

LAMPIRAN A
HASIL PARAMETER KADAR ABU

Perhitungan Penetapan Kadar Abu

No.	Berat Kurs Kosong + Tutup	Berat Serbuk	Berat Kurs + Abu	% Kadar Abu
1	31,2620	2,0004	31,4058	7,19 %
2	31,2618	2,0002	31,4070	7,25 %
3	31,2622	1,9777	31,4038	7,16 %
Rerata ± SD				7,20% ± 0,05

$$\text{Rata-rata} = \frac{7,19 + 7,25 + 7,16}{3} \times 100\% = 7,20\%$$

LAMPIRAN B

HASIL PARAMETER SARI LARUT AIR

Pemeriksaan Kadar Sari Larut Air

No	Berat Ekstrak (g)	Berat Konstan Cawan (g)	Berat Konstan Cawan + Serbuk (g)	Kadar Sari Larut Air (%)
1	5,0042	38,4905	38,5606	1,40
2	5,0025	39,5002	39,5752	1,50
3	5,0020	38,3450	38,4205	1,51
Rerata				1,47

$$\text{Rata-Rata} = \frac{1,40 + 1,50 + 1,51}{3} \times 100\% = 1,47\%$$

LAMPIRAN C

HASIL PARAMETER SARI LARUT ETANOL 70%

Pemeriksaan Kadar Sari Larut Etanol

No	Berat Ekstrak (g)	Berat Konstan Cawan (g)	Berat Konstan Cawan + Serbuk (g)	Kadar Sari Larut Etanol 70% (%)
1	5,0010	38,4905	38,5805	1,80
2	5,0030	39,5002	39,6003	2,00
3	5,0020	38,3450	38,4505	2,11
Rerata				1,97

$$\text{Rata-Rata} = \frac{1,80+2,00+2,11}{3} \times 100\% = 1,97\%$$

LAMPIRAN D

MOISTURE CONTENT

Formula Tanpa Ekstrak Daun Angsana		
Wp (g)	Wa (g)	MC (%)
0,8236	0,0350	4,25
0,7265	0,0283	3,90
0,7550	0,0334	4,42
Rata-rata		4,19 ± 0,27

Formula dengan Ekstrak Daun Angsana 19,89 mg/cm²		
Wp (g)	Wa (g)	MC (%)
2,0587	0,1493	7,25
2,0563	0,1211	5,89
2,0524	0,1114	5,43
Rata-rata		6,19 ± 0,95

Formula dengan Ekstrak Daun Angsana 39,78 mg/cm²		
Wp (g)	Wa (g)	MC (%)
2,1825	0,1650	7,56
2,1780	0,1764	8,10
2,1650	0,0380	8,23
Rata-rata		7,96 ± 0,36

LAMPIRAN E
STATISTIK UJI ANAVA ONEWAY

Oneway

Descriptives

Penurunan Kadar Glukosa Darah

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
negatif	5	344.20	105.791	47.311	212.84	475.56	191	449
E1 6 jam	5	-335.80	92.462	41.350	-450.61	-220.99	-404	-177
E2 6jam	5	-305.40	103.212	46.158	-433.56	-177.24	-489	-241
E1 12 jam	5	-420.00	51.034	22.823	-483.37	-356.63	-492	-367
E2 12jam	5	-538.40	41.241	18.443	-589.61	-487.19	-570	-471
positif	5	-282.80	118.523	53.005	-429.97	-135.63	-449	-116
Total	30	-256.37	298.100	54.425	-367.68	-145.05	-570	449

Test of Homogeneity of Variances

Penurunan Kadar Glukosa Darah

Levene Statistic	df1	df2	Sig.
.671	5	24	.649

ANOVA

Penurunan Kadar Glukosa Darah

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2382058.167	5	476411.633	58.639	.000
Within Groups	194986.800	24	8124.450		
Total	2577044.967	29			

Post Hoc Tests

Multiple Comparisons

Penurunan Kadar Glukosa Darah
Tukey HSD

(I) Kelompok Perlakuan	(J) Kelompok Perlakuan	Mean Differen ce (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
negatif	E1 6 jam	680.000*	57.007	.000	503.74	856.26
	E2 6jam	649.600*	57.007	.000	473.34	825.86
	E1 12 jam	764.200*	57.007	.000	587.94	940.46
	E2 12jam	882.600*	57.007	.000	706.34	1058.86
	positif	627.000*	57.007	.000	450.74	803.26
E1 6 jam	negatif	-680.000*	57.007	.000	-856.26	-503.74
	E2 6jam	-30.400	57.007	.994	-206.66	145.86
	E1 12 jam	84.200	57.007	.681	-92.06	260.46
	E2 12jam	202.600*	57.007	.018	26.34	378.86
	positif	-53.000	57.007	.935	-229.26	123.26
E2 6jam	negatif	-649.600*	57.007	.000	-825.86	-473.34
	E1 6 jam	30.400	57.007	.994	-145.86	206.66
	E1 12 jam	114.600	57.007	.366	-61.66	290.86
	E2 12jam	233.000*	57.007	.005	56.74	409.26
	positif	-22.600	57.007	.999	-198.86	153.66
E1 12 jam	negatif	-764.200*	57.007	.000	-940.46	-587.94
	E1 6 jam	-84.200	57.007	.681	-260.46	92.06
	E2 6jam	-114.600	57.007	.366	-290.86	61.66
	E2 12jam	118.400	57.007	.332	-57.86	294.66
	positif	-137.200	57.007	.194	-313.46	39.06
E2 12jam	negatif	-882.600*	57.007	.000	-1058.86	-706.34

	E1 6 jam	-202.600*	57.007	.018	-378.86	-26.34
	E2 6jam	-233.000*	57.007	.005	-409.26	-56.74
	E1 12 jam	-118.400	57.007	.332	-294.66	57.86
	positif	-255.600*	57.007	.002	-431.86	-79.34
positif	negatif	-627.000*	57.007	.000	-803.26	-450.74
	E1 6 jam	53.000	57.007	.935	-123.26	229.26
	E2 6jam	22.600	57.007	.999	-153.66	198.86
	E1 12 jam	137.200	57.007	.194	-39.06	313.46
	E2 12jam	255.600*	57.007	.002	79.34	431.86

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Penurunan Kadar Glukosa Darah

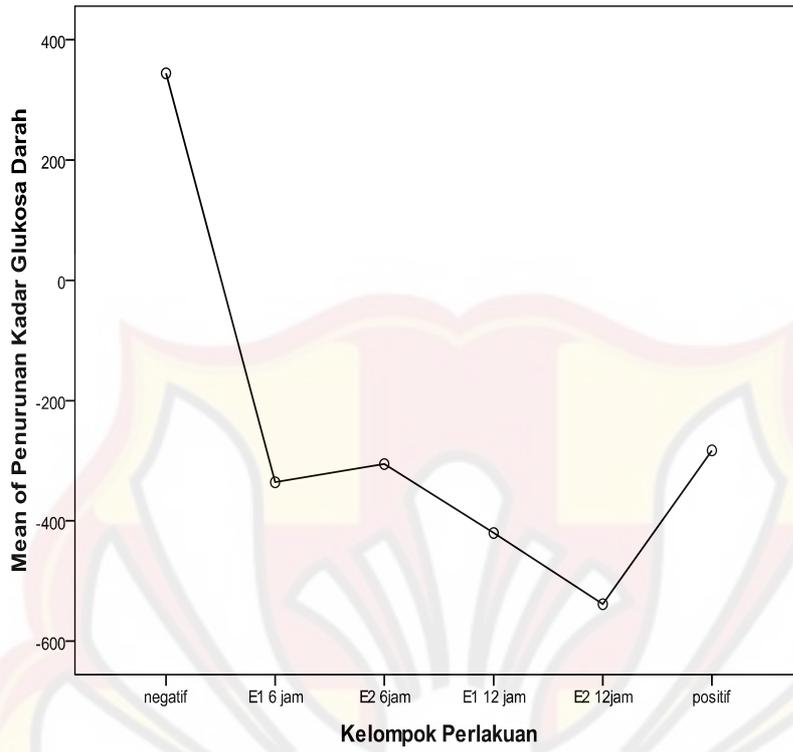
Tukey HSD^a

Kelompok Perlakuan	N	Subset for alpha = 0.05		
		1	2	3
E2 12jam	5	-538.40		
E1 12 jam	5	-420.00	-420.00	
E1 6 jam	5		-335.80	
E2 6jam	5		-305.40	
positif	5		-282.80	
negatif	5			344.20
Sig.		.332	.194	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

Means Plots



LAMPIRAN F
JURNAL PENELITIAN ANTONIUS ET.AL

**TESTING AND TRANSDERMAL'S FORMULATION OF
LEAF EXTRACT *PTEROCARPUS INDICUS* THE SHADE
STREET TO LOWER BLOOD SUGAR RATE**

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ABSTRACT : The aim of this study was to determining the effect of drug's penetration using transdermal patch. Skin is one of the drug's releases routes in which it has many advantages. The advantages are active drug ingredients that are not resistant acid can not hydrolized by stomach acid, preventing the first pass effect in the liver so it can increase the bioavailability of the drug. The composition of the transdermal patches consist of HPMC: PG (35%: 40%, 10%: 40%), HPMC: glycerol (35%: 10%, 10%: 10%) with 2 grams of menthol as an enhancer, and extract of leaves *Pterocarpus indicus* as an active ingredient 0.2 grams for each formulation. The method of this study to test pharmacological used enzymatic method than for the penetration in vitro used Franz diffusion cell method. The dosage of patches formulation with the composition of the HPMC: glycerol (10%: 10%) can penetrates of the drug's releases well, with a linear correlation between the dosage of drug's penetrated against time. Pharmacological effects on extract of leaves *Pterocarpus indicus* dose of 250 mg / kg and 450 mg / kg can be used as an antidiabetic after the seventh day, which was tested on mice experimental animals.

Keywords: *pterocarpus indicus*, transdermal, antidiabetic, penetration

INTRODUCTION

Diabetes or increased blood sugar levels is disease that increasingly getting popular day by day mortality rates are increasingly higher. Diabetes mellitus or commonly called "mother of all diseases" is a disease in which the concentration of glucose (simple sugar) in the blood is high because the body can not release or use insulin adequately. According to the diagnostic criteria Perkeni (Endrokrinologi Indonesian Association) in 2006, is said to have diabetes if a person has a fasting blood glucose > 126 mg/dL and the tests when > 200 mg/dL. Various research necessary to find good way of treatment for decreasing blood sugar with minimal side effects. Based on that conducted various studies to find ways of good sugar treatment to lower blood sugar levels with minimal side effects.

There are many functions from *Pterocarpus indicus* such as extract of the bark in the Philippines are used for diabetes therapy, leprosis and flu. Further more, the young leaves are used to accelerate cook boils and water soaked leaves are used to wash in to get the hair to grow better, water decoction of the

cola tree is also to stop the diarrhea, or a as gargle to heal cancer sores, and even the sap can be for shampoos [Heyne,K.,1987]. Leaf extract Kino and *P. indicus* was also reported to have a property to control the tumor and cancer [Duke,J.A., and Wain,K.K., 1981]. Juice from the root of this plant in Malaysia is used for treatment of syphilis. In Indonesia ,young leaves are used as a treatment ulcher or ulcers [Thomson, Lex A. J.,2006]. Ironically in Indonesia, this plant is only popular as a shade and ornamental plants in urban roadside.

Substances contained in *Pterocarpus indicus* consist of isoflavones, flavones, narin, santalin, angolensin, pterocarpin, pterostilben homopterocarpin, prunetin (prunusetin), formonoetin, isoliquiritigenin, p-hydroxyhydratropic acid, pterofuran, ptercarpol, β -eusdemol [Duke,J.A.,1983] and (-)-epicatechin [Takeuchi.Y.,Kono.Y., Nambata.T.,Terada.N.,et.all.1985 .] that play a role in decreasing blood sugar. Basd on the results of the study in vivo between diabetic ras treated with glibenclamide and antihiperglikemik f bark extracts of *Pterocarpus santalinus* L. (Wich contains lupeol, β -

sitosterol,(-)-epicatechin) at doses of 0.25 g/ kg BW obtained result is more effective than glibenclamide [Rao.K.,Giri.R.,Kesavulu.M.,Apparao.C.,2001], where as *Pterocarpus marsupium* Roxb.have ability to lower blood sugar in experimental animals within five days and have a compound that contained the (-)-epicatechin [Ahmad,R.,Khalid,P.,Khan,M.,Chaube M.,Rastogi,A.,Kidway,J.,1991.]. (-)-epicatechin have hypoglycemic effects due to regenerate beta cells, insulin has the effect of such activities and also converting poinsulin to insulin [Rao.K.,Giri.R.,Kesavulu.M.,Apparao.C., 2001]. *Perocarpus santalinus* L Plant and *Pterocarpus marsupium* Roxb.have been investigated and antihyperglykemik hypoglycemic effect can only be obtained abroad, the using plants alternative *Pterocarpus indicus* Willd that many scattered in the archipelago,which is one clan. Result from various studies angšana leaves can give the effect of blood glucose levels decline. By the Biological Research (1990) about the effects of infusion of leaves of *Pterocarpus indicus* Willd orally 10% no difference with the 50 mg /kg bw of tolbutamide, where as infusion decreased 20% larger

than the effects by tolbutamide in effects decreasing the blood glucose level.This research examined the effects of ethanol extract of leaves of *Pterocarpus indicus* Wild in transdermal preparations.

Intravenous and oral dosage forms of this leaf has less pharmacological effectiveness, where the active ingredients flavonoids from these stocks will experience the hydrolysis in acid (stomach acid). Transdermal formulations of the extract was used to overcome these problems because the network directly into the blood. In the preparation of matrix type patch, the type of polymer used as matrix plays as an important role in the nature of chemical physics and penetrating patch dosage of active ingredients. In this study, using HPMC matrices. The use of menthol as a penetration enhancer for 0.2 ounces to enhance terabsorpsi flavonoids. Propylene glycol at levels of 10% gives the best plasticizer properties of transdermal patches.

MATERIALS AND

METHODS

Plant Material

The plant material used in this study are: Sonokembang

leaves (*Pterocarpus indicus* W.) taken on the road Dinoya, Surabaya, East Java. Section of young leaves on aerated, until dry and then is pulverized as research material. Before being used for research, the plant is determined at the Botanical Garden LIPI Purwodadi Pasuruan, East Java.

Chemicals

Chemicals used in this study, if not stated, the degree of p.a (pro analysis), including: n-hexane, Acetone, Ethanol 70%, Ethanol 96%, Methanol, Acetic Acid glacial, Aluminium chloride, n-butanol, Silica gel 60 GF254, Silica gel 60 for column chromatography, WFI (Water For Injection) (Brataco Chemika Surabaya, Indonesia), Distilled water, HPMC, Gliserol, Propylene glikol, Menthol, Alloxan, Insulin 100 IU, PGA (Gom Arab), Na₂HPO₄, NaH₂PO₄, Rutin, Sodium Hydrochloride.

Specimen

Wistar strain rat skin obtained from male skin rats. Shaved skin of rats with clipped fur, then store in the refrigerator until used.

Research Tools

The tools used for this research is a set of tools

perkolator, gram scales, a set of thin layer chromatography instruments, glass instruments, moisture analyzer MA 30, capillary tube, Oven (Memmert, Germany), porcelain cup, bowls, desiccator, densitometers, a set of tools ash, restrainer, advantage meter, strip test, stirrer, Frans Diffusion Cell penetration tools.

Research Stages:

The first examination of Sonokembang and flavonoid

The first examination of Sonokembang and flavonoid include macroscopic and microscopic leaf slices Sonokembang (*Pterocarpus indicus* W.)

Determination of sample degree

Determination of sample degree are moisture content and ash content determination.

Determination procedure of ash

Angsana leaf powder weight 20 grams, then weight the empty cup. Then the powder inserted into the cup and then heated at temperature of 100°C for one hour. Once completed to ashes, input into the desiccator at temperature of 50°C for one day.

Considering the cup and the powder obtained.

Extraction of Sample

Extraction of sample is using the maceration with cold extraction procedure as follows: considering 150 mg powder and dissolve it in 100 ml ethanol 70%, let it stand at room temperature for one day. After a day of filtered and taken it extract.

Extract Standardization

Parameters of solvent soluble compounds include the levels of certain compounds that dissolve in water and levels of soluble in ethanol.

1. Levels of water soluble compounds

Maceration of 5 grams of extract for 24 hours with 100 ml of water using chloroform LP clogged with pumpkin whipped several times during the six hours and then left for 18 hours. Strain, steamed 20 ml of filtrate to dry in a shallow cup that has been tared, heat the residue at a temperature of 105°C until the weight remains. Calculate the concentration in percent soluble in water, calculated on the initial extract.

2. Soluble content of Ethanol 70%

Maceration of 5 grams of extract for 24 hours with 100 ml

ethanol 70% use the clogged with pumpkin whipped several times during the first six hours and then left for 18 hours. filter by avoid rapid evaporation ethanol. Then steamed 20 ml filtrate to dry in a shallow cup that has been tared, heat the residue at temperature of 105°C until the weight remains. Calculate the concentration in percent soluble in ethanol 70%, calculated on the initial extract.

TLC Examination

Speckled extract on TLC plate 2 µl of GF254, which is used as mobile phase Butanol : Acetic Acid : Water (4:1:5), which has made a day earlier. Chamber saturated with mobile phase and TLC plate inserted to propagate and reach the phase boundary marker.

Testing Blood Glucose

Measurement of blood glucose levels induced in rats before (day 0) and after alloxan induced diabetes and day (2,3,5,7). Before the blood drawn 16-18 hours of fasting rats. The way the treatment of test animals that is 20 white male wistar rats and was made diabetic with alloxan induced by 150 mg/kgBW by intramuscular and then divided randomly into four groups, each of five rats. The first were given Angsana leaf

infusion with doses of 250 mg/kgBW in mouth, the second group were given Angsana leaf infusion with doses 450 mg/kgBW in mouth, the third group as a negative control group were given water 5 ml/kgBW in oral, the fourth group as positive control group were given insulin 12.6 IU/kgBW subcutan. Each group was treated once daily for 7 days. Furthermore, the determination of blood glucose levels is doing day (2,3,5,7) by using enzymatic method, namely ion intermediate hexacyanoferrate (III) ion will be reduced to hexacyanoferrate (II).

Making patches

Making a patch of leaves angšana according with the formula presented in table 1. Angšana leaf levels selected after performing a blood glucose test.

The use of menthol as a penetration enhancer of 0.2 gram. Propylene glycol at levels of 10% gives the best plasticizer properties of transdermal patches.

For each patch with a diameter of 4 cm, derived as follows: leaves of *Pterocarpus indicus* (0.2 grams) along with 0.2 grams of menthol. Solution is added in the base polymer prepared by dissolving HPMC with Propylene glycol and Glycerol in accordance with the formula in 10 ml alcohol. The solution is poured on the aluminium plate and dried at room temperature for 30 minutes to the evaporation of water and get the film layer. After the film layer is formed, wrapped in aluminium foil and stored in desiccator until used. Each formula replicated four times.

Table 1. Composition Leaf Extract patch dosage Angšana

<i>Function</i>	<i>Composition</i>	<i>Formula A</i>	<i>Formula B</i>	<i>Formula C</i>	<i>Formula D</i>
Stabilizing	HPMC	35%	10%	35%	10%
Platisizer	PG	40%	40%	-	-
Platisizer	Glycerol	-	-	10%	10%
Enhancer	Menthol	0.2 gram	0.2 gram	0.2 gram	0.2 gram
The active ingredients	Angšana leaf extract	0.2 gram	0.2 gram	0.2 gram	0.2 gram
Solvent	Alcohol	10 ml	10 ml	10 ml	10 ml

To select the best patch preparation, to be optimized fourth formula above.

Penetration Test in Vitro

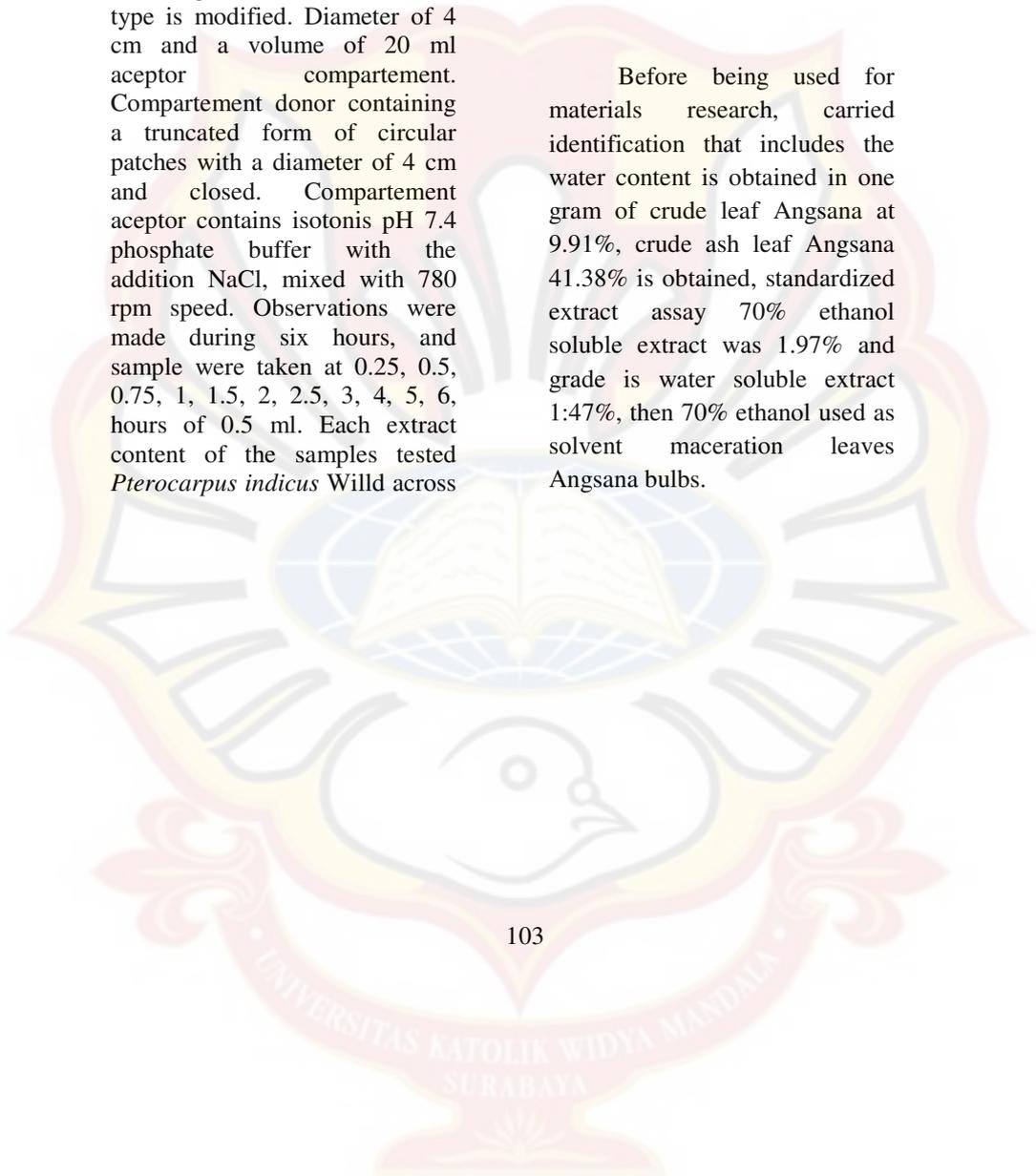
Rat skin obtained from Wistar rats approximately 4 months of age, weight 250-300 gram that had been murdered, her hair shorn using scissors. Skin that has been shaved stored at temperature 4°C in the refrigerator until used.

Penetration tests carried out using a vertical diffusion cell type is modified. Diameter of 4 cm and a volume of 20 ml acceptor compartement. Compartement donor containing a truncated form of circular patches with a diameter of 4 cm and closed. Compartement acceptor contains isotonis pH 7.4 phosphate buffer with the addition NaCl, mixed with 780 rpm speed. Observations were made during six hours, and sample were taken at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, hours of 0.5 ml. Each extract content of the samples tested *Pterocarpus indicus* Willd across

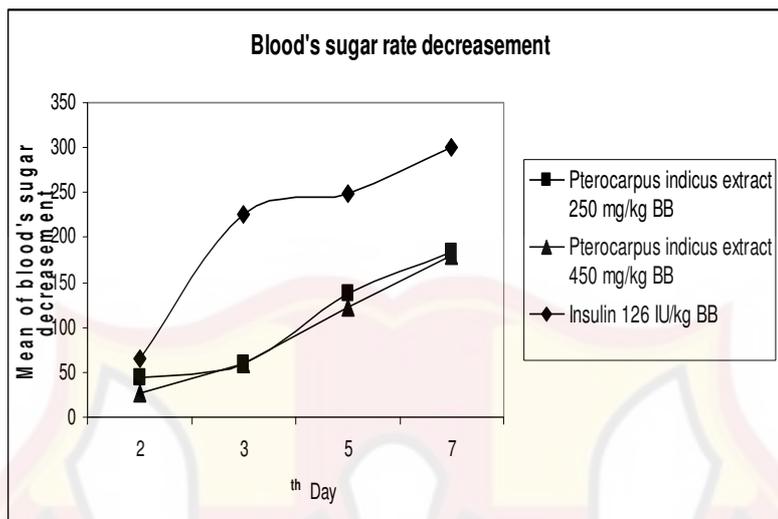
the membrane by using densitometry. Marker used were routine. Observations were obtained from the slope of flux on the plot number of *Pterocarpus indicus* Willd root across the membrane vs time.

RESULTS AND DISCUSSION

Before being used for materials research, carried identification that includes the water content is obtained in one gram of crude leaf Angsana at 9.91%, crude ash leaf Angsana 41.38% is obtained, standardized extract assay 70% ethanol soluble extract was 1.97% and grade is water soluble extract 1:47%, then 70% ethanol used as solvent maceration leaves Angsana bulbs.



Picture 1. Chart of blood's sugar rate decrease



This study aimed to investigate the effect of decreasing blood glucose levels in leaf extracts *Angsana*. A single dose of 250 mg / kg and 450 mg / kg. These doses were obtained from the journal then continued as the dose to the research mg / kg. This research used as a comparison with the dose of insulin 12.6 IU. Observation of the effect of decreasing blood glucose levels in alloxan method, the results of statistical computations using one way anova gained the ability extract dose of 250 mg / kg and doses of 450 mg extract / kg BW have the same effectiveness or there is no significant difference in the

decrease in glucose levels blood, as well as to insulin 12.6 IU no significant difference in the decrease in blood glucose.

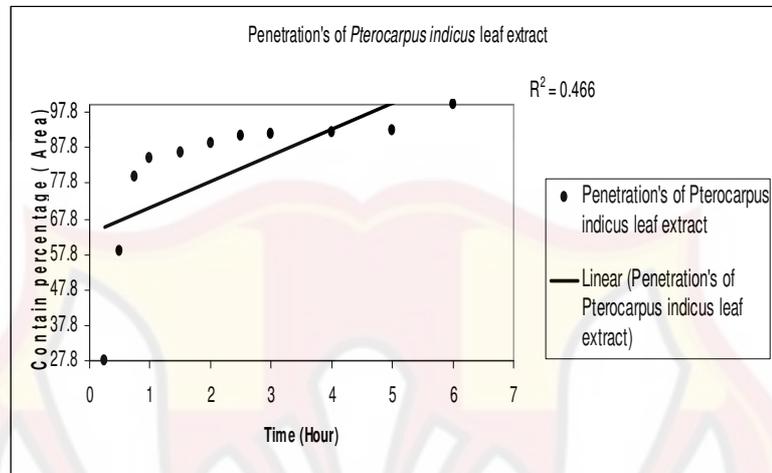
On examination of the content of flavonoids by thin layer chromatography (TLC) using mobile phase n-butanol: acetic acid: water = 4:1:5 and stationary phase silica gel GF254. The observation under UV light at 254 nm for comparison and extracts showed Rf value of each is 0.1575 while the Rf value of *Pterocarpus indicus* extract containing (-)-epicatechin expected around 0.1413. Due to the visible stain on the UV 254 nm. Rf values of

extracts were then used in penetration.

At selected patch formula D obtained results are not broken

patches, thin, and elastic in accordance with the requirements of a good patch.

Picture 2. Penetration's chart of *Pterocarpus indicus* leaf extract



Results of penetration on the D patch formula obtained experimental value of r greater than 0.466 at $\alpha = 0.1$ r theoretical table is 0.458. This shows the linear correlation between the amount of leaf extract of *Pterocarpus indicus* penetration against time.

antidiabetic after 7th day in male rats which given alloxan.

- The penetration rate of leaf extract *Pterocarpus indicus* Willd has linier correlation against time (hours).

CONCLUSION

- The dosage of leaf extract *Pterocarpus indicus* Willd are 250 mg / kg and 450 mg / kg can be used as an

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LAMPIRAN G
DETERMINASI TANAMAN



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SURAT KETERANGAN IDENTIFIKASI
NO.: 913/D.1/2011

Ketua PIPOT Fakultas Farmasi Universitas Surabaya dengan ini menerangkan bahwa material tanaman yang dibawa oleh Saudara :

Antonius – Nrp. 2443007035
(Facultas Farmasi – Unika. Widya Mandala Surabaya)

pada tanggal 4 Januari 2011, ke Pusat Informasi dan Pengembangan Obat Tradisional, berdasarkan buku 'Flora of Java' karangan C.A. Backer & R.C. Bakhuizen van den Brink, jilid I (1963) halaman 615, mempunyai nama ilmiah sebagai berikut:

Marga : *Pterocarpus*
Jenis : *Pterocarpus indicus* Willd.

Klasifikasi tanaman menurut buku 'The Standard Cyclopedia of Horticulture' karangan L.H. Bailey jilid I (1963) halaman 2-4, adalah sebagai berikut:

Divisi : Spermatophyta
Anak divisi : Angiospermae
Kelas : Dicotyledoneae
Anak kelas : Choripatalae
Bangsa : Rosales
Suku : Papilionaceae

Demikian surat keterangan ini dibuat untuk dapat dipergunakan sebagaimana mestinya.

Surabaya, 8 Januari 2011
Ketua PIPOT
Fakultas Farmasi Universitas Surabaya

(Prof. Dr. H. Sutarjadi, Apt.)

LAMPIRAN H

SERTIFIKASI HEWAN COBA

CV. SURABAYA MOUSE SERVICE
WEDORO MASJID NO 20 E RT: 01 RW: 05 WEDORO
KECAMATAN WARU SIDOARJO
TELP. 081938310682 - 031 - 70259110

Yang bertanda tangan di bawah ini :

Nama : M.Syamsul Bahri S.kom

Selaku penanggung jawab pengembangan hewan percobaan menerangkan bahwa yang

Digunakan pada penelitian :

Judul : Efek Hipoglikemik Sediaan Transdermal Ekstrak
Pterocarpus Indicus Willd Dengan Enhancer Mentol Pada
Tikus Diabetes Aloksan.

Peneliti : Antonius

Jurusan : Farmasi

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Demikian surat keterangan ini di buat untuk digunakan sebaik-baiknya.

Sidoarjo, 17-12-2010
Penanggung jawab


M.Syamsul Bahri S.kom)