Phytochemical Screening and Total Phenolic Content in Phyllanthus Niruri and Mimosa Pudica Collected from Kuching, Sarawak (Malaysia)

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ABSTRACT

Phyllanthus niruri and Mimosa pudica are herbal plants that have been traditionally used as medicine to treat numerous ailments. This study has identified the major phytochemicals and quantified the total phenolic content (TPC) in the ethanolic extract of the plants. Plant samples were collected from several locations in Kuching, Sarawak. They were cleaned, dried, and ground prior for analysis. Phytochemical screening revealed the presence of alkaloids, saponins, protein, amino acids, tannins, and flavonoids in P. niruri extract, while only alkaloids, saponins, and flavonoids were identified on M. pudica extract. Folin–Ciocalteu method was used to determine the TPC and the results expressed as mg gallic acid equivalents (GAE) per gram of extract. From the results, P. niruri extract (59.06 mg GAE/g) exhibited slightly higher phenolic content compared to M. pudica extract (44.29 mg GAE/g). These findings emphasize the phytochemical abundance and antioxidant ability of these plants, supporting their effectiveness as medicinal plants.

Keywords--Phyllanthus niruri, Mimosa pudica, phytochemicals, total phenolic content, Sarawak medicinal plants

INTRODUCTION

Phyllanthus niruri is a small annual herb that can grows up to 30-60 cm tall with branched stem at the base and abundant oblong leaves. This plant belongs to Phyllanthaceae family and it grows naturally throughout the tropical and subtropical regions of the world including Malaysia, Indonesia, Thailand, Vietnam, and India [1]. In Malaysia, P. niruri is known as ‘dukong anak’and has been traditionally used to treat cough and kidney disorders [2]. In other regions, P. niruri has also been used in traditional medicine in the management of wide range of ailments including jaundice, diarrhea, asthma, bronchitis, and tuberculosis [3][4].

Mimosa pudica belongs to Fabaceae family and it is an annual/perennial herb that grows in trail. The plant consists of prickly stems and fern-like leaves that gets sensitive when touched. The common name of M. pudica is touch me not and it is known as ‘pokok semalu’ in Malaysia. The plant is native to Central and South America, however now it can be found in many warm regions across the world. The benefits of M. pudica as medicinal plant is well known and it has been used for wound healing, inflammation, diarrhea, urinary disorders, and many more [5]. Furthermore, recent studies revealed that M. pudica to be capable of treating psychological disorders such as depression and mental distress [6].

Sarawak is situated in northwestern Borneo and it is home to numerous indigenous communities, each sustaining unique traditions, languages, and ethnomedicinal knowledge. Although modern medicine is readily accessible nowadays, traditional medicinal practices are still persevered in some villages across Sarawak [7]. P. niruri and M. pudica are among the plants that are still being used as traditional medicine by the local communities. A wide range of bioactive compounds such as tannins, flavonoids, alkaloids, terpenes, and saponins can be found in these plants, which contribute to their pharmacological properties [8][9]. However, the quality and pharmacological activity or herbal plants are often influenced by their geographical origin, including plant variety and growth conditions [10]. Therefore, this study aims to identify the phytochemicals

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and the total phenolic content in the ethanolic extract of P. niruri and M. pudica plants collected from Kuching, Sarawak.

METHODOLOGY

Sample Preparation

Plant samples of P. niruri and M. pudica were collected in Kuching, Sarawak. The plant materials were cleaned and air-dried at room temperature for a few days. The dried specimen was then ground until it turned to a fine powder. An aqueous decoction was performed by immersing 100 g of grounded samples into 1 L of 70% ethanol in a beaker covered with parafilm for 2 to 3 days at room temperature and stirred daily. After a few days, the decoction was filtered before being concentrated under reduced pressure using a rotary evaporator. The plant extracts were stored in the refrigerator for further analysis.

Phytochemical Screening

Several tests were performed to determine the presence of alkaloids, sugar, carbohydrates, glycosides, saponins, protein, amino acids, tannins, steroids, and flavonoids.

Alkaloids: Iodine test and Wagner's test were conducted to detect the presence of alkaloids in the plant extracts. Wagner's reagent was prepared using 1.27g of iodine powder and 2g of potassium iodide solid. The solids were then dissolved in 5 ml of distilled water and slightly stirred. Distilled water was added to make up 100 ml of solution. A few ml of extract was placed in a test tube before adding a few drops of Wagner's reagent. The expected result shows in reddish-brown precipitate, indicating positive results [11]. In the iodine test, 3 ml of extract solution was poured into a test tube, then a few drops of iodine solution were added into the test tube. The mixture was then boiled in a water bath for a few minutes. The blue colour of the mixture will be decolorized upon boiling and reappear when it is cooled which indicates the presence of alkaloids [12].

Sugars and Carbohydrates: To determine the presence of sugars and carbohydrates, two tests were conducted, which are Fehling's test and Molisch's test. Approximately 1 ml of Fehling's solution was poured into a test tube, then 1 ml of plant extract was added. The mixture was boiled in a water bath for a few minutes until the formation of red residue which indicated the presence of sugar [13]. While in Molisch's test, 2 ml of filtrate was poured into a clean test tube. Two drops of alcoholic solution of alpha-naphthol were added then the mixture was shaken well. 1 ml of concentrated sulphuric acid was added by slowly sliding along the side of the test tube and allowing it to stand until violet rings appeared, which showed the presence of carbohydrates [13].

Glycosides: To identify glycosides, Modified Borntrager's test was done to detect the presence of glycosides. A ferric chloride solution was poured into the plant extract. The extract was then boiled for 5 minutes and let cooled. An equal amount of benzene was added into the same test tube until a separation layer appeared. The separated layer was added to an ammonia solution. The color change was predicted to change from rose pink to red which indicates the presence of glycosides [12].

Saponins: An emulsion formation test was done to detect the presence of saponins in the plant extract. Approximately 50 mg of extract was diluted with 20 ml of distilled water in a graduated cylinder. The suspension was shaken well for 15 minutes until a 2 cm layer of foam formed to prove the presence of saponins [11].

Protein and Amino Acids: To determine the presence of protein and amino acids, a biuret test was done to detect the presence of these compounds. An exact amount of 2 ml of plant extract was treated with 20% of $CuSO_4$ solution, then 1 ml of 95% ethanol was added. Excess potassium hydroxide pellets were also added. When the pink color appears, it shows the presence of protein [11].

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Tannins: A ferric chloride test was performed to detect the presence of tannins in the plant extract. A few ml of plant extract was poured into a test tube. A few drops of 0.1% ferric chloride were added to the test tube until the mixture turned brownish-green or blue-black, proving the presence of tannins [14].

Flavonoids: An ammonia test was done to detect the presence of flavonoids in the plant extract. Approximately 5 ml of diluted ammonia solution was added to the plant extract in a test tube. Concentrated H_2SO_4 was also added until the coloration occurs. When the yellow color disappeared, a few drops of 1% aluminum solution were added to the test tube [15].

Steroids: The Liebermann-Burchard test was used to detect the presence of steroids in the plant extract. Approximately 2 ml of acetic anhydride was added to a few ml of plant extract, and 2 ml of H_2SO_4 was also added until the color changed from violet to blue-green, which indicated the presence of steroids [16].

Total Phenolic Content

Total phenolic content in the plant extracts was determined using the Folin-Ciocalteu method [17]. In this assay, 0.4 ml of plant extract was mixed with 2 ml of 10 % Folin Ciocalteu solution and 1.6 ml of 7.5% sodium carbonate in a test tube. The mixture was kept in the dark at room temperature for 2 hours. Then, the absorbance was analyzed using a UV-Vis Spectrometer at 760nm. A calibration curve of gallic acid was prepared, and the results were expressed as mg GAE (gallic acid equivalents)/g dry extract.

RESULTS AND DISCUSSION

The qualitative phytochemical analysis for P. niruri and M. pudica was done to detect the presence of alkaloids, saponins, tannins, sugar and carbohydrates, glycosides, protein, amino acids, flavonoids, and steroids. Table 1 shows the results of the tests that were conducted.

Table 1 Phytochemical screening of P. niruri and M. pudica extract

Phytochemical Compound	Test	Plant sample	(+) positive /(-) negative
Alkaloids	Wagner's test	P. niruri	-
		M. pudica	+
	Iodine test	P. niruri	-
		M. pudica	+
Sugars and Carbohydrates	Fehling's test	P. niruri	-
		M. pudica	-
	Molisch's test	P. niruri	-
		M. pudica	-
Glycosides	Modified Borntrager's test	P. niruri	-
		M. pudica	-
Saponins	Emulsion formation test	P. niruri	+
		M. pudica	+

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Protein and Amino Acid	Biuret test	P. niruri	+
		M. pudica	-
Tannins	Ferric Chloride test	P. niruri	+
		M. pudica	-
Flavonoids	Ammonia test	P. niruri	+
		M. pudica	+
Steroids	Liebermann-Burchard test	P. niruri	-
		M. pudica	-

From the result, we can see that P. niruri has tested positive in certain tests which include the emulsion formation test to detect saponins, the Biuret test to detect protein and amino acid, and the ammonia test to detect flavonoids. Comparing it to a test conducted in Punjab, known to be the origin of this plant, they have tested that P. niruri contains saponins and flavonoids, the same as our obtained result [18]. They have also discovered the presence of alkaloids, phenols, and terpenoids available in this plant. In addition, we have discovered that P. niruri also contains protein and amino acids as the tests were shown to be positive. However, some differences appear in the presence of alkaloids in our research and the one that was conducted in Punjab. This may be caused by the different regions, or different methods used. This might be also because the concentration was too low which causes the results to appear to be negative.

For M. pudica, Wagner's test and Iodine test to detect alkaloids, emulsion formation test to detect saponins, and ammonia test to detect flavonoids had positive results. Compared to the test that was done in India in 2009, they have found even more phytochemical compounds that are present in M. pudica which include terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarin [19]. However, in our research, we could not detect the presence of glycosides and tannins. Several conditions might lead to these results, including certain errors, such as different methods. The geographic factor might also affect the phytochemicals contained in M. pudica. Besides, it may also appear in small amounts, where the test we conducted could not detect the presence of the compounds in M. pudica.

For the quantitative determination of TPC in the plant extracts, the obtained results are presented in Table 3. Based on the data, we can conclude that P.niruri has higher total phenolic content compared to M. pudica. P. niruri has about 59.06 mg GAE/g, while M. pudica has about 44.39 mg GAE/g of TPC. Higher total phenolic content in each of the plant extracts was known to have good bioactivity.

Table 2 Total Phenolic Content in P. niruri and M. pudica extract

Plant extract	Total Phenolic Content (mg GAE/g)
P. niruri	59.06
M. pudica	44.29

However, when compared to a study by Mandal et al. [20], the total phenolic content of M. pudica collected in Nepal was 418.640 mg GAE/g, which is much higher compared to our finding. In another research by Faujan et al. [21], several medicinal plants in Malaysia were analyzed for their TPC, including the shoot of Anacardium occidentale (15.86 mg GAE/g), the shoot of Carica papaya (7.17 mg GAE/g), and Etlingera elatior (9.72 mg GAE/g). For comparison, it is believed that P. niruri and M. pudica collected in Sarawak have

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quite large amounts of phenolics, which may leave many benefits towards human health, and it has also been proven that it is beneficial to cure certain types of diseases.

CONCLUSIONS

In conclusion, the phytochemical screening and total phenolic quantification of P. niruri and M. pudica of Sarawak add more information to their ethnomedicine. This study detected several bioactive compounds in both species, as expected, given the traditional knowledge that these plants help with oxidative stress and other chronic diseases. P. niruri extract showed the presence of alkaloids, saponins, proteins, amino acids, tannins, and flavonoids. Meanwhile, M. pudica extract showed alkaloids, saponins, and flavonoids. TPC analysis indicated stronger antioxidant properties in P. niruri (59.06 mg GAE/g) compared to M. pudica extract (44.29 mg GAE/g), highlighting its superior potential in addressing oxidative stress and related diseases. However, variability in results, such as the absence of certain compounds, might be due to geographical and methodological factors. These findings emphasize the role of these plants as potential low-cost sources of bioactive compounds with antioxidant functions.

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