Isolation And Identification of Secondary Methabolites Compound of *Moringa Oleifera* Lamk Leaf Acetic Ethyl Extract

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**Abstract.** This study aims to isolate and identify secondary metabolite compounds in the ethyl acetate extract of *Moringa* leaves (*Moringa oleifera* Lamk). This isolation includes several stages, namely extraction, fractionation, purification and identification. Identification includes solubility test, UV-VIS spectroscopy test, FTIR and GCMS. Based on the solubility test results, it is known that the isolate is soluble in ethyl acetate solvent and dissolves well in the chloroform solvent, this shows that the isolate obtained is semi-polar and the compound glows under UV light. UV-VIS spectroscopy test results showed a maximum wavelength of 210.52 nm showing the characteristics of the ester group. FTIR spectroscopy data showed wave numbers (cm$^{-1}$) namely: 3450.65 (OH alcohol), 2926.01 and 2560.43 (C-H); 1456.26 and 1382.96 (CH$_2$ and CH$_3$), 1722.43 (C=O); 1616.26 (C = C aromatic); 1039.63 (C-O). The result of GC-MS showed molecular weight of compound is 166. From the results of the analysis it was suggested that the isolate was a phenolic group that are 4-hydroxy benzoyl ethyl esters.

**Keywords:** Isolation, identification, *Moringa oleifera*, spectroscopy, 4-hydroxy benzoyl ethyl esters

1. **Introduction**

The development of traditional medicine is increasing so that many are interested in researching various types of plants, which contains various complex organic compound and nutritious as medicine.

One of the plants that has multiple benefits and is widely cultivated by the community is *Moringa oleifera*, aside from being a vegetable ingredient, almost all parts of this plant have been used as medicine [1]; [2]; [3].

*Moringa oleifera* is rich with minerals, including Calcium, Potassium, Zing, Magnesium, Ferrum, Vitamin B are like Piridoxin, Folic acid and nicotinic acid [4]; [5]; [6]; [7]. *M. Oleifera* leaves low calorific value and can be use in the diet [8]. The pods are fibrous and are valuable to treat digestive problem and thwart colon cancer [9].

The methanol extract showed the highest free radical scavenging activity with IC$_{50}$ value of 49.30 ug/mL in DPPH assay and 11.73 ug/mL in ABT assay [10].

The results of research conducted by *Moringa* leaf extract can cure inflammation of the liver (liver) [11] and can inhibit the development of *Escherechia coli* [12], showing antifungal activity in *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epydermophyton xoccosum*, and *Microscoprum canis*. 

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1. **ISCMTR 2019**

Journal of Physics: Conference Series 1752 (2021) 012053  
doi:10.1088/1742-6596/1752/1/012053

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Moringa oleifera classification [13]:
Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Brassicales
Family : Moringaceae
Genus : Moringa
Species : M. oleifera, Lamk

Moringa root, leaves and bark contain saponins and polyphenols. Besides that, the bark also contains alkaloids and the leaves contain essential oils. The root contains tannins, flavonoids, moringin, moringenin, gum, and pterigospermin [14].

In nature isothiocyanates are in the form of benzyl isothiocyanate (BITC) [Figure 1A], phenethyl isothiocyanate (PEITC) (Figure 1B), or phenyl isothiocyanate (PITC) (Figure 1C) [2]. Isothiocyanates are released from the plant through the action of the myrosinase enzyme after the plant cells are damaged, such as when harvested or when chewed. Based on these facts various studies on isothiocyanates have been carried out.

Figure 1. Structure of [A] Benzyl Isothiocyanate [B] Phenethyl Isothiocyanate, and [C] Phenyl Isothiocyanate [2]

2. Material and Methods

2.1. Material
Moringa oleifera fine powder, several technical organic solvents such as n-hexane, chloroform, acetic ethyl and methanol. Color reagent solutions for identification include Liebermann-Burchard, Wagner, Dragendorf reagents, 10% sulfuric acid solution, iron (III) chloride, whatman filter paper no. 42, ordinary filter paper, silica gel G 60 (70-230 mesh) and aluminum TLC plate coated with silica gel 60 G F254.

2.2. Methods

2.2.1. Extraction. A total of 3 kg of dried Moringa leaf powder was extracted by maceration technique, using ethyl acetate solvent for 3 x 24 hours, so that the content of the chemical compounds in the extracted sample as much as possible into the solvent. The extract was filtered using a Buchner funnel and Whatman paper.

The ethyl acetate masuret obtained was concentrated by using an evaporator to separate the solvent until a thick extract was obtained, with the evaporation method, the solvent was separated below the boiling point so that the compounds contained in the extract were not damaged. The viscous extract obtained is left in free air so that the remaining solvent evaporates.
2.2.2. Group Identification with Wagner, Dragendorf, Liebermann-Burchard and FeCl₃ reagents. The extract is placed on a drip plate, then dropped with Wagner reagent, if chocolate deposits are formed indicating that the extract contains alkaloids, with Dragendorff reagent, if orange is formed indicating the presence of alkaloid compounds, with Liebermann-Burchard reagent if blue is formed, indicating the presence of steroid compounds, and with FeCl₃ reagents, the formation of black indicates flavanoids compounds.

2.2.3. Fractionation. Viscous extract obtained was tested using thin layer chromatography (TLC) with eluent n-hexane: ethyl acetate in various comparisons, which aims to find out the number of compound components contained in the extract that can be seen from the number of stains that appear on the chromatogram, as well as to determine the eluent the best for vacuum liquid chromatography and subsequent TLC.

The fractionation process is carried out in two stages, namely the fractionation stage of flash column chromatography (FCC) and the vacuum liquid chromatography column (VLCC) using silica gel G 60 (70-230 mesh) as a stationary phase and the mobile phase is used by eluent n-hexane which is polished with ethyl acetate as in.

2.2.4. Purification. The solid component obtained is recrystallized using n-hexane solvent. The purity of the compounds obtained was its purity determined by the TLC test with the three-eluent system namely n-hexane: acetone, n-hexane: ethyl acetate, and chloroform: ethyl acetate.

2.2.5. Identification. Identification was continued by solubility and melting point test, then continued with UV-VIS spectroscopy method to determine the wavelength of absorption, FTIR to determine the functional group, and GC-MS to determine the molecular weight of compounds in the presence of molecular ion fragmentation which produces specific fractions of a specific of a compound.

3. Results and Discussions

3.1. Preliminary Test

Maceration results with acetic ethyl solvent, then evaporated until a thick green extract of 54.5 gr thick, were preliminary tested with Lieberman-Burchard, Wagner, Dragendorf, and FeCl₃ reagents, the results in Table 1 and Figure 2.

| Table 1. Preliminary Test Results of n-Hexane Extract |
|------------------------------------------------------|
| Reagent                        | Color After Test | Information      |
| Liebermann-Burchard Wagner     | Blue             | Steroid Positive |
| Dragendorff FeCl₃ 1%            | Brown Precipitate| Alkaloid Positive|
|                                | Orange Black     | Alkaloid Positive|
|                                |                  | Flavonoid Positive|

Figure 2. Preliminary Test With Reagent: (a) Liebermann-Burchard, (b) Wagner (c) Dragendorff, and (d) FeCl₃
3.2. Fractionation

The viscous acetic ethyl extract obtained was then tested by TLC with eluent n-hexane: ethyl acetate with various comparisons so as to obtain n-hexane eluent: acetic ethyl (8: 2) which showed clear and separate appearance of stains, so that it was used in each TLC each fraction, obtained Figures 3 and Figure 4.

![Figure 3. Chromatogram Vacuum Liquid Coulum Chromatography (VLCC) Result](image)

![Figure 4. Chromatogram of the Combined Fraction of VLCC Result](image)

Fraction B as much 1.52 Gr was continued to flash column chromatography, 53 fractions were obtained (Figure 5), then in TLC, the fractions that had the same chromatogram and RF values were combined, obtained the combined fraction B1-B5 (Figure 6).

![Figure 5. Chromatogram of the Flash Column Chromatography (FCC) Results](image)

![Figure 6. Combined Fraction Chromatogram of the FCC Results](image)
3.3. Purification
The combined B4 fraction was evaporated, a green solid was obtained, then its purity was tested with a three-eluent TLC system. The TLC analysis showed one stain on the TLC system of three eluents: (a) n-hexane : acetic ethyl (8 : 2); (b) n-hexane : acetone (6 : 4); and (c) acetic ethyl : chloroform (5 : 5), as shown in Figure 7. From the data obtained it can be concluded that the compounds obtained can be categorized as pure compounds.

![Figure 7. Chromatogram of TLC Three Eluent System, n-hexane : acetic ethyl, (a) 8:2, (b) 6:4 and (c) 1:1](image)

3.4. Identification
Based on the solubility test B4 isolates are not soluble in n-hexane and ethanol, soluble in acetic ethyl and very easily soluble in chloroform means that B4 isolates are semi-polar. Then identification continued with UV-Vis spectroscopy test, FTIR spectroscopy test and GC-MS spectroscopy test.

With UV-Vis spectroscopy, B4 isolates showed a fairly high absorption at a maximum wavelength of 210.52 nm indicating the presence of an ester group. The infra red spectrum of B4 isolate is shown in Figure 8, and the wave number data that related groups are in Table 2.

![Figure 8. Infra Red Spectrum of B4 Isolate](image)

| No. | Wave Number (cm⁻¹) | Function Group Interpretation |
|----|-------------------|-------------------------------|
| 1. | 3450.65           | -OH Strain                   |
| 2. | 2926.01 and 2560.43 | -CH Strain                   |
| 3. | 1456.26           | press –CH from CH₃           |
| 4. | 1382.96           | press –CH from CH₃           |
| 5. | 1722.43           | C=O Strain                   |
| 6. | 1616.26           | C=C (aromatic)               |
| 7. | 1039.63           | C-O Strain                   |

Analysis of B4 Isolate using Gas Chromatography (GC) spectroscopy, obtained one peak that is 1A as in Figure 9. Whereas in the GC-MS spectroscopy test there were peaks of molecules ion with m / z consecutive are 166, 137, 120, 65, shown in Figure 10.
B4 isolate obtained was green with a weight of 0.1 g, in the form of amorphous. The purity test results with TLC system of three eluents namely (a) eluent n-hexane : acetic ethyl (8 : 2), (b) eluent n-hexane : acetone (6 : 4), and (c) eluent acetic ethyl : chloroform (5 : 5), each showing one stain, thus the isolates obtained can be categorized as pure compounds.

The results of the analysis with UV-Vis isolate B4 have a transition $\pi \rightarrow \pi^*$ maximum wavelength of 210.52 nm, referred to as the band of the ester group absorption. This shows that the isolated compound has an ester group.

By Infra-Red Prestige-21 spectroscopy using KBr pellet method showed the absorption band, widened in the area of 3450.65 cm$^{-1}$ which signaled the presence of OH groups Function. In the area of 2926.01 cm$^{-1}$ and 2560.43 cm$^{-1}$ there was a very strong and sharp absorption showing the presence of aliphatic CH groups, followed by absorption bands 1456.26 cm$^{-1}$ which were the aliphatic CH bends of CH$_2$ and uptake in 1382, 96 cm$^{-1}$ which is the CH aliphatic bend of CH$_3$. The uptake in the area of 1722.43 cm$^{-1}$ indicates the presence of C = O groups. Uptake in the region of 1616.26 cm$^{-1}$ indicates the presence of aromatic C = C and absorption in the area of 1039.63 cm$^{-1}$ indicates the presence of a C-O group.

The measurement results by GC-MS spectroscopy produced peaks, with molecular weights of $m/z$ 166 grams/mol. In the mass spectrometer the compound was fragmented into fragment ions with $m/z$ 137, 120, 65, 64. The main fragment ions indicated by $m/z = 120$ were concluded as the most stable fragments in mass spectroscopy. The breakdown of $m/z$ 166 to $m/z$ 120 with the release of the-OC$_2$H$_6$ group (Figure 11) and the breakdown of $m/z$ 166 to the $m/z$ 137 with the release of the C$_2$H$_5$ group (Figure 12).
Based on UV, IR, and GC-MS spectroscopic analysis, and solubility testing, the isolated compound from the ethyl acetate extract of Moringa leaves is suggested as a compound of the phenolic group, namely 4-hydroxy benzoyl ethyl ester with Molecule structure as in (Figure 13).

**Figure 13.** Molecular Structure of 4-Hydroxy Benzoyl Ethyl Ester

4. Conclusion
The secondary metabolite compound which was successfully purified from the *Moringa oleifera* leaf acetic ethyl extract was thought to be a phenoyl ester compound with the molecular formula is C_{10}H_{10}O_{3}, and the structural formula is 4-hydroxy Benzoil Esther Ethyl.

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