Extraction of Phenolic Compound using Natural Deep Eutectic Solvent from Biomass Waste

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Abstract. Phenolic compounds are aromatic compounds known for their bioactive substances which can be found in plants. It has been widely used in various applications due to its antibiotics, anti-inflammatory, anti-allergic, and other benefits. There are various methods to extract the phenolic compounds from plants including ionic liquid, liquid-liquid extraction as well as supercritical extraction. However, all of these methods requires energy extensive, laborious processes, advanced technology, and generate toxic waste. Therefore, there is a growing need to find an alternative green extraction method to reduce the environmental impact while improving the efficiency of the extraction process. Thus, natural deep eutectic solvents (NADES), a combination of two or more components that comes from primary metabolites like organic acids, choline chloride, or sugar, are able to form liquids upon mixing with lower melting point of individual constituents due to hydrogen bond interactions were proposed as alternatives to conventional extraction methods. Therefore, this research determined the suitable combination of NADES solvents (hydrogen bond acceptor/donor ratio) for extraction of phenolic compounds from biomass waste which include young and mature coconut shells and coconut husk, banana peel, empty fruit bunch, and palm oil fruit husk. The extracted compound was analysed using fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) to identify the functional groups and type of phenolic compounds present. The best biomass waste was coconut shell and the best NADES combination was choline chloride and ascorbic acid at 1:2 molar ratio. The FTIR analysis of coconut shell extracted by NADES showed peaks at 3404 cm⁻¹ and 3523 cm⁻¹ indicating O-H stretching followed by 2915 cm⁻¹, 1388 cm⁻¹ to 1473 cm⁻¹, and 1674 cm⁻¹ showing C-H stretching, sp³ C-H band and C=C stretch respectively. As for GC-MS analysis, 26 compounds were detected and four phenolic compounds were identified at peaks 2, 13, 22, and 25. The research was successful in determining the best biomass waste and NADES combination for highest total phenol. The use of NADES was able to extract more phenolic compound from coconut shell than water due to the hydrogen bond between the choline chloride and ascorbic acid.

1. Introduction
Phenolic compounds are secondary metabolites that consist of aromatic rings with a hydroxyl group. They are readily available in the plant kingdom and possess beneficial properties such as antioxidant and anti-inflammatory. The use of phenolic compounds has been sought after for many years because of its benefits for health such as preventing cardiovascular diseases, diabetes, cancer, and obesity [1]. Phenols are also used in food technology as prevention from oxidation and bacterial growth [2]. In synthesis of metal nanoparticles, bioactive compounds like phenolic compounds are used as reducing
agent, making it possible to produce the small material without using toxic chemicals [3]. The source of phenolic compounds can be found easily in nature such as in trees, leaves, or fruits, including biomass waste which the majority comes from agricultural and food waste. The abundance of biomass waste in an agriculture-rich industry like Malaysia has high potential to be utilized for other purposes instead of being disposed to the landfill. This will encourage the wealth generation from waste or known as the circular economy. In order to seize the opportunity, biomass waste can be utilized to extract the phenolic compounds for various applications.

There are many ways to extract phenolic compounds including liquid-liquid extraction, ionic liquids (ILs), and supercritical liquid. However, these techniques involve toxic chemicals, laborious and advance technology which might cause pollution and higher production cost [4]. At the same time, these techniques also have poor biodegradability, sustainability, and biocompatibility of the extraction product. Therefore, this study will use a green method in extracting the active compound from biomass waste. This research will use deep eutectic solvent (DES) to extract the active compound that is trapped inside the biomass waste. DES is a combination of two or more safe components to form a eutectic mixture through hydrogen bond interaction, which has a melting point lower than that of each component. DES combination with lower melting point developed strong hydrogen bond interaction between hydrogen bond donor and acceptor. The strength of hydrogen bond will increase the extraction yield of desired compound [5]. The low viscosity of DES helps in mixing and the high density helps with the separation of the phases in the matrix [6]. Most common DES are based on choline chloride (ChCl), carboxylic acids, urea, citric acid, and other hydrogen-bond donors. Another classification of DES is natural deep eutectic solvent (NADES) that uses natural compounds for both hydrogen donor and acceptor, such as primary metabolites like organic acids and sugars [4].

Therefore, this study focuses to determine the suitable combination ratio of hydrogen bond donor and acceptor that form the best NADES for maximum extraction of phenolic compounds from biomass waste. Then, the extract will undergo the qualitative analysis using GC-MS to identify the unknown active compounds that can be extracted using the selected extraction method. Then the comparison study will be done to differentiate between normal liquid-liquid extraction method with NADES solvent method based on the type of active compounds that can be identified from GC-MS. This result will be of guidance for other researchers to explore further on this type of extraction process so that many important active compounds can be extracted and to be used for various applications.

2. Materials and Methods

The lab grade chemicals used in this experiment were choline chloride, glucose, sucrose, lactic acid, ascorbic acid, hydrochloric acid, and Folin Ciocalteu’s Reagent which were all purchased from BT Science Sdn. Bhd.

The types of equipment used in this experiment were UVILINE 9400 UV-Vis spectrophotometer, oven, centrifuge machine, magnetic stirrer, and pH indicator provided in the Bioprocess Engineering Laboratory in International Islamic University Malaysia (IIUM).

2.1. Screening of different biomass waste

The biomass waste used for screening were banana peel, empty fruit bunch (EFB), palm oil fruit husk, coconut shell, and coconut husk. The samples were dried overnight at 60 °C to ensure the removal of moisture without bioactive compounds degradation. Dried biomass was then ground and stored in air-tight containers. Phenolic compounds were extracted by conventional solvent extraction, using 5 g of biomass waste and 100 mL distilled water, stirred continuously at 90 °C for 20 minutes. The Folin-Ciocalteu method [7] was used to measure the total phenol content. The biomass waste with the highest total phenol content is then used for the remaining of the experiments.

2.2. Screening of different NADES combinations

Different combinations of hydrogen bond donors and hydrogen bond acceptors were used to determine the best combinations for phenolic compound extraction. The heating method was used to develop the natural deep eutectic solvents with some modifications [8]. For screening, the NADES mixtures were placed in a shaking incubator at 65 °C for 30 minutes at 100 rpm. The NADES was then used to extract
phenols from 5 g of biomass waste at room temperature shaker for 20 minutes at 100 rpm followed by filtering. The extracted solution was measured for its total phenol content using the Folin-Ciocalteu method.

2.3. *Fourier-transform infrared spectroscopy (FTIR) analysis*

The NADES, its components, and the extracted solution were analyzed under FTIR to observe the functional groups present. The sample was analyzed in solid phase. FTIR analysis was carried out in transmission mode range of 650 cm\(^{-1}\) to 4000 cm\(^{-1}\).

2.4. *Gas chromatography-mass spectrometry (GC-MS) analysis*

The extracted samples were observed using an Agilent 7890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer. A capillary column HP-5 with 30 m × 0.25 μm and an internal diameter of 0.32 mm was used. Before analyzing the samples, the retention time was locked by changing column pressure using standard samples. A constant pressure model was then used for the entire analysis process. 1 % of sample to 99 % of hexane as a solvent in 1.5 mL vial was filtered from a 2 mm syringe filter was prepared beforehand. The gas chromatography (GC) oven temperature was programmed from 80 to 300 °C via a ramp of 5 °C min\(^{-1}\) and maintained at 80 °C for 2 min and at 300 °C for 5 min. The mass spectrum (MS) was operated in full-scan mode from m/z, 50–700 for qualitative analysis or selected ion monitoring (SIM) mode for quantitative analysis. The inlet and MS transfer line temperatures were maintained at 300 °C and the ion source temperature was 300 °C. Sample injection (1 μL) was done using splitless mode [9].

3. **Results and Discussion**

3.1. *Screening of different biomass waste*

The five different biomass wastes were screened by extraction using water at pH 3. The total phenol extracted was recorded in Table 1. The results show that water extracted young coconut shell had the highest total phenol content among the other five biomass waste. This is because of the abundance of polyphenolic compounds due to its natural property as protection in plants. The shell mainly consists of cellulose and lignin that is similar to wood, making it optimum for phenolic extraction [10].

| No | Biomass waste          | Total phenol (mg GAE/L) |
|----|------------------------|-------------------------|
| 1  | Banana peel            | 739.00                  |
| 2  | Coconut shell          | 2427.50                 |
| 3  | Coconut husk           | 1199.50                 |
| 4  | Empty fruit bunch      | 102.00                  |
| 5  | Palm oil fruit husk    | 150.50                  |

3.2. *Screening of different NADES combinations*

Since coconut shell was proven the best biomass waste with the highest total phenol, different combinations of chemicals with different molar ratios were screened. The eutectic mixture combinations and their total phenol extracted from the coconut shell are shown in Table 2. Based on the result, the best NADES combination was choline chloride with ascorbic acid at 1:2 molar ratio with 3715.67 mg GAE/L total phenol extracted. This is closely followed by glucose and ascorbic acid at 1:2 molar with 3682.67 mg GAE/L total phenol and sucrose with ascorbic acid at 1:2 molar ratio with 3670.00 mg GAE/L total phenol. The pattern here is the presence of ascorbic acid. Even though the use of NADES helps to produce hydrogen bond for optimum phenolic extraction, the use of ascorbic acid affects the polyphenols positively [11]. This is proven by the total phenol amount maintaining at high numbers when using NADES with ascorbic acid.
Table 2. The different chemical combinations and molar ratios of NADES and their total phenol extracted from coconut shell.

| Acceptor  | Donor       | Molar Ratio | Total Phenol (mg GAE/L) | Acceptor  | Donor       | Molar Ratio | Total Phenol (mg GAE/L) |
|-----------|-------------|-------------|-------------------------|-----------|-------------|-------------|-------------------------|
| Water pH 3| NA          |             | 454.00                  | ChCl      | Ascorbic Acid | 2:1         | 3678.00                |
| Water pH 7| NA          |             | 972.00                  | Glucose   | Lactic Acid  | 1:1         | 602.00                  |
| ChCl      | Glucose     | 1:1         | 628.00                  | Glucose   | Lactic Acid  | 1:2         | 442.00                  |
| ChCl      | Glucose     | 1:2         | 548.00                  | Glucose   | Ascorbic Acid | 2:1         | 515.00                  |
| ChCl      | Glucose     | 2:1         | 543.00                  | Glucose   | Ascorbic Acid | 1:1         | 3611.00                |
| ChCl      | Sucrose     | 1:1         | 528.00                  | Glucose   | Ascorbic Acid | 1:2         | 3682.67                |
| ChCl      | Sucrose     | 1:2         | 583.00                  | Glucose   | Ascorbic Acid | 2:1         | 3557.00                |
| ChCl      | Sucrose     | 2:1         | 658.00                  | Sucrose   | Lactic Acid  | 1:2         | 587.50                  |
| ChCl      | Lactic Acid | 1:1         | 1510.50                 | Sucrose   | Lactic Acid  | 2:1         | 434.00                  |
| ChCl      | Lactic Acid | 1:2         | 1497.50                 | Sucrose   | Ascorbic Acid | 1:1         | 3558.50                |
| ChCl      | Lactic Acid | 2:1         | 2353.00                 | Sucrose   | Ascorbic Acid | 1:2         | 3649.50                |
| ChCl      | Ascorbic Acid | 1:1     | 3624.00                 | Sucrose   | Ascorbic Acid | 2:1         | 3670.00                |
| ChCl      | Ascorbic Acid | 1:2     | 3715.67                 | Sucrose   | Ascorbic Acid | 2:1         | 3670.00                |

*NA: not applicable
*ChCl: choline chloride

3.3. Fourier transform infrared spectroscopy (FTIR) analysis

Figure 1 shows the FTIR analysis of a) choline chloride, b) ascorbic acid and c) NADES of choline chloride and ascorbic acid at 1:2 molar ratio. The spectra of choline chloride show a very strong band at 3223 cm⁻¹ that indicates intramolecular hydrogen bond since both hydrogen donor and hydrogen acceptor is present in the compound [12]. The weak peak at 2845 cm⁻¹ also proves the presence of hydrogen bond. The peak at 1482 cm⁻¹ refers to the alkyl group that is present in choline chloride [13]. In figure 1 b), the peaks at 3524 cm⁻¹ and 3407 cm⁻¹ shows the OH stretching that is present in the ascorbic acid. Followed by the peak at 2916 cm⁻¹ for C=C stretching and peak at 3032 cm⁻¹ for sp2 C-H stretching. The band at 1754 cm⁻¹ and 1672 cm⁻¹ corresponds to C=O stretching and C=C stretching which are in line with ascorbic acid properties. From figure 1 c), it shows that NADES is an overlap of choline chloride and ascorbic acid. Changes of vibrations on wavenumbers correspond to the change in bond length which can depict the establishment of hydrogen bond [14]. The OH stretching from choline chloride and ascorbic acid broadens and widens in DES at 3344 cm⁻¹, the shift indicates the formation of hydrogen bond between the two components. The peaks at 2883 cm⁻¹ and 2774 cm⁻¹ shows the presence of C-H stretching and the many peaks at 1709 cm⁻¹ to 1787 cm⁻¹ shows the presence of C=O stretching.

Figure 2 shows FTIR analysis between extract from coconut shell using a) water at pH 3 and b) NADES. Between the two spectra, coconut shell extracted with water (CSW) has fewer peaks than coconut shell extracted with NADES (CSNADES). This is due to the lower amount of different functional groups detected in the extract. Meanwhile, the NADES extracted more compounds from a wider range of functional groups. In figure 2 a), the peak at 3304 cm⁻¹ indicates OH stretching. Since this extract used water as a solvent, large OH stretching may also cause noise and inaccuracy to detect other functional groups. Apart from that, the peak at 1077 cm⁻¹ corresponds to C-O stretching and peak at 1651 cm⁻¹ indicates C=O vibration. Not many peaks are enough to make deductions on functional groups present in the extract. Nonetheless, figure 2 b) shows more characteristic peaks. The OH stretching is evident between peaks 3404 cm⁻¹ and 3523 cm⁻¹. The peak at 2915 cm⁻¹ indicates a C-H stretch and peaks between 1388 cm⁻¹ to 1473 cm⁻¹ corresponds to the sp3 C-H band. A C=O stretch is proven at 1674 cm⁻¹. All of these peaks help to indicate the presence of phenol in the extract which consists of an aromatic ring with a hydroxyl group.
3.4. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was used to detect and compare the compounds extracted from coconut shell using water and NADES. Table 3 shows the compounds present in the extract after going through liquid-liquid extraction with water at pH 3 and NADES. A visual representation is shown in Figure 3. From the table, a total of 26 different compounds from varying chemical groups were detected. From the compounds present in both extracts, carotenoids were more prominent among the other chemical groups. Lycoxanthin, astaxanthin, lycopene, and α-Carotene have dietary benefits, helps reduce blood pressure, and positively affect neurodegenerative diseases [15]. Alkaloids, acids, esters, aldehydes, and ketones with similar antioxidant properties were also present within the extracts.

For liquid-liquid extraction, only one phenolic compound is present, at peak number 13, the 2-Nonaprenyl-6-methoxyphenol. In contrast, NADES was able to extract four phenolic compounds from coconut shell at peak numbers 2, 13, 22, and 25 which are the 1,2,4-Benzencarboxylic acid, 1,2-dimethyl ester, the 2-Nonaprenyl-6-methoxyphenol, the Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane, and the 2',6'-Bis(trimethylsiloxy)acetophenone respectively. Both extraction methods were able to extract a good amount of antioxidants that were trapped within the biomass waste. Nonetheless, NADES was more superior in extracting phenolic compounds than water. This is due to the difference in polarity between water and NADES with phenolic compounds. Even though water is a green solvent that can be utilized for extraction, the hydrogen bond network in water is easily disintegrated with high temperature and pressure [16]. NADES, utilizing the hydrogen bond between choline chloride and ascorbic acid, helps to improve the extraction of phenolic compounds from coconut shell.
Table 3. List of compounds detected in coconut shell extract using GC-MS.

| No | Compound Name                                                                 | Liquid liquid extraction method with water at pH3 | Natural deep eutectic solvent extraction method |
|----|--------------------------------------------------------------------------------|--------------------------------------------------|------------------------------------------------|
| 1  | 1-Methyl-3-phenylindole                                                       | 3351.33                                          | Tungsten, pentacarbonyl(4,5-diethyl-2,2,3,3- trimethyl-1-phenyl-1-phospha-2-sila-5- boracacyclohex-3-ene-P1) , (oc-6,22) |
| 2  | 1,2,4-Benzeneacarbonylic acid, 1,2-dimethyl ester                             | 3429.75                                          | 2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2- yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane |
| 3  | D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4,6-trixo-5-pyrimidiny1]-1- methylbutyl 2,3,4-tris-O-trimethylsilyl]-methyl ester | 3451.79                                          | Lycoxanthin                                      |
| 4  | 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-                            | 3464.34                                          | 5-Aminododecachydro-as-indacen-4-ol             |
| 5  | Decaethylene glycol, bis(pentafluoropropionate)                               | 3474.14                                          |                                             |
| 6  | Zeaxanthin                                                                    | 3498.24                                          |                                               |
| 7  | Rhodovibrin                                                                   | 3521.91                                          |                                               |
| 8  | 2-(5-5-(5-[Cyano-(9,9-dimethyl-1,4-dioxo-7-aza- spiro[4,4]non-7-en-8-yl]-methylene)-3,3- dimethylpyrrolo-2-yldienethyl]-3,3-dimethyl- e1-pyrrolin-5-yldienethyl-4,4,5-trimethyl-e1-pyrroline-5-car bonitrile] | 3584.83                                          |                                               |
| 9  | Lycoxanthin                                                                   | 3594.89                                          | Tris(cyclopentadienyl-co)l-hexapropenylenebenzene |
| 10 | Cyclostirloxiane, hexamethyl-                                                | 3595.12                                          | 5H-Indeno[1,2-b]pyrazin-5-one, 7,8,9-tribromo- N,N'-diethyl-1,2,3,4-tetrahydro-6- (phenoxycarbonyl)- |
| 11 | a-Carotene                                                                    | 3599.8                                          | Dimethoxylycopene                               |
| 12 | 2-Fluoro-5-trifluoromethanobenzic acid, ethyl ester                          | 3601.59                                          |                                             |
| 13 | 2-Nonaprenyl-6-methoxyphenol                                                 | 3609.56                                          |                                             |
| 14 | Aresenour acid, tris(trimethylsilyl) ester                                    | 3699.27                                          |                                             |
| 15 | Rhodoviolascin                                                                | 3709.56                                          |                                             |
| 16 | Silane, [[3a,5a,11a,20S]-pregnane-3,11,17,20,21-penta[1]pentakis[1]pentakis[trimethyl- Astaxanthin | 3726.86                                          |                                             |
| 17 | 4-Methyl-2,4-bis(4'-trimethylsilyl-oxoyloxy)pentene-1                          | 3754.18                                          |                                             |
| 18 | 2,4-IImidazolidinedione, 5-[3,4- bis(trimethylsilyl)oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)- | 3755.57                                          |                                             |
| 19 | 2,3-Dichloro-1,3-bis(norbornadien-2-yl)-1,3-bis[3- trimethylsilylpropoxy]disiloxane | 3756.57                                          | 2,4-IImidazolidinedione, 5-[3,4- bis(trimethylsilyl)oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)- |
| 20 | Tetrahydropirilloxanthin                                                      | 3758.73                                          | 7aH-Cyclopenta[a]cyclopropa[1]cyclusdecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a- dodecachydro-1,3,6,9-pentamethyl-1,2,4,7,10,11-pentaaacetate |
| 21 | 1,4-Dibromo-2-butenenes                                                       | 3774.27                                          | Trimeityl[1-(1,1,3,3,3- tetramethylbutyl)phenoxysilane |
| 22 | 2,2-Bis[4-[(4-chloro-6-(3-ethenylphenoxy)-1,3,5- triazin-2-yl)oxy]phenyl]propene                                             | 3791.46                                          | 3,2,4-diphenyl-1-p-tolyl-cycloprop-2-yl]ester-O-ethyl ester |
| 23 | Pregn-4-ene-3,11,20-trione, 6,17,21-tris(trimethylsilyl)oxy]-, 3,20-bis(O-methylxime), (6u) | 3792.62                                          | Prosta-5,13-dien-1-oc acid, 9,11,15- (trimethylsilyl)oxy]-, trimethylsilyl ester, (5Z,9a,11a,13E,15S)- |
| 24 | Milbemycin B, 5-demethoxy-5-one-6,28-anhydro-25-ethyl-4-ethyl-13-chloro-oxime | 3818.85                                          | 2,5-Bis(trimethylsilyloxy)acetophenone |
| 25 | 1-Methyl-2-phenylbenzimidazole                                                | 3820.75                                          | Astaxanthin                                      |
| 26 | Cyclolabdaester                                                               | 3842.44                                          |                                             |
Figure 3. GC-MS analysis of extracted coconut shell using a) water at pH 3 and b) NADES.

4. Conclusion
This study has been successful in screening the best biomass waste and the best NADES combinations with the highest total phenol. It can be concluded that the coconut shell consists of the highest phenol among the biomass waste and that choline chloride with ascorbic acid at 1:2 molar ratio has the highest phenol extraction capacity. The use of NADES improves the phenolic extraction as compared to using water at pH 3. This is due to the hydrogen bonding between the components and the use of ascorbic acid that positively affects the phenol amount.

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