Phytochemical screening and antioxidant activity of selected underutilized plants in Nadir Plot Taman Botani, Sri Medan, Johor

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Abstract. This study was conducted to determine phytochemical content and antioxidant activity of selected underutilized plants in the Nadir plot in Taman Botani such as *Phyllanthus acidus, Diospyrus dicolour, Flacourtia rukam* and *Anacardium occidentale*. The total phenolic content was analyzed using Folin Ciocalteu and total flavonoid content was analyzed using aluminium chloride. The antioxidant activity was evaluated by DPPH radical scavenging, ABTS radical scavenging and Ferric Reducing Antioxidant Power assay. The results showed that the phenolic content for *Flacourtia rukam* leaves was the highest with 87.17 ± 0.5890 mg GAE/g, *Phyllanthus acidus* leaves had the highest total flavonoid content value with 149.83 ± 0.5768 mg RE/g. DPPH, radical scavenging showed that *Anacardium occidentale* has the lowest IC_{50} value of 57.9132 ± 15.03802 µg/mL. Therefore, it has the strongest antioxidant potential. However, the results for ABTS radical scavenging showed that *Phyllanthus acidus* has the lowest IC_{50} value of 3.7581 ± 5.8748 µg/mL, which has the strongest antioxidant activity. Ferric Reducing Antioxidant Power assay method, *Flacourtia rukam* shows the highest FRAP value with 9.9336 mM Fe^{2+} /L. In conclusion utilising *Phyllanthus acidus, Diospyrus dicolour, Flacourtia rukam* and *Anacardium occidentale* as sources of phytochemicals could offer opportunities for nutraceutical and functional food applications.

1 Introduction

The naturally occurring bioactive substances called phytochemicals, which are primarily divided into two groups, are essential to plant metabolism [1]. Primary phytochemicals, including amino acids, proteins, common sugars, and chlorophyll, are essential to plant life. Conversely, secondary phytochemicals include flavonoids, terpenoids, alkaloids, and steroids that support various processes, including defense systems. The historical use of plants by our ancestors to enhance health is proof of the great range of phytochemicals that

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make plants essential to human life. Since plants containing significant concentrations of these phytochemicals can offer protection against free radical damage, it is imperative to comprehend their antioxidant potential [2-6].

Modern medications unquestionably save lives, but using them carries dangers to one's health as well as possible adverse effects if uncontrolled [7]. Because synthetic pharmaceuticals are made entirely of chemicals, they can have several negative consequences, which makes people look for safer substitutes. Plant-based traditional and folk remedies present a possible substitute, as natural antioxidants are considered less harmful and safer for human health. However, much of the potential of naturally occurring antioxidants in plants still needs to be discovered and utilized.

Taman Botani Johor is an important conservation area in the Johor area that has many plant plots [8]. In this conservation setting, the Nadir plot is particularly important. The underutilization of the Nadir plot, which focuses on uncommon plants that are difficult to find economically, is one example. The risk of extinction associated with neglecting these endangered species emphasizes how urgent it is to conduct adequate management and study.

By concentrating on the Nadir plot in Taman Botani, Sri Medan, Johor, this study seeks to close this gap. The study seeks to ascertain the phytochemical makeup of a few underutilized plants, namely *Phyllanthus acidus*, *Diospyrus dicolour*, *Flacourtia rukam* and *Anacardium occidentale*, highlighting the importance of chemicals such as flavonoids and phenolic compounds in medicine. This study highlights the potential health advantages of the various bioactive substances found in uncommon plants and the significance of preserving these plants.

2 Materials and methods

2.1 Collection of plant

Four specifically chosen medicinal plants (*Phyllanthus acidus, Diospyrus dicolor, Anacardium occidentale, and Flacourtia rukam*) were gathered for this investigation from the Nadir plot. The selection of herbs and shrubs was done with an emphasis on the leaf portion of the plants. With an average of 2 kilogram per plant section, the collected plants were sealed in black plastic bags for phytochemical analysis. The plants that had been delivered were then immediately brought to the lab and kept there at 4°C in preparation for further examination.

2.2 Preparation for plant extraction

The leaf is the plant portion that is used in phytochemical analysis. Tap water was used to wash the leaves. It was then cleaned with distilled water. The air oven was filled with the fresh leaves. At 36°C, the drying process required 72 hours. The moisture content of the leaves was measured after 72 hours. Each plant's dried leaves were processed into a powder using a blender, then sealed in a zip-lock bag and frozen at -20°C.

2.3 Extraction of plant

The extraction process used was cold extraction. A conical flask was filled with 30 g of plant sample. 300 mL of methanol was added to the conical flask and let to soak for 30 minutes at room temperature in order to use it as a solvent. Whatman paper No. 1 was used for filtering the extract. The procedure involved adding methanol to a conical flask three times until the extract was filtered. The methanol extract was mixed all together. To obtain crude methanol

extract, the extract was put on an evaporating dish and dried over a water bath in the fume cupboard. The crude methanol extract was weighed. Next, the yield % was calculated.

2.4 2.4 Qualitative analysis

2.4.1 Test for phenols

A test tube was filled with just a small quantity of the ethanolic extract, 1 mL of water, and one or two drops of iron III chloride ($FeCl_3$). A test result that is blue, green, red, or purple is positive [9].

2.4.2 Test for flavonoids

Each plant sample's aqueous extract was mixed with a few drops of 1% NH_3 solution in a test tube. If flavonoid chemicals are present, a yellow hue is seen [10].

2.5 Determination of total phenolic compound

The Folin-Ciocalteu reagent was used to determine the total phenolic content. One milliliter of plant extract and two milliliters of Folin-Ciocalteu reagent were combined. Following that, the mixture of plant extract and Folin-Ciocalteu reagent was given 2.5 mL of sodium bicarbonate 7.5% and was allowed to sit at room temperature for four minutes. In order to determine the absorbance value, a spectrophotometer was used at 725 nm. The standard utilized was gallic acid.

2.6 Determination of total flavonoid compound

The aluminium colorimetric technique was used to determine the total flavonoid content. After weighing one millilitre of each plant sample extract, the extract was transferred to a ten millilitre volumetric flask and the remaining volume was filled with methanol. Subsequently, 0.5 mL of 5% $NaNO_3$ was added to 1 mL of each plant sample solution, and after 5 minutes, 0.5 mL of 10% $AlCl_3$ was added. Subsequently, 2 mL of 1 M NaOH was added after 6 minutes. Next, distilled water is added to the solution until the 10 mL mark is reached. At room temperature, the solution was incubated for fifteen minutes. Additionally, a sample blank was made ready for comparison. Rutin served as the standard reference for the spectrophotometer that was used to measure the absorbance value at 415 nm.

2.7 Antioxidant activity

Antioxidant activity was determined by 3 assay methods, which are DPPH free radical scavenging assay, ABTS radical cation decolorization assay (2,2 -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), and Ferric reducing antioxidant power (FRAP) assay.

2.7.1 DPPH radical scavenging assay

In a test tube, an aliquot containing 1.0 mL of extract, 2.0 ml of DPPH solution, and a control solution of distilled water was pipetted. In addition, to finish the reaction, the mixture was vortexed and let to sit at room temperature in a dark area for half an hour. Using a blank (methanol) as a reference, the mixture's absorbance was measured at 517 nm using a UV-

VIS spectrophotometer. The following formula was used to calculate the free radical scavenging activity:

$$Inhibition\ (\%): [(Acontrol - Asample) / (Acontrol)] \times 100 \quad (1)$$

2.7.2 ABTS radical scavenging assay

Methanol was added to the ABTS solution to get an absorbance of 0.700 ± 0.002 at 734 nm. A test tube was pipetted with an aliquot containing 2.0 mL of ABTS solution and 0.1 mL of extract or control (distilled water) solution. After vortexing the mixture, it was allowed to sit at room temperature for half an hour in the dark. A UV-VIS spectrophotometer was then used to measure the mixture's absorbance at 734 nm. The following equation was used to determine the ABTS scavenging effect:

$$Inhibition\ (\%): [(Acontrol - Asample) / (Acontrol)] \times 100 \quad (2)$$

2.7.3 Ferric reducing antioxidant power

First, 1.5 mL of FRAP reagent was added with 0.05 mL of extract at various doses (0-100 mg/ml). Subsequently, the mixture was vortexed and incubated in a water bath at 37°C for 20 minutes. Next, a UV-VIS spectrophotometer was used to read the mixture at 593 nm against the standard and the blank (methanol). For the standard calibration curves, ferrous sulphate solution was utilized. Fe (II) concentrations were employed as a reference standard, and the samples' reducing power was contrasted with that of the standard.

3 Result and discussion

3.1 Qualitative phytochemical analysis

Four distinct plant species' leaves were subjected to qualitative phytochemical screening in this study: *Phyllanthus acidus*, *Diospyrus dicolor, Flacourtia rukam*, and *Anacardium occidentale*. The study aimed to evaluate the presence or absence of flavonoid and phenol compounds in the plant samples. These components are recognized as naturally occurring antioxidants with possible physiological and therapeutic applications [11].

The findings of the phytochemical screening are shown in Table 1, which shows that all leaf samples from the chosen plant species contained phenol and flavonoids. It is acknowledged that phenol and flavonoids play a crucial role in giving plants their antioxidant qualities [11]. Methanol was a strategically chosen solvent for the phytochemical screening process because of its high polarity, amphiphilic character, and capacity to extract a broad variety of chemical components from plants.

The identification of components with established physiological and therapeutic properties was made possible by examining plant extracts [12]. As an efficient solvent, methanol made extracting several chemical components from the leaves of *Anacardium occidentale*, *Flacourtia rukam*, *Diospyrus dicolor*, and *Phyllanthus acidus* easier. The constant presence of phenol and flavonoids in all samples points to the possibility that these plant species have antioxidant qualities, which is consistent with the growing body of knowledge on the possible medical benefits of natural antioxidants.

Overall, the qualitative phytochemical screening results demonstrate how abundant bioactive chemicals are in the leaves of the plant species under study. These results lay the

groundwork for additional research into the phytochemical components discovered and their possible uses in physiology and medicine.

Table 1. Phytochemical screening

Test	***Phyllanthus acidus***	***Diospyrus dicolour***	***Anacardium occidentale***	***Flacourtia rukam***
Flavanoids	+++	+	+++	++
Phenols	++	++	+	+++

Note : Keynote Symbols: +++ = Abundant, ++ = Moderate, + = Trace

3.2 Quantitative phytochemical analysis - total phenolic content & total flavonoid content

3.2.1 Total phenolic content

One of the most important steps in determining the amount of phenolic content in plant samples is the Total Phenolic Content (TPC) assay. Plants naturally contain phenolic chemicals, which have redox characteristics and provide them with antioxidant benefits. The Folin Ciocalteu method was used to detect TPC in methanol extracts, and gallic acid was used as a comparative reference [13]. The total polyphenols in the sample extracts were determined more easily with the Folin Ciocalteu reagent. The oxidized phenols were then converted to a combination of blue tungsten and molybdenum oxides, which could be detected spectrophotometrically at 765 nm.

Gallic acid concentration solutions (0, 50, 100, 150, 250, and 500 mg/L) were used to create a standard curve with a coefficient of regression (R_2) of 0.9758 in order to quantify the TPC. The standard curve's equation was found to be $y = 0.0001x + 0.0543$, with an R_2 value of 0.9758. The TPC in the methanolic extracts of *Anacardium occidentale*, *Flacourtia rukam, Diospyrus dicolor*, and *Phyllanthus acidus* varied from 1.677 to 8.717 mg GAE/g using this standard curve.

Table 2. Total Phenolic Content

Sample	**Part**	**Total Phenolic Content (mg GAE/g)**
Phyllanthus acidus	Leaves	39.57 ± 0.5658
Diospyrus dicolour	Leaves	38.17 ± 0.3483
Flacourtia rukam	Leaves	87.17 ± 0.5890
Anacardium occidentale	Leaves	16.77 ± 0.1052

More specifically, Table 2 showed significant differences in the amount of phenols between the various plant species under study. With an 8.717 mg GAE/g (87.17 ± 0.5890 mg GAE/g) TPC value, *Flacourtia rukam* leaves were the highest, followed by *Phyllanthus acidus* leaves (3.957 mg GAE/g; 39.57 ± 0.5658 mg GAE/g), *Diospyrus dicolor* leaves (3.817 mg GAE/g; 38.17 ± 0.3483 mg GAE/g), and *Anacardium occidentale* leaves (16.77 ± 0.1052 mg GAE/g). These results highlight the various profiles of phenolic content found in the investigated plant species, offering important new information about their possible antioxidant capabilities and advancing our knowledge of their physiological and therapeutic uses.

3.2.2 Total flavanoid content

Rutin was used as the standard solution in an aluminium chloride colorimetric test to determine the Total Flavonoid Content (TFC) in methanol extracts. The development of a

stable complex between $AlCl_3$, keto, and the hydroxyl groups of flavones and flavonoids is necessary to evaluate TFC with aluminium chloride. Regression coefficient (R_2) of 0.9801 was obtained by creating a standard curve with standard concentrations of 50, 100, 150, 250, and 500 mg/L. Rutin's standard curve equation was found to be $y = 0.0003x + 0.121$.

By using this standard curve to the methanolic extracts of leaves from *Anacardium occidentale*, *Flacourtia rukam, Diospyrus dicolor,* and *Phyllanthus acidus*, the TFC ranged from 9.703 to 14.983 mg RU/g. Rutin equivalency (RE) in milligrams per gramme was used to express the results. Table 3 shows that the amounts of flavonoids varied significantly among the plants under investigation. At 14.983 mg RU/g (149.83 ± 0.5768 mg RE/g), *Phyllanthus acidus* leaves showed the highest TFC. *Anacardium occidentale* leaves came in second at 12.44 mg RU/g (124.40 ± 0.0728 mg RE/g), *Flacourtia rukam* leaves came in third at 10.103 mg RU/g (101.03 ± 0.1688 mg RE/g), and *Diospyrus dicolor* leaves came in third with 9.703 mg RU/g (97.03 ± 0.3682 mg RE/g). These findings provide valuable insights into the diverse flavonoid content of the studied plant species, contributing to a comprehensive understanding of their potential health benefits and medicinal properties.

Table 3. Total flavonoid content

Sample	Part	Total Flavanoid Content (mg RU/g)
Phyllanthus acidus	Leaves	149.83 ± 0.5768
Diospyrus dicolour	Leaves	97.03 ± 0.3682
Flacourtia rukam	Leaves	101.03 ± 0.1688
Anacardium occidentale	Leaves	124.40 ± 0.0728

3.3 Antioxidant analysis – DPPH, ABTS, FRAP

3.3.1 DPPH

The DPPH radical scavenging assay is a commonly used technique for evaluating antioxidant activity since it is quick and efficient compared to other techniques. The assay employs 2,2-diphenyl-1-picrylhydrazyl, a stable DPPH radical, which yields a violet solution in methanol. The electron-transfer events that underpin the DPPH assay's concept entail an antioxidant's capacity to contribute either an electron or a hydrogen radical to the stable DPPH radical, which is distinguished by its deep violet colour. When free radical scavengers or antioxidant agents reduce DPPH radicals, the corresponding hydrazine, or DPPH-H form, is formed. This causes the solution's colour to change from deep violet to pale yellow [14].

The IC_{50} value was calculated to measure the samples' ability to scavenge radicals; a lower IC_{50} value denotes a higher potential for antioxidant activity. As a point of comparison, the IC_{50} value of standard ascorbic acid was found to be 67.0756 ± 22.0554 µg/mL. The results of this investigation showed that the highest IC_{50} was recorded by *Phyllanthus acidus* at 143.6205 µg/mL, followed by *Diospyrus dicolor* at 120.4454 µg/mL, *Flacourtia rukam* at 97.3910 µg/mL, and *Anacardium occidentale* at 57.9132 µg/mL.

The findings show that *Anacardium occidentale* has the highest potential for antioxidants among the plant extracts under investigation, with the lowest IC_{50} value. This highlights the potential of *Anacardium occidentale* as a natural source of antioxidants and implies that it may be especially useful in scavenging free radicals. The IC_{50} values provide us with a better knowledge of the plant extracts' relative antioxidant capabilities and offer important information on how they might be used to promote health and avoid illnesses related to oxidative stress.

Table 4. IC_{50} of plant samples

Sample	IC_{50} (µg/mL)
Phyllanthus acidus	143.6205 ± 21.4184
Diospyrus dicolour	120.4454 ± 26.3404
Flacourtia rukam	97.3910 ± 20.57187
Anacardium occidentale	57.9132 ± 15.03802

3.3.2 ABTS

The radical scavenging activity of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) is another method used to evaluate antioxidant activity. In this experiment, ascorbic acid was used as the positive control. A lower absorbance suggests that the extracts have a stronger antioxidant ability. The concentration of the sample that can scavenge 50% of the ABTS free radical in the ABTS free radical scavenging procedure is indicated by the IC_{50} value.

The obtained IC_{50} values for *Phyllanthus acidus* leaves, *Anacardium occidentale* leaves, *Diospyrus dicolour* leaves, and *Flacourtia rukam* leaves were 3.7581 µg/mL, 6.3399 µg/mL, 23.6793 µg/mL, and 36.4609 µg/mL, respectively. Among the plant extracts under study, *Phyllanthus acidus* had the lowest IC_{50}, demonstrating its strong antioxidant capacity. The potential of *Phyllanthus acidus* as a strong natural antioxidant is highlighted by the lower IC_{50} value, which indicates that it is especially effective in scavenging ABTS free radicals.

In summary, the results of the DPPH assay are supported by the ABTS radical scavenging activity, which highlights *Phyllanthus acidus*' potent antioxidant qualities. These findings provide important new understandings of the various antioxidant capacities of the plant extracts under investigation. This knowledge may be necessary for the plant extracts' future uses in health promotion and treating disorders linked to oxidative stress.

Table 5. IC_{50} of plant samples

Sample	IC_{50} (µg/mL)
Phyllanthus acidus	3.7581 ± 5.8748
Diospyrus dicolour	23.6793 ± 12.8174
Flacourtia rukam	36.4609 ± 12.6967
Anacardium occidentale	6.3399 ± 5.5149

3.3.3 FRAP

Evaluation of Fe (III) reduction is a critical measure of electron-donating activity, which is an essential process in phenolic compounds' antioxidant effect. By monitoring the reduction of Fe3+ to Fe2+, achieved by donating an electron, the reducing power assay determines the amount of antioxidants in the samples. The production of Perl's Prussian blue at 593 nm is used to quantify the resultant Fe^{2+} complex. A higher absorbance at 593 nm indicates a higher capacity for reductive action. By comparing absorbance changes at 593 nm in test reaction mixtures with those containing a variety of antioxidant combinations, FRAP (Ferric Reducing Antioxidant Power) values are obtained.

With a FRAP value of 9.9336 mM Fe^{2+}/L, *Flacourtia rukam* showed the highest value of all the extracts in this investigation. *Anacardium occidentale* had the lowest FRAP value at 6.5145 mM Fe^{2+}/L, followed by *Diospyrus dicolor* with 8.0272 mM Fe^{2+}/L and *Phyllanthus acidus* with 7.6086 mM Fe^{2+}/L. These findings suggest that the investigated plant extracts had differing degrees of reductive ability, with *Flacourtia rukam* exhibiting the best antioxidant capacity with regard to Fe (III) reduction. These results offer useful data for prospective uses in promoting health and treating illnesses related to oxidative stress. They

also provide significant insights into the various antioxidant pathways present in the plant species.

Table 6. FRAP Value

Sample	**FRAP Value (mM Fe^{2+}/L)**
Phyllanthus acidus	7.6086
Diospyrus dicolour	8.0272
Flacourtia rukam	9.9336
Anacardium occidentale	6.5145

The abundance of phenolic and flavonoid compounds in Flacourtia rukam and Phyllanthus acidus plants suggests a potential correlation with their radical scavenging activity. These plants could be considered promising sources of natural antioxidants, holding significant potential for the treatment of various life-threatening diseases.

4 Conclusion

Phytochemical screening of the selected underutilized plants from Nadir plot which were *Phyllanthus acidus*, *Diospyrus dicolour*, *Flacourtia rukam* and *Anacardium occidentale* revealed tested positive for flavonoid and phenol. Flavonoids and phenols are a major group of compounds that act as primary antioxidants or free radical scavengers. This study proved that the *Flocourtia rukam* contains a high quantity of total phenolic while *Phyllanthus acidus* contain high quantity of total flavonoid compounds. For DPPH assay *Anacardium occidentale* has the lowest IC_{50}. Therefore, it has the strongest antioxidant potential, while different results are shown in ABTS assay where *Phyllanthus acidus* has the lowest IC_{50}. In addition, FRAP assay found Flacourtia rukam shows the highest FRAP value. Utilization of these four medicinal plants will be of advantage to mankind, and an increase in their consumption will help in the prevention of chronic lifestyle diseases. The conducted phytochemical analysis and assessment of antioxidant activity enhance the significance of planting or conserving these plant species.

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