Isolation, characterization, and antibacterial assay of friedelin from *Garcinia latissima* Miq. leaves

N S S Ambarwati¹, B Elya²*, A Malik², M Hanafi³,⁴ and H Omar⁵

¹ Department of Cosmetology, Faculty of Engineering, Universitas Negeri Jakarta, Jl. Rawamangun Muka, East Jakarta, Indonesia, 13220
² Faculty of Pharmacy, Universitas Indonesia, UI Depok Campus, Depok 16424, Indonesia.
³ Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kawasan PUSPIPTEK, Serpong, Tangerang, 15314, Indonesia.
⁴ Faculty of Pharmacy, University of Pancasila, Srengseng Sawah, Jakarta, Indonesia.
⁵ Chemistry Division, Centre for Foundation Studies in Science, University of Malaya, Malaysia

*berna.elya@gmail.com

**Abstract.** The purpose of this study is to isolate *G. latissima* Miq. leaves ethyl acetate extract and to understand its activity against *B. subtilis*. The isolation was performed by open column chromatography, recrystallization, preparative thin layer chromatography and the isolate antibacterial assay by microdilution method. Compound characterization using spectroscopy. The isolated compound was white crystal, soluble in chloroform and insoluble in n-hexane, ethyl acetate. The melting point of the isolated compound was 262-264°C. IR spectrum showed strong signals for C-H stretching of methyl groups and carbonyl oxygen (C=O). The based on NMR data, the chemical shifts of chemical compounds from the isolate was compared with the literature showed that the isolate was friedelin. The MIC of friedelin against *B. subtilis* was 2,500 ppm.

1. **Introduction**

Friedelin has been isolated from *Garcinia prainiana*, and it has been tested that this compound does not have a cytotoxic effect of up to 25 mM. Friedelin is able to help glucose uptake in cells up to 1.8 times compared to insulin, so that it can be used for the treatment of type 2 diabetes [1]. Friedelin has also been isolated from *Garcinia rigida* leaves [2] and *Garcinia celebica* leaves [3]. Here researchers want to do friedelin isolation from the *Garcinia latissima* plant as one of the original plants from Indonesia that has never been studied before.

Indonesia is a vast archipelago country and has tremendous natural wealth [4]. One of nature's riches is the plant, which since prehistoric times has been used as a medicine ingredient [5]. One of the Indonesian plants is *Garcinia latissima* Miq. *Garcinia latissima* Miq. (Clusiaceae) grows on the island of Seram Maluku and Papua, but was cultivated in the Bogor Botanical Garden [6]. Previous research has been done that *G. latissima* Miq. leaves ethyl acetate extract with 2% concentration in dimethyl sulfoxide against *Bacillus subtilis* used agar diffusion method gave inhibition zone diameter 7.68 mm [7]. The minimum inhibitory concentration (MIC) of the extract against *B. subtilis* was 5,000 ppm and the minimum bactericidal concentration (MBC) was 10,000 ppm [7].
The isolation process was carried out in this study to purify the active compounds of fractions which still contain many compounds [8]. With the discovery of a pure compound provides opportunities for the discovery of new drugs [9]. It is also related to the side effects and microbial resistance of the synthesis drug [9].

One way to purify a compound was to use a recrystallization method, recrystallization using suitable solvents [10]. The choice of solvent was based on the polarity properties of the solvent and the solubility of the desired compound [10].

The Thin Layer Chromatography (TLC) is the most common and efficient method used for analysis, detection, and purification of bioactive compounds [11]. The eluent must be optimized in TLC development technique so that was obtained the most suitable mobile phase with an appropriate choice of ratio [11].

2. Materials and methods

2.1. Materials

The leaves of *G. latissima* Miq. were obtained from Bogor Botanical Gardens, West Java, Indonesia and has been determined by The Center for Plant Conservation Bogor Botanical Garden, Indonesian Institute of Sciences (LIPI) [7]. The process of fractionation using silica gel as the stationary phase and mobile phases used n-hexane, ethyl acetate, and methanol, in order of increasing polarity [12]. This research used fraction A from the result of fractionation with a weight of 420 grams [12].

The solvents for recrystallization used analytical grade n-hexane, ethyl acetate, and chloroform (PT Smart Lab Indonesia). The solvents were used according to the polarity index of each different solvent i.e. n-hexane 0.0, chloroform 4.1 and ethyl acetate 4.4 [13]. The thin layer chromatography plate used GF254 (Merck) with mobile phase analytical grade chloroform (PT Smart Lab Indonesia), ethyl acetate (PT Smart Lab Indonesia), and formic acid (Merck) [14]. The melting point determination used melting point apparatus-Stuart ScientificTM, SMP11, UK [16].

Infrared spectroscopy used IRPrestige-21 spectrometer, Shimadzu, Kyoto, Japan [15]. The nuclear magnetic resonance (NMR) spectroscopy used Bruker AscendTM 600 MHz [16].

The 96 well flat bottom tissue culture plate used sterile plates from Biologix, Shandong, China. The thiazolyl blue tetrazolium bromide (tetrazolium salt) used from BBI Life Sciences, Hongkong, China [17]. The solvent for the minimum inhibitory concentration (MIC) determination used dimethyl sulfoxide (DMSO) (8.02912-1000 mL, Merck) [17]. Antibiotic control used erythromycin, control of culture medium, and positive control of growth of *B. subtilis* was used [17].

2.2. Methods

The extraction and fractionation process of *G. latissima* leaves was carried out by researchers in previous research in Laboratory Pharmacognosy-Phytochemistry at Faculty of Pharmacy Universitas Indonesia [3,9]. The extraction process using maceration successively method and the extract used was ethyl acetate extract [7]. The process of fractionation using column chromatography method. We try to dissolve the candidate of isolate in each solvent (14). Preliminary identification of compound is made by the melting point determination (16).

Identification and classification of the compound using infrared spectroscopy using the KBr technique [15]. The powder compound and KBr must be ground to reduce the particle size to fine powder until crystallites and into a disc [18].

Determining the structure of a complete unknown in this study used nuclear magnetic resonance (NMR) spectroscopy which consists of one-dimensional (1D)-$^1$HNMR, $^{13}$CNMR, distortionless enhancement by polarization transfer (DEPT) $^{13}$CNMR and two-dimensional (2D)-NMR-$^1$H-$^{13}$C heteronuclear single-quantum correlation (HSQC) [16].

The antibacterial assay against *Bacillus subtilis* ATCC 6633 was evaluated through the minimum inhibitory concentration (MIC) determination using microdilution technique a 96 well flat bottom tissue culture plate, sterile and by staining with a solution of thiazolyl blue tetrazolium bromide (tetrazolium salt) [17]. In the test, 50 μL of compound solution 20,000 ppm (parts per million) was inoculated in three holes horizontally and then diluted with DMSO to three holes beneath it and so on.
to obtain the compound concentrations of 10,000 ppm, 5,000 ppm, 2,500 ppm, 1,250 ppm, 625 ppm, 312.5 ppm, 156.25 ppm, 78.13 ppm, 39.06 ppm, 19.53 ppm, and 9.77 ppm. Then each hole was added with 10 μL of *B. subtilis* suspension at a concentration of 106 CFU/mL and 40 μL of bacterial growth medium. So that the compound concentration of the test holes of 5,000 ppm, 2,500 ppm, 1,250 ppm, 625 ppm, 312.5 ppm, 156.25 ppm, 78.13 ppm, 39.06 ppm, 19.53 ppm, 9.77 ppm, 4.88 ppm, and 2.44 ppm. The microplates were incubated at 37°C for 24 h [17]. Once the incubation period had lapsed, 10 μL of a solution of thiazolyl blue tetrazolium bromide 0.6 mg/mL was added to the 96 well microplates [19], which were incubated at 37°C for 20 m [20].

2.3. Analytical
Analytical and preparative thin layer chromatography (TLC) was performed on silica gel 60 [14]. In this study, we tried and developed with a mixture of analytical grade chloroform/ethyl acetate/formic acid and the appropriate ratio was (2:0.3:0.2;v/v). If the isolate was pure, one spot will develop in the TLC system [21].

The results of the NMR spectrum are compared with the reference spectrum (structural features identified) [16]. The structure elucidation goal was achieved as a result of 1D- and 2D-NMR spectra to perform an NMR interpretation [16].

In the MIC test, twelve concentrations of the compound with dimethyl sulfoxide (DMSO) solution was analyzed [17].

The MIC was determined visually, and the color changed from light yellow to purple or light red showed there was a bacterial growth. The MIC was defined as the lowest concentration that led to growth inhibition, which was visually observed as no color change [22].

3. Results
The result of TLC analysis of fraction A of *G. latissima* leaves ethyl acetate extract using the best solvent system chloroform: ethyl acetate: formic acid (2: 0.3: 0.2 v/v) was shown in Figure 1 and consists in seven spots.

![Figure 1](image1.png)

**Figure 1.** The TLC profile of fraction A of *G. latissima* Miq. leaves ethyl acetate extract chloroform:ethyl acetate:formic acid (2: 0.3: 0.2 v/v).

The isolate candidate easily dissolved in chloroform but insoluble in n-hexane and ethyl acetate. The isolate solution in chloroform was evaporated at room temperature. The purification of an isolate of recrystallization result was done by preparative thin layer chromatography (PTLC). Isolate from PTLC result gave TLC profile using a mobile phase composed of chloroform, ethyl acetate, and formic acid (2: 0.3: 0.2 v/v) on Figure 2. The TLC profile showed that the spot was the single spot, the possibility the isolate was pure.

![Figure 2](image2.png)

**Figure 2.** The TLC profile of the pure isolate of recrystallization and PTLC result.
The pure isolate melting point was 262-264°C, possessed a sharp melting point. The Fourier transform infrared (FTIR) spectra of the compound were presented in Figure 3, showed some specific peaks on there to identify the functional group.

![Fourier transform infrared (FTIR) spectra of the compound.](image)

**Figure 3.** The Fourier transform infrared (FTIR) spectra of the compound.

The 1H-NMR spectra of the isolate can be seen in Figure 4 with the expansion in Figure 5 (expansion of the region 1.2-2.4 ppm, 600 MHz), Figure 6 (expansion of the region 1.2-1.7 ppm, 600 MHz), and Figure 7 (expansion of the region 0.6-1.1 ppm, 600 MHz).

![1H-NMR spectra of the isolate (CDCl3 (Deuterated Chloroform), 600 MHz).](image)

**Figure 4.** The 1H-NMR spectra of the isolate (CDCl3 (Deuterated Chloroform), 600 MHz).
Figure 5. The expansion of 1H-NMR spectra of the isolate between chemical shift (δ) 1.2 and 2.4 ppm (CDCl3, 600 MHz).

Figure 6. The expansion of 1H-NMR spectra of the isolate between chemical shift (δ) 1.2 and 1.7 ppm (CDCl3, 600 MHz).
Figure 7. The expansion of 1H-NMR spectra of the isolate between chemical shift (δ) 0.6 and 1.1 ppm (CDCl₃, 600 MHz).

The 13C-NMR spectra of the isolate can be seen in Figure 8 with the expansion of the region 0-70 ppm, 150 MHz in Figure 9.

Figure 8. The 13C-NMR spectra of the isolate (CDCl₃, 150 MHz).
Figure 9. The expansion of 13C-NMR spectra of the isolate between chemical shift (δ) 0 and 70 ppm (CDCl₃, 150 MHz).

The DEPT 13C-NMR spectra of the isolate can be seen in Figure 10.

Figure 10. The DEPT 13C-NMR spectra of the isolate (CDCl₃, 150 MHz).

The HSQC spectra of the isolate can be seen in Figure 11 and Figure 12.
Figure 11. The HSQC1 spectra of the isolate (CDCl3, 600 MHz).

Figure 12. The HSQC2 spectra of the isolate (CDCl3, 600 MHz).
The result of MIC microdilution testing system utilizing the tetrazolium salt in Figure 13.

Figure 13. The result of MIC microdilution testing system of the isolate utilizing the tetrazolium salt. Where: MC-medium control, ac-antibiotic control, BC-bacterial control.

4. Discussion

4.1. Isolation and purification of the compounds

The result of the TLC profile of fraction A showed that there are still many spots, so a separation method was necessary. In this research, the recrystallization method and preparative thin layer chromatography method were used, so it was expected to obtain a single TLC profile. After recrystallization and PTLC had been obtained pure isolate indicated by a spot on its TLC result. In this study, TLC has been used as an analytical tool because it has a high separation speed so that it can be used for a single component isolation process from its impurities. TLC is also used to check sample purity because it has a high sensitivity in observing the presence of impurities in pure samples [23].

4.2. Determination of the molecular structure of the compound

The melting point of pure isolate had a sharp melting point (262-264°C), this showed the substance chemically was pure [24].

The approach interpretation of IR spectrum, stretching absorption at a wave number of 2930 cm\(^{-1}\) specific of the C-H functional groups and stretching absorption at a wave number of 1707 cm\(^{-1}\) specific of the C=O functional groups.

The resulting of \(^1\)H-NMR spectrum (600 MHz, CDCl\(_3\)) by chemical shifts (\(\delta\)) showed the spectra at 0.78 (identified as Me-24) chemical shifts, 0.88 (identified as Me-25), 0.94 (identified as Me-29), 0.96 (identified as Me-26, Me-30), 1.09 (identified as Me-27) and 1.10 ppm (identified as Me-28) indicate the presence of 7 methyl groups (type of triterpenes) in singlets (Figure 14) and 1 doublets at \(\delta = 0.88\) (\(\text{J} = 4.56\), Me-23). There was a spectrum at a chemical shift between 1.3 to 2.5 ppm indicating the presence of methylene and methyl groups (CH\(_2\) and CH).

The resulting of chemical shifts (\(\delta\)) of \(^1\)H-NMR, \(^{13}\)C-NMR, and HSQC of the isolate can be tabulated in Table 1. In the \(^{13}\)C-NMR spectrum of the isolate, showed the presence of methyl groups (CH\(_3\)) at chemical shifts (\(\delta\)) 6.8, 14.7, 18.1, 18.3, 20.6, 32.9, 33.6 and 34.9 ppm. The \(^{13}\)C-NMR spectrum and DEPT spectra showed 30 carbons to consist of 8 methyl groups, 11 methylene groups, 4 methylmethones groups, and 7 quaternized atoms. The presence of seven carbon atoms was showed by the peaks represent (\(\delta\)C) at 28.2, 30.1, 37.5, 38.2, 39.4, 42.1, and 213.2 ppm. The chemical shift (\(\delta\)C) appear at 213.2 ppm reflected in the ketone group, corresponding to the FTIR spectrum of the 1713 cm\(^{-1}\) wavenumber. There were no both double bonds and hydroxy group in the structural formula.

The chemical structure supported by the correlation of HSQC1 of the isolate (Figure 11) showed the relationship between \(\delta\)C 22.3 with \(\delta\)H 1.95, 1.72 (2H, ddd). The relationship between \(\delta\)C 41.5 with \(\delta\)H 2.39, 2.29 (2H, ddd), the relationship between \(\delta\)C 58.2 with \(\delta\)H 225 (1H, q), and the relationship between \(\delta\)C 41.2 with \(\delta\)H 1.73, 1.28 (2H, d). Then the relationship between \(\delta\)C 18.2 with \(\delta\)H 1.49, 1.35 (2H, m),
the relationship between δC 52.5 with δH 1.45 (H, d), and the relationship between δC 35.4 with δH 1.46; 1.28 (2H, m).

Direct correlation of molecular structure of HSQC2 the isolate (Figure 12) showed the relationship between δC 6.8 with δH 0.88 (3H, d). The relationship between δC 14.7 with δH 0.78 (3H, s) and the relationship between δC 18.1 with δH 0.88 (3H, s). Then the relationship between δC 34.9 with δH 0.96 (3H, s) and the relationship between δC 32.9 with δH 0.94 (3H, s).

The conclusion based on spectroscopic data and supported by chemical shifts of chemical compounds from the isolate was compared with the literature that the isolate was friedelin (Figure 15), having the formula C30H50O (molecular weight 428). This assumption was reinforced by data of DEPT, HSQC, and chemical shifts data from the literature as in Table 1.

Table 1. Tabulation of chemical shifts from 1H-NMR and 13C-NMR of the isolate spectra compared with Friedelin [25]

| The isolate | Friedelin |
|-------------|-----------|
| C | δ 13C-NMR | δ 1H-NMR | δ 13C-NMR | δ 1H-NMR |
|---|---|---|---|---|
| 1 | 22.3 | 1.95; 1.72 (2H, ddd) | 22.3 | 1.95; 1.71 (2H, ddd) |
| 2 | 41.5 | 2.39; 2.29 (2H, ddd) | 41.5 | 2.37; 2.27 (2H, ddd) |
| 3 | 213.2 | - | 213.2 | - |
| 4 | 58.2 | 2.25 (1H, q) | 58.2 | 2.25 (1H, q) |
| 5 | 42.1 | - | 42.1 | - |
| 6 | 41.2 | 1.73; 1.28 (2H, d) | 41.3 | 1.74; 1.28 (2H, d) |
| 7 | 18.2 | 1.49; 1.35 (2H, m) | 18.2 | 1.49; 1.36 (2H, m) |
| 8 | 52.5 | 1.45 (H, dd) | 53.1 | 1.38 (1H, dd) |
| 9 | 37.5 | - | 37.4 | - |
| 10 | 59.5 | 1.52 (1H, m) | 59.5 | 1.53 (1H, m) |
| 11 | 35.4 | 1.46; 1.28 (2H, m) | 35.7 | 1.45; 1.26 (2H, m) |
| 12 | 30.1 | 1.33; 1.32 (2H, m) | 30.5 | 1.33; 1.32 (2H, m) |
| 13 | 39.4 | - | 39.7 | - |
| 14 | 38.2 | - | 38.3 | - |
| 15 | 32.9 | 1.47; 1.27 (2H, m) | 32.4 | 1.47; 1.27 (2H, m) |
| 16 | 35.4 | 1.52; 1.35 (2H, m) | 36.0 | 1.58; 1.35 (2H, m) |
| 17 | 30.1 | - | 30.0 | - |
| 18 | 42.1 | 1.52 (1H, m) | 42.8 | 1.56 (1H, m) |
| 19 | 35.2 | 1.37; 1.27 (2H, m) | 35.3 | 1.37; 1.22 (2H, m) |
| 20 | 28.2 | - | 28.2 | - |
| 21 | 32.9 | 1.45; 1.30 (2H, m) | 32.8 | 1.50; 1.31 (2H, m) |
| 22 | 39.4 | 1.46; 0.96 (2H, m) | 39.2 | 1.51; 0.95 (2H, m) |
| 23 | 6.8 | 0.88(3H, d) | 7.0 | 0.88 (3H, d) |
| 24 | 14.7 | 0.78 (3H, s) | 14.6 | 0.73 (3H, s) |
| 25 | 18.1 | 0.88 (3H, s) | 17.9 | 0.87 (3H, s) |
| 26 | 20.6 | 1.01(3H, s) | 20.2 | 1.01 (3H, s) |
| 27 | 18.3 | 1.09 (3H, s) | 18.6 | 1.05 (3H, s) |
| 28 | 33.6 | 1.10 (3H, s) | 32.1 | 1.18 (3H, s) |
| 29 | 34.9 | 0.96(3H, s) | 35.0 | 1.00 (3H, s) |
| 30 | 32.9 | 0.94 (3H, s) | 31.8 | 0.94 (3H, s) |

The melting point range of the isolate was 262-264°C compared to the literature melting point range of 262°C-264°C. Friedelin has been isolated from Maytenus robusta leaves [25], from the roots of Terminalia avicennooides [26], from Sacchottis uchi [27], from the roots of Caesalpinia digyna [28], from the roots of Vismia laurentii and Maytenus salicifolia leaves [26].
4.3. Tested for antibacterial activity Against Bacillus subtilis
Friedelin has been reported to have activity as anti-inflammatory, and hepatoprotective [26]. Friedelin has also been reported to be cytotoxic to human tumor cells, reducing gastric acid production, and treating lishmania infections (a protozoan parasite spread by flies) [27]. Friedelin has been tested for anti microbacterial activity with a MIC value of 4.9 ppm [26].

From the result of the antibacterial activity test against B. subtilis with microdilution method, the MIC value of the isolate was 2500 ppm (Figure 13). This value was equal to the MIC value of the fraction A of leaves ethyl acetate extract [12].

5. Conclusion
Friedelin compound from fraction A of G. latissima has been isolated leaves ethyl acetate extract. This compound has activity as an anti-bacterial B. The substance is the same as its fraction activity (the MIC value of the isolate was 2500 ppm).

Acknowledgments
The authors acknowledge the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding this study (with the contract number: 1/SP2H/DRPM/LPPM-UNJ/III/2019).

References
[1] Susanti D, Amirudine M Z A M, Rezali M F and Taher M 2012 Friedelin and Lanosterol from Garcinia prainiana Stimulated Glucose Uptake and Adipocytes Differentiation in 3T3- L1 Adipoctyes Nat. Prod. Res. 1–8
[2] Elya B, He H P, Kosela S, Hanafi M and Hao X J 2011 Triterpenoids from Garcinia rigida Rec. Nat. Prod. 5 56–9
[3] Elfita E, Muharni M, Latief M, Darwati D, Widiyantoro A, Supriyatna S, Baht H H, Dachriyanus D, Cos P, Maes L, Foubert K, Apers S and Pieters L 2009 Antiplasmodial and other constituents from four Indonesian Garcinia spp. Phytochemistry 70 907–12
[4] Agustina C D, Ahmad E, Nugroho D and Siagian H 2012 Political Economy of Natural Resource Revenue Sharing in Indonesia
[5] Yuan H, Ma Q, Ye L and Piao G 2016 The Traditional Medicine and Modern Medicine from Natural Products Molecules 21 1–18
[6] Ambarwati N S S, Malik A, Deborah E A, Arpati SM C H, Hanif M, Elya B and Hanafi M 2017 The Antibacterial Activity of Fractions of Ethyl Acetate Garcinia latissima Miq. Stem Bark Extracts Against Bacillus subtilis and Pseudomonas aeruginosa Asian J. Pharm. Clin. Res. 10 1–4
[7] Ambarwati N S S, Ahmad I, Elya B, Malik A and Hanafi M 2017 Pharmacognostic and Antimicrobial Studies of <i>Garcinia latissima</i> Miq. Leaves (Clusiaceae) Pharmacogn. J. 9 493–8
[8] Sarker S D and Nahar L 2012 An Introduction to Natural Products Isolation <i>Natural Product Isolation Methods and Protocols</i> ed S D Sarker and L Nahar (London: Humaina Press) pp 1–25
[9] Sasidharan S, Chen Y, Saravanan D, Sundram K M, Latha L Y, Bedong-semeling J and Nasi B A 2011 Extraction, Isolation and Characterization of Bioactive Compounds from Plants ’ Extracts Afr. J Tradit Complement Altern Med. 8 1–10
[10] Zala S P, Patel K P, Patel K S, Parmar J P, Sen D J, Parmar J P, Jyoti D, Dev I J D and Scopus C 2012 Laboratory Techniques of Purification and Isolation Int. J. Drug Dev. Res. 4 41–55
[11] Mohd K, Azemian A, Hamil M S R, Bakar A R A, Dharmaraj S, Hamdan M R, Mohamad H, Mat N and Ismail Z 2014 Application of HPTLC and FTIR Profiling Coupled with Chemometrics for The Differentiation of The Varieties of <i>Ficus deltoidea</i> Jack. Asian J. Pharmacetical Clin. Res. 7 110–6
[12] Ambarwati N S S, Elya B, Nur A, Puspitasari N, Malik A and Hanafi M 2018 Activity of Fractions from Garcinia latissima Miq . Leaves Ethyl Acetate Extract as Antibacterial Against Bacillus subtilis and Antioxidant Adv. Sci. Lett. 24 6366–70
[13] Seidel V 2006 Initial and Bulk Extraction Natural Product Isolation ed S D Sarker, Z Latif and A I Gray (New Jersey: Humana Press) pp 27–46
[14] Agrawal V and Desai S 2015 Centrifugally Accelerated Thin Layer Chromatography for Isolation of Marker Compounds and Bioactives J. Pharmacogn. Phytochem. JPP 3 145–9
[15] Liu M, Liu J, Yu Y, Zhou P, Su R, Qi W, Wang L and He Z 2016 Classification and identification of Organic Compounds by Pattern Recognition Based on Near Infrared Spectroscopy Fresenius Environ. Bull. 25 5186–93
[16] Elyashberg M E 2015 Identification and structure elucidation by NMR spectroscopy Trends Anal. Chem. 69 88–97
[17] Caamal-Herrera I O, Carrillo-Cocom L M, Escalante-Réndiz D Y, Aráiz-Hernández D and Azamar-Barrios J A 2018 Antimicrobial and Antiproliferative Activity of Essential Oil, Aqueous and Ethanolic Extracts of Ocimum micranthum Willd Leaves” BMC Complement. Altern. Med. 18 1–9
[18] Simmons I L 1960 The KBr Technique Spex Speak. V 1–8
[19] Biloa Messi B, Ho R and Meli Lannang A 2014 Isolation and biological activity of compounds from Garcinia preussii Pharm. Biol. 52 706–11
[20] Grare M, Fontanay S, Cornil C, Finance C and Duval R E 2008 Tetrazolium Salts for MIC Determination in Microplates: Why? Which Salt to Select? How? J. Microbiol. Methods 75 156–9
[21] Maw S S 2011 Extraction, Isolation and Identification of Chemical Constituents from the Leaves of Clerodendrum inerme Univ. Res. J. 4 161–74
[22] Zubair M, Rizwan K, Rasool N, Afshan N, Shahid M and Ahmed V 2011 Antimicrobial potential of various extract and fractions of leaves of Solanum nigrum Int. J. Phytotherapy 3 63–7
[23] Bele A A and K A K H 2011 An Overview on Thin Layer Chromatography Int. J. Pharm. Sci. Res. 2 256–67
[24] Mann F G and Saunders B C 1978 Practical Organic Chemistry (London: Longman)
[25] Sousa G F, Duarte L P, Alcântara A F C, Silva G D F, Vieira-filho S A, Silva R R and Takahashi J A 2012 New Triterpenes from Maytenus robusta: Structural Elucidation Based on NMR Experimental Data and Theoretical Calculations Molecules 17 13439–56
[26] Mann A, Ibrahim K, Oyewale A O, Amupitan J O, Fatope M O and Okogun J J 2011 Antimycobacterial Friedelan-Terpenoid from The Root Bark of Terminalia Avicennioides Am. J. Chem. 1 52–5
[27] Gomes V, Corrêa G M, Aparecida I, Silva R R and Alcântara A F D C 2013 Pentacyclic Triterpenes and Steroids from the Stem Bark of Uchi (Sacoglottis uchi, Humiriaceae ) Acta Amaz. 43 525–8
[28] Srinivasan R, Chandrasekar M J N and Nanjan M J 2011 Phytochemical Investigations of Caesalpinia digyna Root E-Journal Chem. 8 1843–7