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Antioxidant and Total Phenolic Content of *Catharanthus roseus* Using Deep Eutectic Solvent

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Antioxidant and Total Phenolic Content of *Catharanthus roseus* Using Deep Eutectic Solvent

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Abstract
Deep eutectic solvents as a new type of eco-friendly solvents have attracted attention in chemistry, medicine, and other fields for the extraction and separation of target compounds from medicinal plants. Deep eutectic solvents are easy to prepare and have many advantages as solvents, such as chemical inertness with water, low cost, easy biodegradability, and pharmaceutically acceptable toxicity. In this study, a deep eutectic solvent made up of choline chloride-glycerol (1:2) was used for the extraction of flavonoids from *Catharanthus roseus* plant parts (flower petal, leaves, stem, and root). The highest amount of phenolic content was detected in flower petal, that is, 194.50 mg GAE/g. In DPPH test, the maximum amount of antioxidant activity determined in the flower petal was 73.13%; IC_{50} was calculated by using a linear regression equation; IC_{50} value of the standard, stem, root, leaf, and flower petal was 13.22, 90.44, 83.93, 120.14, 79.49 μg/ml, respectively. The result of this research is that *Catharanthus roseus* has a compatible antioxidant activity. This can be helpful for the treatment of diseases caused by free-radical oxidative stress.

Keywords: *Catharanthus roseus*; Deep eutectic solvent; Antioxidant activity; Total phenolic content.

1. INTRODUCTION

Plants are the essential foundation of medicine [1]. Medicinal plants are the main source of bioactive compounds. In the field of nutrition and food science, bioactive compounds have extra health benefits that promote and maintain consumers' health and prevent chronic illnesses, which are the main topics of discussion nowadays. For the recovery of bioactive compounds from medicinal plants, solid-liquid extraction is used in numerous biochemical, chemical, and pharmaceutical industries. Medicinal plants usually have a minor quantity of active compounds; however, most of the time, the development of a high-performance process justifies its extraordinary value. The effective extraction of bioactive compounds from medicinal plants with great purity and without any loss of activity has resulted in the development of the new extraction process [2].

The deep eutectic solvent (DES) is a new kind of green solvents. DESs have some renowned properties, such as low vapor pressure, high viscosity, easy biodegradability, and high thermal stability. DESs that could be considered as a subcategory of ionic liquids (ILs) are also of great interest. This type of mixture has a lower melting point and potentially lower viscosity than either of the pure components. The most popular ion precursor for DESs is choline chloride (ChCl). This is because ChCl is nontoxic; readily available, thus cheap; and biodegradable. Complexing agents include metal salts, hydrated metal salts, and hydrogen bond donors such as a hydroxide (OH) group. However, hydrogen bond donors are of the most interest since many of them are inexpensive, nontoxic, nonflammable, biodegradable, and versatile—for example, glycerol. Thus, DESs are potentially much cheaper than typical ILs. The DES is a new kind of green solvent and has some renowned properties, such as low vapor pressure, high viscosity, easy biodegradability, and high thermal stability [3, 4].

According to Kaur and Mondal in 2014 [5], most medicinal plants contain a large variety of natural antioxidants, namely, phenols, tannins, and flavonoids, than dietary plants. *Catharanthus roseus* contains significant amounts of phenolic and volatile compounds, including flavonol glycosides and caffeoylquinic acids, which are known for antioxidant activity. It has an important role in the body defense system in acting as antioxidants against reactive oxygen species (ROS), which are unsafe, by forming such goods through normal cell aerobic respiration. The accrual of free radicals can be a reason of pathological conditions such as asthma, ischemia, inflammation, arthritis, neurodegeneration, mongolism, Parkinson's disease, quickening the aging process, and perhaps dementia.

Nowadays, interests have increased in DESs based on choline chloride and their effective use in the extraction and separation of compounds from medicinal plants. Flavonoids are successful compounds being extracted and separated by using DESs from medicinal plants, and DES extraction provides more efficient extraction than other solvents [6, 7].

2. MATERIALS AND METHODS

2.1. Chemicals

These are some chemicals used for analysis in this research. Ethanol was made by a UK company named Fisher Scientific, whereas the rest of the chemicals such as choline chloride, glycerol, DPPH, the Folin-Ciocalteu reagent were produced by Sigma-Aldrich, USA.

2.2. Deep Eutectic Solvent Preparation

Deep eutectic solvents were easily prepared by the heating method. In deep eutectic solvents, hydrogen bond donors mix with hydrogen bond acceptors in a 250 ml reaction flask at 80°C under vigorous agitation for 60 min. After that a homogeneous liquid formed [8]. So in this research choline chloride and glycerol (1:2) were mixed in a 250 ml reaction flask at 80°C for 60 min on a magnetic stirrer.

2.3. Extraction of Plant

Dried plant parts' (root, stem, leaves, and flower petal) powder of *Catharanthus roseus* was used for the solvent extraction procedure. A 10 g plant powder sample was added into a 250 ml flask with 150 ml of a deep eutectic solvent solution. The flask was placed onto a magnetic stirrer at 50°C for 40 min. The solution was then filtered with the help of filter paper. Furthermore, the filtered solution was concentrated using rotary evaporator and then the concentrated extract was collected and kept in a refrigerator at 4°C until the time of the experiment.

2.4. DPPH Assay for Antioxidant Activity

The antioxidant activity of *Catharanthus roseus* plant parts' extracts was assessed by the DPPH assay. The DPPH assay assesses the ability of antioxidants to scavenge free radicals. Antioxidant activity was expressed in percentage inhibition. The following steps were followed to determine antioxidant activity.

The stock solution of standard and all extracts were prepared in ethanol to achieve a concentration of 1 mg/ml. Furthermore, ten different concentrations were prepared from a stock solution (20, 40, 60, 80, 100, 120, 140, 160, 180, 200 μg/ml) in ethanol.

The DPPH assay was performed according to Guerrini in 2016 [9] with minor modification; 2 ml of sample solution from each concentration was transferred to a separate test tube. Then 2 ml of DPPH solution was added. Same steps were followed for a standard solution. The control consisted of ethanol and DPPH in the same volumes without extract. Furthermore, the sample, standard, and control were kept at room temperature in the dark for 30 min. After half an hour, the solution was measured using UV spectrophotometer at 517 nm against blank. The values for the standard and each sample set were taken triplicate, and the average values were calculated. These average values were applied to the following equation to determine the percentage inhibition of standard and sample.

$$\% \text{ inhibition} = (A_{control} - A_{sample})*100/A_{control}$$

where

$A_{control}$ = (Ethanol + DPPH) solution

A_{sample} = (Sample + Ethanol + DPPH) solution

After the calculation of percentage inhibition, IC_{50} (concentration providing 50% inhibition) of standard and sample values were calculated by using linear regression equation between the concentration and percentage inhibition.

2.5. Determination of Total Phenolic Content

The Folin-Ciocalteu reagent was used to assess the total phenolic content of extracts using gallic acid as standard. The total phenolic content test was conducted as described by Genwali in 2013 [10]. The stock solution of standard and all extracts were prepared in ethanol to achieve the concentration of 1 mg/ml. Furthermore, ten different concentrations were prepared from a stock solution (20, 40, 60, 80, 100, 120, 140, 160, 180, 200 μg/ml) in ethanol. One milliliter of each concentration was taken in a test tube, and 5 ml of 10% Folin-Ciocalteu reagent and 4 ml of 7% Na_2CO_3 have been added to make an entire quantity of 10 ml. The combination was mixed well and kept in the dark for 30 min and then the absorbance was measured at 765 nm against blank solution. The experiments have been carried out in triplicate. The average absorbance values of standard were used to plot the calibration curve. The total phenolic content of *Catharanthus roseus* plant parts' extracts was expressed as mg GAE per gram.

2.6. Statistical Analysis

The tests were carried out in three replicates; data were presented as mean ± S.E. Microsoft Excel 2010 was used for statistical and graphical evaluations. Linear correlation coefficient and correlation analyses were done between the concentration of standards and absorbance of the standard that was performed using Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1. DPPH Assay for Antioxidant Activity

The DPPH (2, 2- diphenyl-1-picrylhydrazyl) assay has the ability of an antioxidant that gives an electron or hydrogen radical to DPPH radical, that is, deep violet color with stable free radical. Due to the presence of free radical scavenger an odd electron

gets combined with an antioxidant agent, DPPH radicals get concentrated to corresponding hydrazine, DPPH-H form, and the sample solution changes from deep violet to light yellow color. Absorbance was measured through UV spectrophotometer.

Results indicated that *Catharanthus roseus* root, stem, leaves, and flower petal contained antioxidant activity. The percentage inhibition of *Catharanthus roseus* plant parts, flower petal, root, stem, and leaves, were detected as 73.13 68.64, 64.87, and 61.16%, respectively, and flower petal showed highest antioxidant activity. It is concluded that *Catharanthus roseus* plant has antioxidant activity and would be an amazing source of beneficial antioxidants.

IC_{50} is calculated by using linear regression equation. By using linear regression equation, the IC_{50} value of a standard is 13.22 μg/ml. Low values show higher antioxidant activity, so 13.22 μg/ml IC_{50} value of standard shows that the standard ascorbic acid has higher antioxidant activity. In *Catharanthus roseus* plant parts, the IC_{50} value of root is 83.93 μg/ml, and IC_{50} value of stem is 90.44 μg/ml, whereas leaves have IC_{50} 120.14 μg/ml and flower petal 79.49 μg/ml, which indicated that flower petal has more antioxidant activity than root and then little bit low in stem and lowest antioxidant activity in leaves. The results were compared with the traditional extraction method such as soxhlet extraction and simple maceration using organic solvents. The methanolic extract of leaves of *Catharanthus roseus* showed 78% percentage inhibition at 200 μg/ml, which is higher than DES [11]. The ethyl acetate fraction of *Catharanthus roseus* shoots were found to exhibit the highest antioxidant activity [12]. Antioxidant activity using DESs can be enhanced by improving the drying stage of the extract. Table 1 shows the IC_{50} values of the standard and samples (root, stem, leaves, and flower petal).

3.2. Total Phenolic Content Analysis

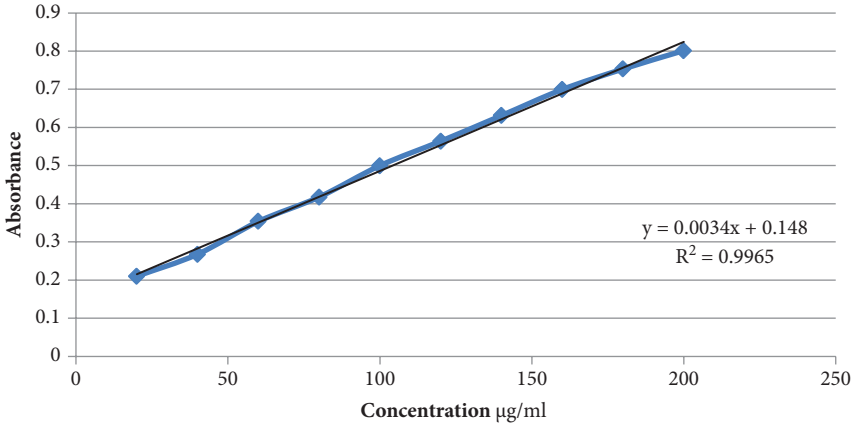
The Folin-Ciocalteu (F-C) method was used to determine the total phenolic content of extracts by using gallic acid as the standard. The calibration curve was constructed by using the absorbance values of standard gallic acid and ten different concentrations of standard dilution. These values were taken in triplicate, and average absorbance value was used for the construction of calibration curve against the different concentrations of the standard. The total phenolic content of the *Catharanthus roseus* plant root, stem, leaves, and flower petal was calculated by using the regression equation of calibration curve ($Y = 0.0034x + 0.148$) of standard gallic acid and stated as mg gallic acid equivalents (GAE) per gram.

Therefore, in the *Catharanthus roseus* plant parts, flower petal showed the highest total phenolic content, then root and stem, and leaves have the lowest total phenolic content. The amount of phenolic content was detected in flower petal, root, stem, and leaves as 194.50, 194.03, 188.46, and 171.13 mg GAE/g, respectively. The results of this research suggest that *Catharanthus roseus* has a compatible antioxidant activity. This can be helpful for the treatment of diseases caused by free-radical oxidative stress. Figure 1 shows the Calibration curve of total phenolic content of standard gallic acid.

Table 1: IC_{50} values of standard and *Catharanthus roseus* plant parts.

Standard & plant component	IC_{50} Value (μg/ml)
Standard	13.22
Stem	90.44
Roots	83.93
Leaves	120.14
Flower petal	79.49

Figure 1: Calibration curve of total phenolic content of standard gallic acid.



4. CONCLUSION

In this study, DPPH and total phenolic content was used to identify the antioxidant activity of *Catharanthus roseus* plant parts. In this study, flower petal showed the highest percentage inhibition and then root and stem, whereas the lowest percentage inhibition has been observed in leaves in DPPH assay test. Therefore, the total phenolic content in *Catharanthus roseus* was also determined in DES extracts. Hence, in the *Catharanthus roseus* plant parts, flower petal showed highest total phenolic content, then root and stem, and leaves have the lowest total phenolic content. The result of this research is that *Catharanthus roseus* has a compatible antioxidant activity. This can be helpful for the treatment of diseases caused by free-radical oxidative stress.

4.1. Future Work

This study has used choline-chloride and glycerol as DESs for extraction, so, a highly recommended future direction would be to use other DESs such as choline-chloride with ethylene glycol and choline chloride with urea and then compare them with hazardous chemicals such as ethanol and methanol for the extraction from *Catharanthus roseus*. Another future direction would be to research on other parts of *Catharanthus roseus* such as seeds and hairy roots using DES extraction, as they are part of *Catharanthus roseus* plant that also may have compounds that can be used for human health.

Author Contributions

All authors contributed equally to this study.

Source of Funding

None.

Conflict of Interest

None.

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