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Deep Eutectic Solvent for extraction of natural antioxidant from a medicinal plant, *Coleus aromaticus*

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Abstract: Antioxidant is a basic requirement for maintaining good health. In this research, natural antioxidant was extracted from *C. aromaticus* by using different types of synthesized deep eutectic solvents (DES). The extracts were analysed using total phenolic content assay and DPPH radical scavenging assay. Finally, HPLC and FTIR methods were employed to check the present of target compounds. From the result obtained, the best DES was ChCl : glycerol with a ratio of 1:4 bearing 97.25 µg/ml of total phenolic content. The bioactive compounds that were successfully identified through HPLC and FTIR assays were Rosmarinic acid, Caffeic acid, Gallic acid and Quercetin. This shows DES is a potential green solvent to extract bioactive compounds present in medicinal plants.

1. Introduction

In this modern era, exposures to free radicals have been gradually increasing due to unhealthy lifestyle and also to other sources such as pollution, smoking and radiation. Free radicals are defined as unpaired electrons that scavenge the body in order to search for other electrons. Exposing to free radicals will fasten the aging process and cause many diseases. This causes several damages to the cells, DNA and proteins in the body and leads to other diseases such as cancer, Parkinson's disease and others [1]. Antioxidants are used to protect the cells and tissues against the toxic effects of the free radicals, prevent other diseases and neutralize the high exposure of free radicals in the body [2]. In fact, an antioxidant is produced naturally in human body, albeit in a very little quantity. Hence, extra antioxidant is required: to be taken up from a diet or a supplement.

Due to the side effects of synthetic antioxidants, many researchers have started exploring the advantages and uses of medicinal plants which have said to be containing natural compounds that can help to improve human health. Due to the toxicity and carcinogenic effects of synthetic antioxidants, the demand of natural antioxidants is becoming higher from time to time. The extraction of antioxidants from herbs such as *C. aromaticus* (*Lemuju*) is essential in order to obtain more natural antioxidants as humans require a lot of antioxidants to fight against free radicals. Plants contain strong antioxidant activity which can trap free radicals by inhibiting the oxidation that causes these harmful particles [3].

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Conventional solvents such as chloroform, methanol, and ionic liquid are used in the extraction of *C. aromaticus*, however these solvents possess many drawbacks such as toxicity and carcinogenic to humans. Because of that health concerns, a safer and a more environmental-friendly green solvent – Deep Eutectic Solvent (DES) is used in this work to extract antioxidant from *C. aromaticus*. DES is tailor made; an ability to design desired kinds of DES by changing the molar ratio of hydrogen bond donor and acceptor. DES has some advantages for instance more biodegradable, cheaper and easier to prepare than that of other solvents; made from renewable compounds [4].

2. Methods

2.1. Preparation of Deep Eutectic Solvents (DES)

The different molar ratio of ChCl to hydrogen bond donor (HBD) as shown in table 1 was mixed in a conical flask at 80°C and mechanically stirred at 250rpm for 2 hours until a homogenous colorless solution was formed [5].

Table 1. Molar ratio of hydrogen bond acceptor to hydrogen bond donor.

No.	ChCl : HBD Molar Ratio	Hydrogen Bond Donor (HBD)
1	1:2	Glycerol
2	1:3	Glycerol
3	1:4	Glycerol
4	2:1	D-glucose
5	1.5:1	D-glucose
6	1:1	D-glucose

2.2. Preparation of Plant Material

Fresh leaves of *C. aromaticus* were collected, thoroughly washed with water and rinsed with distilled water. Then, these leaves were dried in an oven at 60°C for 24 hours [6]. After that, the dried leaves were ground into powder form with the use of a blender grinder.

2.3. Preparation of Leaf Extracts

1g of powdered leaf sample and 10ml of DES were added into a conical flask. The mixture was boiled at 70°C for 1 hour with magnetic agitation at 250rpm [7]. After the extraction was completed, it was vacuum filtered in order to separate the liquid extract and solid residue.

2.4. Determination of Total Phenolic Content (TPC)

Folin-Ciocalteu assay was used for determination of TPC [8].

2.5. Fourier Transform Infrared Spectroscopy (FTIR) Method

For the preparation of liquid samples, one drop of the sample was placed between two plates of sodium chloride and a thin film was formed between the plates. That thin film was then put on the sample chamber and the spectra data were recorded at 3600 – 600 cm⁻¹ with using Perkin Elmer FTIR spectrometer [9].

2.6. 1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) Radical Scavenging Assay

Radical scavenging analysis was carried out using DPPH [10]. All the steps were carried out under dark condition. The color change of the mixture formed was recorded. Then, absorbance was measured at 515nm with the help of UV-Vis spectrophotometer. In this study, gallic acid (commercial antioxidant) was used as a reference standard compound. Finally, percent DPPH scavenging effect was calculated by using the following equation 1:

$$\text{DPPH scavenging effect (\%)} = \frac{A_o - A_i}{A_o} \times 100\% \quad (1)$$

where A_o = Absorbance of control reaction

A_i = Absorbance in presence of test or standard sample [11].

3. Results

3.1. Screening of the best DES for antioxidant extraction

Result for screening process is shown in table 2. Among them, it showed that glycerol-based DES was a suitable solvent for the extraction of antioxidant. Nevertheless, d-glucose-based DES was not very appropriate for this extraction due to the high viscosity of its physical properties.

Table 2. Result for screening of DES

No	ChCl : HBD Molar Ratio	Hydrogen Bond Donor (HBD)	Total Phenolic Content ($\mu\text{g/ml}$)
1	1:2	Glycerol	56.6 $\mu\text{g/ml}$
2	1:3	Glycerol	71.25 $\mu\text{g/ml}$
3	1:4	Glycerol	97.25 $\mu\text{g/ml}$
4	2:1	D-glucose	13.75 $\mu\text{g/ml}$
5	1.5:1	D-glucose	13.25 $\mu\text{g/ml}$
6	1:1	D-glucose	No result
			Too viscous, cannot be filtered

3.2. DPPH Radical Scavenging Assay

DPPH radical scavenging assay is a test to check the reducing ability of the antioxidant presented in our sample. When antioxidant reacts with DPPH, it will become DPPH-H which is lighter in color. Hence, more de-colorization means more reducing ability of our sample which also indicates higher antioxidant capability to scavenge free radicals [12].

The radical scavenging percentage was shown in table 3 by using equation 1. It could be concluded that the free radical scavenging percentage of sample extract was higher than that of gallic acid, which means this plant had a very high research potential for treating diseases.

Table 3. Result of DPPH radical scavenging assay.

	Radical scavenging percentage
Sample	$\frac{0.898 - 0.198}{0.898} \times 100\% = 77.95\%$
Positive control (gallic acid)	$\frac{0.898 - 0.500}{0.898} \times 100\% = 44.32\%$

3.3 High Performance Liquid Chromatography Assay

The components of *C. aromaticus* extracts were determined by using HPLC analysis. By comparing the peak and retention time between the extracts and the standard, the components presented were identified. From Figure 1, it could be seen that there were many peaks presented, which means that *C. aromaticus* is a very potential plant that can be used as a daily supplement. The figure illustrated that rosmarinic acid showed the highest content followed by caffeic acid, quercetin and gallic acid. The result obtained is almost similar to that as in the journal stated by [13]. However, in the research, gallic acid was absent in the leaf extract of *C. aromaticus* but occurred in the stem extract part. While HPLC

results obtained had shown that gallic acid presented in a very little quantity in the extract of *C. aromaticus*.

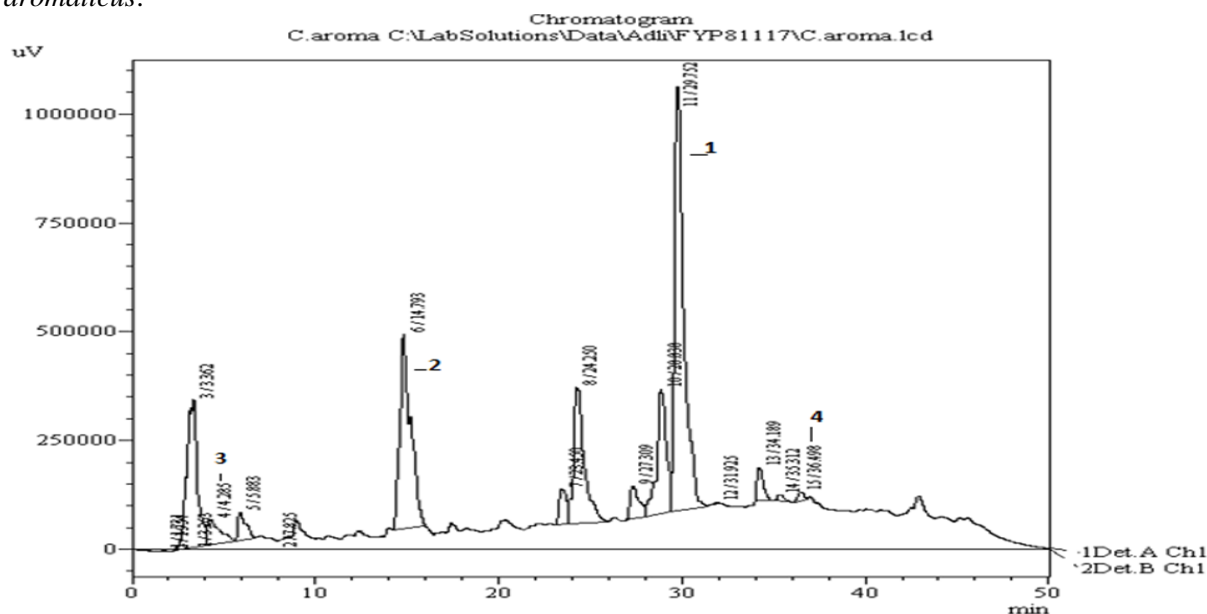


Figure 1. Chromatogram of *C. aromaticus* extract, 1. Rosmarinic acid, 2. Caffeic acid, 3. Gallic acid, 4. Quercetin.

3.4. Fourier Transform Infrared Spectroscopy Assay

From the infrared spectrum obtained, it demonstrated that there were many peaks existed inside the sample extract from *C. aromaticus*. These peaks showed the present of functional groups of the bioactive compounds inside the extract. By comparing the positions of the infrared bands obtained with the standard functional group infrared absorption, the functional groups present could be identified.

From the result obtained, the functional groups that were available inside the sample extract were alcohol/phenol OH stretch, carboxylic acid OH stretch, alkenyl C-H stretch, alkenyl C=H stretch and ester C=O stretch.

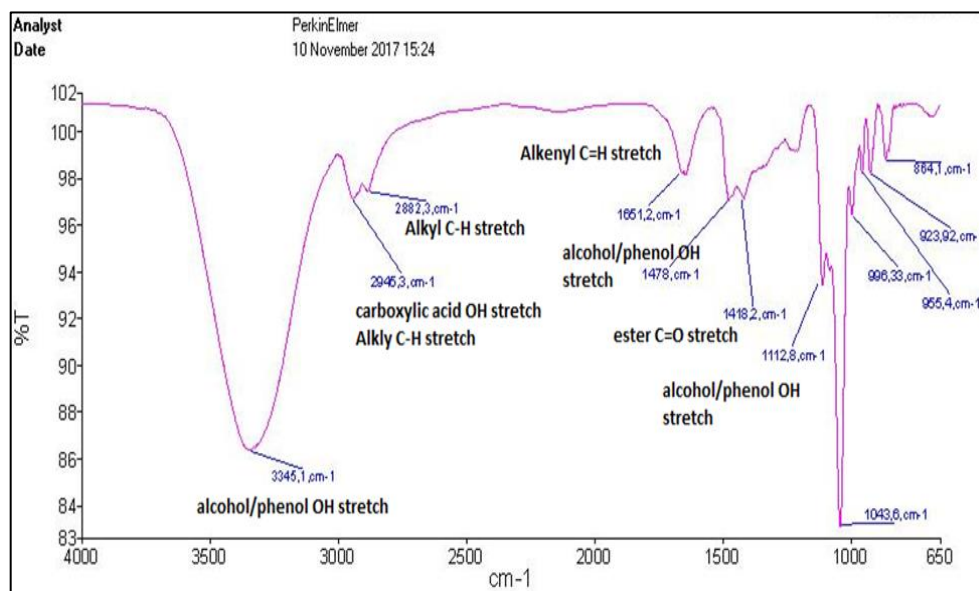


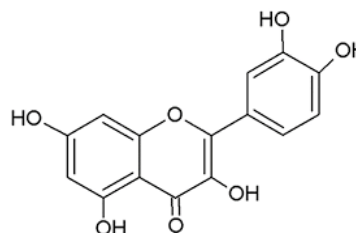
Figure 2. Infrared spectrum of *C. aromaticus* extracts

By comparing the functional groups existed with the structure of rosmarinic acid, caffeic acid, gallic acid and quercetin as illustrated in the table 4 below, it could be confirmed that these bioactive compounds were present inside the sample extract.

Table 4. Structure and functional groups of target bioactive compounds.

Name	Functional group present	Structure
Rosmarinic acid	OH stretch COOH stretch CH stretch C-C stretch	
Caffeic acid	OH stretch COOH stretch CH stretch C-C stretch	
Gallic acid	OH stretch COOH stretch C-C stretch	

Quercetin

OH stretch
COOH stretch
C-C stretch

4. Conclusion

The best DES had also been successfully identified by comparing the total phenolic content extracted from the medicinal plant, *Coleus aromaticus* at fix condition (70°C, 1 hour, 1.10 g/ml solid to solvent ratio). Among them, DES with 1:4 Choline Chloride to Glycerol was the best with extracting the highest total phenolic content which was 97.25 µg/ml. The free radical scavenging ability of the plant extract was higher than that of gallic acid, which was a very good phenomenon to show the potency of *C. aromaticus* in medicinal field. Furthermore, with comparing the chromatogram of extract with chromatogram of different standard running at the same conditions and comparing the position of infrared bands obtained with standard functional group infrared absorption. It could be concluded that rosmarinic acid contributed the most for antioxidant property of *C. aromaticus* followed by caffeic acid, quercetin and gallic acid. This study demonstrated the suitability of ChCl : Glycerol as a new and green solvent for extraction of antioxidant from *C. aromaticus* leaves.

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