Identification of Active Compounds in the Root of Merung (Coptosapelta tomentosa Valeton K. Heyne)

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Abstract. The roots of Merung (Coptosapelta tomentosa Valeton K. Heyne) are a group of shrubs usually found on the margins of secondary dryland forest. Empirically, local people have been using the roots of Merung for medical treatment. However, some researches show that the plant extract is used as a poisonous material applied on the tip of the arrow (dart). Based on the online literature study, there are less than 5 articles that provide information about the active compound of this root extract. This study aimed to give additional information more deeply about the content of active compound of Merung root extract in three fractions, n-hexane (nonpolar), ethyl acetate (semi polar) and methanol (polar). The extract was then analysed using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis of root extract in n-hexane showed there were 56 compounds, with the main compound being decanoic acid, methyl ester (peak 5, 10.13%), 11-Octadecenoic acid, methyl ester (peak 15, 10.43%) and 1H-Pyrazole, 3- (4-chlorophenyl) -4, 5-dihydro-1-phenyl (peak 43, 11.25%). Extracts in ethyl acetate fraction obtained 81 compounds. The largest component is Benzoic acid (peak 19, 22.40%), whereas in methanol there are 38 compounds, of which the main component is 2-Furancarboxaldehyde, 5-(hydroxyl methyl) (peak 29, 30.46%).

1. Introduction
Merung (Coptosapelta tomentosa Valeton K. Heyne) are classified as shrubs and normally found at the edge of the secondary forest. The present of Merung in the wild is very rare but can be abundant in certain areas. This plant is the original plant of Asia continent both tropical and sub-tropical spread from Myanmar to Indo-China, Thailand, Peninsular Malaysia, Sumatra, Bangka, Java and Kalimantan.

Traditionally, people uses Merung root for healing treatments and other uses. Root extract is usually given to children infected with parasitic worms [1], anti-parasites [2], postpartum drugs [3], anti-malaria [4] and others. The abundance of the use of roots as a traditional medicine makes the starting point of some research on the content of active chemical compounds in it. Active compounds are studied as drug candidates or lead compounds to optimize for potentially more potent compounds minimal toxicity.

Several basic studies have reported that the active ingredients contained in Merung Roots include: 1-hydroxy-2-hydroxy methyl anthraquinone [2], anthraquinones (1 and 3-5) and one naphthoquinone (2) [5], flavonoids, tannins, saponins, terpenoids, alkaloids and phenolic [6][7][8]. However, most of the compounds produced are still general so there is still a gap of research that needs to be filled in order for the merit and health benefits of Merung for health can be known more fully.
This is the basis for the necessity of this research is conducted to enrich information content of active compound of Merung root in order to be utilized more broadly, especially in the field of pharmaceutical. In this research will look more deeply information of active chemical compound content of crude extract of Merung root in three fractions, namely \( n \)-hexane (nonpolar), ethyl acetate (semi polar) and methanol (polar). The extracts of the three fractions were then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS).

2. Materials and methods
The materials used were the root of Merung from Mekar Baru Village, Busang District, East Kutai Regency, methanol, \( n \)-hexane and ethyl acetate, all solvents with grade pure analysis (pro analyst). This research is experimental. The obtained data was the result of GC-MS showing chemical components at the root of Merung.

2.1. Place and Time of Research
This research was conducted in research laboratory of Chemical Engineering Department, State Polytechnic of Samarinda and Organic Chemistry Laboratory of Gajah Mada University Yogyakarta in April to August 2017.

2.2. Tools
The tools used for maceration roots and fractionation roots were digital scales, measuring cups, beaker, filter paper, rotary evaporator, separating funnel and glass funnel. While the tools used for the analysis of chemical components was Gas Chromatography-Mass Spectroscopy (70 eV ionization energy, GCMS-QP2010S SHIMADZU). The column used was Rxi 5MS with size 30 m×0.25 mm, thick layer of column 0.25 μm and carrier gas that was Helium.

2.3. Methods
The research will be conducted through the following stages:

2.3.1. Preparation and extraction. The roots were cleaned and chopped into a coarse powder. Rough powder was dried and stored in tightly sealed containers. A total of 100 g of rough powder Merung root was extracted by maceration technique using methanol for 3×24 hours. Every 24 h, the filtrate was filtered and evaporated using a rotary evaporator at 50°C obtained fixed weight.

2.3.2. Partition / fractionation. The methanol extract was then stratified partitioned from \( n \)-hexane and ethyl acetate. Liquid-liquid fractionation of each solvent was carried out three times.

2.3.3. Analysis. The chemical contents of \( n \)-hexane, ethyl acetate and methanol extracts of Merung roots were analysed using Gas Chromatography-Mass Spectroscopy (GC-MS) (ionization energy 70 eV). Individual liquid extract to GC tool. The injector temperature is set to 300 °C. The column temperature is gradient: the initial temperature is 70 °C for 5 min, raised by 5 °C/min to 300 °C, 300 °C for 19 min. Qualitative identification of chemical components is done by comparing the retention time and the resulting mass spectrum with the reference components present in the library.

3. Results
The results of the root component analysis of Merung of each fraction using GC-MS can be seen in Figures 1, 2 and 3. The relative amounts of each component can be determined based on the percentage of the peak region relative to the total peaks.
Figure 1. GC-MS chromatogram of extracts of Merung roots in n-hexane solvent

Figure 2. GC-MS chromatogram of extracts of Merung roots in ethyl acetate solvent

Figure 3. GC-MS chromatogram of extracts of Merung roots in methanol solvent

Based on the results of chromatogram shows there are 56 chemical compounds contained in roots extract n-hexane, 81 compounds in ethyl acetate extract and 38 compounds in root-methanol extract. Each peak analysed its molecular weight by a mass spectrometer. The results of the analysis showed that peak number 11, 11 and 10 of the main compounds of the roots in n-hexane, ethyl acetate and methanol were sequentially. The main constituent compounds of root Merung extract in each fraction can be seen in Tables 1, 2 and 3.

Based on the results of the matching of spectrograph data from each of the main peaks with the library research it can be known chemical compounds.
Table 1. Characterization of \( n \)-hexane soluble compounds

| Peak | Compounds                                      | Retention Time (min) | Area (%) | Accuracy (%) |
|------|-----------------------------------------------|----------------------|----------|--------------|
| 2    | Decanoic acid, methyl ester                   | 19.811               | 0.99     | 91           |
| 5    | Decanoic acid, methyl ester                   | 25.373               | 10.13    | 91           |
| 8    | Tetradecanoic acid, methyl ester              | 30.298               | 4.20     | 91           |
| 10   | Eicosanoic acid, methyl ester                 | 34.727               | 8.39     | 91           |
| 13   | Eicosanoic acid, methyl ester                 | 36.788               | 1.52     | 91           |
| 15   | 11-Octadecenoic acid, methyl ester            | 38.262               | 10.43    | 92           |
| 17   | Octadecanoic acid, methyl ester               | 38.751               | 3.29     | 92           |
| 24   | Hexadecane                                    | 43.696               | 1.14     | 91           |
| 27   | Dodecane, 2,5-dimethyl                        | 45.358               | 1.12     | 91           |
| 29   | Tridecane, 2,5-dimethyl                       | 46.951               | 1.11     | 91           |
| 43   | 1H-Pyrazole, 3-(4-chlorophenyl)-4,5-dihydro-1-phenyl | 52.833             | 11.25    | 65           |

Figure 4. The mass spectrum at peak 5

Figure 5. The mass spectrum at peak 15

Figure 6. The mass spectrum at peak 43
Table 2. Characterization of ethyl acetate soluble compounds

| Peak | Compounds                                      | Retention Time (min) | Area (%) | Accuracy (%) |
|------|-----------------------------------------------|----------------------|----------|--------------|
| 1    | Toluene                                       | 3.561                | 1.37     | 94           |
| 2    | Acetic acid, butyl ester                     | 4.070                | 0.48     | 95           |
| 3    | 2-Furancarboxaldehyde (CAS) Furfural         | 4.519                | 0.87     | 95           |
| 13   | 2,5-Furandione, dihydro-(CAS) Succinic anhydride | 9.851                | 0.74     | 95           |
| 19   | Benzoic acid                                 | 17.537               | 22.40    | 90           |
| 28   | Cinnamic Acid                                | 23.762               | 3.19     | 91           |
| 31   | Benzoic acid, 4-hydroxy                     | 26.402               | 2.20     | 90           |
| 39   | Tetradecanoic acid                           | 31.275               | 0.59     | 93           |
| 44   | 13-Oxabicyclo [10.1.0] tridecane (CAS) Epoxycyclododecane | 34.953               | 4.70     | 80           |
| 45   | Hexadecanoic acid (CAS) Palmitic acid        | 35.700               | 3.16     | 92           |
| 49   | Octadecanoic acid (Stearic acid)             | 39.615               | 2.77     | 93           |

Figure 7. The mass spectrum at peak 19
Table 3. Characterization of methanol soluble compounds

| Peak | Compounds                                              | Retention Time (min) | Area (%) | Accuracy (%) |
|------|--------------------------------------------------------|----------------------|----------|--------------|
| 1    | 2-Propanone, 1-hydroxy (Acetol)                        | 3.488                | 2.08     | 79           |
| 2    | 2-Furancarboxaldehyde (Furfural)                       | 4.543                | 8.51     | 94           |
| 5    | Ethanone, 1-(2-furanyl)                                | 6.408                | 2.36     | 77           |
| 6    | 2-Furanmethanol (Furfuryl alcohol)                     | 6.795                | 3.48     | 79           |
| 18   | 2,5-DIMETHYL-4-HYDROXY-3(2H)-FURANONE                  | 12.208               | 2.44     | 84           |
| 19   | 1,4-Cyclohexanediol (Quinitol)                         | 12.694               | 2.14     | 77           |
| 23   | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl     | 14.496               | 7.01     | 88           |
| 24   | Benzoic acid (Retardex)                                | 15.367               | 6.60     | 93           |
| 29   | 2-Furancarboxaldehyde, 5-(hydroxymethyl)               | 18.031               | 30.46    | 87           |
| 36   | Cyclopentaneacetaldehyde, 2-formyl-3-methyl-alpha,-methylene- (dolichodial) | 29.988 | 6.79 | 78 |

Figure 8. The mass spectrum at peak 29

4. Conclusion

Gas Chromatography-Mass Spectrometry analysis of root extract in n-hexane showed there were 56 compounds, with the main compound being decanoic acid, methyl ester (peak 5, 10.13%), 11-Octadecenoic acid, methyl ester (peak 15, 10.43%) and 1H-Pyrazole, 3- (4-chlorophenyl) 4,5-dihydro-1-phenyl (peak 43, 11.25%). There are 81 compounds in ethyl acetate fraction. The largest component is Benzoic acid (peak 19, 22.40%), whereas in methanol there are 38 compounds, of which the main component is 2-Furancarboxaldehyde, 5-(hydroxyl methyl) (peak 29, 30.46%).

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