Effectivity of custard apple’s (*Annona squamosa*) seed extract in various concentrations on the growth of *Escherichia coli*

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1. **Introduction**

*Escherichia coli* bacteria are naturally present in the human colon. This bacterium has important function in the gut, which is vitamin K synthesis, the conversion of bile pigments, bile acids and the absorption of nutrients in the human gut. It also produces colicin which can protect the digestive tract from pathogenic bacteria. In addition, these bacteria in the environment is a bacterial indicator of sanitation. Bacterial indicator sanitation is a bacterium whose presence in the environment (can also present in drinking water or food) shows the environment was polluted by human waste [1]. It is said to be an indicator of contamination by human waste because it is a normal bacteria in the human digestive system. The presence of these bacteria in foods or drinks may also contain other harmful pathogenic bacteria. Government regulations have determined that food and drink should not contain this bacteria. The Health Minister's Decree requires that the bacteria in the foods should be 0 (zero) per gram of food.
Although in general, the *Escherichia coli* are harmless, some certain strains capable of causing gastroenteritis and acute diarrhea, such as enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), Enteroinvasive *Escherichia coli* (EIEC), Enterohemorrhagic *Escherichia coli* (EHEC) and Enteroaggregative *Escherichia coli* (EAEC). Based on [2] study that examines the bacterial contamination in food hawkers along Jalan Margonda Depok, West Java showed that nearly half of food samples (41%) were contaminated. The results of this study show that there is a connection between the unavailability of a dumpster at the place of sale with the level of contamination. Contamination is also caused because the food is served in a condition not covered so easily infestation of flies. The level of contamination of bacteria in food in Indonesia is reinforced also by [3] research, the results of it is known that *Escherichia coli* contamination on hawkers of 51.8% in food materials and 18.8% of the food served, restaurant 26.3%, and catering services 11.8%. Contamination is mainly due to the bacteria contaminating the foodstuffs, food served, and the hands of the food processor.

In case of disease by the bacteria, one solution that can be used is the utilization of custard apple (*Annona squamosa*). Based on research [4], the aqueous extracts and organic extract solvent from the roots, leaves, fruits, and seeds of custard apple reported to be an insecticide, inhibiting the activity of insect feeding (antifeedant), and repellent to some kind of important pest of agriculture and pest in storage. Research from experts also showed that seed extract from custard apple tested on several models of cancer cells showed significant activity against breast cancer cells. Phytochemical and pharmacological research on custard apple seeds has shown that the major bioactive compound contained in it is acetogenin, which has a strong anti-tumor activity. Acetogenin is recognized as the most potent mitochondrial complex I inhibitor (effective in concentrations up to nanomolar). Acetogenin compounds have a wide ability as pesticides, parasiticides, antimicrobials, inhibitors of cell growth, and anti-cancer. [5] study also showed that active compounds in custard apple’s root extract can increase blood glucose levels, and stimulate insulin release. This is very useful for the treatment of diabetes mellitus. Based on that fact, further research will be conducted on the benefits of custard apple’s seed extract (*Annona squamosa*) on the growth of *Escherichia coli* bacteria.

2. Materials and methods

This research was conducted in Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Pendidikan Ganesha for 3 months.

The basic design used is Completely Randomized Design. The number of samples and replication is determined by formulation \( t (r-1) \geq 20 \) [6]. This study is a true experimental research with a random post-test only design pattern. The treatments were dilution consisting of five treatments, \( P_1 = 10\% \), \( P_2 = 20\% \), \( P_3 = 30\% \), \( P_4 = 40\% \), and \( P_5 = 50\% \).

The population of this study were all *Escherichia coli* bacteria from the culture stock at the Microbiology Laboratory which was reproduced on the NB (Nutrient Broth) medium. While the sample of this study is *Escherichia coli* bacteria that given seed extract of custard apple’s seed with various concentrations.

3. Results and Discussion

3.1 Research Result

The main data was average diameter of inhibition zone of *Escherichia coli* bacteria that was given seed extract of custard apple’s (*Annona squamosa*) with various concentrations. This main data is presented in Table 1.
Table 1. The average diameter of inhibition zone of *Escherichia coli* that given seed extract of custard apple’s (*Annona squamosa*) with various concentrations

| Replication | The average diameter of inhibition zone (mm) |
|-------------|---------------------------------------------|
|             | 10%  | 20%  | 30%  | 40%  | 50%  |
| 1           | 11.1 | 12.0 | 20.0 | 22.5 | 26.3 |
| 2           | 9.5  | 12.1 | 22.3 | 24.3 | 24.8 |
| 3           | 10.0 | 10.8 | 21.8 | 23.4 | 25.4 |
| 4           | 8.8  | 11.1 | 18.5 | 22.3 | 23.5 |
| 5           | 8.9  | 10.5 | 17.8 | 23.3 | 23.8 |
| Subtotal    | 48.1 | 56.4 | 100.3| 115.8| 123.8|
| Average     | 9.66 | 11.30| 20.08| 23.16| 24.76|

Based on data from Table 1, it can be seen that the average diameter of inhibition zone of *Escherichia coli* bacteria that given seed extract of custard apple’s with concentrations of 10%, 20%, 30%, 40%, and 50% in a row is 9.66 mm; 11.30 mm; 20.08 mm; 23.16 mm; and 24.76 mm. To facilitate interpretation data in Table 1, can be described in the form of bar chart in Figure 1.

!![Bar chart of average diameter of inhibition zone Escherichia coli](image)

Figure 1. Bar chart of average diameter of inhibition zone *Escherichia coli*

Figure 1 shows clearly that the higher the concentration of seed extract of custard apple’s after administration toward the growth of bacteria then the diameter of inhibition zone formed also increase. This means that the diameter of *Escherichia coli* inhibition zone was proportional toward the increase of the concentrations seed extract of custard apple’s. It can be understood that the higher the concentrations of seed extract of custard apple’s because it contains antibacterial substances in it will also be higher so that more strongly inhibit the growth of *Escherichia coli*.

The supporting data obtained in this study consist of three types of effectiveness tests among other MIC (Minimum Inhibitory Concentration) test, MBC (Minimum Bactericidal Concentration), test, and Phenol Coefficient test. MIC (Minimum Inhibitory Concentration) test results are presented in Table 2.
Table 2. MIC (Minimum Inhibitory Concentration) test result of seed extract of custard apple’s (Annona squamosa) on growth of Escherichia coli.

| Seed extract of custard apple’s concentrations | K1  | 10%  | 20%  | 30%  | 40%  | 50%  | 60%  | 70%  | 80%  | 90%  | 100% |
|-----------------------------------------------|-----|------|------|------|------|------|------|------|------|------|------|
| K2                                            | 5%  | 10%  | 15%  | 20%  | 25%  | 30%  | 35%  | 40%  | 45%  | 50%  |
| Turbidity                                     | +++ | ++   | +    | -    | -    | -    | -    | -    | -    | -    | -    |

Explanations:
K1 : Initial Concentrations
K2 : Concentration after addition of bacterial suspension
+++ : Very cloudy
++  : Snappy
+   : Slightly murky
-   : Clear

Based on the MIC Test results in Table 2, the test tube at 5% concentration were very cloudy, the test tubes at concentrations of 10% and 15% were still cloudy, and the test tube at 20% concentration were slightly turbid. The clear test tubes begin appeared at concentrations of 25%, 30%, 40%, 45%, and 50%. After performing the MIC test, test tubes of concentrations 25%, 30%, 35%, 40%, 45%, and 50% then grown on NA medium by casting technique and incubated for 24 hours to further observed bacterial growth. The smallest concentration indicating the absence of Escherichia coli bacteria growth was stated as the value of MBC (Minimum Bactericidal Concentration). MBC test results can be seen in Table 3.

Table 3. MBC test results of seed extract of custard apple’s on Escherichia coli bacterial growth

| Seed extract of custard apple’s concentrations | K1  | 50%  | 60%  | 70%  | 80%  | 90%  | 100% |
|-----------------------------------------------|-----|------|------|------|------|------|------|
| K2                                            | 25% | 30%  | 35%  | 40%  | 45%  | 50%  |
| E.coli growth                                  | +   | +    | -    | -    | -    | -    |

Based on Table 3, at concentrations of 25%, and 30%, still indicates the growth of Escherichia coli bacteria, but at concentrations of 35%, 40%, 45%, and 50% have not found bacterial growth. The phenol coefficient test used standard test bacteria was Escherichia coli and standard disinfectant, was phenol. The effectiveness of seed extract of custard apple’s was compared with phenol. The bacteria that was given by phenol and extract were grown on NA medium and incubated for 24 hours. The results of effectiveness testing can be seen in Table 4.
Table 4. Results of phenol coefficient test of seed extract of custard apple’s (*Annona squamosa*) on *Escherichia coli* growth

| Dilutions   | Bacterial growth time (minutes) |
|-------------|---------------------------------|
|             | 5     | 10    | 15    |
| Phenol      |       |       |       |
| 0.25: 20    | -     | -     | -     |
| 0.25: 22.5  | +     | -     | -     |
| 0.25: 25    | +     | -     | -     |
| Seed extract of custard apple’s |       |       |       |
| 0.25 : 100  | +     | -     | -     |
| 0.25 : 112.5| +     | -     | +     |
| 0.25 : 125  | +     | -     | -     |

Note: (+): there was growth, (-): no growth

The highest dilution of the non-lethal phenol within 5 minutes, but deadly within 10 minutes is 0.25: 22.5. The highest dilution of seed extract of custard apple’s which did not kill bacteria within 5 minutes, but deadly within 10 minutes was at 0.25: 125. The coefficient value of phenol was determined by the highest lethal phenol dilution within 10 minutes but not lethal in 5 minutes compared to extract dilution ratio of deadly extract within 10 minutes but not lethal in 5 minutes. Then the phenol coefficient is (0.25: 22.5): (0.25: 125). This means dilution of extract more effectively 5.5 time from phenol dilution. Hypothesis test using one-way ANOVA test. Hypothesis test results can be seen in Table 5.

Table 5. Hypothesis test of inhibition zone diameter of *Escherichia coli*

| Inhibition zone | Sum of Squares | Df | Mean Square | F       | Sig. |
|-----------------|----------------|----|-------------|---------|------|
| Between Groups  | 954.394        | 4  | 238.599     | 164.415 | .000 |
| Within Groups   | 29.024         | 20 | 1.451       |         |      |
| Total           | 983.418        | 24 |             |         |      |

From the result of hypothesis test in Table 5, it can be seen that the significant number is 0.000 <0.05 so $H_0$ was rejected and $H_1$ was accepted. So it can be said that there was an significant different of the various concentrations of seed extract of custard apple’s toward the growth of *Escherichia coli* bacteria. To know the effectiveness of each concentrations of seed extract of custard apple’s in inhibiting the growth of *Escherichia coli* bacteria can be seen in Table 6.

Table 6. Descriptive statistics test results each concentration

| Concentrations | N  | Mean    | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | Lower Bound | Upper Bound |
|----------------|----|---------|----------------|------------|---------------------------------|-------------|-------------|
| 10%            | 5  | 9.660   | 0.93968        | 0.42024    | 8.4932                           | 10.8268     |
| 20%            | 5  | 11.300  | 0.71764        | 0.32094    | 10.4089                          | 12.1911     |
| 30%            | 5  | 20.080  | 1.97408        | 0.88284    | 17.6289                          | 22.5311     |
| 40%            | 5  | 23.160  | 0.79875        | 0.35721    | 22.1682                          | 24.1518     |
| 50%            | 5  | 24.760  | 1.15022        | 0.51439    | 23.3318                          | 26.1882     |
| Total          | 25 | 17.7920 | 6.40123        | 1.28025    | 15.1497                          | 20.4343     |
Table 6 shows the mean diameter of the inhibition zones from small to large are concentrations of 10%, 20%, 30%, 40%, and 50%. The most effective concentration in inhibiting the growth of *Escherichia coli* is a concentration of 50% which is characterized by the largest diameter of the inhibition zone with an average of 24.76 mm.

### 3.2 Discussion

Based on the results of research that has been done and strengthened also with the results of hypothesis test, seed extract of custard apple’s (*Annona squamosa*) effective in inhibiting the growth of *Escherichia coli*, where the concentrations difference is given effect on the growth of *Escherichia coli*. Various concentrations of seed extract of custard apple’s (*Annona squamosa*) were 10%, 20%, 30%, 40%, and 50%. The inhibition zone *Escherichia coli* bacteria due to the concentration of seed extract of custard apple’s can be observed by looking at the magnitude of the constraints zone formed. The diameter of the inhibition zone formed was measured using a caliper to determine how much effect a concentration of seed extract of custard apple’s inhibited the growth of *Escherichia coli*. From the results presented above, the concentration of 50% yielded the widest area of the inhibition zone, which is 24.76 mm.

The inhibition zone was an area around the paper disc that is not overgrown with *Escherichia coli*. The inhibition zone was look like a crystal clear area around the paper disc. The diameter of the inhibition zone formed shows the effectiveness of seed extract of custard apple’s in inhibiting the growth of *Escherichia coli*. The wider the inhibition zone that is formed, the more effective the concentration of seed extract of custard apple’s. The formation of a inhibition zone due to the seed extract of custard apple’s showed that the seed extract of custard apple has an antibacterial active compound that capable to inhibiting and even killing *Escherichia coli*. An antibacterial can act by inhibiting the synthesis of important metabolites in bacteria [7].

Seed of custard apple contain amino acids, protein 42-45%, fat, fatty acids (methyl palmitate, methyl stearate, methyl linoleate), acetogenin active compounds (squamostatin C, D, annonain, annonacin A, annonin I, IV, VI, VIII, IX, XVI, squamostatin A, bulatasin, bulatasinone, squamon, n-coanolin B, neo desasetilurarisin, neo reticulumin A, squamosten A, asimicin, squamocin, sanonacin, anonastatin, neoannonin). Also found squamosisnin A, squamocin B, C, D, E, F, G, H, I, J, K, L, M, N; Skuamostatin B), tannins, saponins, and flavonoids.

Annonaceous compound acetogenins (skumostatin A, B, C, and D and annotemoyin-1 and -2, and glukopiranoidsicholesterly) has cytotoxic ability [8]. According to [9] the Furan chain in the hydrofurhan group on C3 acetogenin compounds has cytotoxic activity. Cytotoxic acetogenin derivatives include asimicin, bulatacin, and squamocin. Acetogenin class of adjacent bis-tetrahydrofurhan (bis-THF), especially asimicin and bulatacin has strong cytotoxic ability. Its cytotoxic effects are stronger than doxorubicin [10]. Asimicin and bulatatin inhibit the NADH-ubiquinone reductase enzyme that is needed in the reaction in mitochondrial respiration [11] and inhibits the process of cell growth in G1 and G2 phase of the eukaryoticyccells [10]. While the squamocin action mechanism is by inhibiting electron transfer in site I (between NADH and ubiquinone) in the electron transport chain in the cell respiration process. Long chain of fatty acids (C32 or C34) that bind the ends of the β-lactone ring inhibit the activity of NADH-ubiquinone oxiireductase [12]. If electron transfer is inhibited, formation of proton gradient is inhibited and energy reserves in that gradient can not form ATP [13]. Other acetogenin derivatives are annonacin, annonacin belongs to the mono-tetrahydrofurhan group. According to research [14], Annonacin also has a cytotoxic ability, especially in cancer cells. Annonacin causes significant cell death by inhibiting the G1 phase of cell growth and inducing apoptotic processes, especially in eukaryotic cells.

Custard apple’s (*Annona squamosa*) seed extract also contains flavonoids, tannins, and saponins. The mechanism of flavonoids as antibacterial compounds is by protein denaturation of the bacterial cell wall by binding to the protein through hydrogen bonding, cytoplasmic membrane damage that can lead to leaking of important metabolites and inactivate enzyme systems of the bacteria. This damage
allows the nucleotides and amino acids to leak out and prevent the entry of active ingredients into cells, this condition can cause bacterial death. Flavonoids also inhibit the synthesis of bacterial macromolecules. According to Cuppetin [15], flavonoids have antioxidant and antibacterial abilities because they can donate their hydrogen atoms, in the form of glucosides (containing glucose side chains) or in free form called aglycons. Flavonoid compounds contain H⁺ ions. This ion will bind to the phosphate group on the cytoplasmic membrane so that the phospholipid will decompose into glycerol, carboxylic acid, and folic acid. Damage caused cytoplasmic membrane phospholipids are not able to retain its shape and cytoplasmic membrane leak.

Tanin (C₇₆H₅₂O₄₆) has the ability to inactivate essential enzymes, the destruction of genetic materials and the cracking of bacterial cell walls that can interfere with cell permeability [16]. Disruption of cell permeability can lead to stunted growth and death in cells [17]. Tanin targets cell wall polypeptides that cause the formation of cell walls to be less than perfect so that bacterial cells become lysed by osmotic or physical pressure [18]. Tanin compounds can induce the formation of bonding compound complexes against microbial enzymes or substrates and the formation of a tannin bonding complex against metal ions can increase the toxicity of tannins.

Saponin compounds can damage the cytoplasmic membrane. Damage to the cytoplasmic membrane may result in the permeability of the cell membrane reduced so that the transport of substances into cells and out of cells becomes uncontrolled. Substances that are inside the cell as an organic ion, enzymes, amino acids, and nutrients can be released from cells [19]. Saponins can inhibit the growth of *Escherichia coli* by the inhibition mechanism of protein synthesis because it accumulates and causes changes in the components of cell constituents [20].

**3.2.1 Effectivity of custard apple’s (Annona squamosa) seed extract on *Escherichia coli*’s growth with MIC (Minimum Inhibitory Concentration)**

The lowest concentration of seed extract of custard apple’s that can inhibit the growth of bacteria is called MIC (Minimum Inhibitory Concentration). The purpose of performing a MIC test is to find out whether a particular antibacterial agent is good for use or not. The MIC test observation indicator is by looking at the mixed conditions of Nutrient Broth, bacterial suspension, and extract on the test sample then looking at the turbidity level of the dilution tube. The dilution started from concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, the concentrations of the extract were included in 1 ml of NB and 1 ml of bacterial suspension, so the concentration becomes half of the initial concentration of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, and 50%. After incubation for 24 hours then observed the level of turbidity. At concentrations of 5% visible turbid reaction tube, 10% concentration and 15% turbid reaction tubes, and at a concentration of 20% test tube start a little turbid, meaning that at concentrations of 5% to 20% still occur bacterial growth process. Seed extract of custard apple’s at these concentrations has not been able to inhibit bacterial growth optimally. The test tubes start to look clear at concentrations of 25% to 50%, meaning that the extract at that concentration was able to inhibit the growth of *Escherichia coli*. This means the MIC value was 25% because at that concentration is the lowest concentration where the test tube was clear or not cloudy. The smaller concentrations of antibacterial agents in MIC tests that are capable of inhibiting bacterial growth, the more susceptible the bacteria are to an antibacterial agent.

**3.2.2 Effectivity of antibacterial of custard apple’s (Annona squamosa) seed extract on *Escherichia coli*’s growth with MBC test (Minimum Bactericidal Concentration)**

MBC (Minimum Bactericidal Concentration) is the lowest concentration of antibacterial material that can kill 99.9% in culture for a prescribed time. Determination of MBC value is done by planting bacteria in liquid hatchery used for MIC test into NA medium and then incubated for 24 hours. An indicator of this test if there is no growth of bacteria on NA media. The clear reaction tubes in the MIC test results used were concentrations of 25%, 30%, 35%, 40%, 45%, and 50%.
After grown for 24 hours in the incubator, a petri dish with the concentration of 25% and 30% is still bacterial growth, but the bacteria growing in petri dish is quite small so it is rather difficult to see in observation. At that concentration is already happening the process of inhibition of growth but has not been able to kill the entire bacteria. A small percentage of bacteria are able to survive, although growth is hampered by the presence of antibacterial ingredients in custard apple’s seed extract. While petri dish at concentration 35%, 40%, 45%, and 50% have not found any more growth of Escherichia coli. At that concentration, bacteria are completely unable to survive. The antibacterial material in the given extract are no longer possible for bacterial cells to perform all life activities. The concentration of 35% is the lowest concentration where there is no bacterial growth, this means the value of MBC is 35%.

3.2.3 Phenol Coefficient Test

Phenol coefficient test is a standard test for comparing the effectiveness of antimicrobial material (in this case seed extract of custard apple’s) with phenol. The results are expressed in phenol coefficients. The phenol coefficient test aims to determine which is more effective between custard apple’s seed extract and phenol in killing bacteria. In the research results, it was found that the highest dilution of phenol non-lethal within 5 minutes, but deadly within 10 minutes was 0.25: 22.5 dilution and dilution highest extract of seed extract of custard apple’s which does not kill bacteria within 5 minutes, but deadly within 15 minutes is at 0.25: 125 dilution. This means the phenol coefficient is 125: 25. Meaning that dilution extract 0.25: 125 is more effective 5-fold than 0.25: 25 phenol dilution. In this test used 3-time intervals so that the duration of exposure varies between 5 minutes, 10 minutes and 15 minutes. It can be seen from the results of the study that at 5 minutes exposure time, both extract and phenol (except phenol with 0.25: 20 dilution) have not been able to kill bacteria. But after 10 minutes and 15 minutes, all extracts (except dilution of the extracts of 0.25: 112.5) and phenol have been able to kill bacteria. The time between 10-15 minutes is a sufficient exposure time so that the antibacterial material can work optimally to kill the test bacteria.

4. Conclusion

The conclusions that can be presented in this research are as follows: (1) there was a difference in inhibition zone of Escherichia coli due to the custard apple’s (Annona squamosa) seed extract with various concentrations. This was derived from hypothesis test results that the number of significance, 0.000 <0.05. (2) concentration of custard apple’s (Annona squamosa) seed extract which was most effective in inhibiting the growth of Escherichia coli was 50% concentration. Recommendations that can be given are (1) need further research on active ingredients in custard apple’s plant, so that the materials can be used well for the benefit of the society, (2) the results of this research is expected to be applied in the community to overcome various health problems, particularly problems with Escherichia coli, (3) this study may be used as additional information in microbiology, botany, and plant physiology activities.

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References

[1] Widiyanti NLPM and Ristiatni NP 2004 J. Ekol. Kesehatan 3
[2] Susanna 2010 J. Kes. Mas. Nas. 5
[3] Djaja IM 2008 J. Makara Kesehatan 12
[4] Febriani A 2011 Prosiding Seminar Nasional dan Lokakarya Forum Komunikasi Perguruan Tinggi Pertanian Indonesia 2-4 September 2013 (Bogor: Fakultas Pertanian Institut Pertanian Bogor)
[5] Mujeeb M, Alam KS, Mohd A, Abhishek M and Aftab A 2009 *The Pharma Research* 2

[6] Gazpersz 1995 *Teknik analisis dalam penelitian percobaan* (Bandung: Penerbit Tarsito)

[7] Pratiwi ST 2008 *Mikrobiologi farmasi* (Jakarta: Penerbit Erlangga)

[8] Chavez D, Mata R, Prieto RI, Hennsen BL 2001 *Physiol. Plant* 111 262

[9] Pradana PY, Suratmo, Retnowati R 2015 *Kimia Student J.* 1 798

[10] Sinha SC, Chen Z, Huang Z, Nakamaru-Ogiso E, Edelstein M 2008 *Alteration J. Med. Chem.* 51 7045

[11] Qayed WS, Aboria AS, Abdel-Rahman HM and Youssef AF 2015 *J. Der. Pharm. Chem.* 7 24

[12] Coothankandaswamy V, Liu Y, Mao S, Brian J, Fakhri M, Mika BJ, Dale GN, Yu-Dong Z 2010 *J. Nat. Prod.*

[13] Coloma AG, Guadano A, Ines C, Diaz RM And Cortes D 2002 Z. Naturforsch 57 1028

[14] Yuan SS, Chang HL, Chen HW, Yeh YT, Kao YH, Lin KW, Wu YC, Su JH 2003 *J. LifeSci* 72 2853

[15] Redha A 2010 *J. Belian* 9 196

[16] Ajizah A 2004 *Sensitivitas Bioscientiae* 1 31

[17] Maliana Y, Khotimah S and Diba FS 2013 *J. Protabiont* 2 7

[18] Sari FP and Sari SM 2011 *Ekstraksi zat aktif antimikroba dari tanaman yodium (Jatropha multifida Linn) sebagai bahan baku alternatif antibiotik alami* (Semarang: Fakultas Teknik Universitas Diponegoro)

[19] Retnowati Y, Bialangi N, Posangi NW 2011 *J. Saintek* 6

[20] Rosyidah K, Nurmuhaimina SA, Komari N, Astuti MD 2010 *J. ALCHEMY* 1 53