



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

Mitayani Purwoko^{1,6}, Harijono Kario Sentono², Bambang Purwanto³, Dono Indarto^{4,5}

¹ Department of Medical Biology, Faculty of Medicine, Muhammadiyah University of Palembang, South Sumatera, Indonesia

² Department of Dermato-Venereology, Faculty of Medicine, Dr Moewardi Hospital, Universitas Sebelas Maret Surakarta, Central Java, Indonesia

³ Department of Internal Medicine, Faculty of Medicine, Dr Moewardi Hospital, Universitas Sebelas Maret Surakarta, Central Java, Indonesia

⁴ Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret Surakarta, Central Java, Indonesia

⁵ Biomedical Laboratory, Universitas Sebelas Maret Surakarta, Central Java, Indonesia

⁶ Faculty of Medicine, Universitas Sebelas Maret Surakarta, Central Java, Indonesia

Corresponding author: Mitayani Purwoko, Department of Medical Biology, Muhammadiyah University of Palembang, Jalan K.H. Balqhi, 13 Ulu, Seberang Ulu I, South Sumatera, Palembang, Indonesia; E-mail: mitayani.dr@gmail.com; Tel.: +6281320074327

Received: 14 Sep 2020 ♦ **Accepted:** 4 Nov 2020 ♦ **Published:** 28 Feb 2022

Citation: Purwoko M, Sentono HK, Purwanto B, Indarto D. Phytochemical evaluation of *Plumbago zeylanica* roots from Indonesia and assessment of its plumbagin concentration. *Folia Med (Plovdiv)* 2022;64(1):96-102. doi: 10.3897/folmed.64.e58086.

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 (P_2O_5), and 16% potassium (K_2O). 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**).

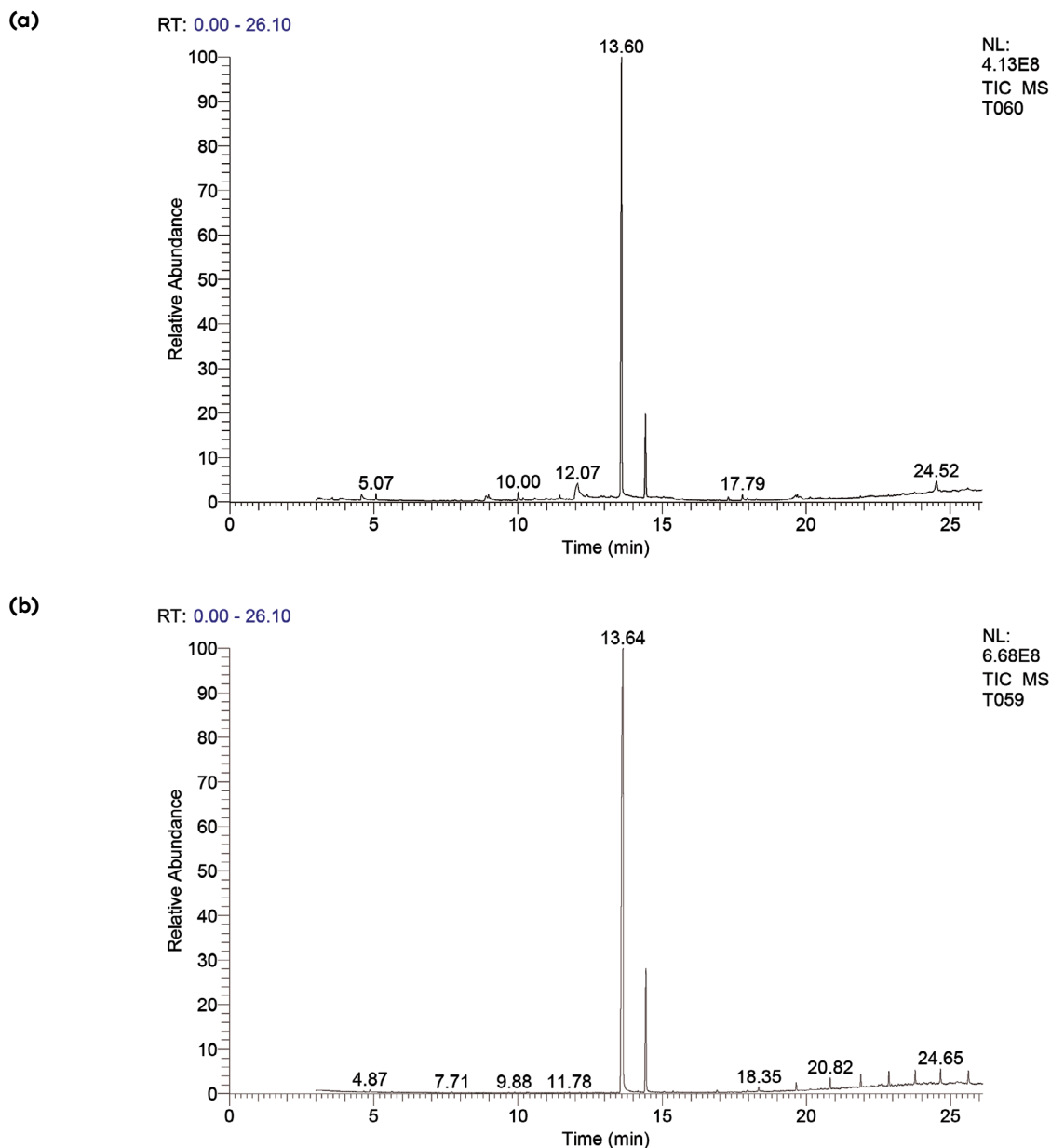


Figure 1. GC-MS examination results. (a) The roots of *P. zeylanica* macerated using absolute ethanol; (b) The roots of *P. zeylanica* macerated using chloroform (CHCl₃).

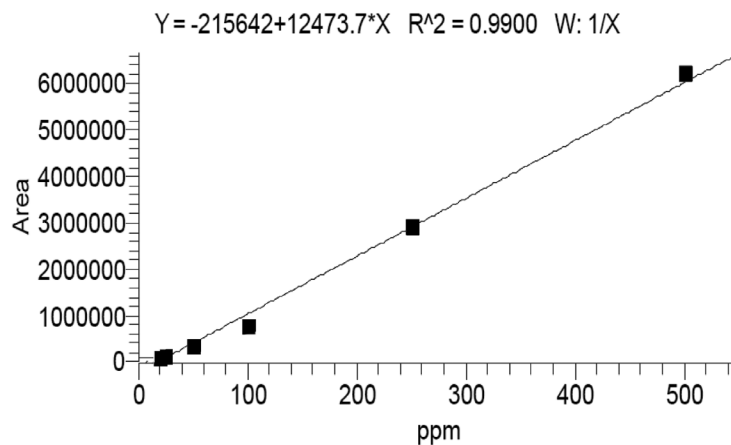


Figure 2. The curve of plumbagin concentration measured by GC-MS.

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

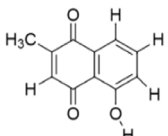
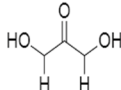
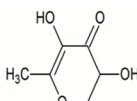
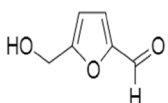
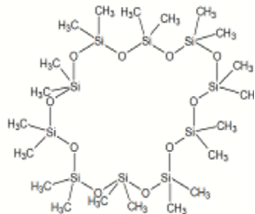
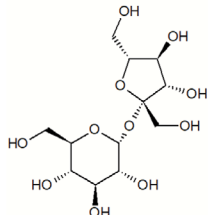
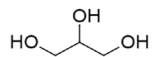


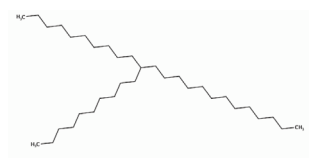
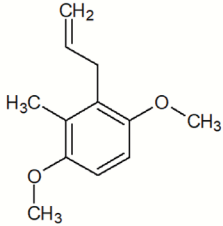
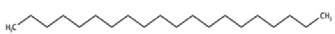
No	Compound name	Structure	Activity on Human Health
1	5-hydroxy-2-methyl-1,4-naphthalenedione		- Antiproliferative ³ - Antimalarial ⁸ - Antibacterial ⁹
2	Dihydroxyacetone		- Skin tanning agent ¹²
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-		Antioxidant ¹³
4	5-Hydroxymethylfurfural		- Antioxidant ¹⁴ - Anti-allergy ¹⁴ - Anti-inflammation ¹⁴ - Antihypoxia ¹⁴ - Antihyperuricemia ¹⁴
5	Cyclodecasiloxane, eicosamethyl-		- Antimicrobial ¹⁵ - Anthelmintic 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.

Acknowledgments

Authors are thankful to the Integrated Laboratory of Chemistry Study Program, Universitas Sriwijaya, Palembang, Indonesia for providing GC-MS examination. Authors are thankful to Biomedical Laboratory of Faculty of Medicine, Universitas Muhammadiyah Palembang, Indonesia for plant extraction service. Authors are thankful to Indonesia Endowment Fund for Education (LPDP), Ministry of Finance, Republic of Indonesia for the scholarship. Authors are thankful to I Gede Alit Pujawan, Muhammad Ainun Najib, and Muhammad Fadhil Rizki Martha for the chemical drawing.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

- Pant M, Lal A, Rana S, et al. *Plumbago zeylanica* L.: a mini review. *Int J Pharm Appl* 2012; 3(3):399–405.
- 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.
- 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.
- Ittiyavirah SP, Ruby R. Effect of hydro-alcoholic root extract of *Plumbago zeylanica* L alone and its combination with aqueous leaf extract of *Camellia sinensis* on haloperidol induced parkinsonism in Wistar rats. *Ann Neurosci* 2014; 21(2):47–50.
- Julaeha E, Herlina T, Mayanti T, et al. [Compounds from ki encok plant (*Plumbago zeylanica*) and the antibacterial activity of dental and mouth disease.] *Chimica et Natura Acta* 2014; 2(2):142–4 [Article in Indonesian].
- 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.
- Subramaniyan V, Paramasivam V. Potential anti-inflammatory activity of *Plumbago zeylanica*. *Asian J Pharm Clin Res* 2017; 10(10):372–5.
- Sumsakul 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.
- Subhash K, Wabale AS, Kharde MN. Phytochemical screening and antimicrobial studies on *Plumbago zeylanica* L. *Adv Biores* 2013; 4(3):115–7.
- 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.
- 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.
- Ciriminna R, Fidalgo A, Pagliaro M. Dihydroxyacetone: An updated insight into an important bioproduct. *Chemistry Open* 2018; 7(3):233–6.
- 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.
- Shapla UM, Solayman M, Alam N, et al. 5-hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. *Chem Cent J* 2018; 12(1):35.
- 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.
- 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.
- 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.
- Swantara MD, Rita WS, Suartha N, et al. Anticancer activities of toxic isolate of *Xestospongia testudinaria* sponge. *Vet World* 2019; 12(9):1434–40.
- Tripathi SK, Panda M, Biswal BK. Emerging role of plumbagin: cytotoxic potential and pharmaceutical relevance towards cancer therapy. *Food Chem Toxicol* 2019; 125:566–82.
- Grodowska K, Parczewski A. Organic solvents in the pharmaceutical industry. *Acta Poloniae Pharmaceutica-Drug Research* 2010; 67(1):3–12.
- Kumar D, Patil PA, Roy S, et al. Comparative toxicity profiles of *Plumbago zeylanica* L. root petroleum ether, acetone and hydroalcoholic extracts in Wistar rats. *Ayu* 2015; 36:329–34.
- Panicker S, Haridasan VK. In vitro cytotoxicity study on U87 cells using root extracts of *Plumbago* species and GC-MS study. *Pharmacogn J* 2018; 10(6s):71–6.

Фитохимическая оценка корней *Plumbago Zeylanica* из Индонезии и оценка концентрации в них плюмбагина

Митаяни Пуруоко^{1,6}, Хариджоно Каро Сентоно², Бамбанг Пуруанто³, Доно Индарто^{4,5}

¹ Кафедра медицинской биологии, Медицинский факультет, Университет „Мохамадия“ Палембанг, Южная Суматра, Индонезия

² Кафедра кожной венерологии, Медицинский факультет, Больница „Д-р Моеуарди“, Университет „Себелас Марет“, Суракатра, Центральная Ява, Индонезия

³ Кафедра внутренних болезней, Медицинский факультет, Больница „Д-р Моеуарди“, Университет „Себелас Марет“, Суракатра, Центральная Ява, Индонезия

⁴ Кафедра физиологии, Больница „Д-р Моеуарди“, Университет „Себелас Марет“, Суракатра, Центральная Ява, Индонезия

⁵ Биомедицинская лаборатория, Больница „Д-р Моеуарди“, Университет „Себелас Марет“, Суракатра, Центральная Ява, Индонезия

⁶ Медицинский факультет, Университет Себелас Марет Суракарта, Центральная Ява, Индонезия

Адрес для корреспонденции: Митаяни Пуруоко, Кафедра медицинской биологии, Медицинский факультет, Университет „Мохамадия“ Палембанг, Джалан К.Х. Баляхи, ул. „Улу“ № 13, Себаранг Улу 1, Южная Суматра, Палембанг, Индонезия; E-mail: mitayani.dr@gmail.com; Тел.: +6281320074327

Дата получения: 14 сентября 2020 ♦ **Дата приемки:** 4 ноября 2020 ♦ **Дата публикации:** 28 февраля 2022

Образец цитирования: Purwoko M, Sentono HK, Purwanto B, Indarto D. Phytochemical evaluation of *Plumbago zeylanica* roots from Indonesia and assessment of its plumbagin concentration. *Folia Med (Plovdiv)* 2022;64(1):96-102. doi: 10.3897/folmed.64.e58086.

Резюме

Введение: *Plumbago zeylanica* — широко распространённое растение во многих тропических странах. В Индонезии это растение, известное как Down Epscock, 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