ATTACHMENT

Production of Extract Isoflavone Metode

Isoflavones are the major polyphenol compound in soybean. Soy isoflavones are mainly glycosides (Genistin and Daidzin) than their aglycones (Genistein and Daidzein). Soy isoflavones were extracted by some methods, maseration and fractination. The soybean was bledded with water then macerated with 30% ethanol and 70% aseton. After being maceration, the isoflavone is being evaporated to eliminate the ethanol. Fractination is conduct by using water and ethil acetat.

Isoflavone level are higher in the extract isoflavone with out peeling off the skin of the soybean rather then extract isoflavone with peeling the skin of the soybean. And the level of extract isoflavone are also determine by the ways of how to extract, the type of liquid for the extraction and the analisis metode that being used. Research had been done to determine which is the best ways to extract isoflavone. The result showed that the highest total isoflavones ((genistein, daidzein, genistin, daidzin) concentration was present in methanolic extract from dehulled soybean. Isoflavone methanolic extract from dehulled soybean could be apply for topical cosmetic and isoflavone acetone extract from dehulled soybean could be used for oral isoflavone.

The way of making extract isoflavone consists of several stages :

1. Extraction of soybean for oral isoflavone

   100 g Soybean seeds extracted with 500 mL of 30% ethanol and 70% aseton maseration of kinetics using rotary shaker with 180 rpm rotation for 4 hours. The extraction was repeated twice and dirotavapor until obtained dry extract.

2. Isoflavone fractination

   Added as much as 1 gram of extract with 100 ml of aquades, fractionation is done based on its polarity level. Subsequent fractionation with a semi-polar solvent (ethylacetate) of 100 ml, thus obtaining an ethyl acetate and water fraction. The result of ethly acetat fractionation will be used. The result of ethyl acetat fractionation were evaporated with a rotary epavorator to dry at a temperature of 40-50 C.

3. How to make isoflavone cream

   The making of isoflavone extract cream was carried out at the chemical laboratory of the Sultan Agung University of Semarang. The cream making consists of extracts and cream base ingredients. 0,1 gram of base cream added with 16 mg of isoflavone.

4. How to apply isoflavone cream

- Prepair the extract isoflavone cream
- Using micro brush 0,1 mm and appy the 0,1 gram isoflavone cream on the dorsal skin of the mice 2 mm thick, apply once a day every morning for two weeks

Histological Slide of Sebum Gland

Hematoxylin and Eosin (H & E) staining is the most common staining technique in histopathology. This uses a combination of two dyes, Hematoxylin and Eosin used for demonstration of nucleus and cytoplasmic inclusions in clinical specimens.[57]

Principle

Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Bluing step converts the initial soluble red color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm.

HEMATOXYLIN & EOSIN (H & E) STAIN PROTOCOL

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No.1½, 22 mm square coverslip

Reagents

1. Harri's Hematoxylin stain

   A = 1 gm hematoxylin in 10 ml ethanol

   B = 20 gm ammonium alum in hot distilled water

   Mix A & B, boil and add 0.5 gm of mercuric oxide and filter.

2. Eosin solution

   Yellow eosin = 1 gm

   Distilled water = 80 ml

   Ethanol = 320 ml

   Glacial Acetic Acid = 2 drops

3. 0.5% HCl
4. Dilute ammonia water

   Procedure

1. Deparaffinize the section : flame the slide on burner and place in the xylene. Repeat the treatment.
2. Hydration : Hydrate the tissue section by passing through decreasing concentration of alcohol baths and water. (100%, 90%, 80%, 70%)
3. Stain in hematoxylin for 3-5 minutes
4. Wash in running tap water until sections “blue” for 5 minutes or less.
5. Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5 minutes.
6. Wash in running tap water until the sections are again blue by dipping in an alkaline solution (eg. ammonia water) followed by tap water wash.
7. Stain in 1% Eosin Y for 10 minutes
8. Wash in tap water for 1-5 minutes
9. Dehydrate in increasing concentration of alcohols and clear in xylene
10. Mount in mounting media
11. Observe under microscope

Results:

Nuclei and other basophilic structures are blue. Cytoplasm and acidophilic structures are light to dark red.

Immunochemistry (IHC)

Immunochemistry is the identification of a certain antigen in a histological tissue section or cytological preparation by an antibody specific to that antigen. Immunohistochemistry refers specifically to histological tissue sections.

Immunohistochemistry Techniques [54]

Immunohistochemistry Techniques uses antibodies, reagents and stains for the diagnosis and research of cancer. The common nuclear counterstains are: Hematoxylin, Light Green, Fast Red, Toliudine Blue and Methylene Blue. Also an Alum-Mordant base on Hematoxylin is used such as Harris's Hematoxylin (now is offered without mercury). Mayer's Hematoxylin is one of the most popular mordants used in immunohistochemistry as well as Gill's Hematoxylins that are classified as 1,2,3.

Immunohistochemistry Techniques uses different methods and approaches. The specimen needs to be well fixed. One of the most popular fixatives is 10% Neutral Formalin and Zinc Formalin. A lso in immunohistochemistry, a transport solution is needed to transport the specimen.

Immunohistochemical techniques detect antigens in tissue sections by means of immunological and chemical reactions. This technique is highly sensitive and

specific and can detect a wide variety of antigens in multiple animal species. This chapter reviews common immunohistochemical methods used in the characterization of normal and pathologic tissue and the reagents used. Pretreatments such as blocking steps for endogenous activities and antigen retrieval are included. Standard procedures on formalin-fixed, paraffin-embedded tissues as well as method standardization for new antibodies and troubleshooting are emphasized.

1. Deparaffinize and rehydrate sections as follows: 2 x 10 min in xylene, 2 min in 100% ethanol, 2 min in 95% ethanol, 2 min in 80% ethanol, 2 min in 70% ethanol, 5 min in tap water and 5 min in distilled water.
2. Block endogenous peroxidases by soaking slides in a solution mixtured with methanol and 30% H2O2 for 10 min at room temperature. Then wash the slides 5 min in distilled water, 5 min in PBS.
3. Heat Induced Epitope Retrieval: Antigen retrieval by microwavl irradiation 2x5min. Then remove the staini ng dish to room temperature and allow the slides to cool for 20 minutes. Note:The AR solution (pH 6.0) is mixture of Sodium Citrate Buffer and Citrate Buffer. Wash the slides 3x5min with PBS.
4. Block in 10% normal goat serum in PBS for 1 hour at 37°C.
5. Incubate sections with primary antibody at appropriate dilution in PBS overnight at 4 °C.
6. Wash the slides 3x5min with PBS.
7. Incubate sections with HRP-conjugated secondary antibody at appropriate dilution in PBS for 30 min at 37°C.

8. Wash the slides 3x3min with PBS.
9. Develop with DAB till the color is appropriate, then rinse the slides under tap water gently for about 1- 2 min.
10. Counterstain in hematoxylin.
11. 2 x 2 min in 100% ethanol, 2 x 5 min in xylene, mount sections with neutral balsam.

Note :

- The color of the reaction is determined by the selection of a precipitating chromogen, usually diaminobenzidine (brown) or aminoethylcarbazole (red), with which the enzyme reacts.[54]

How to culture Propionibacterium Acne[58,59]

Materials and methods

- Sample Collection

The samples were collected from the patients' face (forehead, cheek, and chin). The site of sampling was disinfected by 70% ethanol. To collect the samples, sterile cotton swabs were placed inside a test tube containing 2 mL of physiological serum. To remove and extract the closed comedones and the papules, a lancet was used to make a scratch on the surface of the lesion and the content was then extracted with slight hand pressure.

- Microbial culture

The collected samples were transferred into test tubes containing brain heart infusion broth ; after sterile paraffin was added for creating anaerobic conditions , the samples were incubated at 37 °C for 48 to 72 h. A loop of bacterial suspension was then taken from the liquid medium under sterile conditions next to a fire and was then cultured on plates containing brain heart infusion agar . The plates were placed in anaerobic conditions and were then incubated at 37 °C for 4 to 5 days

Appendix 1. Ethical Clearance

KOMISI BIOETIKA PENELITIAN KEDOKTERAN/KESEHATAN
FAKULTAS KEDOKTERAN
UNIVERSITAS ISLAM SULTAN AGUNG SEMARANG
Sekretariat : Gedung C Lantai I Fakultas Kedokteran Unissula
Jl. Raya Kaligawe Km 4 Semarang, Telp. 024-6583584, Fax 024-6594366

Ethical Clearance

No. 324/IX/2018/ Komisi Bioetik

Komisi Bioetika Penelitian Kedokteran/Kesehatan Fakultas Kedokteran Universitas Islam Sultan Agung Semarang, setelah melakukan pengkajian atas usulan penelitian yang berjudul :

The Effectiveness Different Of Oral Or Topical Soybean Isoflavone On Acne Vulgaris Therapy By Measuring The Amount of Sebum, DHT Level, Activity DHT Reseptor & Histology of Sebacea Gland

Peneliti Utama	: Rockh Edy Shofi Loftyani
Pembimbing	: Prof. Dr. dr. Taufiqurrahman, M.Kes., Sp.And
	DR. Ir. Titiek Sumarawati, M.Kes
Tempat Penelitian	: Laboratorium Patologi Anatomi Rumah Sakit Islam Sultan Agung
	Laboratorium Mikrobiologi Rumah Sakit Islam Sultan Agung
	Laboratorium Farmasi FK Unissula
	Laboratorium Biologi FK Unissula
	Laboratorium Kimia FK Unissula

dengan ini menyatakan bahwa usulan penelitian diatas telah memenuhi prasyarat etik penelitian. Oleh karena itu Komisi Bioetika merekomendasikan agar penelitian ini dapat dilaksanakan dengan mempertimbangkan prinsip-prinsip yang dinyatakan dalam Deklarasi Helsinki dan panduan yang tertuang dalam Pedoman Nasional Etik Penelitian Kesehatan (PNEPK) Departemen Kesehatan RI tahun 2004.

Semarang, 14 September 2018
Komisi Bioetika Penelitian Kedokteran/Kesehatan
Fakultas Kedokteran Unissula
Ketua,

(dr. Sofwan Dahlan, Sp.F(K))

Appendix 2 Bacteri Identification

bioMérieux Customer:

Microbiology Chart Report

Printed Dec 6, 2018 06:56 ICT

Patient Name: acne, -
Location: lmk
Lab ID: acne.

Patient ID: acne.
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Propionibacterium acnes

Source: isolat

Collected: Dec 5, 2018

Comments:	

Identification Information	**Analysis Time:**	5.77 hours	**Status:** Final
Selected Organism	98% Probability **Bionumber:**	**Propionibacterium acnes** 6303001602011	
ID Analysis Messages			

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	-
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	-	37	BGURi	-
39	BGALi	(+)	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	+	57	AlFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	-	62	OPS	+	63	AARAF	-	64	dXYL	-
	GRAM	+		MORPH	-		AERO	-									

Page 1 of 1

Appendix 3 Research Sertification

UNIVERSITAS ISLAM SULTAN AGUNG (UNISSULA)
INTEGRATED BIOMEDICAL LABORATORY
FAKULTAS KEDOKTERAN
Jl. Raya Kaligawe KM.4, Semarang 50112
Tel. +62246583584, email: ibl@unissula.ac.id

Laboratorium Biomedik Terintegrasi

SURAT KETERANGAN SELESAI PENELITIAN

Nomor : 007/IBL-FK-SA/VII/2019
Lampiran : -

Assalamu'alaikum wr. wb.

Yang bertanda tangan di bawah ini :

Nama	:	Dina Fatmawati, S.Si, M.Sc.
Jabatan	:	Kepala Laboratorium Biomedik Terintegrasi
NIK/ NIDN	:	210109143
Menerangkan bahwa	:	
Nama dan NIM	:	Rokh Edy Shofi Loftyani / 168.01.01.08

Benar-benar telah selesai melakukan penelitian di **Laboratorium Biomedik Terintegrasi** Fakultas Kedokteran Universitas Islam Sultan Agung, selama Empat Puluh Sembilan Hari mulai dari 8 Januari 2019 s/d 23 Februari 2019, dengan judul The Effectiveness Different Of Oral Or Topocal Soybean Isoflavone On Acne Vulgaris Therapy By Measuring The Amount Of Sebum, DHT Level, Activity DHT Reseptor & Histology Of Sebacea Gland. Demikian surat keterangan ini dibuat untuk digunakan sebagaimana mestinya.

Wassalamu'alaikum wr. wb.

Semarang, 12 Juli 2019
Mengetahui,
Kepala Lab. Biomedik Terintegrasi
Fakultas Kedokteran Unissula

Dina Fatmawati, S.Si., M.Sc.
NIK 210109143

Appendix 4 Research Result

RSI SULTAN AGUNG
ISLAMIC TEACHING HOSPITAL

LABORATORIUM PATOLOGI ANATOMI
HASIL PEMBACAAN

HASIL PEMBACAAN GLANDULA SEBACEA DAN AKTIFITAS RESEPTOR DHT

	HE	IHC	HE	IHC	HE	IHC	HE	IHC	HE	IHC	TOTAL_HE	TOTAL_IHC	% IHC/HE *100
Control	18	12	12	8	14	10	15	9	15	9	74	48	64.86
Control	15	15	17	3	13	7	18	12	12	8	75	45	60.00
Control	14	8	10	6	20	6	15	11	12	15	71	46	64.79
Control	15	14	14	4	10	12	17	20	17	5	73	55	75.34
Control	14	5	17	8	17	9	15	9	15	16	78	47	60.26
p1.1	11	10	11	5	15	5	16	7	15	4	68	31	45.59
p1.2	12	7	11	4	16	4	16	4	10	10	65	29	44.62
p1.3	11	6	14	4	10	8	12	9	16	9	63	36	57.14
p1.4	17	6	10	3	17	5	6	9	8	8	58	31	53.45
p1.5	19	5	12	6	15	3	9	9	6	9	61	32	52.46
p2.1	10	2	12	3	6	6	14	5	13	6	55	22	40.00
p2.2	11	7	12	3	11	3	12	5	11	6	57	24	42.11
p2.3	12	4	12	2	8	6	15	6	10	5	57	23	40.35
p2.4	11	5	16	3	3	5	14	5	12	7	56	25	44.64
p2.5	14	3	11	6	11	6	15	4	7	6	58	25	43.10
p3.1	9	1	10	1	6	4	10	0	8	0	43	6	13.95
p3.2	9	2	9	2	9	2	9	1	9	0	45	7	15.56
p3.3	8	2	8	1	8	2	8	0	7	0	39	5	12.82
p3.4	10	2	10	2	10	2	7	0	7	0	44	6	13.64
p3.5	11	1	11	1	6	2	7	0	11	0	46	4	8.70
p4.1	7	1	4	0	6	0	2	0	2	0	21	1	4.76
p4.2	6	1	4	2	8	0	7	0	7	0	32	3	9.38
p4.3	5	0	2	0	7	0	2	2	8	0	24	2	8.33
p4.4	5	0	6	1	5	2	6	0	5	0	27	3	11.11
p4.5	7	0	7	0	2	1	3	0	4	0	23	1	4.35

Semarang, 18 Juli 2019

dr.Susilorini, Msi, Med, Sp.PA

Appendix 5. Anatomi Pathology Laboratory RSI Sultan Agung

RSI SULTAN AGUNG
ISLAMIC TEACHING HOSPITAL

LABORATORIUM PATOLOGI ANATOMI

SURAT KETERANGAN

Yang bertanda tangan dibawah ini, Bagian Laboratorium Patologi Anatomi Rumah Sakit Islam Sultan Agung Semarang menerangkan bahwa mahasiswa di bawah ini :

Nama	: Rokh edy shofi loftyani
NIM	: 168.01.01.08
Fakultas/Universitas	: Program Studi Magister Biomedis Fakultas Kedokteran Universitas Sultan Agung Semarang
Judul Penelitian	: The effectiveness different of soybean isoflavone oral and topical on acne vulgaris therapy by measuring the amount of sebum, activity receptor DHT & histology of sebacea gland.

Telah melakukan prosesing jaringan dan pembacaan preparat di Laboratorium Patologi Anatomi Rumah Sakit Islam Sultan Agung Semarang pada bulan Juni 2019 dengan hasil terlampir

Demikian surat keterangan ini kami buat untuk digunakan sebagaimana perlunya.

Semarang, 10 Agustus 2019

dr.Susilorini, Msi.Med,SpPA

Appendix 6. Anatomi Pathology Laboratory UGM

UNIVERSITAS GADJAH MADA
FAKULTAS KEDOKTERAN, KESEHATAN MASYARAKAT, DAN KEPERAWATAN
DEPARTEMEN PATOLOGI ANATOMIK
Gedung Radiopoetro Lantai 4, Jln. Farmako, Sekip Utara, Yogyakarta 55281. Telp/Fax. (0274) 540460

SURAT KETERANGAN

Nomor : 146/UN1/KU.1/PA.2/LT/2019

Yang bertanda tangan di bawah ini:

Nama	:	**Dr.dr. Irianiwati, Sp.PA(K)**
NIP.	:	**19620523 198803 2 002**
Jabatan	:	**Ketua Departemen Patologi Anatomik, Fakultas Kedokteran, Kesehatan Masyarakat, dan Keperawatan, UGM**

menerangkan bahwa Laboratorium Patologi Anatomik Fakultas Kedokteran, Kesehatan Masyarakat dan Keperawatan UGM, telah melakukan pembuatan preparat sebanyak 27 buah dengan pengecatan Androgen Reseptor, pada periode bulan September 2018 untuk mahasiswa nama sebagai berikut :

Nama	:	**dr. Rokh Edi Shofi L**
NIM	:	**MBK.16.8.01.0108**
Mahasiswa	:	**Mahasiswa Program Studi Biomedik (S-2), Fakultas Kedokteran, Universitas Islam Sultan Agung (UNISSULA) Semarang.**
Judul Penelitian	:	***The Effectivenes Different of Oral or Topical Soybean Isoflavone on Acne Vulgaris Therapy By Measuring The Amount of Sebum, DHT Level, Activity DHT Reseptor Histology of Sebacea Gland.***
Pembimbing I	:	**Prof. Dr. Dr. H. Taufiqurrachman N.M.Kes., Sp.And**
II	:	**Dr. Ir. Hj. Titiek Sumarawati, M.Kes**

Demikianlah surat keterangan ini dibuat untuk dapat dipergunakan seperlunya.

Yogyakarta, 16 Mei 2019

Ketua
Departemen Patologi Anatomik

Dr. dr. Irianiwati, Sp.PA(K)
NIP. 19620523 198803 2 002

Appendix 7. Statistical test result

HASIL UJI NORMALITAS

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	KELOMPOK	Statistic	df	Sig.	Statistic	df	Sig.
HE	Kontrol	.179	5	.200*	.984	5	.955
	P1	.105	5	.200*	.999	5	1.000
	P2	.237	5	.200*	.961	5	.814
	P3	.241	5	.200*	.903	5	.427
	P4	.228	5	.200*	.936	5	.636
IHC	Kontrol	.320	5	.104	.809	5	.096
	P1	.269	5	.200*	.894	5	.376
	P2	.221	5	.200*	.902	5	.421
	P3	.237	5	.200*	.961	5	.814
	P4	.241	5	.200*	.821	5	.119
% IHC/HE	Kontrol	.312	5	.125	.828	5	.134
	P1	.232	5	.200*	.906	5	.446
	P2	.209	5	.200*	.942	5	.682
	P3	.282	5	.200*	.886	5	.335
	P4	.232	5	.200*	.908	5	.454

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

HASIL UJI HOMOGENITAS

Test of Homogeneity of Variance

		Levene Statistic	df1	df2	Sig.
HE	Based on Mean	1.438	4	20	.258
	Based on Median	.925	4	20	.469
	Based on Median and with adjusted df	.925	4	13.562	.478
	Based on trimmed mean	1.389	4	20	.273
IHC	Based on Mean	1.614	4	20	.210
	Based on Median	.738	4	20	.577
	Based on Median and with adjusted df	.738	4	8.168	.591
	Based on trimmed mean	1.308	4	20	.301
% IHC/HE	Based on Mean	1.729	4	20	.183
	Based on Median	1.036	4	20	.413
	Based on Median and with adjusted df	1.036	4	11.463	.430
	Based on trimmed mean	1.588	4	20	.216

Oneway OF HE

Descriptives

HE

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kontrol	5	74.200	2.5884	1.1576	70.986	77.414	71.0	78.0
P1	5	63.000	3.8079	1.7029	58.272	67.728	58.0	68.0
P2	5	56.600	1.1402	.5099	55.184	58.016	55.0	58.0
P3	5	43.400	2.7019	1.2083	40.045	46.755	39.0	46.0
P4	5	25.400	4.2778	1.9131	20.088	30.712	21.0	32.0
Total	25	52.520	17.4024	3.4805	45.337	59.703	21.0	78.0

Test of Homogeneity of Variances

HE

Levene Statistic	df1	df2	Sig.
1.438	4	20	.258

ANOVA

HE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7075.840	4	1768.960	183.884	.000
Within Groups	192.400	20	9.620		
Total	7268.240	24			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: HE

LSD

(I) KELOMPOK	(J) KELOMPOK	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kontrol	P1	11.2000*	1.9616	.000	7.108	15.292
	P2	17.6000*	1.9616	.000	13.508	21.692
	P3	30.8000*	1.9616	.000	26.708	34.892
	P4	48.8000*	1.9616	.000	44.708	52.892
P1	Kontrol	-11.2000*	1.9616	.000	-15.292	-7.108
	P2	6.4000*	1.9616	.004	2.308	10.492
	P3	19.6000*	1.9616	.000	15.508	23.692
	P4	37.6000*	1.9616	.000	33.508	41.692
P2	Kontrol	-17.6000*	1.9616	.000	-21.692	-13.508
	P1	-6.4000*	1.9616	.004	-10.492	-2.308
	P3	13.2000*	1.9616	.000	9.108	17.292
	P4	31.2000*	1.9616	.000	27.108	35.292
P3	Kontrol	-30.8000*	1.9616	.000	-34.892	-26.708
	P1	-19.6000*	1.9616	.000	-23.692	-15.508
	P2	-13.2000*	1.9616	.000	-17.292	-9.108
	P4	18.0000*	1.9616	.000	13.908	22.092
P4	Kontrol	-48.8000*	1.9616	.000	-52.892	-44.708
	P1	-37.6000*	1.9616	.000	-41.692	-33.508
	P2	-31.2000*	1.9616	.000	-35.292	-27.108
	P3	-18.0000*	1.9616	.000	-22.092	-13.908

*. The mean difference is significant at the .05 level.

Oneway OF IHC

Descriptives

IHC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kontrol	5	48.200	3.9623	1.7720	43.280	53.120	45.0	55.0
P1	5	31.800	2.5884	1.1576	28.586	35.014	29.0	36.0
P2	5	23.800	1.3038	.5831	22.181	25.419	22.0	25.0
P3	5	5.600	1.1402	.5099	4.184	7.016	4.0	7.0
P4	5	2.000	1.0000	.4472	.758	3.242	1.0	3.0
Total	25	22.280	17.5322	3.5064	15.043	29.517	1.0	55.0

Test of Homogeneity of Variances

IHC

Levene Statistic	df1	df2	Sig.
1.614	4	20	.210

ANOVA

IHC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7271.440	4	1817.860	344.292	.000
Within Groups	105.600	20	5.280		
Total	7377.040	24			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: IHC

LSD

(I) KELOMPOK	(J) KELOMPOK	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kontrol	P1	16.4000*	1.4533	.000	13.369	19.431
	P2	24.4000*	1.4533	.000	21.369	27.431
	P3	42.6000*	1.4533	.000	39.569	45.631
	P4	46.2000*	1.4533	.000	43.169	49.231
P1	Kontrol	-16.4000*	1.4533	.000	-19.431	-13.369
	P2	8.0000*	1.4533	.000	4.969	11.031
	P3	26.2000*	1.4533	.000	23.169	29.231
	P4	29.8000*	1.4533	.000	26.769	32.831
P2	Kontrol	-24.4000*	1.4533	.000	-27.431	-21.369
	P1	-8.0000*	1.4533	.000	-11.031	-4.969
	P3	18.2000*	1.4533	.000	15.169	21.231
	P4	21.8000*	1.4533	.000	18.769	24.831
P3	Kontrol	-42.6000*	1.4533	.000	-45.631	-39.569
	P1	-26.2000*	1.4533	.000	-29.231	-23.169
	P2	-18.2000*	1.4533	.000	-21.231	-15.169
	P4	3.6000*	1.4533	.022	.569	6.631
P4	Kontrol	-46.2000*	1.4533	.000	-49.231	-43.169
	P1	-29.8000*	1.4533	.000	-32.831	-26.769
	P2	-21.8000*	1.4533	.000	-24.831	-18.769
	P3	-3.6000*	1.4533	.022	-6.631	-.569

*. The mean difference is significant at the .05 level.

Oneway OF %IHC/HE

Descriptives

% IHC/HE

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kontrol	5	65.050	6.2136	2.7788	57.335	72.765	60.0	75.3
P1	5	50.652	5.3666	2.4000	43.989	57.315	44.6	57.1
P2	5	42.040	1.9304	.8633	39.643	44.437	40.0	44.6
P3	5	12.934	2.5674	1.1482	9.746	16.122	8.7	15.6
P4	5	7.586	2.9431	1.3162	3.932	11.240	4.4	11.1
Total	25	35.652	22.8353	4.5671	26.226	45.078	4.4	75.3

Test of Homogeneity of Variances

% IHC/HE

Levene Statistic	df1	df2	Sig.
1.729	4	20	.183

ANOVA

% IHC/HE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12169.284	4	3042.321	176.083	.000
Within Groups	345.556	20	17.278		
Total	12514.840	24			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: % IHC/HE

LSD

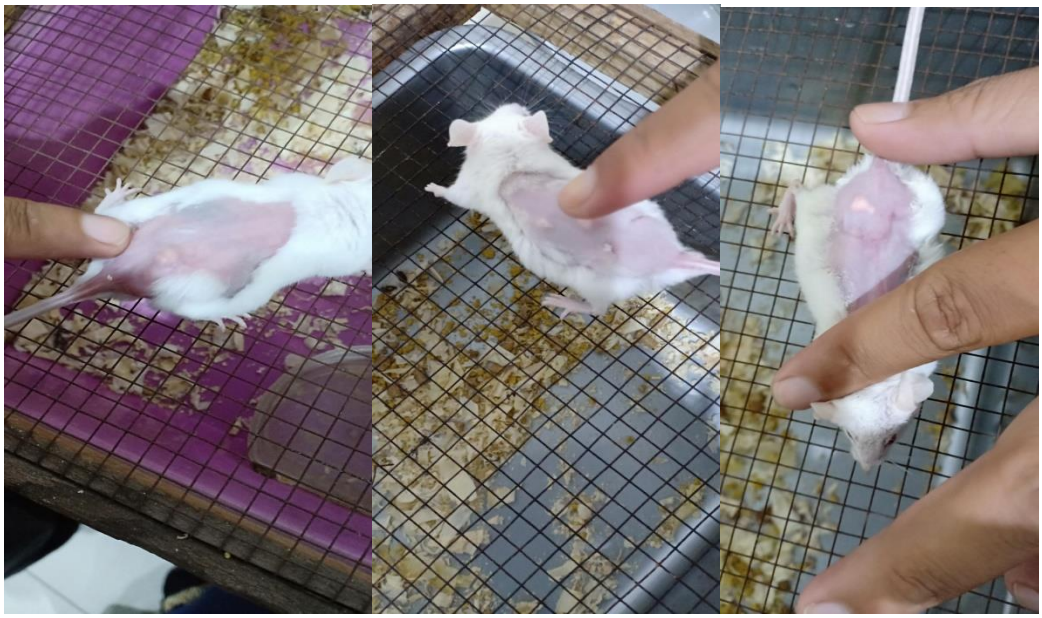
(I) KELOMPOK	(J) KELOMPOK	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kontrol	P1	14.3980*	2.6289	.000	8.914	19.882
	P2	23.0100*	2.6289	.000	17.526	28.494
	P3	52.1160*	2.6289	.000	46.632	57.600
	P4	57.4640*	2.6289	.000	51.980	62.948
P1	Kontrol	-14.3980*	2.6289	.000	-19.882	-8.914
	P2	8.6120*	2.6289	.004	3.128	14.096
	P3	37.7180*	2.6289	.000	32.234	43.202
	P4	43.0660*	2.6289	.000	37.582	48.550
P2	Kontrol	-23.0100*	2.6289	.000	-28.494	-17.526
	P1	-8.6120*	2.6289	.004	-14.096	-3.128
	P3	29.1060*	2.6289	.000	23.622	34.590
	P4	34.4540*	2.6289	.000	28.970	39.938
P3	Kontrol	-52.1160*	2.6289	.000	-57.600	-46.632
	P1	-37.7180*	2.6289	.000	-43.202	-32.234
	P2	-29.1060*	2.6289	.000	-34.590	-23.622
	P4	5.3480	2.6289	.055	-.136	10.832
P4	Kontrol	-57.4640*	2.6289	.000	-62.948	-51.980
	P1	-43.0660*	2.6289	.000	-48.550	-37.582
	P2	-34.4540*	2.6289	.000	-39.938	-28.970
	P3	-5.3480	2.6289	.055	-10.832	.136

*. The mean difference is significant at the .05 level.

Appendix 8. Research foto

P. Acne colony

Anaerobic Jar

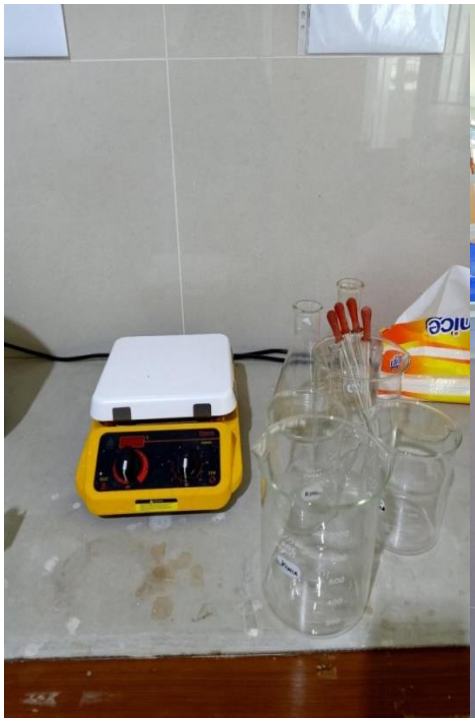


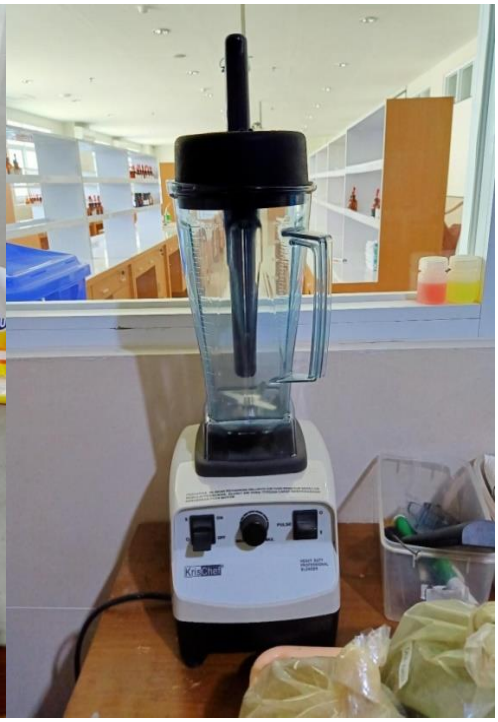
Mice with pustula

P. Acne

Injection in to the intradermal skin of the mice


Separating funnel (corong pisah)

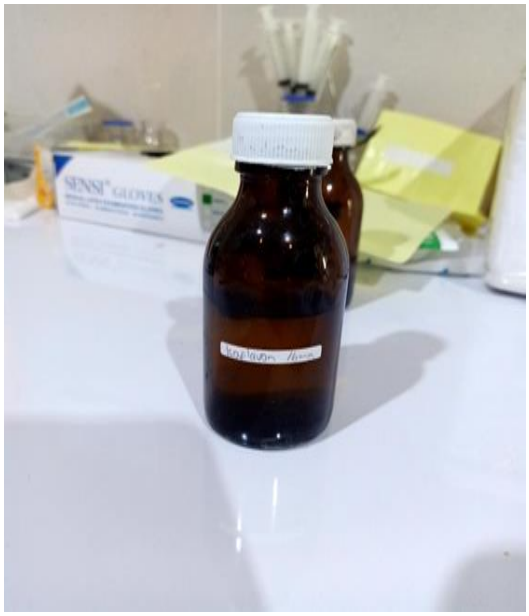

Shaker


Blender

Rotator

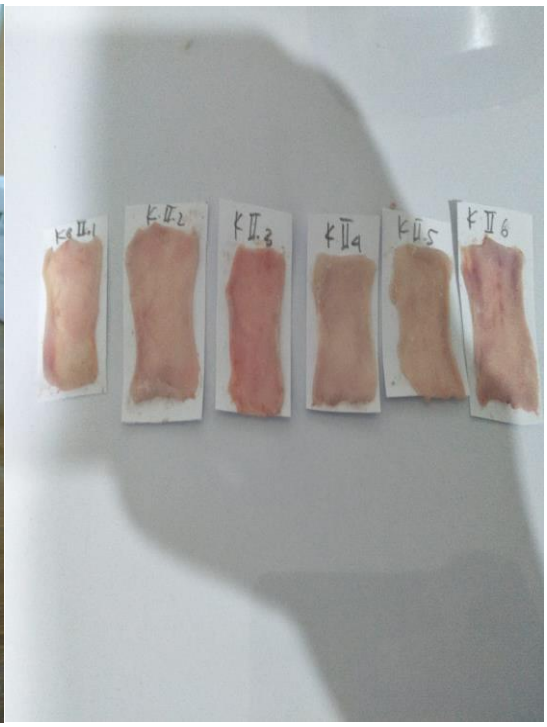
Scale

Gold Standar Therapy



Isoflavone

Doxycyclin



Skin sample

Microscope

Research

Appendix 9. Research schedule

	Mar 2018	Apr 2018	May 2018	July 2018	Agust 2018	Jan 2019	Feb 2019	Mar 2019	Apr 2019	May 2019	June 2019	July 2019	Agust 2019	Sept 2019
Proposal preparation	√	√	√	√										
Proposal exam					√									
Research						√	√	√	√	√	√	√		
Preparation of research reports												√	√	
Thesis results exam														√

Biography

Identity

- Name : Rokh Edy Shofi Loftyani
- Place & date of birth : Kebumen, 2 April 1980
- Religion : Islam
- Gender : Female
- Email : copink192@yahoo.com

Educational Background

- SDN Jaka Sampurna I 1992
- Sekolah Republik Indonesia Tokyo 1995
- SMA Negeri 61 Jakarta 1998
- University Gunadarma Jakarta 2002
- Medical Faculty University Pembangunan Nasional Veteran Jakarta 2009
- Magister Biomedic Medical Faculty University Islam Sultan Agung 2019