Quantitative Evaluation of Various Preparations and Extracts of the Male Contraceptive *Justicia gendarussa* and Identification of a New Aminobenzyl Derivative

Authors
Mariam M. Mnatsakanyan¹, Emerson F. Queiroz¹, Laurence Marcourt¹, Bambang E. W. Prajogo², Jean-Luc Wolfender¹

Affiliations
1 School of Pharmaceutical Sciences, Phytochemistry and Bioactive Natural Products, University of Geneva, University of Lausanne, CMU – 1, Geneva, Switzerland
2 Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Jl. Dharmawangsa Dalam, Surabaya, Indonesia

Key words
UHPLC-TOF-HRMS, gendarusin A, 2-aminobenzyl derivatives, male contraception

Abstract
*Justicia gendarussa* is a medicinal plant found in different regions of Indonesia and used in decoctions by Papuan tribes to reduce male fertility. An enriched ethanolic extract of this plant has been used in the frame of clinical trials in Indonesia to evaluate its male contraceptive activity. Previous studies have indicated that the flavonoid gendarusin A may have a role in the male contraception properties of this plant. In addition, the level of aminobenzyl derivatives was lowered as a safety precaution. In order to obtain a comprehensive chemical profile of the methanolic plant extract, it was analysed by HPLC-PDA-ESI-MS and UHPLC-TOF-HRMS. The same method was also applied to profile extracts of the same plant material from different Indonesian regions, a water decoction used traditionally, and the enriched extract used in clinical trials. This allowed for the dereplication of all previously known flavonoids and newly reported amides, and permitted highlighting the presence of the potentially new aminobenzyl derivative. Targeted isolation of this new amide was performed using medium-pressure liquid chromatography. NMR and HRMS allowed for the establishment of the identity of the novel compound. The same procedure was used to obtain pure standards for quantitative studies. Quantitation of the major compounds was performed for different extracts using HPLC-UV. Significant differences were observed between the samples. Although gendarusin A was the main compound in all samples, it occurred in different amounts in the batches of dried material obtained from different Indonesian regions. The enriched extract contained mainly gendarusin A, as did the traditional decoction, but the level of the aminobenzyl derivatives was significantly lower.

Introduction
*Justicia gendarussa* Burm. F. (Acanthaceae) is a medicinal plant found in Sri-Lanka, India, Malaysia, the Philippines, and Indonesia [1]. Traditionally, the leaves have been used for many illnesses, including rheumatoid gout, arthritis, asthma, vaginal discharges, and headache [2–5], and their anti-inflammatory, analgesic [6], antioxidant, and hepatoprotective activities [7] have also been reported. Particularly remarkable is the male fertility reducing activity of
Fig. 1  HPLC-PDA-ELSD analysis of *J. gendarussa* methanol extract.
J. *gendarussa* leaves, used traditionally by the Indonesian Papuan people for male contraception [8, 9]. Apart from knowledge of it being used as a water decoction, there are, unfortunately, few documented details about the traditional preparations [9, 10].

The male contraceptive properties have gained interest in Indonesia for phytochemical and pharmacological investigations. An ethanolic leaf extract of *J. gendarussa* obtained from the Pacet region (Indonesia) was phytochemically investigated and 6,8-di-C-α-L-arabinocylapigenin (*gendarusin A*) was identified as the major constituent [11, 12]. Also identified was another less abundant flavonoid, C glycoside *gendarusin B* (6-C-α-L-arabinocyl-8-C-β-D-xylocilapigenin) [11, 12], and more recently, unusual 2-amino benzyl derivatives (*justidrusamides A-D*) [13]. O-Substituted aromatic amines [14], β-sitosterol, lupenol, and fridelin [15] were reported to be present in *J. gendarussa* collected in India.

*Gendarusin A* was reported to reversibly inhibit the activity of spermatazoa hyaluronidase activity [11, 12], which is a facilitating enzyme for sperm penetration during in vitro fertilisation [11, 16]; therefore, *gendarusin A* is considered to be the active constituent for the male contraceptive property of *J. gendarussa*. Currently, the *gendarusin A* flavonoid-enriched extract of *J. gendarussa* leaves is reportedly under clinical trials as a male contraceptive in Indonesia [12]. These extracts undergo an extensive “cleaning” standardisation process to improve their safety profile [12, 17].

Given the fact that the plant is also used by Papuans without any pretreatment, we thought comparative chemical profiling of the enriched extracts and the traditional water decoction was important to underline any chemical differences. We compared different *J. gendarussa* samples from other regions for the presence of the active compound and other major constituents to facilitate the future selection of *J. gendarussa* plant specimens with the highest content of the active constituent for cultivation. The extracts of *J. gendarussa* leaves collected in different regions of Indonesia as well as traditional water decoctions and enriched extracts were comparatively profiled for their main active constituent content (*gendarusin A*) and other major chemical markers. In the absence of available certified reference standards, the main active constituent flavonoid (*gendarusin A*), another main flavonoid (*gendarusin B*), and amide constituents (*justidrusamides A-D*) were isolated for use as standards for the quantitation. A detailed comparison of the traditional decoction and the enriched extract was established and the content of the main compounds in *J. gendarussa* from various regions of Indonesia was compared from a quantitative viewpoint.

### Table 1 Linear range, LOD, and LOQ of the three compounds (n = 6).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linearity range (µg/mL)</th>
<th>Calibration equation</th>
<th>LOQ (µg/mL)</th>
<th>LOD (µg/mL)</th>
<th>R²</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1-800</td>
<td>y = 695.45x - 12.682</td>
<td>0.024</td>
<td>0.008</td>
<td>0.999</td>
<td>1.74</td>
</tr>
<tr>
<td>5</td>
<td>1-500</td>
<td>y = 167.95x + 16.155</td>
<td>0.119</td>
<td>0.03</td>
<td>0.997</td>
<td>2.01</td>
</tr>
<tr>
<td>6</td>
<td>1-100</td>
<td>y = 98.195x + 1.316</td>
<td>0.889</td>
<td>0.293</td>
<td>0.998</td>
<td>8.73</td>
</tr>
</tbody>
</table>

*4 - *Justidrusamide A, 5 - *Justidrusamide B, 6 - *Gendarusin A. *h and x are the peak areas and concentrations of the analytes (µg/mL), respectively. The LOQ was defined as the concentrations at which the signal-to-noise ratio was 10, and the LOD was defined as the concentration at which the signal-to-noise ratio was 3.3. *Calibration range correlation factor (R²). *Relative standard deviation (RSD %).
Results and Discussion
In order to obtain a detailed survey of the composition of the leaves of *J. gendarussa*, a preliminary metabolite profiling was performed on the methanol leaf extract (see experiment) using reversed-phase HPLC-PDA-ESI-MS and high-resolution UHPLC-TOF-HRMS (Fig. 1). The evaporative light scattering detector (ELSD) trace revealed that three main constituent peaks, 4 [retention time (RT) 18.62 min], 5 (RT 20.67 min), and 6 (RT 23.31 min), were present in the extract as well as a large amount of very polar constituents, mainly sugars, not retained on C18 (RT 2.6 min). The PDA and MS spectra of these main constituents enabled the dereplication of 6 as the flavonoid C glycoside gendarusin A (UV PDA spectra: λmax 245, 352 nm; HRMS [M - H] at m/z 533.1347) [11, 12] and peaks 4 and 5 as the pair of 2-aminobenzyl derivatives, isomeric justidrusamide A or B [13] (UV PDA spectra: λmax 204, 236 nm; HRMS [M - H] at m/z 368.1365 and 368.1374, respectively).

Other minor constituents still detectable by ELSD were, on the one hand, the isomer of gendarusin A, gendarusin B (7), which exhibited the same m/z ion and chromophore as compound 6 and, on the other hand, three additional 2-aminobenzyl derivatives (1-3) all exhibiting the same chromophore (UV PDA spectra: λmax 204, 236 nm; Fig. 1).

Peaks 2 and 3 displayed [M - H] at m/z 384.1282 and 384.1297 in agreement with the molecular formula C17H22NO9 and were dereplicated as the two other isomeric pairs of justidrusamide C or D [13, 17]. Interestingly, 1 (justidrusamide E) also presented a chromophore of 2-aminobenzyl derivatives and was found to be an isomer of 4 and 5 (HRMS [M - H] at m/z 368.1356). The polarity and online data indicated that 1 (justidrusamide E) was a 2-aminobenzyl derivative that potentially had not been previously described. Since 4, 5, and 6 were the main compounds found in the leaf (Fig. 1), these three compounds were isolated to be used as standards for their quantitation in the traditional decoction, enriched extract used for clinical trials, and leaf extracts of *J. gendarussa* used from different Indonesian regions.

In order to unambiguously identify compound 1 and to obtain enough amount of the standards for the quantitation of 4-6, a crude methanol extract was fractionated. For an efficient and rational isolation, a direct transfer of the analytical HPLC conditions to MPLC was performed using the same reversed-phase material [18]. This procedure resulted in the isolation of six compounds from 3 g of crude methanol extract (1, 2, 3, 4-6). Their full de novo identification was performed based on 1D and 2D NMR and HRMS experiments. In accordance with the dereplication results, the identity of recently reported amides [13] justidrusamide D (2), justidrusamide C (3), justidrusamide A (4), and justidrusamide B (5) and the main flavonoid gendarusin A (6) [11, 12] was confirmed. In addition, 1 was found to be a new 2-aminobenzyl derivative and, as indicated from dereplication, probably a new compound not previously described. Its identification is described below.

Compound 1 was isolated as an amorphous solid. The ESI-HRMS spectrum showed a molecular ion at m/z 368.1356 [M - H]- (calcd. for C17H22NO9, 368.1345; Δppm = 3.0). The NMR data of 1 showed strong similarities with those of justidrusamide D [13]. The data

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**Table 2** Inter-day and intraday precision of the method (n = 6).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inter-day RT RSD</th>
<th>Inter-day Area RSD</th>
<th>Intrayday RT RSD</th>
<th>Intrayday Area RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.5</td>
<td>18.68 0.06 31.63 2.67</td>
<td>18.65 0.03 29.63 12.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>18.67 0.09 1999.4 1.21</td>
<td>18.63 0.04 1466.2 1.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>18.65 0.06 5555.32 2.11</td>
<td>18.59 0.04 5066.7 0.89</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** Recovery studies of justidrusamide A (4), justidrusamide B (5), and gendarusin A (6) from the *J. gendarussa* extract (n = 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Added concentrations (µg/mL)</th>
<th>Recovery</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>50</td>
<td>105</td>
<td>0.55</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>103</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>104</td>
<td>6.83</td>
</tr>
</tbody>
</table>

**Table 4** Retention time reproducibility of justidrusamide A (4), justidrusamide B (5), and gendarusin A (6) in five different *J. gendarussa* leaf preparations extracted and analysed separately (n = 3).

<table>
<thead>
<tr>
<th>Purwodadi</th>
<th>Cibodas</th>
<th>Gempol</th>
<th>Pacet</th>
<th>Water decoction</th>
<th>Enriched extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>18.59 0.04</td>
<td>18.62 0.04</td>
<td>18.60 0.04</td>
<td>18.65 0.36</td>
<td>18.62 0.02</td>
</tr>
<tr>
<td>5</td>
<td>20.69 0.04</td>
<td>20.67 0.12</td>
<td>20.69 0.04</td>
<td>20.70 0.08</td>
<td>20.72 0.02</td>
</tr>
<tr>
<td>6</td>
<td>23.25 0.05</td>
<td>23.31 0.08</td>
<td>23.27 0.06</td>
<td>23.28 0.12</td>
<td>23.30 0.02</td>
</tr>
</tbody>
</table>

*J. gendarussa* leaves from Purwodadi, Cibodas, Gempol, and Pacet regions in Indonesia. Water decoction and enriched extract prepared from *J. gendarussa* leaves. Mean retention time (min).
was indeed consistent with the presence of a 5-hydroxy-2-aminobenzyl alcohol group (δ 6.76 (1 H, dd, J = 8.5, 2.9 Hz, H-4), 7.00 (1 H, d, J = 2.9 Hz, H-6), 7.05 (1 H, d, J = 8.5 Hz, H-3), and 4.94 (2 H, s, H-17)) and a 2,3-dihydroxy-2-(1-hydroxyethyl) butanoic acid group (δ 1.04 (3 H, d, J = 6.4 Hz, CH 3-13), 1.05 (3 H, d, J = 6.4 Hz, CH 3-11), and 3.88 (2 H, q, J = 6.4 Hz, H-10, 12)). Compound 1 differed from 2 at the level of the succinic acid moiety where an additional methine was observed at δ 5.14 (1 H, d, J = 4.5 Hz, H-4') and δC 93.0. The COSY correlation between H-4' and the methylene CH 2-3' and CH 2-2' [δH 2.33 and 2.54] between CH 2-3' and CH 2-2' [δH 2.00 and 2.33] as well as the HMBC correlations from H-4' to CH 2-3' [δC 28.4] and to the ester carbonyl C-1' [δC 174.2] indicated that the succinic acid was cyclised in 1 to form a hydroxy-oxopyrrolidin group. Based on these results, 1 was identified as 5-hydroxy-2-(2-hydroxy-5-oxopyrrolidin-1-yl)benzyl(3 R)-2,3-dihydroxy-2-((R)-1-hydroxyethyl)butanoate, a new 2-aminobenzyl derivative named justidrusamid E (▶ Fig. 2).

The HPLC-UV method developed for the qualitative profiling was used for the quantification of the three main metabolites (4-6) and for comparison of the content of various extracts (see experiment). The method was validated for linearity, limits of detection (LOD), limits of quantification (LOQ), and for precision and accuracy using already isolated and identified compounds (see above) [19]. The linearity range for each compound was measured at a minimum of six different concentrations on each calibration curve (n = 6). Calibration curves were constructed by plotting injected concentrations of the standard analytes (X) vs. their peak areas (Y). Good linearity was observed by the linear regression analyses in a large range of concentrations: 1–800 µg/mL for compound 4 (R² = 0.999), 1–800 µg/mL for compound 5 (R² = 0.998), and 1–100 µg/mL for compound 6 (R² = 0.998) (▶ Table 1).

The LOQ and LOD were calculated by determining the standard deviation of the response and the slope of the linear equation. LOD was found to be 0.008, 0.013, and 0.293 µg/mL for 4, 5, and 6, respectively, and the corresponding LOQ was 0.024, 0.039, and 0.889 µg/mL for 4, 5, and 6, respectively (▶ Table 1).

The precision of the assay was determined by measuring intraday (repeatability) and inter-day (intermediate) variations (expressed as RSD %) at three concentrations included on the calibration curve (▶ Table 2). These solutions were prepared independently from the calibration solutions. Intraday variation was determined by analysing six replicates of known concentrations using the same preparation procedure within a single day (n = 6). Inter-day precision was determined in duplicate on 3 consecutive days (n = 6). The RSD % of the peak areas and retention times were calculated and the results are summarised in ▶ Table 2.

The intraday and inter-day RSD % did not exceed the allowed 15 %, which is in accordance with the bioanalytical method validation guidelines of the Food and Drug Administration [19]. Matrix effects were evaluated using recovery studies by the standard addition method. Known concentrations of standard compounds (4 and 6 at 50 µg/mL, 5 at 20 µg/mL) were spiked into a pre-analysed J. gendarussa methanol crude extract (1000 µg/mL) and reanalysed (▶ Table 3). The mean recovery for all compounds was 95 % and
within the acceptable upper limit of 120% (105, 103, and 104% for compounds 4, 5, and 6, respectively; Fig. 3).

For method reproducibility, the water decoction, enriched extract, and methanol extracts of *J. gendarussa* from various regions were analysed using the validated method. Results expressed as RSD% of the mean RT of compounds 4, 5, and 6 (n = 3) indicated good reproducibility of the method (RSD% < 1%; Fig. 4).

Comparison of the UV profiles of the water decoction and enriched extract (Fig. 3) confirmed that gendarusin A (6) is the major compound in both of the extracts. Furthermore, UV and ELSD results indicated that 4 and 5 (justidrusamides A and B) were at negligible amounts in the enriched extract, while the decoction still contained both in significant amounts. In the ELSD trace of the enriched extract, the peaks corresponding to the 2-aminobenzyl derivatives (1-3) were not detected. These results suggest that the acid-base extraction used for the preparation of the enriched extract for clinical studies removes the amides (see experiment). In the trials, the enriched extract was used once daily at a 450-mg dosage, indicating that the volunteers received 16.40 mg ± 1.26, w/w active compound gendarusin A (6) daily, while compounds 4 and 5 were kept at very low concentrations (0.01 mg ± 0.52 and 0.05 mg ± 0.05, w/w, respectively). Furthermore, as can be seen in Fig. 4, the enriched extract at a 450-mg dosage still contained more active gendarusin A (16.40 mg ± 1.26) than the 1 g dry leaf (12.45 mg ± 0.79) used for the decoction preparation, highlighting that the enriching process during the extraction is an important step to secure a high content of the active compound in the final yield.

The water decoction presented a similar qualitative profile to that of the methanol extracts (Fig. 5, Supporting Information). In order to facilitate future selection of *J. gendarussa* plant specimens with the highest content of the active principle 6, different samples were compared. Dry leaves from various regions in Indonesia (Purwodadi, Gempol, Cibodas, and Pacet) were exhaustively extracted by methanol (see experiment). For quantitation, all samples were prepared at 10 mg/mL through appropriate dilution of the stock solutions in 70/30, v/v MeOH-H2O (see experiment). The HPLC-UV comparison of the methanol extracts revealed that quantitatively all plants contained 1-7 (Fig. 5) and that the influence of the geographic origin did not seem to be very significant in the constituent makeup.

Though the gendarusin A (6) remained the main constituent in all extracts, the amounts were different in all four types of leaves. Purwodadi and Pacet dry leaves contained similarly high amounts of the active compound 6 (Fig. 6), while leaves of the Cibodas had the lowest content. Similar results have been reported elsewhere [17]. By contrast, Pacet and Gempol showed similar amounts of 5 (justidrusamide B). Such variation between the quantitative profiles of *J. gendarussa* leaves might be explained by the effect of cultivation practices in different geographical regions in accordance with previous reports [17].

Therefore, to have the highest antifertility effectiveness, it is necessary to have *J. gendarussa* leaves from the regions highest in...
gendarusin A, i.e., Purwodadi and Pacet. However, more studies on the effects of seasonal variations on the quantitative content of gendarusin A should be undertaken. The gendarusin A content was also found to be higher in the enriched extract containing the pill used for the clinical studies, but lesser than the extracts, which can be explained by the different extraction protocols used.

Overall, J. gendarussa leaves had similar chemical qualitative profiles independent from the geographical regions in Indonesia, however, they were quantitatively different. Cibodas leaves had the lowest amount of the active principle, gendarusin A (6), with about fourfold less flavonoid than the sample collected in Purwodadi, which is most probably due to the geographical region of cultivation (soil, temperature, etc.). The extract used in clinical trials contained mainly gendarusin A, as for the traditional decoction, but the level of the aminobenzyl derivatives was significantly lower. Qualitatively, all compounds isolated from J. gendarussa leaves were found in the traditional preparation (decoction). The water decoction still contained significant, although lesser amounts, of the active compound 6 compared to both the extracts and enriched extracts, which is encouraging for traditional users when taking into consideration their relatively simple method of preparation. Nevertheless, the decoction also contained aminobenzyl derivatives, which were absent in the enriched extract, and therefore the toxicology of these compounds should be considered in order to mitigate any delayed adverse effects amongst traditional users.

Materials and Methods

General experimental procedures

UV spectra were measured on a PerkinElmer Lambda 20 spectrophotometer. NMR spectroscopic data were recorded on a 500 MHz Varian Inova spectrometer. Chemical shifts are reported in parts per million (δ) using the residual CD3OD signal (δH 3.31; δC 49.0) or DMSO-d6 signal (δH 2.50; δC 39.5) as internal standards for 1H and 13C NMR, and coupling constants (J) are reported in Hz. Complete assignment was performed based on 2D experiments (COSY, TOCSY, NOESY, edited-HSQC, and HMBC). ESI-HRMS data were obtained at a Micromass LCT Premier time-of-flight mass spectrometer from Waters with an electrospray ionisation (ESI) interface. Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) using an Analytical...
ried water, (100 mL, pH 3, HCl) 3 × 24 h using a shaker at room temperature (20 ± 3 °C). The plant material residue was washed with running water until it reached a pH of 6. An aliquot of the residue was extracted with 70 % ethanol (3 × 24 h) by maceration and checked for the presence of alkaloids by TLC, using Dragendorff until the results were negative [20]. The filtrate obtained was evaporated at 40 °C using a rotary evaporator (Büchi). This process obtained a 70 % ethanol extract of the J. gendarussa leaves. The dried extract was kept at 4 °C until the analyses were performed. HPLC-PDA-ELSD analyses revealed that the enriched extract obtained was qualitatively similar to the capsules used in the clinical studies (results not shown).

HPLC-PDA-ELSD analysis

HPLC-PDA-ELSD analyses were conducted on an HP 1100 system equipped with a photodiode array detector (Agilent Technologies) connected to an ELSD Sedex 85 (Sedere). The HPLC conditions were as follows: X-Bridge C18 column (250 × 4.6 mm i.d., 5 μm, Waters); solvent system MeOH (B) and H2O (A), both containing 0.1 % FA; gradient elution 0 min 5 % B, 5 to 20 % B in 10 min, 20 % to 100 % B in 30 min. Flow rate 1 mL/min; injection volume 10 μL; sample concentration 10 mg/mL in the mobile phase. The UV absorbance was measured at 254 nm and the UV-PDA spectra were recorded between 190 and 600 nm (step 2 nm). The ELSD detection parameters were as follows: pressure 3.5 bar, 45 °C, split to provide a 500-μL/min flow rate, gain 8. This method was used for the profiling and quantification of the compounds. Leucine enkephalin (Sigma-Aldrich) was used as an internal reference at 2 μg/mL and infused through a Lock Spray probe at a proper dilution of the stock solution in 70/30, v/v MeOH-H2O. All samples were prepared daily before the analyses. All samples were filtered through a 0.45-µm membrane filter and the absorbance was detected at 254 nm. In total, 300 fractions were collected. All fractions were analysed using UPLC-TOF-MS. The purity of compounds 1-7 was estimated using HPLC-ELSD and UPLC-TOF-MS (results not shown), and the ELSD profiles of compounds 4-6 are shown in Fig. 9S, Supporting Information.

Compound characterization

Justirdusamide E (1). Amorphous white powder. [α]D²⁰ + 4.1° (c 0.96, MeOH); UV (MeOH); λmax (log ε) nm: 220 (sh), 276 (4.8). 1H NMR (DMSO-d₆, 500 MHz) δ 1.04 (3 H, m, H-3a), 3.88 (2 H, q, J = 5.5, H-11, 12), 4.87 (2H, s, H-7, 6), 7.00 (1 H, d, J = 2.9 Hz, H-6), 7.05 (1 H, d, J = 8.5 Hz, H-3). 13C NMR (DMSO-d₆, 126 MHz) δ 17.7 (C-11, 13), 24.7 (C-2), 28.4 (C-3), 62.2 (C-6), 68.5 (C-9), 93.0 (C-9), 105.2 (C-1), 156.9 (C-5), 173.8 (C-8), 174.2 (C-1). ESI-HRMS m/z 368.1356 [M - H]- (calcd. for C₁₇H₂₂NO₈, 368.1355).

Preparation of samples for quantitation

Standard stock solutions (1000 μg/mL) of compounds 4, 5, and 6 were prepared separately by accurately weighing 10 mg of each compound, transferring them into a volumetric flask (10 mL), and dissolving them in 10 mL of methanol under sonication (1 min). Working solutions were prepared through serial dilutions of the stock solutions in 70/30, v/v MeOH-H2O in the range of concentrations for the calibration curve covering 1 to 800 μg/mL for each compound [21]. Water decoction, enriched extract, and methanol extract stock solutions were prepared by accurately weighing 200 mg of each sample, transferring them into a volumetric flask (10 mL), and dissolving them in methanol (10 mL) under sonication (1 min). Working solutions (10 mg/mL) were prepared using appropriate dilution of the stock solution in 70/30, v/v MeOH-H2O. All samples were prepared daily before the analyses. All samples were prepared not earlier than a day before the analyses and were kept at 4 °C. All samples were filtered through a 0.45-μm membrane filter before injection into the HPLC system.

Supporting information

HPLC-UV chemical profiles of the J. gendarussa methanol extract vary from various regions in Indonesia and water decoction, 1H NMR spectrum of compound 1, and COSY NMR spectrum of compound 1 are available as Supporting Information.
Acknowledgements

The authors are thankful to the Swiss State Secretariat for Education Research and Innovation (SERI) for the financial support of this project (Grant 2013-0483 to M.M.).

Conflict of Interest

The authors declare no conflict of interest.

References