# Antibacterial activity of water and ethanol extract of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* combination

Bayyinatul Muchtaromah*, Mujahidin Ahmad, Romaidi, Lina Ainun Nazilah, Nayla Alin Naja

Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Indonesia

**Abstract**

Madura has been known as an Indonesian tribe that usually used many recipes of traditional medicine in their daily life. One of the medicinal herbs to increase female fertility is the herbal "Subur Kandungan", which consists of garlic (*Allium sativum*), temu mangga (*Curcuma mangga*), jeringau (*Acorus calamus*). The objective of this study was to determine the phytochemical content and antibacterial activity of *A. sativum*, *C. mangga* and *A. calamus* combination in water and ethanol solvent against *Streptococcus aureus* and *Escherichia coli*. There were three kinds of combinations with different composition ie first combination/C1 (36:36:28); second combination/C2 (40:30:30); third combination/C3 (35:40:45). Clindamycin was as a positive control. The phytochemical screening detected triterpenoids in both extracts, while alkaloids, flavonoids only in ethanol extracts. The highest inhibitory zone of both extracts to *S. aureus* and *E. coli* were discovered in C3 as strong and moderate inhibition. The most effective MIC value of water extract against *S. aureus* was found on C3 ($9.76 \times 10^{10}$) while ethanol extract was obtained by C1 ($5.9 \times 10^8$) at concentrations of 0.39% and MBC at 0.78%. The best MIC value of water extract against *E. coli* was found on C1 ($1.08 \times 10^{10}$) at a concentration of 25% and MBC at 50%, whereas ethanol extract was got on C3 ($9.7 \times 10^9$) at 0.39% & MBC at 0.78%. It could be concluded that "Subur kandungan" herb recipes could be used as antibacterial drugs, which third combination/C3 in the ethanol solvent is the best treatment compare with others.

**Keywords:** antibacterial, *A. calamus, C. mangga*, *A. sativum*, phytochemical

## Introduction

*Staphylococcus aureus* is a gram-positive, non-motile bacteria and a normal flora of the skin, nose, gastrointestinal tract and vagina. *Escherichia coli* is a gram-negative bacteria, a normal flora of the digestive tract that can be found in the vagina during infection. *S. aureus* can infect the reproductive tract with a prevalence rate of 29.8%. *E. coli* can be a pathogen when it reaches tissues outside of the gastrointestinal tract and becomes one of the causes of reproductive tract infection with a prevalence rate of 13.1% (Kamazeri et al., 2012).

*S. aureus* and *E. coli* reported as organisms that were capable to affect aerobic vaginitis (Divya, 2015). Vaginitis occurs due to an imbalance in the growth of normal bacteria characterized by a lack of hydrogen peroxide produced. The condition of vaginitis can have severe consequences of infertility by Lactobacilli. The changes in the pH of the environment of the vagina cause the inflammatory infections of the vagina and discharge of whitish fluid (Razzak et al., 2011). This occurs because vaginal infections cause advanced infection of the portio, cervix, endometrium, and oviduct. In a later manner, vaginitis condition affects the movement and blockage of oviduct as the vital reproductive organs for conception. Anas et al. (2016) reported that microorganisms found from the female reproductive tract of infertile couples in Mojokerto, East Java, Indonesia, were dominated by *S. aureus* (27%) and *E. coli* (27%), with the results of

antibiotic sensitivity tests varying greatly. Through western blotting using s-IgA cervix uteri, *S. aureus* was found in the greatest number. Regarding this condition, it is necessary to solve the problem.

Recently, the use of herbal medicine has been widely adopted in developing and developed countries. Nearly four billion people (80% of the world's population) who live in developing countries depend on herbal medicinal products as the main source of health care and traditional medical practices involving herbal use are seen as an integral part of the culture in the community (Ekor, 2013). One of the Indonesian tribes, Madura, is known with traditional medicine recipes, "Subur Kandungan" herb. This herb is frequently used to increase fertility. The main ingredients of this recipe are 15% garlic (*Allium sativum*), 15% of temu mangga (*Curcuma mangga*), 12% of jeringau (*Acorus calamus*), and other materials up to 100%. The bioactive contents of those herbal recipes are thought to be an important factor in improving female fertility.

Most of the problems associated with the use of traditional and herbal medicines appear primarily from the classification of many of these products as food or dietary supplements in several countries. Thus, evidence of the quality, efficacy, and safety of these herbal medicines is not needed before marketing. In addition, quality testing and production standards tend to be less strict or controlled and in some cases, many traditional health practitioners are not certified or licensed. Scientific information on the content of traditional medicines, appropriate dosage or composition are essential (Kasilo & Trapsida, 2011).

Based on the explanation above, there has never been any research on phytochemical content and the use of a combination of *A. sativum, C. mangga* and *A. calamus* in

∗ Corresponding Author:
Bayyinatul Muchtaromah
Department of Biology, Faculty of Science and Technology,
Universitas Islam Negeri (UIN) Maulana Malik Ibrahim
Malang, Indonesia
Phone: +6281231842316 Fax: +62341558933
e-mail: bayyinatul@bio.uin-malang.ac.id

different compositions and solvents (water and ethanol) as antibacterials against *S. aureus* and *E. coli*. Considering that *E.coli* and *S. aureus* are normal vaginal flora whose existence has a role in changing vaginal pH so that it cannot be completely switched off, it is important to conduct research on the potential combination of *A.* *sativum, C. mangga* and *A. calamus* as antibacterial followed by testing Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (KBM) to obtain the best combination in improving female fertility.

## Methods

### Design of Research

This research was an experimental study using descriptive analysis. The first stage was a qualitative phytochemical test, consisting of alkaloid, flavonoid, triterpenoid, steroid, saponin, and tannin test. The second stage was the inhibitory zone test with a concentration of 100% and 3 replications. The first combination (C1) with the following composition was garlic: temu mangga: jeringau (36:36:28); the second combination (C2) (40:30:30); the third combination (C3) (35:40:45). The third stage was the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) assay, with concentrations of 0, 0.39, 0.78, 1.56, 3.13, 6.25, 12, 25, 50, and 100%, repetition of treatment three times.

### Materials

Symplicia of the garlic bulb, the rhizome of temu mangga and jeringau were obtained from Balai Materia Medica, Malang, Indonesia. All the chemicals used in the present studies were of synthetic grade and were obtained from Merck Specialties Ltd. Bacterial strains *E. coli* and *S. aureus* culture was purchased from Microbiology Laboratory, Medical Faculty, Brawijaya University.

### Extraction by Maceration Method

The procedure for the water and ethanol extraction of garlic, temu mangga and jeringau referred to the procedure on *Centella asiatica* which was previously described by (Muchtaromah et al., 2011; Muchtaromah et al., 2016). 50 g of mix powder of garlic, temu mangga and jeringau was added to 200 mL of ethanol soaked for 24 h, homogenized for 3 h, and filtered with a whatman filter paper grade 1 (Sigma aldrich) on funnel Buchner. The obtained pulp was macerated three times with ethanol to get the clear filtrate. The maceration filtrate was concentrated at 40 ℃ using a rotary evaporator until a thick extract was obtained. The same procedure was carried out on water solvent. The extraction percentage was calculated using the equation (Ahmad, 2009):

$$\% \ Extraction = \frac{m_1 - m_2}{m_1} \times 100$$

Where:
$m_1$ = mass of the sample before extraction
$m_2$ = mass of the sample after extraction

### Phytochemical Screening

The phytochemical examination was performed using standard methods (Tiwari et al., 2011; Ibironke et al., 2010)

### Alkaloids Detection

Each extract was dissolved in a solution of hydrochlo- ric acid then filtered. Dragendroff test: Dragendroff reagent was added (Potassium Bismuth Iodide solution). If there was the formation of red precipitates, it indicated the presence of alkaloids. Mayer Test: Filtrate was added Mayer reagent (Potassium Mercuric Iodide). If there was a yellow precipitate, it indicated the presence of alkaloids.

### Flavonoid Detection

Wilstater Test: The sample was put in a test tube, then dissolved in 1-2 mL of 50% hot methanol and added Mg metal and 0.5 mL of concentrated HCl. If a red or orange solution was formed, it indicated flavonoids.

### Triterpene/steroid Detection

Salkowski test: Extract was treated with chloroform and filtered. The filtrate was treated with several drops of concentrated sulfuric acid, shaken and silenced. The appearance of the golden yellow color indicated the presence of a triterpene.

Liberman Burchard test: Extract was treated with chloroform and filtered. The filtrate was treated with a few drops of acetic anhydride, boiled and cooled. Concentrated sulfuric acid was added. The formation of a brown ring at the intersection indicated the presence of phytosterols/steroid.

### Saponins Detection

Foam Test: as much as 0.5 g of the extract was dissolved and shaken with 2 mL of water, if the resulting foam lasts for ten minutes, then it showed the presence of saponins.

### Tannins Detection

$FeCl_3$ Test: The sample was put into a test tube, then added with 2-3 drops of 1% $FeCl_3$ solution. If the solution was blackish green, it indicated the presence of tannin catechol compound and if it was blackish blue, it indicated the presence of tannin gallate compound.

### Antibacterial Activity of *S. aureus* and *E. coli*

Inhibition zone diameters were measured by diffusion technique (Kirby Bauer method) with 100% concentration. Total 0.1 g of extract using (ethanol, water) individually was diluted with a total volume of 100 μL. Sterile disc paper (6 mm) was inserted into the extract solution and saturated for 30 mins. Then the disk paper was inserted into the agar plate that had been dispersed with bacteria (*S. Aureus, E. coli*) (Vineetha, et al., 2015). Clindamycin was used as a control. The classification of the inhibited zones according to Pan, et al., (2009) was classified as strong (> 6mm), good (3-6 mm) and weak (0-3 mm).

MIC and MFC determination was performed using the microplate dilution method described by (Fatisa, 2013).

Dilution stratification was then performed to produce final concentrations (50%, 25%, 12.5%, 6.25, 3.13%, 1.56%, 0.78%, and 0.39%). Then a 100 μL extract sample was inserted into 96-well plates and added with a 100 μL bacterial suspension adjusted to the previous McFarland standard of 0.5. Steril Mueller Hinton Agar (MHA) was used as a control of sterility and microbial inoculum as a growth control. MIC was the lowest sample concentration that could inhibit the growth of microorganisms, which was seen after overnight incubation at 30 °C for 18 h to 20 h. Bacterial growth was observed visually by comparing sample and control turbidity. MFC was the smallest concentration that could kill microbes, characterized by microbes could not grow on plates, which mean they have died at this concentration. Confirmation of MIC and MFC values was performed by with a streak plate of antibacterial test results in solid dilution.

### Data Analysis

Data of the yield, phytochemical screening, inhibitory zone, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were presented descriptively in the form of figures and tables.

## Results

### Extraction Result

Extraction is the process of attracting the active compound of a material by a suitable solvent and method so that the desired result can be perfectly extracted. Each solvent will attract certain compounds based on the degree of polarity of the compound. The results of maceration of dried powder of garlic, temu mangga and jeringau were presented in table 1.

**Table 1.** The yield of a combination of garlic, temu mangga and jeringau extract

| Extract | Water extract | | Ethanol extract | |
|---------|---------------|------------|-----------------|------------|
| | Yield (%) (w/w) | Appearance | Yield (%) (w/w) | Appearance |
| C1 | 11.360 | Dark brown, concentrated liquid | 11.360 | Dark brown, concentrated liquid |
| C2 | 10.358 | Dark brown, concentrated liquid | 11.473 | Dark brown, concentrated liquid |
| C3 | 7.960 | Brown, concentrated liquid | 11.746 | Dark brown, concentrated liquid |

Table 1 showed that ethanol produced the highest yield of C3 (11.746%) followed by C2 of 11.473%, and C1 of 11.360% while water generated the highest yield of C1 (11.360%) followed by C2 of 10.358%, and C3 of 7.960%. The yield of the extract should depend on the polarity of the solvent used during preparation. Moreover, the solubility of the natural products and the choice of solvent could also determine the yield. A further test was qualitative phytochemical screening.

### Phytochemical Screening

The result of the phytochemical test of a combination of garlic, temu mangga and jeringau were presented at table 2.

The phytochemical test determined the secondary metabolite. Phytochemical test in this study resulted in triterpenoid content in the water extract whereas alkaloid, flavonoids, triterpenoids content in ethanol extract.

### Antibacterial Activity
### Inhibitory Zone Against *S. aureus*

Measurement of the diameter of the inhibition zone around the disk paper using Vernier Caliper. The formation of clear zones around disc paper indicated the inhibition of *S. aureus* growth (Figs. 1a and 1b). All composition of garlic, temu mangga and jeringau extracts had antibacterial activity against *S. aureus* which was indicated by the formation of inhibition zone. The inhibition zone in each treatment was presented in table 3.

Table 3 and Figure 1a showed that the inhibition zone of water extract from the largest in the sequence were C3 (7.81±1.26 mm/strong), C1 (4.01±1.73 mm/good) and C2 (3.26±1.36 mm/good). Clindamycin (C+) had the greatest inhibition zone (33.75±5.26 mm/strong). In the ethanol extract, the largest of inhibition zone was C3 (10.71±1.0 mm/strong), followed by C1 (9.92±0.5 mm/strong) and C2 (4.61± 2.0 mm/good) (Tab. 3 and Fig. 1b). Clindamycin as a positive control had the largest inhibitory zone (37.09± 0.6 mm/strong). According to Pan, et al., (2009), the inhibition zone of 0-3 mm includes the weak category, 3-6 mm good, and ≥6 mm strong.

**Table 2.** Phytochemical screening of combination of garlic, temu mangga and jeringau

| Group of compound | Reagent test | Water extract | | | Ethanol extract | | |
|-------------------|--------------|:---:|:---:|:---:|:---:|:---:|:---:|
| | | C1 | C2 | C3 | C1 | C2 | C3 |
| Alkaloid | Dragendorff | - | - | - | + | + | + |
| | Mayer | - | - | - | - | - | - |
| Flavonoid | Wilstater | - | - | - | + | + | + |
| Triterpenoid | Salkowski | + | + | + | + | + | + |
| Steroids | Lieberman-Burchard | - | - | - | - | - | - |
| Saponin | Foam | - | - | - | - | - | - |
| Tannin | FeCl₃ | - | - | - | - | - | - |

**Table 3.** Inhibition zone of water and ethanol extract against *S. aureus*

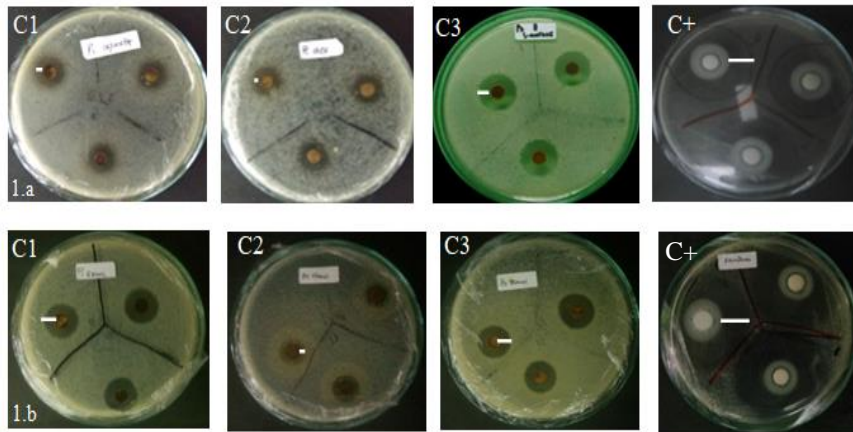| No. | Group | Inhibition Zone (mm)±SD | | | |
|---|---|---|---|---|---|
| | | Water extract | Categorized | Ethanol extract | Categorized by Pan et al (2009) |
| 1. | C1 (28 : 36 : 36) | 4.01±1.73 | Good | 9.92±0.5 | Strong |
| 2. | C2 (30 : 30 : 40) | 3.26±1.36 | Good | 4.61±2.0 | Good |
| 3. | C3 (25 : 40 : 35) | 7.81±1.26 | Strong | 10.71±1.0 | Strong |
| 4. | C+ (Clindamycin) | 33.75±5.26 | Strong | 37.09±0.6 | Strong |



**Figure 1.** Inhibition zone against *S.aureus*(a) on water extract C1 (4.01±1.73mm), C2 (3.26±1.36mm), C3 (7.81±1.26mm), C+ (33.75±5.26 mm (b) on ethanol extract C1(9.92±0.5mm), C2(4.61± 2.0 mm), C3(10.71±1.0 mm) C+(37.09± 0.6 mm).

### MIC and MBC Value Against *S. aureus*

Table 4 presented that bacterial control (C+) had the highest total of a bacterial colony. MIC value of C1 and C3 water extract on *S. aureus* was found at concentration 0.39% with total colony C1 ($6.50 \times 10^{12}$) and C3 ($9.76 \times 10^{10}$) and MBC was at concentration 0.78%. MIC value of C2 with total colony $4.93 \times 10^{10}$ was got at concentrations of 1.56% and MBC at concentration 3.13%.

The MIC value of ethanol extract was obtained consecutively by C1($5.9 \times 10^8$), C2 ($6.9 \times 10^8$) and C3 ($1.87 \times 10^9$) at concentrations of 0.39% and MBC at a concentration of 0.78%. The most effective MIC value was found in C1 with the number of colonies of $5.9 \times 10^8$

**Table 4.** MIC and MBC value of water and ethanol extract against *S. aureus*

| The concentration of test sample | Total colony account of *S.aureus* (CFU/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Water extract | | | Ethanol extract | | |
| | C1 | C2 | C3 | C1 | C2 | C3 |
| 0% (C+) | $1.29 \times 10^{14}$ | $1.42 \times 10^{14}$ | $2.05 \times 10^{14}$ | $1.29 \times 10^{14}$ | $1.42 \times 10^{14}$ | $2.05 \times 10^{18}$ |
| 0.39% | $6.5 \times 10^{12}$ | $1.36 \times 10^{14}$ | $9.76 \times 10^{10}$ | $5.9 \times 10^8$ | $6.9 \times 10^8$ | $1.87 \times 10^9$ |
| 0.78% | 0 | $1.08 \times 10^{13}$ | 0 | 0 | 0 | 0 |
| 1.56% | 0 | $4.93 \times 10^{10}$ | 0 | 0 | 0 | 0 |
| 3.13% | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.25% | 0 | 0 | 0 | 0 | 0 | 0 |
| 12.5% | 0 | 0 | 0 | 0 | 0 | 0 |
| 25% | 0 | 0 | 0 | 0 | 0 | 0 |
| 50% | 0 | 0 | 0 | 0 | 0 | 0 |
| 100% (C-) | 0 | 0 | 0 | 0 | 0 | 0 |

Note :    MIC     MBC

### Inhibitory Zone Against *E. coli*

The water and ethanol extract of *A. sativum, C. mangga,* and *A. calamus* had an antibacterial activity to *E. coli* as shown in the table 5 and figures 2a, 2b.

Table 5 and figure 2a indicated that the highest inhibition zone of water extract was got by C3 of 3.78 mm, followed by C1 of 3.58 mm, and C2 of 3.11 mm. Clindamycin as a positive control had an inhibition zone of 29.42 mm. The highest inhibition zone of ethanol extract to *E. coli* was C3 of 4.26 mm, followed by C1 of 3.65 mm, C2 of 3.26 mm. Clindamycin had an inhibition zone of 33.75 mm (Tab. 5 and Fig. 2b).

**Table 5.** Inhibition zone of water and ethanol extract against *E. coli*

| No | Group | Inhibition Zone (mm)±SD | | | |
|---|---|---|---|---|---|
| | | Water extract | Categorized | Ethanol extract | Categorized |
| 1. | C1 (28 : 36 : 36) | 3.58 ± 0.86 | Good | 3.65 ± 0.4 | Good |
| 2. | C2 (30 : 30 : 40) | 3.11 ± 0.82 | Good | 2.62 ± 0.3 | Weak |
| 3. | C3 (25 : 40 : 35) | 3.78 ± 0.43 | Good | 4.26 ± 0.5 | Good |
| 4. | C+ (Clindamycin) | 29.42 ± 1.97 | Strong | 30.29 ± 2.1 | Strong |
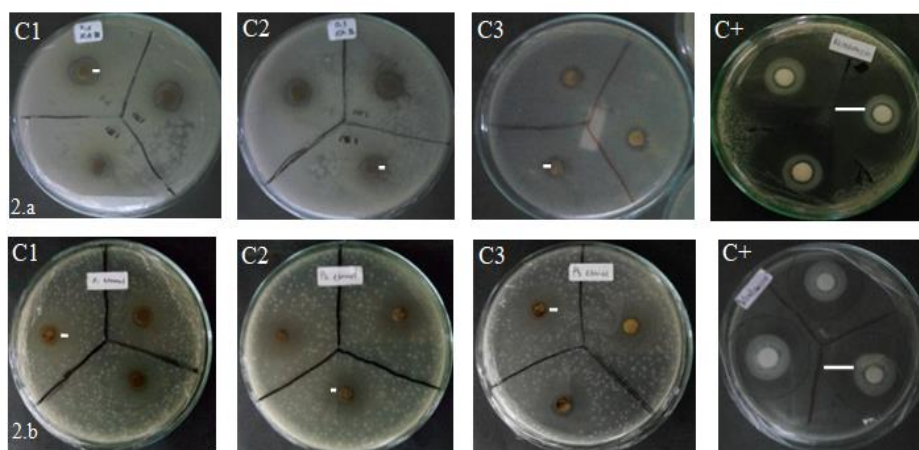


**Figure 2.** Inhibition zone against *E. coli*. (a). on water extract C1 (3.58±0.86mm), C2 (3.11±0.82mm), C3 (3.78±0.43mm), C+ (29.42±1.97mm. (b) on ethanol extract C1 (3.65±0.4mm) and C2 (2.62± 0.3 mm) C3 (4.26±0.5mm), C+ (30.29± 2.1 mm).

### MIC and MBC Value Against *E.coli*

MIC and MBC value of water extract to *E. coli* were found at 25% and 50% concentrations, while the most effective result was found on C1 with the smallest bacterial colony (1.08x10^10). The most effective MIC and MBC in ethanol extract was C3 (MIC 0.39% & MBC 0.78%) with total colony 9.7x10^9, followed by C2 (MIC 1.56% & MBC 3.13%) and C1 (MIC 6.25% & MBC 12.5%) (Tab. 5). It also could be seen that the higher the concentration of the extract the lower the number of bacteria.

**Table 5.** MIC and MBC value of water and ethanol extracts against *E. coli*

| The concentration of test sample | Total Colony Account *E. coli* (CFU/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Water extract | | | Ethanol extract | | |
| | C1 | C2 | C3 | C1 | C2 | C3 |
| 0% (C+) | $2.14 \times 10^{18}$ | $2.35 \times 10^{18}$ | $2.64 \times 10^{18}$ | $2.14 \times 10^{18}$ | $2.35 \times 10^{17}$ | $2.64 \times 10^{18}$ |
| 0.39% | $2.04 \times 10^{14}$ | $1.57 \times 10^{14}$ | $1.27 \times 10^{14}$ | $1.41 \times 10^{18}$ | $1.19 \times 10^{17}$ | $9.70 \times 10^{9}$ |
| 0.78% | $1.35 \times 10^{14}$ | $1.43 \times 10^{14}$ | $1.24 \times 10^{14}$ | $1.18 \times 10^{14}$ | $1.28 \times 10^{13}$ | 0 |
| 1.56% | $1.33 \times 10^{14}$ | $1.33 \times 10^{14}$ | $1.17 \times 10^{14}$ | $1.37 \times 10^{13}$ | $8.70 \times 10^{9}$ | 0 |
| 3.13% | $1.28 \times 10^{14}$ | $1.33 \times 10^{14}$ | $1.07 \times 10^{14}$ | $8.40 \times 10^{12}$ | 0 | 0 |
| 6.25% | $1.17 \times 10^{14}$ | $1.28 \times 10^{14}$ | $8.20 \times 10^{13}$ | $1.23 \times 10^{10}$ | 0 | 0 |
| 12.5% | $8.06 \times 10^{12}$ | $6.70 \times 10^{13}$ | $7.70 \times 10^{12}$ | 0 | 0 | 0 |
| 25% | $1.08 \times 10^{10}$ | $1.60 \times 10^{10}$ | $1.60 \times 10^{10}$ | 0 | 0 | 0 |
| 50% | 0 | 0 | 0 | 0 | 0 | 0 |
| 100% (C-) | 0 | 0 | 0 | 0 | 0 | 0 |

Note:　　　　MIC　　　　　MBC

## Discussion

Table 1 showed that ethanol extract produced higher yields than water extracts. This was probably due to the high polarity of ethanol solvents that could attract various constituents of plants rather than water (Paulsamy & Jeeshna, 2011). There are many steps to get phytochemicals from plants such as milling, homogenization, and extraction. Among these steps, extraction is the main step to restore and isolate phytochemicals from plant material. Extraction efficiency is influenced by the chemical properties of phytochemicals, the extraction method used, the particle size of the sample, the solvent used, and the presence of disturbing substances. Extraction results depend on the solvent with various polarity, pH, temperature, extraction time, and sample composition. Under the same extraction time and temperature, the solvent and sample composition are known as the most important parameters (Mostafa et al., 2018).

The difference in yield due to each combination has a different composition and solvent. In this study, ethanol

extract was able to attract alkaloids, flavonoids, and triterpenoids while water extract was the only triterpenoid. This showed that ethanol extracts were more effective in attracting active ingredients present in all combination. Most of the secondary metabolites in garlic, temu mangga and jeringau were found in higher amounts in ethanol extracts than other solvents. However, flavonoids and alkaloids were rich in ethanol extract. This explained that the level of polarity and nature of species play a major role in extracting secondary metabolites (Ghasemzadeh, 2011; Muchtaromah et al., 2017).

Das et al. (2010) reported that water was a universal solvent, used to extract plant products with antimicrobial activity. Although traditional medicine mainly uses water, plant extracts from organic solvents have been known to provide more consistent antimicrobial activity compared to water extracts. Likewise, flavonoids which dissolve in water (mostly anthocyanins) have not acted as antimicrobials and phenolics which dissolve in water are only important as antioxidant compounds. Kanduč et al. (2016) revealed that water was a solvent, that capable of dissolving many types of chemicals, especially hydrophilic and polar substances. The solubility of a substance in water was determined by the ability of the agent to match the strength of the electric attraction (intermolecular dipole) between water molecules. If a substance was unable to match the attraction between the water molecules, the molecules of the substance were insoluble and will settle in water.

Muhamad et al. (2014) notified that ethanol was a polar solvent, which means it could dissolve polar compounds and ethanol could be mixed with water that was also polar. The important properties were polarities and polar groups of a compound. In principle, a material would be easily soluble in the same solvent polarity so that it would affect the physicochemical properties of the resulting extraction, next ethanol more easily penetrates cell membranes to extract intracellular material from plants. Almost all active compounds of plants that have antimicrobial activity are aromatic or saturated organic compounds, they are most often obtained through initial extraction of ethanol or methanol (Wang, 2010)

Do et al. (2014) revealed that ethanol was a universal solvent so that both polar and nonpolar compounds could be extracted optimally, alongside ethanol being an easy-to-obtain and harmless solvent such as methanol. Ethanol had a low toxicity level and a versatile solvent. Sinambela (2003) stated that the extraction of medicinal plant material with ethanol solvent into a liquid extract or dried extract was mostly done for the purpose of standardization of herbal medicine.

Table 3 and figure 2 informed that the highest inhibition zone of both extracts against *S. aureus* was C3 (35:40:45), followed by C1 (36:36:28) and C2 (40:30:30), while the inhibitory value of ethanolic extract showed higher results than water extract. This result also was judged by better MIC value of ethanolic extract compare to water extract (Tab. 4). The most effective MIC value was found in the C1/ethanolic extract with the number of colonies of $5.9 \times 10^8$. The number of these colonies closed to the number of the existence of normal flora that needed to be maintained. Sánchez et al. (2012) mentioned the normal number of flora bacteria that needed to be maintained ie $10^8$-$10^9$ colonies per mL, Pursuant to Muhamad et al. (2014), the same plant species with different compositions in each combination might produce secondary metabolite compounds in different concentrations and activities or contain different chemical group structures.

This was similar with research of Baljeet et al. (2015), on the antimicrobial test of individual and combination of ethanolic extract from cumin, ginger, and garlic against bacterial strains of *Bacillus subtilis*, *Pseudomonas fluroscens*, *Salmonella typhi* and fungal strains of *Candida albicans* and *Rhizopus azygosporus*. Agar well diffusion assay for antimicrobial activity yielded the inhibitory zone of 12.8 to18.3 mm diameter for cumin, 11.5 to 16.3 mm diameter for ginger and 16.8 to 19.3 mm diameter for garlic extract indicating that garlic was the most effective spice in inhibiting the microbial growth. The combined extracts showed inhibition zones ranging from 12.3 to 19.6 mm in diameter against bacteria and 15.6 to 19.6 mm against fungus. The combined extract of cumin, ginger, and garlic was found to be most effective in inhibiting the microbial growth. The MIC of individual extracts was 12.5 mg/ml against all the tested microorganisms. The MIC of combined extracts fluctuated from 3.8 to 6.7 mg/ml and the most sensitive microbial species in relation to the MIC of combined extracts was *S. Typhi*.

Inhibition of bacterial growth from a combination of water and ethanol extract of garlic, temu mangga and jeringau on *S. aureus* was still far in comparison with positive control (clindamycin). Clindamycin produced a wide inhibitory zone, but the ability of clindamycin to kill *S. aureus* was low, because there was still bacterial growth around the inhibitory zone, meaning that clindamycin could inhibit the bacterial growth only (bacteriostatic). The inhibition zone resulting from the combination of water and ethanol extract was radical because there were no bacteria growing around the clear zone. This means that the combination of water and ethanol extracts could kill *S. aureus* (bactericide). In pursuance of Jennie (2017), clindamycin as an antibacterial inhibited the growth or reproduction of bacteria by inhibiting protein synthesis. *S. aureus* were normal flora bacteria whose existence could not be completely killed, it was more appropriate to use concentrations that could inhibit bacterial growth so that the presence of normal flora bacteria could be maintained (Balasubramanian et al., 2017). MIC and MBC values could provide appropriate concentration information in treating infections, adjusted for the purpose of use.

The content of some active compounds in ethanol extract allowed the inhibition zone to be greater than the water extract (Tab. 2&3). The presence of these active compounds could inhibit or damage vital cell parts such as cell walls, and organelles that were presented in the bacterial cytoplasm. In accordance with Tiwari et al. (2011) triterpenoid compounds could work as antifungal, insecticide, antibacterial, and antiviral. The mechanism of triterpenoid as an antibacterial was to react with the outer membrane of the bacterial cell wall, forming a strong

polymer bond so that it could lead to the destruction of the membrane which was the entrance of the compound entrance. Thus there was a decreasing in permeability of bacterial cell wall and cause bacterial cell deficiency, hence bacterial growth was inhibited or dead.

Several studies on the relationship between flavonoid structure and antibacterial activity had been carried out and these were in close agreement. Also, many research groups had tried to explain the antibacterial mechanism of the actions of selected flavonoids. The activity of quercetin, for example, had at least partially been linked to inhibition of DNA gyrase. Besides, it had been concluded that sophoraflavone G and (-) epigallocatechin gallate inhibited the function of the cytoplasmic membrane and that licochalcones A and C inhibited energy metabolism. Other flavonoids whose mechanism of action had been investigated include robinetin, myricetin, apigenin, routine, galangin, 2,4,2 x - trihydroxy-5 x -methyl chalcone and lonchocarpol A (Cushnie et al., 2005).

Cushnie et al. (2014) reported that the mechanism of antibacterial action (MOA) of alkaloids in the classes of indolizidine, isoquinoline, quinolone, agelasine and polyamine was investigated. In the class indolizidine, alkaloid pergularinin and tylophorinidine, it worked by inhibiting nucleic acid synthesis, because they inhibited the enzyme dihydrofolate reductase in cell-free tests. In the isoquinoline class, two MOAs had been found. Studies with benzophenanthridine and protoberberine isoquinolines showed that it worked by interfering with the Z-ring and inhibiting cell division. Evidence showed that sanguinarine and berberine (a) binded to FtsZ, (b) inhibited FtsZ GTPase activity, (c) inhibited Z ring formation and (d) induced cell extension without affecting DNA replication, nucleoid segregation or membrane structure and without inducing a SOS response. Overexpression and underexpression studies also support this mechanism. Researchers working with isoquinoline undermine phenanthridine proved that these alkaloids acted by inhibiting nucleic acid synthesis through type I topoisomerase inhibition in cell-free tests.

Naturally, quinolone alkaloids did not have a 3-carboxyl group which allowed synthetic quinolones such as fluoro-quinolones to inhibit type II topoisomerase enzymes. Research using alkyl methyl quinolones proved that this was a respiratory inhibitor because they reduced the consumption of $O_2$ in the bacteria treated but did not affect $3H$ uptake. Agelasines were a class of alkaloids from the sea sponge Agelas. Overexpression and affinity studies using alkaloid agelasine D indicated that it worked by inhibiting the enzyme BCG 3185c (dioxygenase), thus disrupting bacterial homeostasis (Tavares et al., 2014).

Based on table 3 & figure 2, *E. coli* (gram negative) resulted in a smaller inhibitory zone than *S. aureus* (gram positive). Jawetz (2005) revealed that the structure of the cell wall of gram-positive bacteria was simpler namely single-layered with a low lipid content (1-4%) making it easier for bioactive ingredients to enter the cell. Gram-negative bacteria are more complex, namely three-layered consisting of an outer layer of lipoprotein, the middle layer of lipopolysaccharide which acts as a barrier to the entry of antibacterial bioactive ingredients, and an inner layer of peptidoglycan with high lipid content (11-12%). In accordance with Muheim et al. (2017), gram-positive bacteria did not have lipopolysaccharides, so that hydrophobic antibacterial compounds could pass through gram-positive cell wall through passive diffusion. Dewi (2013) stated that the acidic acid found in the cell wall of gram-positive bacteria was a water-soluble polymer, which served as a positive ion transport to get out or enter. This water-soluble nature signified that the cell wall of gram-positive bacterial was polar. Polar properties facilitated triterpenoid compound from water extract penetrated gram-positive bacteria cell wall easily.

The greatest MIC value of water extract on *E. coli* was found at C1 ($1.08 \times 10^{10}$) with concentrations of 25%, while the most effective values of extract ethanol on *E. coli* was obtained at C3 ($9.7 \times 10^9$) with a concentration of 0.39% (Tab. 5). The MIC and MBC values were affected by the solvent used. The water was polar, so only the attracted compounds were polar, ie triterpenoid, whereas *E. coli* was a gram-negative bacteria composed of complex cell walls. Lind et al. (2015) revealed that the outer membrane of *E. coli* contained 20 % lipid which was nonpolar. Differences in the nature of polarity between bacterial cell walls and triterpenoid caused MIC and MBC ethanol extracts more effective than water extracts. The number of active compounds attracted to the ethanol solvent caused the MIC and MBC values to be more effective.

Brooks et al. (2008) reported the number of active compounds in the extract was increasing the ability to inhibit bacterial growth. Aside from, the MIC and MBC values indicated by bacterial colonies were inversely proportional to the concentration of the extract. The higher concentration of extract showed lower bacterial count. In accord with Mostafa et al. (2018) the higher the extract the higher the potential to inhibit the bacteria, thus at high concentrations, the number of bacterial colonies decreased. The concentration factor, the type of antimicrobial materials and solvents also determined the ability to inhibit bacterial growth.

## Acknowledgment

## References

Ahmad, A., Abbas, F. M., Alkarkhi, Hena, S., & Khim, L. H. (2009). Extraction, Separation, and Identification of Chemical Ingredients of *Elephantopus scaber* L. Using Factorial Design of Experiment. *International Journal of Chemistry*, *1*(1), 36-49.

Anas, M., Wiyasa, W. A., Riyanto, S., Sardjono, T. W. Aulani'am, & Prawiro, S. R. (2016). Microorganism spectrum of nonspecific vaginitis in women of infertile couples recognized by s-IgA uterine cervix secretion. *Asian Pacific Journal of Reproduction*, *5*(6), 467-47. doi:org/10.1016/j.apjr.2016.10.007

Balasubramanian, D., Harper, L., Hopsin, B. & Torres, V.J. (2017). Minireview. *Staphylococcus aureus* pathogenesis in diverse host environments. *Pathogens and Disease*, *75*(1), 1-1 3.

Baljeet, S. Y., Simmy, G., Ritika, Y., & Roshanlal, Y. (2015). Antimicrobial activity of individual and combined extracts of selected spices against some pathogenic and food spoilage microorganisms. *International Food Research Journal*, *22*(6), 2594-2600.

Brooks, G. F., Butel, J. S., & Morse, S. A. (2008). *Mikrobiologi Kedokteran*. Edited by Jawetz, Melnick, and Adelberg.23rd edition. Jakarta: EGC.

Cushnie, T. P. M., & Lamb, A. J. (2005). Review. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*, 343–356.

Cushnie, T. P. M., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int J Antimicrob Agents, 44*, 377–386. doi:org/10. 1016/j.ijantimicag.2014.06.001

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research, 4*(2), 104-111.

Dewi, A. K. (2013). Isolasi, Identifikasi dan Uji Sensitivitas *Staphylococcus aureus* terhadap Amoxicillin dari Sampel Susu Kambing Peranakan Ettawa ( PE ). *Jurnal Sain Veteriner*, 31(2), 138-150.

Divya, G. A. (2015). Study to Detect ESBL producing *Escherichia coli* Isolates From Women with Genital Tract Infection. Indian *Journal Of Applied Research*, 5 (8).

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju,Y.(2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. *Journal of Food and Drug Analysis*, *22*(3), 296-302.

Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*, *4*, 177.

Fatisa, Y. (2013). Daya Antibakteri Estrak Kulit Dan Biji Buah Pulasan(*Nephelium mutabile*) terhadap *Staphylococcus aureus* dan *Escherichia coli* secara In Vitro. *Jurnal Peternakan*, 10 (1).

Ghasemzadeh, A., Jaafar, H., & Rahmat, A. (2011). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (Zingiber officinale Roscoe.) extracts. *J Med Plant Res*, *5*(7), 1147-1154.

Ibironke, A. A., Sarah, O. N., & Adewale, A. (2010). Anti-microbial activity and phytochemical screening of five selected seeds from Nigeria. *Int J Biomed Pharm Sci*, *4*, 104-106.

Jawetz, E., Melnick, J. L., & Adelberg, E. A. (2005). *Mikrobiologi untuk Profesi Kesehatan* (Review of Medical Microbiology) Diterjemahkam oleh H. Tomang (pp. 317-318). Jakarta: EGC.

Jennie, H. K. (2017). 141 - Macrolides, Ketolides, Lincosamides, and Streptogramins. Anti-infective Therapy. In: Bryskier A. Infectious Diseases (4th Edition). *Elsevier*. *2*, 1217-1229.e1. doi:org/ 10.1016/B978-0-7020-6285-8.00141-6

Kanduč, M., Schlaich, A., Schneck E., & Netz, R. R. (2016). Interactions between Hydrophilic and Hydrophobic Surfaces. *Langmuir, 32* (35), 8767–8782. doi: 10.1021/acs. langmuir. 6b01727

Kamazeri, T. S., Samah, O. A., Taher, M., Susanti, D., & Qaralleh, H. (2012). Antimicrobial activity and essential oils of Curcuma aeruginosa, Curcuma mangga, and Zingiber cassumunar from Malaysia. *Asian Pac J Trop Biomed*, *5*, 202-209.

Kasilo O., & Trapsida, J. M. (2011). Decade of African traditional medicine, 2001–2010. *Afr. Health Mon.(Special Issue), 14*, 25–31

Lind, T. K., Wacklin, H., Schiller, J., Moulin, M., Haertlein, M., & Pomorski, T. G. (2015). Formation and Characterization of Supported Lipid Bilayers Composed of Hydrogenated and Deuterated *Escherichia coli* Lipids. *PLoS ONE*, *10* (12), 0144671. doi:10.1371/ journal.pone.014467

Mostafa, A. A., Abdulaziz, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. S*audi Journal of Biological Sciences, 25*,361–366

Muchtaromah, B., Kiptiyah, K. & Ad, T. K. (2011). Transaminase Enzyme and Liver Histological Profile of Mice Administered Extract of Pegagan (*Centella asiatica* (L.) Urban). *Media Peternakan*, 34, 88–92.

Muchtaromah, B., Ahmad, M., Suyono, S., Romaidi, R., Bahri, S. & Kumalasari, H. P. (2016). Dosage and administration length of *Centella asiatica* (L.) Urban decrease the level of SOD and MDA and improve brain histological condition of rats. *Jurnal Teknologi*, 78, 57–61.

Muchtaromah, B., Ahmad, M., Sabdoningrum, E. K., Afifah, Y. M. & Azzahra, V. L. (2017). Phytochemicals, Antioxidant and Antifungal Properties of *Acorus calamus, Curcuma mangga*, and *Allium sativum* in The Veterinary Medicine International Conference, *KnE Life Sciences*, 93–104. doi: 10.18502/kls.v3i6.1119

Muhamad, N., Muhamed, S. A., Yusoff, M. M., & Gimbun, J. (2014). Influence of Solvent Polarity and Conditions on Extraction of Antioxidant, Flavonoids and Phenolic Content from Averrhoa bilimbi. *Journal of Food Science and Engineering*, *4*,255-260. doi: 10.17265/2159-5828/2014.05.006

Muheim, C., Götzke, H., Eriksson, A. U., Lindberg, S., Lauritsen, I., Morten, H., Nørholm, H., & Daley, D. O. (2017). Increasing the permeability of Escherichia coli using MAC13243. *Scientific Report*, *7* (17629), 1-11. doi:10.1038/s41598-017-17772-6

Pan, X., Chan, F., Wu, T., Tang, H., & Zhao, Z. (2009). The acid, Bile Tolerance And Antimicrobial Property of Lactobacillus acidophilus NIT. J. *Food Control, 20*, 598-602

Paulsamy, S., & Jeeshna, M. V. 2011. Preliminary phytochemistry and antimicrobial studies of an endangered medicinal herb Exacum bicolor Roxb. *Res J Pharm Biol Chem Sci*, *2*(4), 447-457.

Razzak, M., Sabri A., Alaa, H., Al-Charrakh, & AL-Greitty, B. H. (2011). Relationship Between *Lactobacilli* and Opportunistic Bacterial Pathogens Associated with Vaginitis. *North American Journal of Medical Sciences, 3* (4).

Sinambela, J. S. (2003). *Standarisasi Sediaan Obat Herba. Seminar Nasional Tumbuhan Obat Indonesia XXIII*. Jakarta: Universitas Pancasila.

Sánchez, A., Rivera, A., Castillo, F., & Ortiz, S. (2012). Cervical erosion as result of infectious vaginitis. *European Journal of Experimental Biology*, *2* (5).

Tavares, L., Zanon, G., Weber, A. D., Neto, A. T., & Mostardeiro, C. P., (2014). Structure-activity relationship of benzophenanthridine alkaloids from Zan- thoxylum rhoifolium having antimicrobial activity. *PLoS ONE, 9*(5): e97000. doi: 10.1371/journal. pone. 0097000

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening, and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98-106.

Vineetha, N., Vignesh, R. A., & Sridhar, D. (2015). Preparation, Standardization of Antibiotic Discs and Study of Resistance Pattern for First-Line Antibiotics in Isolates from Clinical Samples. *International Journal of Applied Research*, *1*(11), 624-631

Wang, G. X. (2010). In vivo anthelmintic activity of five alkaloids from *Macleaya microcarpa* (Maxim) Fedde against *Dactylogyrus intermedius* in *Carassius auratus*. *Veterinary Parasitology*, *171*, 305–313.