KAJIAN DAUN DAN BUAH KARAMUNTING (*Rhodomyrtus tomentosa* (Aiton) Hassk.) : ANATOMI, HISTOKIMIA, AKTIVITAS ANTIOKSIDAN DAN FITOKIMIA

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INTISARI

Karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk.) telah lama digunakan sebagai obat tradisional di negara-negara Asia Tenggara termasuk Indonesia. *R. tomentosa* memiliki bioaktivitas antara lain antibakteri, antitumor, anti-inflamasi dan antioksidan dengan kandungan senyawa dari golongan floroglucinol, flavonoid, terpenoid, anthracene glycosida, tanin, dan senyawa lainnya. Tujuan penelitian ini adalah mengetahui struktur anatomis dan distribusi secara kualitatif dengan histokimia, menentukan ekstrak dan fraksi yang memiliki aktivitas antioksidan paling tinggi, menelusuri profil senyawa ekstrak dengan *Nuclear Magnetic Resonance* (NMR) dan fraksi dengan *Liquid Chromatography High Resolution Mass Spectra* (LC HRMS) dari daun serta buah *R. tomentosa* dengan umur berbeda. Penelitian ini menggunakan ekstrak etanol daun muda, daun tua, buah hijau, buah merah dan buah ungu. Sampel daun dan buah diambil dari daerah Banjarbaru, Kalimantan Selatan. Pembuatan preparat anatomis daun dan buah menggunakan metode parafin dengan pewarnaan safranin. Analisis histokimia menggunakan reagen spesifik untuk tiap golongan senyawa fenol, flavonoid, tanin, alkaloid dan terpenoid. Uji Kadar flavonoid dan fenol dilakukan dengan metode kolorimetri dan Folin–Ciocalteu, uji kadar antosianin dengan metode pH diferensial. Kapasitas antioksidan ditentukan menggunakan metode *1,1-Diphenyl-2-picrylhydrazyl* (DPPH) dan *Ferric Reducing Antioxidant Power* (FRAP) pada ekstrak dan fraksi. Ekstrak potensial yang terpilih difraksinasi dengan metode Kromatografi Vakum Cair (KVC) menggunakan pelarut campuran dari n-heksan, etil asetat dan etanol dengan perbandingan volume tertentu dan menghasilkan 5 fraksi gabungan. Analisis profil senyawa pada ekstrak dilakukan dengan NMR, hasil spektra ^{1}H NMR diolah dengan *software* MestReNova serta *Analisis Principal Component* (PCA) dengan perangkat lunak *MetaboAnalyst* 5.0. Analisis profil senyawa pada fraksi gabungan dengan metode LC HRMS. Data anatomis dan histokimia dianalisa secara deskriptif, data hasil perhitungan nilai kapasitas antioksidan ekstrak dan fraksi, kadar total fenol, flavonoid dan antosianin dianalisis dengan analisis variansi dan dilanjutkan dengan uji analisis *least significance different* (LSD) untuk melihat hasil perbedaanya. Nilai kapasitas antioksidan dilakukan analisis koefisian korelasi linier sederhana Pearson dengan kadar total fenol, flavonoid dan antosianin. Struktur anatomis

penampang melintang daun *R. tomentosa* terdiri atas bagian epidermis, mesofil, berkas pengangkut, ruang sekretori. Struktur penampang lintang buah terdiri dari lapisan perikarp buah yang dapat dibedakan menjadi eksokarp/lapisan luar, mesokarp, dan endokarp. Distribusi senyawa fenol, flavonoid, tanin, terpenoid dan alkaloid pada daun yaitu di bagian epidermis adaksial dan abaksial, mesofil, xilem, floem, parenkim midrib, ruang sekretori dan trikoma. Distribusi senyawa fenol, flavonoid, tanin, terpenoid dan alkaloid pada buah yaitu bagian eksokarp, mesokarp, endokarp, ruang sekretori, xilem, floem, trikoma, biji. Kapasitas antioksidan tertinggi dengan metode DPPH dan FRAP pada ekstrak etanol buah hijau (1419,75 ± 3,48 dan 1367,59 ± 9,12 µmol *Trolox Equivalent*/g) dan daun muda (1069,38 ± 6,57 dan 1288,94 ± 2,99 µmol *Trolox Equivalent*/g). Kadar total flavonoid tertinggi pada daun muda (96,38 ± 3,96 mg *Quercetin Equivalen*/g), kadar total fenol tertinggi pada buah merah (50,77 ± 7,46 mg *Galic Acid Equivalent*/g) dan kadar antosianin tertinggi pada buah hijau (2,92 ± 0,08 mg/L). Senyawa aromatis hasil spektra ^{1}H NMR dan analisis PCA yang teridentifikasi pada daun muda dan buah hijau adalah *gallic acid, myricetin, myricetin 3-O-rhamnpyranoside, quercetin-3-O-glucoside, quercetin* dan *syringic acid* merupakan golongan flavonoid dan fenol yang berperan sebagai antioksidan, dengan konsentrasi relatif *myricetin* dan *quercetin* lebih tinggi pada daun muda. Hasil fraksinasi ekstrak daun muda menghasilkan 5 fraksi gabungan. Fraksi gabungan 4 (F4) menunjukan kapasitas antioksidan tertinggi (8569,14 ± 0,38 µmol *Trolox Equivalent* /g.) dibandingkan fraksi yang lain dan ekstrak asalnya. Hasil data LC HRMS dianalisis menggunakan *Global Natural Products Social* (GNPS) terdapat 6 senyawa flavonoid yaitu : *myricetin-3- xyloside, myricetin, myricetin-3-rhamnoside, myricitrin, avicularin (quercetin-3-O-α-1-arabinofuranoside, quercetin* dan 1 senyawa fenol yaitu : [6]-Gingerol pada F4. Hasil analisis multivariat pada fraksi diperoleh senyawa *myricitrin* lebih tinggi secara signifikan konsentrasi relatif pada kelompok fraksi semi polar yaitu pada F4 yang berperan sebagai antioksidan kuat.

Kata kunci : antioksidan, flavonoid, histokimia, ^{1}H NMR, *rose myrtle*

STUDY OF KARAMUNTING (*Rhodomyrtus tomentosa* (Aiton) Hassk.) LEAVES AND FRUITS: ANATOMY, HISTOCHEMICAL, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL

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ABSTRACT

Karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk.) has long been used as a traditional medicine in Southeast Asian countries including in Indonesia. *R. tomentosa* has bioactivity, including antibacterial, antitumor, anti-inflammatory, and antioxidant with compounds from the phloroglucinol group, flavonoids, terpenoids, anthracene glycosides, tannins, and other compounds. The aims of this study were: to qualitatively determine the anatomical structure and distribution of compounds using histochemistry, to identify the extract and fraction of leaves and fruit of *R. tomentosa* with the highest antioxidant activity, to explore the profile of extract compounds and fractions using Nuclear Magnetic Resonance (NMR) and Liquid Chromatography High Resolution Mass Spectra (LC HRMS). This study used ethanol extract from young leaves, old leaves, green fruit, red fruit, and purple fruit. The samples were taken from Banjarbaru, South Kalimantan. Anatomical slides preparation of leaves and fruits were made using the paraffin embedding method with safranin staining. Histochemical analysis used specific reagents for each class of phenolic compounds, flavonoids, tannins, alkaloids and terpenoids.The colorimetric and the Folin–Ciocalteu methods were used to determine flavonoid and phenol concentrations, while the differential pH method was used to determine anthocyanin concentration. The antioxidant capacity was determined using the 1,1-difenil-2-pikrilhidrazil (DPPH) and the Ferric Reducing Antioxidant Power (FRAP) methods on the extracts and fractions. The VLC (Vacum Liquid Chomatography) method was used to fractionate the selected potential extract using a mixed solvent of n-hexane, ethyl acetate, and ethanol at a certain volume ratio, producing 5 combined fractions. ^{1}H NMR was used to analyze compound profiles in extracts; NMR spectra were processed with MestReNova software and Principal Component Analysis (PCA) with MetaboAnalyst 5.0 software. The LC HRMS method was used to analyze compound profiles in the combined fraction. Anatomical and histochemical results were analyzed descriptively. The antioxidant capacity of extracts and fractions, as well as concentrations of total phenols, flavonoids, and anthocyanins, were calculated and analyzed using analysis of variance, followed by the Least Significance Different (LSD) analysis test to determine if there would be any differences. The antioxidant capacity value was compared to total phenol, flavonoid,

and anthocyanin concentrations using Pearson's simple linear correlation coefficient. The cross-sectional anatomical structure of *R. tomentosa* leaves consisted of the epidermis, mesophyll, and carrier bundle. Phenolic compounds such as flavonoids, tannins, terpenoids, and alkaloids were distribute in the adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory cavity, and trichomes of the leaves. While in the fruit, they were distributed in the exocarp, mesocarp, endocarp, secretory cavity, xylem, phloem, trichomes, and seeds. The cross-sectional structure of the fruit consists of a fruit pericarp layer which can be divided into exocarp/outer layer, mesocarp, and endocarp. The ethanol extract of green fruit (1419.75 ± 3.48 and 1367.59 ± 9.12 µmol Trolox Equivalent/g) and young leaves (1069.38 ± 6.57 and 1288.94 ± 2.99 µmol Trolox Equivalent/g) had the highest antioxidant capacity when measured by the DPPH and the FRAP methods. Young leaves had the highest total flavonoid concentration (96.38 ± 3.96 mg Quercetin Equivalen/g), red fruit had the highest total phenol concentration (50.77 ± 7.46 mg Galic Acid Equivalent/g) and green fruit had the highest anthocyanin concentration (2.92 ± 0.08 mg/L). Aromatic compounds identified in young leaves and green fruit by ^{1}H NMR spectra and PCA analysis include gallic acid, myricetin, myricetin 3-O-rhamnpyranoside, quercetin-3-O-glucoside, quercetin, and syringic acid, which are flavonoids and phenols that work as antioxidants, with relatively higher concentrations of myricetin and quercetin in young leaves. The fractionation of young leaf extract produced 5 combined fractions. Combined fraction 4 (F4) showed the highest antioxidant capacity (8569.14 ± 0.38 µmol Trolox Equivalent/g.) compared to other fractions. The results of LC HRMS data analysis using Global Natural Products Social (GNPS) contained 6 flavonoid compounds, namely: myricetin-3-xyloside, myricetin, myricetin-3-Rhamnoside, myricitrin, avicularin (quercetin-3-O-α-1-arabinofuranoside, quercetin and 1 phenol compound, namely: [6]-Gingerol at F4. Myricitrin compound has a significantly higher relative concentration in the semi-polar fraction group, namely at F4, which acts as a strong antioxidant.

Keywords: antioxidants, flavonoids, histochemistry, ^{1}H NMR, rose myrtle