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LC-HRMS Analysis of Abelmoschus Manihot Medik from Palu of Central Sulawesi

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© 2024 The Authors. This open access article is distributed under a (CC-BY License) Abstract: Abelmoschus manihot (L.) Medik is one of the traditional medicinal plants from Palu City, Central of Sulawesi with the Malvaceae plant family and commonly is a traditional medicinal plants recognized fot its therapeutic potential, particularly in antioxidant, anti-inflammatory and wound healing applications. This research aims to identify and characterize the chemical compounds present in Abelmoschus manihot (L.) Medik plant from Palu City, Central of Sulawesi using liquid chromatography high resolution mass spectrometry (LC-HRMS). This research was collected fresh plant, designed using ethanol extraction from Abelmoschus manihot (L.) Medik and analyzed using mass spectroscopy (LC-HRMS) with Processing Software - MZMine Ver. 3.9.0. The results of this study provide new insights into the chemical composition of Abelmoschus manihot (L.) Medik and confirm its potential as a source of bioactive compounds that could be futher explored for pharmaceutical development, namely that it contains the main compound Quercetin with a mass of 303.04, Gossypetin; Myricetin with a mass of 319.04, Quercetin 3-Oalpha-L-rhamnoside with a mass of 449.10 Isoquercetin; Hyperoside; Hyperin; Hirsutrin with a mass of 465.10, Gossypol with a mass of 519.20 and rutin with a mass of 291.08.

Keywords: Abelmoschus; LC-HRMS; Palu City

Introduction

Abelmoschus manihot (L.) is a tropical plant in the malvaceae family that has traditionally been known in Central Sulawesi as a traditional medicinal plant (Abdel-Razek et al., 2023). Abelmoschus manihot (L.) Medical consists of two types: red and green. Dark red gedi leaves are one of the favorite plants loved by the people of Palu. The leaves have specifications with the shape of the leaves and the texture of the edges of the wavy leaves (Luan et al., 2020). The leaves of gedi stand out on the surface and have long leaf sticks. The gedi leaves are structured and vary in shape, size, color and pigmentation (Luan et al., 2020). The size of the red gedi leaves has lesaf lengths that reach 10-40 cm with 3-7 strands of leaves. Abelmoschus manihot (L.) Medicine can traditionally be used as a traditional medicine

(Indrawati & Setijorini, 2024; Gurav, 2016). Some studies show that the ability of gedi leaf extract to catch free radicals falls into a very strong category with IC₅₀ which is 3.45 mg/ml (Anggi & Adikusuma, 2019), where the compound's ability is said to have very strong free radical capture activity when the IC50 value is less than 50 µg/mL with activity in counteracting or suppressing the negative oxidative effects in the body with a high force of impediment is very necessary by the body to prevent the formation of free radical within the body (Anggi & Masyita, 2022; Vona et al., 2021). The antioxidant works by donating one of its electrons to an oxidizing compound so that its activity can be inhibited (Chaudhary et al., 2023; Taroreh et al., 2016).

Research carried out on pharmacognostic and phytochemical analysis of leaves, stems and roots of Abelmoschus manihot (L.) Medical showed the results

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of glycosides, stem extracts and wooden skin using ethanol extract revealed the presence of carbohydrates, tannin and glycoside. Research carried out by (Li et al., 2016) on the cost-effective methods performed for the extraction of flavonoids from the flower of Abelmoschus manihot (L.) Medical uses supercritical liquid extraction and the resulting extract has a strong antioxidant and anti-adipogenic effect, so that the possible therapeutic approach of the flower of the Abelmoschus manihot L. Medik can be used for the prevention and treatment of obesity (Chumbhale & Khyade, 2022). Abelmoschus manihot (L.) Medical has a bioactive compound that can reduce the development of multiple myeloma in rats and can increase the resistance of bone marrow (Hou et al., 2020). Especially in the genus Abelmoschus has been ethnomedically showing pharmacological activity and has some phytochemical and nutrient content good to use as food and development of traditional medicine in the pharmaceutical industry based on its chemical composition and biological activity (Wu & Wang, 2023).

results Phytochemistry through GC-MS Abelmoschus manihot leaf extract indicate the presence of phytol, palmitate acid, linoleic acid, dioctil phthalate, tokoferol, Urs-12-en-28-ol, (2E,4E)-2, 4-heptadecadienoic acid and stigmast-4 (Selvaraj et al., 2020) From various backgrounds regarding the content strong of Abelmoschus manihot (L.) Medik, further research is needed on the LC-HRMS analysis of Abelmoschus manihot (L.) Medik from Central Sulawesi, aimed at determining the composition of the chemical compounds contained in Abelmoschus manihot (L.) using the LC-HRMS method.

Method

Chemicals and botanical materials

Abelmoschus manihot (L.) Medik were collected in Palu Central Sulawesi, Indonesia. Abelmoschus manihot (L.) Medik identified from the species used in this study. Parts of the red gedi leaf plant were taken for use in identification at the Central Sulawesi Biological Resources UPT, Tadulako University with No.0083/UN28.23/AL.04/2024.

Preparation of Abelmoschus manihot (L.) Medik ethanol extract

Abelmoschus manihot (L.) Medik were macerated with 2500 mL of ethanol, with the maceration method using 96% ethanol solvent. Simplicia powder was weighed as much as 574 grams then put into a maceration vessel using 1.5 liters of 96% ethanol solvent, covered, then left for 3x24 hours protected from light, stirring occasionally. The extract was filtered using filter paper and then the filtrate was obtained, evaporated using a Rotavapor followed by thickening using a water bath, then calculating the % yield of Abelmoschus manihot (L.) Medik (Anggi, 2021; Arsyad et al., 2023).

LC-HRMS Analysis Settings and the Processing Software

LC-HRMS was carried out at the Core Facility of Mass Spectrometric Analysis at the Biotek Rekayasa Indonesia Laboratory with No. 715/BIOTEK/VI/2024. An UltiMate of liquid chromatograph Thermo Scientific[™] Vanguish[™] UHPLC Binary Pump) with a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) was utilized to determine the flavonoid profile of the Abelmoschus manihot (L.) Medik extract. The chromatographic separation was attained at Column ThermoScientific[™] Accucore[™] Phenyl-Hexyl 100 mm × 2.1 mm ID × 2.6 µm with Eluent A – MS-grade water containing 0.1% formic acid and Eluent B - MSgrade methanol containing 0.1% formic acid of Flow Rate - 0.3 mL/min on a Zorbax Eclipse XDB-C18 reversed-phase column (150 x 3.0 mm, 5 µm with thermostatted on 40 °C. The elution gradient was implemented as follows: from 5% to 95% B for 25 min, followed by an increase to 90% B for 16 min, a decrease to 5% B for 5 min, and re-equilibration under the initial conditions for 0,01 min. The parameters set for the electrospray ionization (ESI) source were as follows: electrospray voltage: 3.30 kV in the negative mode and 4.2 kV in the positive mode; capillary temperature: 320 ⁰C; and S lens RF level: 50. Data were obtained by fullscan datadependent acquisition (FS-dd-MS2) in the positive and negative modes at a resolving power of 70,000 full width at half maximum (FWHM) m/z 200. The following settings of the mass spectrometer were employed: scan range, 66,7-1000 m/z; automatic gain control (AGC), 3e6; injection time, 100 ms; and isolation window, m/z 2.0. The normalized collision energy for the fragmentation of molecular ions was set to 40 eV. Targeted tandem mass spectrometry (MS/MS; dd-MS2) was performed in both positive and negative modes at 15,000 FWHM (m/z 200). AGC for dd-MS2 was set to 1e5, with an injection time of 50 ms and a loop count of 5. In the section of dd settings, the AGC target was programmed at 8e3, and the maximum injection time was set to 100 ms. Data were analyzed using with Processing Software - MZMine Ver. 3.9.0 (Della Vedova et al., 2022; Dwiyanti et al., 2023; Kale et al., 2022; Nunez et al., 2024; Petrova et al., 2023; Rafi et al., 2023).

Result and Discussion

LC-HRMS Analysis of Abelmoschus manihot (L.) Medik From Palu of Central Sulawesi, where the total ion chromatograms (TIC) and full MS scan by spectra (66,7-1000 m/z) at a retention time of 25.00 min obtained for an Abelmoschus manihot (L.) Medik ethanol extract. Figure 1 shows of the total signal response from all the

ions eluting at each retention in the total ion chromatograms (TIC). With respect to extract analysis, LC-HRMS stands out for having a high degree of selectivity, sensitivity, and specificity. Using reversedphase chromatography, the non-targeted LC-HRMS metabolic fingerprints of extract samples were obtained for this study. After brewing the ethanol extract using the LC-HRMS method (Lima et al., 2022), a total of 6 samples were analysed and divided into three research cases. The chromatographic separation was used since a non-targeted technique was designed to produce the richest instrumental. LC-HRMS (Liquid Chromatography - High-Resolution Mass Spectrometry) Test using Instrument: Thermo Scientific[™] Vanguish[™] UHPLC Binary pump coupled with Thermo Scientific[™] Q Exactive[™] Hybrid Quadrupole- Orbitrap[™] high resolution Mass Spectrometer. Mobile Phase inclu with 2 Solutions; Solution A contains water and 0.1% Formic Acid, and Solution B contains Acetonitrile + 0.1% Formic acid. Analytical Column with ThermoScientific™ Accucore[™] Phenyl Hexyl 100 mm x 2,1 mm ID x 2.6 µm. Specifications are instrument flow: 0,3 mL/min, Sample injection volume: 3 µL, analysis running time: 25 minutes, with full MS at 70,000 FWHM Resolution, Dependent data MS2 at 17,600 FWHM, for dividing electron type as positive and negative with heated Electrospray Ionization Compound (H-ESI). Identification by Thermo ScientificTM Compound Discoverer Software and Data were analyzed using with Processing Software - MZMine Ver. 3.9.0.

Table 1. Identified Compounds of Abelmoschus manihot (L.) Medik by Name, Formula, Retention time (Rt), and m/z Values Using LC-HRMS

Compound(s) Name	Formula	Adducts	Found Mass	Calc. Mass Δ	(ppm)	RT	Peak Area	%Are
						(min)		а
Quercetin	C15H10O7	[M+H]+	303.0498	303.0505	2.32	6.35	8257470.50	0.22%
Gossypetin; Myricetin	C15H10O8	[M+H]+	319.0424	319.0454	9.36	0.82	5015.20	0.00%
Gossypetin; Myricetin	C15H10O8	[M+H]+	319.0448	319.0454	2.00	5.67	65054.46	0.00%
Quercetin 3-O-alpha-L-rhamnoside	C21H20O11	[M+H]+	449.1079	449.1084	1.14	6.35	3127788.80	0.08%
Isoquercetin; Hyperoside; Hyperin;	C21H20O12	[M+H]+	465.1029	465.1033	0.78	5.80	268506.40	0.01%
Hirsutrin								
(-)-Gossypol	C30H30O8	[M+H]+	519.2009	519.2019	1.87	12.39	95795.60	0.00%
(-)-Gossypol	C30H30O8	[M+H]+	519.1975	519.2019	8.45	6.18	108000.66	0.00%
Rutin	C27H30O16	[M+H]+	291.0842	291.0869	9.23	0.84	134031.00	0.02%
Total Peak Area							3724830664.00)



Figure 1. Chromatogram LC-HRMS ethanol extract Abelmoschus manihot (L.) Medik



Figure 2. Total ion chromatograms (TIC)) and full MS of Abelmoschus manihot (L.) Medik on LC-HRMS

The ethanol extract of Abelmoschus manihot (L.) Medik From Palu of Central Sulawesi included a range of chemical components (Bro & Smilde, 2014), with the largest amount being quercetin with formula $C_{15}H_{10}O_7$ on calculated mass from formula 303.0505, Gossypetin; Myricetin with formula $C_{15}H_{10}O_8$ on calculated mass

from formula 319.0454, Quercetin 3-O-alpha-L-rhamnoside with formula $C_{21}H_{20}O_{11}$ on calculated mass from formula 303.0505, Isoquercetin; Hyperoside; Hyperin; Hirsutrin with formula $C_{21}H_{20}O_{12}$ on calculated mass from formula 465.1033, Gossypol with formula $C_{30}H_{30}O_8$ on calculated mass from formula 519.2019 and

Rutin with formula $C_{27}H_{30}O_{16}$ on calculated mass from formula 291.0869 (Table 1, Figure 1 and 2).

largest amount being quercetin The of Abelmoschus manihot (L.) Medik, where the quercetin-an aglycone form of flavonoid glycosides obtained from plants-has been shown to be effective against a number of illnesses (Ay et al., 2016; Winata et 2024). Cardiovascular protection, antitumor, al., anticancer, antiulcer, antiallergy, antiviral, antiinflammatory activity, anti-diabetic, gastroprotective, immunomodulatory, neuroprotective and anti-infective are a few of the positive benefits (Anand David et al., 2016; Liao et al., 2022; Tandi et al., 2017; Zhang et al., 2022). Strong evidence exists for quercetin's ability to alter a number of cellular signalling pathways that control inflammation, apoptosis, cell survival and the antioxidant response (Gao et al., 2022; Patala & Anggi, 2022). More extensive epidemiological research and carefully planned clinical trials are required to confirm quercetin's effectiveness in treating and preventing human diseases as wesll as to further elucidate its possible advantages. Abelmoschus manihot (L.) Medik also have amount being Gossypetin; Myricetin, where Myricetin is an essential nutrient that supports immune function and is useful for preserving overall health (Agraharam et al., 2022; Shi et al., 2023). Myricetin's effects on various cell processes, including apoptosis, glycolysis, the cell cycle, energy balance, lipid levels, serum protein concentrations, and osteoclastogenesis, are linked to its health advantages (Imran et al., 2021). Myricetin's has been demonstrated inhibits platelet aggregation by lowering alpha granule secretion and fibrinogen binding. Abelmoschus manihot (L.) Medik also have amount being Quercetin 3-O-alpha-Lrhamnoside, where Quercetin 3-O-alpha-L-rhamnoside is a natural substance possesses the capacity to regulate the inflammatory response and effectively enhance the prognosis of influenza and other ailments, such as autoimmune or viral disorders (Han et al., 2019; Zhang et al., 2022). Isoquercetin of compound on Abelmoschus manihot (L.) Medik, where its then taken up by enterocytes, moved to the liver, released into the bloodstream, and dispersed to various tissues, primarily as metabolic conjugates and from a physiological standpoint, quercetin and isoquercetin have antiinflammatory, anticoagulant, immunomodulatory and antioxidant properties (Chang et al., 2022; Liao et al., 2022; Mehrbod et al., 2018). The components active of Abelmoschus manihot (L.) Medik have the potential as immunomodulatory which can be added to pharmaceutical preparations.

Conclusion

LC-HRMS analysis, bioactive compounds that have of Abelmoschus manihot (L.) Medik are Quercetin with a mass of 303.04, Gossypetin; Myricetin with a mass of 319.04, Quercetin 3-O-alpha-L-rhamnoside with a mass of 449.10 Isoquercetin; Hyperoside; Hyperin; Hirsutrin with a mass of 465.10, Gossypol with a mass of 519.20 and rutin with a mass of 291.08. The components active of Abelmoschus manihot (L.) Medik have the potential as immunomodulatory which can be added to pharmaceutical preparations.

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Conflicts of Interest

The authors declare no conflict of interest of reported research results.

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