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# Phytochemical Screening, Total Phenolic Contents and Antioxidant Activity of *Micromelum minutum* Methanol Extract (Leaves and Branches)

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*Micromelum minutum*, a medicinal plant, has been the subject of increasing research interest due to its potential therapeutic properties. Locally known as the cemumar tree, *Micromelum minutum* belongs to the Rutaceae family, which is primarily found in the tropics. *M. minutum* contains biologically active compounds with potential therapeutic applications for treating various illnesses. This study aimed to screen the phytochemical compounds present in *M. minutum* crude extract, determine the total phenolic content and analyze the antioxidant activity of the crude extracts. The leaves and branches of *M. minutum* were subjected to the cold extraction method, where both samples were macerated in methanol solvent for three days at room temperature. The research then proceeded to phytochemical screening, where the extracts were tested with various tests to identify the phytochemicals present in the crude extracts. Additionally, the total phenolic content of the samples was investigated using Folin-Ciocalteu's reagent, and the reaction was analyzed using UV-Visible spectroscopy. As for the antioxidant activity, the samples were tested to determine the percentage of 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity, which was also analyzed using UV-Visible spectroscopy. The results show that the leaves crude extract had a higher percentage yield compared to the branches, at 6.20% and 3.68%, respectively. Both the leaves and branches crude extracts exhibited the presence of most phytochemicals, including alkaloids, terpenoids, flavonoids, saponins, tannins, quinones, phenols, reducing sugars, and steroids, except for glycosides. The total phenolic content was found to be higher in the leaves crude extracts at 67.96 ± 1.87 mg GAE/g, compared to 43.17 ± 4.67 mg GAE/g in the branches crude extracts. Furthermore, the leaves crude extract demonstrated a higher percentage of DPPH scavenging activity at 65.69 ± 3.86 %, while the branches crude extract had a lower percentage of 30.75 ± 1.15 %. The present of higher phenolic compound in the leaves extract showed their potent antioxidant property and could be the rich source of natural antioxidants. This study concludes that the *M. minutum* leaves extract demonstrates natural antioxidant properties, paving the way for further investigation of its bioactive compounds in the field of pharmaceutical research.

**Keywords**: *Micromelum minutum*; phytochemical; total phenolic content; antioxidant activity; DPPH scavenging assay

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Modern medicinal treatments have been extensively developed by scientists. However, some modern medicines can have harmful side effects, such as kidney failure, and may cause allergies [1]. Additionally, modern medications are often expensive, making them inaccessible to certain populations, particularly in rural areas. As a result, resorting to medicinal herbs as remedies to treat diseases has become a popular choice, as they are generally less harmful and can be as effective as modern treatments [2].

The abundance of medicinal herbs in nature makes them readily accessible for people to use as remedies to treat diseases [3]. According to [4], one such medicinal herb, *Micromelum minutum* (locally known as cemumar), contains flavonoids, coumarins, and alkaloids that have demonstrated significant medicinal potential, including anti-carcinogenic, anti-bacterial, anticoagulant, and antioxidant properties. Many phytochemicals found in *Micromelum* species, such as coumarins, phenylpropanoic acid derivatives, polyoxygenated flavonoids, and alkaloids, have shown significant pharmaceutical properties [5]. Additionally, flavonoids and phenolics exhibit substantial medicinal properties, including anti-inflammatory and antioxidant effects [6]. According to a study by [7], the extract of *Micromelum minutum* contains a significant amount of antioxidant molecules, primarily derived from coumarins, which are derivatives of phenolic compounds.

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The leaves of *M. minutum* are rich in various phytochemicals hold potential in treating disease and valuable as antioxidant agents [8]. To further enhance the scientific understanding of *M. minutum*, this study aims to identify the phytochemical compounds in the crude extracts and analyze the total phenolic content and antioxidant properties of the locally available *M. minutum*.

## EXPERIMENTAL

### Chemicals and Materials

The leaves and branches of *Micromelum minutum* were collected from Kuala Pilah, Negeri Sembilan. The collected plant material was air-dried and sun-dried for a few days to remove moisture. The dried leaves and branches were then ground into a fine powder using a grinder to achieve a consistent powder-like texture. The accurate weight of the fine powder was measured and recorded. Finally, the powdered plant material was transferred into an airtight container for storage until further use in the study.

### Experimental Methods

*Extraction of Crude Extract*

Fine powdered ($W_2$) of leaves (118 g) and branches (124 g) of *M. minutum* were subjected to cold extraction using methanol solvent for 72 hours at room temperature. The plant extracts were then filtered and evaporated under reduced pressure using a rotary evaporator. The crude extract was subsequently transferred to a pre-weighed, airtight bottle. The crude extracts were weighed ($W_1$), and their final weights were recorded to calculate the percentage yield using Equation (1). Finally, the crude extracts were stored in a refrigerator for further analysis.

$$\% \text{ yield extraction} = \frac{W_1}{W_2} \times 100\% \quad (1)$$

*Phytochemical Analysis on Crude Extracts*

The dried crude extracts were conducted a series of phytochemical screening that included tests for alkaloids, flavonoids, phenolics, saponins, terpenoids, steroids, tannins, glycosides, and quinones following standard qualitative procedures with some modifications [9].

*Determination of Total Phenolic Content*

Total phenolic content of dried crude extracts were analyzed using the Folin-Ciocalteu's method, with a slight modification. The dried crude extracts were combined with Folin-Ciocalteu's reagent and $Na_2CO_3$ solution, then incubated for 45 minutes in a dark environment. The absorbance of the dried crude extracts was measured using a UV-Visible spectrophotometer at a wavelength of 765 nm. The calculated results were expressed in milligrams of gallic acid equivalent per gram of extract fresh weight [10,11]. The test was run in triplicates.

*Antioxidant Activity*

The dried crude extracts were further analysed for antioxidant properties by using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. DPPH radical scavenging activity of the *M. minutum* crude extracts was estimated using the method described by [12] with slight modification. Ascorbic acid was used as the positive control, and methanol was the main solvent used to dissolve all the samples. The crude extract samples were tested at eight different concentrations ranging from 7.81 µg/ml to 1000 µg/ml, and they were added to the DPPH solution and incubated under a dark ambience for 30 minutes. The absorbance of the crude extract samples was analyzed at 517 nm using a UV-Visible spectrophotometer. All tests were run in triplicates. The percentage of DPPH scavenging effect was calculated using Equation (2):

$$\% = \frac{[A_{\text{DPPH blank}} - (A_{\text{sample}} - A_{\text{blank sample}})]}{A_{\text{DDPH blank}}} \times 100\% \quad (2)$$

## RESULTS AND DISCUSSION

### Extraction of Crude Extract

The crude extract of *M. minutum* leaves and branches was obtained through maceration extraction using the polar solvent methanol. The percentage yields of the two crude extracts were calculated and presented in **Table 1**.

The crude extract yield obtained from the leaves sample was higher, at 7.32 g and 6.20% yield, compared to the branches sample, which yielded 4.56 g and 3.68%. This suggests the leaves may contain a wider variety and greater quantity of polar compounds compared to the branches [13].

### Phytochemical Screening

Preliminary phytochemical profiling reflects information regarding the diversity of different classes of secondary metabolites in plant extracts such as flavonoids, steroids, saponins, tannins, phenols and flavonoids [14]. **Table 2** showed the presence of most phytochemicals of *M. minutum* crude extracts such as alkaloids, terpenoids, flavonoids, saponins, tannins, quinones, phenols, and steroids, except glycosides.

**Table 1.** The percentage yield of leaves and branches crude extract.

| Crude extract | Weight of the fine powder (g) | Weight of the crude extract (g) | Percentage yield (%) |
|---|---|---|---|
| Leaves | 118 | 7.32 | 6.20 |
| Branches | 124 | 4.56 | 3.68 |

**Table 2.** Phytochemical analysis of *M. minutum* crude extracts.

| Phytochemicals test | Leaves crude extract | Branches crude extract |
|---|---|---|
| Alkaloids | + | + |
| Terpenoids | + | + |
| Flavonoids | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Quinones | + | + |
| Phenols | + | + |
| Steroids | + | + |
| Glycosides | - | - |

(+) : presence (-) : absence

According to [15], there are several main phytochemical compounds that played significant roles especially in medical and pharmaceutical field include alkaloids and phenolics. This study revealed that *M. minutum* crude extracts contain most of the main phytochemical compounds such as flavonoids and phenolics that showed their potent antioxidant property and could be the rich source of natural antioxidants.

**Total Phenolic Content**

The total phenolic content of these extracts from *M. minutum* were determined using the Folin-Ciocalteu assay by constructing a standard curve with gallic acid taking into consideration the relationship between absorbance (765 nm) and concentration (1000 ug/mL) of the extract. The calibration curve generated from the analysis of the standard (gallic acid) was linear with y = 0.0091 x - 0.0278; $R_2$ = 0.997 (**Figure 1**).

Based on the equation obtained from calibration curve, the leaves extract showed the highest phenolic content (67.96 ± 1.87 mg GAE) followed by branches extract (43.17 ± 4.67 mg GAE) (**Table 3**).

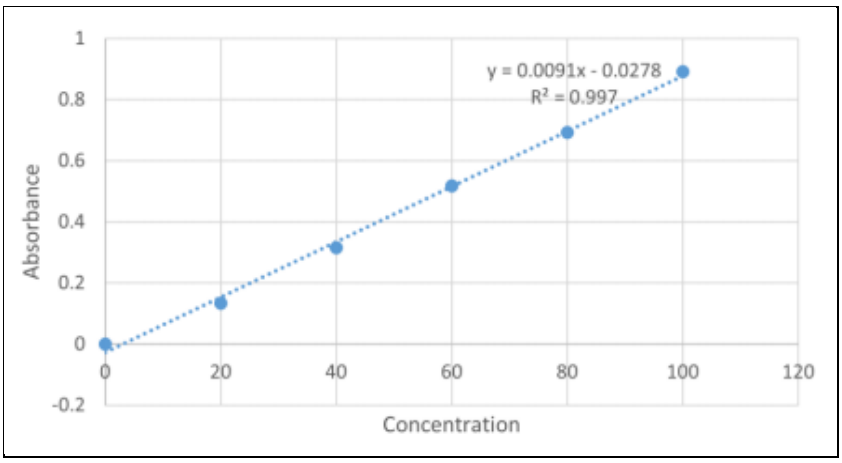


**Figure 1.** Gallic acid standard calibration curve for the quantification of total phenolic content.

**Table 3.** The total phenolic content of leaves and branches *M. minutum* crude extract.

| Type of crude extract | TPC mg GAE/g of extract |
|---|---|
| Leaves | 67.96 ± 1.87 |
| Branches | 43.17 ± 4.67 |

**Table 4.** The percentage inhibition of DPPH radical scavenging activity.

| Type of Sample | Percentage Inhibition (%) |
|---|---|
| Ascorbic acid (Positive control) | 96.59 ± 0.67 |
| *M. minutum* leaves crude extract | 65.69 ± 3.86 |
| *M. minutum* branches crude extract | 30.75 ± 1.15 |

From the results above, it is identified that leaves crude extract has higher content of phenol compared to branches. This is because most phenolic compounds are abundantly found in leaves compared to any other parts of plant as it is one of the crucial compound used in photosynthesis [16]. In addition, a study conducted by [17] indicates that leaves contained higher amount of phenol compounds in order to protect them from photo oxidative damage and environment stresses.

**Antioxidant Assay**

DPPH radical scavenging assay was used to determine the antioxidant activities of *M. minutum* leaves and branches crude extract. It shows that the leaves crude extract had 65.69 ± 3.86 % of DPPH scavenging activity and branches crude extract has 30.75 ± 1.15 % of DPPH scavenging activity. Meanwhile the standard, ascorbic acid possesses the highest radical scavenging activity, 96.59 ± 0.67 %. **Table 4** shows the percentage inhibition of DPPH radical scavenging activity of the samples.

*M. minutum* leaves crude leaves show significant finding between total phenolic content and the antioxidant activity. The present of higher total phenolic content in the leaves extract contribute to the higher antioxidant properties. Other than that, there are several factors can be identified which may affect the value of antioxidant. The factors are including the locality of the plant and heat. According to a study conducted by [18], the locality of a plant where it grows affect the number of secondary metabolites produced in plant where the production of effective compounds is affected due to the humidity changes and temperature of the locality of the herbal plant. In addition, the amount of phenol in the plant sample is also correlate to the antioxidant activity [19].

## CONCLUSION

This study discovers that the leaves *M. minutum* crude extract has higher percentage yield compared to branches crude extracts which is 6.20% and 3.68%, respectively. It showed that leaves crude extracts may contain more compounds as compared to branches. As for phytochemical screening, almost all phytochemicals were found in both crude extracts, except for glycosides. The total phenolic content of leaves is 67.96 ± 1.87 mg GAE/g while the total phenolic content for branches crude extract is 43.17 ± 4.67 mg GAE/g. As for the antioxidant activity, the value of percentage inhibition of antioxidant activity of leaves crude extract is higher which is 65.69 ± 3.86 % while the percentage inhibition of antioxidant activity for branches crude extract is 30.75 ± 1.15 %. The present of higher phenolic compound showed their potent antioxidant property and could be the rich source of natural antioxidants. As conclusion, this study discovered that *M. minutum* leaves extract could be assigned as natural antioxidant and its open door for the bioactive compound identification in the field of pharmaceutical research.

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