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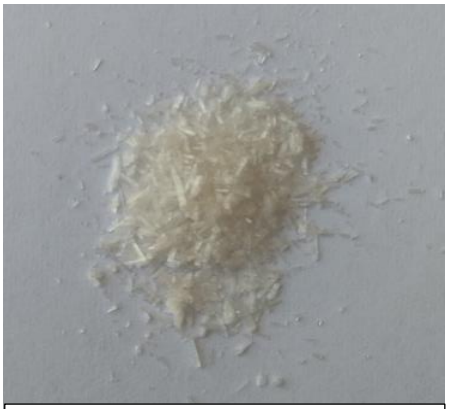
LAMPIRAN

Lampiran 1. Dokumentasi Penelitian

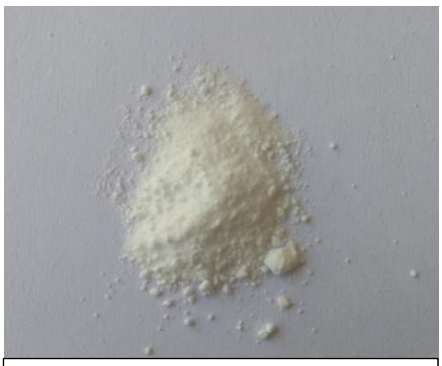
Gambar 6. Sampel uji



Gambar 9. Senyawa arbutin

Gambar 7. Ekstrak *Zingiber zerumbet*

Gambar 10. Ekstrak *Piper cubeba*

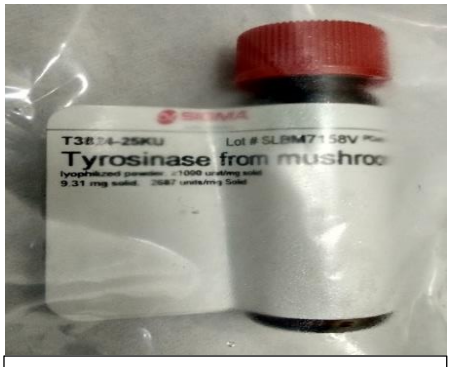


Gambar 8. Senyawa asam kojik

Gambar 11. Ekstrak *Lavandula officinalis*

Gambar 12. Ekstrak *Curcuma xanthorriza*

Gambar 13. Ekstrak curcuma zedoria



Gambar 14. Enzim Tirosinase

Gambar 15. Ekstrak *Piper retrofractum*

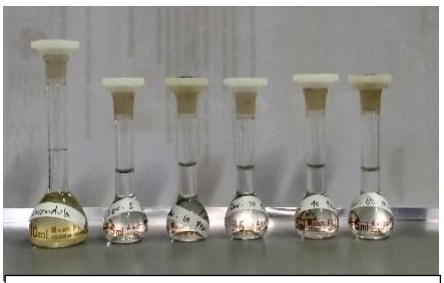
Gambar 16. Ekstrak ocimum basilicum

Gambar 17. Substrak L-Tirosin

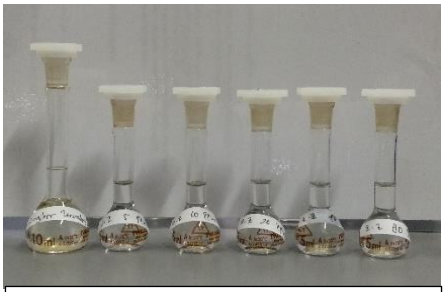
Gambar 18. Pengenceran asam kojik

Gambar 21. Pengenceran arbutin

Gambar 19. Pengenceran ekstrak selasih



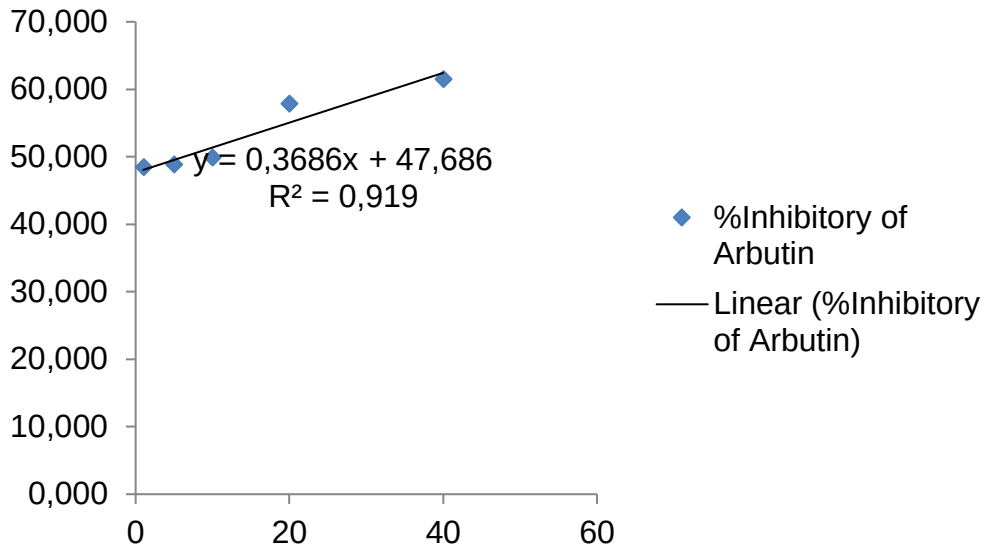
Gambar 22. Pengenceran ekstrak lavender



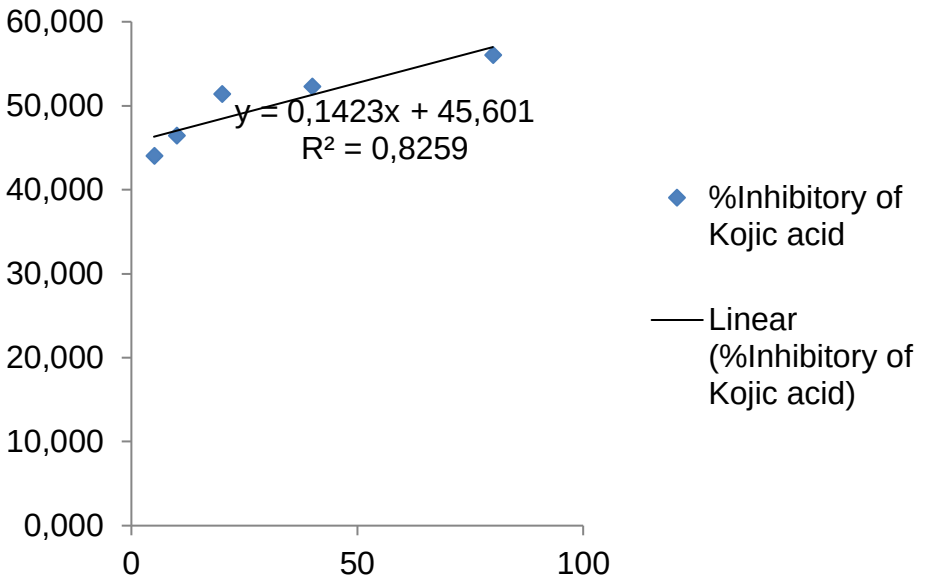
Gambar 20. Pengenceran ekstrak lempuyang

A. Gambar Kurva baku

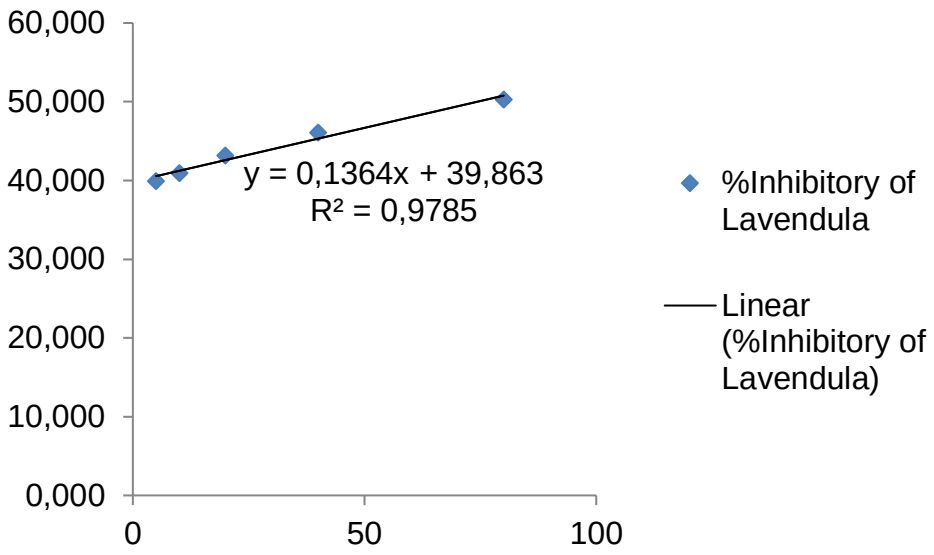
a. Persen penghambatan arbutin (kurva baku)



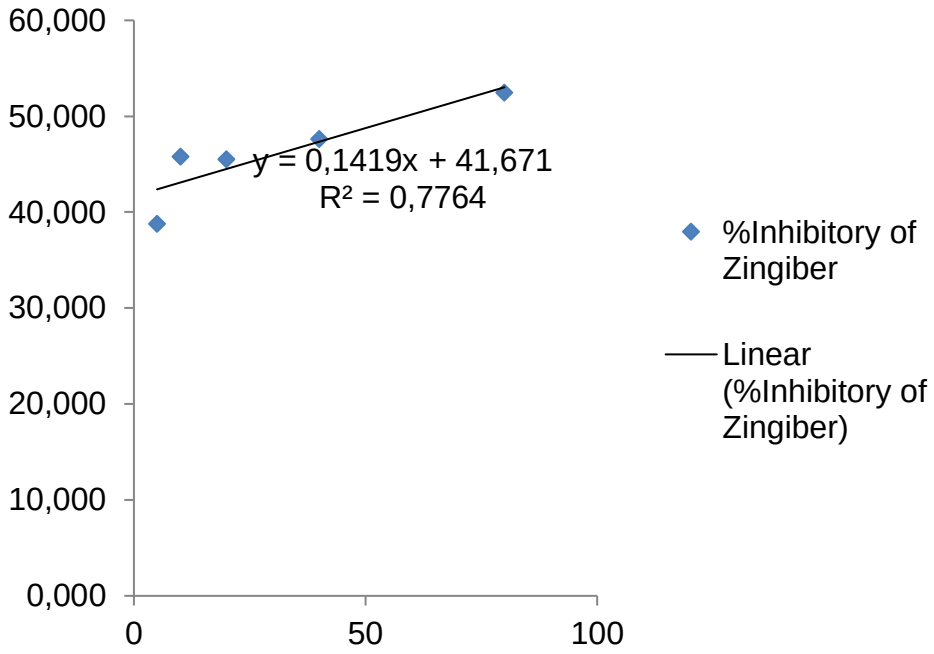
b. Persen penghambatan asam kojik (kurva baku)



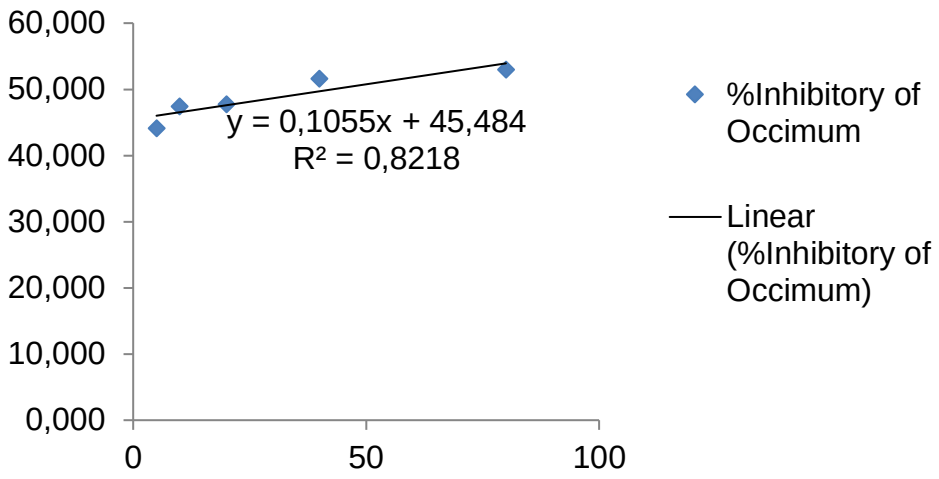
c. Persen penghambatan lavender (kurva baku)



d. Persen penghambatan lempuyang (kurva baku)



e. Persen penghambatan selasih (kurva baku)



Lampiran 2. Principal Component Analysis

Principal Component Analysis: 351.04, 378.05, 630.72, ... 0.79, 3525.88

Eigenanalysis of the Correlation Matrix

Eigenvalue	4.5339	3.7508	2.0308	1.6276	0.6128	0.3382	0.0715	0.0344	0.0000	0.0000
Proportion	0.349	0.289	0.156	0.125	0.047	0.026	0.006	0.003	0.000	0.000
Cumulative	0.349	0.637	0.794	0.919	0.966	0.992	0.997	1.000	1.000	1.000

Eigenvalue	0.0000	-0.0000	-0.0000
Proportion	0.000	-0.000	-0.000
Cumulative	1.000	1.000	1.000

Eigenvectors

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
351.04	-0.129	-0.167	-0.563	0.268	-0.272	0.105	-0.045	-0.507	0.001	0.144
378.05	0.024	-0.448	-0.022	0.327	0.147	0.378	0.351	-0.114	0.401	-0.281
630.72	0.394	-0.143	0.015	-0.305	-0.331	-0.023	0.014	0.053	0.380	0.191
761.88	0.310	0.030	-0.426	-0.242	-0.344	-0.190	0.387	0.101	-0.188	-0.239
904.61	-0.058	-0.142	-0.357	-0.557	0.177	0.596	-0.259	0.210	-0.064	-0.012
1066.64	-0.371	-0.066	0.178	-0.403	-0.188	-0.102	0.010	-0.416	0.270	0.394
1276.88	0.214	-0.444	0.065	0.141	0.059	-0.068	0.109	0.418	0.089	0.547
1446.61	0.106	-0.468	0.239	-0.064	-0.014	0.065	0.077	-0.261	-0.737	0.117
1654.92	-0.425	-0.131	0.116	0.127	-0.267	0.202	-0.220	0.304	-0.053	-0.073
1867.09	0.295	-0.313	0.283	-0.184	0.016	-0.136	-0.406	-0.263	0.158	-0.481
2362.8	0.163	0.355	0.372	-0.102	-0.040	0.526	0.439	-0.189	-0.022	0.060
2850.79	-0.323	-0.181	-0.110	-0.317	0.526	-0.312	0.408	-0.023	0.049	-0.100
3525.88	-0.367	-0.187	0.190	-0.106	-0.506	-0.043	0.261	0.242	-0.012	-0.297

Variable	PC11	PC12	PC13
351.04	-0.447	0.014	0.026
378.05	0.387	-0.025	-0.033
630.72	-0.106	-0.476	-0.446
761.88	0.233	0.441	-0.084
904.61	0.010	-0.031	0.189
1066.64	0.369	0.266	0.099
1276.88	-0.227	0.345	0.245
1446.61	0.139	-0.188	-0.136
1654.92	-0.060	0.342	-0.626
1867.09	-0.276	0.328	0.092
2362.8	-0.366	0.227	-0.042
2850.79	-0.347	-0.020	-0.276
3525.88	-0.209	-0.275	0.436

Lampiran 3. Cluster Analysis of Observations

Cluster Analysis of Observations: 351.04, 378.05, 630.72, ... 79, 3525.88

Euclidean Distance, Complete Linkage

Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	8	93.2860	0.056312	3	8	3	2
2	7	79.7461	0.169873	6	7	6	2
3	6	75.8547	0.202512	1	2	1	2
4	5	63.9875	0.302045	3	4	3	3
5	4	56.4781	0.365027	1	6	1	4
6	3	43.3708	0.474961	1	9	1	5
7	2	42.9483	0.478505	3	5	3	4
8	1	0.0000	0.838722	1	3	1	9

Final Partition

	Number of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster1	9	0.931690	0.301971	0.502087

Lampiran 4. Perhitungan Sampel

a. Perhitungan pengenceran sampel

1) Untuk pembuatan larutan stok sampel ditimbang sebanyak 0,001 gram dalam 10 ml ≈ 100 µg/mL

2) Untuk pembuatan 1 µg/mL

m_1 x V_1 = m_2 x V_2 = 100 µg/mL x V_1 = 1 µg/mL x 5 ml

$$V_1 = \frac{5}{100} = 0{,}05 \text{ ml} \approx 50 \text{ µL}$$

jadi yang dipipet yang sebanyak 0,05 ml dari larutan stok 10 ml

$\approx$ 100 µg/mL, lalu diditambahkan pelarut hingga 5 ml

3) Untuk pembuatan 5 µg/mL

m_1 x V_1 = m_2 x V_2 = 100 µg/mL x V_1 = 5 µg/mL x 5 ml

$$V_1 = \frac{25}{100} = 0{,}25 \text{ ml} \approx 250 \text{ µL}$$

jadi yang dipipet yang sebanyak 0,25 ml dari larutan stok 10 ml

$\approx$ 100 µg/mL, lalu diditambahkan pelarut hingga 5 ml

4) Untuk pembuatan 10 µg/mL

m_1 x V_1 = m_2 x V_2 = 100 µg/mL x V_1 = 10 µg/mL x 5 ml

$$V_1 = \frac{50}{100} = 0{,}5 \text{ ml} \approx 500 \text{ µL}$$

jadi yang dipipet yang sebanyak 0,5 ml dari larutan stok 10 ml

$\approx$ 100 µg/mL, lalu diditambahkan pelarut hingga 5 ml

5) Untuk pembuatan 20 µg/mL

m_1 x V_1 = m_2 x V_2 = 100 µg/mL x V_1 = 20 µg/mL x 5 ml

$$V_1 = \frac{100}{100} = 1 \text{ ml} \approx 1000 \text{ µL}$$

jadi yang dipipet yang sebanyak 1 ml dari larutan stok 10 ml

$\approx$ 100 µg/mL, lalu diditambahkan pelarut hingga 5 ml

6) Untuk pembuatan 40 µg/mL

m_1 x V_1 = m_2 x V_2 = 100 µg/mL x V_1 = 40 µg/mL x 5 ml

$$V_1 = \frac{200}{100} = 2 \text{ ml} \approx 2000 \text{ µL}$$

jadi yang dipipet yang sebanyak 2 ml dari larutan stok 10 ml

≈ 100 µg/mL, lalu diditambahkan pelarut hingga 5 ml

7) Untuk pembuatan 80 µg/mL

m_1 x V_1 = m_2 x V_2 = 100 µg/mL x V_1 = 80 µg/mL x 5 ml

$$V_1 = \frac{400}{100} = 4 \text{ ml} \approx 4000 \text{ µL}$$

jadi yang dipipet yang sebanyak 4 ml dari larutan stok 10 ml

≈ 100 µg/mL, lalu diditambahkan pelarut hingga 5 ml

b. Perhitungan pembuatan substrak L-tirosin

L-tirosin 5 mM

$$mM = \frac{mg}{BM} \; x \; \frac{1000}{v}$$

$$= \frac{mg}{181{,}19} \; x \; \frac{1000}{50 \; mL}$$

$$= 45{,}3 \; mg \approx 0{,}045 \; gram$$

Jadi ditimbang 0,045 gram L-tirosin ditambahkan dengan larutan dapar 6,8 hingga 50 mL

c. Perhitungan persen penghambatan

1. Arbutin

Konsentrasi (µg/mL)	Absorbansi	% inhibitory	IC_{50} (µg/ml)
1	0.163	48.401	
5	0.1617	48.810	
10	0.1583	49.890	6,277
20	0.1331	57.866	
40	0.1217	61.480	
	0.3159 (control)		

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3159-0,163}{0,3159}\ x\ 100 = 48,40$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3159-0,1617}{0,3159}\ x\ 100 = 48,81$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3159-0,1583}{0,3159}\ x\ 100 = 49,89$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3159-0,1331}{0,3159}\ x\ 100 = 57,86$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3159-0,1217}{0,3159}\ x\ 100 = 61,48$

$IC_{50} = \frac{50-a}{b} = \frac{50-47,686}{0,3686} = 6,277$

2. Asam kojik

Konsentrasi (µg/mL)	Absorbansi	% inhibitory	IC_{50} (µg/ml)
5	0.233	41.52	
10	0.2134	46.44	
20	0.1938	51.36	30,913
40	0.1903	52.23	
80	0.1753	56.00	
	0.3984 (control)		

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,233}{0,3984} x\ 100 = 41,52$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2134}{0,3984} x\ 100 = 46,44$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,1938}{0,3984} x\ 100 = 51,36$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,1908}{0,3984} x\ 100 = 52,23$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,1753}{0,3984} x\ 100 = 56,00$

$IC_{50} = \frac{50-a}{b} = \frac{50-45,6}{0,1423} = 30,91$

3. Ekstrak lavender

Konsentrasi (µg/mL)	Absorbansi	% inhibitory	IC_{50} (µg/ml)
5	0.2392	39.96	
10	0.2353	40.94	
20	0.2265	43.15	74,318
40	0.2148	46.08	
80	0.1979	50.33	
	0.3984 (control)		

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2392}{0,3984} x\ 100 = 39,96$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2353}{0,3984} x\ 100 = 40,94$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2265}{0,3984} x\ 100 = 43,15$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2148}{0,3984} x\ 100 = 46,08$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,1979}{0,3984} x\ 100 = 50,33$

$IC_{50} = \frac{50-a}{b} = \frac{50-39,863}{0,1364} = 74,32$

4. Ekstrak lempuyang

Konsentrasi (µg/mL)	Absorbansi	% inhibitory	IC_{50} (µg/ml)
5	0.2437	38.83	
10	0.2158	45.83	
20	0.217	45.53	58,696
40	0.2085	47.67	
80	0.1893	52.48	
	0.3984 (control)		

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2437}{0,3984} x\ 100 = 38,83$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2158}{0,3984} x\ 100 = 45,83$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,217}{0,3984} x\ 100 = 45,53$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,2085}{0,3984} x\ 100 = 47,67$

% inhibitory = $\frac{control-sampel}{control} x\ 100 = \frac{0,3984-0,1893}{0,3984} x\ 100 = 52,48$

$IC_{50} = \frac{50-a}{b} = \frac{50-41,671}{0,1419} = 58,69$

5. Ekstrak selasih

Konsentrasi (µg/mL)	Absorbansi	% inhibitory	IC_{50} (µg/ml)
5	0.2228	44.08	
10	0.2095	47.41	
20	0.2082	47.74	42,805
40	0.1929	51.58	
80	0.1874	52.96	
	0.3984 (control)		

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3984-0,2228}{0,3984}\ x\ 100 = 44,08$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3984-0,2095}{0,3984}\ x\ 100 = 47,41$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3984-0,2082}{0,3984}\ x\ 100 = 47,74$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3984-0,1929}{0,3984}\ x\ 100 = 51,58$

% inhibitory = $\frac{control-sampel}{control}\ x\ 100 = \frac{0,3984-0,1874}{0,3984}\ x\ 100 = 52,96$

$IC_{50} = \frac{50-a}{b} = \frac{50-45,484}{0,1055} = 42,80$

Lampiran 5. Surat determinasi dari UPT Materia Medica Batu

Nomor : 074/164/101.8/2020
Sifat : Biasa
Perihal : Ekstraksi Tanaman

Memenuhi permintaan saudara :
Nama : YUSNITA RIFAI, M.Pharm., Ph.D., Apt.
NIP : 107511172000122001
Fakultas : FAKULTAS FARMASI UNIVERSITAS HASANUDDIN

Perihal Ekstrak Tanaman

Benar telah diekstraksi oleh UPT Materia Medica Batu pada bulan Juni 2020 tanaman sbb: Kemukus (*Piper cubeba* L.); Lavender (*Lavendula officinalis*); Lempuyang gajah (*Zingiber zerumbet*); Kunyit Putih (*Curcuma zedoria*); Cabe Jawa (*Piper retrofractum*); Selasih (*Ocimum basilicum*); Temulawak (*Curcuma xanthorriza*).

Batu, 19 JULI 2020
Kepala UPT Materia Medica Batu

Dra. Hayu RM, Apt, MKes.
NIP.19611102 199103 1 003

Lampiran 6. Pathway Sintesis Melanin