

Phytochemical analysis, total phenolic content and antioxidant activity of two varieties of *Hibiscus sabdariffa* L. leaves

Syaliza Omar^{1,*}, Mohamad Syafiq Sidik¹, Amalina Mamat² and Maimunah Sanny^{3,4}

¹Faculty of Pharmacy, Universiti Sultan Zainal Abidin, 22000 Besut, Terengganu, Malaysia

²Centralised Lab Management Centre (CLMC), Universiti Sultan Zainal Abidin, 22000 Besut, Terengganu, Malaysia

³Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

⁴Laboratory of Food Safety and Food Integrity (FOSFI), Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

Received 24 August 2023

Accepted 10 September 2023

Online 31 December 2023

Keywords:

DPPH; Fourier-transform infrared; *Hibiscus sabdariffa* L.; red roselle; white roselle;

✉*Corresponding author:

Dr. Syaliza Omar

Faculty of Pharmacy, Universiti Sultan Zainal Abidin, 22200, Besut, Terengganu.

Email: syalizaomar@uniswa.edu.my

Abstract

The leaves of roselle (*Hibiscus sabdariffa* L.) are usually used in food and traditional medicine. It also has been recognized as a source of natural antioxidants. Antioxidants are known as molecules that prevent the oxidation process caused by free radical compounds that can harm the human body. Thus, this study aims to characterize the chemical constituents found in ethanolic extracts of red roselle (RR) and white roselle (WR) leaves and determine their antioxidant activity. The phytochemical screening indicated that RR leaf extract contained phenols, tannins, saponins and steroids, while WR leaf extracts consisted of phenols, tannins and reducing sugars. The total phenolic content in RR and WR leaf extracts was 42.07 ± 0.48 mg GAE/g dry weight of leaf and 38.59 ± 1.75 mg GAE/g dry weight of leaf, respectively, while the DPPH radical scavenging activity was 85.65 ± 0.33 % and 83.49 ± 0.13 % at a concentration of 1 mg/ml for RR and WR extracts. Total phenolic content and antioxidant activity showed that RR leaf extract was significantly higher than WR leaf extract. Thin layer chromatography (TLC) separation revealed a few prominent spots in both crude extracts, which were polar in nature. The TLC results can be used as preliminary data to further investigate the compounds. Characterization of the isolated crude compounds in the extracts was done using Fourier-transform infrared (FTIR). The infrared spectrum exhibited the presence of hydroxyl group, carboxylic acids, alkanes, the presence of esters ($-C=O$) and the involvement of aromatic structure of the compound. The study demonstrated the ethanolic extract of two varieties of roselle leaf contains phytochemical constituents that could contribute to the antioxidant activity. The findings of this study will contribute to the development of herbal teas or food supplements to maintain good health. The data from this study will also provide valuable information for future research on the fractionation and purification of potential antioxidant compounds found in the extract.

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1. INTRODUCTION

Hibiscus sabdariffa L. belongs to the Malvaceae family and is commonly known by its vernacular name, 'roselle'. It is cultivated in tropical and subtropical regions around the world (Qi et al., 2005) for its calyces, leaves, seeds and stems which are used as food (Salami and Afolayan, 2021). In Malaysia, roselle is known as asam paya, asam susur, or ribena Malaysia (Mohd-Esa et al., 2010). Generally, the calyx is a plant part of commercial interest. The calyx is dark red in color rich in anthocyanin and has a sour taste. The fresh calyx collected can be processed for various food products such as tea, jam, beverages, food coloring and dyes. In other parts of the world, such as Africa, India and Bangladesh, the leaves are consumed as vegetables and added to soups, salads and

seasonings (Barooah et al., 2023). Just like calyces, roselle leaves also have their own medicinal properties. However, the leaves are disregarded as a source of food or medicine in many countries (Daudu et al., 2015; Wang et al., 2014).

Roselle can be found in many varieties or accessions around the world (Choong et al., 2019; Lyu et al., 2020; Sukkhaeng et al., 2018; Wang et al., 2016). This is generally done through hybridization between different varieties of roselle to produce a new one (Choong et al., 2019). Generally, gamma irradiation is subjected to the seeds through natural mutation of the regional creole varieties, including those of white calyces (Peredo Pozos et al., 2020). The reason for doing so is to improve the plant's genetic diversity, quantitative characteristics and

improve the quality and nutritional value of roselle plants (Hanafiah et al., 2017).

Roselle was studied mainly on calyces. It has been established that the red calyx has variously reported pharmacological activities such as diuretic and hypotensive (Montalvo-González et al., 2022). Another effect observed including its use to treat hypertension, hyperlipidemia, cancer and other inflammatory diseases of the liver and kidneys (Horozic et al., 2023; Riaz and Chopra, 2018). Likewise, it has been used to treat cardiovascular disease and atherosclerosis (Sapian et al., 2023), antiobesity, antioxidant and antidiabetic (Al-Yousef et al., 2020).

While most research works on roselle focus on its calyx, very few research works exist on its leaf. Extracts from roselle leaves have a variety of biological activities, including antioxidant, antitumor, anti-atherosclerotic and antihyperlipidemic activities (Fernández-Arroyo et al., 2011; Guardiola and Mach, 2014). It has been reported that the leaves are rich in flavonoids, which may contribute to their antioxidant capacity (Mohd-Esa et al., 2010; Wang et al., 2014; Wang et al., 2016). Although interest has focused on roselle calyx, roselle leaf, in particular, has not been widely evaluated for its phytochemical constituents that contribute to antioxidant activity. Therefore, this study was conducted to obtain useful information about the chemical compositions and antioxidant activity of two varieties (i.e. red and white) of roselle leaves using a combination of chromatography and spectroscopy methods.

2. MATERIALS AND METHODS

2.1. Plant material collection and extraction

Roselle leaves of red and white varieties (Figure 1) were collected from the local residents (6°5'26.2"N 102°14'20.448"E) in Kota Bharu, Kelantan in January 2023. The samples were addressed as RR (red variety of *Hibiscus sabdariffa* L.) and WR (white variety of *Hibiscus sabdariffa* L.) throughout the article. The leaves were dried for 72 h at 40°C in an incubator before being ground using a blender to a fine powder for analysis. The powdered leaf of red and white roselle (12 g each) was extracted with 70% ethanol using the maceration method with a ratio of solid: liquid (1:20). The mixtures were left at room temperature (25°C) for 48 h. Then, the extract was filtered before the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator to remove ethanol. Then, the extract was subjected to freeze-drying before analysis.

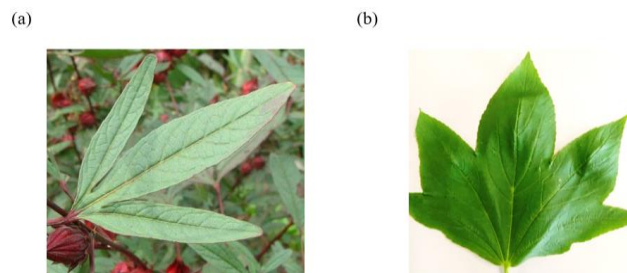


Figure 1: Leaf of two varieties of roselle (*Hibiscus sabdariffa* L.). Red roselle leaf (a), and white roselle leaf (b).

2.2. Phytochemical screening

The presence of phytochemical components in the crude extract was tested using a qualitative phytochemical test. Each phytochemical test produces a color that reveals the presence of a specific phytochemical in the extract.

2.2.1 Test for saponin

Twenty milliliters of distilled water was added to 2 ml of ethanolic extract, and the mixture was shaken for about 15 minutes. Continuous foam formation of at least 1 cm indicates the presence of saponin in the extract (Parekh and Chanda, 2007).

2.2.2 Test for flavonoids

The ethanolic extract (2 ml) was mixed with 2-3 drops of sodium hydroxide solution. After a few drops of 0.01 M hydrochloric acid were added until the yellow color gradually faded to colorless. This color change indicates the presence of flavonoids in the extract (Kumar et al., 2007).

2.2.3 Test for tannins

One milliliter of ethanolic extract was combined with 2 ml of a 5% ferric chloride (FeCl_3) solution. The development of a green or yellow tint indicates the presence of tannins in the extract (Kumar et al., 2007).

2.2.4 Test for steroids (Salkowski's test)

One milliliter of ethanolic extract was extracted and shaken with 1 ml of chloroform. Then, 10 drops of acetic anhydride were added followed by 5 drops of concentrated sulphuric acid along the walls of the test tube. A color change from violet to blue or green color indicates the presence of steroids (Dahanayake et al., 2019).

2.2.5 Test for phenol (FeCl_3 test)

One milliliter of ethanolic extract was dissolved in 2 ml of distilled water. To this solution, a few drops of 10% FeCl_3 solution was added. A dark green color indicates the presence of phenolic compounds (Pooja and Vidyasagar, 2016).

2.2.6 Test for glycosides (Keller-Killiani's test)

Two milliliters of ethanolic extract was dissolved in 0.5 ml of glacial acetic acid. To this solution, 1 ml of concentrated

sulfuric acid is carefully applied to the side wall of the test tube. The liquid interface begins to turn dark blue indicates the presence of cardiac glycosides (Parekh and Chanda, 2007).

2.2.7 Test for alkaloids

One milliliter of Dragendroff's reagent was added to 2 ml of the ethanolic extract. An orange-red precipitate was formed, indicating the presence of alkaloids (Kumar et al., 2007).

2.2.8 Test for reducing sugars (Fehling test)

One milliliter of filtered water was added to 1 ml of the extract, which was then shaken. A few drops of Fehling's solution A and B were added to the filtrate and heated for five minutes. Reducing sugars can be seen as an orange-red precipitate (Shrestha et al., 2015).

2.3. Determination of total phenolic content

At a concentration of 1 mg/ml, 50 µl of each ethanolic leaf extract of RR and WR was added to the falcon conical tube. Each tube received an addition of 100 µl of 10% Follin-Ciocalteu reagent and 100 µl of 7% sodium carbonate. The mixture was then thoroughly shaken and allowed to sit at room temperature (25°C) in the dark for 1 h. The absorbance was then assessed at 760 nm using a microplate reader (Thermo Scientific™ Multiskan™, USA). The amount of gallic acid equivalents (GAE) per gram of dry sample weight (mg/g) represents the overall amount of phenols in the samples (Tahir et al., 2017).

2.4. Determination of antioxidant activity

DPPH(2,2-diphenyl-1-picrylhydrazyl) antioxidant activity analysis was performed using the method adapted from Borah et al., (2020) with slight modifications. Ascorbic acid was taken as a standard (50 µg/ml) and ethanolic extracts of white and red roselle leaf as test solution (2 mg/ml). The 96-well microplate was used in this study. For the preparation of the test solution, 150 µl of 0.2 mM DPPH solution was added to 100 µl of each sample in a microplate, gently mixed, and incubated in a dark condition for 30 min at room temperature (25°C). Negative control was made by mixing 150 µl of 0.2 mM DPPH solution with 100 µl of ethanol. This solution was then allowed to sit at room temperature for 30 min in the dark. The ultraviolet (UV)-visible spectrophotometer (Thermo Scientific™ Multiskan™, USA) was used to assess absorbance at 517 nm. By comparing the absorbance readings of the control and test samples, the inhibitory percentage values were determined. The ability of the sample and standard to inhibit free radicals was calculated as the potential to scavenge the DPPH radical using the following formula:

$$\% \text{ Inhibition of DPPH radicals} = (A_o - A_t / A_o) \times 100\%$$

where A_t denotes the absorbance of the test and standard, and A_o denotes the absorbance of the control.

2.5. Separation of phytoconstituents by TLC

The separation of the components in the extracts was performed using TLC on the stationary phase consisting of a pre-coated silica gel plate. Each of the extracts will be spotted in a line about 1 cm from the bottom of the TLC plate. The mobile phase solvent system, which consists of n-butanol: glacial acetic acid: water (10:1:1), was used to develop the TLC plates in a saturation chamber. The solvent was added to the chromatography tank, covered, and left to saturate for 30 min at room temperature. The plate was removed at the end of the chromatographic development and left to air dry at room temperature. Under ultraviolet light, the separated spots were visualized at wavelengths 265 nm and 360 nm (Brown et al., 2019).

2.6 Fourier-transform infrared (FTIR) analysis

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR, Shimadzu, Nakagyo-ku, Kyoto, Japan) was used to determine the characteristics of chemical functional groups present in the ethanolic extracts of RR and WR leaf. The result of the infrared spectrum revealed absorption peaks that represent chemical fingerprints that correspond to the frequencies of vibrations between the bonds and the atoms that make up the compound. Spectra of extracts were generated using ATR-FTIR connected to a DTGS KBr detector and a Golden Gate Single Reflection Diamond ATR accessory (incident angle of 45°). A small quantity of powdered RR and WR crude extract was used to cover the ATR diamond plate. The pressure clump was swung assembly and screwed down between the opposing base and the tip till the sample was firmly trapped. Spectra were recorded for each sample in the mid-infrared region (4000-400 cm^{-1}) using 16 scans and a resolution of 4 cm^{-1} . After the measurement, the sample was removed, and the plate was cleaned using a non-abrasive tissue with 70% ethanol (Rashid et al., 2023).

2.7 Statistical analysis

All assays were carried out in triplicate. The data were expressed as mean \pm standard deviation. The Statistical Package for Social Science (SPSS) Version 25 software has been used to conduct the statistical analysis (IBM, New York). To ascertain the significance of the difference between extracts, a Student's t-test was used. Values will be regarded as significantly different if the p -value is less than 0.05.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

Phytochemicals are chemical compounds that occur naturally in plants and can have either positive or

negative impacts on health. In this research, qualitative tests were conducted on the ethanolic extracts of RR and WR leaves to identify the presence of saponins, flavonoids, tannins, phenol, glycosides, alkaloids, steroids, and reducing sugar. The conventional qualitative phytochemical test is affordable, simple to do, and needs fewer resources, making it a viable option for initial phytochemical screening (Pandey and Tripathi, 2014). Table 1 shows the phytochemicals found in both extracts. It shows that there were four phytochemicals in the ethanolic extract of RR leaf namely saponins, steroids, tannins and phenols while for the WR leaf extract, three phytochemicals were present namely tannins, phenols and reducing sugars. Our results are in agreement with Oktapiya et al., (2022) who revealed the presence of alkaloids, flavonoids, saponins and tannins in the roselle leaf extract. Interestingly, Yahaya et al., (2017) also found alkaloids, tannins, glycosides, and reducing sugars in the ethanol extract of rosella leaves. Another study by Arvind et al., (2011), discovered that flavonoids, phenolics, triterpenoid, and alkaloids, steroids and cynogenic glycosides were present in the roselle leaves that were extracted in different types of organic solvents.

Table 1: Phytochemical screening of the extracts of RR and WR leaves

Type of the qualitative test	Roselle leaf extract	
	RR	WR
Saponins	+	-
Flavonoids	-	-
Tannins	+	+
Phenols	+	+
Cardiac glycosides	-	-
Alkaloids	-	-
Steroids	+	-
Reducing sugar	-	+

Key: Present (+), Not present (-)

The results of the phytochemical analysis in this research were different from previous studies may be due to the pre-extraction or extraction-related factors. Possibly, the pre-extraction elements including the plant part employed, its origin, the particle size of the powdered leaf, moisture content, drying method, and degree of processing will have an impact on the extraction of phytochemicals from the plant materials (Shaikh et al., 2020). Furthermore, factors relating to extraction, such as the method of extraction used, the solvent selected, the solvent-to-sample ratio, the solvent's pH and temperature, and the duration of the extraction, also have an impact on the extraction of phytochemicals from plant materials (Azwanida, 2015).

3.2 Total phenolic content

The concentration of phenolics in the extracts of RR and WR leaves was determined by Folin–Ciocalteu assay using gallic acid as a standard. In this assay, higher total phenolic content (TPC) value indicates high concentration of phenolics in the samples. Figure 2 shows the TPC values of the extracts of RR and WR leaves. RR extract possessed a higher TPC value (42.07 ± 0.48 mg GAE/g dry weight of leaf) than WR extract (38.59 ± 1.75 mg GAE/g dry weight of leaf). The t-test analysis showed there was a significant difference ($p < 0.05$) between the means of TPC for both extracts. These values were in accordance with the study of Chiu et al., (2015) where they found the TPC value of polyphenolic leaf extract of red roselle was 36.8 ± 2.1 mg GAE/g dry weight of leaf.

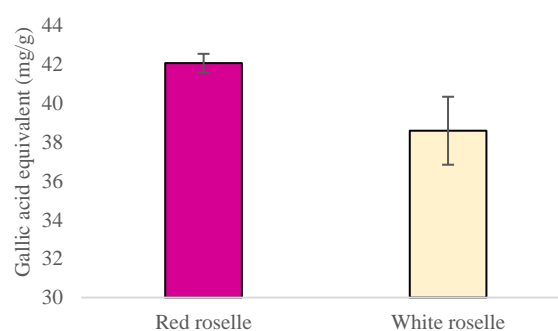


Figure 2: Total phenolic content between the extracts of RR and WR leaves

The variability in TPC values between our study and Chiu et al., (2015) was expected and may be due to several factors. The main differences seen between the studies were the extraction techniques and solvents used. This study used a maceration technique for 48 h with 70% ethanol, while Chiu et al., (2015) extracted leaf powder with absolute methanol at a temperature of 50°C for 3 h. Maceration at room temperature for 48 h was chosen in this study to allow enough time for the solvent to penetrate into the cells and completely destroy the leaf cell components. Although methanol is more polar than ethanol, however, the presence of water in our extraction medium (70% ethanol) allowed higher phenolic solubility thus contributing to higher TPC in this study.

It was clear that the technique of extraction and solvents play an important role in extracting phenolic compounds from samples (Mosić et al., 2020). For example, Zhen et al., (2016) who studied 22 roselle leaf extracts found that TPC values ranged from 18.98 ± 2.7 to 29.9 ± 0.5 mg GAE/g using an ultrasonic water bath for 10 minutes followed by shaking at room temperature overnight with 70% (v/v) methanol/water with 0.1% acetic acid as the extracting solvent. In another study, Zhang et al., (2011) found relatively low TPC values (9.083 ± 0.510 mg) in red roselle leaf extract using reflux with 80%

ethanol for 2 hours. Mohd-Esa et al., (2010) obtained 2.20 ± 0.02 (mg GAE/g) of roselle leaves extracted with 80% methanol and 1.71 ± 0.04 (mg GAE/g) when extracted with distilled water. The results of Mohd-Esa et al., (2010) are very clear that solvents play a role in extracting phenolics. Other factors that contribute to the variation of phenolic content are the variation of the roselle genotype (Zhen et al., 2016), growing conditions in either tropical vs subtropical areas, and the use of fertilizer types when cultivating the crop (Formagio et al., 2015).

3.3 Antioxidant activity

Oxidative stress and free radicals are frequently associated. When oxygen interacts with specific molecules, free radicals are created. Once generated, free radicals pose a hazard due to the potential harm they may do when they interact with vital biological components including DNA and proteins as well as the cell membrane (Martemucci et al., 2022). Antioxidants have the capacity to stop harm from happening before it happens by interacting with free radicals and neutralizing them (Alkadi, 2020). The secondary metabolites produced by plants are known to contribute to antioxidant activities (Hunyadi, 2019).

The radical scavenging activity of the extracts of RR and WR leaves was studied by DPPH assay. The DPPH free radical scavenging assay is a straightforward and popular screening method for finding bioactive compounds with antioxidant capacity (Fenglin et al., 2003). Ascorbic acid was used as a positive control for comparison because of its good radical scavenger ability. Figure 3 shows that RR leaf extract exhibits a high percentage of DPPH scavenging activity which is 85.65 ± 0.33 % compared to WR leaf extract (83.49 ± 0.13 %) at a concentration of 1 mg/ml. Ascorbic acid showed 89.58 ± 0.26 % of DPPH inhibition, a little higher than both extracts. Statistical analysis showed that there was a significant difference ($p < 0.05$) between RR leaf extract, WR leaf extract and ascorbic acid. This data shows that both extracts have substances that may contribute to free radical scavenging action.

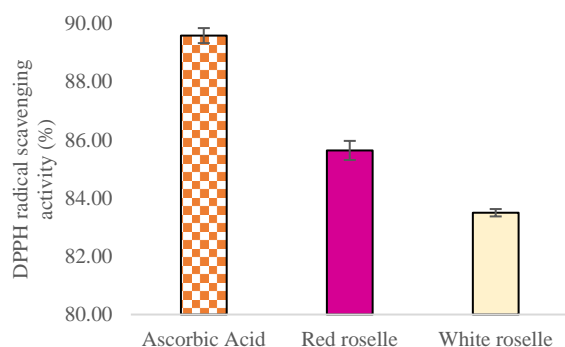


Figure 3: DPPH radical scavenging activity between red roselle and white roselle leaf extracts

A study by Wang et al., (2014) identified five compounds, namely neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, rutin, and isoquercitrin, which can be the main contributors to antioxidant activity in the leaves of red roselle. Our results are in the range with Mohd-Esa et al., (2010) who obtained 89.8 ± 0.33 % DPPH inhibition in 80% methanol extract of red roselle leaves. Although Mohd-Esa et al., (2010) obtained a low TPC value for a similar extract as discussed in section 3.2, the extract showed high DPPH radical scavenging activity, indicating that compounds other than polyphenols have contributed to the antioxidant activity in the extract. Hence, it is possible that phytochemicals other than polyphenols present in RR leaf extract contributed to higher antioxidant activity than in WR leaf extract. As stated in Table 1, steroids and saponins were detected quantitatively in RR leaf extract. Furthermore, steroids (i.e. phytosterols) and saponins extracted from plants have been reported to possess antioxidant activity (Ahmad et al., 2013; Chen et al., 2014; Han et al., 2019). The antioxidant results in this study differ from previous studies might be due to several factors. Antioxidant activities of the plant extracts are mostly owing to the active compounds found in them. It could be due to the extraction factors as discussed in section 3.2. In addition, the drying methods, the variety of roselle, geographical factors and the environment where roselle is grown have an effect on the synthesis of plant molecules with antioxidant properties (Kumar et al., 2015; Zahra et al., 2019).

3.4 Thin layer chromatography

TLC was carried out to isolate and characterize the nature of the compounds in the extracts. Compounds containing a chromophore that absorbs UV light are referred to as UV-active compounds and are visible under the UV light on TLC plates. Retention factor, often known as R_f value, is a ratio used to define how far a component in a combination travels in comparison to how far the solvent travels. R_f value ranges from 0 to 1, with values closer to 1 indicating that the component is more attracted to the solvent than to the stationary phase. TLC of the extracts of RR and WR leaves revealed the presence of several compounds (Figure 4) in the solvent system n-butanol: acetic acid: water (10:1:1) when subjected to UV 254 nm (short wavelength) and 360 nm (long wavelength). RR and WR leaf extracts showed four and three spots at 254 nm, respectively.

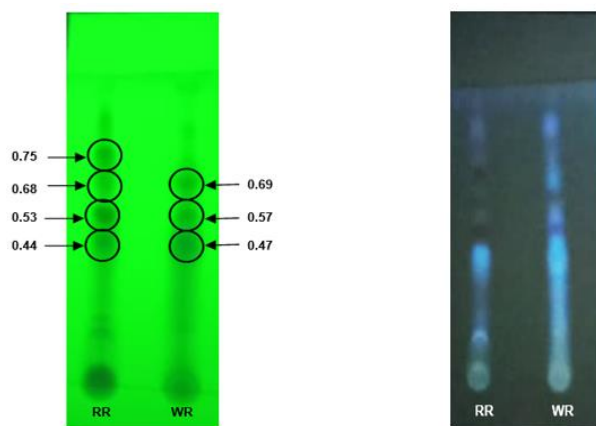


Figure 4: TLC chromatograms and R_f values of RR and WR leaf extracts observed at UV 254 nm (a) and 360 nm (b)

There were many overlapping spots seen in both extracts at 360 nm. Generally, spots seen in the TLC chromatogram correspond to the number of compounds separated by the mobile phase. Compounds having R_f 0.75, 0.68, 0.53 and 0.44 were prominent in the RR extract, while in the WR extract, all spots exhibited clear spots. From the results, the compounds having almost similar values of R_f in both extracts are considered to have the same characteristics in polarity. Therefore, compounds with identical polarity may be present in both extracts of RR and WR leaves. From the R_f value, most of the compounds separated in both extracts were from medium polar compounds. However, to confirm the identity of each compound, advanced methods like high-performance liquid chromatography (HPLC) studies should be carried out. The limitation of this study is that the TLC analysis is only qualitative to determine the number of compounds that could be separated in the extracts of the RR and WR leaves, rather than to identify the presence of any particular compounds in each extract. Furthermore, coextractives such as chlorophyll may interfere with the extracts and that limits the resolution power of TLC. These problems can be solved by a second step known broadly as sample cleanup, that is, isolation of the compounds of interest from the rest of the extract, often carried out on an adsorbent column such as silica gel or microporous resins (Sajewicz et al., 2016).

3.5 FTIR analysis

FTIR spectra of the extracts of RR and WR leaves are shown in Figure 5. The peaks for RR leaf extract at 1728.22 cm^{-1} and 1606.70 cm^{-1} were strong, while the other peaks varied from weak to medium. For WR leaf extract, peaks at 1608.63 cm^{-1} and 1033.85 cm^{-1} were strong while the rest were weak.

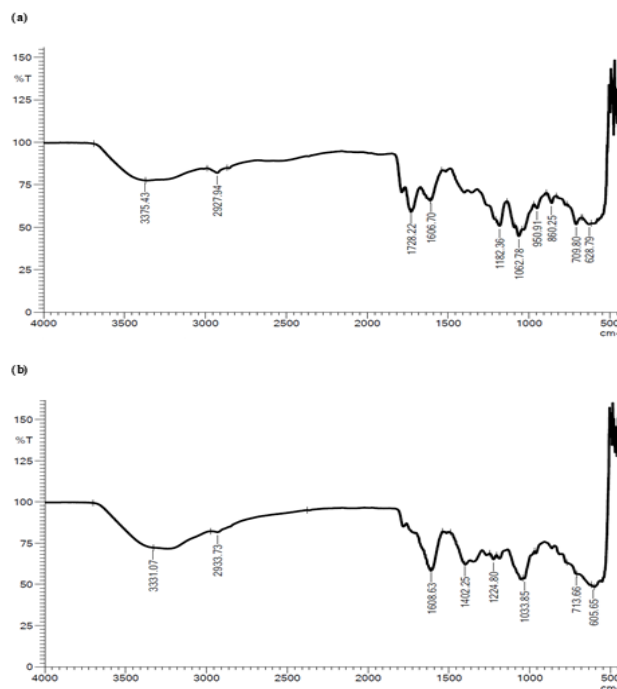


Figure 5: FTIR spectrum data of isolated components in RR (a) and WR (b) leaf extracts.

Tables 2 and 3 show the functional groups of the phytoconstituents in the extracts. There was little difference in functional groups between the extracts of RR and WR leaves. In the RR leaf extract, the peak at 1728.22 cm^{-1} represents a C=O ester, which may be related to triterpenoid saponins and steroid nuclei. This peak was not present in the WR leaf extract. This result follows the phytochemical screening test that found the RR leaf extract in this study contained saponins and sterols.

The peaks at 1606.70 cm^{-1} and 1608.63 cm^{-1} for the extracts of RR and WR leaves were signs of C=C stretching, which may be related to the conjugation of alkenes or aromatic compounds (i.e. benzenoid). The presence of benzenoid was supported by peaks at 709.80 cm^{-1} (RR) and 713.66 cm^{-1} (WR) which were C-H out-of-plane bending vibrations for the substituted benzene ring. A peak at the region $3500\text{--}3650\text{ cm}^{-1}$ in both extracts represents the hydroxyl group. These three peaks indicate the presence of phenol and tannin in the extracts of RR and WR leaves. The identification of benzenoid compounds by FTIR supported the findings from the phytochemical screening, which detected the presence of phenols and tannins (Table 1).

Interestingly, there was a strong peak at 1033.85 cm^{-1} in the WR leaf extract. This peak shows a C-O stretching vibration that may be related to the glycosidic bond. It has been noted that African roselle leaves contained glycosides (Sawabe et al., 2005). This result was supported by a positive result for sugars in WR leaf extract during the phytochemical screening test. Furthermore, O-H bending was present in the WR leaf extract at 1402.25 cm^{-1} , indicating a carboxylic acid group. The carboxylic

acid might be linked to the hydrolyzable tannin structure illustrated by the peaks at 1608.63 cm^{-1} and 713.66 cm^{-1} . In addition, aliphatic C-H stretching was observed at 2927.94 cm^{-1} and 2933.73 cm^{-1} for RR and WR leaf extracts, respectively, representing alkyl groups.

Table 2: FTIR spectra of RR leaf extract

Wave number (cm^{-1})	Theoretical wave number (cm^{-1}) (Merck, 2023)	Functional group	Predicted compound
3375.43	3500-3650	O-H stretching vibration	-hydroxyl group
2927.94	3000-2800	C-H stretching vibrations	-alkane
1728.22	1730-1715	C=O of esters	- α , β -unsaturated ester
1606.70	1650-1600	C=C stretching	-alkene conjugation, aromatic compound
1182.36	1210-1163	C-O stretching ester	ester
1062.78	1085-1050	C-O stretching vibrations	-aliphatic ether, primary alcohol, glucosidic bond
950.91	980-960	C=C bending	-disubstituted alkene (trans)
860.25	840-790	C=C bending	-trisubstituted alkene
709.80	730-665	C=C and C-C bending vibrations	-disubstituted alkene (cis)
	720-680	Out-of-plane C-H bending vibrations	-mono-substituted phenyl structure
628.79	610-690	C-H wagging vibration	-vinyl hydrocarbons

Table 3: FTIR spectra of WR leaf extract

Wave number (cm^{-1})	Theoretical wave number (cm^{-1}) (Merck, 2023)	Functional group	Predicted compound
3331.07	3500-3650	O-H stretching vibration	-hydroxyl group
2933.73	3000-2800	C-H stretching vibrations	-alkane
1608.63	1650-1600	C=C alkene conjugation	-alkene conjugation, aromatic compound
1402.25	1440-1395	O-H bending	-carboxylic acid
1224.80	1250-1020	C-N stretching amine	-amine
1033.85	1085-1050	C-O stretching vibrations	-aliphatic ether, primary alcohol, glucosidic bond
713.66	730-665	C=C and C-C bending vibrations	-disubstituted alkene (cis)
	720-680	Out-of-plane C-H bending vibrations	-mono-substituted phenyl structure
605.63	610-690	C-H wagging vibration	-vinyl hydrocarbons

4. CONCLUSION

In this study, a phytochemical screening test revealed that RR leaf extract contains saponins, tannins, steroids, and phenol while for WR leaf extract, it contains tannin, phenol, and reducing sugars. The total phenolic content and DPPH radical scavenging activity in RR leaf extract were significantly higher than in WR leaf extract. The separation of constituents in both extracts by TLC revealed several spots which were polar in nature. The FTIR analysis identified the presence of several functional groups, which might be related to the compounds detected in the phytochemical tests. These results can be used as supporting evidence that roselle leaf extract has high antioxidant activity and requires further study. Further

studies will focus on the fractionation and purification of potential bioactive compounds present in the extracts.

ACKNOWLEDGEMENT

The authors would like to thank the Centralized Lab Management Centre (CLMC), Universiti of Sultan Zainal Abidin for giving permission to conduct this research at their laboratory facilities.

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