Evaluation and Determination of Total Antioxidant in Anting-Anting (Acalypha indica L.) Leaf Extract

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Abstract. Acalypha indica L. is one of herbal plants found in wet, moderate, tropical areas which grows as a weed. This herb has been used traditionally to treat dysentery, diarrhea, malnutrition, and malaria. But chemical compounds in Acalypha indica L. hasn’t been completely reported yet. This study aims to evaluate chemical compounds and total antioxidant of Acalypha indica L. extract. The extraction of Acalypha indica L. was carried out by maceration method using methanol and ethanol as solvent. The rendemen, water and ash content of Acalypha indica L. extract determined by gravimetric methods. The DPPH method was used to determine total antioxidant and the X-Ray Fluorescence method was used to analyze of the elements contained in the extract. The yield of methanol extract was 14.83%, this was greater than the ethanol extract of Acalypha indica L. which was 5.94%. The water and ash content of the methanol extract were obtained 10.57% and 17.44% respectively, while the ethanol extract was 35.66% and 17.93%. Phytochemical screening of Acalypha indica L. in methanol and ethanol extracts showed that the extracts contained phenolic, flavonoids, steroids, terpenoids and alkaloids compounds. The total antioxidant was obtained 1.59 mg/gDW and 3.11 mg/gDW in methanol and ethanol extract. XRF analysis results showed that methanol and ethanol extract contained elements Mg, Si, Cl, K, Mn and Fe. Based on the result, it can be concluded that Acalypha indica L. extract contained some compounds and elements which is beneficial for health.

1. Introduction

Acalypha indica L. is a medicinal plant belonging to the Euphorbiaceae family. This plant has been widely consumed both as a vegetable and as a traditional medicine to treat asthma, rheumatism and skin diseases. The activity of Acalypha indica L. has been widely reported, such as antibacterial, antifungal, antioxidant, and antidiabetic. The chemical content found in the leaves and roots of Acalypha indica L. include alkaloids, flavonoids, steroids, saponins, tannins, and essential oils [1].

Medicinal plants are widely used in the treatment of metabolic and cellular diseases such as diabetes, cancer, obesity and others. The number of free radicals in the body can produce changes in cells and develop into various diseases. The antioxidant content found in various medicinal plants can neutralize free radicals and modulate the degenerative effects of oxidative stress [2]. The antioxidant content in plant extracts can be determined in vitro using the DPPH method. In this method 2,2-
diphenyl-1-picrylhydroiazyl or DPPH as free radicals is used for the determination of antioxidant activity in plant extracts [3].

Elemental analysis in traditional medicinal plants aims to determine the safety and quality of the medicinal material. The essential elements in medicinal ingredients consist of major-trace (Ca, Cl, H, Mg, N, O, P, K and S), minor-trace (F, I, Fe, Si, Zn) and ultra-trace (Cr, Co, Cu, Mn, Mo, Ni, Se, V). Meanwhile, non-essential elements and/or elements that can be toxic in the body, such as Al, As, Cd, Hg, Pb, Sb, and U, can also contaminate food or medicinal substances sourced from the environment, processing and storage. The determination of the elements in the sample (food, medicinal substances, minerals, other materials) usually uses the ICP AES method or atomic absorption spectrophotometric methods. These methods require the first sample digestion which is time consuming and involves hazardous reagents. Therefore, the XRF method is widely used because it can analyze samples directly in solid form. This method also has good sensitivity and detection limits [4].

The objective of the present studies to determine phytochemical composition, element analysis, and antioxidant activity of medicinal plant *Acalypha indica* L. extract.

## 2. Materials and Method

### 2.1. Materials

The materials used in this study were mercury (II) chloride (HgCl$_2$), potassium iodide, acetic anhydrous, sulfuric acid p.a (H$_2$SO$_4$), methanol p.a, ascorbic acid, 70% ethanol, crystals of iron (III) chloride, distilled water (H$_2$O), sodium hydroxide, chloroform, hydrochloric acid p.a, magnesium powder, iron (III) chloride, acetic anhydride, ethanol 50%, deionized water, DPPH, gallic acid and (*Acalypha indica* L.) leaves.

### 2.2. Equipment

The equipment used in this study were analytical balance, oven FED 720, furnace (Carbolite Gero, UK), desiccator with 24/29 Standard Stopcock, rotary evaporator YAMATO RE301, freeze dryer (Christ, Jerman), XRF and UV-Vis spectrophotometer.

### 2.3. Sample Collection

Samples of *Acalypha indica* L. plants were obtained in the yard and roadside of Siteba, Aia Pacah Padang, West Sumatra, Indonesia. Plant samples were identified by ANDA herbarium, Department of Biology, Andalas University.

### 2.4. Sample preparation

The samples were separated from other plants, insects, and other contaminants. The samples then washed with water and then dried for several days. Samples that have been dry were ground to form a powder.

### 2.5. *Acalypha indica* L. leaf extraction

Extraction of the samples was carried out using a maceration method of 1:20 ratio each using methanol and ethanol 50% for ± 5 days, then filtered using filter paper [5]. The solvents were evaporated using a rotary evaporator for ± 6 hours, then freeze dried for ± 23 hours.

### 2.6. Determination of water content

Each sample was weighed with a porcelain dish. Then the porcelain dish and samples were heated for ± 3 hours at 105°C, then put into a desiccator for 15 minutes and then weight. The samples were heated again until a constant weight was obtained. Water content was calculated based on the difference of the sample weight before and after heating.

### 2.7. Determination of ash content

Each of the following dry samples were weighed with a porcelain crucible. The porcelain crucibles were put into the furnace at 600°C for 3 hours, then weighed. Ash content was determined based on the difference of the sample weight before and after furnaced.
2.8. Phytochemical Screening
Phytochemical screening was carried out on methanol and 50% ethanol extracts of *Acalypha indica* L. leaves. The samples were tested for flavonoids, phenolics, saponins, triterpenoids, steroids, and alkaloids content. Each extract was put into a test tube and then 5 mL chloroform and 5 mL distilled water were added. Then it was shaken and left until it formed two layers, the chloroform and aqueous layer. The chloroform layer was used for triterpenoid and steroid tests, while the aqueous layer was used for the flavonoid, saponin and phenolic tests [6].

2.8.1. Flavonoid test. 1 mL of the aqueous layer was taken into a test tube then added with concentrated hydrochloric acid and a few grains of magnesium powder. The sample was positive for flavonoids when an orange to red solution is formed.

2.8.2. Phenolic test. 1 mL of the aqueous layer was taken and put into the test tube. Then the iron (III) chloride was put into the test tube. The sample was positive for phenolic when a complex was formed with iron (III) chloride and a blue or dark purple color appears.

2.8.3. Saponin test. 1 mL of the aqueous layer was taken and put in a test tube, and shaken vigorously. If foam was formed and did not disappear for 5 minutes after the addition of concentrated hydrochloric acid, the sample tested contains saponins.

2.8.4. Triterpenoids and steroids. The chloroform layer was dropped on three holes of the drop plate. On the first plate hole was added with concentrated sulfuric acid, the second plate hole was added with concentrated sulfuric acid and acetic anhydride and the third plate hole was only a chloroform layer as a comparison. If the first plate hole formed a red or red-purple color then the sample was positive for triterpenoids. In the second plate hole, if a green or green-blue color was formed then the sample was positive for steroids.

2.8.5. Alkaloid test. The sample was crushed using a mortar and a little sand and then added chloroform. 10 mL of the chloroform-ammonia mixture were added and filtered. The filtrate obtained was then added with sulfuric acid. The acid layer was separated, and then Meyer reagent was added. The sample contains alkaloids when a white precipitate was formed.

2.9. Elemental analysis of *Acalypha indica* L. leaf powder and extract.
The elements contained in the powder and extract of the leaves of *Acalypha indica* L. were determined using XRF (X-Ray Fluorescence). The powder and extract of *Acalypha indica* L. leaves were each placed into the sample holder to determine the metal content.

2.10. Determination of total antioxidants in *Acalypha indica* L. extract.

2.10.1. Standard solution. 2 mL of each standard solution of gallic acid 0.001, 0.005, 0.01, 0.02, 0.03, 0.04 mM was pipetted, then put into a dark vial bottle. To the standard solution was added 0.5 mL of 0.3 mM DPPH solution, shaken and left for 15 minutes. The absorbance was measured using a UV/VIS spectrophotometer at the maximum wavelength. The data was used to construct calibration curve.

2.10.2. Sample solution. 2 mL of sample solution each was pipetted, then put into a dark vial bottle. To the sample was added 0.5 ml of 0.3 mM DPPH solution, shaken and left for 15 minutes. The absorbance was measured using a UV/VIS spectrophotometer at the maximum wavelength [3].

3. Results and Discussion

3.1. Extract yield
The methanol and ethanol extracts of *Acalypha indica* L. leaves showed different colors. The color of 50% ethanol extract was reddish brown, while the methanol extract was dark green. The yield of methanol extract was 14.83%, higher than the yield of 50% ethanol extract which was 5.94% (Table
1). The difference in color and extract yield were affected by the polarity of the solvent used for extraction.

3.2. Water content.
Determination of water content was carried out using the gravimetric method. The water content of the 50% ethanol extract was 35%, higher than methanol extract which was 10% (Table 1). The amount of water content in the extract was affected by the solvent used in extraction.

3.3. Ash content.
The determination of the ash content was carried out to determine the quantity of inorganic or mineral compounds in the extract of *Acalypha indica* L. The results showed that the ash content of the extract was ± 17% (Table 1).

| Table 1. Evaluation of *Acalypha indica* L. leaf extract |
|-------------------|-------------------|-------------------|
| Color             | Methanol extract  | Ethanol 50% extract |
| Yield extract (%) | 14.83             | 5.94              |
| Water content (%) | 10.574            | 35.659            |
| Ash content (%)   | 17.345            | 17.934            |

3.4. Phytochemical Screening
Phytochemical screening was carried out to determine secondary metabolite compounds contained in the leaf extract of *Acalypha indica* L. Based on the experimental results, it was found that the leaf extract of *Acalypha indica* L. contained phenolic, flavonoids, steroids, triterpenoids, and alkaloids. The results showed that the same secondary metabolite compounds were present in methanol and 50% ethanol extract (Table 2).

| Table 2. Phytochemical screening of *Acalypha indica* L. leaf extract |
|-------------------|-------------------|-------------------|
| Secondary metabolite | Methanol extract | Ethanol 50% extract |
| Phenolic          | +                 | +                 |
| Flavonoid         | +                 | +                 |
| Alkaloid          | +                 | +                 |
| Triterpenoid      | +                 | +                 |
| Steroid           | +                 | +                 |
| Saponin           | -                 | -                 |

This was also obtained from phytochemical tests using *Acalypha indica* L. without being extracted [6]. Phytochemical test of *Acalypha indica* L. extract from *n*-hexane contained alkaloid; chloroform extract contained flavonoid and alkaloid; and methanol extract contained tannin, flavonoid and alkaloid [7]. The root and leaves of *Acalypha indica* L. was also reported. It contained secondary metabolite alkaloids, catechols, flavonoids, phenolic, saponins, steroids, tannins, and terpenoids compounds [8]. Phytochemical screening of the aqueous and ethanolic extracts of *Acalypha indica* L. leaves showed the presence of various medically active constituents. The phytochemical constituents commonly present in both the leaf extracts include saponins, flavonoids, terpenoids and cardiac glycosides. The ethanolic extract of *Acalypha indica* L. in addition, showed the presence of the phytochemicals such as tannins and steroids [9].

3.5. Elemental analysis with XRF
XRF was used to determine the elements of *Acalypha indica* L. leaf powder and extract. XRF analysis was performed on the simplicia and leaf extract of *Acalypha indica* L. (Table 3)
Figure 1. XRF analysis of *Acalypha indica* L. leaf.

| Element | *Acalypha indica* L. leaf powder (%) | Methanol extract of *Acalypha indica* L. leaf (%) | Ethanol extract of *Acalypha indica* L. leaf (%) |
|---------|-------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Mg      | 5.398                               | 1.458                                         | 0.301                                         |
| Si      | 3.156                               | 1.413                                         | 1.238                                         |
| P       | 4.34                                | 0.46                                          | 0.024                                         |
| S       | 3.284                               | 0.2                                           | 0                                             |
| Cl      | 2.494                               | 2.031                                         | 1.599                                         |
| K       | 24.57                               | 0.603                                         | 10.915                                        |
| Ca      | 54.6                                | 0                                             | 0                                             |
| Ti      | 0.079                               | 0                                             | 0.011                                         |
| Cr      | 0.069                               | 0                                             | 0                                             |
| Mn      | 0.149                               | 0.043                                         | 0.149                                         |
| Fe      | 0.922                               | 0.163                                         | 0.781                                         |
| Ni      | 0.02                                | 0.02                                          | 0.02                                          |
| Cu      | 0.018                               | 0                                             | 0                                             |
| Zn      | 0.059                               | 0                                             | 0                                             |
| Br      | 0.006                               | 0.006                                         | 0.006                                         |
| Rb      | 0.036                               | 0.005                                         | 0.005                                         |
| Sr      | 0.048                               | 0                                             | 0                                             |
| Ag      | 0.698                               | 0                                             | 0                                             |
| Ba      | 0.044                               | 0                                             | 0.004                                         |
| Ce      | 0                                   | 0                                             | 0                                             |
| Eu      | 0                                   | 0                                             | 0                                             |

*Acalypha indica* L. powder consists of major-trace (Ca, Cl, Mg, P, K and S), minor-trace (Fe, Si, Zn) and ultra-trace (Cr, Cu, Mn, Ni) (Figure 1). It contained more essential element than its extract. Ca
was the highest element found in powder of *Acalypha indica* L., followed by K, Mg, P, Si, Cl, and Fe. *Acalypha indica* L. extract contained some elements such as Mg, Si, Cl, K, Mn and Fe. The highest elemental content in methanol extract was Cl, while in the 50% ethanol extract was K. Both extracts of *Acalypha indica* L. gave the same signal (Table 3).

In elemental analysis using the ICP-AES technique, the essential elements such as Ca, K, Na, Mg, Zn, Cu, and Fe were recorded and found in *Acalypha indica* L. The order of concentration was Ca>K>Mg>Na>Fe>Al>Sr>Mn>B>Ca. Some trace elements which having key role in metabolism have also detected such as Al, B, Ba, and Sr. Elements are very important in various complex functions of the body to keep healthy. Calcium has major function in the formation of bones and teeth, control on nerve impulses, muscle concentration, and blood clotting [10].

### 3.6. Total Antioxidant Content.

The determination of the antioxidant content was carried out by the DPPH method, based on the ability of antioxidants to inhibit free radicals by donating hydrogen atoms. The reaction occurs based on the change in the color of the solution. From the two extracts tested, it was found that the antioxidant concentration of the methanol extract was 1.59 mg/gDW lower than 50% ethanol extract 3.11 mg/gDW.

Some previous studies using hexane as a solvent showed very strong antioxidant activity from *Acalypha indica* L. leaves [11, 12, 13, 14]. The difference in polarity of the solvent used for the extraction affected the antioxidant activity of the extract. More polar solvents produced less antioxidant content than semi-polar or non-polar solvents. Another study about antioxidant activity in different types of extracts prepared from the leaves of *Acalypha indica* L. showed that methanolic extract of *Acalypha indica* L. has higher radical scavenging activity (IC₅₀) than chloroform and aqueous plant extracts [15].

### 4. Conclusion

The evaluation results of the methanol and ethanol extracts of *Acalypha indica* L. showed that the extract contained secondary metabolites, essential elements and antioxidant. Based on the data obtained, it can be concluded that the methanol and ethanol extracts of *Acalypha indica* L. leaves have the potential to be used as herbs.

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