**Lampiran 1.** Surat Determinasi Tumbuhan



**Lampiran 2.** Bagan Alir Penelitian Pembuatan Serbuk Simplisia Daun Jeruk Purut (*Citrus hystrix* DC)

Daun jeruk Purut segar

Disortasi basah

Dicuci dengan air yang mengalir

Ditiriskan

6 kg daun

Jeruk purut basah

Dikeringkan

1,8 kg daun

Jeruk purut kering

Sortasi kering

1,5 kg daun

Jeruk purut kering

Dihaluskan dengan menggunakan blender

Disaring dengan ayakan

Dimasukkan dalam wadah tertutup

1,4 kg serbuk

daun jeruk purut

**Lampiran 3.** Tumbuhan Daun Jeruk Purut ( *Citrus hystrix* DC)



Daun Jeruk Purut Segar

Daun Jeruk Purut Segar



Pengeringan Daun Jeruk Purut



Serbuk Simplisia Daun Jeruk Purut

**Lampiran 4.** Lampiran Makroskopik dan Mikroskopik Daun Jeruk Purut

A. Gambar Makroskopik Simplisia Daun Jeruk Purut



**Lampiran 4.** (Lanjutan)

B. Gambar Mikroskopik Serbuk Simplisia Daun Jeruk Purut

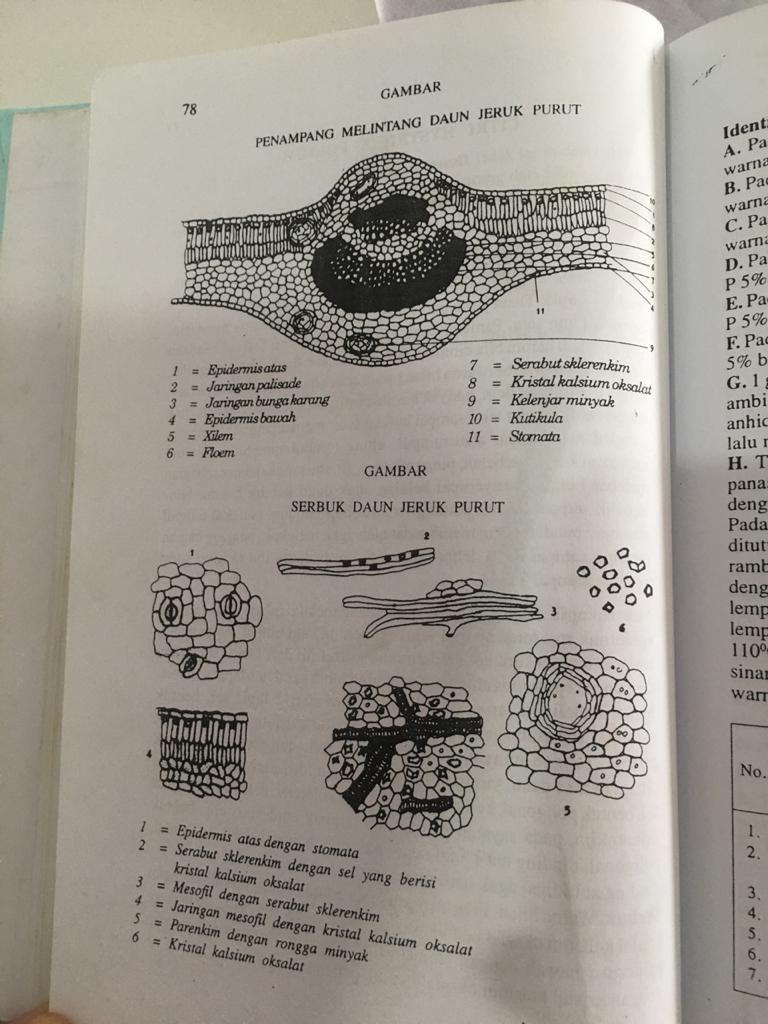


A

C

DD

B



**(MMI-VI, 1995)**

Keterangan :

A=Kristal kalsium oksalat

B=Mesofil dengan serabut sklerenkim

C=Epidermis dengan stomata

D= Jaringan mesofil dengan kristal kalsium oksalat

**Lampiran 5**. Pengujian Karakteristik



Kadar sari larut air



Penetapan kadar air



Penetapan kadar sari larut etanol

**Lampiran 5.**  (Lanjutan)



Penetapan kadar sari larut asam



Penetapan kadar abu total

**Lampiran 6.** Bagan Alir Pembuatan Ekstrak Etanol Daun Jeruk Purut Dengan Metode Maserasi

500 g serbuk simplisia daun jeruk purut

Skrining fitokimia

1. Alkaloid

2. Tanin

3. Flavonoid

4. Saponin

5.Steroid/Triterpenoid

6. Glikosida

Diekstraksi dengan cara maserasi menggunakan pelarut etanol 80%

Dipekatkan dengan alat rotary evaparator

Ekstrak kental 71,9309 gram

Pengujian aktivitas terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*

1. Makroskopis
2. Mikroskopis
3. Kadar air
4. Kadar sari larut air
5. Kadar sari larut etanol
6. Kadar abu total
7. Kadar abu tidak larut asam

Pemeriksaan dan karakterisasi

**Lampiran 7.** Maserasi Daun Jeruk Purut Dan Ekstrak Etanol



Maserasi daun jeruk Purut



Rotary evaporator



Ekstrak daun Jeruk Purut

**Lampiran 8.** Skrining Fitokimia

1. Serbuk daun jeruk purut (*Citrus hystrix* DC)

 ****

**Flavonoid (+)**

**Alkaloid (+)**



**Saponin (+) Steroid/Triterpenoid (+)**

 ****

**Glikosida (-)**

**Tanin (+)**

**Lampiran 8.**  (Lanjutan)

2. Ekstrak Etanol daun jeruk purut (*Citrus hystrix* DC)

 ****

**Flavonoid (+)**

**Alkaloid (+)**



**Steroid/Triterpenoid (+)**

**Saponin (+)**





**Tanin (+) Glikosida (-)**

**Lampiran 9.** Bagan Alir Pengujian Antibakteri

Biakan murni

Diambil 1 ose steril Diambil 1 ose steril

Bakteri *Staphylococcus aureus*

Bakteri *Escherichia coli*

Ditanam pada media Na Ditanam pada media Na

Diinkubasi pada suhu 37ºc Diinkubasi pada suhu

Stok kultur bakteri

37ºc

Diambil dengan jarum ose steril Disuspensikan dalam 10 mL

NaCl 0,9% steril

Dihomogenkan sampai kekeruhan

yang sama dengan Mc. Farland Dipipet 0,1 mL ke tabung reaksi Ditambahkan 9,9 mL NaCl 0,9 %

Suspensi bakteri 108 CFU/mL

steril dan homogenkan Digoreskan ke permukaan media MHA yang telah memadat

Suspensi bakteri 106 CFU/mL

menggunakan swab steril

Dimasukkan kertas cakram yang telah ditetesi ekstrak etanol daun

Jeruk purut

Diinkubasi pada suhu 35-36ºC

selama 18-24 jam

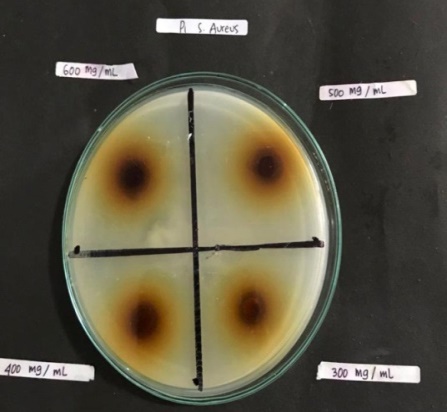
Hasil inkubasi

Diukur diameter zona hambat

Diameter zona hambat bakteri

**Lampiran 10**. Hasil Uji Aktivitas Antibakteri Daya Hambat Ekstrak Etanol Daun Jeruk Purut Terhadap Bakteri *Staphylococcus aures* dan *Escherichia coli*

A. Bakteri *Staphylococcus aureus*

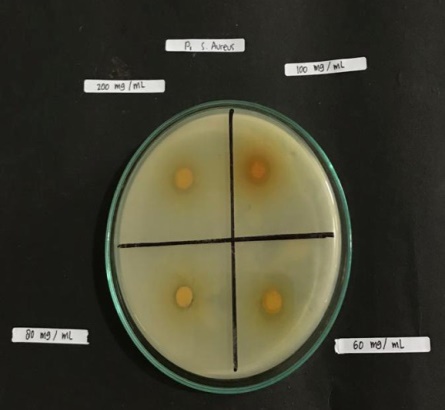
Pengulangan 1 :

B

A

D

C



F

E

H

G



J

I

-

+

Keterangan :

A= 600 mg/mL F= 100 mg/mL

B= 500 mg/mL G= 80 mg/mL

C= 400 mg/mL H= 60 mg/mL

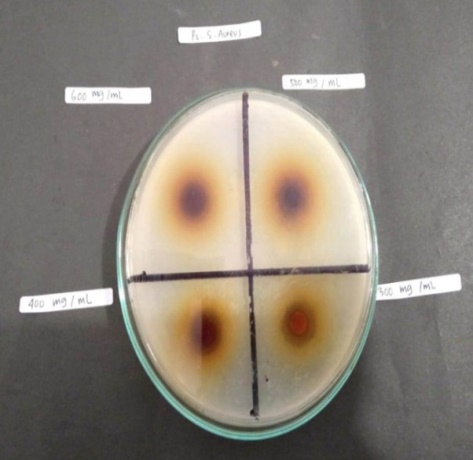
D= 300 mg/mL I= 40 mg/mL

E= 200 mg/mL J= 20 mg/mL

Kontrol (+) = Siproflaksasin Kontrol (-) = Etanol 80%

**Lampiran 10.** (Lanjutan)

Pengulangan 2 :

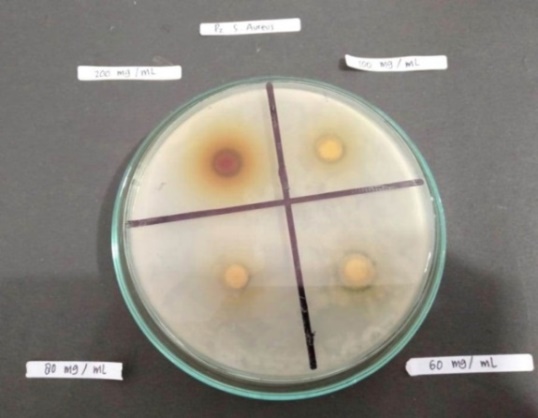


A

B

C

D

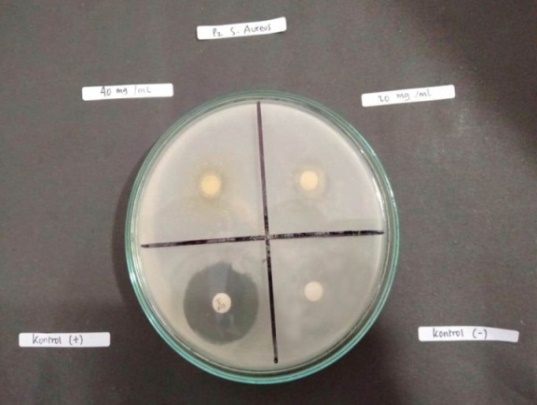


F

E

H

G



J

I

-

+

Keterangan :

A= 600 mg/mL F= 100 mg/mL

B= 500 mg/mL G= 80 mg/mL

C= 400 mg/mL H= 60 mg/mL

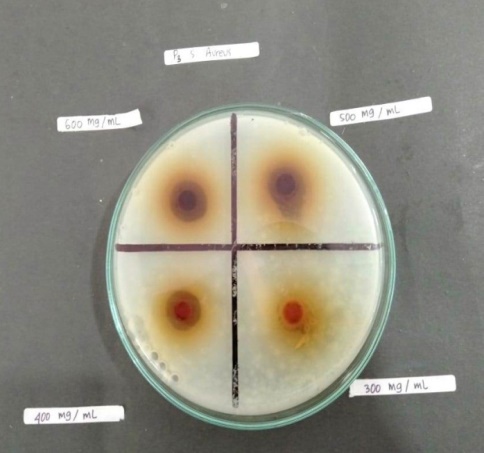
D= 300 mg/mL I= 40 mg/mL

E= 200 mg/mL J= 20 mg/mL

Kontrol (+) = Siproflaksasin Kontrol (-) = Etanol 80%

**Lampiran 10.** (Lanjutan)

Pengulangan 3:

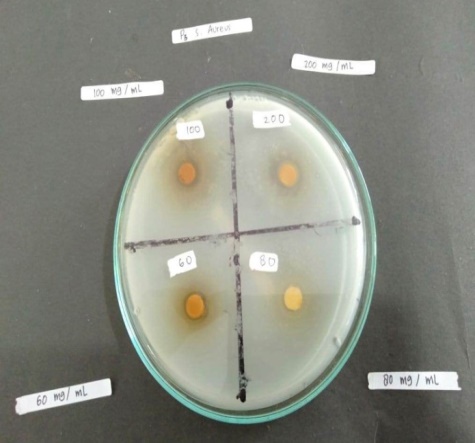


B

A

D

C

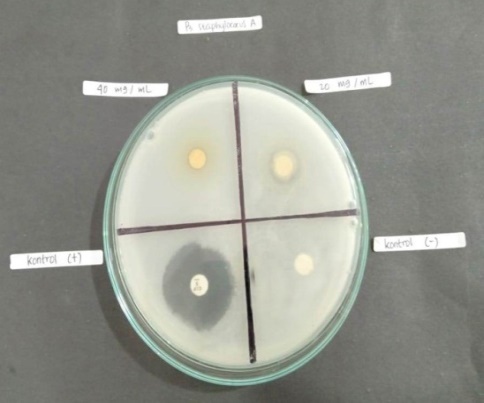


F

E

H

G



I

J

+

-

Keterangan :

A= 600 mg/mL F= 100 mg/mL

B= 500 mg/mL G= 80 mg/mL

C= 400 mg/mL H= 60 mg/mL

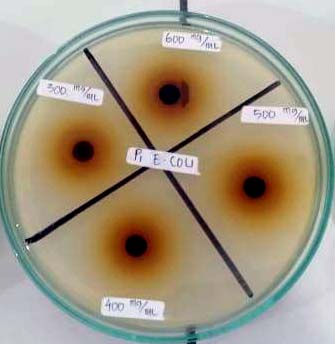
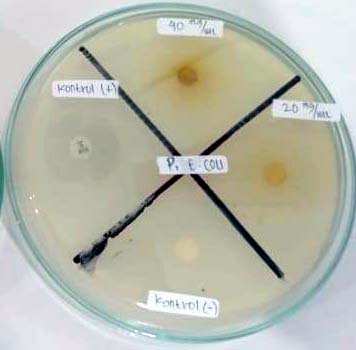
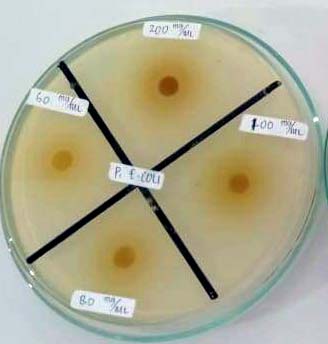
D= 300 mg/mL I= 40 mg/mL

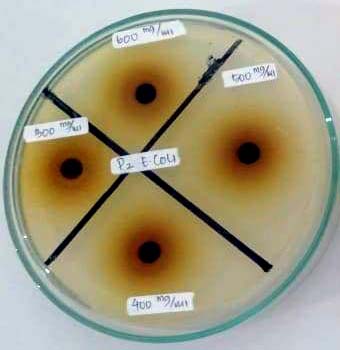
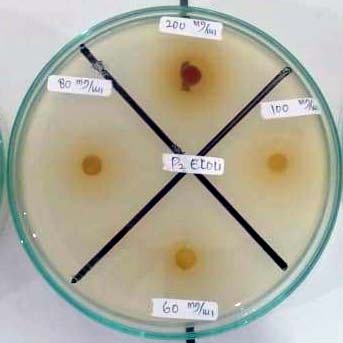
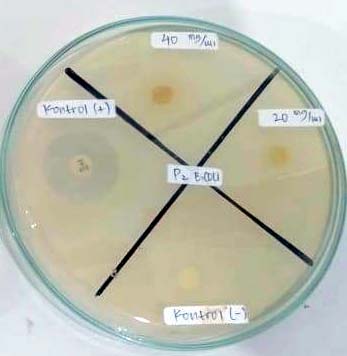
E= 200 mg/mL J= 20 mg/mL

Kontrol (+) = Siproflaksasin Kontrol (-) = Etanol 80%

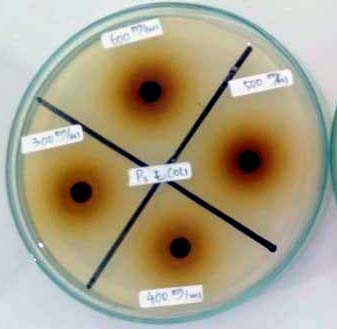
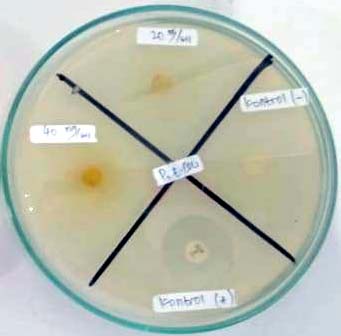
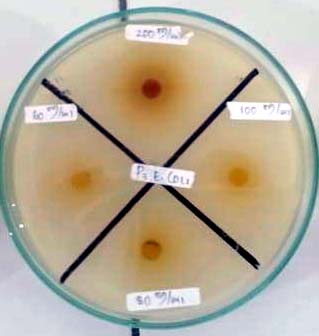
**Lampiran 10.** (Lanjutan)

B. Bakteri *Escherichia coli*

Pengulangan 1.

Pengulangan 2.

Pengulangan 3.



Keterangan :

A= 600 mg/mL F= 100 mg/mL

B= 500 mg/mL G= 80 mg/mL

C= 400 mg/mL H= 60 mg/mL

D= 300 mg/mL I= 40 mg/mL

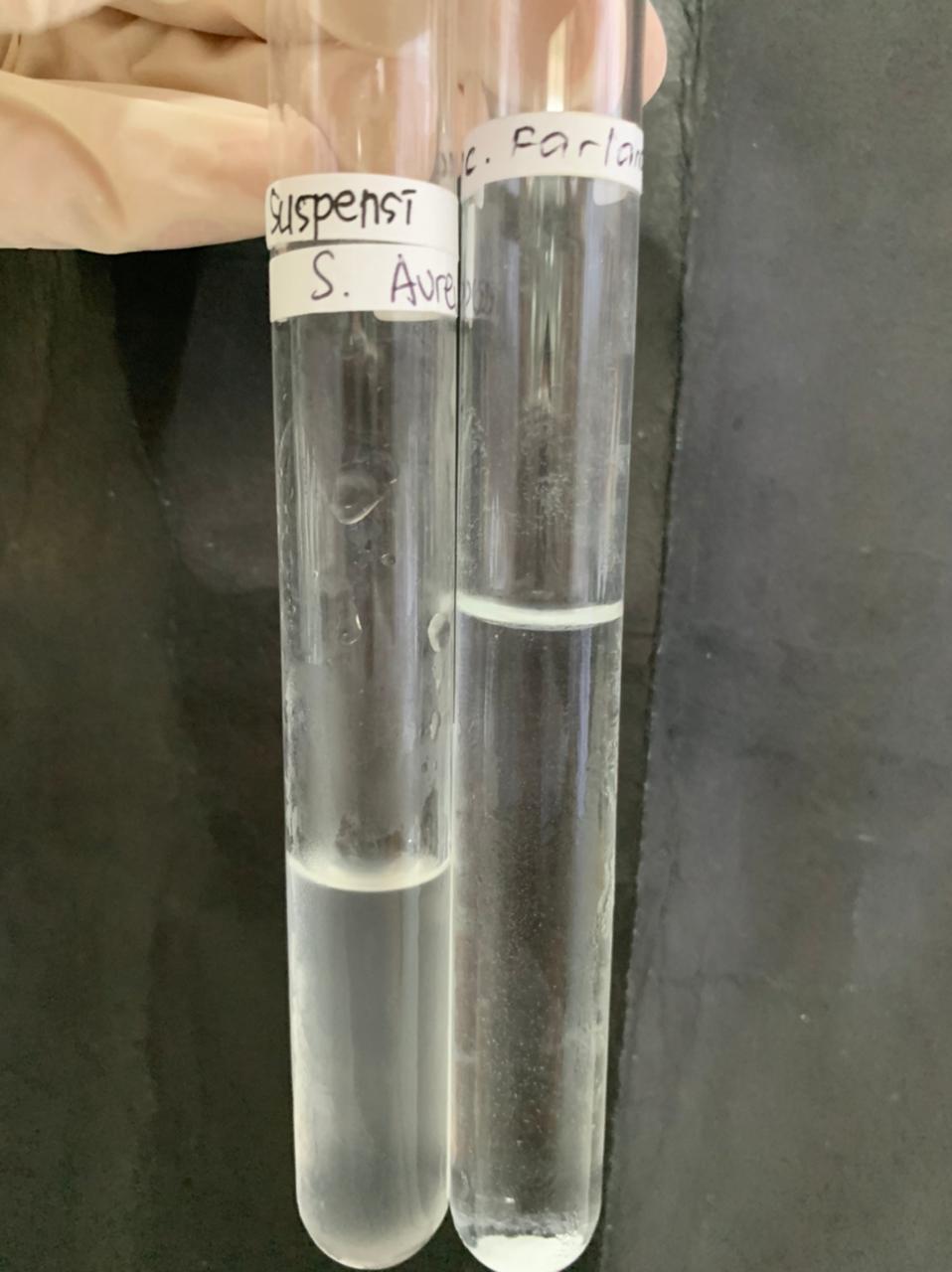
E= 200 mg/mL J= 20 mg/mL

Kontrol (+) = Siproflaksasin Kontrol (-) = Etanol 80%

**Lampiran 11.** Pengujian Antibakteri

****

Uji daya hambat bakteri

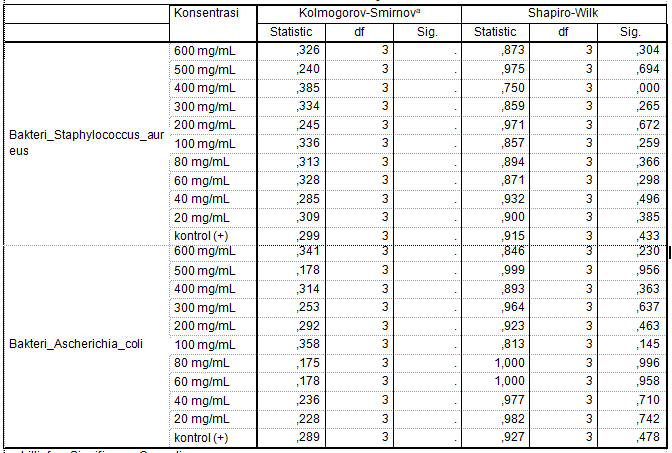
****

Suspensi *Staphylococcus aureus*

****

Suspensi *Escherichia coli*

**Lampiran 12**. Hasil SPSS.



**Lampiran 12.** (Lanjutan)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Descriptives** | | | | | | | | | | | | | | | | |
|  | | | N | Mean | Std. Deviation | | Std. Error | | 95% Confidence Interval for Mean | | | Minimum | | | Maximum | |
| Lower Bound | | Upper Bound |
| Bakteri\_Staphylococcus\_aureus | 600 mg/mL | | 3 | 12,1500 | 1,57877 | | ,91150 | | 8,2281 | | 16,0719 | 11,00 | | | 13,95 | |
| 500 mg/mL | | 3 | 12,0500 | 1,89934 | | 1,09659 | | 7,3318 | | 16,7682 | 10,00 | | | 13,75 | |
| 400 mg/mL | | 3 | 11,7733 | ,04619 | | ,02667 | | 11,6586 | | 11,8881 | 11,72 | | | 11,80 | |
| 300 mg/mL | | 3 | 11,5333 | 1,80716 | | 1,04337 | | 7,0441 | | 16,0226 | 10,25 | | | 13,60 | |
| 200 mg/mL | | 3 | 11,2700 | ,50744 | | ,29297 | | 10,0094 | | 12,5306 | 10,72 | | | 11,72 | |
| 100 mg/mL | | 3 | 11,1133 | 1,40433 | | ,81079 | | 7,6248 | | 14,6019 | 10,12 | | | 12,72 | |
| 80 mg/mL | | 3 | 10,2133 | 1,44383 | | ,83359 | | 6,6267 | | 13,8000 | 9,12 | | | 11,85 | |
| 60 mg/mL | | 3 | 10,1833 | ,16073 | | ,09280 | | 9,7841 | | 10,5826 | 10,00 | | | 10,30 | |
| 40 mg/mL | | 3 | 9,8267 | 1,43977 | | ,83125 | | 6,2501 | | 13,4032 | 8,22 | | | 11,00 | |
| 20 mg/mL | | 3 | 9,4367 | 1,04892 | | ,60559 | | 6,8310 | | 12,0423 | 8,25 | | | 10,24 | |
| kontrol (+) | | 3 | 18,7500 | 2,22205 | | 1,28290 | | 13,2301 | | 24,2699 | 17,00 | | | 21,25 | |
| kontrol (-) | | 3 | ,0000 | ,00000 | | ,00000 | | ,0000 | | ,0000 | ,00 | | | ,00 | |
| Total | | 36 | 10,6917 | 4,17099 | | ,69517 | | 9,2804 | | 12,1029 | ,00 | | | 21,25 | |
| Bakteri\_Ascherichia\_coli | 600 mg/mL | | 3 | 12,9167 | 3,12583 | | 1,80470 | | 5,1517 | | 20,6817 | 10,75 | | | 16,50 | |
| 500 mg/mL | | 3 | 12,5833 | 3,12583 | | 1,80470 | | 4,8183 | | 20,3483 | 9,50 | | | 15,75 | |
| 400 mg/mL | | 3 | 12,0000 | 2,64575 | | 1,52753 | | 5,4276 | | 18,5724 | 10,00 | | | 15,00 | |
| 300 mg/mL | | 3 | 11,6667 | 1,52753 | | ,88192 | | 7,8721 | | 15,4612 | 10,00 | | | 13,00 | |
| 200 mg/mL | | 3 | 10,6667 | 1,04083 | | ,60093 | | 8,0811 | | 13,2522 | 9,50 | | | 11,50 | |
| 100 mg/mL | | 3 | 10,4000 | 6,60076 | | 3,81095 | | -5,9972 | | 26,7972 | 6,10 | | | 18,00 | |
| 80 mg/mL | | 3 | 9,4767 | 1,52500 | | ,88046 | | 5,6883 | | 13,2650 | 7,95 | | | 11,00 | |
| 60 mg/mL | | 3 | 7,4767 | 1,04026 | | ,60059 | | 4,8925 | | 10,0608 | 6,45 | | | 8,53 | |
| 40 mg/mL | | 3 | 6,9167 | 1,23929 | | ,71550 | | 3,8381 | | 9,9952 | 5,80 | | | 8,25 | |
| 20 mg/mL | | 3 | 6,5333 | 1,71561 | | ,99051 | | 2,2715 | | 10,7952 | 4,70 | | | 8,10 | |
| kontrol (+) | | 3 | 17,5833 | 4,54377 | | 2,62335 | | 6,2960 | | 28,8707 | 12,50 | | | 21,25 | |
| kontrol (-) | | 3 | ,0000 | ,00000 | | ,00000 | | ,0000 | | ,0000 | ,00 | | | ,00 | |
| Total | | 36 | 9,8517 | 4,86397 | | ,81066 | | 8,2059 | | 11,4974 | ,00 | | | 21,25 | |
| **Lampiran 12.** (Lanjutan)  **Anova** | | | | | | | | | | | | | | | |
|  | | | | | | Sum of Squares | | Df | | Mean Square | | | F | Sig. | |
| Bakteri\_Staphylococcus\_aureus | | Between Groups | | | | 565,264 | | 11 | | 51,388 | | | 28,262 | ,000 | |
| Within Groups | | | | 43,637 | | 24 | | 1,818 | | |  |  | |
| Total | | | | 608,902 | | 35 | |  | | |  |  | |
| Bakteri\_Ascherichia\_coli | | Between Groups | | | | 623,915 | | 11 | | 56,720 | | | 6,669 | ,000 | |
| Within Groups | | | | 204,122 | | 24 | | 8,505 | | |  |  | |
| Total | | | | 828,037 | | 35 | |  | | |  |  | |

**Lampiran 12.** (Lanjutan)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bakteri\_Staphylococcus\_aureus** | | | | | |
| Duncana | | | | | |
| Konsentrasi | N | Subset for alpha = 0.05 | | | |
| 1 | 2 | 3 | 4 |
| kontrol (-) | 3 | ,0000 |  |  |  |
| 20 mg/mL | 3 |  | 9,4367 |  |  |
| 40 mg/mL | 3 |  | 9,8267 | 9,8267 |  |
| 60 mg/mL | 3 |  | 10,1833 | 10,1833 |  |
| 80 mg/mL | 3 |  | 10,2133 | 10,2133 |  |
| 100 mg/mL | 3 |  | 11,1133 | 11,1133 |  |
| 200 mg/mL | 3 |  | 11,2700 | 11,2700 |  |
| 300 mg/mL | 3 |  | 11,5333 | 11,5333 |  |
| 400 mg/mL | 3 |  | 11,7733 | 11,7733 |  |
| 500 mg/mL | 3 |  | 12,0500 | 12,0500 |  |
| 600 mg/mL | 3 |  |  | 12,1500 |  |
| kontrol (+) | 3 |  |  |  | 18,7500 |
| Sig. |  | 1,000 | ,051 | ,081 | 1,000 |
| Means for groups in homogeneous subsets are displayed. | | | | | |
| a. Uses Harmonic Mean Sample Size = 3,000. | | | | | |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bakteri\_Ascherichia\_coli** | | | | | |
| Duncana | | | | | |
| Konsentrasi | N | Subset for alpha = 0.05 | | | |
| 1 | 2 | 3 | 4 |
| kontrol (-) | 3 | ,0000 |  |  |  |
| 20 mg/mL | 3 |  | 6,5333 |  |  |
| 40 mg/mL | 3 |  | 6,9167 |  |  |
| 60 mg/mL | 3 |  | 7,4767 | 7,4767 |  |
| 80 mg/mL | 3 |  | 9,4767 | 9,4767 |  |
| 100 mg/mL | 3 |  | 10,4000 | 10,4000 |  |
| 200 mg/mL | 3 |  | 10,6667 | 10,6667 |  |
| 300 mg/mL | 3 |  | 11,6667 | 11,6667 |  |
| 400 mg/mL | 3 |  | 12,0000 | 12,0000 |  |
| 500 mg/mL | 3 |  |  | 12,5833 | 12,5833 |
| 600 mg/mL | 3 |  |  | 12,9167 | 12,9167 |
| kontrol (+) | 3 |  |  |  | 17,5833 |
| Sig. |  | 1,000 | ,057 | ,058 | ,057 |
| Means for groups in homogeneous subsets are displayed. | | | | | |
| a. Uses Harmonic Mean Sample Size = 3,000. | | | | | |