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Phytochemical Screening and Free Radical Scavenging Activity of Different Extraction Solvent Polarity of *Euphorbia hirta* L.

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Abstract

Euphorbia hirta is one of the alternative or traditional medicines which are now being used more often to treat a wide range of diseases such as intestinal parasites, diarrhoea, peptic ulcers and asthma by using decoction or infusion. The objective of this study is to evaluate the efficiency of polar and non-polar solvent effects on the determination of total flavonoid content and free radical scavenging activity and test the phytochemical properties (phenolic and flavonoid) in *Euphorbia hirta* and DPPH free radical scavenging activities of flavonoids extracted from different solvents of extraction. Extraction with higher-polarity solvents resulted in substantially higher yields, with ethanol achieving 22.46% compared to only 3.37% for ethyl acetate. Phytochemical screening of ethanol extracts showed the presence of phenolics and flavonoid compounds, but only the phenolic compound showed in the ethyl acetate extract. The value of total flavonoid content is 34.3556 ± 19.0346 mg of quercetin/g extract for ethanol extract, which has higher total flavonoid content compared to ethyl acetate extract, which is 7.6340 ± 1.9363 mg of quercetin/g extract. Ethanol extract demonstrated greater scavenging activity based on IC_{50} values, which it is 0.520281 ± 3.182 µg/ml compare to ethyl acetate extract which has a 43.6095 ± 32.1432 µg/ml IC_{50} value. The study suggests that *E.hirta* has high total flavonoid content and low IC_{50} value, and ethanol extract is a great solvent to use for further development of the plant's products or illness cure.

1. Introduction

Herbal medicine is a method that uses herbs, herbal materials, herbal preparations, and complete herbal products that have plant parts, other plant materials, or mixtures as active components. This definition comes from the

World Health Organisation (WHO). The plant components used to make these herbs include leaves, stems, flowers, roots, and seeds [1][2].

Among those who practice traditional medicine, *Euphorbia hirta* is a very well-liked herb. It is frequently used as a decoction or infusion to treat a variety of conditions, such as intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, and kidney stones. Additionally, the plant is used as an antiseptic to treat wounds, sores, and conjunctivitis, as well as diseases of the skin and mucous membranes such as warts, scabies, tinea, thrush, aphthae, fungal infections, measles, and Guinea worm [3][4]. According to [5], *Euphorbia hirta* has been discovered to have properties of anxiolytic, analgesic, larvicidal, anti-cancer, anti-pyretic, antioxidant, anthelmintic, anti-microbial, and anti-inflammatory.

Numerous studies have been conducted on both modern and traditional method with comparing between the polar solvent [6][7][8]. However, the comparison between polar and non-polar solvents has not been completely studied. Thus, in this study, it is important to know about the efficiency of polar and non-polar solvent effects on the determination of total flavonoid content and free radical scavenging activity. The purpose of the current study was to test the free scavenging activities of flavonoids extracted from different solvents of extraction and the phytochemical properties (phenolic and flavonoid) in *Euphorbia hirta*. The results of this study may increase the total worth of the herb's therapeutic potential.

Alternative medicines, especially *E. hirta* are now being used more often to treat a wide range of diseases. Various research has been proven that plant-based medicines not only have the ability to successfully cure and treat illnesses but also have less side effects and negative impacts than modern medicines. There are several natural bioactive compounds found in plants, such as phenolics, flavonoids, and their derivatives, that are abundant. These substances have drawn interest due to their physiological and bioactive properties, which include antioxidant, anti-allergic, anti-inflammatory, antibacterial, and antidiabetic properties [8].

2. Materials and Methods

2.1 Plant Materials and Preparation

The fresh *Euphorbia hirta* plants were collected from various areas in Universiti Tun Hussein Onn Malaysia. The plants' identity was compared and confirmed by the morphology by the Malaysia Biodiversity Information System (MyBIS). 3kg of fresh plant samples are washed with distilled water to remove dirt and debris and rinsed to remove excess water. The leaves are separated from the plant, and the fresh leaves samples are weighed, and the initial weight is recorded [9]. The fresh leaves are spread on a drying tray; furthermore, the leaf samples are placed inside the hot air oven (Labec) and dried for 120 hours at 30°C to avoid damage of phytochemicals [10]. The moisture content of the leaves is measured, and the weight of the leaves is recorded after 120 hours. The leaves are taken out and ground using a blender. Next, the dried samples are placed in a ziplock bag or an airtight container. The sample is kept in a desiccator at the proper level and used for extraction.

2.2 Sample Extraction

The plant sample was divided into two conical flasks, which are 50g in each conical flask. The powder was extracted by the maceration method [8] with 250ml of ethanol and ethyl acetate for 5 days at room temperature with frequent agitation by using IKA incubator shaker KS 4000 i control. The mixture was filtered with Whatman No. 1 filter paper to remove the impurities and crude. The filtrate was concentrated with EYELA rotary evaporator with vacuum at 40 ℃, which were kept in tiny bottles in the refrigerator at 5 °C [11].

2.3 Determination of Percentage Yield of Ethanol and Ethyl Extract

The following formula was used to determine the yield percentages of the weighted extract yields:

$$Extract\ yield\% = \frac{R}{S} \times 100 \quad (1)$$

where R referred to the weight of extracted plant residues, and S is the weight of the plant raw sample [12].

2.4 Phytochemical Screening

The procedure of phytochemical screening was taken to screen for the existence of phenol and flavonoid compounds in different polarities of *E.hirta* extraction.

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2.4.1 Phenol

The phytochemical screening of the extraction method was carried out in research [13]. The phenol test was carried out by using the ferric chloride ($FeCl_3$) test. By taking 1ml from both the ethanol and ethyl acetate extract to the test tube which were diluted, 1ml of 10% of ferric chloride solution was added to both diluted extract solutions. The presence of phenol was seen as a blue-black colour.

2.4.2 Flavonoid

By using quercetin as a reference, the total flavonoid content was determined by the aluminium chloride method. 1ml of test sample or quercetin and 4 ml of water were added to each test tube. Then, 0.3 ml of 5 % Sodium nitrite and 0.3 ml of 10% of aluminium chloride were added after 5 minutes. After incubation of 6 minutes at room temperature, 1ml of 1M sodium hydroxide was added to the reaction mixture. The distilled water was used to reach the final volume of 10ml. The absorbance of the sample was used to test against the blank at 510 nm by using a spectrophotometer. The entire experiment was done in triplicate for accuracy, and the data were represented in mean ± standard deviation in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight [14].

2.5 Determination of Total Flavonoid Content

By using quercetin as a reference, the total flavonoid content was determined by the aluminium chloride method. 1 ml of test sample or quercetin and 4 ml of water was added to each test tube. Then, 0.3 ml of 5 % Sodium nitrite and 0.3 ml of 10% of aluminium chloride were added after 5 minutes. After incubation of 6 minutes at room temperature, 1ml of 1M sodium hydroxide was added to the reaction mixture. The distilled water was used to reach the final volume to 10ml. The absorbance of sample was used to test against the blank at 510 nm by using a spectrophotometer. The entire experiment was done in triplicate for accuracy, and the data were represented in mean ± standard deviation in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight [14]. The total content of flavonoids in plant extracts was calculated by the following formula:

$$T = C_1 x \frac{V}{M} \quad (2)$$

Where, T = total flavonoid content mg/g-1 of extracts in quercetin equivalent/extracts, C1 = the concentration of quercetin solution established from the calibration curve mg mL-1, V = the volume of extract solution in the form of millilitres, and M = the weight of the pure plant extract in grams [6].

2.6 2,2-Diphenyl-1-Picrylhydrazyl Radical (DPPH) Free Radical Scavenging Activity

The standard methodology was modified in order to evaluate the plant extracts potential to scavenge DPPH free radicals. From the beginning of the steps, 1.8 mL of a 0.004% methanolic solution of DPPH was added to fractional amount of both ethanol and ethyl acetate extracts and standard, which is 200ml, of various concentrations with a range of 30.125 µg/ml to 1000 µg/ml. The absorbance was recorded against a blank at 517 nm with a spectrophotometer after incubation for 30 minutes in darkness at room temperature, which is about 23 ◦ C. The control was the absorption of a blank sample that contained the same quantity of ethanol and ethyl acetate. Quercetin was used as a standard for the experiment. Samples were examined three times [15]. The graph of concentration versus percentage inhibition was used to compute the 50% inhibitory concentrations (IC_{50} values) of the extracts. The percentage of inhibition is used to represent free radical scavenging activity. The sample concentration needed to scavenge 50% of a DPPH free radical is known as the IC_{50} value. The measurements were carried out three times. The IC_{50} values of the extracts denote the concentration at which they have a 50% chance of scavenging free radicals. The IC_{50} of the extract and standards were produced in a visual representation or graph. The following formula was used to compute the percentage of inhibition:

$$1\% = \frac{AC - A0}{AC} \times 100\% \quad (3)$$

where AC referred to the absorbance of the control (200 µL extracts/quercetin) + 1800 µL DPPH solution), AO referred to the absorbance of the sample solution, and 1% defined as the percentage of inhibition [15].
The IC_{50} values of the extract are used to quantify its capability to scavenge radicals. The results were shown as mean values ± standard deviation (n = 3) [16].

2.7 Statistical Analysis

The experiments were conducted in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical analysis was performed using an independent t-test to compare differences between the two groups. A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Percentage Yield

The result for the percentage yield of ethanol extract of *Euphorbia hirta* for 120 hours of maceration is 22.46%, while for the ethyl acetate extract resulted in the lowest percentage yield, which is 3.37% (Table 1).

Table 1 *Percentage yield of ethanol and ethyl acetate extract of E. hirta*

Samples extract	Percentage yield (%)
Ethanol	22.46
Ethyl acetate	3.37

Among the solvents used in the study, the ethanol extraction method showed a higher percentage of yield compared to the ethyl acetate extract. It is because at 20 °C, the evaporation of this material will take a while before a dangerous pollution of the air is attained [17]. This was affecting the percentage yield of the sample extracts in this study. The data presented here are relevant to work demonstrating that ethanol extracts of *E. hirta* yield higher amounts of both phenolics and flavonoids, and also demonstrate greater antioxidant activities than those extracted with ethyl acetate or n-hexane. Together, these data support ethanol as the more efficient solvent both in yield and bioactive recovery [18].

3.2 Phytochemical Screening

Phytochemical screening tests for both ethanol and ethyl acetate extracts of *Euphorbia hirta* were recorded in the table below. The qualitative screening of phytochemical compounds of *E. hirta* showed the presence of phenol and flavonoid in both ethanol and ethyl acetate extracts but there is no flavonoid compound shown in ethyl acetate extract.

Table 2 *Phytochemical screening test for ethanol and ethyl acetate extracts from E.hirta*

Phytochemical compound	Ethanol extract	Ethyl acetate extract
Phenolic	+	+
Flavonoid	+	-

+ shows presence of compound; - shows absence of compound

The qualitative screening of the phytochemical compounds of ethanol and ethyl acetate extracts in *E.hirta* showed the presence of phenol, but only ethanol extracts showed flavonoid compounds. However, based on the research by Karki et al. [19], the ethyl acetate extract of *E. hirta* plants contains flavonoids. Other studies also report flavonoids in *E. hirta* ethyl acetate extracts and in generic leaf or aerial extracts [20][21]. This discrepancy suggests that the absence of flavonoid signal in the revision's ethyl acetate extract is likely methodological (e.g., extraction conditions or sensitivity) rather than a true lack of flavonoids.

3.3 Total Flavonoid Content

The total flavonoid content of the extracts was quantified, where the standard curve is $y = 0.001x + 0.0423$ with $R^2 = 0.9969$. The results indicate that the linear correlation in the detection ranges was satisfactory in Table 3. The value of total flavonoid content is 34.3556 ± 19.0346 mg of quercetin/g extract for ethanol extract, which has higher total flavonoid content compared to ethyl acetate extract, which is 7.6340 ± 1.9363 mg of quercetin/g extract. Another *E. hirta* study also found ethanol to give higher TFC than ethyl acetate and n-hexane, with ethanol extracts containing 28.507 ± 0.464 mg QE/g and showing the highest phenolic and flavonoid levels among solvents. Ethyl acetate extracts of related species sometimes dominate in flavonoids, but in this *E. hirta* dataset, ethanol is consistently superior, supporting the revision's conclusion about ethanol as the better solvent for flavonoids [18]. From the study of [22], the result showed that more polar components were extracted using polar solvents with high reducing and antioxidant properties. Although the extracts in nonpolar solvents were abundant in phenolic acids and flavonoids, it was discovered that they lacked antioxidant and reducing activities.

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Table 3 *Total flavonoid contents in E.hirta extracts. The value is the mean ± standard deviation of mean*

Samples extract	Total flavonoid content (mg QE/g extract)
Ethanol	34.3556 ± 19.0346
Ethyl acetate	7.6340 ± 1.9363

3.4 DPPH Free Radical Scavenging Activity Assay of *E. hirta* Extracts

According to Table 4, it may be assumed that ethanol extract demonstrated greater scavenging activity based on IC_{50} values, which is 0.520281 ± 3.182 µg/ml, compared to ethyl acetate extract, which has 43.6095 ± 32.1432 µg/ml IC_{50} value.

Table 4 *IC_{50} values of DPPH free radical scavenging activity of extracts*

Samples extract	IC_{50} (µg/ml)
Ethanol	0.520281 ± 3.182
Ethyl acetate	43.6095 ± 32.1432

An antioxidant assay based on electron transfer, the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical approach generates a violet solution in methanol. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, is reduced, producing a colourless ethanol solution [23]. The reduction in DPPH radicals' absorbance at 517 nm brought on by antioxidants was used to gauge their capacity for reduction. The abstraction of a hydrogen atom from the antioxidant molecule caused DPPH to turn light yellow. The amount of DPPH decrease will increase with the number of antioxidants present in the extract. A sample's strong scavenging activity is correlated with a high decrease of DPPH [11].

In this research, the scavenging abilities of the ethanol extract of *E. hirta* are higher compared to the ethyl acetate extract, where the IC_{50} value of the ethanol extract is lower value. This result explained that ethanol extracts contain many phenolic and flavonoid compounds [8]. IC_{50} is the most popular and useful way to assess a drug's effectiveness, where it is measured by its half-maximal inhibitory concentration. It provides a measurement of an antagonist medication's efficacy in pharmacological research by indicating the amount of drug required to block a biological process by 50%. The majority of methods for calculating a pharmacological compound's IC50 are based on tests that make use of whole cell systems. Results can vary depending on the experimental cell line utilised and may not distinguish a compound's capacity to block certain interactions, even though they often offer excellent potency information [23].

Another study found that ethanol extracts from *E. hirta* had the lowest IC50 valuations for DPPH and ABTS in a solvent comparison study and therefore exhibited the highest antioxidant capacity when compared to ethyl acetate or n-hexane [18]. Studies on mixed species, including *E. hirta,* also report strong DPPH scavenging for *E. hirta* extracts, with ethyl acetate and methanol extracts reaching up to 92% inhibition at high concentration, and IC50 values around 700 g/L for *E. hirta* ethyl acetate, confirming that polar solvents and higher flavonoid content correlate with better radical scavenging [24].

4. Conclusion

The phytochemical and pharmacological of *Euphorbia hirta* Linn. are among its many potentialities. *E. hirta* Linn. has been utilised as a significant medicinal, therapeutic, and nutritional source for many serious ailments in many regions of the world. *E. hirta* ethanol extract has shown high total flavonoid content and low IC_{50} value, and it is a great solvent to use as further development of the plant's products or for illness cure. Moreover, *E. hirta* is a highly prevalent plant which might be used as an extra income for the rural areas.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Teng Ming Qing, Furzani Pa'ee;* ***data collection:*** *Teng Ming Qing;* ***analysis and interpretation of results:*** *Teng Ming Qing, Furzani Pa'ee;* ***draft manuscript preparation:*** *Teng Ming Qing, Mohamad Haziq Norsam, Kori Muhammad Alkali, Ahmad Arif Ismail,Furzani Pa'ee. All authors reviewed the results and approved the final version of the manuscript.*

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