Screening of Indonesian Edible Plants for Bioactive Constituents and a New Protein Tyrosine Phosphatase 1B Inhibitory Acylbenzene Derivative from Leaves of Indonesian Syzygium polyanthum

Magie Melanie Kapojos,*a,b Delfly Booby Abdjul,a,b,c Hiroyuki Yamazaki,*b Akiho Yagi,b and Ryuji Uchidaa,b

a Faculty of Nursing, University of Pembangunan Indonesia; Bahu, Manado 95115, Indonesia; b Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University; Sendai 981–8558, Japan; and c North Sulawesi Research and Development Agency; 17 Agustus Street, Manado 95117, Indonesia.

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Bioassay screening using Indonesian plants, such as traditional foods (vegetables, spices, and tea) and folk medicinal herbs, identified eight protein tyrosine phosphatase (PTP) 1B inhibitory and two antibacterial plants. The leaves of Syzygium polyanthum (Wight) Walp. were examined in more detail to define PTP1B inhibitory components, resulting in the isolation of a new active acylbenzene (1) along with four related congeners of 1 (2–5) and four oleanane triterpenes (6–9). The structure of 1 was elucidated as 12-oxo-12-(2,3,5-trihydroxy-4-methylphenyl)dodecanoic acid based on its spectroscopic data. The acylbenzenes 1 and 3–5 inhibited PTP1B activity with $IC_{50}$ values ranging between 9.5 and 14 $\mu$M, whereas the triterpenes 7–9 also suppressed this activity with $IC_{50}$ values of 3.3–5.7 $\mu$M.

Key words screening; edible plant; acylbenzene; Syzygium polyanthum; protein tyrosine phosphatase 1B; inhibitor

Introduction

Plants, microorganisms, and marine invertebrates have been a significant source of natural products with structurally and biologically diverse properties, and have contributed to the supply of various pharmaceutical applications for human health.1) In recent years, as the discovery of novel drug candidates from natural origins has been gradually declining, unutilized natural resources in developing countries have been attracting attention in this research field. North Sulawesi in Indonesia is an archipelagic region and still maintains numerous bioresources; therefore, it may be promising to access substances of interest and significance from pristine natural bioresources.2) Thus, it is still attracting attention in this research field. North Sulawesi in Indonesia is an archipelagic region and still maintains numerous bioresources; therefore, it may be promising to access substances of interest and significance from pristine natural environments.2,3) Many types of wild plants that inhabit this area are traditionally utilized as vegetables, spices, tea, and medicinal herbs.4,6)

To identify useful edible plants containing bioactive constituents, thirty samples listed in Table 1 were collected around Manado city (North Sulawesi, Indonesia), and their EtOH extracts were applied to bioassay screening for protein tyrosine phosphatase (PTP) 1B inhibitory activity as well as antibacterial and antymycobacterial activities. PTP1B inhibitors are expected to be potential agents for the treatment and prevention of type 2 diabetes mellitus (T2DM) and obesity5) because this enzyme is regarded as an important negative regulator in the insulin and leptin signaling pathways.6,8) In the present study, the leaf extract of Syzygium polyanthum (Wight) Walp. (Myrtaceae) with potent PTP1B inhibitory effects (71% inhibition) in the in vitro enzyme assay3) was separated by bioactivity-guided purification to obtain five acylbenzene derivatives (1–5)8) that included one new derivative, 12-oxo-12-(2,3,5-trihydroxy-4-methylphenyl)dodecanoic acid (1), and four oleanane-type triterpenes (6–9)9) as shown in Fig. 1. We herein describe the bioactive screening results of Indonesian edible plants and the isolation, structural elucidation, and biological activities of 1–9 from S. polyanthum.

Results and Discussion

Thirty Indonesian edible plants collected at Manado and its surroundings were extracted by EtOH to evaluate their inhibitory activities against PTP1B. Screening results are summarized in Table 1.

The extracts of leaves of Plumeria sp. (No. 1), Cordyline sp. (No. 8), Muntingia calabura (No. 10), unidentified plant (No. 12), and Syzygium polyanthum (No. 27) and fruits of S. aromaticum (No. 28), Coriandrum sativum (No. 29), and Myristica fragans (No. 30) achieved more than 70% inhibition at 50 $\mu$g/mL (Table 1). The leaf extract of another Cordyline sp. (No. 7), unidentified plant (No. 13), and Carica papaya (No. 22) exhibited moderate activity (46–64% inhibition), while aerial parts of unidentified plant (No. 3) modestly inhibited PTP1B activity (22% inhibition) at the same concentration (Table 1).

Screening extracts were also assessed for antibacterial activities against Bacillus subtilis, Staphylococcus aureus, and Escherichia coli and antymycobacterial activities against Mycobacterium smegmatis, M. bovis BCG, M. avium, and M. intracellularare using the paper disk method. The extracts of aerial parts of Drynoglossum piloselloides (No. 19) and leaves of Annona muricata (No. 14) exhibited inhibition zones at 10 $\mu$g/disk against B. subtilis (13 mm) and S. aureus (11 mm), respectively, and none of the plant extracts showed growth inhibition against four mycobacteria in the range of 50 to 100 $\mu$g/mL (Table 1). The test strains of M. smegmatis and M. bovis BCG are alternative microorganisms to detect anti-tuberculous activity8) and M. avium and M. intracellularare are pathogens that cause Mycobacterium avium complex (MAC) disease.11)
Table 1. Bioactive Screening Results for EtOH Extracts of Indonesian Edible Plants

| No. | Scientific name | Part  | Inhibitory rate$^{a}$ | Inhibition zone (mm)$^{b}$ | MIC (µg/mL) |
|-----|-----------------|-------|-----------------------|-----------------------------|-------------|
|     |                 |       | PTP1B | B. subtilis | S. aureus | E. coli | M. smegmatis | M. bovis | BCG | M. avium | M. intracellulare |
| 1   | Plumeria sp.    | Leaves| 82% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 2   | Unidentified    | Aerial| — | — | — | — | >100 | >50 | >100 | >100 |
| 3   | Unidentified    | Aerial| 22% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 4   | Ruellia tuberosa| Aerial| — | — | — | — | >100 | >50 | >100 | >100 |
| 5   | Talinum paniculatum| Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 6   | Peperomia pellucida| Aerial| — | — | — | — | >100 | >50 | >100 | >100 |
| 7   | Cordyline sp.   | Leaves| 64% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 8   | Cordyline sp.   | Leaves| 80% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 9   | Coleus sp.      | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 10  | Muntingia calabara| Leaves| 76% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 11  | Cordyline sp.   | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 12  | Unidentified    | Leaves| 83% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 13  | Unidentified    | Leaves| 46% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 14  | Annona muricata | Leaves| — | — | 11 | — | >100 | >50 | >100 | >100 |
| 15  | Unidentified    | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 16  | Hibiscus rosa-sinensis| Flower| — | — | — | — | >100 | >50 | >100 | >100 |
| 17  | Unidentified    | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 18  | Unidentified    | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 19  | Drymoglossum piloselloides| Aerial| — | 13 | — | — | >100 | >50 | >100 | >100 |
| 20  | Abelmoschus sp. | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 21  | Abelmoschus sp. | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 22  | Carica papaya   | Leaves| 51% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 23  | Clerodendrum minahassae | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 24  | Abelmoschus sp. | Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 25  | Momordica charantia| Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 26  | Diplocycus esculentum| Leaves| — | — | — | — | >100 | >50 | >100 | >100 |
| 27  | Syzygium polyanthum| Leaves| 71% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 28  | Syzygium aromaticum| Fruits| 92% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 29  | Coriandrum sativum| Fruits| 77% inhibition | — | — | — | >100 | >50 | >100 | >100 |
| 30  | Myristica fragrans| Fruits| 86% inhibition | — | — | — | >100 | >50 | >100 | >100 |

$^{a}$ 50 µg/mL; $^{b}$ 10µg/mm disk; $^{c}$ Not active.

Nevertheless, flower parts of *Hibiscus rosa-sinensis* (No. 16), which is recognized as an indigenous anti-tuberculous herb in Indonesia,$^{12}$ was not active in our assays.

The leaves of *Syzygium polyanthum* (No. 27), which is also known as “Daun Salam,” is a familiar herb that is used not only as a food additive and tea with flavor, but also as folk remedies toward several diseases including diabetes.$^{13}$ Moreover, Saifuddin *et al.* reported PTP1B inhibitory activity of the leaves of *Syzygium polyanthum* and the active constituents.$^{7}$ Therefore, we proceeded to further identify the active constituents in the leaves of *Syzygium polyanthum* (No. 27) in the present study.

The EtOH extract from the leaves of *Syzygium polyanthum* was separated into ten fractions using an octadecyl silica (ODS) column. Bioactive fractions were subsequently purified by preparative HPLC (ODS) to afford compounds 1 (2.9 mg), 2 (4.5 mg), 3 (2.7 mg), 4 (1.4 mg), 5 (3.8 mg), 6 (3.9 mg), 7 (1.2 mg), 8 (2.9 mg), and 9 (7.1 mg). Compounds 2–9 were assigned as 10-oxo-10-(2,3,5-trihydroxy-4-methylphenyl)decaneoic acid,$^{7}$ 1-(2,3,5-trihydroxy-4-methylphenyl)hexane-1-one,$^{8}$ 1-(2,3,5-trihydroxy-4-methylphenyl)octane-1-one,$^{8}$ 1-(2,3,5-trihydroxy-4-methylphenyl)decanone-1-one,$^{8}$ oleanolic acid,$^{9}$ ursolic acid,$^{9}$ arjunolic acid,$^{9}$ and asiansic acid,$^{9}$ respectively, by comparing their spectroscopic data with reported values (Fig. 1).

The UV absorption of 1 at 291 nm in CH$_3$OH suggested the presence of a phenyl moiety, and the IR spectrum of 1 indicated the presence of hydroxy and carbonyl groups by bands at 3411 and 1684 cm$^{-1}$, respectively. The $^1$H-NMR spectrum of 1 showed an aromatic proton (δ$_{H}$ 5.88), aryl methyl proton (δ 1.90), and some methylene protons (δ 3.01, 2.27, 1.64, 1.58, and 1.31), and 17 carbon signals in the $^{13}$C-NMR spectrum.
were classified into two carbonyl carbons corresponding to ketone and carboxylic groups ($\delta_{C} 207.6$ and $177.8$), three sp$^2$ oxygenated quaternary carbons ($\delta 165.0, 163.8, 161.3$), two sp$^3$ quaternary carbons ($\delta 105.2$ and $103.6$), one sp$^2$ methine carbon ($\delta 94.8$), eight sp$^3$ methylene carbons ($\delta 44.9, 35.0, 30.7, 30.6, 30.4, 30.2, 26.4, 26.1$), and one methyl carbon ($\delta 7.3$) from the analysis of DEPT135 and heteronuclear multiple quantum coherence (HMHC) spectra. These spectroscopic data of 1 were identical to those of 2, suggesting that the molecular structure of 1 possesses a benzene skeleton with a long acyl chain similar to that of 2.

High resolution-electron ionization (HR-EI)MS of 1 gave its molecular formula as $C_{30}H_{32}O_{6}$ ($m/z$ 352.1873 [M]+, $\Delta$–1.3 mmu), which was suggested to have two more methane units (–CH$_2$CH$_2$–) than that of 2. Additionally, the integral value of the methylene proton at $\delta_{H} 1.31$ (approximately 12H) for 1 was larger than that for 2 (approximately 8H) in their $^1$H-NMR spectra. These marked differences between 1 and 2 demonstrated that compound 1 was a derivative of 2 with a longer acyl chain due to the presence of C$_2$, which was confirmed by the analysis of correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) data (Fig. 2) and named 12-oxo-12-(2,3,5-trihydroxy-4-methylphenyl)dodecanoic acid (1).

The PTP1B inhibitory effects of all isolated compounds, except for 6, were examined using an enzyme assay. Compound 6, oleanolic acid, is a typical PTP1B inhibitor, and, thus, authentic oleanolic acid was used as a positive control in the present study. The IC$_{50}$ values of 1–5, 7–9, and the positive control (oleanolic acid) are listed in Table 2. Compound 1 inhibited PTP1B activity with an IC$_{50}$ value of 9.6 $\mu$M, while 2 exhibited inhibitory activity of 35% at 31 $\mu$M and 3–5 showed IC$_{50}$ values of 14, 9.5, and 9.6 $\mu$M, respectively. The inhibitory activities of 2 and 5 with a C$_{10}$ long chain revealed that the 10'-methyl group in 5 was more favorable for this activity than the 1'-carboxylic acid moiety in 2. Moreover, the longer acyl chain appeared to be more suitable for inhibitory activity. Although the IC$_{50}$ value of 2 was reported as 13.1 $\mu$M in the previous study, our assay method gave the weaker inhibitory activity (35% inhibition at 31 $\mu$M). The oleanane triterpenes 7–9 showed the lowest IC$_{50}$ values (3.3–4.3 $\mu$M) in the present study, and we already reported the PTP1B inhibitory activities of the same type of triterpenes from Lantana camara, which is used to control blood glucose levels in T2DM similar to S. polyanthum. Consequently, the evaluation of medicinal plants containing triterpenes in an in vivo mouse model is ongoing, and the results obtained will be described elsewhere.

In conclusion, we screened thirty Indonesian plant extracts for PTP1B inhibitory, antibacterial, and antymycobacterial activities, identified eight plant extracts that potently inhibited PTP1B, and isolated a new PTP1B inhibitor, 12-oxo-12-(2,3,5-trihydroxy-4-methylphenyl)dodecanoic acid (1), from the leaves of S. polyanthum (No. 27). Continuous efforts to identify and isolate the active compounds of the other selected extracts are currently under way.

### Experimental

**Plant Samples** The edible plants used in the present study were collected in the area of Manado, North Sulawesi, Indonesia. Voucher specimens were preserved in the University of Pembangunan Indonesia (Manado, Indonesia) and North Sulawesi Research and Development Agency (Manado, Indonesia). Each plant sample (12.5–750 mg) was cut into small pieces and extracted with EtOH (approximately 200 mL). After filtration, the solution was concentrated in vacuo to give a crude extract. The extracts obtained were dissolved in CH$_2$OH at a concentration of 5 mg/mL and applied to bioactive screening assays.

**Isolation of New Compound 1** The leaf parts of S. polyanthum were collected at Manado in Indonesia. A voucher specimen was deposited at the Faculty of Nursing, University of Pembangunan Indonesia and North Sulawesi Research and Development Agency as 19F12.

The plant (215 g, dry weight) was cut into small pieces and exhaustively extracted with EtOH. The extract (8.7 g), after evaporation, was divided into ten fractions (Frs. 1–10) with an ODS column (100 g) by stepwise elution with CH$_2$OH in H$_2$O. Fractions 2–5 eluted with 30, 50, and 70% CH$_2$OH were combined (112 mg) and purified by HPLC (column; PEGASIL ODS SP100 (Senshu Scientific Co., Ltd., Tokyo, Japan), i.d. 10 $\times$ 250 mm; mobile phase, 40% CH$_2$OH in H$_2$O containing 0.05% TFA; detection, UV at 210 nm; flow rate, 2.0 mL/min) to give compound 1 (2.9 mg, t$_R$ = 30.3 min).

12-Oxo-12-(2,3,5-trihydroxy-4-methylphenyl)dodecanoic acid (1): Pale yellow oil; $^1$H-NMR (CDCl$_3$) $\delta$: 5.88 (1H, s, H-6), 3.01 (2H, t, $J_{H\text{-}H}$ = 7.3 Hz, H-11'), 2.27 (2H, brs, H$_2$-2'), 1.90 (3H, s, 4-CH$_3$), 1.64 (2H, t, $J_{H\text{-}H}$ = 10.1 Hz, H$_2$-10'), 1.58 (2H, brs, H-3'), 1.31 (10H, brs, H$_2$-4'–H$_2$-9'); $^{13}$C-NMR (CDCl$_3$) $\delta$: 207.6 (s, C-12'), 177.8 (s, C-1'), 165.0 (s, C-3), 163.8 (s, C-5), 161.3 (s, C-2), 105.2 (s, C-1), 103.6 (s, C-4), 94.8 (d, C-6), 44.9 (t, C-11'), 35.0 (t, C-2'), 30.7, 30.6, 30.4, 30.2, 26.4, 26.1 (t, C-3'–C-10'); 7.3 (q, 4-CH$_3$); IR (KBr) cm$^{-1}$: 3411, 2931, 2855, 1684, 1626, 1435, 1207; UV $\lambda_{max}$ (CH$_2$OH) nm (log$\varepsilon$): 223 (3.6), 291 (3.8); HR-EI-MS $m/z$: 352.1873 (Calcd for C$_{20}$H$_{26}$O$_6$: 352.1886); EI-MS $m/z$: 352 [M]+.

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**Conflict of Interest**  The authors declare no conflict of interest.

**Supplementary Materials**  The online version of this article contains supplementary materials.

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