Consistency of Spectra and Antibacterial Activity of The Extract Mixture of *Curcuma longa*, *Zingiber officinale*, and *Syzigium aromaticum*

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Abstract. Finding new antibiotic based on local resources is always a challenging effort, since Indonesia almost totally import the antibiotics. Then, antibiotic based jamu is an alternative. Jamu is a traditional herb prepared from local plants and some rhizomes, such as *Curcuma longa*, *Zingiber officinale*, and *Syzigium aromaticum*. These plants have been reported for their antibacterial capability. This paper is focused on the evaluation of antibacterial of these mixtures and their spectral consistency. The method was initiated by extraction of each plants/rhizome using ethanol and water. Each extract was mixed with equal ratio, and further evaluation for growth inhibiting activity in *Staphylococcus aureus* and *Escherichia coli*. Meanwhile, the spectral analysis was determined using FTIR and UV-Vis spectrophotometry. The result showed that the mixture of ethanol extract gives a slightly better activity than that using water extract. Furthermore, the UV-Vis spectra of the mixture from ethanol extracts indicates different band absorption in 439 and 417 nm, but no absorption observed in water extract in this range. In addition, the ethanol mixture extract also gives new band (FTIR spectra) in between 1010-1045 cm⁻¹, that these are not observed in water extract. In short, it can be summarized that ethanol extraction process give better extraction procedure and provide better antibacterial activity.

1. Introduction
The traditional medicine derived from local herbs, named as jamu, has paid many attentions, especially for their easily prepared and biological activity [1]. Some jamu recipe was derived from rhizome such as *Curcuma longa*, *Zingiber officinale* and other species from this genus. Their function is for healthy drink, diet [2] and antibacterial [3]. The other important herb was *Syzigium aromaticum*. It was reported having activity to kill pathogenic bacteria [4] and anticancer [5].

The bioactivity of the herbs always correlates to their chemical composition. For example, the species of *Curcuma longa* correlate to the curcuminoid molecule [6] contain in the herb. Most of these class molecules are phenolic aromatic compounds with conjugation bond. Meanwhile, the *Zingiber officinale* contains beta-sesquiphellandrene, bisabolene and farnesene [7]. These molecules are classified into terpenoid group with mostly contain aliphatic hydrocarbon and few double bonds in their structure. On the other hand, *Syzigium aromaticum* mostly contain phenolic compound such as eugenol and beta-caryophylene [8]. All the compound groups are categorized as the non-hydrophilic compound and was not easily dissolved in water solvent. However, traditionally, jamu is prepared using water as the main solvent.
This paper reports the consistency of the mixture extract derived from *Curcuma longa*, *Zingiber officinale*, and *Syzigium aromaticum* toward their bioactivity as antibacterial. The extract was prepared using two different solvents i.e. ethanol and water. Meanwhile, their consistency of chemical extracted by both solvents were compared from the FTIR and UV-Vis spectra evaluation to the reference. Recently, the FTIR and UV-VIS methods has been applied to detect the adulteration of traditional plant for herbs and also for chemometric analysis of the main secondary metabolite [9–11]. The specific region, known as the fingerprint area in FTIR spectra [9,10] can be used to detect the consistency the herb sample to the reference. For example the common herb for antibacterial contain a phenolic groups [12]. This has double bond of aromatic and hydroxyl group band absorption. Meanwhile the UV-Vis technique, characterized the common band absorption due to electronic transition from the specific functional groups such as carbon-carbon double bond, double bond in aromatic ring [13], conjugated and non-conjugated double bond, and heterocyclic functional group [11] related their bioactivity [6,7,11,13,14].

2. Experiment section

2.1. Material and chemical

The sample of herbs used for the research was bought from local market in Malang, East Java, i.e. rhizome of *Curcuma longa*, *Zingiber officinale*, and a dried flower of *Syzigium aromaticum*. Meanwhile the chemicals used for the research was ethanol (Merck) and distilled water.

2.2. Instrument for analysis

Some instruments applied for analysis include UV-Vis spectrophotometer (Shimadzu UV-1600 series). Analysis using double beam mode and water was applied as solvent or as a mentioned differently. The FTIR spectrophotometer (Shimadzu 8400S) was operated using potassium bromide pellet.

2.3. Extraction procedure

The extraction procedure using maceration techniques with water and/or ethanol as the solvent. A dried-powder mixture of herbs sample, i.e. *Curcuma longa*, *Zingiber officinale* and *Syzigium aromaticum* were mixed in 250 mL of Erlenmeyer flask. The quantity of each sample was 4.0 g (dry basis). Then, the mixture was added with a 100 mL of water and further stirred until homogeneous mixture was formed and rested at room temperature for 48 h. Then, the mixture was filtered off, and the filtrate was prepared directly as sample for further analysis (FTIR and UV-VIS spectrophotometry) and antibacterial evaluation. Similar separated procedure was also conducted but using ethanol as solvent for extraction.

2.4. Antibacterial activity evaluation

The procedure for testing antibacterial activity using disc diffusion technique. It was undertaken to measure the capability of the sample to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* [15]. The sample used was a mixture extract prepared from ethanol and water extraction process. The variation concentration used for each extract was 0.0; 3.13; 6.25; 12.5; 25.0; 50.0; and 100%, respectively. These sample were loaded into paper disc by immersion. Then, the mixture extracts were put aseptically into the media contain bacteria. It was further inoculated at 37 °C for 24 h. Then, the clean area surround the paper disc was measured as the quantity correlate to the activity of sample inhibit the bacteria growth.
3. Result and discussion
The extraction process of the herbs mixture i.e. *Curcuma longa*, *Zingiber officinale*, and *Syzigium aromaticum* using water as a solvent provide a crude extract with deep-brown in colour. Meanwhile the ethanol extraction give light brown solution (Figure 1). The deep-brown of water extract has strongly smell of eugenol or olive oil. Meanwhile, the ethanol extract has a light smell of olive oil and a spice smell of ginger.

![Figure 1. Schematic process for preparation of the mixture of herbs.](image)

The UV-Vis spectra of the both extract have a pattern band in between 200 and 600 nm (Figure 2). Both extracts also has similar band pattern in two cluster, i.e. in cluster I and cluster II. Cluster I has band absorption in 200-240 nm and this band absorption relate to the electronic transition of the double...
bond electrone in group such as olefinic and aromatic compounds. Meanwhile cluster II has band absorption in 250-310 nm, and this region relate to the electronic transition from non-bonding electrone in carbonyl (C=O) and carboxyl (O=C-OH) group. Both region is predicted correlate to the extracted compound and classified as phenolic, such as eugenol, gingerol, tannin, and tannic acid from the sample. However, in the cluster III (380-480 nm), it was found only the ethanol extract give an absorption. This region generally correspond to the electronic transition of several compound, which contain exention of conjugation from the aromatic compounds. For example in curcuminoid molecules.

**Figure 3.** The FTIR spectra of the extract of water and ethanol from mixture of *Curcuma longa*, *Zingiber officinale*, and *Syzygium aromaticum*.

Moreover, it was detected the different band absorption in FTIR spectra (Figure 3). The ethanol extract give a two absorption band in between 1000-1080 cm\(^{-1}\). These bands correlate to C-O-C bending vibration, that are commonly observed in methoxy-group (-OCH\(_3\)) attached in aromatic compound. However, it was not recorded in the water extract. The rest of the bands in FTIR are mostly similar for both extract. The hydroxyl group is detected at 3470 cm\(^{-1}\), the double bond vibration is detected in about 1650-1620 cm\(^{-1}\) [16].

The consistency of correlation of the extracted compound from the mixture of of *Curcuma longa*, *Zingiber officinale*, and *Syzygium aromaticum* using ethanol and water as solvent, are also relate to their bioactivity to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. The summary of antibacterial activity of both extract in *Escherichia coli* is tabulated in Table 1. Overall, ethanol extract of the herbs mixture has better inhibition than that showed for water extract. The highest activity for *Escherichia coli* growth inhibition was using 100% of ethanol extract, meanwhile the using the same concentration, the water extract of the herbs mixture does not affect significantly.

On the other hand, the antibacterial activity for both extract in *Staphylococcus aureus* is tabulated in Table 2. The similar activity is reported using ethanol extract of the mixture of *Curcuma longa*, *Zingiber officinale*, and *Syzygium aromaticum*. The highest activity is provided from ethanol extract at concentration 100% with inhibition value 11.27±0.01 mm. However, using similar concentration of water extract only provide activity 6.31±0.02 mm. The representative photo during antibacterial evaluation in both bacteria using both extract mixture is depicted in Figure 4.
Table 1. Antibacterial activity of the mixture of ethanol and water extract in *Escherichia coli*.

| No. | Mixture of ethanol extract (%) | Zone inhibition (mm) in *E. coli* | X±SD |
|-----|-------------------------------|-----------------------------------|------|
|     |                               | I       | II      | III     | IV       |       |
| 1   | 100                           | 7.88    | 7.91    | 7.93    | 7.89     | 7.90±0.20 |
| 2   | 50                            | 7.23    | 7.19    | 7.23    | 7.22     | 7.22±0.02 |
| 3   | 25                            | 6.74    | 6.75    | 6.74    | 6.73     | 6.74±0.01 |
| 4   | 12.5                          | 6.22    | 6.18    | 6.19    | 6.19     | 6.19±0.02 |
| 5   | 6.25                          | 6.07    | 6.06    | 6.05    | 6.05     | 6.06±0.01 |
| 6   | 3.13                          | 6.01    | 6.02    | 6.01    | 6.01     | 6.01±0.01 |
| 7   | 0                             | 6.00    | 6.00    | 6.00    | 6.00     | 6.00±0.00 |

| No. | Mixture of water extract (%) | Zone inhibition (mm) in *E. coli* | X±SD |
|-----|-------------------------------|-----------------------------------|------|
|     |                               | I       | II      | III     | IV       |       |
| 1   | 100                           | 6.54    | 6.49    | 6.53    | 6.53     | 6.52±0.02 |
| 2   | 50                            | 6.21    | 6.23    | 6.19    | 6.19     | 6.21±0.02 |
| 3   | 25                            | 6.09    | 6.08    | 6.11    | 6.08     | 6.09±0.01 |
| 4   | 12.5                          | 6.00    | 6.01    | 6.00    | 6.01     | 6.01±0.01 |
| 5   | 6.25                          | 6.00    | 6.00    | 6.00    | 6.00     | 6.00±0.00 |
| 6   | 3.13                          | 6.00    | 6.00    | 6.00    | 6.00     | 6.00±0.00 |
| 7   | 0                             | 6.00    | 6.00    | 6.00    | 6.00     | 6.00±0.00 |

Note: X is the average number, and SD is the standard deviation.

Table 2. Antibacterial activity of mixture of ethanol and water extract in *Staphylococcus aureus*.

| No. | Mixture of ethanol extract (%) | Zone inhibition (mm) in *S. aureus* | X±SD |
|-----|-------------------------------|-------------------------------------|------|
|     |                               | I        | II       | III      | IV       |       |
| 1   | 100                           | 11.29    | 11.26    | 11.27    | 11.27    | 11.27±0.01 |
| 2   | 50                            | 7.97     | 7.97     | 7.95     | 7.97     | 7.97±0.01 |
| 3   | 25                            | 6.34     | 6.35     | 6.34     | 6.34     | 6.34±0.01 |
| 4   | 12.50                         | 6.12     | 6.15     | 6.14     | 6.13     | 6.14±0.01 |
| 5   | 6.25                          | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.00 |
| 6   | 3.13                          | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.00 |
| 7   | 0                             | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.00 |

| No. | Mixture of water extract (%) | Zone inhibition (mm) in *S. aureus* | X±SD |
|-----|-------------------------------|-------------------------------------|------|
|     |                               | I        | II       | III      | IV       |       |
| 1   | 100                           | 6.29     | 6.33     | 6.29     | 6.32     | 6.31±0.02 |
| 2   | 50                            | 6.15     | 6.16     | 6.16     | 6.16     | 6.16±0.01 |
| 3   | 25                            | 6.03     | 6.05     | 6.05     | 6.03     | 6.04±0.01 |
| 4   | 12.5                          | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.01 |
| 5   | 6.25                          | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.00 |
| 6   | 3.13                          | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.00 |
| 7   | 0                             | 6.00     | 6.00     | 6.00     | 6.00     | 6.00±0.00 |

Note: X is the average number, and SD is the standard deviation.
Figure 4. Antibacterial evaluation of extract to *Escherichia coli* and *Staphylococcus aureus*. (A-D) is mixture of water extract and (B-C) is mixture of ethanol extract.

4. Conclusion
In short, the extraction process of the mixture *Curcuma longa*, *Zingiber officinale*, and *Syzygium aromaticum* using water and ethanol solvent provide a different mixture of chemical composition extracted. The water extract mostly provides a deep brown of phenolic-eugenolic smell, meanwhile ethanol extract provides a light brown and spice ginger or curcuminoid smell. The antibacterial activity also in consistence to the chemical extracted from both solvents. This finding suggests to further explored for possible different bioactivity evaluation.

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