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Analysis of phytochemical constituents and antioxidant activity from the fractions of *Luvunga sarmentosa* root extract using LCMS/MS

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Abstract. *Syarpin, Permatasari S, Pujianto DA. 2023. Analysis of phytochemical constituents and antioxidant activity from the fractions of* Luvunga sarmentosa *root extract using LCMS/MS. Biodiversitas 24: 733-740. Luvunga sarmentosa*, one of the native plants of Central Kalimantan, has been used by Dayak people, especially its root, to increase sexual activity, cure sexual dysfunction and increase male fertility. Dayak people boil the stems and roots of *L. sarmentosa* and drink the extract. However, studies on *L. sarmentosa* are still limited to phytochemical content and bioactivity screening of the root extract of *L. sarmentosa*. This research aimed to determine the phytochemical content and antiradical activities of *L. sarmentosa* root fractions. Fractions were obtained using solvents with different polarities. The sample was extracted by maceration using ethanol, and the extract was fractionated by column chromatography using hexane, chloroform, ethyl acetate, and methanol. The chemical content of each fraction was identified using LCMS/MS. The antiradical activity was performed against DPPH using a UV-Visible spectrophotometer. The LCMS/MS analysis results indicated that each fraction contained different percentages of compounds, such as alkaloids, terpenoids, phenylpropanoids, stilbenes, flavonoids, tannins, fatty acids, and steroids. Ostruthin was a compound detected in all fractions with a retention time of 20,917 minutes and was considered a marker compound from the roots of *L. sarmentosa*. The antiradical activity assay showed that the methanol fraction had the best activity with an IC_{50} value of 83.42 ppm. The IC_{50} data shows that increasing the percentage of alkaloid compounds increased their antioxidant activity.

Keywords: Alkaloids, antioxidants, LCMS/MS, *Luvunga sarmetosa*, Ostruthin

INTRODUCTION

In Indonesia, especially Central Kalimantan, various species of plants have been used as herbal medicine. For example, Saluang belum (*Luvunga sarmentosa*), one of the native plants of Kalimantan, increases stamina. The stems and roots are frequently used with various methods of serving. Some of them cut the stems/roots of the plant into small pieces, clean them, brew them with hot water, and drink the extracts. Some people filter before consuming it, depending on the habits of the local community (Fauzi and Widodo 2019).

Luvunga is a genus of the family Rutaceae (Al-Zikri et al. 2016). *Luvunga sarmentosa* is an accepted name based on the POWO. However, the plant lists' data stated that *L. sarmentosa* is unresolved (www.theplantlist.org. 2022). Studies on several species of the genus *Luvunga* have been carried out. The stem and leave extract of *Luvunga scandens* has a cytotoxic activity (Al-Zikri et al. 2016), antibacterial activity (Van et al. 2020), and an aphrodisiac (Fauzi and Widodo 2019). The phytochemical content of several species of the genus *Luvunga* has been reported. Leaf extract of *L. sarmentosa* (Lien et al. 2002) contained triterpenoids. The root extract of *L. scandens* contained isobutyl glycosinolate, ß-sitosterol, and stigmasterol (Sirinut et al. 2017), while tirucallane triterpenes were isolated from the stem extract of *L. scandens* (Al-Zikri et al. 2014).

Identifying secondary metabolites in plants can be performed in several ways, one of which is by using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). Analysis by LCMS/MS has recently been widely used due to its application is not limited to volatile molecules but also highly polar metabolites. Sample preparation is simple and requires a short time to analyze (Dubrow et al. 2022; Rauh 2012; Rojo et al. 2012; Ouyang et al. 2012; Gao et al. 2016). The secondary metabolites can be identified based on several literature databases. Literature databases were NIST mass spectral library from WILEY, Agilent Extractables, and Leachables PCDLs or Compound Discoverer from ThermoScientific, which contains the information on time retention, molecular formula, structure, and molecular weight of the chemical compounds.

Detection methods in chromatography usually use several types of detectors, one of which is mass spectroscopy. This type of mass spectroscopy detector is one of the detectors that can produce data in 3-dimensional form by describing the signal strength and mass spectrum. The resulting mass spectrum provides information on the sample's molecular weight, structure, identity, quantity, and purity (Vogeser and Seger 2016).

LCMS/MS is a beneficial approach in determining a metabolite profile in a sample. Using a separation system with a higher resolution is the key in this method to identify metabolite compounds in the extract. Several previous studies used the LCMS/MS method to identify phytochemical constituents of plants. For example, the secondary metabolites of ethanol extract of Green meniran (*Phyllanthus niruri* Linn) (Widiadnyani 2021), phenolic compounds of the leaves, pulp, and seed of *Eugenia calycina* (Araujo 2020), and phytochemical profile of methanol extract of root and seed shell of *Archidendron bubalinum* from Lampung (Hanafi 2018). Therefore, this study aims to determine the group of compounds in *L. sarmentosa* fractions, identify the secondary metabolites of hexane, chloroform, ethyl acetate, and methanol fractions, and determine their antiradical activity.

MATERIALS AND METHODS

Materials, sample preparation, and extraction

Luvunga sarmentosa roots were collected from Buhut Village, Muara Teweh, Central Kalimantan, Indonesia (Figure 1.). The samples were dried and crushed. A total of 1 kg of the dried roots were macerated with 96% ethanol (500 g in 2 L) for 3 x 24 hours. The macerate was concentrated using a rotary evaporator to obtain concentrated ethanol extract.

Fractionation

The concentrated ethanol extract was fractionated using silica gel G_{60} (50 g) vacuum column chromatography as the stationary phase. Five grams of ethanol extract was eluted with 5 x 250 mL of eluent, consecutively starting from n-hexane, chloroform, ethyl acetate, and methanol. Each fraction was concentrated using a rotary evaporator.

Identification of secondary metabolites by LCMS/MS (Mangurana et al. 2019)

Identifying secondary metabolites in *L. sarmentosa* fractions was conducted using LCMS/MS. The LCMS/MS was carried out in the Advance Laboratory, Bogor Agricultural University, Indonesia. The sample was filtered through a 0.2 µm PTFE membrane. The analysis using LCMS UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific, Accucore C_{18} column, 100 x 2.1 mm, 1.5 m (ThermoScientific), flow rate 0, 2 mL/min. The eluent was water + 0.1% formic acid (A) and acetonitrile + 0.1 % formic acid (B), gradient 0-1 min (5% B), 1-25 min (5-95% B), 25-28 minutes (95%B), 28-30 minutes (5%B). The column temperature was 3°C with an injection volume of 2.0 µL. The analysis used a molecular weight interval of 100-1500 m/z and positive ionization mode. The database used for the qualitative identification of the chemical constituents in the fraction was Compound Discoverer 3.2. The quantitative analysis was calculated from the peak area of identified compounds.

Antioxidant activity testing with UV-Visible Spectrophotometer (Liu et al. 2014)

Twenty mg of 1,1-diphenyl-2-picryl hydrazine (DPPH) was dissolved in methanol to obtain a 200 ppm (0.5 mM) concentration as stock solution. The stock solution of DPPH was diluted using methanol to get a concentration of 40 ppm then the absorbance was measured at a wavelength of 400-800 nm to obtain the maximum wavelength. The optimum operating time was determined from 0 minutes to 60 minutes.

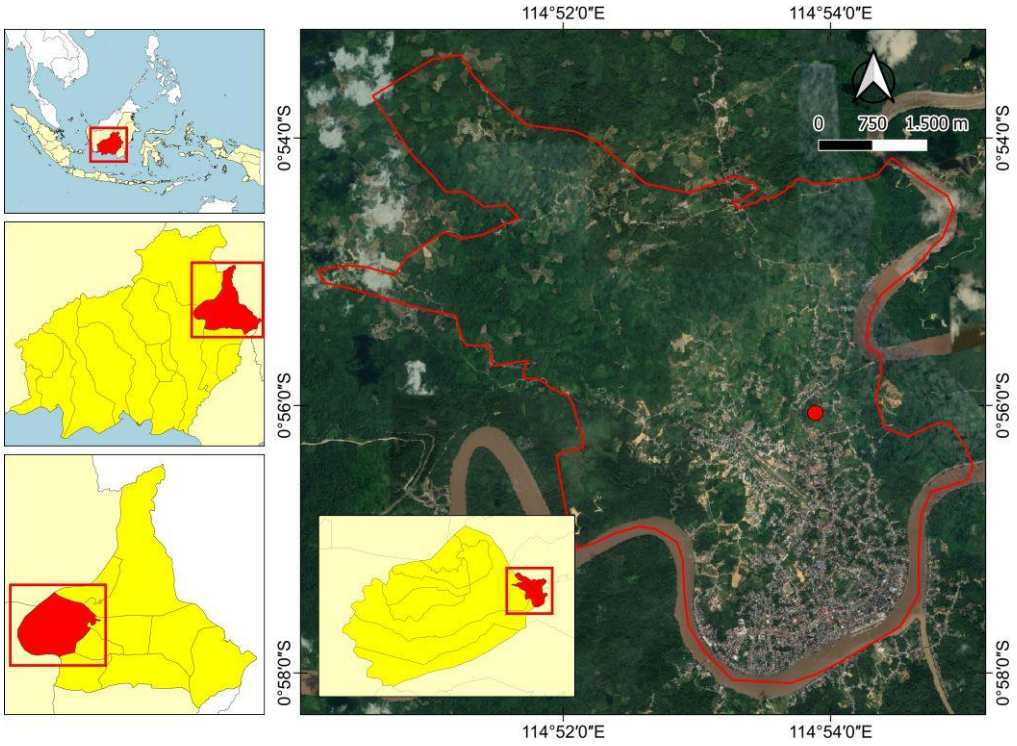

Figure 1. The location of plant collection in Buhut Village, Muara Teweh, Central Kalimantan, Indonesia

Table 1. Category of antioxidant activity

Category	IC_{50} (ppm)
Very strong	< 50
Strong	50-100
Moderate	101-150
Weak	151-200

The concentration of the stock solution of each fraction was 1000 ppm. Next, as much as 1 ml; 1.5 ml; 2 ml; 2.5 ml of stock solution were pipetted into a 25 ml volumetric flask. A volume of 5 ml of 200 ppm DPPH was added to the volumetric flask, followed by methanol addition reaching the volume of 25 ml to obtain fraction concentrations of 40 ppm, 60 ppm, 80 ppm, and 100 ppm. The mixture solutions were left in a dark place. The absorbance was measured with a light spectrophotometer at a 516 nm wavelength from 0 to 60 minutes.

Calculation of the percentage of inhibition

The DPPH free radical inhibition was calculated using formula (1):

$$\%\ \mathbf{inhibition} = \frac{\mathbf{A_{control} - A_{sample}}}{\mathbf{A_{control}}} \mathbf{x100\%}$$

Where $A_{control}$ is the absorbance of the solution without sample and A_{sample} is the absorbance of the sample (Zhang et al. 2018; Widarta et al. 2019).

Determination of IC_{50} value

The ability of fraction as DPPH free radical scavenger was expressed as IC_{50}. The IC_{50} value is the concentration needed to scavenge half of the free radicals. The IC_{50} value was calculated from the linear regression equation Y = AX + B by plotting the concentration of the test solution as the abscissa (X-axis) and the percent inhibition of DPPH as parameters of antioxidant activity as the ordinate (Y-axis). Therefore, in determining the IC_{50} for the sample, the Y value is 50, and the X value obtained is the IC_{50} value. Categories of the antioxidant activity are presented in Table 1 (Mardawati et al. 2008).

RESULTS AND DISCUSSION

Identification of secondary metabolites by LCMS/MS

The root ethanol extract of *Luvunga sarmentosa* was fractionated with different solvent polarity, consecutively hexane, chloroform, ethyl acetate, and methanol. Identification of secondary metabolites in each fraction was carried out using LCMS/MS analysis. The chromatograms of each fraction were analyzed to identify their secondary metabolites (Yunita et al. 2022; Vinaixa et al. 2015). Figure 2 shows the chromatogram of each fraction. Differences in each fraction's LC/MS chromatograms indicated differences in the chemical compounds contained in each fraction. Each peak in the chromatogram has its retention time and molecular weight (Lee et al. 2019; Jannat et al. 2020). The LCMS/MS analysis showed that the number of peaks in the hexane, chloroform, ethyl acetate, and methanol chromatogram were 194, 190, 214, and 76, respectively. The major identified compound groups in each fraction are presented in Figure 3. The difference in the compound content was due to differences in the polarity of the eluent. Polar solvents dissolve polar components, while non-polar solvents dissolve non-polar compounds as, in principle, "like dissolves like" (Harborne 1987).

The highest percentage of the compound in each fraction was alkaloids. Terpenoids and phenyl propanoids are also present in high percentages. Meanwhile, flavonoids were only present in the chloroform fraction, and stilbene was only in the ethyl acetate fraction (0.5%). Steroids, tannins, fatty acids, and metabolites of the phenyl propanoic group, especially the coumarin and chromone groups, were also present in the fractions. It is in line with the previous studies on qualitative phytochemical screening that Saluang root does not contain flavonoids and steroids (Anggriani 2018; Wati et al. 2018). Some fatty acid derivatives were also present in the hexane, chloroform, and ethyl acetate fraction. In contrast, amino acid derivatives were present in a high percentage of methanol fraction after the alkaloids.

Major compounds of saluang belum (*Luvunga sarmentosa*) root

The LCMS/MS analysis of the root of *L.sarmentosa* fractions showed the presence of several identified major compounds. The percentage of all peak areas was calculated, then the identified peak area higher than 1% was considered the major compound in each fraction (shown in Table 2). Ostruthin was the highest compound percentage in hexane, chloroform, and ethyl acetate fraction. Benzyl butyl phthalate was also detected in the n-hexane, chloroform, and ethyl acetate fractions but in a low percentage. Alkaloid compounds and amino derivative compounds dominated the methanol fraction.

The major compounds in the fractions of ethanol root extract of *Luvunga sarmentosa* were alkaloids, phenylpropanoids, and terpenoids. Alkaloids are identified abundantly in all fractions, i.e., 37.6%, 57.9%, 60.3%, and 59.6% in hexane, chloroform, ethyl acetate, and methanol fractions, respectively (Figure 3). The percentage of alkaloids increased as the polarity of eluent increased.

Coumarin derivatives such as 6-geranyl-7-hydroxy-8-methoxy coumarin, scopoletin, (R)-peucedanol, xanthoarnol, and umbelliferone-6-carboxylic acid have been isolated from *L. scandens* (Nguyen et al. 2017). Coumarin is classified as a phenylpropanoid derivative (Deng and Lu 2017). Phenylpropanoid content in hexane, chloroform, ethyl acetate, and methanol were 30.4%, 22.6%, 19.2%, and 6.6%, respectively (Figure 3). In contrast with alkaloids, the percentage of phenyl propanoids decreases as the eluent's polarity increases. It indicated that low-polarity solvents could extract more phenylpropanoid in *L. sarmentosa*.

Terpenoids have high concentrations in all fractions. Terpenoids were present in the hexane fraction (23.7%),

chloroform fraction (12.1%), ethylacetate fraction (11.2%), and methanol fraction (2.6%). These data indicate that increasing solvent polarity decreases the percentage of terpenoids, and it indicated that hexane extracted most of the terpenoids. It is due to most terpenoid compounds being non-polar compounds; therefore, they can be extracted using non-polar solvents such as hexane (Jiang et al. 2016; Namdar et al. 2018). It was in line with previous studies that chemical compounds of the terpenoid group were always extracted using hexane as a solvent (Lee et al. 2019; Ludwiczuk et al. 2017; Delgado-Povedano et al. 2019; Namdar et al. 2018). In contrast, alkaloids could be extracted with various solvents with different polarities.

Based on the LCMS/MS analysis results, Ostruthin was considered the marker compound from the root of *L. sarmentosa* because it was identified in three fractions in high concentration, except for the methanol fraction. The retention time of ostruthin was 20,917 min, with a molecular mass [M+H] of 299,16318 and a molecular formula of $C_{19}H_{22}O_3$ (Figure 4). Ostruthin has been isolated from the roots of *Paramignya trimera*, with antidepressive and anxiolytic effects (Joseph et al. 2018; An et al. 2021). In addition, alkyl triphenylphosphonium ostruthin, a derivative of ostruthin, has the potential as an anti-inflammatory (Vo et al. 2021) and cytotoxic (Dang et al. 2020).

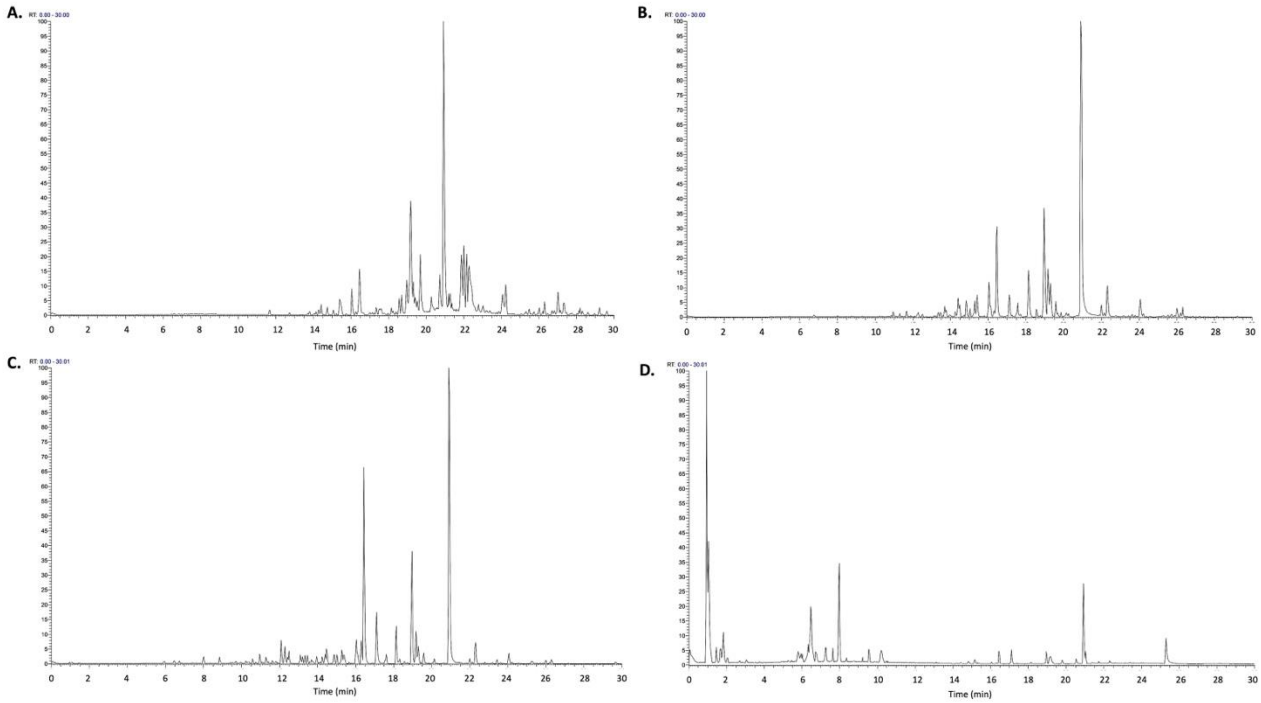

Figure 2. Chromatogram of LCMS/MS, (A) hexane, B) chloroform, C) ethyl acetate, and D) methanol fraction of *L. sarmentosa*

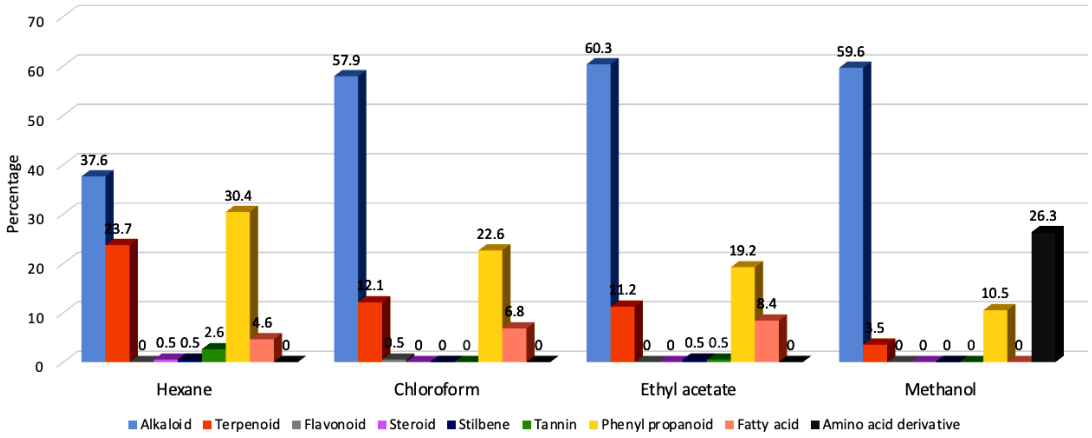

Figure 3. Compound groups of *L.sarmentosa* fractions by LCMS/MS analysis

Table 2. Major compounds of the root fraction of *Luvunga sarmentosa*

Fraction	RT (min)	Percent.	Molecular Formula	m/z	Compound Name	Group of compounds
Hexane	20.917	16.035	$C_{19}H_{22}O_3$	298.16	Ostruthin	Lactone terpenoid
	22.301	13.465	$C_{15}H_{24}$	204.187	(E, E)-alpha-Farnesene	Terpenoid
	21.992	3.675	$C_{19}H_{20}O_3$	296.14	Cryptotanshinone	Terpenoid
	20.710	3.874	$C_{15}H_{22}$	202.17	Curcumin	Phenylpropanoid
	19.183	2.875	$C_{19}H_{20}O_4$	312.14	Benzyl butyl phthalate	-
	19.673	2.558	$C_{16}H_{13}NO_2$	251.094	6-phenyl-3,4-dihydro-1H-2,5-benzoxazocin-1-one	Alkaloid
	16.452	1.929	$C_{14}H_{12}O_3$	228.08	Trioxsalen	Coumarin/phenyl propanoid
	24.235	1.260	$C_{18}H_{23}N$	253.182	Tolpropamine	Alkaloid
	24.055	1.061	$C_{15}H_{20}$	200.156	3,4-Dihydrocadalene	Terpenoid
	16.032	1.004	$C_{14}H_{14}O_3$	230.09	Naproxen	Propionic acid
Chloroform	20.920	33.048	$C_{19}H_{22}O_3$	298.16	Ostruthin	Lactone terpenoid
	18.143	10.323	$C_{19}H_{19}NO_4$	325.13	(*S*)-(+)-Bulbocapnine	Alkaloid
	18.955	9.690	$C_{19}H_{17}NO_4$	323.11	Rutacridone epoxide	Alkaloid
	19.173	3.705	$C_{19}H_{20}O_4$	312.14	Benzyl butyl phthalate	-
	22.304	3.1120	$C_{24}H_{27}NO_4$	393.192	tylophorine	Alkaloid
	16.041	2.576	$C_{14}H_{14}O_3$	230.09	Naproxen	Propionic acid
					Huperzine B	
	14.837	1.235	$C_{26}H_{30}O_8$	470.192	Limonene	Terpenoid
	16.462	1.168	$C_{14}H_{12}O_3$	228.08	Rutacridone epoxide	Coumarin/phenyl propanoid
	15.271	1.022	$C_{15}H_{13}NO_4$	271.084	p-Hydroxyketorolac	Alkaloid
Ethyl acetate	20.908	26.534	$C_{19}H_{22}O_3$	298.16	Ostruthin	Lactone terpenoid
	16.430	16.286	$C_{19}H_{19}NO_4$	325.13	(*S*)-(+)-Bulbocapnine	Alkaloid
	18.946	9.062	$C_{19}H_{17}NO_4$	323.11	Rutacridone epoxide	Alkaloid
	17.113	5.525	$C_{19}H_{17}NO_5$	339.11	Mofezolac	-
	19.175	2.654	$C_{19}H_{20}O_4$	312.14	Benzyl butyl phthalate	-
	14.478	1.127	$C_{19}H_{22}O_5$	330.145	Gibberellin A7	Alkaloid
Methanol	1.054	8.045	$C_{15}H_{11}N_2O_2$	117.079	Betaine	Amino acid derivative-
	6.482	7.164	$C_{12}H_{16}N_2$	188.131	N, N-Dimethyltryptamine	Alkaloid
	6.522	4.585	$C_{13}H_{18}N_2$	202.1466	4-Methyl-α-ethyltryptamine	Alkaloid
	1.06	4.005	$C_7H_7NO_2$	137.0474	Trigonelline	Alkaloid
	1.087	3.604	$C_7H_{13}NO_2$	143.0944	1-Aminocyclohexane carboxylic acid	Amino acid derivative-
	1.077	2.914	$C_5H_9NO_2$	115.0634	L-Proline	Amino acid
	7.264	2.542	$C_{16}H_{19}NO_3$	273.1357	4-(tert-butyl) phenyl 3,5-dimethylisoxazole-4-carboxylate	Alkaloid
	5.805	1.519	$C_{13}H_{15}NO_3$	233.1046	1-Ethyl-6,7-dimethoxy isoquinoline-3-ol	Alkaloid
	7.917	1.501	$C_{12}H_{13}NO_5$	251.0787	N-feruloylglycine	Amino acid
	1.049	1.481	$C_7H_{15}NO_3$	161.1049	DL-Carnitine	Amino acid derivative-
	1.733	1.342	$C_6H_{13}NO_2$	131.0944	L-Isoleucine	Amino acid
	1.085	1.233	$C_5H_{11}N_3O_2$	145.0848	g-Guanidinobutyrate	Amino acid derivative-
	6.483	1.199	$C_{10}H_9N$	143.0733	6-Methylquinoline	Alkaloid
	6.734	1.125	$C_{13}H_{15}NO_2$	217.1098	glutethimide	Alkaloid
	1.09	1.045	$C_6H_{11}NO_2$	129.0788	L (-)-Pipecolinic acid	Amino acid derivative-
	1.049	1.044	$C_{10}H_{19}NO_7$	265.1157	1-[(3-Carboxypropyl) amino]-1-deoxy-beta-D-fructofuranose	Amino acid derivative-

Note: ND stands for Not Detected

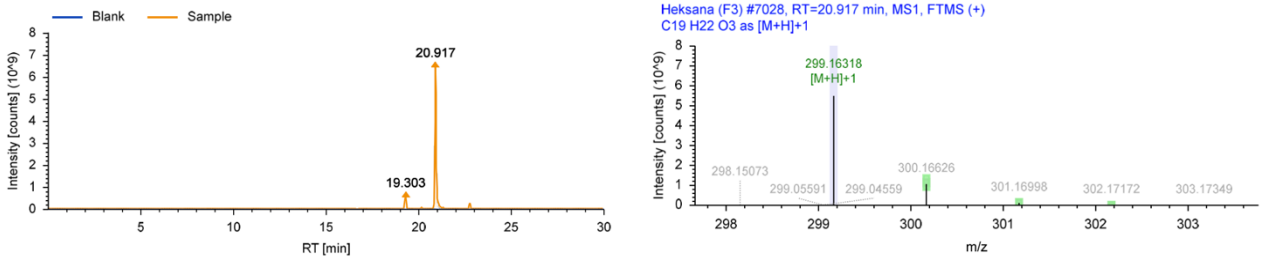

Figure 4. Chromatogram of ostruthin from hexane fraction on positive ESI

The diversity of phytochemical compounds in the *Luvunga* genus

Phytochemical diversity is the source of medicine. Prediction of compound diversity and its potential could be obtained from phylogenetic information (Prasad 2019). However, ecological factors, such as trophic, climatic, edaphic, and topographic variations, affect the chemical content (Defossez 2021). *Luvunga sarmentosa* belongs to the Rutaceae family. There was limited information on the phylogenetic relationship of the *L. sarmentosa*, but not for other species of Luvunga. *Luvunga scandens* were reported to be closely related to *Paramingya trimera* (Ling 2009; Phi 2020). Paramingnya contained coumarin and coumarin glycosides, acridone alkaloids, tirucallane and tirucallane glycosides (terpenoids group), phenols, and flavonoids (Son 2018). This study showed that the major phytochemical compounds in *L. sarmentosa* were alkaloids, phenylpropanoids (coumarin), terpenoids, and a small number of flavonoids. Ostruthin, as the marker compound of *L. sarmentosa*, was also found in the roots of *Paramignya trimera* (Joseph et al. 2018; An et al. 2021).

A previous study by Lien (2002) showed that the leaves of *L. sarmentosa* contained eight apotirucallane triterpenoids (Lien 2002). The root of *L. scandens* has xanthones, coumarin, and triterpene (Zuki 2015). Two tirucallane triterpenes, namely flindissol (1) and 3-oxotirucalla-7,24-dien-21-oic-acid (2), have been isolated from the dichloromethane stem extract of *L. scandens* (Al-Zikri 2014). In addition, the ethyl acetate extract of the root of *L. scandens* contained one new acridone alkaloid (Tran 2019). The results of this study also show that the main phytochemical components of *L. sarmentosa* are alkaloids, phenylpropanoids, and terpenoids which are also found in other species of Luvunga.

Antiradical activity of *Luvunga sarmentosa* root extract fraction

The antiradical activity of each fraction of the root extract of *L. sarmentosa* is presented in Figure 5. It showed that all fractions had the DPPH radical scavenging activity in a concentration dependant manner. The methanol fraction of the root extract of *L. sarmentosa* has the most remarkable ability to inhibit DPPH free radicals. Meanwhile, the chloroform, ethyl acetate, and hexane fractions had lower DPPH free radicals scavenging activity than the methanol fraction. The major phytochemical constituents in methanol extract were alkaloids (59.6%), amino acid derivatives (26.3%), phenylpropanoids (6,6%), and terpenoids (2.6%).

Free radical scavenging activity of the fraction might be related to the presence of metabolites as free radical scavengers, i.e., alkaloids. Alkaloids are reported to have antioxidant properties as free radical scavengers (Gan et al. 2017; Gul et al. 2017); therefore, they protect cells from toxic substances and prevent genetic damage caused by H_2O_2 oxidants. (Andiriyani et al. 2014). In addition, alkaloids are also reported to have other activities, such as antibacterial (Manosalva et al. 2016), anti-inflammatory (Gutiérrez et al. 2014), and anticancer (Nugraha et al. 2019). Some amino acid derivatives were also found in the methanol fraction and probably contributed to the antioxidant activities. Amino acid derivatives are commonly found in plants and animals with biological functions, and several studies reported that some amino acids exhibited radical scavenging activities. Hence, they could be beneficial for the food processing industry as an antioxidant as additives to extend the shelf-life of food or food products and offer beneficial pharmacological effects against cell damage caused by oxidative stress (Pazos et al. 2006; Kim et al. 2017). The IC_{50} value of each fraction of root extract of *L. sarmentosa* is summarized in Table 3.

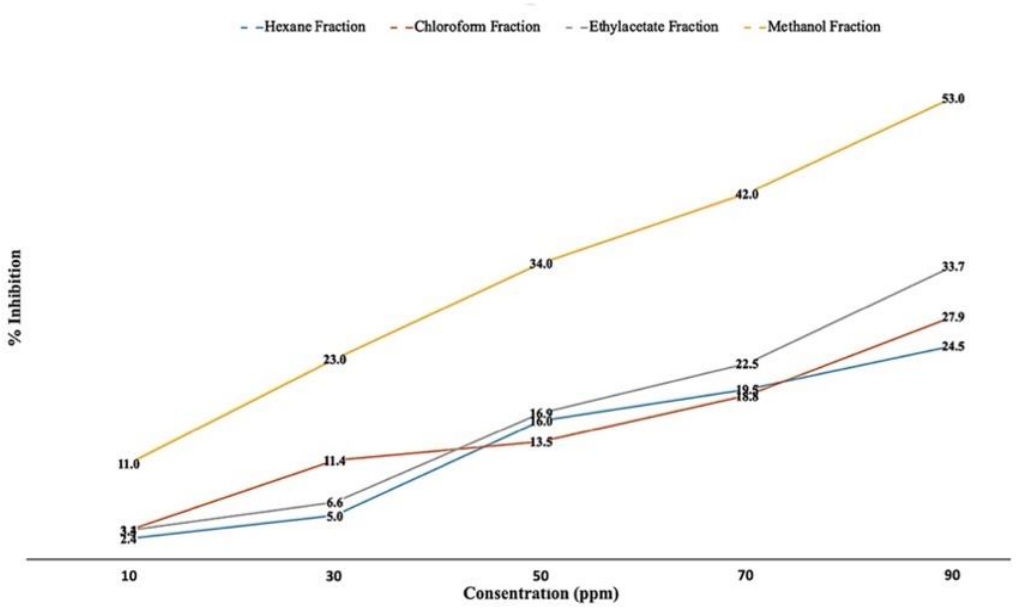

Figure 5. DPPH free radical scavenging activity of *L. sarmentosa* fractions

Table 3. The results of the linear regression equation and IC_{50} for each fraction of the root extract of *Luvunga sarmentosa*

Fraction	Regression equation	IC_{50} (ppm)	Category
hexane	Y = 0.2937X + 1.2012	174.33	Weak
Chloroform	Y = 0.2822X + 0.8986	173.99	Weak
Ethyl acetate	Y = 0.3833X + 2.5681	137.15	Moderate
Methanol	Y = 0.5169X + 6.8812	83.42	Strong

Table 3 shows that the methanol fraction of the root extract of *L. sarmentosa* has strong antiradical activity with an IC_{50} value of 83.42 ppm. On the other hand, ethyl acetate fraction (IC_{50}: 137.15 ppm) has medium antiradical activity. In comparison, chloroform and hexane fractions are categorized as weak free radical scavengers (Mardawati et al. 2008). Furthermore, the IC_{50} value of fractions showed that increasing alkaloid content increases antiradical activity. In contrast, increasing terpenoid and phenyl propanoic compounds decrease antiradical activity.

In conclusion, hexane, chloroform, ethyl acetate, and methanol fractions of *L. sarmentosa* root contain different metabolites; however, all fractions contain alkaloids, phenylpropanoids, and terpenoids. The highest is alkaloids. Ostruthin was considered a marker compound of *L. sarmentosa* root extract. The methanol fraction had the best antiradical activity compared to other fractions, with an IC50 value of 83.42 ppm. Increasing alkaloid content increases antiradical activity.

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REFERENCES

Al-Zikri PNH, Taher M, Susanti D, Ichwan SJA. 2016. Cytotoxic activity of *Luvunga scandens* against human cancer cell lines. Jurnal Teknologi (Sciences and Engineering) 78 (10): 153-157. DOI: 10.11113/jt.v78.7328.

Al-Zikri PNH, Taher M, Susanti D, Rezali MF, Read RW, Sohrab MH, Hasan CM, Ahmad F. 2014. Cytotoxic tirucallane triterpenes from the stem of *Luvunga scandens*. Revista Brasileira de Farmacognosia 24 (5): 561-564. DOI: 10.1016/j.bjp.2014.10.003.

An TC, Ngoc PH, Sang PM, Truc PN, Tien NHT, Nhu TPH, Tam PTT, Hung LN, Trung PV. 2021. Directly purification of Ostruthin from *Paramignya trimera* roots by CPC (Centrifugal Partition Chromatography). Vietnam J Chem 59 (1): 69-72. DOI: 10.1002/vjch.202000117.

Andiriyani MM, Untari EK, Wahdaningsih S. 2014. Pengaruh pemberian ekstrak etanol daun bawang mekah (*Eleutherine americana* Merr.) terhadap kadar malondialdehide tikus wistar jantan pasca paparan asap rokok. Jurnal Fitofarmaka Indonesia 1 (2): 43-50. DOI: 10.33096/jffi.v1i2.189. [Indonesian]

Anggriani MF. 2018. Identifikasi dan potensi senyawa metabolit sekunder pada akar tumbuhan saluang belum (*Luvunga sarmentosa* (Blume) Kurz) sebagai afrodisiak. Jurnal Ilmu Kanderang Tingang 9 (1): 97-102. DOI: 10.37304/jikt.v9i1.9. [Indonesian]

Araujo NM, Arruda HS, Santos FN, Morais DR, Pereirae GA, Pastorea GM. 2020. LC-MS/MS screening and identification of bioactive compounds in leaves, pulp and seed from *Eugenia calycina* Cambess. Food Res Intl 137. DOI: 10.1016/j.foodres.2020.109556.

Dang PH, Dao THX, Le VT, Nguyen CM, Ly TT, Nguyen HX, Le TH, Do TNV, Nguyen MTT, Sun S, Awale S, Nguyen NT. 2020. Synthesis of alkyl triphenylphosphonium ostruthin derivatives as potential cytotoxic candidates. ChemistrySelect 5 (41): 12636-12640. DOI: 10.1002/slct.202003152.

Defossez E, Pitteloud C, Descombes P, Glauser G, Allard PM, Walker TWN, Fernandez-Conradi P, Wolfender JL, Pellissier L, Rasmann S. 2021. Spatial and evolutionary predictability of phytochemical diversity. Proc Nat Acad Sci 118 (3). DOI: 10.1073/pnas.2013344118.

Delgado-Povedano MM, Sánchez-Carnerero CC, Priego-Capote F, Ferreiro-Vera C. 2019. Untargeted characterization of extracts from *Cannabis sativa* L. cultivars by gas and liquid chromatography coupled to mass spectrometry in high resolution mode. Talanta 1-10. DOI: 10.1016/j.talanta.2019.120384.

Deng Y, Lu S. 2017. Biosynthesis and regulation of phenylpropanoids in plants. Crit Rev Plant Sci 36 (4): 257-290. DOI: 10.1080/07352689.2017.1402852.

Dubrow GA, Tello E, Schwartz E, Forero DP, Peterson DG. 2022. Identification of non-volatile compounds that impact consumer liking of strawberry preserves: Untargeted LC-MS analysis. Food Chem 378. DOI: 10.1016/j.foodchem.2022.132042.

Fauzi, Widodo H. 2019. Short communication: Plants used as aphrodisiacs by the Dayak ethnic groups in Central Kalimantan, Indonesia. Biodiversitas 20 (7): 1859-1865. DOI: 10.13057/biodiv/d200710.

Gan J, Feng Y, He Z, Li X, Zhang H. 2017. Correlations between antioxidant activity and alkaloids and phenols of maca (*Lepidium meyenii*). J Food Quality 1-10. DOI: 10.1155/2017/3185945.

Gao W, Kirschbaum C, Grass J, Stalder T. 2016. LC-MS based analysis of endogenous steroid hormones in human hair. J. Steroid Biochem Mol Biol 162: 92-99. DOI: 10.1016/j.jsbmb.2015.12.022.

Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. 2017. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. Sci World J 1-7. DOI: 10.1155/2017/5873648.

Gutiérrez DMA, Bah M, Garduño MLR, Mendoza SOM, Serrano VC. 2014. Anti-inflammatory and antioxidant activities of methanol extracts and alkaloid fractions of four Mexican medicinal plants of Solanaceae. Afr J Tradit Complement Altern Med 11 (3): 259-267. DOI: 10.4314/ajtcam.v11i3.36.

Hanafi, Irawan C, Rochaeni H, Sulistiawaty L, Roziafanto AN, Supriyono. 2018. Phytochemical screening, LC-MS studies and antidiabetic potential of methanol extracts of seed shells of *Archidendron bubalinum* (Jack) I.C. Nielson (Julang Jaling) from Lampung, Indonesia. Pharmacogn J 10 (6) Suppl: s77-s82. DOI: 10.5530/pj.2018.6s.15.

Harborne JB. 1987. Metode Fitokimia. Edisi ke-2. Institut Teknologi Bandung, Bandung.

Jannat B, Ghorbani K, Kouchaki S, Sadeghi N, Eslamifarsani E, Rabbani F, Beyramysoltan S. 2020. Distinguishing tissue origin of bovine gelatin in processed products using LC/MS technique in combination with chemometrics tools. Food Chem 319: 1-6. DOI: 10.1016/j.foodchem.2020.126302.

Jiang Z, Kempinski C, Chappell J. 2016. Extraction and analysis of terpene/terpenoids. Curr Protocols Plant Biol 1: 345-358. DOI: 10.1002/cppb.20024.

Joseph A, Thuy TTT, Thanh LT, Okada M, Biagini G. 2018. Antidepressive and anxiolytic effects of ostruthin, a TREK-1 channel activator. PLOS ONE 13 (8): 1-19. DOI: 10.1371/journal.pone.0201092.

Kim JH, Jang HJ, Cho WY, Yeon SJ, Lee CH. 2017. In vitro antioxidant action of sulphur-containing amino acids. Arabian J Chem 13: 1678-1684. DOI: 10.1016/j.arabjc.2017.12.036.

Lee J, Krotulski AJ, Fogarty MF, Papsun DM, Logan BK. 2019. Chromatographic separation of the isobaric compounds cyclopropyl fentanyl, crotonyl fentanyl, methacryl fentanyl, and para-methyl acryl fentanyl for specific confirmation by LC-MS/MS. J Chromatograph B 1118-1119: 164-170. DOI:10.1016/j.jchromb.2019.04.033.

Lien TP, Kamperdick C, Schmidt J, Adam G, Sung TV. 2002. Apotirucallane triterpenoids from *Luvunga sarmentosa* (Rutaceae). Phytochemistry 60 (7): 747-754. DOI: 10.1016/s0031-9422(02)00156-5.

Ling K, Wang Y, Poon WS, Shaw PC, But PPH. 2009. The Relationship of Fagaropsis and Luvunga in Rutaceae. Taiwania 54 (4): 338-342.

Liu C, Kao C, Wu H, Li W, Huang C, Li H, Chen C. 2014. Antioxidant and anticancer aporphine alkaloids from the leaves of *Nelumbo nucifera* Gaertn. cv. Rosa-plena. Molecules 19 (11): 17829-17838. DOI: 10.3390/molecules191117829.

Ludwiczuk A, Skalicka-Wo´zniak K, Georgiev MI. 2017. Terpenoids. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12-802104-0.00011-1.

Mangurana WOI, Yusnaini, Sahidin. 2019. Analisis LC-MS/MS (Liquid Chromatogaph Mass Spectrometry) dan metabolit sekunder serta potensi antibakteri ekstrak n-Heksana spons *Callyspongia aerizusa* yang diambil pada kondisi tutupan terumbu karang yang berbeda di perairan Teluk Staring. Jurnal Biologi Tropis 19 (2): 131-141. DOI: 10.29303/jbt.v19i2.1126. [Indonesian]

Manosalva L, Mutis A, Urzúa A, Fajardo V, Quiroz A. 2016. Antibacterial activity of alkaloid fractions from *Berberis microphylla* G. Forst and study of synergism with ampicillin and cephalothin. Molecules 21 (1): 76. DOI: 10.3390/molecules21010076.

Mardawati E, Filianty F dan Harta H. 2008. Kajian aktivitas antioksidan ekstrak kulit manggis (*Garcinia mangostana* L.) dalam rangka pemanfaatan limbah kulit manggis di Kecamatan Puspahiang Kabupaten Tasikmalaya. Jurnal Industri Teknologi Pertanian. [Indonesian]

Namdar D, Mazuz M, Ion A, Koltai H. 2018. Variation in the compositions of cannabinoid and terpenoids in *Cannabis sativa* derived from inflorescence position along the stem and extraction methods. Ind Crops Prod 113: 376-382. DOI: 10.1016/j.indcrop.2018.01.060.

Nguyen TP, Minh NP, Dat TB, Le TD, Tran Nguyen MA, Pham TNT, Tri DM. 2017. Limonoid from the rhizomes of *Luvunga scandens* (Roxb.) Buch. Ham. Nat Prod Res 31 (19): 2281-2285. DOI: 10.1080/14786419.2017.1303690.

Nugraha AS, Damayanti YD, Wangchuk P, Keller PA. 2019. Anti-infective and anti-cancer properties of the Annona species: Their ethnomedicinal uses, alkaloid diversity, and pharmacological activities. Molecules 24 (23): 1-31. DOI: 10.3390/molecules24234419.

Ouyang Z, Furlong MT, Wu S, Sleczka B, Tamura J, Wang H, Suchard S, Suri A, Olah T, Tymiak A, Jemal M. 2012. Pellet digestion: a simple and efficient sample preparation technique for LC-MS/MS quantification of large therapeutic proteins in plasma. Bioanalysis 4 (1): 17-28. DOI: 10.4155/bio.11.286.

Pazos M, Andersen, ML, Skibsted LH. 2006. Amino acid and protein scavenging of radicals generated by iron/hydroperoxide system: a electron spin resonance spin trapping study. J Agric Food Chem 54: 10215-10221. DOI: 10.1021/jf062134n.

Phi TCM, Chu HH, Le NT, Nguyen DB. 2020. Phylogenetic relationship of Paramignya trimera and its relatives: an evidence for the wide sexual compatibility. Sci Rep 10. DOI: 10.1038/s41598-020-78448-2.

Prasad MA, Zolnik CP, Molina J. 2019. Leveraging phytochemicals: the plant phylogeny predicts sources of novel antibacterial compounds. Future Sci OA 5 (7). DOI: 10.2144/fsoa-2018-0124.

Rauh M. 2012. LC-MS/MS for protein and peptide quantification in clinical chemistry. J Chromatograph B 883-884: 59-67. DOI: 10.1016/j.jchromb.2011.09.030.

Rojo D, Barbas C, Rupérez FJ. 2012. LC-MS metabolomics of polar compounds. Bioanalysis 4 (10): 1235-1243. DOI: 10.4155/bio.12.100.

Sirinut P, Petchkongkeaw A, Romsaiyud A, Prateeptongkum S, Thongyoo P. 2017. Phytochemical constituents from the root of *Luvunga scandens* and biological activity evaluation. Nat Prod Commun 12 (9): 1483-1484. DOI: 10.1177/1934578x1701200926.

Son NT. 2018. Notes on the genus Paramignya: Phytochemistry and biological activity. Bulletin of Faculty of Pharmacy, Cairo University 56 (1): 1-10. DOI: 10.1016/j.bfopcu.2017.12.001.

Tran NMA, Do THT, Truong LH, Le DT, Phan MN, Pham NKT, Mai DT, Nguyen TP. 2019. Acridone alkaloids from the rhizomes of *Luvunga scandens* (Roxb.) Buch. Ham. Nat Prod Rese 33 (15). DOI: 10.1080/14786419.2018.1492577.

Van HT, Nguyen DGM, Huynh NTA, Le VS. 2020. Antibacterial activities of ethanolic extract of four species of Rutaceae family. Plant Sci Today 7 (3): 463-468. DOI: 10.14719/pst.2020.7.3.784.

Vinaixa M, Schymanski EL, Neumann S, Navarro M, Salek RM, Yanes O. 2015. Mass spectral databases for LC/MS and GC/MS-based metabolomics: state of the field and future prospects. TrAC Trends Anal Chem 1-33. DOI: 10.1016/j.trac.2015.09.005.

Vo NT, Kusagawa E, Nakano K, Moriwaki C, Miyake Y, Haruyama S, Fukuhara S, Nguyen NT, Dang PH, Nguyen MTT, Kataoka T. 2021. Biological evaluation of alkyl triphenylphosphonium ostruthin derivatives as potential anti-inflammatory agents targeting the nuclear factor κB signaling pathway in human lung adenocarcinoma A549 cells. Biochem 1 (2): 107-121. DOI: 10.3390/biochem1020010.

Vogeser M, Seger C. 2016. Mass spectrometri methods in clinical diagnostic-state of the art and perspectives. Trends Anal Chem Trac (84): 1-4. DOI: 10.1016/j.trac.2016.04.018.

Wati H, Muthia R, Jumaryatno R, Hayati F. 2018. Phytochemical screening and aphrodisiac activity of *Luvunga sarmentosa* (Bl.) Kurz ethanol extract in male Wistar Albino Rats. Res J Pharm Biol Chem Sci 9 (4): 931-937.

Widarta IWR, Wiadnyani AAIS. 2019. Effect of drying methods on the antioxidant activity of avocado leaves. Jurnal Aplikasi Teknologi Pangan 8 (3): 80-85. DOI: 10.17728/jatp.3361. [Indonesian]

Widiadnyani NKE, Astawa INM, Yasa IWPS, Sukrama IDM. 2021. Phytochemical test and identification of active compounds with LC-MS/MS in green meniran leaf (*Phyllanthus niruri* Linn). Bali Med J 10 (1): 132-136. DOI: 10.15562/bmj.v10i1.2208.

Yunita E, Kurniati T, Rosa FL, Melati P, Lestari N. 2022. Short Communication: Analysis of detected metabolite compounds from the crude extract of Rimau Gerga Lebong oranges fruit (*Citrus reticulata* 'RGL') using LC-QTOF-MS/MS. Biodiversitas 23 (7): 3778-3783. DOI: 10.13057/biodiv/d230754.

Zhang D, Wan Y, Hao J, Hu R, Chen C, Yao X, Zhao W, Liu Z, Li L. 2018. Evaluation of the alkaloid, polyphenols, and antioxidant contents of various mulberry cultivars from different planting areas in eastern China. Ind Crops Prod 122: 298-307. DOI: 10.1016/j.indcrop.2018.05.065.

Zuki M. 2015. Study on Chemical Constituents, Antioxidant and Cytotoxic Activities of *Dracaena umbratica* Ridl. and *Luvunga scandens* Buch-Ham. [Masters Thesis]. Universiti Teknologi MARA, Malaysia.