

Bioactivity Evaluation of *Leucas zeylanica* and its Phytochemicals

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Abstract *Leucas zeylanica* belongs to the Lamiaceae family has high medicinal value. Present study focused on the isolation and bioactivities of aerial part of *L. zeylanica*. The phytochemicals screening results showed that terpenoids, flavonoids, tannins, saponins, phenols and alkaloids are present in aerials part of *L. zeylanica*. Vacuum liquid chromatography (VLC) and column chromatography (CC) techniques were used in fractionation and purification of the crude extracts. The structure of isolated compound was elucidated by Infrared (IR) and Nuclear Magnetic Resonance (¹H, ¹³C NMR) spectroscopies. A compound was successfully isolated from the chloroform extract and identified as vanillin. Hexane crude extract contained abundance of terpenoids while methanol crude extract was abundance in phenols, tannins and flavonoids. The antioxidant activity of the crude extracts was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. Methanol extract was found to show good scavenging activity with IC50 value of 145.9 µg/mL. This is in accordance to the results showing that methanol extract showed the highest total phenolic content and total flavonoid content which were 133.0 mg GAE/g and 238.0 mg QE/g, respectively. The anthelmintic activity of the crude extracts was also tested by using adult earthworms *Lumbricus rubellus* and it was found that the activity is dose dependent for methanol and chloroform extracts. The result showed that methanol extract at concentration of 50 mg/mL exhibited better activity than other extracts but not comparable with the reference drug Albendazole.

Keywords: *Leucas zeylanica*, phytochemicals, antioxidant, anthelmintic.

Introduction

Leucas zeylanica which is locally known as ketumbit has high medicinal value. This plant grows in many tropical and temperate countries except for areas with very low temperature. This species is belongs to Lamiaceae family which also known as mint family. Lamiaceae family has about 236 genera and around 7,173 species and they are mostly annual or perennial herbs or shrubs having aroma such as thyme, spearmint and rosemary [1, 2]. In India, indigenous tribes used it for treating fever, jaundice, snake and scorpion bites [3, 4]. In addition, recent research found that the aqueous extract of the leaves exhibited antidiabetic effect, in which it reduced the blood glucose level in alloxan-induced diabetic rats significantly. It also provides stomach ache relief especially due to roundworms for children [5]. It is found that the lipophilic extract of *L. zeylanica* contains phytosterols, fatty acids, sesquiterpenes and diterpenes [6]. The presence of flavonoids, steroids and alkaloids are confirmed in the acetone and ethanolic extracts of *L. zeylanica* [7]. The bioactivity studies reported on this plant are antibacterial and antidiabetic activities [5, 8]. Based on literature survey, there is less study done on the phytochemicals and bioactivity of this species. This species gets lesser attention in various studies than other species in genus *Leucas*. Although being used as a traditional medicine by locals as anthelmintic and wound healing agent, its other pharmacological properties are yet to be scientifically studied and confirmed. Lacking of data on their biological activities, hence get less opportunities to promote its uses. It requires more scientific studies to unveil its pharmacological properties. Thus, it is important to thoroughly studied the phytochemicals and bioactivity of *L. zeylanica*.

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Materials and methods

Plant Materials

The plant was collected from Pulau Gajah, Pengkalan Chepa, Kelantan in October 2019. The voucher number of this plant is UKMB40376. The aerial parts were dried and finely grounded before proceeding for extraction.

Extraction and Isolation

The dried sample powder of the aerial parts of *L. zeylanica* (610.0 g) was macerated with *n*-hexane at room temperature for three days. The same procedure repeated by using solvents chloroform and methanol on the same sample. The crude extracts were collected and filtered under gravity filtration. The filtrate then was evaporated by a rotary evaporator at 40°C. The chloroform crude extract (4.10 g) was fractionated by vacuum liquid chromatography (VLC) on silica gel 230-400 mesh (100.0 g) and further isolated using column chromatography (CC) successfully isolated vanillin (**1**) (9.1 mg).

Characterization of Isolated Compound

The ¹H NMR (300 MHz) and ¹³C NMR (100 MHz) spectra were recorded on Bruker Advance 300 spectrometer using deuterated chloroform (CDCl₃) as solvents. The infrared spectra were recorded on Perkin-Elmer series 1650 spectrophotometer with KBr pellet for solid samples. The GC analyses were performed using Hewlett Packard HP 6890 series II A gas chromatograph equipped with Ultra 1 column (25 m long, 0.33 μm thickness, and 0.20 mm inner diameter). The GC-MS chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer.

Phytochemicals Screening

Phytochemicals screening was performed on the crude extracts to test for the presence of terpenoids, flavonoids, tannins, saponins, phenols and alkaloids. For terpenoids, concentrated sulphuric acid was added to form a layer and brown colouration at the interface indicated presence of terpenoids. Dilute sodium hydroxide turned extract solution to yellow and became colourless when dilute hydrochloric acid added indicated presence of flavonoids. The presence of tannins was confirmed when extract solution turned green after 1 % ferric chloride solution was added. Saponin was tested positive when foam layer on top persisted for more than 15 minutes after distilled water was added to the extract solution and shaken vigorously. Formation of green colour solution after distilled water and 10 % ferric chloride solution was added indicated presence of phenols [9]. Mayer's reagent was added to extract solution and the formation of cream precipitate indicated the presence of alkaloids.

Antioxidant Activity

Antioxidant capacity was measured using DPPH scavenging assay with slightly modification [10]. Total phenolic content (TPC) and total flavonoid content (TFC) were tested using Folin-Ciocalteu and Aluminium chloride colorimetric methods, respectively [11, 12]. Absorbance was measured at 760 nm for TPC and 510 nm for TFC.

Anthelmintic Activity

Anthelmintic assay was prepared according to Gupta & et al. [13]. The test subjects were adult earthworms *Lumbricus rubellus* with width between 3-5 mm and length of 8-12cm. They were acquired from a local shop. The reference drug used was Albendazole. The crude extracts were dissolved in 5% dimethyl sulphoxide (DMSO) before subjected to sonication. Three earthworms were placed in each aqueous solutions of crude extracts at concentration 50, 25 and 10 mg/ml. The time for the earthworms to become motionless was recorded as paralysis time and the time when the earthworms no longer respond to applied external stimuli was recorded as the death time.

Results and discussion

Phytochemicals screening showed there was abundance of terpenoids present in hexane and chloroform extracts while in methanol extract, small amount of terpenoids was present as shown in Table 1. Chloroform extract also contained small amount of flavonoids and phenols. In methanol extract, flavonoids, tannins, saponins and phenols are present abundantly. A very small amount of

alkaloids was found to present in methanol extract.

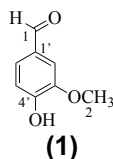
Isolation using column chromatography successfully isolated one compound elucidated as vanillin (**1**) (9.1 mg, 2.67 %) a white needle solid with a melting point of 77-78°C with a R_f value of 0.40 in *n*-hexane-diethyl ether (1:1); IR ν_{\max} cm^{-1} : 3164 (O-H), 3027 (sp^2 C-H), 2946 (sp^3 C-H), 2857, 2747 (aldehyde C-H), 1662 (C=O), 1586, 1464 (C=C aromatic), 1151 (C-O); ^1H NMR (CDCl_3 , 300 MHz) (Appendix 2): δ 3.98 (3H, s, H-2), δ 6.25 (1H, br. s, 4'-OH), δ 7.06 (1H, d, $J=8.4$ Hz, H-5'), δ 7.43-7.45 (2H, m, H-2', H-6'), δ 9.84 (1H, s, H-1); ^{13}C NMR (CDCl_3 , 75 MHz) (Appendix 3): δ 56.2 (C-2), δ 108.7 (C-2'), δ 114.3 (C-5'), δ 127.7 (C-6'), δ 129.9 (C-1'), δ 147.1 (C-4'), δ 151.7 (C-3') and δ 191.0 (C-1). GC-MS with positive mode, electron ionization gave m/z 152 (74 %, M^+), 151 (100 %, $\text{M}^+ - \text{H}$), 137 (4 %, $\text{M}^+ - \text{CH}_3$), 123 (20 %, $\text{M}^+ - \text{CHO}$).

Table 1. Phytochemicals screening of aerial parts of *L. zeylanica*

Test	Crude Extract		
	Hexane	Chloroform	Methanol
Terpenoids	+++	++	+
Flavonoids	-	+	+++
Tannins	-	-	+++
Saponins	-	-	++
Phenols	-	+	+++
Alkaloids	-	-	+

- : absent, + : trace amount, ++ : average amount, +++ : appreciable amount.

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Free radical scavenging activity of crude extracts was tested by using DPPH assay at concentrations of 62.5, 125, 250, 500 and 1000 ppm (Table 2). Methanol extract exhibited good antioxidant capacity compared to the others extract with low SC_{50} value of 145.9 ppm. This data was in line with the TPC and TFC results of methanol extract. This proved correlation of phenolics and flavonoids content with good scavenging activity. Both hexane and chloroform extracts do not possess significant TPC value while methanol extract has TPC value of 133.0 mg GAE/g extract. Meanwhile, hexane extract also does not possess significant TFC as it does not fall into calibration range. It can be seen the increasing pattern of TPC and TFC value with the solvent polarity. This also can be seen from the scavenging activity of hexane extract which showed high SC_{50} value, 10128.5 ppm. Chloroform and methanol extract showed TFC of 55.5 mg QE/g and 238.0 mg QE/g, respectively. The presence of phenolic compounds with high number of hydroxyl groups which able to donate hydrogen to stabilize the free radicals [11]. The paralysis time and death time of each group for anthelmintic study are summarized in Table 3. Control was observed for more than 8 hours and no paralysis or death were occurred. Methanol extract has the greatest anthelmintic strength as compared to other extracts. Based on

previous study, the presence of tannin in methanol extracts increase the anthelmintic capacity [14]. For chloroform extract at 25 and 10 mg/mL, no death was occurred. Hexane extract at all concentrations did not incurred death nor paralysis on earthworms.

Table 2. SC₅₀ value, TPC and TFC of crude extracts

Extract	DPPH SC ₅₀ (ppm)	TPC (mg GAE/g)	TFC (mg QE/g)
Hexane	10128.5	-	-
Chloroform	2695.8	-	55.0
Methanol	145.9	133.0	238.0
Gallic acid	14.2	-	-

Table 3. Paralysis and death time of earthworms

Group	Treatment	Concentration (mg/mL)	Effect	
			Paralysis time ± SD (min)	Death time ± SD (min)
Control	Drugless	5 % DMSO in distilled water	-	-
Test	Methanol	50	17.0 ± 1.7	41.0 ± 3.6
		25	41.7 ± 2.1	108.0 ± 4.6
		10	125.0 ± 4.6	237.3 ± 0.6
	Chloroform	50	134.7 ± 7.6	217.0 ± 9.2
		25	401.0 ± 1.7	-
		10	-	-
	Hexane	50	-	-
		25	-	-
		10	-	-
Standard	Albendazole	20	17.3 ± 1.5	23.0 ± 2.6

Conclusions

In conclusion, this study successfully isolated one compound from the aerial parts of *L. zeylanica* which elucidated as vanillin. Besides the antioxidant and anthelmintic activity of this genus have been successfully carried out and have found prominent results. Very little phytochemicals study has been carried out on the genus of *Leucas* especially on *L. zeylanica* though it has been used by local peoples to treat roundworms for children. Therefore, more phytochemical studies are required to validate the claimed of their traditional uses.

Conflicts of interest

There is no conflict of interest regarding this study.

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References

- [1] R. M. Harley, "Checklist and key of genera and species of the Lamiaceae of the Brazilian Amazon," *Rodriguesia*, 63(1), 129, 2012.
- [2] R. R. Raja, "Medicinally potential plants of Labiatae (Lamiaceae) family: An overview," *Research Journal of Medicinal Plant*, 6(3), 203, 2012.
- [3] M. Khanam, M. "A critical study of the genus *Leucas* R. Br. (Lamiaceae) from Bangladesh," *Bangladesh Journal of Plant*, 12(1), 1, 2005.
- [4] D. L. Jain, A. M. Baheti, S. R. Jain, and K.R. Khandelwal, "Use of medicinal plants among tribes in Satpuda region of Dhule and Jalgaon districts of Maharashtra—An ethnobotanical survey," *Indian Journal of Traditional Knowledge*, 9(1), 152, 2010.
- [5] M. Dutta, M. Uddin, "Antidiabetic potentiality of aqueous leaf extract of *Leucas zeylanica* in alloxan-induced diabetic rats," *Journal of Innovations in Pharmaceutical and Biological Sciences*, 3(4), 121, 2016.
- [6] M. Napagoda, J. Gerstmeier, H. Butschek, S. Lorenz, "Lipophilic extracts of *Leucas zeylanica*, a multi-purpose medicinal plant in the tropics, inhibit key enzymes involved in inflammation and gout," *Journal of Ethnopharmacology*, 224, 474, 2018.
- [7] B. Radhika, T. Srilekha, S. Chaitanya, K. Sandhya, "Pharmacognostic and preliminary phytochemical evaluation of the leaves of *Leucas zeylanica*," *International Journal of Biomedical Investigation*, 1(1), 102, 2018.
- [8] F. Abdullah, S. N. Nasir, D. K. Han, S. Appalasamy, et al., "Potential of *Leucas zeylanica* extract to eliminate *E. coli* and *S. aureus* in *Corbicula fluminea* ("Etak") tissue," *Malaysian Journal of Fundamental and Applied Sciences*, 15(4), 597, 2019.
- [9] S. Handayani, R. Arianingrum, W. Haryadi, "Vanillin structure modification of isolated vanilla fruit (*Vanilla Planifolia* Andrews) to form vanillinacetone," *Proceedings at 14th Chemical Congress 2011*, 252, 2011.
- [10] S. E. Hashim, H.M. Sirat, K. H. Yen, I. S. Ismail, S. N. Matsuki, "Antioxidant and alpha glucosidase inhibitory constituents from *Hornstedtia* species of Malaysia," *Natural Products Communication*. 10(9), 9, 2015.
- [11] C. Sulaiman, I. Balachandra, "Total phenolics and total flavonoids in selected Indian medicinal plants," *Indian Journal of Pharmaceutical Sciences*. 74, 258, 2012.
- [12] S. Chandra, S. Khan, B. Avula, H. Lata, M. H. Yang, M. A. Elsohly, I. A. Khan, "Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study," *Evidence-Based Complementary and Alternative Medicine*, 2014(4), 253875, 2014.
- [13] J. K. Gupta, K. K. Agrawal, A. Verma, K. Singh, "Investigation of in-vitro anthelmintic activity of *L. lavandulifolia*, *L. cephalotes* and *L. aspera*," *Journal of Pharmacy Research*, 5(1), 212, 2012.
- [14] R. Ramani, H. B. Karra, B. M. Boddupalli, R. N. Aniseti, "Pharmacognostical, phytochemical and anthelmintic evaluation of *Leucas indica* (L)," *Pharmacognosy Journal*, 2(10), 317, 2009.