Identification and determination of phenolic acids content in mango “golek” leaves ethanol extract

A Khasan(1), E Fachriyah(1), D Kusrini(1)

(1) Organic chemistry Laboratory, Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Semarang 50275, Indonesia
E-mail: enny.fachriyah@live.undip.ac.id

Abstract. Ethanol extract from mango “golek” leaves (Mangifera indica L) was obtained by maceration in ethanol 96%. Phenolic acid was isolated from the extract by hydrolysis with and without a hydrolyzing agent (TH). Two different hydrolyzing agents were used separately, i.e., sulfuric acid (HA) and sodium hydroxide (HB). Identification of phenolic acid was made with TLC and High-performance liquid chromatography (HPLC). Determination of phenolic acid content was carried out using TLC Scanner. Standard phenolic acid was used as a control. Phenolic acid compounds were obtained in the HA, HB and TH isolate namely gallic acid. The total gallic acid content was 35.88% consisting of HA, HB and TH fraction of 3.46%; 12.42%; 19.98%.

1. Introduction
Secondary metabolism is the product of plant interactions with the environment. Secondary metabolism has many benefits, for example as drugs, antioxidants, aromas, fragrances, dyes, insecticides, and pheromones. Secondary metabolites can be classified according to their biosynthesis or carbon frame building blocks. Phenolic compounds are a group of very large and complex secondary metabolites found in plants. One group of phenolic compounds is phenolic acids [1].

Phenolic acid is a large family of natural compounds that are widely distributed in plants, with properties that are very important and useful for both plant producers and human health [1]. Phenolic acid compounds get more attention in recent years because of their effects on human health. As a polyphenol, phenolic acid is a very strong antioxidant and has antibacterial activity [2], antiviral [3], anti-inflammatory [4] and anticancer [5].

The derivative of hydroxybenzoic acid and hydroxycinnamic acid is a type of phenolic acid which is widely found in plants [6]. Phenolic acid is a compound composed of phenols and carboxylic acids. Phenolic acid can be in the form of compounds C6-C3, C6-C2, and C6-C1. C6-C3 phenolic acid is derived from a hydroxycinnamic acid and C6-C1 is derived from hydroxybenzoate. Although the basic framework remains the same, phenolic acids differ in number and position of hydroxyl groups on the aromatic ring [1].

Mango (Mangifera indica L.) is a plant belonging to the Anacardiaceae family in the order of Sapindales and grows in many parts of the world, especially in tropical countries. More than 1000 varieties of mangoes are available throughout the world. Of the varieties available, only a few grow on a commercial and commercial scale. At present, mangoes are cultivated in an area of about 3.7 million ha worldwide. Mango fruit occupies the 2nd position as a tropical plant, under bananas regarding production and the area used [7].
Several studies have shown the efficacy of mango leaves as antidiabetic [8], antioxidant [9], anti-inflammatory [10], and antibacterial [11]. The content of secondary metabolites in mango leaves is flavonoids [12], phenolic acids [13], alkaloids, tannins [14]. According to Saleem [13], phenolic acids in mango leaves are 4-hydroxybenzaldehyde acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, and gallic acid.

Mango has many varieties, one of which is the Golek mango variety. In this study identification and determination of phenolic acids will be carried out in the mango leaves of the Golek variety.

2. Materials and method

2.1. Tools

Standard research glassware, 254 nm UV lamp, a set of macerator tools and buchii evaporator rotation tool, analytical balance, vial bottle, TLC plate, drop pipette, TLC Scanner Camag 3 and Alliance HPLC by Waters with PDA and UV/Vis Detector.

2.2. Material

The main material of the study was Golek mango leaves. Materials including pro-analysis materials (p.a) are NaOH, H₂SO₄, NaHCO₃, HCl, methanol, chloroform, gallic acid, ferulic acid, pyrogallol acid, caffeic acid, and salicylic acid. Materials included in the technical material are methanol, n-hexane, distilled water, ammonia, amyl alcohol, Mg powder, acetic acid anhydride, Meyer reagent, Dragendroff reagent, ether, anhydrous Na₂SO₄, GF₂54 silica gel plates, and FeCl₃.

2.3. Method

2.3.1. Sample preparation

Mango leaf samples obtained around the Semarang area. Mango leaves are cleaned with water and dried by air without being exposed to sunlight and mashed.

2.3.2. Phytochemical screening

The dried powder of mango leaves and ethanol extract were then tested by phytochemical screening to determine the chemical content of the compounds including alkaloids, flavonoids, saponins, tannins, quinones, triterpenoids and steroids.

2.3.3. Extraction

Mango leaf powder as much as 2 kg then macerated with n-hexane, every 24 hours the solvent was replaced with a new one until the filtrate was colorless. The n-hexane extract was then concentrated using a rotary evaporator to obtain concentrated n-hexane extract. Mango leaf pulp is dried by air and macerated with ethanol, every 24 hours the solvent is replaced until the colorless filtrate. Ethanol extract was then concentrated using a rotary evaporator to obtain concentrated ethanol extract. Concentrated ethanol extract was dissolved in ethanol, then added with distilled water in a ratio of 1:1 and allowed to stand for 24 hours to remove chlorophyll. The mixture is then filtered to separate between chlorophyll and ethanol-water extract.

2.3.4. Isolation of phenolic acid

Phenolic acid is isolated in three methods, namely acid hydrolysis (HA), base hydrolysis (HB) and without hydrolysis (TH). Acid hydrolysis is carried out using 2 N H₂SO₄ by heating for 2 hours at 60°C. Base hydrolysis is carried out using 1 N NaOH in a dark room for 24 hours. After the hydrolysis process is completed, the three fractions are then extracted using ether and anhydrous Na₂SO₄. The extraction results are then dried and dissolved in methanol [15].

2.3.5. Separation of phenolic acid

The separation of phenolic acid was carried out in the HA, HB, and TH fractions using thin layer chromatography (TLC) with eluent ethyl acetate: glacial acetic acid: chloroform (2:1:7) using stationary silica gel 60 GF₂₅₄. The stains obtained are then compared with standard phenolic acids. The
standard phenolic acids used are gallic acid, salicylic acid, caffeic acid, ferulic acid, and pyrogallol. Stains that have an Rf value are parallel to the comparative identified by TLC and quantitative analysis with the TLC scanner Camag 3.

2.3.6. Identification with TLC scanner and HPLC

Analysis with the TLC Scanner was carried out by varying the concentration of standard phenolic acid solutions that were parallel to the stain. The concentrations used were 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm. Then TLC was carried out with eluent ethyl acetate: glacial acetic acid: chloroform (2: 1: 7). The results of the TLC are then scanned by using the TLC Scanner Camag 3.

Identification with HPLC was carried out based on research from Seal [16] with a mobile phase of acetonitrile and 1% acetic acid (1: 9). A 250 ppm sample solution was prepared by dissolving 3 mg of gallic acid samples in 12 mL of methanol. Then a standard 100 ppm gallic acid solution was prepared by dissolving 5 mg of standard gallic acid in 50 mL methanol.

3. Results and discussion

3.1. Sample preparation

7 kg of wet mango leaves are washed, then dried by air-drying which aims to maintain secondary metabolite compounds in the leaves. Obtained as much as 2 kg of dried leaves then mashed which aims to enlarge the surface so that all the compounds contained can be extracted perfectly in the maceration process.

![Figure 1. A dry powder of mango golek leaves](image)

3.2. Phytochemical screening

Phytochemical screening test from mango leaves is the first step in this study. Phytochemical screening aims to find out the secondary metabolites in mango leaves. The method used is a method of color reaction and precipitation. Phytochemical screening tests were carried out on dried mango leaf powder. Phytochemical screening tests carried out on mango leaf powder include alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, and steroids. Phytochemical test results of mango leaves can be seen in table 1.

3.3. Extraction

Mango leaf powder as much as 2 kg was macerated with n-hexane solvent to remove non-polar compounds. Then the residue is air dried and re-macerated with 96% ethanol. Ethanol solvents are universal solvents so they can dissolve compounds contained in mango leaves and are good solvents for preliminary extraction.

Maceration with n-hexane and ethanol was carried out until the solution was clear. The filtrate from maceration results with both solvents and then evaporated with a rotary evaporator so that it gets concentrated extract. Concentrated extracts of n-hexane obtained from maceration were 47.13 grams (2.35%). A concentrated extract of ethanol as much as 195.6 grams (9.78%).
3.4. Isolation of phenolic acid

Phenolic acid is isolated in three isolation methods, namely acid hydrolysis (HA), base hydrolysis (HB), and without hydrolysis (TH). Acid hydrolysis aims to take phenolic acid from the form of glycosides. Base hydrolysis aim to take phenolic acid from the ester form. The isolation results of phenolic acids from acid hydrolysis fraction (HA), base hydrolysis (HB), and without hydrolysis (TH) were 0.77g; 0.93g; 0.78g.

| Table 1. The results of the chemical screening test of mango Golek leaves |
|-----------------|-------------------|
| Test            | Result |
| Alkaloid        | +      |
| Flavonoid       | +      |
| Triterpenoid    | -      |
| Steroid         | -      |
| Saponin         | +      |
| Tanin           | +      |
| Kuinon          | +      |
| Phenol          | +      |

3.5. Separation of phenolic acid

Separation of phenolic acids was carried out by TLC with silica gel GF254 stationary phase and an eluent mixture of ethyl acetate: chloroform: acetic acid (2:7:1) as the mobile phase. At this stage, the HA fraction produced four stains, the HB fraction four stains, and TH 4 stains fraction. The results of the Rf calculation show that there are stains on each fraction having similarities to Rf. It can be concluded that in all three fractions contain the same compounds.

Further identification was carried out by comparing with standard phenolic acids. It was found that the stains that had similar Rf from the three fractions had similarities with Rf as well as standard phenolic acid, namely gallic acid with Rf = 0.12.

![Image of TLC results](image)

**Figure 2.** Results of TLC Fraction TH, HA, HB and phenolic acid standard with eluent mixture of ethyl acetate: chloroform: acetic acid (2: 7: 1) at a wavelength of 254 nm Identification with TLC Scanner and HPLC

Identification of phenolic acids was carried out by TLC Scanner at 254 nm wavelength and sample area that had been carried out by TLC and eluted with the stationary phase of silica gel and...
eluent mixture of ethyl acetate: chloroform: acetic acid (2:7:1). The scanning process is carried out under conditions:

- Slit dimension: 6.0 x 0.45 µm.
- Wavelength: 254 nm.
- Scanning speed: 20 mm/s.
- Data resolution: 100 µm/step.
- Measurement mode: Absorption

Identification is carried out with a standard gallic acid solution. Chromatogram results show that at one peak each fraction has an Rf value that is almost the same as standard gallic acid. The Rf value of each fraction and the standard gallic acid solution can be seen in table 2.

![TLC Scanner Chromatogram with gallic acid comparator](image)

**Table 2.** The Rf value of the TLC scanner results

| No | compound name | Rf  |
|----|---------------|-----|
| 1  | G50           | 0.09|
| 2  | G100          | 0.09|
| 3  | G250          | 0.10|
| 4  | G500          | 0.11|
| 5  | G1000         | 0.13|
| 6  | TH            | 0.12|
| 7  | HA            | 0.10|
| 8  | HB            | 0.12|

In these results, it can be concluded that the standard retention time of gallic acid has similarities with one of the Rf of each of these fractions which is 0.12. Data from analysis using HPLC support this result.

Analysis using HPLC phenolic acid and gallic acid isolates showed a similar chromatogram. HPLC Chromatogram Phenolic acid isolate showed a peak at 4.982 minutes. HPLC analysis for standard gallic acid showed a peak that was almost the same as phenolic acid isolates, which was 4.976 minutes. Chromatogram analysis of HPLC standard phenolic acid and gallic acid isolates can be seen in Figure 4.
The Uv-Vis spectrum profile from the peak shows similarities, where there is an absorption at a wavelength of 226.4 nm and 271.4 nm. From the data above it can be concluded that the three fractions proved to contain gallic acid. The structure of gallic acid can be seen in Figure 5.

![HPLC chromatogram](image)

**Figure 4.** HPLC chromatogram (a) phenolic acid isolate (b) standard gallic acid

![Structure of gallic acid](image)

**Figure 5.** Structure of gallic acid
TLC scanner is used to determine gallic acid levels obtained from mango leaf ethanol extract. Determination of the content was carried out by the linear equation of the standard curve obtained from the correlation of the concentration and the area of the standard compound. Correlation curve of concentration and area of standard compound gallic acid can be seen in Figure 6. Gallic acid curve equation \( y = 21.734x + 314.99 \)

![Figure 6. Gallic acid calibration curve](image)

From this equation is used to determine the weight of the compound in each sample. From the weight of the compound can be used to determine levels of gallic acid in the three fractions. The total gallic acid content was 35.88 % which consisted of HA, HB and TH fractions, each of which was 3.47%; 12.43%; 19.98%.

4. Conclusion
The results of research conducted on manga golek leaves can be concluded that the isolation of phenolic acids by hydrolysis method obtained by the fractions of HA, HB, and TH respectively 0.77g; 0.78g; 0.93g. Mango leaves contain phenolic acids, namely gallic acid. The total gallic acid content was 35.88 % which consisted of HA, HB and TH fractions, each of which was 3.47%; 12.43%; 19.98%.

Acknowledgment
Upon completion of this research, thanks were given to the lecturer of the chemical department of the FSM Diponegoro University, especially in the field of organic chemistry for guidance and direction during the research. Thanks are also conveyed to PT. Kromtekindo Utama that has helped the analysis process.

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