Lichen *Dirinaria Applanata* Microwave Assisted Extraction Using Deep Eutectic Solvents and Binary Solvents

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Abstract. Ionic liquids and volatile organic solvents are both intended to be replaced with deep eutectic solvents (DES) in industrial and laboratory procedures. Low cost, simple manufacturing, biodegradability, strong solubilization strength, and low toxicity are a few advantages of using DES. One of the most crucial areas in green chemistry is the emergence of DES. Lichens, on the other hand, produce Secondary Metabolites with distinctive properties (SM). These SM exhibit a range of biological actions, such as anti-tumor, anti-inflammatory, and antioxidant qualities. This study focuses on the Microwave Assisted Extraction (MAE) of the SM from the lichen *Dirinaria applanata* utilizing a Binary Solvent (BS) of acetone and ChCl:Urea and DES constructed of chloroform and urea. 1,4-butanediol. The advantage of MAE is faster extraction times and less solvent usage. At 100 W, the MAE was run for 60, 120, 180, 240, and 300 s. The separation of the numerous Secondary Metabolites (SM) from the lichen *Dirinaria applanata* was observed using thin layer chromatography (TLC). The greatest yield (%) of MAE employing ChCl:Urea after 240 s was 22.95%. The ChCl:1,4-butanediol extraction yield (%) when employing a binary solvent (BS) is comparable to that of acetone-based normal soaking extraction (NSE). Using BS and DES as solvents in MAE offers a more affordable option than the standard soaking extraction method that uses acetone. To identify and describe the secondary metabolites obtained from the lichen *Dirinaria applanata*, more investigation must be conducted.

Keywords: Deep Eutectic Solvent · Ionic liquid · Lichen · Green chemistry · binary solvent

1 Introduction

The quantitative and qualitative analysis of active compounds from plants usually relies on the choice of extraction method [1]. The first step of any medicinal plant study is the extraction of bioactive compounds. The result and outcome of the study are dependent on the extraction [2]. Factors such as the matrix properties, solvent, temperature, and time, influence the extraction processes [3].

The emergence of DES is one of the most important areas in green chemistry. DES is composed of hydrogen bond donors (HBDs) and acceptors (HBAs), and the hydrogen

S. Hadisaputra et al. (Eds.): NiTRIC 2022, AHCPS 4, pp. 13–19, 2023.
https://doi.org/10.2991/978-94-6463-130-2_3

bonding interaction is believed to play an important role in forming eutectic systems [4]. A DES system is a mixture of two components, a hydrogen bond donor (HBD), such as choline chloride, which is the most used, and a hydrogen bond acceptor (HBA), such as urea or oxalic acid [5]. The important features of a DES system are non-flammability, adjustable viscosity, and very low volatility [6]. These properties give DES their ability to extract a wide range of compounds, both polar and nonpolar, and have been suggested as alternatives to conventional volatile organic compounds and ionic liquids [7].

Lichens are interesting organisms due to their stable nature; they are also self-supporting symbionts involving algae and fungi or cyanobacteria. They can survive even under very harsh environments, such as under direct sunlight and high altitude, because they can produce unique characteristic secondary metabolites. These active compounds, called secondary metabolites, have various biological activities such as antiviral, anti-tumor, anti-inflammatory, and antioxidant activities [8].

MAE is simply the use of microwave energy to heat the solid sample along with the solvents to split the compounds of interest into the solvent. MAE effectively reduces both the operational time and the solvents used when compared to conventional extraction methods [9].

The need to reduce or limit the use of toxic volatile organic solvents cannot be over-emphasized. This research is particularly focused on utilizing the environmentally benign nature of DES to extract the secondary metabolites of lichen *Dirinaria applanata* and to study the effect of DES on extraction efficiency.

2 Materials and Methods

A. *Chemicals and Materials*

A focused monomode domestic microwave apparatus was used for the microwave-assisted extractions. The sample was irradiated with a microwave in a closed system using a domestic microwave with programmable heating power and irradiation time.

Chemicals used are Choline chloride, ChCl (Sigma-Aldrich Chemicals), 1,4 butanediol (Acros Organics), Urea (R & M Chemicals), and Acetone (Bendosen Laboratory Chemicals).

B. *Sample Collection*

Dirinaria applanata was first collected at University Kebangsaan Malaysia (UKM), Malaysia. The sample was cleaned and shredded into smaller parts prior to drying. After cleaning and identification, the sample was then air-dried for 24 h and then ground into smaller particles with a size of approximately 0.86 ± 0.20 mm. The selected sieved ground samples were weighed before extraction [10].

C. *Preparation of Deep Eutectic Solvents*

The DES used were prepared by mixing choline chloride, ChCl (98% purity) with 1,4-butanediol (99% purity) at a molar ratio of 1:5 and ChCl (98% purity) with urea

Table 1. Processing Parameters for MAE

Parameter	Value
Solvent to solid ratio (DES extraction)	5 mL to 0.1 g
Sample size	approx. 0.2 mm
Microwave power	100 W
Irradiation time	60 s, 120 s, 180 s, 240 s, 300 s
DES to acetone ratio (BS extraction)	1:9, 2:8, 3:7, 6:4, 5:5 in mL

(99% purity) at a molar ratio of 1:2 according to Bi et al. 2013 [7]. The mixture was continuously stirred at 80 ^{0}C until a homogenous mixture was obtained. The solution was then kept in a Scott bottle once cooled down.

D. *Preparation of Binary Solvents*

The BS system is made of acetone as an organic solvent with DES made up of ChCl:1,4-butanediol. The BS systems were prepared at a ratio of 1:9, 2:8, 3:7, 4:6, 5:5 DES to acetone ratio. The mixtures were stirred to homogenize the two solvents.

E. *Normal Solvent Extraction*

This extraction process was conducted at room temperature using acetone as the organic solvent. The extraction process was carried out by soaking 0.2 g of dried *Dirinaria applanta* in 10 ml of acetone for 14 h. The liquid crude extract was then filtered and collected after 14 h (optimum) prior to chromatographic analysis.

F. *Microwave-Assisted Extraction*

The microwave-assisted extraction was carried out using a monomode domestic microwave apparatus. Lichen *Dirinaria applanata* was mixed with DES, and then the mixtures were subjected to microwave heating. After the microwave irradiation, the obtained extracts were filtered to remove the lichen residue, and the filtrate was allowed to cool down to remove the temperature. The organic solvent was then allowed to evaporate in a fume hood, after which the extracts were weighed, and the yield (%) recorded (Table 1).

$$\text{Yield}\,(\%) = \frac{mass\ of\ the\ extract}{mass\ of\ lichen} \times 100\% \tag{1}$$

G. *Liquid-Liquid Extraction*

Due to the low volatility of DES, it is difficult to get the crude extract after MAE. Liquid-liquid extraction using organic solvents was used to separate the SM from the

Table 2. Yield (%) of Extraction for MAE

Sample	Mass of extract	Yield (%)
NSE with acetone	39.7 mg	19.85%
MAE after 300 s (ChCl:Urea)	43.3 mg	21.65%
MAE after 240 s (ChCl:Urea)	45.9 mg	22.95%
MAE after 180 s (ChCl:Urea)	29.8 mg	14.9%
MAE after 120 s (ChCl:Urea)	28 mg	14%
MAE after 60 s (ChCl:Urea)	26.4 mg	13.2%
MAE after 120 s (BS at 9:1)	42 mg	21%
MAE after 120 s (BS at 8:2)	39.5 mg	19.75%
MAE after 120 s (BS at 7:3)	39.6 mg	19.80%
MAE after 120 s (BS at 6:4)	39.33 mg	19.67%
MAE after 120 s (BS at 5:5)	39.2 mg	19.6%

DES/H_20 layer. Two layers were formed consisting of organic solvent with the SM and DES/H_20 with the lichen residue. The organic solvent layer was removed and allowed to evaporate under a fume hood. The organic solvents used are Ethyl acetate for ChCl:Urea and Chloroform for ChCl:1,4-butanediol. The yield (%) for extraction was then recorded.

H. *Thin Layer Chromatography*

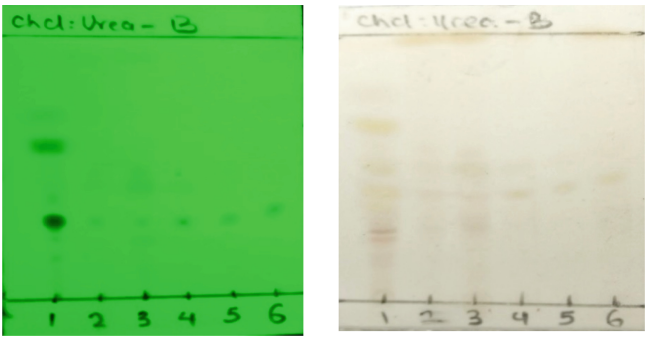
An aluminium TLC plate, silica gel coated with fluorescent indicator F_{254}, was used as a stationary phase to observe the presence of atranorin in liquid raw extracts. Migration distances and spots are determined under UV light with a wavelength of 254 nm. In the chamber, hexane/diethyl ether/formic acid are combined and used as a mobile phase system with a ratio of (130:80:20).

3 Results and Discussion

A. *Yield (%) of Extraction*

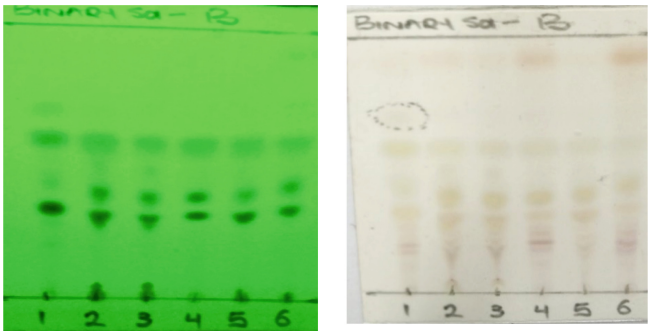
Due to the low volatility of DES, it is difficult to get the crude extract after MAE. Liquid-liquid fractionation using organic solvents was used to remove the SM from the DES/H_2O. Two layers were formed, organic solvent with the SM and DES/H_2O with the residue. The organic solvent layer was removed and allowed to evaporate under a fume hood (Table 2).

After liquid-liquid fractionation, the organic solvent layer containing the secondary metabolites was removed and allowed to evaporate under a fume hood. The yield (%) of MAE using ChCl:Urea and MAE using (BS of acetone and ChCl:1,4-butanediol) was calculated from the mass of the extract and the mass of the sample.



NSE 300s 240s 180s 120s 60s NSE 300s 240s 180s 120s 60s

Fig. 1. TLC for MAE using ChCl:Urea



NSE 300s 240s 180s 120s 60s NSE 300s 240s 180s 120s 60s

Fig. 2. TLC for MAE using Binary Solvent

B. *Qualitative Analysis Using TLC*

Qualitative analysis using TLC was used to determine the presence of the secondary metabolites from lichen extracts after MAE using DES and BS. The spots were visualized under short UV light at 254 nm as well as after spraying with diluted H_2SO_4 (Figs. 1 and 2).

The above results show that Deep Eutectic Solvents (DES) made up of ChCl:Urea and ChCl:1,4-butanediol synthesized by mixing the two components at elevated temperatures along with mixing until a homogenous solution was successfully formed. The TLC chromatogram shows that a wide range of secondary metabolites of lichen *Dirinaria applanata* can be extracted using DES-based MAE. Our finding agrees to previous reports [11, 12]. MThe secondary metabolites of lichen *Dirinaria applanata* were extracted in a relatively high yield (%). Moreover, application of DES in extraction process offers a greener and environmentally route in extraction [13]. This suggests that DES can be useful solvent in the MAE of the secondary metabolites from lichen *Dirinaria applanate* as well as from other organisme.

4 Conclusion

DES can replace the volatile and environmentally harmful organic solvents that are used in Normal Solvent Extraction. This extraction method is a green approach for the extraction of secondary metabolites from plant matrices.

Acknowledgements. Authors thank University of Sule Lamido, Kafin Hausa, Nigeria for supporting this research.

Conflict of Interests. All authors declare to have no conflict of interests.

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