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The Bioactivity Potential of *Acmella paniculata* Plant Extract in Antioxidant Activity by Two Different Extraction Methods

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ABSTRACT

Widely used synthetic chemicals like heavy metals act as active compounds in personal care products such as shampoo, soap and cosmetics. These hazardous chemicals cause harmful threats to the environment and unintentionally lead to dangerous exposure towards human health. Thus, demands on natural organic compounds have increased nowadays. Bioactive compounds from plant extracts are well known to have intrinsic biological values that are relevant in promoting human health. There are numerous ways to extract these unique and important compounds involving techniques that are simple, environmentally friendly and efficient. The aim of this study is to highlight the biological potential of compounds from the local plant, Acmella paniculata in antioxidant activity. This small flowering shrub species is also known locally as Subang nenek. The first phase of this study involved the leaf extraction of A. paniculata performed by a common method known as Soxhlet extraction using methanol solvent and a simple centrifuged method using deep eutectic solvent (DES) which is a green solvent. Soxhlet method is a conventional procedure that takes longer time and involves specific equipments. However, this method has been used for many years as standard method due to its consistent results. Meanwhile, DES based centrifugation method is a new, innovative method for plant extraction with a fast procedure and minimal used of equipment. The second phase involved phytochemical screening and antioxidant activity testing. The phytochemical screening for A. paniculata leaf extract showed noticeable

secondary metabolites such as alkaloids, tannins, terpenoids and steroids. This plant showed good pharmacological values in antioxidant activity using DPPH free radical scavenging assay. The antioxidant percentage of DES extract showed higher potential results (56.11±3.54) compared to the Soxhlet extract (39.15±23.99) in leaf. Overall, the DES extraction is promising method in increasing the yield of antioxidant compounds as well as provided a simple and rapid technique in plant extraction.

Keywords: Acmella paniculata; Soxhlet Method; DES; Antioxidant; Leaf Extract

INTRODUCTION

Products for health care commonly contain active synthetic chemicals which can be a harmful threat to the environment and human health. The accumulation of these chemicals in soil and water will eventually lead to acid rain and the contamination of multiple land areas and water sources [1]. The toxic chemicals found in cosmetic products are usually formaldehyde, parabens, triclosan and benzalkonium chloride [2]. These chemicals can even be toxic in a small amount of consumption.

Acmella paniculata (A. paniculata) also known as 'Subang nenek' is a small shrub plant that grows naturally in damp areas such as nearby lakes, ponds and river banks. Genus *Acmella* is widely distributed in tropical and subtropical regions of the world including Malaysia, Indonesia and India. *A. paniculata* is locally used for toothaches, mouth ulcer, leukorrhea and other health concerns. This is because it has anti-cancer, anti-inflammatory, antioxidant and antimicrobial activities [3].

Different parts of *A. paniculata* have shown various pharmacological activities. Secondary metabolites like phenols, flavonoids, alkaloids, tannin, terpenoids and steroids contribute to the various bioactivities [4]. The leaf extract of *A. paniculata* known to potentially exhibit antimicrobial, antimalarial and antioxidant bioactivities [5]. *A. paniculata* consists of main contributors like spilanthol and acmellonate that decrease toothaches, stimulate saliva secretion and create an anaesthetic effect [6]. The flower, leaf and roots have active metabolites making it a highly potential medicinal

plant with important phytochemical constituents appropriate for human health.

This study involved two types of extraction methods. Soxhlet extraction is a conventional method that originated in 1879 by Franz von Soxhlet [7, 8]. Soxhlet method is a continuous hot extraction achieved by heating a selected solvent from the round bottom flask, which evaporates through the condenser. The evaporated solvent will then drip into the plant material and permeating through it. This extracting activity will help in the bioactive compounds that encounter with the solvent. The plant material is typically held in a porous container within a siphonable chamber [9]. Soxhlet extraction is the most favourable conventional extraction technique and is still used as a standard comparison for current new extraction techniques. Nevertheless, the solvents employed in these conventional extraction methods are known to be hazardous for the environment and human health such as the alcohol group that know to be flammable, volatile and toxic at high concentration [9]. Hence, the demand for novel technologies with less to no usage of organic solvents has grown. This is because it offers certain advantages over the existing conventional extraction procedures that use excessive organic solvents [10]. Deep eutectic solvent (DES) is a new class of green electrolyte materials with properties analogous to that of ionic liquids [11]. DES is an environmentally friendly alternative for plant extraction. DES had gained attention as a promising option for the extraction and separation of different desired compounds from natural sources such as plants [12]. The method used is a new and straightforward process that involved steps of soaking, centrifugation, filtration and re-centrifugation.

Both Soxhlet and DES extraction can be used for a wide range of compounds, including organic and inorganic substances. Most of these compounds (phenolics, flavonoids and terpenoids) can be extracted using both methods [13]. Both methods extract specifically from highly bioactive plants. However, the DES method is particularly a natural and organic approached that is considered safer. The bioactivities of leaf extract from the *A. paniculata* were identified based on the two types of extraction. The *A. paniculata* phytochemical composition and antioxidant activity was analysed using qualitative chemical tests and DPPH assay respectively.

METHODOLOGY

Plant identification of *A. paniculata*

The living specimen of *A. paniculata* was collected and cultivated in the Flora Garden of the School of Biological Sciences, Universiti Sains Malaysia (USM). It was then authenticated by a taxonomist at the Herbarium of the School of Biological Sciences, USM. The voucher specimen (USMP 11924) was kept at USM for proper preservation purposes.

Preparation of *A. paniculata*

The leaves of *A. paniculata* were selected and separated for analysis. The leaves were washed and air-dried. The leaf part was then dried in an oven for 24 h below 40 °C. The part was ground into fine powder and stored in labelled bottles wrapped with black paper. Silica bead gel packets were placed in the bottles to absorb moisture. It was kept at room temperature until further use [14].

Extraction of *A. paniculata* using Soxhlet method

This method was done accordingly with some modifications [15]. Powdered *A. paniculata* leaf (10 g) was placed in a Soxhlet apparatus. The extraction was carried out using 200 ml of methanol for 5 h. Methanol was separated from the mixture using a rotary evaporator. The crude leaf extract was obtained. The extraction was performed in triplicate and the yield was expressed as mean ± SD. The crude extracts were diluted with 5 % dimethyl sulfoxide (DMSO) at a concentration of 30 %, 40 % and 50 % (v/v) prepared for antioxidant testing.

Extraction of *A. paniculata* using DES-centrifuged method

This method was done accordingly with slightly modifications [16]. As much as 0.2 g of powdered *A. paniculata* was mixed with 10 ml of DES (CA/Gly) at different concentrations (30 %, 40 %, 50 % (v/v)). The DES was diluted using deionized water. The samples were vortexed then soaked. Next, it was centrifuged at 5,000 rpm for 5 min. The samples were filtered using gravity filtration where the powdered leaf *A. paniculata* was

discarded and the liquid filtrate was obtained. It was centrifuged again. The supernatants were obtained in triplicates where the yield will be expressed as mean ± SD.

Phytochemical screenings

The phytochemical constituents were screened leaf plant extracts from both Soxhlet and DES extraction methods.

i. Detection of tannins

The tannin detection was conducted with some modifications. A volume of 0.1 ml of extract solution was mixed with 2 ml of 2 % $FeCl_3$ solution in a test tube. The formation of the black colour showed the presence of tannins [4].

ii. Detection of steroids

An amount of 1 ml plant extract was mixed with 2 ml of chloroform. The H_2SO_4 solution was added carefully. The reddish-brown colour produced indicated the presence of steroid molecules in the extract [9].

iii. Detection of terpenoids

The terpenoid detection was conducted with some modifications. An amount of 0.2 ml plant extract solution was mixed with 2 or 3 ml of pure chloroform solution. Then 3 ml of concentrated H_2SO_4 solution had been added and gently mixed. The formation of dark red or brown colour showed the presence of terpenoids [9].

iv. Detection of alkaloids

The Wagner's test is conducted for alkaloid detection with some modifications. A total of 1 ml of the plant extract was added and shaken with 1 ml of potassium iodide (Wagner's reagent) in a test tube. The formation of a reddish-brown precipitate indicated the presence of alkaloids in the extract [9].

Antioxidant test

The free radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) with slightly modifications [17]. A total of 3 ml

of 0.1 mM of methanolic DPPH solution was added into 1 ml of the plant extracts. The mixture was shaken vigorously and left for 30 min at room temperature in the dark. The absorbance value was measured at 517 nm of wavelength. Free radical scavenging activity (RSA) was expressed as inhibition percentage and was calculated using Eq. (1):

$$Antioxidant\ Activity\ (\%) = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \times 100 \quad (1)$$

The DPPH assay method was based on the reduction of methanolic DPPH solution. The presence of the hydrogen donating antioxidant (dark blue colour) was converted to the non-radical form of (yellow colour) diphenyl-picryl hydrazine. The extractions were performed in triplicates and the yield was expressed as mean ± SD.

RESULTS AND DISCUSSION

Phytochemical compounds of *Acmella paniculata* leaf extract

Phytochemical screening is a preliminary test conducted to detect the presence of non-nutrient bioactive compounds found in various type of plants. Due to their antioxidant qualities, these substances lessen the risk of oxidative damages brought on by free radicals formed during normal metabolism [18]. In this present study, phytochemical compounds were found in the leaf extract of *A. paniculata* using both extraction methods. The compounds of *A. paniculata* are listed in Table 1.

Table 1: Phytochemical compounds of *Acmella paniculata* obtained from leaf extract using Soxhlet and DES-centrifuged methods.

Acmella paniculata extract (Soxhlet method)	
Tannin	Present
Alkaloid	Present
Terpenoid	Present
Steroid	Absent
Acmella paniculata extract (DES method)	
Tannin	Absent
Alkaloid	Absent
Terpenoid	Present
Steroid	Present

The Soxhlet method for methanol leaf extract showed the positive response for tannins, alkaloids and terpenoids, however, only steroids were found negative in the extract. Methanol is extremely polar and so it is able extract polar compounds in higher yields [19, 20]. The absence of steroid may be due to it being degraded as the process involves high temperature during extraction [21].

In contrast, the leaf extract from DES method showed the presence of steroids and terpenoids while tannin and alkaloids were absent. Steroids were present maybe due to the stability of room temperature during soaking. Steroids are part of fat groups therefore it tends to dissolve in non-polar solvents. Hence, steroids prefer to be extracted in polar and semi-polar solvents [22, 23]. Steroids are present by using this method because of DES have multiple interactions such as hydrogen bonding, dipole-dipole, dipole-induced dipole, Van der Waals (dispersion) and ion-dipole interactions that allow extraction of polar and non-polar components [24]. Next, terpenoid compounds also can be attracted to semipolar or even polar solvents as it possesses a hydroxyl group attached to the hydrocarbon chain [23]. The results found that both steroids and terpenoids are present in leaf extract of *A. paniculata* but not at an extremely high concentration. As for absence of tannin and alkaloids, the efficiency and characterization of DES to attract these compounds are still being investigated as the DES used as the extraction method is still relatively new.

Generally, the extraction of solute and the solvents are affected through polarity, chemical structure, extraction concentration, vapour pressure and acid-base properties [25]. DES present remarkable flexibility as the alternations of DES characteristics such as the molar ratio of the individual components, temperature and water content can affect the physico-chemical properties (fluidity, conductivity and polarity) [26, 27]. DES comprises of a mixture between citric acid and glycerol which is more natural, safe and environmentally friendly. DES is highly adaptable as the individual components involved can be ionic or non-ionic, polar or non-polar. Thus, the versatility of DES can dissolve a wider range of compounds compared to organic solvents [28].

Antioxidant activity of *Acmella paniculata* leaf extract

Antioxidant activity translates to the capability of compounds to neutralize and eliminate free radicals and reactive oxygen species (ROS). ROS can be both hazardous and advantageous in biological systems depending on the concentration. At lower concentrations, these reactive species lead to positive effects in physiological processes such as cellular signalling pathways and immune function. While higher concentrations cause oxidative stress through cell, protein, and DNA damage. Therefore, the balance of these reactive species is crucial. Antioxidants are free radical scavengers that donate electrons to ROS preventing oxidative damaging of cells [29]. Multiple antioxidant plant secondary metabolites like polyphenols and flavonoids are essential for plant acclimatisation and environmental adaption as well as human health when ingested through fruits and vegetables. The balance between the production and scavenging of reactive oxygen species (ROS) will be affected by any environmental challenges, such as pollution, nutrient deficiency, temperature and water changes. The availability of ROS essential for normal plant growth and signal transduction. Therefore, keeping the oxidative equilibrium is essential for plant stress adaptation [30].

The antioxidant activity of *A. paniculata* leaf extract obtained from both Soxhlet and DES-centrifuged methods is as presented in Table 2 accordingly. The Figure 1 shows the linear graph of comparison antioxidant activity of extract based on different concentrations and methods.

Table 2: Antioxidant activity of *A. paniculata* leaf extract by Soxhlet and DES-centrifuged methods

Acmella paniculata leaf DPPH assay		
Concentration	RSA (%)	
	Soxhlet method	DES method
30%	21.20±27.99	30.86±17.30
40%	26.97±19.55	54.48±4.27
50%	39.15±23.99	52.29±3.54

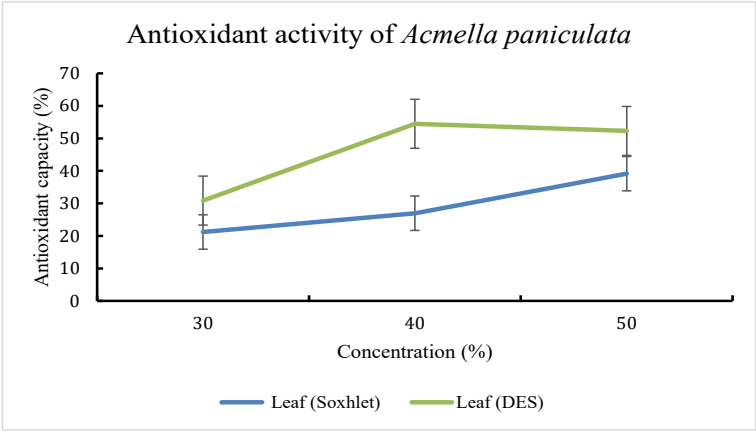

Figure 1: Antioxidant activity of *A. paniculata* leaf extracts obtained from Soxhlet and DES-centrifuged methods

DES leaf extract showed a slightly higher antioxidant capacity with a percentage of radical scavenger values ranging from 30-50 % compared to Soxhlet extracts which range from 20-40 %. The antioxidants activity of *A. paniculata* might be due to the high presence of valuable bioactive compounds such as phenolics, flavonoids, alkaloids, tannins and steroids as found in the previous phytochemical test [31]. Overall, DES indicates a higher antioxidant activity compared to Soxhlet method for the leaf extract.

Comparison of Soxhlet and DES extraction method

Soxhlet extraction can be work with a wide range of solvents effectively extracting various types of compounds, producing a higher and valuable extraction product [32]. However, it uses a complex apparatus

setup. It also requires a large volume of solvent consumption for continuous reflux through the sample. In this study, an absolute methanol was used as the solvent extractor which can be toxic. Moreover, Soxhlet method is a time-consuming extraction process as it takes approximately 6 to 8 h of extraction and involved multiple extraction cycles [33].

In contradict, the DES extraction method only takes 2 to 4 h of extraction and uses DES as the solvent extractor. Overall, it uses a simple apparatus setup which is a centrifuging machine. DES as an extraction solvent that is multifunctional and safe with a green affect compared to DMSO and methanol. An amount of 5 % DMSO was selected as an extract dilutor after the separation process between plant extract and methanol. It can be classified as harmful if the concentration rises above 10 % [34]. However, the negative potential of DMSO was eliminated as it is safe to the cells at 5 %. At the side of DES, it is mostly having high viscosity which commonly required dilution [35]. The DES in this study used only water as the dilutor.

The benefits of DES as a solvent extractor

The DES extraction method may be more favourable as DES itself provides a more versatile, flexible and environmentally friendly outcome [36, 37]. The extraction process requires less extraction time [21]. This method also only required a simple centrifuge machine. DES is a new class of solvent that have emerged as a green alternative for compounds extraction. DES is typically made from renewable resources, such as sugars and amino acids. It is also biodegradable that its typical structure can be degrade or digest by bacteria and other microorganisms. In this study, DES is formed by mixing two compounds of citric acid and glycerol. When these compounds are mixed by forming a eutectic mixture, it has a melting point that is lower than the melting point of any of the individual compounds, thus exhibit stability within the structure. DES has a number of advantages over conventional solvents as it is non-toxic, biodegradable, and non-flammable. DES is also able to dissolve a wide range of compounds, including organic and inorganic substances making it the suitable option than conventional solvent [28].

CONCLUSION

The study had demonstrated that leaf extract from *A. paniculata* leaf exhibit the presence of bioactive compounds such as steroid, terpenoids and alkaloids which may be responsible for the detected activity of antioxidant. The DES-centrifuged method has proven to be highly potential and possesses a simple and rapid extraction for plant. The used of DES itself as the solvent extractor also making the overall extraction process safe for the environment in contrary with the used of methanol in Soxhlet method.

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