Phytochemical Evaluation of *Plumbago Zeylanica* Roots from Indonesia and Assessment of its Plumbagin Concentration

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Abstract

Introduction: *Plumbago zeylanica* grows widely in many tropical countries. In Indonesia, this plant, known as Daun Encok, has some beneficial effects on human health.

Aim: This exploration study aimed to identify the plumbagin compound in *P. zeylanica* roots from Indonesia.

Materials and methods: Dried roots of *P. zeylanica* were manually ground and then the powder was macerated using ethanol and chloroform for 24 hours at room temperature. All extracts of *P. zeylanica* were then analyzed using gas chromatography-mass spectrometry (GC-MS). Plumbagin concentration was measured by comparing the extract with pure plumbagin.

Results: GC-MS analysis of ethanol extract and chloroform extract of *P. zeylanica* roots showed the presence of plumbagin as the highest peak. Plumbagin concentration in ethanol extract was 13%, while in chloroform extract it was 81%.

Conclusions: The chloroform extract of *P. zeylanica* root from Indonesia demonstrates a higher concentration of plumbagin compared to ethanol extract.

Keywords

chloroform root extract, GC-MS, medicinal plant

List of Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| GC-MS        | Gas Chromatography-Mass Spectrometry; |
| ppm          | part per million; |
| ml           | milliliter; |
| μL           | microliter; |
| g            | gram; |
| NIST/EPANIH  | National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health; |
| RH           | relative humidity; |
| MF           | molecular formula; |
| MW           | molecular weight; |
| RT           | retention time |
INTRODUCTION

Plumbago zeylanica, which is commonly known as Daun Encok or Ki Encok in Indonesia, belongs to the family Plumbaginaceae. This plant grows in many countries and is recognized by the community as having many benefits on human health; for example, in treating joint pain and skin diseases. Several previous studies have found other benefits of this plant, namely as a nephroprotective agent, anti-proliferation of cancer cells, anti-parkinsonism agent, antibacterial agent, analgesic, and anti-inflammatory. However, only a few of those studies were conducted using Plumbago zeylanica from Indonesia.

The best known bioactive marker in P. zeylanica is plumbagin. Plumbagin is effective as an antiproliferative, antimalarial, and antibacterial agent. In a previous study, GC-MS was used to find the compound contained in ethanolic extract and diethyl extract of P. zeylanica root, but they did not find plumbagin. Plumbagin concentration in ethanolic extract of root part has been found to be low (1.9%). There is no publications about plumbagin contained in P. zeylanica plants from Indonesia.

AIM

This study aimed to identify the plumbagin compound contained in the root part of P. zeylanica plants from Indonesia.

MATERIALS AND METHODS

Plantation of P. zeylanica

The P. zeylanica seeds were obtained from Akhyar Flora, Bekasi, West Java, Indonesia and then were grown in pots 25-40 cm in diameter filled with soil. In order to stimulate seed sprout, it was watered twice a day with tap water and was supplemented with a commercial fertilizer containing 16% nitrogen (6.5% nitrate-N and 9.5% ammonium-N), 16% phosphate (P₂O₅) and 16% potassium (K₂O). After 7 months, grown P. zeylanica plants had some big roots that could be used for the next extraction process. The P. zeylanica plant was authenticated by a botanist, Mrs. Susi Dewiyeti, S.Si, M.Si, which was deposited at the Department of Biology, Faculty of Education, Universitas Muhammadiyah Palembang, South Sumatera, Indonesia.

P. zeylanica roots were cut from their plants in the afternoon and washed thoroughly under tap water to remove soils and other debris. Cleaned roots were air-dried for 18 days in room temperature by exposing them to air flow and indirect sunlight. The average of relative humidity in Palembang remained constant (94.9% RH) during those days and the average of sun rays was 5.6 hours per day. Finally, air-dried roots were mechanically ground to make powder using a blender.

Extraction of P. zeylanica roots

A total of 10 g of root powder was soaked in either 100 ml ethanol (Emsure®, Merck, Germany) or 100 ml chloroform (Emsure®, Merck, Germany) for 24 hours. These root powder solutions were filtered with filter paper and the filtered solutions were concentrated using a rotary evaporator (B-One Rotary Evaporator Model RE-1000VN) with 52 rpm at 60°C for 30 minutes for the ethanol extract and 10 minutes for the chloroform extract. The extract yield of 100 g roots powder with ethanol solvent was 4.6% whilst 1.7% was for chloroform solvent. All crude extracts were finally stored in the refrigerator at 4°C until further analysis.

Chemical analysis of P. zeylanica extracts using gas chromatography-mass spectrometry

The ethanol and chloroform extracts of P. zeylanica roots was analysed using Thermo Scientific Trace 1310 Series (Thermo Fisher Scientific, San José, CA, USA) and a Thermoscientific A1 1310 automatic injector (San José, CA, USA). The chemical analysis was conducted in a ZB-5MS (30 m × 0.25 mm × 0.25 µm) column (Phenomenex®, Torrance, CA, USA) and the injector temperature was set at 300°C. The injection mode was splitless and the injection volume was 1 µL. The plumbagin compound was identified and matched with the peak of main EI MS library (mainlib) from NIST/EPA/NIH. Chemical structure of all components was made by Chem Draw software (PerkinElmer, US).

RESULTS

The raw extracts of P. zeylanica roots were examined by GC-MS. GC-MS examination results for the two samples are shown in Fig. 1 below. GC-MS for each extract showed 50 peaks and all peaks are known. But, in this article, only 10 highest % area were shown in Tables 1, 2 and 3.

Details on the compound of each extract are shown in Tables 1, 2 and 3. Based on Table 1 and Table 2, there were 2 similar compounds: 5-hydroxy-2-methyl-1,4-naphthalenedione (plumbagin) and 2-Allyl-1,4-dimethoxy-3-methyl-benzene.

Because of the highest GC-MS curves for both extracts based on Table 1 and Table 2, there were 2 similar compounds: 5-hydroxy-2-methyl-1,4-naphthalenedione (plumbagin), we then confirmed the presence of plumbagin using pure plumbagin (Sigma Aldrich, lot #SLBZ1960). The purchased plumbagin as an internal control contains 5-hydroxy-2-methyl-1,4-naphthalenedione. The final concentration of plumbagin in chloroform solvent was about four times higher than the ethanol solvent (Table 4) based on the formula below (Fig. 2).
Figure 1. GC-MS examination results. (a) The roots of *P. zeylanica* macerated using absolute ethanol; (b) The roots of *P. zeylanica* macerated using chloroform (CHCl3).

Figure 2. The curve of plumbagin concentration measured by GC-MS.
Table 1. Chemical compounds in ethanol extract of *P. zeylanica* roots (sorted by low to high RT)

| No. | Compound name                               | MF   | MW  | RT (% area) |
|-----|--------------------------------------------|------|-----|-------------|
| 1   | Dihydroxyacetone                            | C₃H₆O₃ | 90  | 4.57        | 1.47        |
| 2   | Glycerin                                    | C₃H₇O₃ | 92  | 8.89        | 1.02        |
| 3   | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | C₄H₈O₃ | 144 | 8.98        | 1.39        |
| 4   | 5-Hydroxymethylfurfural                     | C₅H₇O₃ | 126 | 10.00       | 1.13        |
| 5   | Sucrose                                     | C₁₂H₂₂O₁₁ | 342 | 12.07       | 7.07        |
| 6   | 5-hydroxy-2-methyl-1,4-naphthalenedione     | C₁₁H₈O₃ | 188 | 13.60       | 56.88       |
| 7   | 2-Allyl-1,4-dimethoxy-3-methylbenzene       | C₁₂H₁₆O₂ | 192 | 14.42       | 9.59        |
| 8   | 11-decyltetradecasane                       | C₁₂H₂₀ | 478 | 19.64       | 0.99        |
| 9   | 11-decyltetradecasane                       | C₁₄H₃₀ | 478 | 24.52       | 3.26        |
| 10  | Cyclodecasiloxane, eicosamethyl-            | C₂₀H₆₀O₁₀Si₁₀ | 740 | 25.61       | 1.01        |

MF: molecular formula; MW: molecular weight; RT: retention time

Table 2. Chemical compounds in chloroform extract of *P. zeylanica* roots (sorted by low to high RT)

| No. | Compound name                               | MF   | MW  | RT (min) | % area |
|-----|--------------------------------------------|------|-----|----------|--------|
| 1   | 5-hydroxy-2-methyl-1,4-naphthalenedione    | C₁₁H₈O₃ | 188 | 13.64    | 71.97  |
| 2   | 2-Allyl-1,4-dimethoxy-3-methylbenzene      | C₁₂H₁₆O₂ | 192 | 14.44    | 11.15  |
| 3   | Eicosane                                   | C₂₀H₄₂ | 282 | 18.35    | 0.67   |
| 4   | Heneicosane                                | C₂₁H₄₄ | 296 | 19.65    | 0.68   |
| 5   | Eicosane                                   | C₂₀H₄₂ | 282 | 20.82    | 0.87   |
| 6   | Eicosane                                   | C₂₀H₄₂ | 282 | 21.88    | 0.97   |
| 7   | Eicosane                                   | C₂₀H₄₂ | 282 | 22.86    | 0.99   |
| 8   | Eicosane                                   | C₂₀H₄₂ | 282 | 23.77    | 1.20   |
| 9   | Tetraatriacontane                          | C₂₄H₃₀ | 478 | 24.65    | 1.46   |
| 10  | Tetraatriacontane                          | C₂₄H₃₀ | 478 | 25.62    | 1.55   |

MF: molecular formula; MW: molecular weight; RT: retention time

DISCUSSION

Plumbagin is known as an anticancer agent. Plumbagin demonstrates the activation of autophagy, apoptosis, and cell cycle arrest in some cancers. Plumbagin also exhibits the capability of angiogenesis inhibition in some cancers.¹⁹ The selection of chloroform as solvents in this study was based on the results of the previous study. Plumbagin is naphthoquinone. Quinone in *Plumbago zeylanica* roots only detected in extract dissolved with chloroform.² Chloroform or trichloromethane belongs to the Class 2 solvent that is non-genotoxic carcinogens for the animal. Chloroform has a possible toxicity that is reversible.²⁰

A previous study mentioned that the concentration of plumbagin in ethanol extract of *Plumbago zeylanica* root was only 1.9%.¹¹ In this study, we aimed to identify the plumbagin content in the roots of *Plumbago zeylanica* from Indonesia. We found a higher concentration of plumbagin that was extracted using chloroform. A high concentration of plumbagin in chloroform extract is never been published before, but the amount of 81% concentration in this study (Table 4) is similar to plumbagin concentration in petroleum ether extract of *P. zeylanica* in a previous study.²¹ This study found 56.88% area of plumbagin in GC-MS result of ethanolic extract (Table 1). This area is higher than the previous study which only found plumbagin in 7.94% area of ethanolic extract.²² Some previous study used cultivated plants from India, while this study used cultivated plants from Indonesia. The geographical location of cultivation may give different results of the phytochemical contents.

CONCLUSIONS

It can be inferred from the current study that plumbagin concentration is four times higher in chloroform extract compared to ethanol extract as revealed through the GC-
### Table 3. Bioactive compounds identified using ethanol and chloroform extracts of *P. zeylanica* roots

| No | Compound name                                      | Structure | Activity on Human Health                  |
|----|----------------------------------------------------|-----------|------------------------------------------|
| 1  | 5-hydroxy-2-methyl-1,4-naphthalenedione            | ![Structure](image1) | - Antiproliferative<sup>3</sup>  
- Antimalarial<sup>8</sup>  
- Antibacterial<sup>9</sup>  |
| 2  | Dihydroxyacetone                                   | ![Structure](image2) | - Skin tanning agent<sup>12</sup>       |
| 3  | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | ![Structure](image3) | Antioxidant<sup>13</sup>               |
| 4  | 5-Hydroxymethylfurfural                           | ![Structure](image4) | - Antioxidant<sup>14</sup>  
- Anti-allergy<sup>14</sup>  
- Anti-inflammation<sup>14</sup>  
- Antihypoxia<sup>14</sup>  
- Antihyperuricemia<sup>14</sup>  |
| 5  | Cyclodecasiloxane, eicosamethyl-                   | ![Structure](image5) | - Antimicrobial<sup>15</sup>  
- Antihelmintic<sup>15</sup>  
- Antioxidant<sup>15</sup>  
- Hepatoprotective<sup>15</sup>  |
| 6  | Sucrose                                            | ![Structure](image6) | Hyperlipidemic agent<sup>16</sup>     |
| 7  | Glycerin                                           | ![Structure](image7) | Skin hydrating agent<sup>17</sup>     |
| 8  | Heneicosane                                        | ![Structure](image8) | Anticancer<sup>18</sup>               |
| 9  | Tetracontane                                       | ![Structure](image9) | Anticancer<sup>18</sup>               |
| 10 | 11-decyltetrasoste                                | ![Structure](image10) | Unknown                                |
| 11 | 2-Allyl-1,4-dimethoxy-3-methyl-benzene             | ![Structure](image11) | Unknown                                |
| 12 | Eicosane                                           | ![Structure](image12) | Unknown                                |
Identification of Plumbagin in Plumbago Zeylanica Roots

**Table 4. The concentration of plumbagin in each sample**

| Solvent type   | Sample weight (g) | Sample volume (ml) | Concentration ppm |
|---------------|-------------------|--------------------|-------------------|
| Chloroform    | 0.0079            | 81                 | 805340            |
| Ethanol       | 0.0102            | 13                 | 128718            |

MS technique. Hence, chloroform solvent is suggested to be used to exploit the potential of plumbagin from *P. zeylanica* as herbal medicine.

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### Conflict of Interest

The authors declare no conflict of interest.

### REFERENCES

1. Pant M, Lal A, Rana S, et al. Plumbago zeylanica L.: a mini review. Int J Pharm Appl 2012; 3(3):399–405.
2. Rajakrishnan R, Lekshmi R, Benil PB, et al. Phytochemical evaluation of roots of Plumbago zeylanica L and assessment of its potential as a nephroprotective agent. Saudi J Biol Sci 2017; 24:760–6.
3. Ito C, Matsui T, Takano M, et al. Anti-cell proliferation effect of naphthoquinone dimers isolated from Plumbago zeylanica. Nat Prod Res 2018; 32(18):2127–32.
4. Ittyavirah SP, Ruby R. Effect of hydro-alcoholic root extract of Plumbago zeylanica L alone and its combination with aqeous leaf extract of Camellia sinensis on haloperidol induced parkinsonism in Wistar rats. Ann Neurosci 2015; 17(2):269–75.
5. Sumsakul W, Plengsuriyakarn T, Chaijaroenkul W, et al. Antimicrobial studies on Plumbago zeylanica Linn. J Med Plant Res 2011; 5(9):1756–61.
6. Thanigavelan V, Venkatachalam K, Venkatachalam L, et al. Hydroalcoholic extract of Plumbago zeylanica Linn root bark exhibit analgesic and anti-inflammatory activities in experimental rat models. Am J Pharm Health Res 2014; 2(4):209–221.
7. Subramaniam V, Paramasivam V. Potential anti-inflammatory activity of Plumbago zeylanica. Asian J Pharm Clin Res 2017; 10(10):372–5.
8. Sumkasuk W, Plengsuriyakarn T, Chaijaroenkul W, et al. Antimalarial activity of plumbagin in vitro and in animal models. BMC Complement Med Ther 2014; 14(1):15.
9. Subhash K, Wabale AS, Kharde MN. Phytochemical screening and antimicrobial studies on Plumbago zeylanica L. Adv Biomes 2013; 4(3):115–7.
10. Ajayi GO, Olagunju JA, Ademuyiwa O, et al. Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of Plumbago zeylanica Linn. J Med Plant Res 2011; 5(9):1756–61.
11. Dohare B, Jain B, Khare S, et al. Comparative estimation of plumbagin in aerial and root part of Plumbago zeylanica using UV-visible spectrophotometric. UKJPB 2015; 3(3):9–14.
12. Ciriminna R, Fidalgo A, Pagliaro M. Dihydroxyacetone: An updated insight into an important bioprocess. Chemistry Open 2018; 7(3):233–6.
13. Yu X, Zhao M, Liu F, et al. Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose-histidine Maillard reaction products. Food Res Int 2013; 51(1):397–403.
14. Shapla UM, Solayman M, Alam N, et al. 5-hydroxymethylfurural (HMF) levels in honey and other food products: effects on bees and human health. Chem Cent J 2018; 12(1):35.
15. Bratty MA, Makeen HA, Alhazmi HA, et al. Phytochemical, cytotoxic, and antimicrobial evaluation of the fruits of Miswak Plant, Salvadora persica L. J Chem 2020; Article ID 4521951.
16. Police SB, Harris JC, Lodder RA, et al. Effect of diets containing sucrose vs. D-tagatose in hypercholesterolemic mice. Obesity 2009; 17(2):269–75.
17. Milani M, Sparavigna A. The 24-hour skin hydration and barrier function effects of a hyaluronic 1%, glycerine 5%, and Centella asiatica stem cells extract moisturizing fluid: an intra-subject, randomized, assessor-blinded study. Clin Cosmet Investig Dermatol 2017; 10:311–5.
18. Swantara MD, Rika WS, Suartika N, et al. Anticancer activities of toxic isolate of Xestospongia testudinaria sponge. Vet World 2019; 12(9):1434–40.
Фитохимическая оценка корней Plumbago Zeylanica из Индонезии и оценка концентрации в них плюмбагина

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Резюме

Введение: Plumbago zeylanica — широко распространённое растение во многих тропических странах. В Индонезии это растение, известное как Down Encock, благотворно влияет на здоровье человека.

Цель: Это исследование направлено на выявление ингредиента плюмбагина в корнях P. zeylanica из Индонезии.

Материалы и методы: Высушенные корни P. zeylanica измельчали вручную, а затем порошок замачивали в этаноле и хлороформе на 24 часа при комнатной температуре. Затем все экстракты P. zeylanica анализировали с помощью газовой хроматографии/масс-спектрометрии (GC-MS). Концентрацию плюмбагина измеряли путём сравнения экстракта с чистым плюмбагином.

Результаты: GC-MS анализ этанольного экстракта и хлороформенного экстракта корней P. zeylanica показал наличие плюмбагина в высоких пределах. Концентрация плюмбагина в этанольном экстракте составила 13%, а в хлороформном — 81%.

Заключение: Хлороформенный экстракт из корней P. zeylanica из Индонезии обнаруживает более высокую концентрацию плюмбагина, чем этанольный экстракт.

Ключевые слова
хлороформенный экстракт корня, GC-MS, лекарственное растение