Trop J Nat Prod Res, September 2022; 6(9):1440-1442 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Identification of Chemical Compounds in *Plumbago zeylanica* Linn Leaves from Indonesia

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ARTICLE INFO

Article history:
Received 13 July 2022
Revised 24 July 2022
Accepted 21 September 2022
Published online 01 October 2022

ABSTRACT

Plumbago zeylanica L., commonly known as leadwort or Ki Encok plant in Indonesia, has some beneficial effects on human health, such as anticancer, anti-microbial, anti-inflammatory, wound healing, and anti-diabetic. Therefore, this experimental study aims to evaluate the chloroform extracts of *P. zeylanica* L. leaves for the isolation of the phytoconstituents. The crushed form of the leaves from South Sumatera (6.5 g) was macerated in 65 ml of chloroform for 5 days, while a total of 80 g leaves powder from Central Java was dissolved in 800 ml of chloroform. The extracts were filtered and evaporated to dryness using a rotary evaporator. Subsequently, the identification of secondary metabolites was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). In conclusion, the leaves of *P. zeylanica* L. contain *β*-sitosterol, lupeol, plumbagin, phytol, and lauric acid.

Keywords: Beta-sitosterol, Gas chromatography, Leadwort, Lupeol, *Plumbago zeylanica*.

Introduction

Plumbago zeylanica L. (*P. zeylanica* L.) plant, which originally comes from South Asia, has spread worldwide in several tropical and subtropical countries with different local names such as doctor bush, Ceylon leadwort, chitrak, and the Indonesian name "Ki Encok". This plant has been recognized by Indian communities for thousands of years and used for traditional medicine as anti-microbial, anti-inflammatory, wound healing, anti-diabetic, central nervous system stimulant, anti-fertility, larvicidal, anti-tumour, and anti-viral agents.[1]
The preliminary phytochemical screening of the leaves revealed the presence of saponin, phenolic, flavonoids, alkaloids, terpenoids, sterols, amino acids, tannin, anthraquinones, cholesterols, and quinones.[2] Furthermore, various secondary metabolites have been identified, including plumzeylanone, plumbagin, droseron, zeylanone, and zeylanone epoxide, which are responsible for the aforementioned therapeutic effects.[3] The majority of previous studies on *P. zeylanica* L. were conducted in South Asian countries such as India.[4-6] There are only a few investigations into the secondary metabolite content of the plant native to Indonesia. The Indonesian archipelago is comprised of hundreds of islands, and the geographical characteristics of these islands might vary. Besides, previous studies mostly used the roots[4-7] and not the leaves, hence, the secondary metabolites in the leaves are relatively unknown.

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Citation: Purwoko M, Indarto D, Purwanto B, Soetrisno S, Kariosentono H. Identification of Chemical Compounds in *Plumbago zeylanica* Linn Leaves from Indonesia. Trop J Nat Prod Res. 2022; 6(9):1440-1442. http://www.doi.org/10.26538/tjnpr/v6i9.17

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

One of the disadvantages of using roots as the material is that the plant will die culminating in the need to grow them again. The rationale of this study is to provide information on the secondary metabolites of *P. zeylanica* L. leaves to serve as a scientific foundation for their use in herbal medicine. In addition, previous studies demonstrated that geographical and climate circumstances affect the secondary metabolite composition.[8,9] Due to the paucity of information regarding the content and the effect of geographical conditions, there is a need to conduct an experiment using *P. zeylanica* L. native to two distinct regions in Indonesia.

Materials and Methods

Plumbago zeylanica L. plants
Two types of *P. zeylanica* L. plants were treated, the first one was cultivated in October 2019 in Palembang, South Sumatra (PZSS). This plant was identified by a botanist, Mrs. Susi Dewiyeti, from the Faculty of Education, Universitas Muhammadiyah Palembang, South Sumatera, Indonesia with deposit voucher number 141. The leaves were picked in the afternoon of January 2020, then washed under tap water until clean, and air dried in an open room for 18 days, without exposure directly to sunlight. Next, the dried leaves were crushed with a blender to make powder. The second material was *P. zeylanica* L. leaf powder, purchased from Herbadream Store in Surakarta, Central Java (PZCJ).

Leaves extraction
The crushed form of PZSS (6.5 g) was macerated in 65 ml chloroform (Emsure®, Merck, Germany) for 5 days, while a total of 80 g leaves powder of PZCJ was dissolved in 800 ml Chloroform. The extracts were filtered and evaporated to dryness using rotary evaporator (B-One Rotary Evaporator Model RE-1000VN) at 60°C, 52 rpm, for 20 minutes. Finally, the filtrates were stored in the refrigerator before further analysis.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis was conducted using a Thermo Scientific Trace 1310 Series (Thermo Fisher Scientific, San Jose, CA, USA) with a Trace ISQ LT mass detector (San Jose, CA, USA) equipped with a Thermo Scientific AI 1310 automatic injector (San Jose, CA, USA). The chromatographic conditions were as follows: a ZB-5MS (30 m x 0.25 mm x 0.25 μm) column from Phenomenex® (Torrance, CA, USA) with an injector temperature of 300°C. The injection mode was splitless, and the volume was 1 μL, while the Main EI MS Library (mainlib) from NIST/EPA/NIH was used for identification purposes.

Results and Discussion

The 10 highest peaks in chromatograms of chloroform extracts from PZSS are presented in Table 1. One of the secondary metabolites found was phytol which is very beneficial to health. It has several benefits, such as acting as an antioxidant, causing apoptosis, reducing inflammation, and killing bacteria.[10] Besides, dodecanoic acid or commonly known as Lauric acid, was found in the chloroform extract. It is also found in virgin coconut oil and has antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium,* and *Escherichia coli*.[11,12]

A total of 10 compounds with the highest peaks were identified in the chloroform extracts of PZCJ as shown in Table 2. Based on further analysis using the EI MS Library, both chloroform extracts similarly consist of 1-undecanol, dodecyl acrylate, tributyl acetylcitrate, Decyl propanoate, and 3-(2-oxopropyl) cyclopentane-1-one compounds. However, the proportion of these five compounds in PZSS was lower than in PZCJ. The highest proportion of chemical compounds in the chloroform extract of PZSS was 1-Undecanol (Table 1) and in PZCJ was Dodecylacrylate (Table 2). One of the secondary metabolites found in the chloroform extract of PZCJ was β-sitosterol (Table 2). This metabolite is known to have biological actions as anti-diabetic, analgesic, anti-inflammatory, and anticancer.[13-15] A molecular docking study reported that β-sitosterol has activity against Protein Kinase C.[16] This conclusion is not coincidental with the results of Dhalani et al. (2020), who identified γ-sitosterol, Taraxasterol, and Lanosterol as non-polar molecules in *P. zeylanica* L. leaves, but not β-sitosterol.[17] This study also found Lupeol in the chloroform extract of PZCJ as shown in Table 2. It is known as a multi-target agent, similar to Plumbagin, and has biological activities as anticancer and anti-inflammatory.[18,19] Furthermore, a small amount of Plumbagin was found in the chloroform extract of PZCJ (Table 2). Previous studies reported that the leaf part of *P. zeylanica* L. from Tamil Nadu, India contained Plumbagin up to 0.00007%.[20] This compound is known to have anti-cancer and anti-inflammatory activities.[21]

According to previous reports, latitude and altitude affect the secondary metabolites of *Lavandula angustifolia* Mill. The composition in some volatile chemical substances and essential oils reduced as the latitude increased.[22] Secondary metabolites in a plant are also affected by climate and edaphic factors.[23] In this study, Central Java and South Sumatera are located on different islands. The differences in chemical compounds between the chloroform extract of PZSS and PZCJ are presumably caused by climate, weather, and soil type.

Conclusion

Plumbago zeylanica L. is a useful medicinal plant with various secondary metabolites, which has some pharmacological properties for treatment of human diseases. The leaves of *P. zeylanica* obtained from Central Java contain β-sitosterol, lupeol, and plumbagin, all of which have anti-cancer and anti-inflammatory properties, while those from South Sumatera contain phytol and lauric acid. Variable climatic and environmental growth conditions culminate in a vast phytochemical diversity of these resources. The method of determining geographical factors for the existence of secondary metabolites can assist in choosing places for collecting plant material with the necessary chemical profile.

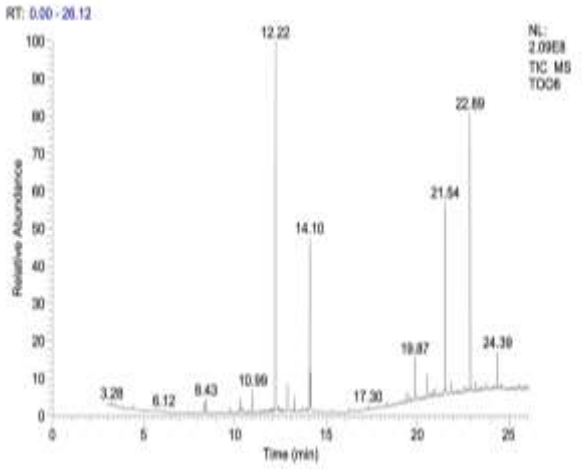

Figure 1: Chromatogram of chloroform extracts of PZSS.

Table 1: Compounds contained in chloroform extracts of *P. zeylanica* L. leaves from South Sumatera (sorted from high to low % area)

No	Chemical compounds	Formula	RT[a] (min)	% area
1	1-Undecanol	$C_{11}H_{24}O$	12.22	25.58
2	Hexanedioic acid,bis (2-ethylhexyl) ester	$C_{22}H_{42}O_4$	22.89	24.47
3	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	21.54	17.63
4	Dodecyl acrylate	$C_{15}H_{28}O_2$	14.10	15.58
5	Phytol	$C_{20}H_{40}O$	19.86	3.91
6	Decyl propanoate	$C_{13}H_{26}O_2$	14.17	3.46
7	Dodecyl dodecanoate	$C_{24}H_{48}O_2$	24.39	3.26
8	Dodecanoic acid (lauric acid)	$C_{12}H_{24}O_2$	12.88	2.26
9	Dibutyl decanedioate	$C_{18}H_{34}O_4$	20.51	2.05
10	3-(2-oxopropyl) cyclopentane-1-one	$C_8H_{12}O_2$	10.99	1.81

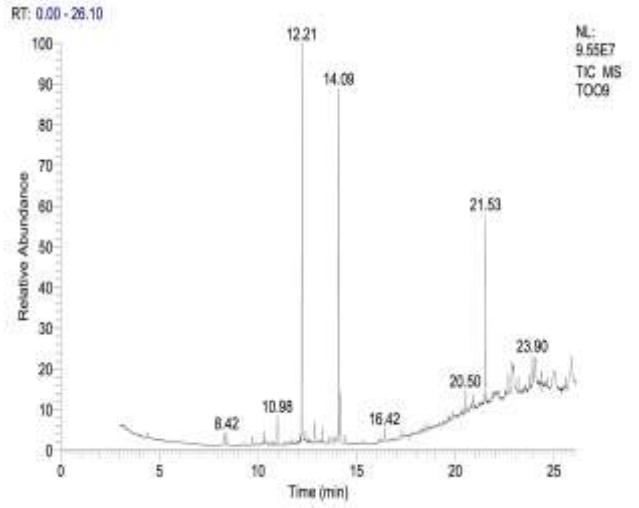

Figure 2: Chromatogram of GC-MS analysis of chloroform extracts of PZCJ.

Table 2: Compounds contained in chloroform extracts of PZCJ (sorted from high to low % area)

No	Chemical compounds	Formula	RT[a] (min)	% area
1	Dodecyl acrylate	$C_{15}H_{28}O_2$	14.09	32.28
2	1-Undecanol	$C_{11}H_{24}O$	12.21	27.70
3	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	21.53	18.39
4	Decyl propanoate	$C_{13}H_{26}O_2$	14.16	4.51
5	1-Heptatriacontanol	$C_{37}H_{76}O$	24.04	4.39
6	3-dodecanoyloxypropyl dodecanoate	$C_{27}H_{52}O_4$	22.68	2.98
7	1,3-dihydroxypropan-2-yl hexadecanoate	$C_{19}H_{38}O_4$	23.90	2.98
8	Lupeol	$C_{30}H_{50}O$	25.87	2.47
9	3-(2-oxopropyl)cyclopentane-1-one	$C_8H_{12}O_2$	10.98	2.31
10	Beta Sitosterol	$C_{29}H_{50}O$	22.88	1.98
11	5-hydroxy-2-methyl-1,4-naphthalenedione (Plumbagin)	$C_{11}H_8O_3$	13.56	0.35

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are grateful to Lembaga Pengelola Dana Pendidikan the Ministry of Finance Republic of Indonesia for the scholarship.

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