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Antioxidant activity and ^{1}H NMR profiling of leaves and fruits of *Rhodomyrtus tomentosa* from South Kalimantan, Indonesia

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Abstract. *Kuntorini EM, Triyasmono L, Astuti MD. 2024. Antioxidant activity and ^{1}H NMR profiling of leaves and fruits of* Rhodomyrtus tomentosa *from South Kalimantan, Indonesia. Biodiversitas 25: 2020-2027. Rhodomyrtus tomentosa* (Aiton) Hassk is a flowering plant belonging to Myrtaceae, native to southern and southeastern Asia. This study aims to evaluate and compare the antioxidant activity of leaves and fruits ethanol extracts of *R. tomentosa* (Ait.) Hassk from two locations, namely Banjar and Batola, South Kalimantan Province, Indonesia. The samples included old and young leaves and three stages of fruit maturity, namely green, red, and purple. ^{1}H NMR spectroscopy was used to identify the chemical profile of extracts, which comprised organic acids, carbohydrates, amino acids, and phenolic compounds. Antioxidant capacity was assessed with DPPH, which showed intensity differences in aromatic shifts in leaves and fruits for both regions. Young leaves and green fruits had higher intensity than old leaves and red and purple fruits, correlating with antioxidant capacity. Analysis of ^{1}H NMR spectra of young and old leaf extracts identified vital metabolites, such as gallic acid, myricetin 3-O-rhamnopyranoside, myricetin, quercetin, quercetin-3-O-glucoside, as well as syringic acid, in regional chemical shifts of 10.0-6.0 ppm as aromatic compound area. The differences in spectral signal intensity revealed higher metabolite levels, specifically carbohydrates and amino acids, in red and purple fruits. However, green fruits had higher quantities of aromatic compounds.

Keywords: DPPH, metabolite profiling, myricetin, *Rhodomyrtus tomentosa*

INTRODUCTION

An antioxidant is a bioactive compound capable of counteracting the harmful effects of free radicals and oxidative stress induced by reactive mechanisms. The oxidative stress causes cellular damage and degeneration, along with chronic degenerative diseases development, including diabetes, cancer, cardiovascular diseases, and neurovascular diseases (Masisi et al. 2016). The reliance on synthetic antioxidants, such as 4-hexyl-resorcinol, to meet the daily requirement of antioxidants has roused concern due to the associated detrimental health effects and toxicological implications. Consequently, there is an increasing emphasis on applying natural antioxidants derived from bioactive plant components (Maskam et al. 2014; Ismandari et al. 2020). Medicinal plants are the most potent sources of natural antioxidants. They may contain phytochemical compounds such as flavonoids, phenolic acids, tannins, terpenoids, and alkaloids, which have considerable antioxidant activity for maintaining health. Researchers have shown that plant chemical compounds possess antioxidant activity to prevent diseases induced by free radicals. As a result, they may become part of an effective preventive defense strategy against various human diseases. Natural antioxidants extracted from medicinal plants are inexpensive, safe, and do not have toxic effects compared to synthetic antioxidants (Maskam et al. 2014).

Rose Myrtle (*Rhodomyrtus tomentosa* (Ait.) Hassk) is an evergreen perennial shrub with diverse structural compounds and biological activities (Zhao et al. 2019). In traditional Chinese, Vietnamese, and Malaysian medicine, all parts of the plant (leaves, roots, flowers, fruits) have long been used to treat diarrhea, stomach pain, gynecopathy, and wound healing. Parts of the roots and trunks are used for stomach diseases and after childbirth. Local Indonesians use the crushed leaves of *R. tomentosa* to treat wounds (Hamid et al. 2017). *Rhodomyrtus tomentosa* is an anti-inflammatory, anti-diarrheal, anti-dysentery medication in Thailand (Vo and Ngo 2019). The main components of *R. tomentosa* include triterpenoids, flavonoids, meroterpenoids of phenols, and microelements (Kusuma et al. 2016; Zhao et al. 2019).

Recent studies have focused on exploring the functional characteristics of *R. tomentosa* extracts, renowned for pharmacological properties and traditional applications in treating various ailments (Yang et al. 2023). The medicinal properties of this plant and its components have been extensively investigated. Fruits are rich in flavonoids, phenols, polysaccharides, and other bioactive compounds, including piceatannol, a stilbene component with anti-leukemia potential (Lai et al. 2013, 2015). It is used to produce wines and beverages (Yin et al. 2021). Currently, *R. tomentosa* is mainly studied for the phytochemical components found in leaves, flowers, and stems due to their

potent antioxidant, anti-bacterial, and anti-inflammatory activities, as well as the ability to reduce DNA damage (Wu et al. 2015; Kusuma et al. 2016; Vo and Ngo 2019, Ridlo et al. 2020; Hu et al. 2022). Kuntorini et al. (2022) reported previously that green fruits of *R. tomentosa* from Banjarbaru showed potent antioxidant activity, with IC_{50} values against DPPH and FRAP were 1419.75±3.48 as well as 1367.59±9.12 µmol TE/g DW. Ethanol extracts showed the highest values of TFC in the young leaves and green fruits, namely 96.375±3.96 and 95.731±5.42 mg QE/g DW.

The limited use of *R. tomentosa* in South Kalimantan, particularly its leaves and fruits, is attributed to a lack of knowledge about the extraction methods and potential benefits. Despite several advantages, this plant is a pest due to its rapid growth. This study continues a previous one as part of a series of research roadmaps. There has been no comprehensive study on the metabolic and antioxidant profiles of leaves and fruits at various stages of maturity. This examination represents an inaugural systematic analysis of metabolites contained in *R. tomentosa* leaves and fruits obtained from two locations, using combined NMR spectroscopy, particularly to identify antioxidant components. The study effectively shows the suitability and effectiveness of the NMR method in analyzing the metabolites of plants.

MATERIALS AND METHODS

Plant materials

Samples of young and mature leaves (2nd-6th and 7th-12th order from the shoot) of *R. tomentosa* were collected. Red, purple, and green fruits were also collected (Figure 1). Munsell Color Charts were used as a reference for plant tissue color (Wilde 1977). The samples were obtained from natural habitats in Martapura, Banjar District (3°26′00.6″S, 114°51′30.5″E), and Marabahan, Batola District (3°09′16.0″S, 114°37′19.8″E), South Kalimantan in June-July 2023. The samples were identified and authenticated in the Herbarium Bogoriense, National Research and Innovation Agency, with certificate number 1007/IPH.1.01/If.07/IX/2023.

Procedures

Crude ethanol extract preparation

Fruits and different leaf ages were selectively harvested from apical branches and dried at 40°C in an oven, followed by grinding at ambient temperature. Approximately 500 g of each ground material was macerated in 1000 mL ethanol (SmartLab, Indonesia) for 72 h. The solvent was replaced every 24 hours (Nurcholis et al. 2021; Kuntorini et al. 2022). Finally, extracts from the same samples were pooled, thoroughly mixed, filtered to remove cell debris, dried using a rotary evaporator, and stored in refrigeration for further analysis. The extract yield was calculated by weight of ethanol extract/weight of dried leaves/fruits) × 100%.

Antioxidant analysis

Following the methods of Purwakusumah et al. (2016) and Kuntorini et al. (2022), DPPH (2,2-diphenyl picrylhydrazyl) radical scavenging activity was assessed by diluting ethanol extracts in absolute methanol to obtain the appropriate concentration. The sample (2 mL) was added with 2 mL of 0.17 mM DPPH (Sigma-Aldrich, Germany) and then incubated in the dark for 30 mins at ambient temperature. The absorbance of the samples was measured at wavelength of 516 nm utilizing UV/Vis Spectrophotometer (UV/Vis Spectrophotometer, Genesystm 10 Series, USA). The free radical scavenging activity was quantified in micromoles of Trolox (Sigma-Aldrich, Germany) equivalents per unit of Dry Weight (DW) (µmol TE/g).

Sample preparation for [1]H-NMR

The [1]H NMR samples were prepared using a modified methodology from previous studies (Kim et al. 2010; Gogna et al. 2015; Mishra et al. 2019). Approximately 25 mg of crude extract was placed into a 2 mL Eppendorf tube along with 1 mL of NMR solvent, comprising 0.5 mL of methanol-d4, 0.5 mL of KH_2PO_4 buffer, and pH 6.0 containing 0.001% TMSP (Trimethyl Silypropionic acid sodium, Sigma-Aldrich). The mixture was vortexed and sonicated for 1 min, followed by homogenization and centrifugation for 1 min at 10,000 rpm. The supernatant was transferred to the NMR tube for subsequent examination using [1]H NMR.

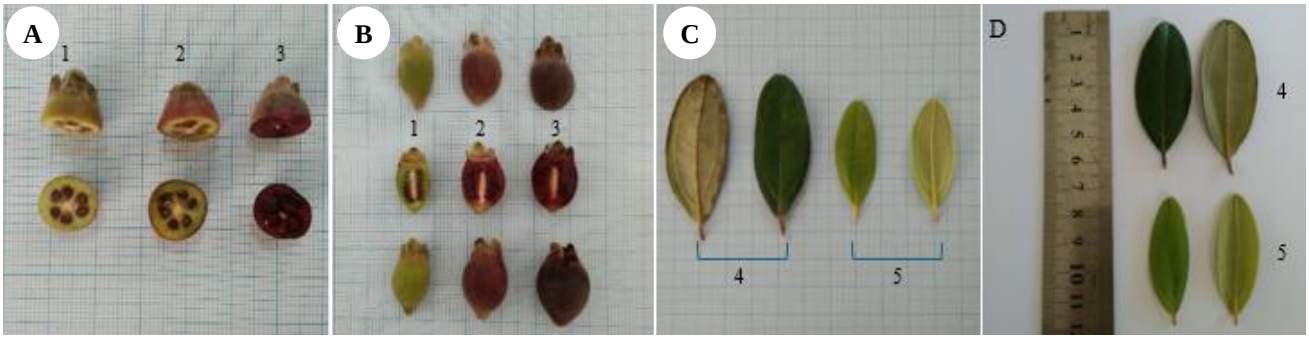

Figure 1. Fruits and leaves of *Rhodomyrtus tomentosa*. A. Fruits from Batola; B. Fruits from Banjar; C. Leaves from Batola; D. Leaves from Banjar; 1. Green fruits; 2. Red fruits; 3. Purple fruits; 4. Old leaves; 5. Young leaves

^{1}H NMR spectroscopy

The ^{1}H NMR analysis was conducted with a 500 MHz spectroscopy (JEOL JNM ECZ500R) at 25°C. The parameters applied included 128 scans over 10 mins, a relaxation delay of 1.5 mins, an X_angle of 60°, and a pre-saturation mode set at 4.27 ppm. An internal lock was also established using deuterated solvent, and the spectral width was measured from 0 to 10 ppm.

Data analysis

Mean values and standard deviations (mean±SD) were calculated based on three replications. Quantitative data underwent statistical analysis using a One-Way Analysis of Variance (ANOVA) using Microsoft Excel® and IBM SPSS Statistics 21 software. Significant differences were determined at $p<0.05$, and significant differences among treatments were analyzed further using the LSD method.

The ^{1}H NMR spectra were analyzed using MestReNova software, including processes of manual phasing, baseline modification, and calibration to internal standard solution (TMSP) signals at a chemical shift of 0.0 ppm. The observed NMR resonance multiplicities were designated in line with the established convention. The symbols used in this context were s: singlet, dd: doublet of doublets, d: doublet, t: triplet, and m: multiplet (Mishra et al. 2019). Additionally, the identification of metabolites was conducted by comparing with database derived from prior studies (Kim et al. 2010; Ali et al. 2011; Nuringtyas et al. 2012; Gogna et al. 2015; Cerulli 2018; Mishra et al. 2019). Semi-quantitative signals were examined by comparing their areas to the TMSP signal, serving as an internal reference. Subsequently, the ^{1}H NMR signals were adjusted to total intensity to produce data suitable for multivariate analysis.

RESULTS AND DISCUSSION

Yield and antioxidant capacity of ethanol extracts

The extract yields were varied (Table 1); the highest was obtained from purple fruits (15.24% w/w), whereas green fruits produced the lowest yield (5.63% w/w). Maceration, a non-thermal extraction method, was used to prevent the degradation of secondary metabolite compounds without heating. In the maceration process, the solvent entered the cells through diffusion, causing the dissolution of cellular contents due to concentration disparities between the extracellular and intracellular fluid until a solute concentration equilibrium was obtained (Harbone 1987).

The solvent used in the extraction process is to extract the active ingredients. Ethanol effectively extracted sterol, flavonoid, phenolic, and alkaloid (Wardani et al. 2019). The yield of extract indicates the amount of extracted chemical compounds. The yields of the samples from two regions with antioxidant capacity were not subjected to statistical analysis. The extract yield of leaf and fruit from the two areas was not significantly different.

Antioxidants bind to free radicals and thus prevent many diseases, including various Non-Communicable Diseases (NCDs), from damaging healthy cells. Its activity can be measured by several in vitro experiments - one of the most simple, rapid, and widespread is DPPH measurements (Rondevaldova et al. 2022). The ethanol extracts of *R. tomentosa* samples were rich in hydroxyl compounds that can reduce DPPH to DPPH-H through hydrogen donation. An increase in 1,1-diphenyl-2-picryl-hydrazyn level resulted in a color change from dark purple to pink or yellow. It can be observed using a spectrophotometer so that the free radical scavenging activity of the sample can be quantified (Sayuti and Yenrina 2015; Ismandari et al. 2020).

Purwakusumah et al. (2016) stated that antioxidant capacity expressed as trolox equivalents are more meaningful and descriptive than that expressed as percent inhibition. Trolox (6-hydroxy-2,5,7,8 tetramethyl croman-2-carboxylic) is an analog of water-soluble vitamin E or α-tocopherol. However, assessing antioxidant activity based on percent inhibition focuses solely on the minimal percentage inhibition of antioxidant compounds against radicals or metal radicals.

The antioxidant capacity of *R. tomentosa* leaves and fruits ranges from 260.58±0.91 µmol TE/g to 2795.33± 9.07 µmol TE/g (Table 1). The findings showed that ethanol extracts possessed similar antioxidant capacity from samples from different locations. The ethanol extract of green fruits' had the highest DPPH radical scavenging capacity, i.e., with respective values of 2360.35±6.86 µmol TE/g (Banjar District) and 2795.33±9.07 µmol TE/g (Batola District). Conversely, the lowest values were observed in purple fruits, i.e., 260.58±0.91 µmol TE/g and 364.05±3.82 µmol TE/g, respectively, which were lower than those reported by Lai et al. (2015), i.e., 431.17±14.5 µmol TE/g. These results of DPPH radical scavenging capacity were higher than that of grapes, blueberries, blackberries, bananas, oranges, mangoes, kiwis, and apples (8.79-92.60 µmol TE/g) (Wu et al. 2015). This study suggested the high antioxidant potential of the purple fruits and other plant parts of *R. tomentosa*. Maskam et al. (2014) stated that the fruit extract of *R. tomentosa* had potent antioxidant properties due to phenol compounds. Purified extracts of anthocyanin from *R. tomentosa* fruits have strong antioxidant activity, including the ability to scavenge DPPH radicals (IC_{50}: 6.27±0.25 g/mL) (Cui et al. 2013).

The antioxidant capacity of *R. tomentosa* extract from the two locations differed, with the extract's antioxidant capacity from the Batola district being higher than that of the Banjar district that was due to the higher content of phenolics and flavonoids (aromatic compounds) (Figures 2.A and 2.B). Ethanol extracts of green fruits and young leaves had higher concentrations of aromatic compounds. Table 1 showed that green fruits and young leaves had higher antioxidant capacity than red and purple fruits at both sample sites, with extract from Batola having higher antioxidant capacity and spectral signal intensity than Banjar (Figure 2). The antioxidant content of plants has a linear correlation with the phenolic and flavonoid content of the samples (Zargoosh et al. 2019).

Table 1. Extract yield (%, w/w) and DPPH antioxidant scavenging capacity of *Rhodomyrtus tomentosa* leaves and fruits ethanol extract

Sample	Extract yield (%, w/w)		DPPH radical scavenging capacity (µmol TE/g DW)	
	Banjar	Batola	Banjar	Batola
Young leaves	9.66	8.064	1143.01±3.43^d	1429.53±9.07^d
Old leaves	12.296	11.49	836.20±9.07^c	881.24±5.94^c
Green fruits	5.63	6.05	2360.35±6.86^e	2795.33±9.07^e
Red fruits	9.85	7.76	415.06±2.99^b	443.47±2.06^b
Purple fruits	14.35	15.24	260.58±0.91^a	364.05±3.82^a

Note: The data represent the mean±standard deviation. Data were evaluated using one-way ANOVA and the LSD test with = 0.05. Numbers followed by different superscript letters in the same column are significantly different

The difference in the antioxidant capacity of the extracts from the two regions was due to factors affecting plant compound content. The environmental conditions of the two areas are relatively the same in terms of temperature, soil pH, and humidity. The sample collection from the Batola and Banjar regions around the Banjarbaru area continues previous studies.

The effect of habitat on the amount of secondary metabolites in different herbs has been studied previously. Habitat could be a factor affecting the quantity and accumulation of secondary metabolites. The location where a plant grows can influence the process of producing chemical compounds due to its temperature and humidity. The mechanisms underlying environmental effects on accumulating secondary metabolites are not adequately understood. Zargoosh et al. (2019) showed that the environment strongly influences the production of metabolites, including enzymes.

The study by Ene-Obong et al. (2018) on *Monodora myristica* showed increased antioxidant activity because of increasing flavonoids, phenolics, and vitamin C content. Conversely, *Ricinodendron heudelotii*, with the lowest phenolic and vitamin C content, had the least DPPH-reducing ability. It showed that the antioxidant activity and free radical scavenger depended on the location of the hydroxyl group and other structural characteristics. Wu et al. (2015) measured substantial antioxidant activity in *R. tomentosa* fruit extract containing high flavonoids through various methods, including DPPH, FRAP, inhibition of lipid peroxidation, superoxide anion radical activity, and hydroxyl radicals. The results show that leaves and fruits containing flavonoids and phenols have similar antioxidant activity.

There is a direct correlation between antioxidant activity and total phenolic content (Idris et al. 2022). Total Flavonoids Contents (TFC) and Total Phenolics Contents (TPC) play important roles as electron donors, chain breakers, and free radical catchers in the antioxidant mechanism. Therefore, the flavonoid content in the plant is directly proportional to its antioxidant activity. Likewise, the presence of phenolic compounds also correlates with their antioxidant activity due to their redox properties as hydrogen donors and reducing agents that can capture free radicals (Van Hung 2016). Phenolic compounds can be divided into simple phenols, phenolic acids, hydroxycinnamic acid derivatives, and flavonoids (Lin et al. 2016). Several studies have shown that phenolic compounds such as flavonoids (especially flavonols and anthocyanins) and hydroxycinnamic acids are the most widespread and diverse polyphenols. In addition, flavonoids are the most widely found phenolic compounds in all parts of the plant. Phenolic compounds also provide other health benefits such as antimicrobial, anti-inflammatory, cytotoxic, antitumor, and antiallergic activities (Limmongkon et al. 2017; Zhang et al. 2018; Yin et al. 2021). As in leaf and fruit extracts of *R. tomentosa*, the high content of flavonoids and phenols is directly proportional to its antioxidant activity (Zhao et al. 2019).

Compounds identification of *R. tomentosa* leaves and fruits by ^{1}H NMR spectra

The chemical properties of *R. tomentosa* ethanol extract in leaves and fruits were analyzed for antioxidant activity by ^{1}H NMR spectroscopy. This non-destructive spectroscopy method requires a simple sample preparation process and short duration, which is significantly advantageous for analyzing complex mixtures such as food extracts. The ^{1}H NMR spectra of the samples are presented in Figure 2.

NMR spectroscopy, which ascertains the magnetic resonance of molecular nuclei in the presence of external magnetic fields (Hatada and Kitayama 2004), has been widely used to identify secondary metabolites due to its capability to generate a particular and distinctive spectrum for each compound. NMR spectroscopy is widely used for metabolomic investigations in various organisms due to its non-invasive and quantitative character, robustness, and reproducibility (Deborde et al. 2017; Mishra et al. 2019). Furthermore, the number of identified compounds can be correlated with the number and type of NMR signals (Leiss et al. 2011). The NMR metabolomics methods simplify compound identification by comparing sample signals to those generated by the same compounds in prior reports using CD_3OD-D_2O as the solvent (Kim et al. 2010; Ali et al. 2011; Nuringtyas et al. 2012; Gogna et al. 2015; Cerulli 2018; Mishra et al. 2019). Aqueous methanol is a common extraction solvent capable of extracting a broad spectrum of polar and non-polar compounds. Various solvents can impact the chemical shift of substances in NMR. Therefore, multiple publications were consulted to conduct a comparative analysis of potentially identifiable signal changes, with the coupling constant as a crucial parameter used to authenticate correspondence between signals in the data and references (Kim et al. 2010).

Table 2. ^{1}H NMR chemical shifts (δ) and coupling constants (Hz) of putative metabolites of *Rhodomyrtus tomentosa* leaves and fruits extracts in MeOH-d4

Compound	Chemical shifts δ (ppm) and coupling constants (Hz)		Reference
Amino acids			
Aspartate	2.68	(dd, J= 3.0; 17.0 Hz)	Cerulli et al. (2018)
Glutamic acid	2.07	(m); 2.36 (m)	Kim et al. (2010)
Leucine	0.96	(d, J= 6.85 Hz); 0.98 (d, J= 5.92 Hz)	Leyva et al. (2021)
Methionine	2.15 m; 2.65	(t, J= 6.08; 6.08 Hz)	Ali et al. (2011)
Organic acids			
Fumaric acid	6.56	(s)	Kim et al. (2010)
Malic acid	4.34	(dd, J= 6.6 ; 4.7 Hz)	Kim et al. (2010)
Succinic acid	2.56	(s)	Kim et al. (2010)
Sugars			
Mannitol	3.77	(d, J= 3.28 Hz)	Nuringtyas et al. (2012)
β-glucose	4.48	(d, J= 7.79 Hz)	Nuringtyas et al. (2012)
α-glucose	5.11	(d, J= 3.84 Hz)	Nuringtyas et al. (2012)
Sucrose	5.39	(d, J= 3.91 Hz)	Nuringtyas et al. (2012)
Aromatics compounds			
Gallic acid	7.03	(s)	Ali et al. (2011)
Myricetin	6.28	(d, J= 1.99 Hz); 7.32 (s)	Ali et al. (2011)
Myricetin 3-O- rhamnpyranoside	6.98	(s)	Cerulli et al. (2018)
Quercetin-3-O- glucoside	6.18	(d, J= 2.08 Hz); 6.37 (d, J= 2.04 Hz)	Cerulli et al. (2018)
Quercetin	6.25	(s); 7.63 (d, J= 2.22 Hz); 7.51 (s)	Liu et al. (2017)
Syringic acid	3.89	(s)	Ali et al. (2011)
Other compounds			
α -Linolenic acid	1.16	(t, J=7.04 ; 7.04); 1.29 (m)	Rizzuti et al. (2013)
Choline	3.20	(s)	Ali et al. (2011)
Sterol	0.68	(s)	Liu et al. (2017)

Note: s: Singlet; d: Doublet, dd: Double doublet; t: Triplet; m: Multiplet

The ^{1}H NMR spectra were divided into three regions based on chemical shifts (δ), namely aliphatic compounds (amino and organic acids including terpenoids), carbohydrates, and aromatic compounds detected within the chemical shifts of 0.5-3.0 ppm, 3.1-6.0 ppm, and >6 ppm, respectively (Kim et al. 2010). Figure 2 depicts the analysis and comparison of various developmental phases of the ^{1}H NMR spectra of leaves and fruit extracts from two locations.

The ^{1}H-NMR analysis results showed the presence of primary and secondary metabolite compounds, with amino acids (chemical shift of 2.0-0.5 ppm) and carbohydrates (chemical shift of 5.0-3.0 ppm) classified as primary metabolites and aromatic compounds (chemical shift >6 ppm) as secondary metabolites. A gradual decline in phenolic content was observed in the aromatic regions of the NMR spectra all through the maturation phases of leaves and fruits. Young leaves and green fruit samples showed higher signal intensities in the aromatic region than old, red, and purple leaves (Figures 2.A and 2.B).

Distinct attributes in NMR spectra derived from leaves and fruits were observed in samples from Batola and Banjar, particularly in the 5.0-3.0 ppm chemical shift range. The chemical shift was more substantial in fruits than in leaves, suggesting fruits as the primary producers of the anomeric content of glucose, α-glucose, and fructose. Intensity and diversity varied between leaves and fruit samples for chemical shifts of approximately 5.31 ppm (sucrose). Intensity variations were also identified in the aromatic shifts (6.5-7.5 ppm) detected in leaves and fruits from both locations. Higher intensities were recorded in young leaves and green fruits compared to older leaves, red and purple fruits, consistent with their antioxidant activity.

The ^{1}H NMR spectra of ethanol extracts from young and old leaves showed the presence of aromatic compounds with a regional chemical shift ranging from 10.0 to 6.0 ppm. The identified flavonoid compounds included gallic acid, myricetin, myricetin 3-O-rhamnopyranoside, quercetin-3-O-glucoside, and syringic acid (Table 2). Young leaves showed a significant concentration of these compounds, corresponding to the comparatively high antioxidant capacity observed. It was consistent with the study by Gogna et al. (2015) regarding total phenol content, flavonoid, and antioxidant activity in young and old leaves and papaya seeds (*Carica papaya* L.) using NMR spectroscopy.

A comparison of the ^{1}H NMR spectral analysis of extracts from purple, red, and green fruits showed almost identical signal diversity among the three samples (Figure 2). However, in terms of signal intensity or integral, red and purple fruits showed more pronounced results for carbohydrates and amino acids than green fruits, which showed a higher signal intensity for aromatic compounds. The disparities in spectral signal intensities suggested that red and purple fruits contained more metabolites, particularly carbohydrates and amino acids, while green fruits had higher aromatic compounds. According to Lacy et al. (2014), the concentration of metabolites could affect the intensity of spectral signals in NMR analysis. The advantage of the NMR method is its ability to concurrently yield qualitative and quantitative data, as the signal intensity is directly proportional to the molar concentration of the compound (Pauli et al. 2005).

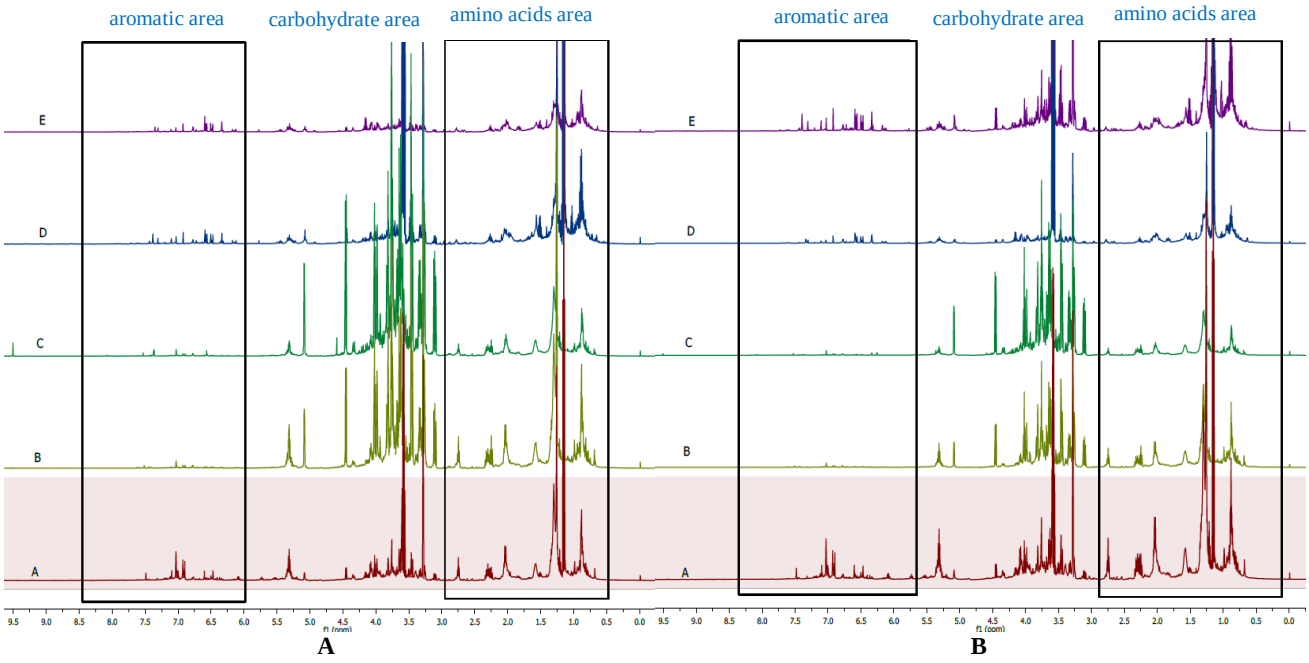

Figure 2. Representative ^{1}H NMR spectra of: A. Fruits and leaves of *Rhodomyrtus tomentosa* (Ait.) Hassk. extracts were collected from Banjar; and B. Fruits and leaves were collected from Batola. A: Young leaves; B: Old leaves; C: Purple fruits; D: Red fruits; E: Green fruits

In this study, despite containing lower concentrations of carbohydrates and amino acids than red and purple fruits, the ethanol extracts of green fruits showed a higher concentration of aromatic compounds. The results of antioxidant capacity (Table 1) showed a higher antioxidant capacity in green fruits than in red and purple fruits at both sample locations. Batola had better antioxidant capacity and spectral signal intensity than Banjar. Ali et al. (2011) reported similar consistent metabolite profiles in various stages of fruit development in grape cultivars (*Vitis* spp.) based on the ^{1}H NMR spectra obtained. The green stage comprises the highest phenol concentrations, which decrease gradually as fruits ripen while accumulating more amino acids and sugars.

Despite the benefits of applying ^{1}H-NMR in metabolomics studies, challenges arise in chemical identification due to overlapping signals across various regions, specifically in the 5.0-3.0 ppm range corresponding to sugar compounds. This study faced difficulties in discerning signals in the sugar region (except for glucose and sucrose), limiting the detection of certain substances. However, 20 potential compounds were identified through the ^{1}H NMR spectra analysis (Table 2). The detected amino acid region showed distinct signals corresponding to leucine, glutamate, methionine, and aspartate, characterized by chemical shifts from 3.0 to 0.5 ppm. The chemical shifts of organic acid compounds, including malic, fumaric, and succinic acids, were observed in the 3.0-2.0 ppm range. Sugars, such as mannitol, β-glucose, α-glucose, and sucrose, were commonly detected in the 5.00-3.50 ppm chemical shift. The less crowded regions at 10.0-6.0 ppm showed several phenolics, namely gallic acid, myricetin, myricetin 3-O-rhamnpyranoside, quercetin-3-O-glucoside, quercetin, and syringic acid. The remaining compounds identified were α-linolenic acid, choline, and sterols.

In conclusion, this study identified intensity differences in both regions' aromatic shift (6-7.5 ppm) of leaves and fruits. Young leaves and green fruits had greater intensity, correlating with the obtained antioxidant capacity. The ^{1}H NMR spectra of young and old leaf extracts for the regional chemical shift 10.0-6.0 ppm, particularly in the aromatic compound area, showed gallic acid, myricetin, myricetin 3-O-rhamnpyranoside, quercetin-3-O-glucoside, quercetin, and syringic acid. The difference in spectral signal intensity showed that red and purple fruits contained higher quantities of primary metabolites (specifically carbohydrates and amino acids), while green fruits comprised more aromatic compounds. It signified the potential of *R. tomentosa* leaves and fruits as promising antioxidant agents, although further studies may be needed to determine their role in nutritional applications.

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