BS-95, F2-BS-50, F3-BS-50, F5-BS-50, F6-BS-50 and Dok-wan® tablets were 10.11 min, 10.19 min, 9.32 min, 8.30 min, 7.52 min and 22.40 min respectively.

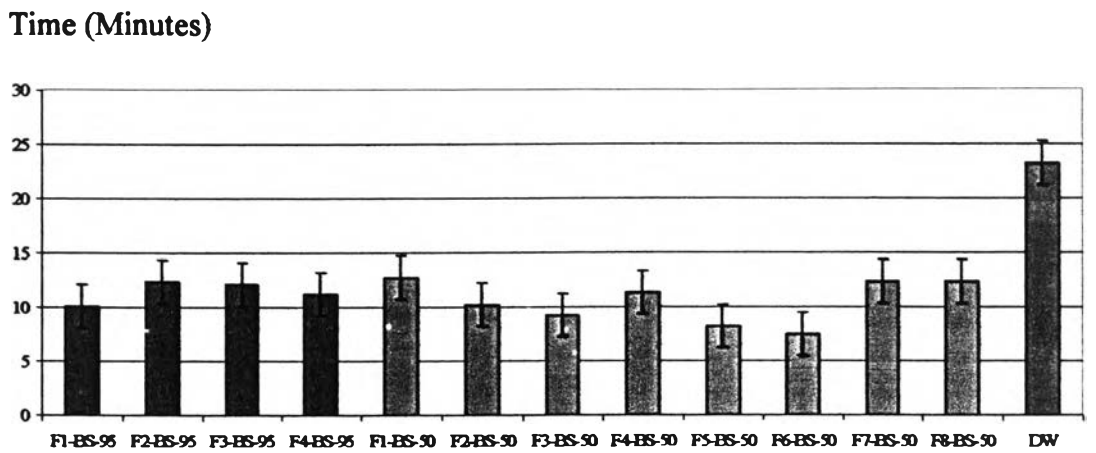


Figure 38. Disintegration time of *Butea superba* 50% and 95% ethanolic extract tablets compared to Dokwhan® tablets (DW).

Table 33. Overall physical examination of formulation of *Butea superba* extract tablets and Dokwhan® tablets (DW).

Formulation	Ingredients	Appearance	%Friability	Hardness(kp)	%Weight variation	Disintegration time(min)
F1-BS-95	*Butea superba* extract, Tapioca starch, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	0.49	5.82	0.88	10.11
F2- BS-95	*Butea superba* extract, Corn starch, Ac-Di-Sol® ,Aerosil®, Stearic acid	Good	0.37	11.60	0.55	12.37
F3- BS-95	*Butea superba* extract, Tapioca starch, Corn starch, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	8.05	2.50	0.48	12.18
F4- BS-95	*Butea superba* extract, Tapioca starch, Corn starch, PVP K-30, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	3.67	5.48	0.29	11.23
F1- BS-50	*Butea superba* extract,Tapioca starch, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	8.58	3.70	0.08	12.72
F2- BS-50	*Butea superba* extract, Corn starch, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	3.19	4.00	2.02	10.19
F3- BS-50	*Butea superba* extract,Tapioca starch, Corn starch, AvicelPH101,Ac-Di-Sol®, Aerosil®, Stearic acid	Good	4.45	2.54	0.87	9.23
F4- BS-50	*Butea superba* extract, Tapioca starch,Corn starch, AvicelPH101, Ac-Di-Sol®, Aerosil®,Stearic acid	Good	5.56	5.94	1.24	11.41
F5- BS-50	*Butea superba* extract, Tapioca starch, Corn starch, AvicelPH102, Ac-Di-Sol®,Aerosil®,Stearic acid	Good	0.68	5.72	0.47	8.30
F6- BS-50	*Butea superba* extract, Tapioca starch, Corn starch, PVP K-30, Ac-Di-Sol®, Aerosil®,Stearic acid	Good	5.40	4.10	0.25	7.52
F7- BS-50	*Butea superba* extract, Tapioca starch, Corn starch, PVP K-30, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	5.31	5.25	0.24	12.35
F8- BS-50	*Butea superba* extract, Tapioca starch, Corn starch, PVP K-90, Ac-Di-Sol®, Aerosil®, Stearic acid	Good	2.32	3.72	1.62	12.53
DW	*Butea superba*, *Elephantopus scaber*, *Ficus fovedata* wall.	Good	0.86	2.35	1.67	22.40

4.6 The uniformity of dosage form

From the conventional tablet testing and result obtained as physical appearance, friability, hardness, weight variation and disintegration time to select the best of *Butea superba* extract tablets formulation as shown in Table 22. They were F1-BS-95 and F5-BS-50, because both formulations have properties of good appearance, friability less than 1%, hardness value due to specification, weight variation less than 5% and disintegration time interval of 8.0-11.0 min. In addition, in the granule testing topic F1-BS-95 and F5-BS-50 had uniformity of color, smell odor , taste acceptance. Moreover the moisture content was not more than 9% to made dry granule hygroscopic and percent of Carr's index was in the level of fair flowing.

For the content uniformity of *Butea superba* extract tablets, medicarpin was found in liquid extract, dry granule extract and extract tablets as shown in Figure 40 and 41. Dokwhan® tablets did not have medicarpin in the formulation as demonstrated in the HPLC chromatogram in Figure 39.

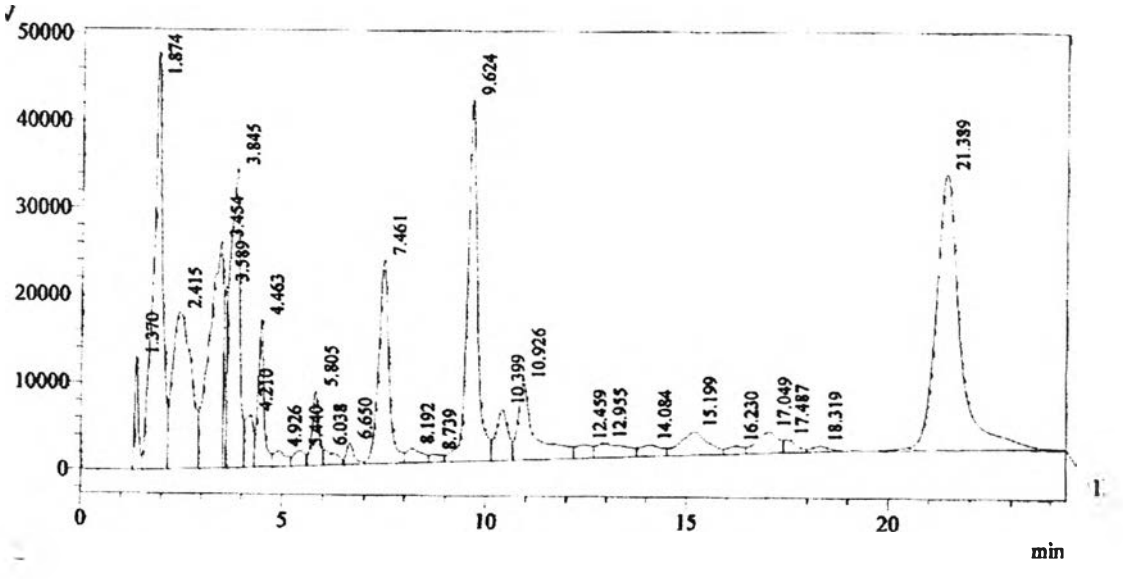


Figure 39 HPLC chromatogram of Dok-wan® tablets.

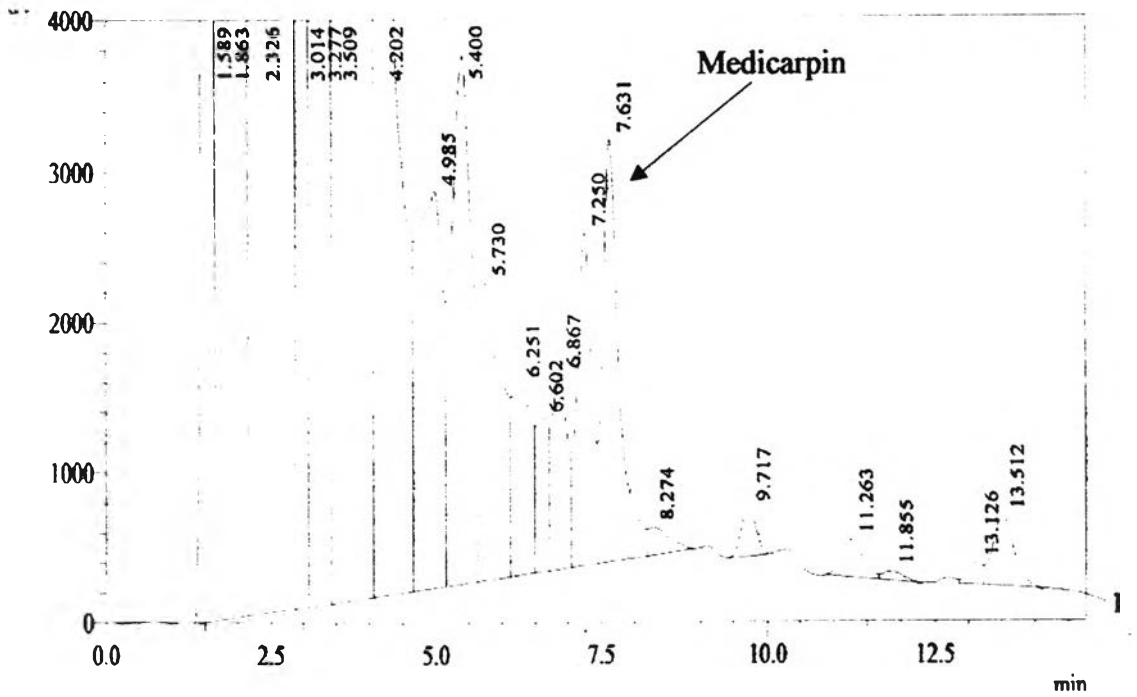


Figure 40. HPLC chromatogram of Butea superba 95% ethanolic extract tablet (F1-BS-95).

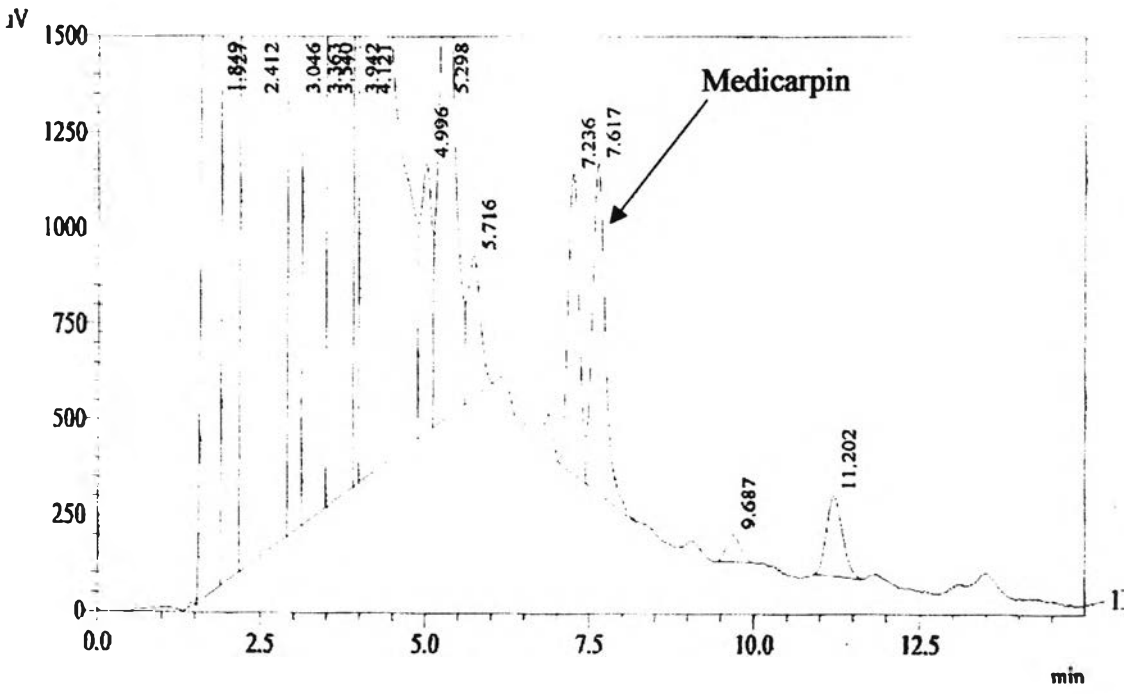


Figure 41. HPLC chromatogram of Butea superba 50% ethanolic extract tablet (F5-BS-50).

The content uniformity of freshly prepared *Butea superba* extract tablets, are shown in Table 34. and 35. The formulation of F5-BS-50 had of 98.82% of the label amount of medicarpin. The percentage of standard deviation (%RSD) was 6.93. The formulation of F1-BS-95 had of 99.09% of the label amount of medicarpin. The percentage of standard deviation (%RSD) was 2.92.

Table 34. Content uniformity of 50% ethanolic *Butea superba* extract tablets.

No. Tablet	Tablet weight (mg)	Amount of medicarpin (mg)	%w/w of medicarpin	% Label amount
1	431.2	0.06819	0.01716	106.56
2	433.6	0.06718	0.01681	104.39
3	432.8	0.05451	0.01366	84.83
4	436.3	0.05863	0.01458	90.54
5	438.2	0.06968	0.01729	107.37
6	434.3	0.06128	0.01531	95.08
7	435.1	0.06249	0.01558	96.75
8	433.7	0.06338	0.01585	98.43
9	436.6	0.06657	0.01654	102.71
10	434.9	0.06558	0.01636	101.59
		Mean = 0.063767 SD = 0.00446 RSD = 6.994	Mean=0.015851 SD = 0.00106 RSD = 6.687	Mean = 98.83 SD = 6.85232 RSD = 6.933

Table 35. Content uniformity of 95% ethanolic *Butea superba* extract tablets.

No. Tablet	Tablet weight (mg)	Amount of medicarpin (mg)	%w/w of medicarpin	% Label amount
1	431.3	0.21585	0.05430	103.49
2	432.4	0.21745	0.05456	103.99
3	434.5	0.19788	0.04947	94.28
4	434.5	0.19537	0.04878	92.97
5	434.7	0.20466	0.05108	97.36
6	434.8	0.19995	0.04989	95.09
7	434.1	0.20631	0.05156	98.27
8	435.5	0.21123	0.05262	100.29
9	436.3	0.21864	0.05437	103.62
10	433.3	0.21289	0.05331	101.61
		Mean=0.208023 SD = 0.00799 RSD =3.84	Mean=0.051953 SD = 0.00209 RSD = 4.02	Mean = 99.09 SD = 3.8979 RSD = 2.92

The result passed the specification of general monograph of USP29, in which the content uniformity of the tablets was within the range of 90-100 % of the label amount and percentage of standard deviation (%RSD) was close to 6.

The determinated amount of medicarpin in fluid extract, dry granules extract and extracts tablet form 50% ethanol extracts were 0.04125 %w/w, 0.01610 %w/w and 0.01585 %w/w, respectively. Those from 95% ethanolic *Butea superba*

fluidextracts were 0.15089 %w/w, 0.05246 %w/w and 0.05195 %w/w, respectively. The comparisons were shown in Figure 42.

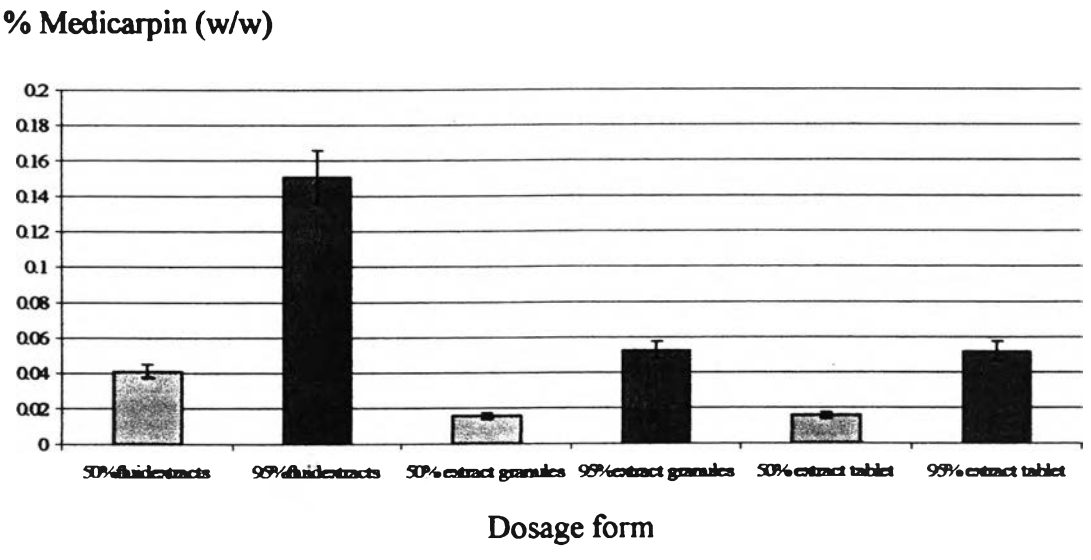


Figure 42. The comparisons of medicarpin were 50% and 95% ethanolic *Butea superba* extracts in 3 dosage form.

4.6 Dissolution study

The extraction method of *Butea superba* indicated that medicarpin was poorly soluble in water whereas it could be soluble in mixture of water and alcohol system. It was reported that, was the partially organic dissolution medium, particularly water-alcohol solvent system could be used (Corrigan, 1991). From preliminary study, the chosen dissolution medium for this experiment was the mixture of water-ethanol at the ratio of 80:20, because the concentration of ethanol was not too high in the medium or rapidly evaporated at 37±0.5° C.

From the previous investigations, carbohydrate, sugar and organic acids (citric acid and malic acid) in herbal extracts of *Eschscholtzia californica* Cham., may be responsible for increased hygroscopicity and poor processing behaviour of the extract include dissolution profile (Schiller et al., 2002). Gum and resin were found in *Baphicacanthus cusia* extract which had influence in dissolution study (Heng et al., 2004).

The dissolution at 30 minutes of F5-BS-50 and F1-BS-95 formulations (n=6) were in the range of 93.45-101.0% and 94.65-103.26% label amount (Table 36. in Appendix B). The percent of dissolution at 30 minutes of both formulations were sufficiently high profile as shown in Figure 43 and 44.

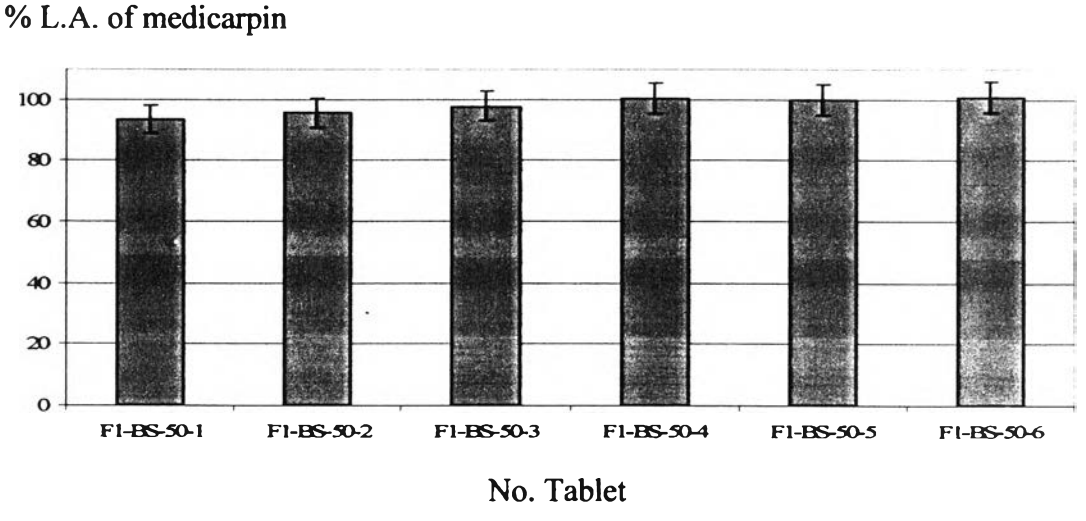


Figure 43. Dissolution of 50% ethanolic *Butea superba* extract tablets (F5-BS-50).

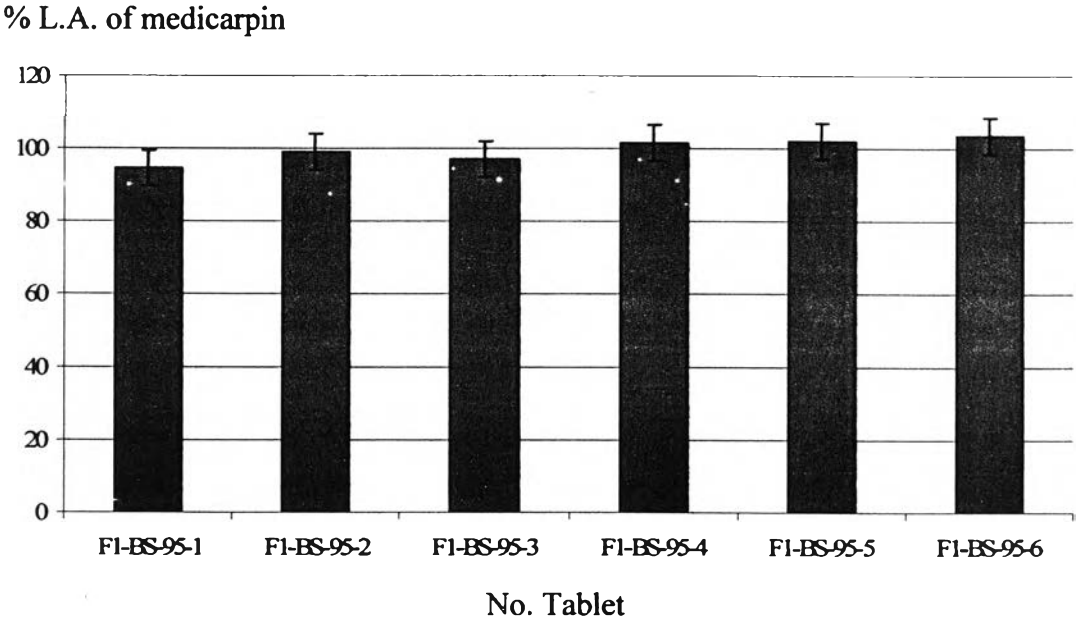


Figure 44. Dissolution of 95% ethanolic *Butea superba* extract tablets (F1-BS-95).

5. Stability study

In the manufacturing industry, *Butea superba* extract tablets produced from 50% ethanol fluidextract would be preferred than those of 95% ethanol fluidextract, because 50% ethanol was diluted from 95% ethanol resulted in save cost production. Low concentration of ethanol was hardly flammable or explosion. The process was easy to handle and storage could be done at room temperature. Solvent from the process could be recovered by distillation and adjusted concentration by 95% ethanol. There was less toxicity to operator and low pollution in the environment. Fluidextracts produced from 50% ethanol had better taste than 95% ethanol. And finally, the color and odor of 50% ethanol extract as brown and smell like sugar were better than those of 95% ethanol as red-purple and smell like alcohol.

From the stated reasons, *Butea superba* extract tablets produced from 50% ethanol fluidextract were of interest to be scaled-up and subjected to stability study.

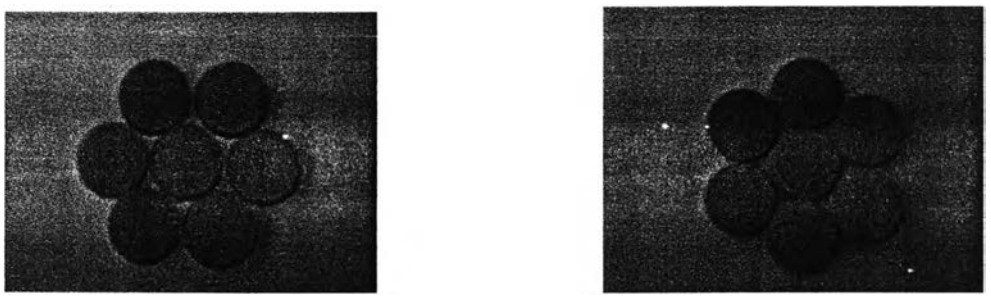
USP 29 / NF 24 defined factors affecting product stability as the primary environment factors that can reduce stability include exposure to adverse temperatures, light, humidity, oxygen, and carbon dioxide. The major dosage form factor that influence drug stability include particle size (in emulsion and suspension), pH, solvent system composition (% free water and overall polarity), compatibility of anions and cations, primary container, specific chemical additives and molecular binding and diffusion of drug and excipients.

In dosage forms, the reaction as hydrolysis, epimerization, decarboxylation, dehydration, oxidation, photochemical decomposition, ionic strength, pH effect, interionic compactibility, solid state stability and temperature usually cause loss of active drug content, and they usually do not provide obvious visual of olfactory evidence of their occurrence.

From the stability study of St. John's wort tablet carried out under three different storage conditions for 6 months (as 25±2° C and 60±5% RH, 40±2° C and 75±5% RH) and 5 months (50° C), the hardness and friability were not changed. The marker compounds (hypericin and pseudohypericin) were significantly decreased with time (Shah et. al., 2005).

Medicarpin as flavonoid substance was decomposed by alkaline hydrolysis (Markham, 1982; Faraj and Vasanthan, 2004). *Butea superba* extract tablets were investigated without excipients showing alkaline property such as magnesium stearate. Medicarpin may be stable in extract tablet on the stability study.

Butea superba extract tablets of formulation of F5-BS-50 were packed under plastic bags and storage in glass desiccators. The containers were stand alone under ambient temperature for 1 year and 10 months. The color of tablet was browner than the freshly prepared one as shown in Figure 45. The hardness and friability were similar to freshly prepared and percentage of the remaining of medicarpin are not different initial preparation. The content was about 97.91%.



a) b)

Figure 45. Physical appearance of F5-50 *Butea superba* extract tablets as a) Freshly prepared and b) stored 1 year and 10 months.

From the data obtained as shown in Table 34., the amount of medicarpin in freshly preparation were found to be 98.82%. After storage for 1 year and 10 months the amount was slightly decreased to 97.91% as shown in Figure 46. and Table 38. Both freshly prepared and stored tablets were not different in content of medicarpin.

The physical testing was performed on both freshly prepared and stored tablets. The friability test of freshly prepared tablet and stored tablet were found to be 0.68% and 0.55% respectively as shown in Figure 47. Hardness of freshly prepared tablet and stored *Butea superba* extract tablets were found to be 5.72 kp and 5.85 kp, respective and showed in Figure 48. The disintegration time of freshly prepared tablets was less than tablets after the storage (8.30 compared to 8.55 min) as shown in

Figure 49. This was probably that table stored in the desiccators could adsorb moisture. Moreover carbohydrate, sugar, resin and organic acid (citric acid and malic acid) were not hygroscopicity (Schiller et al., 2002; Heng et al., 2004). Therefore tested tablets showed unchanged physical properties.

Table 38. Content uniformity of 50% ethanolic *Butea superba* extract tablets 1 year and 10 months.

No. Tablet	Tablet weight (mg)	Amount of medicarpin (mg)	% Label amount
1	435.5	0.06487	100.36
2	437.8	0.06697	103.02
3	434.7	0.05741	88.99
4	434.5	0.05758	89.30
5	429.4	0.05655	88.68
6	435.9	0.06564	101.41
7	436.7	0.06636	102.34
8	431.3	0.06399	99.92
9	436.6	0.06457	99.67
10	433.1	0.06781	105.45
		Mean = 0.06317 SD = 0.00407 RSD = 6.44	Mean = 97.91 SD = 6.056 RSD = 6.18

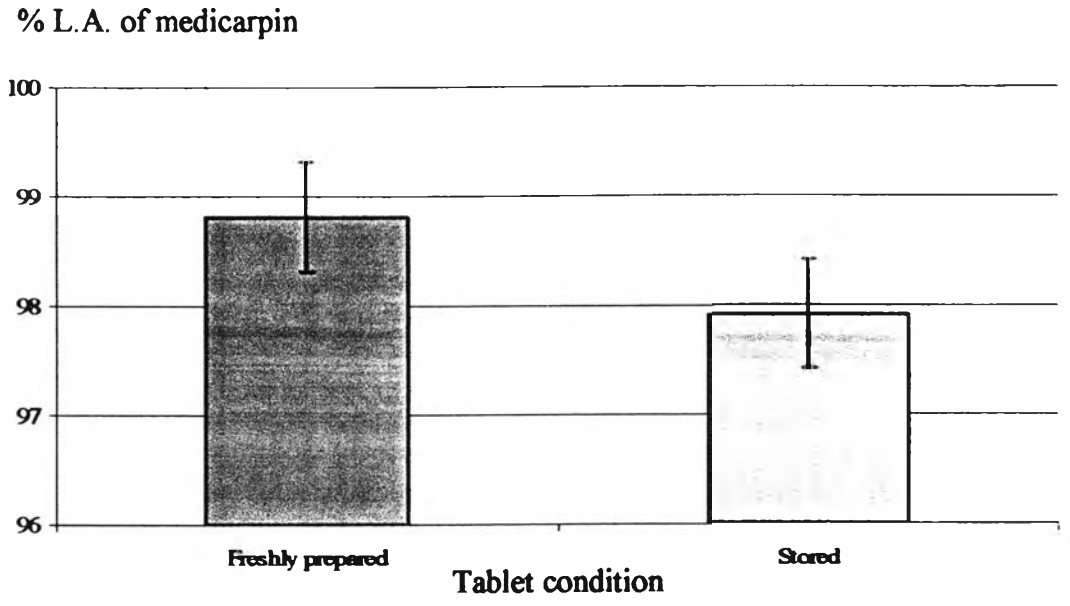


Figure 46. Content uniformity of *Butea superba* extract tablet, freshly prepared and stored tablets.

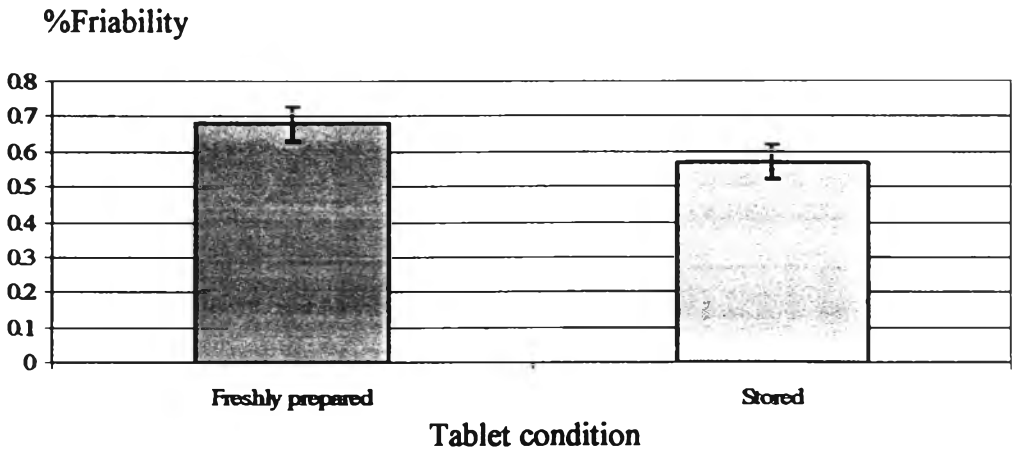


Figure 47. Friability of *Butea superba* extract tablets, freshly prepared and stored tablets.

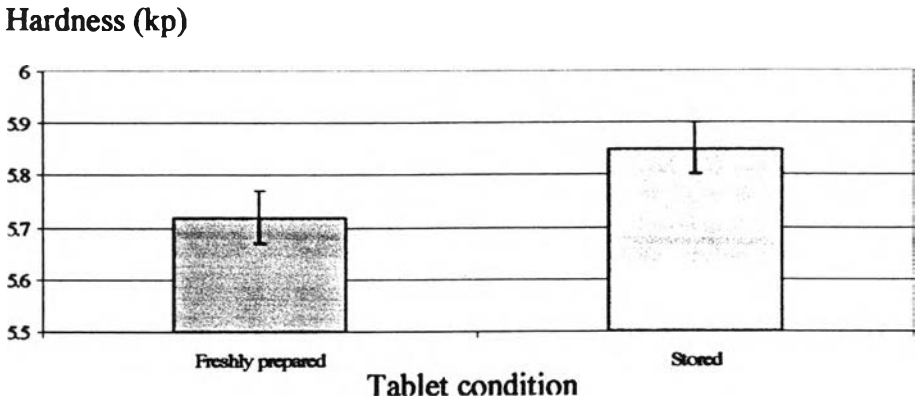


Figure 48. Hardness of *Butea superba* extracts, freshly prepared and stored tablets.

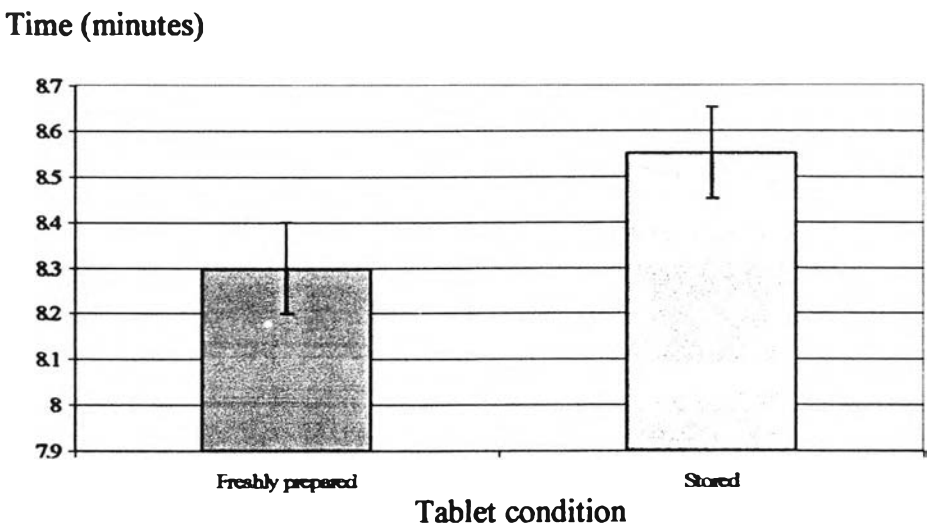


Figure 49. Disintegration time of *Butea superba* extract tablets, freshy prepared and stored tablets.