Preliminary Phytochemical Screening and Fluorescence Characterization of Several Medicinal Plants Extract from East Java Indonesia

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Abstract. The preliminary phytochemical screening and fluorescence characterization of several medicinal plant extracts from East Java Indonesia have been carried out. Some medicinal plants used in this study are Annona muricata, Strobilanthes crispus, Piper ornatum and Andrographis paniculata. The objective of this research is to find out the result of phytochemical screening and characterization of several medicine plants by fluorescence spectroscopy. The plant part in the form of leaves is extracted using maceration method. The extraction process was carried out for 3 days with ethanol solvent in room temperature. The result of preliminary phytochemical screening showed the existence of secondary metabolite compounds in several medicinal plant extracts, among others, alkaloid, saponin, terpenoid, steroid, tannin and flavonoid. The fluorescence characterization spectra obtained shows that several medicinal plant extracts have optimum absorption at wavelength of excitation of 323 – 342 nm and wavelength of emission of 662 – 685 nm.

1. Introduction
Indonesia is famous for its biodiversity, and one of which is medicinal plants. Since long time ago, Indonesian people have known and used it as another treatment to overcome health problems. The use and utilization of medicinal plants has increased significantly in recent years. The public is increasingly aware of the importance of medicinal plants for health. Some of the advantages of using medicinal plants include abundant availability, easily obtained, inexpensive, harmless, effective and does not cause many side effects for users. The use of medicinal plants is broad enough to treat several types of diseases therapeutically[1]. Types of diseases that can be treated using medicinal plants include diabetes mellitus, liver, heart, tumors, cancer and others. The choice of medicinal plants in treating several dangerous
diseases has been proven over the past few years so that it is used as a basis for finding new therapeutically effective drugs such as anticancer, antitumor, antimicrobial, antioxidant and antihepatotoxic compounds[2–5].

According to data from the World Health Organization (WHO), most people from developing countries choose traditional methods of treatment that come from medicinal plants. The bioactive compounds contained will be the best source for getting various types of drugs. However, this must be further investigated to discuss further, protection, and effectiveness[6]. Medicinal plants have some bioactive components that deliver physical activity in our body. Some organic compounds contained in medicinal plants include, alkaloids, saponins, terpenoids, steroids, tannins and flavonoids[7]. These compounds have many benefits for human body. Primary and secondary living metabolism have a role to produce that bioactive component. Secondary metabolites compounds are very diverse chemical for therapeutic treatment in humans, medicine, farming and other utilization[8].

In this study four different types of medicinal plants were used from Indonesian East Java. The four plants are *Annona muricata*, *Strobilanthes crispus*, *Piper ornatum* and *Andrographis paniculata*. The choice of medicinal plants is based on several properties and benefits of these medicinal plants. In addition, the existence of these four types of plants is often found around and its availability is quite abundant.

Phytochemical screening is a method of bioactive compounds identification that is unknown in plant extracts through qualitative analysis[3]. Phytochemical screening is a preliminary stage in a phytochemical study that aims to provide an overview of the class of compounds contained in plants that are being studied. Phytochemical screening method is done by looking at the color testing reaction using a color reagent[9]. Knowledge of the chemical components contained in medicinal plants needs to be studied. This information will be significant for the synthesis of complex active components of chemical compounds contained in medicinal plants. Phytochemical screening in medicinal plants, in addition to being used to identify active compounds that are beneficial to the body’s health (positive effects of herbal medicines) can also be used to identify active compounds that cause toxins (negative effects of herbal medicines). This causes the phytochemical screening process of medicinal plants to be important before conducting further analysis[10–12].

To determine the character of the bioactive compounds contained in plant extracts, one of the methods that can be used is the fluorescence spectrophotometry method[13]. Characterization using the fluorescence spectrophotometric method can be used to determine the ability of some extracts of medicinal plants in absorbing and releasing ultraviolet radiation. The principle of the analysis of this method is by measuring the absorption of the sample of light energy entering the wavelength of its excitation and releasing the energy of the light at its emission wavelength[14].

In this research, preliminary phytochemical screening and fluorescence characterization have been carried out on several medicinal plants such as *Annona muricata*, *Strobilanthes crispus*, *Piper ornatum* and *Andrographis paniculata* from the East Java Indonesia.

### 2. Material and methods

#### 2.1. Material

The materials used in this study include the leaves of several types of medicinal plants which were macerated using ethanol pa (Merck) solvent. Preliminary phytochemical screening is done using the following ingredients: aquaest, chloroform (Merck), anhydrous acetic acid (Merck), H₂SO₄, HCl (Merck), FeCl₃ 1%, Mg powder, (Merck), Dragendorf, Mayer, and Liebermann-Burchard reagents and others.

Plants materials consist of fresh leaves, *Annona muricata*, *Strobilanthes crispus*, *Piper ornatum* and *Andrographis paniculata* were obtained from Batu, East Java, Indonesia.
Some types of plant material collected by the leaves. Leaf samples were air dried and protected from direct sunlight for less than 5 days. The dried leaf samples are mashed using a blender. Furthermore, dry powder or simplicia is stored in a closed container at room temperature.

Medicinal plant extracts were extracted using maceration methods. A total of 50 gram of simplicia was macerated with 500 ml of ethanol solvent in the maceration vessel. Maceration process is carried out for 3 days and stirred for a certain time interval. The maceration results were filtered and then evaporated at 40 °C. Extracts of medicinal plants obtained were ready for phytochemical screening and characterized by fluorescence spectrophotometry.

2.2. Phytochemical testing of several medicinal plants extracts

Ethanol extracts from several medicinal plants were screened for the following phytochemical constituents: alkaloids, saponins, terpenoids, steroids, tannins and flavonoids. Standard methods were used in testing the presence of bioactive compounds in medicinal plant extracts[15,16].

2.2.1 Alkaloids test. 2 mL of plant extract was dissolved in 8 mL of distilled water. Solution is then filtered and added with 1% HCl then put into two different test tubes. In the first test tube, 2-3 drops of Dragendorff reagent are added. To the second test tube 2-3 drops of Mayer’s reagent are added. Plant extracts contain alkaloid compounds if the test result produces a brown, red or orange red precipitate (Dragendorff reagent) and white or yellowish precipitate (Mayer reagent).

2.2.2 Saponins test. 2 mL of plant extract is dissolved in 8 mL of distilled water and shaken until homogeneous. Solution is then filtered and placed into a test tube. After the solution is cold, it is shaken vigorously. Plant extracts contain saponin compounds if the test result produces a stable foam.

2.2.3 Terpenoids test. 2 mL of plant extract was dissolved in 8 mL of distilled water. Solution is then filtered and added with 2 mL chloroform then placed into a test tube. 2 mL H₂SO₄ is added through the test tube wall. Plant extracts contain terpenoid compounds if the test result produces brown or orange rings in a layer of two solvents.

2.2.4 Steroid test. 2 mL of plant extract was dissolved in 8 mL of distilled water. Solution is then filtered and added with 2mL chloroform and anhydrous acetic acid then placed in a test tube. 2 mL H₂SO₄ was added through the test tube wall. Plant extracts contain steroid compounds if the test result produces a blue or green color.

2.2.5 Tannins test. 2 mL of plant extract was dissolved in 8 mL of distilled water. Solution is then filtered and added with 2-3 drops of 1% FeCl₃ then placed into a test tube. Plant extract contain tannin compounds if the test result produces blue or blackish green color.

2.2.6 Flavonoids test. 2 ml of plant extract was dissolved in 8 mL of distilled water. Solution is then filtered and added with Mg powder and placed into a test tube. 4-5 drops of concentrated HCl was added through the test tube. Plant extracts contain flavonoid compounds if the test result produces a pink scarlet color.
2.3. **Fluorescence Characterization of Several Medicinal Plant Extracts**

20 µL plant extracts were dissolved in 100 mL of demineralized water in a measuring flask. 10 µL of the solution is taken and dissolved in 50 mL of demineralized water. The sample solution is taken sufficiently then placed into a cuvette and ready to be measured. Sample measurements were carried out using the Perkin Elmer LS 55 fluorescence spectrophotometer. This instrumentation was linked with Winlab FL software. The sample measurements were carried out at the maximum wavelength obtained from the results of the previous measurements[14].

3. **Result**

Phytochemical test resulted from ethanol extracts from several medicinal plant extracts is presented in Table 1. This table shows the presence of active phytochemical constituent compounds which are organic compounds contained in several extracts of medicinal plants that have been studied.

| No | Type of plant | Alkaloid | Saponin | Terpenoid | Steroid | Tannins | Flavonoid |
|----|---------------|----------|---------|-----------|---------|---------|-----------|
| 1  | *A. muricata* | +        | +       | +         | -       | +       | +         |
| 2  | *S. Crispus*  | +        | +       | +         | -       | +       | +         |
| 3  | *P. ornatum*  | -        | -       | +         | +       | +       | +         |
| 4  | *A. paniculata* | +   | -       | +         | -       | +       | +         |

Phytochemical test results showed that all types of plants studied contained terpenoids, tannins, and flavonoids. Saponin compounds were not found in *Annona muricata* and *Piper ornatum* plants. Only *Piper ornatum* plants contain steroid compounds and do not contain alkaloid compounds.

**Table 2. Fluorescence Characterization of several medicinal plants extract.**

| No. | Plants   | Excitation | Emission |
|-----|----------|------------|----------|
|     |          | Wavelength (nm) | Fluorescence Intensity (%) | Wavelength (nm) | Fluorescence Intensity (%) |
| 1   | *A. muricata* | 342        | 573.65   | 682      | 567.71   |
| 2   | *S. Crispus*  | 328        | 638.74   | 662      | 618.02   |
| 3   | *P. ornatum*  | 330        | 648.12   | 683      | 473.03   |
| 4   | *A. paniculata* | 323       | 654.67   | 685      | 511.96   |

Fluorescence characterization of several medicinal plant extracts is presented in Table 2. In this table, fluorescence characterization is based on two processes namely the excitation process and the emission process. Each process has its maximum wavelength determined based on its fluorescent intensity value.
Fluorescence spectrum resulted from several extracts of medicinal plants are presented in Figure 4 below:

Figure 4. Fluorescence spectrum of several medicinal plants extracts.

In Figure 4, there is an overall fluorescence spectrum of two processes, namely the energy absorption process (the excitation process) and the release of energy (the emission process) of ultraviolet radiation. In this spectrum, there are differences in the characteristics of each medicinal plants extracts. The difference can be seen from the maximum wavelength and fluorescence intensity. In this study, four types of medicinal plants were used with different phytochemical components.

4. Discussion
Preliminary phytochemical screening of ethanol extracts from several medicinal plants has been carried out. The results of phytochemical analysis indicated the presence of phytochemical constituents...
contained in extracts of medicinal plants. The secondary metabolites component contained in the extracts of medicinal plants included the following compounds: alkaloids, saponins, terpenoids, steroids, tannins and flavonoids.

Almost all extracts of medicinal plants studied contain alkaloids, except for extracts of medicinal plants of *Piper ornatum*. In contrast, steroid compounds were only found in *Piper ornatum* medicinal plant extracts but were not found in other medicinal plant extracts. Saponin compounds were found in the extracts of medicinal plants *Strobilanthes crispus* and *Piper ornatum* and were not found in the extracts of medicinal plants *Andrographis paniculata* and *Annona muricata*.

Results of fluorescence characterization studies of several medicinal plant extracts showed the absorption of ultraviolet radiation at the excitation wavelength. In the excitation process, the maximum wavelength is generated in the ultraviolet area (200-400 nm). The four types of medicinal plants studied gave the results of excitation spectra that had different maximum wavelengths. This difference is caused by the content of different compounds in each of these medicinal plant extracts. In the excitation spectrum the shortest wavelength (323nm) is produced by the extract of the medicinal plant *Andrographis paniculata* and the farthest wavelength (342nm) is produced by the extract of the medicinal plant *Annona muricata*. This is influenced by the different compound components of the two medicinal plant extracts. From the results of phytochemical screening tests, it is known that the two extracts of these medicinal plants have the same compound content in them, namely the presence of alkaloid compounds, terpenoids, tannins and flavonoids as well as not containing steroid compounds. The difference is that there is no saponin content in *Andrographis paniculata* extracts. The presence of saponin compounds in the *Annona muricata* extract is possible to cause a shift in wavelength. Saponin compounds have two basic chemical structures, glycone and aglycone. The combination of these two structures allows the saponin compound to absorb ultraviolet radiation at longer wavelengths so that this plant extract produces the farthest excitation wavelength compared to the three other medicinal plant extracts. This process continues with the process of re-emitting ultraviolet radiation by releasing a certain amount of energy. This process was carried out to return to a stable state that was ground state. This process was known as the emission process. In the emission process, the maximum wavelength is generated in the visible light area (400-800 nm). The four types of medicinal plants studied gave different maximum wavelength emission spectra results. This difference is caused by the content of different compounds in each of these medicinal plant extracts. In the shortest wavelength emission spectra (662nm) produced by the extract of the medicinal plant *Strobilanthes crispus* and the farthest wavelength (685nm) produced by the extract of the medicinal plant *Andrographis paniculata*. This is influenced by the different compound components of the two medicinal plant extracts.

Another parameter that needed to be considered was the value of the intensity of the fluorescence produced in each fluorescence process. The intensity of fluorescence indicates the number of compound components contained in extracts of medicinal plants that can absorb or emit ultraviolet radiation. The difference in the value of fluorescence intensity during the excitation and emission processes shows the ability of each medicinal plant extract to absorb ultraviolet radiation. Of the four types of medicinal plants studied, *Piper ornatum* extracted medicinal plants had the highest absorption ability of ultraviolet radiation indicated by a difference in the value of fluorescence intensity of 175.09%. The lowest absorption ability of ultraviolet radiation produced by the extract of the medicinal plant *Annona muricata* with a difference in the value of fluorescence intensity of 5.94%. The phytochemical component of the flavonoid and tannin compounds contained in the extracts of medicinal plants made the extract to absorb ultraviolet radiation. The area of absorption of ultraviolet radiation was broad enough to cover the regions of UV A and UV B. This can be seen in the fluorescence spectrum produced by some extracts of medicinal plants. At the same time, the maximum intensity of extract absorption at its wavelength can be known.

The results of fluorescence characterization indicated that some medicinal plants have a specific fluorescence spectrum both when absorbing (excitation) and releasing (emitting) radiation of ultraviolet. In the excitation process, the highest fluorescence intensity was produced by the extract of the medicinal plant *Andrographis paniculata* with a wavelength of 323 nm. While in the emission process, the highest
fluorescence intensity was produced by the extract of the medicinal plant *Strobilanthes crispus* with a wavelength of 662 nm. In this study, fluorescence characterization showed that several extracts of medicinal plants can absorb ultraviolet radiation specifically at wavelength 323 - 342 nm and release ultraviolet radiation at wavelength 662-685 nm.

5. Conclusion
The results concluded that the preliminary screening of phytochemicals from some medicinal plants in general contained terpenoid compounds, tannins and flavonoids. Almost all extracts of medicinal plants studied contained alkaloids, except for extracts of medicinal plants *Piper ornatum*. In contrast, steroid compounds were only found in extracts of medicinal plants *Piper ornatum* but not found in extracts of other medicinal plants studied in this study. In the extracts of medicinal plants *Strobilanthes crispus* and *Piper ornatum*, phytochemical components of the compound saponins were found, but not in the extracts of medicinal plants *Andrographis paniculata* and *Annona muricata*. Fluorescence characterization showed that several extracts of medicinal plants can absorb ultraviolet radiation specifically at wavelength 323 - 342 nm and release ultraviolet radiation at wavelength 662-685 nm.

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