

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

GC-MS: Gas Chromatography-Mass Spectrometry;

ppm: part per million;

ml: milliliter;

µL: microliter;

g: gram;

NIST/EPA/NIH: 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.¹ Several previous studies have found other benefits of this plant, namely as a nephroprotective agent, antiproliferation of cancer cells, anti-parkinsonism agent, antibacterial agent, analgesic, and anti-inflammation.²⁻⁷ 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, anti-malarial, and antibacterial agent.^{3,8,9} In a previous study, GC-MS was used to find the compound contained in ethanol extract and diethyl extract of *P. zeylanica* root, but they did not find plumbagin.^{2,10} Plumbagin concentration in ethanolic extract of root part has been found to be low (1.9%).¹¹ 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 and big enough 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) with a Trace ISQ LT mass detector (San José, CA, USA) (GC-MS) and a Thermo Scientific AI 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**.

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 showed 5-hydroxy-2-methyl-1,4-naphthalenedione (or 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**).

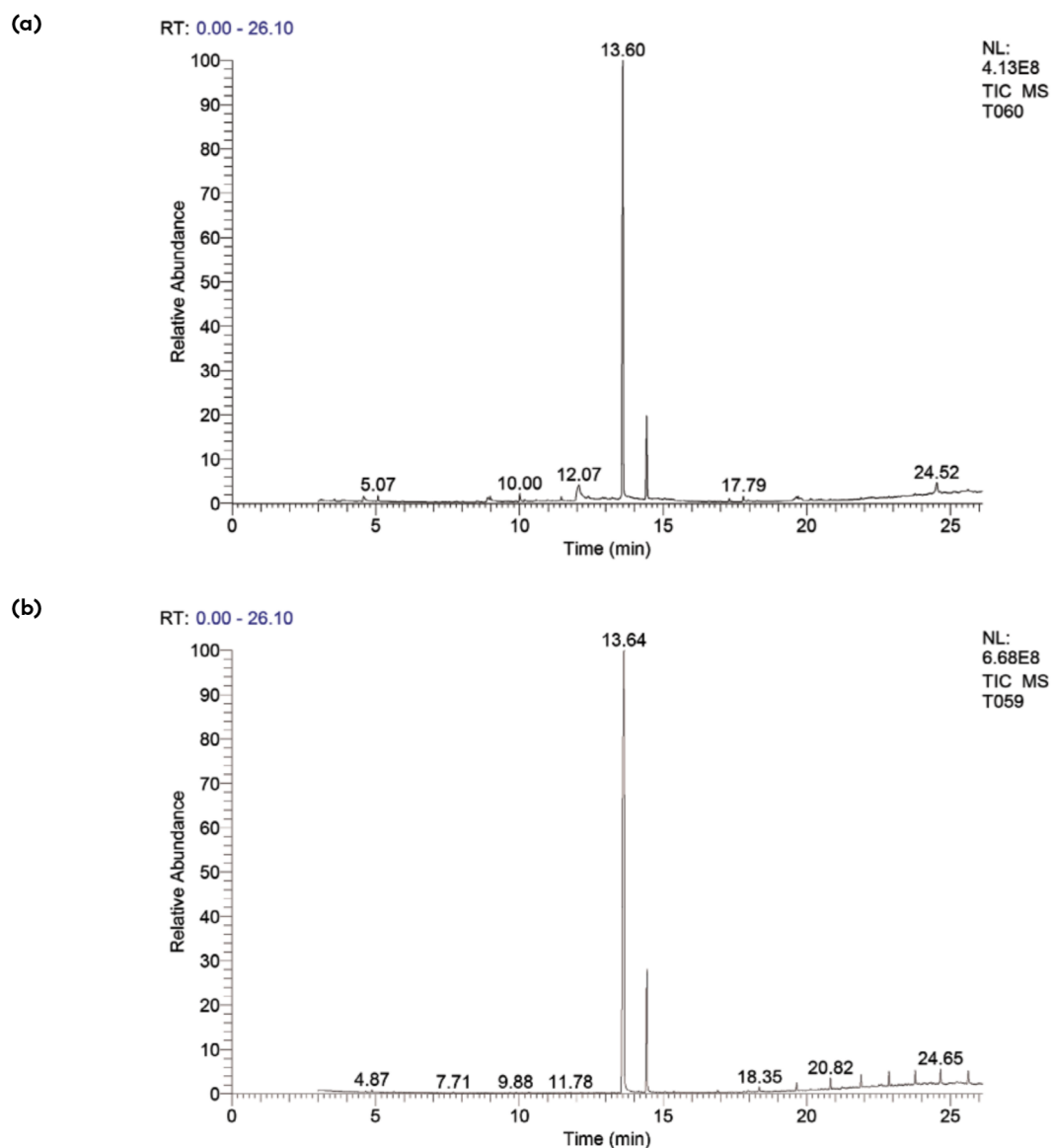


Figure 1. GC-MS examination results. (a) The roots of *P. zeylanica* macerated using absolute ethanol; (b) The roots of *P. zeylanica* macerated using chloroform (CHCl₃).

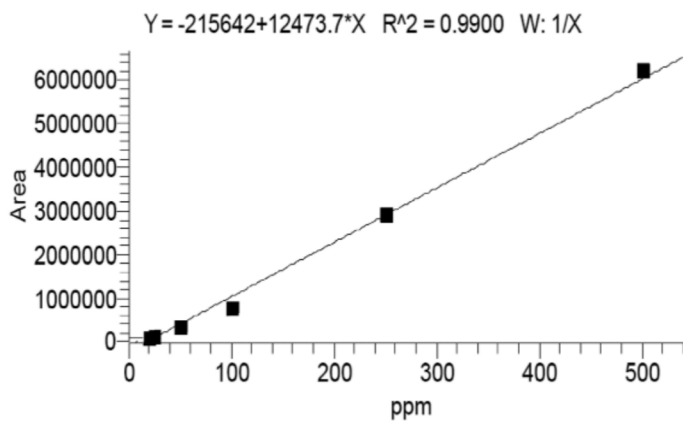


Figure 2. The curve of plumbagin concentration measured by GC-MS.

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	C ₃ H ₆ O ₃	90	4.57	1.47
2	Glycerin	C ₃ H ₈ O ₃	92	8.89	1.02
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144	8.98	1.39
4	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	10.00	1.13
5	Sucrose	C ₁₂ H ₂₂ O ₁₁	342	12.07	7.07
6	5-hydroxy-2-methyl-1,4-naphthalenedione	C ₁₁ H ₈ O ₃	188	13.60	56.88
7	2-Allyl-1,4-dimethoxy-3-methylbenzene	C ₁₂ H ₁₆ O ₂	192	14.42	9.59
8	11-decyltetracosane	C ₃₄ H ₇₀	478	19.64	0.99
9	11-decyltetracosane	C ₃₄ H ₇₀	478	24.52	3.26
10	Cyclodecasiloxane, eicosamethyl-	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	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	C ₁₁ H ₈ O ₃	188	13.64	71.97
2	2-Allyl-1,4-dimethoxy-3-methyl-benzene	C ₁₂ H ₁₆ O ₂	192	14.44	11.15
3	Eicosane	C ₂₀ H ₄₂	282	18.35	0.67
4	Heneicosane	C ₂₁ H ₄₄	296	19.65	0.68
5	Eicosane	C ₂₀ H ₄₂	282	20.82	0.87
6	Eicosane	C ₂₀ H ₄₂	282	21.88	0.97
7	Eicosane	C ₂₀ H ₄₂	282	22.86	0.99
8	Eicosane	C ₂₀ H ₄₂	282	23.77	1.20
9	Tetratriacontane	C ₃₄ H ₇₀	478	24.65	1.46
10	Tetratriacontane	C ₃₄ H ₇₀	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

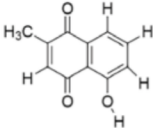
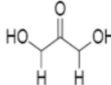
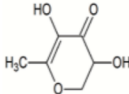
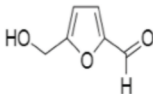
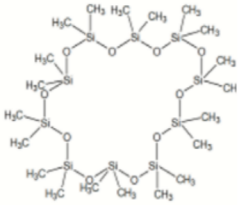
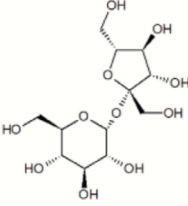
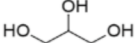


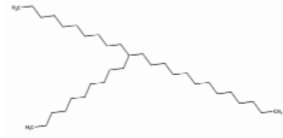
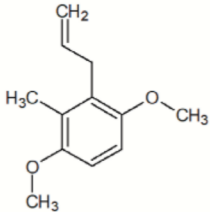

No	Compound name	Structure	Activity on Human Health
1	5-hydroxy-2-methyl-1,4-naphthalenedione		- Antiproliferative ³ - Antimalarial ⁸ - Antibacterial ⁹
2	Dihydroxyacetone		- Skin tanning agent ¹²
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-		Antioxidant ¹³
4	5-Hydroxymethylfurfural		- Antioxidant ¹⁴ - Anti-allergy ¹⁴ - Anti-inflammation ¹⁴ - Antihypoxia ¹⁴ - Antihyperuricemia ¹⁴
5	Cyclodecasiloxane, eicosamethyl-		- Antimicrobial ¹⁵ - Antihelminthic ¹⁵ - Antioxidant ¹⁵ - Hepatoprotective ¹⁵
6	Sucrose		Hyperlipidemic agent ¹⁶
7	Glycerin		Skin hydrating agent ¹⁷
8	Heneicosane		Anticancer ¹⁸
9	Tetratriacontane		Anticancer ¹⁸
10	11-decyltetracosane		Unknown
11	2-Allyl-1,4-dimethoxy-3-methyl-benzene		Unknown
12	Eicosane		Unknown

Table 4. The concentration of plumbagin in each sample

Solvent type	Sample weight	Sample volume	Concentration	
	(g)	(ml)	%	ppm
Chloroform	0.0079	5	81	805340
Ethanol	0.0102	5	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.

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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 хлороформенном – 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
