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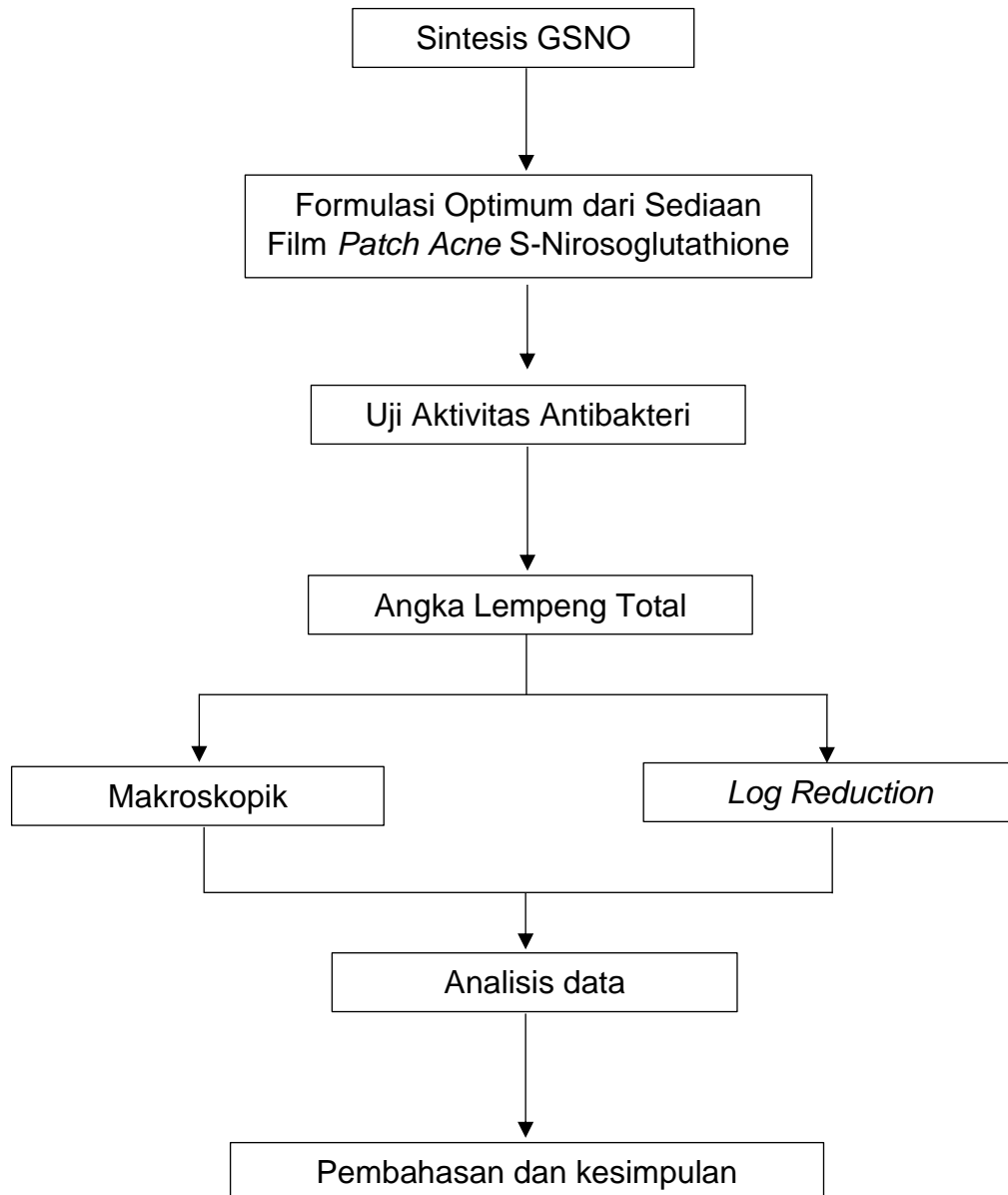Roy, D. 2014. Comprehensive Biotechnology. *Probiotics.* pp: 591-602

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LAMPIRAN

Lampiran 1. Skema Kerja Penelitian

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                    ┌─────────────────────┐
                    │    Sintesis GSNO     │
                    └─────────────────────┘
                               │
                               ▼
          ┌──────────────────────────────────────┐
          │   Formulasi Optimum dari Sediaan      │
          │ Film Patch Acne S-Nirosoglutathione   │
          └──────────────────────────────────────┘
                               │
                               ▼
              ┌─────────────────────────────┐
              │   Uji Aktivitas Antibakteri  │
              └─────────────────────────────┘
                               │
                               ▼
              ┌─────────────────────────────┐
              │    Angka Lempeng Total       │
              └─────────────────────────────┘
                       │             │
                       ▼             ▼
          ┌──────────────┐      ┌──────────────┐
          │ Makroskopik  │      │ Log Reduction │
          └──────────────┘      └──────────────┘
                       │             │
                       └──────┬──────┘
                              ▼
                  ┌──────────────────┐
                  │   Analisis data  │
                  └──────────────────┘
                              │
                              ▼
              ┌─────────────────────────────┐
              │ Pembahasan dan kesimpulan    │
              └─────────────────────────────┘
```

Lampiran 2. Komposisi Media

***Mueller Hinton Agar* (MHA)**

Beef Extract Powder	2.0 g
Acid digest of casein	17.5 g
Pati	1.5 g
Agar	17.0 g
Aquades	ad 1 L

pH 7,3 ± 0,1 pada 25°C.   (Zimbro *et al.*, 2009)

Mueller Hinton Broth (MHB)

Beef Extract Powder	2 g
Acid Digest of Casein	17.5 g
Pati	1.5 g
Aquades	ad 1 L

pH 7,3 ± 0,1 pada 25°C.   (Zimbro *et al.*, 2009)

Lampiran 3. Tabel Hasil Uji Penelitian

Tabel 2. Hasil perhitungan jumlah koloni pada kontrol negatif

Replikasi	Jumlah koloni		
	10^{-1}	10^{-2}	10^{-3}
1	0	0	0
2	0	0	0
3	0	0	0
Rata-rata±SD	**0**	**0**	**0**

Tabel 3. Hasil perhitungan jumlah koloni *blank* film 20 mg/mL

Replikasi	Jumlah koloni			CFU/mL
	10^{-6}	10^{-7}	10^{-8}	
1	112	78	29	$1,8x10^{10}$
2	117	87	55	$3,2x10^{10}$
3	115	80	32	$2,0x10^{10}$
Rata-rata±SD	**114,7±2,5**	**81,7±4,7**	**38,7±14,2**	**$2,3x10^{10}±7,3x10^{9}$**

Tabel 4. Hasil perhitungan jumlah koloni sediaan film *patch* GSNO 5 mg/mL

Replikasi	Jumlah koloni			CFU/mL
	10^{-1}	10^{-2}	10^{-3}	
1	334	216	85	533.000
2	303	168	50	334.000
3	309	186	73	458.000
Rata-rata±SD	**315,3±16,4**	**190±24,2**	**69,3±17,8**	**$4,4x10^{5}±1,0x10^{5}$**

Tabel 5. Hasil perhitungan jumlah koloni sediaan film *patch* GSNO 10 mg/mL

Replikasi	Jumlah koloni			CFU/mL
	10^{-1}	10^{-2}	10^{-3}	
1	277	131	17	142.500
2	207	115	6	95.500
3	226	122	12	121.000
Rata-rata±SD	**236,7±36,2**	**122,7±8,0**	**11,7±5,5**	**$1,2x10^{5}±3,2x10^{4}$**

Tabel 6. Hasil perhitungan jumlah koloni sediaan film *patch* GSNO 20 mg/mL

Replikasi	Jumlah koloni			CFU/mL
	10^{-1}	10^{-2}	10^{-3}	
1	63	35	3	6.300
2	48	13	4	4.800
3	57	29	7	5.700
Rata-rata±SD	**56±7,6**	**25,7±11,4**	**4,7±2,1**	**$5,6x10^{4}±754,9$**

Lampiran 4. Perhitungan Angka Lempeng Total

Sediaan film *patch* konsentrasi 5 mg/mL

Pengenceran 10^{-2}

Replikasi 1 $\quad\quad$ Cfu/mL $\quad\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{216 \text{ x } 10^2}{0,1}$$

$$= 2,2 \text{ x } 10^5 \text{ CFU/mL}$$

Replikasi 2 $\quad\quad$ Cfu/mL $\quad\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{168 \text{ x } 10^2}{0,1}$$

$$= 1,7 \text{ x } 10^5 \text{ CFU/mL}$$

Replikasi 3 $\quad\quad$ Cfu/mL $\quad\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{186 \text{ x } 10^2}{0,1}$$

$$= 1,9 \text{ x } 10^5 \text{ CFU/mL}$$

$\overline{x} \quad\quad\quad = \dfrac{(2,2 \text{ x } 10^5)+(1,7 \text{ x } 10^5)+(1,9 \text{ x } 10^5)}{3} = 1,9 \text{ x } 10^5 \text{ Cfu/mL}$

Pengenceran 10^{-3}

Replikasi 1 $\quad\quad$ Cfu/mL $\quad\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{85 \text{ x } 10^3}{0,1}$$

$$= 8,5 \text{ x } 10^5 \text{ CFU/mL}$$

Replikasi 2 $\quad\quad$ Cfu/mL $\quad\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{50 \text{ x } 10^3}{0,1}$$

$$= 5,0 \text{ x } 10^5 \text{ CFU/mL}$$

Replikasi 3 Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{73 \times 10^3}{0,1}$$

$= 7,3 \times 10^5$ CFU/mL

$\overline{x}$ $\qquad = \dfrac{(8,5 \times 10^5)+(5,0 \times 10^5)+(7,3 \times 10^5)}{3} = 6,9 \times 10^5$ Cfu/mL

Maka nilai CFU/mL pada sediaan film *patch* GSNO konsentrasi 5 mg/mL

CFU/mL $\quad = \dfrac{(1,9 \times 10^5)+(6,9 \times 10^5)}{2} = 4,4 \times 10^5$ cfu/mL

Sediaan film *patch* GSNO konsentrasi 10 mg/mL

Pengenceran 10^{-2}

Replikasi 1 Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{115 \times 10^2}{0,1}$$

$= 1,2 \times 10^5$ CFU/mL

Replikasi 2 Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{131 \times 10^2}{0,1}$$

$= 1,3 \times 10^5$ CFU/mL

Replikasi 3 Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{122 \times 10^2}{0,1}$$

$= 1,2 \times 10^5$ CFU/mL

Maka,

$\overline{x}$ $\qquad = \dfrac{(1,2 \times 10^5)+(1,3 \times 10^4)+(1,2 \times 10^5)}{3} = 1,2 \times 10^5$ Cfu/mL

Pengenceran 10^{-3}

Replikasi 1 $\quad$ Cfu/mL $\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{17 \times 10^3}{0,1}$$

$$= 1{,}7 \times 10^5 \text{ CFU/mL}$$

Replikasi 2 $\quad$ Cfu/mL $\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{6 \times 10^3}{0,1}$$

$$= 6{,}0 \times 10^4 \text{ CFU/mL}$$

Replikasi 3 $\quad$ Cfu/mL $\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{12 \times 10^3}{0,1}$$

$$= 1{,}2 \times 10^5 \text{ CFU/mL}$$

$\bar{x} \qquad = \dfrac{(1,7 \; x \; 10^5)+(6,0 \; x \; 10^4)+(1,2 \; x \; 10^5)}{3} = 1{,}2 \times 10^5 \text{ Cfu/mL}$

Maka nilai CFU/mL pada sediaan film *patch* GSNO konsentrasi 10 mg/mL

CFU/mL $\qquad = \dfrac{(1,2 \; x \; 10^5)+(1,2 \; x \; 10^5)}{2} = 1{,}2 \times 10^5 \text{ cfu/mL}$

Sediaan film *patch* GSNO konsentrasi 20 mg/mL

Pengenceran 10^{-1}

Replikasi 1 $\quad$ Cfu/mL $\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{63 \times 10^1}{0,1}$$

$$= 6{,}3 \times 10^3 \text{ CFU/mL}$$

Replikasi 2 $\quad$ Cfu/mL $\quad = \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{48 \times 10^1}{0,1}$$

$$= 4,8 \times 10^3 \text{ CFU/mL}$$

Replikasi 3   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{57 \times 10^1}{0,1}$$

$$= 5,7 \times 10^3 \text{ CFU/mL}$$

$\overline{x}$ $= \dfrac{(6,3 \times 10^3)+(4,8 \times 10^3)+(5,7 \times 10^3)}{3} = 5,6 \times 10^3 \text{ Cfu/mL}$

Pengenceran 10^{-2}

Replikasi 1   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{13 \times 10^2}{0,1}$$

$$= 1,3 \times 10^4 \text{ CFU/mL}$$

Replikasi 2   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{35 \times 10^2}{0,1}$$

$$= 3,5 \times 10^4 \text{ CFU/mL}$$

Replikasi 3   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \frac{29 \times 10^2}{0,1}$$

$$= 2,9 \times 10^4 \text{ CFU/mL}$$

$\overline{x}$ $= \dfrac{(1,3 \times 10^4)+(3,5 \times 10^4)+(2,9 \times 10^4)}{3} = 2,6 \times 10^4 \text{ Cfu/mL}$

Maka nilai CFU/mL pada sediaan film *patch* GSNO konsentrasi 20 mg/mL

CFU/mL $= \dfrac{(5,6 \times 10^3)+(2,6 \times 10^4)}{2} = 1,6 \times 10^4 \text{ cfu/mL}$

***Blank* Film**

Pengenceran 10^{-7}

Replikasi 1   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \dfrac{\textbf{78 x } \mathbf{10^7}}{\textbf{0,1}}$$

$= 7,8 \times 10^9$ CFU/mL

Replikasi 2   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \dfrac{\textbf{87 x } \mathbf{10^7}}{\textbf{0,1}}$$

$= 8,7 \times 10^9$ CFU/mL

Replikasi 3   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \dfrac{\textbf{80 x } \mathbf{10^7}}{\textbf{0,1}}$$

$= 8,0 \times 10^9$ CFU/mL

$\overline{x}$ $= \dfrac{(\mathbf{7,8 \, x \, 10^9}) + (\mathbf{8,7 \, x \, 10^9}) + (\mathbf{8,0 \, x \, 10^9})}{\mathbf{3}} = 8,2 \times 10^9$ Cfu/mL

Pengenceran 10^{-8}

Replikasi 1   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \dfrac{\textbf{29 x } \mathbf{10^8}}{\textbf{0,1}}$$

$= 2,9 \times 10^{10}$ CFU/mL

Replikasi 2   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \dfrac{\textbf{5,5 x } \mathbf{10^8}}{\textbf{0,1}}$$

$= 5,5 \times 10^{10}$ CFU/mL

Replikasi 3   Cfu/mL $= \dfrac{\textbf{Jumlah koloni x Total faktor dilusi}}{\textbf{Volume yang dicuplik ke cawan petri}}$

$$= \dfrac{32 \times 10^8}{0,1}$$
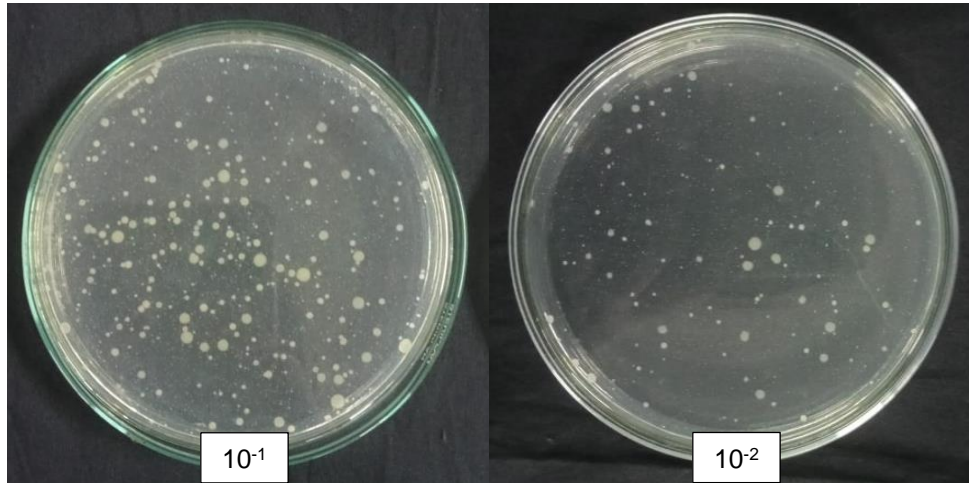
$$= 3,2 \times 10^{10} \text{ CFU/mL}$$

$\overline{x}$ $= \dfrac{(2,9 \times 10^{10}) + (5,5 \times 10^{10}) + (3,2 \times 10^{10})}{3} = 3,9 \times 10^{10} \text{ Cfu/mL}$
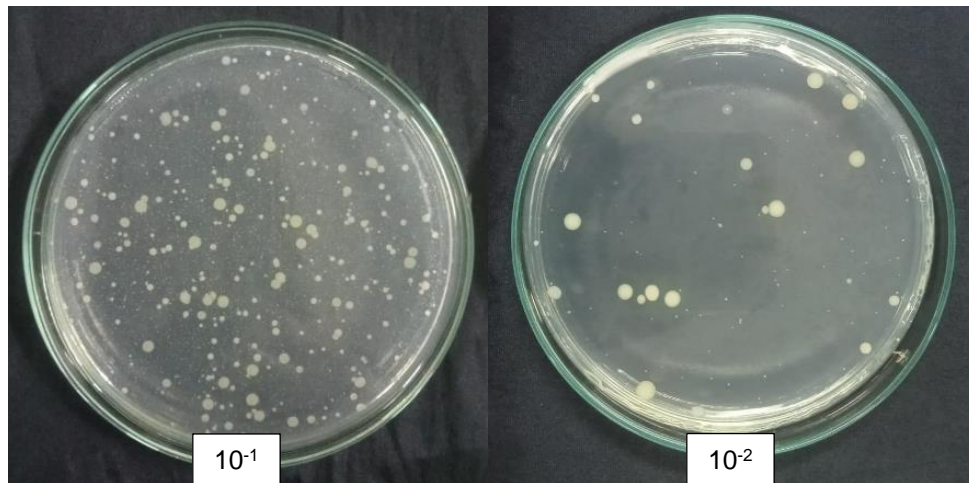
Maka nilai CFU/mL pada *blank* film konsentrasi 20 mg/mL

CFU/mL $= \dfrac{(8,2 \times 10^9) + (3,9 \times 10^{10})}{2} = 2,3 \times 10^{10} \text{ cfu/mL}$

Lampiran 5. Hasil Pengamatan Makroskopis

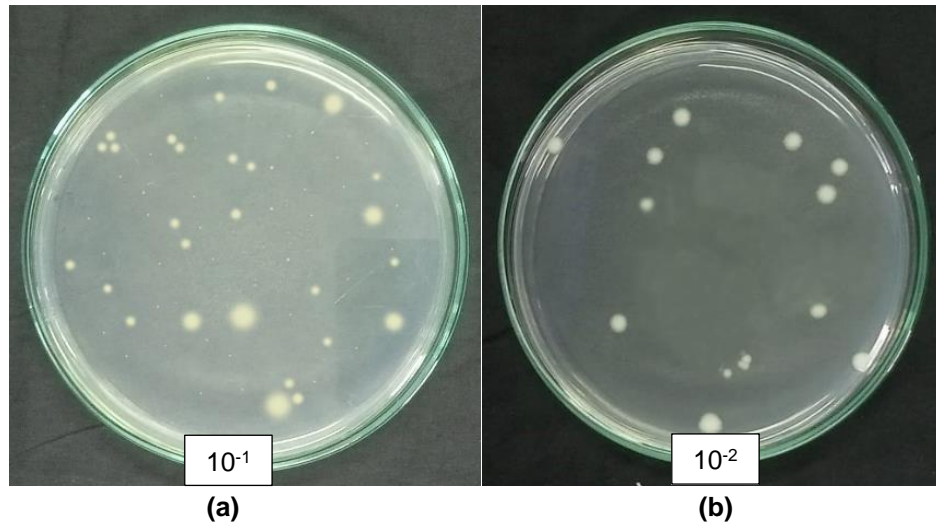(a) 10^{-1} (b) 10^{-2}

Gambar 12. Sediaan film *patch* GSNO konsentrasi 5 mg/mL

(a) 10^{-1} (b) 10^{-2}

Gambar 13. Sediaan film *patch* GSNO konsentrasi 10 mg/mL

Gambar 13. Sediaan film *patch* GSNO konsentrasi 20 mg/mL

Lampiran 5. Data Hasil Analisis Statistika

Pada konsentrasi sediaan film *patch* GSNO 5 mg/mL

Uji Normalitas

Tests of Normality

	Sediaan	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Log reduction 24 jam	Blank film	.	3	.	.	3	.
	GSNO film	.231	3	.	.980	3	.730

a. Lilliefors Significance Correction

Independent Sample T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Log reduction 24 jam	Equal variances assumed	6.515	.063	25556.548	4	.000	1482891666	58023.94142	1482730566	1483052767
	Equal variances not assumed			25556.548	2.000	.000	1482891666	58023.94142	1482642009	1483141323

Pada konsentrasi sediaan film *patch* GSNO 10 mg/mL

Uji Normalitas

Tests of Normality

	Sediaan	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Log reduction 24 jam	Blank film	.	3	.	.	3	.
	GSNO film	.189	3	.	.998	3	.906

a. Lilliefors Significance Correction

Independent Sample T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Log reduction 24 jam	Equal variances assumed	4.742	.095	109187.480	4	.000	1483213666	13584.10018	1483175951	1483251382
	Equal variances not assumed			109187.480	2.000	.000	1483213666	13584.10018	1483155219	1483272114

Pada konsentrasi sediaan film *patch* GSNO 20 mg/mL

Uji Normalitas

Tests of Normality

	Sediaan	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Log reduction 24 jam	Blank film	.	3	.	.	3	.
	GSNO film	.219	3	.	.987	3	.780

a. Lilliefors Significance Correction

Independent Sample T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Log reduction 24 jam	Equal variances assumed	5.953	.071	53721504.83	4	.000	2.34167E+10	435.88989	2.34167E+10	2.34167E+10
	Equal variances not assumed			53721504.83	2.000	.000	2.34167E+10	435.88989	2.34167E+10	2.34167E+10

Lampiran 6. Dokumentasi

Gambar 15. Hasil Sintesis GSNO

Gambar 16. Autoklaf

Gambar 17. Proses Penyiapan Suspensi Bakteri Uji dan Preparasi Sampel

Gambar 18. Proses Pengujian ALT