Identification of metabolite compounds from ethanolic extract of the Red Gedi Leaves (Abelmoschus manihot L. Medik) by LC-ESI-MS

by Juliet Tangka

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Identification of metabolite compounds from ethanolic extract of the Red Gedi Leaves (*Abelmoschus manihot* L. Medik) by LC-ESI-MS

Juliet Tangka[1,2], Elisabeth N. Barung[2], Diana Lyrawati[3]*, Djoko W. Soeatmadji[4], Nurdiana[5]

[1]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.
[2]Department of Pharmacy, Politeknik Kesehatan, Kementerian Kesehatan Manado, Manado, Indonesia.
[3]Department of Pharmacy, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.
[4]Department of Internal Medicine, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.
[5]Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.
*Corresponding Author E-mail: **juliet.tangka@gmail.com, elisabeth.barung1225@gmail.com, diana.l@ub.ac.id, djokowahono@ub.ac.id, nurdianafarmako.fk@ub.ac.id**

ABSTRACT:
Abelmoschus manihot L. Medic, commonly called "red gedi", is an endemic species of Minahasa, Indonesia. The leaves of red gedi have been widely used in ethnomedicine and functional food as an antidiabetic. In this study, the ethanolic extract of the red gedi leaves was characterized by using liquid chromatography coupled to electrospray ionization-tandem mass spectrometry (LC–ESI-MS). Compounds identified were phenolic acid derivates, flavonoids, terpenoids, phytosterols, alkaloids, and lignans. The most abundant flavonoids in the extract sample were quercetin derivatives. In total, 38 metabolite compounds were identified in red gedi leaves and were reported for the first time, including alpha spinasterol which is newly identified in this particular Abelmoschus species.

KEYWORDS: *Abelmoschus manihot* L. Medik, bioactive compounds, quercetins, LC-ESI-MS.

1. INTRODUCTION:

The Abelmoschus manihot. L Medik) from Malvaceae grows endemically in North Sulawesi and known as "gedi". The leaves of gedi empirically used by the locals to manage cardiovascular disease as an antidiabetic, antihypertension and anticholesterolemia [1, 2, 3]. Others reported some active flavonoids derived from the flowers and the whole plants of gedi used as traditional medicine[4, 5]. However, little is known about the metabolite compounds of the gedi leaves and their pharmacologic activities, in particular the red gedi. The leaves of gedi has been reported to contain β-sitosterol[5], eikodekana[6], and heptadecanoic acid[7]. The flavonoid identified from ethyl acetate extract of gedi leaves were flavonoids of auron class i.e. 3',4,6-trihydroxy, 4-alkoksi auron with functional groups aliphatic C-H, free -OH, alcohol C-O, aromatic C=C, aromatic C-He, ether C-O and dan C=O also substituted OH at C-4, C-6 and C-3' as well as OR at C-4 as reported by Theodora[8].

Ethanolic (96%) extract of gedi contained total flavonoid 41.56%[9]. Specifically in red gedi, it was reported that the total phenolic, flavonoids, and tanin were 1003 mg/kg, 722 mg/kg and 1029 mg/kg, respevtively[2]. In this study, we aimed to identify the metabolite compounds of the red gedi leaves following etanol extraction using liquid chromatography coupled with mass spectrometry.

2. MATERIAL AND METHODS:

2.1. Plant material:

The leaves of red gedi *Abelmoschus manihot* L. Medik were collected during August 2018 from North Tondano Plantation, North Sulawesi, Indonesia. Determination of the plants were performed at the Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences, Bogor, Indonesia (Letter B-3177/IPH.3./KS/IX/2018 on September 18, 2018).

2.2. Chemical and reagents:

Formic acid and methanol were obtained from Merck KGaA (Darmstadt, Germany), used for chromatographic analysis. All solutions were prepared with ultra-pure water (Millipore, MA, USA). The other solvents and reagents in this study were of analytical grade.

2.3. Extraction:

The extract was obtained by dynamic maceration extraction technique using 96% ethanol solvent. Fifty grams of the dried red leaves *Abelmoschus manihot* L. Medic, put into a 1-liter Erlenmeyer, then added 500 mL 96% ethanol (1:10) and placed on a digital hot plate magnetic stirrer. Extraction was performed at 30 to 40^{0}C, stirred at 200 rpm for 6 hours. Macerate was collected by filtering the solution and transferred into a closed vessel. The macerate was then evaporated first at 50^{0}C using a rotary evaporator, followed by oven evaporation at 80^{0}C until a thick extract obtained without the smell of ethanol. The final extract was refrigerated and stored until used for further experiments.

2.4. Identification of active compounds:

Characterization of compounds of ethanolic extract of red leaves *Abelmoschus manihot* L.Medik was conducted using an UHPLC system connected to a Triple Quadrupole Mass Spectrometer LCMS-8040 (Shimadzu, Kyoto, Japan). The specific configuration included LabSolutions Ver. 5.00 Chromatography Workstation, using a column Shimadzu Shim-pack FC-ODS III (2.0 mm (I.D.) x 150 mm,3 µm), mobile phase A solution containing 0.1% formic acid in water, mobile phase B absolute methanol. Separation of compounds was carried out with gradient elution profile 0/0 at 0 min, 15:85 at 5 min, 20:80 at 20 min, 90:10 at 24 min. Mass spectra were simultaneously acquired using electrospray ionization (ESI) in the positive ionization modes, and full-scan mass spectra were acquired at a mass-to-charge ratio (m/z) of 50–1000. Fragmentation method used was at low energy CID with flow rate 0.5 mL/min; column temperature 35°C; and injection volume 1 µL. Other settings were used at values obtained by automatic adjustment.

Primary raw data of LC-ESI-MS assay was analyzed by data alignment, peak findings, peak integration, and retention time (Rt) correction using a LabSolutions Ver. 5.00 Chromatography Workstation, compared with commercial compounds based on the NIST/EPA/NIH mass spectral library.

3. RESULTS AND DISCUSSION:

3.1. Identification of metabolite compounds:

In this study, we identified in total 38 phenolic- and non-phenolic compounds of ethanolic extract red gedi leaves *Abelmoschus manihot* L.Medik, based on retention time and the LC-ESI-MS profiles (Figure 1 and Table 1). Identification of major peaks was based on their molecular mass[10]. Chemical formulas were identified based on m/z precursor and fragment ions. Spectra data were compared to that of the NIST/EPA/NIH mass spectral library. The LC-ESI-MS and similar methods based on chromatographic profiles are commonly used for compounds identification[11-14].

Fig.1. LC-MS/MS peak chromatograms (positive ion mode) of the ethanolic extract of red gedi leaves *Abelmoschus manihot* L.Medik.
The peaks are labeled according to the compounds listed in Table 1.

Identified phenolic compounds from ethanolic extract of red gedi were of 6 phenolic acids and 14 flavonoids. The phenolic acids were two hydroxybenzoic acid derivates (vanilic acid, ficusol) dan four hydroxycinnamic acid derivates (p-coumaric acid, caffeic acid methyl ester, ferulic acid and syringic acid). The flavonoids were of mostly as flavonols (quercetin, myricetin, gossypetin, hibiscetin, glycoside –O-, hyperin, kaempferitrin and isoquercetin) and some of anthocyanin flavonoids (sambicyanin and cyanidin 3-O-rutinoside).

The non-phenolic compounds identified in the ethanolic extract of red gedi leaves were many. They were 1 of hydroxybutanedioic acid (malic acid), 9 terpenoids, 2 alkaloids (moupinamide, daphniphylline), two steroids (α-spinasterol, stigmasterol) and 4 lignans. The terpenoids were of sesquiterpenes (farnesol, hibiscone A, B, gmelofuran, hibisquinone A) and triterpenoids (myriceric acid A, B, C and hibicusin). The lignans were boehmenan, erythrocarolignan E, coumarinolignan (aquillochin), and phenylpropanoidlignan (syringaresinol). All the compounds in red gedi leaves of the species *Abelmoschus manihot* L.Medik were for the first time identified and reported in this study.

The identified compounds were not all uniquely found in in red gedi leaves. For instance, quercetin (compound 13) identified based on peak m/z 302.0427 and characteristic peak at m/z 303.0460, 304.0469 and 304.0494 were also found in other species. Similar peaks were reported in extract of other plants including *Capparis spinosa*[14, 15]. The quercetin derivatives (compound 22, 23 and 24), i.e., quercetin-3-O-rhamnoside, hyperin and isoquercetin respectively, were also reported by others earlier[14, 16-18]. The quercetin-3-O-rhamnoside (compound 22), showed precursor ion at peak m/z 448,1006, however, was identified first time in this genus.

Several identified compounds of gedi leaves may explain the antidiabetic properties of the extract. Further studies using in silico and in vitro, followed by in vivo studies, as commonly performed [19-23], will serve as pharmacological evidence of such properties.

Table 1. Peak assignment of metabolites in ethanolic extract of red gedi leaves *Abelmoschus manihot* L.Medik using LC-ESI-MS in positive ion mode

Peak No	RT (min)	Molecular Formula	Calculated mass (M)	Experimental mass $[M+H]^+$ *m/z*	Tentative Identified Compounds	PubChem CID
1	1.473	$C_6H_6O_5$	134.0215	135.0249	Malic acid	525
2	1.839	$C_9H_8O_3$	164.0473	165.0507	p-Coumaric acid	637542
3	2.799	$C_8H_8O_4$	168.0423	169.0456	Vanillic acid	8468
4	5.043	$C_{10}H_{10}O_4$	194.0579	195.0613	Ferulic acid	445858
5	5,044	$C_{10}H_{10}O_4$	194.0579	195.0613	Caffeic acid methyl ester	689075
6	5.177	$C_9H_{10}O_5$	198.0528	199.0562	Syringic acid	10742
7	5.967	$C_{15}H_{26}O$	222.1984	223.2017	Farnesol	445070
8	7.338	$C_{11}H_{14}O_5$	226.0841	227.0875	Ficusol	100955863
9	7.616	$C_{15}H_{20}O_2$	232.1463	233.1497	Hibiscone A	102239770
10	8.004	$C_{15}H_{18}O_3$	246.1256	247,1289	Gmelofuran	156117
11	8.011	$C_{15}H_{20}O_3$	248.1412	249.1446	Hibiscone B	102090463
12	8.246	$C_{15}H_{14}O_4$	258.0892	259.0926	Hibiscoquinone A	442745
13	11.427	$C_{15}H_{10}O_7$	302.0427	303.0460	Quercetin	5280343
14	11.502	$C_{18}H_{19}NO_4$	313.1314	314.1348	Moupinamide	5280537
15	11.514	$C_{15}H_{10}O_8$	318.0376	319.0409	Myricetin	5281672
16	11.518	$C_{15}H_{10}O_8$	318.0376	319.0409	Gossypetin	5280647
17	12.038	$C_{15}H_{10}O_9$	334.0325	335.0358	Hibiscetin	15559735
18	15.635	$C_{29}H_{48}O$	412.3705	413.3739	α-Spinasterol	5281331
19	15.638	$C_{29}H_{48}O$	412.3705	413.3739	Stigmasterol	5280794
20	17.423	$C_{21}H_{20}O_9$	416.1107	417.1141	Aquillochin	
21	17.456	$C_{22}H_{26}O_8$	418.1628	419.1661	Syringaresinol	443023
22	22.616	$C_{21}H_{20}O_{11}$	448.1006	449.1039	Quercetin -3 –O-rhamnoside	5353915
23	24.027	$C_{21}H_{19}O_{12}$	463.0882	464.0916	Hyperin	5281643
24	24. 032	$C_{21}H_{20}O_{12}$	464.0955	465.0988	Isoquercetin	5280804
25	24.762	$C_{30}H_{48}O_4$	472.3553	473.3586	Myricerol	
26	25.891	$C_{21}H_{20}O_{13}$	480.0904	481.0937	Gossypetin -3-glucoside	44259979
27	25.913	$C_{21}H_{20}O_{13}$	480.0904	481.0937	Myricetin-3-glucoside	44259426
28	25.961	$C_{21}H_{20}O_{14}$	496.0853	497.0887	Hibiscetin-3-glucoside	44259992
29	29.67	$C_{32}H_{49}NO_5$	527.3611	528.3644	Daphniphyllin	21627122
30	33.483	$C_{27}H_{30}O_{14}$	578.1636	580, 1678	Kaempferitrin	5486199
31	33.569	$C_{27}H_{31}O_{15}^+$	581.1501	582.1535	Sambicyanin	44256719
32	34.063	$C_{27}H_{31}O_{15}^+$	595.1657	596.1691	Cyanidin-3-O-rutinoside	441674
33	36.983	$C_{39}H_{52}O_7$	632.3713	633.3747	Myriceric acid A	
34	37.042	$C_{39}H_{58}O_7$	634.3870	635.3903	Myriceric acid B	15767724
35	46.229	$C_{40}H_{40}O_{12}$	712.2520	713.2553	Boehmenan	5274624
36	46.253	$C_{40}H_{42}O_{13}$	730.2625	731.2659	Erythro Carolignan E	5274622
37	49.702	$C_{48}H_{60}O_9$	780.4237	781. 4271	Hibicusin	5274618
38	49.883	$C_{48}H_{60}O_{10}$	796.4186	797,4220	Myriceric acid C	15767725

4. CONCLUSION:

In this study, the ethanolic extract of the red gedi leaves was characterized by using liquid chromatography coupled to electrospray ionization-tandem mass spectrometry (LC–ESI-MS). Compounds identified were phenolic acid derivates, flavonoids, terpenoids, phytosterols, alkaloids, and lignans. In total, 38 metabolite compounds were identified in red gedi leaves and were reported for the first time, including alpha spinasterol which is newly identified in this particular Abelmoschus species. The most abundant flavonoids in red gedi extract were quercetin derivatives. This study may serve as the basis for further research elucidating the pharmacological activities underlying the use of the plant as traditional medicine for cardiovascular disease.

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