Chemical components of different parts of *Strychnos ligustrina*, a medicinal plant from Indonesia

A A D Rahayu¹*, A I Prihantini¹, Krisnawati¹, Y M M A Nugraheni²

¹Research and Development Institute of Technology Non-Timber Forest Products, Jl. Pejanggik No.128 Cakranegara / Jl. Dharma Bhakti No.7, PO BOX 1054, Ds. Langko, Kec. Lingsar, Kab. Lombok Barat – NTB 83371, Indonesia
²Forest Tree Seed Technology Research and Development Institute, Jl. Pakuan Ciheuleut PO BOX 105, Tegalega, Bogor 16129, Indonesia

*Corresponding email: anita13rahayu@gmail.com

Abstract. *Strychnos ligustrina* Blume is one of the potential plants for medicine that has been used by the local community in Indonesia for traditional medicine. Some studies showed that *S. ligustrina* parts had different activities, such as antimalarial, antibacterial, antimicrobial, antioxidant, and anticancer activities. Triterpenoids, phenolics, tannins, alkaloids, and flavonoids were detected in this species. Different plant parts and grow sites may affect the composition of chemical components. Therefore, the present study investigated the chemical components in different parts of *S. ligustrina* from some grow sites. The chemical components of different plant parts were analyzed by GC-MS and showed 51 compounds in total. The major constituents identified as mome inositol, heptadecene-(8)-carbonic acid-(1), palmitic acid, quinic acid, and stearic acid. The main active constituent of the antimalarial drug, strychnine, was also detected in this study. The PCA and cluster analysis of chemical components resulted in distinguished plant parts into three groups, whereas had shown no difference among the sites. In addition, the crucial compounds of this species that potential for antimalarial, strychnine, are only found in the leaf and stem. It has been shown that the leaf and stem are potential plant parts for the antimalarial agent.

1. Introduction

Indonesia is one of the countries that has mega biodiversity, especially the richest diversity of plant species worldwide [1]. The diversity of medicinal plants in the country also has been recognized globally and has a high economic value of the trading that is predicted to be US$5 trillion by 2050 [2]. *Strychnos ligustrina* Blume syn. *S. lucida* R. Br. is one of the medicinal plants that has been used as a source of traditional medicine in Bali and West Nusa Tenggara [3] that is included in the Least Concern species [4]. *S. ligustrina* is a small tree, it can reach 30 cm in diameter and up to 12 m in height with a bitter taste of all parts of the plant [3].

The local community has used *S. ligustrina* as a traditional medicine to treat some diseases such as malaria, fever, toothache, stomach ache, diabetes, and heart disease [5]. Furthermore, some researches had proven that different parts of this species showed different activities. The leaf is potential for antibacterial, that against *Staphylococcus aureus* [6] and anticancer activity against MOLT-3 cells [7], the seed showed has antibacterial activity against *S. aureus* ATCC 25923 and *Salmonella thyphii* [8], the wood of this species is potential for antimalarial [1] and antimicrobial against *Bacillus subtilis* ATCC
6683, B. cereus and S. pyogenes; the stem bark showed has an antioxidant and anticancer activity that inhibited MOLT-3 cells [7].

Previous publications have reported that the chemical components that had been found in S. ligustrina were triterpenoids [1,7,8], phenolics [1,7], tannins, alkaloids [1,8] such as strychnine and brucine, and flavonoids [1]. However, the composition of the compounds of medicinal plants in one species may depend on several factors, i.e. different parts of plants [9], different stages of fruit maturation [10], the environmental condition of the location where the plants are growing [11], and the cultivar of the species [12].

Based on previous studies about the quantity of the chemical components in plant parts in different condition of the growth site, S. ligustrina that grow in several sites may affect the differences of the chemical components. In addition, there are no reports that evaluate the profile of the chemical components in different parts of this species. Thus, this study aimed to investigate the chemical composition in several parts of S. ligustrina from different grow sites.

2. Materials and methods

2.1. Plant material

Four parts of S. ligustrina (leaf, root, stem, and seed) were used as samples. The samples were collected from several sites in West Bali National Park, Indonesia, in June 2015. Leaf, root and stem were collected from Teluk Terima (TT), Prapat Agung (PA), and Lampu Merah (LM), while the seed sample was collected from Teluk Terima (TT), Banyu Wedang (BW), Sumber Bathok (SB) and Palengkong (PL).

2.2. Sample extraction

The samples (leaf, root, stem, and seed) were dried and to be crushed into a homogenous powder for extraction. The powdered samples were diluted with alcohol 70%.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

Chemical components were analyzed in Chemical Analysis Laboratory, Faculty of Mathematics and Natural Sciences of Mataram University using GC-MS QP2010 Ultra (Shimadzu Co. Ltd. Kyoto, Japan) with semi-polar column RTX sMs and 30 m in length. The oven temperature was held with an initial temperature at 40°C for 3 min and programmed to reach 260°C in 19.33 min. Helium was used as the carrier gas, at a flow rate of 2.77 mL/min. The injection of sample in split mode with a ratio of 51.0. The chemical components were determined by the relative area peak and were identified using retention indices as well as Wiley 7.0 mass-spectral libraries. The compounds with the similarity index of the percentage of peak area were more than 85% were determined as the chemical components of S. ligustrina.

2.4. Statistical analysis

The Principal Component Analysis (PCA) was used to evaluate the variation of chemical components among four parts of S. ligustrina and different sites. The chemical components with the maximum percentage of peak area less than 1% in all parts were not used in PCA. PCA and Pearson correlation coefficients were performed using XLSTAT (Addinsoft).

3. Results and discussion

3.1. Chemical components of plant parts

In the present study, the location of collected samples and the content of chemical components from the four parts of S. ligustrina in different sites can be seen in Tables 1 and 2. The content of chemical compounds indicated variations among the plant parts in different sites. The compound with the highest percentage of peak area in leaf, root, and stem was mome inositol (18.52-50.16%). This result is similar
to the previous study that found mome inositol (23.51%) as a dominant compound in Strychnos nux-vomica. This compound has a biological activity for antioxidants [13].

Heptadecene-(8)-carbonic acid-(1) was the highest percentage of the compound contained in the seed from Banyu Wedang (BW) and Palengkong (PL) that accounted for 29.12% and 20.27%, respectively. This compound has a potential activity as an antioxidant [14]. On the other hand, seed from Sumber Batok (SB) and Teluk Terima (TT) contained 4,4,6A,6B,8A,11,12,14B-octamethyl-1,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A (25.29%) and brucine (43.95%), respectively, as a dominant compound. Brucine had also been found in S. nux-vomica that showed an anti-inflammatory [15] and analgesic activity [16].

Table 1. Geographical sites of S. ligustrina sampled from different parts of West Bali National Park, Indonesia.

| Site code | Sample | Location       | District, Province | Latitude (S)       | Longitude (E)       | Altitude (m) | Soil structure |
|-----------|--------|----------------|--------------------|--------------------|--------------------|--------------|---------------|
| TLse      | seed   | Teluk Terima   | Buleleng, Bali     | 8°08'44" - 8°09'05" | 114°32'00" - 114°32'25" | 2 - 57       | Granular      |
| PLse      | seed   | Palangkong     | Buleleng, Bali     | 8°09'08" - 8°09'24" | 114°33'51" - 114°34'28" | 29 - 66      | Granular      |
| BWse      | seed   | Banyu Wedang   | Buleleng, Bali     | 8°09'34" - 8°09'39" | 114°34'03" - 114°34'16" | 16 - 25      | Granular      |
| SBse      | seed   | Sumber Batok   | Buleleng, Bali     | 8°10'11" - 8°10'14" | 114°30'26" - 114°31'40" | 59 - 90      | Granular      |

| Site code | Sample | Location       | District, Province | Latitude (S)       | Longitude (E)       | Altitude (m) | Soil structure |
|-----------|--------|----------------|--------------------|--------------------|--------------------|--------------|---------------|
| LML, LM, LMrs | leaves, root, stem | Lampu Merah | Buleleng, Bali | 8°05'43"       | 114°26'34"        | 22           | No data       |
| PAI, PAR, PAS   | leaves, root, stem | Prapat Agung | Buleleng, Bali | 8°09'12"       | 114°26'49"        | 40           | No data       |
| TTI, TTR, TTs | leaves, root, stem | Teluk Terima | Buleleng, Bali | 8°09'04"       | 114°31'08"        | 37           | No data       |

Table 2. The percentage of peak area of 51 phytochemicals of different parts of S. ligustrina in different sites.

| No | Compound name | TT 1 | PA 1 | LM 1 | TT r | PA r | LM r | TT s | PA s | LM s | BW se | SB se | PL se | TT se |
|----|---------------|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|
| 1  | Bicyclo (2.2.1) heptane, -5-(ethyl-1-amine) | bdl  | nd   | 0.17 | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 2  | n-Propylbenzen e | nd   | nd   | 0.06 | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 3  | 1,1,2- Diethoxyisop etane | nd   | nd   | 0.09 | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 4  | 1-Ethyltoluene | nd   | 0.36 | 0.36 | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 5  | 1-Methyltoluene | 0.34 | nd   | nd   | 0.29 | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 6  | 1,2,3-Trimethylben zene | 0.53 | 0.54 | 0.58 | nd   | 0.40 | nd   | 0.42 | 0.40 | nd    | nd    | nd    | nd    | nd    |
| 7  | 1,2,4-Trimethylben zene | nd   | nd   | nd   | 0.46 | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 8  | 1,3,5- Trimethylben zene | nd   | nd   | 0.14 | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 9  | Cyclopropyl carbinol 2,3-Dihydro-3,5. | nd   | nd   | nd   | 0.42 | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| No | Compound name                          | TT 1 | PA 1 | LM 1 | TT r | PA r | LM r | TT s | PA s | LM s | BW se | SB se | PL se | TT se |
|----|----------------------------------------|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|
| 11 | Glycerose                              | nd   | nd   | nd   | 4.35 | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 12 | Pyrocatechol                           | 0.90 | 1.60 | 1.11 | 0.44 | 2.31 | 2.25 | 1.06 | 2.32 | 2.01 | nd    | nd    | nd    | nd    |
| 13 | 1,2,3-Propanetriol, monoacetate        | nd   | nd   | nd   | 3.12 | nd   | nd   | nd   | nd   | nd   | 0.51  | nd    | nd    | nd    |
| 14 | Nonoic acid                            | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 0.74  | nd    | nd    | nd    |
| 15 | 1,2,3,4-Tetrahydroxybutane             | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 16 | Phenol, 4-ethenyl-2-methoxy-           | nd   | nd   | 0.21 | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 17 | Methyl 2-formylbenzoate                | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 0.44 | nd   | nd    | nd    | nd    | nd    |
| 18 | 2-Amino-9-(3,4-dihydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-3,9-dihydropurine | nd   | nd   | nd   | 9.72 | 10.30 | bdl  | 10.29 | bdl  | bdl  | bdl   | bdl   | bdl   | bdl   |
| 19 | Levoglucosan                           | 1.30 | 1.55 | 0.92 | 1.14 | 1.77 | 2.39 | 1.90 | 2.31 | 1.91 | nd    | nd    | nd    | nd    |
| 20 | Lauric acid                            | bdl  | nd   | bdl  | nd   | nd   | nd   | 0.42 | nd   | 0.39 | nd    | nd    | nd    | nd    |
| 21 | Quinic acid                            | nd   | 3.62 | 5.26 | 6.48 | 8.81 | 6.28 | 8.74 | 6.91 | 6.67 | 2.20  | 5.47  | 3.31  | 2.09  |
| 22 | Methyl, alpha-D-glucopyranoside        | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 3.25  | nd    | nd    | nd    |
| 23 | Myristinol                             | 50.16| 48.20| 56.56| 32.86| 29.67| 29.90| 28.12| 18.52| 27.52| 6.27  | 8.47  | 10.00 | 4.94  |
| 24 | Neophytadiene                          | 0.26 | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 0.55 | nd    | nd    | nd    | nd    |
| 25 | Methyl palmitate                      | 0.99 | 0.99 | 1.61 | nd   | 0.57 | 0.85 | 0.65 | 0.94 | 1.87 | 1.45  | 0.75  | 0.93  | 0.62  |
| 26 | Palmitic acid                          | 6.22 | 6.41 | 5.46 | 4.89 | 6.69 | 8.80 | 3.84 | 8.83 | 7.25 | 17.23 | 8.34  | 11.49 | 7.82  |
| 27 | 2-Methyl octadec-9-enoate             | nd   | nd   | nd   | nd   | nd   | nd   | 3.13 | nd   | 1.61 | nd    | nd    | nd    | nd    |
| 28 | Methyl octadec-11-enoate              | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 29 | Methyl octadec-7-enoate               | nd   | nd   | nd   | 0.86 | nd   | nd   | 1.36 | nd   | nd   | nd    | nd    | nd    | nd    |
| 30 | Methyl 11-octadecenoate               | 1.50 | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 31 | Methyl oleate                          | 1.49 | 1.55 | nd   | 1.28 | 1.74 | nd   | 2.03 | 3.32 | nd   | 1.75  | nd    | nd    | nd    |
| 32 | Phytol                                | 2.05 | 3.82 | nd   | nd   | nd   | nd   | nd   | nd   | 1.34 | nd    | nd    | nd    | nd    |
| 33 | Phytol isomer                         | nd   | nd   | 2.70 | nd   | nd   | nd   | nd   | 0.55 | nd   | nd    | nd    | nd    | nd    |
| 34 | Methyl stearate                       | nd   | nd   | nd   | nd   | 0.27 | 0.28 | nd   | 0.54 | 0.41 | 0.81  | nd    | 0.43  | 0.38  |
| 35 | Heptadecenoic-(8)-carboxylic acid-(1)  | 10.92| 10.93| nd   | 10.13| 12.66| nd   | 8.51 | 13.25| nd   | 29.12 | 15.03 | 20.57 | 14.24 |
| 36 | Cyclopentadecane, 2-hydroxy-           | nd   | nd   | nd   | nd   | 14.00| nd   | nd   | nd   | nd   | nd    | nd    | nd    | nd    |
| 37 | Oleic acid                            | nd   | nd   | 8.75 | nd   | nd   | nd   | 3.13 | nd   | 1.61 | nd    | nd    | nd    | nd    |
In this study, brucine, *Sophoridane* (except mome inositol and quinic acid) were also found in other plant parts. Those fatty acids (14.00% of 2-hydroxy-10,11,12,12A-tetrahydrofuran-2-yl)-3,9-dihydro-puri; 14.00% of cyclopentadecanone, 2-hydroxy-was only obtained in root from Lampu Merah (LM), while 4,4,6A,6B,8A,11,12,14B-octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A (25.29%) was found in seed from SB. Furthermore, oleic acid was only obtained in leaf (8.75%) and stem (11.75%) from LM.

In addition, there were the main compounds that were used for therapy in genus *Strychnos*, brucine and strychnine. Those compounds were usually found in Semen Strychni, the seed of *Strychnos nux-vomica* [18] that accounted for more than 70% of alkaloids constituent [19]. In this study, brucine

| No | Compound name | TT l | PA l | LM l | TT r | PA r | LM r | TT s | PA s | LM s | BW s | SB s | PL s | TT s |
|----|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 39 | Stearic acid  | 4.98 | 5.86 | 4.14 | 3.34 | 4.73 | 5.47 | 3.44 | 5.62 | 5.77 | 13.75 | 4.62 | 7.39 | 4.75 |
| 40 | Sophoridane   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 0.49 |
| 41 | Matrine       | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 1.93 |
| 42 | cis-Octadec-9-enal | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 1.02 |
| 43 | Di-(9-Octadecenoyl)-glycerol | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 0.91 |
| 44 | 2-Monopalmitin | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 1.18 |
| 45 | Bis(2-ethylhexyl) phthalate | nd   | 1.23 | 1.19 | nd   | 2.70 | 7.11 | nd   | 1.49 | nd   | nd   |
| 46 | Isooctyl phthalate | 1.11 | nd   | nd   | 5.09 | nd   | 2.60 | 2.49 | nd   | nd   | nd   |
| 47 | 2-Monoolein   | bdl  | nd   | nd   | bdl  | 0.86 | nd   | nd   | 1.60 | nd   | 3.44 |
| 48 | 1,3-cis-9-Octadecenoyl-1,3-propanediol | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 2.59 |
| 49 | 4,4,6A,6B,8A,11,12,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 25.29 |
| 50 | Strychnine    | nd   | 0.89 | 1.75 | nd   | nd   | 2.02 | 2.78 | nd   | nd   | nd   |
| 51 | Brucine       | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | nd   | 43.95 |

Note: TT l: Teluk Terima leaf; LM s: Lampu Merah stem; PA l: Prapat Agung leaf; BW s: Banyu Wedang seed; LM l: Lampu Merah leaf; SB se: Sumber Batok seed; TT r: Teluk Terima root; PL se: Palengkong seed; PA r: Prapat Agung root; TT se: Teluk Terima seed; LM r: Lampu Merah root; TT s: Teluk Terima stem; bdl: below detection level; and PA s: Prapat Agung stem; nd: not detected.

The compounds with a percentage of peak area minimum of 1% are referred to as major components. From the analysis, it was identified 20 major compounds (Figure 1). The total percentage of major components in all plant parts was in the range of 58-88%. The major components in the four plant parts showed slight variations among the sites. Overall, all plant parts contained mome inositol, palmitic acid, heptadecene-(8)-carboxylic acid-(1), stearic acid, quinic acid, and methyl palmitate. Those fatty acids (except mome inositol and quinic acid) were also found in other *Strychnos* species (*S. nux-vomica*, *S. ignatii*, and *S. icaja*) [17].

A large percentage of some compounds were only found in specific parts or sites. In root and stem from TT, and root from Prapat Agung (PA) were found in the range of 9.72-10.30% of 2-amino-9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-3,9-dihydro-puri. A around 14.00% of cyclopentadecanone, 2-hydroxy-was only obtained in root from Lampu Merah (LM), while 4,4,6A,6B,8A,11,12,14B-octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A (25.29%) was found in seed from SB. Furthermore, oleic acid was only obtained in leaf (8.75%) and stem (11.75%) from LM.
(43.95%) was only detected in seed from TT, while strychnine could not be found in this plant part. Strychnine was found in a small amount (0.89-2.78%) in leaf and stem from PA and LM (Figure 1). This result was similar to another study that found a low concentration of strychnine in the extract of \textit{S. ligustrina} wood in different percentages of ethanol and 100% aquades solvent (< 4%). On the other hand, brucine was found in high concentration, in the range of 11.62-24.96% [1]. A similar result was also found in \textit{S. nux-vomica}. In this species, the concentration of brucine was higher than strychnine in the seed, whereas in the stem, the concentration of brucine was lower than strychnine [20]. It showed that the concentration of strychnine and brucine was may vary in different plant parts.

**Figure 1.** The component of major compounds in four plant parts of \textit{S. ligustrina}

3.2. \textit{Principal Component Analysis (PCA)}

Table 3 represented there was a significant positive and negative correlations between some compounds of \textit{S. ligustrina}. 2-Amino-9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-3,9-dihydro-puri had a positive and significant correlation with 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (0.889), levoglucosan had a positive and significant correlation with pyrocatechol (0.923) and had a negative and significant correlation with 2-monopalmitin (-0.871), while 2-monopalmitin had a positive and significant correlation with 2-monoolein. Moreover, palmitic acid had a positive and significant correlation with stearic acid (0.944), cyclopentadecanone, 2-hydroxy- had a positive and significant correlation with bis(2-ethylhexyl) phthalate (0.905).
The table below shows the correlation coefficient matrix for the phytochemical compounds of *S. ligustrina*.

| Var   | C1   | C2   | C3   | C4   | C5   | C6   | C7   | C8   | C9   | C10  | C11  | C12  | C13  | C14  | C15  | C16  | C17  | C18  | C19  | C20  |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| C1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C2    | 0.038| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C3    | 0.889| 0.126| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C4    | 0.208| 0.923| 0.278| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C5    | 0.594| 0.556| 0.637| 0.579| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C6    | 0.042| 0.459| 0.105| 0.490| 0.030| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C7    | 0.622| 0.175| 0.617| 0.088| 0.204| 0.156| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C8    | 0.364| 0.330| 0.467| 0.473| 0.406| 0.617| 0.333| 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |
| C9    | 0.339| 0.666| 0.303| 0.490| 0.428| 0.169| 0.511| 0.136| 1    |      |      |      |      |      |      |      |      |      |      |      |      |
| C10   | 0.060| 0.595| 0.051| 0.641| 0.413| 0.629| 0.173| 0.712| 0.569| 1    |      |      |      |      |      |      |      |      |      |      |      |
| C11   | 0.203| 0.374| 0.158| 0.403| 0.137| 0.505| 0.056| 0.075| 0.201| 0.405| 1    |      |      |      |      |      |      |      |      |      |      |
| C12   | 0.296| 0.255| 0.231| 0.154| 0.165| 0.330| 0.739| 0.187| 0.626| 0.591| 0.122| 1    |      |      |      |      |      |      |      |      |      |
| C13   | 0.310| 0.270| 0.394| 0.385| 0.426| 0.427| 0.443| 0.944| 0.156| 0.681| 0.024| 0.100| 1    |      |      |      |      |      |      |      |      |
| C14   | 0.282| 0.775| 0.357| 0.871| 0.463| 0.776| 0.074| 0.539| 0.370| 0.629| 0.188| 0.274| 0.359| 1    |      |      |      |      |      |      |      |
| C15   | 0.240| 0.604| 0.039| 0.528| 0.305| 0.217| 0.052| 0.038| 0.403| 0.529| 0.905| 0.067| 0.087| 0.356| 1    |      |      |      |      |      |      |
| C16   | 0.801| 0.022| 0.590| 0.274| 0.310| 0.114| 0.592| 0.398| 0.308| 0.047| 0.164| 0.240| 0.369| 0.371| 0.311| 1    |      |      |      |      |      |
| C17   | 0.275| 0.549| 0.187| 0.662| 0.424| 0.618| 0.084| 0.481| 0.493| 0.564| 0.166| 0.243| 0.359| 0.812| 0.181| 1    |      |      |      |      |      |
| C18   | 0.010| 0.343| 0.158| 0.386| 0.046| 0.325| 0.118| 0.035| 0.204| 0.140| 0.083| 0.122| 0.120| 0.398| 0.158| 0.164| 0.166| 1    |      |      |      |
| C19   | 0.285| 0.510| 0.335| 0.401| 0.233| 0.273| 0.669| 0.146| 0.774| 0.482| 0.176| 0.795| 0.078| 0.398| 0.023| 0.077| 0.352| 0.176| 1    |      |      |      |
| C20   | 0.203| 0.343| 0.158| 0.386| 0.336| 0.387| 0.198| 0.011| 0.280| 0.111| 0.063| 0.122| 0.106| 0.567| 0.158| 0.164| 0.627| 0.083| 0.176| 1    |      |      |      |

Note:
- C1=2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one;
- C2=pyrocatechol;
- C3=2-amino-9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-3,9-dihydro-puri;
- C4=levoglucosan;
- C5=quinic acid;
- C6=mono inositol;
- C7=methyl palmitate;
- C8=apalmitic acid;
- C9=methyl oleate;
- C10=heptadene-(8)-carbonic acid-(1);
- C11=cyclpentadecanone, 2-hydroxy;  
- C12=oleic acid;
- C13=stearic acid;
- C14=2-monopalmitin;
- C15=bis(2-ethylhexyl) phthalate;
- C16=isoctyl phthalate;
- C17=2-monoolein;
- C18=4,4,6A,6B,8A,11,12,14B-octamethyl-, 1,4,4A,5,6A,6B,7,8,8A,9,10,11,12,12A,14,14A;
- C19=strychnine;
- C20=brucine

*Values in bold are different from 0 with a significance level alpha = 0.05

Furthermore, the important chemical compounds of *S. ligustrina*, strychnine and brucine, also correlated with other compounds. Strychnine had a positive correlation with methyl palmitate (0.669), methyl oleate (0.774), and oleic acid (0.795). Brucine had a positive correlation with 2-monopalmitin (0.567) and 2-monoolein (0.627). In addition, strychnine had a little negative correlation with brucine (-0.176). It seems that the increase in the amount of strychnine in plant parts may was followed by the decline of the amount of brucine.

The principal component analysis of 20 compounds of *S. ligustrina* in different parts showed the total of first and second factors or components accounted for the highest relative variances. Relative variances of first and second factors were 34.51% and 22.52%, respectively, and comprised 57.04% of the total variance (Table 4). The compounds with the highest factor load for the first factor were pyrocatechol, levoglucosan, heptadene-(8)- carbonic acid-(1), and 2-monopalmitin. Moreover, 2,3-
dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 2-amino-9-(3,4-dihydroxy-5-hydroxymethyl tetrahydro-furan-2-yl)-3,9-dihydro-puri, and methyl palmitate were the compounds that accounted for the highest factor load for the second factor.

**Table 4.** Principal component analysis of chemical components of *S. ligustrina* in different parts.

| Chemical components                                      | F1     | F2     |
|----------------------------------------------------------|--------|--------|
| 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one       | -0.198 | 0.882  |
| Pyrocatechol                                             | -0.839 | -0.196 |
| 2-Amino-9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-    | -0.291 | 0.833  |
| furan-2-yl)-3,9-dihydro-puri                            |        |        |
| Levoglucosan                                             | -0.892 | 0.047  |
| Quinic acid                                              | -0.638 | 0.330  |
| Mome inositol                                            | -0.682 | -0.034 |
| Methyl palmitate                                         | -0.102 | -0.881 |
| Palmitic acid                                            | 0.660  | -0.422 |
| Methyl olate                                             | -0.610 | -0.588 |
| Heptadecene-(8)-carbonic acid-(1)                        | 0.844  | 0.166  |
| Cyclopentadecanone, 2-hydroxy-                           | -0.296 | -0.186 |
| Oleic acid                                               | -0.435 | -0.582 |
| Stearic acid                                             | 0.562  | -0.426 |
| 2-Monopalmitin                                           | 0.910  | -0.103 |
| Bis(2-ethylhexyl) phthalate                             | -0.485 | -0.270 |
| Isooctyl phthalate                                       | -0.247 | 0.766  |
| 2-Monoolein                                              | 0.793  | -0.065 |
| 4,4,6A,6B,8A,11,12,14B-Octamethyl-                        | 0.222  | 0.014  |
| 1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A            |        |        |
| Strychnine                                               | -0.522 | -0.570 |
| Brucine                                                  | 0.457  | 0.015  |
| Eigenvalue                                               | 6.903  | 4.506  |
| Variability (%)                                          | 34.514 | 22.528 |
| Cumulative (%)                                           | 34.514 | 57.042 |

The PCA biplot diagram presents the correlation among plant parts in different sites (Figure 2). Seed from all locations located to the right of the vertical axis PC1 (positive values). It showed that the chemical composition of the seed was similar. Root and stem from TT located to the left of the vertical axis PC1 (negative values) and positive value PC2. They placed closely therefore those showed similarity in their content composition. Leaf from PA and LM, root, stem from PA and LM located to the left of the vertical axis PC1 (negative values) and leaf from TT was positioned close to the right of the vertical axis PC1 (positive values). They showed similarities in their pattern of chemical composition (figure 2).

In the previous study on *Thymus caramanicus* showed that the major components varied between populations that related to environmental factors such as altitude and temperature [11]. Other environmental factors include humidity, rainfall, solar radiation, and macronutrient and micronutrients of soil also correlated to compositions of *Tithonia diversifolia* [21]. It seems that environmental factors have a significant influence on the chemical composition of plants. However, this result showed that different sites were not affected in chemical composition, while the difference in chemical composition
of *S. ligustrina* was found in plant parts. The similarity of the environmental conditions such as altitude and soil structure of sites may cause the similarity of the chemical components among plant parts.

![Figure 2](image1.png)

**Figure 2.** Biplot of the first two factors for different parts of *S. ligustrina* in different sites based on chemical compounds.

The hierarchical cluster in Figure 3 represented the dissimilarities and relationship among *S. ligustrina* parts from different sites based on Euclidean distances from the percentage of the peak of chemical components. The results showed that *S. ligustrina* studied was classified into three groups on dissimilarities of 32.63%. The first group contained seeds from all sites, while the second group consisted of root and stem from TT. The third group comprised of root and stem from LM and PA, also leaf from all sites. It presented that the chemical components between seed and leaf were different, while root and stem performed insignificant differences in the chemical components.

The first group of *S. ligustrina* samples had 2-monopalmitin and 2-monoolein that did not find in the second and third groups. Furthermore, the first group was distinct from other groups because it did not contain pyrocatechol and levoglucosan (Figure 1). It showed that those compounds distinguished the seeds from the other parts. Three phytochemical compounds that differentiate between the second and third groups. The third group contained methyl oleate, bis (2-ethylhexyl) phthalate, and strychnine that did not found in the second group (Figure 1). However, those compounds did not differentiate among leaf, root and stem, but could be used to discriminate between seed and the other parts.

![Figure 3](image2.png)

**Figure 3.** Hierarchical cluster analysis for different parts of *S. ligustrina* in different sites based on chemical compounds.
Strychnine was an important compound in *S. ligustrina* which has antimalarial activity [1]. Based on the results of this study, strychnine was only detected in the leaf and stem (Figure 1). No study explained strychnine in the root of this species. However, the study of *S. nux-vomica* appeared strychnine not only in leaf and stem but also in root [22]. Another study showed the extract of *S. ligustrina* seed detected alkaloids as one of a chemical content [8]. It seems that strychnine as an alkaloid compound was contained in its seed. Strychnine was not detected in root and seed may be caused by the very small amount of this compound in this part so it could not be detected by GC-MS analysis.

**Conclusion**
The major constituents identified in plant parts of *S. ligustrina* as mome inositol, heptadecene-(8)-carbonic acid-(1), palmitic acid, quinic acid, and stearic acid. Strychnine is the main active constituent of antimalarial drug detected in leaf and stem in a low concentration, but not be found in root and seed part. As the result showed, the chemical components did not distinct in different sites. The differences in chemical components could be seen in different plant parts. Furthermore, leaf and stem that contained strychnine, may have potential plant parts to be used as the antimalarial agent.

**Acknowledgments**
The authors are grateful to Gipi Samawandana, Ogi Setiawan, and the officer of West Bali National Park for supporting the sample collection. The funding was provided from the Research and Development Institute of Technology Non-Timber Forest Product of the Ministry of Environment and Forestry, Indonesia.

**References**

[1] Manurung H, Sari R K, Syafii W, Cahyaningsih U and Ekasari W 2019 Antimalarial activity and phytochemical profile of ethanolic and aqueous extracts of Bidara laut (*Strychnos ligustrina* Blum) *Wood J. Korean Wood Sci. Technol.* 47 587-596

[2] Cahyaningsih R, Brehm J M and Maxted N 2021 Setting the priority medicinal plants for conservation in Indonesia *Genet. Resour. Crop Evol.* 68 2019-50

[3] Setiawan O and Rostiwiati T 2014 Bidara Laut (*Strychnos lingustrina* Blume) syn. *S. lucida* R. Br: HHBK Potensial di NTB dan Bali Bidara Laut (*Strychnos lingustrina Blume*) syn. *S. lucida* R. Br: Sumber Bahan Obat Potensial di Nusa Tenggara Barat dan Bali (vol 1) ed T Rostiwiati and P Setio (Bogor: Forda Press)

[4] Hidayat S, Zuhud E A M and Widyatmoko D 2020 IOP Conf. Ser. on Earth and Environmental Science vol 528 (Bogor: IPB University/IOP Publishing Ltd) p 12017

[5] Wahyuni N 2014 Etnobotani bidara Laut (*Strychnos lingustrina* Blume. syn. *S. lucida* R. Br) di NTB dan Bali Bidara Laut (*Strychnos lingustrina Blume*) syn. *S. lucida* R. Br: Sumber Bahan Obat Potensial di Nusa Tenggara Barat dan Bali (vol 1) ed T Rostiwiati and P Setio (Bogor: Forda Press) chapter 3 pp 13-22

[6] Suriaman E and Khasanah S 2017 Skrining aktivitas antibakteri daun kelor (*Moringa oleifera*), daun bidara laut (*Strychnos ligustrina* Blume), dan amoxicilin terhadap bakteri patogen *Staphylococcus aureus*. *J. Biota*. 3 21-25

[7] Da Costa Sarmento N, Worachartcheewan A, Pingaew R, Prachayasittikul S, Ruchirawat S and Prachayasittikul V 2015 Antimicrobial, antioxidant and anticancer activities of *Strychnos lucida* R. Br. *Afr J. Tradit. Complement. Altern. Med.* 12 122-127

[8] Sumiati E 2014 Uji aktivitas antibakteri ekstrak kloroform dan ekstrak etanol biji bidara laut (*Strychnos ligustrina* Bl) terhadap *Staphylococcus aureus* ATCC 25923 dan *Salmonella typhi* *Biogenesis J. IIm. Biol.* 2 1-10

[9] Hazrati S, Ebadi M T, Mollaei S and Khurizadeh S 2019 Evaluation of volatile and phenolic compounds, and antioxidant activity of different parts of *Ferulago angulata* (schlecht.) *Boiss Ind. Crops Prod.* 140 111-589

[10] Reidel R V B, Cioni P L, Majo L and Pistelli L 2017 Evolution of volatile emission in *Rhus*
coriaria organs during different stages of growth and evaluation of the essential oil composition Chem. Biodivers.

[11] Bigdeloo M, Hadian J and Nazeri V 2017 Composition of essential oil compounds from different populations of Thymus caramanicus Jalas J. Appl. Res. Med. Aromat. Plants 7 95-98

[12] Nowicka P, Wojdylo A and Laskowski P 2019 Principal component analysis (PCA) of physicochemical compounds’ content in different cultivars of peach fruits, including qualification and quantification of sugars and organic acids by HPLC Eur. Food Res. Technol. 245 929-938

[13] Suganthy M and Gajendra C 2020 Chemical characterization of Strychnos nux-vomica L. leaves for biopesticidal properties using GC-MS Int. J. Chem. Stud. 8 1112-1116

[14] Putri Y 2020 Identifikasi senyawa bioaktif madu di beberapa daerah Sumbawa dengan menggunakan gas chromatography Food and Agro-industry J. 1 27-32

[15] Yin W, Wang T-S, Yin F-Z and Cai B-C 2003 Analgesic and anti-inflammatory properties of brucine and brucine N-oxide extracted from seeds of Strychnos nux-vomica J. Ethnopharmacol. 88 205-214

[16] Yu G et al. 2019 Brucine alleviates neuropathic pain in mice via reducing the current of the sodium channel J. Ethnopharmacol 233 56-63

[17] Frédérich M, Choi Y H, Angenot L, Harnischfeger G, Lefeber A W and Verpoorte R 2004 Metabolomic analysis of Strychnos nux-vomica, Strychnos icaja and Strychnos ignatii extracts by 1H nuclear magnetic resonance spectrometry and multivariate analysis techniques. Phytochemistry 65 1993-2001

[18] Gu L, Wang X, Liu Z, Ju P, Zhang L, Zhang Y, Ma B, Bi K and Chen X 2014 A study of Semen Strychni-induced renal injury and herb–herb interaction of Radix Glycyrrhizae extract and/or Rhizoma Ligustici extract on the comparative toxicokinetics of strychnine and brucine in rats Food Chem Toxicol 68 226-233

[19] Chen J, Hou T, Fang Y, Chen Z-p, Liu X, Cai H, Lu T-l, Yan G-j and Cai B-c 2011 HPLC determination of strychnine and brucine in rat tissues and the distribution study of processed semen strychni Yakugaku Zasshi 131 721-729

[20] Varaprasad V and Kumar R B 2017 Preparation of non-toxic dose of aqueous extract of Strychnine from the stem pieces of Strychnos-nux-vomica J. Pharm. Sci. Res. 9 762-765

[21] Sampaio B L, Edrada-Ebel R and Da Costa F B 2016 Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: a model for environmental metabolomics of plants Sci. Rep. 6 1-11

[22] Bandopadhyay J and De B 1997 Seasonal variation of strychnine and brucine in vegetative parts of Strychnos nux-vomica Int. J. Pharmacogn. 35 349-353